Template/ Pro forma for Submission

NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	**GBPNI/NMHS-2017-18/HSF-04/600**	**Date of Submission:**	3	1	1	2	2	0	2	1
			d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

FELLOWSHIP TITLE (IN CAPITAL)

"Himalayan Research Fellowship Programme"

Sanctioned Fellowship Duration: *from* (**28/03/2018**) *to* (**28/02/2021**).

Extended Fellowship Duration (if applicable): *from* (1/03/2021) *to* (31/12/2021).

***Submitted to*:**

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***Submitted by*:**

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NMHS-Final Technical Report (FTR) *template*

NMHS- Institutional Himalayan Fellowship Grant

DSL: *Date of Sanction Letter*

2	8	0	3	**2**	**0**	**1**	**8**
d	d	m	m	y	y	y	y

DFC: *Date of Fellowship Completion*

3	1	1	2	**2**	**0**	**2**	**1**
d	d	m	m	y	y	y	y

Part A: CUMULATIVE SUMMARY REPORT

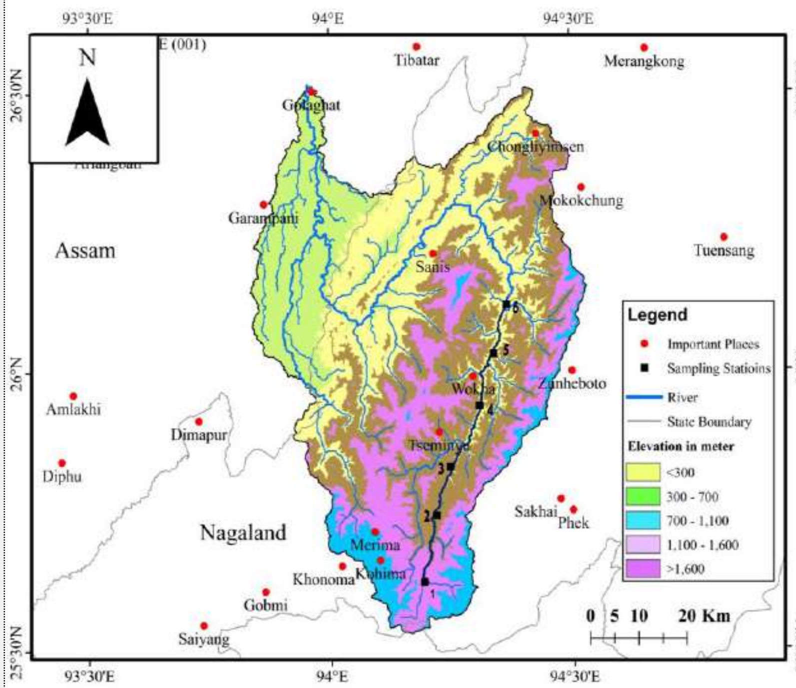
1. Details Associateship/Fellowships

1.1 Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	**GBPNI/NMHS-2017-18/HSF-04/600**
Name of the Institution/ University:	Assam Agricultural University
Name of the Coordinating PI:	Dr. Sarada Kanta Bhagabati Dr. Rajdeep Dutta
Point of Contacts (Contact Details, Ph. No., E-mail):	

1.2 Research Title and Area Details

i.	Institutional Fellowship Title:	**Study on Limnological profile and Fish Diversity of River Doyang, Northeastern Himalayan Region, Nagaland.**					
ii.	IHR State(s) in which Fellowship was implemented:	**Nagaland**					
iv.	Scale of Fellowship Operation	Local:		Regional:		Pan-Himalayan:	√

iii.	Study Sites covered	 Map of the study area.
v.	Total Budget Outlay (Crore) :	INR 0.8034840

1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration	
		From	To
Research Associates			
Sr. Research Fellow	1	**1/08/2018**	**31/12/2021**
Jr. Research Fellows			
Project Fellows			

2. Research Outcomes

2.1. Abstract

Background: Doyang is the largest river of Nagaland and approximately lies between 25°40´44´´ and 26°13´74´´ N Latitude and between 94°14´31´´ and 94°0´54´´ E Longitude. The Doyang river flows through a length of about 152 km within the state of Nagaland, almost dividing the state into two equal halves, traversing different climatic and geomorphological terrains and receiving tributaries from the districts of Kohima, Zunheboto, Mokokchung and Wokha. On the riparian valleys, there are about 65 villages directly or indirectly availing its resources which makes it one of the most socially, culturally and economically important river of the state.

Aims and Objective: Keeping these views in mind, a field level study was conducted for a period of 2 years commencing from January, 2019 to December, 2020 along 152 km stretch in the Doyang River system with six sampling stations, elevation ranging from 867 MSL to 263 MSL, to evaluate the diversity of fish fauna, their conservation status, habit characterization and any anthropogenic stress on the river Doyang. In addition to these a laboratory-based toxicity study was also conducted for one year from January, 2021 to December, 2021, to evaluate any anthropogenic stress caused by the used of the agricultural pesticides to the fish diversity in the agricultural field adjacent to the river Doyang.

Methodology: The materials used and methodology followed in different aspects like study of the river, collection, preservation and identification of Ichthyofauna; sampling of physico-chemical parameters; qualitative and quantitative analyses of plankton samples and identification of anthropogenic factor (including toxicity study) effecting fish fauna. Regular field trips were conducted in six selected stations at monthly intervals for evaluation of Ichthyofaunal biodiversity, collection of water samples for physico-chemical and biological analysis. All the analysis was carried out following standard protocols.

Results: Overall study showed that the water and soil parameters of the Doyang river was congenial for fishes, however Principal Component analysis showed that during winter and monsoon, PC1 was largely and positively affected by pollution indicating parameters, whereas during post monsoon and pre monsoon, PC1 was largely and positively affected by the other physico chemical parameters. This may be due to pollutants affecting water quality in rivers have temporal and spatial variations. Moreover, station 2, 3 and 4 also showed few anthropogenic activities like over fishing, sand and boulder mining from the river bed. During the present investigation a total of 52 fish species belonging to 28 genera, 11 families and 5 orders are recorded from 6 selected sampling stations of the river Doyang, Nagaland India. Among the orders, the Cypriniformes formed the largest group with a contribution of 3 (27.27 %) families, 19 (67.56%) genera and 35 (67.32%) species. The order Perciformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 3 (27.27 %) families, 3 (10.71%) genera and 9 (17.30%) species followed by Siluriformes with 3 (27.27%) family, 4 (14.29%) genera and 6 (11.53%) species and symbranchyformes and Baloniformes with 1 (9.09) family, 1 (3.57%) genus and 1 (1.92%) species each. According to IUCN conservation status (2021), 52 species recorded shows that the highest species were recorded under least concern (LC) category with a total no of 39 and contributed 75 %. under LC category, the major species contribution is from the family Cyprinidae with 20 (38.46 %) followed by Channidae 5 (9.61 %), Nemacheilidae 4 (7.6 %), Silorhynchidae and Sissoridae with 2 (3.86 %) each, Bagaridae, Amblycepitidae, Bedidae, Anabantidae, Mastacembalidae and Belonidae with 1 species

contributed 1.92 % each. Under near threatened (NT) category Cyprinidae and Sissoridae contributed 3 (5.76%) and 1 (1.92 %) species respectively. Like that, the family Cyprinidae represented the vulnerable (VU) category with 2 (3.84 %) species each. One species which contributed 1.92 % under Cyprinidae family represented the endangered (EN) category. A total of 6 nos, 2 (3.84%) from cyprinidae, 1 (1.92%) from each Nemacheilidae, Sissoridae, Channidae and Bedidae respectively falls under the not evaluated (NE) category of IUCN conservation status. For the first time Cytochrome Oxidase subunit I (COI) of mitochondrial gene sequences of Thirty-eight (38) (approx. 800%) of the total collected fish species sequences was generated and successfully submitted to NCBI gene data base and accession number was obtained. During the present study a total of 30 genera of plankton was recorded out of which phytoplankton consist of 18 genera under 3 family namely Chlorophyceace, Bacillariophyceae and Cyanophyceae and Zooplankton of 12 genera under 3 family namely Cladocera, Rotifera and Copepoda. According to Palmer's index of pollution the total score of Algal Genus Pollution Index (AGPI) of sites S1& S2< S3< S4<S5, S6 were calculated to be 2, 5, 7 and 9 respectively. The total scores of S1 and S2 showed 4 indicating probable lack of organic pollution while S5 and S6 showed moderate pollution due to anthropogenic factors or human interference. The study also implied that primary productivity of the river was found to be in the lower side with the average value ranging from with the average for GPP (0.116 g C m^{-3} d^{-1}) and NPP (0.057 g C m^{-3} d^{-1}). Anthropogenic factors encountered during the regular sampling in the Doyang river system are Constant dumping of solid waste like polythene bags, paper waste and domestic sewage in the river, removal of sand gravel and boulders from the river bed, alteration of river course, use of pesticide for protection of agricultural crops from pest and insects in the adjoining paddy fields of the river system, electric fishing, blasting and poisoning in the river.

A laboratory-based toxicity test was also conducted based on the use of the commercial insecticide in the adjoining paddy fields which might create an anthropogenic stress on the ichthyofaunal diversity. Therefore, the aim of the study was to evaluate the toxicity effects of commercial-grade Imidacloprid (Premise, 30.50%, SC) to standard non targeted test organism, *Cyprinus carpio* var. *communis* (Common carp) using biological endpoints like histological analysis, haematological parameters, serum biochemical analysis, antioxidant responses, neurotoxicity (AChE activity), genotoxicity (Micronucleus test) and gene expression study. The current study reveals that 96hr LC_{50} value of commercially available Imidacloprid was 208.38 ppm (173.66 - 262.37) with 95% confidence interval. Effect of 96hr LC_{50} concentration was determined by exposing test fish to above said concentration under laboratory static renewal test and analysis was carried out on every 24, 48, 72, and 96hr, whereas for 28 days chronic exposure semi static renewal test was deployed with 3 sub lethal concentrations $LC_{50}/8$ (T_1= 26.04 ppm), $LC_{50}/10$ (T_2=20.83 ppm,) and $LC_{50}/12$ (T_3=17.36 ppm) which were selected based on the calculated 96hr LC_{50} value and analysis was carried on 7th, 14th, 21st and 28th day. Behavioural alterations like jumping movement, restlessness, hyperventilation, hyperactivity, gulping, coughing and corkscrew swimming at surface and bottom of the tank were observed. Enhanced mucus secretion, loss of buoyancy and string of faeces hanging from anus or on the tank was also observed during acute exposure to 96hr LC_{50} concentration for 24, 48, 72, and 96 hrs. Marked histological alterations in liver like exocrine pancreatic acini, hepatic degeneration and mononuclear infiltration were observed; in gills epithelial lifting, oedema, telangiectasis in secondary lamellae, lamellar fusion while in kidney expansion of Bowman's space, cloudy swelling of epithelial cells, necrosis of several renal tubules and multiple focal areas of inter-tubular haemorrhage were observed during both acute and chronic exposure. Results showed that immune-haematological parameters like haemoglobin (Hb), packed cell volume (PVC), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Nitroblue tetrazolium bursts activity (NBT), lysozyme activity (LA) altered significantly (*$p<0.05$) during both acute and chronic exposure. Serum biochemical parameters like Glucose, Cholesterol, Phospholipid, Triglyceride, HDL, VLDL, Magnesium, AST, ALT increased significantly whereas protein, albumin, globulin, A:G ratio, LDL significantly decreased during both acute and chronic exposure to IMI. Significant induction in oxidative stress enzymes (SOD, CAT, GPx, AST and ALT) and Oxidative stress biomarkers (ROS, MDA) in liver, gill and brain tissues were also observed during acute exposure, whereas in chronic exposure the same was observed in dose and time dependent manner. Significant reduction in brain AChE enzyme activity due to inhibition of acetylcholine esterase and DNA damage through significant induction of micronuclei formation in the erythrocyte of fish blood was clearly observed. Upregulation of HSP70 and CYP1A gene in both liver and gill tissues of exposed fish were observed on 7th,14th, 21st and 28th day in dose and time dependent manner.

Conclusion and Recommendation: River Doyang is enjoying its Ichthyofaunal diversity but in few stations like 1 and 2 diversity is quite less might be due to some anthropogenic factors like river mining, destructive fishing and might be due to use of insecticide in adjoining paddy fields, which enters the river system as agricultural runoff. Furthermore, the information will also help researchers and policy makers to aid them in their efforts in effective management of the important river system. Frequent exploration has to be undertaken to explore the ichthyofaunal of the said river system and identify the anthropogenic activities creating threat to fish fauna and its habitat. Awareness and training programmes have to be conducted for the local people regarding the conservation of the river and their use in livelihood generation.

2.2. Objective-wise Major Achievement

S. No.	Cumulative Objectives	Major achievements (in bullets points)
1.	To systematically study and record ichthyo faunal biodiversity of selected river systems of North Eastern Himalayan region	• During the present investigation a total of 52 fish species belonging to 28 genera, 11 families and 5 orders are recorded from 6 selected sampling stations of the river Doyang, Nagaland India. • Among the orders, the Cypriniformes formed the largest group with a contribution of 3 families, 19 genera and 35 species. The order Perciformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 3 families, 3 genera and 9 species followed by Siluriformes with 3 family, 4 genera and 6 species and symbranchyformes and Baloniformes with 1 family, 1 genus and 1 species each. • For the first time Cytochrome Oxidase subunit I (COI) of mitochondrial gene sequences of Thirty-eight (38) (approx. 80%) of the total collected fish species sequences was generated and successfully submitted to NCBI gene data base and accession number was obtained. • During the present study a total of 30 genera of plankton was recorded out of which phytoplankton consist of 18 genera under 3 family namely Chlorophyceace, Bacillariophyceae and Cyanophyceae and Zooplankton of 12 genera under 3 family namely Cladocera, Rotifera and Copepoda. • During the present study, a total of 18 genera of phytoplankton were recorded. Three majors groups of phytoplankton viz. Chlorophyceace represented by 9 genera, Bacillariophyceae represented by 5 genera and Cyanophyceae represented by 4 genera were found in the different stations along the Doyang river system. • During the present study, a total of 12 genera of zooplanktons belonging to three categories of zooplankton viz. Cladocera represented by 6 genera, Rotifera represented by 4 genera, Copepoda represented by 2 genera was collected from the Doyang river.

2.	To assess conservation status of the fish species of selected river systems based on field surveys	• The IUCN conservation status (2021) of the 52 recorded species shows that the highest species were recorded under least concern (LC) category with a total no of 39 and contributed 75 %. under LC category, the major species contribution is from the family Cyprinidae with 20 (38.46 %) followed by Channidae 5 (9.61 %), Nemacheilidae 4 (7.6 %), Silorhynchidae and Sissoridae with 2 (3.86 %) each, Bagaridae, Amblycepitidae, Bedidae, Anabantidae, Mastacembalidae and Belonidae with 1 species contributed 1.92 % each. • Under near threatened (NT) category Cyprinidae and Sissoridae contributed 3 (5.76%) and 1 (1.92 %) species respectively. • Like that, the family Cyprinidae represented the vulnerable (VU) category with 2 (3.84 %) species each. • One species which contributed 1.92 % under Cyprinidae family represented the endangered (EN) category. • A total of 6 nos, 2 (3.84%) from cyprinidae, 1 (1.92%) from each Nemacheilidae, Sissoridae, Channidae and Bedidae respectively falls under the not evaluated (NE) category of IUCN conservation status (2021).

3.	Habitat characterization of selected fish species of the river system	• The geomorphology of river Doyang is divided into three zones upper, middle and lower zone along with mean gradient river bank and riparian zone. • The river beds in the upper and middle zones were hard and rocky and mostly composed of boulders, cobbles and gravels etc. • In the lower zone, the river bed was soft due to the presence of sand and clayey type of soil. • These uneven distributions of the sediment were greatly influenced by the slope gradient of the river bed. • The upper zone with a mean inclination of 0.52m/sec had fast flow regime hence the large boulders were dislodged and carried lower down the river. • Similarly, the middle zone with a mean slope gradient of 0.43 m/sec had a strong flow regime which powers the transfer of smaller rocks and gravels within it. • While the lower zone of the river, with the reduction in the mean slope gradient 0.27 m/sec of the river bed was mostly composed of sand, silt and clay. • River bank was more stable in the upper zone of the river due to armouring by the rocky sediments, though in the middle and lower zone of the river, the river banks were partly stable. • The river also carried and deposited large and medium wood debris on the riverbed and bank in the upper zone, while in the middle and lower zone of the river smaller wood debris were observed mostly. • The Riparian zones were primarily composed of woody forest and shrubs in the upper and middle zone though it was sparse in the lower zone of the river. • Human habitations on river banks were the main source of discharging the sewage, farmyard washings, agricultural waste, pesticides etc. into the river system. • However, the human population size was found to be small in the upper zone and moderate and sparse in the middle and lower zones of the river respectively. • Moreover, river mining in huge quantity using Bulldozer and dumper was seen in almost all the three zones. Other major pollution of the river was not encountered and it was also evident from the physico-chemical analysis of water samples. •

4.	To identify anthropogenic factors affecting ichthyo fauna of the river systems (if any) and to find out the mitigation measures (if required)	• According to Palmer's index of pollution the total score of Algal Genus Pollution Index (AGPI) of sites S1, S2< S3< S4<S5, S6 were calculated to be 2, 5, 7 and 9 respectively. The total scores of S1 and S2 showed 4 indicating probable lack of organic pollution while S5 and S6 showed moderate pollution due to anthropogenic factors or human interference • Water quality index (WQI) developed using 15 physico-chemical parameters of water provides a positive relationship with the seasonal changes. Maximum WQI values were recorded during monsoon season from all the six stations followed by post monsoon (winter) and pre-monsoon. The WQI value showed a mixed pattern of changes in all the seasons. WQI of the upstream stations from 1 to 2 is lower than the downstream stations, i.e., 5 and 6 showing the increase in pollution level while moving downstream of the river. • PCA analysis also showed that winter and monsoon, PC1 was largely and positively affected by pollution indicating parameters, whereas during post monsoon and pre monsoon, PC1 was largely and positively affected by the other physico chemical parameters. • Anthropogenic factors encountered during the regular sampling in the Doyang river system are • Constant dumping of solid waste like polythene bags, paper waste and domestic sewage in the river, • Rremoval of sand gravel and boulders from the river bed, • Aalteration of river course, • Use of pesticide for protection of agricultural crops in the adjoining paddy fields of the river system • Electric fishing • Blasting • Use of poison in the river side were also frequently reported by the locals.

2.3. Outputs in terms of Quantifiable Deliverables*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, and Reason thereof:
1.	A database of fish species of the river ecosystem under study indicating their true conservation status and	Taxonomic and molecular characterisation of fish fauna of the river covering its diversity, distribution, Habitat suitability anthropogenic	Taxonomic identification of 52 fish species and molecular characterisation of 38 species out of 52 has been done during the period	

	habitat with supporting photographs.	factor affecting if any		
2.	Identification of anthropogenic stress factors affecting ichthyo fauna of the river ecosystem (if any) and its possible mitigation measures (if required).	Any kind of anthropogenic factors affecting fish and their habitat are being constantly monitored.	• Constant dumping of solid waste like polythene bags, paper waste and domestic sewage in the river. • Constant removal of sand gravel and boulders from the river bed. • Alteration of river course. • Use of pesticide for protection of agricultural crops in the adjoining paddy fields of the river system. • Electric fishing, blasting and poisoning in the river side were also frequently reported by the locals. • The study will also provide a better understanding of the ichthyofauna and ecology of the river and gives base line information that can be used in creating better conservation strategies. • Furthermore, the information will also help non taxonomist, researchers and policy makers to aid them in their efforts in effective management of the important river system.	Additional laboratory-based toxicity study was being carried on commercial grade Imidacloprid, which was frequently used in large quantity in the adjoining paddy field to protect agricultural crops from insects and pests. This test was conducted to observe any negative effects of this pesticide on non-target model test organism which might be acting factor anthropogenic factor.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number/ Brief Details	Remarks/ Enclosures
1.	New Methodology developed:	-	
2.	New Models/ Process/ Strategy developed:	-	

S. No.	Particulars	Number/ Brief Details	Remarks/ Enclosures
3.	New Species identified:	-	
4.	New Database established:	• Total number of fish fauna • Conservation status of fish • Planktons data (Phyto and Zooplankton) • Palmer index • 15 physico-chemical water quality data. • Water quality index • Soil parameters • Primary productivity of the river system. • Toxicology data base for the commercially available pesticide Imdacloprid, Premise (30.50%SC) for the region.	**ANNEXURE I (PART B COMPREHENSIVE REPORT)**
5.	New Patent, if any:	-	
	I. Filed (Indian/ International)	-	
	II. Granted (Indian/ International)	-	
	III. Technology Transfer (if any)	-	
6.	Others, if any:		

3. Technological Intervention

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology	-	
2.	Diffusion of High-end Technology in the region	-	
3.	Induction of New Technology in the region	-	
4.	Publication of Technological / Process Manuals	-	
	Others (if any)		

4. New Data Generated over the Baseline Data

S. No.	New Data Details	Existing Baseline	Additionality and Utilisation of New data (*attach supplementary documents*)
1.	**52 fish species**	**Imnatoshi and Ahmed, 2013, 46 fish species**	**ANNEXURE I (Table 2a)**
2.	38 out of 52 identified species has been barcoded.	**First time**	**ANNEXURE I (Table 10)**
3	Diversity indices of fish fauna		**ANNEXURE I (Table6, 7 and 8)** **Figure 4, 5 and 6.**
4	Conservation status of the fish fauna	**Imnatoshi and Ahmed, 2013, 46 fish species**	**ANNEXURE I (Table 9)**
5	Plankton diversity	**No report earlier**	**ANNEXURE I (Table 11)**

6	Diversity indices of plankton	**No report earlier**	**ANNEXURE I (Table 14, Figure 12)**
7	Palmer index has been developed for the said river system	**No report earlier**	**ANNEXURE I (Table 16)**
8	15 water quality parameters	**Lkr, *et al*., 2020**	**ANNEXURE I (Figure 13 to 27)**
9	Water quality Index	**Lkr, *et al*., 2020**	**ANNEXURE I (Figure 28)**
10	Soil Parameters	**Lkr, *et al*., 2020**	
11	Primary Productivity	**No report**	**ANNEXURE I (Table 28, Figure 29)**
12	Toxicological data for the commercial insecticide, Imidacloprid Premise (30.50%SC)	**No report for that region**	**ANNEXURE I (ANTHROPOGENIC FACTORS 3.12)**

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Beneficiaries
1.	Sustainable Development Goals (SDGs)	-		
2.	Climate Change/INDC targets	-		
3.	International Commitments	-		
4.	National Policies	-		
5.	Others collaborations	-		

6. Financial Summary (Cumulative)*

*Please attach the **consolidated and audited Utilization Certificate (UC) and Consolidated and *Year-wise* Statement of Expenditure (SE)** separately, *ref*. **Annexure I.**

7. Quantification of Overall Research Progress

S. No.	Parameters	Total (Numeric)	Attachments* with remarks
1.	IHR State(s) Covered:	*1*	**Figure 1 (ANNEXURE I)**
2.	Fellowship Site/ LTEM Plots developed:		
3.	New Methods/ Model Developed:		
4.	New Database generated:	*11*	**ANNEXURE I**
5.	Types of Database generated:	*3*	**ANNEXURE I**
6.	No. of Species Collected:	*52*	**ANNEXURE I (Table 2)**
7.	New Species identified:	-	
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	• *1. MFSc* • *1. PhD*	**APPENDIX I**
9.	No. of SC Himalayan Researchers benefited:		
10.	No. of ST Himalayan Researchers benefited:		
11.	No. of Women Himalayan Researchers empowered:		
12.	No. of Knowledge Products developed:		

13.	No. of Workshops participated:		
14.	No. of Trainings participated:	2	***APPENDIX 2***
15.	Technical/ Training Manuals prepared:		
	Others (if any):		

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/ Enclosures***
		National	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)	1			***APPENDIX 3***
2.	Book Chapter(s)/ Books:				
3.	Technical Reports/ Popular Articles				
4.	Training Manual (Skill Development/ Capacity Building)				
5.	Papers presented in Conferences/ Seminars		1		***APPENDIX 4***
6.	Policy Drafts (if any)				
7.	Others (specify)	1 (Under Review)	2 (Under Review)		***APPENDIX 5***

9. Recommendation on Utility of Research Findings, Replicability and Exit Strategy

9.1 Utility of the Fellowship Findings

S. No.	*Research Questions Addressed*	*Succinct Answers (within 150–200 words)*
1.	How is the ichthyofaunal biodiversity status of the river system under study?	• During the present investigation a total of 52 fish species belonging to 28 genera, 11 families and 5 orders are recorded from 6 selected sampling stations of the river Doyang, Nagaland India. • Among the orders, the Cypriniformes formed the largest group with a contribution of 3 (27.27 %) families, 19 (67.56%) genera and 35 (67.32%) species. The order Perciformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 3 (27.27 %) families, 3 (10.71%) genera and 9 (17.30%) species followed by Siluriformes with 3 (27.27%) family, 4 (14.29%) genera and 6 (11.53%) species and symbranchyformes and Baloniformes with 1 (9.09) family, 1 (3.57%) genus and 1 (1.92%) species each.

2.	How is the conservation status of the fish species of the selected river system?	• The IUCN conservation status of the 52 recorded species shows that the highest species were recorded under least concern (LC) category with a total no of 39 and contributed 75 %. under LC category, the major species contribution is from the family Cyprinidae with 20 (38.46 %) followed by Channidae 5 (9.61 %), Nemacheilidae 4 (7.6 %), Silorhynchidae and Sissoridae with 2 (3.86 %) each, Bagaridae, Amblycepitidae, Bedidae, Anabantidae, Mastacembalidae and Belonidae with 1 species contributed 1.92 % each. • Under near threatened (NT) category Cyprinidae and Sissoridae contributed 3 (5.76%) and 1 (1.92 %) species respectively. • Like that, the family Cyprinidae represented the vulnerable (VU) category with 2 (3.84 %) species each. • One species which contributed 1.92 % under Cyprinidae family represented the endangered (EN) category. • A total of 6 nos, 2 (3.84%) from cyprinidae, 1 (1.92%) from each Nemacheilidae, Sissoridae, Channidae and Bedidae respectively falls under the not evaluated (NE) category of IUCN conservation status (2021).
3.	What are the suitable habitat requirements for healthy growth, reproduction and survival of the ichthyofauna?	• All the studied water and soil parameters estimated were within permissible limit, except in some stretches of the sampling sites where anthropogenic activities has been observed. • Relative abundance of Cypriniformes was estimated highest in all the sampling sites. Perciformes were the second most dominating order. No invasive species were recorded in the sampling sites. However, local people commented on the presence of some exotic species in the river.
4.	What are the anthropogenic factors that are affecting ichthyofauna of the river ecosystem and how? How to mitigate these effects?	• Constant dumping of solid waste like polythene bags, paper waste and domestic sewage in the river, • Removal of sand gravel and boulders from the river

		bed, • Alteration of river course, • Use of pesticide for protection of agricultural crops in the adjoining paddy fields of the river system causing toxic condition in the aquatic environment • Electric fishing • Blasting • Use of poison in the river side were also frequently reported by the locals. **Mitigation measures:** • Frequent exploration has to be undertaken to explore the ichthyofaunal of the said river system and identify the anthropogenic activities creating threat to fish fauna and its habitat. • Awareness and training programmes have to be conducted for the local people regarding the conservation of the river and their use in livelihood generation.

9.2 Recommendations on Replicability and Exit Strategy:

Particulars	Recommendations
Replicability of Fellowship, if any	

Exit Strategy:	DNA barcoding has become very much important in developing countries like India because of rapid introduction of invasive and pest species, which in turn lead to the extinction of the important indigenous fauna of the region. Earlier studies reports that there is decline in fish fauna owing to various factors like introduction of alien species and anthropogenic factors like river mining in large scale, destructive fishing, poisoning, use of pesticides/ insecticides in the agricultural crops in the adjoining areas. Since DNA barcoding has not been carried out previously in the river Doyang, thus this study aims to accurately identify and catalogue the ichthyofaunal of Doyang river system and improve the quality of taxonomic information by providing records of novel barcode sequences as well as species descriptions for the said river system. The study will also provide a better understanding of the ichthyofauna and ecology of the river and gives base line information that can be used in creating better conservation strategies. Furthermore, the information will also help non taxonomist, researchers and policy makers to aid them in their efforts in effective management of the important river system. Moreover, the toxicity study shows IMI (Premise 30.50%SC) is a moderately hazardous insecticide to non-target aquatic organism, whose NOEC values lies below 17.36 ppm. During both acute and chronic exposure of IMI caused deleterious alterations to histological structures of liver, gills and kidney and induces significant changes on haemato-immunological parameters, oxidative defence and stress parameters of the test fish. Significant changes in brain AChE enzyme activity and micronucleus formation in erythrocytes were also observed during 96 hours and 28 days acute and chronic exposure. Results clearly indicates that IMI even at sublethal concentrations (T1=26.04 ppm, T2= 20.83 ppm, T3=17.36 ppm) can significantly act as potential immunosuppressor, oxidative stress enhancer and can trigger neurotoxic as well as genotoxic effects. Furthermore, if the exposure to such concentrations is continued for longer duration (beyond 28 days) it might cause anaemic condition, reduced growth, cellular abnormalities and even mortality which needs further investigation. Also, based on the increasing possible use of imidacloprid, we would also suggest additional toxicity studies of other commercial products containing imidacloprid as an active ingredient in non-target aquatic organism. Moreover, the observed parameters can also be useful in monitoring long term effects of IMI and determining water quality criteria for control policies and conservation strategies for aquatic as well as human health.

(Signature of HRA/HJRF/HPF)

(S.K. Bhagabati)
(**NMHS FELLOWSHIP COORDINATOR**)

(B. Kalita)
(**HEAD OF THE INSTITUTION**)

Place: ………………..
Date: ……../……/……

EXECUTIVE SUMMARY

- The present geomorphologic studies of Doyang river system revealed that in upper zone the erosion process was found predominated, with mean gradient of 0.52m/sec. The middle region was characterized by a gradient of 0.43 m/sec with reduced velocity of water current. In the lower zone of the river, sedimentation and river bed aggradation were observed, with an average gradient of 0.27 m/sec. The vegetation of riparian zone was predominantly covered by woody forest and shrubs on both sides of the river banks. Human habitations on river banks were the main source of discharging the sewage, farmyard washings, agricultural waste, pesticides etc. into the river system.
- Fifty-two (52) fish species have been collected from all the six sampling sites of Doyang river system. Voucher specimen of the collected fish species are maintained at Fish Museum of Dept. of AEM, College of Fisheries, AAU, Raha with unique specimen code. During the present investigation a total of 52 fish species belonging to 28 genera, 11 families and 5 orders are recorded from 6 selected sampling stations of the river Doyang, Nagaland India. Among the orders, the Cypriniformes formed the largest group with a contribution of 3 (27.27 %) families, 19 (67.56%) genera and 35 (67.32%) species. The order Perciformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 3 (27.27 %) families, 3 (10.71%) genera and 9 (17.30%) species followed by Siluriformes with 3 (27.27%) family, 4 (14.29%) genera and 6 (11.53%) species and symbranchyformes and Baloniformes with 1 (9.09) family, 1 (3.57%) genus and 1 (1.92%) species each. Diversity indices calculated for ichthyofauna indicates that station 6 to be more diverse whereas least diverse station is found to be station 1.
- The IUCN conservation status of the 52 recorded species shows that the highest species were recorded under least concern (LC) category with a total no of 39 and contributed 75 %. under LC category, the major species contribution is from the family Cyprinidae with 20 (38.46 %) followed by Channidae 5 (9.61 %), Nemacheilidae 4 (7.6 %), Silorhynchidae and Sissoridae with 2 (3.86 %) each, Bagaridae, Amblycepitidae, Bedidae, Anabantidae, Mastacembalidae and Belonidae with 1 species contributed 1.92 % each. Under near threatened (NT) category Cyprinidae and Sissoridae contributed 3 (5.76%) and 1 (1.92 %) species respectively. Like that, the family Cyprinidae represented the vulnerable (VU) category with 2 (3.84 %) species each. One species which contributed 1.92 % under Cyprinidae family represented the endangered (EN) category. A total of 6 nos, 2 (3.84%) from cyprinidae, 1 (1.92%) from each Nemacheilidae, Sissoridae, Channidae and Bedidae respectively falls under the not evaluated (NE) category of IUCN conservation status (2021).
- For the first time Cytochrome Oxidase subunit I (COI) of mitochondrial gene sequences of Thirty-eight (38) (approx. 78%) of the total collected fish species sequences was generated and successfully submitted to NCBI gene data base and accession number was obtained.

- During the present study a total of 30 genera of plankton was recorded out of which phytoplankton consist of 18 genera under 3 family namely Chlorophyceace, Bacillariophyceae and Cyanophyceae and Zooplankton of 12 genera under 3 family namely Cladocera, Rotifera and Copepoda. During the present study, a total of 18 species of phytoplankton were recorded. Three majors' groups of phytoplankton viz. Chlorophyceace represented by 9 species, Bacillariophyceae represented by 5 species and Cyanophyceae represented by 4 species were found in the different stations along the Doyang river system. During the present study, a total of 12 species of zooplanktons belonging to three categories of zooplankton viz. Cladocera represented by 6 species, Rotifera represented by 4 species, Copepoda represented by 2 species was collected from the Doyang river.During the study period the Margalef's richness index (d) was found to be highest at station 1 with a value of 2.925 and with a lowest value of 1.946 at station 6 whereas Pielou's evenness index (J') was found to be highest at station 6 (0.9321) and lowest at Station 2 (07214). Shannon-Weinner index (H') was found to be highest at station 6 (2.415) and lowest at station 2 (2.158). Like that, the highest value of Simpson index (1-ƛ) was found to be at station 6 (0.905) and lowest at station 2 (0.8624). According to Palmer's index of pollution the total score of Algal Genus Pollution Index (AGPI) of sites S1, S2< S3< S4<S5, S6 were calculated to be 2, 5, 7 and 9 respectively. The total scores of S1 and S2 showed 4 indicating probable lack of organic pollution while S5 and S6 showed moderate pollution due to anthropogenic factors or human interference.
- During the present study, analysis of various water quality variables of the river was conducted at six selected sites at monthly interval basis. The range for all the water and soil parameters were: Surface water temperature (17.3˚C to 25.5˚C), Water Depth (0.76m to 1.67m), Water velocity (0.19 m/sec to 0.75 m/sec), Water pH (6.30 to 7.8), Dissolved oxygen (DO) (6.3 to 11.41 ppm), Total dissolved solids (53 ppm to 177 ppm), Turbidity (2.14 NTU to 83 NTU), Conductivity (71 μS/ cm to 499 μS/cm), Alkalinity (41 ppm to 199 ppm), Hardness (81.01 ppm to 194.19 ppm), Biochemical Oxygen Demand (BOD) (0.81 ppm to 18.93 ppm), Chemical Oxygen Demand (COD) (1.60 ppm to 31.40 ppm), Ammonia (0.01 ppm to 0.039 ppm), Nitrate (0.087 ppm to 0.245 ppm), Phosphate (0.041 ppm to 0.129 ppm), Soil pH (5.72 to 6.70), Organic Carbon (0.42% to 2.32%) and Organic matter in (0.72% to 4.0%) The present study also reflected seasonal variations in water quality variables of Doyang river which exhibited considerable seasonal and spatial variations of different parameters.
- In the study we have seen that, during winter and monsoon, PC1 was largely and positively affected by pollution indicating parameters, whereas during post monsoon and pre monsoon, PC1 was largely and positively affected by the other physico chemical parameters. This may be due to pollutants affecting water quality in rivers have temporal and spatial variations and should be investigated based on each river's environmental conditions. We also observed that different stations are having different contributions towards the total variance. The reason for these changes can be found in different environmental conditions and human activities around the river from one place to another.

- Water quality index (WQI) developed using 15 physico-chemical parameters of water provides a positive relationship with the seasonal changes. Maximum WQI values were recorded during monsoon season from all the six stations followed by post monsoon (winter) and premonsoon. The WQI value showed a mixed pattern of changes in all the seasons. WQI of the upstream stations from 1 to 2 is lower than the downstream stations, i.e., 5 and 6 showing the increase in pollution level while moving downstream of the river.
- The study implied that primary productivity of the river was found to be in the lower side with the average value ranging from with the average for GPP (0.116 g C m-3 d-1) and NPP (0.057 g C m-3 d-1).
- All the studied Physico-chemical parameters of soil and water were estimated within permissible limit, except in some stretches of the sampling sites where anthropogenic activities has been observed.
- Relative abundance of Cypriniformes was estimated highest in all the sampling sites. Perciformes were the second most dominating order. No invasive species were recorded in the sampling sites. However, local people commented on the presence of some exotic species in the river.
- Anthropogenic factors encountered during the regular sampling in the Doyang river system are Constant dumping of solid waste like polythene bags, paper waste and domestic sewage in the river, removal of sand gravel and boulders from the river bed, alteration of river course, use of pesticide for protection of agricultural crops in the adjoining paddy fields of the river system leading to the toxicity effects in the non-targets aquatic animals like fish, electric fishing, blasting and poising in the river side were also frequently reported by the locals. Laboratory static renewal test (USEPA, 2002) was carried out to find out the median lethal concentration (LC_{50}). Following the range finding test six different test concentrations with a spacing factor of 1.6 (50 ppm, 80.00 ppm, 128.00 ppm, 204.80 ppm, 327.68 ppm and 524.28 ppm) were selected for the final acute toxicity experiment. Percent mortality was plotted against log concentration of IMI and a curve was obtained. From the curve, 96 hrs. LC_{50} value was calculated to be 208.38 ppm (208380 µg/l) which indicates the chemical to be “moderately hazardous”.
- The present findings when compared to the study done by Bayer Crop Science, 2013 in analytical grade of IMI on common carp, it reveals that the 96hr LC_{50} ratio to be >1. Thus, from the above findings we observe that commercial grade of Imidacloprid (Premise, 30.5%SC) is more toxic than the analytical one. Effect of 96hr LC_{50} concentration (208.38 ppm) was determined by exposing test fish under laboratory static renewal system and analysis being carried out on every 24, 48, 72, and 96hr. Semi static renewal system was deployed for 28 days chronic toxicity test, where 3 sublethal concentrations $LC_{50}/8$ (T_1= 26.04 ppm), $LC_{50}/10$ (T_2=20.83 ppm,) and $LC_{50}/12$ (T_3=17.36 ppm) was selected based on the above calculated 96hr LC_{50} value and analysis was carried on 7th, 14th, 21st and 28th day.
- Upon acute exposure to 96hr LC_{50} concentration for 24, 48, 72, and 96 hr behavioural alterations like jumping movements, restlessness, hyperventilation, hyperactivity, gulping, coughing and corkscrew swimming at surface and bottom of the tank was observed. Enhanced

mucus secretion, loss of buoyancy and string of faeces hanging from anus or on the tank were also reported. All fishes displayed normal behaviour with no apparent external alterations in morphology during chronic exposure.

- Marked histological alterations in liver like exocrine pancreatic acini, hepatic degeneration, mononuclear infiltration; in gill, epithelial lifting and oedema, telangiectasis, lamellar fusion and in kidney expansion of Bowman's space, cloudy swelling of epithelial cells, necrosis of several renal tubules and multiple focal areas of inter-tubular haemorrhage was observed during both acute and chronic exposure to IMI.
- Results showed that immune-haematological variables like haemoglobin (Hb), packed cell volume (PVC), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), Nitroblue tetrazolium activity (NBT) and Lysozyme activity (LA) was significantly altered during acute exposure whereas during chronic it altered in dose and time dependent manner.
- Serum biochemical parameters like Glucose, Cholesterol, Phospholipid, Triglyceride, HDL, VLDL, Magnesium, AST, ALT was significantly increased whereas protein, albumin, globulin, A:G ratio, LDL, were significantly decreases during both acute and chronic exposure of *C. carpio* to IMI.
- Significant induction in oxidative stress enzymes (SOD, CAT, GPx, AST and ALT) and Oxidative stress biomarkers (ROS, MDA) in liver, gill and brain tissues was observed during acute exposure, whereas in chronic exposure it followed dose and time dependent variations.
- Significant reduction in brain AChE enzyme activity due to inhibition of acetylcholine esterase activity, whereas significant DNA damage through induction of micronuclei formation in the erythrocyte of fish blood was observed during both acute and chronic exposure.
- Significant upregulation of HSP70 and CYP1A gene in both liver and gill tissues of exposed fish was observed on 7th,14th, 21st and 28th day in dose and time dependent manner when compared to the control group.

The assessment of ichthyofaunistic resources of Doyang river system and further categorization of the species in the present investigation will definitely provide an important baseline date for conservation of the fish species in their natural habitat. The database of the fish and fisheries with the information embodied in the thesis will offer good opportunities to the conservationist, policy makers, entrepreneurs and fish culturist to take up appropriate measures for conservation of fish species in their natural habitat, so that the rich fish germplasm resources of the river system can flourish unabated. With this premise, fewer commendations could be forwarded for insitu conservation of the dwindling ichthyofauna.

Furthermore, DNA barcoding has become very much important in developing countries like India because of rapid introduction of invasive and pest species, which in turn lead to the extinction of the important indigenous fauna of the region. Earlier studies reports that there is decline in fish fauna owing to various factors like introduction of alien species and anthropogenic factors like river mining in large scale, destructive fishing, poisoning, use of pesticides/ insecticides in the agricultural crops in the adjoining areas. Since DNA barcoding has not been carried out previously in the river Doyang, thus this study aims to accurately identify and catalogue the ichthyofaunal of Doyang river system and improve the quality of taxonomic information by providing records of novel barcode sequences as well as species descriptions for the said river system. The study will also provide a better understanding of the ichthyofauna and ecology of the river and gives base line information that can be used in creating better conservation strategies. Furthermore, the information will also help non taxonomist, researchers and policy makers to aid them in their efforts in effective management of the important river system.

Moreover, the toxicity study shows IMI (Premise 30.50%SC) is a moderately hazardous insecticide to non-target aquatic organism, whose NOEC values lies below 17.36 ppm. During both acute and chronic exposure of IMI caused deleterious alterations to histological structures of liver, gills and kidney and induces significant changes on haemato-immunological parameters, oxidative defence and stress parameters of the test fish. Significant changes in brain AChE enzyme activity and micronucleus formation in erythrocytes were also observed during 96 hours and 28 days acute and chronic exposure. Results clearly indicates that IMI even at sublethal concentrations (T1=26.04 ppm, T2= 20.83 ppm, T3=17.36 ppm) can significantly act as potential immunosuppressor, oxidative stress enhancer and can trigger neurotoxic as well as genotoxic effects. Furthermore, if the exposure to such concentrations is continued for longer duration (beyond 28 days) it might cause anaemic condition, reduced growth, cellular abnormalities and even mortality which needs further investigation. Also, based on the increasing possible use of imidacloprid, we would also suggest additional toxicity studies of other commercial products containing imidacloprid as an active ingredient in non-target aquatic organism. Moreover, the observed parameters can also be useful in monitoring long term effects of IMI and determining water quality criteria for control policies and conservation strategies for aquatic as well as human health.

1. INTRODUCTION

Water, the most vital and precious resources and the most abundant compound on the Earth surface also regarded as one of nature's greatest gift to Earth. Water accounts to more than 70% of the planet Earth, of which only 2.5% is freshwater and as little as 0.3% are accessible in the form of lakes and rivers. River, also known as Blood of the Earth is a fundamental resource not only for human life but also for the flourishment of the flora and fauna. Rivers are large natural stream of water draining into an ocean, lake, or other bodies of water (Kumar and Dua, 2009). Even though, they account to only about 0.0001% of the total amount of water on Earth, rivers are vital carriers of water and nutrients around the Earth (Wetzel, 2001).

India is blessed and bestowed with a large number of rivers and their tributaries. It is bestowed with 15 major, 45 medium and more than 102 minor rivers, with a total length of 45,000 km covering a catchment area of 3.12 million km^2 (Ayyappan *et al.,* 2017). River is the lifeline and bloodline of India. Most of the nation's population is dependent upon the river either directly or indirectly. Agriculture is the mainstay of India. Hence, a vast portion of the country's population is dependent upon the river for their agricultural purposes. Rivers serve the purpose of irrigation, potable water, cheap transportation, hydroelectricity and provides livelihood to millions of people around the globe (Gleick, 1993 and Smith and Gleick, 2012).

With the worldwide concern about freshwater becoming a scarce resource in the near future, developing countries have increased and emphasised their interest towards water quality monitoring programs (Debels *et al.,* 2005). Water quality index (WQI) is one of the most effective ways to determine the health status of an aquatic ecosystem (Kannan, 1991; Sinha and Shrivastava, 1994; Pradhan *et al.,* 2001). Water quality gives information about the various concentrations of solutes. Water quality is described by its physical, chemical and microbiological characteristics (Venkatesharaju *et al.,* 2010). The polluted river water initially alters the physico-chemical characteristics and subsequently affects the biotic components of the system. Thus, it is necessary to study or monitor the water quality status of aquatic ecosystem, which act as an alarm to tackle potential future catastrophe. Water quality emphasises on the physico-chemical parameters of water to detect the pollution status and suitability of various aquatic organisms for a particular water body (Sabbir *et al*., 2010).

Aquatic ecosystems have undergone detrimental changes in the past years, mainly as a result of anthropogenic activities. The quality of river waters and lakes changes with the seasons and geographic areas, even in the absence of pollutants (Chitmanat and Traichaiyaporn, 2010). In comparison to tropical systems, temperate rivers experience greater seasonal temperature fluctuations, less distinct rainy and dry seasons and more seasonally variable light intensity (Junk, 1999; Tockner *et al.,* 2000; Wahl *et al.,* 2008). Biodegradable organic matters present in water are often non-toxic, but the consumption of oxygen during its degradation prevents the water from supporting fish life (Trivedi, 1992). The quality of water in rivers is highly inconsistent in nature due to environmental conditions such as basin lithology, vegetation and climate (Awasthi and Tamot, 2010 and Sharma and Walia, 2015). The quality of an aquatic environment is dependent upon various factors such as physical, chemical, biological and meteorological factors. Water quality is dependent and determined by the amalgamation of various factors in various ways and magnitude (Rahman M.S., 1992). The determination of water quality is a prime concern today for the purpose of both human use as well as organisms as they are directly related to the health status of living organisms.

Fishes play a vital role in the upliftment of economy of our nation. Fish is one of the aquatic biota that plays an important role in the stabilization of water ecosystem and also for the people along the stream (Pracheil, 2010). High levels of fish diversity indicate high quality of waters ecosystem, so that the level of fish diversity can be used as an indicator to estimate water quality and level of pollution present in the waters (Ngodhe *et al.,* 2013). Freshwater ecosystems are among the most productive and diverse ecosystems and are estimated to support over 10,000 species of fish (Nelson, 1994).

India is one of the richest biodiversity heritage sites of the World (Gadgil, 1996). The North Eastern region of India in particular is a host to unique aqua bodies with the main river systems, their tributaries, hill streams. NE India is mapped under the biodiversity hotspots (Kottelat and Whitten's 1996). Conservation International have listed Northeast India as part of two of the 34 biodiversity hotspots, the Himalayas and Indo-Burma (Roach, 2005). The Himalayan region is home to one of the world's highest mountains and deepest gorges. The hills and the valleys of this region gives rise to large number of torrential hill streams, which in turn leads to big rivers and ultimately become part of Ganga- Brahmaputra-Barak–Chindwin–Kolodyne–Gomati- Meghna system (Kar, 2005).

The study of freshwater fish fauna in India goes back to Hamilton (1822), who studied fish fauna of the river Ganges and its tributaries. Documentation and listing of fish fauna and its diversity from different region of India was mainly carried out by Jerdon (1848). Also, Day (1875) recorded the freshwater fish diversity of the Indian region. The study of rivers and the fish fauna in rivers of India is crucial, so as to negotiate with the agricultural production pressure and the nutritional deficit that the country has as a result of its huge population. Various studies have been done on the water quality and fish diversity status of the rivers in India but in case of the studies related to the North-Eastern region of India, very minimal work have been done. The present literature available symbolise that-studies related to major rivers in the North-Eastern region like the Brahmaputra, the Barack, rigorous works have been done relating to the water quality status and the fish fauna availability but in contrast to its tributaries, and other small hill streams and rivers, studies have been found to be very minimal. Thousands and thousands of people, villages and towns, urban or rural, factories and industries are dependent upon the rivers as their lifeline for various purposes such as drinking purposes, agricultural activities, and also for food security. Therefore, the present study and was undertaken with a view to determine the present status of ichthyofaunal diversity, conservation status, habitat characterisation and any anthropogenic factors effecting the riverine system.

Primary productivity is a prime factor which determines the productivity of an aquatic ecosystem. Primary productivity serves an important role in the aquatic ecosystem particularly from fisheries point of view. The primary productivity of an aquatic ecosystem is the illustration of its biological production. It is the rate at which radiant energy is transformed to organic substances by photosynthetic organisms and the chemosynthetic activities of the producer organisms. Primary productivity of lotic ecosystem is influenced by various factors such as photoperiod, water level, turbidity and rainfall (Gupta, 1982; Verma and Datta, 1989). Light and nutrient are the limiting factors for primary productivity in an aquatic ecosystem. Most of the organic matters in an aquatic ecosystem is generated within the water by phytoplankton which is then utilised by the consumers. The basis of the ecosystem functioning is the biological production of autotrophs which is manipulated by primary productivity of an aquatic body (Mohanty *et al.,* 2014; Odum *et al.,* 1971). World ecologists have given emphasis on the importance of primary productivity as an important functional attribute of the biosphere due to its influence on the rate of multiplication and growth of living organism in an ecosystem (Carvalho and Eyre, 2012).

In addition to these works, a laboratory-based toxicity study was conducted based on the preliminary market survey of pesticide shops during the year 2018-19 and the survey revealed that IMI in the brand name of Premise (30.50% SC) was widely used insecticide among the agricultural farmers of the region. They were using this product for controlling of control sucking pests as tick, plant hoppers, whiteflies, and leafhoppers on agricultural crops. Imidacloprid have relatively lengthy half-life in soil (28-1250 ppm) and is considered as a potential surface and ground water contaminant as only 1.60% to 28% is actually absorbed by crops, while the rest enters into the different water tables of the aquatic environment through various sources like rainfall, drainage water, spray drift and accidental drift (Robin and Stork, 2003; Anderson *et al.,* 2015; Frew *et al.,* 2018; Gunal *et al.,* 2020). Global reports suggest that environmental concentration (mainly from 9 countries) of IMI ranges from 0.001 to 320 µg/l (Thunnisen, 2020; Morrissey *et al.,* 2015). However, systematic inventories are very scarce from other regions like India, where there is an increasing trend of IMI use in agricultural and domestic field @ 640 mg/kg to 15250 mg/kg (Bayers Crop Science, 2013). As a result, the possible negative effects of IMI exposure in non-target creatures, such as humans, animals, and especially aquatic animals like fish, are gaining attention (Ozdemir *et al.,* 2018).

Thus, IMI was chosen because of its widespread usage, lack of linkage to aquatic toxicity in the region, or emerging use in the agricultural activities with zero past monitoring. Although data are available on the toxicological effects of Imidacloprid but still there is huge knowledge gap to clarify some aspects of diagnosis of neonicotinoid poisonings in non-targeted standard organism like fish and agricultural or domestic practitioner. Thus, hypothesis was tested to determine toxicity effect of commercial-grade Imidacloprid (Premise, 30.50%, a.i) on non-target animals, fish. The purpose of the study was to evaluate both acute and chronic toxicity effects of IMI, using a standard non target test animal, *Cyprinus carpio* var*. communis* (OECD, 2019)

2. METHODOLOGY ADPOTED

The materials used and methodology followed in different aspects like geomorphology of the river, collection, preservation and identification of Ichthyofauna; sampling of physico -chemical parameters; qualitative and quantitative analyses of plankton samples. Regular field trips were conducted in six selected stations at monthly intervals for a period of two years from January, 2019 to December, 2020, covering Pre-monsoon (January-April), Monsoon (May-August) and Post-monsoon (September-December) seasons for collection of Ichthyofauna, water samples for physico-chemical and biological analysis and to study the fishing techniques and gears used in Doyang river system.

The investigation was divided into six broad aspects viz

i) Hydrobiology of the river Doyang.
ii) Collection and documentation Ichthyofauna in Doyang river system, Nagaland.
iii) Taxonomic and molecular characterisation of the collected fish species.
iv) Water and soil parameters, planktonic and productivity analysis of water sample and their relationship with the abundance of ichthyofauna.
v) Habitat characterisation and identification of anthropogenic factors affecting the ichthyofaunal of the said river system.
vi) Laboratory based toxicity study to evaluate the toxicity effects of insecticide on fish diversity used in adjoining agricultural field of the river system.

2.1 Hydrobiology of the river Doyang

The hydrobiological studies were carried out following the works of Dikshit (1990), Kumar et al (1990), Borah and Goswami (2006) and Prasad and Biswas (2011). The hydrobiology of the Doyang river was evaluated by repeated visit, survey and scientific observations on the spot along the course of the river. Emphasis was given on the fluvial characteristics, river bed aggradation, degradation and instability of the river bank. Riparian vegetation in the catchment areas of the river was also recorded. The detailed course of the river passing through different regions and the riparian zone of the river was studied.

2.2 Documentation of Ichthyofauna

2.2.1 Collection of Icthyofauna

The collection of Icthyofauna of the river Doyang and its tributaries was made from six selected stations along the course of the river. The location map of all the selected stations is shown in fig.2. Field trips were conducted at regular intervals to collect the fish fauna from their natural habitat by employing local fishermen. Besides spot collections from six selected station, the fishes were also obtained from different fish landing centers, local fish markets and fishermen along the rivers/ tributaries and from several approachable areas of the river by using different types of nets namely cast nets, gill nets, triangular scoop nets and a variety of locally made fishing traps. The best way to collect fish for scientific or taxonomic studies to catch them alive through fishing net, a trap or any other devices locally adopted except poisoning with toxic chemicals or dynamiting. After catching the specimen alive, some of the important characters e.g. colour of the fish, bands, spots or stripes if any was noted down in the field book prior to preservation of the specimen in formalin. The coloured photograph of all the live fish specimen was also taken in the field for preparation of an atlas of the fish germplasm resources of the river, since the samples gets discoloured after preservation.

At the time of collection of fishes, maximum care was taken to keep the external morphology intact for taxonomic studies. Collection from different place was packed separately. One field label was attached to each lot with detailed information indicating locality, altitude, name of the rivers / streams / water bodies, date, time, name of collector, fields collection number, etc. The labels were written on stout paper with the pencil. The permanent labels were written with ink after identification with registration number and deposited in the Fish Museum, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon.

2.2.2 Preservation of the specimen

The live specimen collected in the field were fixed in a solution of dilute formalin The small fishes were fixed and preserved in 4-5 % formalin solution. Whereas, for larger fishes, 9-10 % formalin was used for better fixation as described by Ayappan and Satyamurthi (1960). For fishes ranging from 10-30 cm length, an incision on the mid ventral line of the abdomen was made with a knife/scissor without damaging the alimentary tract. For fishes more than 30 cm length, undiluted concentrated formalin were injected in several places along the abdomen. In addition, depending on size 1 – 2 incision along the belly were also made. Where the abdomen is not rounded but sharp and keeled, the incision were made on the left side of the fish. Small sized fishes were immersed in the solution as it is. The live specimen immersed in solution die slowly expanding its fins and rays which helps during identification. The specimens were soaked in formalin solution for at least 4-5 hours. After bringing them in the laboratory, these fishes were removed and put into fresh formalin solution.

2.2.3 Identification

For identification of fish species, standard measurements and counts were followed as described by Jayaram (1999). Measurements were made with a digital caliper to the nearest 0.1mm and body proportions were expressed as percentage of standard length (SL) and head length (HL). Different body measurements followed representing Cypriniformes and Siluriformes species are depicted in fig.3. Transverse scales were counted as scales between lateral line and dorsal fin origin and between lateral line and pelvic fin origin. For small specimens counting of fins and scales were made under binocular microscope, preferably after using a temporary surface stain (pen ink).

Morphometric analysis for identifying the fishes was carried out generally with reference to Day (1978a), Menon (1987), Talwar and Jhingran (1991), Jayaram (1999) and Vishwanath (2002). For further confirmation the following literature were followed, Hamilton-Buchanan (1822), Pandey (1970), Mishra (1976a,76b), Menon et al. (1977), Tilak & Hussain (1977), Day (1978b), Roberts (1980, 1994), Jayaram (1981, 1991), Sen & Jayaram (1982), Menon (1992), Nelson (1994), Needham (2000), Bendangkokba and Ahmed (2007) and Ao et al. (2008).

Pectoral fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping was isolated following phenol: chloroform method. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking its integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial COI gene using

Fish F1 & amp; R1 Primer with the help of a thermal cycler (Eppendorf AG 22331 Hamburg). PCR product is then sequenced at Eurofin Scientific Laboratory. The generated barcodes were submitted to NCBI and accession number were obtained for the individual fish species.

2.3 Ecological studies of the river

It includes seasonal and station wise analysis of physico – chemical parameter of the river water and the variations of river biota both qualitatively and quantitatively. For ecological studies collection of samples was done monthly intervals from all the six selected stations between 8 to 10 a.m below 30 cm/ 1 foot below the water surface and about 2 meters away from the shore for a period of two years during January 2019 to December, 2020. The meteorological status of the study area during the specific study period was collected from the State Agriculture Department. Data such as atmospheric temperature and rainfall data were collected.

2.3.1 Physico-Chemical parameters

Different physio-chemical parameters like Surface Water Temperature (SWT), Water Depth, Water Velocity, Water pH, Dissolve Oxygen (DO), Total Dissolve Solid (TDS), Conductivity, Alkalinity, Turbidity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammonia, Nitrate and Phosphorous were analysed used standard protocol APHA (2019). The sediment samples were collected on seasonal interval, air dried and analyzed for pH, organic matter, organic carbon, nitrogen, potassium and phosphorus as per standard methodology (Jhingran, 1992; Walky & Black, 1934)

2.3.2 Productivity

The primary productivity of the river Dzii was estimated through oxygen production and consumption by phytoplankton using light and dark bottle method of Garder and Gran (1927). The value obtained through light and dark bottle was expressed in g C m^{-3} d^{-1}.

2.4 Planktons

Collection of plankton samples was done by slowly filtering 50 litres of water samples collected from the six selected stations along the river (with 0.5 to 1 m depth) through the plankton net (silk cloth no. 25). The filtrate obtained in the plankton net test tube after separating the suspended particles and flock vegetation was preserved in 5% formalin solution in specimen tubes with proper labelling in the field. Planktons were collected from different stations in the morning and evening hours to avoid diurnal migration of most zooplanktons like Copepods, Cladocera and Rotifers.

For qualitative analysis of planktonic sample, phytoplankton and zooplanktons were stained with lugol's solution and Polyvinyl alcohol- glycerol eosin stains and identified under the compound microscope by dropping 4 to 6 drops of 5% formalin in a slide and identified. For identification the works of Edmondson (1959), Needham and Needham (1972), Koste (1978), Michael and Sharma (1988) were followed. Other references that were espoused for the study and analysis of planktons includes Charkraborty et al. (1959), Dobriyal et al. (1983), Yosuf (1989) and Sharma and Sharma (1999, 2000,2001, 2009). For quantitative analysis of planktons, the filtrate was concentrated to 25 ml each time and preserved in 5% formalin solution. Quantitative analysis was done for both phytoplankton and zooplankton by using Sedgwick-Rafter counting cell and its density expressed in units per litre.

In addition to this an additional toxicity study was conducted to determine the detrimental effect of insecticide in the ichthyofauna diversity used in the adjoining paddy field of the Doyang river system. Methodology and approach followed during the analysis is given below.

2.5 Toxicity Analysis

2.5.1 Location of the experiment

The experiment was performed at the Laboratory of Department of Aquatic Environment Management (AEM), College of Fisheries, Assam Agricultural University, Raha, Nagaon, Assam. The geographical locations reported for the laboratory is 26°.21'.55'' N latitude and 92°.50'.67'' E longitude.

2.5.2 Ethical concern

The usage of experimental fish is in accordance with current laws in India. In compliance with the guidelines of the Institutional Animal Ethics Committee, College of Fisheries, Assam Agricultural University, care and treatment of collected fish samples were performed prior to the experiment. The experimental protocol and end points were carried out according to the guidelines laid by the said committee.

2.5.3 Test chemical

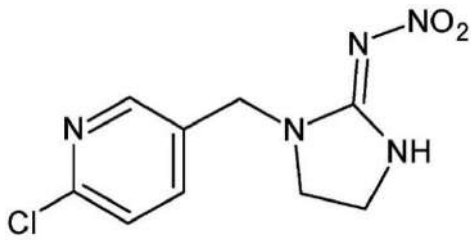


FIG 4: CHEMICAL STRUCTURE OF IMIDACLOPRID

Imidacloprid (IMI)	
Chemical formula	$C_9H_{10}ClN_5O_2$
IUPAC ID	*N*-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl} nitramide
Molar mass	255.661
Appearance	Colourless crystals
Melting point	136.4 to 143.8 °C (277.5 to 290.8 °F; 409.5 to 416.9 K)
Solubility in water	0.51 g/l (20 °C)

A locally available commercial formulation of Imidacloprid (Premise 30.50% SC, *N*-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-y l} nitramide, was obtained from local pesticide shop, Nagaon, Assam, under the trade name Premise, systematic insecticide registered and manufactured by Bayer Crop Science limited, Thane west, India. Imidacloprid product Premise 30.50% SC contains 30.50% Imidacloprid as active ingredient and some solvent such as Xanthane Gum 13.00%, Trihydroxy propane 10.00%, Ethoxylated polymethacrylate in propylene glycol and water 4.50%, Ethoxylated derivative of styrylated phenols 1.50%, Phenol methoxy methanol 1.00%, Blend of methylisothiazolinone and its chloro derivative 1.00%, water solution of polymethyl siloxane 1.00% and water demineralised Q.S.

2.5.4 Preparation of stock and working solutions

Commercial grade Imidacloprid (Premise, 30.50% SC, 305 ppm) product was purchased from a local pesticide shop and maintained as a stock solution. During the experiment 4.91 ml, 7.86 ml, 12.59 ml, 20.14 ml, 32.22 ml, and 51.55 ml of stock solution was diluted in approximately 30 litres of water in order to obtain working concentrations of 50 ppm, 80 ppm, 128 ppm, 204.80 ppm, 327.68 ppm and 524.28 ppm respectively for range finding and definitive test. For acute or lethal exposure required quantity of stock solution was diluted in 30 litres water to obtain the above concentration. Similarly, for chronic or sublethal test required quantity of stock solution was diluted in 80 litres water to obtain the working concentration of LC_{50} /8, LC_{50} /10, and LC_{50} /12. Furthermore, the concentrations of the insecticide were maintained by changing the water every after 24 hrs.

2.5.5 Test Fish

Cyprinus carpio var. *communis* (Linnaeus, 1778).

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Subfamily: Cyprininae

Genus: *Cyprinus*

Species: *carpio*

Common carp, *Cyprinus carpio* is the most widely cultured freshwater fish species in the world (Xing *et al*., 2012). It exhibits high tolerance to the environmental stress and has become a widely used model species for fish toxicological studies (OECD, 2019, William *et al*., 2008).

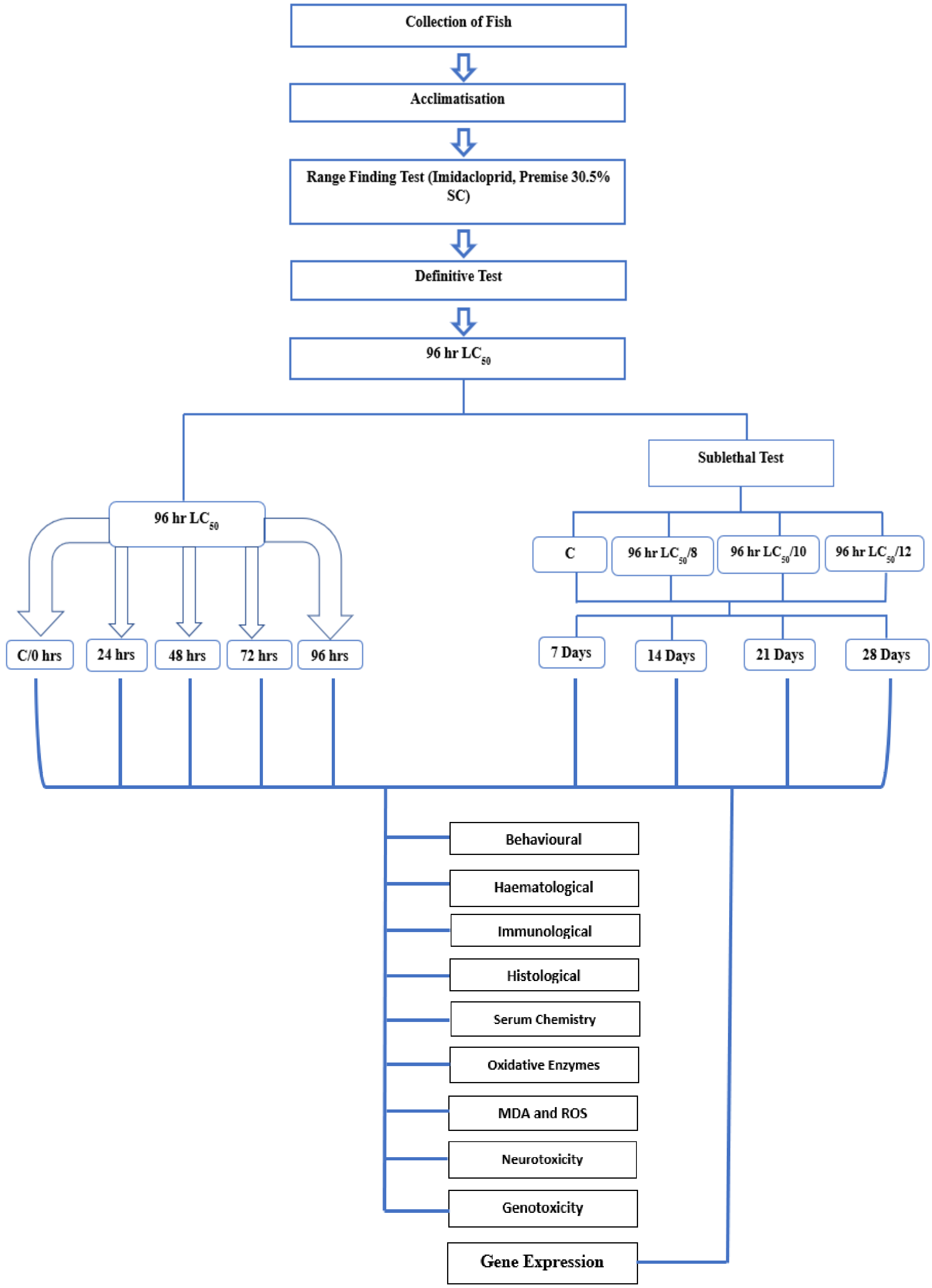
2.5.6 Fish species and acclimatization

The common carp fingerlings, *C. carpio* var *communis* (n=900) were obtained from the Fish Farm, College of Fisheries, Assam Agricultural University, Raha, Nagaon. Mean length and weight of the fishes with was 4.15± 0.4 cm and 0.935±0.23gm respectively. The fishes were acclimatized for 21 days in large cement cistern (1000L capacity) containing dechlorinated tap water with constant aeration. The photo period was maintained at 12:12hr dark/light cycle. During acclimatization, the fishes were fed twice *ad libitum* with commercial feed containing 35% crude protein and zero mortality was recorded. Feeding was suspended 24 hrs prior to the commencement of the experiment. The average values of physiochemical parameters of the water medium used in the toxicity tests were, water temperature 25±1°C; pH 7.4±0.3; DO 5.8 ±0.2 ppm; Total Alkalinity 117±5.35 as $mgCaCO_3/l$ and Total hardness 103±4.73 as $mgCaCO_3/l$, which were in accordance with (USEPA, 2002) specifications.

2.5.7 Experimental Design

After the initial acclimatization period, fish with no clinical symptoms were selected to determine the 96-h LC_{50} value of IMI in static renewal system in laboratory as per standard methods (USEPA, 2002).

The range finding test was carried out prior to the definitive test to determine the concentration of the test solution. All experiments were conducted in 100L rectangular glass aquaria (120 X 45 X 80 cm). Commercially available IMI, Premise, 30.50%SC (305 ppm) was taken as stock solution. The experimental concentration was prepared by dissolving the stock solution in appropriate quantity of distilled water. Of the calculated 96hr LC_{50} value, 1/8, 1/10 and 1/12 of 96hr LC_{50} of IMI concentrations were selected for sublethal toxicity studies (Sprague, 1971). Completely Randomised Block design (CRD) was followed throughout the experiment for all statistical inference. During the experiment total number of observation (n), n= 10 individuals per replicate aquarium, 3 aquariums per treatment) for both acute and chronic toxicity analysis was maintained. The complete flow through chart of the whole experimental set up is shown below.



DIAGRAMMATIC REPRESENTATION OF THE EXPERIMENTAL SET UP OF ACUTE AND CHRONIC EXPOSURE OF IMIDACLOPRID IN COMMON CARP.

2.5.8 Acute Toxicity test

Range finding Test and definitive Test

Range finding test was carried out for 96 hours following the procedure of Solbe (1995). Three numbers of Common carp, *Cyprinus carpio* fingerlings were stocked in each aquarium with the following graded concentrations of commercially available Imidacloprid, Premise (30.50%SC), 0 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm and 1000ppm.

Laboratory static renewal test (USEPA, 2002) was carried out to find out the median lethal concentration (LC_{50}). Following the range finding test, six different test concentrations with a spacing factor of 1.6 (50.00 ppm, 80.00 ppm, 128.00 ppm, 204.80 ppm, 327.68 ppm and 524.28 ppm) were selected for acute toxicity experiment. Ten fingerlings of average length and weight 4.17± 0.4 cm and 0.952±0.18 gm respectively was randomly distributed and placed in 21 nos. of glass aquarium of size 100L (120 X 45 X 80 cm) containing 30 litres of test solution. The control and each treatment were run in triplicate. The aquariums were covered with net in order to prevent the fish from jumping out of the aquarium throughout the experimental period. The behavioural pattern of the fishes was closely monitored throughout the experimental period for 96 hrs. 96hr LC_{50} of commercially available Imidacloprid, Premise was calculated using probit analysis Sarmah *et al*. (2020) and obtained 96 hr LC_{50} value was then used for further downstream studies.

2.5.8.1 Calculation of Percent Mortality

Abbotts Formula (1925) was used for getting the exact mortality which could be obtained by subtracting the natural mortality in the control group from the experimental group.

P= (Om-Cm/ 100-Cm) x 100

Where,

P=Corrected mortality,

Om= Observed mortality

Cm=Control mortality (all percentage)

2.5.9 Chronic/Sublethal Toxicity Analysis

The sublethal concentration of Imidacloprid, Premise 30.50 % SC was selected based on the 96hr LC_{50} value obtained during the above experiment. To evaluate the sublethal effect, fishes were divided into four main groups each with 100 individuals per aquaria of capacity 100 L (150 X 60 X 80 cm) containing 60 litres of test solution. Group one was kept in pesticide-free water and treated as the control. Fish in groups two, three and four were subjected to three sublethal concentrations of IMI that is 1/8 LC_{50} (Treatment I), 1/10 LC_{50} (Treatment II) and1/12 LC_{50} (Treatment III) for a period of 28 days, respectively. Test medium was renewed every day to avoid dilution owing to active ingredient degradation (Lavanya *et al*., 2011; Hemalatha *et al*., 2016). The experimental aquaria were aerated and fishes were hand fed daily with formulated diet containing 35% crude protein up to apparent satiation of the fish. Zero mortality with no behavioural abnormalities was recorded during the whole experimental period. The whole experimental setup including control group was run in triplicate. At every 7^{th}, 14^{th}, 21^{st} and 28^{th} day of exposure period, 10 fishes were randomly selected form each group for haematological, histological, serum biochemistry, oxidative stress enzymes, AChE activity, micronucleus tests and gene expression study.

2.5.10 Analysis of different biomarkers

Different bio markers like Behavioural, Hemato-immunological parameters (Haemoglobin content (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), Mean Corpuscular Haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Mean Corpuscular Volume (MCV), Nitro blue tetrazolium activity (NBT) and Lysozyme activity (LA), Histological analysis of liver gills and kidney, Serum bio chemical parameters like Glucose (GLU), Total Protein (TP), Albumin (ALB), Globulin (GLO), ALB:GLO ratio, Triglyceride (TG), Cholesterol (CHO), High Density lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), Magnesium (MG), Phospholipid (PL), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Different Antioxidant Enzyme like Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx), Oxidative stress biomarkers like Reactive Oxygen Species level (ROS), Malondialdehyde (MDA), Neurotoxic assay like brain Acetylcholinesterase activity, Genotoxic assay like micronucleus test and Gene expression study for HSP70 and CYP1A genes were carried out following standard protocols.

3. KEY FINDINGS AND RESULTS

Doyang is the largest river of Nagaland and approximately lies between 25°40´44´´ and 26°13´74´´ N Latitude and between 94°14´31´´ and 94°0´54´´ E Longitude (Figure I). The Doyang river flows through a length of about 152 km within the state of Nagaland, almost dividing the state into two equal halves, traversing different climatic and geomorphological terrains and receiving tributaries from the districts of Kohima, Zunheboto, Mokokchung and Wokha. On the riparian valleys, there are about 65 villages directly or indirectly availing its resources which makes it one of the most socially, culturally and economically important river of the state.

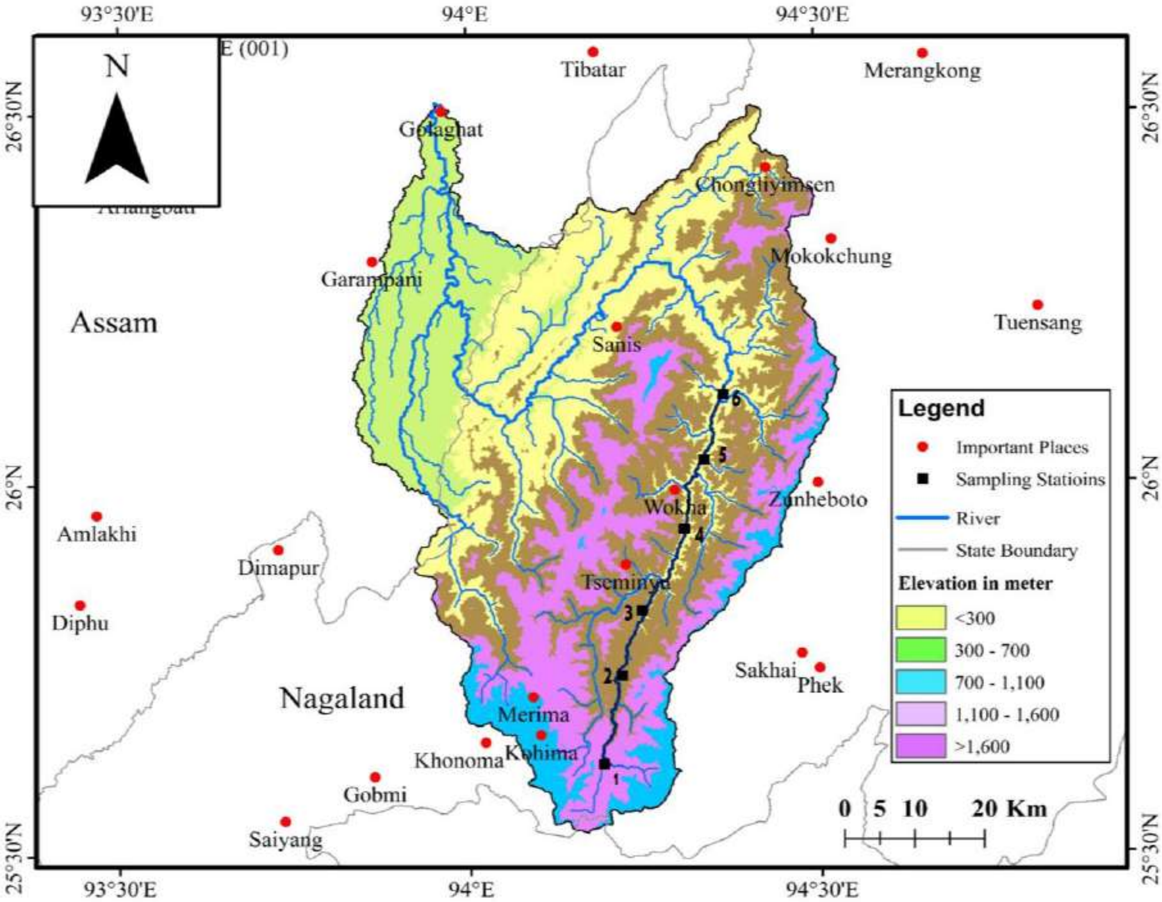


Fig 1. Map of the Study Area

Table 1. Detailed characterization of each sampling site of the Doyang river system.

Sampling station	Name of the site	River	Latitude	Longitude	Elevation (mtr)
1	Mithelephe Kohima	Doyang	25°37'20.7"N	94°11'27.1"E	867
2	Kohima-meluri Road	Doyang	25°39'19.6"N	94°11'11.6"E	754
3	Chakabhama Nagaland	Doyang	25°41'10.6"N	94°11'04.7"E	695
4	Wokha Mokochung Road	Doyang	25°32’65"N	94°36’43"E	610
5	Longidang, Nagaland	Doyang	26°01'24.0"N	94°21'29.9"E	410
6	Mukhami, Nagaland	Doyang	26°06'54.0"N	94°23'11.5"E	285


Photograph of station 1


Photograph of station 2


Photograph of station 3


Photograph of station 4


Photograph of station 5


Photograph of station 6

3.1 Ichthyofaunal diversity of the river Doyang, Nagaland, India.

During the present investigation a total of 52 fish species belonging to 28 genera, 11 families and 5 orders are recorded from 6 selected sampling stations of the river Doyang, Nagaland India. The number and percentage composition of families, genera and species under different orders are shown (Table 2a). Among the orders, the Cypriniformes formed the largest group with a contribution of 3 (27.27 %) families, 19 (67.56%) genera and 35 (67.32%) species. The order Perciformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 3 (27.27 %) families, 3 (10.71%) genera and 9 (17.30%) species followed by Siluriformes with 3 (27.27%) family, 4 (14.29%) genera and 6 (11.53%) species and symbranchyformes and Baloniformes with 1 (9.09) family, 1 (3.57%) genera and 1 (1.92%) species each (Table 3 and Figure 2).

Among the recorded families which are shown in the Table 4 and Figure 3, Cyprinidae contributed 16 (57.15%) genera and 28 (53.83%) species. Nemachailidae and Sissoridae represented with 2 (7.14%) genera each and 5 (9.62%) and 4 (7.69) species each respectively. Psilorhynchidae, Channidae, Badidae, Bagaridae, Amblycephilidae, Anabantidae, Mastacembalidae and Belonidae with 1 (3.57%) genera and 2 (3.85%), 6 (11.54%), 2 (3.85%), 1 (1.92%), 1 (1.92%),1 (1.92%),1 (1.92%),1 (1.92%) families each respectively.

Whereas Imnatoshi (2013) from the same river reported 46 species belonging to 30 different genera under 14 families and 5 orders. The order Cypriniformes was represented by a maximum number of 26 species (65%) followed by Siluriformes 7 species (17.5%), Perciformes 4 species (10%), Synbranchiformes 2 species (5%) and Beloniformes 1 species (2.5%) (Table 2b).

Table No 2a. Collected fish species from River Doyang with their conservation status as IUCN (2021).

Sl. No	Order	Family	Species	Local Name	Standard Length (cm)	IUCN Status (2021)
1	Cypriniformes	Cyprinidae	*Opsarius bendelisis*	*Tawa*	*11.19*	LC
2			*Barilius barila*	*Zer*	*5.77*	LC
3			*Opsarius barna*	*Zer*	*8.96*	LC
4			*Garra naganensis*	*Anget*	*6.57*	LC
5			*Garra gotyla*	*Anget*	*6.87*	LC
6			*Garra kempi*	*Anget*	*6.40*	LC
7			*Garra lamta*	*Anget*	*6.54*	LC
8			*Garra annandelei*	*Anget*	*6.02*	LC
9			*Garra lissorhynchus*	*Anget*	*4.50*	LC
10			*Devario aequipinnatus*	*Zer*	*8.15*	LC
11			*Danio danquila*	*Zer*	*4.59*	LC
12			*Pethia ticto*	*TsutoZer*	*3.27*	LC
13			*Pethia conchonicus*	*TsutoZer*	*2.84*	LC
14			*Puntius sophore*	*TsutoZer*	*4.13*	LC
15			*Puntius chola*	*TsutoZer*	*2.53*	LC
16			*Salmostoma bacaila*	-	*7.32*	LC
17			*Tor putitora*	*TsutoZer*	*8.95*	EN
18			*Tor tor*	*TsutoZer*	*12.54*	DD
19			*Neolissochilus hexagonolepis*	*Seben*	*16.33*	NT
20			*Neolissochilus hexastichus*	*Seben*	*9.10*	NT

21			*Schizothorax richardsonii*	*Seben*	*14.05*	VU
22			*Schizothorax labiatus*	*Seben*	*7.79*	NE
23			*Tariqilabeo latius*	*Tongtsu*	*9.83*	LC
24			*Labeo pangusia*	*Tongtsu*	*9.45*	NT
25			*Labeo fimbriatus*	*Tongtsu*	*7.32*	LC
26			*Cyprinion semiplotom*	*Tongtsu*	*6.43*	VU
27			*Bangana dero*	*Tongtsu*	*17.26*	LC
28			*Esomus dendricus*	*Zer*	*4.20*	LC
29		Silorhynchidae	*Psilorhynchus homoleptera*	*Mern-ngo*	6.68	LC
30			*Psilorhynchus balitora*	*Mern-ngo*	5.13	LC
31		Nemacheilidae	*Schistura fasciata*	*Retong*	*4.94*	LC
32			*Schistura maculosa*	*Retong*	*4.32*	LC
33			*Schistura naganensis*	*Retong*	*5.96*	LC
34			*Schistura Corica*	-	*3.62*	NE
35			*Paracantocobitis botia*	*Retong*	*7.07*	LC
36	Siluriformes	Sissoridae	*Glytothorax caviae*	*Ajang*	*7.27*	LC
37			*Glytothorax striatus*	*Ajang*	*8.90*	NT
38			*Glytothorax barmanensis*	*Ajang*	*9.98*	LC
39			*Oreoglanis spp*	-	*6.10*	-
40		Bagridae	*Olyra kempi*	*Nenak*	*11.37*	LC
41		Amblycepitidae	*Amblyceps apangi*	-	*3.74*	LC
42	Perciformes	Channidae	*Channa aurantimaculata*	*Alopungo*	*8.08*	DD
43			*Channa punctatus*	*Alopungo*	*8.93*	LC

44			*Channa orientalis*	*Alopungo*	*6.34*	LC
45			*Channa stewarti*	*Alopungo*	*4.23*	LC
46			*Channa gachua*	*Alopungo*	*5.71*	LC
47			*Channa striatus*	*Alopungo*	*6.18*	LC
48		Badidae	*Badis badis*	*Ak ngo*	*3.20*	LC
49			*Badis assamensis*	*Ak ngo*	*3.56*	DD
50		Anabantidae	*Anabas testiduneus*	-	*4.19*	LC
51	Symbranchiformes	Mastacembalidae	*Mastacembelus armatus*	Kongsha	18.40	LC
52	Baloniformes	Belonidae	*Xenontodon cancila*	Rongsenngo	9.43	LC

EN= Endangered, VU= Vulnerable, NT= Near Threaten, LC= Least Concern, NE= Not Evaluated.

Schistura spp (unidentified)	*Retong*	*4.10*
Schistura spp (unidentified)	*Retong*	*3.96*
Garra spp (unidentified)	*Anget*	*4.56*

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Table 2b. Comparative study of the ichthyofaunal diversity of the Doyang River system.

Order	Family	Species	IUCN Status (2021)	Imnatoshi (2013)	Present study
Cypriniformes	Cyprinidae	*Opsarius bendelisis*	LC	√	√
		Barilius barila	LC	√	√
		Opsarius barna	LC	√	√
		Garra naganensis	LC	√	√
		Garra gotyla	LC	√	√
		Garra kempi	LC	**X**	√
		Garra lamta	LC	**X**	√
		Garra annandelei	LC	**X**	√
		Garra lissorhynchus	LC	√	√
		Garra gravelyi	NA	√	**X**
		Devario aequipinnatus	LC	√	√
		Danio danquila	LC	√	√
		Pethia ticto	LC	√	√
		Pethia conchonicus	LC	**X**	√
		Puntius sophore	LC	√	√
		Puntius chola	LC	√	√

		Salmostoma bacaila	LC	X	√
		Tor putitora	EN	√	√
		Tor tor	DD	X	√
		Neolissochilus hexagonolepis	NT	√	√
		Neolissochilus hexastichus	NT	√	√
		Schizothorax richardsonii	VU	X	√
		Schizothorax labiatus	NE	X	√
		Tariqilabeo latius	LC	√	√
		Labeo pangusia	NT	√	√
		Labeo bata	LR-nt	√	X
		Labeo rohita	NA	√	X
		Catla Catla	NA	√	X
		Cirrhinus mrigala	NA	√	X
		Cyprinus carpio	NA	√	X
		Ctenopharyngodon idellus	NA	√	X
		Hypopthalmichthys molitrix	NA	√	X
		Labeo fimbriatus	LC	√	√
		Cyprinion semiplotom	VU	X	√
		Bangana dero	LC	√	√

		Esomus dendricus	LC	√	√
	Silorhynchidae	*Psilorhynchus homoleptera*	LC	√	√
		Psilorhynchus balitora	LC	**X**	√
	Nemacheilidae	*Schistura fasciata*	LC	**X**	√
		Schistura maculosa	LC	**X**	√
		Schistura naganensis	LC	**X**	√
		Schistura Corica	NE	**X**	√
		Schistura prashadi	VU	√	**X**
		Neonoemacheilus assamensis	NA	√	**X**
		Paracantocobitis botia	LC	√	√
		Lepitocephalichthys guntea	NA	√	
Siluriformes	Sissoridae	*Glytothorax caviae*	LC	√	√
		Glytothorax striatus	NT	**X**	√
		Glytothorax barmanensis	LC	**X**	√
		Glyptothorax telchitta	Ln RT	√	**X**
		Oreoglanis spp	-	**X**	√
	Claridae	*Clarius batrachus*	LC	√	**X**
	Siluridae	*Kryptopterus indicus*	CR	√	**X**

	Bagridae	*Olyra kempi*	LC	√	√
		Olyra longicauda	NA	√	**X**
	Amblycepitidae	*Amblyceps apangi*	LC	√	√
Perciformes	Channidae	*Channa aurantimaculata*	DD	**X**	√
		Channa punctatus	LC	√	√
		Channa orientalis	LC	√	√
		Channa stewarti	LC	**X**	√
		Channa gachua	LC	**X**	√
		Channa striatus	LC	**X**	√
	Badidae	*Badis badis*	LC	√	√
		Badis assamensis	DD	**X**	√
	Anabantidae	*Anabas testiduneus*	LC	√	√
Symbranchiformes	Mastacembalidae	*Mastacembelus armatus*	LC	√	√
	Synbranchidae	Monopterus albus	NA	√	**X**
Baloniformes	Belonidae	*Xenontodon cancila*	LC	√	√
			Total	**46**	**52**

Plate 1: Fish species recorded from the river Doyang

Tor putitora

Tor tor

Neolissochilus hexagonolepis

Neolissochilus hexasticus

Labeo fimbriatus

Cyprinion semiplotum

Labeo pangusia

Schizothorax labiatus

	
Puntius sophore	*Pethia conchonicus*
	
Barilius barila	*Tariqilabeo latius*
	
Salmostoma bacaila	*Devario aquipinnatus*
	
Schistura nagaensis	*Schistura maculosa*

Schistura fasciata

Barilius bandelisis

Glyptothorax striatus

Glyptothorax caviae

Garra lissorhynchus

Garra lamta

Garra naganensis

Olyra kempi

	
Glyptothorax burmanensis	*Channa aurantimaculata*
	
M. armarus	*Badis badis*
	
Cabdio morar	*Garra annadelei*
	
Oreoglanis sp.	*Schistura Khugae*

	
Danio danguila	*Channa orientalis*
	
Bangana dero	*Garra gotyla*
	
Garra kempi	*Garra spp.*
	
Amblyceps apangi	*Psilorynchus balitora*

Table 3. Number of families, genera and species under various orders.

SI. No	Orders	Family		Genus		Species	
		No. of families	% of families	No of Genus	% of Genus	No of Species	% of Species
1	Cypriniformes	3	27.27	19	67.86	35	67.32
2	Siluriformes	3	27.27	4	14.29	6	11.53
3	Perciformes	3	27.27	3	10.71	9	17.30
4	Symbranchiformes	1	9.09	1	3.57	1	1.92
5	Baloniformes	1	9.09	1	3.57	1	1.92
Total		**Total**	11	**100**	28	**100**	52

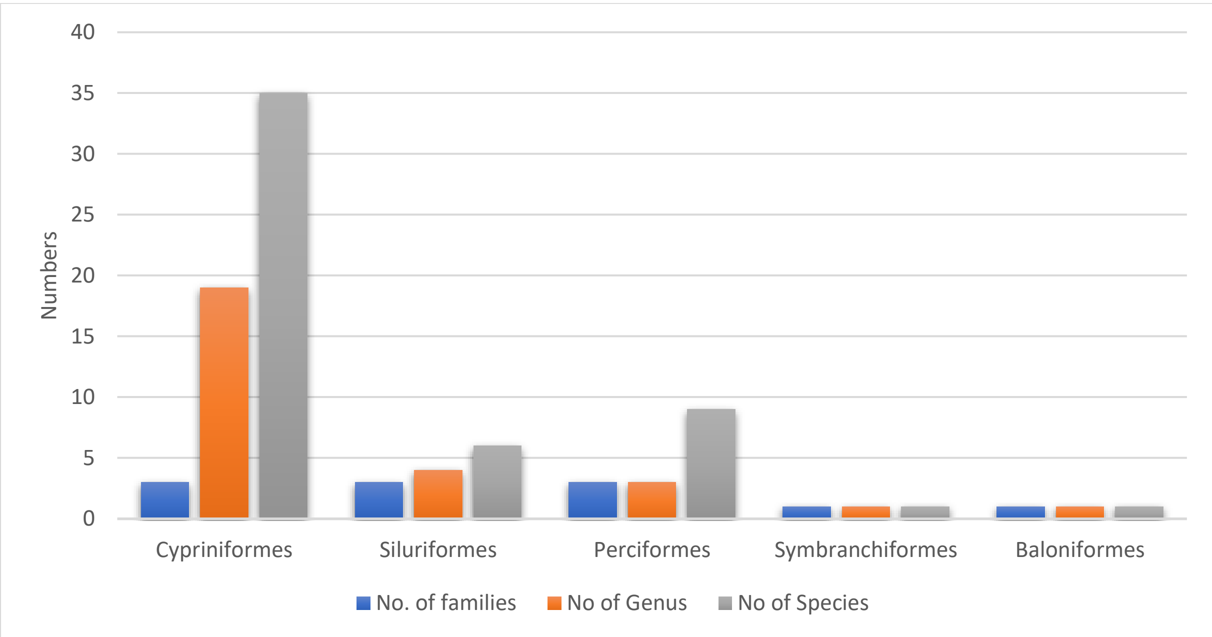


FIGURE 2: NUMBER OF FAMILIES, GENERA AND SPECIES UNDER VARIOUS ORDERS

Table 4: Number and percentage composition of genera and species of fishes under various families

Sl. No.	Family	Genera		Species	
		Number of genera	% of genera	Number of species	% of species
1.	Cyprinidae	16	57.15	28	53.85
2.	Silorhynchidae	1	3.57	2	3.85
3.	Nemacheilidae	2	7.14	5	9.62
4.	Sissoridae	2	7.14	4	7.69
5.	Bagridae	1	3.57	1	1.92
6.	Amblycepitidae	1	3.57	1	1.92
7.	Channidae	1	3.57	6	11.54
8.	Badidae	1	3.57	2	3.85
9.	Anabantidae	1	3.57	1	1.92
10.	Mastacembalidae	1	3.57	1	1.92
11.	Belonidae	1	3.57	1	1.92
Total		**28**	**100**	**51**	**100**

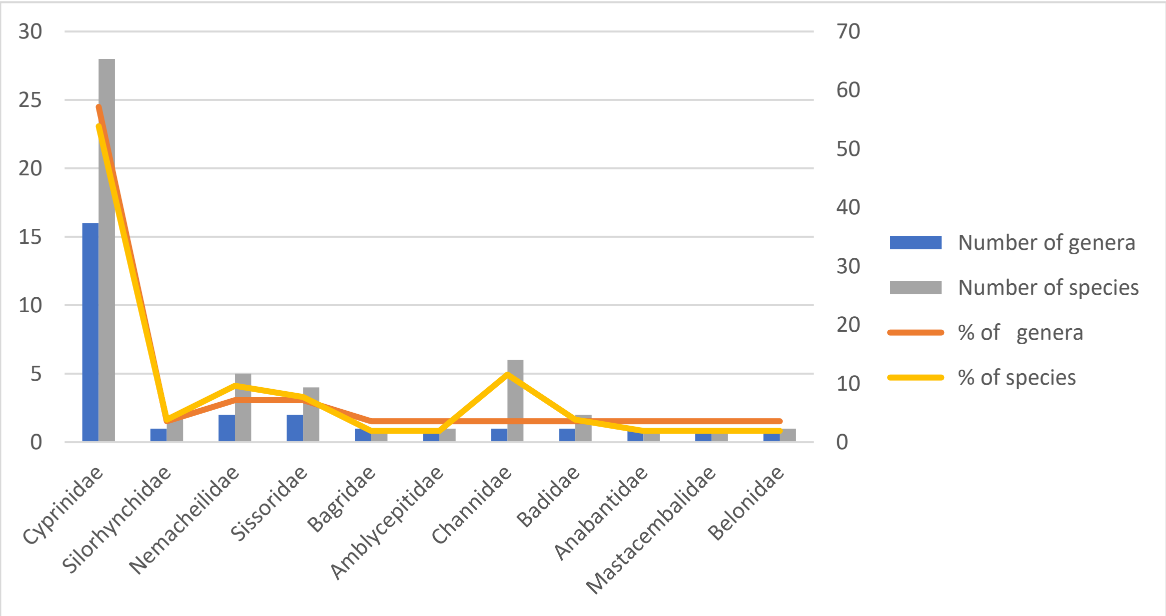


FIGURE 3: NUMBER AND PERCENTAGE COMPOSITION OF GENERA AND SPECIES OF FISHES UNDER VARIOUS FAMILIES

Table 5: Station wise total individuals of species recorded from the river Doyang in three different seasons

Sl. No.	Species	Season																		Abundance	Relative abundance (%)
		Pre-Monsoon						Monsoon						Post-Monsoon							
		S_1	S_2	S_3	S_4	S_5	S_6	S_1	S_2	S_3	S_4	S_5	S_6	S_1	S_2	S_3	S_4	S_5	S_6		
1	*Neolissochilus hexagonolepis*	0	2	2	4	0	0	0	0	1	2	0	0	0	4	6	6	8	10	45	2.68
2	*Neolissochilus hexasticus*	0	0	2	3	0	0	0	0	2	4	0	0	0	0	3	5	0	0	19	1.13
3	*Schizothorax richardsonii*	1	2	4	0	0	0	1	2	3	0	0	0	5	3	7	0	0	0	28	1.66
4	*Schizothorax labiatus*	1	2	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	10	0.59
5	*Tor tor*	0	0	0	1	3	4	0	0	0	0	2	3	0	0	0	4	4	5	26	1.54
6	*Tor putitora*	0	0	3	4	3	5	0	0	0	2	1	2	0	0	0	7	6	9	42	2.50
7	*Tarqilabeo latius*	0	0	4	6	4	7	0	0	0	2	3	4	0	0	6	4	10	14	64	3.81
8	*Labeo pangusia*	0	0	2	4	0	7	0	0	0	0	0	3	0	0	4	3	8	7	38	2.26
9	*Labeo fimbriatus*	0	0	3	0	6	8	0	0	2	0	2	4	0	0	0	6	4	5	40	2.38

10	*Cyprinion semiplotum*	0	0	0	3	5	3	0	0	0	0	3	1	0	0	0	3	5	4	27	1.60
11	*Bangana dero*	0	0	0	0	7	4	0	0	0	0	0	2	0	0	0	3	5	7	28	1.66
12	*Esomus dandricus*	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	6	9	0.53
13	*Psilorhynchus homoleptera*	0	0	3	5	6	8	0	0	3	0	2	3	0	0	0	4	6	8	48	2.85
14	*Psilorhynchus balitora*	0	0	2	4	7	11	0	0	0	0	3	5	0	0	3	4	7	13	59	3.51
15	*Schistura fasciata*	0	2	0	0	3	6	0	2	0	0	0	0	3	5	4	0	0	0	25	1.48
16	*Schistura maculosa*	2	4	2	0	4	0	2	4	0	0	0	0	4	3	5	0	0	0	30	1.78
17	*Schistura nagensis*	2	3	3	0	0	0	2	3	3	0	0	0	3	6	3	0	0	0	28	1.66
18	*Schistura Corica*	0	2	3	0	0	0	0	2	3	0	0	0	2	4	5	0	0	0	21	1.25
19	*Paracanthocobitis botia*	0	0	0	3	2	5	0	0	0	3	2	5	0	0	4	6	4	7	41	2.44
20	*Glypthothorax caviae*	0	0	4	2	0	2	0	0	2	2	0	0	0	0	5	4	0	0	21	1.25
21	*Glypthothorax striatus*	0	2	3	0	0	0	0	2	3	0	0	0	0	0	4	3	0	0	17	1.01
22	*Glypthothorax barmanichus*	1	3	1	0	2	0	1	3	1	0	2	0	3	2	0	0	0	0	19	1.13

23	*Oreoglanis*	0	0	0	0	1	3	0	0	0	0	0	2	0	0	0	0	0	5	11	0.65
24	*Olyra kempi*	0	0	0	2	2	0	0	0	0	2	2	0	0	0	3	0	5	4	20	1.19
25	*Amblyceps apangi*	0	0	0	2	4	1	0	0	0	2	0	0	0	0	3	0	4	6	22	1.31
26	*Garra naganensis*	2	3	2	0	0	0	2	3	2	0	0	0	0	3	4	0	0	0	21	1.25
27	*Garra gotyla*	0	0	4	3	0	4	0	0	2	3	0	2	0	0	0	2	5	4	29	1.72
28	*Garra lamta*	0	0	2	2	0	0	0	0	2	2	0	0	0	0	3	6	3	7	27	1.60
29	*Garra kempi*	0	0	0	3	3	0	0	0	0	3	3	0	0	0	3	0	5	3	23	1.36
30	*Garra annandalei*	0	0	0	4	2	3	0	0	0	2	2	3	0	0	4	3	7	5	35	2.08
31	*Garra lissorhynchus*	0	0	3	5	4	6	0	0	3	4	3	4	0	0	3	7	5	8	55	3.27
32	*Devario aequipinnatus*	0	0	4	7	5	8	0	0	2	3	6	7	0	0	5	7	7	13	74	4.40
33	*Danio dangila*	0	0	0	2	4	0	0	0	0	2	0	2	0	0	0	0	4	6	20	1.19
34	*Pethia ticto*	0	0	0	4	5	7	0	0	0	4	5	7	0	0	7	4	6	4	53	3.15
35	*Pethia conchonicus*	0	0	0	2	5	4	0	0	0	2	2	4	0	0	12	7	10	15	63	3.75
36	*Puntius sophore*	0	0	0	0	4	3	0	0	0	0	4	3	0	0	0	0	7	9	30	1.78

37	*Puntius chola*	0	0	0	2	4	5	0	0	0	0	3	2	0	0	0	5	0	7	28	1.66
38	*Salmostoma bacaila*	0	0	0	3	3	5	0	0	0	4	2	3	0	0	0	4	9	7	40	2.38
39	*Opsarius bendelisis*	0	5	4	4	3	7	0	6	3	4	5	6	0	0	5	8	6	14	80	4.76
40	*Opsarius barna*	0	0	0	0	4	2	0	0	0	0	0	0	0	0	0	3	0	6	15	0.89
41	*Barilius barila*	0	0	3	4	5	6	0	0	0	2	7	2	0	0	0	6	8	12	55	3.27
42	*Channa aurantimaculat a*	0	0	4	3	0	0	0	0	3	4	0	0	3	5	3	0	0	0	25	1.48
43	*Channa punctatus*	0	0	0	0	0	6	0	0	0	0	3	8	0	0	0	0	8	7	32	1.90
44	*Channa orientalis*	2	3	0	2	0	0	2	3	0	2	0	0	3	4	5	0	0	0	26	1.54
45	*Channa stewartii*	0	0	3	0	0	0	0	0	3	0	0	0	0	0	4	0	3	0	13	0.77
46	*Channa gachua*	0	0	0	0	0	5	0	0	0	4	10	12	0	0	0	0	7	5	43	2.56
47	*Channa striatus*	0	0	0	0	3	3	0	0	0	0	7	6	0	0	0	0	5	7	31	1.84
48	*Badis badis*	0	0	0	0	2	5	0	0	0	0	0	3	0	0	0	3	4	5	22	1.31
49	*Badis assamensis*	0	0	0	0	4	3	0	0	0	0	0	5	0	0	0	4	4	6	26	1.54

50	*Mastacembalus armatus*	0	2	2	4	0	0	0	2	2	0	3	0	0	0	3	3	4	8	33	1.96
51	*Xenontodon cancila*	0	0	2	3	0	0	0	0	0	0	0	3	0	0	0	4	7	5	24	1.42
52	*Anabas testudineus*	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	5	6	18	1.07
		12	37	78	100	119	159	10	32	45	64	87	116	30	42	126	138	205	279	1679	

The seasonal species abundance of the river Doyang is represented in Table 5. The highest species abundances were recorded in post monsoon season at station 6 with 279 individuals followed by 205 in station 5 in post-monsoon and 159 (16.94%) in pre-monsoon season in station 6. The lowest was reported in station 1 with 10 number individuals in monsoon season. The most dominant species were the *Opsarius bendelisis* (4.76%) followed by *Devario aequipinnatus* (4.40%) and *Pethia conchonicus* (3.70%).

3.2 Diversity indices

Diversity indices of the river Doyang are shown in three different seasons. In pre monsoon season (Table 6; Figure 4) the Margalef's richness index (d) was found to be highest at station 4 with a value of 6.29 and with a lowest value of 2.81 at station 1 whereas Pielou's evenness index (J') was found to be highest at station 2 (0.949) and lowest at Station 6 (0.909). Shannon-Weinner index (H') was found to be highest at station 6 (3.37) and lowest at station 1 (2.02). Simpson index (1-ƛ) was found to be highest at station 5 (0.963) and lowest at station 1 (0.86).

Table 6: Station wise diversity indices of the river in pre - monsoon season

Station	**Margalef's richness index (d)**	**Pielou's evenness index (J')**	**Shannon-Weinner Index (H')**	**Simpson index (1-λ')**
Station 1	2.817	0.944	2.02	0.86
Station 2	3.6	0.949	2.588	0.92
Station 3	5.96	0.935	3.24	0.959
Station 4	6.29	0.928	3.32	0.961
Station 5	6.277	0.928	3.36	0.963
Station 6	6.116	0.909	3.37	0.962

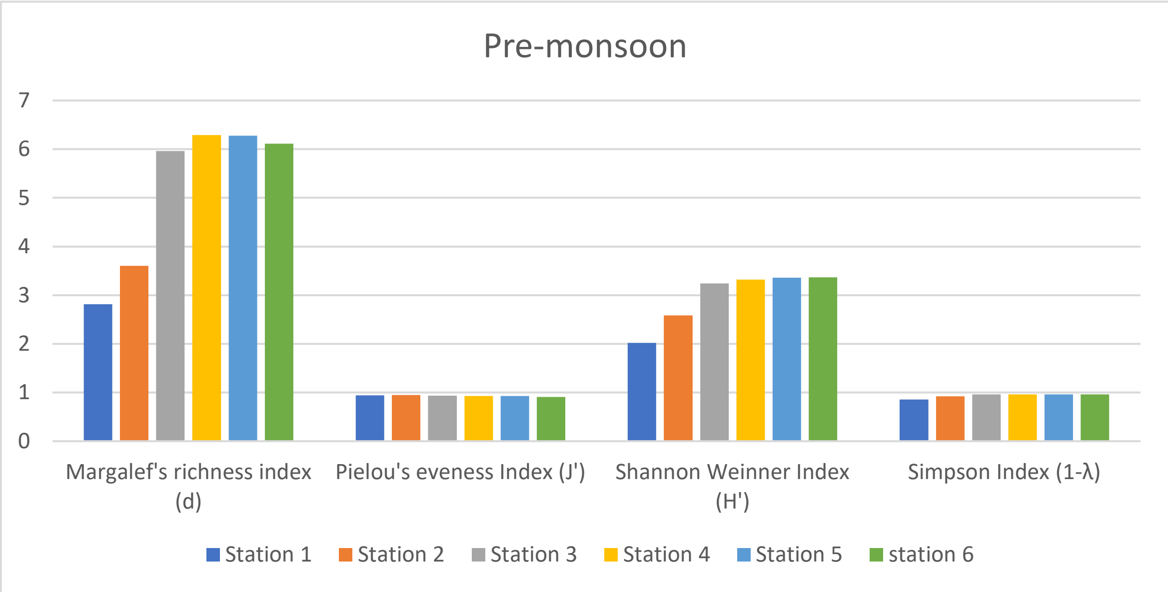


FIGURE 4: STATION WISE DIVERSITY INDICES OF THE RIVER DOYANG IN PRE – MONSOON SEASON

In monsoon season (Table 7; Figure 5) the Margalef's richness index (d) was found to be highest at station 6 with a value of 5.89 and with a lowest value of 2.17 at station 1 whereas Pielou's evenness index (J') was found to be highest at station 1 and 3 (0.957) and lowest at Station 5 (0.861). Shannon-Weinner index (H') was found to be highest at station 4 (3.086) and lowest at station 1 (1.74). Like that, the highest value of Simpson index (1-ƛ) was found to be at station 6 (0.954) and lowest at station 1 (0.82).

Table 7: Station wise diversity indices of the river in monsoon season

Station	Margalef's richness index (d)	Pielou's evenness index (J')	Shannon-Weinner Index (H')	Simpson index (1-λ)
Station 1	2.17	0.957	1.74	0.82
Station 2	2.885	0.932	2.328	0.894
Station 3	4.72	0.957	2.901	0.943
Station 4	5.29	0.952	3.086	0.952
Station 5	5.374	0.861	3.069	0.946
Station 6	5.89	0.863	3.22	0.954

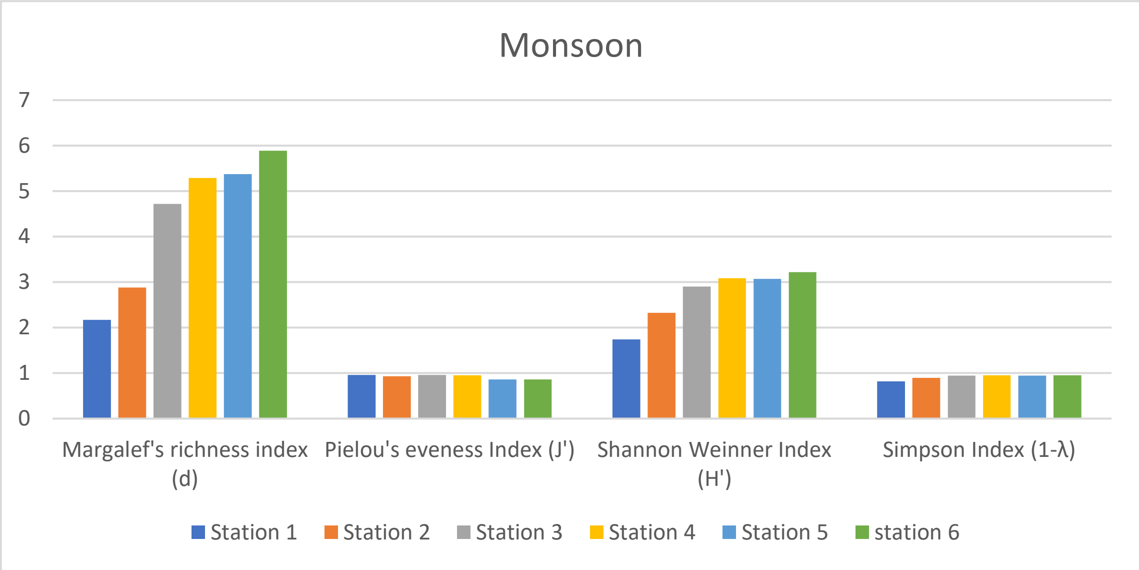


FIGURE 5: STATION WISE DIVERSITY INDICES OF THE RIVER DOYANG IN MONSOON SEASON

In post monsoon season (Table 8; Figure 6) the Margalef's richness index (d) was found to be highest at station 6 with a value of 6.51 and with a lowest value of 2.352 at station 1 whereas Pielou's evenness index (J') was found to be highest at station 1 (0.971) and lowest at Station 6 (0.922). Shannon-Weinner index (H') was found to be highest at station 6 (3.55) and lowest at station 1 (2.168). Like that, the highest value of Simpson index (1-ƛ) was found to be at station 6 (0.969) and lowest at station 1 (0.88).

Table 8: Station wise diversity indices of the river in post monsoon season

Station	**Margalef's richness index (d)**	**Pielou's evenness index (J')**	**Shannon-Weinner Index (H')**	**Simpson index (1-λ)**
Station 1	2.352	0.971	2.168	0.88
Station 2	2.675	0.958	2.356	0.901
Station 3	5.58	0.931	3.26	0.95
Station 4	5.886	0.943	3.34	0.962
Station 5	6.38	0.95	3.5	0.968
Station 6	6.51	0.922	3.55	0.969

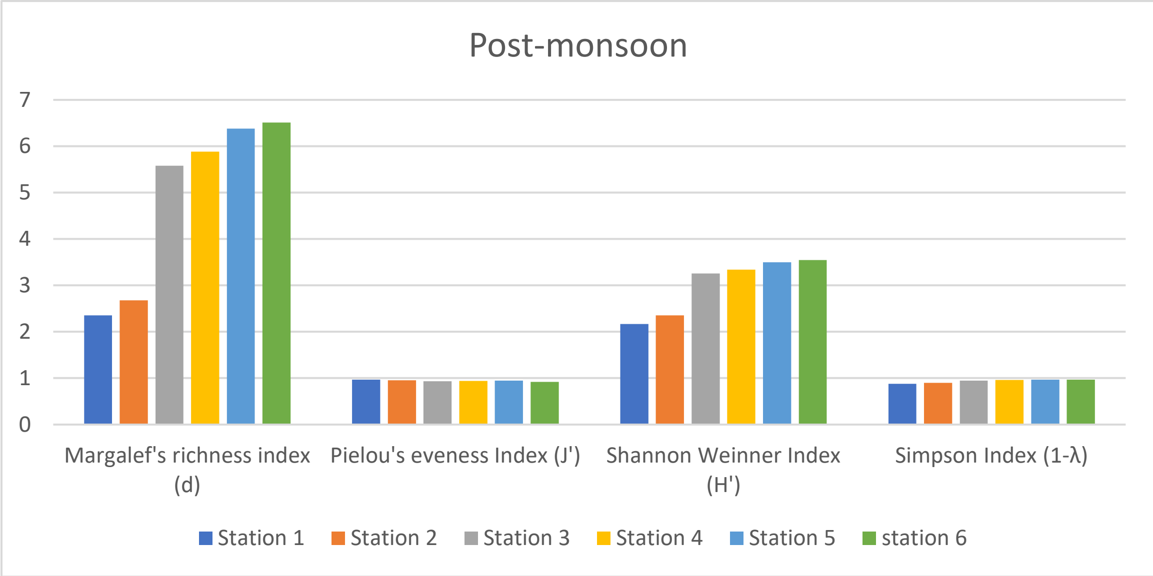


FIGURE 6: STATION WISE DIVERSITY INDICES OF THE RIVER DOYANG IN POST MONSOON SEASON

3.3 Local fishing techniques and gears

Selection of fishing methods and gears are influenced by various factors such as physiographic of the water body, nature of fish stock, characteristics of the raw material from which gear are fabricated and standard of living (Choudhury, 1992). Therefore, variation in application of gear can be observed in different rivers, which have characteristic of their own due to unique nature of the water resources of the region (Gurumayum and Choudhury, 2009). As such, the different techniques and gears prevalently used along the length of Doyang river system are described herewith. The pictorial representations of various fishing gears are used in the river system are given below:

	
Tsüteptsü (Scoopnet)	
	

	
Atep Kago	Longr kago
	
	

3.4 Conservation status of the fish fauna of the river Doyang

The IUCN conservation status of the 52 recorded species with their number under different category are shown in Table 9 and Figure 7. The highest species were recorded under least concern (LC) category with a total no of 39 and contributed 75%. under LC category, the major species contribution is from the family Cyprinidae with 20 (38.46 %) followed by Channidae 5 (9.61 %), Nemacheilidae 4 (7.6 %), Silorhynchidae and Sissoridae with 2 (3.86 %) each, Bagaridae, Amblycepitidae, Bedidae, Anabantidae, Mastacembalidae and Belonidae with 1 species contributed 1.92 % each. Under near threatened (NT) category Cyprinidae and Sissoridae contributed 3 (5.76%) and 1 (1.92 %) species respectively.

Table 9: Number (percentage) of species belonged to each family under different categories of IUCN conservation status

Order	**Family**	**NT**	**LC**	**VU**	**EN**	**NE**	**Total**
Cypriniformes	Cyprinidae	3	20	2	1	2	28
	Silorhynchidae	0	2	0	0	0	2
	Nemacheilidae	0	4	0	0	1	5
Siluriformes	Sissoridae	1	2	0	0	1	4
	Bagaridae	0	1	0	0	0	1
	Amblycepitidae	0	1	0	0	0	1
Perciformes	Channidae	0	5	0	0	1	6
	Bedidae	0	1	0	0	1	2
	Anabantidae	0	1	0	0	0	1
Symbranchiformes	Mastacembalidae	0	1	0	0	0	1
Baloniformes	Belonidae	0	1	0	0	0	1
Total		4	39	2	1	6	52

Like that, the family Cyprinidae represented the vulnerable (VU) category with 2 (3.84 %) species each. One species which contributed 1.92 % under Cyprinidae family represented the endangered (EN) category. A total of 6 nos, 2 (3.84%) from cyprinidae, 1 (1.92%) from each Nemacheilidae, Sissoridae, Channidae and Bedidae respectively falls under the not evaluated (NE) category of IUCN conservation status (2021).

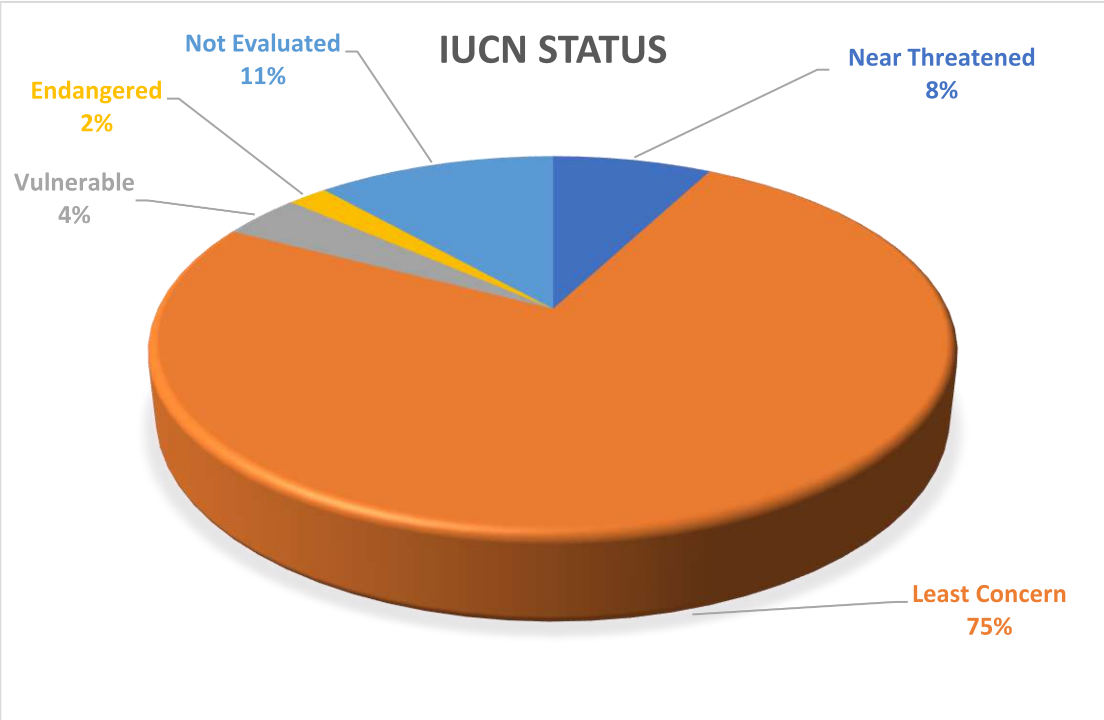


FIGURE 7: IUCN CONSERVATION STATUS OF THE FISH FAUNA EXPRESSED IN PERCENTAGE.

3.5 Molecular characterization of fish fauna of the selected river system

Total of 38 number of fish species (73.07%) COI gene sequence have been generated from the said river system and successfully submitted to the NCBI data whose gene accession number is being listed in Table 10.

Table 10: DNA barcoding of the species recorded with accession number.

Sl. No	Name of the Species	Accession Code
1)	*Tor tor*	OK087606
2)	*Tor putitora*	MN563577
3)	*Neolissochilus hexagonolepis*	OK090918
4)	*Schizothorax labiatus*	OK254887
5)	*Labeo pangusia*	OK036592
6)	*Tariqilabeo latius*	MN830285
7)	*Esomus dendricus*	OK176529
8)	*Pethia conchonicus*	MN830291
9)	*Garra naganensis*	OK036586
10)	*Garra gotyla*	OK091000
11)	*Garra kempi*	OK036441
12)	*Garra annadelei*	OK090934
13)	*Opsarius bendelisis*	MT755011
14)	*Devario aequipinnatus*	MN830286
15)	*Schistura fasciata*	MT755012
16)	*Schistura maculosa*	OK036587
17)	*Schistura naganensis*	MN830289
18)	*Schistura corica*	OK103746
19)	*Glytothorax striatus*	MN830287
20)	*Channa aurantimaculata*	MN830290
21)	*Channa punctatus*	OK091125 OK462977
22)	*Channa orientalis*	OK091025
23)	*Channa stewarti*	OK091602
24)	*Channa gachua*	OK091663
25)	*Badis assamensis*	MN830288
26)	*Mastacembelus armatus*	MT755010
27)	*Neolissochilus hexastichus*	OK462979
28)	*Cyprinion semiplotum*	OK462972
29)	*Salmostoma bacaila*	OK462971
30)	*Puntius sophore*	OK462978
31)	*Garra lissorhynchus*	OK462974

32)	*Barilius Barila*	OK462980
33)	*Opsarius barna*	OK462973
34)	*Psilorhynchus homoleptera*	OK462976
35)	*Psilorhynchus balitora*	OK462981
36)	*Acanthocobitis botia*	OK462975
37)	*Badis Badis*	OK462983
38)	*Amblyceps apangi*	OK462982

3.6 Planktons

During the present study a total of 30 genera of plankton was recorded out of which phytoplankton consist of 18 genera under 3 family namely Chlorophyceace, Bacillariophyceae and Cyanophyceae and Zooplankton of 12 genera under 3 family namely Cladocera, Rotifera and Copepoda. Detailed of which is shown in the Table 11 and Figure 8 and 9.

Table 11: Average monthly variations of Plankton analysis during the study period.

		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Chlorophyceace													
Spirogyra		10	8	10	0	0	5	0	2	2	3	5	10
Volvox		9	8	0	8	7	0	3	0	4	8	4	3
Cladocera		11	0	6	7	6	0	0	0	0	2	2	0
ulothrix		12	9	5	6	11	3	0	3	3	2	0	2
Chlorella		0	10	5	0	9	2	1	0	2	5	2	4
oedogoniun		6	7	7	0	0	0	5	2	0	3	7	0
Chlamydomonas		3	5	2	9	2	1	0	2	2	0	4	8
Cloisterium		2	0	3	5	2	3	2	1	1	2	2	2
Zygnema		3	3	3	6	3	0	2	3	0	7	7	3
Total		56	50	41	41	40	14	13	13	14	32	33	32
Bacillariophyceae													
Navicula		8	0	4	1	0	0	2	2	2	2	3	2
Nitzschia		5	4	5	1	5	1	0	1	0	2	1	0
Diatoma		2	7	0	3	4	2	2	0	2	4	0	3
Melosira		6	8	3	6	3	0	1	2	0	2	2	5
Gomphonema		3	8	5	5	1	1	0	2	2	0	4	0
Total		24	27	17	16	13	4	5	7	6	10	10	10
Cyanophyceae													
Spirulina		0	7	2	0	5	1	1	1	0	2	3	0
Anabaena		7	0	2	3	3	2	0	2	2	1	2	1
Nostoc		8	11	2	8	0	0	2	0	3	3	0	2
Oscillatoria		1	0	9	0	2	1	1	2	0	0	1	3
Total		16	18	15	11	10	4	4	5	5	6	6	6
Cladocera													

Moina		0	4	0	2	2	0	2	0	1	0	1	0
Daphnia		6	0	4	2	2	0	0	3	0	1	2	1
Bosmina		0	5	2	3	4	0	2	0	2	2	0	2
Alona		5	0	1	4	2	2	0	0	1	1	2	3
Pleroxqs		0	6	4	0	3	0	0	1	2	0	1	1
Diaphanosoma		3	0	2	3	1	1	0	0	0	4	2	1
Total		14	15	13	14	14	3	4	4	6	8	8	8
Rotifera													
Branchionous		0	3	2	0	2	2	0	1	0	2	0	1
Cephalodella		5	0	1	4	3	0	2	0	3	0	4	3
Tolyarpha		8	4	2	3	2	0	2	2	2	2	3	0
Asplanchna		0	5	2	3	4	1	0	2	0	2	0	3
Total		13	12	7	10	11	3	4	5	5	6	7	7
Copepoda													
Cyclops		0	6	4	1	1	0	0	3	2	2	2	2
Diamtomus		7	0	0	2	2	3	2	0	0	2	0	0
Total		7	6	4	3	3	3	2	3	2	4	2	2
All total		130	128	97	95	91	31	32	37	38	66	66	65

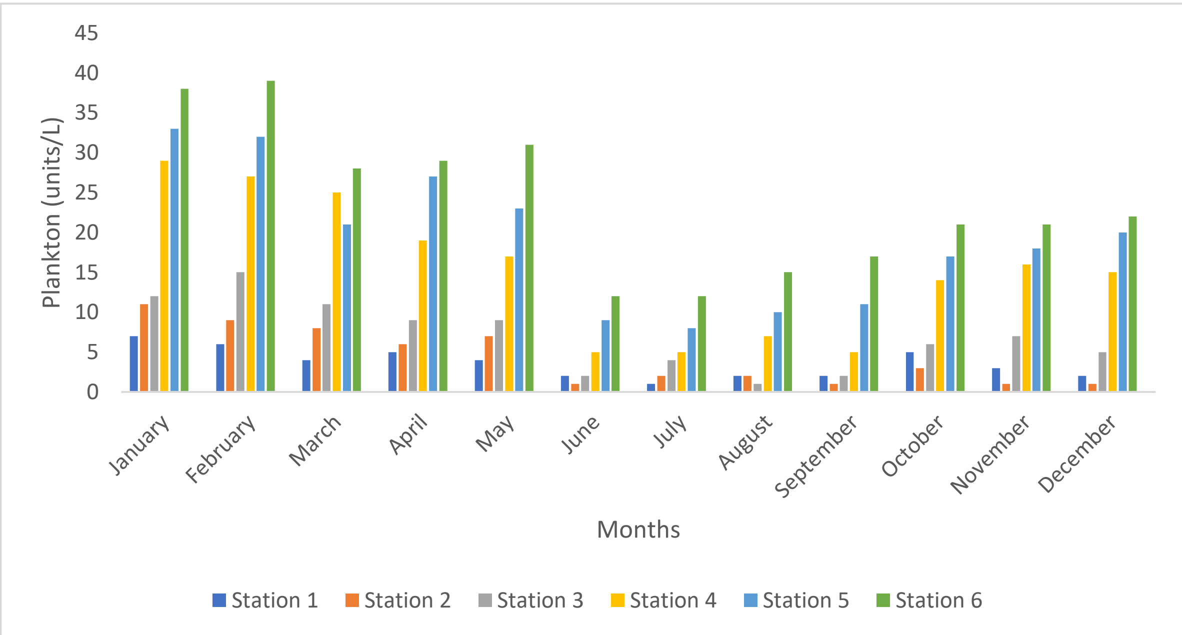


Figure 8: Diagrammatic representation of average monthly variations of planktons from the study area.

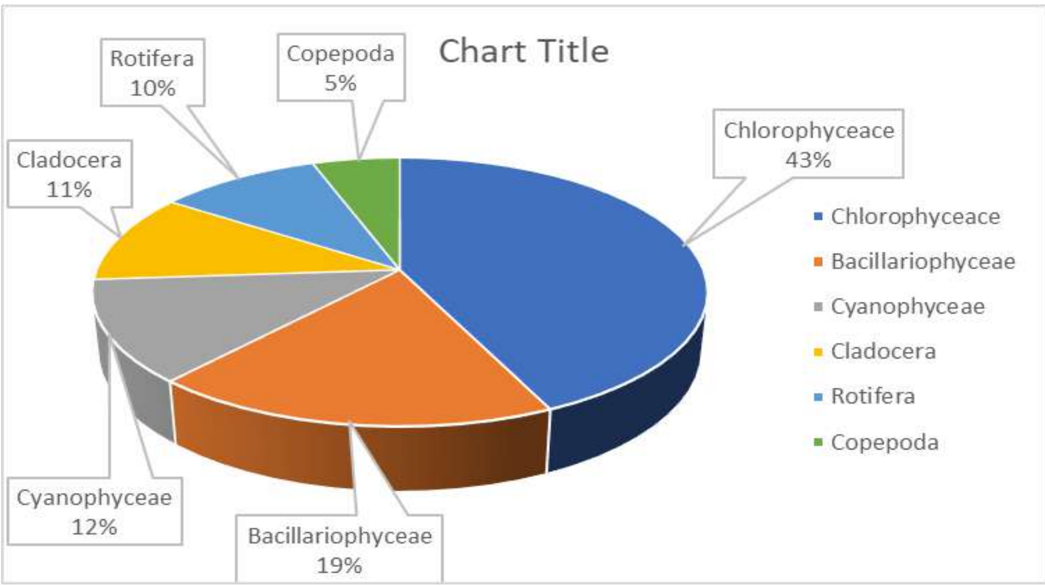


Figure 9. Diagrammatic representation of percentage composition different groups of planktons.

3.6.1 Qualitative composition of Phytoplankton:

During the present study, a total of 18 species of phytoplankton were recorded. Three majors groups of phytoplankton viz. Chlorophyceace represented by 9 species, Bacillariophyceae represented by 5 species and Cyanophyceae represented by 4 species were found in the different stations along the Doyang river system.

The monthly and station wise variations of phytoplankton are depicted is Table 12 and Figure 10. The mean qualitative richness of phytoplankton ranged from 1.0-23.0 nos. The maximum phytoplankton richness 23.0 species was collected in the month of Febuary (station VI). The minimum phytoplankton 0 species was observed in the month of June.

Table 12: Average monthly variation of phytoplankton during the present study.

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
January	3	7	10	17	20	20
February	3	5	8	15	18	23
March	1	4	6	15	12	19
April	4	3	5	15	18	19
May	4	6	4	11	15	20
June	0	1	1	3	7	9
July	1	2	3	3	4	7
August	1	1	0	5	4	9
September	1	0	2	3	7	11
October	3	1	3	6	7	11
November	1	1	4	8	11	9
December	1	1	2	6	10	12

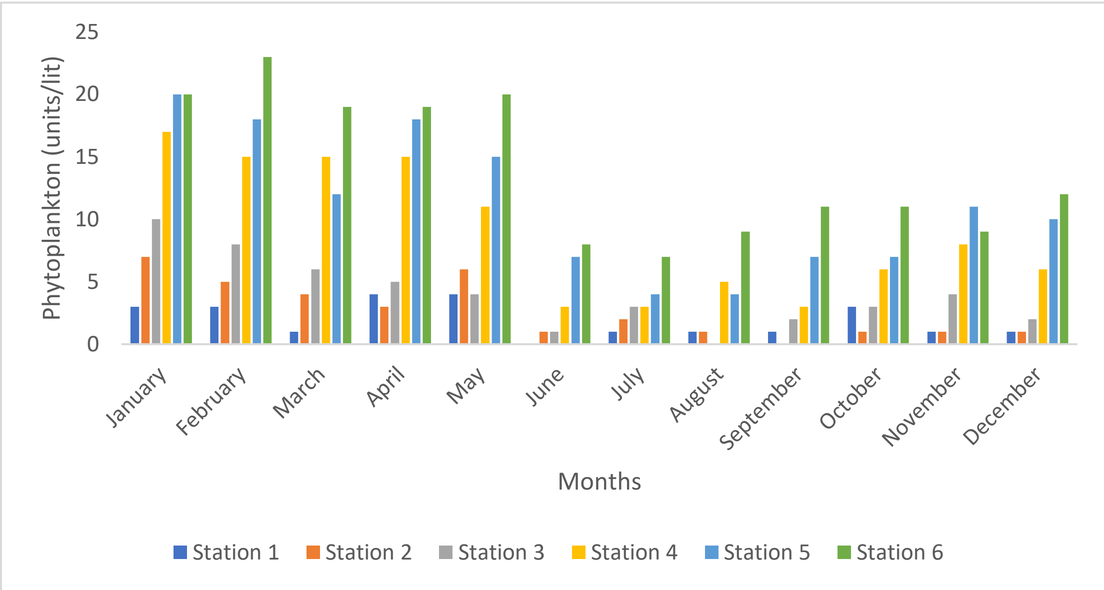


Figure10: Diagrammatic representation of monthly variation of phytoplankton.

3.6.2 Qualitative composition of Zooplankton

During the present study, a total of 12 species of zooplanktons belonging to three categories of zooplankton viz. Cladocera represented by 6 species, Rotifera represented by 4 species, Copepoda represented by 2 species was collected from the Doyang river.

Among the zooplankton, a total of four species was observed in all the six stations. The monthly and station wise variations of zooplanktons are depicted is Table 13 and Figure 11. The qualitative richness of zooplankton ranged from 0.0-18.0 species. The maximum zooplankton richness 18.0 species (fig.20) was collected in the month of January (station VI). The mean minimum zooplankton 0 species was observed in the month of May (Station I), June, July, November and December (Station II) and September (Station III).

Table13 : Monthly analysis of Zooplankton analysis from the study area.

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
January	4	4	2	12	13	18
February	3	4	7	12	14	16
March	3	4	5	10	9	9
April	1	3	4	4	9	10
May	0	1	5	6	8	11
June	2	0	1	2	2	4
July	0	0	1	2	4	5
August	2	1	1	2	6	5
September	1	1	0	2	4	6
October	2	3	3	8	10	9
November	2	0	3	8	7	12
December	1	0	3	9	10	10

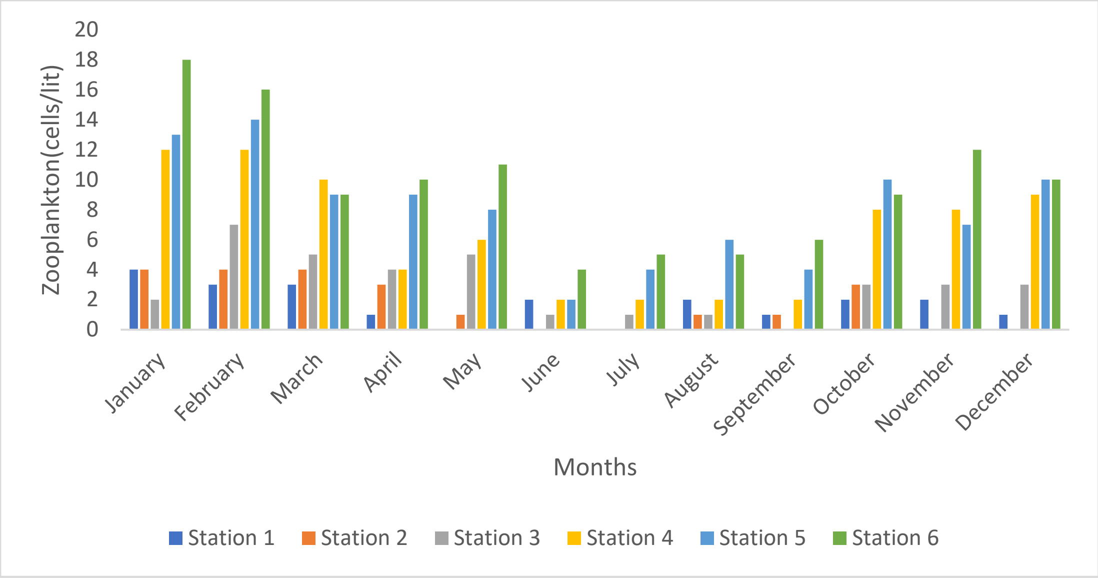


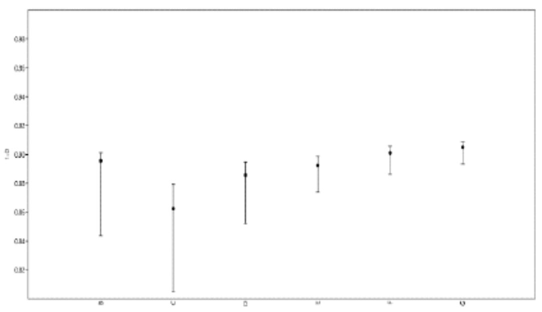
Figure 11: Diagrammatic representation of monthly variation of Zooplankton.

3.6.3 Plankton Diversity Index

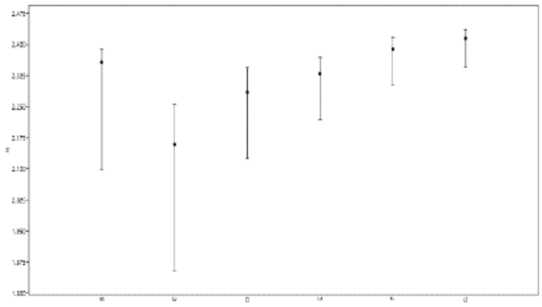
During the study period the Margalef's richness index (d) was found to be highest at station 1 with a value of 2.925 and with a lowest value of 1.946 at station 6 whereas Pielou's evenness index (J') was found to be highest at station 6 (0.9321) and lowest at Station 2 (07214). Shannon-Weinner index (H') was found to be highest at station 6 (2.415) and lowest at station 2 (2.158). Like that, the highest value of Simpson index (1-ƛ) was found to be at station 6 (0.905) and lowest at station 2 (0.8624) (Table 14; Figure 12)

Table 14: Station wise diversity indices of plankton from the Doyang river system.

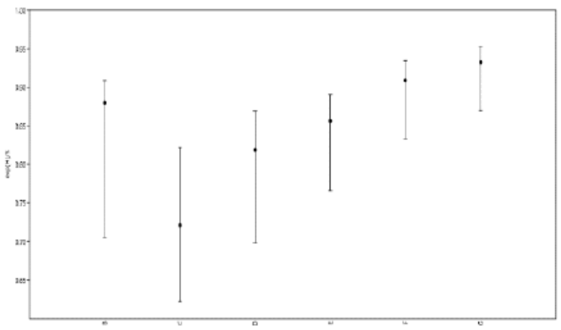
Sl. No	Index	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
1	Simpson	0.8956	0.8624	0.8858	0.8923	0.901	0.905
2	Shannon	2.357	2.158	2.285	2.33	2.389	2.415
3	Evenness	0.8796	0.7214	0.8189	0.8561	0.9085	0.9321
4	Margalef	2.925	2.784	2.489	2.109	2.024	1.946



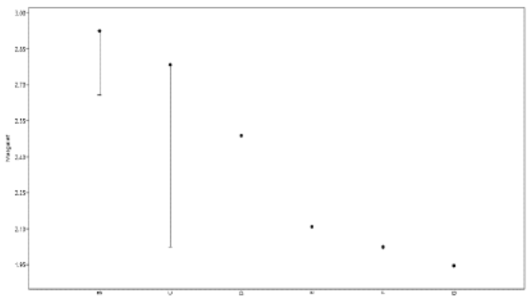
Shannon index



Simpson index



Evenness index



Margalef index

Figure 12. Graphs obtained from the Past 3 Software

3.6.4 Palmer Index

Palmer (1969), first made the list of algae genera and species which indicate organic pollution (Table 15). According to Palmer, scores of 20 or more are indication of high organic pollution. The pollution tolerant genera belonging to three groups of algae from six sites of Doyang river system was recorded (Table 16). By using Palmer's index of pollution for rating of water samples as high, moderate and low organically polluted at six sites of Doyang river system were tested. The total score of Algal Genus Pollution Index (AGPI) of sites S1, S2< S3< S4<S5, S6 were calculated to be 2, 5, 7 and 9 respectively (Table 2). The total scores of S1 and S2 showed 4 indicating probable lack of organic pollution while S5 and S6 showed moderate pollution due to anthropogenic factors or human interference according to Palmer, Closterium was found to be the most active participant in most of the sites which may be the good indicator of contaminated water. *Navicula, Nitzschia,* were recorded repeatedly in station 3 and 4 and consider as indicators of pollution in view of the results of Palmer pollution index.

Table 15: Algal genus pollution index (Palmer, 1969).

Genus	Pollution Index
Anacystis	1
Ankistrodesmus	2
Chlamydomonas	4
Chlorella	3
Closterium	1
Cyclotella	1
Euglena	5
Gomphonema	1
Lepocinclis	1
Melosira	1
Micractinium	1
Navicula	3
Nitzschia	3
Oscillatoria	5
Pandorina	1
Phacus	2
Phormidium	1
Scenedesmus	4
Stigeoclonium	2
Synedra	2

Following numerical values for pollution classification of Palmer (1969), 0-10= Lack of organic pollution 10-15= Moderate pollution 15-20= Probable high organic pollution 20 or more = Confirms high organic pollution.

Table 16. Pollution index of Algal genera level according to Palmer, (1969) at Six sites of river Doyang.

Genus	Pollution Index (Palmer, 1969)	S1	S2	S3	S4	S5	S6
Chlorophyceace							
Spirogyra	-	+	+	+	+	+	+
Volvox	-	+	+	+	+	+	+
Cladocera	-	+	+	+	+	+	+
Ulothrix	-	+	+	+	+	+	+
Chlorella	3	-	-	-	-	-	+(3)
Oedogoniun	-	+	+	+	+	+	+
Chlamydomonas	4	-	-	-	-	+(4)	-
Closterium	1	+(1)	+(1)	+(1)	-	-	-
Zygnema	-	+	+	+	+	+	+
Bacillariophyceae							
Navicula	3	-	-	+(3)	+(3)	-	-
Nitzschia	3	-	-	+(3)	+(3)	+	+
Diatoma	-	-	-	+	+	+	+
Melosira	1	-	-	+(1)	+(1)	-	-
Gomphonema	1	+(1)	+(1)	+(1)	-	-	+(1)
Cyanophyceae							
Spirulina	-	+	+	+	+	+	+
Anabaena	-	+	+	+	+	+	+
Nostoc	-	+	+	+	+	+	+
Oscillatoria	5	-	-	-	-	+(5)	+(5)
Total Score		**2**	**2**	**5**	**7**	**9**	**9**

3.7 Physico-chemical properties of water

The physico-chemical properties of an aquatic body greatly influence the survival, growth and reproduction of aquatic flora and fauna. The natural factors which determine the water quality in the river are the precipitation, dissolution of rocks and evaporation crystallization process (Allen, 1995). During the present investigation seasonal variations of different water quality parameters (viz. water temperature, water depth, water velocity, pH, dissolved oxygen, total dissolved solids, conductivity, alkalinity, hardness, turbidity, BOD, COD, ammonia, nitrate and phosphorous) of the river Doyang were estimated. The values are presented in the figure below

3.7.1 Surface Water temperature:

Surface Water temperature affects the growth and reproduction of living organisms. It has a great impact on water density. During the study period the maximum water temperature was recorded as 24.85˚C in station 2 during pre-monsoon and minimum 17.75˚C was recorded in station 1 during winter (Figure 13).

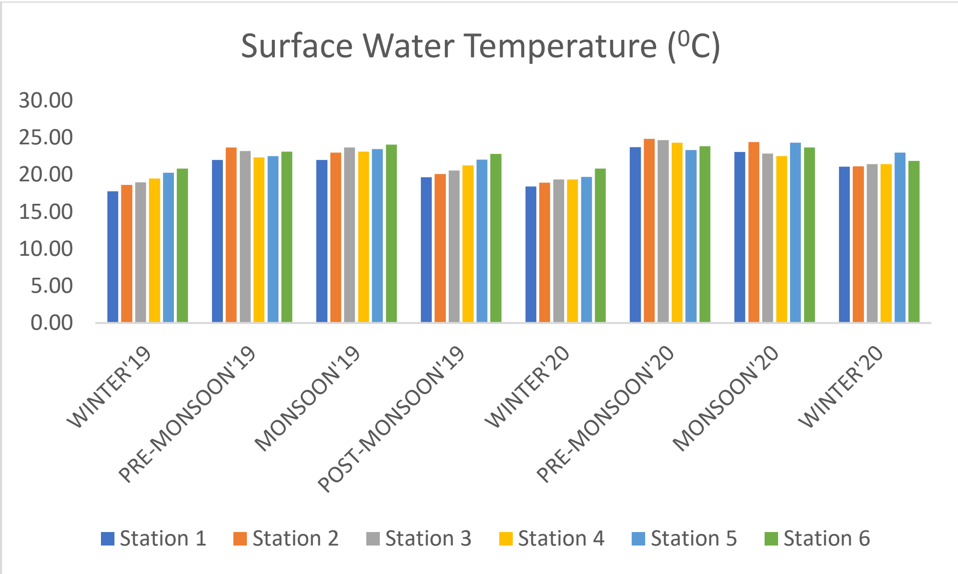


FIGURE 13: SEASONAL VARIATION OF WATER TEMPERATURE (˚C) AT SIX SELECTED STATIONS.

3.7.2 Water Depth

Water depth affects the abundance of living organisms. It has a great impact on growth and reproduction in freshwater ecosystem. During the study period the maximum water temperature was recorded as 1.48 m at station 6 during monsoon and the minimum 0.78m was recorded at station 1 during winter (Figure 14)

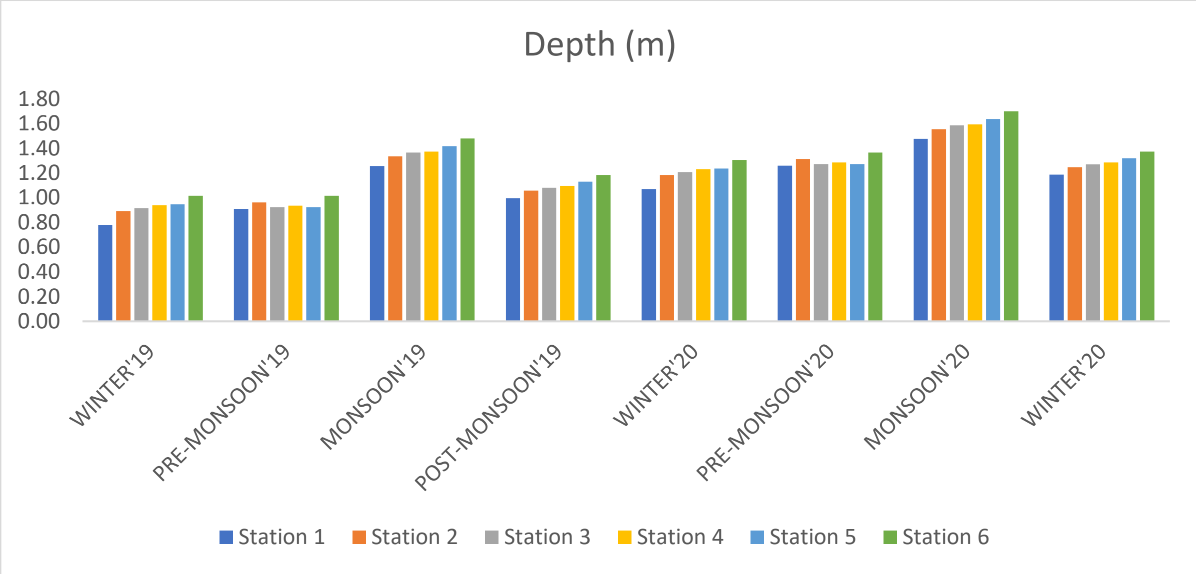


FIGURE 14: SEASONAL VARIATION OF WATER DEPTH (METER) AT SIX SELECTED STATIONS

3.7.3 Water velocity

The water velocity of a river changes along the course of river and is determined by factors such as the gradient, the volume of water, the shape of river channel and the amount of friction created by the bed, rocks and plants. During the present study period, the maximum water velocity was recorded 0.88 m/sec at station 1 during monsoon and the minimum water current was recorded 0.20 m/sec at station 6 during winter (Figure 15).

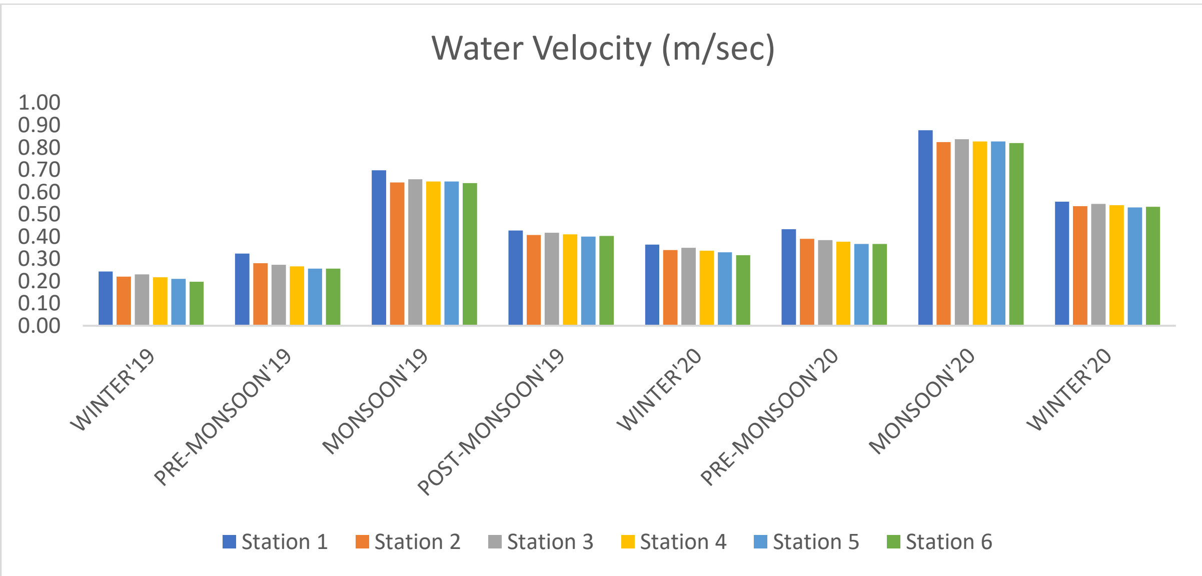


FIGURE 15: SEASONAL VARIATION OF WATER VELOCITY (METER PER SEC) AT SIX SELECTED STATIONS

3.7.4 Water pH

It is a measure of the acidity of the water based on its hydrogen ion concentration and can be defined as the negative logarithm of the hydrogen ion concentration. During the period of investigation, the maximum water pH 7.6 was recorded at station 3 and 5 during pre-monsoon respectively, whereas a minimum water pH of 6.47 was recorded at station 6 during pre-monsoon (Figure 16).

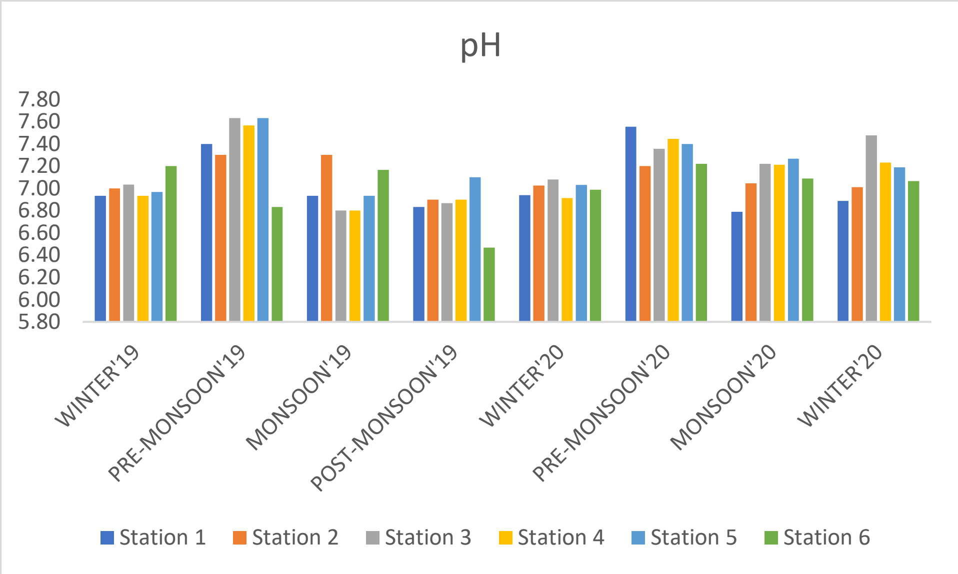


FIGURE 16: SEASONAL VARIATION OF WATER PH AT SIX SELECTED STATIONS

3.7.5 Dissolved oxygen (DO)

Dissolved oxygen concentration of water is affected by diffusion and aeration, photosynthesis, respiration and decomposition. During the period of investigation, the monthly dissolved oxygen was found to be highest during winter with a high value of 10.63 ppm at station 2 and the lowest value of 7.2 ppm was recorded at station 1 in during monsoon. (Figure 17).

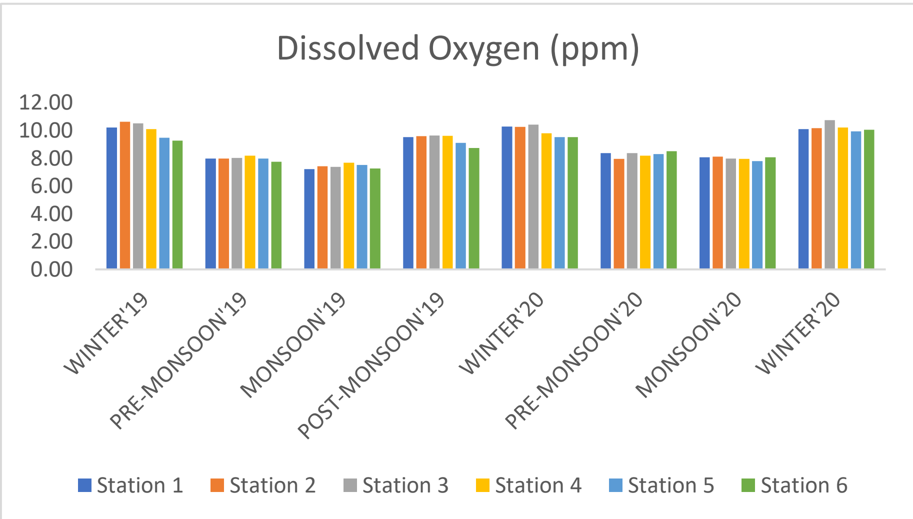


FIGURE 17: SEASONAL VARIATION OF DISSOLVED OXYGEN (PPM) AT SIX SELECTED STATIONS

3.7.6 Total dissolved solids

Total dissolved solids are a measure of the inorganic salts mainly calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates and organic substances contained in a liquid in molecular ionized or micro-granular (colloidal sol) suspended form. During the study period the total dissolved solids was recorded with a highest value of 158.33 ppm at station 4 during monsoon and lowest 56.33 ppm at station 5 during winter respectively (Figure 18).

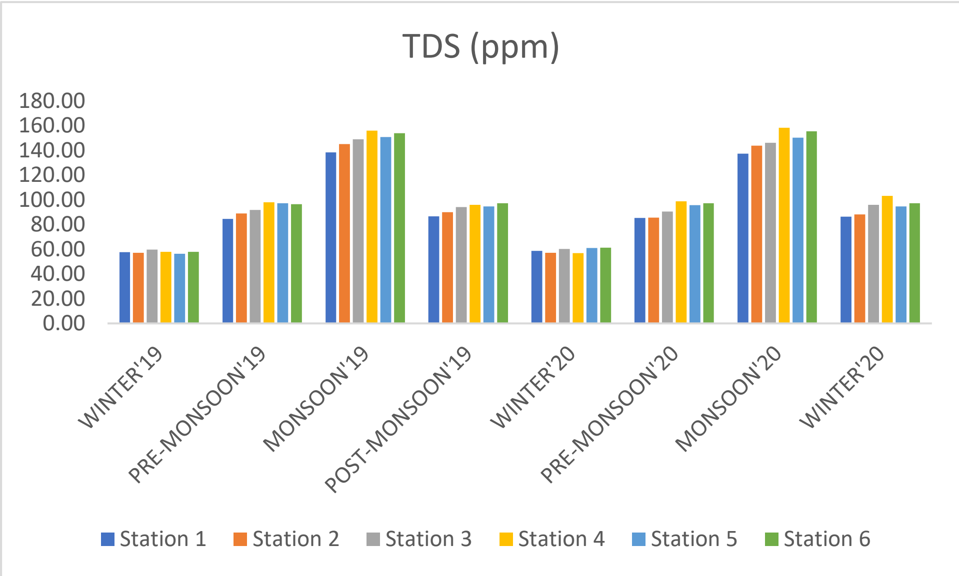


FIGURE 18: SEASONAL VARIATION OF TOTAL DISSOLVED SOLID (ppm) AT SIX SELECTED STATIONS

3.7.7 Turbidity

Total dissolved solids are a measure of the inorganic salts mainly calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates and organic substances contained in a liquid in molecular ionized or micro-granular (colloidal sol) suspended form. During the study period the total dissolved solids was recorded with a highest value of 49.71 NTU at station 6 during monsoon and lowest 5.06 NTU at station 3 during winter (Figure 19).

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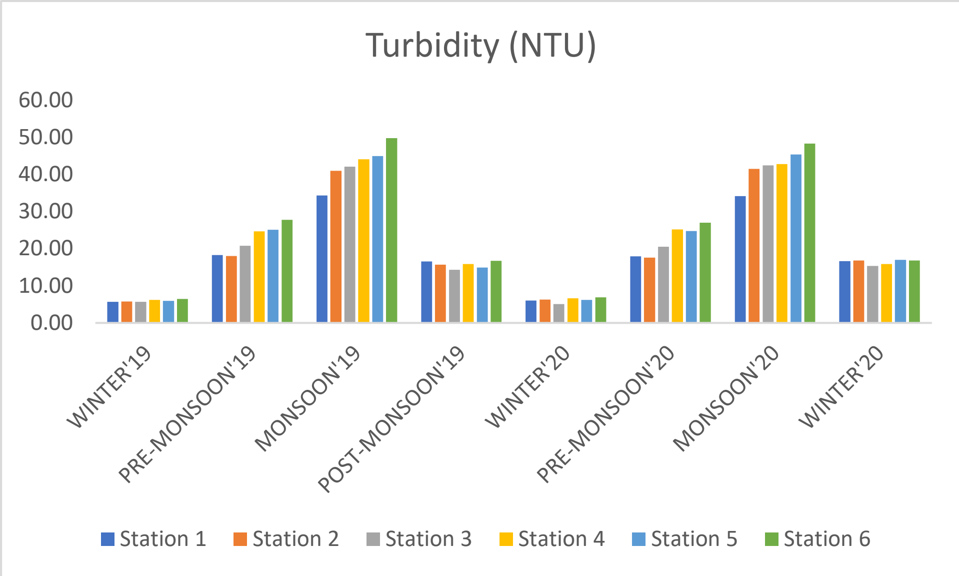


FIGURE 19: SEASONAL VARIATION OF TURBIDITY (NTU) AT SIX SELECTED STATIONS

3.7.8 Conductivity

Conductivity is a measure of water's capacity to pass electrical flow. It is directly related to the concentration of dissolved salts and inorganic materials such as alkalis, chlorides, sulphides and carbonate compounds. The maximum value 425.33 µS cm^{-1} of specific conductivity was recorded at station 2 during monsoon and minimum value 90.02 µS cm^{-1} was recorded at station 2 during winter (Figure 20).

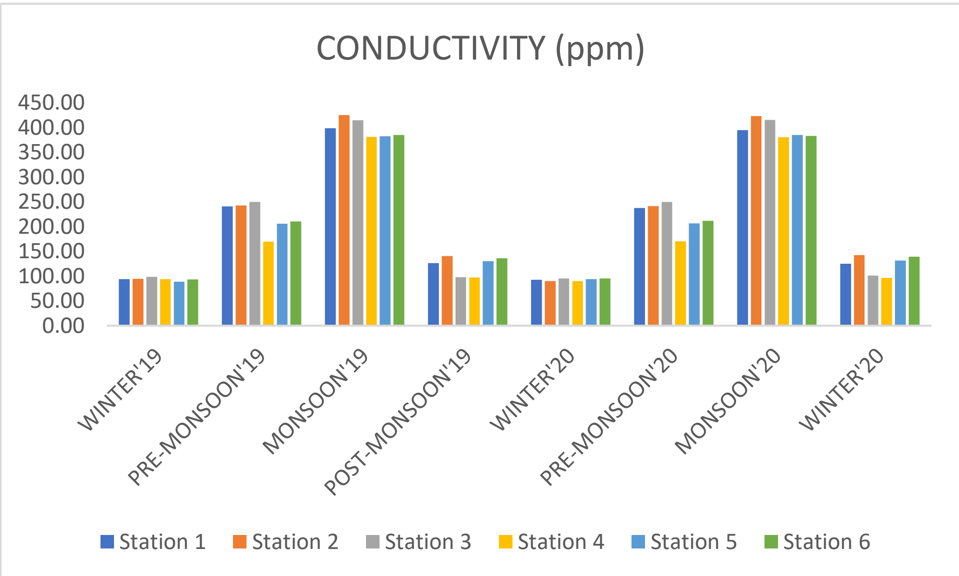


FIGURE 20: SEASONAL VARIATION OF CONDUCTIVITY (µScm) AT SIX SELECTED STATIONS.

3.7.9 Alkalinity

Alkalinity is a measure of the water's ability to neutralize acidity. It tests the level of bicarbonates, carbonates and hydroxides in water. During the present investigation the maximum alkalinity value was recorded 180.63 at station 2 and 3 during monsoon and the minimum value 41 mg l^{-1} was recorded at station 4 during post monsoon (Figure 21).

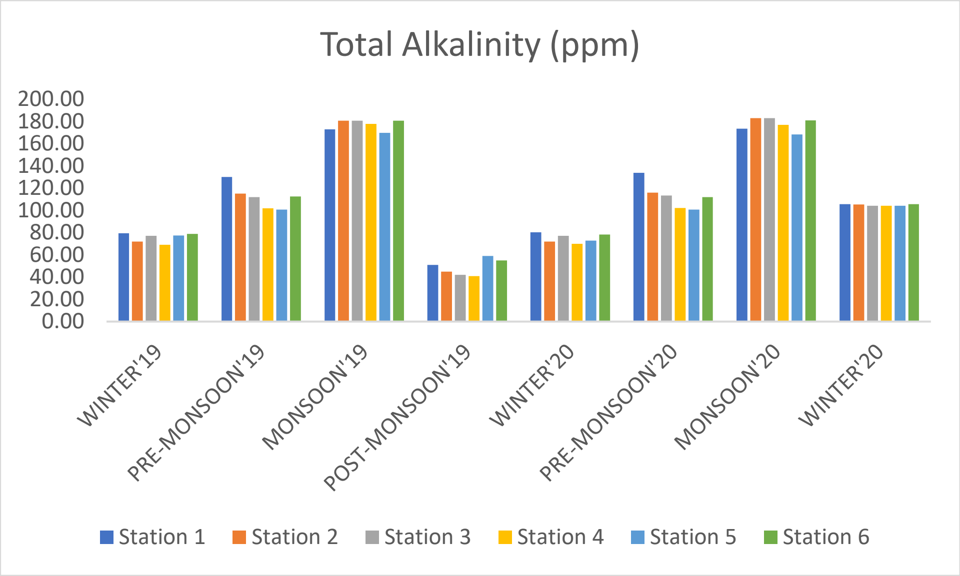


FIGURE 21: SEASONAL VARIATION OF ALKALINITY (ppm) AT SIX SELECTED STATIONS

3.7.10 Hardness

Hardness of water is the measure of the quantities of calcium and magnesium salts mainly present in the water. Different divalent salts are involved in the hardness of water, but calcium and magnesium constitute the most common source. During the present investigation the maximum hardness value was recorded 173.84 ppm at station 3 during premonsoon and the minimum value 87.38 mg l^{-1} was recorded at station 3 during winter (Figure 22).

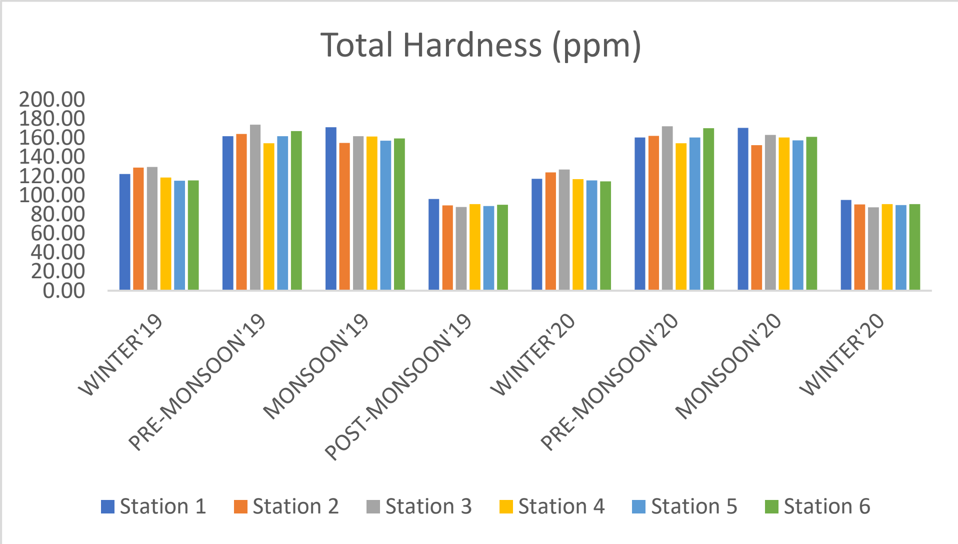


FIGURE 22: SEASONAL VARIATION OF HARDNESS (ppm) AT SIX SELECTED STATIONS

3.7.11 BIOCHEMICAL OXYGEN DEMAND (BOD).

Biochemical Oxygen Demand is the amount of dissolve oxygen needed by aerobic biological organism to break down organic material present in a given water sample at certain temperature over a specific time period. During the present investigation the maximum BOD value was recorded 14.26 mg l^{-1} at station 6 during pre-monsoon and the minimum value 1 ppm was recorded at station 3 during winter (Figure 23).

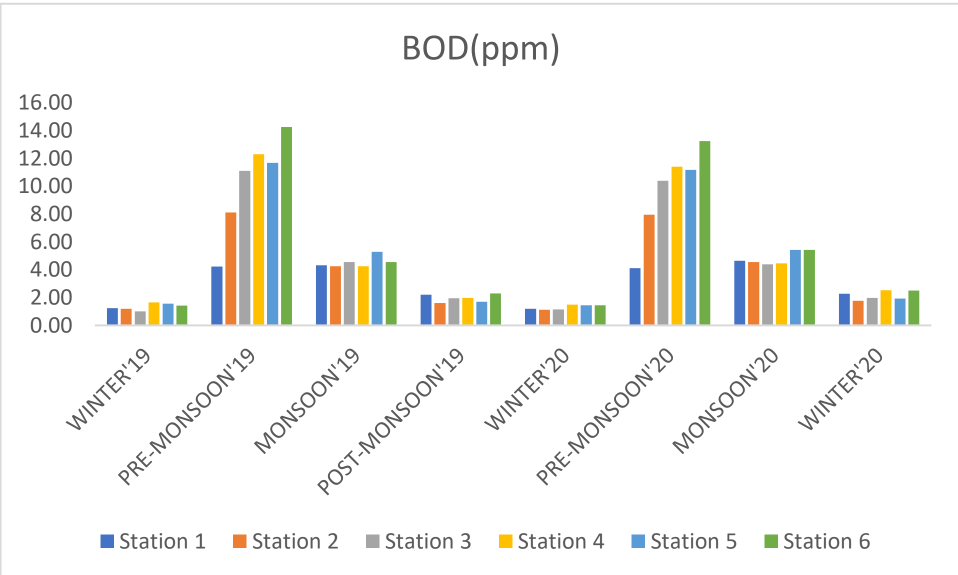


FIGURE 23: SEASONAL VARIATION OF BIOCHEMICAL OXYGEN DEMAND (BOD)(ppm) AT SIX SELECTED STATIONS

3.7. 12 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is an indicative measure of the amount of oxygen consumed during reactions in a solution. It is also a useful test to check the pollution load of a water sample. During the present investigation the maximum hardness value was recorded 23.87 at station 6 during pre-monsoon and the minimum value 1.98 ppm was recorded at station 1 during winter (Figure 24).

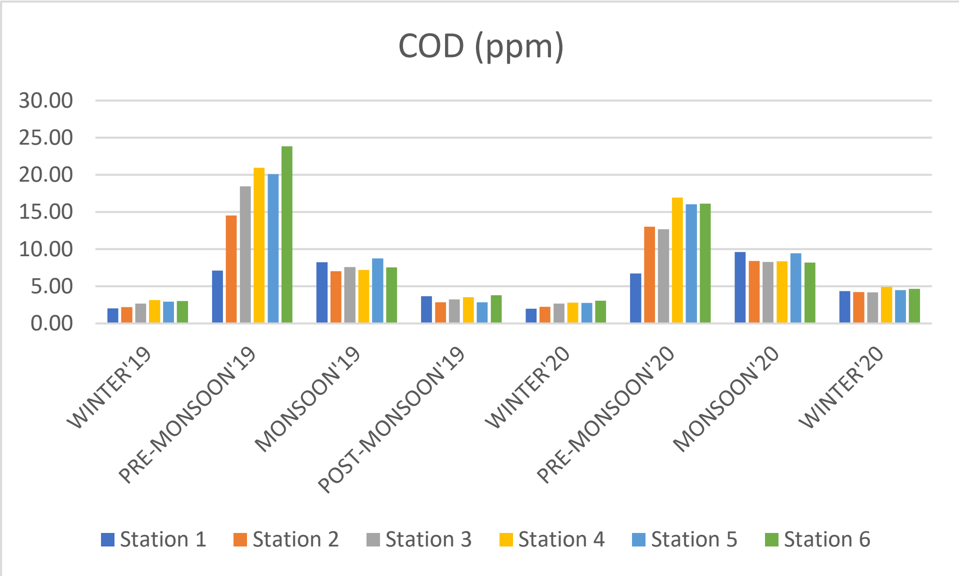


FIGURE 24: SEASONAL VARIATION OF CHEMICAL OXYGEN DEMAND (BOD)(ppm) AT SIX SELECTED STATIONS

3.7.13 Ammonia

Total Ammonia is the measure of the sum of both the ionised (NH_4^+) as well as the unionised (NH_3) ammonia. Ionised ammonia is not toxic but unionised form of ammonia is highly toxic. Ammonia is also an important pollutant as they are relatively common and can be toxic causing lower reproduction and growth and even death. During the present investigation the maximum ammonia value was recorded 0.03 ppm at station 6 during pre, on and post monsoon and the minimum value 0.01 mg l^{-1} was recorded at station 1 and 3 during winter (Figure 25).

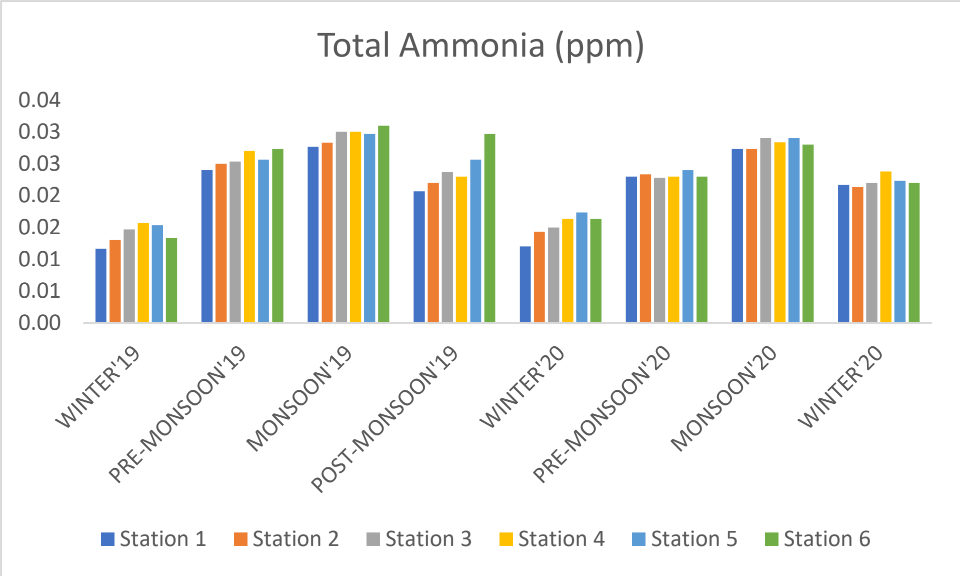


FIGURE 25: SEASONAL VARIATION OF AMMONIA (ppm) AT SIX SELECTED STATIONS

3.7.14 Nitrate

Nitrate is a critical nutrient for the growth of algae and help in accelerating eutrophication. Nitrate in surface water is an important factor to determine the pollution status and anthropogenic load of river water (Johnes and Burt, 1991). Agricultural runoff is also rich in nitrate. During the present investigation the maximum nitrate value was recorded 0.23 ppm at station 5 during monsoon and the minimum value 0.07 ppm was recorded at station 3 during winter (Figure 26).

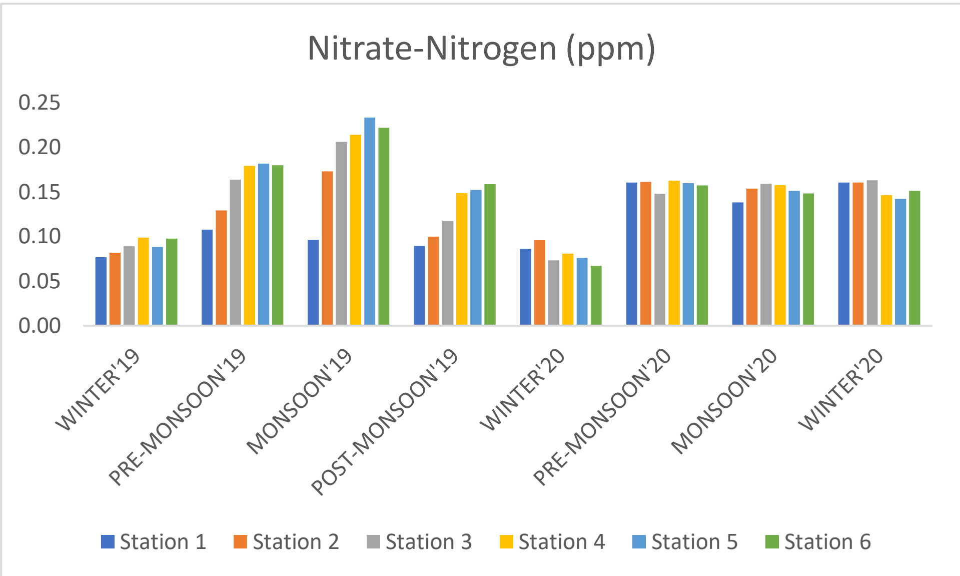


FIGURE 26: SEASONAL VARIATION OF NITRATE CONTENT (ppm) AT SIX SELECTED STATIONS

3.7.15 Phosphate

Phosphate is present in natural waters as soluble phosphate and organic phosphates (Ravindra *et al.* 2003). Phosphorous is a limiting nutrient in freshwater system, hence it is an important parameter as it regulates the phytoplankton production in the presence of nitrogen (Stickney, 2005). Phosphates availability in water depends upon the organic matter present in the bottom water and also the type of microorganism present in the system. Soils which are slightly acidic favour the release and their availability of phosphate in the aquatic system. During the present investigation the maximum phosphate value was recorded 0.12 ppm at station 6 during pre-monsoon and minimum value 0.05 ppm was recorded at station 1 and 2 during winter and post monsoon (Figure 27).

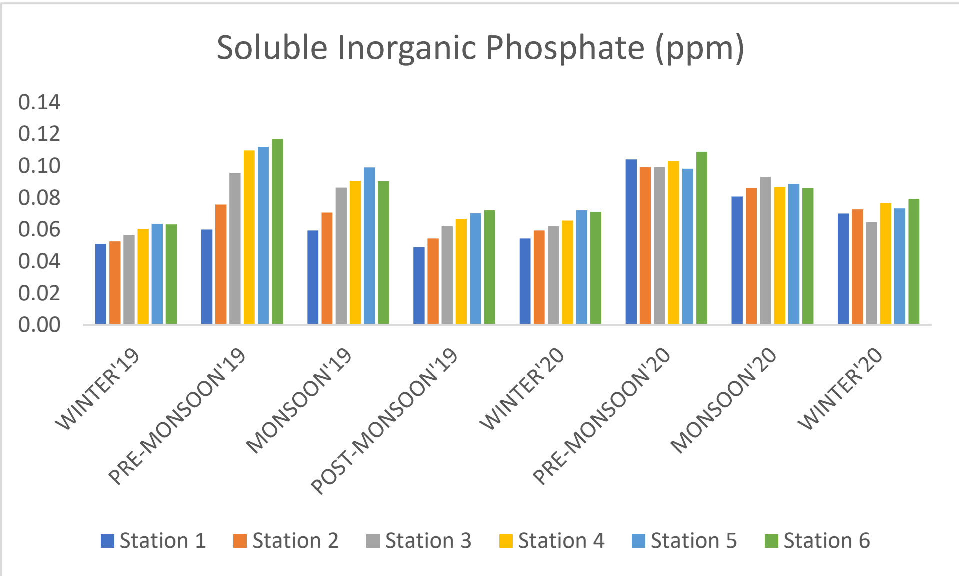


FIGURE 27: SEASONAL VARIATION OF PHOSPHATE (ppm) AT SIX SELECTED STATIONS (AVERAGE DATA FOR TWO YEARS)

3.7 (a) Sediment Parameters of River Doyang

1. Sediment pH: Sediment pH measures the acidic and alkaline condition of the river bed which has a direct or indirect influence on water pH and nutrient circulation. The findings of present study indicate that sediment pH varied between 5.72 (Post-Monsoon, 2019) to 6.90 (Pre-monsoon, 2020).

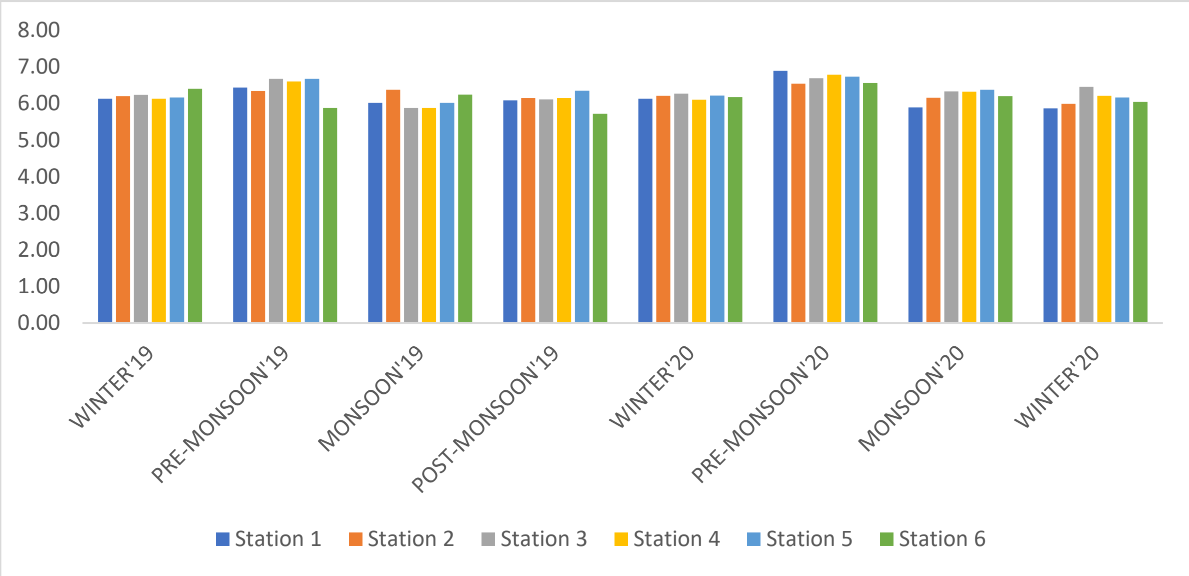


FIGURE 27 (a): SEASONAL VARIATION OF SEDIMENT pH AT SIX SELECTED STATIONS

2. Sediment Organic Carbon: In present investigation Sediment Organic Carbon percentages were found within the range of 0.42-2.32%, minimum during winter and maximum during monsoon season.

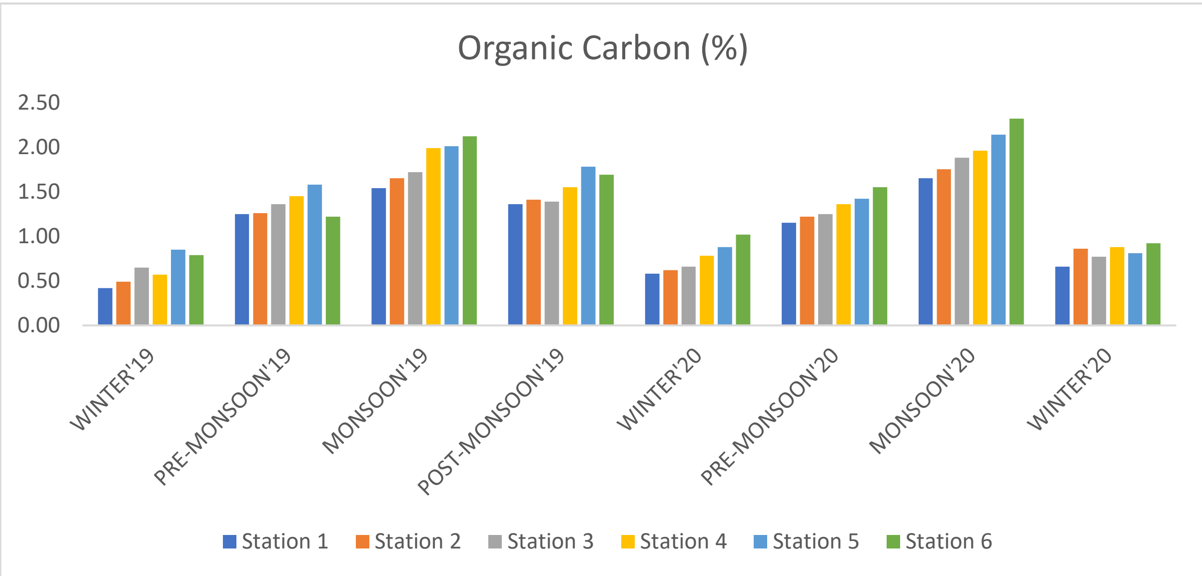


FIGURE 27 (b): SEASONAL VARIATION OF SEDIMENT ORGANIC CARBON (%) AT SIX SELECTED STATIONS

3. Sediment Organic Matter: Sediment organic matter of the present investigation ranged from 0.72 to 4.00 %.

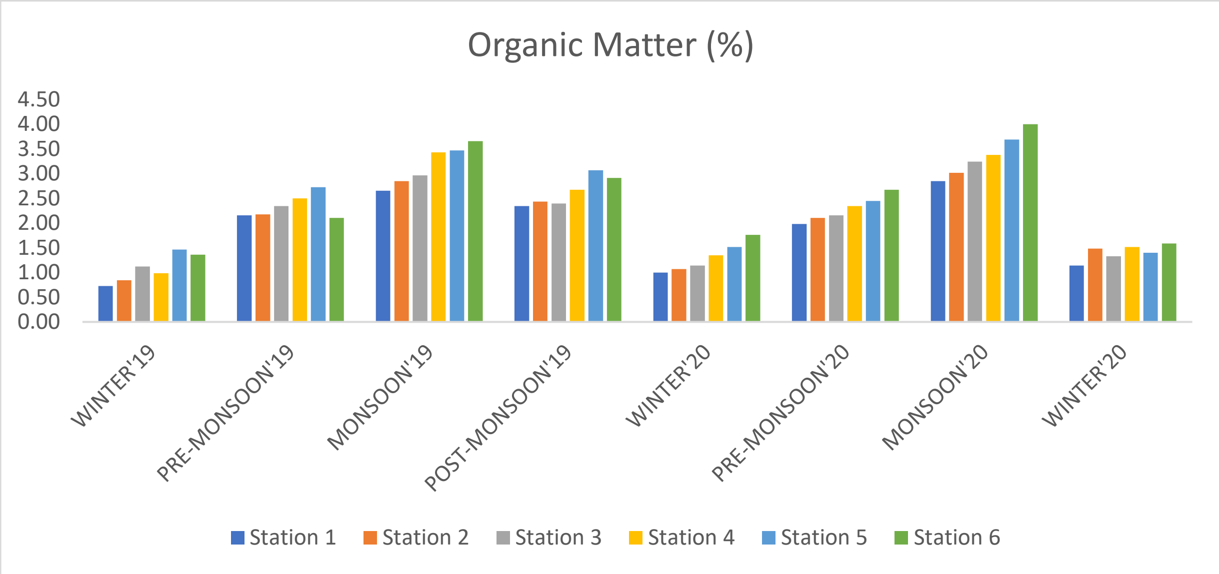


FIGURE 27: SEASONAL VARIATION OF SEDIMENT ORGANIC MATTER (%) AT SIX SELECTED STATIONS

3.8 Water Quality Index (WQI) calculation

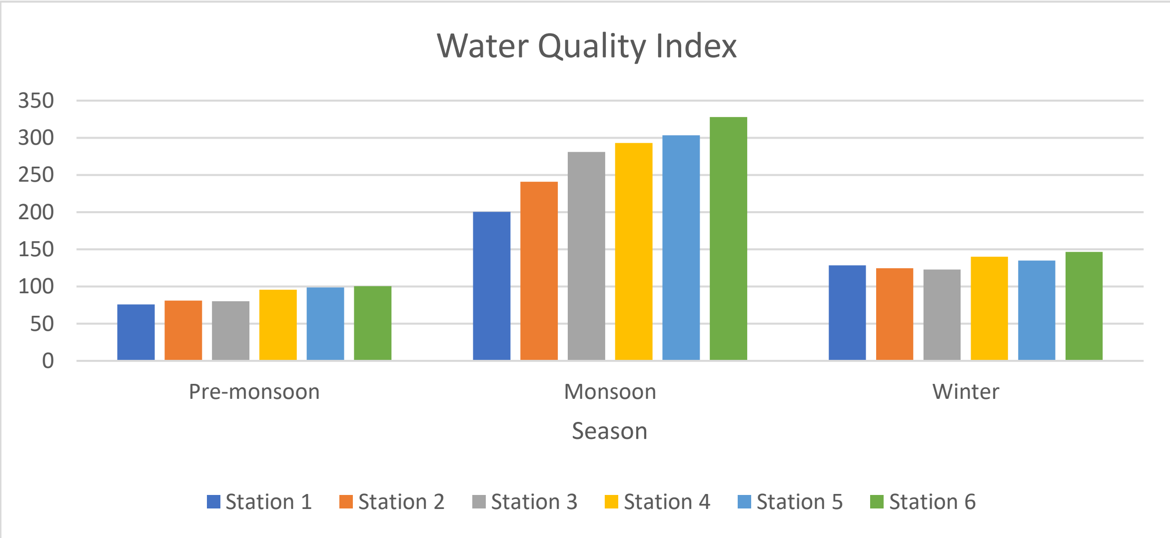
The calculation of WQI using Weighted Arithmetic Index involves the estimation of 'unit weight' assigned to each physicochemical parameter selected. Different units and dimensions of the selected parameters are transformed into a common scale using the assigning units. Table shows the drinking water quality standards and the unit weights assigned to each parameter used for the calculation of WQI.

The overall values of WQI of the water samples from all the six sampling stations for each season are presented in Figure. WQI were observed to have a positive relationship with the seasonal changes. Maximum WQI values were recorded during monsoon season from all the six stations followed by post monsoon (winter) and premonsoon. The WQI value showed a mixed pattern of changes in all the seasons. WQI of the upstream stations from 1 to 2 is lower than the downstream stations, i.e., 5 and 6 showing the increase in pollution level while moving downstream of the river (Table 17 and Figure 28

Table 17. WQI range, status and possible usage of the water sample (Brown et al., 1972)

WQI	Water Quality Status (WQS)	Possible usage
0-25	Excellent	Drinking, irrigation and industrial
26-50	Good	Drinking, irrigation and industrial
51-75	Poor	Irrigation and industrial
76-100	Very Poor	Irrigation

FIGURE 28. Water quality index for Doyang river system depicting the current health status of the river



3.9 Primary productivity

Primary productivity is an important biological phenomenon in nature on which the entire aquatic diverse array of life is dependent upon, either directly or indirectly. It involves the trapping of radiant energy from the sun and then transforming it into biochemical energy by photosynthetic apparatus. Primary productivity can therefore be defined as the weight of new organic matter created through the assimilation of carbon. In freshwater ecosystem, phytoplankton and macrophytes are two important factors responsible for the photosynthetic fixation of carbon.

Gross Primary Productivity (GPP)

Gross Primary Productivity (GPP) is the amount of energy usually expressed as carbon biomass, that a primary producer generates in a given unit time. It is expressed in unit of mass per unit area per unit time. During the present study GPP was found to range between 0.091 to 0.157 g C m^{-3} d^{-1}, recording a maximum value in the month of January, while minimum value was recorded in April (Table 18 and Figure 29).

Net Primary Productivity (NPP)

Net Primary Productivity (NPP) is the amount of organic matter that is retained in the plant tissues after respiration (Chattopadhyay, 1998). The NPP value of the river Doyang ranged between 0.043 to 0.071 g C m^{-3} d^{-1}, showing a maximum value during February, and a minimum during the month of July. Thus, the study implied that primary productivity of the river was found to be in the lower side with the average value ranging from with the average for GPP (0.116 g C m-3 d-1) and NPP (0.057 g C m-3 d-1) (Table 18 and Figure 29).

Table 18. Average monthly Primary productivity data during different months of the study period in g C $m^{-3}d^{-1}$.

Months	Gross Primary Productivity	Net Primary Productivity
January	0.157	0.071
February	0.132	0.074
March	0.148	0.069
April	0.091	0.049
May	0.120	0.056
June	0.109	0.049
July	0.094	0.043
August	0.094	0.053
September	0.120	0.056
October	0.105	0.053
November	0.101	0.056
December	0.124	0.060

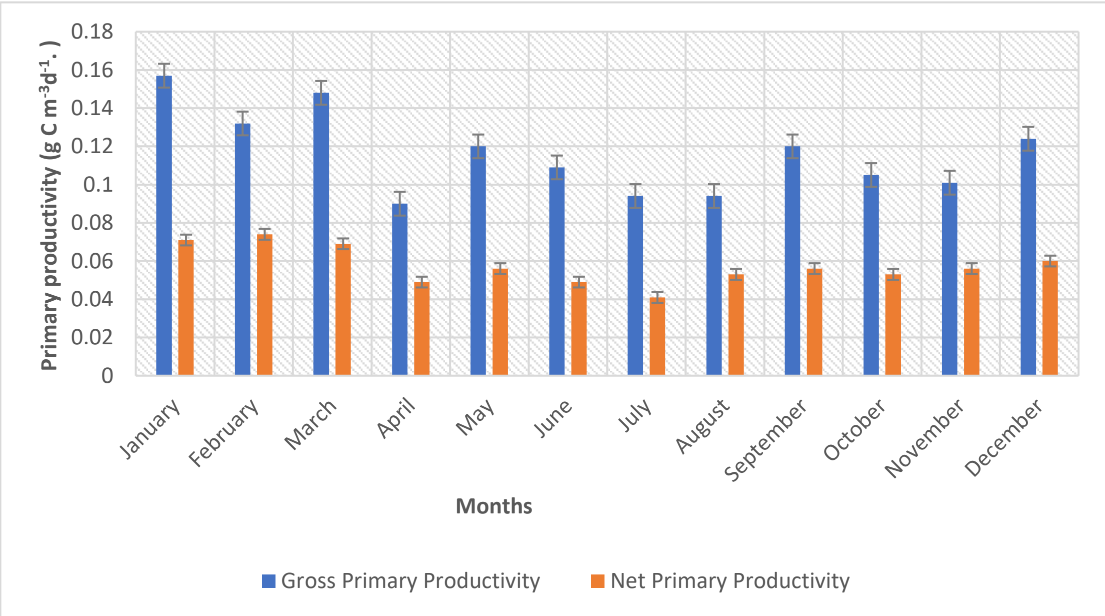


FIGURE 29. AVERAGE MONTHLY VARIATION OF PRIMARY PRODUCTIVITY OF RIVER DOYANG DURING THE STUDY PERIOD

3.10 Principal component analysis

Principal Component Analysis can be used for dimensionality reduction in a data set by retaining those characteristics of the data set that contribute most to its variance, by keeping lower order principal components and ignoring higher order ones. It is very useful in the analysis of data corresponding to large number of variables. It has been widely used as they are unbiased methods which can indicate associations between samples and variables (Wenning and Erickson, 1994). In the PCA method, eigenvalues are normally used to determine the principal components (PCs).

In winter, PC1 has a total variance of 44.72% and it was positively affected by, TDS, Nitrate and Turbidity. Parameters of station 3 is somewhat closely related to this principal component. PC1 is negatively affected by free pH, alkalinity, hardness and electrical conductivity. PC2 showed a total variance of 24.24% and it was positively affected by surface water temperature, phosphate, TDS, Nitrate, Turbidity and is negatively affected by DO and water velocity. Station 2 parameters were away from the origin, which indicates its contribution to total variance is more than the other two stations, which denotes its greatest share of changes occurring in water quality parameters in winter (Figure 30).

In pre monsoon, PC1 showed a total variance of 58.64 % and is positively affected by turbidity, water velocity, surface water temperature and ammonia. PC1 is more highlighting the physical parameters of water during premonsoon, which indicates, physical parameters are mainly affecting the overall data set during pre-monsoon. PC1 is negatively affected by DO. PC2 is showing a total variance 13.66%. PC2 is positively affected by BOD, COD, SWT, and negatively affected by hardness (Figure 31).

During monsoon, PC1 showed a total variance of 34.19% and positively affected by BOD_3, COD, phosphate and water temperature and negatively affected by TDS and electrical conductivity. PC2 showed a total variance of 24.02.76% and is positively affected by DO. Parameters of station 1 is more towards the center than the other stations, this indicates lesser contribution towards total variance (Figure 32).

During post monsoon, PC1 has a total variance of 52.47%, it is positively affected by velocity, TDS, water temperature, and is negatively affected by DO. PC2 is having total variance of 21.95 % and is positively affected by pH. PC2 is not affected negatively by the parameters. Parameters of station 2 and 3 is more scattered away from the center, indicating that these two stations are more contributing towards total variance (33).

In this study we have seen that, during winter and monsoon, PC1 was largely and positively affected by pollution indicating parameters, whereas during post monsoon and pre monsoon, PC1 was largely and positively affected by the other physico chemical parameters. This may be due to pollutants affecting water quality in rivers have temporal and spatial variations and should be investigated based on each river’s environmental conditions (Zeinalzadeh and Rezaei 2017). We also observed that different stations are having different contributions towards the total variance. The reason for these changes can be found in different environmental conditions and human activities around the river from one place to another.

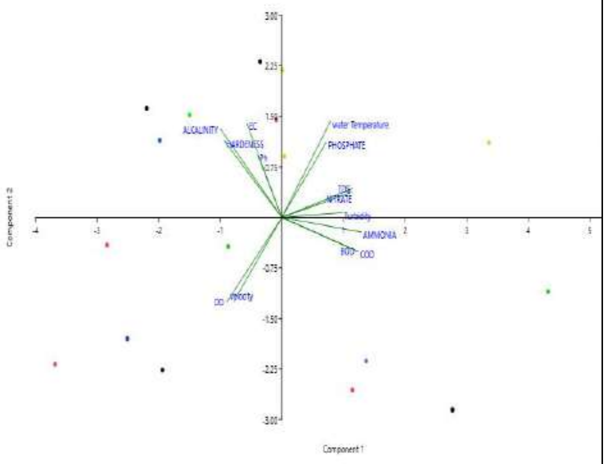


Fig 30: Coordinates of principal component analysis of winter (dots station 1 red, station 2 blue and station 3 black, station 4 green, station 5 yellow and station 5 pink

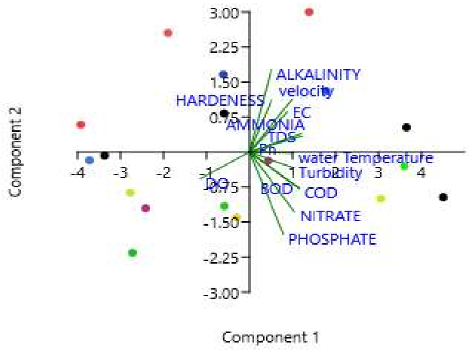


Fig 31: Coordinates of principal component analysis of Pre-monsoon (dots station 1 red, station 2 blue and station 3 black, station 4 green, station 5 yellow and station 5 pink

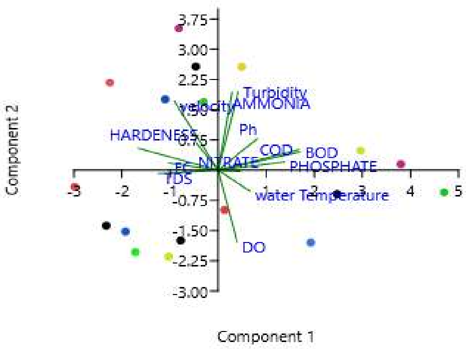


Fig 32: Coordinates of principal component analysis of Moonsoon (dots station 1 red, station 2 blue and station 3 black, station 4 green, station 5 yellow and station 5 pink

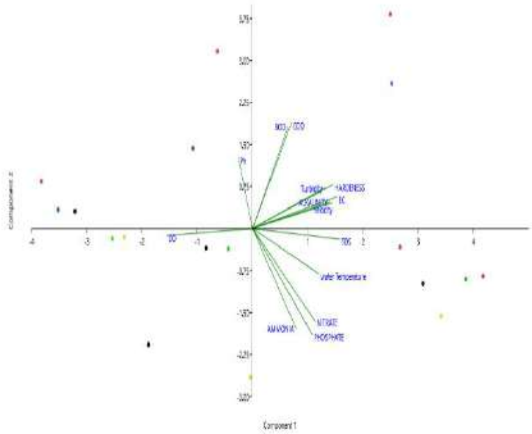


Fig 33: Coordinates of principal component analysis of Post Monsoon (dots station 1 red, station 2 blue and station 3 black, station 4 green, station 5 yellow and station 5 pink

3.11 Geomorphology of the River:

The geomorphology of river Doyang is divided into three zones upper, middle and lower zone along with mean gradient river bank and riparian zone which is depicted in table- 19.

The river beds in the upper and middle zones were hard and rocky and mostly composed of boulders, cobbles and gravels etc. In the lower zone, the river bed was soft due to the presence of sand and clayey type of soil. These uneven distributions of the sediment were greatly influenced by the slope gradient of the river bed. The upper zone with a mean inclination of 0.52m/sec had fast flow regime hence the large boulders were dislodged and carried lower down the river. Similarly, the middle zone with a mean slope gradient of 0.43 m/sec had a strong flow regime which powers the transfer of smaller rocks and gravels within it. While the lower zone of the river, with the reduction in the mean slope gradient 0.27 m/sec of the river bed was mostly composed of sand, silt and clay. River bank was more stable in the upper zone of the river due to armouring by the rocky sediments, though in the middle and lower zone of the river, the river banks were partly stable. The river also carried and deposited large and medium wood debris on the riverbed and bank in the upper zone, while in the middle and lower zone of the river smaller wood debris were observed mostly. The Riparian zones were primarily composed of woody forest and shrubs in the upper and middle zone though it was sparse in the lower zone of the river. Human habitations on river banks were the main source of discharging the sewage, farmyard washings, agricultural waste, pesticides etc. into the river system. However, the human population size was found to be small in the upper zone and moderate and sparse in the middle and lower zones of the river respectively. Moreover, river mining in huge quantity using Bulldozer and dumper was seen in almost all the three zones. Other major pollution of the river was not encountered and it was also evident from the physico-chemical analysis of water samples.

Table 19: Hydrobiology of river Doyang River

Parameters	Upper zone		Middle zone		Lower Zone	
	Station 1	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Station 6**
River Bed	Hard, Rocky, boulders and Sand	Rocky, boulders and sand.	Cobbles, pebbles and Sand	Sandy and Rocky	Rocky and sandy Bottom	Rocky and sandy Bottom
Sediment Type	Cobles pebbles and gravels	Rocky and Sandy	Sandy and pebbles	Sand and pebbles	Sand and pebbles	Sand and pebbles
Mean Gradient	0.53m/sec	0.51m/sec	0.44m/sec	0.41m/sec	0.26m/sec	0.29m/sec

River Bank	Not Stable	Stable	Not Stable	Moderately stable	Stable	Stable
Wood debris in River bank	Shrubs, Grass wooden debris	None	Not observed	Not observed	Shrubs, Grass wooden debris	Shrubs, Grass wooden debris
Riparian Zone	Woody forest Shrubs and Grass	Woody forest Shrubs and Grass	Shrubs, and Grass woods on both side	Woody forest Shrubs and Grass	Shrubs, and Grass woods on both side	Shrubs, and Grass woods on both side
Human habitation	No Habitation	Moderate on both side	Moderate on both side	No Habitation	No Habitation	No Habitation

Fish species obtained station wise for habitat characterisation:

Stations	Average Water quality Parameter	Fish recorded
1	S. W. temp: 20. 33 W. velocity: 0.47 m/sec pH : 7.7 DO : 9.5	*Tor tor, Schizothorax spp, Neolissochilus spp. Garra spp., Glypthothorax spp*
2	S. W. temp: 20.86 W. velocity: 0.50 m/sec pH : 7.56 DO : 9.3	*Tor spp, Garra spp, Glypthothorax spp*
3	S. W. temp: 21.35 W. velocity: 0. 41m/sec pH : 7.5 DO : 8.7	*Psilorhynchus spp, Glypthothorax spp*
4	S. W. temp: 22 W. velocity: 0.39 m/sec pH : 7.6 DO : 8.5	*Except Schizothorax spp, Neolossochilus spp all are present.*
5	S. W. temp : 24.50 W. velocity : 0. 26m/sec pH : 7.3 DO : 7.8	*Except Schizothorax spp, Neolossochilus spp all are present.*
6	S. W. temp: 24 W. velocity: 0.29 m/sec pH : 7.2 DO : 7.4	*Except Schizothorax spp, Neolossochilus spp all are present.*

<u>Habitat of fishes with supporting photographs:</u>

	
Station 1	Station2
	
Station 3	Station 4
	
Station 5	Station 6

3.12 Anthropogenic Factors

The major anthropogenic factors observed during the regular sampling in the Doyang river system are listed below along with the supporting photographs.

- Constant dumping of solid waste like polythene bags, paper waste and domestic sewage in the river.
- Constant removal of sand gravel and boulders from the river bed.
- Alteration of river course.
- Use of pesticide for protection of agricultural crops in the adjoining paddy fields of the river system leading to the toxicity effects in the non-targets aquatic animals like fish.
- Electric fishing, blasting and poising in the river side were also frequently reported by the locals.


Dumping of solid waste like polythene bags, paper waste and domestic sewage in the river


Removal of sand gravel and boulders from the river bed

Alteration of river course

Agricultural Land adjoining the river system

Agricultural Land adjoining the river system

Common insecticide use in the agricultural land

3.13 Acute toxicity Test (LC_{50})

All the procedures conformed to international standards for animal experimentation and were approved by Institutional Animal ethics Committee, College of Fisheries, Assam Agricultural University, Raha, Nagaon, India.

From range finding test seven (7) concentrations were selected for the final definitive test. The test concentrations were 0 ppm, 50.00 ppm, 80.00 ppm, 128.00 ppm, 204.80 ppm, 327.68 ppm and 524.28 ppm with a spacing factor of 1.6 (Table 20), where the control treatment showed 0 % mortality. The acute toxicity test conducted to find out the 96 hours LC_{50} concentration of Imidacloprid to *Cyprinus carpio* showed 0% mortality at 50 ppm. The mortality rate increased with increase in the concentration of Imidacloprid and 100% mortality was recorded at 327.68 ppm and 524.28 ppm (Table 20). Percent mortality was plotted against log concentration of Imidacloprid and a probit response curve was obtained. The results obtained from the curve showed that the 96hr LC_{50} value of commercial product of Imidacloprid (Premise, 30.50% SC) for common carp fingerlings, *Cyprinus carpio* was 208.38 ppm (208380 µg/l) (173.66 ppm - 262.37 ppm) with 95% confidence interval (Fig. 34a and 34b). Initially mortality rate was higher for first 24hrs of exposure followed by a decrease in the mortality rate till 48 hours, post 48 hours of exposure the mortality rate declined to 0% while narrowing 96 hours.

Table 20. Percentage mortality of test fish common carp fingerlings when exposed to various concentrations of commercially available Imidacloprid (Premise, 30.50% SC) during the 96 hr LC_{50} experiment.

Concentration (ppm)	Initial Number of fish	Cumulative count of death of fish with time of exposure				Percentage Mortality (%)
		24hr	48hr	72hr	96hr	
0.00	30	0	0	0	0	0
50.00	30	0	0	0	0	0
80.00	30	0	1	2	3	10
128.00	30	0	3	3	6	20
204.80	30	1	4	5	10	33.33
327.68	30	13	11	6	30	100
524.28	30	17	13	30	30	100

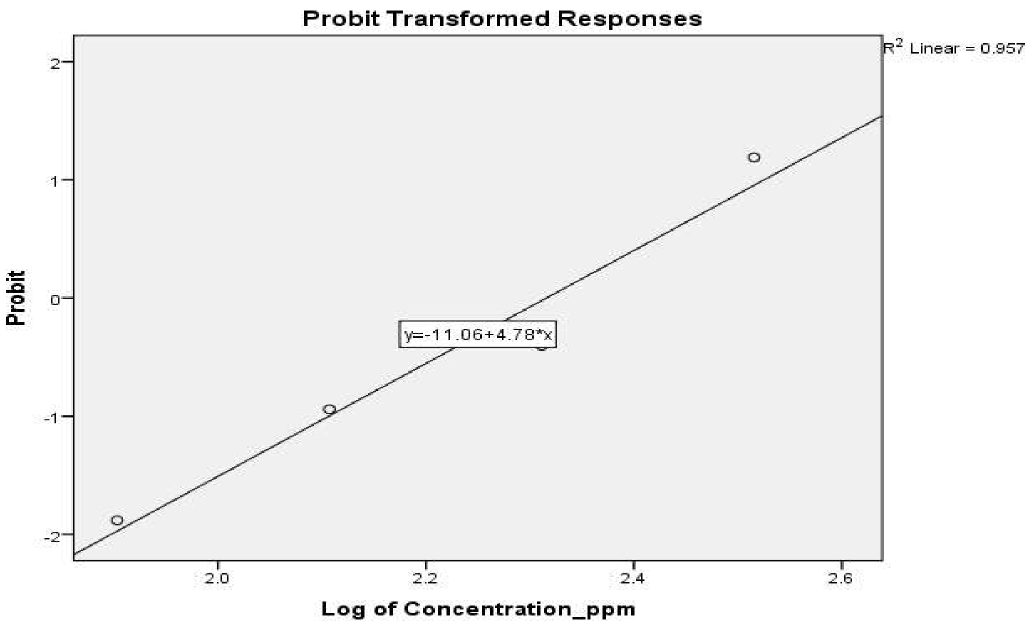


FIGURE 34A. REGRESSION LINE FOR LOG CONCENTRATION VERSUS MORTALITY OF *Cyprinus carpio* FOLLOWING EXPOSURE TO 96 hrs COMMERCIAL FORMULATION OF IMIDACLOPRID (PREMISE 30.50% a.i).

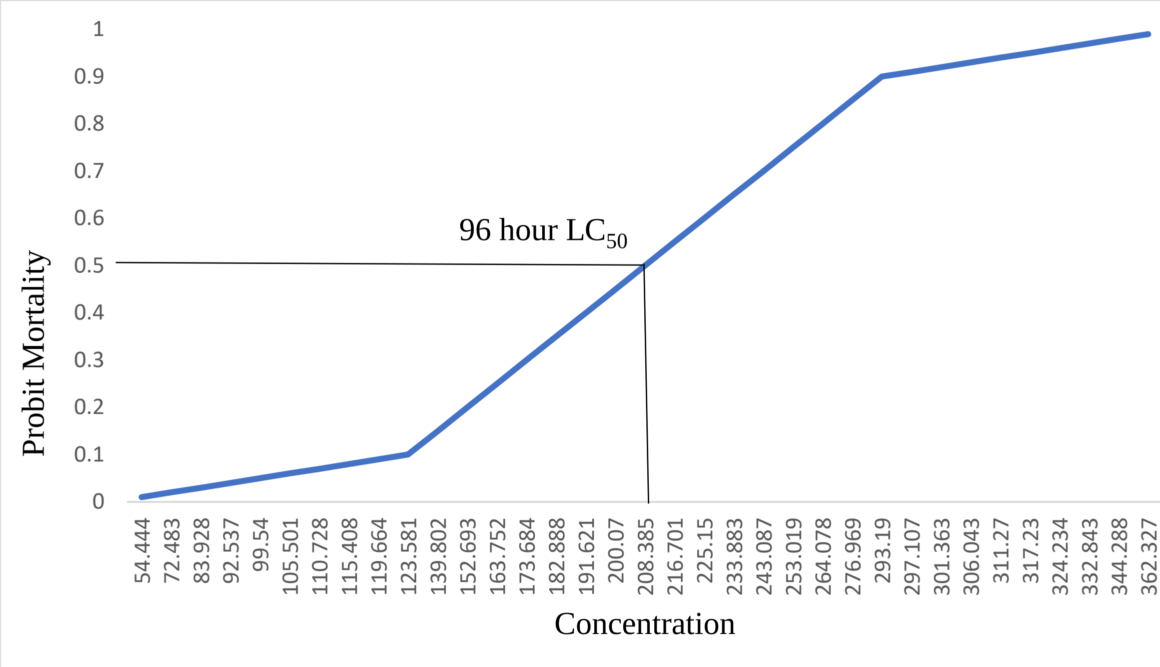


FIGURE 34b. CONCENTRATION (ppm) MORTALITY CURVE OF *Cyprinus carpio* FINGERLINGS EXPOSED IMIDACLOPRID (PREMISE, 30.50% SC) FOR LC_{50} DETERMINATION. MEAN MORTALITY PERCENTAGE ±S.E.M; n=30, WHERE n= NUMBER OF FISH INDIVIDUALS IN EACH TREATMENT (10 INDIVIDUALS PER REPLICATE AQUARIUM, 3 AQUARIUMS PER TREATMENT).

Imidacloprid is classified as Class II (moderately hazardous) by WHO. Results of the present findings indicates that 208.38 ppm (208380µg/l) is the 96hr LC_{50} value of the commercial product of imidacloprid, Premise (30.50%SC) for freshwater fish *Cyprinus carpio*. The obtained value is similar to other 96 hr LC_{50} concentration reported by several workers for analytical grade of Imidacloprid which are listed in Table 21.

Table 21. 96 hr LC_{50} values of Imidacloprid to different fish species.

Test Species of Fish	**96 LC_{50} value (ppm)**	**Reference**
Zebra fish (*Brachydenio rerio*)	241 ppm	Tisler *et al*., 2009
Oncorhynchus mykiss	211 ppm	Tisler *et al*., 2009
Lepomis machrochirus	105 ppm	Tisler *et al*., 2009
Cyprinus carpio	280 ppm	Bayer Crop Science, 2013
Labeo rohita	550 ppm	Qadir *et al.,* 2015
Cyprinus carpio larvae	129 ppm	Islam *et al*., 2019

3.14 Sublethal toxicity

The aim of the present study was to assess the adverse effects of long term (28 days) sublethal exposure of commercial grade of Imidacloprid (Premise 30.50%, SC) to common carp (*Cyprinus carpio)* fingerlings, using multiple biomarkers like haemato-immunological parameters, histopathological alterations, serum biochemical indices, antioxidant enzymes, ROS, MDA, neurotoxicity (AChE activity), DNA damage (micronucleus test) and gene expression. The adverse effects of sublethal concentrations can alter certain biological process rather than quantitative estimation of mortality as the end point and facilitate development of bioindicators to monitor pollutant and more specifically insecticides adverse effects (Sepeci-Dincel *et al*., 2009)

Three pesticide concentrations T_1 (LC_{50}/8), T_2 (LC_{50}/10) and T_3 (LC_{50}/12) were selected for the sublethal toxicity analysis based on the 96 hr LC_{50} value (208.38 ppm or 208380 µg/l) which was determined in the acute toxicity experiment. All the results of the present study suggest that exposure of common carp fingerlings to a sublethal concentration of LC_{50}/8 (26.04 ppm), LC_{50}/10 (20.83 ppm,), LC_{50}/12 (17.36 ppm) of commercial grade imidacloprid Premise (30.50%SC) for 28 days showed varying degree of toxic effects on different biomarkers, with no apparent external alterations in morphology during our semi static renewal experiment. All fishes displayed normal behaviour.

3.15 Behavioural analysis

3.15.1 Acute toxicity

Behaviour is a visible reaction of an organism to a stimulus on the whole-organism organization level. However, based on biochemical reactions and exerting consequences on the population and biocoenosis levels, behaviour can be regarded as highly integrative (Little *et al*., 2001; Jenssen, 1997 and Pablos *et al*., 2011). The control fish behaved in a natural manner, they were active with well-coordinated movements and they were alert to the slightest disturbance compared to toxic environment relatively where reduced activity was exhibited during early hours of pesticide exposure. The intensity of the behavioural activities of the fish decreased with increasing concentration and duration of exposure. In the present investigation, the fishes of control group displayed normal behaviour (Table

4) with very active and well-coordinated movements. They showed alertness at the slightest disturbance, but when exposed to 96 hr LC_{50} concentration of Imidacloprid during 96 hr acute toxicity experiment they showed varying degree of behavioural alterations which are listed in Table 5. Jumping movements, restlessness, hyperventilation, hyperactivity, gulping, coughing and corkscrew swimming at surface and bottom of the tank were recorded during the exposure period. Enhanced mucus secretion, loss of buoyancy and string of faeces hanging from anus or on the tank were also observed (Table 22). They slowly became sluggish with short jerky movements, frequent surfacing, and gulping of air and erratic movements. Finally, they settled down at the bottom with the loss of equilibrium, loss of reflex and rolling of body, convulsions prior to death or moribund. Furthermore, the fishes displayed frequent surfacing in an attempt to avoid the toxic environment. However, behavioural irregularities that showed by the exposed fish to IMI changes with respect to time.

Table 22. Description and definition of fish clinical signs on definite intervals of time on LC_{50} concentration exposed to Imidacloprid. (OECD, 2019).

Clinical sign	Definition	0-Normal (Absent)	1-Minor	2-Medium	3-Major	24hrs	48 hrs	72 hrs	96 hrs
Loss of buoyancy	Floating at surface or sinking to the bottom	Normal	-----	---	Floating at the surface	3	3	3	3
Hyperactivity	Increase in spontaneous activity	Normal (calm)	-----	----	Erratic swimming	3	3	3	3
Hyperventilation	Increased frequency of opercular ventilatory movement.	Normal	---	-----	Fast opercular movement	3	3	3	3
Gulping	Mouth (and operculum) movement at water surface, resulting intake of water and air.	None	----	-----	Piping	3	3	3	3
Coughing	Fast reflex expansion of mouth and operculae not at water surface - assumed to clear ventilatory channels	None	----	----	Gasping, abnormal opercular activity, yawn	0	2	3	3
Skin Colour		None	----	----	Pale	0	0	0	3
Corkscrew swimming	Rotation around long axis; erratic movements, often in bursts	Normal	---------	---------	Rotation around long axis	0	2	3	3
Mucus secretion	Excess mucus production	None	-----	------	Excess mucus production	3	3	3	3

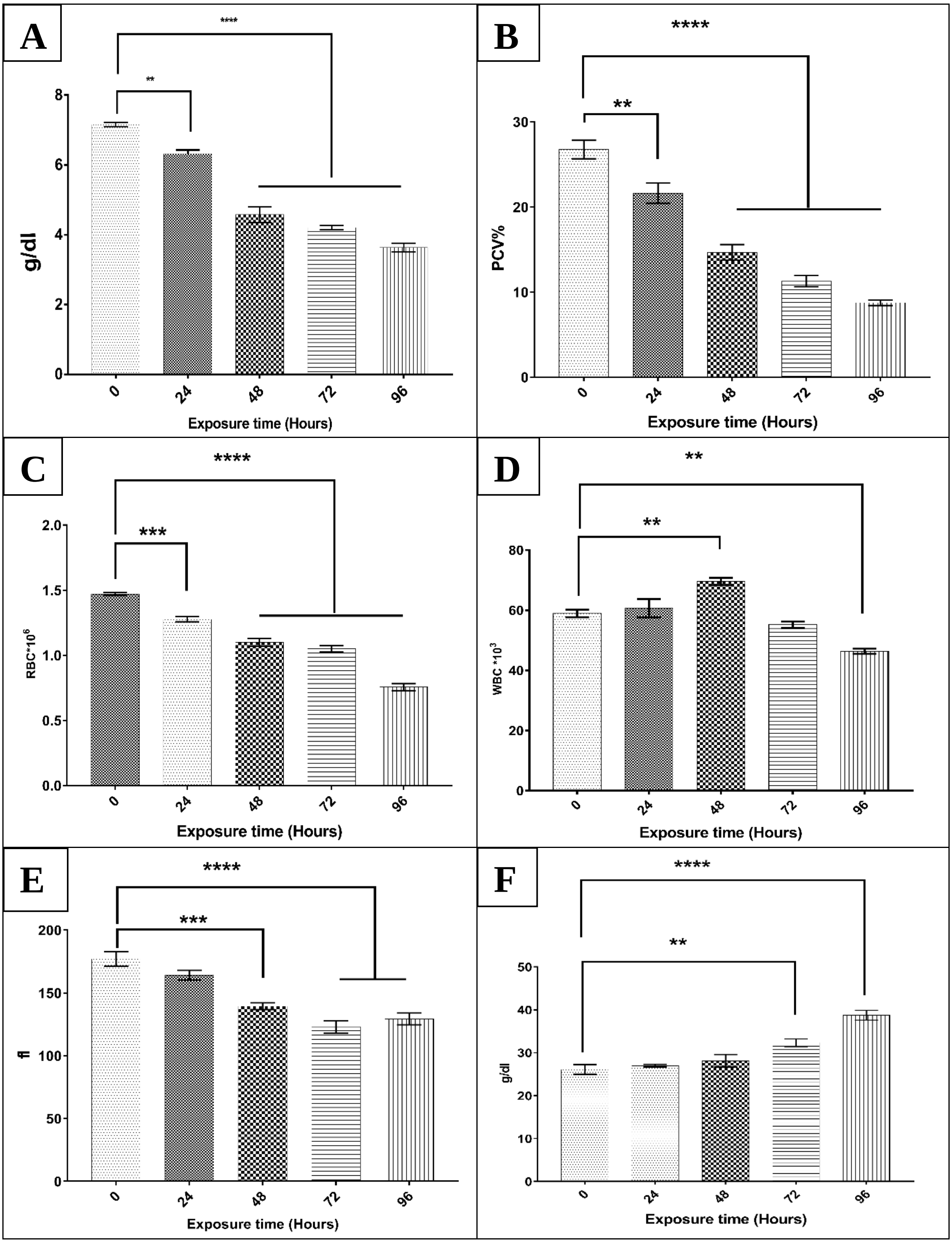
Loss of schooling / shoaling behaviour	Individual fish show loss of aggregating and social interactions	None	-----	------	Isolation, social isolation	0	3	3	3
Faecal (anal) casts	String of faeces hanging from anus or on tank floor	None	-----	------	String of faeces hanging from anus or on tank floor	0	0	3	3
Haemorrhage	Petechias (pinhead sized spots) and/or haematoma (area of blood) due to intradermal or sub-mucus bleeding	None	-----	------	Petechias (pinhead sized spots)	0	0	0	0
Exophthalmia	Swelling within orbital socket(s) resulting in bulging of one or both eyes	None	-----	------	Pop eye, protruding eyeball	0	0	0	0

3.16 Haematology

3.16.1 Acute toxicity

Changes in the haematological parameters are considered as valuable indices of the physiological status of an organism in response to toxicant (Nwani *et al.*, 2013). The results showed a significant decrease in the value of red blood cells (RBC), haemoglobin content (Hb), packed cell volume (PCV) and mean cell volume (MCV) in the exposed fishes when compared to control groups.

During acute exposure to IMI, RBC content value significantly decreases (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) from 1.47±0.01($10^6/mm^3$) (0 hrs) to 0.75±0.02 ($10^6/mm^3$) (96 hrs) (Fig 35A). Similarly, Hb, PCV and MCV decreases significantly (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) from 7.16±0.055 (g/dl) (0 hrs) to 3.64±0.12 (g/dl) (96 hrs), 26.76±1.09 (%) (0 hrs) to 8.75±0.34 (%) (96 hrs), 177.13±5.97 (fl/cell) (0 hrs) to 129.32±4.63 (fl/cell) (96 hrs) (Fig 35B, 35C, 35E) respectively. For WBC there was initially significant increase in count till 48 hr 58.89±1.20 ($10^3/mm^3$) (0hrs) to 69.58±1.23 ($10^3/mm^3$) (48hrs) and later decrease to 46.35±0.83 ($10^3/mm^3$) (96hrs) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) (Fig 31D). A significant increase (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) 26.13±1.15 to 38.74±1.14 (g/dl) was observed in Mean corpuscular haemoglobin content (MCHC) between 0 to 96 hrs (Fig 31E.). However, no significant difference was observed in Mean Corpuscular Haemoglobin (MCH) (pg/cell) between 0 to 96 hrs (Fig 35F).


A
****
**
8
6
4
2
0
g/dl
0
24
48
72
96
Exposure time (Hours)
B
****
**
30
20
10
0
PCV%
0
24
48
72
96
Exposure time (Hours)
C
****
***
2.0
1.5
1.0
0.5
0.0
RBC*10^6
0
24
48
72
96
Exposure time (Hours)
D
**
**
80
60
40
20
0
WBC *10^3
0
24
48
72
96
Exposure time (Hours)
E
****
***
200
150
100
50
0
fl
0
24
48
72
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Exposure time (Hours)
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g/dl
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Exposure time (Hours)

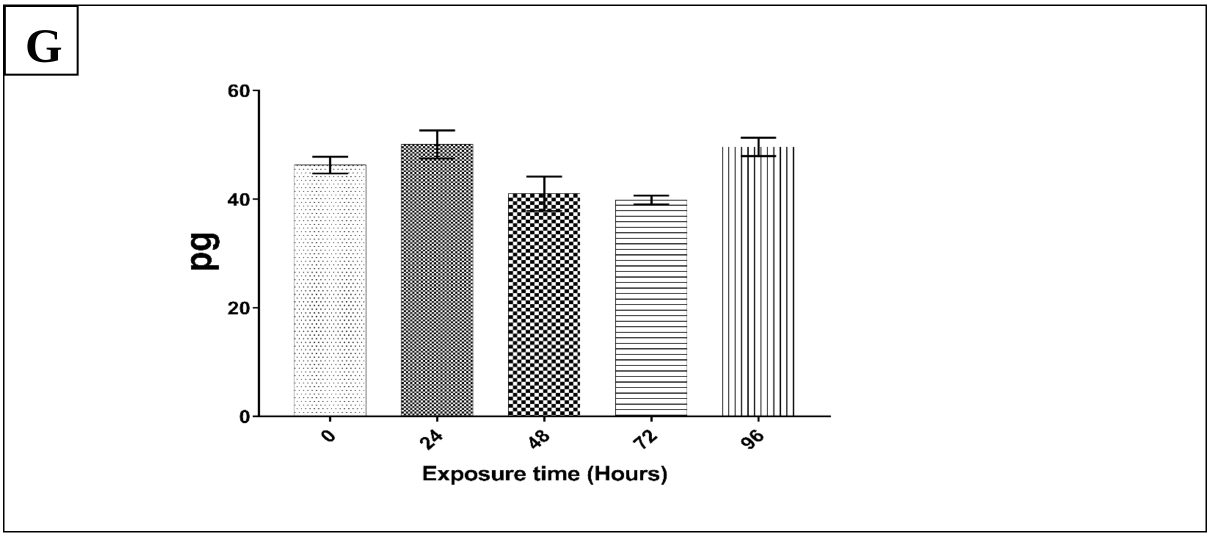
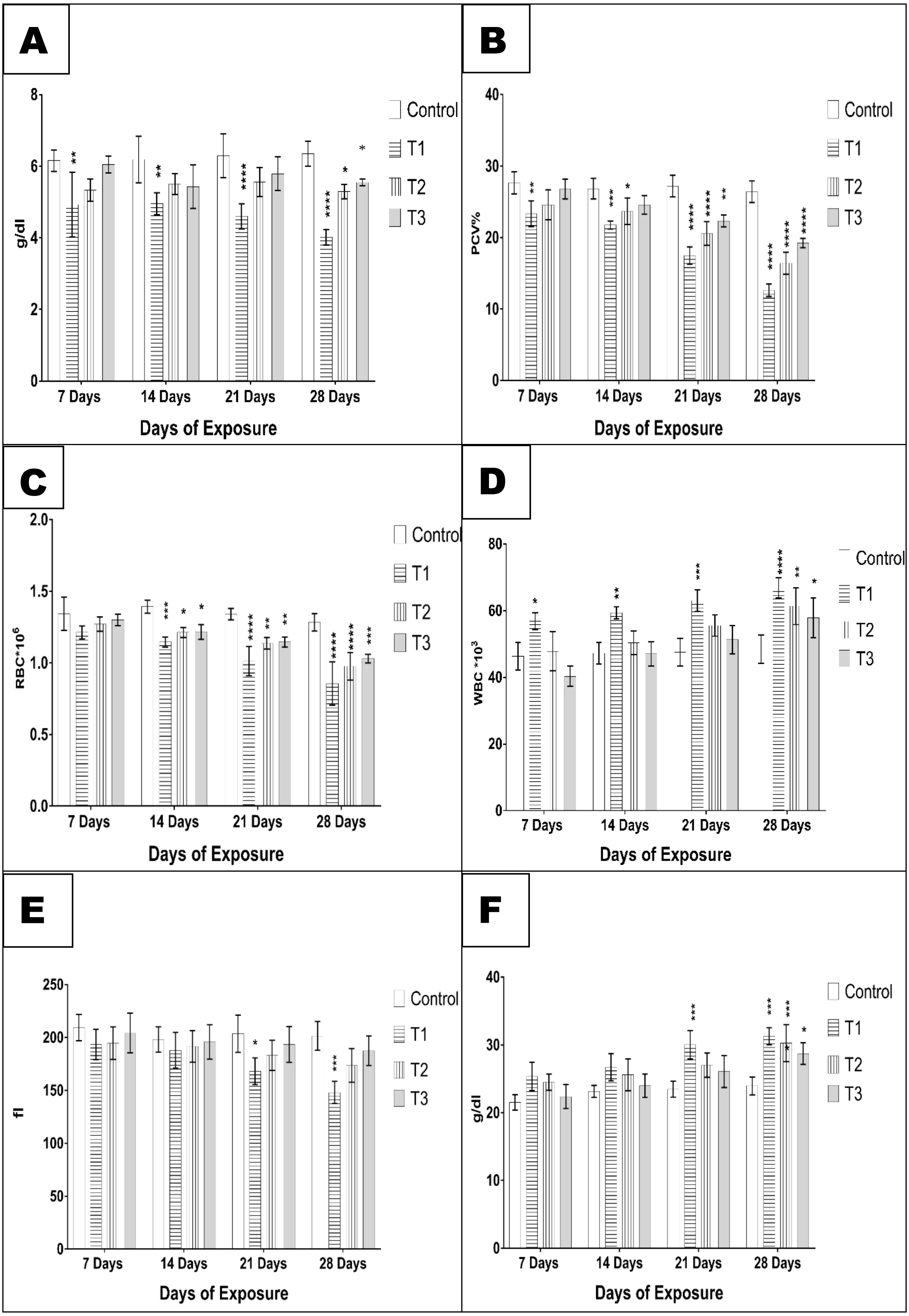


FIGURE 35. EFFECT OF 96HR LC_{50} CONCENTRATION ON DIFFERENT HAEMATOLOGICAL PARAMETERS IN *Cyprinus carpio*. **A**. HAEMOGLOBIN CONTENT (g/dl), **B.** PACKED CELL VOLUME (%), **C**. RED BLOOD CELLS (RBC, X 10^6/mm), **D**. WHITE BLOOD CELLS (WBC, X 10^3/mm), **E**. MEAN CORPUSCULAR VOLUME (MCV, fl) **F**. MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC, g/dl), **G**. MEAN CORPUSCULAR HAEMOGLOBIN (MCH, pg). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.16.2 Sublethal toxicity

The haematological parameters during sublethal exposure to IMI are presented in Figure 36. Haemoglobin (g/dl) content was significantly reduced in T1 on day 7, 14, 21and 28 followed by T1 and T2 on day 28 (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). PVC (%) decreased statistically in T1 on day 7, 14 21 and 28 followed by T2 on day 14, 21 and 28 and T3 on day 21 and 28 (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). Similarly, RBC (10^6/mm^3) showed significant reduction from day 14 on all the three treatment groups throughout experimental period (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) when compared to that of control. WBC (10^3/mm^3) was significantly increased in T1 on day 7, 14, 21 and 28 followed by T2 and T3 on day 28. MCV (fl/cell) value was found to be significantly reduced on day 21 and 28 in T1, whereas MCHC (g/dl) index was significantly induced only in T1 on day 21 and in T1, T2 and T3 on day 28. However, no statistically difference was observed in increased value of MCH (pg/cell) when compared to that of control


A
g/dl
Control
T1
T2
T3
7 Days
14 Days
21 Days
28 Days
Days of Exposure
B
PCV%
Control
T1
T2
T3
7 Days
14 Days
21 Days
28 Days
Days of Exposure
C
RBC*10^6
Control
T1
T2
T3
7 Days
14 Days
21 Days
28 Days
Days of Exposure
D
WBC *10^3
Control
T1
T2
T3
7 Days
14 Days
21 Days
28 Days
Days of Exposure
E
fl
Control
T1
T2
T3
7 Days
14 Days
21 Days
28 Days
Days of Exposure
F
g/dl
Control
T1
T2
T3
7 Days
14 Days
21 Days
28 Days
Days of Exposure

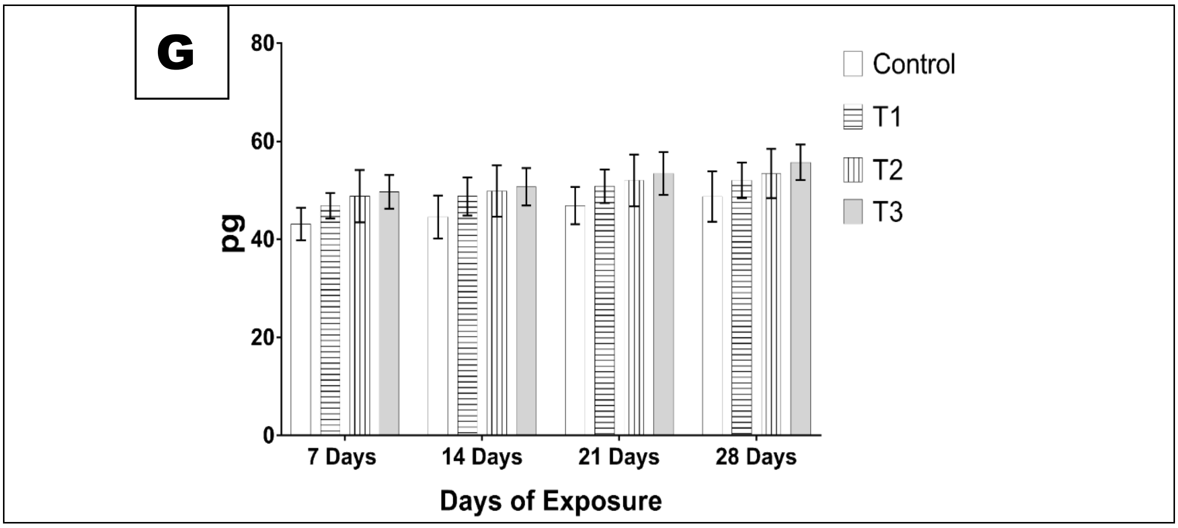


FIGURE 36. VARIATIONS IN DIFFERENT HAEMATOLOGICAL PARAMETERS OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.50% SC) (T1= $LC_{50}/8$ (26.04 ppm), T2=$LC_{50}/10$ (20.38 ppm) AND T3=$LC_{50}/12$ (17.36 ppm). **A**. HAEMOGLOBIN CONTENT (g/dl). **B**. PACKED CELL VOLUME (%). **C**. RED BLOOD CELLS (RBC, X 10^6/mm). **D**. WHITE BLOOD CELLS (WBC, X10^3/mm), **E**. MEAN CORPUSCULAR VOLUME (MCV, fl). **F**. MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC, g/dl). **G**. MEAN CORPUSCULAR HAEMOGLOBIN (MCH, pg). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.17 Nitro blue tetrazolium (NBT)

In teleost fish, the innate immune system is the first mechanism activated in defense against invading pathogens, and it is considered more important than the specific system, playing an important role for host survival. The nitroblue tetrazolium (NBT) assay is an indication of respiratory burst due to oxidative radical production from monocytes and neutrophils (Anderson *et al.* 1995), and widely used to evaluate the non-specific immune response and stress mechanisms of toxicants. Besides, it is the nonspecific immune parameter that undergoes alteration as a result of infection, toxicity, diet, stressors, temperature fluctuation or pollution. Lysozyme is an important non-specific immune indicator and defense molecule of fish innate immune system released from lysosomes of neutrophils and macrophages under stressor-localized factors or unexpected environmental changes (Gallin, 1982; Chipman and Sharon, 1969). Lyzozyme activity is usually regulated to improve the immune defense of fish on contact with increasing pathogens and other various stress factors (Zhao *et al.,* 2010).

3.17.1 Acute toxicity

The change in immunological parameters for every 24 hrs when exposed to 96 LC_{50} concentration of commercially formulated Imidacloprid is provided in Fig 37. The results showed significant decrease in both NBT (mg NBT formation/ml) and LA (U/ml) activity from 2.53±0.29 (0 hrs) to

1.07±0.10 (96hrs) (Fig 37A), and from 654.14±6.74 (0 hrs) to 266.04±2.30 (96hrs) (Fig 37B) respectively during the study period (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

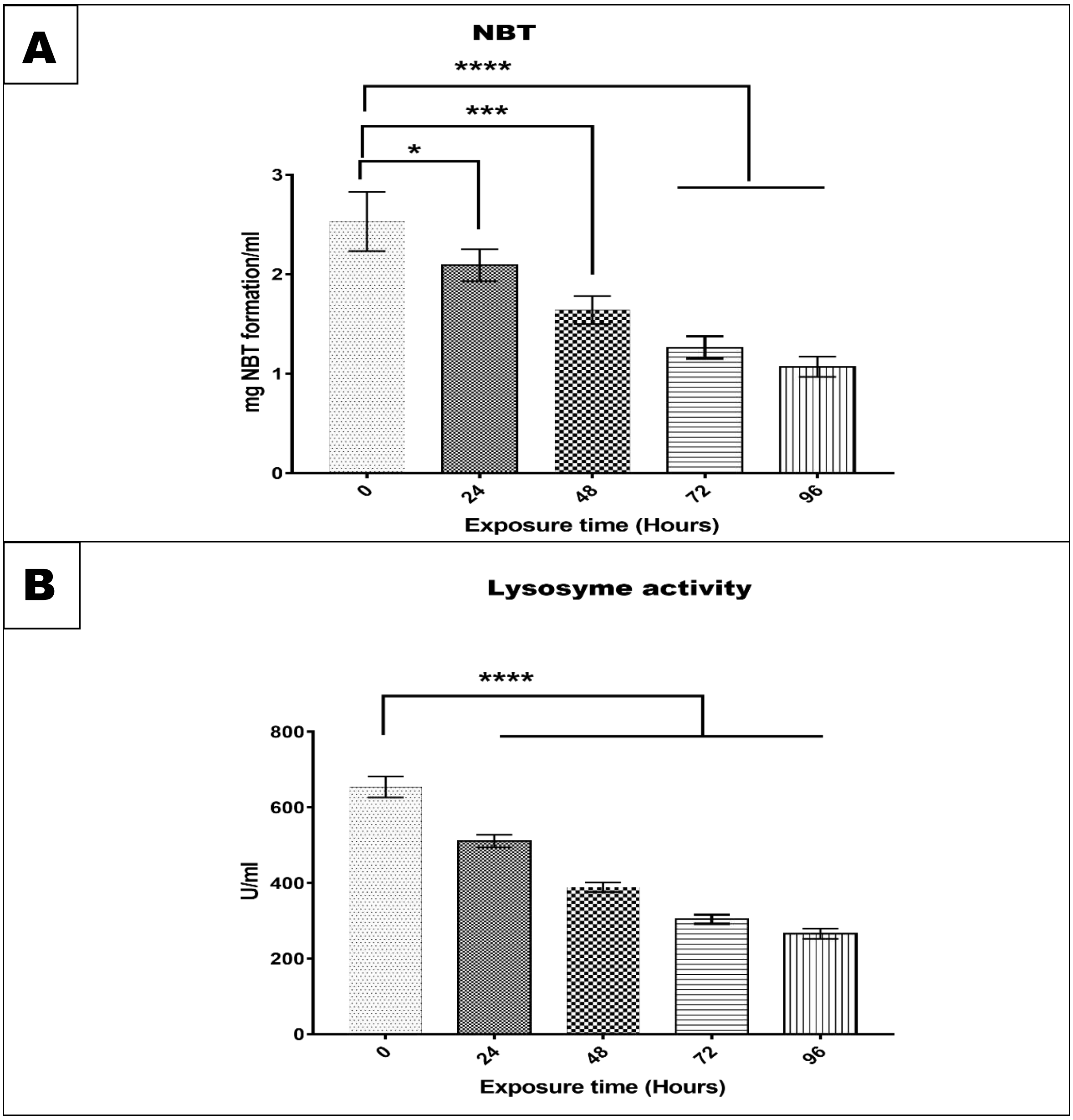


FIGURE 37. EFFECT OF 96hr LC_{50} CONCENTRATION ON DIFFERENT IMMUNOLOGICAL PARAMETERS IN *Cyprinus carpio*. **A**. NITROBLUE TETRAZOLIUM (NBT, mg NBT FORMATION/ml). **B**. LYSOSOMAL ACTIVITY (LA, U/ml). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.

3.17.2 Sublethal toxicity

Sublethal effects of IMI on different immunological parameters are shown in Figure 38. NBT (mg NBT formation/ml) activity did not show any significant changes at day 7 and 14 in any of the three treatments, but significant reduction was recorded on day 21 and 28 in T1 and T2 (Fig 38A). Lysozyme activity (U/ml) was found to be significantly reduced in all the groups from day 14 till end of the exposure period (28 days) except T3 which did not show any significant reduction at day 14 (Fig 38B) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

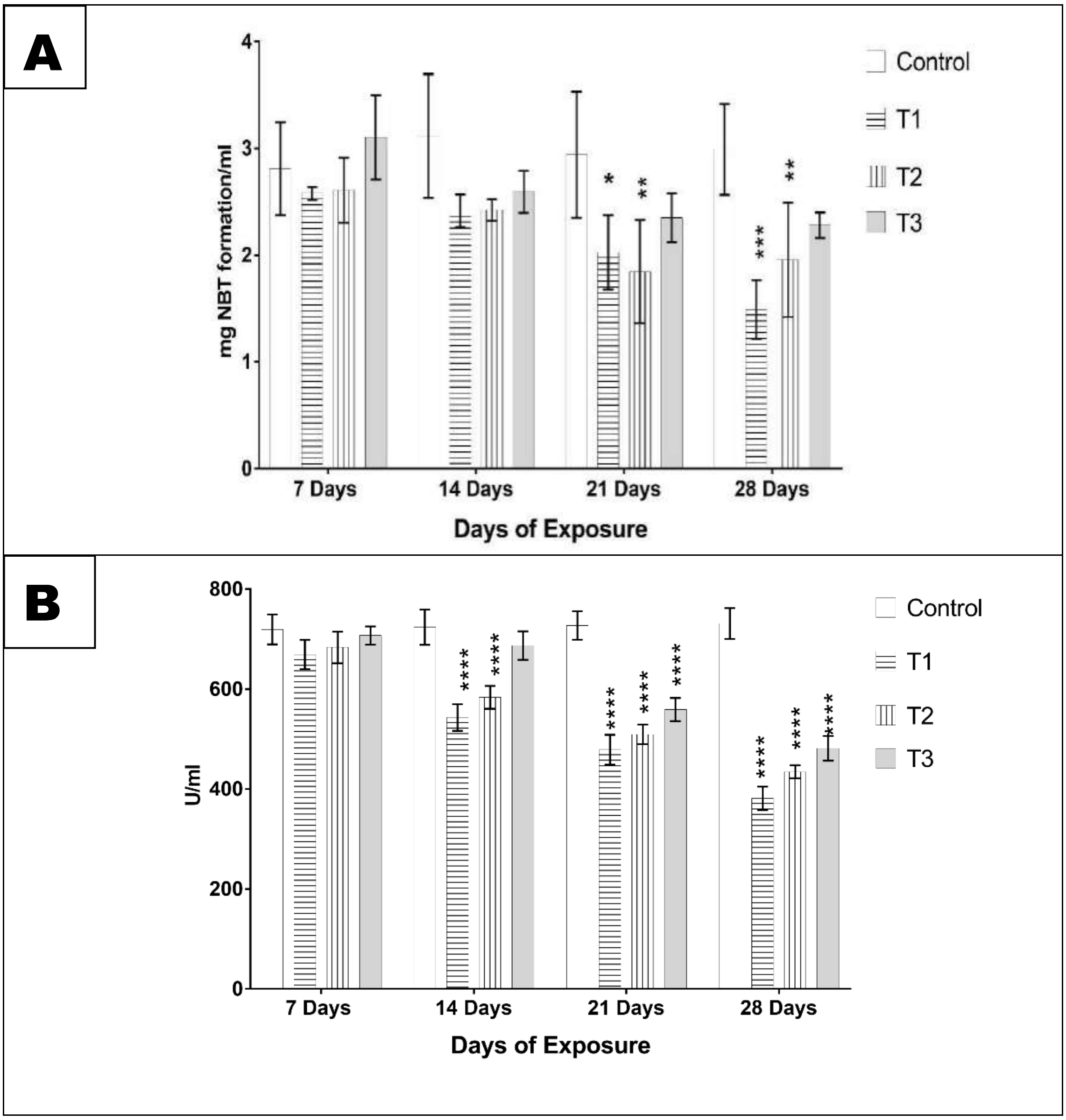


FIGURE 38. VARIATIONS IN DIFFERENT IMMUNOLOGICAL PARAMETERS OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.5%SC) (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.38 ppm) AND T3=LC_{50}/12 (17.36 ppm). **A**. NITROBLUE TETRAZOLIUM (NBT, mg NBT FORMATION/ml). **B**. LYSOSOMAL ACTIVITY (LA, U/L). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.18 Histological studies.

3.18.1 Acute toxicity

The histological examination indicated moderate to high incidence of tissue damages on gill, liver and kidney by IMI in common carp after exposure to lethal concentration (LC_{50}) (208.38 ppm) for 96hr. Histopathological changes recorded in liver, gill and kidney are summarized in Fig 39, 40 and 41 respectively. Individuals in the control group did not show any histological changes in liver, gill and kidney of the examined tissues (Fig 39A, 40A and 41A).

Liver tissues of the exposed fish indicated exocrine pancreatic acini and vascular congestion (Fig 39B), severe diffuse fatty type vacuolization and necrosis of hepatocytes (Fig 39C and 39D). However, examination of hepatic tissues from exposed fish showed disruption of hepatic tissue organisation, vascular congestion, diffuse fatty type vacuolization (Fig 39E) and also mononuclear cell infiltration induced inflammation of liver and passive hyperaemia (Fig 39F).

Gill tissue of the exposed fish showed congestion and dilation of primary and secondary lamellar blood capillaries, the gill arch exhibited oedema with inflammatory infiltrates (Fig 40B), epithelial hyperplasia, fusion, desquamation, necrosis or complete rupture of the epithelial layer and clubbed tips of the secondary lamellae (Fig 40C), alongside with lamellar epithelial lifting, oedema, and capillaries telangiectasia were evident in the secondary lamellae with bulbus end (Fig 40D). Multiple focal areas of oedema and telangiectasia of secondary lamellae were also very distinct (Fig 40E) and epithelial lifting and oedema of the secondary lamellae was prominent (Fig 40F).

The kidney tissues of exposed fish to LC_{50} concentration for 96 hr revealed different degrees of changes like, expansion of Bowman's space, contraction of the glomerulus (Fig 41B), Inter-tubular congestion, complete necrosis of several renal tubules (Fig 41C), expansion of Bowman's space and cloudy swelling of epithelial cells of renal tubules (Fig 41D), complete necrosis of several renal tubules, multiple focal areas of inter-tubular haemorrhage (Fig 41E), necrosis in the tubular epithelium, renal epithelium contains intra luminal acidophilic substances with multiple focal areas of inter-tubular haemorrhage (Fig 41F).

3.18.2 Sublethal toxicity

The general histological examination indicated low to moderate incidences of IMI damages on different tissues of common carp after exposure to three different sublethal concentrations (T1= 96hr LC_{50}/8 (26.04 ppm), T2=96 hr LC_{50}/10 (20.38 ppm,) and T3=96 hr LC_{50}/12 (17.36 ppm)) for 28 days. Histopathological changes recorded in liver, gill and kidney during the exposure period are shown in Fig 39, 40 and 41. Individuals in the control group did not display any histological changes in any of the examined liver, gill and kidney tissues (Fig 39A, 40A, 41A). The liver tissue of the exposed fish showed low to moderate hydropic degeneration and cellular infiltration (Fig 39D and 39F) in T1, T2 and T3 treatments after 28 days of exposure. Similarly, gills and kidney of the exposed fishes showed low to moderate incidence of telangiectasis (40E), epithelial lifting and oedema (40F) in gills and expansion of Bowman's space (41B) and cloudy swelling of epithelial cells (41D) in kidney compared to the control group.

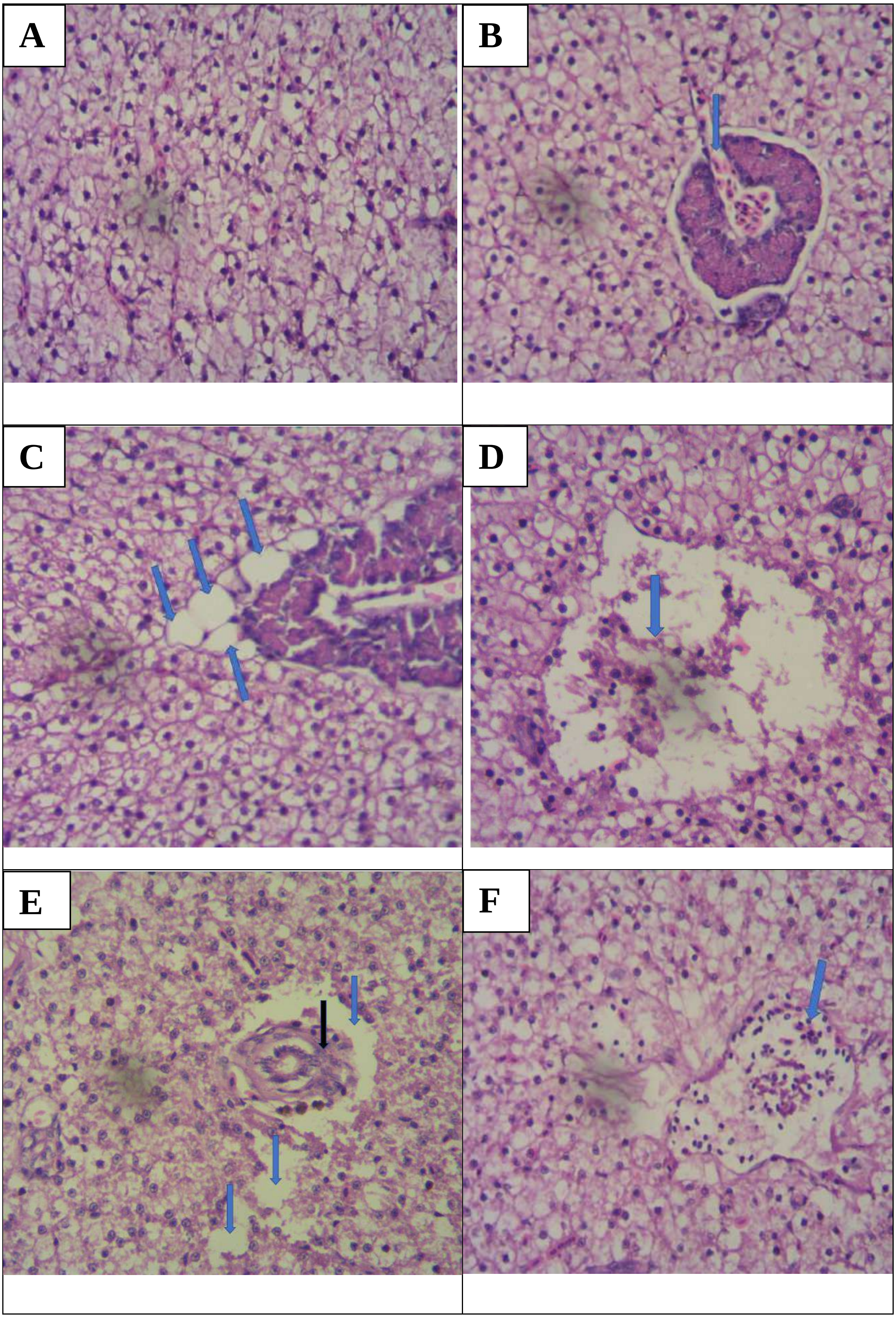

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FIGURE 39. REPRESENTATIVE PHOTOMICROGRAPHS OF THE LIVER TISSUES OF COMMON CARP (*CYPRINUS CARPIO)* (H & E STAIN, 400X). **A**. CONTROL (400X) LIVER, NO CHANGES OBSERVED. **B**. EXOCRINE PANCREATIC ACINI AND VASCULAR CONGESTION (ARROW). **C**. SEVERE DIFFUSE FATTY TYPE VACUOLIZATION. **D**. NECROSIS OF HEPATOCYTES AND EXOCRINE PANCREATIC ACINI. **E**. DISRUPTION OF HEPATIC TISSUES ORGANISATION, VASCULAR CONGESTION, DIFFUSE FATTY TYPE VACUOLIZATION. **F**. MONONUCLEAR CELL INFILTRATION THAT INDUCED INFLAMMATION OF LIVER AND PASSIVE HYPERAEMIA.

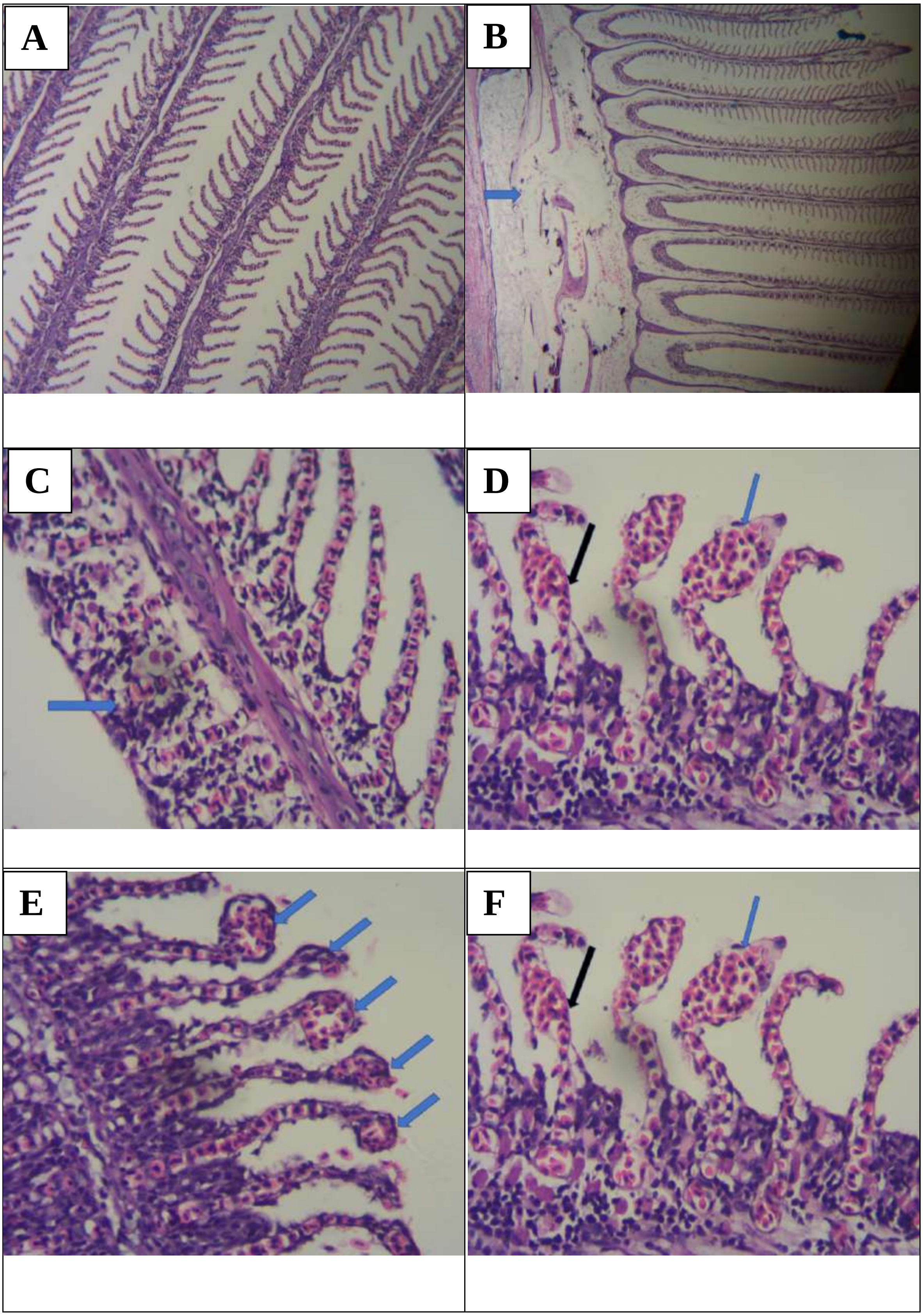
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FIGURE. 40. REPRESENTATIVE PHOTOMICROGRAPHS OF THE GILL TISSUES OF COMMON CARP (*CYPRINUS CARPIO)* (H & E STAIN, 400X). **A**. CONTROL (100X) GILL. **B**. THE GILL ARC EXHIBITED OEDEMA, INFLAMMATORY INFILTRATES, CONGESTION OF BLOOD CAPILLARIES. **C**. EPITHELIAL HYPERPLASIA, FUSION, NECROSIS, METAPLASTIC DESQUAMATION, MUCOUS MALFORMATION. **D**. MULTIPLE FOCAL AREAS OF OEDEMA AND TELANGIECTASIA OF SECONDARY LAMELLAE WITH BULBOUS ENDS. **E** CAPILLARIES TELANGIECTASIA IN THE SECONDARY LAMELLAE. **F**. EPITHELIAL LIFTING AND OEDEMA OF SECONDARY GILL LAMELLAE.

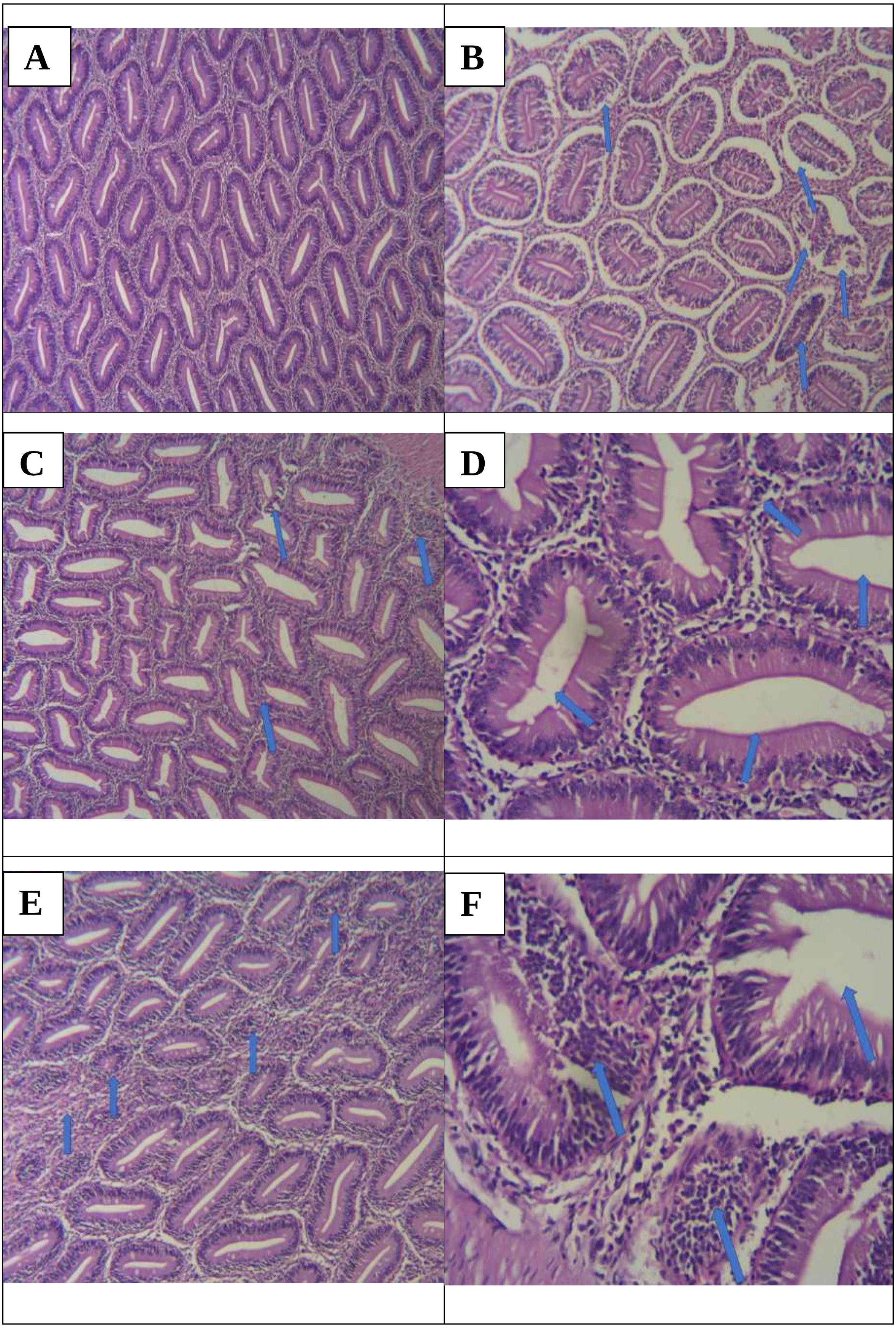
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FIGURE 41. REPRESENTATIVE PHOTOMICROGRAPHS OF THE KIDNEY TISSUES OF COMMON CARP (*CYPRINUS CARPIO)* (H & E STAIN, 100X, 400X). **A**. CONTROL (100X) KIDNEY, NO CHANGES OBSERVED. **B**. EXPANSION OF BOWMAN'S SPACE, CONTRACTION OF THE GLOMERULUS **C**. INTER-TUBULAR CONGESTION, COMPLETE NECROSIS OF SEVERAL RENAL TUBULES. **D**. EXPANSION OF BOWMAN'S SPACE AND CLOUDY SWELLING OF EPITHELIAL CELLS OF RENAL TUBULES **E**. COMPLETE NECROSIS OF SEVERAL RENAL TUBULES, MULTIPLE FOCAL AREAS OF INTER-TUBULAR HAEMORRHAGE. **F**. NECROSIS IN THE TUBULAR EPITHELIUM, RENAL EPITHELIUM CONTAINS INTRA LUMINAL ACIDOPHILIC SUBSTANCES, MULTIPLE FOCAL AREAS OF INTER-TUBULAR HAEMORRHAGE.

3.19 Serum biochemical analysis

3.19.1 Acute toxicity

Biochemical analysis can provide valuable information for monitoring the health conditions of fishes. Biochemical changes mainly depend on the age of fish species, the cycle of maturity and health condition (Priya *et al*., 2015). Moreover, analysis of serum biochemical constituents' levels showed useful information in detection and diagnosis of metabolic disturbances and diseases in fishes (Jamalzadeh *et al.,* 2009).

Table 23 depicts the changes in different serum biochemicals parameters of common carp fingerlings exposed to IMI 96 hr LC_{50} concentration and showed significant changes (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) in all the 14 serum biochemical indices observed during the study period. Glucose (GLU) (m/dl) content varies from 45.80±0.02 from 0 hrs to 177.48±1.48 (96 hrs) upon acute exposure to 96hr LC_{50} concentration for 96 hrs. Similarly, other parameters like Total Protein (TP) (g/dl) 4.26±0.17 from 0 hrs to 1.83±0.01 (96 hrs), Albumin (ALB) (g/dl) 1.57±0.01 from 0 hrs to 0.47±0.01 (96 hrs), Globulin (GLO) (g/dl) 2.67±0.02 from 0 hrs to 1.32±0.01 (96 hrs), ALB:GLO ratio 0.58±0.01 from 0 hrs to 0.35±0.01 (96 hrs), Triglyceride (TG) (mg/dl) 138.07±1.50 from 0 hrs to 218.22±1.89 (96 hrs), Cholesterol (CHO) (mg/dl) 146.43±1.36 from 0 hrs to 226.73±1.62 (96 hrs), High Density lipoprotein (HDL) (mg/dl) 115.29±1.71 from 0 hrs to 182.79±1.39 (96 hrs), Low Density Lipoprotein (LDL) (mg/dl) 2.38±0.04 from 0 hrs to 0.18±0.01 (96 hrs), Very Low-Density (VLDL) (mg/dl) 27.61±1.44 from 0 hrs to 42.15±1.51(96 hrs), Magnesium (MG) (mg/dl) 3.35±0.01 from 0 hrs to 10.52±0.05 (96 hrs), Phospholipid (PL) (mg/dl) 194.73±1.63 from 0 hrs to 252.51±1.44 (96 hrs), Alanine aminotransferase (ALT) (U/L) 48.69±0.70 from 0 hrs to 85.55±0.86 (96 hrs) and Aspartate aminotransferase (AST) (U/L) 31.23±0.23 from 0 hrs to 117.74±1.37 (96 hrs) varies from 0 to 96hrs upon exposure to 96 hr LC_{50} concentration.

3.19.2 Sublethal toxicity

The sublethal effect of IMI on common carp fish in terms of blood serum parameters Glucose (GLU), Total Protein (TP), Albumin (ALB), Globulin (GLO), ALB:GLO ratio, Triglyceride (TG), Cholesterol (CHO), High Density lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), Magnesium (MG), Phospholipid (PL), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) are summarized in Table 24. The GLU (mg/dl) value recorded in increasing trend but significant increase was found in all the three concentrations on 28th days of exposure period (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). The TP (g/dl), ALB (g/dl), GLO (g/dl), TG (mg/dl) and VLDL (g/dl) were found to be in decrease trend but significant decrease in TP and GLO was observed in all the three treatments in 28 days of exposure period, TG and VLDL where significantly reduced in T1 on 21 days and in T1 and T2 on 28th days whereas, ALB was significant reduced in all treatment from 14th days onwards when compared to the control (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). The other serum parameters like CHO (mg/dl), PL (mg/dl), LDL (mg/dl), HDL (mg/dl), MG (mg/dl), AST (U/L) and ALT (U/L) were observed in the increasing trend. The CHO, PL, LDL, HDL and MG were recorded significantly higher in T1and T2 on 21 days followed by T1, T2 and T3 on 28 days (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). AST and ALT activities were recorded significantly higher in T1 on 7 days followed by other treatments T1, T2 on 14 days, T1, T2 and T3 on 21 and 28 days (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

Table 23. 24-hour variability on different serum biochemical parameters of Cyprinus carpio upon exposure to 96hr LC_{50} concentration of commercial imidacloprid (LC_{50} value i.e., 208.38ppm).

Serum Biochemical Parameters	**Period of Exposure (hrs)**				
	0	**24**	**48**	**72**	**96**
Glucose (mg/dl)	45.80±0.02^{a}	106.41±0.23^{b}	141.26±0.10^{c}	159.88±0.47^{d}	177.48±1.48^{e}
Total Protein (g/dl)	4.26±0.17^{d}	2.61±0.03^{c}	2.39±0.06^{bc}	2.14±0.02^{ab}	1.83±0.01^{a}
Albumin (g/dl)	1.57±0.01^{e}	1.29±0.03^{d}	1.07±0.01^{c}	0.73±0.02^{b}	0.47±0.01^{a}
Globulin (g/dl)	2.67±0.02^{d}	1.34±0.02^{c}	1.29±0.01^{b}	1.12±0.01^{a}	1.32±0.01^{bc}
A:G ratio	0.58±0.01^{b}	0.96±0.02^{e}	0.81±0.01^{d}	0.65±0.01^{c}	0.35±0.01^{a}
Total Cholesterol (mg/dl)	146.43±1.36^{a}	167.23±1.25^{b}	185.23±1.57^{c}	203.53±1.95^{d}	226.73±1.62^{e}
Phospholipid (mg/dl)	194.73±1.63^{a}	213.04±2.55^{b}	223.23±1.75^{c}	236.57±1.98^{d}	252.51±1.44^{e}
Triglycerides (mg/dl)	138.07±1.50^{a}	160.64±1.57^{b}	174.29±1.41^{c}	194.83±1.64^{d}	218.22±1.89^{e}
Low Density Lipoprotein (LDL) (mg/dl)	2.38±0.04^{a}	1.58±0.02^{b}	0.54±0.01^{c}	0.37±0.01^{d}	0.18±0.01^{e}
High Density Lipoprotein (HDL) (mg/dl)	115.29±1.71^{a}	131.72±2.79^{b}	148.42±0.81^{c}	163.18±1.62^{d}	182.79±1.39^{e}
Very Low-Density Lipoprotein (VLDL) (mg/dl)	27.61±1.44^{e}	32.12±0.94^{d}	34.73±1.82^{c}	37.86±1.07^{b}	42.15±1.51^{a}
Magnesium (mg/dl)	3.35±0.01^{a}	5.78±0.02^{b}	6.68±0.02^{c}	8.37±0.03^{d}	10.52±0.05^{e}
AST (U/L)	31.23±0.23^{a}	57.05±1.51^{b}	74.12±1.94^{c}	93.11±2.94^{d}	117.74±1.37^{e}
ALT (U/L)	48.69±0.70^{a}	31.42±1.29^{b}	51.79±1.61^{c}	67.51±1.44^{d}	85.55±0.86^{e}

Values are mean ± S.E; n=5. Value with different alphabet superscripts differ significantly ($p<0.05$) between duration of exposure.

Table 24. Variations in different serum biochemical parameters of test fish *Cyprinus carpio* for 28 days during exposure to sublethal concentrations of commercial Imidacloprid (Premise 30.50%SC) (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.38 ppm) and T3=LC_{50}/12 (17.36 ppm).

	Duration of Exposure in Days															
	7 Days				**14 Days**				**21 Days**				**28 Days**			
Parameters	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Glucose(mg/dl)	36.86 ± 2.08	41.24 ± 2.16	38.05 ± 3.41	37.18 ± 3.87	34.45 ± 2.04	43.52 ± 3.57	41.53 ± 3.53	40.08 ± 2.03	35.33 ± 3.49	45.36 ± 2.13	43.74 ± 3.04	42.53 ± 2.51	34.36 ± 2.33	48.52 ± 2.06***	46.32 ± 3.69*	45.38 ± 2.03*
Protein(g/dl)	3.61±0.14	3.08±0.29	3.47±0.10	3.54±0.17	3.92±0.22	3.16±0.14*	3.31±0.04	3.43±0.18	3.67±0.13	2.95±0.11*	3.06±0.19	3.23±0.32	3.44±0.17	2.51±0.19**	2.57±0.10**	2.50±0.10**
Albumin(g/dl)	1.36±0.01	1.26±0.07	1.28±0.02	1.33±0.06	1.57±0.08	1.13±0.05***	1.24±0.06**	1.27±0.06*	1.67±0.09	1.06±0.05***	1.16±0.05**	1.19±0.06**	1.42±0.05	0.97±0.05****	1.05±0.05***	1.07±0.05**
Globulin(g/dl)	2.25±0.15	1.82±0.22	2.19±0.10	2.20±0.11	2.36±0.15	2.03±0.09	2.07±0.09	2.15±0.24	2.00±0.22	1.89±0.06	1.90±0.14	2.04±0.26	2.02±0.12	1.64±0.24**	1.68±0.08*	1.83±0.12*
A:G ratio	0.63±0.04	0.71±0.06	0.59±0.03	0.61±0.01	0.69±0.02	0.56±0.00	0.60±0.05	0.61±0.09	0.83±0.14	0.56±0.01**	0.61±0.02*	0.63±0.05	0.72±0.02	0.59±0.15**	0.61±0.05*	0.63±0.09
Total Cholesterol(mg/dl)	151.33±7.47	157.45±7.34	155.40±6.88	152.89±6.04	153.50±5.93	177.31±9.13	169.37±8.06	160.87±7.28	154.22±6.91	192.07±7.85**	174.16±7.29	169.41±7.01	153.77±6.08	197.67±8.55***	179.15±7.35*	173.55±7.03
Phospholipid (mg/dl)	200.47±5.46	204.94±5.36	203.44±5.02	201.61±4.41	202.06±4.33	219.44±6.67	213.64±5.88	207.44±5.32	202.58±5.05	230.21±5.73**	217.13±5.32*	213.67±5.12	202.25±4.44	234.30±6.24***	220.78±5.36**	216.69±5.13*
Triglyceride(mg/dl)	137.36±6.91	122.98±5.49	125.55±5.89	129.08±5.64	133.45±7.41	112.54±5.10	117.83±6.69	121.62±5.22	135.09±6.65	104.54±5.08**	113.31±5.28*	115.88±6.48	127.59±6.22	96.86 ± 4.39**	107.83±4.82*	109.47±4.55

LDL (mg/dl)	3.38±0.18	3.56±0.18	3.43±0.16	3.40±0.16	3.43±0.15	3.94±0.20	3.67±0.13	3.50±0.12	3.31±0.14	3.82±0.17	3.75±0.23	3.69±0.15	3.63±0.15	4.03±0.19**	3.87±0.16*	3.84±0.18
HDL (mg/dl)	120.48±5.91	129.29±6.06	126.86±5.54	123.67±4.76	123.38±4.30	150.87±7.92*	142.14±6.59	133.05±6.36	123.89±5.44	167.34±6.66****	147.75±6.01**	142.55±5.56	124.63±4.68	174.27±7.48****	153.71±6.23**	147.82±5.95*
VLDL (mg/dl)	27.47±1.38	24.60±1.10	25.11±1.18	25.82±1.13	26.69±1.48	22.51±1.02	23.57±1.34	24.32±1.04	27.02±1.33	20.91±1.02**	22.66±1.06	23.18±1.30	25.52±1.24	19.37±0.88**	21.57±0.96*	21.89±0.91
Magnesium (mg/dl)	3.35±0.16	3.76±0.18	3.54±0.14	3.48±0.13	3.41±0.15	3.89±0.19	3.73±0.15	3.67±0.16	3.43±0.14	4.17±0.20*	3.97±0.17*	3.89±0.15	3.37±0.17	4.31±0.21**	4.17±0.18**	4.07±0.16*
AST(U/L)	120.65±3.88	147.24±6.60*	141.44±5.82	139.92±6.02	123.81±4.66	157.52±6.27**	150.48±7.50*	146.57±6.37	127.76±4.81	179.87±6.61****	163.57±6.79**	161.43±7.50**	122.85±5.03	194.87±7.76****	181.30±7.53****	178.54±8.23****
ALT(U/L)	57.85±2.64	71.58±2.76*	63.19±2.88	61.41±2.87	52.88±2.62	78.41±4.07***	67.55±3.26*	63.34±2.93	61.54±3.03	89.38±4.15***	73.59±3.05**	67.46±2.97	55.92±2.70	93.76±4.24****	82.33±3.58***	77.32±3.42*

Values are mean ± S.E; n=5. Statistical significance represents (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) was analysed under Two-way ANOVA using Graph pad prism version 7.0.

3.20 Oxidative stress enzymes

3.20.1 Acute toxicity

Insecticides may induce oxidative stress, leading to the generation of free radicals and causing lipid peroxidation, and may be the underlying molecular mechanism that gives rise to pesticide induced toxicity. Increased lipid peroxidation and oxidative stress can affect the activities of a number of protective antioxidants that are known to be sensitive indicators of increased oxidative stress (Agarwal *et al*., 1991; Almeida *et al*., 2010; Yonar *et al*., 2012). In the present study, different oxidative stress enzymes like SOD, CAT, GPx, ALT and AST have been evaluated in the liver, gill and brain tissue of the fish. The data of SOD, CAT, GPx, AST and AST of liver, gill and brain is shown in Fig (42, 43 and 44).

In *C. carpio* treated with LC_{50} concentration (226.487ppm) IMI for 24, 48, 72 and 96 hr showed significant increase in SOD activity in the liver, gills and brain tissues when compared to the control/0hr. (from 2.11±0.11, 0 hr to 15.42±0.86 to 96 hr for liver; from 1.80±0.12, 0 hr to 11.65±0.20 for gills; from 2.71±0.13, 0hr to 12.21±0.40, 96 hr for brain) (Fig. 42A, 43A and 44A) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) respectively.

In *C. carpio* treated with LC_{50} concentration (226.487ppm) IMI for 24, 48, 72 and 96 hr exhibited significant increase in CAT activity (Table 14) in the liver, gills and brain tissues when compared to the control/0hr. (from 3.03±0.13, 0hr to 12.82±0.59, 96 hr for liver; from 3.59±0.17, 0hr to 13.34±0.67, 96 hr for gills; From 4.41±0.18, 0hr to 11.71±0.40, 96 hr for brain) (Fig. 42B, 43B and 44B) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) respectively.

The increase in GPx activity in the liver, gills and brain tissues for 24, 48, 72, 96 hr was significantly higher in IMI exposed fish with LC_{50} concentration (226.487ppm) when compared with control (from 2.81±0.26, 0hr to 14.24±0.57, 96 hr for liver; from 2.47±0.19, 0hr to 8.57±0.29, 96 hr for gill; from 6.57±0.25, 0 hr to 39.32±0.62, 96 hr for brain) (Fig. 42C, 43C and 44C). (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

Similarly, significantly increased value of AST and ALT was also recorded in the liver and gills of IMI exposed fish for 24hr, 48hr, 72hr and 96hr when compared with control which is depicted in Table 14, (Fig 42D, 43D and 44D) and (Fig 42E, 43E and 44E) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

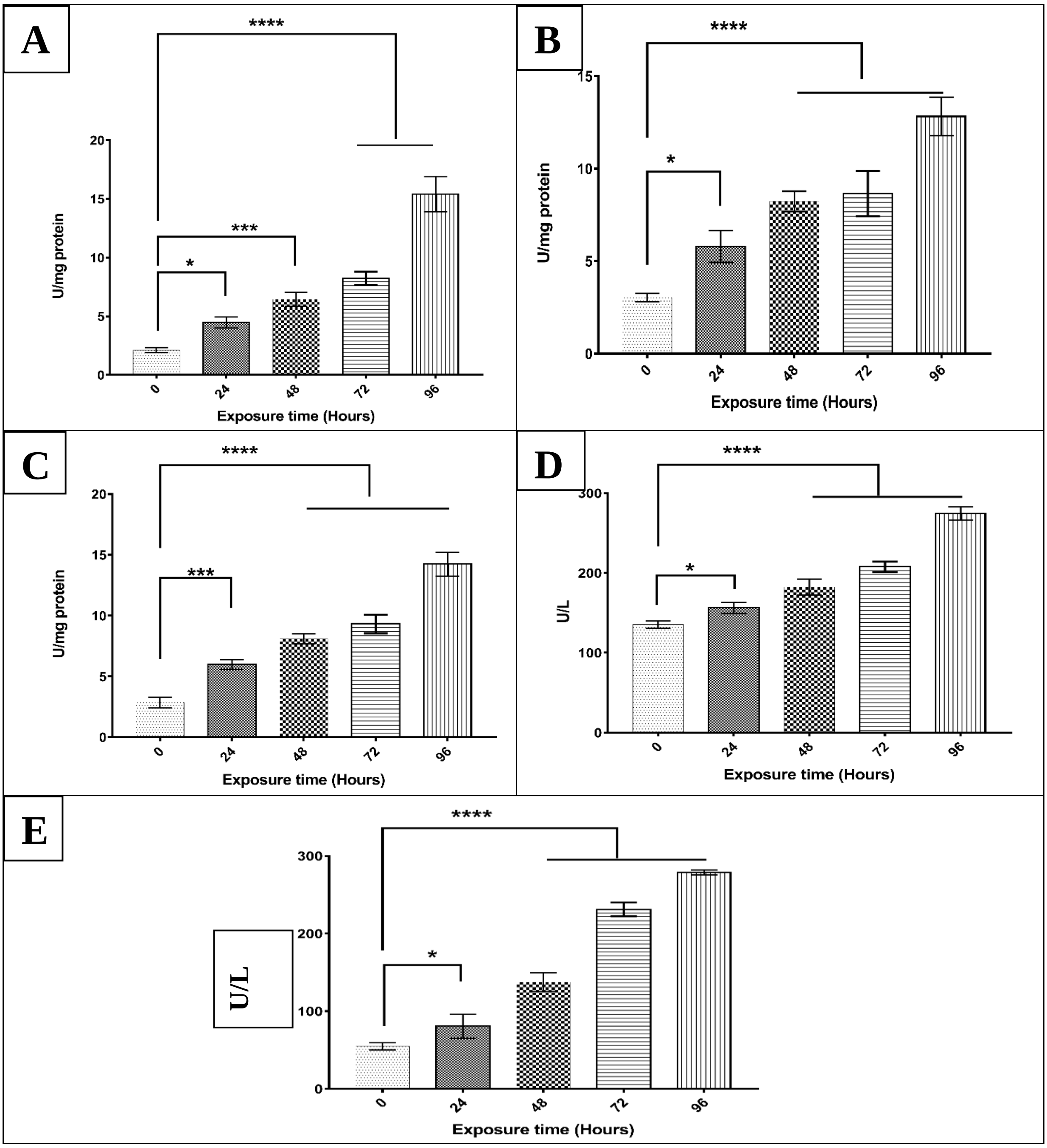

FIGURE 42. EFFECT OF 96hr LC_{50} CONCENTRATION ON DIFFERENT ANTIOXIDANT ENZYMES ACTIVITIES OF LIVER IN *Cyprinus carpio*. A. SODIUM OXIDASE DISMUTASE (SOD, U/mg protein). B. CATALASE (CAT, U/mg protein). C. GLUTATHIONE PEROXIDASE (GPX, U/mg protein). D. ALANINE AMINOTRANSFERASE (ALT, U/L), E. ASPARTATE AMINOTRANSFERASE (AST, U/L). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

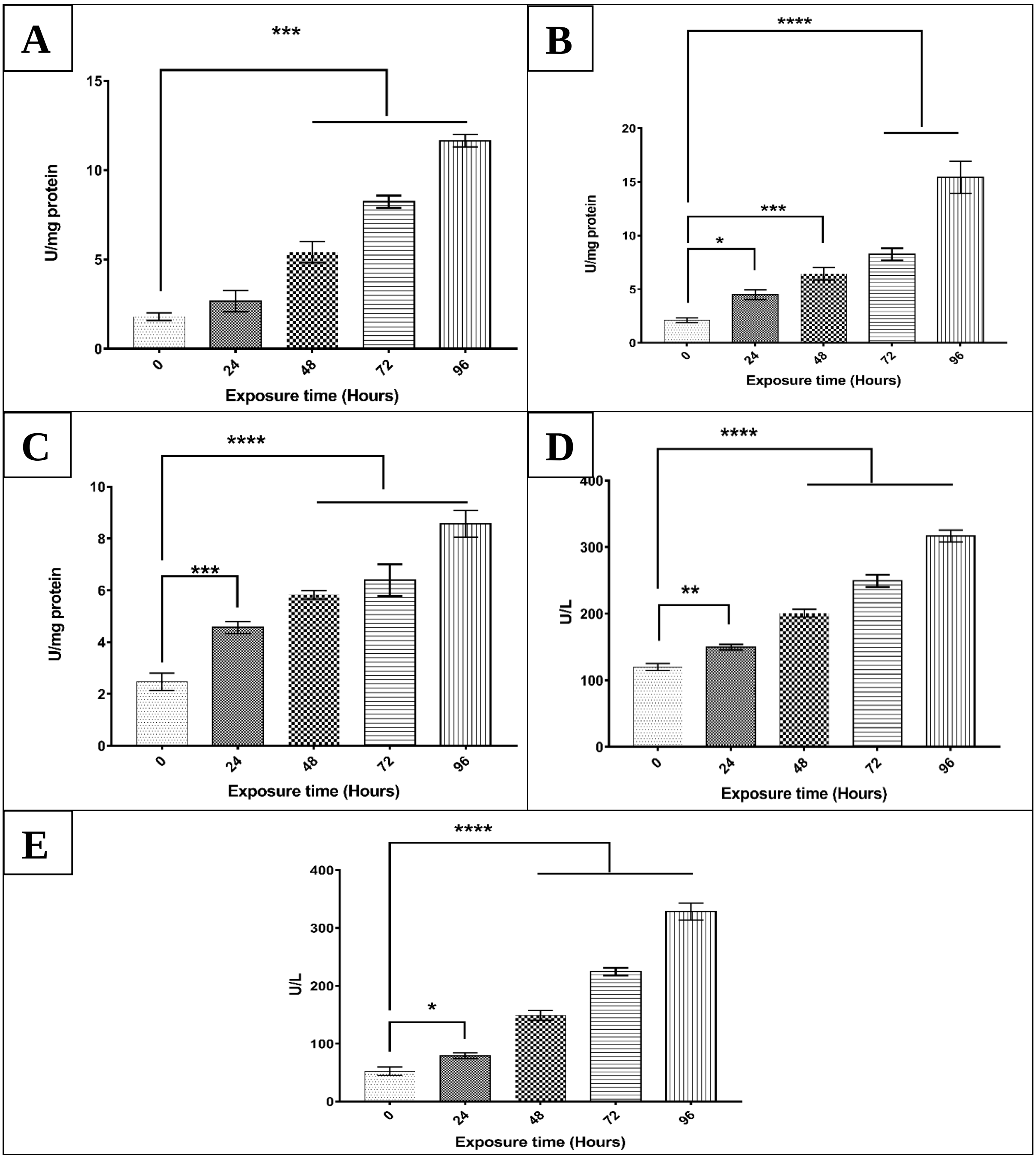

FIGURE 43. EFFECT OF 96hr LC_{50} CONCENTRATION ON DIFFERENT ANTIOXIDANT ENZYMES ACTIVITIES OF GILL IN *Cyprinus carpio*. A. SODIUM OXIDASE DISMUTASE (SOD, U/mg protein). B. CATALASE (CAT, U/mg protein). C. GLUTATHIONE PEROXIDASE (GPX, U/mg protein). D. ALANINE AMINOTRANSFERASE (ALT, U/L), E. ASPARTATE AMINOTRANSFERASE (AST, U/L). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

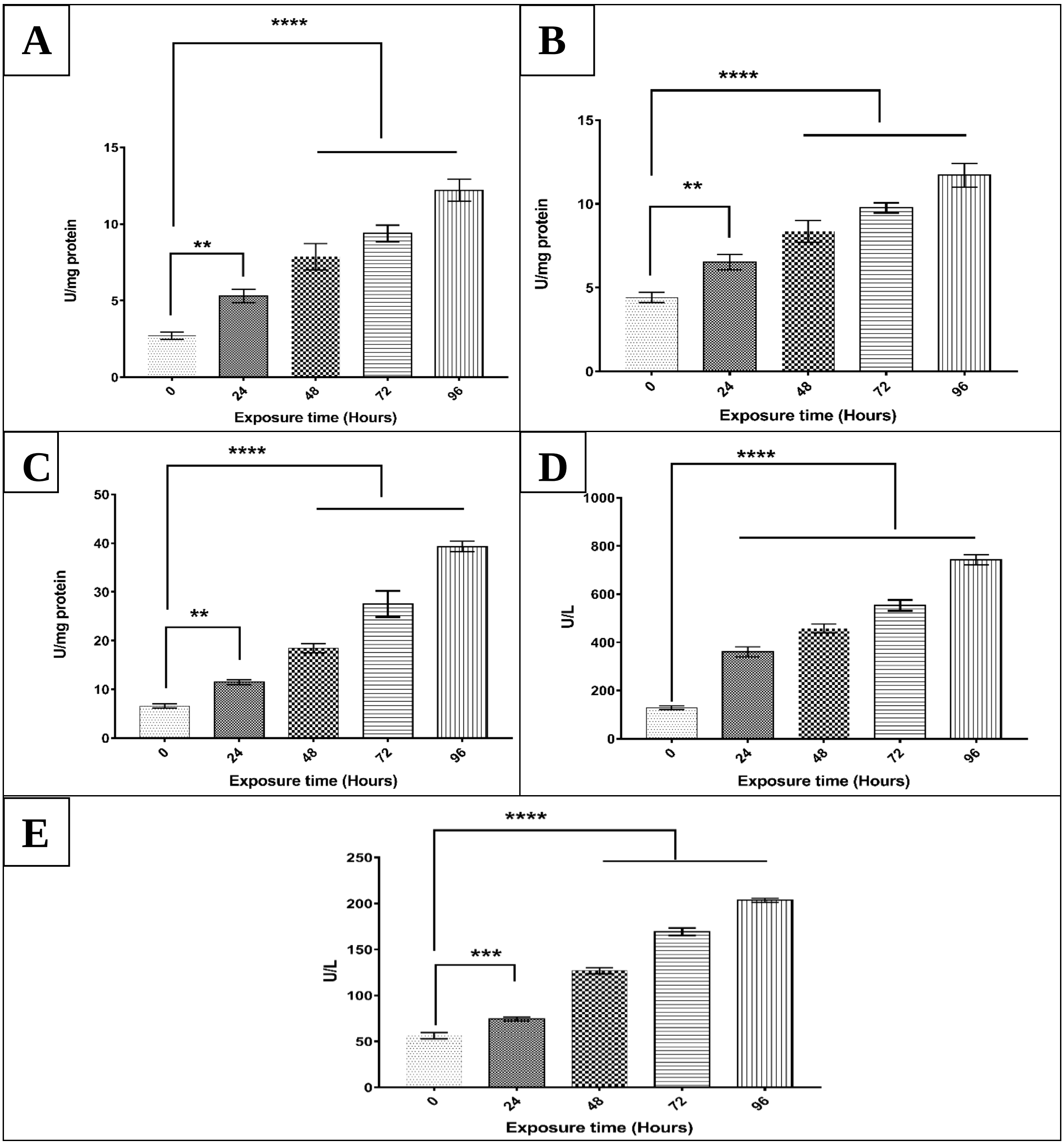

FIGURE 44. EFFECT OF 96HR LC_{50} CONCENTRATION ON DIFFERENT ANTIOXIDANT ENZYMES ACTIVITIES OF BRAIN IN *Cyprinus carpio*. A. SODIUM OXIDASE DISMUTASE (SOD, U/mg protein). B. CATALASE (CAT, U/mg protein). C. GLUTATHIONE PEROXIDASE (GPX, U/mg protein). D. ALANINE AMINOTRANSFERASE (ALT, U/L), E. ASPARTATE AMINOTRANSFERASE (AST, U/L). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.20.2 Sublethal toxicity

Antioxidant enzymes such as SOD, CAT, GPx, AST and ALT in liver, gill and brain tissues of *C. carpio* upon chronic exposure to three sublethal concentration of IMI for 28 days showed significant alteration with increase in concentration and time period shown in figure (45, 46 and 47).

SOD of liver showed significant increase on day 21 and 28 in all the treatment groups but SOD in gill was significantly increase on day 21 in T1 and in all the three treatment groups on day 28 when compared to the control. In brain SOD was significantly increased in T1 on day 7 followed by significant increase in all the treatment groups on day 14, 21 and 28 (Fig. 45A, 46A and 47A) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

Significant increase of CAT on day 21 in T1 in both liver and gills, followed by significant increase in all the treatment groups on day 28 was reported for both liver and gill tissue of exposed fishes. In brain, CAT was significantly increased in T1 on day 14 followed by significant increase in all the three-treatment groups on day 21 and 28 (Fig 45B, 46B, and 47B) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

GPx activity was significantly increased in liver on day 7 in T1 treatment group followed by significant increased in all the three treatment groups on day 14, 21 and 28 when compared to control. In gills, GPx activity was found to be significantly increased right from 7th day till 28 days in all the treatment groups. However, in brain, GPx was significantly raised on day 14th in T1 group followed by significant rise in all the other three groups on day 21 and 28(Fig. 45C, 46C and 47C) (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

Both Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activity in liver, gill and brain tissues shows significant increased in all the three treatment groups from day 14th onwards when compared to the control (Fig. 45D, 46D and 47E) .

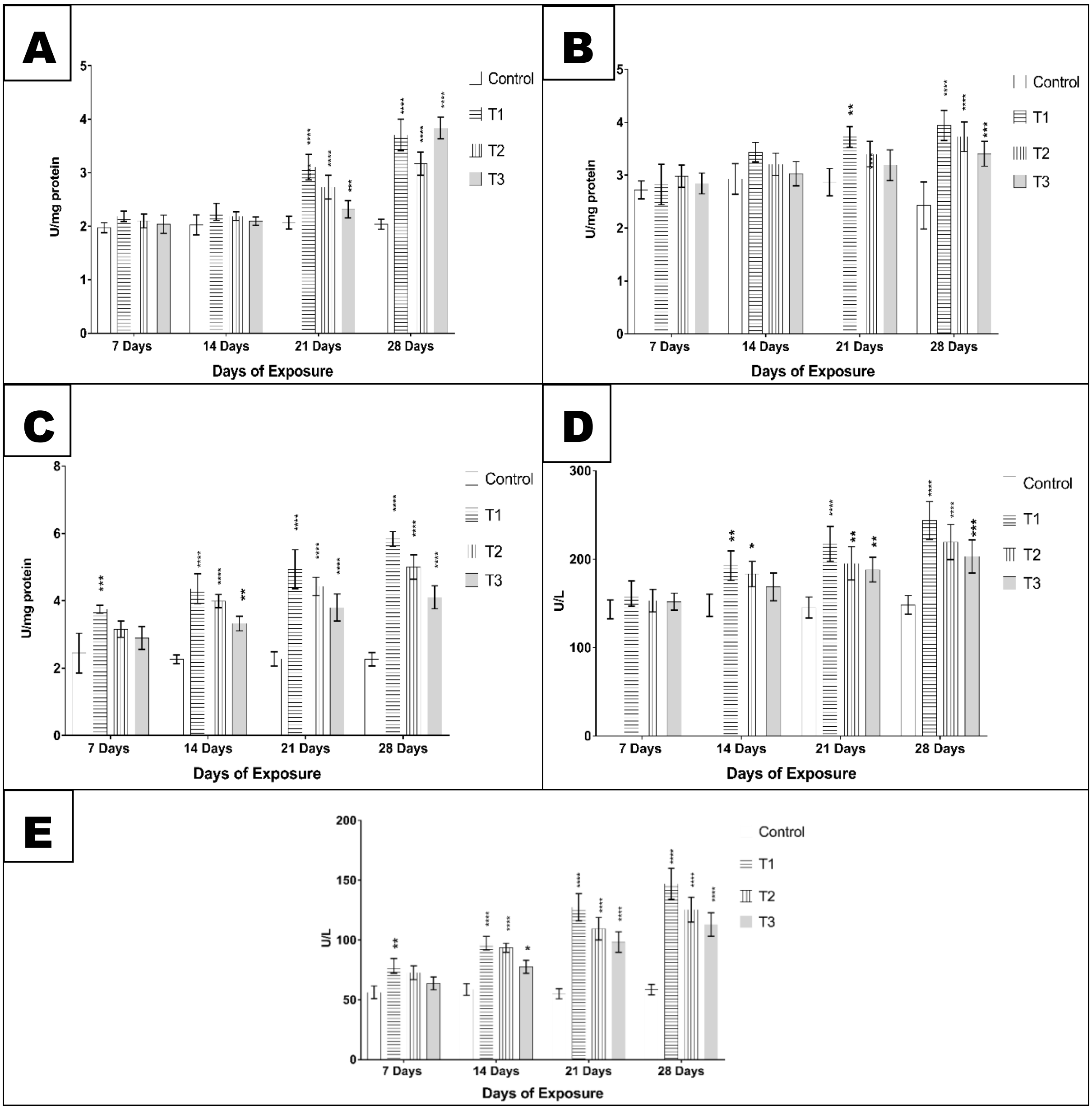

FIGURE 45. VARIATIONS IN DIFFERENT ANTIOXIDANT ENZYMES IN LIVER OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.50%SC) (T1= $LC_{50}/8$ (26.04 ppm), T2=$LC_{50}/10$ (20.38 ppm) AND T3=$LC_{50}/12$ (17.36 ppm). A. SUPEROXIDASE DISMUTASE (SOD, U/mg protein). B. CATALASE (CAT, U/mg protein). C. GLUTATHIONE PEROXIDASE (GPX, U/mg protein). D. ALANINE AMINOTRANSFERASE (ALT, U/L), E. ASPARTATE AMINOTRANSFERASE (AST, U/L). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

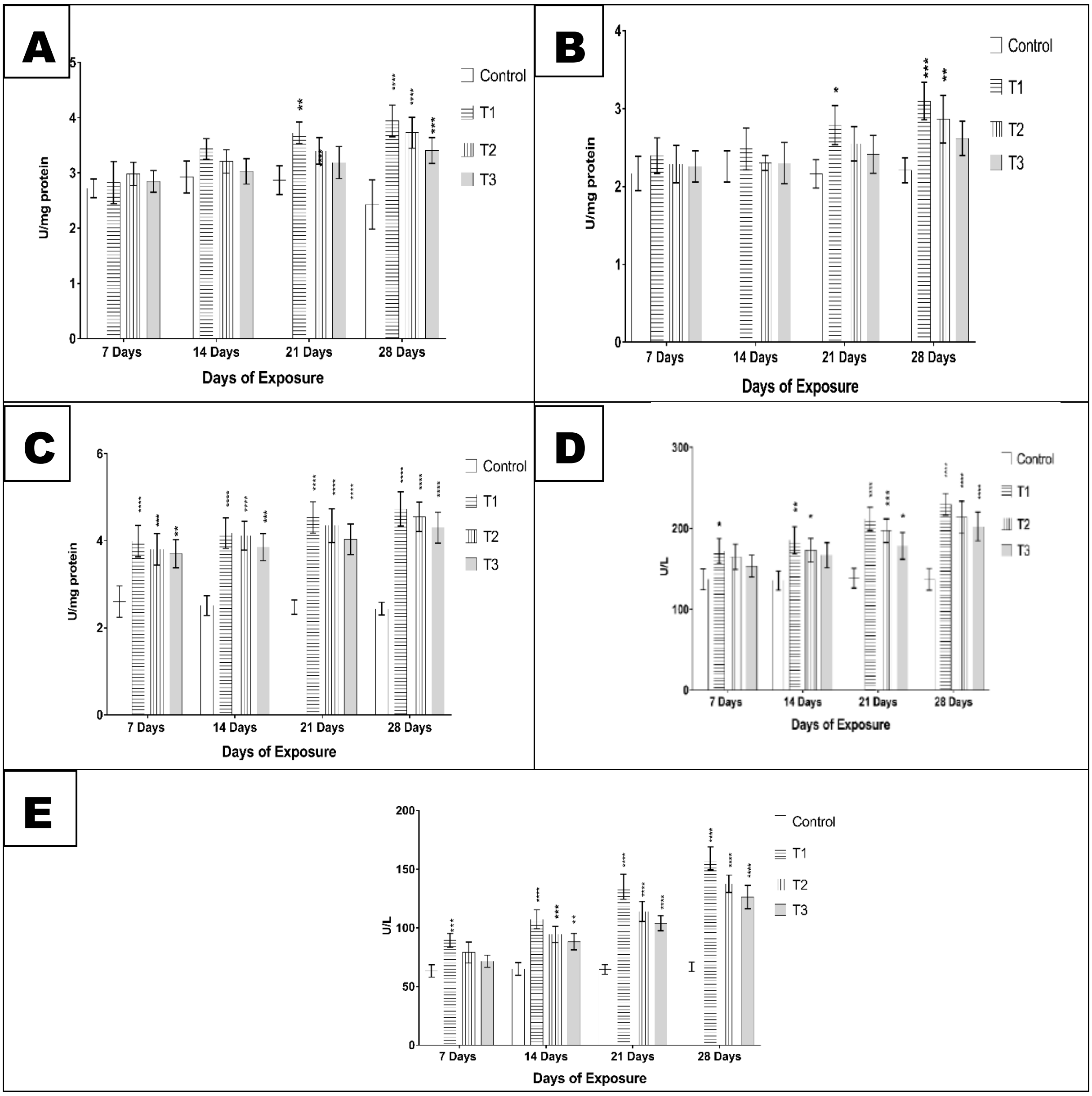

FIGURE 46. VARIATIONS IN DIFFERENT ANTIOXIDANT ENZYMES IN GILLS OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.50%SC) (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.38 ppm) AND T3=LC_{50}/12 (17.36 ppm). A. SUPEROXIDASE DISMUTASE (SOD, U/mg protein). B. CATALASE (CAT, U/mg protein). C. GLUTATHIONE PEROXIDASE (GPX, U/mg protein). D. ALANINE AMINOTRANSFERASE (ALT, U/L), E. ASPARTATE AMINOTRANSFERASE (AST, U/L). VALUES ARE MEAN ± S.E; N=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

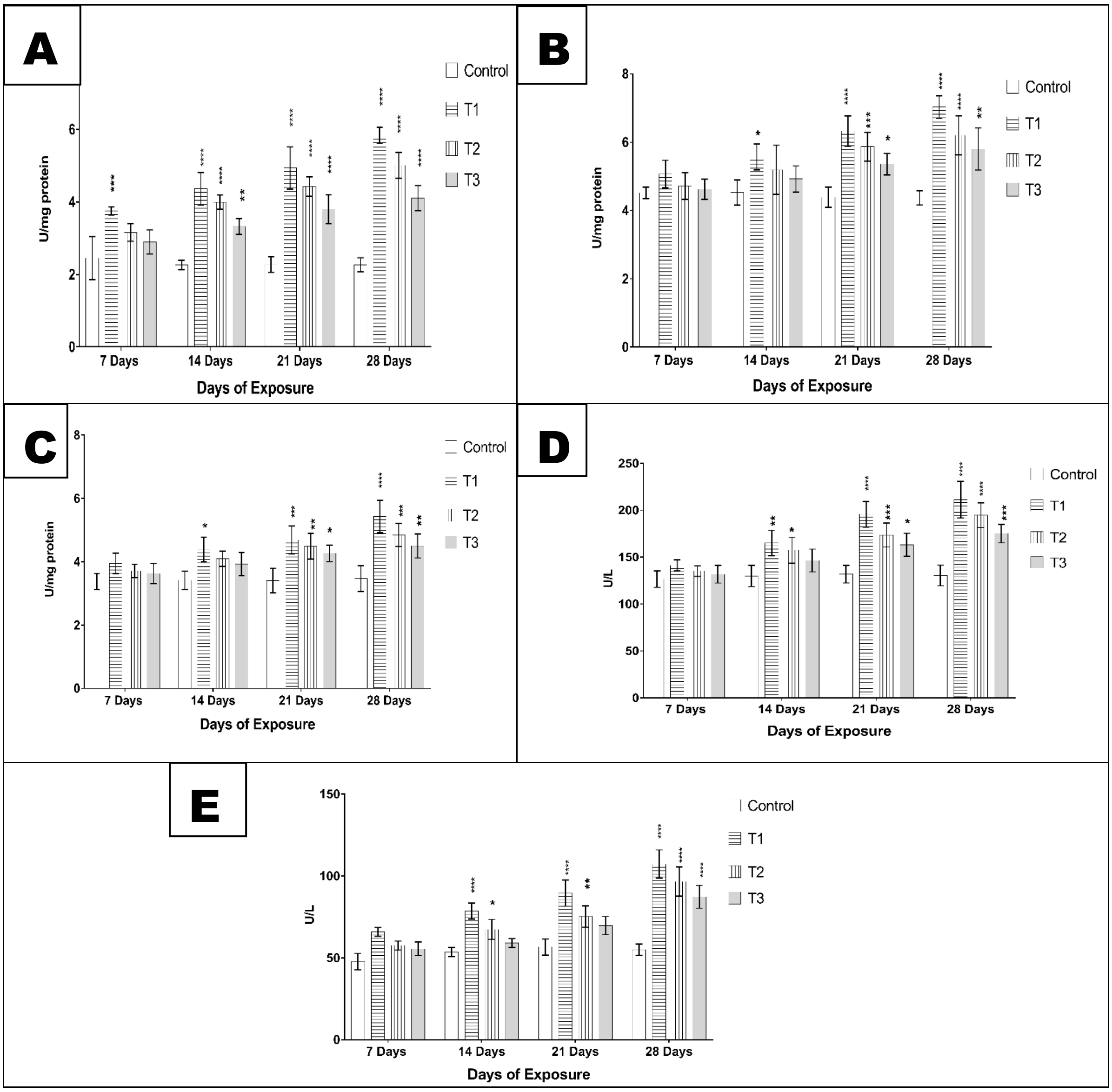

FIGURE 47. VARIATIONS IN DIFFERENT ANTIOXIDANT ENZYMES IN BRAIN OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.50%SC) (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.38 ppm) AND T3=LC_{50}/12 (17.36 ppm). A. SUPEROXIDASE DISMUTASE (SOD, U/mg protein). B. CATALASE (CAT, U/mg protein). C. GLUTATHIONE PEROXIDASE (GPX, U/mg protein). D. ALANINE AMINOTRANSFERASE (ALT, U/L), E. ASPARTATE AMINOTRANSFERASE (AST, U/L). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.20 Oxidative Stress markers

ROS level and MDA content

3.20.1 Acute toxicity

The changes in ROS levels and MDA content for every 24 hrs of *C. carpio* when exposed to 96 hr LC_{50} concentration of commercially formulated Imidacloprid is shown in Fig 48 and 49. The ROS (Fluorescent intensity/mg protein) level significantly increases from 243.16±7.75 (0hrs) to 520.89±12.46 (96hrs) in liver and from 411.19±6.12 (0hrs) to 791.08±14.12 (96 hrs)in gills (Fig. 48A and 48B), whereas MDA (nmol/mg protein) content significantly increases from 8.25±0.42 (0hrs) to 22.58±0.74 (96hrs) in liver and from 5.92±0.32 (0hrs) to 17.57±0.74 (96 hrs) in gills (fig 49A and 49B) upon acute exposure to IMI. (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

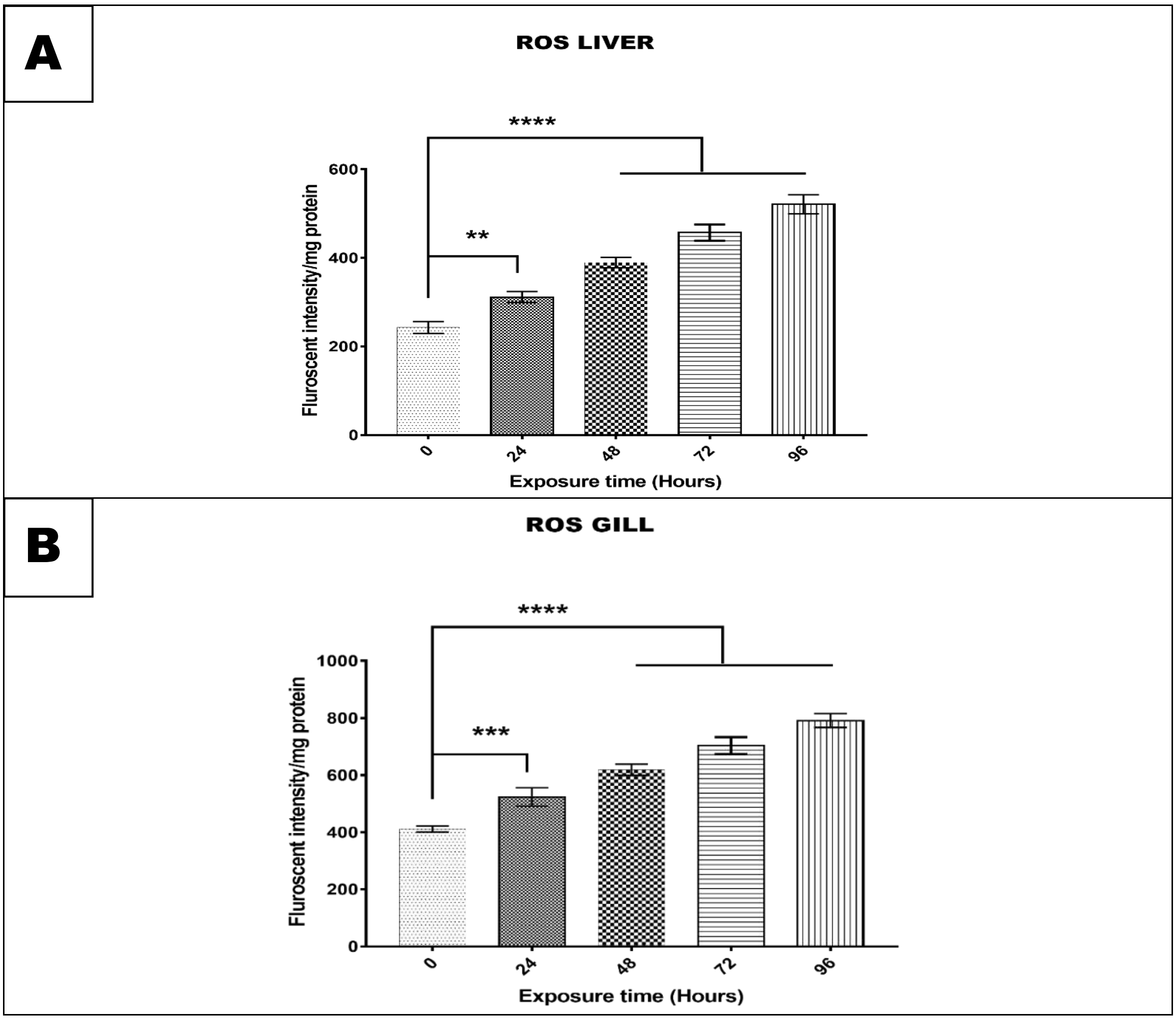

FIGURE 48. EFFECT OF 96hr LC_{50} CONCENTRATION ON DIFFERENT STRESS BIOMARKERS IN *Cyprinus carpio* A. REACTIVE OXYGEN LEVEL IN LIVER (ROS, FLUORESCENT INTENSITY/mg PROTEIN). B. REACTIVE OXYGEN LEVEL IN GILL (ROS, FLUORESCENT INTENSITY/mg PROTEIN). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

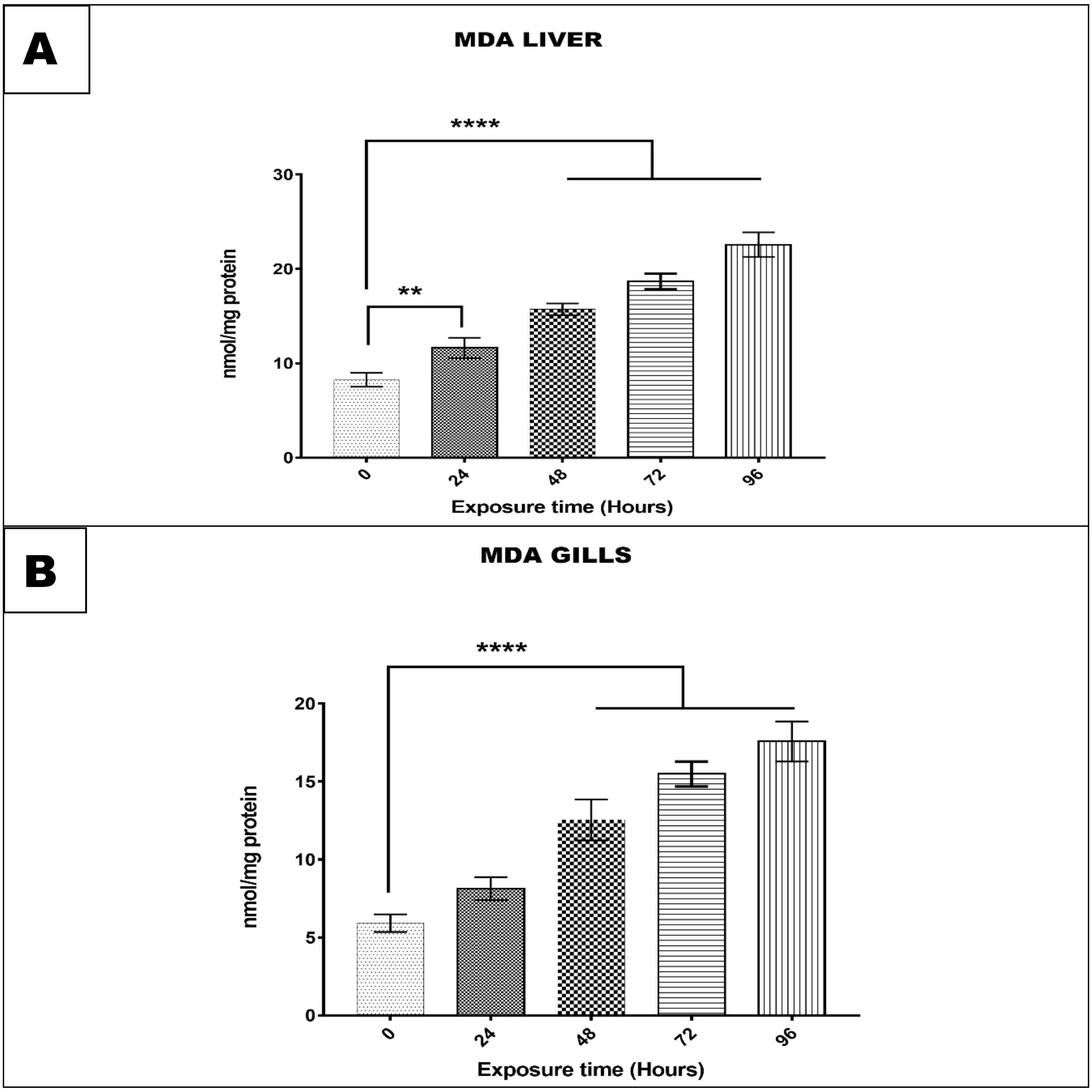

FIGURE 49. EFFECT OF 96hr LC_{50} CONCENTRATION ON DIFFERENT STRESS BIOMARKERS IN *Cyprinus carpio*. A. MALONALDEHYDE CONTENT IN LIVER (MDA, nmol/mg PROTEIN). B. MALONALDEHYDE CONTENT IN GILL (MDA, nmol/mg PROTEIN). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.20.2 Sublethal toxicity

The level of ROS level and lipid peroxidation caused by IMI in the liver and gills tissues of common carp assessed using MDA content are shown in Fig (50 and 51). ROS (Fluorescent intensity/mg protein) level in liver with no significant increase was recorded at day 7, significant increase in T1 at day 14 and 21 and in all the treatments at day 28 when compared to the control whereas in gill significant increase in all the treatments during entire exposure period was observed (Fig. 50A and 50B). The MDA (nmol/mg protein) content in liver and gill tissues of common carp with test concentration of IMI was not significantly different at day 7, whereas significant difference was recorded on T1 at day 14 in gills and liver and almost all treatments showed significant difference at day 21 and 28 in both liver and gill tissue of exposed fish. (Fig 51A and 51B). (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

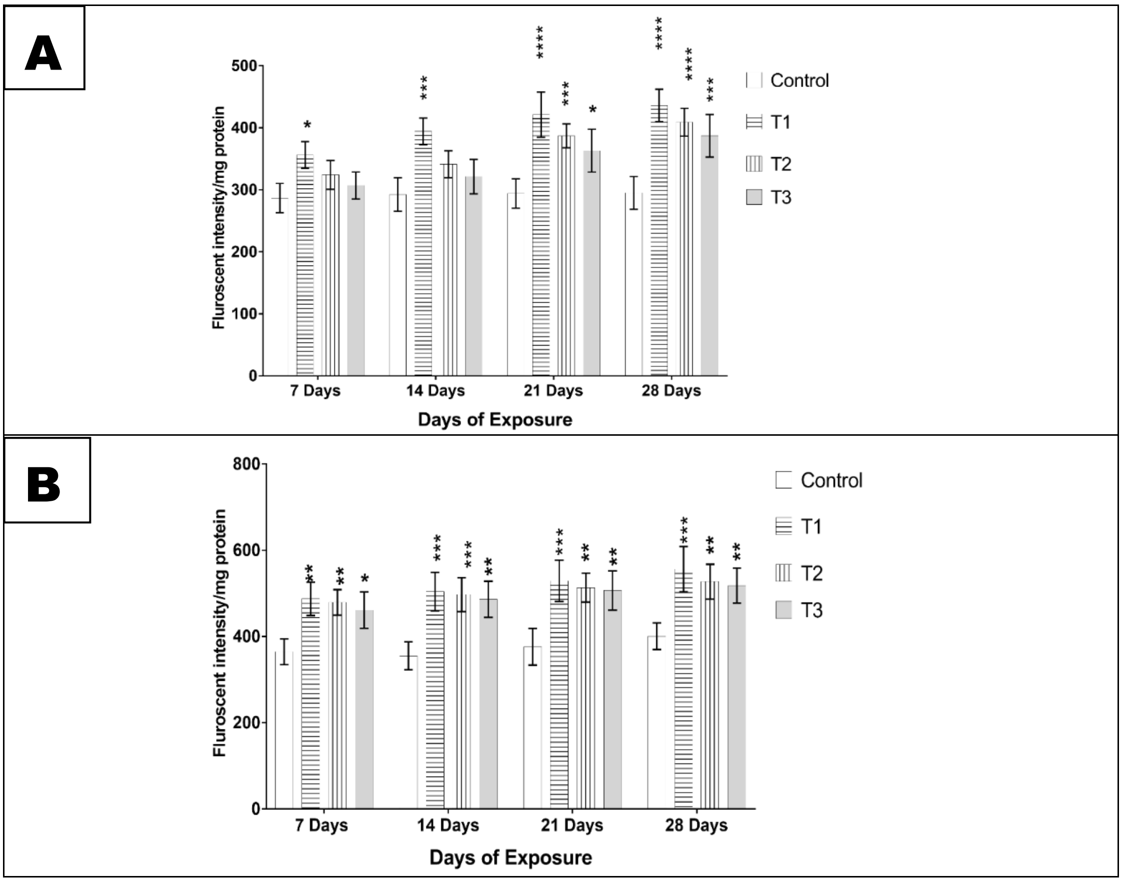

FIGURE 50. VARIATIONS IN LIVER AND GILL REACTIVE OXYGEN SPECIES (ROS) LEVEL OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.5%SC) (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.83 ppm) AND T3=LC_{50}/12 (17.36 ppm). A REACTIVE OXYGEN LEVEL IN LIVER (ROS, FLUORESCENT INTENSITY/mg PROTEIN). B. REACTIVE OXYGEN LEVEL IN GILL (ROS, FLUORESCENT INTENSITY/mg PROTEIN). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

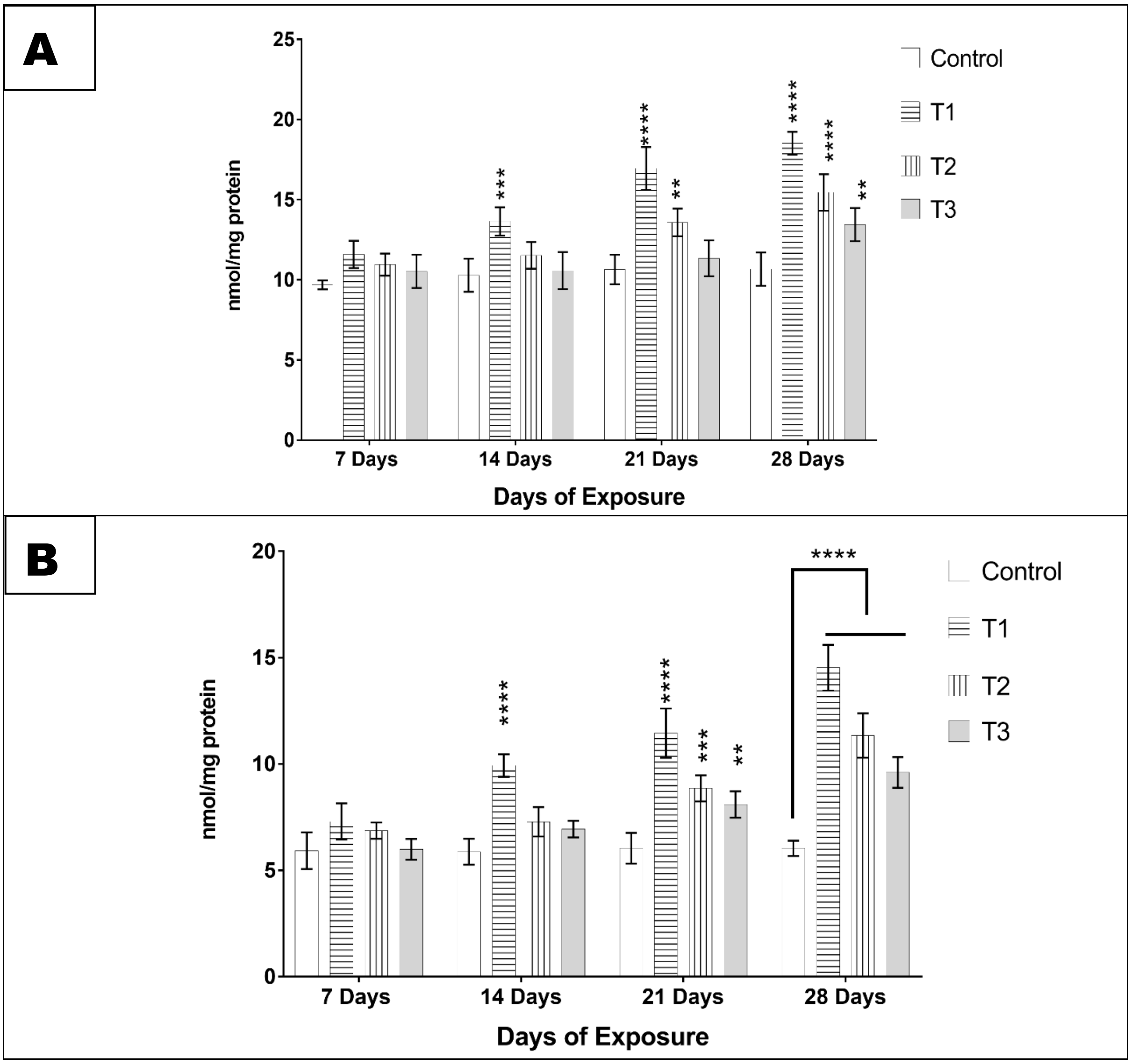

FIGURE 51. VARIATIONS IN LIVER AND GILL MALONALDEHYDE (MDA) CONTENT OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.5%SC) (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.83 ppm) AND T3=LC_{50}/12 (17.36 ppm). A. MALONALDEHYDE CONTENT IN LIVER (MDA, nmol/mg PROTEIN). B. MALONALDEHYDE CONTENT IN GILL (MDA, nmol/mg PROTEIN). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.21 Neurotoxic assay

Acetylcholinesterase activity (AChE)

3.21.1 Acute toxicity

Brain tissue of common carp exposed to 96 hr LC_{50} of imidacloprid shows a significant decrease in brain AChE enzyme activity (EU/mg protein) from 0.73±0.015 (0 hrs) to 0.55±0.02 (24 hrs) and 0.73±0.015 (0 hrs) to 0.19±0.01 (96 hrs), (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) (Fig 52) in brain AChE enzyme activity.

3.21.2 Sublethal toxicity

Similarly, during sublethal exposure to 28 days for three different concentrations of Imidacloprid (Premise 30.5%SC; T1= LC_{50}/8 (26. 04 ppm), T2=LC_{50}/10 (20.83 ppm,) and T3=LC_{50}/12 (17.36 ppm) exposed to brain tissue for AChE activity (EU/mg protein) shows significant reduction (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001) (Figure 53) in values when compared to control group. Lowest value for AChE activity 0.43 was recorded on day 28 at sublethal concentration of T_1 followed by 0.50 on day 28 for T2 when compared to control group of fishes.

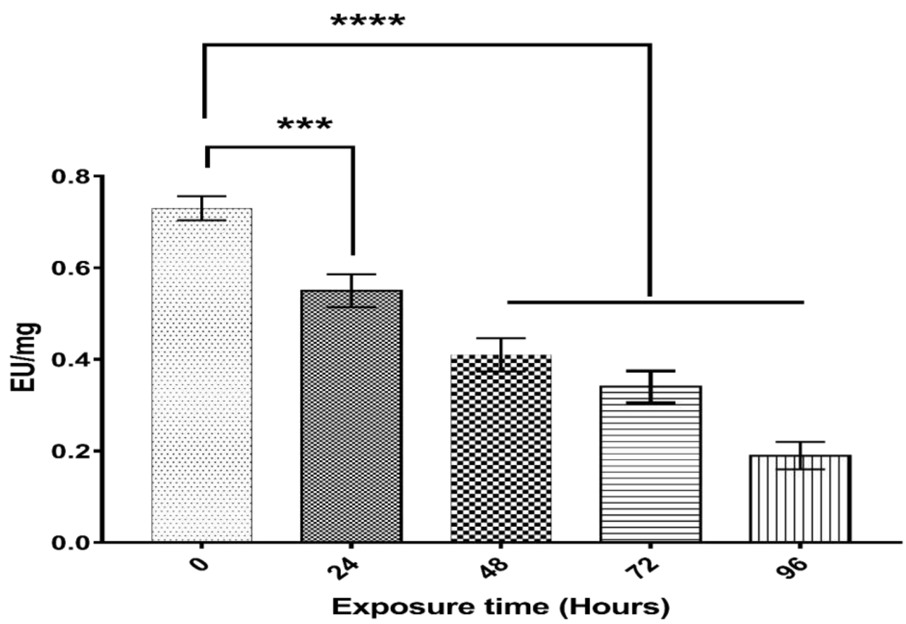

FIGURE 52. EFFECT OF 96hr LC_{50} CONCENTRATION ON BRAIN ACETYLCHOLINE ESTERASE ENZYME ACTIVITY IN *Cyprinus carpio*. ACETYLCHOLINE ESTERASE ACTIVITY (ACHE ACTIVITY, EU/mg). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

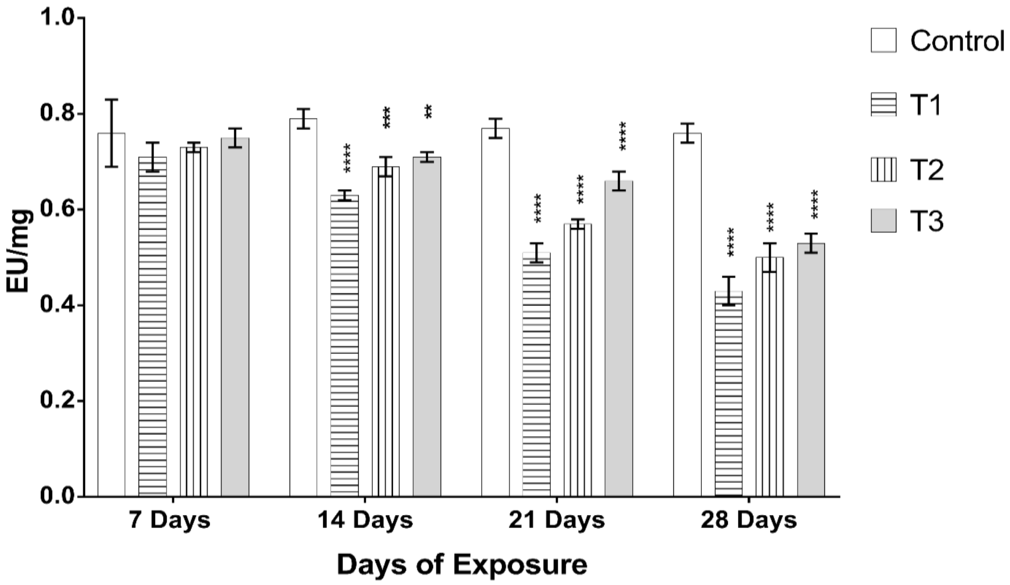

FIGURE 53. VARIATIONS IN BRAIN ACETYLCHOLINE ESTERASE ENZYME ACTIVITY OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.5%SC) (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.83 ppm) AND T3=LC_{50}/12 (17.36 ppm). ACETYLCHOLINE ESTERASE ACTIVITY (ACHE ACTIVITY, EU/mg). VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0.

3.22 Genotoxic assay

Micronucleus test

3.22.1 Acute toxicity

Micronucleus (MNi) having no connection with the main nucleus look similar in colour and intensity as that of main nucleus and having an area less than 1/3rd of the main nucleus was scored. The erythrocytes of common carp were elliptical in shape with a centrally located oblong nucleus. The frequency of MNi induced upon acute exposure to IMI (96 hr LC_{50} concentration) is shown in Figure 54. Highest number of MNi formation (1.94 ± 0.04 %) was obtained upon acute exposure to LC_{50} concentration for 96 hrs. The observed MNi showed similar features as described by Schmid (1975). MNi frequency for the 96hr LC_{50} concentration treated fish significantly increases at 72hrs and 96 hrs when compared to 0hr, 24hr and 48hr of exposure.

3.22.2 Sublethal toxicity

During sublethal exposure a significant effect of duration on induction of MNi was observed for all the three concentrations (T1= LC_{50}/8 (26.04 ppm), T2=LC_{50}/10 (20.83 ppm,) and T3=LC_{50}/12 (17.36 ppm). The frequency of MNi induced upon sublethal exposure to three different concentrations of IMI (96 hr LC_{50} concentration) shown in Figure 55. Highest MNi frequency of 1.16 % was observed on day 14 and 21 at sublethal T1 concentration followed by 0.85 % on day 21 at sublethal T2. Lowest MNi frequency was recorded 0.34 % on day 7th day at sublethal concentration T3. Cyclophosphamide

(20mg/kg body weight) used as Positive control was able to induce significant MNi frequency in comparison to negative control (Figure 56).

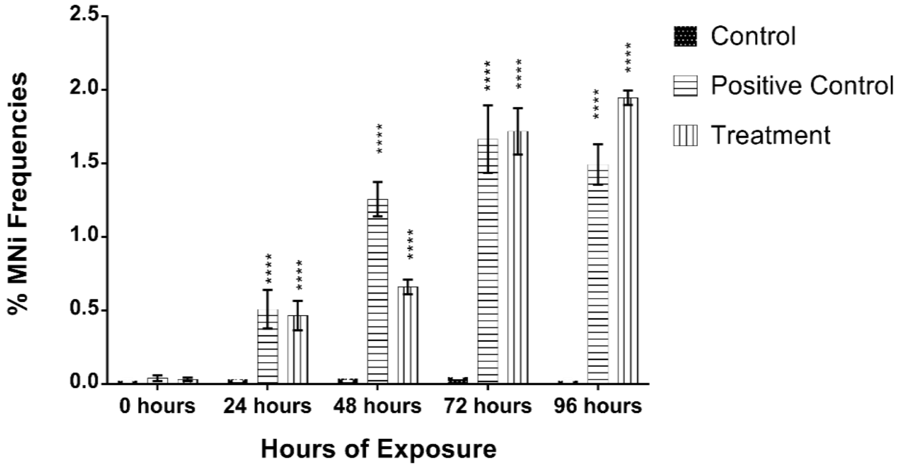

FIGURE 54. EFFECT OF 96hr LC_{50} CONCENTRATION OF IMI ON VARIATIONS IN MNI FREQUENCY IN RELATION TO DIFFERENT EXPOSURE TIME USING CYCLOPHOSPHAMIDE 20mg/kg BODY WEIGHT AS POSITIVE CONTROL. VALUES ARE MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WERE ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0

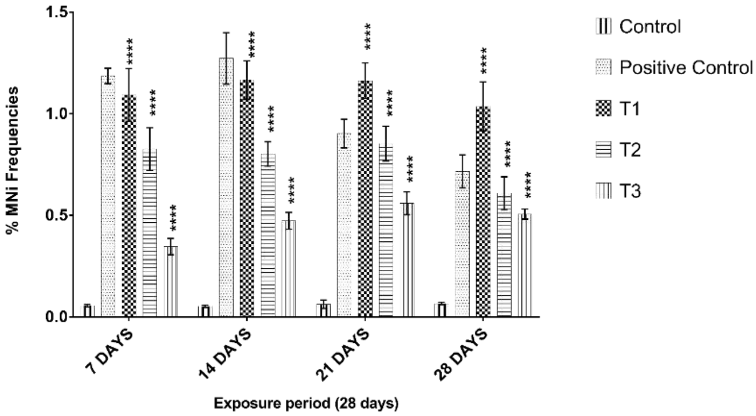

FIGURE 55. VARIATIONS IN MNI FREQUENCY IN ERYTHROCYTE OF TEST FISH *Cyprinus carpio* FOR 28 DAYS DURING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF COMMERCIAL IMIDACLOPRID (PREMISE 30.5%SC) (T1= $LC_{50}/8$ (26.04 ppm), T2=$LC_{50}/10$ (20.83 ppm) AND T3=$LC_{50}/12$ (17.36 ppm). CYCLOPHOSPHAMIDE 20mg/kg BODY WEIGHT WAS USED AS POSITIVE CONTROL. VALUES ARE EXPRESSED IN MEAN ± S.E; n=5; ERROR BARS INDICATE STANDARD ERROR. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WERE ANALYSED UNDER TWO-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.

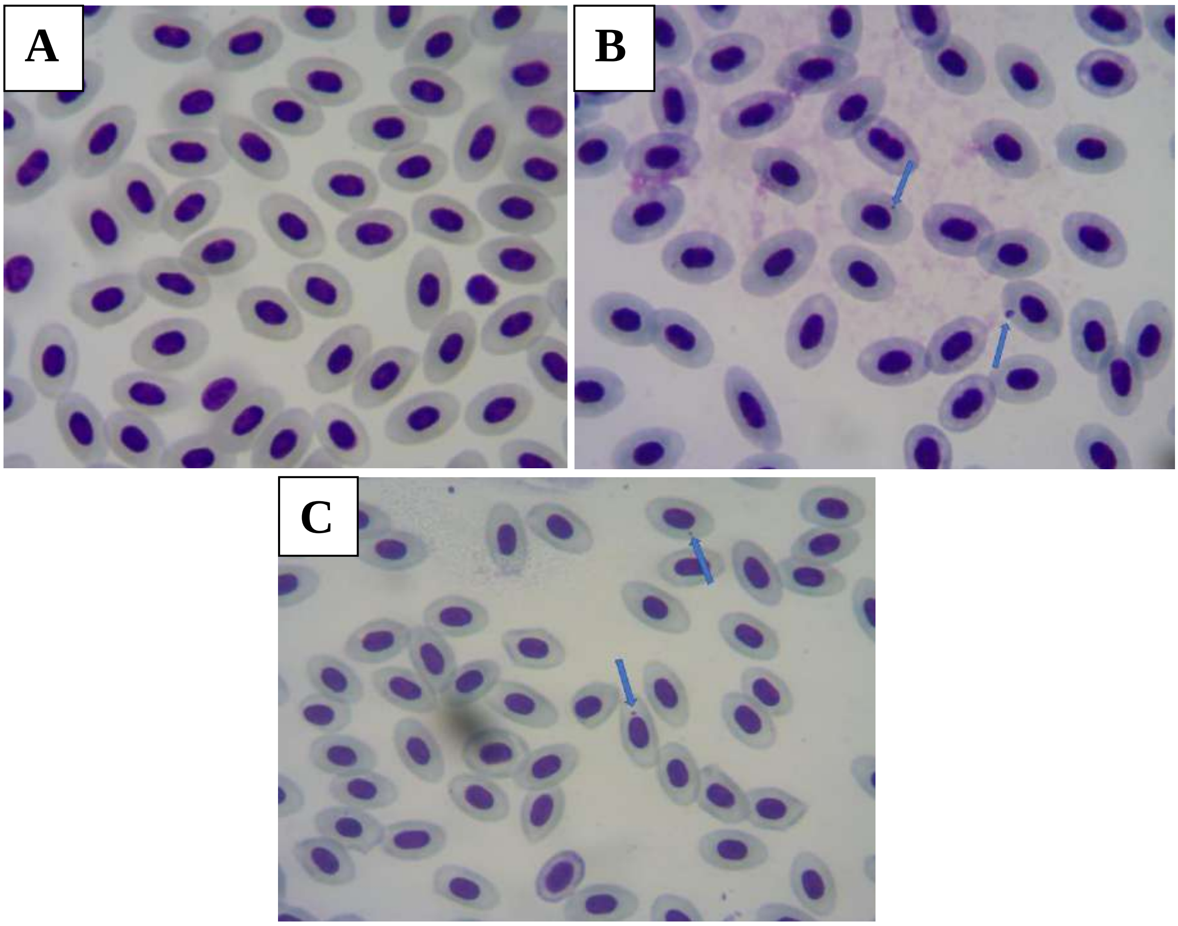

FIGURE: 56 A. NORMAL ERYTHROCYTE CELLS OF COMMON CARP. **B**. MICRONUCLEI FORMATION IN THE ERYTHROCYTE AFTER EXPOSURE TO CYCLOPHOSPHAMIDE (20mg/kg BODY WEIGHT). **C**. MICRONUCLEI FORMATION IN THE ERYTHROCYTE AFTER EXPOSURE TO IMI.

3.23 Gene Expression Analysis

3.23.1 Sublethal toxicity

The gills and liver samples of both test and control fish were analysed for 2 different gene expression heat shock protein (HSP70) and Cytochrome P450 (CYP1A). cDNA samples were quantified using Nanodrop to find out the concentration of each sample. qPCR amplification was carried out using gene specific primers. The results were analysed and the fold changes in the expression of the gene in different samples are shown in Figure 57, 58, 59 and 60). The beta actin gene was used as internal control (Housekeeping gene) in gene expression for the normalization.

The results were analysed and the fold changes in the expression of the gene (HSP70 and CYP1A) in gill and liver samples are shown in Figures (57, 58, 59 and 60). The mRNA level of HSP70 in gill was significantly upregulated in treatment T1 and T2 on day 7, 14, 21 and 28 (fig 57A, 58A, 59A and 60A), whereas HSP70 in the liver was significantly upregulated in all the treatments T1, T2 and T3 on day 7, 14, 21 and 28 except T3 on day 7 (57B, 58B, 59B and 60B) ($p<0.0001$). Similarly, CYP1A gene in gill and liver was significantly upregulated in treatment T1 and T2 on days 7, 14, 21 and 28 (57C, 58C, 59C and 60C) and T1 on day 7, T1 and T2 on day 14 and 21, and T1, T2 and T3 on day 28 (57D, 58D, 59D and 60D) respectively (p<0.0001).

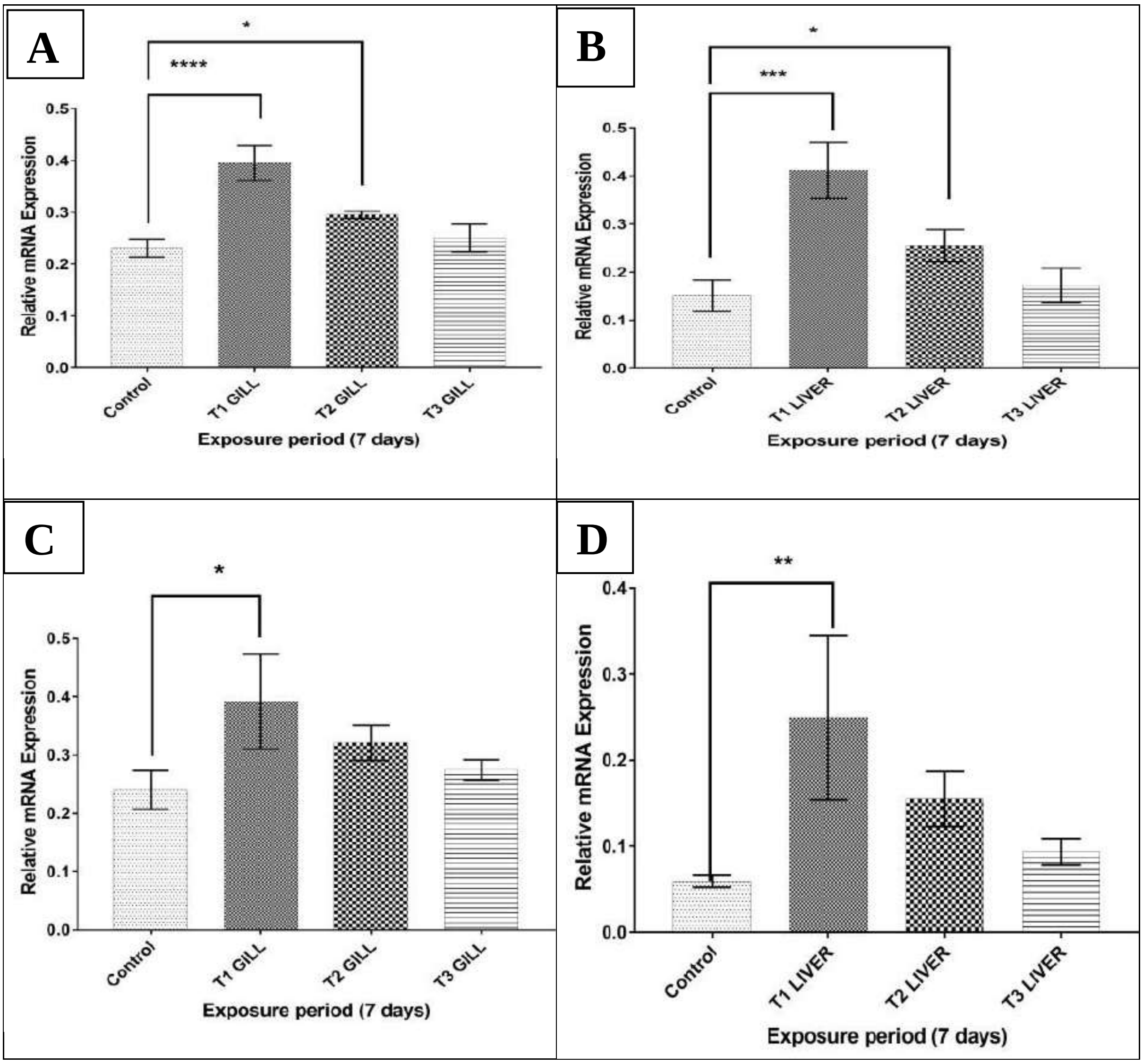

FIGURE 57. EFFECT OF T_1 (26. 04 ppm), T_2(20.83 ppm), T_3 (17.36 ppm) DOSES OF IMI EXPOSURE FOR 7^{th} DAYS ON mRNA TRANSCRIPT LEVELS OF HSP70 AND CYPIA IN THE GILLS AND LIVER OF COMMON CARP. VALUES REPRESENT THE MEAN ±SE; n=5; ERROR BARS INDICATE STANDARD DEVIATION. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0. **A**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF GILLS. **B**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF LIVER. **C**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF GILLS. **D**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF LIVER.

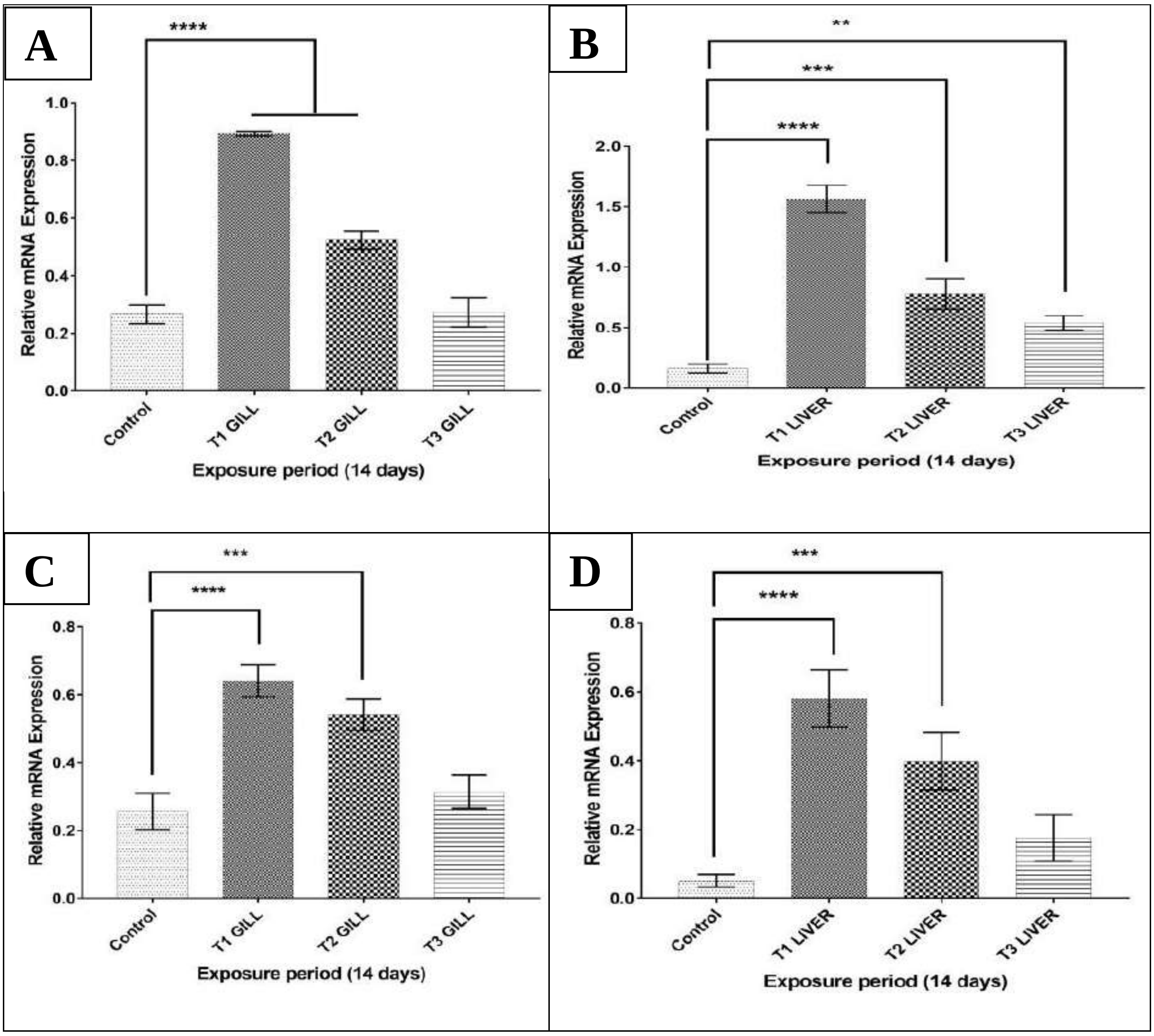

FIGURE 58. EFFECT OF T_1 (26. 04 ppm), T_2(20.83 ppm), T_3 (17.36 ppm) DOSES OF IMI EXPOSURE FOR 14th DAYS ON mRNA TRANSCRIPT LEVELS OF HSP70 AND CYPIA IN THE GILLS AND LIVER OF COMMON CARP. VALUES REPRESENT THE MEAN ±SE; n=5; ERROR BARS INDICATE STANDARD DEVIATION. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0. **A**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF GILLS. **B**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF LIVER. **C**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF GILLS. **D**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF LIVER.

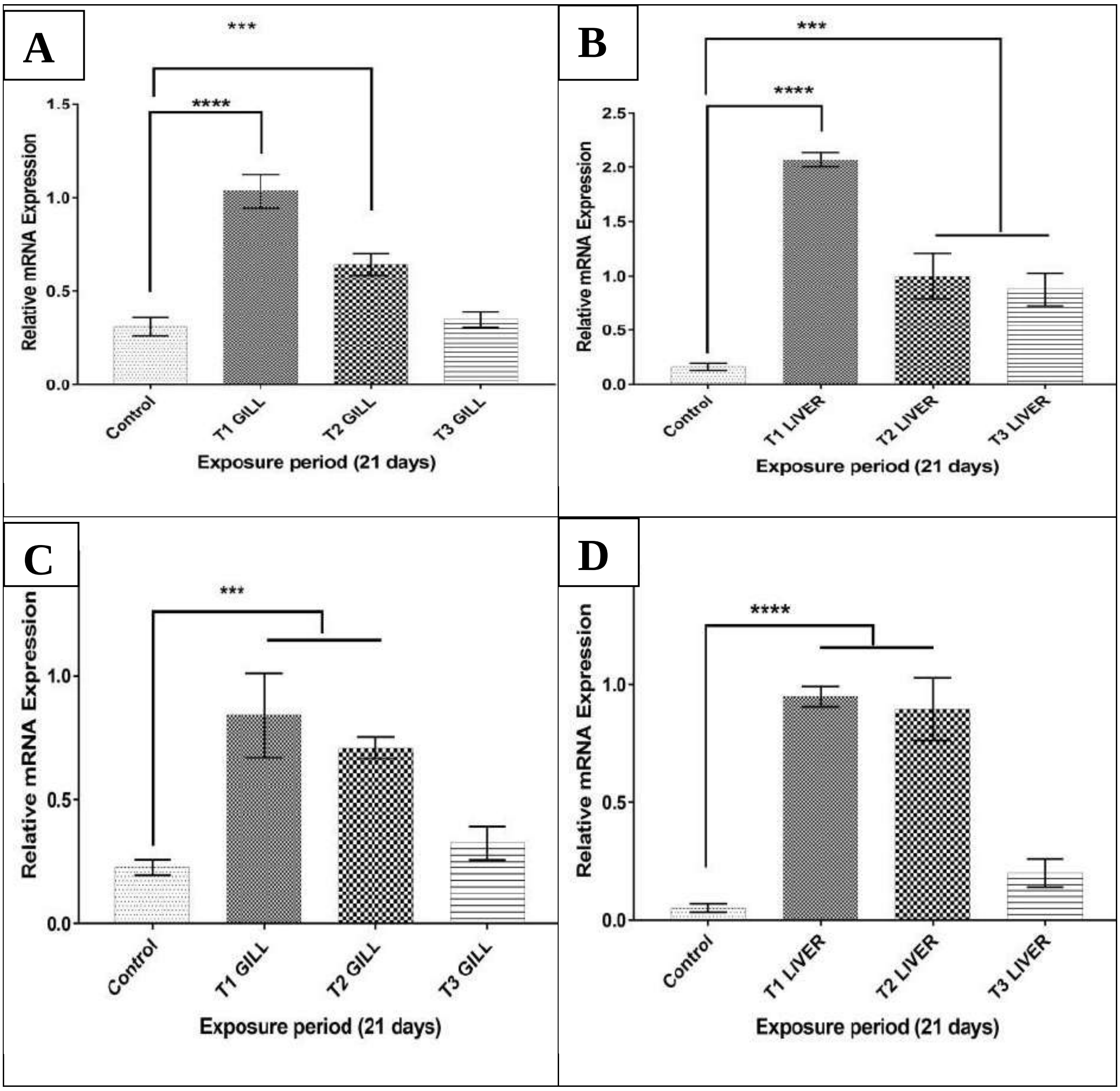

FIGURE 59. EFFECT OF T_1 (26. 04 ppm), T_2(20.83 ppm), T_3 (17.36 ppm) DOSES OF IMI EXPOSURE FOR 21st DAYS ON mRNA TRANSCRIPT LEVELS OF HSP70 AND CYPIA IN THE GILLS AND LIVER OF COMMON CARP. VALUES REPRESENT THE MEAN ±SE; n=5; ERROR BARS INDICATE STANDARD DEVIATION. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0. **A**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF GILLS. **B**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF LIVER. **C**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF GILLS. **D**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF LIVER.

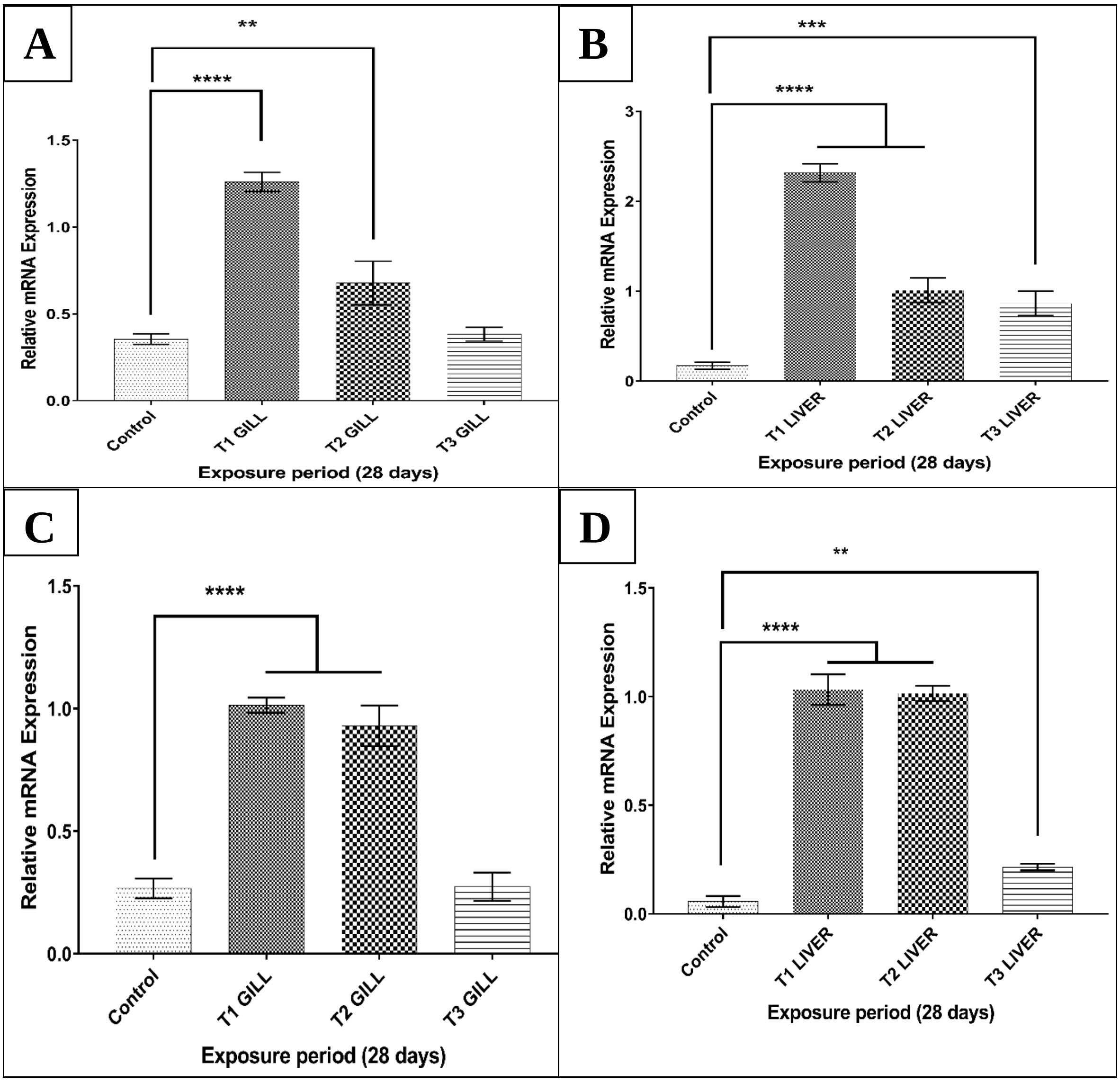

FIGURE 60. EFFECT OF T_1 (26. 04 ppm), T_2(20.83 ppm), T_3 (17.36 ppm) DOSES OF IMI EXPOSURE FOR 28th DAYS ON mRNA TRANSCRIPT LEVELS OF HSP70 AND CYPIA IN THE GILLS AND LIVER OF COMMON CARP. VALUES REPRESENT THE MEAN ± SE; n=5; ERROR BARS INDICATE STANDARD DEVIATION. STATISTICAL SIGNIFICANCE REPRESENTS (*P<0.05, **P<0.01, ***P<0.001 AND ****P<0.0001) WAS ANALYSED UNDER ONE-WAY ANOVA USING GRAPH PAD PRISM VERSION 7.0. **A**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF GILLS. **B**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF HSP70 OF LIVER. **C**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF GILLS. **D**. REPRESENTS THE RELATIVE mRNA EXPRESSION LEVELS OF CYPIA OF LIVER.

4.0 OVERALL ACHIEVEMENTS

- Fifty-two (52) fish species have been collected from all the six sampling sites of Doyang river system.
- Voucher specimen of the collected fish species are maintained at Fish Museum of Dept. of AEM, College of Fisheries, AAU, Raha with unique specimen code.
- During the present investigation a total of 52 fish species belonging to 28 genera, 11 families and 5 orders are recorded from 6 selected sampling stations of the river Doyang, Nagaland India.
- Among the orders, the Cypriniformes formed the largest group with a contribution of 3 (27.27 %) families, 19 (67.56%) genera and 35 (67.32%) species. The order Perciformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 3 (27.27 %) families, 3 (10.71%) genera and 9 (17.30%) species followed by Siluriformes with 3 (27.27%) family, 4 (14.29%) genera and 6 (11.53%) species and symbranchyformes and Baloniformes with 1 (9.09) family, 1 (3.57%) genus and 1 (1.92%) species each.
- Diversity indices calculated for ichthyofauna indicates that station 6 to be more diverse whereas least diverse station is found to be station 1.
- The IUCN conservation status of the 52 recorded species shows that the highest species were recorded under least concern (LC) category with a total no of 39 and contributed 75 %. under LC category, the major species contribution is from the family Cyprinidae with 20 (38.46 %) followed by Channidae 5 (9.61 %), Nemacheilidae 4 (7.6 %), Silorhynchidae and Sissoridae with 2 (3.86 %) each, Bagaridae, Amblycepitidae, Bedidae, Anabantidae, Mastacembalidae and Belonidae with 1 species contributed 1.92 % each. Under near threatened (NT) category Cyprinidae and Sissoridae contributed 3 (5.76%) and 1 (1.92 %) species respectively. Like that, the family Cyprinidae represented the vulnerable (VU) category with 2 (3.84 %) species each. One species which contributed 1.92 % under Cyprinidae family represented the endangered (EN) category. A total of 6 nos, 2 (3.84%) from cyprinidae, 1 (1.92%) from each Nemacheilidae, Sissoridae, Channidae and Bedidae respectively falls under the not evaluated (NE) category of IUCN conservation status (2021).
- For the first time Cytochrome Oxidase subunit I (COI) of mitochondrial gene sequences of Thirty-eight (38) (approx. 78%) of the total collected fish species sequences was generated and successfully submitted to NCBI gene data base and accession number was obtained.
- During the present study a total of 30 genera of plankton was recorded out of which phytoplankton consist of 18 genera under 3 family namely Chlorophyceace, Bacillariophyceae and Cyanophyceae and Zooplankton of 12 genera under 3 family namely Cladocera, Rotifera and Copepoda.
- During the present study, a total of 18 species of phytoplankton were recorded. Three majors groups of phytoplankton viz. Chlorophyceace represented by 9 species, Bacillariophyceae

represented by 5 species and Cyanophyceae represented by 4 species were found in the different stations along the Doyang river system.

- During the present study, a total of 12 species of zooplanktons belonging to three categories of zooplankton viz. Cladocera represented by 6 species, Rotifera represented by 4 species, Copepoda represented by 2 species was collected from the Doyang river.
- During the study period the Margalef's richness index (d) was found to be highest at station 1 with a value of 2.925 and with a lowest value of 1.946 at station 6 whereas Pielou's evenness index (J') was found to be highest at station 6 (0.9321) and lowest at Station 2 (07214). Shannon-Weinner index (H') was found to be highest at station 6 (2.415) and lowest at station 2 (2.158). Like that, the highest value of Simpson index (1-ƛ) was found to be at station 6 (0.905) and lowest at station 2 (0.8624).
- According to Palmer's index of pollution the total score of Algal Genus Pollution Index (AGPI) of sites S1, S2< S3< S4<S5, S6 were calculated to be 2, 5, 7 and 9 respectively. The total scores of S1 and S2 showed 4 indicating probable lack of organic pollution while S5 and S6 showed moderate pollution due to anthropogenic factors or human interference.
- Water quality index (WQI) developed using 15 physico-chemical parameters of water provides a positive relationship with the seasonal changes. Maximum WQI values were recorded during monsoon season from all the six stations followed by post monsoon (winter) and premonsoon. The WQI value showed a mixed pattern of changes in all the seasons. WQI of the upstream stations from 1 to 2 is lower than the downstream stations, i.e., 5 and 6 showing the increase in pollution level while moving downstream of the river.
- Principal component analysis shows that during winter and monsoon, PC1 was largely and positively affected by pollution indicating parameters, whereas during post monsoon and pre monsoon, PC1 was largely and positively affected by the other physico chemical parameters. This may be due to pollutants affecting water quality in rivers have temporal and spatial variations and should be investigated based on each river's environmental conditions. We also observed that different stations are having different contributions towards the total variance. The reason for these changes can be found in different environmental conditions and human activities around the river from one place to another.
- The study implied that primary productivity of the river was found to be in the lower side with the average value ranging from with the average for GPP (0.116 g C m-3 d-1) and NPP (0.057 g C m-3 d-1).
- All the studied Physico-chemical parameters of water and soil were estimated within permissible limit, except in some stretches of the sampling sites where anthropogenic activities has been observed.
- Relative abundance of Cypriniformes was estimated highest in all the sampling sites. Perciformes were the second most dominating order. No invasive species were recorded in the sampling sites. However, local people commented on the presence of some exotic species in the river.

- Anthropogenic factors encountered during the regular sampling in the Doyang river system are Constant dumping of solid waste like polythene bags, paper waste and domestic sewage in the river, removal of sand gravel and boulders from the river bed, alteration of river course, use of pesticide for protection of agricultural crops in the adjoining paddy fields of the river system leading to the toxicity effects in the non-targets aquatic animals like fish, electric fishing, blasting and poising in the river side were also frequently reported by the locals.
- Laboratory static renewal test (USEPA, 2002) was carried out to find out the median lethal concentration (LC_{50}). Following the range finding test six different test concentrations with a spacing factor of 1.6 (50 ppm, 80.00 ppm, 128.00 ppm, 204.80 ppm, 327.68 ppm and 524.28 ppm) were selected for the final acute toxicity experiment. Percent mortality was plotted against log concentration of IMI and a curve was obtained. From the curve, 96 hrs. LC_{50} value was calculated to be 208.38 ppm (208380 µg/l) which indicates the chemical to be "moderately hazardous".
- The present findings when compared to the study done by Bayer Crop Science, 2013 in analytical grade of IMI on common carp, it reveals that the 96hr LC_{50} ratio to be >1. Thus, from the above findings we observe that commercial grade of Imidacloprid (Premise, 30.5%SC) is more toxic than the analytical one.
- Effect of 96hr LC_{50} concentration (208.38 ppm) was determined by exposing test fish under laboratory static renewal system and analysis being carried out on every 24, 48, 72, and 96hr. Semi static renewal system was deployed for 28 days chronic toxicity test, where 3 sublethal concentrations $LC_{50}/8$ (T_1= 26.04 ppm), $LC_{50}/10$ (T_2=20.83 ppm,) and $LC_{50}/12$ (T_3=17.36 ppm) was selected based on the above calculated 96hr LC_{50} value and analysis was carried on 7th, 14th, 21st and 28th day.
- Upon acute exposure to 96hr LC_{50} concentration for 24, 48, 72, and 96 hr behavioural alterations like jumping movements, restlessness, hyperventilation, hyperactivity, gulping, coughing and corkscrew swimming at surface and bottom of the tank was observed. Enhanced mucus secretion, loss of buoyancy and string of faeces hanging from anus or on the tank were also reported. All fishes displayed normal behaviour with no apparent external alterations in morphology during chronic exposure.
- Marked histological alterations in liver like exocrine pancreatic acini, hepatic degeneration, mononuclear infiltration; in gill, epithelial lifting and oedema, telangiectasis, lamellar fusion and in kidney expansion of Bowman's space, cloudy swelling of epithelial cells, necrosis of several renal tubules and multiple focal areas of inter-tubular haemorrhage was observed during both acute and chronic exposure to IMI.
- Results showed that immune-haematological variables like haemoglobin (Hb), packed cell volume (PVC), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), Nitroblue tetrazolium activity (NBT) and Lysozyme activity (LA) was significantly altered during acute exposure whereas during chronic it altered in dose and time dependent manner.

- Serum biochemical parameters like Glucose, Cholesterol, Phospholipid, Triglyceride, HDL, VLDL, Magnesium, AST, ALT was significantly increased whereas protein, albumin, globulin, A:G ratio, LDL, were significantly decreases during both acute and chronic exposure of *C. carpio* to IMI.
- Significant induction in oxidative stress enzymes (SOD, CAT, GPx, AST and ALT) and Oxidative stress biomarkers (ROS, MDA) in liver, gill and brain tissues was observed during acute exposure, whereas in chronic exposure it followed dose and time dependent variations.
- Significant reduction in brain AChE enzyme activity due to inhibition of acetylcholine esterase activity, whereas significant DNA damage through induction of micronuclei formation in the erythrocyte of fish blood was observed during both acute and chronic exposure.
- Significant upregulation of HSP70 and CYP1A gene in both liver and gill tissues of exposed fish was observed on 7^{th},14^{th}, 21^{st} and 28^{th} day in dose and time dependent manner when compared to the control group.

6.0 Exit Strategy and Sustainability.

DNA barcoding has become very much important in developing countries like India because of rapid introduction of invasive and pest species, which in turn lead to the extinction of the important indigenous fauna of the region. Earlier studies reports that there is decline in fish fauna owing to various factors like introduction of alien species and anthropogenic factors like river mining in large scale, destructive fishing, poisoning, use of pesticides/ insecticides in the agricultural crops in the adjoining areas. Since DNA barcoding has not been carried out previously in the river Doyang, thus this study aims to accurately identify and catalogue the ichthyofaunal of Doyang river system and improve the quality of taxonomic information by providing records of novel barcode sequences as well as species descriptions for the said river system. The study will also provide a better understanding of the ichthyofauna and ecology of the river and gives base line information that can be used in creating better conservation strategies. Furthermore, the information will also help non taxonomist, researchers and policy makers to aid them in their efforts in effective management of the important river system.

Moreover, the toxicity study shows IMI (Premise 30.50%SC) is a moderately hazardous insecticide to non-target aquatic organism, whose NOEC values lies below 17.36 ppm. During both acute and chronic exposure of IMI caused deleterious alterations to histological structures of liver, gills and kidney and induces significant changes on haemato-immunological parameters, oxidative defence and stress parameters of the test fish. Significant changes in brain AChE enzyme activity and micronucleus formation in erythrocytes were also observed during 96 hours and 28 days acute and chronic exposure. Results clearly indicates that IMI even at sublethal concentrations (T1=26.04 ppm, T2= 20.83 ppm, T3=17.36 ppm) can significantly act as potential immunosuppressor, oxidative stress enhancer and can trigger neurotoxic as well as genotoxic effects. Furthermore, if the exposure to such concentrations is continued for longer duration (beyond 28 days) it might cause anaemic condition, reduced growth, cellular abnormalities and even mortality which needs further investigation. Also, based on the increasing possible use of imidacloprid, we would also suggest additional toxicity studies of other commercial products containing imidacloprid as an active ingredient in non-target aquatic organism. Moreover, the observed parameters can also be useful in monitoring long term effects of IMI and determining water quality criteria for control policies and conservation strategies for aquatic as well as human health.

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APPENDIX 1

Assessment of Productivity and Fish Diversity of Dzii River of Kohima, Nagaland.

A Thesis
Submitted to the
Assam Agricultural University

In partial fulfilment of the requirements for the Degree of
MASTER OF FISHERIES SCIENCE
IN
AQUATIC ENVIRONMENT MANAGEMENT

By
KEDOLHOUSE KUOTSU
18-FMR-07

DEPARTMENT OF AQUATIC ENVIRONMENT MANAGEMENT
COLLEGE OF FISHERIES
ASSAM AGRICULTURAL UNIVERSITY
Raha-782103, Nagaon, Assam
August, 2020

M.F.Sc. Thesis

In Vivo* Evaluation of Toxicity effects of a Neonicotinoid Insecticide Imidacloprid on Freshwater Cypriniform *Cyprinus carpio* var. *communis

A Thesis

Submitted to the

Assam Agricultural University

In partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

AQUATIC ENVIRONMENT MANAGEMENT

By

HEMANTA POKHREL

2018-FDR-08

DEPARTMENT OF AQUATIC ENVIRONMENT MANAGEMENT

COLLEGE OF FISHERIES

ASSAM AGRICULTURAL UNIVERSITY

Raha-782103, Nagaon, Assam

2021

Ph. D Thesis

APPENDIX 2

ICAR-Central Institute of Fisheries Education
(University under Sec.3 of UGC act 1956)
Indian Council of Agricultural Research
Panch Marg, Off Yari Road, Versova, Andheri(W), Mumbai - 400 061

Certificate

This is to certify that
Hemanta Pokhrel
participated in the Skill Development Programme on
Molecular Taxonomy and DNA Barcodes
at
Central Institute of Fisheries Education, Mumbai
during 5-14 February 2019

(Aparna Chaudhari)
Course Director

14 February 2019

(Gopal Krishna)
Director/Vice Chancellor

NFDB MANAGE

College of Fisheries, Assam Agricultural University (AAU)
Raha, Assam
In Partnership with
National Institute of Agricultural Extension Management (MANAGE)
(An Organization of Ministry of Agriculture & Farmers Welfare, Govt. of India)
Rajendranagar, Hyderabad
Sponsored by
National Fisheries Development Board (NFDB)
Ministry of Animal Husbandry, Dairying & Fisheries, Govt. of India
Rajendranagar, Hyderabad

ID. No: ACADP/CoF, AAU, Raha-18/2019-20

Certificate of Participation

*This is to Certify that Mr./Ms.......*Hemanta Pokhrel*.........participated in a 30 days Skill Development Certificate training Course on* ***"Aqua Clinics & Aquapreneurship Development Programme (AC&ADP)"*** *conducted at College of Fisheries, Assam Agricultural University (AAU), Nagaon, Assam from 27.08.2019 to 25.09.2019.*

Dr. Saravanan Raj
Director (Agril. Extn.)
MANAGE

Dr. S. Borthakur
Nodal Officer
CoF, AAU, Raha

Dr. K.K. Tamuli
Professor & Dean
CoF, AAU, Raha

Executive Director (Tech)
NFDB

Smt V. Usha Rani, IAS
Director General
MANAGE

APPENDIX 3

B-4706
[1-8]

RESEARCH ARTICLE | Indian Journal of Animal Research

Effects of Imidacloprid on Histology and mRNA Levels of HSP70 and CYP1A Gene to a Standard Non-targeted Test Animal, *Cyprinus carpio*

Hemanta Pokhrel, Raktim Sarmah, Sarada Kanta Bhagabati, Rajdeep Dutta, Abdul Malik Ahmed, Dipanka Nath, Lawonu Mudoi, Lucy Ingtipi, Arnab Narayan Patowary, Binod Kalita

10.18805/IJAR.B-4706

ABSTRACT

Background: Imidacloprid (IMI) belongs to the class of neuro active insecticides, used to control pest and insects in agricultural crops. Reports suggest that IMI has highly toxic consequences on bees, humans and non-targeted aquatic animals. Hence the present study aims to investigate the toxicity effects of commercial-grade Imidacloprid (IMI) (Premise, 30.5%, a.i) using histological and transcriptional changes in heat shock protein 70 (HSP70) and cytochrome P4501A(CYP1A) genes in liver and gills of non-targeted aquatic animal, *Cyprinus carpio*.

Methods: Three different sublethal concentrations of IMI (T1=26.04 mg/L, T2= 20.38 mg/L, T3=17.36 mg/L) were selected and common carp fingerlings were exposed for 28 days where histological alterations and expression study of HSP70 and CYP1A in gills and liver of exposed fishes being studied on 7, 14, 21 and 28 days interval using standard procedure.

Result: Marked histological alteration like hydropic degeneration and cellular infiltration in liver; telangiectasis in secondary lamellae, epithelial lifting and oedema in gills were recorded. Moreover, significant upregulation of HSP70 and CYP1A gene in the gills and liver in a dose and time dependent manner was observed ($P<0.05$). As a result of the current investigation, it is obvious that IMI could be a possible toxicant that affects fish at the tissue and gene level in dosage and time dependent ways, potentially influencing other physiological processes in the long term.

Key words: CYP1A, Cyprinus carpio, HSP70, Imidacloprid, Sublethal.

INTRODUCTION

A neonicotinoid insecticide, imidacloprid (IMI) is used all over the world to control sucking pests like aphids, plant hoppers, whiteflies, and leafhoppers on crops (Ozdemir *et al.*, 2018). IMI is an agonist of post-synaptic nicotinic acetylcholine receptors that binds to activates nicotine that causes impairments in the central nervous system. Moreover, excessive formation of reactive oxygen species (ROS) is also caused by high levels of Imidacloprid toxicity, resulting in oxidative stress (Ge *et al.*, 2015). Since the European Food Safety Authority confirmed its harmful effects on bees and farmers, the usage of IMI has been a contentious subject. IMI was chosen because of its widespread usage, lack of linkage to aquatic toxicity in the region, or emerging use with past monitoring. After application, it can spread due to storms, agricultural run-off and spray drift into the aquatic environment. As a result, the possible negative effects of IMI exposure in non-target creatures, such as humans, animals, and especially aquatic animals, are gaining attention (Ozdemir *et al.*, 2018).

HSPs are evolutionary conserved molecular chaperones that, among other things, transport, fold, and assemble faulty or misfolded proteins. The HSP70 (70-kDa) family of stress proteins is the most common and well-studied. It has been shown to be activated in vitro and in vivo in response to a variety of environmental stresses, including oxidative stress (Clayton *et al.*, 2005). Several isozymes of a CYP450 family in fish are also highly inducible by various toxic substances, play a significant role in xenobiotic metabolism. As a result, over the last decade, a growing number of researches have looked into the idea of employing CYP450 enzymes and HSPs as biomarkers of cellular deleterious effects. The stimulation and subsequent accumulation of CYP450 enzymes and HSPs are linked to the production of reactive oxygen species (ROS), as a result of which they can be employed as oxidative stress biomarkers (Li *et al.*, 2015). Histopathological alterations

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APPENDIX 4

Ministry of Environment, Forest & Climate Change

Implemented by giz Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH

On behalf of: Federal Ministry for the Environment, Nature Conservation and Nuclear Safety of the Federal Republic of Germany

Zoological Survey of India

Participation Certificate

First International Symposium on Aquatic Biodiversity of the North Eastern Region of India

Presented to:

Hemanta Pokhrel

for their presentation on the study

Ecological Integrity and Fish Diversity of Dzii and Doyang River System, Northeastern Himalayan Region Nagaland, India

Authors: Hemanta Pokhrel, Kedolhouse Kuotsu, Sarada Kanta Bhagabati, Rajdeep Dutta, Abdul Malik Ahmed, Dipanka Nath, Raktim Sarmah and, Lowanu Prasad Mudoi

at the First International Symposium on Aquatic Biodiversity of the North Eastern Region of India held on October 26th and 27th 2021. We thank you for your contribution towards conservation of Aquatic Biodiversity in the North Eastern Region of India.

Dr Uwe Scholz, Project In-Charge, GIZ NERAQ Project	Prof. Dandadhar Sarma, Department of Zoology, Gauhati University	Dr Dhriti Banerjee, Director, Zoological Survey of India

APPENDIX 5

- Kedolhouse Kuotsu, Hemanta Pokhrel, Sarada Kanta Bhagabati, Rajdeep Dutta, Abdul Malik Ahmed, Dipanka Nath, Raktim Sarmah, Lowanu Prasad Mudoi. (2021). Ecological Integrity and Fish diversity of Dzii and Doyang river system, Northeastern Himalayan region Nagaland, India. *Indian Journal of Animal Science* (Under Review, Naas 6.2)
- Hemanta Pokhrel, Raktim Sarmah, Sarada Kanta Bhagabati, Rajdeep Dutta, Abdul Malik Ahmed, Dipanka Nath, Lucy Ingtipi, Lawonu Prasad Mudoi, A. N. Patowary and Binod Kalita. (2021). In-vivo evaluation of toxicity effects of a Neonicotinoid Insecticide, Imidacloprid using multiple biomarker response to a standard non targeted test organism, *Cyprinus carpio*. *Achieves of Environmental Contamination and Toxicology*. (Under Review, NAAS 8.5)
- Hemanta Pokhrel, Raktim Sarmah, Sarada Kanta Bhagabati, Rajdeep Dutta, Abdul Malik Ahmed, Dipanka Nath, Lucy Ingtipi, Lawonu Prasad Mudoi, A. N. Patowary and Binod Kalita. (2021). Sublethal toxicity effects of Imidacloprid on multiple biomarkers response to standard non-targeted test animal, *Cyprinus carpio*. *Drug and Chemical Toxicology*. (Under Review, NAAS 8.4)

Project ID: HSF2017-18/I-16/04

FINAL TECHNICAL REPORT

OF THE PROJECT

ON

"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

Submitted to:
Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies (NMHS)
G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal, Almora 263643, Uttarakhand

Submitted by:

Dr. Sarada Kanta Bhagabati
Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103

Template/Pro forma for Submission

NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	**HSF2017-18/I-16/04**

Date of Submission:								

PROJECT TITLE

"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

Sanctioned Fellowship Duration: from (28/03/2018) to (28/02/2021).

Extended Fellowship Duration: from (1/03/2021) to (31/12/2021).

***Submitted to*:**

Er. Kireet Kumar

Scientist 'G' and Nodal Officer, NMHS-PMU

National Mission on Himalayan Studies, GBP NIHE HQs

Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi

E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

***Submitted by*:**

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[Contact No.: 7896250516]

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NMHS-Final Technical Report (FTR)

NMHS- Institutional Himalayan Fellowship Grant

DSL: Date of Sanction Letter

2	8	0	3	2	0	1	8

DFC: Date of Fellowship Completion

3	1	1	2	2	0	2	2

Part A: <u>CUMULATIVE SUMMARY REPORT</u>

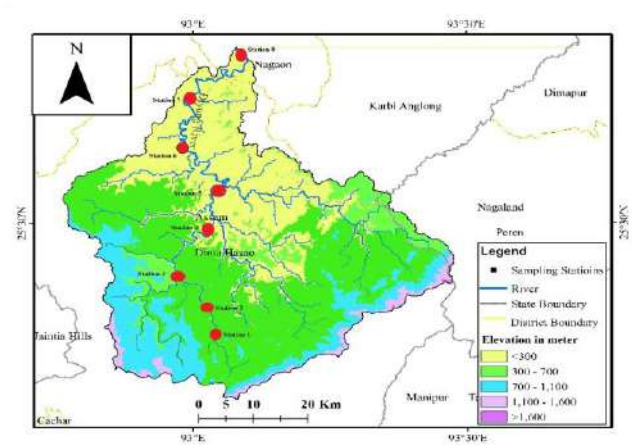
1. Details Associateship/Fellowships

1.1 Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	HSF2017-18/I-16/04
Name of the Institution/ University:	College of Fisheries, Assam Agriculture University
Name of the Coordinating PI:	1. Dr. Rajdeep Dutta Assistant Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103 2. Dr. S.K. Bhagabati, Associate Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103
Point of Contacts (Contact Details, Ph. No., E-mail):	1. Email ID: drrajdeepdutta@gmail.com : sskbk2002@gmail.com Ph No: 9854757790 & 7896250516

1.2 Research Title and Area Details

i.	Institutional Fellowship Title:	Study of Fish Germplasm of River Diyung, North Eastern Himalayan Region with special reference to their Habitat and Conservation status.

ii.	IHR State(s) in which Fellowship was implemented:	**Assam**					
iv.	Scale of Fellowship Operation	Local:		Regional:	Yes	Pan-Himalayan:	
iii.	Study Sites covered (*site/location maps to be attached*)	Assam 					
v.	Total Budget Outlay (Crore) :	INR 0.80 Cr					

1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration	
		From	To
Research Associates			
Sr. Research Fellow			
Jr. Research Fellows		01/08/2018	31/12/2021
Project Fellows			

2. Research Outcomes

2.1. Abstract

The freshwater ecosystem is home to a diverse, delicate, and endemic biota, representing roughly 6% of all species. India is a hotspot of freshwater fish diversity and contributes a large

number of endemic biological resources to the world. In addition, Indian waterways are home to 11.7% of the world's fish species, with 295 endemic fish species found only in India is recognized by the IUCN. The Eastern Himalayan region encompassing Northeast India is considered one of the hotspots of freshwater fish diversity in the world. Among North-East states, Assam is also very rich in its ichthyofaunal diversity. Bhattacharjya *et al*., 2003 reported a total of 217 fish species belonging to 104 genera, 37 families, and 10 orders from wetlands and other water bodies of Assam. But in recent times, due to many anthropogenic factors, the precious and unique indigenous ichthyofauna of Assam are facing a great threat.

Dima Hasao the hill district of Assam is bestowed with a number of rivers like Diyung, Jatinga, Mahur, Jiri, Dhansiri and Jinam. The Diyung is the largest river of Dima Hasao District originating from the Barail range, which flows a distance of 240 km before joining in Kopili, a tributary of the mighty river Brahmaputra at Dayangmuk in Kabi Anglong. The river gets its name as Doiang after it enters into Karbi Anglong district. Geographically it is located between 92º44′30″E and 93º30′E longitudes and 25º10″N and 25º50″ N latitudes. The area being under sub-tropical monsoon climate, the flow regime of the Diyung River is determined by southwest monsoon rainfall.

- ***Objectives:***

1. To investigate the species diversity in River Diyung, North Eastern Himalayan Region, Assam using traditional and molecular taxonomic tools.
2. To characterize the habitat of the fish species of the river.
3. To assess the conservation status of fish species and to identify anthropogenic factors affecting fish diversity.

Methodology:

Objective 1: Fish samples were collected from the river Diyung at its 8 sampling stations on monthly intervals and the length and weight of the fish species were recorded. Photography of the fish specimens and their habitat were done. The fish samples were preserved and brought to the laboratory in 10% formalin. The fishes were identified using standard keys (Jayaram, 2006; Vishwanath & Nebeshwar, 2009; Kottelat, 2013).

DNA Barcoding

Pectoral fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping was isolated following the phenol: chloroform method. The concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking their integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial COI gene using

Fish F1&R1 Primer with the help of a thermal cycler (Eppendorf AG 22331 Hamburg). The PCR product is then sequenced at Eurofin Scientific Laboratory. The generated barcodes were submitted to NCBI and accession numbers were obtained for the individual fish species.

Objective 2:

Macro-habitat

Fish longitudinal distribution over various environmental gradients is controlled by Macrohabitat. The microhabitat properties of a stream include channel gradient, stream depth, stream breadth, riparian cover, and bank stability. Generally, stream habitat characteristics were measured in each study site. Habitat use data were collected from a 100 m reach in all study sites. The study locations were chosen based on habitat diversity (with pools and riffles). Segregation of stream habitats was based on (Aadland, 1993) and habitat guild was followed using the methods by Arunachalam (2000) and Arunachalam & Madhusoodanan Nair (1997c).

Microhabitat

Microhabitat is defined as physical sites occupied or exploited by life stages of a fish species that have certain characteristics (such as depth, water column velocity, cover type, and substrate type). Microhabitat analysis was performed in all the eight (8) study sites in order to assess the variability in microhabitats used. Among the fish species, some fish were not evaluated because of their low numbers. At each bank, sampling was done in the upstream direction for short distances. When the fishes were located, species were determined and was recorded. Substrate types were recorded for each habitat by visual methods. Each stretch in the study site was quantified for depth, flow, and substrate characteristics. A number of transects usually 8-10 were taken across the stream channel and depth, water velocity, and dominant substrates were measured.

Physicho-chemical parameters

Water and sediment samples were collected from 8 different stations of river Diyung from January, 2019 to May, 2021. Some of the physical parameters like depth, air & surface water temperature, water velocity, TDS & EC were determined on the spot. Other parameters like turbidity, dissolved oxygen, pH, total alkalinity, total hardness, nitrate, nitrite, ammonia of the water samples were carried out in the laboratory as per APHA (2018). The sediment samples were collected on a seasonal interval, air-dried, and analyzed for pH, organic matter, and organic carbon, as per standard methodology (Jhingran, 1992; Walky & Black, 1934).

Plankton and periphyton samples were identified with the help of standard literature Edmondson (1959), Needham & Needham (1966) and ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967).

Objective 3: Information regarding anthropogenic factors was evaluated with the help of field surveys and conservation status was evaluated using IUCN, 2021 guidelines.

Results:

Objective 1: During the present investigation, a total of 81 fish species belonging to 10 orders, 24 families, and 52 genera were recorded from the studied river. DNA barcodes were generated for 42 numbers of fish species from River Diyung and submitted to NCBI and for 45 numbers fish species accession were obtained.

Objective 2:

The habitat structure was measured and categorized into various categories for measuring fish habitats. In the 8 selected sites of the present study, the major habitat types like shallow pool, slow riffle, fast riffle, raceways, medium and deep pools were identified. Almost all the selected study sites were represented by diverse habitat types which have shown significant heterogeneity. The study has shown that fish species preferred more riffles, deep pools, raceways and medium pools than other habitat types It has also shown that dominant cyprinids used different types of habitats but more preferred were fast riffle, slow riffle, shallow and deep pools.

Analysis of seasonal variation data of hydrobiological parameters of River Diyung reveals anthropogenic stress in upper stretches. Parameters like BOD_3 and COD within the permissible limit indicate the non-polluted condition of the river but slightly in the higher site during monsoon and post-monsoon seasons in lower stretches. Water turbidity the of Diyung river was found to be higher in the lower stretches of the river during monsoon seasons which might be due to erosion of the riverbank, and surface runoff from agricultural fields. Other water quality parameters like pH, dissolved oxygen, alkalinity, and hardness were found within the permissible limit. Analysis of seasonal variation of sediment parameters in the Diyung river shows sediment pH acidic to alkaline in nature. Other parameters like sediment N, P & K show seasonal variation during the study period.

A total of 35 genera of plankton were recorded from River Diyung during the study period. The population of phytoplankton was represented by 26 genera belonging to Chlorophyceae (12 genera), Bacillariophyceae (7 genera), Cyanophyceae (6 genera) and Euglenophyceae (1 genera). The zooplankton population was represented by Rotifera (4 genera), Cladocera (3 genera), and Copepoda (2 genera). The population density of plankton varied from season to season. The average minimum plankton density was found to be 21.33±3.68 units/L and a maximum of 626.67±13.10 units/L. It was observed that the values of BOD_3 & COD were on the higher side during the monsoon and post-monsoon season in lower stretches of the river (Diyungmukh and Digandu PT-II) which might be the indication of organic load during those seasons. Palmers' index also showed a similar trend. By using Palmer's index of pollution for the rating of water samples as lack of organic pollution, moderate and high organic pollution at all the stations were tested. The total score of the algal Genus Pollution Index (AGPI) of the sites S1<S2<S3<S4=S5<S6<S7<S8 were calculated to be 9, 8, 12, 13, 13, 20, 21 and 22 respectively.

Sharpe increased in the total score of 31 in station 5 indicating high organic pollution due to urban

S. No.	Objectives	Major achievements (in bullets points)
		•

waste influx according to Palmer (1969). *Navicula*, *Nitzcha* and *Synedra* were recorded in the lower stations of the Diyung river consider as indicators of pollution in view of the results of Palmer's index.

Objectives 3. According to the Red List of Freshwater Fishes published by IUCN (2021) more than half of the existing fish species (76.54 %) of this river were found to be in the least concern (LC) category, while 11.11 % of fish species were recorded as near threatened (NT), only 2.44 % as data deficient (DD), 2.44% as Vulnerable, 1.23% Endangered (EN) and 6.13% not Evaluated (NE). This is the first full record of fish species from the entire stretch of river Diyung

Different anthropogenic factors like continuous sand and stone quarries, overexploitation of fishes using destructive fishing methods, and sewage disposal, were recorded during the sampling period.

Conclusion: From the study, it can be summarized that River Diyung provides suitable habitat for rich ichthyofaunal diversity consisting of both warm and coldwater species. A total of 81 fish species were recorded during the study period, which is reported for the first time from the entire stretches of the river. Among all the stretches middle and lower stretches showed maximum fish diversity. The river water in the middle stretch was found to be highly congenial for aquatic life due to which higher fish diversity was recorded in that stretch. River Diyung which harbors rich ichthyofaunal diversity of both cold and warm water fish species imparting nutritional security and providing recreational fisheries even is not exempted from anthropogenic activities (sand and boulder mining, electrical fishing practice, river poisoning, overfishing, etc.) in recent years. Identifying and quantifying the impact of these multitudes of stressors led by human activities will give an insight into the scientific intervention in support of the conservation of aquatic resources.

Recommendation: Habitat destruction activities in rivers should be strictly prohibited. In-*situ* conservation of threatened fish species like mahseer and other commercially important indigenous fish species should be implemented. Development of ornamental and sports fishery could be undertaken.

2.2. Objective-wise Major Achievements

1.	To investigate the species diversity in River Diyung, North Eastern Himalayan Region, Assam using traditional and molecular taxonomic tools.	• During the present study, a total of **81 species belonging to 52 genera, 24 families, and 10 orders** were recorded from different stretches of the River Diyung 81 fish species. Among these orders Cypriniformes formed the largest group with a contribution of 20.85% families and 42 (51.85%) species. The order Siluriformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 7 (29.16%) families and 17 (20.98%) species followed by Anabantiformes with 4 (16.66%) families and 9 (11.11%) species, Synbranchiformes with 2 (8.33%) families and 4 (4.93%) species, Osteoglossiformes with 1 (4.16%) family and 2 (2.64%) species, Perciformes with 1 (4.16%) families and 2 (2.46%) species, Beloniformes with 1 (4.16%) family and 2 (2.46%) species. The smallest group was formed by orders Gobiiformes, Anguilliformes and, Clupeiformes with 1 (4.16%) family and 1 (1.23%) species. Among the families Cyprinidae contributed 33 (40.74%) species, Bagridae represented with 6 (7.4%) species, Channidae with 4 (4.93%) species. Mastacembelidae, Sissoridae and Nemacheilidae, and Psilorhynchidae with 3 (3.70%) species. • DNA barcodes generated: **42 fish species and 45 sequences** from River Diyung. • **The first full record of ichthyofaunal diversity** of entire stretches of Diyung river.

2.	To characterize the habitat of the fish speci	• The results of the present study show that a great
2	To characterize the habitat of the fish species of the river.	• In the 8 selected sampling sites of the present study, the major habitat types like shallow pools, slow riffle, fast riffle, raceways, medium and deep pools were identified. Almost all the selected study sites were represented by diverse habitat types which have shown significant heterogeneity. The study has shown that fish species preferred more riffles, deep pools, raceways and medium pools than other habitat types. It has also shown that dominant cyprinids used different types of habitats but more preferred were fast riffle, slow riffle, shallow and deep pools. • Variations in habitat composition of selected sites were noticed. Even we have found variation between the study sites of the same stretch of the river. • The present study clearly reveals that the fish species and their abundance are strongly correlated in habitat complexity and heterogeneity. • Station-4 shows a high species diversity than other stations of the Diyung River. This is because of having more habitat complexity in contrast to other sites. Maximum habitat heterogeneity was found in Dehangi Bazar point (DBP) whereas minimum habitat heterogeneity was recorded from Syamagram (SR) and Lower Halflong Bridge (LHB) where the lowest number was recorded because shallow pools, slow rifles and deep pools were not found • To study the habitat of the river, water and sediment samples were collected from 8 different stations starting from its origin up to the confluence point. • Turbidity was found to be higher during the study, especially in monsoon and post-monsoon in lower stretches. • Electrical conductivity and total dissolved solids were found to be higher during monsoon and post-monsoon seasons in lower stretches. DO, total alkalinity and total hardness were found to be higher during the winter season.

3.	To assess the conservation status of fish species and to identify anthropogenic factors affecting fish diversity.	• According to the Red List of Freshwater Fishes published by IUCN (2021) more than half of the existing fish species 57 (76.54 %) of this river were found to be in the least concern (LC) category, while 9 (11.11%) fish species were recorded as near threatened (NT), only 2 (2.44%) as data deficient (DD), 2 (2.44%) as Vulnerable, 1(1.23%) Endangered (EN) and 3 (6.13%) not Evaluated (NE) • In the present study, it was observed that anthropogenic activities like the extraction of sand and gravel from riverbed destructive fishing methods viz. dynamiting, electrofishing, liming, use of Ichthyotoxic plants, etc. (authors' pers. obs.) along the rivers and streams are responsible in the declining fish population in major Rivers of the district. These methods are highly responsible for decreasing the trend of threatened endemic fish species in the northeastern region.

2.3. Outputs in terms of Quantifiable Deliverables*

Sl. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved
1.	Number of fish species available in the river systems under study with their proper taxonomic identification and indicating their true conservation status	Taxonomic and molecular characterization of the fish fauna of the river covering its diversity and distribution.	Checklist of Fish species (New database): 81 Museum specimens: 81 DNA barcodes: 42 species
2.	An updated inventory/catalogue (soft copy) of fish species of the river ecosystems under study indicating their habitat with supporting photographs.		Different micro and macro-habitat of fishes were identified. Dataset on the environmental health of the river: 1 GIS Map: 1
3	Identification of anthropogenic stress factors affecting ichthyofauna of the	Any kind of anthropogenic factors affecting fish and their habitat are being	Anthropogenic factors encountered during the regular sampling in the

	river ecosystems (if any) and its possible mitigation measures (if required).	constantly monitored.	river Diyung system are i. Continuous Sand and Boulder mining. ii. Destructive fishing methods iii. Construction of the bridge, iv. Washing clothes and bathing

(*) As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number/ Brief Details	Remarks/ Attachment
1.	New Methodology developed	-	
2.	New Models/ Process/ Strategy developed	-	-
3.	New Species identified	-	-
4.	New Database established		• Total number of fish fauna • Conservation status of fish • Plankton data (Phyto and Zooplankton) • Palmer index • 15 physico-chemical water quality data. • Data on 3 parameters of sediment quality.
5.	New Patent, if any	-	-
	I. Filed (Indian/ International)	-	-
	II. Granted (Indian/ International)	-	-
	III. Technology Transfer (if any)	-	-

S. No.	Particulars	Number/ Brief Details	Remarks/ Attachment
6.	Others (if any) DNA barcoding of fish species	42	Species-specific DNA barcodes of 42 fish species (45 sequences) from River Diyung were generated, submitted to NCBI and accession number was obtained for the first time.

3. Technological Intervention

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology	-	-
2.	Diffusion of High-end Technology in the region	-	-
3.	Induction of New Technology in the region	-	-
4.	Publication of Technological / Process Manuals	-	-

4. New Data Generated over the Baseline Data

S. No.	New Data Details	Status of Existing Baseline	Additionality and Utilisation New data
1.	Morphological identification & molecular characterisation of the fish fauna of River Diyung	No earlier record of fish fauna from entire stretches of River Diyung is available	A total of 81 fish species have been recorded from entire stretches of river and generated 45 mitogenome sequences for 42 species from River Diyung for the first time. In the present study, *Tor putitora* which is an endangered species has been recorded in good numbers during the study periods.
2.	Habitat study and fish assemblage	No earlier report on habitat study and fish assemblage	It will helpful for the future researcher.
3.	Seasonal variation of hydrobiological & parameters	No earlier report on a hydrobiological study of River Diyung is available	The new data will be helpful in understanding the impact of anthropogenic factors on the ecosystem integrity of the river. It will be also helpful in devising future fisheries development strategies in this river.
4.	Sediment characteristic of River Diyung	No earlier report on sediment characteristics of River Diyung is available	The new information will be helpful for future researchers working in this region
4.	Plankton diversity	No earlier report is available	-
5.	Diversity indices of plankton	No earlier report is available	-
6.	Palmer index has been developed for the said river system	No report earlier is available	-

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Beneficiaries
1.	Sustainable Development Goals (SDGs)	Life below water		
2.	Climate Change/INDC targets			
3.	International Commitments			
4.	National Policies			
5.	Other's collaborations			

6. Financial Summary (Cumulative)*

*Please attach the **consolidated and audited Utilization Certificate (UC) and Consolidated and *Year-wise* Statement of Expenditure (SE)** separately, *ref*. **Annexure I.**

7. Quantification of Overall Research Progress

S. No.	Parameters	Total (Numeric)	Attachments* with remarks
1.	IHR State(s) Covered:	1	
2.	Fellowship Site/ LTEM Plots developed:	8	Photographs of sampling sites and map of study area attached (Annexures-I & II)
3.	New Methods/ Model Developed:		
4.	New Database generated:		
5.	Types of Databases generated:		
6.	No. of Species Collected:	81 fish species collected. 45 mitogenome sequences from 42 species have been generated and to NCBI database and accession number received	

7.	New Species identified:		
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	SRF:01 PhD:01 (Pursuing)	
9.	No. of SC Himalayan Researchers benefited:		
10.	No. of ST Himalayan Researchers benefited:		
11.	No. of Women Himalayan Researchers empowered:		
12.	No. of Knowledge Products developed:		
13.	No. of Workshops participated:		
14.	No. of Trainings participated:	01	Appendix-3
15.	Technical/ Training Manuals prepared:		
	Others (if any):		

***** Please attach the soft copies of supporting documents word files and data files in excel.

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		Nation al	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)		1		Appendix-1
2.	Book Chapter(s)/ Books:				
3.	Technical Reports/ Popular Articles				
4.	Training Manual (Skill Development/ Capacity Building)				
5.	Papers presented in Conferences/ Seminars		1		Appendix-2
6.	Policy Drafts (if any)				
7.	Others (specify)		1(Under review)		Appendix-4

* 1 Research papers are communicated and under peer review.

9. Recommendation on Utility of Research Findings, Replicability and Exit Strategy

9.1 Utility of the Fellowship Findings

S. No.	Research Questions Addressed	Succinct Answers (within 150–200 words)
1.	How many fish species found in the River Diyung?	During the present study, a total of 81 fish species belonging to 52 genera, 24 families, and 10 orders were recorded from different stretches of River Diyung**.** The total fish species identified in this study, account for 37.5 percent of the total number of fish species in the Brahmaputra River basin (Bhattacharjya *et al.* 2003). The most dominant species of their relative abundance were *Opsarius bendelisis, Pethia ticto*, *P. conchonius*, *Psilorhynchus balitora*, *Devario aequipinnatus*, *Barilius barila*, *Salmostoma Bacaila*, *Puntius sophore*, *Paracanthocobitis botia, G. lissorhynchus*, *Garra nasuta*, *G. annadalei*, *Mastacembelus armatus*, *Tariqilabeo latius*, *Danio dangila*, *Chagunius chagunio*, *Glossogobius giuris etc.* Eleven common groups of fishes were recorded during this study where Minnows and barbs (30.49 %) were found to be the most prominent group in the Diyung River followed by catfishes (20.73 %), carps (13.41 %), perch (9.76 %), loach (7.32 %), eels (6.10 %) and snakehead (4.88%). The contribution of feather backs, gars, clupeids, and mudskipper was 2.44%, 2.44%, 1.44%, and 1.44% respectively
	What are the suitable habitats of the fish species in the river	Habitat preferences of fishes was based on flow, depth and substrate categories which showed significant variation and few species have shown an overlap in their habitat preference. As far as flow is concerned, most of the fish species preferred moderate water flow and is followed by the slow category. The fishes like *Osteobrama cotio cunma, Bagarius bagarius, Amblypharyngodon mola* etc. extensively preferred

2		medium pool type of habitats while as *Labeo bata* and *Systoma sarana, Cirrhinus mrigala, Cavasius cavasius, Wallago attu, Chanda nama, Ompok bimaculatus, Rita rita* etc. extensively used raceway habitats. Species like *Sperata aor, Mastacembelus armatus, Chagunius chagunio, Tor Putitora, Botia rostrata, Garra nasuta, Labeo dyocheilus, L. pangusia* etc. are mostly found in the deep pools with more habitat area. Semi torrential fishes of the genus *Lepidicephalacthyes, Schistura, Acanthocobitis, Crossocheilus, Amblyceps, Psilorhynchus, Olyra, Botia* need boulder, sand and pebble for their shelter. The fishes like *Glyptothorax* striatus, *Glytothorax trilineatus, Bangana dero, Psilorhynchus, Schistura etc.* mostly occur in fast flow whereas, *Devario aequipinnatus, Danio dangila* preferred medium to low-velocity habitats. Cyprinid fish like *Barilius* species are common in pools, runs and riffles type of habitats. The small size *Puntius* like fishes were found great number in shallow pools habitats. *Mystus cavasius, Mystus teengara, Cirrhinus reba, Mystus vittatus, Cirrhinus mrigala, Xenentodon cancila* etc. were found in the habitats ranging from sandy to muddy substrates.
3	How is the conservation status of the fish species collected during the study?	9 fish species (11.11%) viz. *Neolissochilus hexagonolepis*, *Neolissochilus hexastichus, Labeo pangusia, Notopterus chitala, Ompok bimaculatus, Glyptothorax striatus, Bagarius bagarius, Ailia coila* and *Anguilla* were recorded as near threatened (NT), only 2 (2.44%) viz. *Badis assamensis* and *Tor tor* as data deficient (DD), 2 (2.44%) viz. *Botia rostrata* and *Wallago attu* as Vulnerable, 1 (1.23%) *Tor putitora* Endangered (EN), 4 (6.13%) *Opsarius ngawa, Psilorhynchus nahlongthai, Schistura fasciata, Strongylura leura*

		not Evaluated (NE) and more than half of the existing fish species 76.54 % of this river were found to be in the least concern (LC) category.
4	How different anthropogenic factors affecting the fish diversity of river?	The destructive method of fishing kills both target and non-target fishes. The population of migratory species such as *Neolissochilus hexagonolepis, Tor tor,* and *T putitora* has been found in declined trend in all the studied Rivers. Extraction of sand and gravel from river beds has a direct and indirect negative impact on semi torrential migratory groups of fishes such as *Shistura* sp*,* *Lepidocephalichthys sp*, and mahseer sp. These species generally bury themselves under pebble and sand. Sand mining and damaging of the riparian vegetation may cause habitat destruction for these species. Migratory species also use the sand bed as a breeding ground. During the investigation period it was observed that sand and stone mining is common in upper stretches of the rivers such as Diyung. Unfortunately, the *Dimasa* people also practice destructive fishing like poisoning, dynamiting, and electrofishing. Although these techniques are highly efficient in catching fish, they do not spare even other aquatic invertebrates as well as tiny fishes which are not even considered fit for consumption. As such, the entire aquatic ecology is disturbed by the use of such fishing methods

9.2 Recommendations on Replicability and Exit Strategy:

Particulars	Recommendations
Replicability of Fellowship, if any	
Exit Strategy:	➢ The ichthyofaunal resources of river Diyung of Northeast India exhibit a combination of both torrential and plain water forms, together with cold as well as warm water species. ➢ In the hill district, the river offers a lot of potential for recreational fishing and ecotourism, which will boost the local economy. ➢ The creation of ornamental fish-culture units with full technical support is intended to have a multiplier effect on aquaculture enterprises in the area. It will not only improve the socioeconomic position of the district's rural residents but will also save the fish from extinction. ➢ It has also been found that the relative abundance of some of the important species including mahseer (*Tor*, *Neolissochilus*) along with other coldwater species is in a declining state as reported by the fishers and local stakeholders. Therefore, it is critical to save this threatened species from the extinction in near future. ➢ In order to protect the habitats requirements of migratory and other hill stream fishes, sand and boulder extraction activities from river beds should be completely forbidden. ➢ Existing state fishery legislation limiting fishing during the breeding season and the use of other damaging fishing gear, among other things, should be properly enforced.

(**NMHS FELLOWSHIP COORDINATOR**)

(Signed and Stamped)

(**HEAD OF THE INSTITUTION**)

(Signed and Stamped)

Place: ………………..

Date: …../……/……..

PART B: COMPREHENSIVE REPORT

EXECUTIVE SUMMARY

The Executive Summary of the fellowship should not be more than 3–5 pages, covering all essential features in precise and concise manner as stated in Part A (Cumulative Fellowship Summary Report) and Part B (Comprehensive Report).

n of N (n = Sequential number; N= Total no. of fellowships granted to the

Fellowship Report No.:

Researchers Details

Type of Fellowship (HRA/HJRF/HJPF)	Name of Himalayan Researcher	Date of Joining	Date of Resignation**	Research Title	Name of the PI & Designation
(*in case of continuation of fellowship*)					

*If the appointed researcher resigned in the mid of the fellowship duration, then also mention the name of the Himalayan researcher who carried forward the fellowship.

1 INTRODUCTION

North East India is one of the world's 36 biodiversity hotspots region for freshwater fish diversity (Kottelet and Whitten, 1996). The Himalayan biodiversity hotspot region stretches over 3000 km in Pakistan, Nepal, Bhutan, Northwestern and northeast India and includes the world's highest mountains and deepest gorges. Assam, NE India forms the part of the eastern Himalaya while Kumaon Garhwal hills, Northwest Kashmir form the western Himalaya (IUCN, 2021). The Eastern Himalayas Northeast region gives rise to numerous distinct habitats and ecosystems *viz*. rivers, streams, wetland, canals etc. Amongst many rivers, the mighty Brahmaputra flows through the States of Arunachal Pradesh and Assam, covering 900 km and with 42 tributaries. These rivers, in mountainous course pass through the gorge, carved out by erosional activities forming V-shaped valleys. Upon reaching the plains they form flat valleys, oxbow lakes, floodplain wetlands etc. In the mountainous course, the water is rough and turbulent but in plains, they exhibit a contrasting phenomenon as marked by forming menders and regular changes in directions.

The Diyung, situated at the Dima Hasao district of Assam, NE India is a rain-fed river that traverses an approximate length of 240 km through dense tropical deciduous forests and is joined by several streams, namely the Brashang, Didaola, Kholong, Rubi, Abhung, Dihamlai and Dilaima and finally ends up into the Kopili River (a major southern tributary to the Brahmaputra river) at Diyungmukh. Although, the considerable studies relevant to fish taxonomy, fish biology, and ecology, conservation, etc. have been carried out so far in NE regions but seem to be scanty in relation to Diyung River.

Structural characteristics of the lotic environment are closely associated with the occurrence of fish species. The importance of habitats and the relationship between fish and habitat are of major concern to fishery biologists. A common use of fish habitat indicates the physical and chemical characteristics of the environment, excluding biological attributes. Fish habitat is defined as "Habitat for fish is a place or for migratory fishes, a set of places in which a fish, a fish population or fish assemblage can find the physical and chemical features needed for life, such as suitable water quality, migration routes, spawning grounds, feeding sites, resting sites and shelter from enemies and adverse weather" (Orth & White, 1993). Habitat features have been identified as major determinants in the n distribution and abundance of fishes from earlier times (Shelford, 1911) and later individual fish species as well as entire assemblages were studied for behaviour patterns in streams of North America (Winn, 1958; Smart & Gee, 1979; Baker & Ross, 1981). Fish species diversity is correlated with habitat complexity (Gorman & Karr, 1978; Schlosser, 1982) of depth, flow and substrate types.

Extensive studies on freshwater fishes in India are available, but most of them are either concerned with taxonomy (Datta Munshi & Srivastava, 1988; Talwar & Jhingran, 1991; Menon, 1992, Jayaram 1981) or with capture fisheries or aquaculture (see reviews by Jhingran, 1975). Studies on fish assemblage structure and their habitat requirements in Indian streams are lacking though few initiatives started in the 1980s in south Indian (Arunachalam *et al.,* 1988, 1997). A new approach concerns the study of fish assemblage structure, habitat requirements of individual fish species in the assemblage structure and habitat preference of assemblage members in Western Ghats stream of Peninsular India but such type of studies are not taken in northeastern part of India.

Freshwater fishes are deemed threatened for being sensitive to any quantitative and qualitative changes in their habitat (Moyle, 1992; Duncan, 2001). The fish richness and abundance in any water body are the functions of geomorphic, biotic and abiotic factors (Brown *et al*., 2011). The geomorphic factors include connectivity, habitat type etc. and the biotic factor includes migration, foraging, interaction in the food chain etc. and Dissolved oxygen, Temperature, Nutrients, Salinity etc. are the important abiotic factors (Menegotto *et al*., 2019; Rau *et al.,* 2019). These Physico-chemical parameters singly or synergistically change the water chemistry, flow regime nutrient dynamics and thus regulate the ecological process (Ji, 2008). Therefore, fish assemblage structure is the indicator of water quality assessment, flow regime, and ecological integrity of any ecosystem (Fu *et al.,* 2003).

The rivers in India experiencing serious threats to aquatic biodiversity therefore flagship projects are being executed on biodiversity conservation using various methods and strategies. Anthropogenic activities such as damming, habitat modification and habitat fragmentation, pollution, and water abstraction have caused tremendous and devastating effects on the freshwater ecosystem and fish diversity resulting in reduced fish resources and a rise in threat to endemic fishes to the extent of imperilment.

1.1 Brief summary of the activities under taken by the researcher (max. 1000 words)

The methodology used for achieving Objective 1

During the collection of data, both primary and secondary sources were considered. Primary data were collected from fishermen, lessee, and riparian communities of the river about species occurrence, type, and abundance every month during the study period according to the objectives of the study. Fish specimens and water samples were collected from river Diyung by conducting one sampling per month from the 8 selected stations from January 2019 to May 2022. Catching of fish at the sampling site was done by using cast nets, gill nets, hooks and lines of different sizes, and some indigenous traps with the help of local skilled fishermen. Catching operation was done in the early morning or evening because at those hours the fishermen and fish landing zone is found to be more active than at other times of the day. At the time of collection date and locality were recorded. The present study period was across four seasons i.e. Pre-monsoon, Monsoon, Retreating monsoon & Winter).

3. I. 6 Preservation and identification

Some of the fishes were identified at the fishing site itself and the rest unidentified specimens were brought to the laboratory. During the collection of the specimen guidelines of the National Biodiversity Authority, Govt. of India (Biological Diversity Act, 2002) was followed. Identification of the fish sample was done up to species level followed by Talwar and Jhingran (1991), Nath and Dey (1997, 2000), Jayaram (1999, 2010), Viswanath, *et al.* (2007) and Das and Biswas (2008). Valid scientific names were taken from Eschmeyer's Catalog of Fishes and FishBase (Froese & Pauly, 2019). The fishes were photographed with a digital camera immediately before preservation. The collected specimens were preserved in 5-6% aqueous formaldehyde solution and later the fish species were deposited in the Fish museum of the Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon. Current conservation status was evaluated according to the Red data list of the International Union for Conservation of Nature and Natural Resources (IUCN, 2017).

DNA Barcoding

Pectoral fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping was isolated following the phenol: chloroform method. The concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking their integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial COI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendorf AG 22331 Hamburg). The PCR product is then sequenced at Eurofin Scientific Laboratory. The generated barcodes were submitted to NCBI and accession numbers were obtained for the individual fish species.

Objective 2:

Macro-habitat

Fish longitudinal distribution over various environmental gradients is controlled by Macrohabitat. The microhabitat properties of a stream include channel gradient, stream depth, stream breadth, riparian cover, and bank stability. Generally, stream habitat characteristics were measured in each study site. Habitat use data were collected from a 100 m reach in all study sites. The study locations were chosen based on habitat diversity (with pools and riffles). Segregation of stream/site habitats was based on Aadland (1993) habitat guild was followed using the methods by Arunachalam (2000) and Arunachalam & Madhusoodanan Nair (1997c)

Microhabitat

Microhabitat is defined as physical sites occupied or exploited by life stages of a fish species that have certain characteristics (such as depth, water column velocity, cover type, and substrate type). Microhabitat analysis was performed in all the eight study sites in order to assess the variability in microhabitats used by fishes. Among the fish species, some fish were not evaluated because of their low numbers. At each bank, sampling was done in the upstream direction for short distances. When the fishes were located, species were determined and recorded. Substrate types were recorded for each habitat by visual methods. Each stretch in the study site was quantified for depth, flow, and substrate characteristics. A number of transects usually 8-10 were taken across the stream channel and depth, water velocity, and dominant substrates were measured.

Physicho-chemical parameters

Water and sediment samples were collected from 8 different stations of river Diyung from January, 2019 to May, 2021. Some of the physical parameters like water depth, air & surface water temperature, water velocity, TDS & EC were determined on the spot. Other parameters like Turbidity, Dissolved oxygen, pH, Total alkalinity, Total hardness, Nitrate, Nitrite, Ammonia of the water samples were carried out in the laboratory as per APHA (2018) and CPCB (2001). The sediment samples were collected on a seasonal interval, air-dried, and analyzed for pH, organic matter, and organic carbon, as per standard methodology (Jhingran, 1992; Walky & Black, 1934). Plankton and periphyton samples were identified with the help of standard literature Edmondson (1959), Needham & Needham (1966) and the ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967).

Objective 3: Information regarding anthropogenic factors was evaluated with the help of field surveys and conservation status was evaluated using IUCN, 2021 guidelines

1.2 Details of Scientific data collected and Equipment's Used (max 500 words)

a. Air & water temperatures were measured using a mercury thermometer.

b. Water velocity was measured using a current meter.

c. Parameters like pH, conductivity, TDS of the river water were measured *in-situ* using a digital soil & water testing kit (Systronics India Limited/371).

d. DO, Alkalinity & Hardness values were estimated by Titration method.

e. BOD bottles were incubated in BOD incubators.

f. For estimation of COD, water samples were digested in a KEL PLUS Automatic COD digestion system/ KES 08 L CAC.

g. Parameters like Nitrate, nitrite, total ammonia and soluble inorganic phosphate were determined using uv-visible spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202).

h. Available nitrogen was estimated by alkaline potassium paramagnet method in kjeldhal flask.

i. The available potassium was estimated by flame photometer.

j. Latitude & longitude of the stations were recorded using a GPS instrument.

k. Photography of the fish specimens and stations were done using a digital camera.

l. The morphometric measurements & weight of the collected fish specimens were recorded using a vernier calliper and a pan balance respectively.

m. DNA isolation from pectoral fin clippings of the fishes was done using Phenol-Chloroform method.

n. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306).

o. Integrity of DNA samples were checked using an Electrophoresis system (Biorad)

p. Amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg).

q. Plankton samples were collected using a plankton net.

r. Plankton & periphyton samples were observed under a Microscope.

1.3 Primary Data Collected (max 500 words)

i. The morphometric measurements & weight of the collected fish

ii. Latitude & longitude of the study stations of both the rivers

iii. Museum fish specimens

iv. Air & water temperatures

v. Water velocity

vi. Water pH

vii. Dissolved oxygen concentration of river water

viii. Conductivity of river water

ix. TDS of river water
x. Total alkalinity of river water
xi. Total hardness of river water
xii. Biological Oxygen Demand$_3$ (BOD_3) of the river water
xiii. Chemical Oxygen Demand (COD) of the river water
xiv. Nitrogen-nitrate
xv. Nitrogen-nitrite
xvi. Total ammonia
xvii. Sediment pH
xviii. Sediment organic matter
xix. Sediment organic carbon
xx. Plankton biomass

2 KEY FINDINGS AND RESULTS

3.I Major Research Findings

3.1.1 Ichthyofaunal Diversity of Diyung River

During the study, a total of 81 species belonging to 52 genera, 24 families, and 10 orders were recorded from different stretches of the River Diyung. In the present study, the order Cypriniformes formed the largest group with a contribution of (20.85%) families, 24 (46.15%) genera, and 42 (51.85%) species. The order Siluriformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 7 (29.16%) families and 17 (20.98%) species followed by Anabantiformes with 4 (16.66%) families and 9 (11.11%) species, Synbranchiformes with 2 (8.33%) families and 4 (4.93%) species, Osteoglossiformes with 1 (4.16%) family and 2 (2.64%) species, Perciformes with 1 (4.16%) families and 2 (2.46%) species, Beloniformes with 1 (4.16%) family and 2 (2.46%) species. The smallest group was formed by orders Gobiiformes, Anguilliformes, and Clupeiformes with 1 (4.16%) family and 1 (1.23%) species. Among the families Cyprinidae contributed 33 (40.74%) species, Bagridae represented with 6 (7.4%) species, Channidae with 4 (4.93%) species. Mastacembelidae, Sissoridae and Nemacheilidae, and Psilorhynchidae with 3 (3.70%) species. The maximum number of species belong to the genus *Garra* (five species) followed by *Opsarius and Channa* (four species) then *Labeo*, *Psilorhynchus* and Mystus (three species each). The various fish species were categorized based on their structural modification after Nath and Dey (2000) — 16 (19.75%) Torrential (T); 24 (29%) species Semi torrential forms (ST); **15 (18.51%)** species belonging to Migratory form (MF), and **The remaining 26 (32.09%)** species are in Plain water form. Eleven common groups of fishes were recorded during this study where Minnows and barbs (30.49 %) were found to be the most prominent group in the Diyung River followed by catfishes (20.73 %), carps (13.41 %), perch (9.76 %), loach (7.32 %), eels (6.10 %) and snakehead (4.88%). The contribution of feather backs, gars, clupeids, and mudskipper was 2.44%, 2.44%, 1.44%, and 1.44% respectively. The value of the Shannon-Weiner diversity index calculated based on fish assemblage for eight sampling stations of the river ranged between 2.78 to 3.74. As far as the diversity indices are concerned Dehangi Bazar Point (S4) and Diyungmukh confluence zone (S8) exhibited the highest H' value (3.742 and 3.738 respectively) while Syamagram (S1), the least (2.784). The Margalef richness index (D) value showed variation and highest being recorded from Station 4 (7.15) and lowest from Station 1 (3.404). However, the evenness index was highest in station 8 (0.8749) and lowest in station 5 (0.8011). The highest value of D and H' were observed during the post-monsoon season were as evenness values during pre-monsoon seasons.

Among all the 81 morphologically identified species a total of 45 sequences were generated from 42 species. All the 45 successfully amplified sequences were cross-referenced to GeneBank and NCBI databases. Fourty five sequences (100%) belonging to 42 species showed species sequence similarity of >97% when cross-referenced in the GeneBank. Using NCBI, 45 sequences belonging to 42 species could be matched to species level. Species level match for all the sequences from morphologically identified species, barcoding gap could not be made.

3.1.2: Water Quality & Sediment Parameters of River Diyung:

A total of fifteen (14) water quality parameters and six (3) sediment parameters were tested at 8 different stations by covering the whole stretch of the Diyung for a period of 29 months from January 2019 to May 2021.

Data on seasonal variation of water quality parameters of River Diyung January, 2019 to May 2021 is depicted in Appendix - 1. **Comparison of water quality parameters of the study rivers with congenial values for fishes:**

Sl. No	Parameter	Value (range)	Congenial Limit	Remark
1.	Surface Water Temperature (^{0}C)	17.55-30.70		Suitable for both cold and warm water fishes.
2.	Turbidity (NTU)	2.57-241.50	20-30	Turbidity exceeds permissible limit

				in the lower stretches of the river mainly station 6, 7 & 8 during monsoon and post monsoon sesons.
3.	pH	7.32-7.32	7-8.5	Water pH was found to be neutral to alkaline conditions during the study period.
4.	Dissolved Oxygen (ppm)	5.77-8.70	>5	Average DO values were found to be within an acceptable range.
5.	Total Alkalinity (ppm)	49.36-81.48	80-200	Alkalinity values were found to be not congenial for fishes
6.	Total Hardness (ppm)	57.32-88.65	75-150	Hardness values were found to be not congenial for fishes
7.	Electrical Conductivity (µS/cm)	99.82-173.64	50-1500	Found to be within an acceptable range
8.	Total Dissolved Solids (ppm)	62.69-119.43	<400	Found to be within an acceptable range
9.	Biochemical Oxygen Demand (ppm)	2.09-24.02	<10	BOD values of stations 6, 7 & 8 were found in a higher range than the congenial limit during monsoon indicating anthropogenic stress in these stations.
10.	Chemical Oxygen Demand (ppm)	3.01-45.99	<20	COD values of stations 6, 7 & 8 were found in a higher range than the congenial limit during monsoon indicating anthropogenic stress in these stations
11.	Nitrate-nitrogen (ppm)	0.011-0.421	0.10-3.00	Found to be within the acceptable range
12.	Nitrite Nitrogen (µg/L)	0.002-0.047	0-0.50	Found to be within the acceptable range
13.	Soluble Inorganic Phosphate (ppm)	0.02-0.12	0.05-0.4	Found to be more than acceptable range
14.	Total Ammonia (ppm)	0.290-0.215	0-1.0	Found to be more than acceptable range

3.1.3. Plankton Biomass of River Diyung:

A total of 35 genera of plankton were recorded from River Diyung during the study period. The population of phytoplankton was represented by 26 genera belonging to Chlorophyceae (12 genera), Bacillariophyceae (7 genera), Cyanophyceae (6 genera) and Euglenophyceae (1 genera). The zooplankton population was represented by Rotifera (4 genera), Cladocera (3 genera), and Copepoda (2 genera). The population density of plankton varied from season to season. The average minimum plankton density was found to be 21.33±3.68 units/L and a maximum of 626.67±13.10 units/L. Palmers' index also showed a similar trend. By using Palmer's index of pollution for the rating of water samples as lack of organic pollution, moderate and high organic pollution at all the stations were tested. The total score of the algal Genus Pollution Index (AGPI) of the sites S1<S2<S3<S4=S5<S6<S7<S8 were calculated to be 9, 8, 12, 13, 13, 20, 21 and 22 respectively.

3.1.3. To assess the conservation status of fish species and to identify anthropogenic factors affecting fish diversity.

According to the Red List of Freshwater Fishes published by IUCN (2021), 9 fish species (11.11%) viz. *Neolissochilus hexagonolepis*, *Neolissochilus hexastichus, Labeo pangusia, Notopterus chitala, Ompok bimaculatus, Glyptothorax striatus, Bagarius bagarius, Ailia coila* and *Anguilla* were recorded as near threatened (NT), only 2 (2.44%) fish species viz. *Badis assamensis* and *Tor tor* as data deficient (DD), 2 fish species (2.44%) viz *Botia rostrata* and *Wallago attu* as Vulnerable, 1 (1.23%) fish *Tor putitora* as Endangered (EN), 4 (6.13%) *Opsarius ngawa, Psilorhynchus nahlongthai, Schistura fasciata, Strongylura leura* as not Evaluated (NE) and more than half of the existing fish species 76.54 % of this river were found to be in the Least Concern (LC) category.

The destructive method of fishing kills both target and non-target fishes. The population of migratory species such as *Neolissochilus hexagonolepis, Tor tor,* and *T putitora* has been found in declined trend in all the studied Rivers. Extraction of sand and gravel from river beds has a direct and indirect negative impact on semi torrential migratory groups of fishes such as *Shistura* sp*, Lepidocephalichthys* sp, and mahseer sp . These species generally bury themselves under pebble and sand. Sand mining and damaging of the riparian vegetation may cause habitat destruction for these species. Migratory species also use the sand bed as a breeding ground. During the investigation period, it was observed that sand and stone mining is common in upper stretches of the rivers such as Diyung. Unfortunately, the *Dimasa* people also practice destructive fishing like poisoning, dynamiting, and electrofishing. Although these techniques are highly efficient in catching fish, they do not spare even other aquatic invertebrates as well as tiny fishes which are not even considered fit for consumption. As such, the entire aquatic ecology is disturbed by the use of such fishing methods

(Signature of HRA/HJRF/HPF)

(**NMHS FELLOWSHIP COORDINATOR**)

(Signed and Stamped)

(**HEAD OF THE INSTITUTION**)

(Signed and Stamped)

Place: ………………..

Date: ……../……/……

Executive Summary:

1. During the present study, a total of 81 fish species belonging to 52 genera, 24 families, and 10 orders were recorded from different stretches of River Diyung **(Table 2)**. In the present study, the order Cypriniformes formed the largest group with a contribution of 42 (51.85%) species. sssThe order Siluriformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 17 (20.98%) species followed by Anabantiformes with 9 (11.11%) species, Synbranchiformes with 4 (4.93%) species, Osteoglossiformes with 2 (2.64%) species, Perciformes with 2 (2.46%) species, Beloniformes with 2 (2.46%) species **(Fig-2).** The results of the current study would be valuable as baseline data for any forthcoming assessment of fish diversity. Among the families Cyprinidae represented 33 (40.74%) species, Bagridae represented 6 (7.4%) species, Channidae 4 (4.93%) species. Mastacembelidae, Sissoridae and Nemacheilidae and Psilorhynchidae 3 (3.70%) species and Botiidae, Notopteridae, Badidae, Belonidae, Schilbeidae, Ailiidae, Siluridae, and Ambassidae with 2 (2.47%) species and remaining families with 1 (1.23%) species each in the total fish population **(Fig-3).**
2. The dominant species were *Opsarius bendelisis*, *Pethia ticto*, *P. conchonius*, *Psilorhynchus balitora*, *Devario aequipinnatus*, *Barilius barila*, *Salmostoma bacaila*, *Puntius sophore*, *Paracanthocobitis botia*, *Garra lissorhynchus*, *G. nasuta*, *G. annadalei*, *Mastacembelus armatus*, *Tariqilabeo latius*, *Danio dangila*, *Chagunius chagunio*, *Glossogobius giuris*, *Channa gachua*, *Channa punctata*, *Psilorhynchus homaloptera*, *Badis assamensis*, *Labeo bata*, *L. dyocheilus*, *Schistura fasciata*, *Cirrhinus reba*, *Chanda nama*, *Sperata aor*, *Xenentodon cancila*.
3. Eleven common groups of fishes were recorded during this study where Minnows and barbs (30.49 %) were found to be the most prominent group in the Diyung River followed by catfishes (20.73 %), carps (13.41 %), perch (9.76 %), loach (7.32 %), eels (6.10 %) and snakehead (4.88%). The contribution of feather backs, gars, clupeids, and mudskipper was 2.44%, 2.44%, 1.44%, and 1.44% respectively **(Fig-4).**
4. According to the Red List of Freshwater Fishes published by IUCN (2021) more than half of the existing fish species (76.54 %) of this river were found to be in the Least Concern (LC) category, while 11.11 % of fish species were recorded as Near Threatened (NT), only 2.44 % as Data Deficient (DD), 2.44% as Vulnerable, 1.23% Endangered (EN) and 6.13% Not Evaluated (NE) **(Fig-5).**
5. The value of the Shannon-Weiner diversity index calculated based on fish assemblage for eight sampling stations of the river ranged between 2.78 to 3.74. As far as the diversity indices

are concerned Dehangi Bazar Point (S4) and Diyungmukh confluence zone (S8) exhibited the highest H' value (3.742 and 3.738 respectively) while Syamagram (S1), the least (2.784). The Margalef richness index (D) value showed variation and highest being recorded from Station 4 (7.15) and lowest from Station 1 (3.404). However, the evenness index was highest in station 8 (0.8749) and lowest in station 5 (0.8011). The highest value of D and H' were observed during the post-monsoon season as evenness values during pre-monsoon seasons **(Table-3 &4).**

6. The hierarchical cluster analysis technique was used to find the similarity in species abundance and composition. The cluster analysis categorized the fish species into two distinct groups**.** Group 1 comprised sites S6, S7, and S8 representing the lower stretches of the river. Group 2 comprised stations S1, S2, S3, S4 and S5, all of which were located in the middle and upper stream. Fish assemblage in relation to environmental parameters of Diyung river is plotted on axis 1 and axis 2 by CCA analysis with Eigenvalue calculated higher at Axis 1 (93.44%) and Axis 2 with (5.06%).
7. Among all the 81 morphologically identified species a total of 45 sequences were generated from 42 species. All the 45 successfully amplified sequences were cross-referenced to GeneBank and NCBI databases. Fourty-five (45) sequences (100%) belonging to 42 species showed species sequence similarity of >97% when cross-referenced in the GeneBank.

INTRODUCTION

North East India is one of the world’s 36 biodiversity hotspots region for freshwater fish diversity (Kottelet and Whitten, 1996). The Himalayan biodiversity hotspot region stretches over 3000 km in Pakistan, Nepal, Bhutan, Northwestern and northeast India and includes the world’s highest mountains and deepest gorges. Assam, NE India forms the part of the eastern Himalaya while Kumaon Garhwal hills, Northwest Kashmir form the western Himalaya (IUCN, 2021). The Eastern Himalayas Northeast region gives rise to numerous distinct habitats and ecosystems *viz*. rivers, streams, wetland, canals etc. Amongst many rivers, the mighty Brahmaputra flows through the States of Arunachal Pradesh and Assam, covering 900 km and with 42 tributaries. These rivers, in mountainous course pass through the gorge, carved out by erosional activities forming V-shaped valleys. Upon reaching the plains they form flat valleys, oxbow lakes floodplain wetlands. In the mountainous course, the water is rough and turbulent but in plains, they exhibit a contrasting phenomenon as marked by forming menders and regular changes in directions.

The Diyung, situated at the Dima Hasao district of Assam, NE India is a rain-fed river that traverses an approximate length of 240 km through dense tropical deciduous forests and is joined by several streams, namely the Brashang, Didaola, Kholong, Rubi, Abhung, Dihamlai and Dilaima and finally ends up into the Kopili River (a major southern tributary to the Brahmaputra river) at Diyungmukh. Although, the considerable studies relevant to fish taxonomy, fish biology, and ecology, conservation, etc. have been carried out so far in NE regions but seem to be scanty in relation to Diyung River.

Structural characteristics of the lotic environment are closely associated with the occurrence of fish species. The importance of habitats and the relationship between fish and habitat are of major concern to fishery biologists. A common use of fish habitat indicates the physical and chemical characteristics of the environment, excluding biological attributes. Fish habitat is defined as "Habitat for fish is a place or for migratory fishes, a set of places in which a fish, a fish population or fish assemblage can find the physical and chemical features needed for life, such as suitable water quality, migration routes, spawning grounds, feeding sites, resting sites and shelter from enemies and adverse weather" (Orth & White, 1993). Habitat features have been identified as major determinants in the n distribution and abundance of fishes from earlier times (Shelford, 1911) and later individual fish species as well as entire assemblages were studied for behaviour patterns in streams of North America (Winn, 1958; Smart & Gee, 1979; Baker & Ross, 1981). Fish species diversity is correlated with habitat complexity (Gorman & Karr, 1978; Schlosser, 1982) of depth, flow and substrate types.

Extensive studies on freshwater fishes in India are available, but most of them are either concerned with taxonomy (Datta Munshi & Srivastava, 1988; Talwar & Jhingran, 1991; Menon, 1992, Jayaram 1981) or with capture fisheries or aquaculture (see reviews by Jhingran, 1975). Studies on fish assemblage structure and their habitat requirements in Indian streams are lacking though few initiatives started in the 1980s in south Indian (Arunachalam *et al.,* 1988, 1997). A new approach concerns the study of fish assemblage structure, habitat requirements of individual fish species in the assemblage structure and habitat preference of assemblage members in Western Ghats stream of Peninsular India but such type of studies are not taken in northeastern part of India.

Freshwater fishes are deemed threatened for being sensitive to any quantitative and qualitative changes in their habitat (Moyle, 1992; Duncan, 2001). The fish richness and abundance in any water body are the functions of geomorphic, biotic and abiotic factors (Brown *et al*., 2011). The geomorphic factors include connectivity, habitat type etc. and the biotic factor includes migration, foraging, interaction in the food chain etc. and Dissolved Oxygen, Temperature, Nutrients, salinity etc. are the important abiotic factors (Menegotto *et al*., 2019; Rau *et al.,* 2019). These Physico-chemical parameters singly or synergistically change the water chemistry, flow regime nutrient dynamics and thus regulate the ecological process (Ji, 2008). Therefore, fish assemblage structure is the indicator of water quality assessment, flow regime, and ecological integrity of any ecosystem (Fu *et al.,* 2003).

The rivers in India experiencing serious threats to aquatic biodiversity therefore flagship projects are being executed on biodiversity conservation using various methods and strategies. Anthropogenic activities such as damming, habitat modification and habitat fragmentation, pollution, and water abstraction have caused tremendous and devastating effects on the freshwater ecosystem and fish diversity resulting in reduced fish resources and a rise in threat to endemic fishes to the extent of imperilment.

METHODOLOGY ADOPTED

The materials used and methodology followed in different aspects like geomorphology of the river, collection, preservation, and identification of Ichthyofauna; sampling of physico- chemical parameters; qualitative and quantitative analyses of plankton samples. Regular field trips were conducted in eight selected stations at monthly intervals for a period of 29 months from January 2019 to May 2020, covering Pre-monsoon (March-May), Monsoon (June-August), and Post-monsoon (September-November), and Winter (December-February) seasons for collection of Ichthyofauna, water samples for Physico-chemical and biological analysis.

Diyung river system.

The investigation was divided into six broad aspects viz

i) Geomorphology of river Diyung.
ii) Collection and documentation Ichthyofauna in Diyung River, Assam.
iii) Taxonomic and molecular characterization of the collected fish species.
iv) Physico-chemical parameters of water sample, plankton relationship with the abundance of ichthyofauna.

Geomorphology of the river Diyung

2.1 Study Area

Diyung is the largest river of the district that originated near the Hempeo Peak (Barail Ranges) at about 1700 m MSL, in the south-western part of the Dima Hasao district of Assam, India. Traversing an approximate length of 240 km, it flows northeast through dense tropical deciduous forests and is joined by several streams, namely the Brashang, Didaola, Kholong, Rubi, Abhung, Dihamlai, and Dilaima. Before draining into the Kopili River (a major southern tributary to the Brahmaputra drainage in Assam) at Diyungmukh, along the northern border of Dima Hasao, the Diyung is joined by the Mahur and Langting rivers, respectively, from the southeastern part of the district. It is a hill-stream river with a pool and riffle type of reach. The micro-habitats of the river are generally dominated by pools and riffles. The substrate is dominated by gravels, boulders, cobbles, sand, muds, and harbors lots of commercially important fish species throughout the year.

Sampling Site:

The present study encompassed 240 km of Diyung River covering the entire stretch from upstream to downstream. The river was thoroughly surveyed and eventually split into eight distinct sampling stations spanning the river's upstream, midstream, and downstream stretches. Sampling sites were chosen so that they represented the general habitat conditions within the area and could be regularly accessed. The study was carried out for a period of 29 months starting from January 2019 to May 2021. The study sites were Syamagram (Station-1), Lower Haflong Bridge (Station-2), Samparidisha Village (Station-3), Dihingi Bazar Point (Station- 4), Thaijuwari Village (Station-5), Kungkruwari Village (Station-6), Digandu PT-II (Station-7) and Diyungmukh (Station-8).

Station 1

The sampling station at Syamagram is located near New Halflong railway station, Dima Hasao district at a Latitude of 25°08ˈ12"N and Longitude of 93°01ˈ42"E with an elevation of 482 m above MSL. This sampling site is located near the rivers upper stretches. The channel width varies between 8-10 m with a water depth of about 0.20-0.60 m. Although the average water flows at this site is around 1.05 m/s but during the dry seasons, the water flow is relatively low. The river bed is characterized by rocky, boulders, cobbles, and gravels in this place. Cast nets and traps are commonly operated for fishing **(Plate-2).**

Station 2

The second station is located at the Lower Halflong Bridge of the Diyung River (Dima Hasao), about 9 km downstream from the first station (Syamagram), at a latitude of 25°11ˈ58"N and a longitude of 93°01ˈ2"E, with an elevation of 340 m above mean sea level. The channels width varies between 20-30 m with a water depth of about 0.25-1.50 m. Although the average water flow at this site is around 0.97 m/s. The sampling station is located on the outskirts of Halflong, and the riverbed is characterized by rocks, cobbles, pebbles, and gravel. Gill nets, cast nets, and indigenous traps were used to catch fish specimens **(Plate-2).**

Station 3

In a distance of 7 km from the 2nd station, the 3rd station was chosen at Samparidisha Village (Dima Hasao) at a latitude of 25°14′12″N and a longitude of 93°00′35″E at an elevation of 298 m above MSL. The channels width varies between 30-35m with a water depth of about 0.50-2.70m. Although the average water flow at this site is around 0.96 m/s. The riverbed is rocky at this site, and the sediments are made up of stones and gravel. Cast nets, gill nets, poles, and lines, as well as traditional methods like bamboo tarps, are used for fishing at this location **(Plate-2).**

Station 4

This station is located at Dihingi Bazar Point (Dima Hasao) at a latitude of 25^{0}25′24″N and a longitude of 92^{0}59′34″E, at an elevation of 148 m above MSL and roughly 30 km from the 3rd station. The river widens downstream of this station, and the amount of boulders and cobbles decreases dramatically. The channels width varies between 50-75m with a water depth of about 0.30-4.20m. Although the average water flow at this site is around 1.03 m/s. This section of the river has a stony bottom with gravel, pebbles, and sand. Local fishermen and fish markets near the river provided specimens, and fish were taken using a cast net, pole and line, gill nets, and indigenous traps **(Plate-2).**

Station 5

The sampling station is located at Thaijuwari Village (Langyen) Dima Hasao, at a Latitude of 25^{0}32′21″N and Longitude of 92^{0}59′06″E, at an elevation of 126 m above MSL and a distance of about 15 km from the 4th station. The channels width varies between 35-40m with a water depth of about 0.20-4.60m. Although the average water flow at this site is around 0.95m/s. The station is characterized by sandy soil with pebbles and gravel. From this point of the river, the habitat is changed and the number of pebbles and gravels is quite less. Fish specimens were collected with the help of local fishermen using different types of nets and gears like gill net, cast net, longline, and traditional methods like bamboo tarps **(Plate-2).**

Station 6

The sampling station is located at Purana Kungkruwari Village, Dima Hasao situated at a Latitude of 25^{0}34′58″N and Longitude of 92^{0}56′38″E, at an elevation of 124 m above MSL and a distance of about 18 km from the 5th station. The channels width varies between 30-45m with a water depth of about 0.80-5.30m. The average water flow at this site is around 0.91m/s. The riverbed is sandy and muddy at this location. Local fishermen assisted in the collection of fish specimens, which were taken using various nets and gears such as gill nets, cast nets, long lines, dragnets, and traditional methods such as bamboo tarps **(Plate-2).**

Station 7

The 7th station was selected at Digandu PT-II, which is located at an altitude of 25^{0}34′34″N and a longitude of 92^{0}57′44″E, at an elevation of 132 m above MSL and roughly 32 km from the 6th station. The channels width ranges from 50-75m, with a water depth of 0.30-5.60m. At this site, the average water flow at this site is around 0.87m/s. The riverbed is sandy and muddy at this location. At this point, the river bed is characterized by sandy and muddy soil. Various types of nets, such as gill nets, cast nets, long lines, and dip nets, were used to catch fish specimens **(Plate-2).**

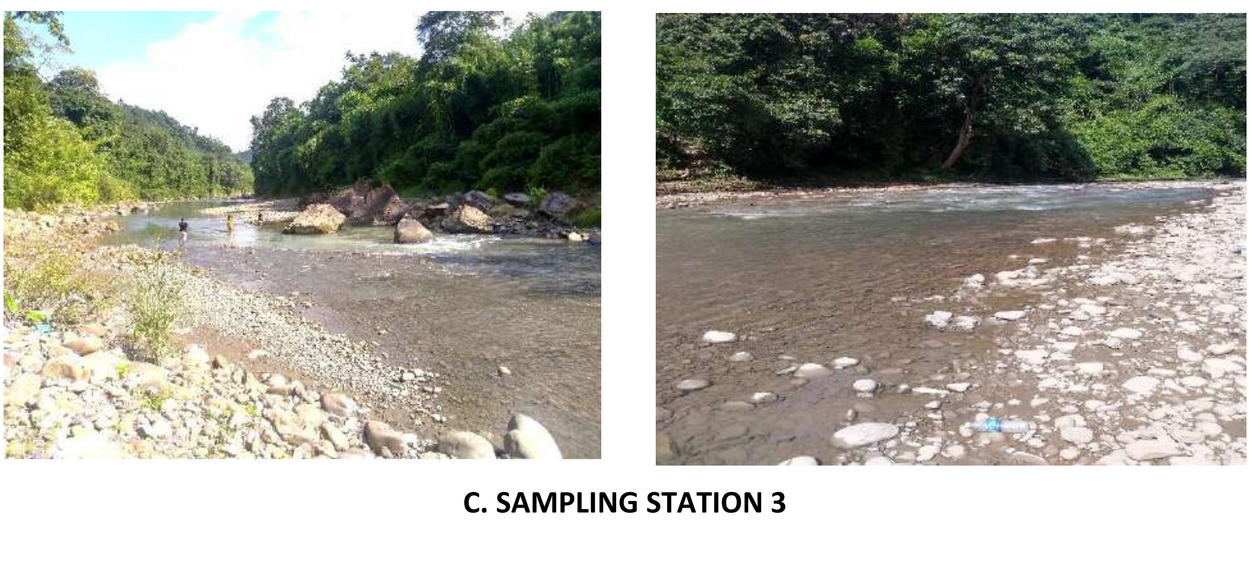
Station 8

The sampling station is located at Diyungmukh, Karbi Anglong, at an elevation of 25°48ˈ27"N and Longitude of 92°55ˈ44"E, at an altitude of 84 m above MSL and a distance of about 35 km from the 7th station. The channels width varies from 60-90m, with a water depth of 0.20-6.30m. At this site, the average water flow at this site is around 0.84m/s. The river in this station joins River Kopili, a tributary of the mighty Brahmaputra. At this point, the river bed is characterized by sandy and muddy soil. Fish specimens were collected from local fishermen and fish markets adjacent to the river which were caught by using a cast net, longline, gill nets, dragnets and dip nets. The fish mainly comprises warm water species **(Plate-2).**

SITE VIEW OF THE SAMPLING STATIONS

A. SAMPLING STATION 1

B. SAMPLING STATION 2



C. SAMPLING STATION 3


D. SAMPLING STATION 4


E. SAMPLING STATION 5


F. SAMPLING STATION 6

G. SAMPLING STATION 7

H. SAMPLING STATION 8

2.2 Collection of data

During the Study data were considered. Primary data were collected from fishermen, lessee, and riparian communities of the river about species occurrence, type, and abundance every month during the study period according to the objectives of the study.

2.3 Sample collection

Fish specimens and water samples were collected from river Diyung by conducting one sampling per month from the 8 selected stations for a period of 29 months. Catching of fish at sampling site was done by using cast net mesh size 4 -10 and 11 – 14 mm), gill net (15 – 20 mm and 10-15 mm), with the help of local skilled fishermen. Fish catching operation was done in the early morning or evening because at those hours the fishermen and fish landing zone is found to be more active than at other times of the day. At the time of collection, maximum care was taken to keep the external morphology intact for taxonomic studies.

2.4 Preservation and identification

Some of the fishes were identified at the fishing site itself and the rest unidentified specimens were brought to the laboratory for identification. During the collection of the specimen guidelines of the National Biodiversity Authority, Govt. of India (Biological Diversity Act, 2002) was followed. Identification of the fish samples were done up to species level followed by Talwar and Jhingran (1991), Nath and Dey (1997, 2000), Jayaram (1999, 2010), Viswanath, *et al.* (2007) and Das and Biswas (2008). Valid scientific names were taken from Eschmeyer's Catalog of Fishes and FishBase (Froese & Pauly, 2019). The fishes were photographed with a digital camera immediately prior to preservation. The collected specimens were preserved in 5-6% aqueous formaldehyde solution and later the fish species were deposited in the Fish museum of Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon. Current conservation status was evaluated according to the Conservation Assessment and Management Plan (CAMP, 1998) workshop and the Red data list of the International Union for Conservation of Nature and Natural Resources (IUCN, 2021).

2.3 Habitat studies

Macro-habitat

Fish longitudinal distribution over various environmental gradients is controlled by Macrohabitat. The microhabitat properties of a stream include channel gradient, stream depth, stream breadth, riparian cover, and bank stability. Generally, stream habitat characteristics were measured in each study site. Habitat use data were collected from a 100 m reach in all study sites. The study locations were chosen based on habitat diversity (with pools and riffles). Segregation of stream/site habitats was based on (Aadland, 1993) and this method was so suitable in the stream sites cited in India and the habitat guild was followed using the methods by Arunachalam and Madhusoodanan Nair (1997c) and Arunachalam (2000).

Microhabitat

Microhabitat is defined as physical sites occupied or exploited by life stages of a fish species that have certain characteristics (such as depth, water column velocity, cover type, and substrate type). Microhabitat analysis was performed in all the fourteen study sites in order to assess the variability in microhabitats used by fishes. Among the fish species, some fish were not evaluated because of their low numbers. At each bank, sampling was done in the upstream direction for short distances. When the fishes were located, species were determined and was recorded. Substrate types were recorded for each habitat by visual methods. Each stretch in the study site was quantified for depth, flow, and substrate characteristics. A number of transects usually 8-10 were taken across the stream channel and depth, water velocity, and dominant substrates were measured.

2.3.1 Physico-chemical parameters

Different physio-chemical parameters like Surface Water Temperature (SWT), Water Depth, Water Velocity, Water pH, Dissolved oxygen (DO), Total Dissolved solid (TDS), Conductivity, Alkalinity, Turbidity, Biological Oxygen Demand$_3$ (BOD_3), Chemical Oxygen Demand (COD), Ammonia, Nitrate and Phosphorous were analyzed used standard protocol APHA (2019).

2.4 Planktons

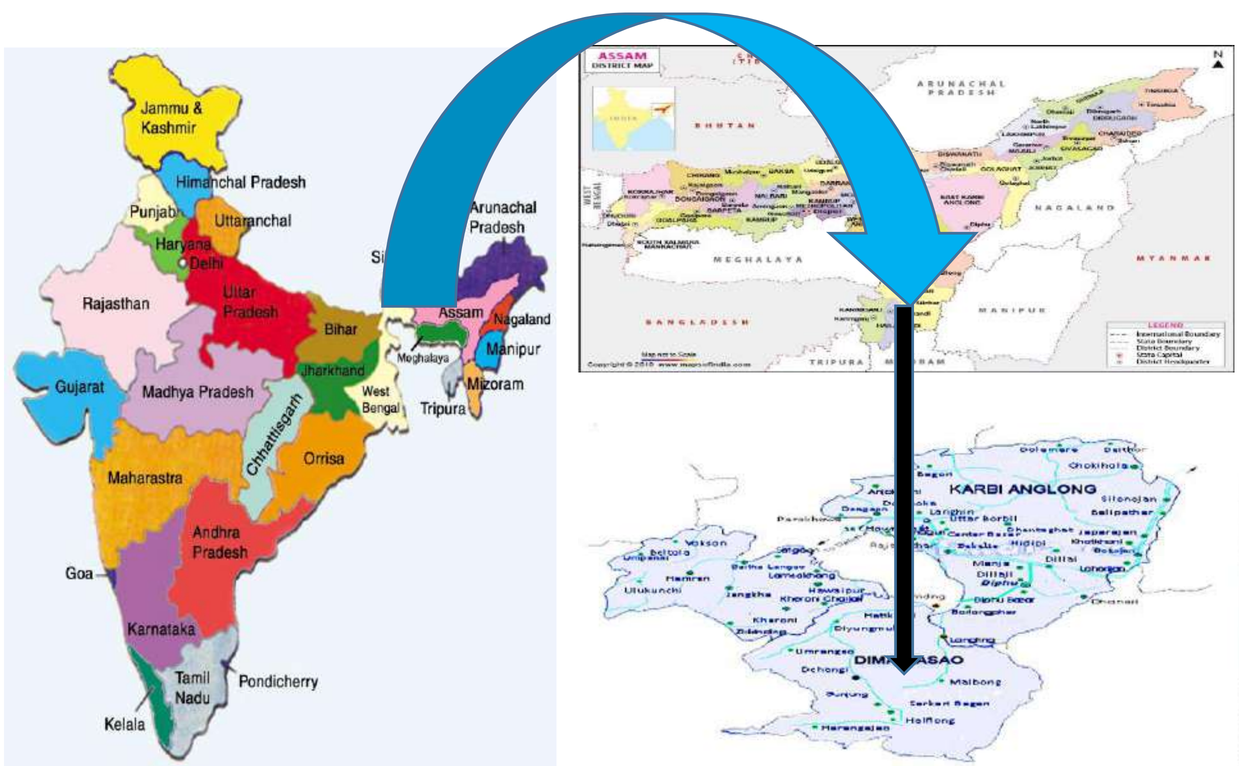
Collection of plankton samples were done by slowly filtering 50 liters of water samples collected from the six selected stations along the river (with 0.5 to 1 m depth) through the plankton net (silk cloth no. 25). The filtrate obtained in the plankton net test tube after separating the suspended particles and flock vegetation was preserved in 5% formalin solution in specimen tubes with proper labeling in the field. Planktons were collected from different stations in the morning hours to avoid the diurnal migration of most zooplanktons.

For qualitative analysis of the planktonic sample, phytoplankton and zooplanktons were stained with lugol's solution and identified under the compound microscope by dropping 2-3 drops of 5% formalin in a slide and identified. For identification, the works of Edmondson (1959), Needham and Needham (1972), Koste (1978), Michael and Sharma (1988) were followed. Other references that were espoused for the study and analysis of planktons include Chakraborty *et al.* (1959), Dobriyal *et al.* (1983), Yosuf (1989), and Sharma and Sharma (1999, 2000, 2001, and 2009). For quantitative analysis of planktons, the filtrate was concentrated to 25 ml each time and preserved in a 5% formalin solution. Quantitative analysis was done for both phytoplankton and zooplankton by using Sedgwick-Rafter counting cell and its density expressed in units per liter.

Statistical Analysis:

Species diversity can be defined as the number of species found in a given area within a certain time period. The Margalef's richness index (D), Shannon-Weiner diversity index (H), and Pielou's evenness index (J) were employed to measure the spatial-temporal variation of fish species diversity in this study. The K-Dominance plot was constructed by ranking the species in decreasing order of abundance to relate species richness and abundance (Hammer *et al*., 2001). Canonical correspondence analysis (CCA) was utilized to determine the link between fish diversity and ecological parameters using PAST software version 4.03 (Abell *et al*., 2008).

KEY FINDINGS AND RESULTS



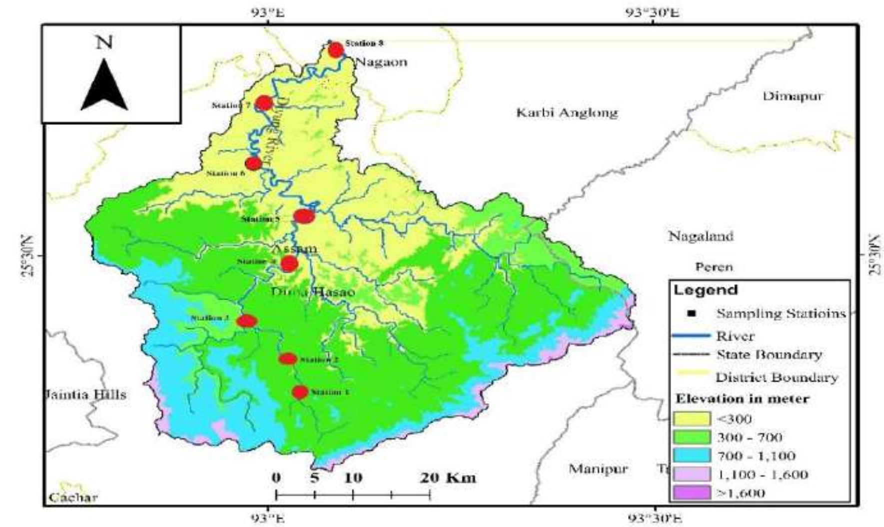


Figure 1: Map of the study area.

Table 1. Detailed characterization of each sampling site of Diyung River.

Sl No	Site/Code	Latitude & Longitude	Elevation (m MSL)	Channel Width (m)	Water Depth range (m)	Average flow m/s	Reach type	Riparian vegetation
1	Syamagram (SR)	25°08ˈ12"N 93°01ˈ42"E	482	8-10	0.20-0.6	1.05	Riffle	Meso riparian
2	Lower Halflong Bridge (LHB)	25°11ˈ58"N 93°01ˈ21"E	340	20-30	0.25-1.5	0.97	Riffle, Shallow Pool	Meso riparian
3	Samparidisha Village (SV)	25°14′12″N 93°00′35″E	298	30-35	0.5-2.7	0.96	Riffle, Shallow Pool	Meso riparian
4	Dihingi Bazar Point(DBP)	25°25′24″N 92°59′34″E	148	50-75	0.3-4.2	1.03	Riffle, shallow & Deep pool	Meso riparian
5	Thaijuwari Village (TJV)	25°32′21″N 92°59′06″E	126	35-40	0.2-4.6	0.95	Riffle, run, shallow pool	Meso riparian
6	Purana Kungkruwari Village (PKV)	25°34′58″N 92°56′38″E	117	30-45	0.8-5.3	0.91	Run & Raceway	Meso riparian
7	Digandu PT-II (DP)	25°34′34″N 92°57′44″E	80	50-75	0.3-5.6	0.87	Raceway, run & pool	Meso riparian
8	Diyungmukh (DM)	25°48ˈ27"N92°55ˈ44"E	70	60-90	0.2-6.3	0.84	Run & Raceway	Meso riparian

Ichthyofaunal diversity of river Diyung, Assam

The occurrence, diversity, distribution and habitat use of fish provides essential information on exploitation, conservation, and management measures. Fish are the most studied group of animals and the most accurate predictors of spatial trends (Abell *et al.,* 2008). Freshwater fish fauna all around the world is in imperilment due to high levels of endemism and human pressure (Magurran *et al.,* 2009). During the present study, a total of 81 fish species belonging to 52 genera, 24 families, and 10 orders were recorded from different stretches of River Diyung (Table 2). In the present study, the order Cypriniformes formed the largest group with a contribution of 5 (20.85%) families and 42 (51.85%) species. The order Siluriformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 7 (29.16%) families and 17 (20.98%) species followed by Anabantiformes with 9 (11.11%) species, Synbranchiformes with 4 (4.93%) species, Osteoglossiformes with 2 (2.64%) species, Perciformes with 2 (2.46%) species, Beloniformes with 2 (2.46%) species **(Fig-2).** The results of the current study would be valuable as baseline data for any forthcoming assessment of fish diversity. The total fish species identified in this study, account for 37.5 percent of the total number of fish species in the Brahmaputra River basin (Bhattacharjya *et al.* 2003). These findings are found in parallel with several studies on the fish biodiversity in different types of freshwater bodies of India, where they reported Cypriniformes and Siluriformes as the most prevailing orders (Dey *et al.,* 2021; Dey *et al*., 2018; Medda & Dey, 2021; Baro, 2015). Among the families Cyprinidae represented 33 (40.74%) species, Bagridae represented 6 (7.4%) species, Channidae 4 (4.93%) species. Mastacembelidae, Sissoridae and Nemacheilidae and Psilorhynchidae 3 (3.70%) species and Botiidae, Notopteridae, Badidae, Belonidae, Schilbeidae, Ailiidae, Siluridae, and Ambassidae with 2 (2.47%) species and remaining families with 1 (1.23%) species each in the total fish population **(Fig-3).** The dominance of Cyprinid fishes has been reported from other rivers of India like the Sankosh River, (Baro *et al.,* 2015), Khowai river (Mandol *et al.,* 2015), the Brahmaputra river (Sarma *et al*., 2012; Baishya *et al.,* 2016), the Ranganadi river (Koushik *et al.* 2016). No exotic fish species were recorded from the entire stretches of the rivers during the investigation periods which indicates that the rivers are in good condition.

Table 2: List of Fishes in Diyung River, Assam.

Sl. No	Order	Family	Species	Common Name	Vernacular name	IUCN 2021	Availability	Group name
1	Cypriniformes	Cyprinidae	*Tor tor*	Deep bodied mahseer	Nah yung	DD	VR	Carp
2			*Tor putitora*	Golden Mahseer	Nah suur	EN	TYS	Carp
3			*Neolissochilus hexagonolepis*	Copper mahseer	Nah msang	NT	R	Carp
4			*Neolissochilus hexastichus*	McClleland Boker	Nah msang	NT	VR	Carp
5			*Garra annandalei*	Annandale garra	Nah loh	LC	TYL	Minnow & barbs
6			*Garra gotyla gotyla*	Nilgiris garra	Nah loh	LC	TYL	Minnow & barbs
7			*Garra nasuta*	Khasi garra	Nah loh	LC	TYL	Minnow & barbs
8			*Garra lamta*	*Lamta garra*	Nah loh	LC	TYL	Minnow & barbs
9			*Garra lissorhynchus*	Khasi garra	Nah loh	LC	TYL	Minnow & barbs
10			*Opsarius bendelisis*	Hamilton's Barila	Nah hajeng	LC	TYL	Minnow & barbs
11			*Opsarius ngawa*	-	Nah hajeng	NE	R	Minnow & barbs
12			*Opsarius barna*	Barna baril	Nah hajeng	LC	TYL	Minnow & barbs
13			*Opsarius tileo*	Tileo baril	Puthi	LC	R	Minnow & barbs
14			*Barilius barila*	Bared trout	Nah hajeng	LC	TYL	Minnow & barbs

15			*Pethia ticto*	Two spot barb	Puthi	LC	TYL	Minnow & barbs
16			*Pethia conchonius*	Rosy barb	Puthi	LC	TYL	Minnow & barbs
17			*Puntius sophore*	Soft fin swamp barb,	Puthimah	LC	TYL	Minnow & barbs
18			*Systomus sarana*	Olive barb	Puthi	LC	R	Minnow & barbs
19			*Devario devario*	Bengal danio	Nah hajengs	LC	R	Minnow & barbs
20			*Devario aequipinnatus*	Giant danio	Nah hajeng	LC	TYL	Minnow & barbs
21			*Danio dangila*	Moustached da nio	Nah belang	LC	TYL	Minnow & barbs
22			*Salmostoma bacaila*	Large rose belly Minow		LC	TYL	Minnow & barbs
23			*Chagunius chagunio*	Chenguni	Nah gung gashaodzi	LC	TYL	Minnow & barbs
24			*Osteobrama cunma*	Cunma	-	LC	R	Minnow & barbs
25			*Tariqilabeo latius*	Stone roller	-	LC	TYL	Carps
26			*Labeo bata*	Bata	Nah bon	LC	TYS	Carps
27			*Labeo dyocheilus*	Brahmaputra labeo	Nah wah	LC	TYS	Carps
28			*Labeo pangusia*	*Pangusia Labeo*	-	NT	TYS	Carps
29			*Bangana dero*	*Kalaban*	-	LC	TYS	Carps
30			*Cirrhinus reba*	Reba carp	-	LC	TYS	Carps
31			*Cirrhinus mrigala*	Mrigal carp	-	LC	TYL	Carps
32			*Cabdio morar*	Morar	-	LC	TYS	Minnow & barbs

33			*Amblypharyngodon mola*	Mola carplet	-	LC	TYL	Minnow & barbs
34		Psilorhynchidae	*Psilorhynchus homaloptera*	Torrent stone carp	Nahlohkhibru	LC	TYS	Minnow & barbs
35			*Psilorhynchus balitora*	Balitora minnow	Nahlohkhibru	LC	TYL	Minnow & barbs
36			*Psilorhynchus nahlongthai*	-	-	NE	VR	Minnow & barbs
37		Botiidae	*Botia rostrata*	Gangetic loach	Nah hola	VU	R	Loach
38			*Botia dario*	Bengal loach	Nah hola	LC	VR	Loach
39		Nemacheilidae	*Paracanthocobitis botia*	Mottled zipper loach	Nah rani	LC	TYL	Loach
40			*Schistura fasciata*	-	Nah londre	NE	TYL	Loach
41			*Schistura sp.*	-	-		VR	Loach
42		Cobitidae	*Lepidocephalichthys guntea*	Guntea loach	Nah rani	LC	TYS	Loach
43	Osteoglossiformes	Notopteridae	*Notopterus synurus*	Bronze featherback	-	LC	R	Featherback
44			*Notopterus chitala*	Humped Featherback	Nah ma	NT	VR	Featherback
45	Anabantiformes	Badidae	*Badis assamensis*	Assamese Chameleon fish	Nah daokha	DD	TYS	Minnow & barbs
46			*Badis badis*	Dwarf Chameleon fish	Nah daokha	LC	TYS	Minnow & barbs
47		Channidae	*Channa marulius*	Giant snakehead	Gozar	LC	VR	Snakehead
48			*Channa gachua*	Dwarf snakehead	Borga	LC	TYS	Snakehead
49			*Channa punctata*	Spotted snakehead	-	LC	TYS	Snakehead
50			*Channa striata*	striped snakehead	-	LC	R	Snakehead

51		Anabantidae	*Anabas testudineus*	Climbing perch	-	LC	R	Perch
52	Gobiiformes	Gobiidae	*Glossogobius giuris*	Tank goby/bare eye goby	-	LC	TYL	Mudskipper
53		Osphronemidae	*Trichogaster fasciata*	Giant gourami	-	LC	TYL	Perch
54			*Trichogaster lalius*	Dwarf Gourami	-	LC	TYS	Perch
55	Perciformes	Ambassidae	*Chanda nama*	Elongated glass parchlet fish	-	LC	TYL	Perch
56			*Parambassis ranga*	Indian glassy fish	-	LC	TYS	Perch
57	Siluriformes	Bagridae	*Mystus cavasius*	Gangetic Mystus	-	LC	R	Catfish
58			*Mystus tengara*	Tengara catfish	-	LC	R	Catfish
59			*Mystus vittatus*	Striped dwarf catfish	-	LC	TYS	Catfish
60			*Rita rita*	Rita	Nah gagol	LC	R	Catfish
61	Siluriformes		*Sperata aor*	long-whiskered catfish	Nah gree	LC	TYS	Catfish
62			*Olyra kempi*	Long tail catfish	-	LC	R	Catfish
63		Siluridae	*Wallago attu*	Helicopter catfish	-	VU	R	Catfish
64			*Ompok bimaculatus*	Butter catfish	Nah blai	NT	R	Catfish
65		Sisoridae	*Glyptothorax trilineatus*	Three-lined catfish	Nah phikhauri	LC	TYS	Catfish
66			*Glyptothorax striatus*			NT	VR	Catfish
67			*Bagarius bagarius*	Devil catfish	Nah phi	NT	R	Catfish
68			*Clupisoma garua*	Bachcha	Nah shing	LC	R	Catfish
			Gagata cenia	Clawn catfishs	-	LC	R	Catfish

69		Ailiidae	*Ailia coila*	Gangetic ailia	-	NT	R	Catfish
70		Erethistidae	*Erethistes hara*	Kosi Hara	-	LC	VR	Catfish
71		Schilbeidae	*Eutropiichthys murius*	Indus garua	-	LC	VR	Catfish
72			*Eutropiichthys Vacha*	Batchwa Vacha	-	LC	VR	Catfish
73		Amblycepitidae	*Amblyceps apangi*	Indian torrent catfish	-	LC	TYL	Catfish
74	Beloniformes	Belonidae	*Xenentodon cancila*	Needlefish	Nah gongela	LC	R	Gar
75			*Strongylura leura*	Banded Needlefish		NE	TYL	Gar
76	Synbranchiformes	Mastacembelidae	*Mastacembelus armatus*	Tire-track spiny eel	Nah dang	LC	R	Eel
77			*Macrognathus aral*	one-stripe spiny eel	Nah dang	LC	TYS	Eel
78			*Macrognathus aculeatus*	Lesser spiny eel	Nah dang	LC	R	Eel
79		Synbranhidae	*Monopterus cuchia*	Gangetic Mud eel	Nam nah	LC	R	Eel
80	Anguilliformes	Anguilidae	*Anguilla bengalensis*	India Mottlet eel	Nah ner	NT	R	Eel
81	Clupeiformes	Clupeidae	*Gudusia chapra*	Indian River Shad		LC	VR	Shad

VR-Very rare, R= rare, TYS- Throughout the year in small amounts, TYL- Throughout the year in large amounts, NT- Near threatened, EN-Endangered, VU- Vulnerable, NE- Not evaluated, DD- Data deficient, LC- Least concern

	
Garra lissorhynchus	*Garra annadalei*
	
Garra nasuta	*Garra gotyla gotyla*
	
Garra lamta	*Opsarius bendelisis*
	
Barilius barila	*Opsarius barna*
	
Opsarius tileo	*Opsarius ngawa*

Pethia conchonius

Systoma sarana

Puntius sophore

Pethia ticto

Devario aequipinnatus

Devario devario

Danio dangila

Cirrhinus mrigala

Cirrhinus reba

Bangana dero

Labeo pangusia

Labeo dyocheilus

Labeo bata

Chagunius chagunio

Tor tor

Tor putitora

Tariquilabeo latius

Neolissochilus hexastichus

Neolissochilus hexagonolepis

Salmostoma bacaila

Cabdio morar

Psilorhynchus homaloptera

Psilorhynchus nalongthai

Psilorhynchus balitora

Osteobrama cunma

Amblypharyngodon mola

Schistura fasciata

Schistura sp.

Botia rostrata

Botia derio


Lepidocephalichthys gunta


Macrognathus aral


Macrognathus punchalus


Mastacembelus armatus


Channa punctata


Channa striatus


Channa gachua


Channa marulius


Wallagu attu


Ompok bimaculatus

Mystus vittatus

Mystus cavasius

Mystus teengara

Erethristis hara

Amblyceps apangi

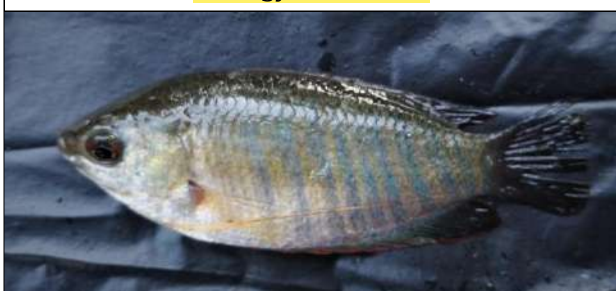
Olyra kempi

Ailia coilia

Clupeisoma garua

Sperata aor

Glyptothorax striatus

	
Glyptothorax trilineatus	*Bagarius bagarius*
	
Rita rita	
	
Strongylura leiura	*Xenentodon cancila*
	
Tricogaster fasciata	*Tricogaster lalia*
	
Anabas testudieus	*Pseudambassis baculis*

Canda nama

Parambasis ranga

Notopterus notopterus

Notopterus chitala

Badis badis

Badis assamensis

Gudusia chapra

Glossogobius giuris

Anguilla bengalensis

Monopterus cuchia

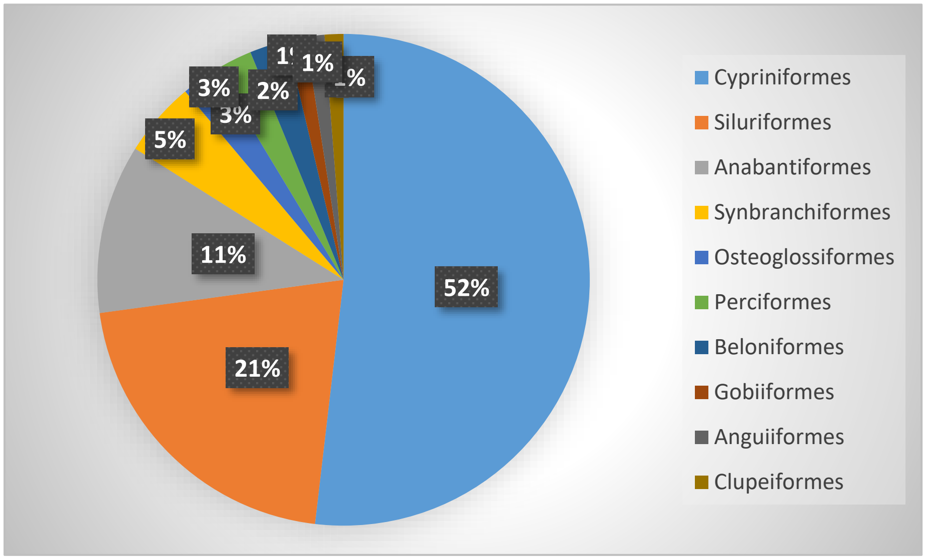


Figure 2: Composition of fish species under different orders available in Diyung river.

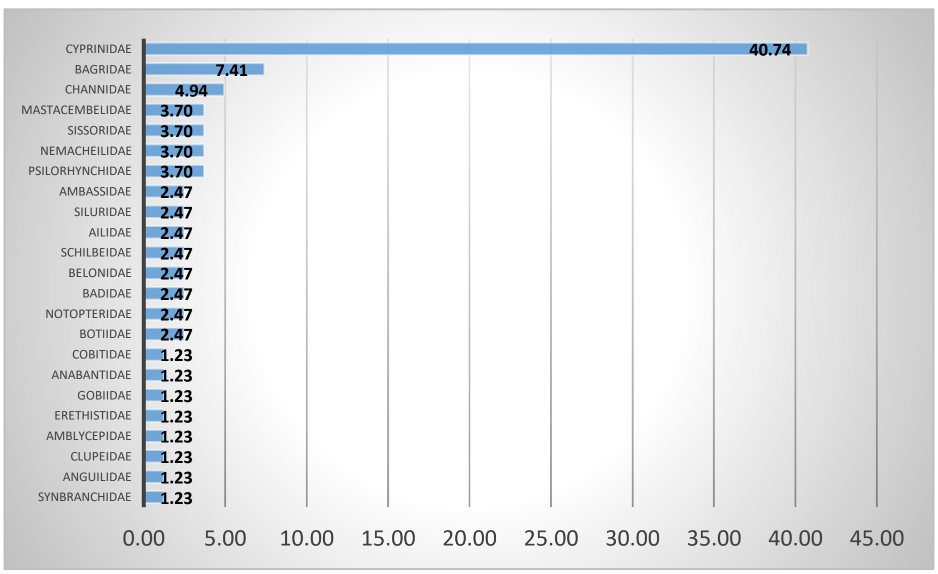


Figure3: Contribution of the family to the fish population in Diyung river.

The dominant species were *Opsarius bendelisis* (RA, 4.74%), *Pethia ticto* (RA, 4.73%), *P. conchonius* (RA, 4.45%), *Psilorhynchus balitora* (RA, 3.92%), *Devario aequipinnatus* (RA, 3.67%), *Barilius barila* (RA, 2.73%), *Salmostoma bacaila* (RA, 2.49%), *Puntius sophore* (RA, 2.34%), *Paracanthocobitis botia* (RA, 2.27%), *Garra lissorhynchus* (RA, 2.02%), *G. nasuta* (RA, 1.80%), G. annadalei (RA, 1.73%), *Mastacembelus armatus* (RA, 1.78%), *Tariqilabeo latius* (RA, 1.76%), *Danio dangila* (RA, 1.75%), *Chagunius chagunio* (RA, 1.71%), *Glossogobius giuris* (RA, 1.68%), *Channa gachua* (RA, 1.61%), *Channa punctata* (RA, 1.61%), *Psilorhynchus homaloptera* (RA, 1.52%), *Badis assamensis* (RA, 1.51%), *Labeo bata* (RA, 1.49%), *L. dyocheilus* (1.30%), *Schistura fasciata* (RA, 1.42%), *Cirrhinus reba* (RA, 1.34%), *Chanda nama* (RA, 1.34%), *Sperata aor* (RA, 1.25%), *Xenentodon cancila* (RA, 1.20%).

Eleven (11) common groups of fishes were recorded during this study where Minnows and barbs (30.49 %) were found to be the most dominant group in the Diyung River followed by catfishes (20.73 %), carps (13.41 %), perch (9.76 %), loach (7.32 %), eels (6.10 %) and snakehead (4.88%). The contribution of feather backs, gars, clupeids, and mudskipper was 2.44%, 2.44%, 1.44%, and 1.44% respectively (Fig. 4). According to the Red List of Freshwater Fishes published by IUCN (2021) more than half of the existing fish species (76.54 %) of this river were found to be in the least concern (LC) category, while 11.11 % of fish species were recorded as near threatened (NT), only 2.44 % as data deficient (DD), 2.44% as Vulnerable, 1.23% Endangered (EN) and 6.13% not Evaluated (NE) (Figure-5). Very rare (VR) fish made up 13.5% of the total fish composition in Diyung River, and rare (R) fish made up roughly 30.86% of the available species. Furthermore, approximately one-third of the entire fish population (32.10%) was available in large quantities throughout the year (TYL), while only 23.46% of fish were present in small quantities throughout the year (TYS) **(Figure-6).**

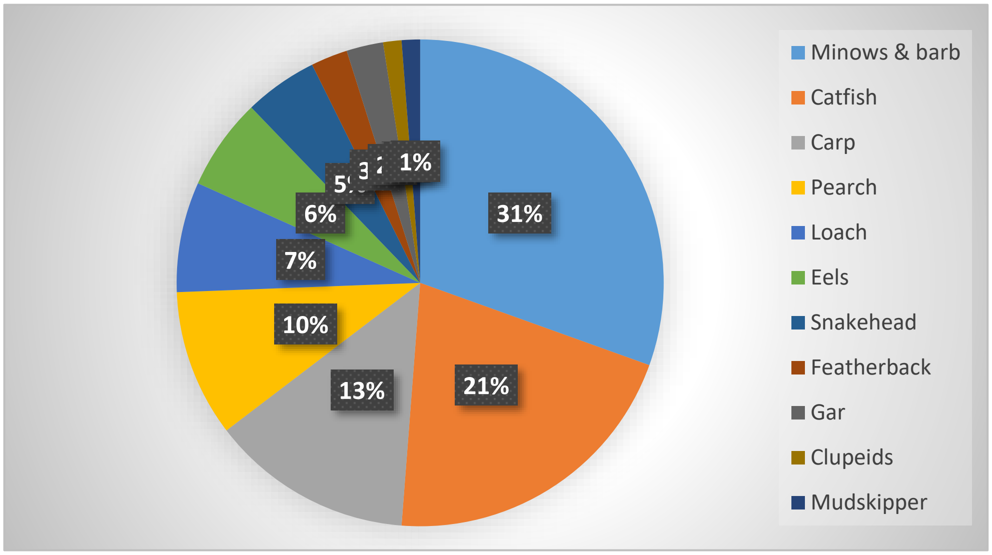


Figure 4: Percentage composition of common groups of fish documented in Diyung river

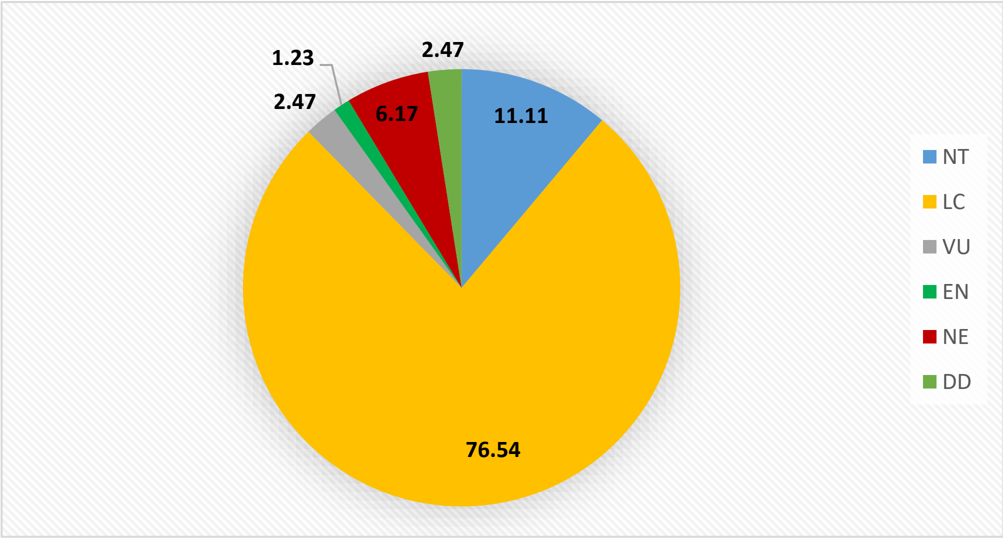


Figure 5: Percentage composition of fish species belonging to the categories of IUCN (ref. 2) in Diyung river.

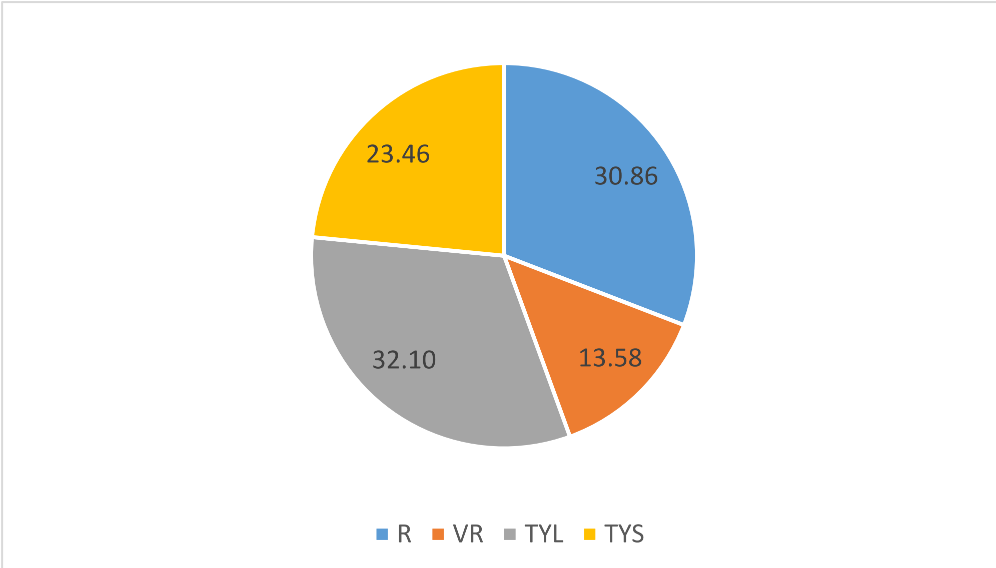


Figure 6: Percentage composition of fish on the basis availability in Diyung river.

A baseline study conducted by Sarabjit (2016) at Diyung river recorded 78 fish species. Compared with the previous study, a fifteen number of fish species viz. *Puntius chola, Rasbora rasbora, Raiamas bola, Psilorhynchus arunachalensis, P. amphicephalus, P. nudithoracicus, Pangio pangia, Schistura chindwinica, S. macrocephalus, Glyptothorax botius, G. radiolus, G. telchitta, Nangra assamensis, Pseudecheneis sulcata, P. viriosa.* On the other hand, nineteen species viz. *Neolissochilus hexastichus, Amblyceps apangi, Mystus teengara, Danio dangila, Pethia ticto, Gudusia chapra, Garra lamta, Systomus sarana, Anabas testudineus, Monopterus cuchia, Trichogaster lalius, T. fasciata, Badis assamensis, Strongylura leura, Erethistes hara, Ailia coilia, Glyptothorax trilineatus, Wallago attu, Psilorhynchus nahongthai and P. homaloptera* are being recorded in the present study, which was not reported in the previous study.

Sarabjit (2016), in his study in a river, recorded twelve species under the threatened category, including seven near threatened, one endangered, and four vulnerable species. The status of seven NT species viz. *Chitala chitala, Anguilla bengalensis, Tor tor, Neolissochilus hexagonolepis, Glyptothorax striatus, Bagarius bagarius, Ompok bimaculatus*, is still found under the NT category except for *Tor tor* which presents IUCN (2021), status is data deficient. Among the four vulnerable species viz. *Devario assamensis, Botia rostrata, Schistura chindwinica, and Schistura macrocephalus* were recorded in the previous study, only one species i.e *Botia rostrata* was retrieved in the present study.

Fish Diversity Indices

According to Clarke & Warwick (2001), the diversity indices are used to characterize species abundance in the community and are the quantitative estimates of biological variability that are used to compare communities of different habitats. The Spatio-temporal variation of diversity indices among the selected sampling sites of the River Diyung is shown in (Table-3 & 4). The value of the Shannon-Weiner diversity index calculated based on fish assemblage for eight sampling stations of the river ranged between 2.78 to 3.74. As far as the diversity indices are concerned Dehangi Bazar Point (S4) and Diyungmukh confluence zone (S8) exhibited the highest H' value (3.742 and 3.738 respectively) while Syamagram (S1), the least (2.784). The Margalef richness index (D) value showed variation and highest being recorded from Station 4 (7.15) and lowest from Station 1 (3.404). However, the evenness index was highest in station 8 (0.8749) and lowest in station 5 (0.8011). The highest value of D and H' were observed during the post-monsoon season were as evenness values during pre-monsoon seasons. The main causes of the differences occurring in the biodiversity indexes among stations and seasons may be due to seasonal variation of nutrients affecting the coexistence of many fish species (Huh & Kitting, 1985), variations in atmospheric air currents and environmental conditions (Hossain *et al*., 2012) seasonal fish migrations (Ryer & Orth, 1987) and variations in water regimes in different seasons.

Table 3: Details of fish diversity indices for different sampling stations in Diyung River.

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
Taxa_S	19	35	44	52	45	40	45	48
Individuals	198	496	872	1252	682	524	752	1108
Shannon_H	2.784	3.334	3.587	3.742	3.585	3.52	3.666	3.738
Evenness_e^H/S	0.8519	0.801	0.821	0.869	0.801	0.844	0.869	0.874
Margalef	3.404	5.478	6.351	7.15	6.743	6.229	6.644	6.704

Table 4: Details of fish diversity indices for different seasons in Diyung River

	Monsoon	Post-monsoon	Pre-monsoon	Winter
Taxa_S	69	78	62	54
Dominance_D	0.021	0.017	0.025	0.036
Simpson_1-D	0.979	0.982	0.975	0.963
Shannon_H	4.042	4.176	3.909	3.623
Evenness_e^H/S	0.824	0.834	0.804	0.693
Margalef	9.405	9.963	8.536	7.828

Cluster Analysis (Bray- Curtis Similarity Index)

The hierarchical cluster analysis technique was used to find the similarity in species abundance and composition. The cluster analysis categorized the fish species into two distinct groups **(Figure- 7).** Group 1 comprised sites S6, S7, and S8 representing the lower stretches of the river. Thirteen fish species (*Opsarius bendelisis, Pethia ticto, P. conchonius, Puntius sophore, Devario devario, Salmostoma bacaila, Cirrhinus reba, Paracanthocobitis botia, Channa gachua, C. punctata, Osteobrama cunma, Labeo bata and Mastacembelus armatus*) were recorded in group 1. Group 2 comprised stations S1, S2, S3, S4 and S5, all of which were located in the middle and upper stream. Eleven species *(Tor putitora*, *Garra gotyla*, *G. nasuta, barilius barila, Devario aequipinnatus, Danio dangila, Tariqilabeo latius, Labeo dyocheilus, Psilorhynchus homaloptera, P. balitora* and *Schistura fasciata*) were found in cluster 2. The species showing more than 1% relative abundance is only shown here.

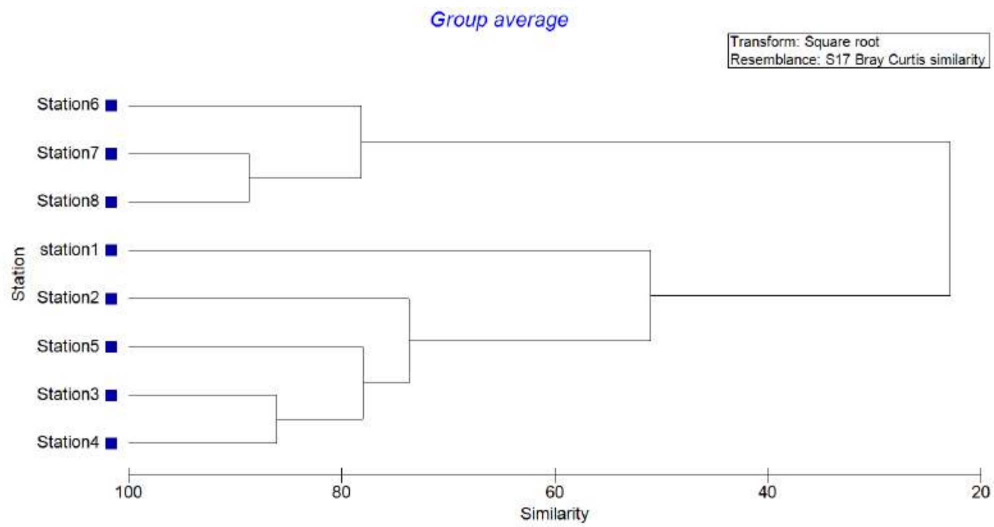


Figure7: Dendrogram clustering of Bray- Curtis similarity index.

Canonical Correspondence Analysis (CCA)

A multivariate method- CCA was used to establish the relationship between fish abundance and environmental parameters. A total of 9 environmental parameters were used. Fish assemblage in relation to environmental parameters of Diyung river is plotted in axis 1 and axis 2 by CCA analysis with Eigenvalue calculated higher at Axis 1 (93.44%) and Axis 2 with (5.06%) **(Fig. 8).**

The fish assemblage structure is dependent on the interaction of multiple ecological processes over changing the temporal and spatial scale (Poff *et al*., 1997). These factors act indigently and constrain the presence and distribution of fishes through a hierarchy of nested environmental filters. Fish abundance and distribution are the resultant of a multitude of stream variables and Physico-chemical regimes of water such as water depth, water-flow velocity, substrate, canopy and thermal regime, Dissolved oxygen, transparency etc. (Raveendar, 2018; Tesfays, 2019). In our study*, Cirrhinus mrigala, Mastacembelus armatus, Xenentodon cancila, Glossogobius giuris, Channa punctata, Mystus vittatus, Pethia ticto* and *Salmostoma bacaila* showed a positive relationship with depth, temperature and turbidity. Dissolved Oxygen (DO) and velocity showed a positive correlation with *Tor putitora, Schistura fasciata, Paracanthocobitis botia, Devario devario, Garra lissorhynchus, G. gotyla, D. aequipinnatus, G. lissorhynchus, Opsarius bendelisis, Psilorhynchus homaloptera, P. balitora* and *Barilius barila*. Raveendar *et al.* (2008) was observed a positive correlation between fish assemblage with total alkalinity, dissolved oxygen, pH, transparency, and specific conductivity in a reservoir. Environmental parameters like DO, pH, water depth, TDS, alkalinity, Conductivity, and Hardness were found to be positively correlated with the fish assemblage. This pattern has been observed in flood plain wetlands by Sarkar *et al*. (2020). The species *Channa gachua, T. fasciata, B. dario, L. bata, Osteobrama cotio* and *P. conchonius* did not show any defined relationship with the above environmental parameter. Water flow is the dominant factor determining the distribution of aquatic life forms in a river and these organisms develop life-history mechanisms to sustain in response to altered flow regimes was observed by Akhi *et al.* (2020) which substantiate our findings with respect to *Garra lissorhynchus, G. gotyla, D. aequipinnatus, G. lissorhynchus Opsarius bendelisis, Psilorhynchus homaloptera, P. balitora* and *Barilius barila*. These species evolved morphologically and physiologically to adapt to these fast-flowing waters. Hui *et al.* (2019) found that chemical parameters water temperature, salinity, dissolved oxygen are the main factors in structuring fish assemblage. Morias *et al*. (2009) also recorded that water inflow is the most deciding factor in changing the biotic and abiotic regime with an important role in the distribution and abundance of ichthyoplankton. Silva and Poliance, (2020) came to the decision in their study on the Amazon floodplain that water hydrology strongly influences the fish assemblage structure and distribution.

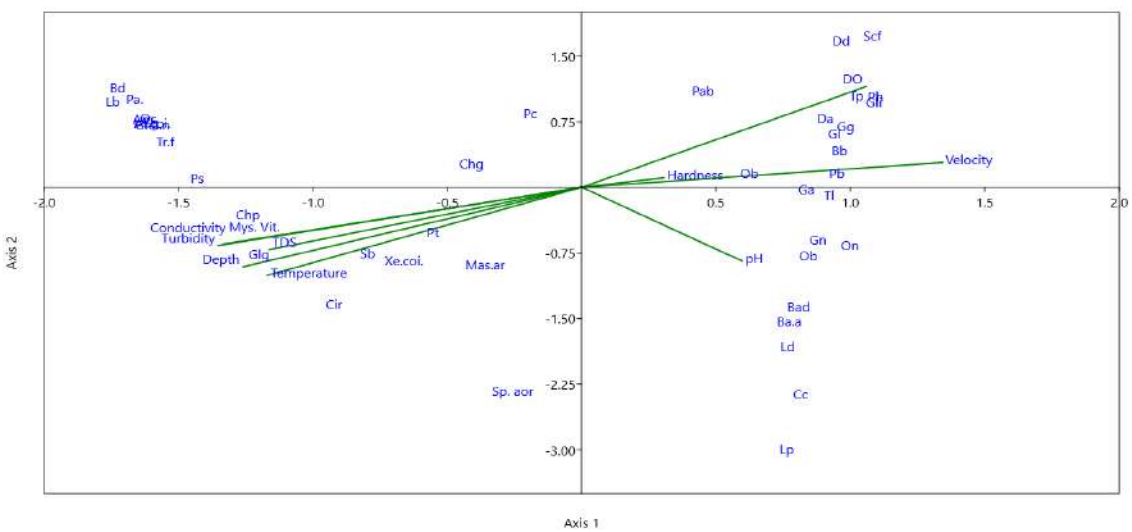


Figure 8: Relationship between fish assemblage of Diyung River with environmental parameters across sampling periods.

K-DOMINANCE (Spatial) CURVE

The cumulative dominance curve (K- dominance curve) is expressed as a percentage of abundance in a sample. On a logarithmic scale, the plot is displayed against the species rank 'K.' By ranking the species in descending order of abundance, the dominance curve was plotted to evaluate the dominance of individual species between different sampling sites and seasons.

In site-wise plot curve for the S4- Dehangi Bazar point lies on the lower side, extended further and rise slowly due to high density of species and also the curve reaches 100% cumulative due to more number of species as evident in X-axis. Generally in an undisturbed ecosystem, the K dominance curve is S-shaped showing a gentle slope with medium starting point reaching 100% cumulative. Similar curve pattern is seen in S-4 indicating high species diversity with little ecological disturbance compared to that of other sites. K- Dominance curve is represented in **fig 9.** Habitat complexity structure the fish assemblage and leads to different ecological processes and spatial habitat complexity gives rise to various microhabitats and increase the fish diversity and abundance (Poff *et al.*,1990), and loss of habitat complexity results in biotic homogenization.

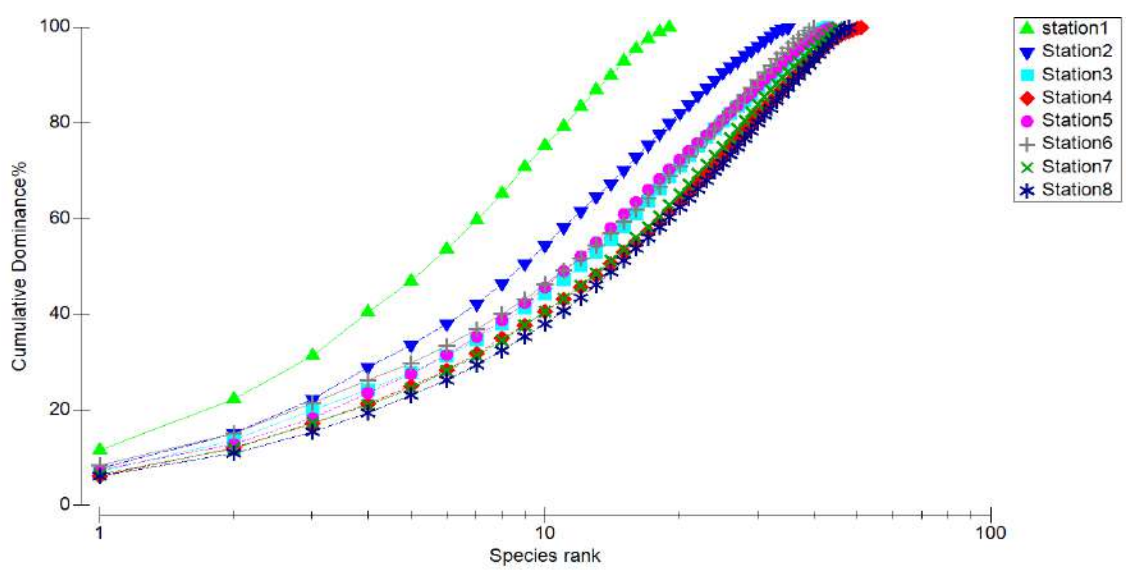


Figure 9: Station wise K- dominance curve for species biomass

K-DOMINANCE (Temporal) CURVE

In the Temporal plot, the post-monsoon curve lies on the lower side extended further and rises slowly due to the high density of species, reaching 100% cumulative due to more species forming more or less an S-shaped curve **(Figure-10).** The highest species abundance in the post-monsoon might be linked with higher aggregation of fish due to reduced water levels in the river which enhanced fish capturing. The river bed featured numerous deep pools exposed to fishing during post-monsoon. In the post-monsoon, the river water expands the horizon by inundating the adjoining areas and providing more space for fish to forage leading to declined abundance in the river. The seasonal changes can influence the fish aggregation and assemblage pattern (Kumar *et al*., 2020; Kautza *et al*., 2012; Akhi *et al*., 2020).

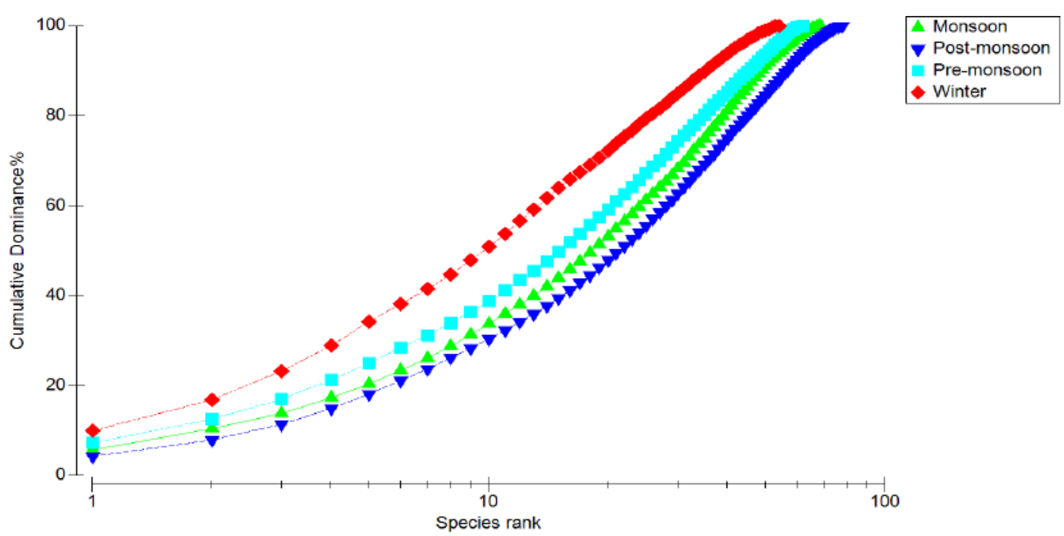


Figure 10: Seasonal K- dominance curve for species biomass

Table 4: DNA Barcoding of the Species Recorded With Accession Number

Sl No	Voucher Specimen	Organism	GeneBank Accession number
1	*COFAAEMMF 4001*	*Devario aequipinnatus*	OK287082
2	*COFAAEMMF4096*	*Schistura fasciata*	OL434416
3	*COFAAEMMF 4007*	*Opsarius bendelisis*	MN830278, MT709057
4	*COFAAEMMF 4018*	*Opsarius barna*	OL440723
5	*COFAAEMMF 4004*	*Opsarius ngawa*	MN830280
6	*COFAAEMMF 4077*	*Bangana dero*	OK021561
7	*COFAAEMMF 4027*	*Psilorhynchus balitora*	OK091104
8	*COFAAEMMF 4076*	*Psilorhynchus nahlongthai*	OK021558
9	*COFAAEMMF 4006*	*Psilorhynchus homaloptera*	MN830283
10	*COFAAEMMF 4003*	*Tor putitora*	MN830279
11	*COFAAEMMF 4012*	*Tor tor*	MT709053
12	*COFAAEMMF4097*	*Garra kempi*	OL440722
13	*COFAAEMMF 4002*	*Garra annadalei*	MN830281
4	*COFAAEMMF 4016*	*Garra lissorhynchus*	OK245258
15	*COFAAEMMF 4009*	*Garra nasuta*	MT709056
16	*COFAAEMMF 4014*	*Garra gotyla gotyla*	MW326668
17	*COFAAEMMF 4005*	*Pethia conchonius*	OK310735
18	*COFAAEMMF 4010*	*Botia rostrata*	MT709055
19	*COFAAEMMF 4012*	*Neolissochilus hexagonolepis*	OK147870
20	*COFAAEMMF 4023*	*Chagunius chagunio*	OK021579, OK236772
21	*COFAAEMMF 4078*	*Cirrhinus mrigala*	OK030550
22	*COFAAEMMF 4025*	*Tariqilabeo latius*	OK091167
23	*COFAAEMMF 4031*	*Lepidocephalichthys guntea*	OK244697
24	*COFAAEMMF 4012*	*Puntius sophore*	OK255709
25	*COFAAEMMF 4022*	*Salmostoma bacaila*	OK244556
26	*COFAAEMMF 4092*	*Barilius barila*	OL415195
27	*COFAAEMMF4094*	*Labeo pangusia*	OL425818
28	*COFAAEMMF 4032*	*Notopterus notopterus*	OK135728
29	*COFAAEMMF 4047*	*Macrognathus aral*	OK236376
30	*COFAAEMMF 4036*	*Channa punctata*	OK091002
31	*COFAAEMMF 4053*	*Clupeisoma garua*	OK244652
32	*COFAAEMMF 4042*	*Sperata aor*	MW326669
33	*COFAAEMMF 4043*	*Wallago attu*	OK256189
34	*COFAAEMMF4087*	*Amblyceps apangi*	OK245256
35	*COFAAEMMF4091*	*Glyptothorax striatus*	OL413480
36	*COFAAEMMF 4008*	*Glyptothorax trilineatus*	MT709054
37	*COFAAEMMF4084*	*Gagata cenia*	OK090943
38	COFFAEMMF4080	*Erethistes hara*	OK104034
39	COFFAEMMF4034	*Channa marulius*	OL658834
40	COFFAEMMF4036	*Danio dangila*	OL658836
41	COFFAEMMF4024	*Osteobrama cotio*	OL658838
42	COFFAEMMF4046	*Mastacembelus armatus*	OL658838

Habitat Utilization of Fishes

The habitat structure was measured and categorized into various categories for measuring fish habitats. In the 8 selected sites of the present study, the major habitat types like shallow pool, slow riffle, fast riffle, raceways, medium and deep pools were identified **(Table-5).** Almost all the selected study sites were represented by diverse habitat types which have shown significant heterogeneity. The study has shown that fish species preferred more riffles, deep pools, raceways and medium pools than other habitat types It has also shown that dominant cyprinids used different types of habitats but more preferred were fast riffle, slow riffle, shallow and deep pools **(Table-6)**

Macro and microhabitats combine to create the total habitat available for organisms. Macrohabitat controls the general pattern of species distribution and abundance which governs the flow of energy through the system and also controls the distribution and abundance of microhabitat. However, the riparian cover showed no significant relationship between stream gradient and fish density. Habitat area and habitat diversity were important attributes in determining fish diversity. Significant statistical relationships were found between species richness and habitat diversity. Likewise, the habitat area was significantly related to total fish density. High habitat diversity was associated with high species diversity in Dehangi bazar point (S4) and in Diyungmukh (S8) in river Diyung among the 8 selected sites because of the development of structurally complex channel with large pool, riffles and run habitats.

Table5: General habitat characteristics and channel morphology in river Diyung, Assam.

Sl No	Site/Code	Latitude & Longitude	Elevation (m)	Width (m)	Depth range (m)	Average flow m/s	Habitat type	substrate
1	Syamagram (SR)	25°08ˈ12"N 93°01ˈ42"E	388	8-10	0.20-0.6	1.05	Riffle	Mixed with boulder, gravel, cobble& sand
2	Lower Halflong Bridge (LHB)	25°11ˈ58"N 93°01ˈ21"E	340	20-30	0.25-1.5	0.97	Riffle, Shallow Pool	Mixed with boulder, gravel, cobble & sand
3	Samparidisha Village (SV)	25^{o}14′12″N 93^{o}00′35″E	298	30-35	0.5-2.7	0.96	Riffle, Shallow Pool	Cobble, pebble is predominantly, boulder is also present
4	Dihingi Bazar Point(DBP)	25^{0}25′24″N 92^{0}59′34″E	148	50-75	0.3-4.2	1.03	Riffle, shallow & Deep pool	Predominant with cobble, pebble, and gravel and also sand and mud
5	Thaijuwari Village (TJV)	25^{0}32′21″N 92^{0}59′06″E	126	35-40	0.2-4.6	0.95	Riffle, run, shallow pool	Mixed with sand, gravel, mud, pebble and cobble
6	Purana Kungkruwari Village (PKV)	25^{0}34′58″N 92^{0}56′38″E	117	30-45	0.8-5.3	0.91	Run & Raceway	Predominant with sand and mud,
7	Digandu PT-II (DP)	25^{0}34′34″N 92^{0}57′44″E	80	50-75	0.3-5.6	0.87	Raceway, run & pool	Predominant with sand and mud,
8	Diyungmukh (DM)	25°48ˈ27"N 92°55ˈ44"E	70	60-90	0.2-6.3	0.84	Run & Raceway	Predominant with sand and mud,

Table 6: Habitat structure in Samparidisha Village (LHB) and Lower Halflong Bridge (LHB) of Diyung River

Habitat	Length (m)		Mean width (m)		Habitat Area (m^2)	
	SR	LHB	SR	LHB	SR	LHB
Pool 1	13	15	8	18	104	270
Pool 2	-	10	-	22	-	220
Run	12	12	7	17	84	204
Riffle 1	43	27	10	24	430	640
Riffle 2	32	36	9	13	288	468

Table7: Habitat Samparidisha Village (SV) and Dihingi Bazar Point (DBP) of Diyung River

Habitat	Length (m)		Mean width (m)		Habitat Area (m^2)	
	SV	DBP	SV	DBP	SV	DBP
Pool 1	14	20	18	25	252	500
Pool 2	9	12	20	21	180	252
Pool 3	15	18	15	25	225	450
Run	8	10	17	23	136	230
Riffle 1	28	20	14	22	392	440
Riffle 2	24	14	16	15	360	210
Raceway	-	6	-	21	0	126

Table 8: Habitat structure in Thaijuwari Village (TJV) and Purana Kungkruwari (PKG) of Diyung River

Habitat	Length (m)		Mean width (m)		Habitat Area (m^2)	
	TJV	PKV	TJV	PKV	TJV	PKV
Pool 1	12	4	22	16	264	64
Pool 2	11	12	26	15	286	180
Pool 3	15	13	16	19	240	208
Run	13	17	21	15	273	255
Run	16	18	23	22	368	396
Riffle 1	12	20	18	18	216	360
Riffle 2	16	10	13	23	208	230
Raceway	5	18	17	16	85	288
Raceway	-	20	-	19	-	380

Table 9: Habitat structure in Digandu PT-II (DP) and Diyungmukh (DM) of Diyung River.

Habitat	Length (m)		Mean width (m)		Habitat Area (m^2)	
	DP	DM	DP	DM	DP	DM
Pool 1	15	15	30	31	450	465
Pool 2	13	6	35	42	455	210
Pool 3	24	-	22	-	528	-
Run	25	20	27	31	675	620
Run	-	25	-	26	-	650
Raceway	22	15	25	22	550	375
Raceway	-	17	-	36	-	612

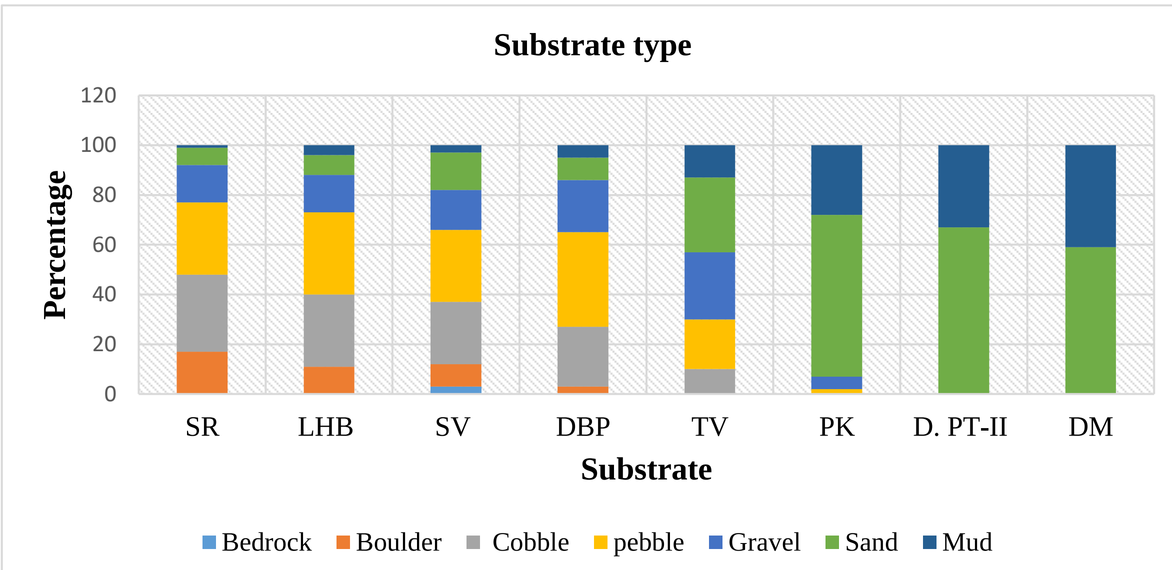


Figure 11: Different types of substrate (%) in eight study sites.

The percentage distribution of diversified habitats showed a considerable variation among the study sites. All study sites were represented by microhabitat heterogeneity phenomenon. All fish species collected from various sites of selected rivers showed variation in size and occupied different habitat types. Maximum habitat heterogeneity was found in Dehangi Bazar point (DBP) whereas minimum habitat heterogeneity was recorded from Syamagram (SR) and Lower Halflong

Bridge (LHB) where the lowest number was recorded because shallow pools, slow rifles and, deep pools were not found.

Habitat preferences of fishes were based on flow, depth and substrate categories which showed significant variation and few species have shown an overlap in their habitat preference. As far as flow is concerned, most of the fish species preferred moderate water flow and is followed by the slow category. The fishes like *Osteobrama cotio cunma, Bagarius bagarius, Amblypharyngodon mola* etc. extensively preferred medium pool type of habitats while as *Labeo bata* and *Systoma sarana, Cirrhinus mrigala, Cavasius cavasius, Wallago attu, Chanda nama, Ompok bimaculatus, Rita rita* etc. extensively used raceway habitats. Species like *Sperata aor, Mastacembelus armatus, Chagunius chagunio, Tor Putitora, Botia rostrata, Garra nasuta, Labeo dyocheilus, L. pangusia* etc. are mostly found in the deep pools with more habitat area. Semi torrential fishes of the genus *Lepidicephalacthyes, Schistura, Acanthocobitis, Crossocheilus, Amblyceps, Psilorhynchus, Olyra, Botia* need boulder, sand and pebble for their shelter.

The fishes like *Glyptothorax* striatus, *Glytothorax trilineatus, Bangana dero, Psilorhynchus, Schistura etc.* mostly occur in fast flow whereas, *Devario aequipinnatus, Danio dangila* preferred medium to low-velocity habitats. Cyprinid fish like *Barilius* species are common in pools, runs and riffles type of habitats. The small size *Puntius* like fishes were found great number in shallow pools habitats. *Mystus cavasius, Mystus teengara, Cirrhinus reba, Mystus vittatus, Cirrhinus mrigala, Xenentodon cancila* etc. were found in the habitats ranging from sandy to muddy substrates.

Studies showed that fishes have developed various types of morphological and behavioral adaptations in order to exploit specific habitat type. Substrate type is an important factor that influences the distribution of some specialized forms like loaches, Sisorids, Bagrids, Nemacheilines and Balitorids. These fish species prefer riffle and glide habitat types and they have developed some morphological characteristics such as ventral mouth with suckers, butterfly like spread pectoral and pelvic fins and possesses adhesive pads etc. Surface living fishes like *Devario aequipinnatus Salmostoma bacaila, Opsarius bendelisis, Opsarius barna, Barilius barila Danio dangila* etc. were observed to resides nearby shady places of vegetation's as they form the hub of terrestrial insects as their food and were not dependent on substrates. Streamlined fishes like *Tor tor*, *T. Putitora,* etc. have developed some special adaptations like slender bodies, narrow caudal peduncle and deep caudal fins in order to bear higher current velocity. The bottom dwellers have developed special characters like suker in mouth region of *Garra gotyla, G. nasuta* etc. in order to make easy attachment to substrate.

Table 10: Phytoplankton composition of the 8 stations observed during the present study.

	Genus	Station1	Station2	Station3	Station4	Station5	Station6	Station7	Station8
	Chlorophyceae								
1	CLOSTERIUM	-	p	-	p	p	p	-	p
2	COSMARIUM	p	p	-	p	p	p	-	p
3	ZYGNEMA	-	-	p	p	p	p	p	p
4	PEDIASTRUM	P	p	-	p	p	p	p	p
5	PANDORINA	-	-	-	-	p	p	p	p
6	EUDORINA	-	p	p	p	p	-	p	p
7	OOCYSTIS	p	p	p	p	p	p	p	p
8	CLADOPHORA	p	p	p	p	p	p	p	p
9	ULOTHRIX	-	-	-	p	p	p	p	-
10	VOLVOX	p	p	p	p	p	p	-	p
11	SPIROGYRA	p	p	p	p	-	p	p	-
12	CHLORELLA	-	-	p	p	-	p	p	p
	Bacillariophyceae								
13	TABELLARIA	p	p	p	p	p	p	p	p
14	FRAGILARIA	p	p	p	p	p	p	p	p
15	NAVICULA	p	-	p	-	p	p	p	p
16	NITZSCHIA	-	p	-	p	p	p	p	p
17	MELOSIRA	p	-	p	p	p	p	p	p
18	CYCLOTELLA	p	-	p	p	-	p	p	p
19	FRUSTULIA	-	p	p	p	p	p	-	p
	Cyanophyceae								
20	SYNEDRA	p	p	p	p	p	p	p	p
21	OSCILLATORIA	-	-	-	-	-	p	p	p
22	ANABENA	p	p	p	p	-	p	p	p
23	MERISMOPEDIA	-	p	p	p	p	-	p	p
24	SPIRULINA	p	p	p	p	p	p	-	p
25	NOSTOC	p	-	-	p	p	p	p	p
	Euglenophyceae								
26	PHACUS	p	p	p	p	p	-	p	p

Table 11: Zooplankton composition of the 8 stations observed during the present study.

	Genus	Station1	Station2	Station3	Station4	Station5	Station6	Station7	Station8
	Rotifera								
27	BRACHIONUS	p	-	-	p	p	p	p	p
28	KERATELLA	p	p	p	p	p	p	p	p
29	LECANE	p	p	p	-	p	p	p	p
30	POLYARTHRA	p	-	-	p	-	p	p	p
	Copepod								
31	CYCLOPS	p	-	-	p	p	p	p	p
32	DIAPTOMUS	p	p	p	p	p	p	p	p
	CLADOCERA	p	p	p	p	p	p	p	p
33	DAPHINIA	p	p	p	p	p	p	p	p
34	MOINA	p	p	p	p	p	p	p	p
35	BOSMINA	p	p	p	p	p	p	p	P

Table 12: Algal genus pollution index (Palmer, 1969)

Genus	**Pollution Index**	**Genus**	**Pollution Index**
Anacystis	1	Micractinium	1
Ankistrodesmus	2	Navicula	3
Chlamydomonas	4	Nitzschia	3
Chlorella	3	Oscillatoria	5
Closterium	1	Pandorina	1
Cyclotella	1	Phacus	2
Euglena	5	Phormidium	1
Gomphonema	1	Scenedesmus	4
Lepocinclis	1	Stigeoclonium	2
Melosira	1	Synedra	2

Following numerical values for pollution classification of Palmer (1969), 0-10= Lack of organic pollution 10-15= Moderate pollution 15-20= Probable high organic pollution 20 or more = Confirms high organic pollution

Table 13: Pollution index of Algal genera according to Palmer, (1969) at 8 stations of Diyung River

Genus	Station1	Station2	Station3	Station4	Station5	Station6	Station7	Station8
Chlorophyceae								
CLOSTERIUM	0	1	0	1	1	1	0	1
COSMARIUM	+	+	-	+	+	+	-	+
ZYGNEMA	-	-	+	+	+	+	+	+
PEDIASTRUM	+	+	-	+	+	+	+	+
PANDORINA	0	0	0	0	1	1	1	1
EUDORINA	-	+	+	+	+	-	+	+
OOCYSTIS	+	+	+	+	+	+	+	+
CLADOPHORA	+	+	+	+	+	+	+	+
ULOTHRIX	-	-	-	+	+	+	+	-
VOLVOX	+	+	+	+	+	+	-	+
SPIROGYRA	+	+	+	+	-	+	+	-
CHLORELLA	0	0	3	3	0	3	3	3
Bacillariophyceae								
TABELLARIA	+	+	+	+	+	+	+	+
FRAGILARIA	+	+	+	+	+	+	+	+
NAVICULA	3	0	3	0	3	3	3	3
NITZSCHIA	0	3	0	3	3	3	3	3
MELOSIRA	1	0	1	1	1	1	1	1
CYCLOTELLA	1	1	1	1	0	1	1	1
FRUSTULIA	-	+	+	+	+	+	-	+
Cyanophyceae								
SYNEDRA	2	2	2	2	2	2	2	2
OSCILLATORIA	0	0	0	0	0	5	5	5
ANABENA	+	+	+	+	-	+	+	+
MERISMOPEDIA	-	+	+	+	+	-	+	+
SPIRULINA	+	+	+	+	+	+	-	+
NOSTOC	+	-	-	+	+	+	+	+
Euglenophyceae								
PHACUS	2	2	2	2	2	0	2	2
Total	**9**	**8**	**12**	**13**	**13**	**20**	**21**	**22**

Plankton Diversity and Biomass

Plankton is the most sensitive component of the aquatic ecosystem, as it signals environmental perturbations. Phytoplankton is an important component of the food chain because it is the source of primary productivity and also serves as a biological indicator of water quality in pollution studies. Fish require protein, lipids, carbs, mineral salts, and water in the proper proportions, which zooplankton delivers (Jabeen and Barbhuya, 2018). Plankton research and monitoring are useful for determining the physicochemical and biological characteristics of water for any purpose.

A total of 35 genera of plankton were recorded from River Diyung during the study period. The population of phytoplankton was represented by 35 genera belonging to Chlorophyceae (12 genera), Bacillariophyceae (7 genera), Cyanophyceae (6 genera) and Euglenophyceae (1genera) **(Table 10).** Zooplankton population was represented by Rotifera (4 genera), Cladocera (2 genera) and Copepoda (3 genera) **(Table 11).**

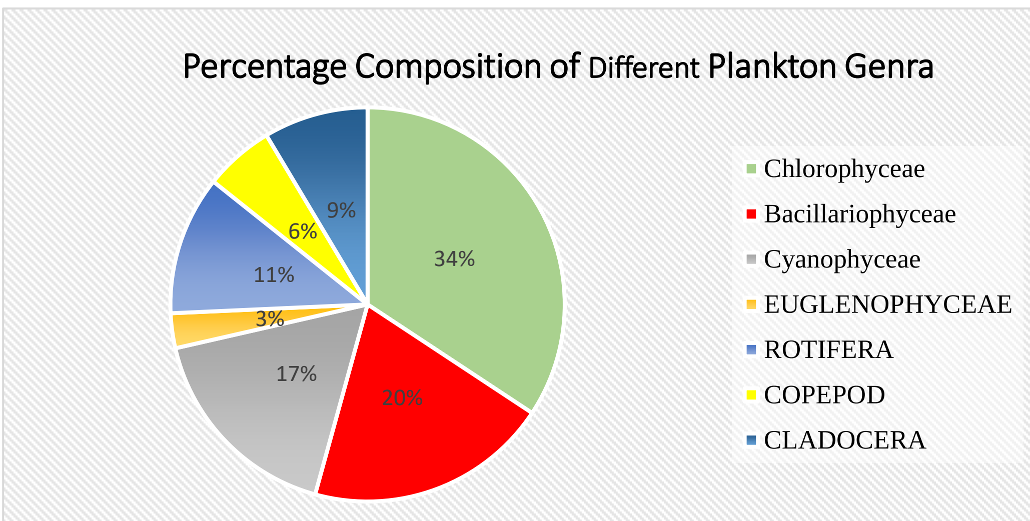


Figure 12: Percentage contribution of different plankton genera in river River Diyung recorded during the study period.

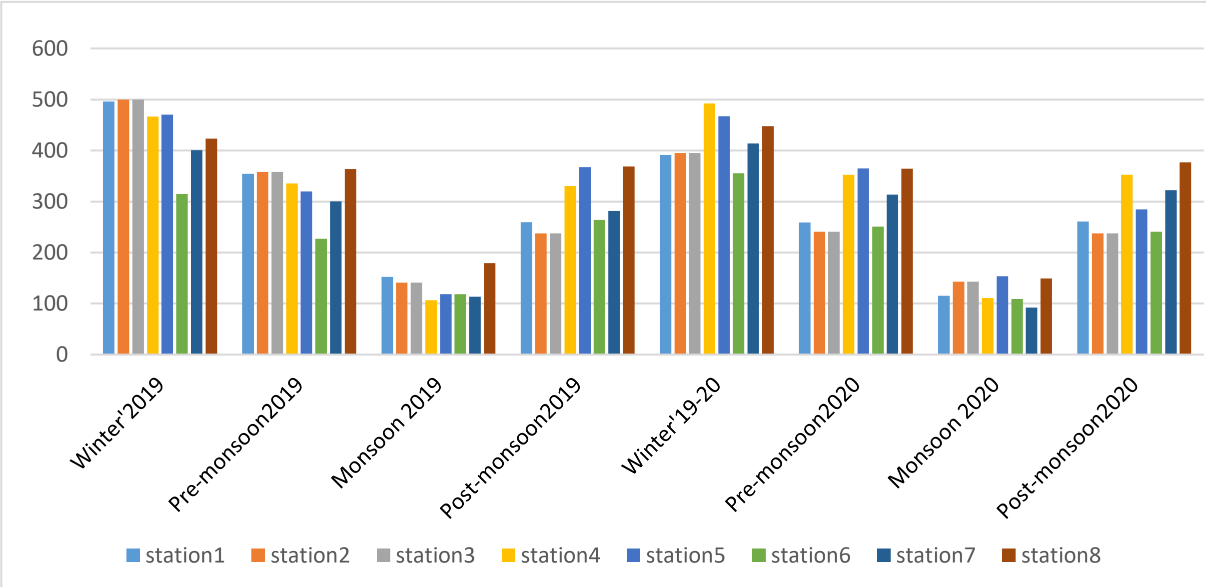


Figure 13: Seasonal variation of plankton density at stations 1 to 8 during the study period.

Palmer (1969) was the first to compile a list of algae taxa and species that indicate the presence of organic contamination. Scores of 20 or more, according to Palmer, indicate high levels of organic contamination (Table 12). At all of the stations, water samples were rated as absence of organic pollution, moderate organic pollution, and high organic pollution using Palmer's indicator of pollution. The total score of Agal Genus Pollution Index (AGPI) of the sites S2<S1<S3<S4<S5<S6<S7<S8<S8 were calculated to be 8, 9, 12, 13, 13, 20, 21 and 22 respectively (Table 13 and Figure 14). It was observed that the total score of S1 and S2 showed below 10 which indicates lack of organic pollution. Slight increase increase in total score of 12, 13, 13 in station 3,4 and 5 indicating moderate organic pollution. *Navicula, Nitzcha* and *Synedra* were recorded repeatedly in lower stations of Diyung river and consider as indicators of pollution in view of results of Palmer’s index.

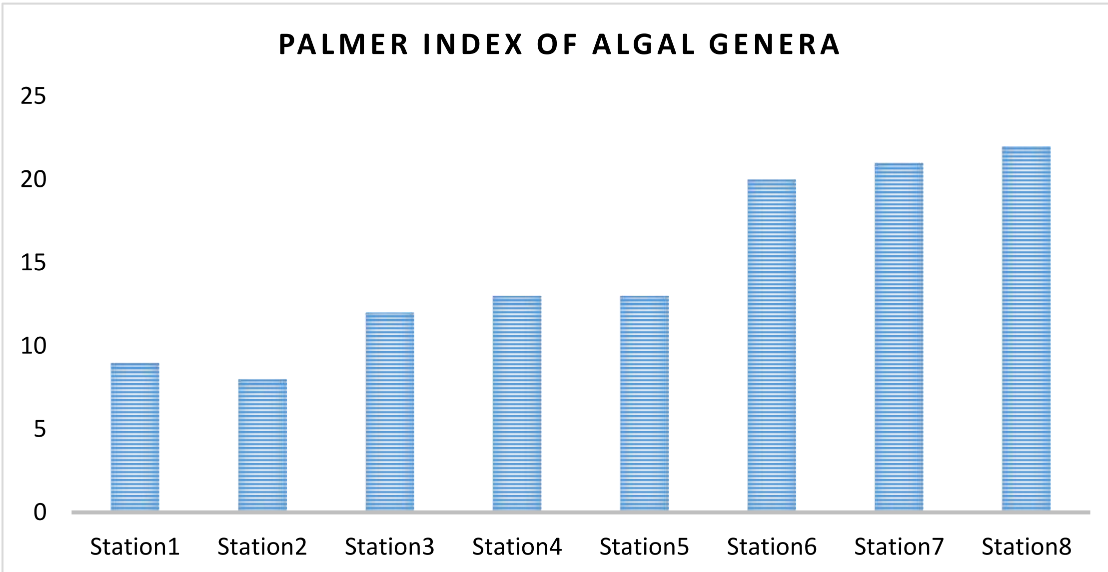


Figure14: Figure shows the palmer Index of Algal Genera

Physical parameters of water:

Surface water temperature (^{0}C)

The seasonal Surface water temperature variation during the study period is shown is **(Fig. 15).** The minimum seasonal water temperature was found as 17.55 ^{0}C in the winter season (2019) at station 1 and the maximum 30.07 ^{0}C was found at station 8 during monsoon season (2019). Water temperature affects the growth and reproduction of living organisms it has also a significant impact on the density of water.

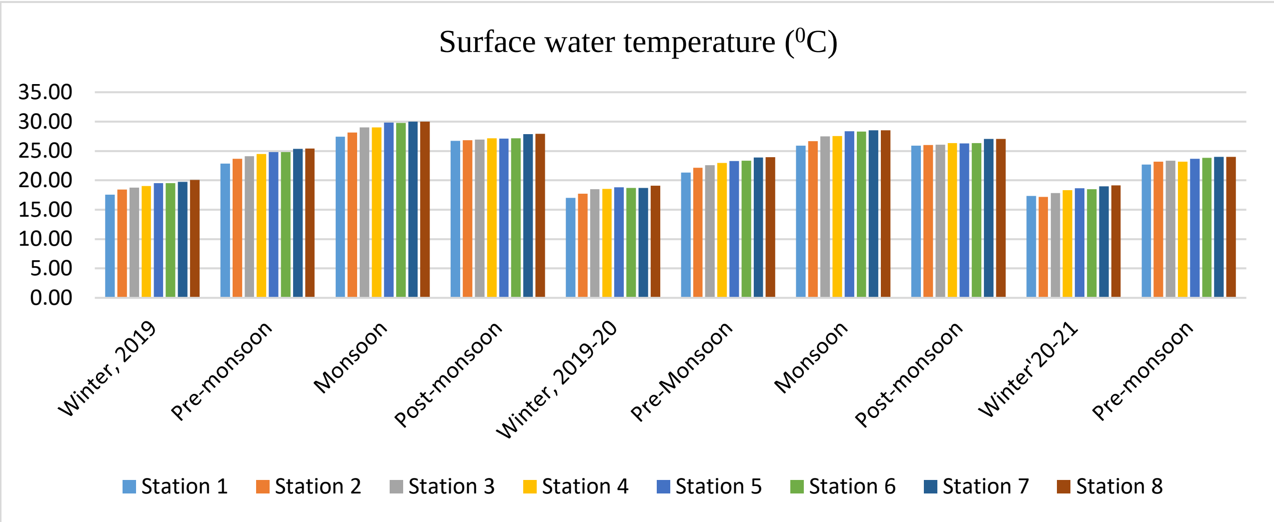


Figure 15: Seasonal variation of surface water temperature (˚c) at eight selected stations (January' 2019 to May' 2021).

Station	Minimum (˚c)	Maximum (˚c)
Station 1	**17.55 (Winter' 2019)**	27.42 (Monsoon' 2019)
Station 2	18.43 (Winter' 2019**)**	28.17 (Monsoon' 2019)
Station 3	18.75 (Winter' 2019)	29.00 (Monsoon' 2019)
Station 4	19.05 (Winter' 2019)	29.03 (Monsoon' 2019)
Station 5	19.50 (Winter' 2019)	29.83 (Monsoon' 2019)
Station 6	19.53 (Winter' 2019)	29.78 (Monsoon' 2019)
Station 7	19.75 (Winter' 2019)	30.05 (Monsoon' 2019)
Station 8	20.08 (Winter' 2019)	**30.07 (Monsoon' 2019)**

Air Temperature (^{0}C)

The air temperature variation during the study period is shown is **(Fig 16).** The minimum seasonal air temperature was found as 19.15 ^{0}C in the winter season (2019-20) at station 1 and the maximum 31.65 ^{0}C was found at station 8 during the monsoon season (2020).

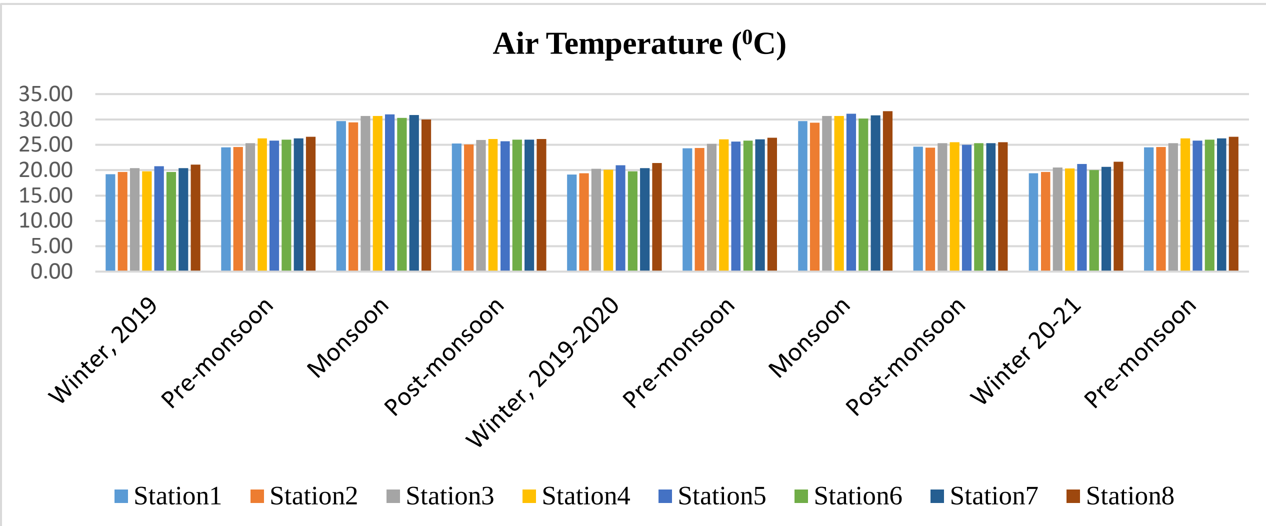


Figure 16: Seasonal variation of air temperature (˚c) at eight selected stations (January' 2019 To May' 2021).

Station	Minimum (˚c)	Maximum (˚c)
Station 1	**19.15 (Winter' 19-20)**	27.42 (Monsoon' 2019)
Station 2	19.41 (Winter' 19-20)	28.17(Monsoon' 2019)
Station 3	20.25 (Winter' 19-20)	29.00(Monsoon' 2019)
Station 4	19.80 (Winter' 2019)	30.67 (Monsoon' 2019)
Station 5	20.75 (Winter' 2019)	31.10 (Monsoon' 2020)
Station 6	19.63 (Winter' 2019)	30.30 (Monsoon' 2019)
Station 7	20.38 (Winter' 2019)	30.85 (Monsoon' 2019)
Station 8	21.13 (Winter' 2019)	**31.65 (Monsoon' 2020)**

Water Velocity (m/s):

During the present study, the seasonal variations in mean water velocity are shown in the (Fig 17). The seasonal mean water current was found maximum at Station 4 during Monsoon' 2020 with an average value of 1.66 m/se and minimum was recorded at station 7 during winter' 2019 with an average velocity of 0.33 m/s.

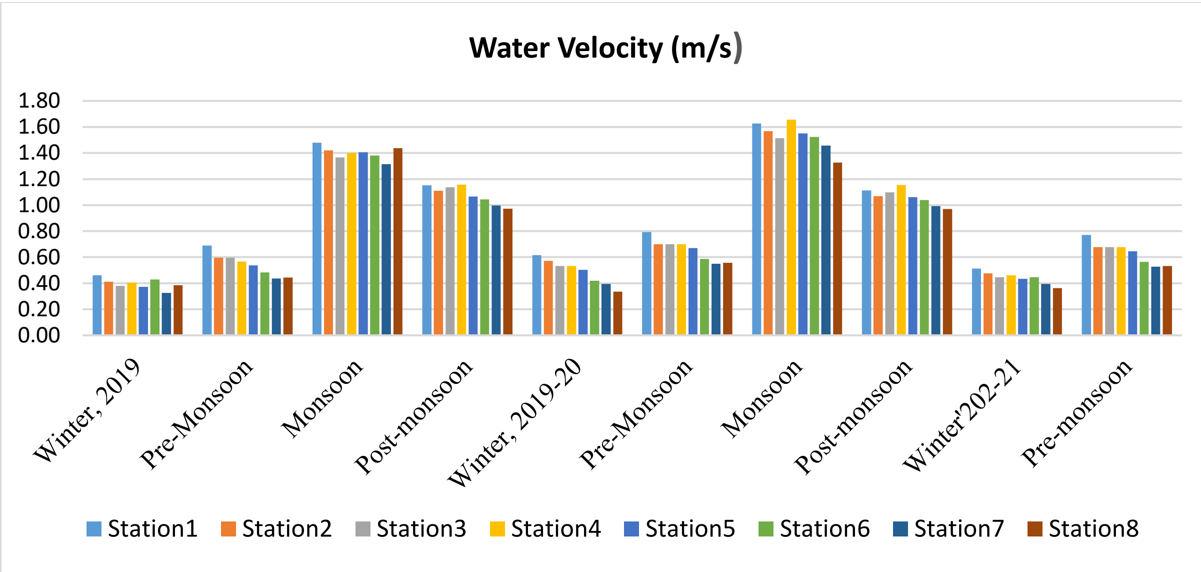


Figure 17: Seasonal variation of water velocity (m/s) at eight selected stations (January 2019 to May' 2021).

Station	**Minimum (m/s)**	**Maximum**
Station 1	0.46 (Winter' 2019)	1.63 (Monsoon'2020)
Station 2	0.41 (Winter' 2019)	1.57 (Monsoon'2020)
Station 3	**0.38 (Winter' 2019)**	1.51(Monsoon'2020)
Station 4	0.41 (Winter' 2019)	**1.66 (Monsoon'2020)**
Station 5	0.37 (Winter' 2019)	1.55 (Monsoon'2020)
Station 6	0.42 (Winter'19-20)	1.52 (Monsoon'2020)
Station 7	**0.33 (Winter' 2019)**	1.46(Monsoon'2020)
Station 8	0.34 (Winter' 19-20)	1.44 (Monsoon' 2019)

Turbidity (NTU)

Water turbidity recorded in River Diyung at all the selected sampling sites ranged from **2.57 NTU–241.50 NTU.** High value of seasonal turbidity was found during monsoon season (2020) at station 8 with value of 215.37 NTU while the lowest seasonal turbidity was recorded with value of 3.92 during winter 2019. The higher value during monsoon seasons might be due to high load of suspended solids, agricultural runoff and sediment loads along with rainwater influx.

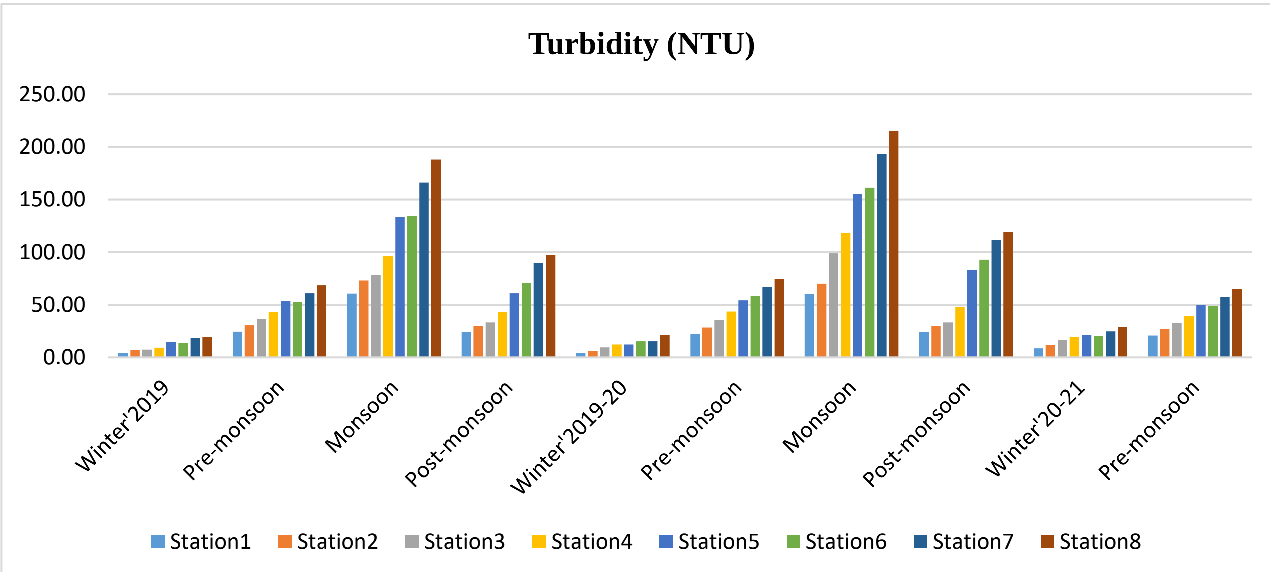


Figure 18: Seasonal variation of dissolved oxygen (mg/l) at eight selected stations (January 2019 To May' 2021).

Station	Minimum	Maximum
Station 1	**3.92 (Winter' 2019)**	60.17 (Monsoon'2020)
Station 2	5.92 (Winter'19-20)	73.01 (Monsoon'2019)
Station 3	7.45 (Winter'2019)	98.85 (Monsoon'2020)
Station 4	9.37(Winter'2019)	118.23 (Monsoon'2020)
Station 5	12.22 (Winter'19-20)	155.37 (Monsoon'2020)
Station 6	13.88 (Winter'2019)	161.38 (Monsoon'2020)
Station 7	15.41 (Winter'19-20)	193.54 (Monsoon'2020)
Station 8	19.32 (Winter'2019)	**215.37 (Monsoon'2020)**

Chemical parameters of water:

Dissolved Oxygen (mg/l)

Dissolved oxygen concentration of water is affected by diffusion and aeration, photosynthesis, respiration and decomposition. **.** Maximum value of seasonal Dissolved oxygen was found during winter season (19-20) at station 1 with value of 8.70 while the lowest seasonal DO was recorded with value of 5.77 during monsoon 2019 at station 7. High value of DO concentration during the winter season may be attributed to lower temperature.

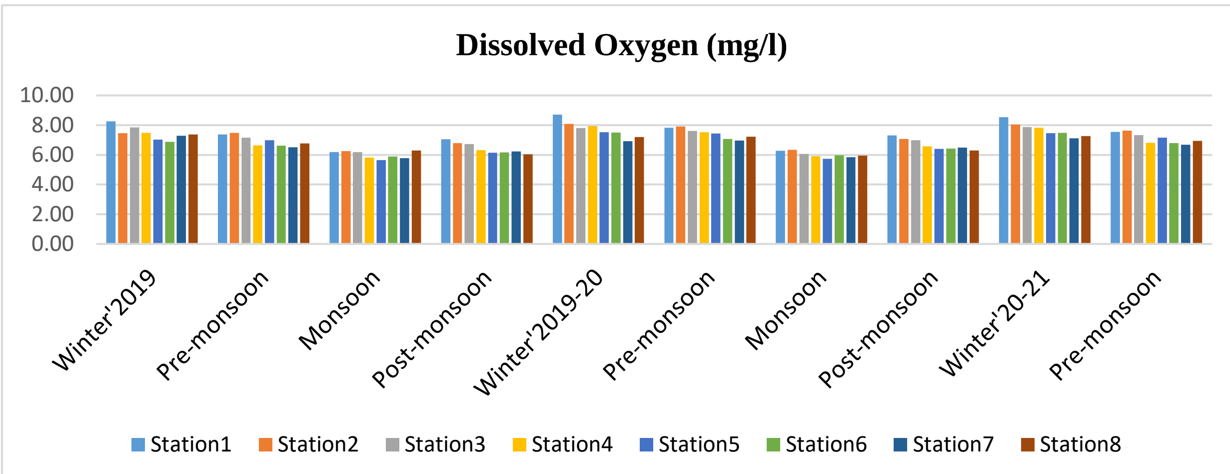


Figure 19: Seasonal variation of dissolved oxygen (mg/l) at eight selected stations (January' 2019 To May' 2021).

Station	**Minimum**	**Maximum**
Station 1	**6.20 (Monsoon' 2019)**	**8.70 (Monsoon'19-20)**
Station 2	6.26 (Monsoon' 2019)	**8.09** (Monsoon'2020)
Station 3	**6.07 (Winter'2020)**	**7.85 (Winter'2019)**
Station 4	5.83 (Monsoon 2019)	7.94 (Winter'19-20)
Station 5	5.65 (Mpnsoon'2019)	7.53 (Winter'19-20)
Station 6	5.88 (Monsoon'2019)	7.50 (Winter' 19-20)
Station 7	**5.77 (Monsoon'2019)**	7.29 (Winter'2019
Station 8	5.94 (Monsoon' 2020)	7.37 (Winter'2019)

pH:

PH recorded in River Diyung at all the selected sampling sites is shown in (Fig 20..). PH value of the entire site remained neutral to alkaline in all the 8 seasons during the investigation period with maximum value of 7.32 during Monsoon’ 2020 at station 3 and minimum value during Monnsoon’2019 having value 7.13 at station 5.

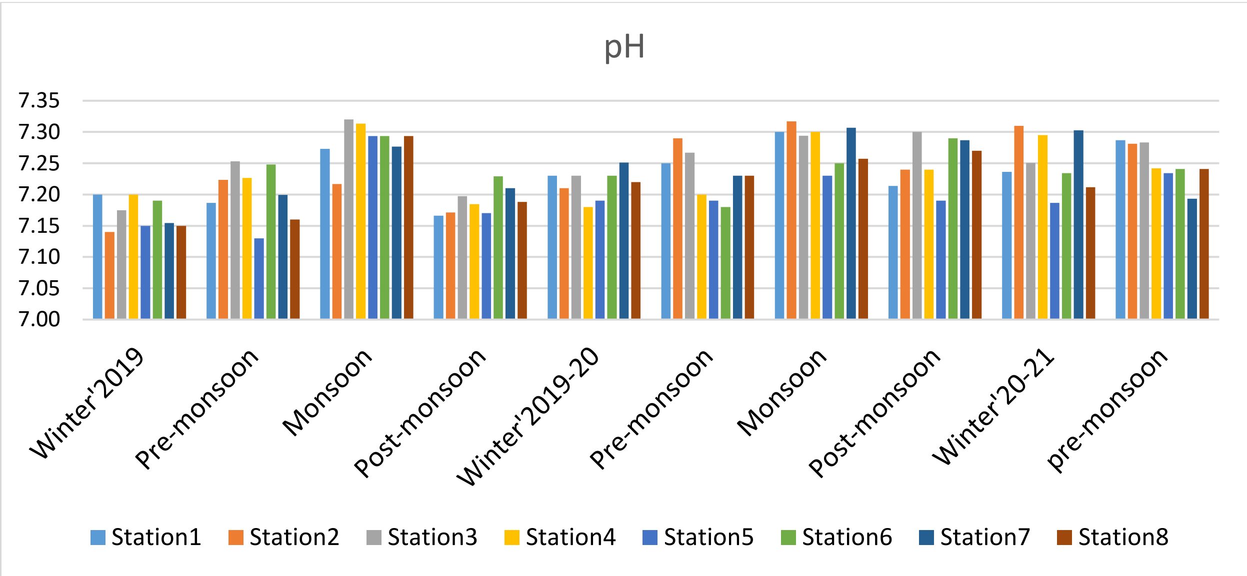


Figure 20: Seasonal variation of pH at eight selected stations (January’ 2019 to May’ 2021).

Station	Minimum	Maximum
Station 1	7.17 (Post-monsoon 2019)	7.30(Monsoon’2020)
Station 2	7.14 (Winter’2019)	7.31(Monsoon’2020)
Station 3	7.18 (Winter’2019)	**7.32(Monsoon’2020)**
Station 4	7.18 (Post-monsoon 2019)	7.31(Monsoon’2020)
Station 5	7.13 (Mpnsoon’2019)	7.29(Monsoon’2020)
Station 6	7.18 (Pre-monsoon’2020)	7.29 (Post-monsoon’2020)
Station 7	7.15(Winter’2019)	7.31 (Monsoon’2020)
Station 8	7.15(Winter’2019)	7.29 (Monsoon’2019)

Hardness (mg/l)

Station wise seasonal variations in hardness were shown in (Fig 21). The seasonal variations of hardness was found highest with value 88.65 in Monsoon season (2020) and minimum was recorded during monsoon season (2019) at station 3 with value of 57.32.

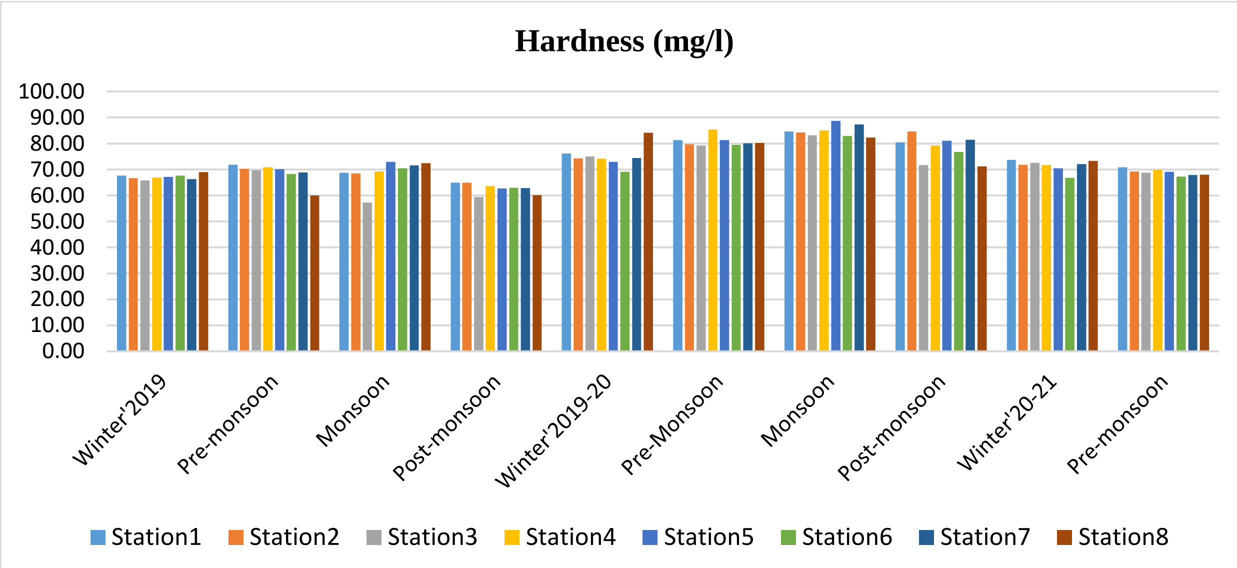


Figure 21: Seasonal variation of hardness (mg/l) at eight selected stations. (January' 2019 to May' 2021).

Station	**Minimum**	**Maximum**
Station 1	64.93(Post-monsoon' 2019)	84.570 (Monsoon'2020)
Station 2	64.89(Post-monsoon' 2019)	84.578 (Post-onsoon'2020)
Station 3	**57.32 (Monsoon'2019)**	83.150 (Monsoon'2020)
Station 4	63.52(Post-monsoon'2019)	85.345 (Pre-monsoon'2020)
Station 5	62.69(Post-monsoon'2019)	**88.657 (Monsoon'2020)**
Station 6	67.61 (Pre-monsoon'2020)	82.850(Monsoon'2020)
Station 7	62.78 (Post-monsoon'2019)	87.357(Monsoon'2020)
Station 8	60.04 (Pre-monsoon'2019)	84.150 (Winter'19-20)

Total Alkalinity (mg/l)

Station wise seasonal variations in alkalinity were shown in (**Fig- 22)**. The seasonal variations of alkalinity was found highest with value 81.48 in Monsoon season (2020) and minimum was recorded during winter season (2019) at station 1 with value of 49.36.

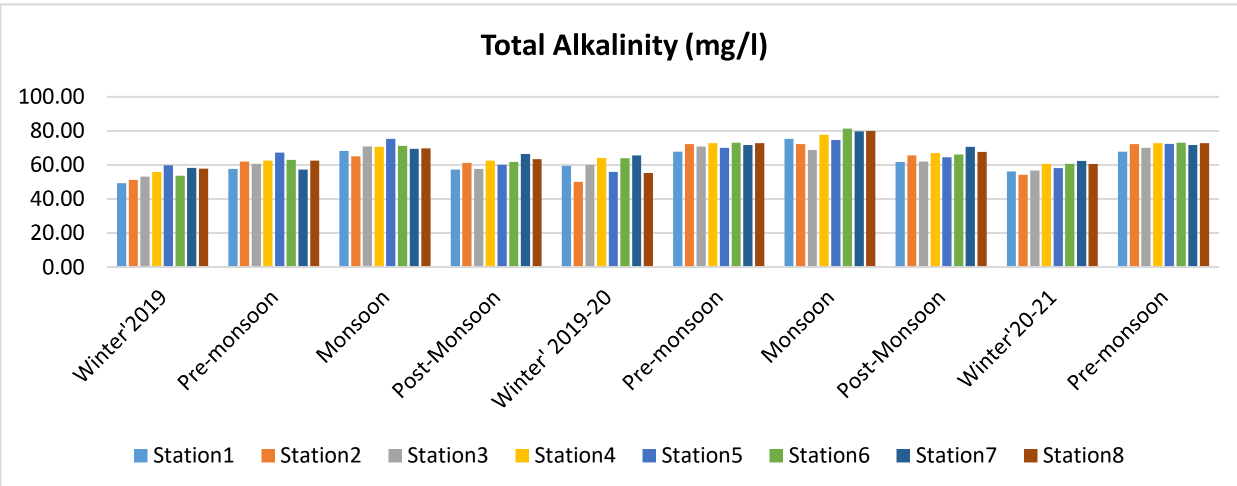


Figure 22: Seasonal variation of alkalinity (mg/l) at eight selected stations. (January' 2019 to May' 2021).

Station	Minimum	Maximum
Station 1	**49.36 (Winter' 2019)**	75.33(Monsoon'2020)
Station 2	50.22 (Winter' 19-20)	72.28(Monsoon'2020)
Station 3	53.23(Winter' 2019)	70.83 (Monsoon'2019)
Station 4	55.86(Winter' 2019)	77.83 (monsoon'2020)
Station 5	56.00 (Winter' 19-20)	75.45 (Monsoon'2019)
Station 6	53.79 (Winter' 2019)	**81.48 (Monsoon'2020)**
Station 7	57.38 (Pre-monsoon'2019)	79.72(Monsoon'2020)
Station 8	55.21 (Winter' 19-20)	80.00(Monsoon'2020)

Conductivity (uS/cm)

Electrical conductivity of water was measured to find the concentration of salt/ion content in the River. Seasonal maximum value of EC was observed in monsoon seasons (2020) with value 173.64 uS/cm at station 8 and minimum value with 99.82 uS/cm of EC was recorded during winter seasons 2019 at station 1.

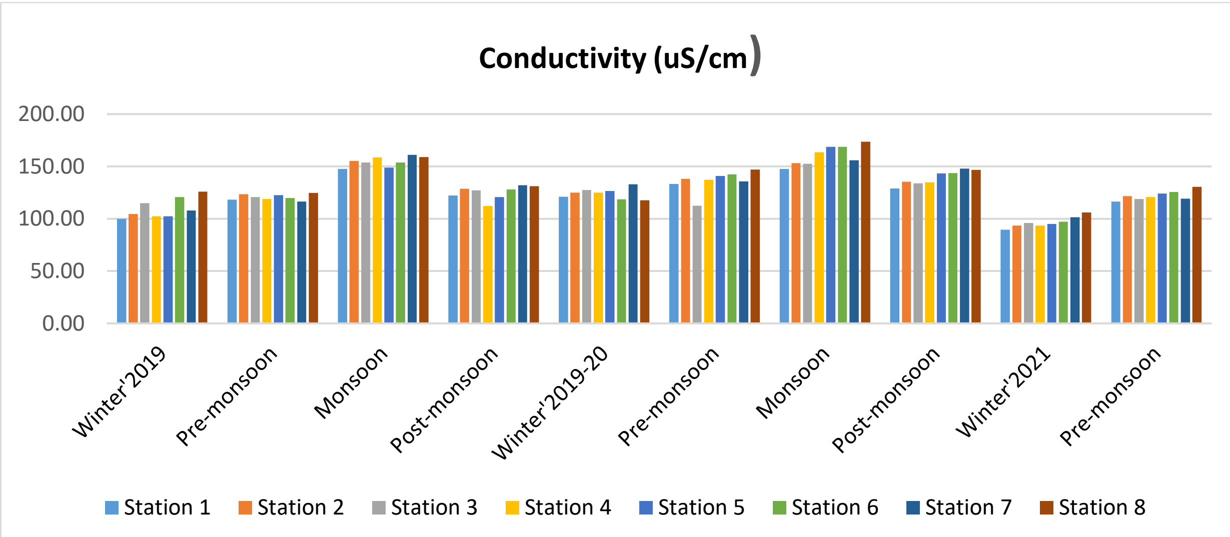


Figure 23: Seasonal variation of conductivity (uS/cm) at eight selected stations (January' 2019 to May' 2021).

Station	**Minimum**	**Maximum**
Station 1	**99.82 (Winter' 2019)**	147.77(Monsoon'2020)
Station 2	104.47(Winter' 2019)	155.13(Monsoon'2019)
Station 3	112.40 (Pre-monsoon'2020)	153.59 (Monsoon'2019)
Station 4	102.39(Winter' 2019)	163.65(monsoon'2020)
Station 5	102.46(Winter' 19-20)	168.68(Monsoon'2020)
Station 6	118.63(Winter'19-20)	168.62(Monsoon'2020)
Station 7	108.02(Winter' 2019)	161.05(Monsoon'2019)
Station 8	117.68(Winter' 19-20)	**173.64(Monsoon'2020)**

Total Dissolved Solid (mg/l)

The seasonal average total dissolved solids variations are shown in (**Fig 24).** The maximum TDS value was recorded during monsoon' 2019 of 119.43 and minimum value was recorded during winter 2019-20 with value of 62.69.

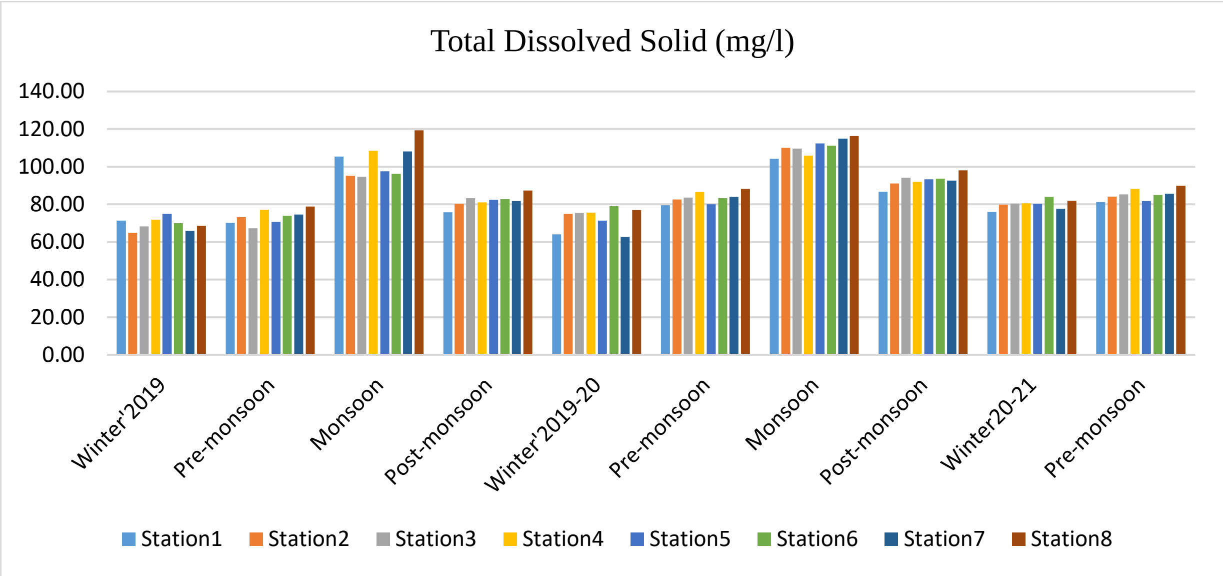


Figure 24: Seasonal variation in total dissolved solids (mg/l) of selected stations (January' 2019 To May' 2021).

Station	**Minimum**	**Maximum**
Station 1	64.02 (Winter' 19-20)	105.34 (Monsoon'2019)
Station 2	64.96 (Winter' 2019)	110.06 (Monsoon'2020)
Station 3	67.28 (Pre-monsoon'2019)	109.63 (Monsoon'2020)
Station 4	71.85 (Winter' 2019)	108.53 (monsoon'2019)
Station 5	70.71 (Pre-monsoon' 19-20)	112.48 (Monsoon'2020)
Station 6	70.07(Winter'2019)	111.20 (Monsoon'2020)
Station 7	**62.69 (Winter'19-20)**	115.00 (Monsoon'2020)
Station 8	68.64 (Winter' 2019)	**119.43(Monsoon'2019)**

Biochemical Oxygen Demand$_3$ (BOD_3)

The seasonal variation of Biochemical Oxygen Demand are shown in **Fig 25.** The maximum BOD_3 value was recorded during monsoon' 2020 at station 6 with value 24.02 and minimum value was recorded during winter 2019 with value of 2.09 at station 1.

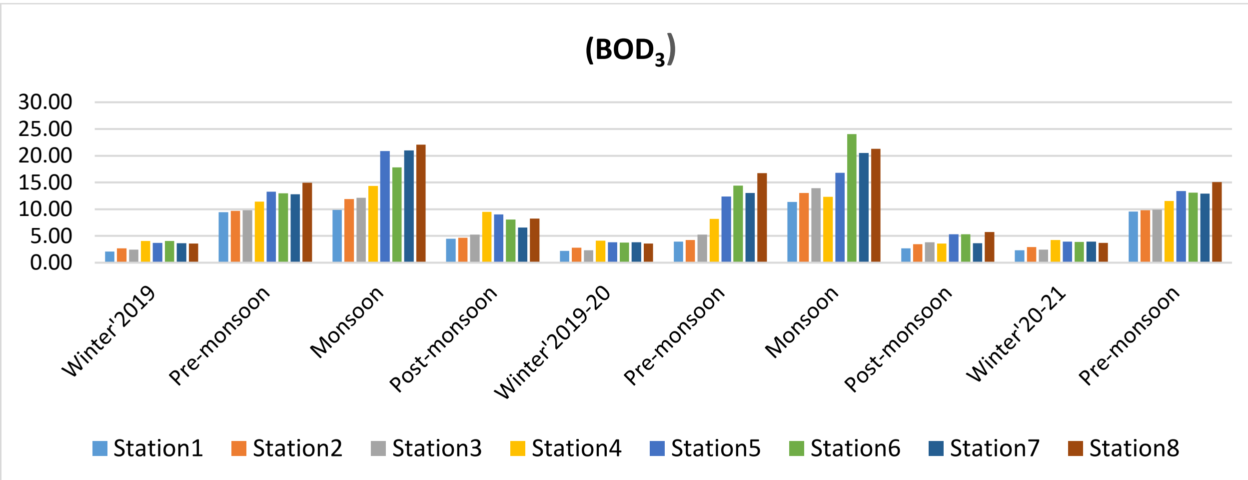


Figure 25: Seasonal variation in BOD_3 (mg/l) of selected stations (January' 2019 to May' 2021).

Station	Minimum	Maximum
Station 1	**2.09 (Winter' 2019)**	11.33 (Monsoon'2020)
Station 2	2.69 (Winter' 2019)	13.01 (Monsoon'2020)
Station 3	2.31 (Winter'19-20)	13.95 (Monsoon'2020)
Station 4	4.05 (Winter' 2019)	14.33 (Monsoon'2019)
Station 5	3.69 (Winter' 2019)	20.88 (Monsoon'2019)
Station 6	3.73 Winter'19-20)	**24.02 (Monsoon'2020)**
Station 7	3.67 Winter' 2019)	20.99 (Monsoon'2019)
Station 8	3.58 Winter' 2019)	22.06 (Monsoon'2019)

Chemical Oxygen Demand (COD) (mg/l)

The seasonal variation of total dissolved solids are shown in (**Fig-26).** The maximum COD value was recorded during monsoon' 2020 at station 8 with value 45.99 and minimum value was recorded during winter 2019-20 with value of 3.01 at station 1.

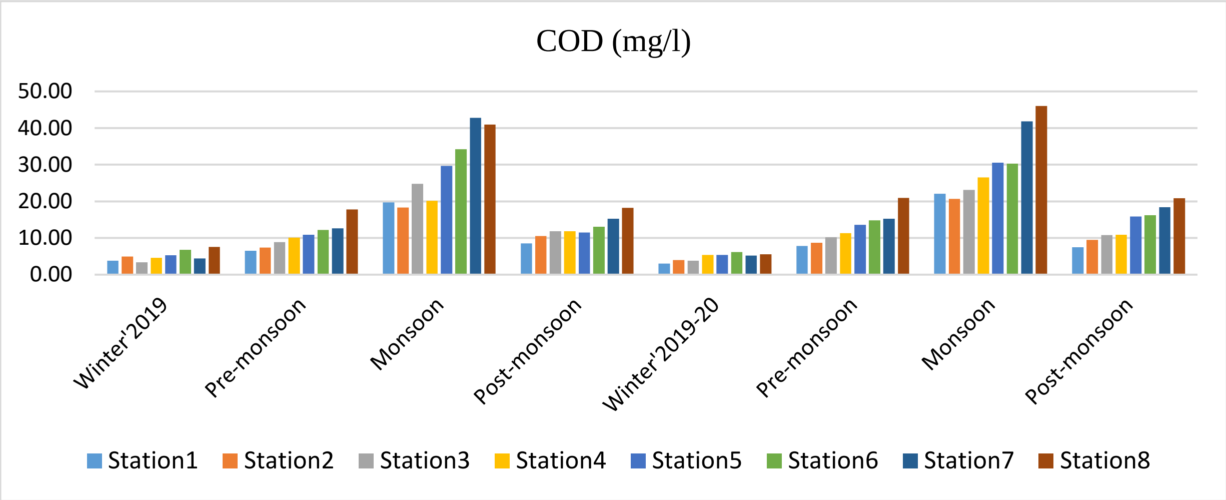


Figure 26: Seasonal variation in COD (mg/l) of selected stations (January' 2019 to May 2021).

Station	**Minimum**	**Maximum**
Station 1	**3.01 (Winter' 19-20)**	22.09 (Monsoon'2020)
Station 2	3.95 (Winter' 19-20)	20.69(Monsoon'2020)
Station 3	3.38 (Winter'2019)	24.81 ((Monsoon'2019)
Station 4	4.60 (Winter'2019)	26.55(monsoon'2020)
Station 5	5.29 Winter'2019)	30.54(Monsoon'2020)
Station 6	6.17(Winter'19-20)	34.24(Monsoon'2019)
Station 7	4.43(Winter' 2019)	42.81(Monsoon'2019)
Station 8	5.58(Winter' 19-20)	**45.99 (Monsoon'2020)**

Nitrate Nitrogen (NO_3):

The seasonal variation of Nitrate Nitrogen as shown in (**Fig 27).** The maximum NO_3 value was recorded during mmonsoon2019 at station 8 with a value of 0.421 and minimum value was recorded during winter 2019 with the value of 0.011 at station 1.

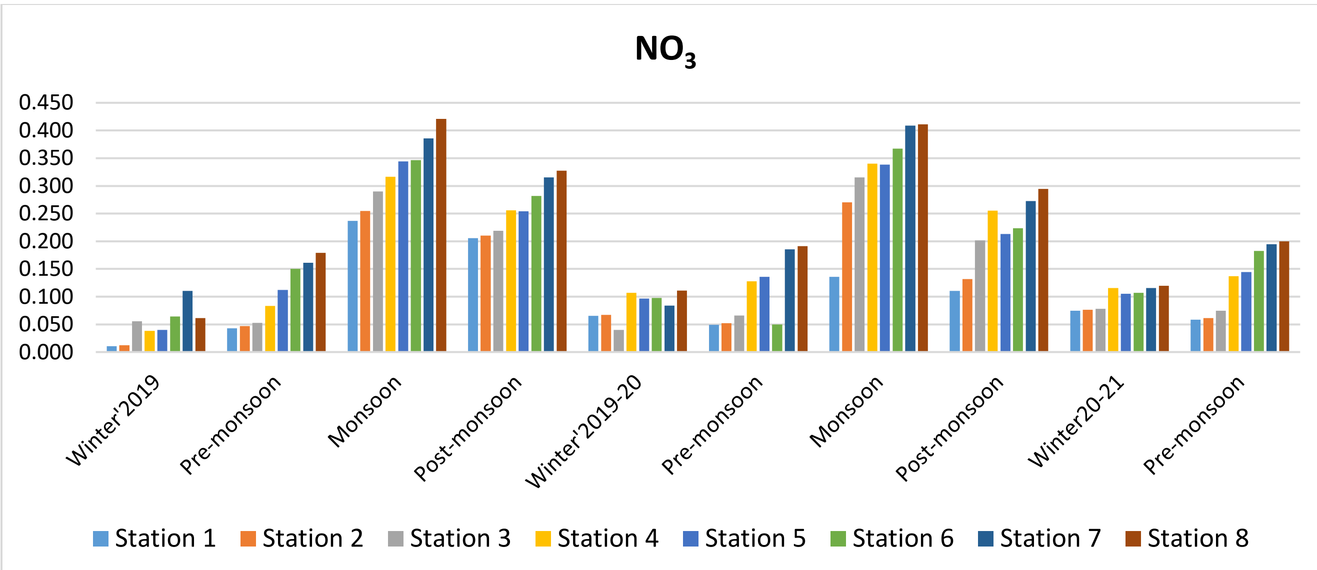


Figure 27: Seasonal variation in $\mathbf{NO_3}$ (mg/l) of selected stations (January 2019 to May2021).

Station	**Minimum**	**Maximum**
Station 1	**0.011(Winter'2019)**	0.237 (Monsoon'2019)
Station 2	0.013(Winter'2019)	0.270 (Monsoon'2020)
Station 3	0.040(Winter' 19-20)	0.315(Monsoon'2020)
Station 4	0.038 (Winter'2019)	0.340(Monsoon'2020)
Station 5	0.040 (Winter'2019)	0.344(Monsoon'2019)
Station 6	0.050 ('Pre-monsoon 19-20)	0.368(Monsoon'2020)
Station 7	0.084 (Winter' 19-20)	0.409(Monsoon'2020)
Station 8	0.062 (Winter' 19-20)	**0.421(Monsoon'2019)**

Nitrite Nitrogen (NO_2)

The seasonal variation of Nitrate Nitrogen are shown in (**Fig 29).** The maximum NO_2 value was recorded during monsoon' 2020 at station 8 with value 0.047and minimum value was recorded during winter 2019 with value of 0.002 at station 1.

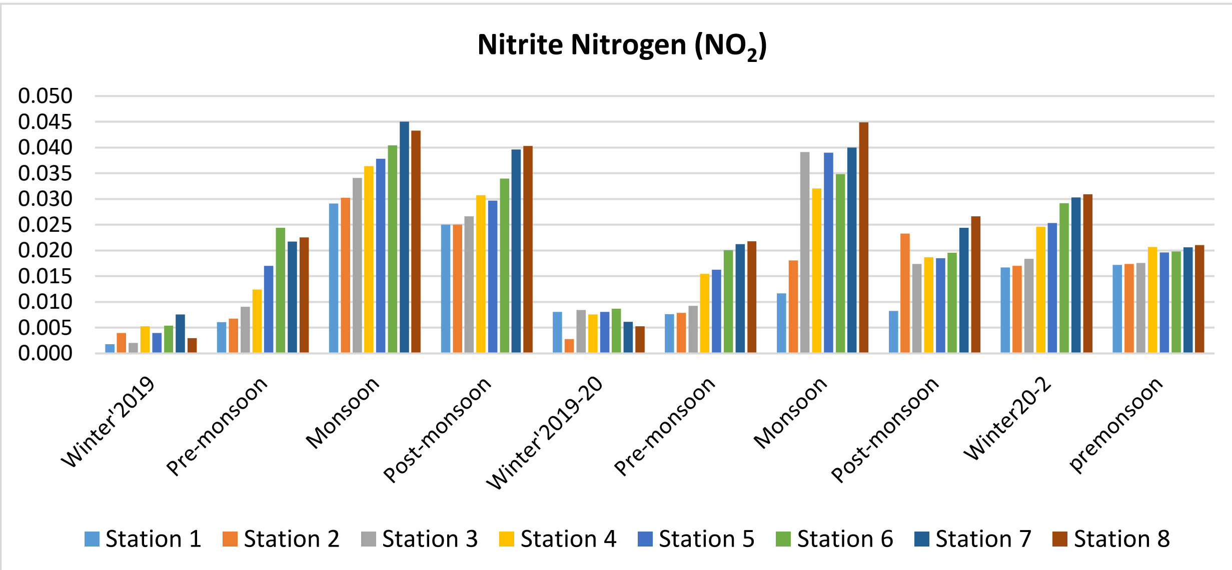


Figure 28: Seasonal variation in **NO_2** (mg/l) of selected stations (January' 2019 to May' 2021).

Station	**Minimum**	**Maximum**
Station 1	**0.002 (Winter'2019)**	0.029(Monsoon'2019)
Station 2	0.003 (Winter'19-20)	0.030(Monsoon'2019)
Station 3	0.002(Winter'2019)	0.039 (Monsoon'2020)
Station 4	0.005(Winter'2019)	0.036 (Monsoon'2019)
Station 5	0.004(Winter'2019)	0.039(Monsoon'2020)
Station 6	0.005(Winter'2019)	0.040(Monsoon'2019)
Station 7	0.006 (Winter'19-20)	0.045(Monsoon'2019)
Station 8	0.003 (Winter'2019)	**0.047(Monsoon'2020)**

Total Ammonia (NH_4):

The seasonal variation of Total Ammonia are shown in (**Fig 29).** The maximum NH_4 value was recorded during monsoon' 2020 at station 8 with value 0.2157 and minimum value was recorded during winter 2019-20 with value of 0.290 at station 1.

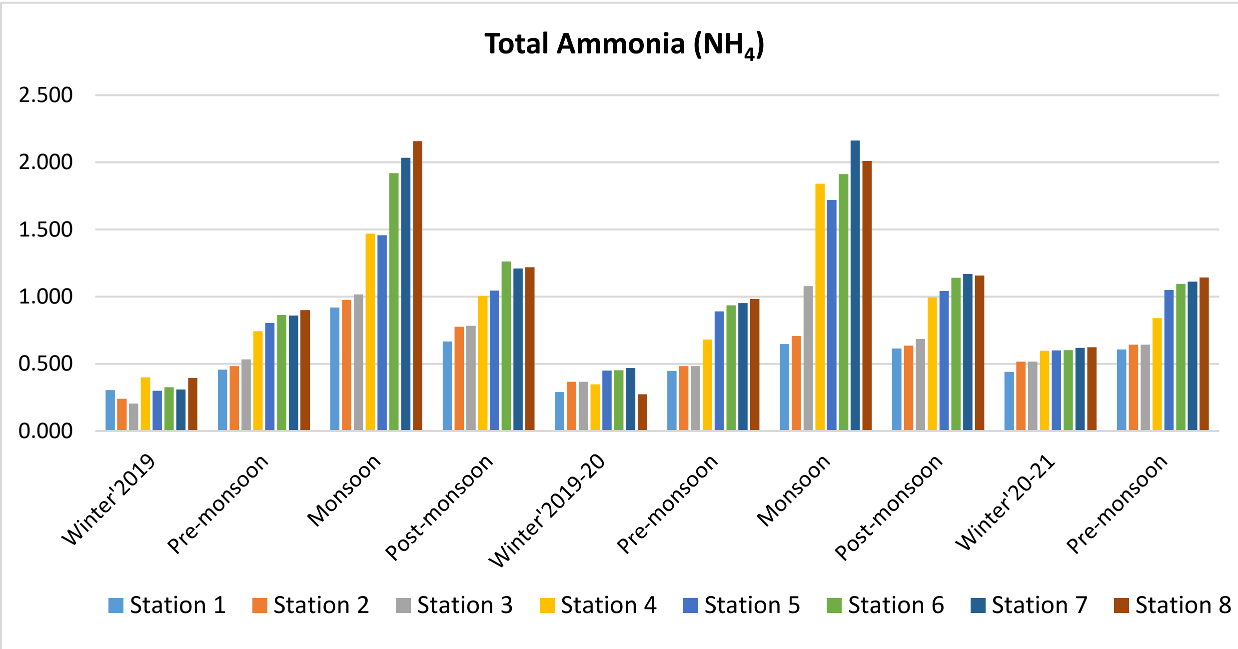


Figure 29: Seasonal variation in NH_4 (mg/l) of selected stations (January' 2019 to May' 2021).

Station	**Minimum**	**Maximum**
Station 1	**0.290 (Winter'19-20)**	0.919 (Monsoon'2019)
Station 2	0.240 (Winter'2019)	0.975 (Monsoon'2019)
Station 3	0.205 (Winter'2019)	1.078 (Monsoon'2020)
Station 4	0.347 Winter'19-20)	1.841 (Monsoon'2020)
Station 5	0.300 (Winter'2019)	1.720 (Monsoon'2020)
Station 6	0.325 (Winter'2019)	1.920 (Monsoon'2019)
Station 7	0.310 (Winter'2019)	2.163 (Monsoon'2019)
Station 8	0.273 (Winter'19-20)	**2.157 (Monsoon'2020)**

Soil parameters

Sediment pH: Sediment pH measures the acidic and alkaline condition of the river bed which has a direct or indirect influence on water pH and nutrient circulation. The findings of the present study indicate that sediment pH varied between 5.85 (Pre-Monsoon, 2019) to 7.26 (Monsoon, 2020).

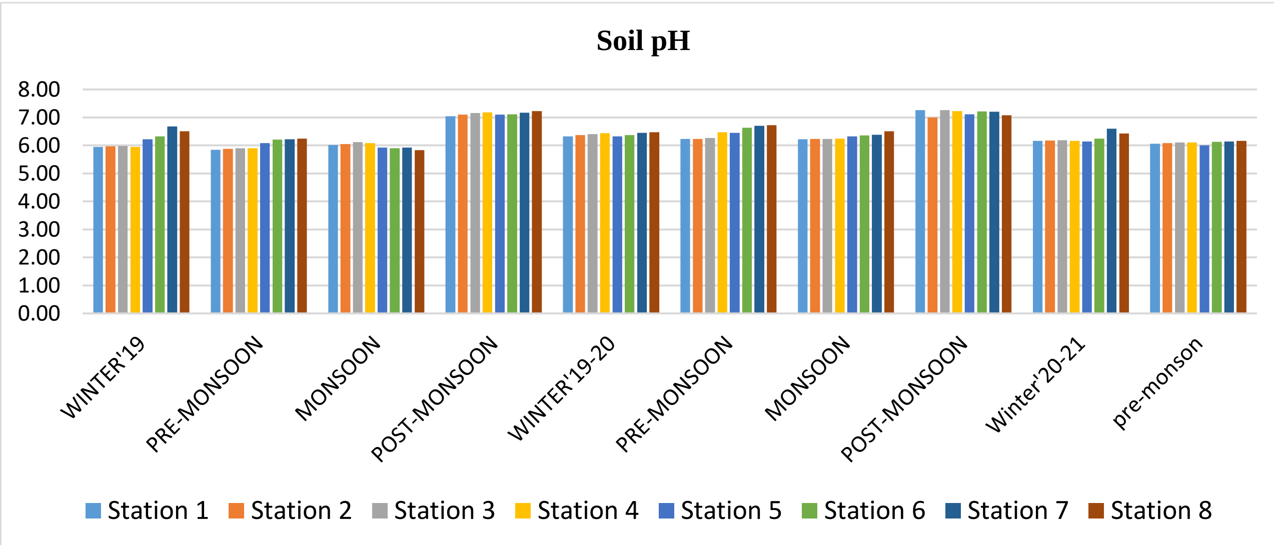


Figure 30: Seasonal Variation of Sediment pH at Station 1-8

Station	**Minimum**	**Maximum**
Station 1	**5.85 (Pre-monson'2019)**	**7.26 (Monsoon'2020)**
Station 2	5.88(Pre-monsoon'2019)	7.10(post-Monsoon'2019)
Station 3	5.90(Pre-monsoon'2019)	7.26(post-Monsoon'2020)
Station 4	5.90(Pre-monsoon'2019)	7.23(post-Monsoon'2020)
Station 5	5.92(Monsoon'2019)	7.11(post-Monsoon'2020)
Station 6	5.90(Monsoon'2019)	7.22(post-Monsoon'2020)
Station 7	5.93(Monsoon'2019)	7.20(post-Monsoon'2020)
Station 8	5.83(Monsoon'2019)	7.23(Post-Monsoon'2019)

Sediment Organic Carbon:

In the present investigation Sediment, Organic Carbon percentages were found within the range of 0.29-2.63%, minimum during winter and maximum during Monsoon season.

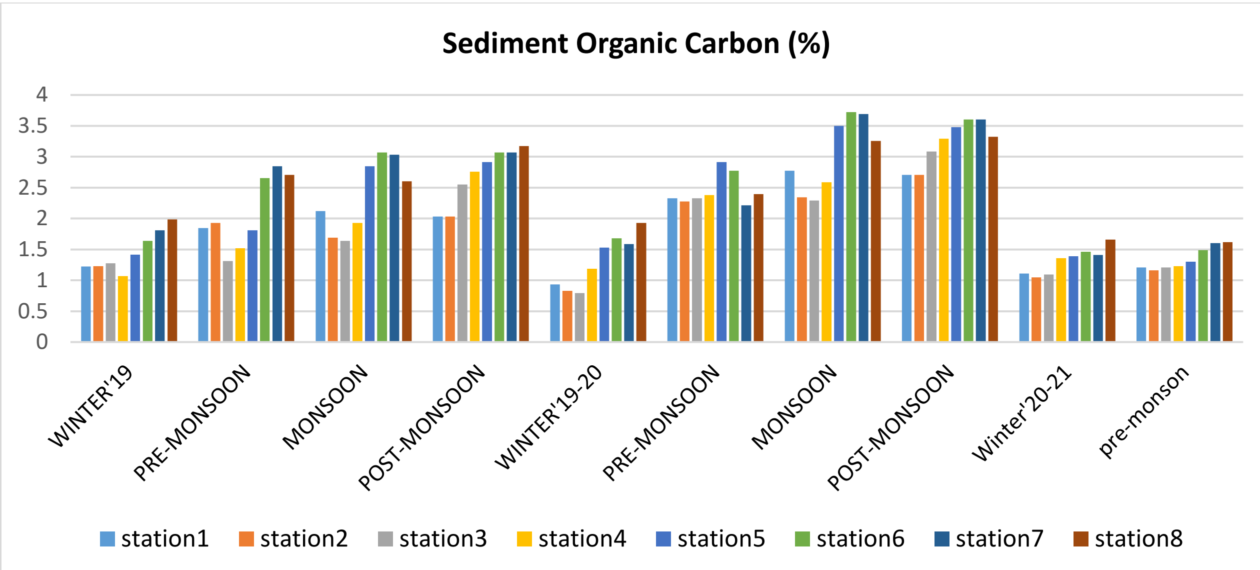


Figure 31: Seasonal Variation of Sediment Organic Carbon at Station 1-8

Station	**Minimum**	**Maximum**
Station 1	0.63 (Winter’19-20)	1.57(Monsoon’2020)
Station 2	**0.58 (Winter’19-20)**	1.57(Monsoon’2020)
Station 3	0.584 (Winter’2019)	1.87(Monsoon’2020)
Station 4	0.69 (Winter’19-20)	1.91 (Monsoon’2019)
Station 5	0.83 (Winter’19-20)	1.71 (Monsoon’2020)
Station 6	0.88 (Winter’19-20))	1.78(Monsoon’2019)
Station 7	0.902 (Winter’2019)	1.78(Monsoon’2019)
Station 8	1.12 (Winter’19-20)	**1.90 (Monsoon’2020)**

Sediment Organic Matter: Sediment organic matter of the present investigation ranged from 0.93 to 3.68 %.

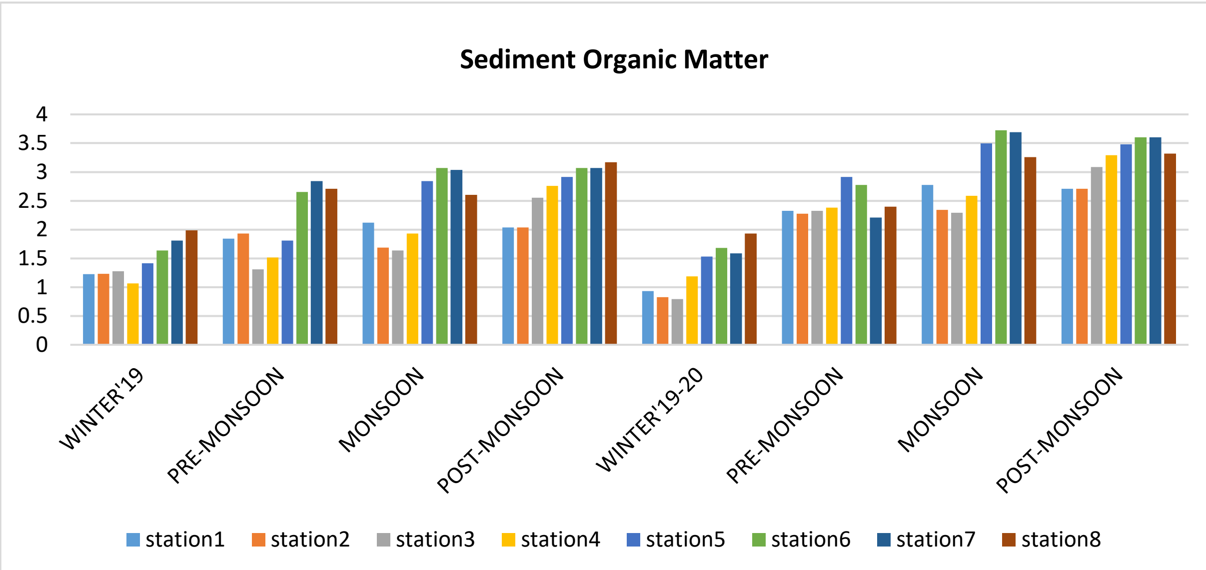


Figure 32: Seasonal Variation of Sediment Organic Carbon at Station 1-8

Station	Minimum	Maximum
Station 1	0.93096(Winter'19-20)	2.77564(Monsoon'2020)
Station 2	0.82752(Winter'19-20)	2.34464(Monsoon'2020)
Station 3	0.79304(Winter'19-20)	3.08388 (Post-monsoon2020)
Station 4	1.067124(Winter'2019)	3.29076(Post-monsoon2020)
Station 5	1.41712(Winter'2019)	3.49972(Monsoon'2020)
Station 6	1.6378(Winter'2019)	3.72384(Monsoon'2020)
Station 7	1.58608(Winter'19-20)	3.68936(Monsoon'2020)
Station 8	1.93088(Winter'19-20)	3.3212(Post-Monsoon'2020)

Conservation status of fish diversity

Environmental degradation results from damming, water pollution and naturally occurring changes are known to affect the fish. Indiscriminate exploitation of fish, pollution threats by various sources like industrial, agricultural and other harmful wastes lead to vanish the fish. Assessing and monitoring the status of fish of a particular region is very crucial for its conservation, because the local population of fish reflects the uniqueness of local habitat conditions and ultimately depicts the global diversity. Hence, much attention should be given to conserve the local fish population. Therefore, sustainable conservation strategies should be formulated to conserve the species which are facing the threats presently or in near future. Decline in the biodiversity are far greater in freshwater than in the most affected terrestrial ecosystems (Sala *et al.,* 2000). In the present study, out the 81 fish species, 9 (11.11%) viz. *Neolissochilus hexagonolepis*, *Neolissochilus hexastichus, Labeo pangusia, Notopterus chitala, Ompok bimaculatus, Glyptothorax striatus, Bagarius bagarius, Ailia coila* and *Anguilla* were recorded as near threatened (NT), only 2 (2.44%) *Badis assamensis* and *Tor tor* as data deficient (DD), 2 (2.44%) *Botia rostrata* and *Wallago attu* as Vulnerable, 1 (1.23%) *Tor putitora* Endangered (EN), 4 (6.13%) *Opsarius ngawa, Psilorhynchus nahlongthai, Schistura fasciata, Strongylura leura* not Evaluated (NE) and more than half of the existing fish species 76.54 % of this river were found to be in the least concern (LC) category.

The present study shows that many species are in tremendous stress in the selected rivers and which may be due to over and indiscriminate fishing, destruction of fish habitats, sand and boulder extraction and lack of knowledge on fish ecology. In the present study it is clear that the species like *Neolissochilus hexagonolepis*, *Neolissochilus hexastichus, Labeo pangusia, Notopterus chitala, Ompok bimaculatus, Glyptothorax striatus, Bagarius bagarius, Ailia coila* and *Anguilla* are in critical condition which needs the protection of habitats. These species were represented only in a few sites thereby they were characterized by low abundance, narrow distribution, and high treats. Although, *Tor putitora* is an endangered species the abundance was fairly good but it is still in high risk. *Tor tor* is a data deficient species due to less abundance and hence high risk.

Major Anthropogenic factors:

Sand and Stone quarries: Stone quarrying and Sand mining from the Diyung rivers is a man-made activity responsible for water pollution and as well as habitat destruction for fish species. Semi torrential fishes of the genus *Lepidicephalacthyes, Schistura, Acanthocobitis, Crossocheilus, Amblyceps, Pillaia, Botia* are mostly at risk due to sand and pebble mining, because these fishes takes shelter under sand, pebble and crevices1

Over exploitation of fishes using destructive fishing methods: Dima Hasao has witnessed a significant decrease in wild fish production due the over exploitation of fishes. Hill stream fishes are not in abundance but somehow are available and seem to take shelter under the crevices of rocks and so catching hill stream fishes is not easy, so local people have resorted to destructive fishing methods. The generally used plant derivatives or chemicals in streams or rivers to catch fishes in large scale within short period. The common plants used as fish sedatives are *Ru-gjao phang* **(***Millettia pachycarpa*), *Ru panthao* (*Randia spinosa*), *Agurdukha (Croton caudatus), Suji (Acacia pinnata), Jengreng (Albizzia)* and *Mejen* (*Zanthoxylum alatum*).

Exit Strategy and Sustainability.

- The ichthyofaunal resources of river Diyung of Northeast India exhibit a combination of both torrential and plain water forms, together with cold as well as warm water species.
- In the hill district, the river offers a lot of potential for recreational fishing and ecotourism, which will boost the local economy.
- The creation of ornamental fish-culture units with full technical support is intended to have a multiplier effect on the area's aquaculture enterprises in the area. It will not only improve the socioeconomic position of the district's rural residents but will also save the fish from extinction.
- However, it has been found that the relative abundance of some of the important species including mahseer (*Tor*, *Neolissochilus*) along with other coldwater species is in a declining state as reported by the fishers and local stakeholders. As a result, it is critical to save this threatened species from the extinction in near future.
- In order to protect the habitats requirements of migratory and other hill stream fishes, sand and boulder extraction activities from river beds should be completely forbidden.

- Existing state fishery legislation limiting fishing during the breeding season and the use of other damaging fishing gear, among other things, should be properly enforced.

Acknowledgement.

I, Abdul Malik Ahmed (HJRF_002) would like to acknowledge National Mission on Himalayan Studies (NMHS) (Project ID: GBPNI/NMHS-17-18/HSF-04/600), Ministry of Environment Forest and Climate Change (MoEFCC) and Nodal institute GBPNIHESD, Kosi-Katarmal, Almora, for all the financial support provided to carry out the present study under the Himalayan Research Fellowship Programme.

Appendix-1

College of Fisheries, Assam Agricultural University (AAU)
Raha, Assam
In Partnership with
National Institute of Agricultural Extension Management (MANAGE)
(An Organization of Ministry of Agriculture & Farmers Welfare, Govt. of India)
Rajendranagar, Hyderabad
Sponsored by
National Fisheries Development Board (NFDB)
Ministry of Animal Husbandry, Dairying & Fisheries, Govt. of India
Rajendranagar, Hyderabad

ID. No: ACADP/CoF, AAU, Raha-24/2019-20

Certificate of Participation

This is to Certify that Mr./Ms...... **Abdul Malik Ahmed** .....*participated in a 30 days Skill Development Certificate training Course on* ***"Aqua Clinics & Aquapreneurship Development Programme (AC&ADP)"*** *conducted at College of Fisheries, Assam Agricultural University (AAU), Nagaon, Assam from 27.08.2019 to 25.09.2019.*

Dr. Saravanan Raj, Director (Agril. Extn.), MANAGE
Dr. S. Borthakur, Nodal Officer, CoF, AAU, Raha
Dr. K.K. Tamuli, Professor & Dean, CoF, AAU, Raha
Executive Director (Tech), NFDB
Smt V. Usha Rani, IAS, Director General, MANAGE

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21 Days National Training Course (NTC-2020)

Organized by
National Agriculture Development Cooperative Ltd. (NADCL)
Baramulla (J & K) 193103
(Established Under Self Reliant Co-operative Act, 1999: Under Ministry of Cooperatives)
Sub-office: HMT, Zainakote, Srinagar- 190012
www.nadclag.in; naagdevcoopltd2018@gmail.com
Co-Organized by
Agro-Environmental Developmental Society (AEDS)
Rampur, Uttar Pradesh - 244901

Certificate

This is to certify that

Abdul Malik Ahmed, Research Scholar, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Assam

successfully completed 21 Days National Training Course (NTC-2020) on "Preparing and Management of Result Oriented Research Projects in Agriculture, Horticulture, Animal Husbandry and Allied Sectors" organized by National Agriculture Development Cooperative Ltd. (NADCL) Baramulla (Jammu and Kashmir) co-organized by Agro-Environmental Developmental Society (AEDS) Rampur (Uttar Pradesh) during 26th May to 15th June 2020.

Dr. Ratna Nashine
Organizing Director
Professor and Dean, COA & RS
(IGKV, Raipur) Chhattisgarh

(P. Qamar-ud-Din Shah)
Chairman
NADCL, Baramulla (J & K)

Appendix-2

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DocID: https://connectjournals.com/03895.2021.24.1101 | eISSN 0976-1780

LENGTH-WEIGHT RELATIONSHIP OF FOUR FISH SPECIES (FAMILY-CYPRINIDAE) FROM RIVER DIYUNG, BRAHMAPUTRA DRAINAGE NORTH EASTERN, INDIA

A. M. Ahmed*, R. Dutta, S. K. Bhagabati, H. Pokhrel, D. Nath, R. Sarmah and L. P. Mudoi

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ABSTRACT : The present investigation was carried out on 307 fish species collected from river Diyung of Assam, India for the first time to observe length-weight relationship for four fresh water fish species namely, *Garra lissorhynchus* (McClelland, 1842), *Barilius barila* (Hamilton, 1822), *Opsarius bendelisis* (Hamilton, 1807) and *Tariqilabeo latius* (Hamilton, 1822). The estimated allometric coefficient 'b' values ranged from 2.78-2.90 and all the LWRs were highly significant with p<0.01. Among these, the LWRs estimates for two species was not yet been reported as per FishBase (Froese and Pauly, 2019).

Key words : Brahmaputra, biomass, Borail range, freshwater, length-weight.

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INTRODUCTION

Assam is drained by numerous rivers, which run into total length of 4820 km (Das *et al*, 2017; Das *et al*, 2018) including Brahmaputra and Barak, the two major rivers of the state (Borah *et al*, 2014a). Diyung is the longest river of Dima Hasao district of Assam (25.18°N, 93.03°E), which originates from Borail mountain range and traverses a course of 240 km before joining Kopili, a southern tributary of river Brahmaputra. These rivers harbor rich repository of fish species and numerous studies on their biology, population parameters, length-weight relationships etc. has been carried out over the years. A length-weight relationship is an important tool in fishery management. LWRs can be used for estimating the weight corresponding to a given length and so to determine fish condition (Forese, 2006; Lee Cren, 1991). It establishes the mathematical relationship between length and weight of the fish and measures the variation from the expected weight for length of individual fish or relevant groups of individuals as indicators of general well-being, fatness, gonadal development etc. (Le Cren, 1951). It provides basic information in fish biology and is useful in determining the weight and biomass from known length data, from length-frequency distribution enables compare species growth from different regions (Koutrakis and Tsikliras, 2003). The LWRs can be used as a character for differentiation of small taxonomic units, as the exponent b may be different for fish of different localities, sexes and stages of developmental process but constant for fishes similar in these aspects (Lee Cren, 1951). The establishment of LWR is often needed for the calculation of production and biomass of a fish population (Anderson *et al*, 1983) and estimation of it and relative condition factors enables estimation of the population of the same species from different localities. The knowledge on LWRs for most tropical and subtropical fish species is still scarce (Ecountin *et al*, 2005). Recently, various attempts have been made to study LWRs of indigenous freshwater fish species from Brahmaputra basin (Borah *et al*, 2018; Nath *et al*, 2019; Borah *et al*, 2020), Barak basin (Nath *et al*, 2017), Ganga basin (Baitha *et al*, 2018) and from rivers of peninsular India (Borah *et al*, 2019). To our knowledge, no LWR data are available for *Garra lissorhynchus* and *Barilius barila* in FishBase (2019).

MATERIALS AND METHODS

Fish specimens were collected from 6 different sampling stations *viz.* Syamagram (25°08′12°N 93°01′42°E), Diyang Hranghkol (25°10′02.5°N 93°01′50.3°E), Lower Haflong Bridge (25°11′37°N 93°01′07°E), Dihingi Bazar Point

Appendix- 3

Participation Certificate

First International Symposium on Aquatic Biodiversity of the North Eastern Region of India

Presented to:

A. M. Ahmed

for their presentation on the study

Assessment of Ichthyofaunal Biodiversity of Diyung River of North Eastern Himalayan Region, Assam

Authors: A. M. Ahmed, R. Dutta, S. K. Bhagabati, H. Pokhrel, L. P. Mudoi, D. Nath & R. Sarmah

at the First International Symposium on Aquatic Biodiversity of the North Eastern Region of India held on October 26th and 27th 2021. We thank you for your contribution towards conservation of Aquatic Biodiversity in the North Eastern Region of India.

Dr Uwe Scholz, Project In-Charge, GIZ NERAQ Project

Prof. Sarbeswar Sarma, Department of Zoology, Gauhati University

Dr Dhriti Banerjee, Director, Zoological Survey of India

Appendix-4

Abdul Malik Ahmed, Rajdeep Dutta, Sarada Kanta Bhagabati, Hemanta Pokhrel, Lawonu Prasad Mudoi, Raktim Sarmah, Dipanka Nath & Imtiaz Ahmed (2022). Fish Species Composition, Distribution, and Community Structure of a Himalayan Biodiversity Hotspot River Diyung, North East India. *Pakistan Journal of Zoology*, NAAS 6.8)

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Project ID: HSF2017-18/I-16/04

FINAL TECHNICAL REPORT

OF THE PROJECT

ON

"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

सत्यमेव जयते

प्रकृतिः रक्षति रक्षिता

Nature Protects if She is Protected

Submitted to:

Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies (NMHS)
G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal, Almora 263643, Uttarakhand

ASSAM AGRICULTURAL UNIVERSITY

Submitted by:

Dr. Sarada Kanta Bhagabati
Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103

Std. Doc.: NMHS/FG-FTR

National Mission on Himalayan Studies (NMHS) 2020

Template/Pro forma for Submission

NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	**HSF2017-18/I-16**	**Date of Submission:**								
			d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

PROJECT TITLE

"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

Sanctioned Fellowship Duration: from (28/03/2018) to (28/02/2021).

Extended Fellowship Duration: from (1/03/2021) to (31/12/2021).

***Submitted to*:**

Er. Kireet Kumar

Scientist 'G' and Nodal Officer, NMHS-PMU

National Mission on Himalayan Studies, GBP NIHE HQs

Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi

E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

***Submitted by*:**

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Ph. 9435562939

E-mail: sskbk2002@gmail.com

NMHS-Final Technical Report (FTR)

NMHS- Institutional Himalayan Fellowship Grant

DSL: Date of Sanction Letter

DFC: Date of Fellowship Completion

3	**1**	**1**	**1**	**2**	**0**	**2**	**2**
d	d	m	m	y	y	y	y

2	**8**	**0**	**3**	**2**	**0**	**2**	**2**
d	d	m	m	y	y	y	y

Part A: CUMULATIVE SUMMARY REPORT

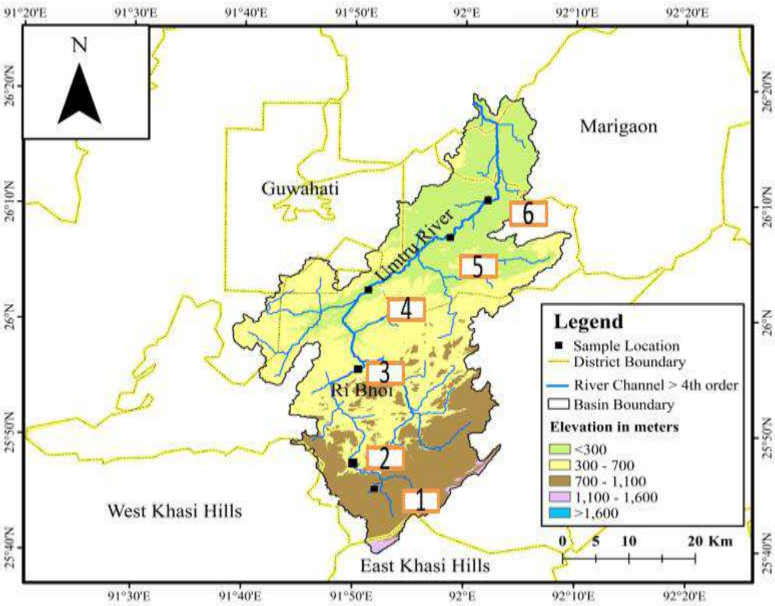
1. Details Associateship/Fellowships

1.1 Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	HSF2017-18/I-16/04
Name of the Institution/ University:	College of Fisheries, Assam Agriculture University
Name of the Coordinating PI:	1. Dr. Rajdeep Dutta Assistant Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103 2. Dr. S.K. Bhagabati, Associate Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103
Point of Contacts (Contact Details, Ph. No.,	• Email ID: drrajdeepdutta@gmail.com

E-mail):	: sskbk2002@gmail.com • Ph No: 9854757790 & 7896250516

1.2 Research Title and Area Details

<table>
<tr><td>i.</td><td>Institutional Fellowship Title:</td><td colspan="6">Study of hydrobiological status and fish diversity of river Umtrew Meghalaya and Assam</td></tr>
<tr><td>ii.</td><td>IHR State(s) in which Fellowship was implemented:</td><td colspan="6">Assam</td></tr>
<tr><td>iv.</td><td>Scale of Fellowship Operation</td><td>Local:</td><td></td><td>Regional:</td><td>Yes</td><td>Pan-Himalayan:</td><td></td></tr>
<tr><td>iii.</td><td>Study Sites covered (site/location maps to be attached)</td><td colspan="6">

Map of the Study Area</td></tr>
<tr><td>v.</td><td>Total Budget Outlay (Crore) :</td><td colspan="6">INR 0.8034840.00</td></tr>
</table>

1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration	
		From	To
Research Associates			
Sr. Research Fellow	1	1/12/2018	31/12/2021
Jr. Research Fellows			
Project Fellows			

2. Research Outcomes

2.1. Abstract

- ***Background*:** North eastern India is covered with a large network of rivers, which is having a total length of 19,150 km, out of which Assam is having 4820 km of rivers and in Meghalaya it is 5,600 km (Gurumayum and Choudhury, 2007). In Assam, mainly Barak and Brahmaputra rivers along with their tributaries are contributing to the riverine system of the state and in Meghalaya, mainly the tributaries of Brahmaputra contribute to the riverine network of the state. Umtrew river is one of the most important river system and is provide livelihood to many people, supports a diverse flora and fauna, and serves an important resource for the people living on the banks of the river. But the river is getting polluted day by day due to urbanization and industrialization along its stretch. Keeping all these points into consideration, NMHS sponsored medium grant project an attempt has been made to study the hydrobiological status and fish diversity of river Umtrew.
- ***Aims:***

1. To assess the pollution and hydrological status of the river.
2. To study the ichthyofaunal diversity and conservation of indigenous and endemic species of North East Himalaya.

- ***Objectives:***
- To study the temporal and seasonal variation of the hydrobiological profile of the Umtrew river system.
- To study the degree of anthropogenic stress on the river by using index of biotic integrity(IBI)
- To study the fish diversity of the river.
- To identify the anthropogenic factor affecting the river and find out the mitigation measures.
- To study the effect of weather change on the river (If any)

- **Methodologies:**

Objective 1: Water and sediment samples were collected from 6 different stations of river Umtrew from January, 2019 to May, 2021. Some of the physical parameters like depth, air & surface water temperature, water velocity, TDS & EC were determined on the spot. Other parameters like Turbidity, Dissolved oxygen, pH, Total alkalinity, Total hardness, Nitrate, Nitrite, Ammonia, Soluble Inorganic Phosphate of the water samples were carried out in the laboratory as per APHA (2005). The sediment samples were collected on seasonal interval, air dried and analysed for pH, organic matter, organic carbon, nitrogen, potassium and phosphorus as per standard methodology (Jhingran, 1992; Walky & Black, 1934). To study the pollution status of the river, water samples from the 6 stations was collected on monthly interval and pollution status of the river was assessed in terms of Biochemical oxygen Demand$_3$ (BOD_3), Chemical Oxygen Demand (COD) using standard protocol. Different pollution indices was developed with the help of water quality parameters. Heavy metal analysis was done using standard protocol given by Trievesy *et al*., 1987. Palmer's pollution index was also developed using qualitative and quantitative analysis of plankton population.

Objective 2: Based on the collected primary and secondary data during the study period the anthropogenic factors was determined. Index of biological integrity was analyzed with standard protocol given by Karr,1981.

Objective 3: Fish samples were collected from both the rivers of 6 different stations of river Umtrew on monthly intervals and length and weight of the fish species were recorded. Photography of the fish specimens and their habitat were done. The fish samples were preserved and brought to the laboratory in 10% formalin. The fishes were identified using standard keys (Jayaram, 2006; Vishwanath & Nebeshwar, 2009; Kottelat, 2013). Plankton and periphyton samples were identified with the help of standard literatures Edmondson (1959), Needham & Needham (1966) and ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967).

DNA Barcoding

Pectoral fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping was isolated following phenol: chloroform method. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking its integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg). The PCR product is then sequenced at Eurofin Scientific Laboratory. The generated barcodes were submitted to NCBI and accession number were obtained for the individual fish species.

Objective 4: Anthropogenic factors were observed during sampling period in different sampling station from higher to lower stretch.

Objective 5: Monthly rainfall data and monthly average air temperature data was collected from the Indian meteorological department for tow year.

Results:

Objective 1: The seasonal variation of water quality parameters of Umtrew river depicted that river faces more pollution in the middle and downstream. During the monsoon season the river water faces more stress compare to other season. Pollution indicating parameters like BOD_3 and COD crossed maximum permissible limit of drinking and fish culture. Principal component analysis also indicate that the river water got more effected by pollution indicating parameters during monsoon season. Water quality index (WQI) showed Umtrew river water in not suitable for drinking and fish culture. Metal content in the river water is within the permissible limit but long term used of this water may causes health hazard on the local people.

Objective 2: Umtrew river exhibits many anthropogenic activities like construction dam, mining activities, industrial waste, lavatory waste etc. River bed distraction practices like bolder mining and sand mining practices are observed during the sampling period. River faces different type of pollutant produces nearby industries and human anthropogenic activities. Three dams were constructed on the Umtrew river which are not only responsible for alteration of the river quality but also quantity of the river water. These dams are also responsible for interdict fish migration pattern. Fishes that were collected from the river mostly omniverse by their feeding habits. Beside these activities river habited distraction like bolder miming and send mining. Overall percentage of omnivores fish in the river is 48.97% and percentage of carnivores and herbivores fishes 24.48% and 26.53% respectively. Percentage of omnivores fishes more than 45% refers as degraded environment according to Karr *et al*., 1981.

Objective 3: During the present investigation, a total of 49 fish species belonging to 10 orders, 20 families and 36 genera were recorded from the studied river. DNA barcodes were generated for 18 numbers of fish species from River Umtrew, submitted to NCBI and 18 numbers of accession numbers were obtained. Among the recorded fish species from River Umtrew, 1 species are assessed as critically rear (2.04%), 4 are near threatened (8.16%), 2 are vulnerable (4.08%) and other 42 species are least concerned (85.71%) according to IUCN (2021). One additional species is not evaluated and one species is data deficit.

A total of 24 genera of plankton were identified from the river Umtrew river during study period. A total of 14 genera of plankton were recorded from River Umtrew during the study period. Population of phytoplankton was represented by 14 genera belonging to Chlorophyceae (5 genera), Bacillariophyceae (5 genera) and Cyanophyceae (5 genera). Zooplankton population was represented by Rotifera (3 genera), Cladocera (2 genera) and Copepoda (3 genera). The population density of plankton varied from season to season.

Objective 4: Umtrew river is the major river system of Maghalaya. At present three dams were contracted on the river and that are Umiam dam, Kyrdemkulai dam and Umtrew dam. These dams are responsible to change the water quality as well as the quantity. Besides that, the river also facing waste water that generated from the Shillong town and nearby population on the upstream. In downstream areas the river faces Byrnihat industrial areas. Sand mining and bolder mining is also practiced in these areas which is mostly responsible for alteration of fish habited. These areas is also facing a thick human population.

Objective 5: There is no significant changes being observed during the study period by weather change.

Conclusion: Umtrew river system is one of the major river system in Meghalaya. In the upper state of the east khasi hill many mining practices are going on. Presently three dams were constructed on the Umtrew river which are mainly responsible for the migration of the hill stream fishers. This is the reason hill stream fishes are found scanty in upper stream. In the middle stream water effected by different type of house hold and industrial waste. Many sand and bolder mining activities are practicing in this middle stream areas. The water quality parameters indicates that river water is not suitable for drinking and fish culture. The water required proper treatment before use.

Recommendations: Sand and bolder mining activities on river bed should immediately stop and implement proper legislative rules. Wate water should treat properly before releasing into the river. River water volume should maintain in each season.

2.2. Objective-wise Major Achievements

S. No.	Objectives	Major achievements (in bullets points)
1.	To study the temporal and seasonal variation of the hydrobiological profile of the Umtrew river system.	• Studied the water quality of the river for 2.5 year all long the stretches. Study the seasonal variation of the water, • Water Quality Index was developed • Principal component analysis of the water quality parameter for season. • Different type of pollution indies were developed with the help of water quality parameters. • Heavy metal analysis was done for tow season both soil and water. • Different type of hazard index were developed • Research paper: Correspondence 2
2.	To study the degree of anthropogenic stress on the river by using index of biotic integrity(IBI) To study the fish diversity of the river.	• Three dam were constructed • Sand and bolder mining practices are going on. • Facing the water generated by Shillong town, industries and different households • IBI index indicate river having a poor water quality

3.	To study the fish diversity of the river.	• 49 fish species belonging to 10 orders, 20 families and 36 genera were recorded from the studied river. Cyprinidae was the most dominant family • DNA barcodes generated: 18 fish species from River Umtrew. • Conservation status: - Critically rare (2.44%), near threatened (8.16%), vulnerable (4.08%) and least concerned (81.63%) according to IUCN (2021). One additional species is not evaluated and one species is data deficit. • Species recorded most are omnivore by their feeding habits • Research paper: Communicated 1 •
4	To identify the anthropogenic factor affecting the river and find out the mitigation measures.	• Anthropogenic factors encountered during the regular sampling in the Umtrew river system are: ➢ Hydro-electric dam: 3 nos ➢ Sand and bolder mining ➢ Water generated by Shillong town and byornihat industrial area ➢ Washing cloths, bathing and lavatory waste
5	To study the effect of weather change on the river (If any)	• No significant change observed

2.3. Outputs in terms of Quantifiable Deliverables*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved
1.	First-hand information on environmental health of the selected river will be generated which will act as an important baseline information for future climate change related studies.	Dataset of the ecological status of Umtrew River.	Dataset on environmental health of the river: 1 GIS Map: 1
2.	An updated biodiversity status of the river ecosystem	Taxonomic and molecular characterisation of fish fauna of the river covering its diversity, distribution,	Checklist of Fish species Museum specimens: 49 DNA barcodes: 18
3.	Information on trophic level structure of the river ecosystem		We gathered all the available information regarding the feeding habits of 49 collected fish species belonging to 10 orders, 20 families and 36 genera. Based the individual food items trophic level structure of

			Umtrew river was determined. The trophic level of the river ranges from 2.0±0.00 to 4.5±0.80. the trophic level was dominated by mid-level carnivore (24.4%) followed by omnivores (48.97%) and herbivores (26.53%).
4	Identification of anthropogenic stress factors affecting the river ecosystem (if any) and its possible mitigation measures.	Any kind of anthropogenic factors affecting fish and their habitat are being constantly monitored.	•Anthropogenic factors encountered during the regular sampling in the Umtrew river system are: • Hydro-electric dam: 3 nos • Sand mining and bolder mining • Industrial pollutant • Water generate by Shillong town • Washing cloths and

			bathing (Annexure V)

(*) As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number/ Brief Details	Remarks/ Attachment
1.	New Methodology developed	-	
2.	New Models/ Process/ Strategy developed	-	-
3.	New Species identified	-	-
4.	New Database established	6	• Total number of fish fauna • Pollution Status • Fish biodiversity • Plankton data (Phyto and Zooplankton) • Palmer index Seasonal variation of water quality parameters.
5.	New Patent, if any	-	-
	I. Filed (Indian/ International)	-	-
	II. Granted (Indian/ International)	-	-
	III. Technology Transfer (if any)	-	-

S. No.	Particulars	Number/ Brief Details	Remarks/ Attachment
6.	Others (if any) DNA barcoding of fish species	59	Species specific DNA barcodes of 18 fish species from River Umtrew was generated, submitted to NCBI and accession number obtained for the first time.

3. Technological Intervention

S. FNo.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology		
2.	Diffusion of High-end Technology in the region		
3.	Induction of New Technology in the region		
4.	Publication of Technological / Process Manuals		

4. New Data Generated over the Baseline Data

S. No.	New Data Details	Status of Existing Baseline	Additionality and Utilisation New data
1.	Morphological identification & molecular characterisation of fish fauna of River Umtrew	No earlier record of fish fauna from River Umtrew is available	We have recorded 49 fish species and generated mitogenome sequences for 18 species from River Umtrew for the first time.
2.	Seasonal variation of hydrobiological & parameters	No earlier report on hydrobiological study of River Umtrew is available	The new data will be helpful in understanding the impact anthropogenic factors on ecosystem integrity of the river. It will be also helpful in devising future fisheries development strategies in this river.
3	Heavy metal content in river water and sediment	No earlier report is available	The new data is helpful for the future impact on the nearby population
4.	Sediment characteristic of River Umtrew	No earlier report on sediment characteristic of River Umtrew is available	The new information will be helpful for future researchers working in this region
5.	Plankton diversity	No report earlier	

6.	Diversity indices of plankton	No report earlier	
7.	Palmer index has been developed for the said river system	No report earlier	
8	Pollution indices	No report earlier	

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Beneficiaries
1.	Sustainable Development Goals (SDGs)			
2.	Climate Change/INDC targets			
3.	International Commitments			
4.	National Policies			
5.	Other's collaborations			

6. Financial Summary (Cumulative)*

*Please attach the **consolidated and audited Utilization Certificate (UC) and Consolidated and *Year-wise* Statement of Expenditure (SE)** separately, *ref*. **Annexure I.**

7. Quantification of Overall Research Progress

S. No.	Parameters	Total (Numeric)	Attachments* with remarks

1.	IHR State(s) Covered:	1	
2.	Fellowship Site/ LTEM Plots developed:	6	Photographs of sampling sites and map of study area attached (Annexure- I & II)
3.	New Methods/ Model Developed:		
4.	New Database generated:		
5.	Types of Databases generated:		
6.	No. of Species Collected:	49(DNA barcodes of 18 fish species submitted and accession number received	Annexure-III
7.	New Species identified:		
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	JRF:01 Masters: 1 PhD:01 (Pursuing)	
9.	No. of SC Himalayan Researchers benefited:		
10.	No. of ST Himalayan Researchers benefited:		
11.	No. of Women Himalayan Researchers empowered:		
12.	No. of Knowledge Products developed:		
13.	No. of Workshops participated:		

14.	No. of Trainings participated:		
15.	Technical/ Training Manuals prepared:		
	Others (if any):		

***** Please attach the soft copies of supporting documents word files and data files in excel.

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)	1*	*	0.40	Annexure VI
2.	Book Chapter(s)/ Books:				
3.	Technical Reports/ Popular Articles				
4.	Training Manual (Skill Development/ Capacity Building)				
5.	Papers presented in Conferences/ Seminars				
6.	Policy Drafts (if any)				
7.	Others (specify)		1	International Symposium on Aquatic Biodiversity of the north eastern (Annexure VII)	

* 2 Research papers are communicated and under peer review.

9. Recommendation on Utility of Research Findings, Replicability and Exit Strategy

9.1 Utility of the Fellowship Findings

S. No.	Research Questions Addressed	Succinct Answers
1.	How is the hydrobiological status of the river under study	The water quality parameter were found under the optimum range during the study period. The values pollution indicating parameters for fish culture like of BOD and COD were found higher then the optimum level. The study also indicates that river water is mostly detreated during monsoon period. The study also indicates that the pollution pressure is more in the middle and downstream. Water quality index value indicates that the river water needs proper treatment before use for drinking and fish culture. Though the heavy metal pollution in the river water under the range but the long-time exposure of this water may occur a serious health issue to the nearby people.
2	How many fish species are available in the entire stretch of the river	The fish diversity of the Umtrew river comprised with 49 numbers of species comes under the 36 genera, 20 families and 10 orders. The present study reveals that the highest species was found during the post monsoon period. The recorded 10 order comprised namely Cypriniformes, Siluriformes, Anabantiformes, Syubranchiformes, Perciformes, Gobiformes, Mugliformes, Clupiformes, Beloniformes, Osteoglossiformes. The Cypriniformes one of

		the most dominant order as compare to all the order recoded in the present study comprising of Cypriniformes (42%), Siluriformes (24%), Anabantiformes (10%), Syubranchiformes (6%), Perciformes (4%), Osteoglossiformes (4%), Gobiformes (2%), Mugliformes (2%), Clupiformes (3%), Beloniformes (3%), Cypriniformes is the most dominated species in the brahamaputra basin (Sarma *et al*., 2012). The most abundant family of the fishes, Cyprinidae was reported by 11 species contributing 22% of the fish diversity in the Umtrew river.
3	How is the trophic level structure of the river ichthyofauna?	The trophic level index indicates that most of the fishes are omnivore (48.97%) with their feeding habit and rest of that 24.48% comes under in carnivore and 26.5%3 are comes under in herbivore category. According to Karr (1981) the Umtrew river environment is comes under poor category since more than 45% fish species are comes under omnivore.
4	What are the anthropogenic factors that are affecting the biota of the river and how?	In the present study fish faunal diversity was found scanty in the stations existing in the higher altitude. This is because the river facing barrier with two major and one small dam along its stretch. These dams are mainly responsible for the fish migration pattern. Beside that the river water is also contaminated with different type of pollution like acid mine drainage, lavatory waste, waste generated from the Shillong town, different

		industrial waste, anthropogenic activities like sand mining and bolder mining etc.
5	Is there any effect of weather change on the river ecosystem	No any significant effect on river water being observed during study period.

9.2 Recommendations on Replicability and Exit Strategy:

Particulars	Recommendations
Replicability of Fellowship, if any	
Exit Strategy:	• Umtrew river facing tremendous pollution pressure from Shillong town, industrial areas and various anthropogenic activities. The waste water before releasing into the river should be treated properly and continues water quality monitoring system should be establish in these areas. • There should be continuous flow of water nearby dam side to maintain the optimum water depth and dam should design with fish migration facilities. • Illegal construction near the river side should impose strict regulation. • Sand and bolder mining practices should prohibit to conserved the habited. • Strict fishy laws should be implemented.

(**NMHS FELLOWSHIP COORDINATOR**)

Place: …………………

Date: …../……/……..

(**HEAD OF THE INSTITUTION**)

PART B: COMPREHENSIVE REPORT (including all sanctioned positions of Researchers)

Based on the Fellowship Proposal submitted/approved at the time of sanction, the co-ordinating Principal Investigator shall submit a comprehensive report including report of all individual researchers.

The comprehensive report shall include an **Executive Summary** and it should have separate chapters on (1) **Introduction** (2) **Methodologies, Strategy and Approach** (3) **Key Findings and Results** (4) **Overall Achievements** (5) **Impacts of Fellowship in IHR** (6) **Exit Strategy and Sustainability** (7) **References/ Bibliography** and (8) **Acknowledgements** (It should have a mention of financial grant from the NMHS, MoEF&CC).

Further, description of Technical Activities, List of Trainings/ Workshops/ Seminars with details of trained resources, list of New Products developed under the fellowship, Manual of Standard Operating Procedures (SOPs) developed, Technology developed/Transferred etc should be enclosed as Appendix.

Report (hard copy) should be submitted to:

Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies (NMHS)
G.B. Pant National Institute of Himalayan Environment (GBP NIHE)
Kosi-Katarmal, Almora 263643, Uttarakhand

Report (soft copy) should be submitted at:

E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

PART B: COMPREHENSIVE REPORT

EXECUTIVE SUMMARY

The Executive Summary of the fellowship should not be more than 3–5 pages, covering all essential features in precise and concise manner as stated in Part A (Cumulative Fellowship Summary Report) and Part B (Comprehensive Report).

n of N *(n = Sequential number; N= Total no. of fellowships granted to the Institute/ University)*

Fellowship Report No.:

Researchers Details

Type of Fellowship (HRA/HJRF/HJPF)	Name of Himalayan Researcher	Date of Joining	Date of Resignation**	Research Title	Name of the PI & Designation
(*in case of continuation of fellowship*)					

*If the appointed researcher resigned in the mid of the fellowship duration, then also mention the name of the Himalayan researcher who carried forward the fellowship.

1 INTRODUCTION

1.1 Background/ Summary of the Associateship / Fellowship Study undertaken

Our survival on Earth depends on three basic resources – water, air and soil, nature's three valuable gifts to mankind. Among these, water is the most important component as it forms the basic medium for origin of life. The amount of water in Earth is abundant, but the amount of portable water is a tiny fraction of the total water present in the world. Geographically, India is a vast nation with an area of 329 million hectares, which is almost 2.4% of the worlds area and it has 4% of the fresh water resources of the world. According to WHO (2006), only 0.007% of water on earth is readily available for consumption purpose. Though the freshwater resources are highly vulnerable for human use, still these resources are being over used, polluted and wasted with little regards irrespective of human health and ecological consequences (Lavado *et al.,* 2004.It is becoming very critical to examine in terms of population growth because the renewable freshwater resources are finite in nature. The freshwater resources are having immense importance in our lives and well-being, but despite this we are increasingly beginning to take these precious resources as being infinite and, for granted. According to WHO (2019) 1 in 3 people globally do not have access to safe drinking water. The demand of water is increasing on a very steep rate mainly due to growing industrialization and exploding population. Moreover, considerable part of this limited amount of water is polluted by sewage, industrial waste and wide range of chemicals (Malmqvist and Rundle, 2002; Nilsson *et al*., 2005; Sabater and Stevenson, 2010; Belenguer *et al.,* 2014). According to Gawande *et al*., (2016) Water quality index is the most useful technique to estimate the pollution status of the water in a single number. However, Shekhar *et al.,*2008 estimated plankton using Shannon index to identify the water quality status of river Bhadra (Mysore) affected by the paper mill and steel mill.Water quality and pollution status of Chambal river in Madya pradesh studied by Saksena *et al.,*2008.In 2016

River Umtrew is formed by union of two streams, one originating from the Sohpetbneng Peak near Mawrong village and the second one is the outflow of the Umium dam. The two streams converge near Nongkhyllem Wildlife sanctuary and it flows across Byrnihat town in Meghalaya. The river then enters Assam through Sonapur, where it is known as Digaru and finally debouches into mighty river Brahmaputra near Chandrapur. Through its course, the river provides livelihood to many people, supports a diverse flora and fauna, and serves an important resource for the people living on the banks of the river. But the river is getting polluted due to industrialization and urbanization along its stretch. Burnihat Industrial area, situated in the banks of the river. There are many industries in this area including the chemical industries, PVC industries, cement industries, distilleries, food and beverage industries etc. and these industries are huge amount of effluent and are ultimately discharged into the river. In a report about Industrial waste water generation, it was mentioned that Chemical industries can generate waste water upto 97.8 MLD and that for distillery and food and beverage industries it is 37 and

6.5 MLD, respectively (Ministry of water resources, 2018). The District hospital, Sonapur is also situated near the river, and every day the wastes are getting dumped into the river. Environment is a delicately balanced system and it should be protected, and protecting it is not a regional issue. While thinking globally, we must act locally, and for this awareness among the locals is also necessary. Thus, a proper study is very much required in the present location, where there is little awareness about the harm which is done to the river due to the unfavorable anthropogenic activities. Keeping all these aspects in view, through this NMHS sponsored project an attempt has been made to study hydrobiological status and ichthyofaunal diversity of Umtrew river.

1.2 Baseline and Scope of the Associateship / Fellowship

During the fellowship pregame the first hand information were collected from the all stretches of the river. Water quality seasonal variation of physico-chemical, Plankton composition of the Umtrew river was investigate during time period from January 2019 to May 2021 With the help of the water quality parameters river health status was studied. Species specific DNA barcodes were for fish fauna of River Umtrew during the project for the first time. Morphological identification of the indigenous fish fauna of the river supported by molecular characterization will provide a complete dataset on ichthyofaunal diversity. Their was many anthropogenic activities being observed which affecting the river water as well as biota during the study period. This information will definitely help for the future implementation of the river management policies.

Overview of the Major Issues to be addressed

Some of the major issues addressed through this project are:

Construction of dams: For survival of different aquatic organism, it is very necessary to maintain the quantity or volume of the river along the year. In Umtrew river there are three dams along the entire stretch of the river. These dams obstruct the continuous flow of the water mainly during dry season. The fish species migration also brings effected by these dams.

i) Acid mine Drainage: These practices were observed during the study period. Due to this AMD the Umtrew river water loses species diversity by the addition of different pollutant in the river water.

ii) Water pollution: Pollution is another biggest concern for the River Umtrew. The waste water that produces from different sources like towns, industrial areas and household waste are ultimately released into the river. Beside that many anthropogenic activities like bathing, washing cloth, lavatory waste is released by nearby population. The waste water that discharges from the upstream areas become the source of the downstream areas. Used of this was can create acute as well as chronic health hazard.

iii) Habitat degradation: Umtrew river exerting many unlawful colonies like exploitation for unauthorized purposes such as agriculture, sand mining, bolder mining etc. In the floodplain areas of the Umtrew river it is very necessary that only the permitted activities and structure are allowed is these areas are very vital for river's health.

Public unawareness: Management of large waterbodies like river cannot be done by the government alone. The local resident should have some responsibility toward the management of the river. Due to ignorance to the local people the river water condition come under threat.

iv) Unavailability of alternative options: One of the best ways of conservation of indigenous fish fauna of natural aquatic ecosystems is promotion of aquaculture to reduce sole dependency of fish on these natural resources and thereby providing the fisherfolks with alternative fish centric livelihood options. But unfortunately, the local tribal people are not aware about scientific fish farming practices. So, they are very much dependent on fishing in rivers/streams for their food fishes.

1.3 Brief summary of the activities under taken by the researcher

[Providing full details of Field study, experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs (**Data, table and figures should be**

attached as separate source file (.docx, .xls, jpg, .jpeg, .png, .shp, etc.)].

2 METHODOLOGIES, STARTEGY AND APPROACH

2.1 Methodologies used for the study

i) **Methodology used for achieving Objective 1:** Water and sediment samples were collected from 6 different stations of river Umtrew from January, 2019 to May, 2021. Some of the physical parameters like depth, air & surface water temperature, water velocity, TDS & EC were determined on the spot. Other parameters like Turbidity, Dissolved oxygen, pH, Total alkalinity, Total hardness, Nitrate, Nitrite, Ammonia, Soluble Inorganic Phosphate of the water samples were carried out in the laboratory as per APHA (2005). The sediment samples were collected on seasonal interval, air dried and analysed for pH, organic matter, organic carbon, nitrogen, potassium and phosphorus as per standard methodology (Jhingran, 1992; Walky & Black, 1934). To study the pollution status of the river, water samples from the 6 stations was collected on monthly interval and pollution status of the river was assessed in terms of Biochemical oxygen Demand$_3$ (BOD_3), Chemical Oxygen Demand (COD) using standard protocol. Different pollution indices was estimated with the help of water quality parameters. Heavy metal analysis was done using standard protocol given by Trievesy *et al*., 1987. Palmer's pollution index was also estnuimated using qualitative and quantitative analysis of plankton population.

ii) **Methodology used for achieving Objective 2:** Based on the collected primary and secondary data during the study period the anthropogenic factors were determined. Index of biological integrity was analyzed with standard protocol given by Karr,1981.

iii) **Methodology used for achieving Objective 3:** Fish samples were collected from both the rivers of 6 different stations of river Umtrew on monthly intervals and length and weight of the collected fish species were recorded. Photography of the fish specimens and their habitat were done. The fish samples were preserved and brought to the laboratory in 10% formalin. The fishes were identified using standard keys (Jayaram, 2006; Vishwanath &

Nebeshwar, 2009; Kottelat, 2013). Plankton and periphyton samples were identified with the help of standard literatures Edmondson (1959), Needham & Needham (1966) and ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967).

DNA Barcoding Pectoral: Fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping were isolated following phenol: chloroform method. Concentration of the DNA samples were measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking its integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg). The PCR product is then sequenced at Eurofin Scientific Laboratory. The generated barcodes were submitted to NCBI and accession number were obtained for the individual fish species

iv) **Methodology used for achieving Objective 4:** Anthropogenic factors were observed during sampling period in different sampling stations from higher to lower stretch.

v) **Methodology used for achieving Objective 5:** Monthly rainfall data and monthly average air temperature data were collected from the Indian meteorological department for two year.

**2.2 Details of Scientific data collected and Equipment's Used **

a. Air & water temperatures were measured using a mercury thermometer.
b. Water velocity was measured using a current meter.
c. Parameters like pH, conductivity, TDS of the river water were measured *in-situ* using a digital soil & water testing kit (Systronics India Limited/371).
d. DO, Alkalinity & Hardness values were estimated by Titration method.
e. BOD bottles were incubated in BOD incubators.
f. For estimation of COD, water samples were digested in a KEL PLUS Automatic COD digestion system/ KES 08 L CAC.

g. Parameters like nitrate, nitrite, total ammonia and soluble inorganic phosphate were determined using uv-visible spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202).
h. Available nitrogen was estimated by alkaline potassium paramagnet method in kjeldhal flask.
i. The available potassium was estimated by flame photometer.
j. Heavy metal was estimated using Atomic Adsorption Spectrophotometry (AAS).
k. Latitude & longitude of the stations were recorded using a GPS instrument.
l. Photography of the fish specimens and stations were done using a digital camera.
m. The morphometric measurements & weight of the collected fish specimens were recorded using a vernier calliper and a pan balance respectively.
n. DNA isolation from pectoral fin clippings of the fishes was done using Phenol-Chloroform method.
o. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306).
p. Integrity of DNA samples were checked using an Electrophoresis system (Biorad)
q. Amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg).
r. Plankton samples were collected using a plankton net.
s. Plankton & periphyton samples were observed under a Microscope.

2.2 Primary Data Collected

i. The morphometric measurements & weight of the collected fish
ii. Latitude & longitude of the study stations of both the rivers
iii. Fish specimens for museum
iv. Air & water temperatures
v. Water velocity
vi. Water pH
vii. Dissolved oxygen concentration of river water
viii. Conductivity of river water
ix. TDS of river water

x. Total Alkalinity of river water

xi. Total hardness of river water

xii. Biological oxygen demand$_3$ (BOD_3) of the river water

xiii. Chemical oxygen demand (COD) of the river water

xiv. Nitrogen-nitrate

xv. Nitrogen-nitrite

xvi. Total ammonia

xvii. Soluble inorganic phosphate

xviii. Plankton biomass

2.3 Details of Field Survey arranged

Regular field survey in the study river was conducted during the entire duration of the project for collection of fish specimens, water samples, sediment samples, plankton and periphyton samples. During survey, primary and secondary data were also collected pertaining to the objectives of the project.

2.4 Strategic Planning for each Activities

Health status of the river: Six sampling station were selected from the different elevations for estimation of water quality parameters, Plankton and periphyton of Umtrew river for different seasons.

Fish Diversity: Fishes were collected from entire stretches of the river using cast net, gill net hook and line etc.

2.5 Activity-wise Timeframe followed using Gantt/ PERT Chart

Activities	Months																								
	1	2	3	4	5	6	7	8	9	10	11	12	Etc.				18				24		27		35	36
Recruitment of Project Staff																										
Preparati																										

on & Procurements																										
Initial survey																										
Assessment of Ichthyofaunal diversity																										
Evaluation of Environmental Health																										
Annual Report																										
Final Report Preparation & Submission																										

3 KEY FINDINGS AND RESULTS

3.1 Major Research Findings

3.1.1: Water Quality & Sediment Parameters of River Umtrew:

A total of fifteen (15) water quality parameters were tested at 12 different stations by covering the whole stretch of the Umtrew river for a period of 29 moths from January, 2019 to May, 2021.

Data on seasonal variations of water quality parameters of River Umtrew from January, 2019 till May, 2021 is depicted on Appendix - 1. Data on seasonal variation of sediment parameters of River Umtrew from January, 2019 till May, 2021 is depicted on Appendix - 1.

Comparison of water quality parameters of the study rivers with congenial values for fishes:

Sl. No	Parameter	Result	Congenial Limit	Remark
1.	Surface Water Temperature (^{0}C)	15.75 - 32.00		Suitable for both cold and warm water fishes.
2.	Turbidity (NTU)	2.75-56.11	20-30	Turbidity exceeds permissible limit from station 4-12.
3.	pH	6.4 -7.80	7-8.5	Water pH was under alkaline condition during the study period.
4.	Dissolved Oxygen (ppm)	5.43 -10. 52	>5	Average DO values were found to be within acceptable range.
5.	Total Alkalinity (ppm)	19.50 - 108.66	80-200	Alkalinity values were found under congenial for fishes
6.	Total Hardness (ppm)	26.02-75.31	75-150	Hardness values were not under congenial for fishes
7.	Electrical Conductivity (µS/cm)	45.82-115.71	50-1500	EC values are within acceptable range
8.	Total Dissolved Solids (ppm)	21.5 - 205.66	<400	Found to be within acceptable range
9.	Biochemical Oxygen Demand (ppm)	7.14-36.20	<10	BOD values of station 8-12 were found in higher range than the congenial limit during monsoon indicating

				anthropogenic stress in these stations
10.	Chemical Oxygen Demand (ppm)	13.93 - 54.28	<20	COD values of station 8-12 were found in higher range than the congenial limit during monsoon indicating anthropogenic stress in these stations
11.	Nitrate-nitrogen (ppm)	0.113 - 0.333	0.10-3.00	Found to be within acceptable range
12.	Nitrite Nitrogen (µg/L)	0.016 - 0.09	0-0.50	Found to be within acceptable range
13.	Soluble Inorganic Phosphate (ppm)	0.14 -1.81	0.05-0.4	Found to be more than acceptable range
14.	Total Ammonia (ppm)	0.130 - 0.897	0-1.0	Found to be more than acceptable range

3.1.2 Ichthyofaunal Diversity of Umtrew river:

This project is bringing out first ever information on ichthyofauna of River Umtrew. During the present investigation, a total of 49 fish species belonging to 36 genera, 20 families and 10 orders were recorded from 6 selected sampling stations of the river Umtrew, Maghalaya India. The number and percentage composition of order and family under are shown (Table 3 and 4). Among the orders, the Cypriniformes formed the largest group with a contribution of 4 (20.00 %) families, 15 (41.66%) genera and 21 (42.85%) species. The order Siluriformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 5 (25 %) families, 10 (27.77%) genera and 12 (24.48 %) species followed by Anabantiformes with 1 (5%) family, 1 (2.7%) genera and 5 (10.20%) species, Syubranchiformes with 1 (5%) family, 1 (2.7%) genera and 3 (6.12%) species, Perciformes and Osteoglossiformes with 1 (5%) family, 1 (2.7%) genera and 2 (4.08%) species each and Gobiformes, Mugliformes, Clupiformes, Beloniformes with 1 (5%) family, 1 (2.7%) genera and 1 (2.04%) each.

3.1.3. Plankton Biomass of River Umtrew:

A total of 24 genera of plankton were identified from the Umtrew river. Five genera belong to the Chlorophyceae family, five to the Bacillariophyceae family, four to the Cynophyceae family, two to the Rotifera family, two to the Cladocera family, Copepod and Copepod nauplii, and fish eggs and larvae. During the research period, plankton density varied from 8 to 69 uL^{-1}.

According to Palmer, scores of 20 or more are indication of high organic pollution. The pollution tolerant genera belonging to three groups of algae from six sites of Umtrew river system was recorded. By using Palmer's index of pollution for rating of water samples as high, moderate and low organically polluted at six sites of Umtrew river system were tested. The total score of Algal Genus Pollution Index (AGPI) of sites S1<S2<S3< S6<S5< S4 are calculated to be 3,5, 8, 10,11 and 13 respectively. The total score of S1, S2 and S3 indicating probable lack of organic pollution in the riverwhile S4, S5 and S6 showed moderate pollution in the river due to anthropogenic factors or human interference according to Palmer, Chlorella, Nitzschia and Synedra Closterium were found to be the most active participant in most of the sites which may be the good indicator of contaminated water. Oscillatoria was recorded repeatedly in station 4, 5 and 6 and consider as indicators of pollution in view of the results of Palmer pollution index.

3.1.4. Anthropogenic factors affecting the river ecosystem:

- **Extraction of sand form the river bank**
- **Constraction dams for hydroelectric project**
- **Industrial area near river bank**
- **Human interference**
- **Irregular fishing activity**

3.2 Key Results :

- During the present investigation a total of 49 fish species belonging to 36 genera, 20 families and 10 orders are recorded from 6 selected sampling stations of the river Umtrew, Maghalaya India. Among the orders, the

Cypriniformes formed the largest group with a contribution of 4 (20.00 %) families, 15 (41.66%) genera and 21 (42.85%) species.

- DNA barcodes generated and NCBI accession no obtained for 18 species from Umtrew river for the first time.
- The highest species were recorded under least concern (LC) category with a total no of 42 and contributed 85.71%. under LC category, the major species contribution is from the family Cyprinidae with 8 (18.32 %) followed by Bagridae and Danionidae 5 (10.20%) each, Channidae 4 (8.16 %), Mastacembelidae 3 (6.12 %), Daninidae 2 (4.08%).Sisoridae, Ailidae, Claridae, Cobidae, Botidae, Nandidae, Ambassidae, Notoptaridae, Belonidae, Osphronemida, Gobidae, Muglidae and Clupidae 1 (2.38%). Under near threatened (NT) category Cyprinidae 2 (4.08%), Sissoridae and Alidae contributed 1 (2.38%) each. Like that, the family Siluridea and Botidae represent vulnerable category with 1 (2.38%) species each. One species which contributed 2.28% under family Cyprinidae represent the critically care category.
- The fishes that were found in the present study are mostly come under omnivores by their feeding habit.
- Surface water temperature regime of both the rivers is congenial for both hill stream and warm water fishes.
- Tubidity values was increased during the monsoon season.
- River showed alkaline condition of its water during all season.
- The BOD values increasing in the lowest reach and it extremely high than the permissible limit. COD shoed similar trend.
- Principal Componant Analysis showed water is more affected by pollution indicating parameters during monsoon season.
- Water quality index value indicates that river water need proper treatment before using for drinking and fish culture.

- Other pollution parameters indicates that river water is in slightly to heavy polluted category.
- The eutrophication index EPI value is indicating the river water is no eutrophic on the other hand Carlson's trophic index CTSI value indicated the river is oligotrophic.
- Heavy metal indices showed long-term use of river water may causes health hazard.
- Palmer index values indicates that organic pollution increases in the middle and downstream areas.

3.3 Conclusion of the study undertaken

- The present study revealed physico-chemical and biological parameters of the study Umtrew river water of Umtrew (Digaru) clearly displayed seasonal variation. The physicochemical parameters revealed that environmental integrity of station 1,2,3 is maintained compared to station 4,5 and 6. Water pollution assessment parameters of the river like BOD_3, COD, ammonia clearly indicated that there is variation among the environments of six stations, and station 4,5 and 6 are more polluted than station 1, 2 and 3, this requires constant monitoring and management in order to protect the river from getting further deteriorated. Factors like industrial discharge from Burnihat Industrial area, agricultural runoff and domestic release are, may be the main causes for the pollution status of the river.
- Principal Component Analysis showed that, parameters like BOD3, COD, ammonia, alkalinity, turbidity and TDS are having more effect on the principal components, which indicates that these parameters are playing a distinctive role in characterizing the dataset and are having more effect in determining the overall condition of the stations in terms of water quality. Changes in parameters affecting principal components may be due to pollutants affecting water quality in rivers have temporal and spatial variations and should be investigated based on each river's environmental conditions.

- The study river Umtrew is very rich in indigenous fish germplasm. This river is the habitat of many endangered, vulnerable and near threatened fish species. Therefore, conservation plans should be developed for in-situ conservation of these precious indigenous fish species.

4 OVERALL ACHIEVEMENTS

4.1 **Achievements on Objectives**

1. **Objective 1:** To study the temporal and seasonal variation of the hydrobiological profile of the Umtrew river system.

Achievements:

- Data set on physico-chemical parameters of water is generated of the study.
- GIS Maps of the study river developed.
- Pollution indices was estimated for the river water
- Heavy metal for the river was assessed.

2. **Objective 2:** To study the degree of anthropogenic stress on the river by using index of biotic integrity(IBI)

Achievements:

- Different types of anthropogenic stress were observed during the study
- List of the different industries and their waste.
- Index of biotic intracity was developed with the help of the fish pullulation.

3. **Objective 3:** To study the fish diversity of the river.

Achievements:

- Checklist of fish species of River Umtrew (A total number of 49 fish species) successfully generated.
- Species specific DNA barcodes generated for 18 fish species from River Umtrew, submitted to NCBI.
- Museum specimens of 49 fish species from these rivers are maintained at NMHS Fish Museum, Dept. of AEM, College of Fisheries, AAU, Raha.

- Conservation status of indigenous fish species of both the study rivers presented as per IUCN (2021) guidelines.

- Plankton variation was studied and Plamer index were estimated for six stations.

4. **Objective 4**: To identify the anthropogenic factor affecting the river and find out the mitigation measures.
   Achievements:

   - Different anthropogenic factors like sand mining, bolder mining, dams, unauthorized construction, washing clothes, taking bath etc. were recorded during the study period and their mitigation measures are discussed.

- **Objective 5:** To study the effect of weather change on the river (If any)
  Achievements:

- No significant changes being observed during the study period by weather change.

4.2 Establishing New Database/Appending new data over the Baseline Data

- This project is bringing out first ever information on ichthyofauna of River Umtrew. During the present investigation**,** a total of 49 fish species belonging to 36 genera, 20 families and 10 orders are recorded from 6 selected sampling stations of the river Umtrew, Maghalaya India. The number and percentage composition of order and family under are shown. Among the orders, the Cypriniformes formed the largest group with a contribution of 4 (20.00 %) families, 15 (41.66%) genera and 21 (42.85%) species. The order Siluriformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 5 (25 %) families, 10 (27.77%) genera and 12 (24.48 %) species followed by Anabantiformes with 1 (5%) family, 1 (2.7%) genera and 5 (10.20%) species, Syubranchiformes with 1 (5%) family, 1 (2.7%) genera and 3 (6.12%) species, Perciformes and Osteoglossiformes with 1 (5%) family, 1 (2.7%) genera and 2

(4.08%) species each and Gobiformes, Mugliformes, Clupiformes, Beloniformes with 1 (5%) family, 1 (2.7%) genera and 1 (2.04%) each.

- This report also describes first-hand information on physico-chemical properties of water and sediment from River Umtrew. Average surface water temperature varied from 15.75-32.00 ^{0}C, water velocity from 0.11-4.07 m/sec, turbidity from 2.75-56.11 NTU, pH from 6.4-7.8, dissolved oxygen from 5.43-10.52 ppm, total alkalinity from 19.56-108.66 ppm, total hardness from 26.20-75.31 ppm, electrical conductivity from 45.82-115.71 µS/cm, TDS from 21.50-2605.66 ppm etc.
- Different pollution indices indicated the river water is polluted. Plankton population was studied and palmer index was estimated. The index also indicate that river water is polluted in the middle and lower stretch.

4.3 Generating Model Predictions for different variables

- No

4.4 Technological Intervention

- No

4.5 On-field Demonstration and Value-addition of Products

- No

4.6 Developing Green Skills in IHR

- No

4.7 Addressing Cross-cutting Issues

- No

5 IMPACTS OF FELLOWSHIP IN IHR

5.1 Socio-Economic Development (max. 500 words, in bullet points)

5.2 Scientific Management of Natural Resources In IHR (max. 500 words, in bullet points)

5.3 Conservation of Biodiversity in IHR (max. 500 words, in bullet points)

5.4 Protection of Environment (max. 500 words, in bullet points)

5.5 Developing Mountain Infrastructures (max. 500 words, in bullet points)

5.6 Strengthening Networking in IHR (max. 700 words, in bullet points)

6 EXIT STRATEGY AND SUSTAINABILITY

6.1 How effectively the fellowship findings could be utilized for the sustainable development of IHR (max. 1000 words)

6.2 Efficient ways to replicate the outcomes of the fellowship in other parts of IHR (max. 1000 words)

6.3 Identify other important areas not covered under this study, but needs further attention (max. 1000 words)

6.4 Major recommendations for sustaining the outcomes of the fellowship in future (500 words in bullets)

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8 ACKNOWLEDGEMENTS

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(Signature of HRA/HJRF/HPF) (**NMHS FELLOWSHIP COORDINATOR**)

Place: ………………..
Date: ……../……/……

(**HEAD OF THE INSTITUTION**)

Executive Summary:

1. The common features in respect of water quality of river Umtrew (Digaru) were: Alkaline pH (6.4 -7.80), Moderate Turbidity (2.75-56.11 NTU), low Total alkalinity (19.50 -108.66 mgL^{-1}), Soft to moderately hard water (26.02-75.31 mgL^{-1}), optimum DO (5.43 -10. 52 mgL^{-1}), congenial TDS value (21.5 -205.66 mgL^{-1}), Electrical Conductivity (45.82-115.71 μScm^{-1}), Nitrate-Nitrogen (0.016 -0.09mgL^{-1}), Lower Nitrite-Nitrogen values (0.113-0.333 μgL^{1}) and Poor values of Soluble Inorganic Phosphate (Avg. 0.130 -0.897 mgL^{-1}).
2. Season wise summary of WQI value of water samples obtain from six different stations revealed that most of the water samples are fall into unsuitable water category i.e WQI>100. The maximum WQI value was observed at 269.14 during monsoon season in station 6 with an average value of 165.16±53.92. The EI value is indicating the river water is not eutrophic on the other hand CTSI value indicated the river is oligotrophic. However, the OPI and COI value was indicates that the water of the river comes into slightly to heavily polluted category in different station and in different season.
3. The MPN index in the Umtrew (Digaru) river ranged from 5 to 1400+ per 100 ml. The highest value was reported during the winter, and the lowest during the monsoon
4. The heavy metal investigation shows that pollution load index, degree of contamination, heavy metal toxicity load and median lethal toxicity were in the lower range whereas water quality index, Heavy metal pollution index, Evaluation index and Contamination factor were found to be in higher range. Human health risk assessment for oral exposure, Pb (2.8×10^{-2}), Cd (2×10^{-3}) and Ni (1×10^{-4}) was found to be equal or higher than the standard value of Incremental lifetime cancer risk (ILCR) ($\leq 1\times 10^{-4}$).
5. Principal component analysis indicated total variance of 49.57% in winter and it was positively affected by TDS, ammonia, EC, turbidity, BOD, COD, nitrite and nitrate. In pre monsoon, PC1 showed a total variance of 63.25% and it is positively related with turbidity, surface water, surface water, TDS, BOD and COD. During monsoon, PC1 has a total variance of 62.24% and positively affected by BOD, COD, turbidity, ammonia, TDS, EC, nitrite and temperature. During post monsoon period PC1 has total variance of 65.29%. It is positively affected by surface water temperature and turbidity and negatively affected by hardness and alkalinity.
6. A total of 49 fish species under 36 genera, 20 families and 10 orders were recorded. Among them Cypriniformes (42%) is the dominated order followed by Siluriformes (24%). As per IUCN status 1 species falls under critically endangered, 4 species are near threatened, 2 species under vulnerable and 42 species are of least concern. A significant correlation between species distribution and environmental variables was also reported. The trophic level ranges from 2.0±0.00 to 4.5±0.80. The trophic level was dominated by mid-level carnivore (24.4%) followed by omnivores (48.97%) and herbivores (26.53%).

7. The total score of Algal Genus Pollution Index (AGPI) of sites S1<S2<S3< S6<S5< S4 are calculated to be 3,5, 8, 10,11 and 13 respectively. The total score of S1, S2 and S3 indicating probable lack of organic pollution while S4, S4 and S6 showed moderate pollution due to anthropogenic factors or human interference

Annexure I

STUDY AREA

Meghalaya is a hill state of NE India, covers a total area of 22,720 km^2, with latitudes ranging from 20.1° N to 26.5° N and longitudes ranging from 85.49° E to 95.52° E. To the north and northeast, it is bordered by Assam, India, while to the south and southwest, it is bordered by Bangladesh. The river Umtrew is originated by the confluence of two streams, one of which originates near Mawrong hamlet on the Sohpetbneng Peak, and the other one is the Umium dam's outflow. These two streams meet at the Nongkhyllem Wildlife Sanctuary and run through Byrnih, a small town of Meghalaya. The river then enters into the state Assam at Sonapur, where it is known as Digaru until merging with the Brahmaputra near Chandrapur, Karup dist. of Assam. The Umtrew (Digaru) river basin is located between 25°35′15″ and 26°14′18″N latitude and 91°35′17″ to 92°00'15"E longitude in north-eastern India. River Digaru is the name given to the northern plain section of the Umtrew river when it enters into Assam. It covers a distance of about 30 km from the Umtrew hydroelectric power station, and along its periphery numerus industries like cement, drinks, iron, PVC are located. At a latitude of $26^{0}13^{/}51.8^{//}$N and a longitude of $91^{0}37^{/}28.8^{//}$E, it meets the Kopili river, a tributary of the Brahmaputra.

Table 1: Details of the different sampling stations with their longitude, latitude

Station No.	**Station Name**	**River Name**	**Longitude**	**Latitude**	**Elevation**
1	Zero Point	Umtrew, Meghalaya	$25^{0}43^{/}05.4^{//}$N	$91^{0}51^{/}28.1^{//}$E	738.5
2	Kyrdemkulai Dam	Umtrew, Meghalaya	$25^{0}44^{/}27.5^{//}$N	$91^{0}48^{/}31.9^{//}$E	692.2
3	Umtrew Dam	Umtrew, Meghalaya	$26^{0}00^{/}27.6^{//}$N	$91^{0}52^{/}02.7^{//}$E	122.8
4	Bornihat Bazer	Umtrew, Meghalaya	$26^{0}02^{/}31.7^{//}$N	$91^{0}52^{/}02.7^{//}$E	64.9
5	Digaru Bridge	Digaru, Assam	$26^{0}07^{/}13.9^{//}$N	$91^{0}58^{/}39.6^{//}$E	60.3
6	Digaru Kopili Confluence Point	Kopili, Assam	$26^{0}13^{/}51.8^{//}$N	$91^{0}37^{/}28.8^{//}$E	58.5

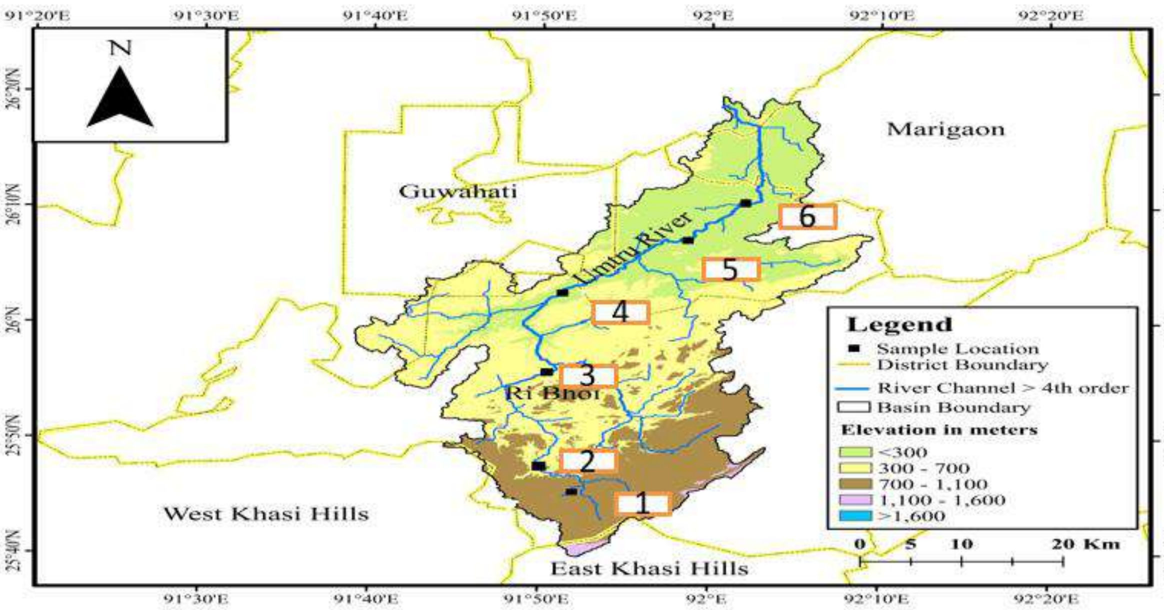

Fig 1:- Map of Umtrew River indicating Sampling Sites.

PLATE 2: STATION 1 ZERO POINT (EAST KHASI HILL, RI BHOI DISTRICT, MAGHALYA)

PLATE 3: STATION 2 KYRDEMKULAI DAM (EAST KHASI HILL, RI BHOI DISTRICT, MAGHALYA

PLATE 4: STATION 3 UMTREW HYDRO ELCTRIC PROJECT (RI BHOI DISTRICT, MAGHALYA

PLATE 5: STATION 4 BURNUHAT INDUSTRIAL AREA (RI BHOI DISTRICT, MAGHALYA)

PLATE 6: STATION 5 DISTRICT HOSPITAL, SONAPUR(KAMPUR DISTRICT,ASSAM)

PLATE 7: STATION 6 DIGARU KOPILI CONFLUENT POINT (BIGNI GHAT, KAMRUP, ASSAM)

Table 1.1: Morphological Characteristics of the Different stations of Umtrew river:

Station No.	Station Name	Morphology
Station 1	Zero Point	Rocky bottom, Water current fast, one side of the river bank is covered by dense forest.
Station 2	Kyrdemkulai Dam	Sandy bottom, Water is mostly stagnant due to dam contraction.
Station 3	Umtrew Dam	Rocky to coarse sandy river bottom, Water current is mostly affected by the dam.
Station 4	Bornihat Bazer	Sandy bottom with little water current, Water mostly affect by human activity establish beside the side of the river bank
Station 5	Digaru Bridge	Sandy bottom with little water current
Station 6	Digaru & Kopili Confluence Point	Sandy and muddy bottom with large water spread area.

Annexure II

METHODOLOGY

Water Quality Analysis:

Water samples for estimation of water quality parameters and the plankton samples were collected on monthly basis from January 2019 to May 2021 in the selected stations of river Umtrew (Digaru)(Fig.1). Physical parameters of water like surface water temperature, water velocity were estimated using the standard methods. Different chemical parameters of the water samples (pH, dissolved oxygen, alkalinity etc.) were analysed using standard procedures (APHA, 1989). TDS and Electrical Conductivity were estimated using Digital NEPHELO –TURBIDITY METER 132 (Systronics), Systronics digital conductivity meter 306. BOD_3 and COD were estimated using standard methods given by CPCB, 2011. Nitrate, Nitrite and Ammonia were evaluated using standard methods of APHA, 2005.

Water Quality Index:

The Water Quality Index (WQI) was designed to assess the combined impact of various water quality parameters on overall water quality. In the present study index was calculated on seven set of important water quality parameters and they are pH, Eclectic Conductivity (EC), Total Dissolved Solid (TDS), Biochemical Oxygen Demand (BOD_3), Dissolved Oxygen (DO), Total Hardness (TH) and Total Alkalinity (TA) were selected to generate the WQI. To calculate WQI 'weighted arithmetic index method' (Brown *et al*., 1970) was used, and the equation is as follows:

$$WQI = \frac{\Sigma\ QnWn}{\Sigma Wi}$$

where Qn is the quality rating of n^{th} water quality parameter, Wn is the unit weight of n^{th} water quality parameter and Wi is the relative wight of the water quality parameters.

The quality rating Q_n is calculated using the equation

$$Q_n = \frac{(V\ a - V\ i)}{(V\ s - V\ i)} \times 100$$

Where, V_a is the actual amount of n^{th} parameter present,

V_i is the ideal value of the parameter [V_i = 0, except for pH (V_i = 7) and DO (V_i = 14.6 mgl^{-1})],

V_s is the standard permissible value for the n^{th} water quality parameter.

Unit weight (W_i) is calculated using the formula

W_i = 1/Si

where 1 is the constant of proportionality

Table 2 : WQI range, Status, and possible usages of water sample (Brown *et al.*,1970)

WQIQI	Water quality status (WQS)	Possible usage	Grade
0-25	Excellent	Drinking, irrigation and industrial	A
26-50	Good	Drinking, irrigation and industrial	B
51-75	Poor	Irrigation and industrial	C
76-100	Very Poor	Irrigation	D
Above 100	Unsuitable for drinking and fish culture	Proper treatment required before use	E

Statistical analysis:

At first water quality data were divided into four seasons winter, pre monsoon, monsoon and post monsoon. To identify the factor which is responsible for water quality variation, PCA was applied. Through the field measurement, principal component analysis can help to identify sources of water contaminant and provides a better understanding of the effective pollution variables in different river reaches (Zeinalzadeh and Rezaei, 2017). In the present study PAST software was used to determine the principal component.

PCA derives information on the most significant quality parameters due to the spatial and seasonal variation. Water quality data with non-normal distribution were logarithmically transferred. Estimated parameters that were the main gradient of PCA were selected for WQI calculation. The correlation matrix was used to examine the link between water quality parameters and the index score. For the results of WQI in different season one way ANOVA was followed post-hoc turkey multiple comparison was used at $p< 0.05$.

Carlson Trophic State Index (C-TSI)

Acetone was used to estimate chlorophyll.a and a spectrophotometer was used to quantify it. The absorbance at 660 nm and 620 nm was measured in a spectrophotometer after chlorophyll was removed in 80 percent acetone. The absorption co-efficient was used to calculate the quantity of chlorophyll. The amount of chlorophyll in the extract, measured in milligrams of chlorophyll per gram of tissue, was determined using the equation below:

$$\text{mgchla/g tissue} = 12.7(A_{660}) - 2.69\,(A_{620}) \times \frac{10}{1000\times10} \qquad (3)$$

The trophic state index(TSI) of Carlson was calculated using the following formula

- TSI for Chlorophyll-a (CA)TSI = 9.81In Chlorophyll-a (ug/ L) +30.6
- TSI for Secchi depth (SD)TSI = 60-14.41In Secchi depth (Meters)

- TSI for Total phosphorus (TP)TSI = 14.42 In Total phosphorous (ug/l) + 4.15

where TSI is Carlson Trophic State Index and In is Natural logarithm.

Carlson's trophic state index (CTSI) = [TSI (TP)+TSI(CA)+TSI(SD)]/3

TP and Chlorophyll-a in micrograms per litre, SD transparency in meters.

Based on the values of CTSI the lakes are classified as oligotrophic (low productive), mesotrophic (moderately productive) and eutrophic (highly productive). The range of the Carlson'strophic state index values and classification of lakes are presented in the Table 3.

Table 3: Carlson's trophic state index values and classification of lakes (Prasad and Siddaraju, 2012)

TSI value	Trophic Status	Attributes
< 30	Oligotrophic	Clear water, oxygen throughout the year in the hypolimnion
30-40	Oligotrophic	A lake will still exhibit oligotrophy, but some shallower lakes will become anoxic during the summer
40-50	Mesotrophic	Water moderately clear, but increasing probability of anoxia during the summer
50-60	Eutrophic	Lower boundary of classical eutrophy: Decreased transparency, warm-water fisheries only
60-70	Eutrophic	Dominance of blue-green algae, algal scum probable, extensive macrophyte problems
70-80	Eutrophic	Heavy algal blooms possible throughout the summer, often hypereutrophic
>80	Eutrophic	Algal scum, summer fish kills, few macrophytes

Comprehensive Pollution Index (CPI)

CPI was computed using several mathematical models and calculates the pollution degrees by the suitable technique based on the evaluation of single factor index and considering the combined effect of all components examined (Guo, 2006). The CPI can be expressed as follows:

$$CPI=\frac{1}{n}\sum_{i=n}^{n} PI \quad (4)$$

PI_i is calculated according to the following equation:

$$PI = \frac{Ci}{Si}$$

where Ci is measured concentration of parameter number in water; Si is permitted limitation of parameter number according to environmental standard.

CPI is classified into five categories:

1. Category 1: CPI from 0 to 0.20 (clean);
2. Category 2: CPI from 0.21 to 0.40 (sub clean);
3. Category 3: CPI from 0.41 to 1.00 (slightly polluted);
4. Category 4: CPI from 1.01–2.00 (medium polluted);
5. Category 5: CPI 2.01 (heavily polluted).

In this study, we calculate CPI by using 13 water parameters: Temperature, DO, pH, Alkalinity, Hardness, Turbidity, TDS, BOD_3, COD, Nitrate, Nitrite, TDS, Phosphate and Eclectic Conductivity. These parameters were analysed in the Umtrew River water during the study period.

Organic Pollution Index (OPI)

To determine OPI; COD, DO, dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphate (DIP) are the four metrics used to determine OPI.OPI index was developed by dividing the values of four parameters, COD, DIN, DIP, and DO (Yadav *et al*., 2018). The organic pollution index (OPI) is calculated using the equation below.

$$OPI = \frac{COD}{COSs} + \frac{DIN}{DINs} + \frac{DIP}{DIPs} + \frac{DO}{DOs} \quad (5)$$

where, according to the environmental standard, CODs, DOs, DINs and DIPs are the limited concentrations of COD and DO; DINs is total limited concentration of nitrite; and DIPs is the limited concentration of phosphate. OPI is classified into four categories: excellent (OPI <0); good (OPI 0–1); polluted (1–4), extremely polluted (4–5).

Heavy metal analysis:

50 ml of sample was taken in a 100 ml beaker and 5 ml of concentrated HNO_3 was added to it. Then the solution was heated and the volume was reduced to 20 ml. After cooling the volume of the solution was made up to 50 ml. This was the extract for estimating metal content (Trievesy *et al*., 1987). Then toxic metals are determined with the help of AAS (Agilent Spectra AAS 220).

Heavy Metal Pollution Index (HPI):

The cumulative impact of individual heavy metals on surface water quality is represented by this index (Sheykhi and Moore, 2012). Each heavy metal is given a rating for this index based on its relative importance, which is specified as inversely proportional to the recommended standard value for each heavy metal. It is calculated as follows:

$$HPI=\frac{\sum_{i=1}^{n} WiQi}{\sum_{i=1}^{n} Wi} \quad (6)$$

Where Wi is the unit weight of the i^th heavy metal, Q is the sub index for the i^th heavy metal, and n is the number of heavy metals which is equals to 5 (Cu, Zn, Pd, Cd and Ni) for the present study. The sub index (Qi) is computed as:

$$Q=\sum_{i=1}^{n} \frac{| Mi-Ii|100}{Si-Ii} \quad (7)$$

Where Mi (µg/L) is the examined value of the heavy metal. In eq. (7) Si and Ii is the standard and ideal values, respectively, for the drinking water taken from USEPA (2009) for the heavy metals (µg/L). A value of HPI below 100 represent low pollution of the heavy metals. While 100 is the threshold value at which harmful health consequences are probable. An HPI value greater than 100 indicates the water is unsuitable for the consumption.

Heavy metal evaluation index (HEI):

HEI present the overall surface water quality with respect to heavy metals content, and computed by the following equation: (Al-Ani *et al*.,1987; Ameh,2013).

$$HEI\sum_{i=n}^{n} \frac{Mi}{MACi} \quad (8)$$

Where MACi is the monitored value and maximum admissible concentration of the ith heavy metal. Classification of surface water quality based upon HEI are: <10 for low, 10-20 for moderate and >20 is high pollution (Kumar *et al*., 2019).

Degree of contamination (C_d):

Degree of contamination (C_d) indicates the collective detrimental impact of the heavy metals on surface water (Backman *et al*.,1998) and determined as:

$$C_d=\sum_{i-1}^{n} Cfi \quad (9)$$

$$Cfi= \frac{Mi}{MACi}-1 \quad (10)$$

Where *Cfi* is the contamination factors for the ith heavy metal. The categories used to represent heavy metal pollution on the basis of C_d are: <1 for low, 1-3 for moderate and > 3 for high pollution of heavy metals in the surface water body (Backman *et al*, 1998).

Heavy Metal toxicity load (HMTL):

The HMTL index assesses the amount of heavy metals present in water sources that have an effect on human health (Saha and Paul, 2018). It is determined by multiplying examined content of heavy metals with the Hazard Intensity. Calculation is as follows:

$$\text{HMTL}=\sum_{i=1}^{n} Mi \times HISi \qquad (11)$$

Where *HISi* is the hazard intensity score of the i^{th} heavy metal adopted from ATSDR (2017).

Median Lethal Toxicity (MLT):

Median lethal concentration is also known as LC50 referred the dose that kills 50 % of the total population. The median lethal dose (MLD) value is inversely proportional to the toxicity i.e the more the MLD value lesser the toxicity (Chinedu *et al*.,2013). Heavy metals that present in the water gets entered into the aquatic animals and human health by direct ingestion, dermal absorption or by inhalation. Besides that, bioaccumulation of heavy metal in the aquatic organism can enter into the different tropic levels by biomagnification. The LC50 values of heavy metals was calculated by the presence of the ions. In present study the MLD value was determined by the MLD of its commonly occurring salts. The calculation is as follows:

$$MLD(metal) = \frac{MLDms \times Mm.m}{ms.mm} \qquad (12)$$

Where MLDms is the median lethal toxicity of the metal salt. M_{mm} is the molar mass of metal and ms_{mm} id the molar mass of the metal salt.

Median Lethal toxicity (MLT) can be defend as the amount of metal present in 1000L of water in units of its LD50 (mg/kg. rat or mouse, oral).

$$MLD(metal) = \frac{Amount\ of\ the\ metal\ (mg/kl)}{MLD\ (\frac{mg}{kg} rat\ and\ mouse.orel)} \qquad (13)$$

In terms of metal concentration in water, equation as follows:

$$MLD(metal) = \frac{Amount\ of\ metal\ (\mu g/l)}{MLD\ (\frac{mg}{kg} rat\ or\ mouse.orel)} \qquad (14)$$

Sum of the all individual MLT defined as Total Median Lethal Toxicity (TMLT).

Assessment of Human Health Risk:

Human health risk assessment is a numerical method to assess the probability of the health effect of the hazardous chemical on human (Liu *et al*., 2014). In the present study, non-carcinogenic human health hazards were assessed by ingestion and dermal exposure of heavy metals that found in the Umtrew river surface water. To find the probable non cancer health risk through orel and dermal exposure of surface water, Hazard Quotients (HQ) and Hazard index (HI)was calculated in the present study. In this study adult population are assumed as the most targeted group. Chronic Daily Intake (CDI) of heavy metal by oral and dermal adsorption was calculated by following equation according to US Environment Protocol Agency (USEPA, 2004).

$$CDI_{oral} \,(\text{mg kg}^{-1}\text{ day}^{-1}) = \frac{Chm \times DI \times ABS \times EF \times ED}{BW \times AT} \quad (15)$$

$$CDI_{dermal} \,(\text{mg kg}^{-1}\text{ day}^{-1}) = \frac{Chm \times SA \times Kp \times ABS \times ET \times EF \times ED \times CF}{BW \times AT} \quad (16)$$

In equation 15 and 16 C_{hm} represents heavy metal concentration, DI is daily average intake, SA indicates skin surface area, K_p represents permeability coefficient, ET is exposure time, EF exposure frequency, ED represent exposure time, CF is conversion ratio, BW is average body weight, ABS and AT is absorption factor and average time respectively.

The HQ is the ratio between the calculated mean chronic daily intake (CDI) of heavy metals to the oral reference dose (R_fD) for the same heavy metal through oral and dermal adsorption of water was calculated using the following formula:

$$HQ_{oral} = \frac{\text{CDI oral}}{\text{RfD oral}} \quad (17)$$

$$HQ_{dermal} = \frac{\text{CDI dermal}}{\text{RfD dermal}} \quad (18)$$

.

The HI represents the total non-carcinogenic health risks posed by various heavy metals found in water. HI is calculated using standard formula given by USEPA guideline. Calculation are as follows.

$$HI_{oral} = \sum_{i=1}^{n} HQ\ oral = HQ_{Co} + HQ_{Zn} + HQ_{Pb} + HQ_{Cd} + HQ_{Ni} \quad (19)$$

$$HI_{dermal} = \sum_{i=1}^{n} HQ\ dermal = HQ_{Co} + HQ_{Zn} + HQ_{Pb} + HQ_{Cd} + HQ_{Ni} \quad (20)$$

To estimate the potential non carcinogenic human health risk minimal value was taken as 1.0. The HI value is <1.0 it indicates that the hazardous health effects on communities are unanticipated. On the contrary, if the value of HI is >1.0 it indicates local residents in the study area may be exposed to non-carcinogenic health risks (Mohammadi *et al*., 2019).

Carcinogenic Health Risk:

Incremental lifetime cancer risk (ILCR) was estimated due to presence of potential carcinogen like Pb, Cd and Ni. Incremental lifetime cancer risk was calculated by multiplying CDI of oral and dermal and Cancer Slop Factor (CSF) (Mohammadi *et al*.,2019). Calculation is as follows:

$$ILCR = CDI \times CSF \quad (21)$$

The permissible limits are considered to be 10^{-6} and $<10^{-4}$ for a single carcinogenic element and multi element carcinogens.

Analysis of plankton samples:

Plankton samples were collected by filtering 100-200 litres of river water using 28 mm mesh nylobolt plankton net Santhanam *et al.* (1987). The collected plankton samples were preserved in 3-4 % formalin in separate plankton tubes. In laboratory, from the known volume plankton sample counting was done by using Sedgwick Rafter Plankton counting cell (Sharma and Saini 2005).

Measuring Biodiversity of Plankton

Shannon – Wiener (H) of plankton was calculated using the formula of Shannon (1949)

$$\text{Shannon index } (H) = -\sum_{i=1}^{s} \mathrm{P}i \ln \mathrm{P}i$$

Where, n = individuals of one particular species found

N= total number of individuals found

P_i = proportion of (n/N)

$\ln P_i$ = Natural log of proportion of (n/N)

Σ = sum of the calculations, and s is the number of species.

Annexure III

RESULTS

Atmospheric variables:

Atmospheric temperature and Rainfall:

The temperature of the atmosphere in the study area i.e the entire stretch oh Umtrew river was fluctuated between 6.6^{0}c (January 2019) to 28.1^{0}c (August 2019). Rainfall ranged from 22.8 mm to 429.7 mm during the study period. The lowest rainfall occurred in January 2019 and the highest was is in July 2019. In 2020 the temperature fluctuated between 6.5 (January) to 28.6 ^{0}c (August) and rainfall ranges from 14.5 to 627.7 mm. Lowest rainfall observed in January and lowest was observed in July.

Table 4 : Monthly atmospheric temperature and rainfall pattern during the study period (Source: Indian Council of Agricultural Research for NEH Region, Umium, Meghalaya).

Months	TOTAL MONTHLY RAINFALL (mm)	MEAN MONTHLY MAXMUM TEMPRATURE (°c)	MEAN MONTHLY MINIMUM TEMPRATURE (°c)	TOTAL MONTHLY RAINFALL (mm)	MEAN MONTHLY MAXMUM TEMPRATURE (°c)	MEAN MONTHLY MINIMUM TEMPRATURE (°c)
January	22.8	21.2	6.6	14.5	18.8	6.5
February	13.6	22.6	8.9	54.0	20.8	7.9
March	19.9	25.1	11.9	75.4	24.6	11.3
April	180.9	26.6	15.2	223.5	26.2	14.3
May	203.3	28.1	18.0	578.5	26.1	17.0
June	380.4	28.1	19.9	463.5	27.0	20.4
July	429.7	27.9	20.4	627.7	26.7	20.9
August	396.7	29.6	20.6	197.6	28.6	21.1
September	290.6	27.1	19.3	695.2	27.1	20.0
October	259.1	25.1	16.1	484.6	26.9	18.1
November	33.2	24.2	12.8	79.0	24.7	10.9
December	24.8	20.5	6.9	25.4	21.2	8.3

Physical Parameters of Water

Water velocity:

Water velocity of a river depends upon many factors like drainage of water from catchment area, rainfall pattern of the area, etc. During the study period, the velocity ranged from 0.225 msec^{-1} in station 3(Winter 19 to 4. 077 (Monsoon 19) msec^{-1} in station 1.

Table 5: Maximum and Minimum values of water velocity

	Min	Max
Station 1	3.17 (Winter 2020)	4.077 (Monsoon 2019)
Station 2	0.110 (Pre monsoon 2020)	0.340 (Monsoon 2020)
Station 3	0.225 (Winter 2019)	1.25 (Monsoon 2020)
Station 4	0.460 (Winter 2019)	1.88 (Monsoon 2020)
Station 5	0.475 (Winter 2019)	1.78 (Monsoon 2020)
Station 6	0.525 (Winter 2019)	1.807 (Monsoon 2020)

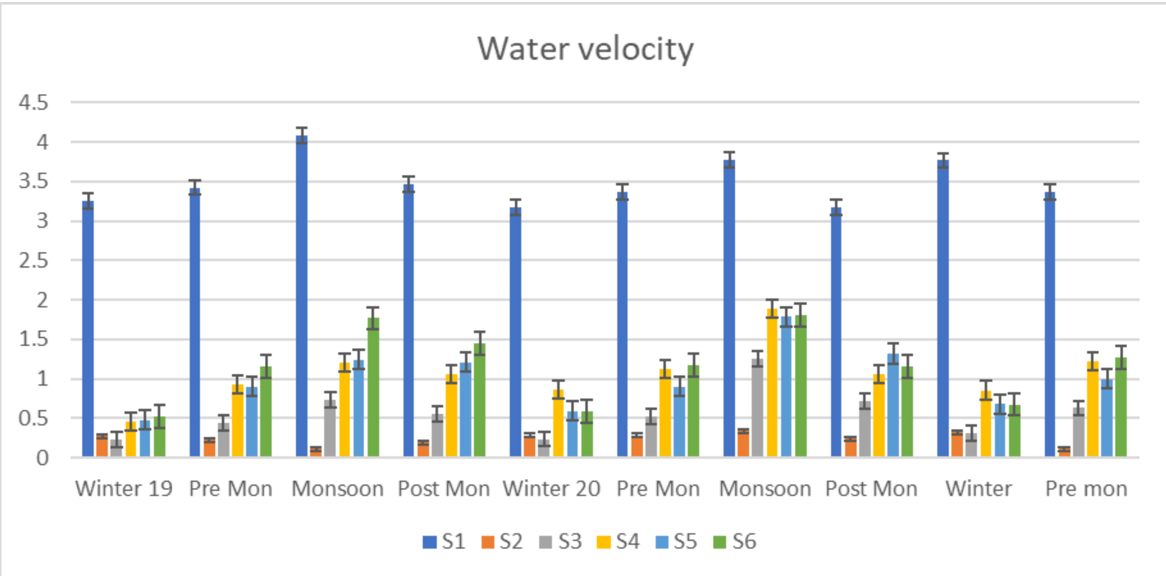

FIGURE 2: MONTHLY VARIATION OF WATER VELOCITY AT SIX SELECTED STATION

Surface Water Temperature:

Water quality of aquatic ecosystem depends on the surface water temperature up to a huge extent. Surface water temperature of river Umtrew (Digaru) showed a sharp seasonal variation, the overall surface water temperature varied between 15.75 ^{0}C (Winter 19) in station 1 and 32 ^{0}C (Monsoon 19) in station 5 during the study period.

Table 6: Maximum and Minimum values of surface water temperature

	Min	Max
Station 1	15.75 (Winter 2019)	23.89 (post Monsoon 2020)
Station 2	17.00 (Winter 2019)	25.00 (Monsoon 2019)
Station 3	18.00 (Winter 2019)	28.33 (Monsoon 2020)
Station 4	19.00 (Winter 2019)	30.00 (Monsoon 2020)
Station 5	19.00 (Winter 2019)	32.00 (Monsoon 2019)
Station 6	20.50 (Winter 2019)	30.32 (Monsoon 2020)

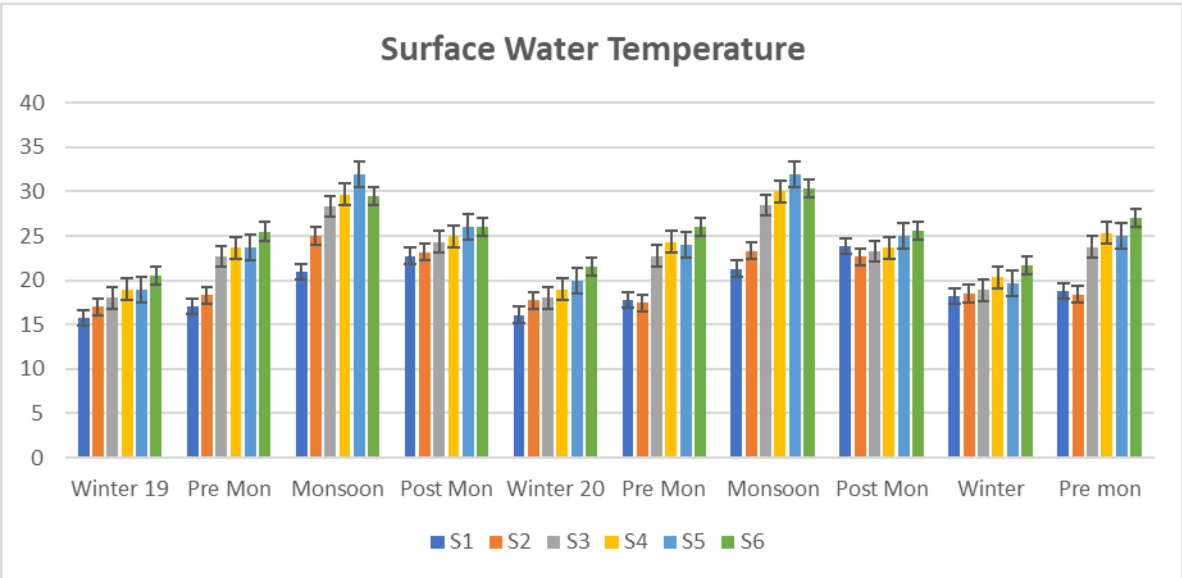

FIGURE 3: MONTHLY VARIATION OF SURFACE WATER TEMPARATURE AT SIX SELECTED STATION

Turbidity:

Turbidity is caused by particles suspended or dissolved in water that scatter light making the water appear cloudy or murky. Particulate matter can include sediment - especially clay and silt, fine organic and inorganic matter, soluble coloured organic compounds, algae, and other microscopic organisms. The turbidity of Umtrew river was found to vary between 2.75 NTU (Winter 19) in station 1 and 56.11 NTU (Monsoon 20) in station 4.

Table 7: Maximum and Minimum values of turbidity

	Min	Max
Station 1	2.75 (Winter 2019)	9.43 (Monsoon 2020)
Station 2	6.04 (Winter 2020)	17.7 (Monsoon 2019)
Station 3	6.75 (Winter 2019)	24.83 (Monsoon 2020)
Station 4	6.15 (Winter 2019)	56.11 (Monsoon 2020)
Station 5	9 (Winter 2019)	50.93 (Monsoon 2020)
Station 6	8.65 (Winter 2019)	53.32 (Monsoon 2020)

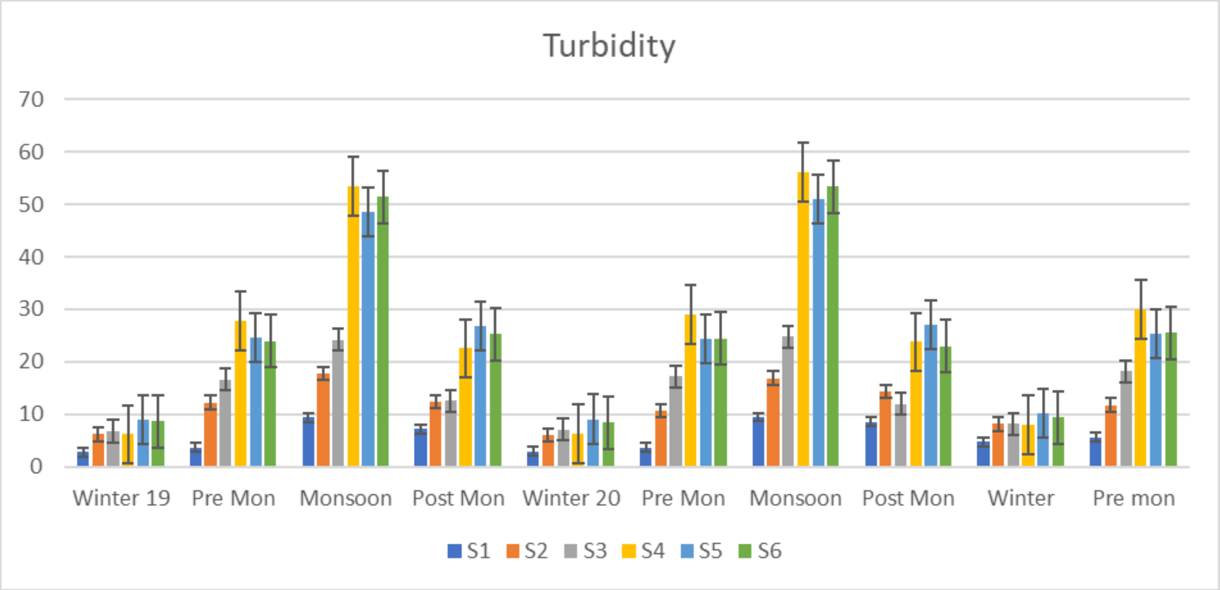

FIGURE 4: MONTHLY VARIATION OF TURBIDITY AT SIX SELECTED STATION

Chemical parameters of water:

Water pH:

pH stands for power of Hydrogen and it measures the intensity of acidity and alkalinity of water by quantifying the concentration of hydrogen ions. pH of the river ranged between 6.4 and 7.80 during the study period. Maximum pH was observed in station 1 during monsoon period and minimum was observed during post monsoon period in station 2.

Table 8: Maximum and Minimum values of pH

	Min	Max
Station 1	7.26 (Post monsoon 2020)	7.80(Monsoon 2019)
Station 2	6.4 (Pot monsoon 2019)	7.7 (Monsoon 2019)
Station 3	6.9 (Pot monsoon 2020)	7.66 (Pre Monsoon 2019)
Station 4	7.07 (Post Monsoon 2019)	7.60 (Pre Monsoon 2021)
Station 5	6.9(Pot monsoon 2019)	7.80 (Monsoon 2019)
Station 6	7.1 (Winter 2021)	7.53 (Pre Monsoon 2020)

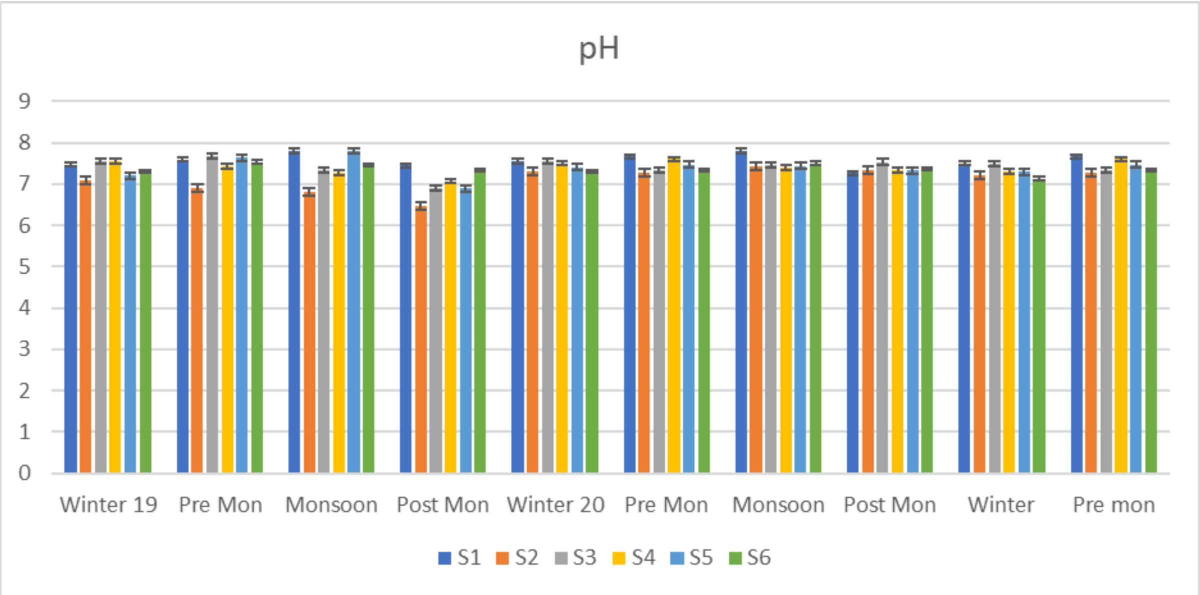

FIGURE 5: MONTHLY VARIATION OF pH AT SIX SELECTED STATION

Dissolved oxygen:

Level of dissolved oxygen in water refers to the level of free, non-compound oxygen present in water. For assessing water quality, DO is an important parameter, and it is having a major influence on the organisms living within a body of water, as it can harm them if its concentration is too high or too low. Dissolved oxygen of Umtrew (Digaru) river was recorded to fluctuate between 5.43 mgL^{-1} and 10.52 mgL^{-1}, minimum was in the monsoon 2019 and maximum was in winter 2019.

Table 9: Maximum and Minimum values of dissolved oxygen

	Min	Max
Station 1	8.41 (Pre Monsoon 2019)	10.52 (Winter 2021)
Station 2	5.50 (Monsoon 2019)	7.57 (Winter 2020)
Station 3	5.70 (Monsoon 2020)	9.35 (Winter 2021)
Station 4	5.50 (Monsson 2020)	9.06 (Winter 2021)
Station 5	5.56 (Monsoon 2020)	8.45 (Winter 2019)
Station 6	5.43 (Monsoon 2019)	8.75 (Winter 2019)

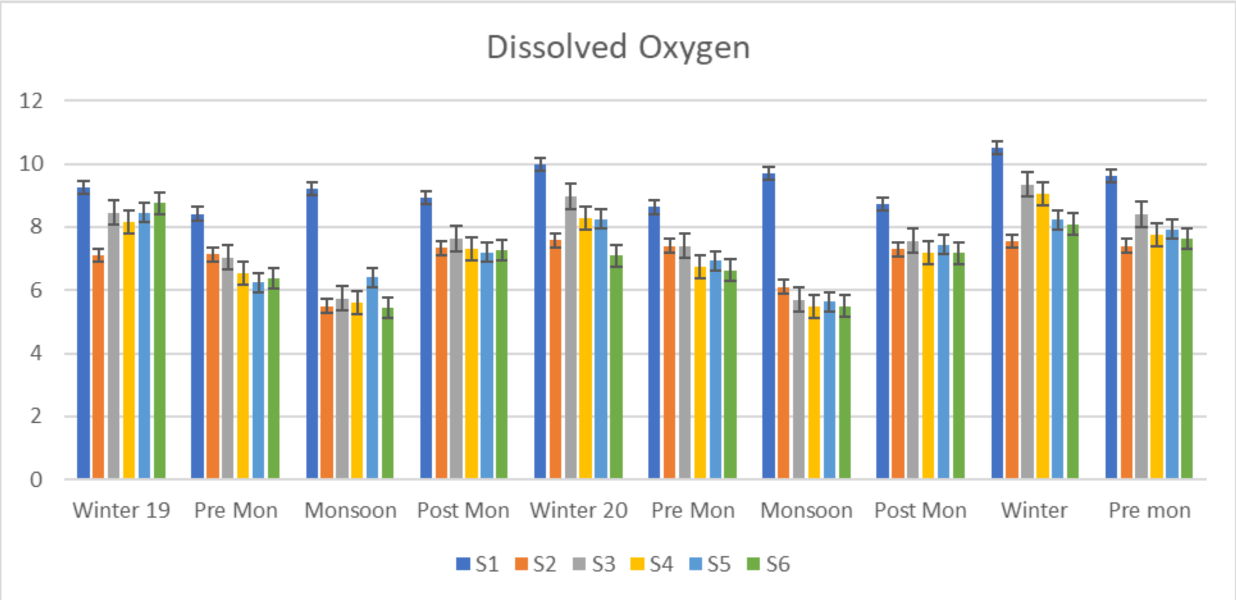

FIGURE 6: MONTHLY VARIATION OF DISSOLVED OXYGEN AT SIX SELECTED STATION

Total Alkalinity :

Total alkalinity is the sum of titratable bases in water. In most waters, bicarbonate, carbonate are the predominant bases that contribute to alkalinity. It is the buffering capacity of a water body and thus maintain a fairly stable pH level. During present study, maximum alkalinity was observed 108.66 mgL^{-1} in station 1 and 19.50 mgL^{-1} in station 5.

Table 10: Maximum and Minimum values of total alkalinity

	Min	Max
Station 1	42.46 (Winter 2020)	108.66 (Monsoon 2019)
Station 2	44.00 (Winter 2019)	67.00 (winter 2020)
Station 3	17.66 (Winter 2021)	34.66 (Monsoon 2020)
Station 4	19.50 (Winter 2019)	55.67 (Monsoon 2020)
Station 5	19.50 (Winter 2020)	50.66 (Monsoon 2020)
Station 6	21.50 (Winter 2020)	52.66 (Monsoon 2019)

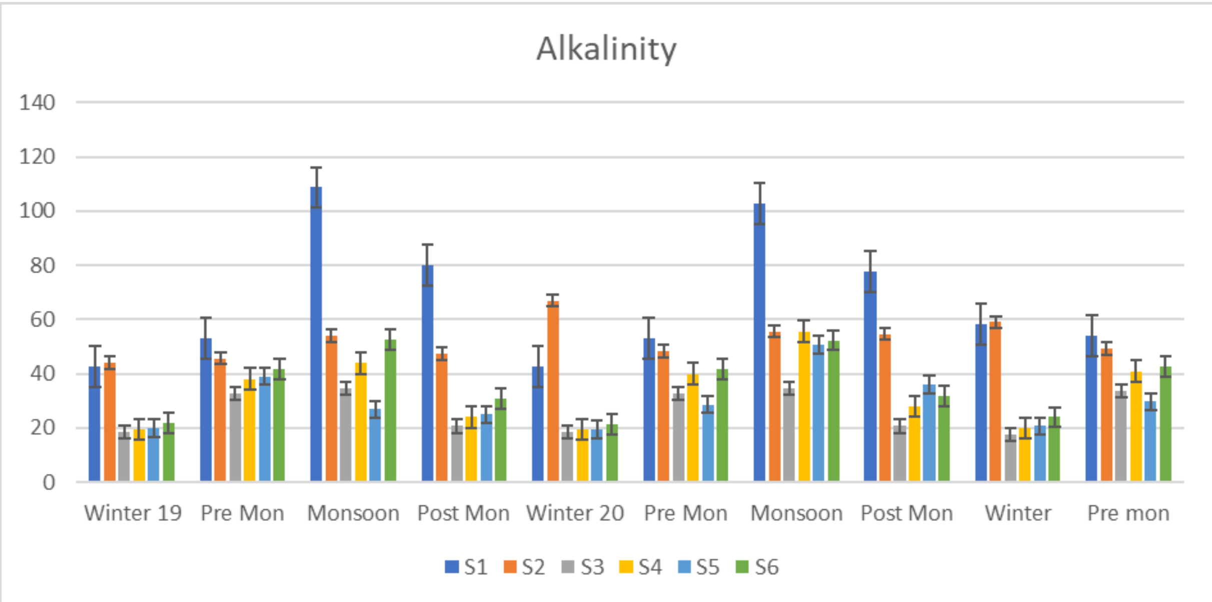

FIGURE 7: MONTHLY VARIATION OF ALKALINITY AT SIX SELECTED STATION

Total Hardness:

Total hardness is the sum of concentration of calcium and magnesium in water. Other divalent cations also contribute to hardness, but their concentration in natural waters are usually low. During the study period, the during winter (26.02 mgL^{-1}), highest value of total hardness was recorded and the lowest value was recorded during post monsoon (75.31 mgL^{-1}).

Table 11: Maximum and Minimum values of total hardness

	Min	Max
Station 1	42.42 (Post Monsoon 2020)	55.75 (Winter 2020)
Station 2	44.42 (Monsoon 2019)	58.05 (Winter 2020)
Station 3	26.02 (Post Monsoon 2019)	52.12 (Pre Monsoon 2021)
Station 4	26.69 (Post Monsoon 2019)	48.76 (Pre Monsoon 2021)
Station 5	28.35 (Post Monsoon 2019)	50.71 (Pre Monsoon 2020)
Station 6	33.29 (Post Monsoon 2019)	75.31 (Winter 2020)

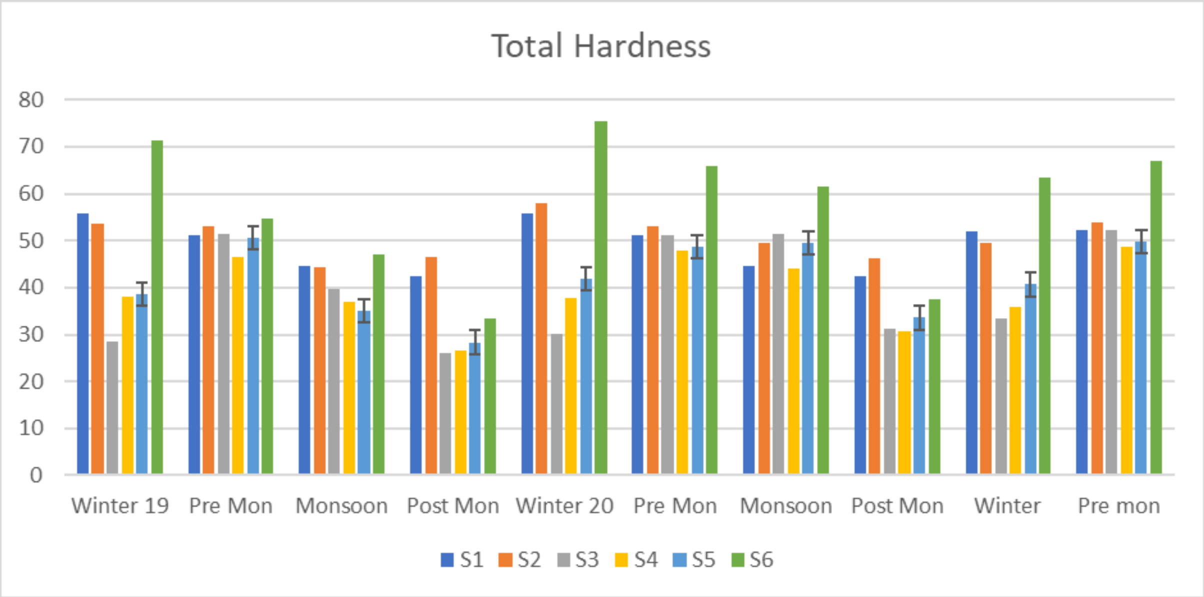

FIGURE 8: MONTHLY VARIATION OF TOTLA HARDNESS AT SIX SELECTED STATION

Electrical conductivity:

Electrical Conductivity (EC) in natural waters is the simplified measure of the water's ability to conduct electric current. Mostly this is determined by dissolved salts found in the body of water. It also provides an indirect indicator of ion concentration, such as Nitrate, Sulphate, Phosphate, Magnesium, Potassium, Calcium, and Iron. In the present study EC was maximum in station 2 (monsoon 20) and minimum in station 1 (Winter 20).

Table 12: Maximum and Minimum values of electrical conductivity

	Min	Max
Station 1	45.82 (Winter 2020)	76.35 (Monsoon 2020)
Station 2	54.31 (Pre Monsoon 2021)	245.55 (Monsoon 2020)
Station 3	98.50 (Pre Monsoon 2019)	137.11 (Monsoon 2020)
Station 4	106.10 (Pre Monsoon 2019)	108.327 (Monsoon 2020)
Station 5	100.69 (Winter 2019)	124.15 (Monsoon 2020)
Station 6	115.71 (Pre Monsoon 2019)	127.60 (Monsoon 2020)

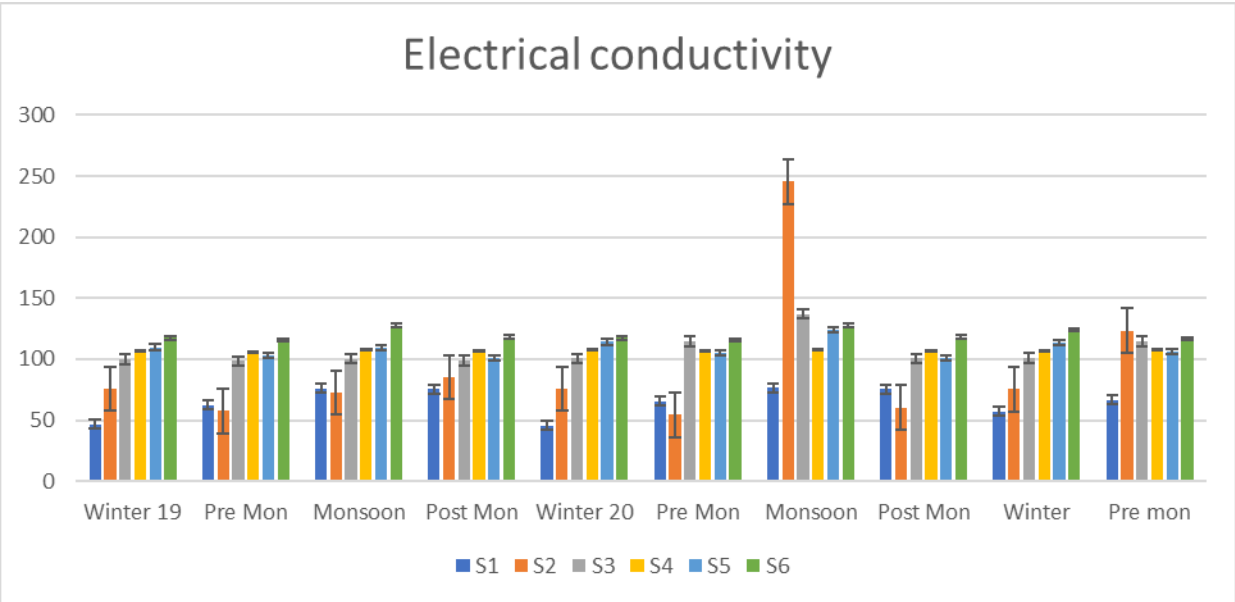

FIGURE 9: MONTHLY VARIATION OF ELECTRICAL CONDUCTIVITY AT SIX SELECTED STATION

Total Dissolved Solids:

Total dissolved solids (TDS) is the term used to describe the inorganic salts and small amounts of organic matter present in solution in water. Calcium, magnesium, sodium, and potassium cations and anions of carbonate, hydrogen carbonate, arsenic, sulfate, and nitrate are typically the principal constituents. In the present study the TDS values ranges from 21.5 mgL^{-1} to 205.66 mgL^{-1}.

Table 13: Maximum and Minimum values of total dissolved solids

	Min	Max
Station 1	21.50 (Winter 2019)	51.66 (Monsoon 2019)
Station 2	22.40 (Winter 2020)	130.72 (Monsoon 2020)
Station 3	26.00 (Winter 2019)	146.33 (Monsoon 2020)
Station 4	48.50 (Winter 2019)	190.00 (Monsoon 2020)
Station 5	53.00 (Winter 2019)	205.33 (Monsoon 2020)
Station 6	55.50 (Winter 2020)	205.66 (Monsoon 2020)

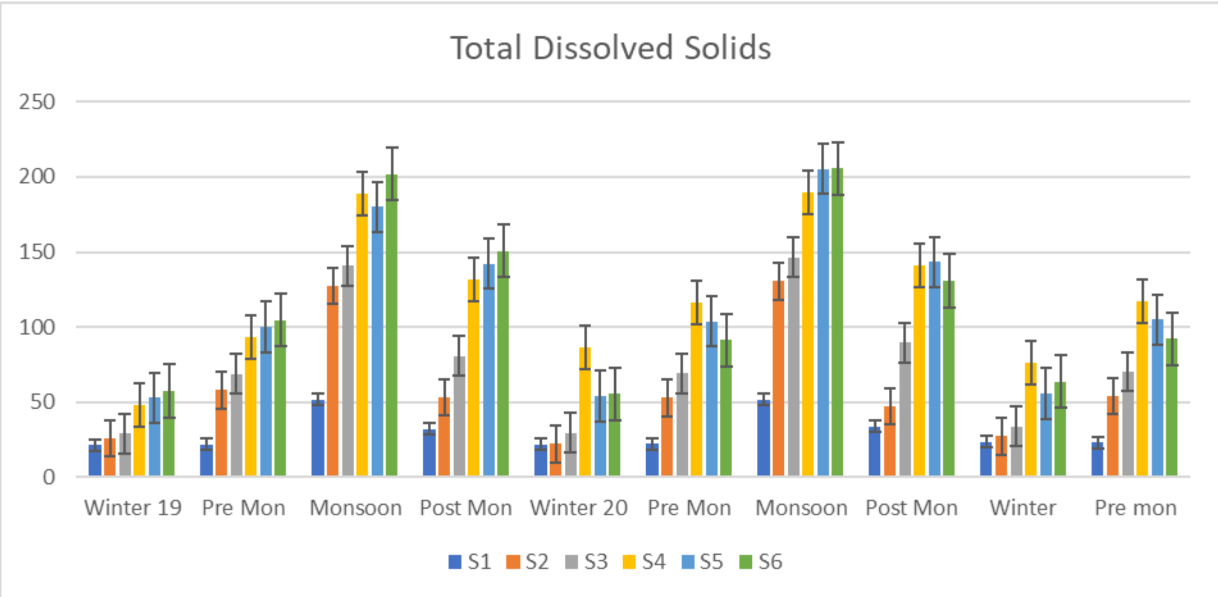

FIGURE 10: MONTHLY VARIATION OF TDS AT SIX SELECTED STATION

Pollution Indicating Parameters

Biological Oxygen Demand (BOD):

Biological Oxygen Demand (BOD) is a pollution indicating parameter and its increase indicates decomposition of organic matter (Boyd and Tucker, 1998). It measures oxygen required for aerobic oxidation of decomposable organic matter and certain inorganic materials in water, polluted waters and wastewater under controlled conditions of temperature and incubation period (CPCB, 2011). BOD of Umtrew (Digaru) river fluctuated between 7.14 mgL^{-1} to 36.20 mgL^{-1} .

Table 13: Maximum and Minimum values of BOD

	Min	Max
Station 1	7.64 (Winter 2020)	10.33 (Monsoon 2019)
Station 2	7.14 (Winter 2019)	10.55 (Monsoon 2019)
Station 3	8.42 (Winter 2019)	22.25 (Monsoon 2020)
Station 4	10.75 (Winter 2019)	27.50 (Monsoon 2020.)
Station 5	13.40 (Winter 2019)	31.70 (Monsoon 2019)
Station 6	10.49 (Winter 2019)	26.50 (Monsoon 2020)

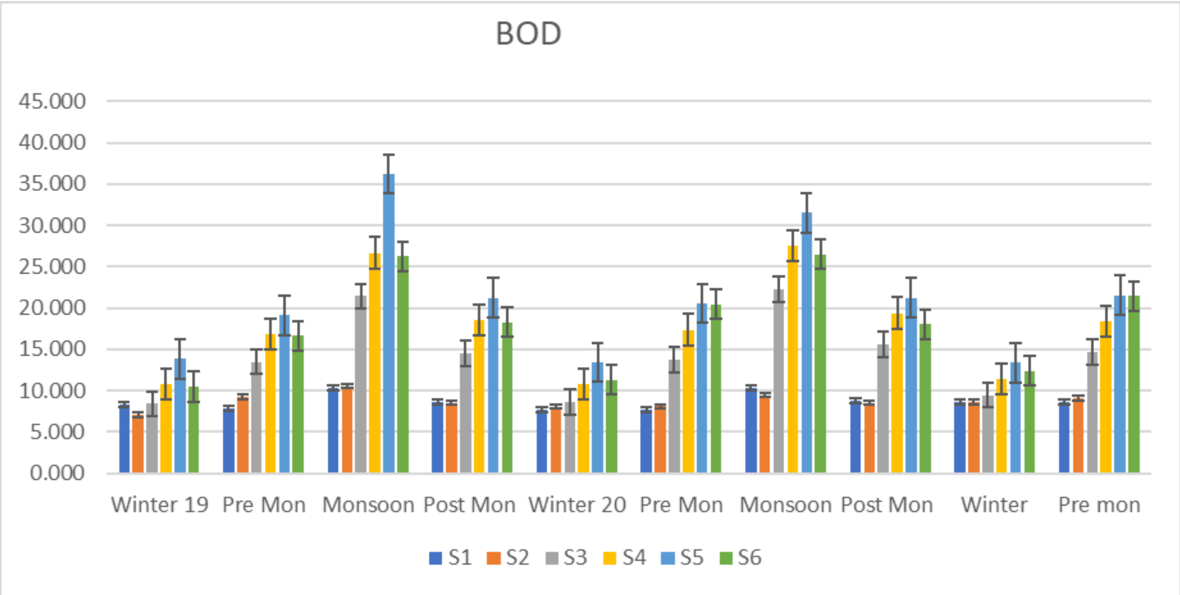

FIGURE 11: MONTHLY VARIATION OF BOD AT SIX SELECTED STATION

Chemical Oxygen Demand (COD):

Chemical Oxygen Demand (COD) is an approximate indicator of the amount of oxygen that a determined solution will absorb through a reaction. In terms of water quality, COD is useful by offering a metric to assess the effect of an effluent on the receiving body (Ali and Sreekrishnan, 2001). During the study period, COD of Umtrew (Digaru) river fluctuated between 13.93 mgL^{-1} to 54.28 mgL^{-1}.

Table14: Maximum and Minimum values of COD

	Min	Max
Station 1	13.93 (Winter 2020)	21.58 (Monsoon 2019)
Station 2	15.92 (Winter 2019)	24.09 (Monsoon 2019)
Station 3	14.85 (Winter 2019)	38.05 (Monsoon 2020)
Station 4	17.95 (Winter 2019)	48.10 (Monsoon 2020)
Station 5	23.40 (Winter 2019)	53.68 (Monsoon 2020)
Station 6	24.16 (Winter 2020)	54.28 (Monsoon 2019)

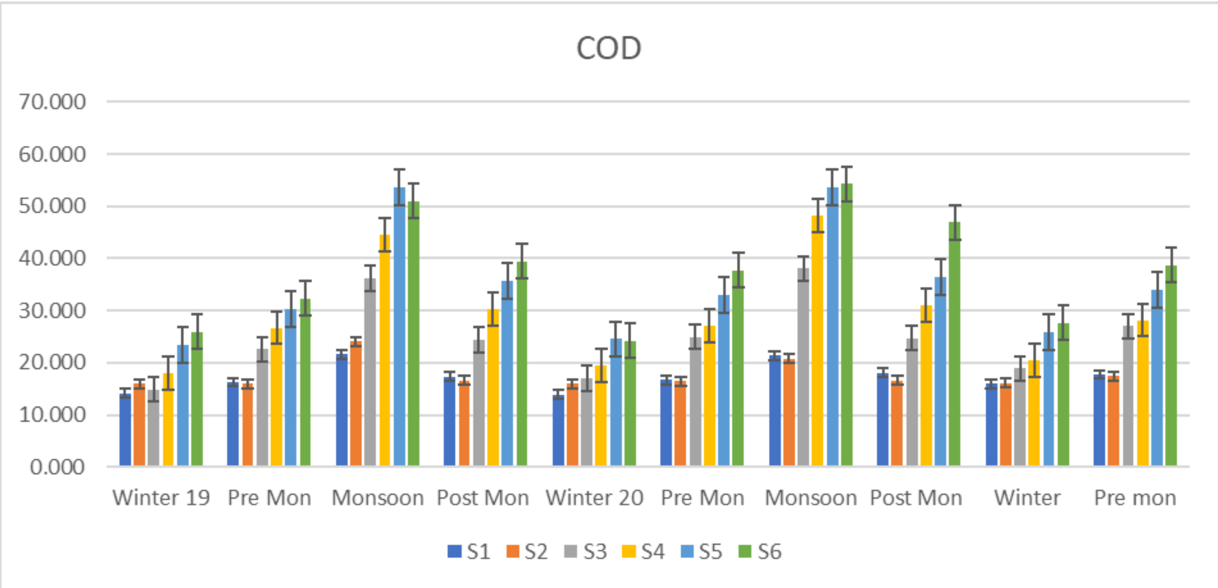

FIGURE 12: MONTHLY VARIATION OF COD AT SIX SELECTED STATION

Nitrate-Nitrogen:

Nitrate (NO3-N) is a common form of inorganic combined nitrogen in natural waters and aquaculture systems. Most of the nitrate found in unpolluted waters is the end product of nitrification. Nitrate is the major form of nitrogen used by phytoplanktons. In the present study maximum was found in station 4 and minimum was found in station 1.

Table 15: Maximum and Minimum values of nitrate nitrogen

	Min	Max
Station 1	0.135 (Winter 2019)	0.247 (Monsoon 2019)
Station 2	0.145 (Pre Monsoon 2020)	0.270(Monsoon 2019)
Station 3	0.113 (Winter 2019)	0.257 (Pre Monsoon 2020)
Station 4	0.217 (Post Monsoon 2020)	0.333 (Pre Monsoon 2020.)
Station 5	0.150 (Winter 2019)	0.327 (Monsoon 2020)
Station 6	0.150 (Winter 2019)	0.270 (Monsoon 2019)

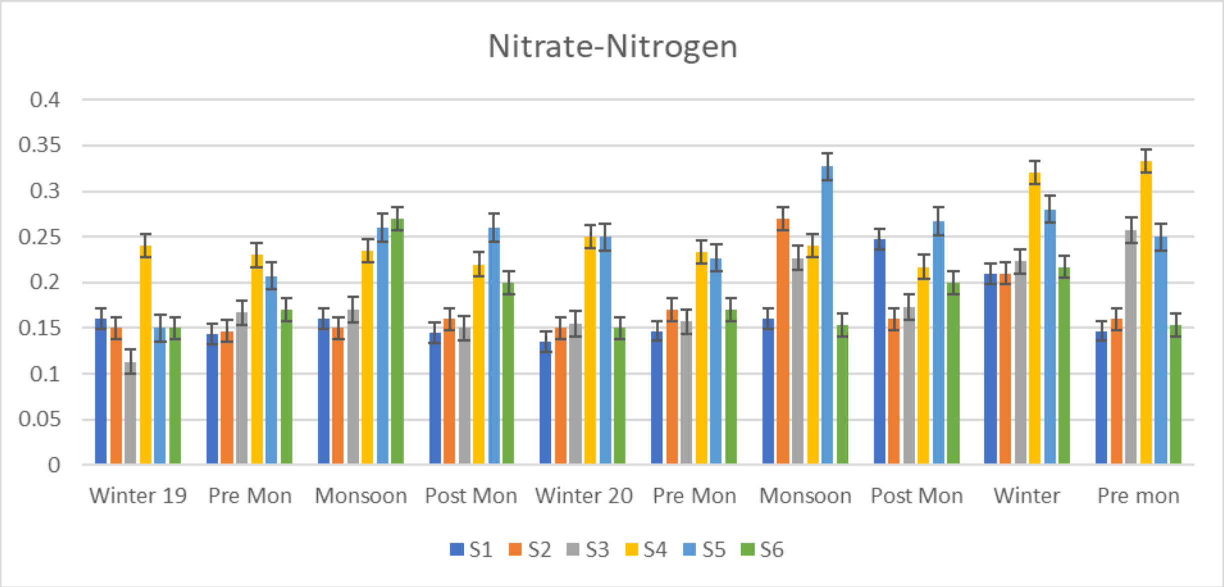

FIGURE 13: MONTHLY VARIATION OF NITRATE NITROGEN AT SIX SELECTED STATION

Nitrite – Nitrogen:

Nitrite (NO2-N) is a naturally occurring intermediate product in two bacteria mediated process involving transformation of nitrogen in water and soil. Nitrite accumulation can be toxic to fishes. During the study period, Nitrite concentration of the river Umtrew (Digaru) ranged between 0.016 to 0.076 mgL^{-1} lowest value was recorded in the station 1 and the highest value was recorded in the station 3.

Table16: Maximum and Minimum values of nitrite nitrogen

	Min	Max
Station 1	0.016 (Pre-Monsoon 2019)	0.05 (Post Monsoon 2019)
Station 2	0.03 (Pre Monsoon 2019)	0.05 (Monsoon 2020)
Station 3	0.043 (Winter 2019)	0.076 (Post Monsoon 2019)
Station 4	0.025 (Pre monsoon 2020)	0.09 (Post Monsoon 2019)
Station 5	0.026 (Pre Monsoon 2020)	0.09 (Post Monsoon 2019)
Station 6	0.049 (Pre Monsoon 2019)	0.068 (Winter 2021)

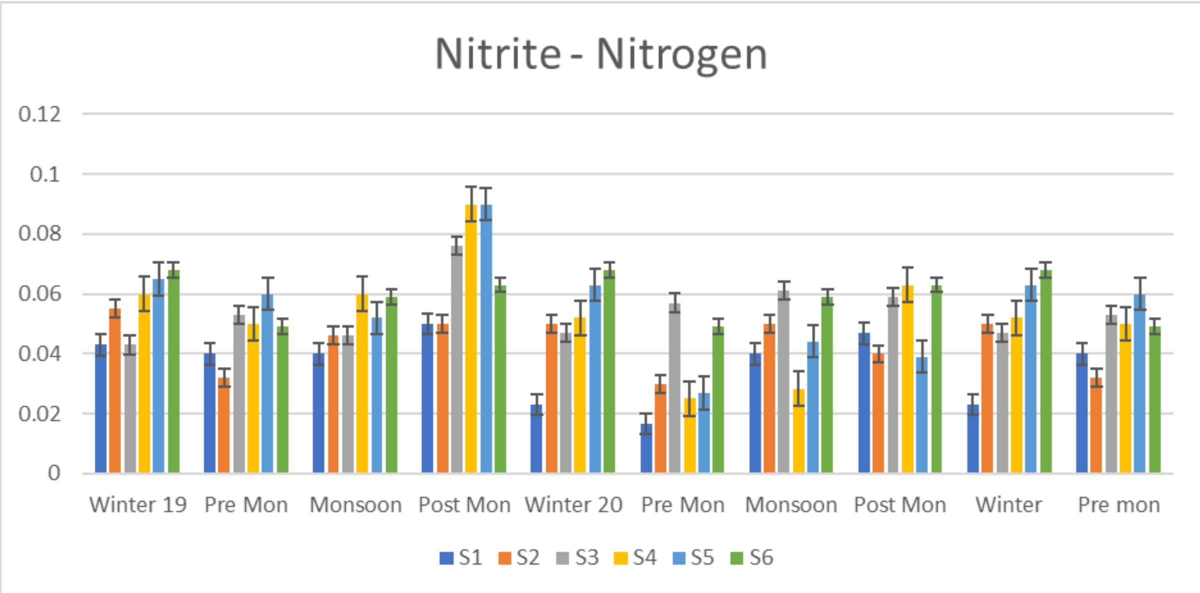

FIGURE 14: MONTHLY VARIATION OF NITRITE NITROGEN AT SIX SELECTED STATION

Total ammonia:

Ammonia is the principal nitrogen waste product that crustaceans and most fish excrete. Some fish excrete large quantities of urea, but most of it gets easily hydrolyzed in the water into ammonia and carbon dioxide. Ammonia is also produced during decomposition of nitrogen containing organic matter. Ammonia accumulation in aquaculture is undesirable because unionized ammonia is harmful to aquatic animals (Boyd and Tucker, 1998). In present study total ammonia was recorded maximum 1.81 mgL^{-1} during monsoon season and minimum was found 0.14 mgL^{-1} during winter.

Table 16: Maximum and Minimum values of total ammonia

	Min	Max
Station 1	1.32 (Pre Monsoon 2019)	0.303 (Monsoon 2019)
Station 2	0.145 (Winter 2020)	0.283 (Monsoon 2020)
Station 3	0.463 (Post Monsoon 2019)	1.23 (Monsoon 2020)
Station 4	0.65 (Winter 2019)	1.90 (Monsoon 2019)
Station 5	0.66 (Winter 2020)	3.06 (Monsoon 2019)
Station 6	0.68 (Winter 2019)	1.81 (Monsoon 2019)

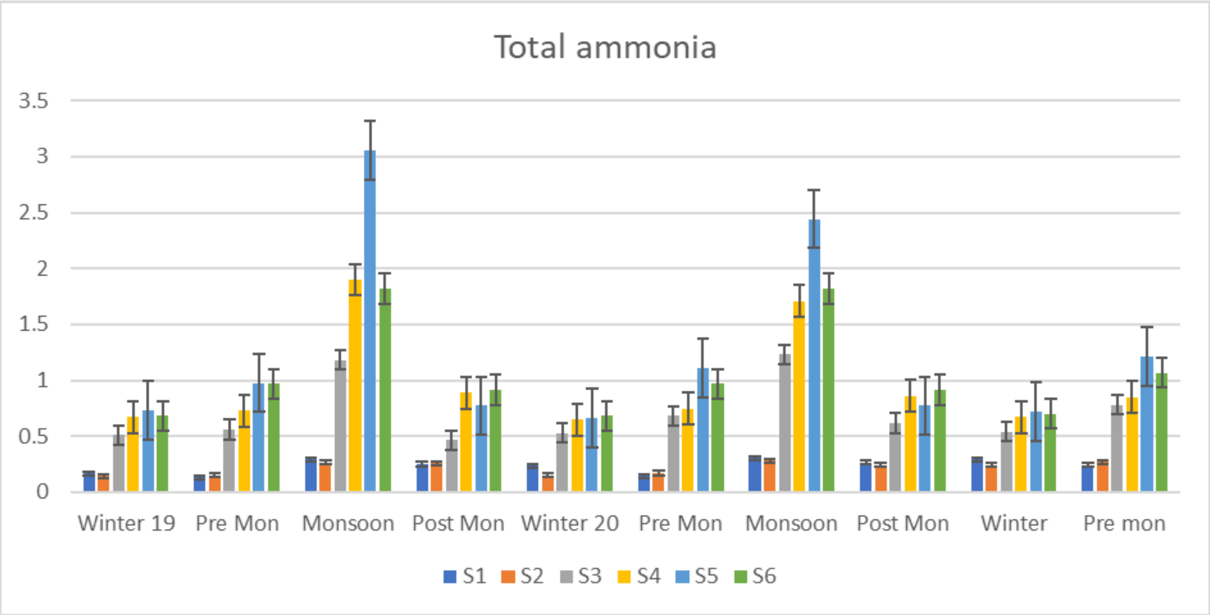

FIGURE 15: MONTHLY VARIATION OF TOTAL AMMONIA AT SIX SELECTED STATION

Soluble inorganic phosphate:

Nearly all of the phosphorus (P) present in water is in the form of phosphate (PO4) and primarily present in surface water as bound to living or dead particulate matter and contained in soil as insoluble Ca3(P04)2 and adsorbed phosphates on colloids except under highly acidic conditions. It is an important plant nutrient, as it is often low in supply and promotes the growth of plants (algae) and its role in increasing productivity is well recognized (Bhatnagar and Devi, 2013). During the present study phosphate was highest found during monsoon (0.897 mgL^{-1}) and minimum was found during post monsoon period (0.130 mgL^{-1ss}).

Table 17: Maximum and Minimum values of soluble inorganic phosphate

	Min	Max
Station 1	0.138 (Post Monsoon 2020)	0.261 (Monsoon 2019)
Station 2	0.159 (Winter 2020)	0.338 (Monsoon 2020)
Station 3	0.131 (Post Monsoon 2020)	0.308 (Monsoon 2019)
Station 4	0.140 (Winter 2020)	0.310 (Monsoon 2020)
Station 5	0.130 (Post Monsoon 2020)	0.484 (Monsoon 2020)
Station 6	0.161 (Post Monsoon 2020)	0.897 (Monsoon 2019)

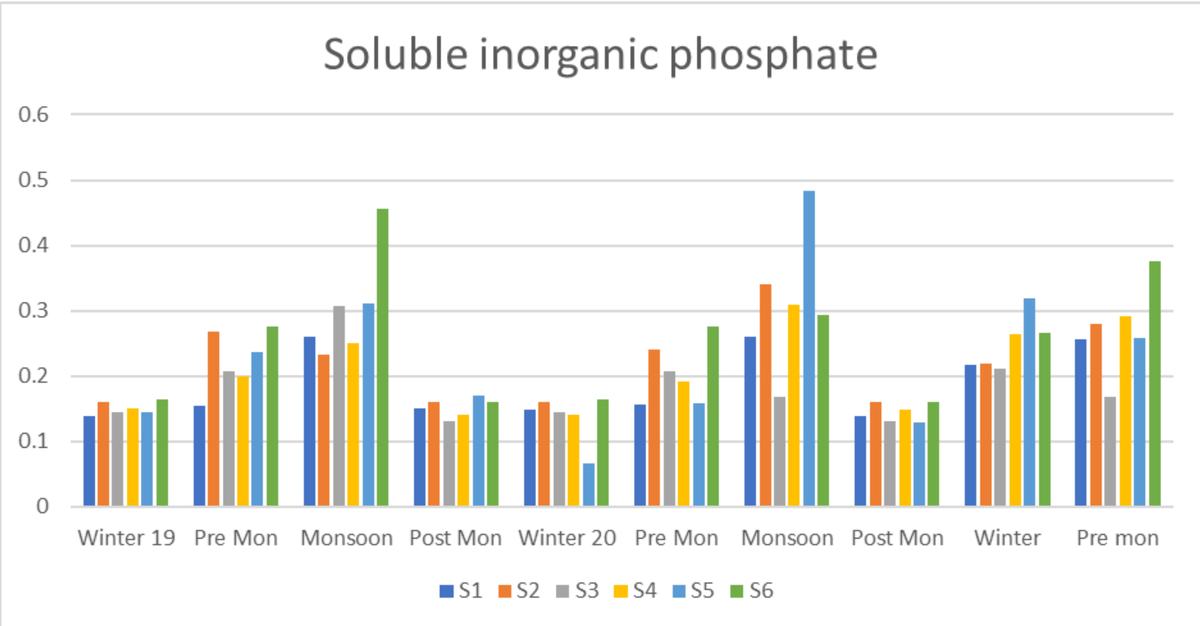

FIGURE 16: MONTHLY VARIATION OF SOLUBLE INORGANIC PHOSPHATE AT SIX SELECTED STATION

3.3 Water Quality Index:

The calculation of WQI was done by following the ‘weighted arithmetic index’ method and the estimation of the ‘unit weight' assigned to each Physico-chemical parameter included for the computation. By assigning the unit weight the different units and dimensions are covered to be single scale. The drinking water quality criteria and unit weights are given to each parameter to calculate the WQI were shown in Table 18. The observed values of the specified physico-chemical parameters in all sampling sites for each season are tabulated corresponding with WQI values . The greatest impacting parameters in the WQI scores were determined to be DO and BOD, out of the seven parameters evaluated for this study. Season wise summary of WQI value of water samples obtain from six different station revealed that most of the water samples are fall into unsuitable water category (WQI>100). The maximum WQI value was observed at 269.14 during monsoon season in station 6 with an average value of 165.16±53.92. Increased surface run-off from surrounding urban agglomerations and direct discharge from stormwater drains along roadways close to the river is primarily responsible for the river's water unsuitability during the monsoon season [16]. WQI values for stations 1 and 2 are range from 62.15-120.84. In these two stations the river is facing less anthrophonic stress and river water is running without barriers and having the ability to clean itself. In station 3 water passes through Umtrew dam, which results from a stagnant water body. The water quality at this location may be poor because of its motionless state and the river lost self-assimilation capacity of the riverine ecosystem. Station 4, 5 and 6 is the most polluted stations along with the entire starch or the Umtrew river. Station 4 and 5 are highly pollutant because of pollutant received from industries of the Byornihat industrial area, Meghalaya. In station 6 a huge population and socioeconomic pressures in the form of river bed encroachment and river water exploitation for different anthropogenic activities have a major impact on water quality.

The index value showed that during the winter and monsoon season, the Umtrew river water was come under unsuitable category (Fig 2.). During the pre-monsoon season, the water quality of the sampling locations was found to be unsuitable to poor. In the post-monsoon season, only station 2 water quality was found poor and rest of the season the river water quality of the river fall in the unsuitable category.

Table 18 : Relative weights (Wn) of the parameters used for WQI determination.

Parameters	ICMR/BIS standard (Vs)	Unit weight (Wn)
pH	6.5–8.5	0.232
Eclectic Conductivity	300	0.005
TDS	500	0.003
BOD	5	0.34

DO	5	0.34
Total Hardness(TH)	300	0.005
Alkalinity	120	0.145

Table 19: Calculation of WQI at Station 1

Parameters	Station 1							
	Winter		Pre -Monsoon		Monsoon		Post Monsoon	
	Wn	WnQn	Wn	WnQn	Qn	QnXWn	Wn	WnQn
pH	80	18.56	166	38.512	92	21.344	106	24.592
Eclectic Conductivity	18.9	0.094	24.19	0.12095	25.08	0.1254	25.76	0.1288
TDS	4.2	0.0126	6.066	0.018198	6.696	0.020088	6.066	0.018198
BOD	171.2	58.208	163.2	55.488	211	71.74	165.2	56.168
DO	85.263	28.9	62.84211	21.36632	59.68421	20.29263	57.26316	19.46947
Total Hardness(TH)	15	0.075	16.3	0.0815	13.73	0.06865	14.58667	0.072933
Total Alkalinity	48.7	7.0615	54.70833	7.932708	90	13.05	67.21667	9.746417
WQI= $\frac{\Sigma QnWn}{\Sigma Wi}$	105.6086		115.4389		118.3559		102.9867	
Garde	E		E		E		E	

Table 20 : Calculation of WQI at Station

Parameters	Station 2							
	Winter		Pre -Monsoon		Monsoon		Post Monsoon	
	Wn	WnQn	Wn	WnQn	Qn	QnXWn	Wn	WnQn
pH	160	37.12	-20	-4.64	80	18.56	-100	-23.2
Eclectic Conductivity	25.51333	0.127567	19.21667	0.096083	24.16	0.1208	28.46	0.1423
TDS	6.2	0.0186	11.6	0.0348	25.466	0.076398	10.666	0.031998
BOD	135.2	45.968	184	62.56	211	71.74	170.8	58.072
DO	70.52632	23.97895	77.57895	26.37684	94.73684	32.21053	75.47368	25.66105
Total Hardness(TH)	14.18	0.0709	17.64667	0.088233	14.8	0.074	15.51333	0.077567
Total Alkalinity	58.33333	8.458333	38	5.51	45	6.525	39.44167	5.719042
WQI= $\frac{\Sigma QnWn}{\Sigma Wi}$	108.1704		84.13641		120.8474		62.15323	
Garde	E		D		E		C	

Table 21 : Calculation of WQI at Station 3

Parameters	Station 3							
	Winter		Pre -Monsoon		Monsoon		Post Monsoon	
	Wn	WnQn	Wn	WnQn	Qn	QnXWn	Wn	WnQn
pH	140	32.48	120	27.84	80	18.56	-20	-4.64
Eclectic Conductivity	35.46667	0.177333	33.43333	0.167167	24.16	0.1208	33.02	0.1651
TDS	6.8	0.0204	13.8	0.0414	25.466	0.076398	16.132	0.048396
BOD	164.2	55.828	270	91.8	211	71.74	289.8	98.532
DO	68.42105	23.26316	78.63158	26.73474	94.73684	32.21053	72.31579	24.58737
Total Hardness(TH)	10.01	0.05005	17.19333	0.085967	14.8	0.074	8.673333	0.043367
Total Alkalinity	19.16667	2.779167	27.21667	3.946417	45	6.525	17.21667	2.496417
WQI= $\frac{\Sigma QnWn}{\Sigma Wi}$	107.101		140.7623		120.8474		113.3015	
Garde	E		E		E		E	

Table 22 : Calculation of WQI at Station 4

Parameters	Station 4							
	Winter		Pre -Monsoon		Monsoon		Post Monsoon	
	Wn	WnQn	Wn	WnQn	Qn	QnXWn	Wn	WnQn
pH	80	18.56	80	18.56	66	15.312	12	2.784
Eclectic Conductivity	37.4	0.187	36.12	0.1806	32.83333	0.164167	35.85333	0.179267
TDS	10.6	0.0318	18.666	0.055998	28.132	0.084396	26.4	0.0792
BOD	233	79.22	336	114.24	428.6	145.724	370	125.8
DO	74.73684	25.41053	83.89474	28.52421	92.31579	31.38737	75.78947	25.76842
Total Hardness(TH)	11.34333	0.056717	15.45667	0.077283	13.23333	0.066167	8.896667	0.044483
Total Alkalinity	20	2.9	31.66667	4.591667	28.88333	4.188083	20	2.9
WQI= $\frac{\Sigma QnWn}{\Sigma Wi}$	118.0991		155.3549		184.0432		147.248	
Garde	E		E		E		E	

Table 23 : Calculation of WQI at Station 5

Parameters	Station 5							
	Winter		Pre -Monsoon		Monsoon		Post Monsoon	
	Wn	WnQn	Wn	WnQn	Qn	QnXWn	Wn	WnQn
pH	80	18.56	120	27.84	52	12.064	-20	-4.64
Eclectic Conductivity	37.73333	0.188667	34.42	0.1721	35.36667	0.176833	33.56667	0.167833
TDS	11.2	0.0336	20	0.06	37.8	0.1134	28.466	0.085398
BOD	296.8	100.912	382	129.88	413.6	140.624	424.6	144.364
DO	71.57895	24.33684	87.05263	29.59789	93.68421	31.85263	76.84211	26.12632
Total Hardness(TH)	12.01	0.06005	16.90333	0.084517	12.54333	0.062717	9.45	0.04725
Total Alkalinity	19.16667	2.779167	32.5	4.7125	36.66667	5.316667	20.83333	3.020833
WQI= $\frac{\Sigma QnWn}{\Sigma Wi}$	137.262		179.7636		177.7666		158.104	
Garde	E		E		E		E	

Table 24: Calculation of WQI at Station 6

Parameters	Station 5							
	Winter		Pre -Monsoon		Monsoon		Post Monsoon	
	Wn	WnQn	Wn	WnQn	Qn	QnXWn	Wn	WnQn
pH	140	32.48	100	23.2	146	33.872	66	15.312
Eclectic Conductivity	44.14333	0.220717	38.57	0.19285	36.01	0.18005	39.38667	0.196933
TDS	11.8	0.0354	20.8	0.0624	40.932	0.122796	30.132	0.090396
BOD	188.8	64.192	332.6	113.084	630.6	214.404	365.8	124.372
DO	64.21053	21.83158	86.31579	29.34737	99.36842	33.78526	76.21053	25.91158
Total Hardness(TH)	21.95667	0.109783	18.25	0.09125	13.56667	0.067833	11.09667	0.055483
Total Alkalinity	21.66667	3.141667	34.71667	5.033917	38.33333	5.558333	25.83333	3.745833
WQI= $\frac{\Sigma QnWn}{\Sigma Wi}$	114.0291		159.8241		269.1498		158.5834	
Garde	E		E		E		E	

Table 25:. Summary of WQI of the Umtrew River

Sampling station	Winter		Pre -Monsoon		Monsoon		Post Monsoon	
Station 1	105.60	Unsuitable	115.43	Unsuitable	118.35	Unsuitable	102.98	Unsuitable
Station 2	108.17	Unsuitable	84.13	Very Poor	120.84	Unsuitable	62.15	Poor
Station 3	107.101	Unsuitable	140.76	Unsuitable	120.84	Unsuitable	113.30	Unsuitable
Station 4	118.09	Unsuitable	155.35	Unsuitable	184.04	Unsuitable	147.24	Unsuitable
Station 5	137.26	Unsuitable	179.76	Unsuitable	177.76	Unsuitable	158.10	Unsuitable
Station 6	114.02	Unsuitable	159.82	Unsuitable	269.14	Unsuitable	158.58	Unsuitable
Average	115.045		139.213		165.1683		123.72	

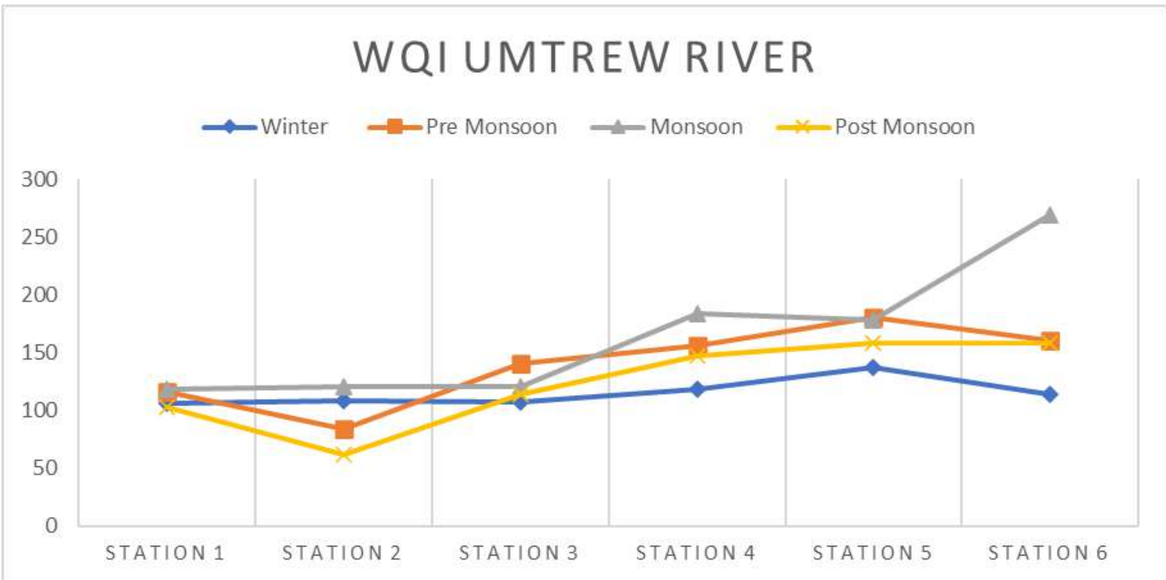

Figure 17: WQI rating of variouse sampleing sites of Umtrew river

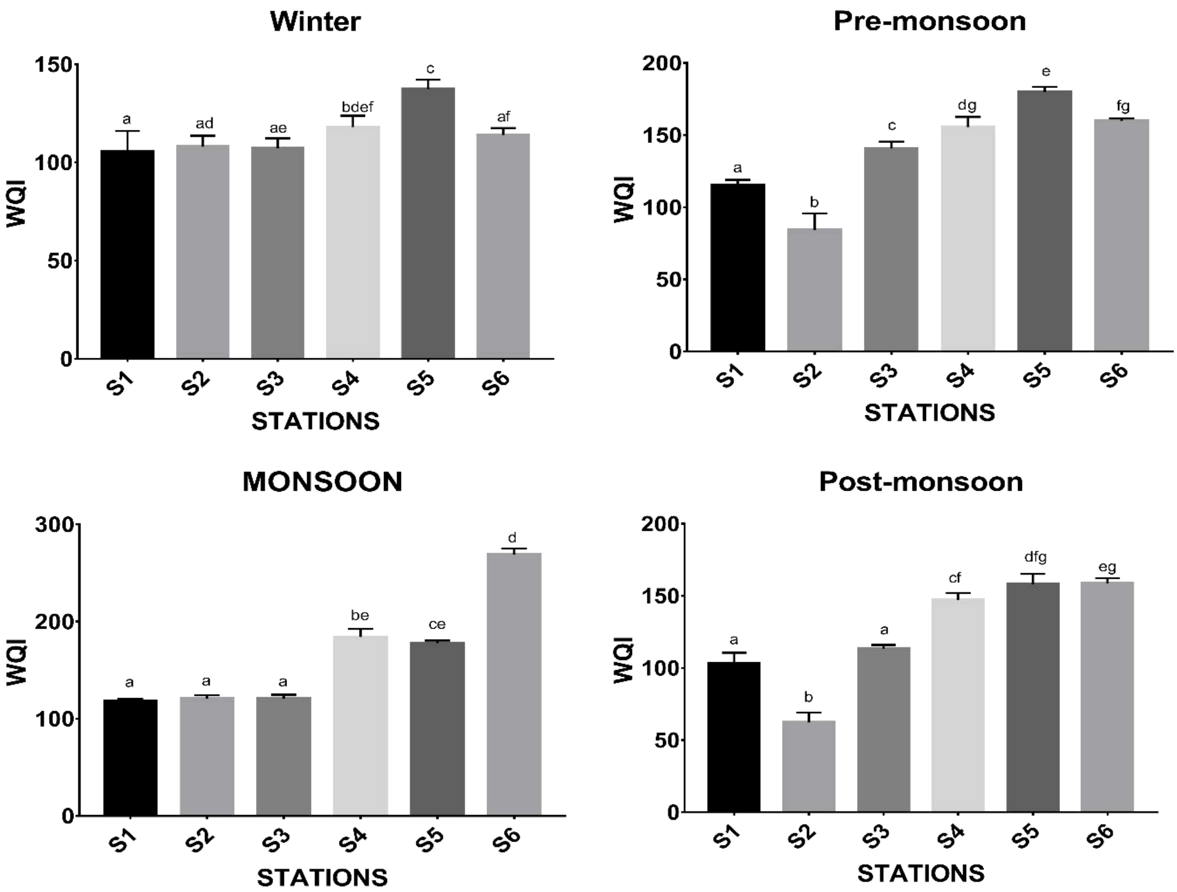

Figure 18: Water quality index scores in the sampleing station (P < 0.05)

Station 4 which is located nearby industrial and residencial area, water quality influenced by electric conductivity (EC), total disolved solid (TDS) and BOD_3. Many infustracture, market, dumping zones, sand mininig activities are widely practice in the downstream area of the Umtrew river. The WQI result sugguested that there is an overlap between the station 4 and station 5 physico chemical and biological parameters.The station 6 water is mainly influence by the station 4 and station 5. The WQI for the study period showing significantly increasing trend from the upstream to its downstream. The water quality station 1, station 2 and station 3 did not show a significant difference. In station 4 and station 5 and station 6 showing significantly poor water quality during winter, monsoon and post monsoon as this areas are facing more presure from anthropoganic and industrial waste (Fig 4).

Parameters selection by the PCA analysis:

The principal components are usually determined using eigenvalues in the PCA approach (PCs). In the present study shows a remarkable change of slop after the fourth eigenvalues. In the present study, the first five PCs were used for further analysis as suggested by Vega *et al*., 1998. The eigenvalues explained 68.8%, 83.8%, 81.8% and 83.1% of total variance of information contain in the original data set for winter, per monsoon, monsoon and post monsoon respectively. The figure 2 shows the temporal and spatial variation of the water quality parameters of the first two component.

In winter, PC1 has total variance of 49.57% and it was positively affected by TDS, ammonia, EC, turbidity, BOD, COD, nitrite and nitrate. PC1 underline the impotence of pollution attribute resulting from the effect of runoff and effluent. PC1 is negatively affect by alkalinity, DO and water velocity. PC2 showed the total variance of 19.36%. It is positively related with water velocity, alkalinity and DO. There is no correlation between hardness and phosphate with surface temperature, CO_2 and pH. Station 1 and 2 is most affected by water velocity, alkalinity and DO. BOD and COD, nitrate, nitrite, ammonia and phosphate are in higher side in station 6 and station 5 and in on the other hand turbidity, temperature, conductivity and CO_2 are found higher in station 4 and 5

In pre monsoon, PC1 showed a total variance of 63.25% and it is positively related by turbidity, surface water, surface water, TDS, BOD and COD. PC1 is more highlighting physical parameters of water during pre-monsoon with pollution indictor like COD and BOD, which affecting the overall dataset. PC1 is negatively affected by water velocity and DO. PC2 showing the total variance of 19.63% and is mostly affected by pH and water velocity. There is no correlation between pH and water velocity with CO_2, hardness and phosphate. Station 1 is mostly influence by parameters like DO, alkalinity and water velocity. Station 3 is less influence by the parameters like nitrite, nitrite, COD, BOD, surface water, temperature and ammonia but station 4 and 5 is most affected by these parameters.

During monsoon, PC1 has a total variance of 62.24% and positively affected by BOD, COD, turbidity, ammonia, TDS, EC, nitrite and temperature. PC1 is mainly focus more the pollution parameters, therefore this component seems to measure the prepondence of BOD and COD over other parameters. PC1 is negatively affected by DO and alkalinity. PC2 showed a total variance 19.63% and positively affected by water velocity and nitrite. There is no corelation between water velocity with CO_2, water temperature and TDS. Station 1 water quality is mostly influence DO, alkalinity and hardness. Station 5 and station 6 water was mostly influence parameters are BOD, COD, phosphate, nitrite, nitrate, ammonia, pH and turbidity. TDS, surface water temperature and CO_2 is mostly influencing the station 4 water quality.

During post monsoon period PC1 has total variance of 65.29%, It is positively affected by surface water temperature and turbidity and negatively affected by hardness and alkalinity. PC2 having a variance of 17.98% and is positively affected by water velocity and pH. PC2 is affected negatively by pH and water velocity. The station 1 water quality most influence by pH, water velocity, DO and alkalinity. For station 2 and 3 the parameter that mainly influence the water quality is hardness, BOD, COD, ammonia, nitrite, nitrate, EC, turbidity and temperature influence the water quality in station 4 and station 6.

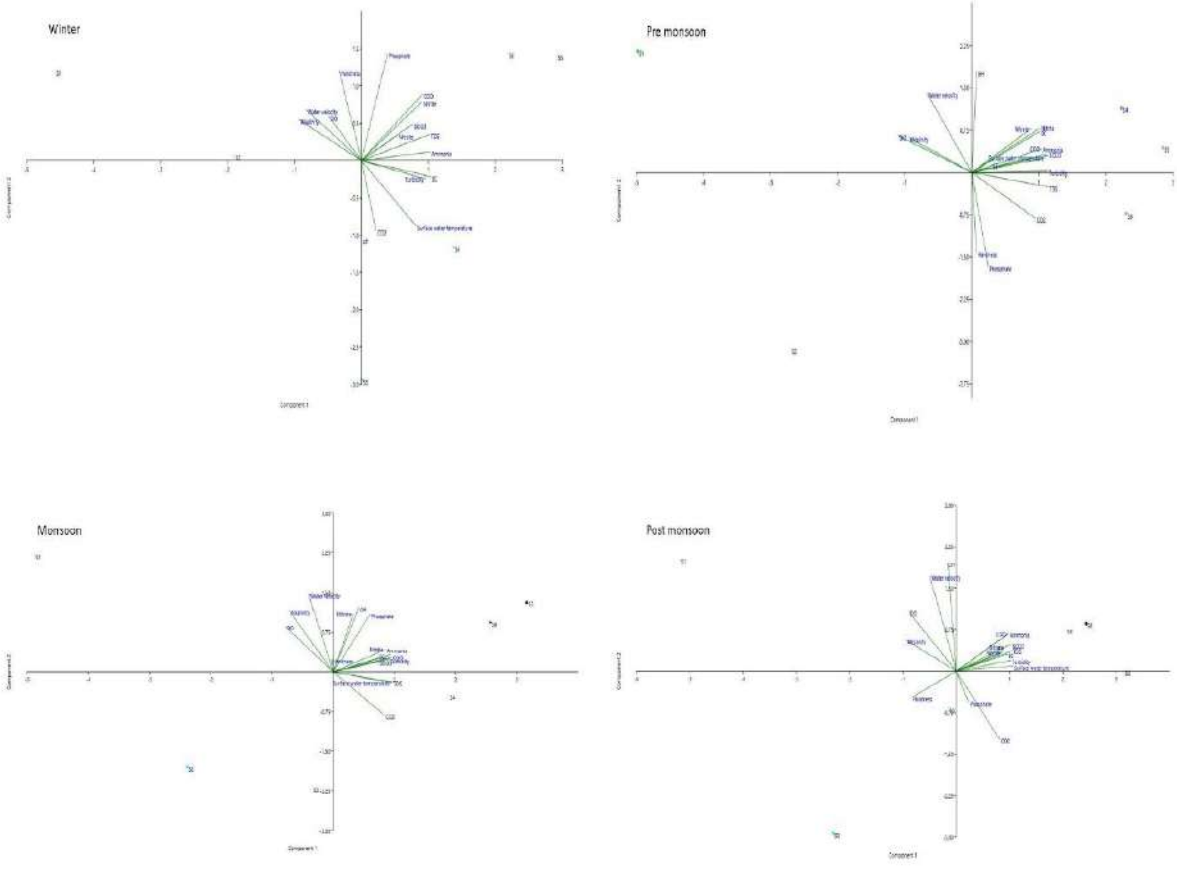

Figure 19: Coordinates of the principal component axis based on monitored variables and station.

Eutrophication Index:

The surface water of the Umtrew river was found to be zero eutrophic. The index values for different season for all station was <1 which indicated the surface water was zero eutrophic with less nutrient and it can be adversely affect human as well as biological diversity .

Table 26 : Eutrophication Index for the Winter, Pre-Monsoon, Monsoon and Post Monsoon

Season							
	Station 1	Station 2	Station 3	Sation 4	Station 5	Station 6	Eutrophication
Winter	0.016	0.028	0.019	0.036	0.047	0.080	NO
Pre Monson	0.023	0.027	0.060	0.074	0.124	0.122	NO
Monsoon	0.040	0.049	0.051	0.064	0.098	0.127	NO
Post Monsoon	0.027	0.023	0.049	0.080	0.101	0.084	NO
Average	0.026	0.032	0.044	0.063	0.093	0.103	
SD±	0.008	0.010	0.015	0.016	0.028	0.021	

Organic Pollution Index

The OPI values for the Umtrew river surface water ranges from 2.21 (Winter) to 3.89 (monsoon). The OPI values ranges for winter 2.21-3.03, pre monsoon 2.22-2.87, monsoon 2.30-3.89 and post monsoon 2.31-3.42. The average value was found highest during the monsoon period, which indicated the organic pollution come from mainly agricultural runoff along with rain water. The OPI values found comparatively less in the stations present in higher altitude (i.e Station 1, 2 and 3), since these stations exhibit less anthropogenic pressure as compare to stations present in lower altitude.

Table 27 : Organic Pollution Index for the Winter, Pre-Monsoon, Monsoon and Post Monsoon

Season								
	Station 1	Station 2	Station 3	Sation 4	Station 5	Station 6	Average	Status
Winter	2.55	2.21	2.24	2.45	2.82	3.0	2.55	Polluted
Pre Monson	2.4	2.22	2.43	2.6	2.69	2.87	2.55	Polluted
Monsoon	2.5	2.3	2.93	3.35	3.89	3.63	3.10	Polluted
Post Monsoon	2.38	2.31	2.67	2.9	3.2	3.42	2.83	Polluted

Comparative Pollution index:

The CPI data shows the worth of the whole river throughout the season with no significant differences. The CPI values of Umtrew river ranges from 0.75 to 5.06 with average values 2.11. According to CPI classification, this river was slightly, medium and strongly polluted. The CPI values during rainy season ranges from 1.36 to 5.06 indicate strongly polluted in all station. However, in the upstream station (1 and 2) the CPI values ranges from .75 to 1.95 i.e the water was slightly and medium polluted in these stations.

Table 28: Comprehensive Pollution Index for the Winter, Pre-Monsoon, Monsoon and Post Monsoon

Station	Season	OPI value	Description
Station 1	Winter	0.75	Slightly Polluted
	Pre Monson	0.98	Slightly Polluted
	Monsoon	1.36	Medium Polluted
	Post Monsoon	1.10	Medium Polluted
Station 2	Winter	0.99	Slightly Polluted
	Pre Monson	1.46	Medium Polluted
	Monsoon	1.95	Medium Polluted
	Post Monsoon	1.50	Medium Polluted
Station 3	Winter	1.22	Medium Polluted
	Pre Monson	1.94	Medium Polluted
	Monsoon	2.68	Heavily Polluted
	Post Monsoon	1.67	Medium Polluted
Station 4	Winter	1.11	Medium Polluted
	Pre Monson	2.86	Heavily Polluted
	Monsoon	2.86	Heavily Polluted
	Post Monsoon	2.55	Heavily Polluted
Station 5	Winter	1.42	Medium Polluted
	Pre Monson	2.67	Heavily Polluted

	Monsoon	5.06	Heavily Polluted
	Post Monsoon	2.93	Heavily Polluted
Station 6	Winter	1.38	Medium Polluted
	Pre Monson	2.59	Heavily Polluted
	Monsoon	4.95	Heavily Polluted
	Post Monsoon	2.77	Heavily Polluted
Average		2.11	Heavily Polluted

Carlson Trophic Index or Trophic State Index (C-TSI):

The C-TSI was calculated at the entire sampling site during winter, pre monsoon, monsoon and post monsoon. The results of trophic index was found to 12.37 to 7.34, 12.53 to 4.39, 10.24 to 4.2 and 9.18 to 5.07 in winter, pre monsoon, monsoon and post monsoon respectively. whereas the average C-TSI was ranges from 10.76 to 5.71 i.e. C-TSI <30-40, is an indication oligotrophic condition of the river which means water was clear and it could be support life of the aquatic flora and fauna.

Table 29 :Carlson Trophic State Index the Winter, Pre-Monsoon, Monsoon and Post Monsoon

Season							
	Station 1	Station 2	Station 3	Sation 4	Station 5	Station 6	Trophic Status
Winter	11.09	12.37	9.24	7.34	8.63	8.64	Oligotrophic
Pre Monson	12.53	11.41	7.96	4.39	7.39	8.82	Oligotrophic
Monsoon	10.24	8.08	4.21	5.84	6.19	4.83	Oligotrophic
Post Monsoon	9.18	9.17	6.98	5.26	5.07	5.53	Oligotrophic
Average	10.76	10.26	7.10	5.71	6.82	6.96	Oligotrophic
SD±	1.22	1.70	1.85	1.07	1.33	1.79	

Heavy Metal Analysis:

Heavy metal concentration (Pb, Zn, Ni, CU and Cd) on the surface water of the Umtrew river is depicted in table 30. Among the estimated heavy metals highest mean value was detected for Pb (0.112 mgL^{-1}) followed by Zn (0.077 mgL^{-1}), Ni (0.061 mgL^{-1}), Cu (0.013 mgL^{-1}) and Cd (0.004 mg /l). Comparing the heavy metal values with the permissible limit of drinking water given by USEPA

(2009) we found the average content of Ni and Cd on the higher side. On the other hand, the average content of Cu, Zn and Pb was found on the lower side of the permissible limit.

Table 30 : Heavy metal concentration (mgL^{-1}) in Umtrew river Meghalaya:

Site	Season	Copper	Zinc	Lead	Cadmium	Nickel
	Winter	0.034	0.033	0.012	0.002	0.232
	Summer	0.005	0.008	0.15	0.005	0.012
	Min	0.005	0.033	0.012	0.002	0.012
	Max	0.034	0.033	0.15	0.005	0.232
S1	Avg	0.0195	0.0205	0.081	0.0035	0.122
	Winter	0.042	0.026	0.15	0.002	0.045
	Summer	0.014	0.0043	0.12	0.007	0.024
	Min	0.014	0.0043	0.12	0.002	0.024
	Max	0.042	0.026	0.15	0.007	0.045
S2	Avg	0.028	0.01515	0.135	0.0045	0.0345
	Winter	0.027	0.017	0.13	0.003	0.0255
	Summer	0.011	0.007	0.11	0.006	0.0312
	Min	0.011	0.007	0.11	0.003	0.0255
	Max	0.027	0.017	0.13	0.006	0.0312
S3	Avg	0.019	0.012	0.12	0.0045	0.02835
Mean		0.013	0.077	0.112	0.004	0.061

Contamination Factor (CF) and Pollution Load Index (PLI):

The CF values and PLI values of the estimated heavy metals presented in table 31. The CF values reflect the degree of heavy metal pollution in the water. Low contamination is indicated by CF<1, moderate contamination (1≤CF≤3), high contamination (3≤CF≤ 6) and extremely high contamination is indicated by CF>6 (Abdullah *et al*., 2011). Results depicts that CF value for Cu and Zn are less than 1, Whereas Pb value is greater than 6 indicating extremely high contamination of Pb in the river water. Moreover, CF values for Cd and Ni were found to be in the range of 0.66 to 2.3 and 0.34 to 3.31 respectively, indicating moderate contamination of the metals.

A Pollution load index (PLI) refers the degree of association of heavy metal to water. The PLI value greater than 1 implies metal pollution whereas PLI of less than 1 indicates no substantial contamination of the heavy metals (Abdullah *et al*., 2011). In the present study the PLI values are less than 1 therefore it signifies that there is no substantial contamination of heavy metal in the said river system.

Table 31 : Pollution Load Index of Umtrew river surface water

Stations	Season	Contamination Factor (CF)					PLI
		Copper	Zinc	Lead	Cadmium	Nickel	
1	Winter	0.017	0.011	1.2	0.666667	3.314286	0.10
1	Summer	0.0025	0.002667	15	1.666667	0.171429	0.02
2	Winter	0.021	0.008667	15	0.666667	0.642857	0.16
2	Summer	0.007	0.001433	12	2.333333	0.342857	0.04
3	Winter	0.0135	0.005667	13	1	0.364286	0.08
3	Summer	0.0055	0.002333	11	2	0.445714	0.05

Heavy Metal Pollution Index:

Heavy metal pollution index is a key factor that determines the overall heavy metals pollution in the waterbody. It is calculated based on weighted arithmetic quality (Singh and Kamal, 2017). HPI value in the present study was calculated to be 168.05. The threshold value of HPI for drinking water is 100 (US EPA, 2009). Further more Prasad *et al*., 2001 reported that HPI value less than 100 represent water with no heavy metal pollution. Since, the calculated HPI value of the Umtrew river system is more then 100, therefore it can be conclude saying that the water might be polluted with heavy metal and not fit for drinking purpose.

Table 32 : HPI for the values heavy metal in surface water based on USEPA (2009) guidelines for drinking water

Heavy Metals	Mean (µg/l)	Highest permitted value for drinking Water	Maximum Desirable value	Sub index(Qi)	Unit Weight Wi-(K/Si)	Wi*Qi
Cu	13.76	1300	40	13.76	0.000769231	0.010584615
Zn	77.36	5000	3000	1.5472	0.0002	0.00030944
Pd	112	50	100	224	0.02	4.48
Cd	4.1	5	500	82	0.2	16.4
Ni	61.76	15	-	411.7333333	0.066666667	27.44888889
∑Wi= 0.2876; ∑Wi × Qi=48.33978294 and HPI= 168.0589362						

Heavy Metal Evaluation Index (HEI) and Degree of Contamination (C_d)

Heavy metal evaluation index (HEI)is a method of estimating the water quality with focus on heavy metals in drinking water (Ghaderpoori *et al*., 2018). Kumar *et al*., 2019 stated that HEI index less than 10 refers to low heavy metal pollution. In the present study the HEI value was found to be 2.73, which is indicative of low heavy metal pollution. For, degree of contamination, value was calculated to be less than 1, which also suggest that Umtrew river system is less contaminated with heavy metal pollution (Backman. *et al*., 1998).

Table 33 : Heavy metal evaluation index (HEI) and degree of contamination (C_d) for various heavy metals in surface waters.

Heavy Metals	Mean (µg/l)	Maximum Desirable value	Mi/MAC	$Cf=\frac{Mi}{Si}-1$
Cu	13.76	40	0.344	-0.656
Zn	77.36	3000	0.025786667	0.97421333
Pd	112	100	1.12	0.12
Cd	4.1	500	0.0082	-0.9918

Ni	61.76	50	1.2352	0.2352
HEI=∑ Mi/MAC=2.733186667 C_d= ∑ *Cf*=-2.266813333				

Heavy Metal Toxicity Load (HMTL):

Heavy metal toxicity load (HMTL) measures the pollution load or content of heavy metal in a water body and provide us with information that what percentage of heavy metals that need to be removed from water to make it safe for human drinking purpose. In the present study the HMTL values for the calculated heavy metals are in the lower scale than the permissible limit provided by Agency for Toxic Substances and Disease Registry (ATSDR). Since the concentration are under the permissible limit, therefore at present there is no any urgent need of any kind of water treatment of the study area to meet up the quality of drinking water standards as provided by the regulatory authority

Table 34 : Heavy metal toxicity load (HMTL) of surface water bodies following ATSDR (2017) relative toxicity level of heavy metals responsible for human health hazard

Heavy Metals	Hazard Intensity Score	Mean value(mgL^{-1})	Recoded HMTL (mgL^{-1})	Permissible Limit (mgL^{-1})	% age removal of heavy metals to reduce Pollution Load PTL
Copper	1013	0.013	13.169	101.3	-
Zinc	915	0.077	70.455	4575	-
Lead	1531	0.112	171.472	2	-
Cadmium	1320	0.004	5.28	132	-
Nickel	996	0.061	60.756	69.72	-

Total Median Lethal Toxicity (TMLT):

The total median lethal toxicity TMLT is the summation of the median lethal toxicity (MLT) of the individual heavy metal in a water body. The average MLT values for the metals reviewed in the present study is given in Table 35. Ni reflect the highest value for MLT whereas, Cd was on the lower side. The toxicity value is also express by median lethal dose MLD unit (mg/kg).

Table 35 : Median Lethal Toxicity (MLT) of heavy metal in Umtrew river surface water:

Heavy Metals	Salt for LD50	Molecular Wight	Atomic Wight	LD50	LD50 metal (oral rat)	heavy Metal µg/l	MLT
Cu	CuCl2	159.6	63.55	482	191.9	13.76	0.071

Zn	ZnCl2	136.286	65.38	350	167.9	77.36	0.460
Pd	Pbcl2	278.1	207.2	201	149.7	112	0.748
Cd	CdCl2	183.32	112.41	88	53.96	4.1	0.075
Ni	NiCl2	129.6	58.69	105	47.5	61.76	1.300
∑MLT=2.654							

Human Health Risk Assessment:

The assessment of metal toxicity indicates the adverse effect cause by heavy metal in human health by the presence of toxic substances in it. In the present study the heavy metal risk is estimated by oral and dermal exposure as per the standard given by USEPA (2004). The reference does for oral and dermal and cancer slop factor (CSF) are presented in table 36.

Non-carcinogenic health risk:

In present study adults were considered as targeted group though the oral ingestion and dermal exposure. The values were measured based on HQ and HI. The calculated CDI_{oral} value were 5.7×10^{-4} ,3.2×10^{-4}, 4.6×10^{-4}, 1.7×10^{-4} and 2.5×10^{-4} mg kg^{-1} day^{-1} for Cu, Zi, Pb, Cd and Ni respectively. The decreasing trend of calculated CDI_{oral} values are Cu> Pb> Zi> Ni> Cd which reads as Cu to be highest with (5.7×10^{-4}) and Cd to be least with (1.7×10^{-4}) . The calculated HQ_{oral} values Cu, Zi, Pb, Cd and Ni were 1.4×10^{-5}, 1.07×10^{-5}, 3.3×10^{-3}, 3.4×10^{-4} and 1.2×10^{-4} respectively. In the present study the calculated HQ_{oral} values for the heavy metals were less than 1, which signifies that-values were within the acceptable range with no carcinogenic health risk. However, HI_{oral} value was found to be 1.4 $x10^{-3}$. Similar kind of findings were also reported by Mohammadi *et al*., (2019) where he clearly says that HQ_{oral} and HI_{oral} value less than one does not pose any potential or carcinogenic health risk to the local dwellers.

The estimated CDI_{dermal} values for Cu, Zi, Pb, Cd and Ni were 2.3×10^{-6}, 8.07×10^{-5}, 1.9×10^{-7}, 7.1×10^{-7} and 2.1×10^{-6} respectively. The calculated values are shown in decreasing trend with, Zn being the highest with (8.07×10^{-5}) and Pb being the lowest with (1.95×10^{-7}), Zn> Cu> Ni> Cd> Pb. However, the calculated HQ_{dermal} values were 1.9×10^{-7}, 4.2×10^{-5}, 4.6×10^{-7}, 1.4×10^{-3} and 3.9×10^{-7} for Cu, Zi, Pb, Cd and Ni respectively. The HI dermal values was found to be 3.8×10^{-3} . Since both HQ_{dermal} and HI_{dermal} values in the present study were below 1, thus it can be concluded saying that all these metals were within the acceptable range of non-carcinogenic health risk.

Table 36 : Reference Dose (RfD) and Cancer Slop Factor (CSF) for different metal:

Element	RfD_{oral}	RfD_{dermal}	CSF(mg/kg/day)
Cu	40	12	-
Zn	300	1.9	-
Pd	1.4	0.42	8.5
Cd	0.5	0.0005	6.1
Ni	20	5.4	0.84

Source: Incremental Lifetime Cancer Risk Dose (2004)

Table 37 : Assessment of Possible Health Risk Caused by Heavy Metal by the surface waterbody of Umtrew river.

Elements	CDI_{oral}	CDI_{dermal}	HQ_{oral}	HQ_{dermal}	$ILCR_{oral}$	$ILCR_{dermal}$
Cu	5.7×10^{-4}	2.35×10^{-6}	1.4×10^{-5}	1.9×10^{-7}		
Zn	3.2×10^{-4}	8.07×10^{-5}	1.07×10^{-5}	4.2×10^{-5}		
Pd	4.6×10^{-4}	1.95×10^{-7}	3.3×10^{-3}	4.6×10^{-7}	$2.8x10^{-2}$	$1.6 x10^{-6}$
Cd	1.7×10^{-4}	7.15×10^{-7}	3.4×10^{-4}	1.4×10^{-3}	$2 x10^{-3}$	$4.3 x10^{-6}$
Ni	2.5×10^{-4}	2.15×10^{-6}	1.2×10^{-4}	3.9×10^{-7}	$1 x10^{-4}$	$1.8 x10^{-6}$
			HI_{oral}=1.4 $x10^{-3}$	HI_{dermal}=3.8 $x10^{-3}$		

Carcinogenic Health Risk:

Heavy metals (Pb, Cr (VI), Cd, and Ni contamination can potentially enhance the risk of human's cancer (Tani *et al*., 2005; Cao *et al*., 2014). In the present study the calculated ILCR values for oral and dermal exposure were 2.8×10^{-2}, 2×10^{-3}, 1×10^{-4} and 1.6×10^{-6}, 4.3×10^{-6}, 1.8×10^{-6} for Pd, Cd and Ni respectively, which can be considered to have the potential to create cancer risks to human when one is exposed. The ILCR value for any heavy metal less than 1×10^{-6} are considered to be insignificant and where risk of cancer is negligible, whereas, ILCR value above 1×10^{-4} are considered as harmful and pose risk for cancer. The appropriate standard for all heavy metals is 1×10^{-5} (Cao *et al*., 2014; Yang *et al*., 2014). The results depicts that among all the calculated heavy metals Pd has highest chance for cancer risk and Ni has least chance for cancer risk by the oral ingestion.

Analysis for total coliform:

Total coliforms are a kind of Gram-negative spoorless bacteria that can digest lactose, generate acid, and gas without needing oxygen. Total coliforms come mostly from human and animal excreta and are used as a key indicator of whether a water supply has been contaminated by excreta. Total coliforms are now the most widely used index in all over the world to assess the contamination of household drinking water. Total coliforms testing is one of the most significant indices for evaluating the hygienic quality of drinking water. It has a wide range of hygienic implications. coliform MPN index for presumptive tests per 100 ml of river water varied from 7 to 1400+ throughout the research period, whereas confirmatory tests ranged from 5 to 1400+. During the research period, station-level variations in the MPN index are listed below:

Table 36: Average maximum and minimum values

Stations	**Ranges(^{0}C)**	**Maximum**	**Minimum**
Station 1	7±1.41to 30±10.80	July	December
Station 2	8±3.55 to 40±4.08	June	November
Station 3	7±0..81 to 35±7.07	June	December
Station 4	35±12.24 to 1400±37.41	June	December
Station 5	200±8.16 to 1400±35.59	July	November
Station6	350±90.92 to 1400±37.41	June	January

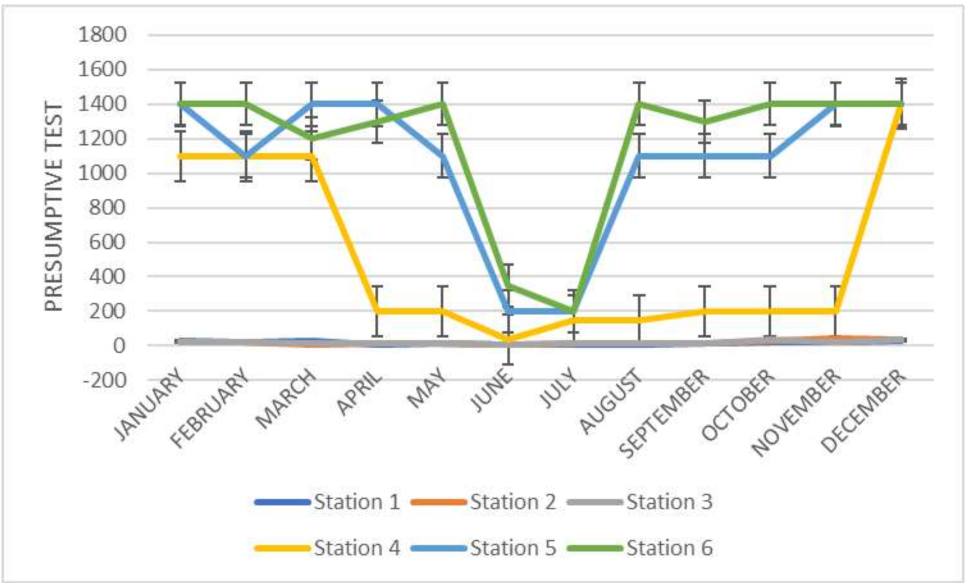

FIG 20: AVARAGE MONTHLY VARIATION OF MPN INDEX (PRESUMPTIVE TEST) AT DIFFERENT STATIONS DURING THE STUDY PERIOD

Table 37: Average maximum and minimum values

Stations	**Ranges(^{0}C)**	**Maximum**	**Minimum**
Station 1	6± 0.94 to 15±0.47	November	July
Station 2	5±1.24 to 15±0.81	November	July
Station 3	6±0.47 to 25±094	October	June
Station 4	7±0.81 to 35±0.81	October	June
Station 5	35±1.69 to 1400±23.57	October	June
Station6	200± 1.69 to 1400±47.14	October	June

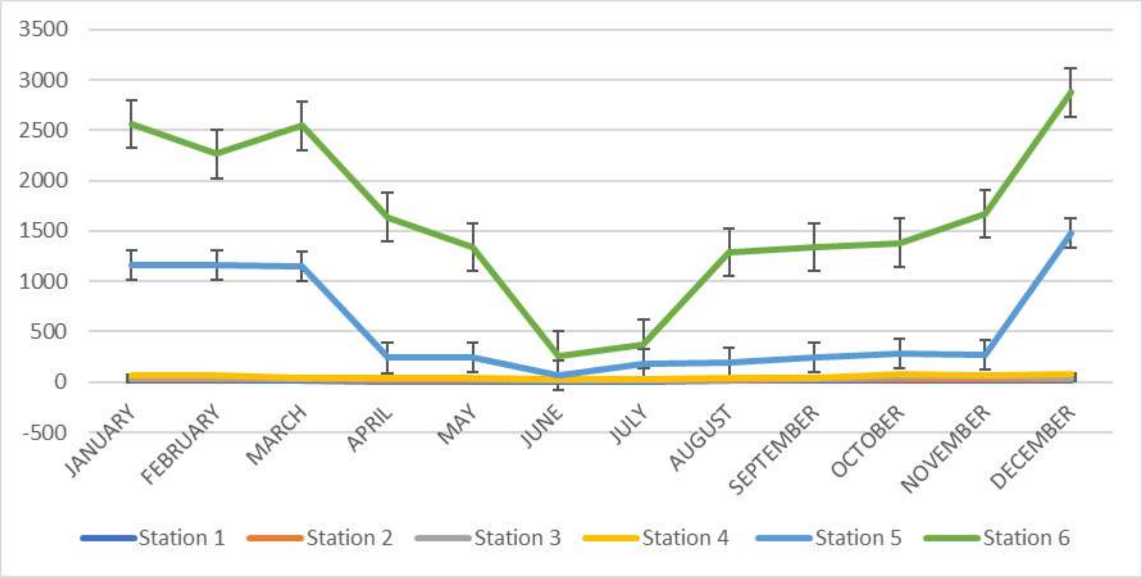

FIG 21: MONTHLY VARIATION OF MPN INDEX (CONFERMATIVE TEST) AT DIFFERENT STATIONS DURING THE STUDY PERIOD

The risk of contracting a waterborne illness increases as the amount of pathogenic microorganism contamination increases. The link, however, is not intrinsically simple and is greatly reliant on factors such as infectious dosage and host susceptibility (WHO 1993). In the current investigation, the total coliform was determined using a three-tube technique. The MPN index in the Umtrew (Digaru) river ranged from 5 to 1400+ per 100 ml. The highest value was reported during the winter, and the lowest during the monsoon, due to fecal discharge dilution caused by excessive rains. The fecal count is quite high in forth, fifth and sixth stations. MPN index ranged from 23 to 1600+ per 100 ml. in industrial district of Borneo's Northwest coast (Leong *et al*. 2018). According to Shah and Joshi (2017), the MPN index of the Sabarmati River ranges from 0 to 10,000 per ml. According to Shah and Joshi (2017), the MPN index of the Sabarmati River ranges from 0 to 10,000 per ml. In the current study, stations 4, 5 and 6 had higher coliform counts, indicating more pollution in these stations when compared to station 1, 2 and 3. This might be attributed to a higher rate of fecal matter discharge because stations 4, 5 and 6 are located near densely inhabited and semi-urban areas.

Plankton

Phytoplankton is an important component of the food chain since it is essential for primary production and also serves as a biological indicator of water quality in contamination studies. Since, population density and variety of plankton in a water body varies from place to place and aquatic systems within the same area, they are critical factors in enforcing sustainable management standards (Verme and Prakash, 2020).

The present study was conducted 12 months from January 19 to December 21. A total of 24 genera of plankton were identified from the river Umtrew river. Five genera belong to the Chlorophyceae family, five to the Bacillariophyceae family, four to the Cynophyceae family, two to the Rotifera family, two to the Cladocera family, Copepod and Copepod nauplii, and fish eggs and larvae. During the research period, plankton density varied from 8 to 69 uL^{-1}.

Phytoplankton:

In the present investigation 14 genera of phytoplankton population was observed in the all six stations. A total of genera recorded in station 1 and 2 was 13 and in station 3 was 14. Number of genera was recorded in station 4, 5 and 6 were 10, 8 and 9 respectively.

Composition and monthly distribution of phytoplankton:

In station 1, total number of 13 genera were recorded during the study period. The percentage composition of Chlorophyceae, Bacillariophyceae and Cyanophyceae were 52.33%, 24.55% and 23.22% respectively.

In station 2, 13 genera were recorded, the percentage composition of Chlorophyceae, Bacillariophyceae and Cyanophyceae were 53.75%, 25.28% and 20.97% respectively.

At station 3, 13 genera of plankton were observed. In this station, the percentage composition of Chlorophyceae, Bacillariophyceae and Cyanophyceae were 48.00%, 25.41% and 26.59% respectively.

In station 4, total number of 10 genera were recorded during the study period. The percentage composition of Chlorophyceae, Bacillariophyceae and Cyanophyceae were 42.00%, 30.71% and 27.29% respectively.

In station 5, 8 genera were recorded, the percentage composition of Chlorophyceae, Bacillariophyceae and Cyanophyceae were 45.24 %, 29.84% and 24.92% respectively. (T-).

In station 6, total number of 9 genera were recorded during the study period. The percentage composition of Chlorophyceae, Bacillariophyceae and Cyanophyceae were 43.23%, 29.66% and 27.11%, respectively.

Phytoplankton Density:

The phytoplankton density at station 1 was found to ranged from 30-69 $cellsl^{-1}$.. During the study period Chlorophyceae was the dominant genera (38.42%) followed by Bacillariophyceae (33.50%) and Cyanophyceae (28.07%).

The total phytoplankton density of the station 2 ranged from 20-59 $cellsl^{-1}$. During the study period Chlorophyceae was dominant genera (40.91%) followed by Bacillariophyceae (31.73%) and Cyanophyceae (27.34%).

The phytoplankton density of station 3 was found to be varying between 15-48 $cellsl^{-1}$. Chlorophyceae was dominant genera (41.01%) followed by Bacillariophyceae (33.98%) and Cyanophyceae (25%).

The phytoplankton density at station 4 was found to ranged from 10-42 $cellsl^{-1}$. During the study period Chlorophyceae was the dominant genera (38.42%) followed by Bacillariophyceae (33.50%) and Cyanophyceae (28.07%).

In Station 5 plankton density was ranged from 8-39 $cellsl^{-1}$. During the study period Chlorophyceae was the dominant genera (43.37%) followed by Bacillariophyceae (31.12%) and Cyanophyceae (25.49%).

The total phytoplankton density of the station 6 ranged from 13-38 $cellsl^{-1}$. During the study period Chlorophyceae was dominant genera (40.63%) followed by Bacillariophyceae (33.01%) and Cyanophyceae (26.34%).

Table 38: Phytoplankton composition of the 6 stations observed during the present study

Phytoplankton	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Station 6**
Chlorophyceae						
Volvox	+	+	+	+	+	+
Ulothrix	+	+	+	+	+	+
Chlorella	-	+	+	+	+	+
Cladophora	+	-	+	-	-	-
Chlamydomonas	-	+	+	+	-	-
Bacillariophyceae						
Navicula	+	+	+	+	+	-
Fragillaria	+	+	+	-	-	+
Nitzschia	+	-	+	+	+	-
Synedra	-	+	+	+	-	+
Melosira	+	+	+	-	+	-
Cynophyceae						
Anabaena	+	+	+	+	+	+
Oscillatoria	-	-	-	+	+	+

Nostoc	+	+	+	-	-	+
Spirulina	+	+	+	+	+	-

Table 38: Monthly distribution of different phytoplankton genera at Station 1

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Chloro- Phyceae												
Volvox	+	+	+	+	+	+	-	-	+	+	+	+
Ulothrix	+	+	+	+	-	+	+	-	+	+	+	+
Chlorella	+	+	+	+	-	-	+	+	-	+	+	+
Cladophora	+	+	+	+	+	-	-	+	+	+	+	+
Bacillariophyceae												
Navicula	+	+	+	-	+	-	-	-	+	+	+	+
Fragilleria	+	+	+	-	+	-	+	+	+	+	+	+
Nitzschia	+	+	-	+	-	-	+	+	+	+	+	+
Synedra	+	+	+	+	+	+	-	-	+	+	+	+
Melosira	+	+	+	+	+	+	-	+	+	+	+	+
Cynophyceae												
Anabaena	+	+	+	-	+	-	-	+	+	+	+	+
Oscillatoria	+	+	+	-	+	-	+	+	+	+	+	+
Nostoc	+	+	-	+	+	+	+	-	+	+	+	+
Spirulina	+	+	+	+	+	+	+	+	+	+	+	+

Table 39: Monthly distribution of different phytoplankton genera at Station 2

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Chloro- Phyceae												
Volvox	+	+	+	+	+	+	-	-	+	+	+	+
Ulothrix	+	+	+	+	-	+	+	-	+	+	+	+
Chlorella	+	+	+	+	-	-	+	+	-	+	+	+
Cladophora	+	+	+	+	+	-	-	+	+	+	+	+
Chlamydomonas	+	+	+	-	+	-	-	+	+	+	+	+
Bacillariophyceae												
Navicula	+	+	+	-	+	-	-	-	+	+	+	+
Fragilleria	+	+	+	-	+	-	+	+	+	+	+	+
Nitzschia	+	+	-	+	-	-	+	+	+	+	+	+
Synedra	+	+	+	+	+	+	-	-	+	+	+	+
Melosira	+	+	+	+	+	+	-	+	+	+	+	+
Cynophyceae												
Anabaena	+	+	+	-	+	-	-	+	+	+	+	+
Oscillatoria	+	+	+	-	+	-	+	+	+	+	+	+
Nostoc	+	+	-	+	+	+	+	-	+	+	+	+
Spirulina	+	+	+	+	+	+	+	+	+	+	+	+

Table 40: Monthly distribution of different phytoplankton genera at Station 3

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Chloro- Phyceae												
Volvox	+	+	+	+	+	+	-	-	+	+	+	+
Ulothrix	+	+	+	+	-	+	+	-	+	+	+	+
Chlorella	+	+	+	+	-	-	+	+	-	+	+	+
Cladophora	+	+	+	+	+	-	-	+	+	+	+	+
Chlamydomonas	+	+	+	-	+	-	-	+	+	+	+	+
Bacillariophyceae												
Navicula	+	+	+	-	+	-	-	-	+	+	+	+
Fragilleria	+	+	+	-	+	-	+	+	+	+	+	+
Nitzschia	+	+	-	+	-	-	+	+	+	+	+	+
Synedra	+	+	+	+	+	+	-	-	+	+	+	+
Melosira	+	+	+	+	+	+	-	+	+	+	+	+
Cynophyceae												
Anabaena	+	+	+	-	+	-	-	+	+	+	+	+
Oscillatoria	+	+	+	-	+	-	+	+	+	+	+	+
Nostoc	+	+	-	+	+	+	+	-	+	+	+	+
Spirulina	+	+	+	+	+	+	+	+	+	+	+	+

Table 41: Monthly distribution of different phytoplankton genera at Station 4

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Chloro- Phyceae												
Volvox	+	+	+	+	+	+	-	-	+	+	+	+
Ulothrix	+	+	+	+	-	+	+	-	+	+	+	+
Chlorella	+	+	+	+	-	-	+	+	-	+	+	+
Chlamydomonas	+	+	+	-	+	-	-	+	+	+	+	+
Bacillariophyceae												
Navicula	+	+	+	-	+	-	-	-	+	+	+	+
Nitzschia	+	+	-	+	-	-	+	+	+	+	+	+
Synedra	+	+	+	+	+	+	-	-	+	+	+	+
Cynophyceae												
Anabaena	+	+	+	-	+	-	-	+	+	+	+	+
Oscillatoria	+	+	+	-	+	-	+	+	+	+	+	+
Spirulina	+	+	+	+	+	+	+	+	+	+	+	+

Table 42: Monthly distribution of different phytoplankton genera at Station 5

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Chloro- Phyceae												
Volvox	+	+	+	+	+	+	-	-	+	+	+	+
Ulothrix	+	+	+	+	-	+	+	-	+	+	+	+
Chlorella	+	+	+	+	-	-	+	+	-	+	+	+
Bacillariophyceae												
Navicula	+	+	+	-	+	-	-	-	+	+	+	+
Nitzschia	+	+	-	+	-	-	+	+	+	+	+	+
Melosira	+	+	+	+	+	+	-	+	+	+	+	+
Cynophyceae												
Anabaena	+	+	+	-	+	-	-	+	+	+	+	+
Spirulina	+	+	+	+	+	+	+	+	+	+	+	+

Table 43: Monthly distribution of different phytoplankton genera at Station 6

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Chloro- Phyceae												
Volvox	+	+	+	+	+	+	-	-	+	+	+	+
Ulothrix	+	+	+	+	-	+	+	-	+	+	+	+
Chlorella	+	+	+	+	-	-	+	+	-	+	+	+
Bacillariophyceae												
Fragilleria	+	+	+	-	+	-	+	+	+	+	+	+
Synedra	+	+	+	+	+	+	-	-	+	+	+	+
Cynophyceae												
Anabaena	+	+	+	-	+	-	-	+	+	+	+	+
Oscillatoria	+	+	+	-	+	-	+	+	+	+	+	+
Nostoc	+	+	-	+	+	+	+	-	+	+	+	+

Palmer Index

Palmer (1969), first made the list of algae genera and species which indicate organic pollution . According to Palmer, scores of 20 or more are indication of high organic pollution. The pollution tolerant genera belonging to three groups of algae from six sites of Umtrew river system was recorded . By using Palmer's index of pollution for rating of water samples as high, moderate and low organically polluted at six sites of Umtrew river system were tested. The total score of Algal Genus Pollution Index (AGPI) of sites S1<S2<S3< S6<S5< S4 are calculated to be 3,5, 8, 10,11 and 13 respectively . The total score of S1, S2 and S3 indicating probable lack of organic pollution while S4, S4 and S6 showed moderate pollution due to anthropogenic factors or human interference according to Palmer, Chlorella, Nitzschia and Synedra Closterium was found to be the most active participant in most of the sites which may be the good indicator of contaminated water. Oscillatoria was recorded repeatedly in station 4, 5 and 6 and consider as indicators of pollution in view of the results of Palmer pollution index.

Table 44 : Algal genus pollution index (Palmer, 1969).

Genus	Pollution Index
Anacystis	1
Ankistrodesmus	2
Chlamydomonas	4
Chlorella	3
Closterium	1
Cyclotella	1
Euglena	5
Gomphonema	1
Lepocinclis	1
Melosira	1
Micractinium	1
Navicula	3
Nitzschia	3
Oscillatoria	5
Pandorina	1
Phacus	2
Phormidium	1
Scenedesmus	4
Stigeoclonium	2
Synedra	2

Following numerical values for pollution classification of Palmer (1969), 0-10= Lack of organic pollution 10-15= Moderate pollution 15-20= Probable high organic pollution 20 or more = Confirms high organic pollution.

Table 45. Pollution index of Algal genera level according to Palmer, (1969) at Six sites of river Umtrew.

Phytoplankton	Pollution Index (Palmer, 1969)	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
Chlorophyceae							
Volvox	_	+	+	+	+	+	+
Ulothrix	_	+	+	+	+	+	+
Chlorella	3	-	+(3)	+(3)	+(3)	+(3)	+(3)
Cladophora	_	+	-	+	-	-	-
Chlamydomonas	_	-	+	+	+	-	-
Bacillariophyceae							
Navicula	_	+	+	+	+	+	-
Fragillaria	_	+	+	+	-	-	+
Nitzschia	3	+(3)	-	+(3)	+(3)	+(3)	-
Synedra	3	-	+(2)	+(2)	+(2)	-	+(2)
Melosira	_	+	+	+	-	+	-
Cynophyceae							
Anabaena	_	+	+	+	+	+	+
Oscillatoria	3	-	-	-	+(5)	+(5)	+(5)
Nostoc	_	+	+	+	-	-	+
Spirulina	_	+	+	+	+	+	-
Total		**3**	**5**	**8**	**13**	**11**	**10**

Zooplankton:

A total of 8 genera of zooplankton were recorded from all the six stations. The zooplankton population comprised of Rotifera (2 genera), Cladocera (3 genera) and Copepoda (3 genera). The total number of 7 genera were recorded from all the 3 stations during the study period.

Composition and Monthly Distribution of Zooplankton:

At station 1, the total genera of zooplankton recorded were 8. The percentage contribution of Rotifera, Cladocera and Copepoda were 36.53%, 34.61% and 28.84%. At station 2, the total number of genera of zooplankton recorded was 8. The percentage contribution of Rotifera, Cladocera and Copepoda were 40%, 31.81% and 21.18%. At station 3, the total number of genera of zooplankton recorded was 8. The percentage contribution of Rotifera, Cladocera and Copepoda were 43.01%, 30.10% and 26.88%. At station 4, the total number of genera of zooplankton recorded was 7. The percentage contribution of Rotifera, Cladocera and Copepoda were 37.68%, 28.98% and 33.33%. At station 5, the total genera of zooplankton recorded were 6. The percentage contribution of Rotifera, Cladocera and Copepoda were 52.94%, 31.37% and 15.68%.At station 6, the total genera of zooplankton recorded were 5. The percentage contribution of Rotifera, Cladocera and Copepoda were 47.38%, 29.82% and 22.80%.

Density of zooplankton:

Station 1 Rotifera was dominant group (27.14%) followed by Cladocera (38.57%) and Copepoda (34.28%). At station 2 Rotifera was dominant group (30.98%) followed by Cladocera (34.50%) and Copepoda (34.50%). At station 3 Rotifera was dominant group (32.52%) followed by Cladocera (34.95%) and Copepoda (32.52%). At station 4 study Rotifera was dominant group (27.36%) followed by Cladocera (34.73%) and Copepoda (37.89%). At station 5 Rotifera was dominant group (36.48%) followed by Cladocera (37.83%) and Copepoda (27.67%). At station 5 Rotifera was dominant group (32.53%) followed by Cladocera (37.34%) and Copepoda (30.12%).

Table 46: Zooplankton composition of the 6 stations observed during the present study

	Station 1	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Station 6**
Rotifera						
Branchionous	+	+	+	+	+	+
Keratella	+	+	+	-	+	-
Cladocera						
Moina	+	+	+	+	+	+
Daphnia	+	+	+	+	-	+
Copepods						
Copepod nauplii	+	+	+	+	-	-
Fish egg	+	+	+	+	+	-
Fish larvae	+	+	+	+	+	+

Table 46: Monthly distribution of different Zooplankton genera at Station 1

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Rotifera												
Branchionous	+	+	+	+	+	+	-	-	+	+	+	+
Keratella	+	+	+	+	+	+	-	+	+	-	+	+
Cladocera												
Moina	+	+	+	+	+	-	+	-	+	-	+	+
Daphnia	+	+	+	+	+	-	-	+	+	+	+	+
Copepods												
Copepod nauplii	+	+	+	+	-	-	+	+	-	+	+	+
Fish egg	-	-	+	+	+	+	+	+	+	+	-	-
Fish larvae	-	-	+	+	+	+	+	+	-	-	+	-

Table 48: Monthly distribution of different Zooplankton genera at Station 2

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Rotifera												
Branchionous	+	+	+	+	+	+	-	-	+	+	+	+
Keratella	+	+	+	+	+	+	-	+	+	-	+	+
Cladocera												
Moina	+	+	+	+	+	-	+	-	+	-	+	+
Daphnia	+	+	+	+	+	-	-	+	+	+	+	+
Copepods												
Copepod nauplii	+	+	+	+	-	-	+	+	-	+	+	+
Fish egg	-	-	+	+	+	+	+	+	+	+	-	-
Fish larvae	-	-	+	+	+	+	+	+	-	-	+	-

Table 49: Monthly distribution of different Zooplankton genera at Station 3

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Rotifera												
Branchionous	+	+	+	+	+	+	-	-	+	+	+	+
Keratella	+	+	+	+	+	+	-	+	+	-	+	+
Cladocera												
Moina	+	+	+	+	+	-	+	-	+	-	+	+
Daphnia	+	+	+	+	+	-	-	+	+	+	+	+
Copepods												
Copepod nauplii	+	+	+	+	-	-	+	+	-	+	+	+
Fish egg	-	-	+	+	+	+	+	+	+	+	-	-
Fish larvae	-	-	+	+	+	+	+	+	-	-	+	-

Table 40: Monthly distribution of different Zooplankton genera at Station 4

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Rotifera												
Branchionous	+	+	+	+	+	+	-	-	+	+	+	+
Cladocera												
Moina	+	+	+	+	+	-	+	-	+	-	+	+
Daphnia	+	+	+	+	+	-	-	+	+	+	+	+
Copepods												
Copepod nauplii	+	+	+	+	-	-	+	+	-	+	+	+
Fish egg	-	-	+	+	+	+	+	+	+	+	-	-
Fish larvae	-	-	+	+	+	+	+	+	-	-	+	-

Table 51: Monthly distribution of different Zooplankton genera at Station 5

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Rotifera												
Branchionous	+	+	+	+	+	+	-	-	+	+	+	+
Keratella	+	+	+	+	+	+	-	+	+	-	+	+
Cladocera												
Moina	+	+	+	+	+	-	+	-	+	-	+	+
Copepods												
Fish egg	-	-	+	+	+	+	+	+	+	+	-	-
Fish larvae	-	-	+	+	+	+	+	+	-	-	+	-

Table 52: Monthly distribution of different Zooplankton genera at Station 6

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Rotifera												
Branchionous	+	+	+	+	+	+	-	-	+	+	+	+
Cladocera												
Moina	+	+	+	+	+	-	+	-	+	-	+	+
Daphnia	+	+	+	+	+	-	-	+	+	+	+	+
Copepods												
Fish larvae	-	-	+	+	+	+	+	+	-	-	+	-

Fish Diversity:

During the present investigation a total of 49 fish species belonging to 36 genera, 20 families and 10 orders are recorded from 6 selected sampling stations of the river Umtrew, Maghalaya India. The number and percentage composition of order and family under are shown (Table 53 and 54). Among the orders, the Cypriniformes formed the largest group with a contribution of 4 (20.00 %) families, 15 (41.66%) genera and 21 (42.85%) species. The order Siluriformes also contributed a major portion to the total number and percentage composition of the recorded fish fauna of the river with 5 (25 %) families, 10 (27.77%) genera and 12 (24.48 %) species followed by Anabantiformes with 1 (5%) family, 1 (2.7%) genera and 5 (10.20%) species, Syubranchiformes with 1 (5%) family, 1 (2.7%) genera and 3 (6.12%) species, Perciformes and Osteoglossiformes with 1 (5%) family, 1 (2.7%) genera and 2 (4.08%) species each and Gobiformes, Mugliformes, Clupiformes, Beloniformes with 1 (5%) family, 1 (2.7%) genera and 1 (2.04%) each.

The IUCN conservation status of the 49 recorded species with their number under different category are shown in Table 55. The highest species were recorded under least concern (LC) category with a total no of 42 and contributed 85.71%. under LC category, the major species contribution is from the family Cyprinidae with 8 (18.32 %) followed by Bagridae and Danionidae 5 (10.20%) each, Channidae 4 (8.16 %), Mastacembelidae 3 (6.12 %), Daninidae 2 (4.08%).Sisoridae, Ailidae, Claridae, Cobidae, Botidae, Nandidae, Ambassidae, Notoptaridae, Belonidae, Osphronemida, Gobidae, Muglidae and Clupidae 1 (2.38%). Under near threatened (NT) category Cyprinidae 2 (4.08%), Sissoridae and Alidae contributed 1 (2.38%) each. Like that, the family Siluridea and Botidae represent vulnerable category with 1 (2.38%) species each. One species which contributed 2.28% under family Cyprinidae represent the critically care category. According to CAMP report among 49 species 14 are vulnerable (28.57%), 17 are lower risk near threaten (34.69%), 4 are lower risk least concern (8.16%) and 14 are not evaluated (34.69%). Ramanujan *et al*., 2010 studied the icthyophonal diversity of the Khasi hills and found 68 species belonging 45 genera, 20 families and 6 orders. Where Cyprinidae was the most dominant group dominated by 30 species

In the present study fish faunal diversity was found scanty in the stations existing in the higher altitude. This is because the river facing barrier with two major and one small dam along its stretch. These dams are mainly responsible for the fish migration pattern. Beside that the river water is also contaminated with different type of pollution like acid mine drainage, lavatory waste, waste generated from the Shillong town, different industrial waste, anthropogenic activities like sand mining and bolder mining etc. The trophic level index indicates that most of the fishes are omnivore (48.97%) with their feeding habit and rest of that 24.48% comes under in carnivore and 26.5%3 are comes under in herbivore category. According to Karr *et al*., 1987) the Umtrew river environment is comes under poor category since more than 45% fish species are comes under omnivore.

Table 53: Composition of fish community by order:

SL No	Taxa	Number of species	Percentage (%)
1	Cypriniformes	21	42.85
2	Siluriformes	12	24.48
3	Anabantiformes	5	10.20
4	Synbranchiformes	3	6.12
5	Perciformes	2	4.08
6	Osteoglossiformes	2	4.08
7	Gobiformes	1	2.04
8	Mugliformes	1	2.04
9	Clupiformes	1	2.04
10	Beloniformes	1	2.04

Table 54:Composition of fish community by family:

SL number	Taxa/Family	Number of species	Percentage (%)
1	Cyprinidae	11	22.44
2	Bagridae	5	10.20
3	Danionidae	5	10.20
4	Channidae	4	8.14
5	Mastacembelidae	3	6.12
6	Siluridae	2	4.08
7	Botidae	2	4.08
8	Sisoridae	2	4.08
9	Aridae	2	4.08
10	Daninidae	2	4.08
11	Notopteridae	2	4.08

12	Belonidae	1	2.04
13	Nandidae	1	2.04
14	Osphronemida	1	2.04
15	Claridae	1	2.04
16	Cobidae	1	2.04
17	Ambasidae	1	2.04
18	Gobidae	1	2.04
19	Muglidae	1	2.04
20	Clupide	1	2.04

Table 55 :Fish fauna of Umtrew river, their taxonomic status, trophic level, feeding habits, relative abundance, CAMP and IUCN status.

SL No.	Species	Family	Order	Trophic Level (Based on food Items)	Feeding Habit	CAMP	IUCN status
1	*Sperata aor*	Bagridae	Siluriformes	3.6±0.53	Carnivorous	**CAMP Status**	LC
2	*Mystus vittatus*	Bagridae	Siluriformes	3.1±0.1	Carnivorous	NE	LC
3	*Mystus cavasius*	Bagridae	Siluriformes	3.4±0.5	Carnivorous	VU/N	LC
4	*Rita rita*	Bagridae	Siluriformes	3.7±0.57	Carnivorous	VU	LC
5	*Mystus tengara*	Begridae	Siluriformes	3.20.40	Carnivorous	LRnt	LC
6	*Wallago attu*	Siluridae	Siluriformes	3.7±o.56	Carnivorous	NE	VU
7	*Heteropneustes fossilis*	Siluridae	Silurifomes	3.6±0.3	Omnivorous	VU	LC
8	*Bagarius bagarius*	Sisoridae	Siluriformes	3.7±0.59	Carnivorous	LRnt	NT
9	*Gagata cenia*	Sisoridae	Siluriformes	3.3±0.5	Omnivorous	VU	LC
10	*Allia coilia*	Ailidae	Silurifomes	3.6 ±0.6	Omnivorous	NE	NT
11	*Clupisoma garua*	Ailidae	Siluriformes	3.7±0.59	Carnivorous	NE	LC
12	*Clarias batrachus*	Claridae	Siluriformes	3.4 ±0.50	Omnivorous	VU	LC
13	*Channa marulius*	Channidae	Anabantiformes	4.5±0.80	Carnivorous	NE	LC
14	*Channa punctata*	Channidae	Anabantiformes	3.8±0.70	Omnivorous	LRnt	LC

15	*Channa striata*	Channidae	Anabantiformes	3.4±0.45	Omnivorous	LRnt	LC
16	*Channa gachua*	Channidae	Anabantiformes	3.8±0.62	Omnivorous	LRlc	LC
17	*Macrognathus pancalus*	Mastacembelidae	Synbranchiformes	3.1±0.33	Omnivorous	VU	LC
18	*Macrognathus aral*	Mastacembelidae	Synbranchiformes	3.1±0.33	Omnivorous	LRnt	LC
19	*Mastacembelus armatus*	Mastacembelidae	Syubranchiformes	2.8±0.27	Herbivorous	LRnt	LC
20	*Puntius sophore*	Cyprinidae	Cypriniformes	2.6±0.1	Omnivorous	NR	LC
21	*Cirrhinus mrigala*	Cyprinidae	Cypriniformes	2.2±0.12	Herbivorous	LRnt	LC
22	*Systomus sarana*	Cyprinidae	Cypriniformes	2.9±0.2	Herbivorous	LRnt	CR
23	*Osteobrama curma*	Cyprinidae	Cypriniformes	2.9±0.3	Herbivorous	VU	LC
24	*Amblypharyngodon mola*	Cyprinidae	Cypriniformes	3.2±0.4	Herbivorous	VU	LC
25	*Petihia ticto*	Cyprinidae	Cypriniformis	2.2±0.0	Herbivorous	LRlc	LC
26	*Neolissochilus hexagonolepis*	Cyprinidae	Cypriniformes	3.0±0.37	Omnivorous	LRnt	NT
27	*Cirrihinus reba*	Cyprinidae	Cypriniformes	3.6±0.59	Herbivorous	VU	LC
28	*Garra gotyla*	Cyprinidae	Cypriniformes	2.0 ±0.00	Herbivorous	VU	LC
29	*Garra annandalei*	Cyprinidae	Cypriniformes	2.0 ±0.00	Herbivorous	VU	LC
30	*9 Labeo dyocheilus*	Cyprinidae	Cypriniformes	2.0 ±0.0	Herbivorous	LRnt	NT
31	*Salmostoma bacaila*	Danionidae	Cypriniformes	3.2±0.40	Omnivorous	VU	LC
32	*Salmophasia balookee*	Danionidae	Cypriniformes	3.2 ±0.4	Omnivorous	LRlc	LC

33	*Danio dangila*	Daninidae	Cypriniformes	3.0±0.4	Omnivorous	NE	LC
34	*Deverio aequipinatus*	Daninidae	Cypriniformes	2.9±0.33	Herbivorous	NE	LC
35	*Barilius bendelisis*	Danionidae	Cypriniformes	3.4 ±0.4	Omnivorous	LRnt	LC
36	*barilius barila*	Danionidae	Cypriniformes	3.2 ±0.4	Omnivorous	LRnt	LC
37	*Lepidocephalichthys guntea*	Cobidae	Cypriniformes	2.7±0.2	Herbivorous	VU	LC
38	*Barilius barna*	Danionidae	Cypriniformes	3.4 ±0.4	Omnivorous	NE	LC
39	*Botia rostrata*	Botidae	Cyriniformes	3.4 ±0.4	Omnivorous	LRnt	VU
40	*Botia Dario*	Botidae	Cyriniformes	3.2 ±0.4	Omnivorous	NE	LC
41	*Nandus nandus*	Nandidae	Perciformes	3.9±0.63	Omnivorous	LRnt	LC
42	*Chanda nama*	Ambassidae	Perciformes	3.6±0.54	Carnivorous	LRnt	LC
43	*Notopterus synurus*	Notoptaridae	Osteoglossiformes	3.5±0.0	Carnivorous	NE	LC
44	*Notopterus notopterus*	Notopteridae	Osteoglossiformes	3.5 ±0.0	Carnivorous	NE	LC
45	*Xenentodon cancila*	Belonidae	Beloniformes	3.9±0.62	Omnivorous	NE	LC
46	*Trichogaster fasciata*	Osphronemida	Anabantiformes	3.1±0.3	Omnivorous	LRnt	LC
47	*Glossogobius giuris*	Gobidae	Gobiformes	3.7±0.2	Omnivorous	NE	LC
48	*Rhinomugil corsula*	Muglidae	Mugliformes	2.4±0.2	Herbivorous	LRnt	LC
49	*Gudusia chapra*	Clupidae	Clupiformes	3.1±0.3	Omnivorous	VU	LC
LC- Least concern, VU- Vulnerable, NT- Near threatened, LRnt- Lower risk-near threatened, LRlc- Lower risk-least concern, NE- Not							

evaluated

Table 56 : DNA Barcoding of the Species Recorded With Accession Number

Sl. No.	Species Name	NCBI Accession Code
1.	*Cirrhinus mrigala*	MW326659
2.	*Labeo Gonius*	MW326660
3.	*Channa punctata*	MW326661
4.	*Wallago attu*	MW326662
5.	*Channa marulius*	MW326663
6.	*Nandus nandus*	MW326664
7.	*Puntius sophore*	MW326665
8.	*Macrognathus aral*	MW326666
9.	*Notopterus synurus*	MW326667
10.	*Heteropneustes fossilis*	OK103921
11.	*Lepidocephalichthys guntea*	OK091024
12.	*Labeo dyocheilus*	OK091027
13.	*Osteobrama cotio*	OM491192
14.	*Gagata cenia*	OM491193
15.	*Cirrihinus reba*	OM491194
16.	*Mastacembelus armatus*	OM491195
17.	*Deverio aequipinatus*	OM491196
18.	*Sperata aor*	OM491197

Patterns of species composition in relation to environmental variables

The main pattern shown by the CCA is a longitudinal gradient in species composition. Forward selection and Monte Carlo permutation (199 iteration) allowed to identify 14 environmental variables accounting for 50% of the variance explained by 15 variables: width, velocity, oxygen, depth, mean % of leaves wood and % rocks. These variables were considered as the best predictors of the species environmental relationships in the Umtrew River. For data analysis, we considered the first two axes expressing the highest variability of species data. In the CCA performed, axis 1 (eigenvalue = 0.48) and axis 2 (eigenvalue = 0.14) expressed 72.01% of the cumulative variance in the species data. Monte Carlo permutation tests showed that both axes were significant ($p = 0.005$). From these selected variables, DO, water velocity, pH, turbidity, BOD, COD, width, Depth, Sand clay and mud and mean % of mixed leaves-wood in the substrate seem to be the important variables explaining longitudinal change in species composition both upstream and downstream gradient. In the present study, high mean % of leaves-wood in the substrate, sand clay and mud reflect a habitat located in river system. Based on CCA axis 1, there is a high correlation between position of sampling sites and position along an upstream-downstream gradient. In the river west Africa basin (Essetchi et sl., 2003), Ntem River (Cameroon), Kamdem Tohan & Teugels (1998) ound a similar result in *Tilapia guineensis, Brycinus macrolepidotus* and *Barbus* species.

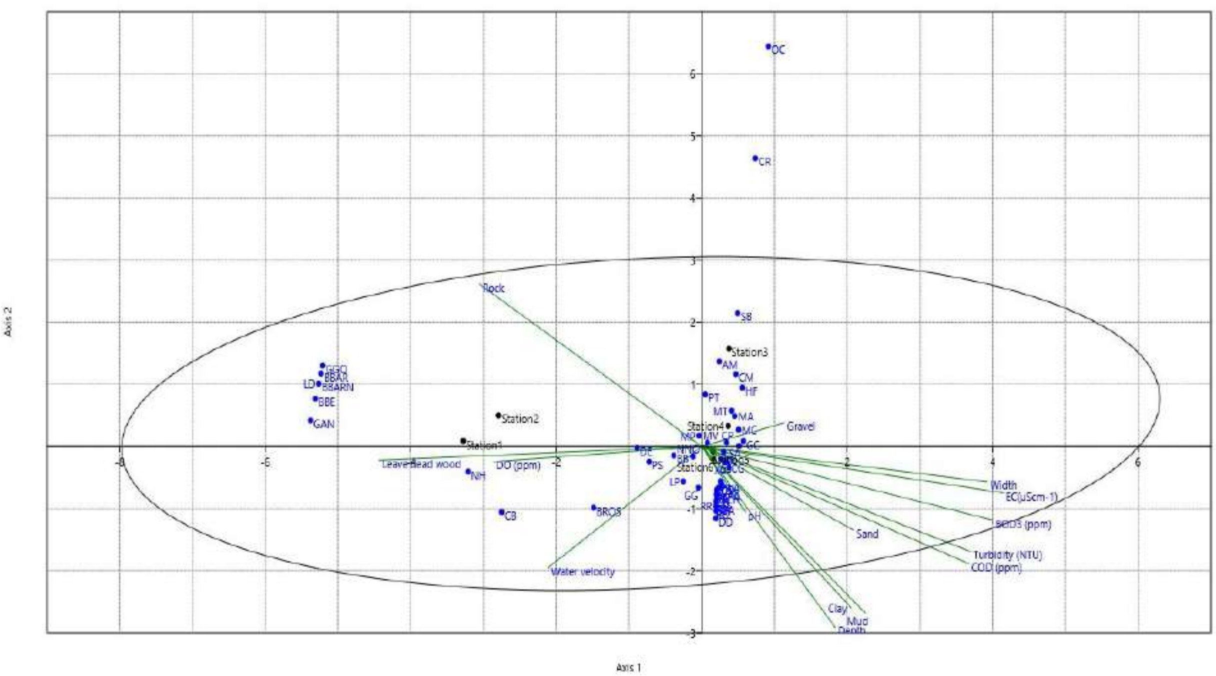

Fig 22: Canonical correspondence analysis showing correlation between species composition and environmental variables. SA- *Sperata aor, CM-Channa marulius, CS-Channa striata, CGChanna gachua, HF-Heteropneustes fossilis, MP-Macrognathus pancalus, NP-Notopterus Chitala. MV-Mystus vittatus, NNO-Notopterus notopterus*, *MC-Mystus cavasius, WA-Wallago attu, XC-Xenentodon cancila, MA-Macrognathus aral, PS-Puntius sophore, CM-Cirrhinus mrigala, TF-Trichogaster fasciata, BB-Botia Dario, AM-Amblypharyngodon mola, SS-Systomus sarana, NN-Nandus nandus, MT-Mystus tengara, PT-Puntius ticto,, OC-Osteobrama cotio,* SB-Salmostoma *bacailla, NH-Neolissochilus hexagonolepis, AC-Ailia coila, DE-Devario aequipinnatus, GG-Glossogobius giuris, RC-Rhinomugil corsula, CG-Clupisoma garua, MAA-Mastacembelus armatus, LP-Lepidocephalichthys guntea, BBA-Bagarius bagarius, RR-Rita rita, GC-Gagata cenia, CN-Chanda nama, CR-Cirrihinus reba, GCH-Gudusia chapra, SB-Salmostroma balookee. DD-Danio dangila, CB-Clarias batrachus, GGP-Garra gotyla, GAN=Garra annandalei, LD-Labeo dyocheilus, BBE-Barilius bendelisis, BBAR-barilius barila, BBARN-Barilius barna, BROS-Botia rostrata.*

Species distribution

Fishes were collected in all 8 sampling sites. Species composition differed from one site to another. To study the longitudinal distribution of fishes, we used data from the main river sites only, and followed the upper-lower gradient. Species composition was seen more in site 1, decreases from sites 2 to 4 and finally increases from site 5 to 6 (Fig. 3). The tendency for species composition to increase from the source to the mouth of the river has been widely observed by community ecologists. However, following the upper-lower gradient of the main channel of the Umtrew River, we observed an irregular distribution of fishes. This is most likely due to the impact of different anthropogenic factors. In site 2 and 3, two dams (Kyrdemkulai and Umtrew) dam has been built built to retain water for generation of electricity and various uses. The immediate consequence of the construction of a dam, whatever its size in an aquatic ecosystem, is the modification of the hydrological regime, creating thus a lentic environment. This artificial lacustrine habitat is unfavourable to rheophilic species that migrate to the upper part of the river. This possibility of migration should account for the relatively high number of species sampled in sampling site 3 located immediately above the dam. This last sampling site seems to be less disturbed by human activities. Moreover, the discharge of industrial waste and motor oils in sampling site 4 should constitute a real source of pollution which seriously affects the ecosystem and consequently threatens fish biodiversity in the Umtrew River. This is confirmed by the presence of highly tolerant species towards poor environmental condition. Besides some airbreathing fishes having high tolerant capacity to poor environmental condition like H. fossils other fish species were relatively less abundant in sampling site 4. Apart from the two major factors linked to human activities (dam construction and industrial pollution), degradation of the forest along the Umtrew River for creating space for industrial estate should be noted.

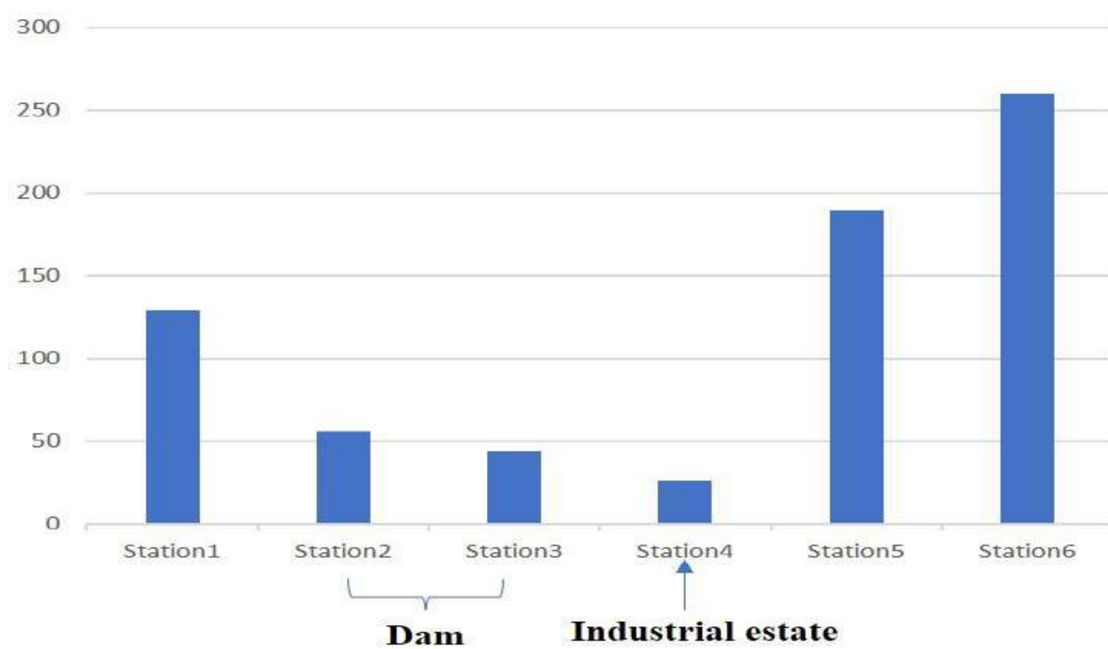

Fig 23. Longitudinal distribution of freshwater fish species along the main channel of the Umtrew River.

Diversity indices:

Pre Monsoon Season:

The ichthyofaunal diversity indices of the Umtrew river are given for the three seasons. The Simpson was found to be highest at station 5 with a value of 0.962 and lowest values was found in station 1 with a value of 0.656 in the pre monsoon season . In case of Shannon index the highest value was also found in the station 6 (3.403) and lowest value was for the station 1 (1.213). However, the Evenness index of the season pre monsoon season was found highest of the station 2 (0.9187) and lowest value was found in station 6 (0.8352). The highest value for Margalef's richness index was for the station 6 (6.871) and lowest was for the station 1 (1.443).

Table 57: Station wise diversity indices of the Umtrew river in pre - monsoon season

	Simpson_1-D	Shannon_H	Pielou's evenness Index(E)	Margalef's richness index (d)
Station 1	0.6563	1.213	0.8409	1.443
Station 2	0.8927	2.313	0.9187	3.53
Station 3	0.9143	2.609	0.8493	4.219
Station 4	0.9479	3.175	0.7973	6.717
Station 5	0.962	3.368	0.8532	6.738
Station 6	0.9619	3.403	0.8352	6.871

Monsoon Season:

During the monsoon season the highest Simpson index value was found station 6 (0.959) and lowest was found for station 1 (0.875) . The Shannon index value was also highest in the station 6 (3.314) and lowest found for the station 1 (2.079). Pielou's evenness Index value for pre monsoon season was found 1.00 in the station 1 and 2 on the other hand the lowest value was calculated 0.882 in station 5. Highest richness index value was observed for the station 6 (7.486) and lowest was for station 1 (3.366).

Table 58: Station wise diversity indices of the Umtrew river in Monsoon season

	Simpson_1-D	Shannon_H	Pielou's evenness Index(E)	Margalef's richness index (d)
Station 1	0.875	2.079	1	3.366
Station 2	0.8889	2.197	1	3.641
Station 3	0.9363	2.799	0.9659	5.434
Station 4	0.9584	3.271	0.908	7.313
Station 5	0.9568	3.276	0.8824	7.413
Station 6	0.9593	3.314	0.8869	7.486

Post Monsoon:

The Simpson was found to be highest at station 6 with a value of 0.966 and lowest values was found in station 1 with a value of 0.850 in the pre monsoon season. In case of Shannon index the highest value was also found in the station 6 (3.493) and lowest value was for the station 1 (2.112). However, the Evenness index of the season pre monsoon season was found highest of the station 2 (0.935) and lowest value was found in station 6 (0.8653). The highest value for Margalef's richness index was for the station 4 (7.129) and lowest was fr the station 1 (3.004).

Table 59: Station wise diversity indices of the Umtrew river in Post Monsoon season

	Simpson_1-D	Shannon_H	Pielou's evenness Index(E)	Margalef's richness index (d)
Station 1	0.850	2.112	0.8262	3.004
Station 2	0.9434	2.93	0.9359	5.482
Station 3	0.9523	3.185	0.8634	6.678
Station 4	0.9564	3.311	0.831	7.129
Station 5	0.9639	3.431	0.8586	6.815
Station 6	0.9662	3.493	0.8653	6.854

A biological diversity is a mathematical measure of a community's diversity. Biodiversity indices are useful tools for determining rarity and frequency of species in a population. In the most ecological research, the Shannon-weinner index (H′) ranges between 1.5 and 3.5, with a value greater than 3.0 indicating greater diversity (Magurran, 2004). In the present study, The Shannon- weinner index values was ranges from 1.213 to 3.493. The lowest value was found in the station 1 during pre-monsoon season and the highest value was observed during the post monsoon season in station 6. Since the greater index value represent greater diversity (Magurram, 2004), the fish diversity was highest during post monsoon season on the other side the less diversity was found during pre-monsoon season. The index values also varies station to station. Stations that existing in the upper starches were less index value the lower station in all season and it may be due to the effect on dam.

According to Mohammad *et al*., 2019 the bigger the Simpson index (1-λ) value, the greater the diversity. The Simpson index ranges from 0 (poor diversity) to high diversity (1). In river Umtrew the index value was ranges from 0.656 to 0.966. The highest value was observed during the post monsoon season in station 6 and the lowest value was observed during pre-monsoon season in station 1. The index value also shows the maximum diversity was observed during the post monsoon period.

Pielou's evenness index (e) expresses how equally individuals are scattered among the various species (Clarke and Warwick, 2001). Pielou's evenness index for the Umtrew river was found ranges from 0.826 to 1. During the monsoon season the index value was found to be the highest and the lowest value was observed during post monsoon season. The index value for the station 1 and 2 during the monsoon season was indicate these two-stations having highest equally distributed individuals as compare to other station.

Margalef's richness index(d) is the simplest index to measure the biodiversity, which simply count of the number of different species in a given area. During the study period, Margalef's richness index was highest during monsoon period at station 5 and lowest was found in the station 1 during the pre-monsoon period. Station 5 is situated near the dam site where during the monsoon period the water was released. In these areas water depth was optimum during the monsoon period so that fishermen can use their fishing gears in these areas effectively on the other hand station 1 is situated in the higher altitude so there is no effective fishing activity observed in the areas situated in the higher altitude areas. Umtrew hydroelectric project also inhabit the fish migration patter. During monsoon period when was is released the fish can migrate to upstream areas to downstream or vice versa

Androgenic Stress:

Industrial area near river bank **Human interference**

Umtrew dam for hydroelectric project **Extraction of sand form the river bank**

	
Extraction of bolder form the river bank	**Damping area on river bank**
	

Irregular release of water from dams	**Fishing**

ANNEXURE IV

Photographs of Collected & Identified Fish Species

	
Heteropneustes fossilis	*Xenentodon cancila*
	
Puntius sophore	*Sperata aor*

	
Cirrhinus mrigala	*Channa marulius*
	
Garra annandalei	*Labeo dyocheilus*
	
Salmostoma bacaila	*Danio dangila*
	
Barilius barila	*Lepidocephalichthys guntea*
	
Notopterus synurus	*Chitala chitala*
	
Trichogaster fasciata	*Glossogobius giuris*

	
Macrognathus aral	*Macrognathus pancalus*
	
Botia dario	*Channa gachua*
	
Nandus nandus	*Mystus cavasius*
	
Mastacembelus armatus	*Puntius sophore*
	
Cirrhinus mrigala	*Systomus saranas*

	
Systomus sarana	*Amblypharyngodon mola*
	
Neolissochilus hexagonolepis	*Garra gotyla*

	
Notopterus chitala	*Channa punctata*
	
Wallago attu	*Mystus vittatus*
	
Mystus cavasius	*Rita rita*

	
Mystus tengara	*Wallago attu*
	
Bagarius bagarius	*Gagata cenia*
	
Channa gachua	*Channa punctata*
	
Channa striata	*Channa gachua*
	
Botia rostrata	*Chanda nama*
	
Gudusia chapra	*Allia coilia*

	
Cirrihinus reba	

Annexure V

LIST OF THE INDUSTRIES

Nature of Industrial activities	**Numbers**	**Type of pollutant**
Construction	4	Contraction waste,
Gasoline/fuel	2	Waste oil
Iron/alloy	29	Hot metal pre-treated sludge, dust and debris
Coke/drinks	2	Plastic bottle
Mining	5	Slurry, tailings, rock and other discards
Plastic	3	Microplastic pollution, other plastic waste
Food Processing	4	Different type of solid and liquid waste
Wire	1	Stainless steel waste wire
Cement	4	Waste oil, solvents, plastic, ETP sludge, other sludge
Hospitals	1	Waste containing infectious material
Paper	1	Black liquor, woody residue, fly ash
Cosmetics	1	Packaging materials
Liquor	1	BOD, COD, TDS, Potassium and sulphate
Plywood	3	
Agarbatti	1	
Carbide chemical	1	Toxic gasses like phenol

Annexure VI

B-4921
[1-8]

RESEARCH ARTICLE

Indian Journal of Animal Research

Fish Diversity, Conservation Status and its Relationships with Environmental Variables in Umtrew River System, Northeast, India

L.P. Mudoi, H. Pokhrel, S.K. Bhagabati, R. Dutta, A.M. Ahmed, R. Sarmah, D. Nath

ABSTRACT

Background: River water and its faunal diversity are regarded as an integral part of the environmental stability and river ecosystem. To know the abundance and diversity of fin fishes in relation to their environmental parameters, a hilly river Umtrew in Meghalaya and Assam was investigated during January, 2019 to December, 2020.

Methods: Fish, plankton and water samples were collected regularly at monthly intervals for a period of 24 months. Standard protocols were followed for analysis of the collected samples.

Result: A total of 49 fish species under 36 genera, 20 families and 10 orders were recorded. Among them Cypriniformes (42%) is the dominated order followed by Siluriformes (24%). As per IUCN status 1 species falls under critically endangered, 4 species are near threatened, 2 species under vulnerable and 42 species are of least concern. A significant correlation between species distribution and environmental variables was also reported. Our findings clearly oppose the tendency for species composition to increase from the source to the mouth of the river, which is probably prohibited by two major anthropogenic activities. These activities could constitute in the future a real threat for the fish population and other aquatic organisms.

Key words: Diversity, Environmental variables, Palmer, Pollution, Threatened.

INTRODUCTION

Protection of the natural environment is one of the prime focus for preservation and conservation of living species. Unfortunately, throughout the World and in India in particular, aquatic ecosystems are continuously altered by human activities (Ahmed *et al.*, 2013). This alteration is thought to play an important role in fish community structures and in other aquatic organisms (Resh *et al.*, 1988; Poff and Ward, 1989) and may be responsible for extinction of numerous species.

Hilly river ecosystem and biodiversity is very important due to the source of origin of different colourful fin fishes which live in diversified habitats consisting of clay, sand, rock, gravel, stone and boulders. Habitat characteristics are correlated with presence and absence of fishes (Gorman and Karr, 1978). Again, water qualities of a river influence the fish diversity (Kow *et al.* 2016).

Among such Umtrew river system is one of the major river systems in Meghalaya and Assam, India, which supports a diverse range of flora and fauna making the river a valuable resource for the region. However, the river is facing tremendous pressure through many anthropogenic factors like construction of dams and constant dumping of municipal and industrial waste generated by industries (Pranjit *et al.*, 2012). Thus, looking into the pathetic condition and with a hope for restoration of the river natural ecosystem present study was undertaken to evaluate the fish diversity and abundance in relationship to its environmental variables. This study might also be helpful to the researchers, fish managers and policy makers to understand the water quality and ichthyofaunal status of hill streams for its better management to maintain and sustain the running water ecosystem and its resources.

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MATERIALS AND METHODS

Study area

The river Umtrew is originated by the confluence of two streams, one of which originates near Mawrong hamlet on the Sohpetbneng Peak and the other one is the Umium dam's outflow (Fig 1). These two streams meet at the Nongkhyllem Wildlife Sanctuary and run through Byrnih, a small town of Meghalaya. The river then enters into the state Assam at Sonapur, where it is known as Digaru until merging with the Brahmaputra near Chandrapur, Kamrup of Assam. River. The Digaru river covers a distance of about 30 km

Annexure VI

Ministry of Environment, Forest & Climate Change

Implemented by giz Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH

On behalf of: Federal Ministry for the Environment, Nature Conservation and Nuclear Safety of the Federal Republic of Germany

Zoological Survey of India

Participation Certificate

First International Symposium on Aquatic Biodiversity of the North Eastern Region of India

Presented to:

Lawonu Prasad Mudoi

for their presentation on the study

Estimating Pollution Load From Continuous Discharge and Monthly Flow in Umtrew River

Authors: Rajdeep, D., Sarada, K.B, and Lawonu Prasad Mudoi

at the First International Symposium on Aquatic Biodiversity of the North Eastern Region of India held on October 26th and 27th 2021. We thank you for your contribution towards conservation of Aquatic Biodiversity in the North Eastern Region of India.

Dr Uwe Scholz, Project In-Charge, GIZ NERAQ Project

Prof. Dandadhar Sarma, Department of Zoology, Gauhati University

Dr Dhriti Banerjee, Director, Zoological Survey of India

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Project ID: HSF2017-18/I-16/04

FINAL TECHNICAL REPORT

OF THE PROJECT

ON

"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

Submitted to:
Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies (NMHS)
G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal, Almora 263643, Uttarakhand

Submitted by:

Dr. Sarada Kanta Bhagabati
Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103

Template/Pro forma for Submission

NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	**HSF2017-18/I-16/04**

Date of Submission:								
	d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

PROJECT TITLE
"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

Sanctioned Fellowship Duration: from (28/03/2018) to (28/02/2021)

Extended Fellowship Duration: from (1/03/2021) to (31/12/2021)

***Submitted to*:**

Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies, GBP NIHE HQs
Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi
E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

***Submitted by*:**

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NMHS-Final Technical Report (FTR)

NMHS- Institutional Himalayan Fellowship Grant

DSL: Date of Sanction Letter

2	8	0	3	2	0	1	8
d	d	m	m	y	y	y	y

DFC: Date of Fellowship Completion

3	1	1	2	2	0	2	1
d	d	m	m	y	y	y	y

Part A: <u>CUMULATIVE SUMMARY REPORT</u>

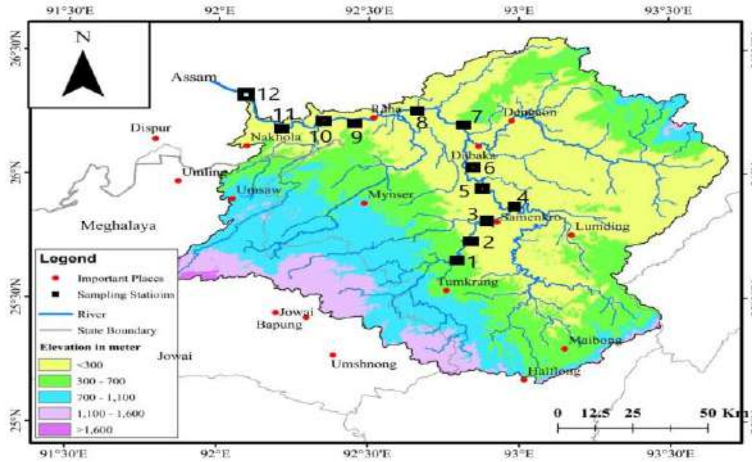
1. Details Associateship/Fellowships

1.1 Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	HSF2017-18/I-16/04
Name of the Institution/ University:	College of Fisheries, Assam Agriculture University
Name of the Coordinating PI:	1. Dr. Rajdeep Dutta Assistant Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103 2. Dr. S.K. Bhagabati, Associate Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103
Point of Contacts (Contact Details, Ph. No., E-mail):	1. Email ID: drrajdeepdutta@gmail.com : sskbk2002@gmail.com 2. Ph No: 9854757790 & 7896250516

1.2 Research Title and Area Details

i.	Institutional Fellowship Title:	Study of Ecological Status, Fish Diversity and Plankton Diversity of River Kopili, North-Eastern Himalayan Region, Assam.					
ii.	IHR State(s) in which Fellowship was implemented:	**Assam**					
iv.	Scale of Fellowship Operation	Local:		Regional:	Yes	Pan-Himalayan:	

iii.	Study Sites covered	Assam  Map of the Study Area
v.	Total Budget Outlay (Crore):	INR 0.8034840 Cr

1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration	
		From	To
Research Associates			
Sr. Research Fellow			
Jr. Research Fellows	2	01/08/2018	31/12/2021
Project Fellows			

2. Research Outcomes

2.1. Abstract

- ***Background***: The Eastern Himalayan region encompassing the Northeast India is considered as one of the hotspots of freshwater fish diversity in the world. Among North East states, Assam is also very rich in its ichthyofaunal diversity. Bhattacharjya *et al*., 2003 reported a total of 217 fish species belonging to 104 genera, 37 families and 10 orders from wetlands and other waterbodies of Assam. But in recent times, due to many anthropogenic factors the precious and unique indigenous ichthyofauna of Assam are facing a great threat. Keeping all these aspects in mind, through this NMHS sponsored medium grant project an attempt has been made to study ichthyofaunal diversity as well as ecosystem integrity of Kopili river, one of the most important South bank tributaries of mighty Brahmaputra River.

- ***Aims:***

1. Conservation of indigenous and endemic ichthyofauna of North East Himalaya
2. Pollution status and hydrobiological status of the river.

- ***Objectives:***

1. To study the ecological status of river in terms of physico-chemical characters of water and sediment.
2. To study fish and plankton diversity of the river.
3. To study about pollution status of the river.
4. To identify anthropogenic factors threatening the fish diversity of the river and to find out mitigation measures.

- **Methodologies:**

Objective 1: Water and sediment samples were collected from 12 different stations of river Kopili from January, 2018 to May, 2021. Some of the physical parameters like depth, air & surface water temperature, water velocity, TDS & EC were determined on the spot. Other parameters like Turbidity, Dissolved oxygen, pH, Total alkalinity, Total hardness, Nitrate, Nitrite, Ammonia, Soluble Inorganic Phosphate of the water samples were carried out in the laboratory as per APHA (2018). The sediment samples were collected on seasonal interval, air dried and analyzed for pH, organic matter, organic carbon, nitrogen, potassium and phosphorus as per standard methodology (Jhingran, 1992; Walky & Black, 1934).

Objective 2: Fish samples were collected from both the rivers of 12 different stations of river Kopili on monthly intervals and length and weight of the fish species were recorded. Photography of the fish specimens and their habitat were done. The fish samples were preserved and brought to the laboratory in 10% formalin. The fishes were identified using standard keys (Jayaram, 2006; Vishwanath & Nebeshwar, 2009; Kottelat, 2013). Plankton and periphyton samples were identified with the help of standard literatures Edmondson (1959), Needham & Needham (1966) and ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967).

DNA Barcoding

Pectoral fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping was isolated following phenol: chloroform method. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking its integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendorf AG 22331 Hamburg). The PCR product is then sequenced at Eurofin Scientific Laboratory. The generated barcodes were submitted to NCBI and accession number were obtained for the individual fish species.

Objective 3: To study the pollution status of the river, water samples from the 12 stations was collected on monthly interval and pollution status of the river was assessed in terms of Biochemical oxygen $Demand_3$ (BOD_3), Chemical Oxygen Demand (COD) and fecal coliform count using standard protocol. Palmer's pollution index was also assessed.

Objective 4: Based on the collected primary and secondary data during the study period the anthropogenic factors was determined.

Results:

Objective 1: Analysis of seasonal variation data of hydrobiological parameters of River Kopili reveal anthropogenic stress in middle and lower stretches. Parameters like BOD_3 and COD crossed the permissible limit indicating probable pollution load. Water pH of Kopili river was found to be highly acidic during 2019 which might be due to the impact of acid mine drainage from NC hill districts; but after 2019 the water become alkaline in nature, which might be due to the ban imposed by NGT on rat hole mining in the NC hills. Analysis of seasonal variation of Kopili river shows sediment pH acidic to alkaline in nature. Other parameters like organic carbon, organic matter, sediment N, P & K shows seasonal variation during the study period.

Objective 2: During the present investigation, a total of 108 fish species belonging to 12 orders, 31 families and 63 genera were recorded from the studied river. DNA barcodes were generated for 59 numbers of fish species from River Kopili, submitted to NCBI and 63 numbers of accession numbers were obtained. Two (2) fish species were recorded for the first time from Brahmaputra drainage during the present study. One number of exotic species (*Cyprinus carpio*) was recorded from Kopili river during the study period. Among the recorded fish species from River Kopili, three species are assessed as endangered (2.78%), 10 are near threatened (9.26%), three are vulnerable (2.78%) and other 90 species are least concerned (83.33%) according to IUCN (2021). One additional species is not evaluated and one species is data deficit.

A total of 46 genera of plankton were recorded from River Kopili during the study period. Population of phytoplankton was represented by 35 genera belonging to Chlorophyceae (17 genera), Bacillariophyceae (10 genera), Cyanophyceae (7 genera) and Euglenophyceae (1genera). Zooplankton population was represented by Rotifera (5 genera), Cladocera (3 genera) and Copepoda (3 genera). The population density of plankton varied from season to season. The average minimum plankton density was found to be 21.33±3.68 units/L and maximum 626.67±13.10 units/L.

Objective 3: It was observed that the values of BOD3, COD & fecal coliform count was in higher side during the monsoon and post monsoon season in all the stations, which might be the indication of pollution threats during those seasons. Palmer's index also showed similar trend. By using Palmer's index of pollution for rating of water samples as lack of organic pollution, moderate and high organic polluted at all the stations were tested. The total score of Agal Genus Pollution Index (AGPI) of the sites S1<S2<S4<S5<S3<S11<S7<S10<S8<S6<S12 were calculated to be 8, 9, 15, 18, 19, 20, 21, 21, 23, 24, 24 and 25 respectively. It was observed that the total score of S1 and S2 showed below 10 which indicates lack of organic pollution. Sharpe increase in total score of 18 in station 4 indicating high organic pollution due to tourist influx according to Palmer (1969). *Navicula*, *Nitzcha* and *Synedra* were recorded repeatedly in lower stations of Kopili river and consider as indicators of pollution in view of results of Palmer's index.

Objective 4: Different anthropogenic factors like construction of hydro-electric dam, continuous sand mining, construction of bridge pillars, bathing, washing cloths etc were recorded during the sampling period.

Conclusion: The river water in upper stretch i.e., in NC hilly region was found to be highly acidic in nature during 2018-19, due to which no fish species was recorded at that time in that stretch. But, after the ban imposed by NGT on rat hole mining the pH of water become alkaline in nature since 2020. Due to which many cold-water fishes are obtained from that region. A total of 108 fish species was recorded during the study period, which include *Pethia stoliczkanus* & *Sistura khugae* which are reported for the first time from Brahmaputra drainage. Ecosystem integrity of the river is threatened by anthropogenic activities in middle and lower stretches.

Recommended: Habitat destruction activities in rivers of should be strictly prohibited. *In-situ* conservation of commercially important as well as indigenous fish species should be implemented.

2.2. Objective-wise Major Achievements

S. No.	Objectives	Major achievements (in bullets points)
1.	To study the ecological status of river in terms of physico-chemical characters of water and sediment.	• To study the ecological status of the river, water and sediment samples were collected from 12 different stations starting from its origin upto the confluence point. • Water & sediment pH was found to be highly acidic during 2019. • Turbidity, electrical conductivity, total dissolved solids were found to be higher during monsoon and post-monsoon seasons. • DO, total alkalinity and total hardness was found to be higher during winter season. • During the study period it was observed that the values of total alkalinity was quite low (12.67-76.00 mg/L) than the recemented values of total alkalinity for fishes. • Research paper: Published 1

2.	To study fish and plankton diversity of the river.	• 108 fish species belonging to 12 orders, 31 families and 63 genera were recorded from the studied river. Cyprinidae was the most dominant family comprising of 42 species, followed by Bagridae (8), Sissoridae (6), Channidae (5), Ambasidae (4), Siluridae (4) species. Each of Mastacembelidae, Schilbeidae, Osphronemidae, Nemacheilidae, Botiidae contained three species whereas families viz. Psilorhynchidae, Cobitidae, Notopteridae, Badidae contained 2 species each. On the other hand, rest of the families contained single species. • DNA barcodes generated: 59 fish species from River Kopili. • Conservation status: - endangered (2.78%), near threatened (9.26%), vulnerable (2.78%) and least concerned (83.33%) according to IUCN (2021). One additional species is not evaluated and one species is data deficit. • Two (2) fish species were recorded for the first time from Brahmaputra drainage during the present study. • Research paper: Published 1

3.	To study about pollution status of the river.	• The values of BOD_3, COD and fecal coliform count were in higher side during the monsoon and post monsoon season in all the stations, which might be the indication of pollution threats during those seasons. • Palmer's index also showed similar trend. The total score of Agal Genus Pollution Index (AGPI) of the sites S1<S2<S4<S5<S3<S11<S7<S10<S8<S6<S12 were calculated to be 8, 9, 15, 18, 19, 20, 21, 21, 23, 24, 24 and 25 respectively. • It was observed that the total score of S1 and S2 showed below 10 which indicates lack of organic pollution. • Sharpe increase in total score of 18 in station 4 indicating high organic pollution due to tourist influx according to Palmer (1969). *Navicula*, *Nitzcha* and *Synedra* were recorded repeatedly in lower stations of Kopili river and consider as indicators of pollution in view of results of Palmer's index.
4	To identify anthropogenic factors threatening the fish diversity of the river and to find out mitigation measures.	• Anthropogenic factors encountered during the regular sampling in the Kopili river system are: ➢ Hydro-electric dam: 2 nos ➢ Acid mine drainage ➢ Sand mining, ➢ Construction of bridge, ➢ Bricks industry near river side, ➢ Turbidity ➢ Washing cloths and bathing

2.3. Outputs in terms of Quantifiable Deliverables*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved
1.	First-hand information on environmental health of the selected river will be	Dataset of the ecological status of Kopili River.	Dataset on environmental health of the river: 1 GIS Map: 1

	generated which will act as an important baseline information for future climate change related studies.		
2.	An updated biodiversity status of the river ecosystem	Taxonomic and molecular characterisation of fish fauna of the river covering its diversity, distribution,	Checklist of Fish species (New database): 1 Museum specimens: 108 DNA barcodes: 59
3.	Information on trophic level structure of the river ecosystem		We gathered all the available information regarding the feeding habits of 108 collected fish species belonging to 12 orders, 31 families and 63 genera. Based on the individual food items trophic level structure of Kopili river was determined. The trophic level of the river ranges from 2.0±0.00 to 4.5±0.80. The trophic level was dominated by mid-level carnivore (39.81%) followed by high-level carnivore (25%), omnivores (23.15%) and herbivores (12.03%).
4	Identification of anthropogenic stress factors affecting the river ecosystem (if any) and its possible mitigation measures.	Any kind of anthropogenic factors affecting fish and their habitat are being constantly monitored.	•Anthropogenic factors encountered during the regular sampling in the Kopili river system are: • Hydro-electric dam: 2 nos • Acid Mine Drainage • Sand mining, • Dam construction • Acid mine drainage • Construction of bridge,

			• Bricks industry ear river side, • Washing cloths and bathing

(*) As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number/ Brief Details	Remarks/ Attachment
1.	New Methodology developed	-	-
2.	New Models/ Process/ Strategy developed	-	-
3.	New Species identified	-	-
4.	New Database established	6	• Total number of fish fauna • Conservation status of fish • Plankton data (Phyto and Zooplankton) • Palmer's index • 15 physico-chemical water quality data. • 6 chemical sediment quality data
5.	New Patent, if any	-	-
	I. Filed (Indian/ International)	-	-
	II. Granted (Indian/ International)	-	-
	III. Technology Transfer (if any)	-	-
6.	Others (if any) DNA barcoding of fish species	59	Species specific DNA barcodes of 59 fish species from River Kopili was generated, submitted to NCBI and accession number obtained for the first time.

3. Technological Intervention

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology		
2.	Diffusion of High-end Technology in the region		
3.	Induction of New Technology in the region		
4.	Publication of Technological / Process Manuals		

4. New Data Generated over the Baseline Data

S. No.	New Data Details	Status of Existing Baseline	Additionality and Utilisation New data
1.	Morphological identification & molecular characterisation of fish fauna of River Kopili	No earlier record of fish fauna from River Kopili is available	We have recorded 108 fish species and generated mitogenome sequences for 59 species from River Kopili for the first time. One exotic fish species was recorded from River Kopili during our study. During the present study, two (2) fish species identified from River Kopili are the first records of their occurrence from Brahmaputra drainage.
2.	Seasonal variation of hydrobiological & parameters	No earlier report on hydrobiological study of River Kopili is available	The new data will be helpful in understanding the impact anthropogenic factors on ecosystem integrity of the river. It will be also helpful in devising future fisheries development strategies in this river.
3.	Sediment characteristic of River Kopili	No earlier report on sediment characteristic of River Kopili is available	The new information will be helpful for future researchers working in this region

4.	Plankton diversity	No report earlier	
5.	Diversity indices of plankton	No report earlier	
6.	Palmer index has been developed for the said river system	No report earlier	

5. **Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations**

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Beneficiaries
1.	Sustainable Development Goals (SDGs)			
2.	Climate Change/INDC targets			
3.	International Commitments			
4.	National Policies			
5.	Other's collaborations			

6. **Financial Summary (Cumulative)***

*Please attach the **consolidated and audited Utilization Certificate (UC) and Consolidated and *Year-wise* Statement of Expenditure (SE)** separately, *ref*. **Appendix I.**

7. **Quantification of Overall Research Progress**

S. No.	Parameters	Total (Numeric)	Attachments* with remarks
1.	IHR State(s) Covered:	1	
2.	Fellowship Site/ LTEM Plots developed:	12	Photographs of sampling sites and map of study area attached (Annexure- I & II)
3.	New Methods/ Model Developed:		
4.	New Database generated:		
5.	Types of Databases generated:		
6.	No. of Species Collected:	108(DNA barcodes of 59 fish species submitted and accession number received	Annexure- III
7.	New Species identified:		
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	JRF:02 PhD:01	
9.	No. of SC Himalayan Researchers benefited:		

10.	No. of ST Himalayan Researchers benefited:		
11.	No. of Women Himalayan Researchers empowered:		
12.	No. of Knowledge Products developed:		
13.	No. of Workshops participated:		
14.	No. of Trainings participated:		
15.	Technical/ Training Manuals prepared:		
	Others (if any):		

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)		1*		Annexure-IV
2.	Book Chapter(s)/ Books:				
3.	Technical Reports/ Popular Articles				
4.	Training Manual (Skill Development/ Capacity Building)				
5.	Papers presented in Conferences/ Seminars				
6.	Policy Drafts (if any)				

* 2 Research papers are communicated and under peer review.

9. Recommendation on Utility of Research Findings, Replicability and Exit Strategy

9.1 Utility of the Fellowship Findings

S. No.	Research Questions Addressed	Succinct Answers (within 150–200 words)
1.	How is the pollution status of the river under study?	The upper stretch of Kopili river was found to be polluted during 2019; but after 2019 the river became unpolluted. The lower stretch is found to be moderately polluted as BOD_3 and COD values found to be in higher limit than recommended level.
2	How many fish species are found in the River Kopili?	During the present investigation 108 fish species were recorded.
3	How is the trophic level structure of the river ecosystem?	The trophic level of the river dominated by mid-level carnivores (39.81%) followed by high level carnivores (25%), omnivores (23.15%) and herbivores (12.03%). Annexure- XI
4	What are the anthropogenic factors that are affecting the river ecosystem and how?	Coal mining, dams, sand mining, bridge piers are the anthropogenic factors recorded during the study period.

9.2 Recommendations on Replicability and Exit Strategy:

Particulars	Recommendations
Replicability of Fellowship, if any	
Exit Strategy:	➢ The water of Kopili river during 2018-19 was found to be highly acidic in nature and after continuous netting also fish could not be retrieved from Karbi-Anglong region. The reason of highly acidic condition may be the coal mining activities in the NC hills. After October 2019, when pipeline of hydro-electric dam busted the river water started changing from acidic to alkaline condition and fish species could be retrieved. ➢ Previous reports reported that there is no fish species present in Kopili river, but in this report a total of 108 fish species which include cold water fish species from NC hilly areas. So, conservation efforts of indigenous ichthyofauna of the study river should be considered. ➢ Sand mining activities from river bed should be totally prohibited in order to conserve the microhabitat requirement of hill stream fishes. ➢ State fishery laws prohibiting fishing during breeding season, use of destructive fishing gears etc. should be strictly followed.

(S. K. Bhagabati)

(NMHS FELLOWSHIP COORDINATOR)

(B. Kalita)

(HEAD OF THE INSTITUTION)

Place: ………………..
Date: …../……/……..

PART B: COMPREHENSIVE REPORT

EXECUTIVE SUMMARY

Fellowship Report No.:

Researchers Details

Type of Fellowship (HRA/HJRF/HJPF)	Name of Himalayan Researcher	Date of Joining	Date of Resignation**	Research Title	Name of the PI & Designation
HJRF	Nilangana Kalita	01/08/2018	28/02/2019		Dr. R. Dutta
HJRF	Dipanka Nath	25/10/2019		Ecosystem integrity & fish diversity of River Kopili	Dr. R. Dutta

1 INTRODUCTION

1.1 Background/ Summary of the Associateship / Fellowship Study undertaken

The river is a lotic ecosystem flowing under the influence of gravity and confluence into the sea and some into lakes. Rivers are important pathways for the flow of energy, matter and organisms through the landscape (Kagalou *et al*., 2002). Rivers also play a major role in assimilation or transportation of the municipal and industrial wastewater discharges continuously or occasionally or seasonally. Most of the ancient civilizations grew along the banks of the rivers. At present, most of the industries agricultural land, populated cities and towns can be found near bank of the rivers. River, a symbol of India's age-old cultural heritage and civilization, occupies a unique position in the ethos of Indian people. There are 15 major, 45 medium and more than 102 minor rivers in India with a total length of 45,000 km covering a catchment area of 3.12 million km2. Among the major river system, the Brahmaputra is the second largest river of India traversing 900 km in the country (Handbook of Fisheries and Aquaculture). In India, river systems are traditionally classified, according to their origin - into Himalayan and Peninsular rivers, or according to the direction of flow-into East flowing and West flowing rivers (NCIWRDP 1999; Amarasinghe *et al*., 2005).

The North-Eastern part of India is rich in riverine resources with a total length of 19,150 km. Riverine fisheries plays an important role in the region in terms of providing livelihood and nutritional security to many fisherfolks. The state of Assam alone has 4820 km stretch of riverine resources (approx. 2,05,000 ha) mainly contributed by two main rivers basin Brahmaputra and Barak basins along with their 53 tributaries. A few reports are available on hydrobiology and fisheries of Brahmaputra and Barak basin (Jhingran, 1991; Biswas, 1998; Biswas, Baruah, 2000 & Baruah & Biswas, 2002; Bailung & Biswas 2018). Different factors relating to decline of fisheries in the Brahmaputra River basin have been discussed by Yadava and Sugunan (1992). A few assessments of surface water quality of river

Brahmaputra were conducted by Saikia and Gupta (2012). Although many studies were carried out on Brahmaputra and Barak River basins, but works relating to water quality assessment on both the river basins are very scanty, especially on Brahmaputra River which is regarded as the lifeline for Assam. Irresponsible/ destructive fishing techniques, water pollution, habitat degradation is identified as some of the key threats to the indigenous fish germplasm of the state. Many indigenous fish species of the state are rapidly entering into the categories of Vulnerable, Endangered, Threatened due to these threats. Keeping all these aspects in view, through this NMHS sponsored project an attempt has been made to study ichthyofaunal diversity as well as ecosystem integrity of Kopili river.

1.2 Baseline and Scope of the Associateship / Fellowship

During this NMHS project first-hand information on fish fauna of River Kopili was generated. Species specific DNA barcodes were for fish fauna of River Kopili during the project for the first time. Morphological identification of the indigenous fish fauna of the river supported by molecular characterization will provide a complete dataset on ichthyofaunal diversity. The project has investigated the seasonal variation of physico-chemical water & sediment quality parameters, plankton composition of River Kopili from January 2019 to May 2021. The updated new data will be helpful in understanding anthropogenic stress factors affecting ecosystem integrity of the river. This information will be very helpful in planning future fisheries development strategies of this river.

1.3 Overview of the Major Issues to be addressed

Some of the major issues addressed through this project are:

i) **Construction of dams:** Dams can impact fish biodiversity, fish stocks and fisheries indirectly by modifying and/or degrading upstream and downstream aquatic environments, including: thermal stratification; downstream flow alteration; release f trapped sediments from reservoir to the river etc. There are two dams present on Kopili river of which one dam is under construction. Hazardous situation was occurred during November, 2019 when pipeline of dam busted a havoc situation was created; which damaged not only the river ecosystem but also its riparian zone.

ii) **Acid mine Drainage:** The rivers run reddish due to a phenomenon called Acid Mine Drainage (AMD), caused by active and abandoned mines, coal storage sites and overburdened rocks. Leaching of heavy metals and the washing down of the soil removed to reach the coal seams add to the pollution in the rivers. Due to this AMD the river water of Kopili became highly acidic during 2019-19; due to which not a single fish species was retrieved from NC hill areas during that period.

iii) **Water pollution:** Pollution of river water also affecting the indigenous ichthyofauna. Agricultural chemicals, industrial effluents, untreated sewage etc. are affecting the riverine water quality which ultimately affecting the fish fauna.

iv) **Habitat degradation:** Hill stream fishes require special conditions for their growth and survival. These fishes are specially adapted to utilize the unique hill stream environment. Fast flowing stream water current and presence of sand, pebbles, cobbles, rocks, boulders etc. in the stream bed are indispensable for growth, survival and reproduction of these fishes. But mining activities in the river beds are greatly affecting the habitat of these fishes. Sand mining of river beds destroyed the habitat of these fishes which lead to extinction of some of these precious ichthyofauna.

v) **Public unawareness:** The civil society of the sate quite are unaware about the importance of conservation of indigenous ichthyofauna. Due to their ignorance about the value of biodiversity of indigenous fish germplasm, precious fish fauna from different waterbodies of the state are facing different forms of anthropogenic threats.

vi) **Unavailability of alternative options:** One of the best ways of conservation of indigenous fish fauna of natural aquatic ecosystems is promotion of aquaculture to reduce sole dependency of fish on these natural resources and thereby providing the fisherfolks with alternative fish centric livelihood options. But unfortunately, the local tribal people are not aware about scientific fish farming practices. So, they are very much dependent on fishing in rivers/streams for their food fishes.

2 METHODOLOGIES, STARTEGY AND APPROACH

2.1 Methodologies used for the study

i) **Methodology used for achieving Objective 1:** Twelve (12) stations were selected covering the entire stretch of the river based on elevation. Water & sediment samples were collected from these stations during different seasons of the year. Samples were collected between 10-11 am. Some of the physical parameters like depth, air & water temperature, water velocity, pH, conductivity, TDS etc. were determined *in-situ*. pH, conductivity, TDS of the river water were measured *in-situ* using a digital soil & water testing kit (Systronics India Limited/371). Other parameters like DO, alkalinity, hardness, BOD_3, COD, nitrate, nitrite, total ammonia, soluble inorganic phosphate of the water samples were carried out in the laboratory as per APHA (2018) and CPCB (2001). Soil samples were collected quarterly by Ekman's dredge separately from three sampling station for the estimation of different soil parameters (Jackson, 1973). Then the samples were dried in room temperature and pulverized to a fine size and sieved through a standard sieve and it was used for estimation of pH, organic carbon, organic matter and available soil N, P and K in the laboratory. Sediment parameters like sediment pH, sediment organic carbon, sediment organic matter, and sediment N, P and K were estimated quarterly adopting standard procedures (Jhingran, 1992; Walky & Black, 1934).

ii) **Methodology used for achieving Objective 2:** Twelve (12) stations were selected covering the entire stretch of the river based on elevation and fish specimens were collected during different seasons of the year. Photography of the fish specimens and their habitat were done. The morphometric measurements were recorded. The fish samples were preserved and brought to the laboratory in 10% formalin for further analysis. The fishes were identified using standard keys (Jayaram, 2006; Vishwanath & Nebeshwar, 2009; Kottelat, 2013). Species specific DNA barcodes of the fish species were generated as per the standard methodology of Ward *et al*. (2005). Pectoral fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping was isolated following phenol: chloroform method. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking its integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg). The PCR product is then sequenced at Eurofin Scientific Laboratory. The generated barcodes were submitted to NCBI and accession number were obtained for the individual fish species.

Plankton samples were collected in duplicate by filtering 100-200 liters of river water using 28 mm mesh nylobolt plankton net as described by Santhanam *et al.* (1987). The collected plankton samples were preserved in 3-4 % formalin in separate plankton tubes. In laboratory, from the known volume plankton sample counting was done by using Sedgwick Rafter Plankton counting cell (Sharma and Saini, 2005). Plankton were identified at genera level using the identifying keys of Edmondson (1959), Needham & Needham (1966) and ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967). Plankton biomass in terms of density was determined using plankton density (Units/L) a Sedgwick Rafter Cell as per the methodology of Sharma and Saini (2005).

iii) **Methodology used for achieving Objective 3:** Water pollution studies of the river was carried out in terms of BOD_3, COD and fecal coliform count as per CPCB Guide Manual: Water and Waste Water Analysis (2011).

iv) **Methodology used for achieving Objective 4:** Based on the results obtained from objective 1, 2 & 3 anthropogenic factors affecting the river ecosystem was determined and mitigation measures was suggested.

2.2 Details of Scientific data collected and Equipments Used

a. Air & water temperatures were measured using a mercury thermometer.
b. Water velocity was measured using a current meter.
c. Parameters like pH, conductivity, TDS of the river water were measured *in-situ* using a digital soil & water testing kit (Systronics India Limited/371).
d. DO, Alkalinity & Hardness values were estimated by Titration method.
e. BOD bottles were incubated in BOD incubators.

f. For estimation of COD, water samples were digested in a KEL PLUS Automatic COD digestion system/ KES 08 L CAC.

g. Parameters like Nitrate, nitrite, total ammonia and soluble inorganic phosphate were determined using uv-visible spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202).

h. Available nitrogen was estimated by alkaline potassium paramagnet method in kjeldhal flask.

i. Available Sediment Phosphorus was estimated in spectrophotometer.

j. The available potassium was estimated by flame photometer.

k. Latitude & longitude of the stations were recorded using a GPS instrument.

l. Photography of the fish specimens and stations were done using a digital camera.

m. The morphometric measurements & weight of the collected fish specimens were recorded using a vernier calliper and a pan balance respectively.

n. DNA isolation from pectoral fin clippings of the fishes was done using Phenol-Chloroform method.

o. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306).

p. Integrity of DNA samples were checked using an Electrophoresis system (Biorad)

q. Amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg).

r. Plankton samples were collected using a plankton net.

s. Plankton & periphyton samples were observed under a Microscope.

t. Quantitative analysis of plankton was done using Sedgwick Rafter Cell.

2.3 Primary Data Collected

i. The morphometric measurements & weight of the collected fish
ii. Latitude & longitude of the study stations of both the rivers
iii. Museum fish specimens
iv. Air & water temperatures
v. Water velocity
vi. Water pH
vii. Dissolved oxygen concentration of river water
viii. Conductivity of river water
ix. TDS of river water
x. Total Alkalinity of river water
xi. Total Hardness of river water
xii. Biological Oxygen Demand$_3$ (BOD_3) of the river water
xiii. Chemical Oxygen Demand (COD) of the river water
xiv. Nitrogen-nitrate

xv. Nitrogen-nitrite
xvi. Total ammonia
xvii. Soluble inorganic phosphate
xviii. Sediment pH
xix. Sediment organic matter
xx. Sediment organic carbon
xxi. Sediment available nitrogen
xxii. Sediment available potassium
xxiii. Sediment available phosphorus
xxiv. Plankton biomass

2.4 Details of Field Survey arranged

Regular field survey the study river was conducted during the entire duration of the project for collection of fish specimens, water samples, sediment samples, plankton & periphyton samples. During those surveys, primary and secondary data were also collected pertaining to the objectives of the project.

2.5 Strategic Planning for each Activities

- **Ecosystem Integrity Study:** Water, sediment, plankton and periphyton samples were collected from 12 different stations covering the whole stretch of Kopili river during different seasons of the year. While selecting the stations it was ensured that every station represents different elevations. Water and plankton samples were collected from each station on monthly intervals while sediment samples were collected seasonally.
- **Fish Biodiversity Study:** Twelve (12) stations were selected covering the entire stretch of the river. Fish samples were collected using gill net and cast net with the help of local people. Sometimes survey was also conducted on local fish market.

2.6 Activity-wise Timeframe followed using Gantt/ PERT Chart

Activities	Months																									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Etc.**				**18**				**24**		**27**		**35**	**36**
Recruitment of Project Staff	■																									
Preparation & Procurements	■	■																								
Initial survey			■	■																						
Assessment of Ichthyofaunal diversity				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Evaluation of Environmental Health				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Annual Report												■									■					
Final Report Preparation & Submission																									■	■

3 KEY FINDINGS AND RESULTS

3.1 Major Research Findings

3.1.1: Water Quality & Sediment Parameters of River Kopili:

A total of fifteen (15) water quality parameters and six (6) sediment parameters were tested at 12 different stations by covering the whole stretch of the Kopili river for a period of 29 moths from January, 2019 to May, 2021.

Data on seasonal variation of water quality parameters of River Kopili from January, 2019 till May, 2021 is depicted on Annexure - V. Data on seasonal variation of sediment quality parameters of River Kopili from January, 2019 till May, 2021 is depicted on Annexure - VI.

Comparison of water quality parameters of the study rivers with congenial values for fishes:

Sl. No	Parameter	Result	Congenial Limit	Remark
1.	Surface Water Temperature (^{0}C)	18.33-31.50		Suitable for both cold and warm water fishes.
2.	Turbidity (NTU)	0.40-115.18	20-30	Turbidity exceeds permissible limit from station 4-12.
3.	pH	3.50-7.71	7-8.5	Water pH was found to be acidic to alkaline condition during the study period.
4.	Dissolved Oxygen (ppm)	4.17-10.15	>5	Average DO values found to be within acceptable range. But during monsoon season values<5 were recorded.
5.	Total Alkalinity (ppm)	12.67-76.00	80-200	Alkalinity values found to be not congenial for fishes
6.	Total Hardness (ppm)	40.31-72.20	75-150	Hardness values found to be not congenial for fishes
7.	Electrical Conductivity (µS/cm)	49.03-211.45	50-1500	Found to be within acceptable range
8.	Total Dissolved Solids (ppm)	42.10-160.35	<400	Found to be within acceptable range
9.	Biochemical Oxygen Demand (ppm)	0.33-19.61	<10	BOD values of station 8-12 were found in higher range than the congenial limit during monsoon indicating anthropogenic stress in these stations
10.	Chemical Oxygen Demand (ppm)	0.53-32.82	<20	COD values of station 8-12 were found in higher range than the congenial limit during monsoon indicating anthropogenic stress in these stations
11.	Nitrate-nitrogen (ppm)	0.016-0.637	0.10-3.00	Found to be within acceptable range
12.	Nitrite Nitrogen (µg/L)	0.0016-0.064	0-0.50	Found to be within acceptable range
13.	Soluble Inorganic Phosphate (ppm)	1.21-3.31	0.05-0.4	Found to be more than acceptable range
14.	Total Ammonia (ppm)	0.155-2.285	0-1.0	Found to be more than acceptable range

3.1.2 Ichthyofaunal Diversity of Kopili River:

This project is bringing out first ever information on ichthyofauna of River Kopili. During the present investigation, a total of 108 fish species belonging to 12 orders, 31 families and 63 genera were recorded from the studied river. Order wise composition shows dominance of Cypriniformes (48.15%) followed by Siluriformes (25.00%), Anabantiformes (11.11%), Perciformes (3.70%), Synbranchiformes (3.70%), Clupiformes and Osteoglossiformes comprised of 1.85% each and other order contains 0.93% each. Three species are assessed as endangered (2.78%), 10 are near threatened (9.26%), three are vulnerable (2.78%) and other 90 species are least concerned (83.33%) according to IUCN (2021). One additional species is not evaluated and one species is data deficit.

Cyprinidae was the most dominant family comprising of 42 species, followed by Bagridae (8), Sissoridae (6), Channidae (5), Ambasidae (4), Siluridae (4) species. Each of Mastacembelidae, Schilbeidae, Osphronemidae, Nemacheilidae, Botiidae contained three species whereas families viz. Psilorhynchidae, Cobitidae, Notopteridae, Badidae contained 2 species each. On the other hand, rest of the families contained single species. One (1) fish species: *Badis* sp could be identified only up to genera level from River Kopili. One exotic fish species (*Cyprinus carpio*) recorded from River Kopili during the present study. Photographs of collected and identified fish species shown in Annexure- VII.

3.1.3. Plankton Biomass of River Kopili:

A total of 46 genera of plankton were recorded from River Kopili during the study period. Population of phytoplankton was represented by 35 genera belonging to Chlorophyceae (17 genera), Bacillariophyceae (10 genera), Cyanophyceae (7 genera) and Euglenophyceae (1genera). Zooplankton population was represented by Rotifera (5 genera), Cladocera (3 genera) and Copepoda (3 genera).

The population density of plankton varied from season to season. The average minimum plankton density was found to be 21.33±3.68 units/L in monsoon 2019 in station 1 and maximum in winter 2019-20 in station 12 (626.67±13.10 units/L).

Palmer (1969) first made the list of algae genera and species which indicate organic pollution. According to Palmer, scores of 20 or more are indication of high organic pollution. By using Palmer's index of pollution for rating of water samples as lack of organic pollution, moderate and high organic polluted at all the stations were tested. The total score of Agal Genus Pollution Index (AGPI) of the sites S1<S2<S4<S5<S3<S11<S7<S10<S8<S6<S12 were calculated to be 8, 9, 15, 18, 19, 20, 21, 21, 23, 24, 24 and 25 respectively. It was observed that the total score of S1 and S2 showed below 10 which indicates lack of organic pollution. Sharpe increase in total score of 18 in station 4 indicating high organic pollution due to tourist influx according to Palmer (1969). Navicula, Nitzcha and Synedra were recorded repeatedly in lower stations of Kopili river and consider as indicators of pollution in view of results of Palmer's index.

Plankton biomass of River Kopili is shown in Annexure- VIII.

3.1.4. Anthropogenic factors affecting the river ecosystem:

- Coal mining
- Sand mining
- Hydro-electric Dams

- Sand Mining
- Bridge Piers
- Turbidity

Details of anthropogenic factors are discussed in annexure- IX

3.1.5. Pollution Status of River Kopili is discussed in annexure -X.

3.2 Key Results

- During the present investigation, a total of 108 fish species belonging to 12 orders, 31 families and 63 genera were recorded from the studied river. Cyprinidae was the most dominant family comprising of 42 species, followed by Bagridae (8), Sissoridae (6), Channidae (5), Ambasidae (4), Siluridae (4) species. Each of Mastacembelidae, Schilbeidae, Osphronemidae, Nemacheilidae, Botiidae contained three species whereas families viz. Psilorhynchidae, Cobitidae, Notopteridae, Badidae contained 2 species each. On the other hand, rest of the families contained single species.
- DNA barcodes generated for 59 fish species and 63 NCBI accession no obtained from River Kopili for the first time.
- Three species are assessed as endangered (2.78%), 10 are near threatened (9.26%), three are vulnerable (2.78%) and other 90 species are least concerned (83.33%) according to IUCN (2021). One additional species is not evaluated and one species is data deficit.
- One fish species *Pethia stoliczkanus* was recorded for the first time Brahmaputra drainage during the present study.
- The trophic level of the river dominated by mid-level carnivores (39.81%) followed by high level carnivores (25%), omnivores (23.15%) and herbivores (12.03%).
- Surface water temperature regime of both the rivers is congenial for both hill stream and warm water fishes.
- Turbidity of Kopili river water found to be higher from station 4 and maximum during monsoon season may be due to the surface run-off from catchment areas due to raining.
- pH was found to be acidic in nature during 2018-2019 in NC hilly areas might be due to unregulated rat hole mining happed in NC hills. But after 2019 NGT banned on rat hole mining the

pH of Kopili river water started becoming alkaline in nature. In the lower stretches of Kopili river average water pH found to be congenial for fishes.

- The river showed a characteristics of low alkalinity high hardness condition during the study period.
- Average BOD_3 values of all the other stations than hilly areas of River Kopili found to exceed acceptable limit (<10ppm) indicating anthropogenic stress in these stations.
- Except Station 1, 2, 3 of River Kopili, COD values of all other stations of River Kopili found to exceed acceptable limit (≤20 ppm) indicating probable pollution load in these stations.
- The values of Nitrate, Nitrite, Ammonia and Phosphate was found to be congenial for fishes.
- A total of 46 genera of plankton were recorded from River Kopili during the study period. Population of phytoplankton was represented by 35 genera belonging to Chlorophyceae (17 genera), Bacillariophyceae (10 genera), Cyanophyceae (7 genera) and Euglenophyceae (1genera). Zooplankton population was represented by Rotifera (5 genera), Cladocera (3 genera) and Copepoda (3 genera).
- Different sediment parameters like organic carbon, N, P and K varied seasonally.
- Different anthropogenic factors like hydro-electric dams, sand mining, coal mining, construction of bridge piers, high turbidity etc. were recorded.

3.3 Conclusion of the study undertaken

- The study River Kopili is very rich in indigenous fish germplasm. This river is the habitat of many endangered, vulnerable and near threatened fish species. Therefore, conservation plans should be developed for in-situ conservation of these precious indigenous fish species. In addition, fish species having international acclaim as Sport Fish like Mahseers are recorded from this river. Therefore, certain locations of this river can be promoted as Angling Destinations for amateur Anglers. Further studies are required to understand the biology of indigenous fish species of the river having ornamental value. Hatcheries can be established at suitable places covering all the elevations of the river course to undergo induced breeding of the indigenous fish species particularly Mahseers
- Two most important anthropogenic factors viz. dams and coal mining in NC hill areas creating threats to the ecosystem including bot biotic and abiotic factors of Kopili river. Coal mining reduced after 2019 as NGT banned on illegal rat hole mining due to which river is flourishing in terms of its biotic components including plankton and fish diversity. So, alternative method should be considered for power generation.

- ❖ Ecosystem integrity showed that pollution indicator parameters like BOD_3 and COD was found to the in higher limit than congenial for fisheries point during monsoon and post-monsoon season might be due to the organic load carried by the surfaces run-off from catchment areas as a result of rain.

4 OVERALL ACHIEVEMENTS

4.1 Achievements on Objectives

i. **Objective 1:** To study the ecological status of river in terms of physico-chemical characters of water and sediment.

Achievements:

a) Dataset on physico-chemical parameters of water and sediment is generated of the study.
b) Dataset on physico-chemical parameters of sediment is also generated of Kopili river.
c) GIS Maps of the study river developed.
d) One research paper published.

ii. **Objective 2:** To study fish and plankton diversity of the river.

Achievements:

a) Checklist of fish species of River Kopili (A total number of 108 fish species) successfully generated.
b) Species specific DNA barcodes generated for 59 fish species from River Kopili, submitted to NCBI and 63 accession number obtained.
c) Museum specimens of 108 fish species from these rivers are maintained at NMHS Fish Museum, Dept. of AEM, College of Fisheries, AAU, Raha.

d) Conservation status of indigenous fish species of both the study rivers presented as per IUCN (2021) guidelines.

e) One fish species (*Pethia stoliczkanus*) reported for the first time from Kopili river, Brahmaputra River drainage.

f) A total of 46 genera of plankton were recorded from River Kopili during the study period. Population of phytoplankton was represented by 35 genera belonging to Chlorophyceae (17 genera), Bacillariophyceae (10 genera), Cyanophyceae (7 genera) and Euglenophyceae (1genera). Zooplankton population was represented by Rotifera (5 genera), Cladocera (3 genera) and Copepoda (3 genera).

g) Palmer's pollution index is also developed.

iii. **Objective 3:** To study about pollution status of the river.

Achievements:

a) Pollution status of Kopili river was studied based on the parameters viz BOD & COD.

iv. Objective 4: To identify anthropogenic factors threatening the fish diversity of the river and to find out mitigation measures**.**

Achievements:

a) Different anthropogenic factors like sand mining, coal mining, dams, constructions of bridge piers, turbidity, washing clothes, taking bath etc. were recorded during the study period and their mitigation measures are discussed

4.2 Establishing New Database/Appending new data over the Baseline Data

- This project is bringing out first ever information on ichthyofauna of River Kopili. During the present investigation, a total of 108 fish species belonging to 12 orders, 31 families and 63 genera were recorded from the studied river. Three species are assessed as endangered (2.78%), 10 are near threatened (9.26%), three are vulnerable (2.78%) and other 90 species are least concerned (83.33%) according to IUCN (2021). One additional species is not evaluated and one species is data deficit. Cyprinidae was the most dominant family comprising of 42 species, followed by Bagridae (8), Sissoridae (6), Channidae (5), Ambasidae (4), Siluridae (4) species. Each of Mastacembelidae, Schilbeidae, Osphronemidae, Nemacheilidae, Botiidae contained three species whereas families viz. Psilorhynchidae, Cobitidae, Notopteridae, Badidae contained 2 species each. On the other hand, rest of the families contained single species.

- This report also describes first hand information on physico-chemical properties of water and sediment from River Kopili. Average surface water temperature varied from 18.50-31.50 ^{0}C, water velocity (0.78-2.65 m/sec), turbidity (0.16-114.84 NTU), pH (3.50-7.54), dissolved oxygen (4.17-10.22 ppm), total alkalinity (12.67-76.00 ppm), total hardness (40.31-72.20 ppm), electrical conductivity (49.03-211.45 µS/cm), TDS (42.10-160.3.5 ppm) etc. Sediment parameters like pH, organic carbon, organic matter, nitrogen, potassium & phosphorus also varied significantly among seasons.

- A total of 46 genera of plankton were recorded from River Kopili during the study period. Population of phytoplankton was represented by 35 genera belonging to Chlorophyceae (17 genera), Bacillariophyceae (10 genera), Cyanophyceae (7 genera) and Euglenophyceae (1genera). Zooplankton population was represented by Rotifera (5 genera), Cladocera (3 genera) and Copepoda (3 genera). This is also first-hand information on the plankton biomass of River Kopili.

4.3 **Generating Model Predictions for different variables**

- No

4.4 **Technological Intervention**

- No

4.5 **On-field Demonstration and Value-addition of Products**

- No

4.6 Developing Green Skills in IHR

- No

4.7 Addressing Cross-cutting Issues

- No

5 IMPACTS OF FELLOWSHIP IN IHR

5.1 Socio-Economic Development

The project findings can help in developing strategies for better management of fisheries resources of IHR:

- Many hill streams of IHR are home to popular Sport fish like Mahseer. These streams can be identified, conserved and managed on scientific principles to promote these streams as Sports Fishing destinations.

- Many fish species of IHR like Chocolate mahseer, Golden Mahseer, Kalabans are also very high market demand. Culture of these fish species need to promoted in IHR for harnessing their culture potential.

- Many fish species of IHR have high demand in international market as ornamental fish. These fish species should be identified, their breeding biology should be studied systematically and their captive breeding protocol should be developed to promote their export as ornamental fish.

5.2 Conservation of Biodiversity in IHR

- Indigenous fish fauna of IHR are very unique. Many of these fish species are endemic to this region only. Habitat of these precious indigenous ichthyofauna of IHR should be studied and *in-situ* conservation measures should be initiated for protecting these precious resources.

- Fish species categorised as endangered, threatened, vulnerable should be prioritised and special conservation programmes should be launched.

- The present study generated very important information on geographic distribution of many important fish species of IHR. The extended geographic range and habitat information will help in developing conservation strategies of these fish species.

- Mitogenome sequences of indigenous fish fauna generated during the project will help the other researchers of IHR working in this field in concrete identification of fish fauna of this region which is very much essential for conservation of ichthyofaunal biodiversity of this region.

- Fishery rules/laws of IHR states should be strictly implemented for conservation of fish diversity of IHR.

5.3 Protection of Environment

- Habitat destruction of indigenous fish population of IHR is one of the major identified threats. During the present study also, it came up as one of the biggest threats to the indigenous fish germplasm. Boulder mining, sand mining activities are rapidly destroying the habitat of the fishes. These activities need to be controlled without further delay in order protect the habitat of indigenous fishes.

- Identified stretches of rivers and streams can be declared as protected zones preventing any kind of fishing activities in these areas. Fish sanctuaries should be declared in the IHR for protecting the habitat of indigenous fishes.

- Destructive fishing techniques should be discouraged. The ill impacts of these techniques on fish population and its habitat should be taught to the fisherfolks by organising various awareness campaigns.

- Mass awareness programmes need to be conducted in IHR to sensitise the people about conservation indigenous fish germplasm and their habitat.

5.4 Developing Mountain Infrastructures

- The project has developed research infrastructure for fish biodiversity study which can be also utilised for future similar research works of this region.

5.5 Strengthening Networking in IHR

- All the project findings will be available in public domain. These findings can be effectively utilised in better understanding of aquatic ecosystems of IHR.

- Information on ichthyofauna generated during the project can be utilised by other researchers of IHR for identification, habitat ecology study of the indigenous fish species.

- The information generated during the study can be effectively utilised for developing conservation model of indigenous fishes of IHR

- The literatures and resources developed during the project can be utilised by other IHR regions for training, awareness, outreach activities of fisherfolks.

6 EXIT STRATEGY AND SUSTAINABILITY

6.1 How effectively the fellowship findings could be utilized for the sustainable development of IHR

This project has documented first-hand information on fish diversity, plankton biomass, water as well as sediment characteristics, pollution status and anthropogenic factors of River Kopili, which originates from South-Western part of Shillong peak, Borail range. The study revealed that, the river was not suitable for fish species during 2018-19 as the water pH was highly acidic in nature in NC hill areas. But since late 2019 the pH of water starts increasing to alkaline point and became the habitat for some important endangered, vulnerable and near threatened fish species; whose *in-situ* conservation measures can be initiated in the river. Presence of Mahseer in the study rivers also opened scope for development of sports fishing activities in these rivers which in turn can promote eco-tourism in this region. The study showed that habitat destruction of indigenous fish fauna in the form of sand mining from river bed and hydro-electric dams are a serious concern threatening indigenous fish population; hence strong measures need to be initiated in the entire IHR controlling such activities which may pose serious threat to the indigenous fish germplasm of the region.

6.2 Identify other important areas not covered under this study, but needs further attention

- Study on biology of indigenous fishes with special reference to reproductive biology
- Development of captive breeding protocol of selected fish species. Species included in concerned categories like Endangered, Vulnerable, threatened should be studied with priority and efforts should be made to develop captive breeding protocol of these fish species.

6.3 Major recommendations for sustaining the outcomes of the fellowship in future

- Conservation efforts of indigenous ichthyofauna of the study rivers will continue with community participation. The village community of the study rivers voluntarily prohibited use of destructive fishing techniques in the entire river stretch. Some of the selected stretches of the river are also declared as "No Fishing Zone". Such community participation will sustain the conservation efforts in future also.
- Boulder/Sand mining activities from river bed should be totally prohibited in order to conserve the microhabitat requirement of hill stream fishes.
- State fishery laws prohibiting fishing during breeding season, use of destructive fishing gears etc. should be strictly followed.
- Ranching should be conducted.

- Small scale hill aquaculture should be promoted for socio-economic upliftment of local tribal people of the region.

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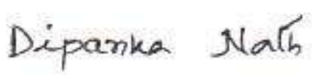


(Signature of HRA/HJRF/HPF)

(S. K. Bhagabati)

(NMHS FELLOWSHIP COORDINATOR)

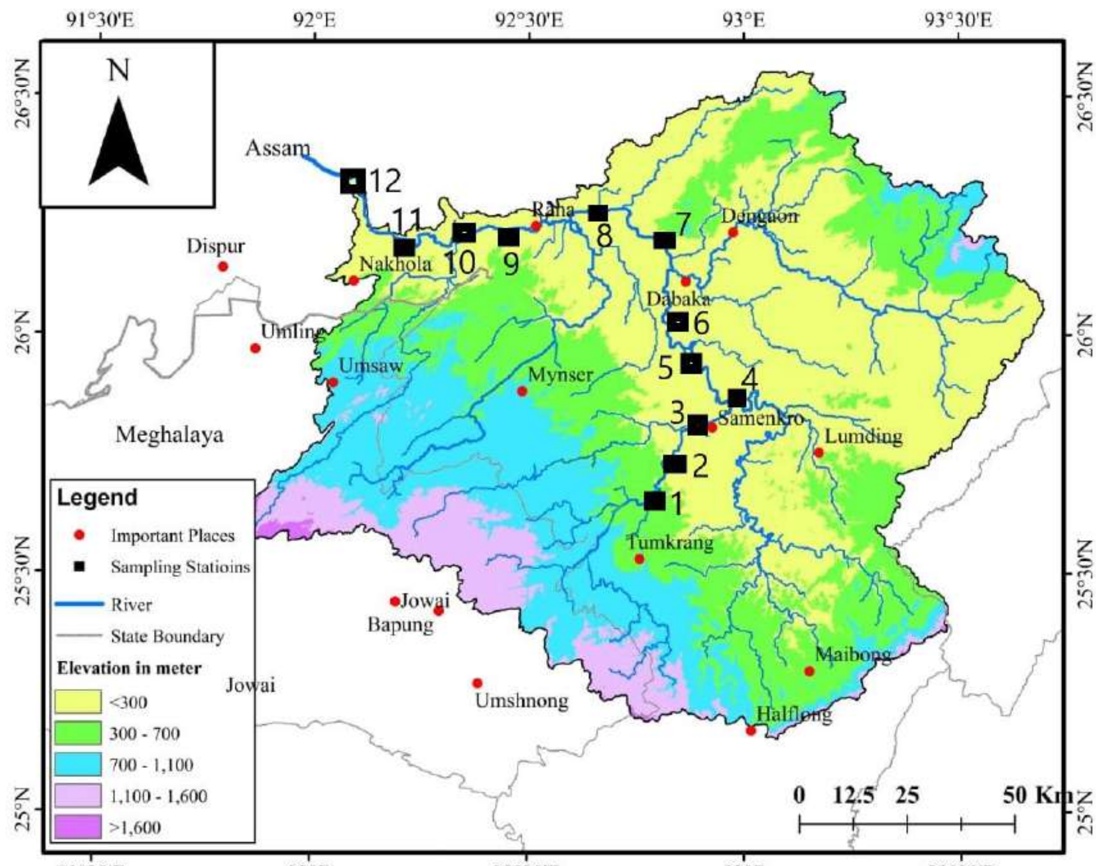
(B. Kalita)

(HEAD OF THE INSTITUTION)

Place: ………………..
Date: ……../……/……

ANNEXURE I

MAP SHOWING THE STUDY AREA OF RIVER KOPILI



ANNEXURE II
DESCRIPTION OF THE STUDY AREA

Station 1: This station is situated at a latitude of 25°30'54"N and longitude of 94°43'0"E. This station is located below the Kopili Hydro-Electric Power Dam at Dima Hasao district. This station is characterized by average water velocity of 1.67 m/sec and average depth of about 1.5 m.

Station 2: Station two is located at a latitude of 25°35'52"N and longitude of 92°44'54"E. This station is situated at 29 Kilo, Dima Hasao district. The distance between the station 1 and station 2 is 19 km. Average water velocity and depth of this site found to be 1.65 m/sec and 1.7 m respectively.

Station 3: This station is situated at a latitude of 25°42'39"N and longitude of 92°49'01"E. It is located at Panimur, Karbi-Anglong district of Assam. This station is known for picnic spot, where water velocity was higher than other stations (1.75m/sec). The distance between station 2 and station 3 is 19 km.

Station 4: This station is located at Doyangmukh, Karbi-Anglong, where the river Doiang joints Kopili at a latitude of 25°42'39"N and longitude of 92°49'01"E. The distance between station 3 and 4 is 9.65 km. The average water velocity recorded was 1.65 m/sec and depth to be 1.8 m.

Station 5: This station is located at Kheroni, Karbi-Anglong, at a latitude of 25°50'92.5"N and longitude of 92°53'18.6"E. The distance between station 4 and 5 is 37 km.

Station 6: This station is situated at a latitude of 26°00'18''N and longitude of 92°45'34''E. It is located at Tumpreng, Karbi-Anglong district of Assam. The distance between station 5 and station 6 is 43 km.

Station 7: This station is situated at a latitude of 26°09'48''N and longitude of 92°38'38''E. It is located at Ghilani, Nagaon district of Assam. The distance between station 6 and station 7 is 24 km.

Station 8: This station is located at Chaparmukh, Nagaon, at a latitude of 26°11'54"N and longitude of 92°31'16"E. The distance between station 7 and 8 is 22.5 km.

Station 9: This station is located at Dharamtul, Nagaon, Assam at a latitude of 26°09'55"N and longitude of 92°21'12"E. The distance between station 8 and 9 is 20 km.

Station 10: This station is situated at a latitude of 26°10'41''N and longitude of 92°13'07''E. It is located at Mayang, Morigaon district of Assam. The distance between station 9 and station 10 is 21 km.

Station 11: This station is situated at a latitude of 26°12'03''N and longitude of 92°01'16''E. It is located at Kalangpar, Morigaon district of Assam. The distance between station 10 and station 11 is 34 km.

Station 12: This station is located at Burha Mayang, Morigaon, where the river Kopili confluence with the mighty river Brahmaputra at a latitude of 26°14'57''N and longitude of 91°57'26''E. The distance between station 11 and 12 is 10 km.

ANNEXURE III

DETAILS OF THE FISH SPECIES COLLECTED

Sl No	Species	Common Name	IUCN 2021	Ornamental Value	NCBI Accession NO
1.	*Tor putitora*	Golden Mahseer	EN	+	OK018132
2.	*Neolissochilus hexagonolepis*	Copper mahseer	NT	+	OK017906
3.	*Garra annandalei*	Annandale garra	LC	+	OK017427
4.	*Garra gotyla gotyla*	Nilgiris garra	LC	+	OK092316
5.	*Garra nasuta*	Khasi garra	LC	+	
6.	*Garra lamta*	Lamta garra	LC	+	
7.	*Garra lissorhynchus*	Khasi garra	LC	+	OK299112
8.	*Garra kempi*	Kempi garra	LC	+	OL436248
9.	*Opsarius bendelisis*	Hamilton's Barila	LC	+	OL434973
10.	*Opsarius barna*	Barna baril	LC	+	OK300050
11.	*Barilius barila*	Bared trout	LC	+	OK091134
12.	*Pethia stoliczkanus*		LC	+	OM009242 OM009249
13.	*Puntius chola*	Swamp barb	LC	+	
14.	*Pethia ticto*	Two spot barb	LC	+	
15.	*Pethia conchonius*	Rosy barb	LC	+	OK310720
16.	*Puntius sophore*	Soft fin swamp barb,	LC	+	MZ798434

17.	*Systomus sarana*	Olive barb	LC	+	
18.	*Chagunius chagunio*	Chenguni	LC	+	OK087620
19.	*Osteobrama cunma*	Cunma	LC	+	OL685186
20.	*Tariqilabeo latius*	Stone roller/gangetic latia	LC	+	OK017171 OK310717
21.	*Labeo bata*	Bata	LC	-	
22.	*Labeo calbasu*	Orangefin labeo	LC	+	OL305727
23.	*Labeo gonius*	Kuria labeo	LC	-	OL440716
24.	*Labeo dyocheilus*	Brahmaputra labeo Ghora mach	LC	+	
25.	*Labeo pangusia*	Pangusia Labeo	NT	-	OK017452
26.	*Labeo boga*		LC	-	
27.	*Labeo fimbriatus*	Fringed-lipped peninsula carp	LC	-	
28.	*Labeo rohita*	Rohu Labeo	LC	-	
29.	*Labeo catla*	Catla	LC	-	
30.	*Cirrhinus reba*	Reba carp	LC	+	OK104079
31.	*Cirrhinus mrigala*	Mrigal carp	LC	-	OK287079
32.	*Cyprinious carpio*	Common carp	LC	-	
33.	*Bengala elanga*	Bengala barb	LC	+	
34.	*Cabdio morar*	Morar	LC	+	
35.	*Amblypharyngodon mola*	Mola carplet	LC	+	
36.	*Psilorhynchus homaloptera*	Torrent stone carp Homaloptera minow	LC	+	OL450426

37.	*Psilorhynchus balitora*	Balitora minnow	LC	+	OL450427
38.	*Botia rostrata*	Gangetic loach	VU	+	
39.	*Botia dario*	Bengal loach	LC	+	
40.	*Paracanthocobitis botia*	Mottled zipper loach	LC	+	OL434974
41.	*Schistura fasciata*	-	NE	+	OK103854
42.	*Schistura khugae*		VU	+	
43.	*Schistura reticulata*		EN	+	OK103914
44.	*Lepidocephalichthys guntea*	Guntea loach	LC	+	OK305931
45.	*Lepidocephalichthys annandalei*	Annandale loach	LC	+	OK310736
46.	*Esomus danricus*	Flying barb	LC	+	OK135729
47.	*Danio rerio*	Zebra Danio	LC	+	
48.	*Devario devario*	Bengal danio	LC	+	
49.	*Devario aequipinnatus*	Giant danio	LC	+	OK012603
50.	*Danio dangila*	Moustached danio	LC	+	OL693658
51.	*Salmostoma bacaila*	Large rose belly Minow	LC	+	OK091001
52.	*Salmostoma phulo*	Finescale razorbelly minnow	LC	+	OL693681
53.	*Notopterus synurus*	Bronze featherback	LC	+	OK090941
54.	*Notopterus chitala*	Humped Featherback	NT	+	
55.	*Badis assamensis*	Assamese Chameleon fish	DD	+	
56.	*Badis badis*	Dwarf Chameleon fish	LC	+	MZ672109

57.	*Channa marulius*	Giant snakehead	LC	+	OL440718
58.	*Channa stewartii*	Assamese snakehead	LC	+	
59.	*Channa gachua*	Dwarf snakehead	LC	+	
60.	*Channa punctata*	Spotted snakehead	LC	+	OL440717
61.	*Channa striata*	striped snakehead	LC	+	OK305960
62.	*Anabas testudineus*	Climbing perch	LC	+	MZ798424
63.	*Glossogobius giuris*	Tank goby/bare eye goby	LC	+	
64.	*Chanda nama*	Elongated glass parchlet fish	LC	+	MZ965047
65.	*Parambassis baculis*	Himalayan glassy perchlet	LC	+	
66.	*Parambassis ranga*	Indian glassy fish	LC	+	
67.	*Laubuka laubuca*	Indian glass barb	LC	+	
68.	*Trichogaster fasciata*	Giant gourami	LC	+	
69.	*Trichogaster lalius*	Dwarf Gourami	LC	+	OK306904 OK310734
70.	*Trichogaster labiosa*	Thick lipped gourami	LC	+	
71.	*Mystus cavasius*	Gangetic Mystus	LC	+	
72.	*Mystus tengara*	Tengara catfish	LC	+	OK306011
73.	*Mystus bleekeri*	Day's mystus	LC	+	
74.	*Mystus vittatus*	Striped dwarf catfish	LC	+	
75.	*Rita rita*	Rita	LC	+	MZ798284
76.	*Sperata aor*	long-whiskered catfish	LC	+	OL440720

77.	*Sperata seenghala*	Giant river-catfish	LC	-	OK287085
78.	*Olyra kempi*	Long tail catfish	LC		
79.	*Clarias magur*	Walking Catfish	EN	+	
80.	*Heteropneustes fossilis*	Stinging catfish	LC	+	OK091662
81.	*Wallago attu*	Helicopter catfish	VU	-	OK302918
82.	*Ompok bimaculatus*	Butter catfish	NT	+	OL693800
83.	*Ompok pabo*	Pabo catfish	NT	+	
84.	*Ompok pabda*	Pabdah catfish	NT	+	
85.	*Glyptothorax striatus*		NT	+	OL435102
86.	*Glyptothorax telchitta*		LC	+	
87.	*Bagarius bagarius*	Devil catfish	NT	+	
88.	*Clupisoma garua*	Bachcha	LC	+	OK300431
89.	*Gagata cenia*	Indian gagata	LC		OK091600
90.	*Gagata gagata*	Gangetic gagata	LC		OM011979
91.	*Ailia coila*	Gangetic ailia	NT	+	OK091007
92.	*Erethistes hara*	Kosi Hara	LC	+	OK305937
93.	*Eutropiichthys murius*	Indus garua	LC	+	
94.	*Eutropiichthys vacha*	Batchwa Vacha	LC	+	OK303069
95.	*Pachypterus atherinoides*	Indian potasi	LC	+	
96.	*Amblyceps apangi*	Indian torrent catfish	LC	+	OK298953

97.	*Chaca chaca*	Squarehead catfish	LC	+	
98.	*Xenentodon cancila*	Needlefish	LC	+	
99.	*Mastacembelus armatus*	Tire-track spiny eel	LC	+	OL693657
100.	*Macrognathus aral*	one-stripe spiny eel	LC	+	OK301273
101.	*Macrognathus aculeatus*	Lesser spiny eel	LC	+	
102.	*Monopterus cuchia*	Gangetic Mud eel	LC	-	
103.	*Anguilla bengalensis*	India Mottlet eel	NT	-	
104.	*Gudusia chapra*	Indian River Shad	LC	-	
105.	*Setipinna phasa*	Gangetic hairfin anchovy	LC	+	
106.	*Rhinomugil corsula*	Corsula	LC	-	OK092292
107.	*Nandus nandus*	Gangetic leaffish	LC	+	OL440719
108.	*Tetradon cutcutia*	Ocellated pufferfish	LC	-	

ANNEXURE IV

PUBLICATIONS

1. Published Research Paper:

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Assessment of water quality status of river Kopili, in Karbi Anglong district of Assam using water quality index

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DOI: https://doi.org/10.22271/chemi.2020.v8.i5e.10629

Abstract
River Kopili, regarded as one of the most important Southern bank tributaries of the mighty river Brahmaputra, is facing a tremendous pollution threat from its riparian areas, especially in its upper stretches due to anthropogenic activities. The Central Pollution Control Board (CPCB) in one of its report has placed the Kopili river in 4th rank among the 56 most polluted river of North-East region. The results of the present study showed that water quality index [1] of the river ranges from poor to unsuitable quality of water for drinking in almost all the five sampling stations. The water quality found to be deteriorated during winter season with an average WQI value of 81.88 as compared to pre-monsoon and monsoon season with an average value of 67.13 and 80.12 respectively. According to the water quality index, station 4 and 5 are recorded to be the most polluted stations among the study area. Thus, all the above analysis showed that the riverine ecosystem is moderately polluted, which may degrade further if proper management of the riverine ecosystem is not initiated timely, which is the need of the hour to sustain the environmental integrity of the river Kopili.

Keywords: Kopili river, Brahmaputra, environmental integrity, water quality index

Introduction

River is a lotic ecosystem flowing under the influence of gravity and confluence into the sea and some into lakes. Rivers are regarded as one of the most important resources in the world as well as in India in particular. Rivers are important pathways for the flow of energy, matter and organisms through the landscape [2]. Rivers also play a major role in assimilation or transportation of the municipal and industrial wastewater discharges continuously or occasionally or seasonally. Most of the ancient civilizations grew along the banks of the rivers.
The water quality of a river is the composite of several interrelated compounds, which are subjected to local and temporal variations and also affected by the volume of water flow [3]. Surface water resources are more vulnerable to pollution than ground water resources [4] especially in developing countries where the heavy industrialization, increasing urbanization, and adaptation of modern agricultural practices play an important role in improving the living standard but at the same time cause severe environmental damage [5], and declining quality of life for many people [6]. Water pollution affects their physico-chemical characteristics and microbiological quality [7]. Therefore, constant monitoring of a river system is required to evaluate the effects of environmental factors on water quality for proper utilization and sustainable development of the resources.
Water Quality Indices (WQI) provides a single value to the water quality of a source on the basis of one or the other system which translates the list of constituents and their concentrations present in a sample into a single value. The application of WQI helps to understand the overall water quality status of individual sampling stations at a certain time [8] and its suitability for various beneficial uses. The concept of indices to represent gradation in water quality was first proposed by [9], since then numerous water quality indices have been formulated that can easily evaluate the overall water quality of an area promptly and efficiently. The general WQI developed by [1] has undergone much improved modification suitable for a different purpose.

2. Published Research Paper:

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TECHNICAL NOTE

Applied Ichthyology | DWK | WILEY

First record of a barb, *Pethia stoliczkana* (Day) from Brahmaputra drainage, Assam, India

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Abstract

In this communication, occurrence of cyprinid fish species, *Pethia stoliczkana* (Day, 1871) has been reported for the first time from River Kopili, Brahmaputra drainage, Assam, India. A total number of eight specimens of the species were recorded from the river during the study. Conventional, as well as molecular taxonomic tools, confirmed the species as *P. stoliczkana*. The Mitogenome sequence of *P. stoliczkana* generated during the present study is the first such information from an Indian river.

1 | INTRODUCTION

Pethia is a native genus of tiny freshwater fish from South-East Asia belonging to the family Cyprinidae. The name *Pethia* is of Sinhalese origin and refers to tiny cyprinid fishes (Pethiyagoda et al., 2012). Combination of several characters and character states like small adult size, absence of rostral barbel, absence of maxillary barbel (minute if present), serrated and stiff last dorsal fin with 8 branched rays, anal fin with 3 unbranched and 5 branched rays, complete, incomplete or interrupted lateral line with 19–24 scales; black oblong mark above pectoral fin and one dark black blotch on 17th–19th scales of lateral line differentiate it from other South and South-East Asian Cyprinids (Pethiyagoda et al., 2012). *Pethia* currently has 40 recognized species around the globe, of which 28 species are from India (Shangningam et al., 2019).

The species *Pethia stoliczkana* was described by Day (1871), from Chindwin basin, Myanmar. Kottelat (2001) reported the geographical distribution of the species as Myanmar, Thailand and Laos. Further studies by Vishwanath and Juliana (2004) reported occurrence of *P. stoliczkana* in Chindwin basin, Manipur, India. IUCN (2021) reported the conservation status of the fish species as Least Concern (LC). However, no mitogenome sequence information of *P. stoliczkana* is available from Indian rivers.

During a recent exploration of fish fauna of River Kopili, eight numbers of barbs were collected; which were later identified and confirmed as *P. stoliczkana* with the help of conventional and molecular taxonomic tools. This study reports morphometric and molecular characteristics of *P. stoliczkana* from the Brahmaputra drainage for the first time and extends its geographical range.

2 | MATERIALS AND METHODS

Kopili is a southern tributary of the mighty River Brahmaputra which originates from South-Western part of Shillong Peak, Borail Himalayan range, Meghalaya, India at an elevation of 1600 m MSL. The river traverses a total length of 290 km before debouching into River Brahmaputra and has a catchment area of 16,420 km^2. During the study, in the month of November 2021, eight specimens of *Pethia* were collected from Kopili river (location: 29 Kilo, Dima Hasao district, Assam, India; 25°35′52″N 92°44′54″ E) using a cast net (mesh size 10–15 mm) following the guidelines of National Biodiversity Authority, Government of India (Figure 1a,b). The species is locally known as "Puthi". After collection, the specimens were photographed in fresh condition (Figure 3a) and fixed in 10% formalin solution (Figure 3b). Pectoral fin clippings from two fresh fish specimens were collected and preserved in absolute alcohol (Merck) for DNA barcoding (Ward et al., 2009). The fish specimens were identified following standard literature Sen (1985), Jayaram (1991 & 1994), Talwar and Jhingran (1991) and

ANNEXURE V

Details of the Physico-Chemical Parameters of River Kopili

Physical parameters of water:

1. Surface Water temperature:

Water temperature is of enormous significance as it regulates various abiotic characteristics and biotic activities of an aquatic ecosystem which is recognized by many authors (Mc Combie, 1953; Hutchinson, 1957; Jana, 1973; Chari, 1980; Kataria et al., 1995; Iqbal and Katariya, 1995; Sharma and Sarang, 2004; Radhika et al., 2004The minimum and maximum surface water temperature of Kopili river ranges from 18.50 (winter, 2019) to 31.50 (Monsoon, 2020).

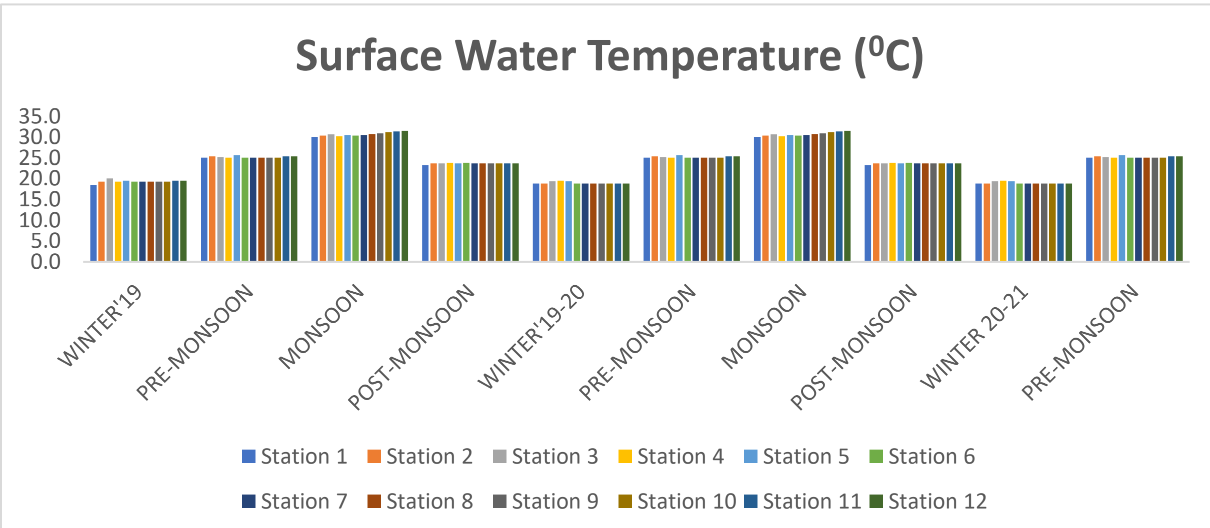


Figure: Seasonal variation of surface water temperature at station 1-12

Stations	Minimum	Maximum
1	18.50±0.50 (Winter, 2019)	30.00±0.0.82 (Monsoon, 2020)
2	18.83 ± 0.60 (Winter, 2019-20)	30.33±1.31 (Monsoon, 2020)
3	19.33±0.20 (Winter, 2020-21)	30.67±1.43 (Monsoon, 2020)
4	19.25±0.20 (Winter, 2019)	30.17±1.65 (Monsoon, 2020)
5	19.33±0.50 (Winter, 2019-20)	30.50±1.87 (Monsoon, 2020)
6	18.83±0.60 (Winter, 2020-21)	30.33± 1.65 (Monsoon, 2020)
7	18.83±0.60 (Winter, 2020-21)	30.50±1.87 (Monsoon, 2020)
8	18.83±0.60 (Winter, 2020-21)	30.70±0.88 (Monsoon, 2020)
9	18.83±0.60 (Winter, 2020-21)	30.90± 0.70(Monsoon, 2020)
10	18.88±0.60 (Winter, 2020-21)	31.17±0.62 (Monsoon, 2020)
11	18.83±0.60 (Winter, 2020-21)	31.33±0.47 (Monsoon, 2020)
12	18.83±0.60 (Winter, 2020-21)	31.50±0.41 (Monsoon, 2020)

2. Water Velocity: Water velocity of Kopili river shows a seasonal variation being the lowest in winter and highest during monsoon season.

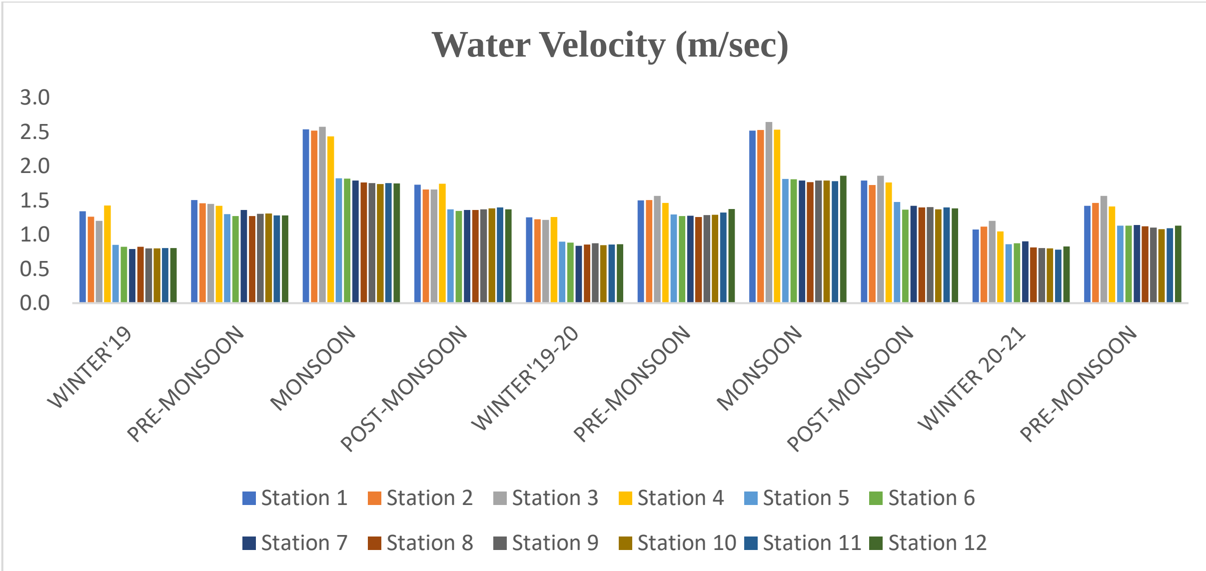


Figure: Seasonal variation of water velocity at station 1-12

Stations	Minimum	Maximum
1	1.08±0.16 (Winter, 2020-21)	2.54±0.30(Monsoon, 2020)
2	1.12± (Winter, 2020-21)	2.53±0.20 (Monsoon, 2020)
3	1.20±0.02 (Winter, 2019)	2.65±0.20 (Monsoon, 2020)
4	1.05±0.12 (Winter, 2020-21)	2.54±0.21 (Monsoon, 2020)
5	0.85±0.10 (Winter, 2019-20)	1.82±0.32 (Monsoon, 2020)
6	0.83±0.07 (Winter, 2019)	1.82±0.11 (Monsoon, 2020)
7	0.79±0.05 (Winter, 2019)	1.79±0.09 (Monsoon, 2020)
8	0.81±0.02 (Winter, 2020-21)	1.77± 0.02(Monsoon, 2020)
9	0.80±0.07 (Winter, 2019-20)	1.79±0.03 (Monsoon, 2020)
10	0.80±0.06 (Winter, 2019-2)	1.79± 0.08(Monsoon, 2020)
11	0.78±0.04 (Winter, 2019)	1.78±0.02 (Monsoon, 2020)
12	0.81±0.02 (Winter, 2019)	1.86±0.15 (Monsoon, 2020)

3. **Turbidity:** Turbidity depends on the presence or absence of clay silt, dissolved organic and inorganic matter, turbid water received from the catchment area, plankton and other microscopic organisms. (Mishra and Saksena, 1991; Singh, 1999; Kulshrestha and Sharma, 2006). Turbidity of Kopili river water ranges between 0.16 NTU to 114.84 NTU.

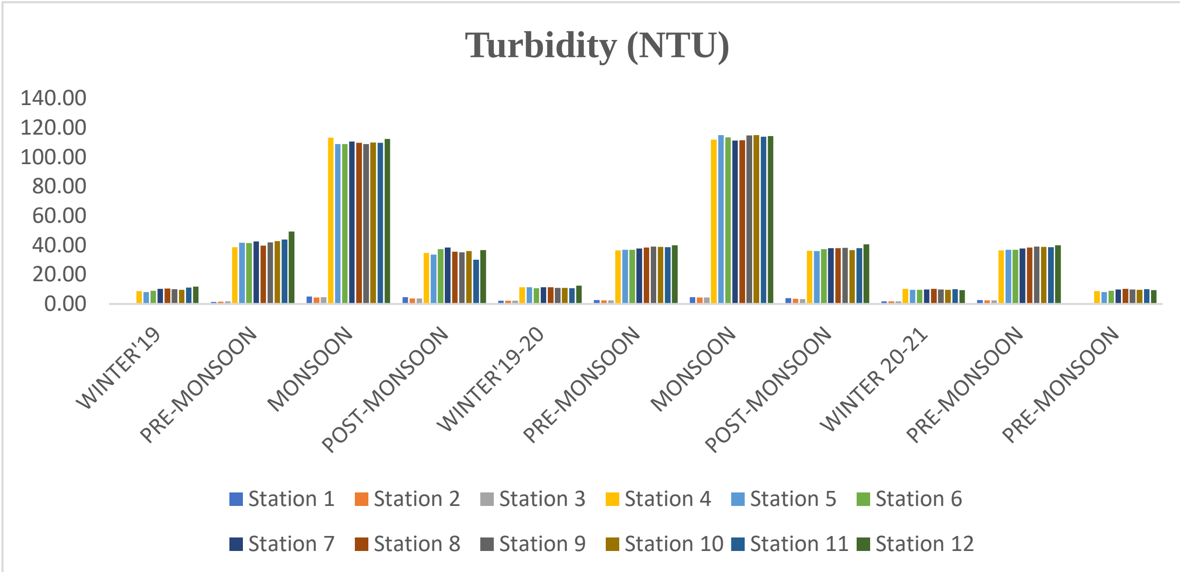


Figure: Seasonal variation of Turbidity at station 1-12

Stations	Minimum	Maximum
1	0.40±0.04 (Winter, 2019)	5.09±0.40 (Monsoon, 2019)
2	0.47±0.02 (Winter, 2019)	4.51±0.81(Monsoon, 2020)
3	0.16±0.10 (Winter, 2019)	4.58±0.85 (Monsoon, 2020)
4	8.88±2.38 (Winter, 2019)	113.43±29.56 (Monsoon, 2020)
5	8.23±2.03 (Winter, 2019)	114.98±24.89 (Monsoon, 2020)
6	9.08±2.38 (Winter, 2019)	113.59±20.83 (Monsoon, 2020)
7	9.98±2.12 (Winter, 2020-21)	111.30±21.03 (Monsoon, 2020)
8	10.30±3.72 (Winter, 2020-21)	111.56±18.72 (Monsoon, 2020)
9	9.80±3.53 (Winter, 2020-21)	114.84±23.93 (Monsoon, 2020)
10	9.56±2.44 (Winter, 2019)	115.18±22.20 (Monsoon, 2020)
11	10.03±3.79 (Winter, 2020-21)	113.89±22.94 (Monsoon, 2020)
12	9.54±3.32 (Winter, 2020-21)	114.42±22.88 (Monsoon, 2020)

Chemical parameters of water

1. Water pH: pH is a measure of the acidic and alkaline condition of a water body that affects its productivity (Welch, 1952). pH of water is important because all physico-chemical reactions of water in an aquatic body take place at a definite pH which plays an important role in the productivity of river. The river water pH of Kopili falls under acidic to alkaline conditions. Lowest pH of water was found to be 3.50±0.71 (post-monsoon, 2019) and highest during post monsoon, 2019 (7.54±0.12). Acidic condition of water found in the upper stretches of river i.e. in Karbi-Anglong district during 2019. But after 2019 the river water became alkaline in nature, which might be due the ban imposed by NGT in rat hole mining since 2019.

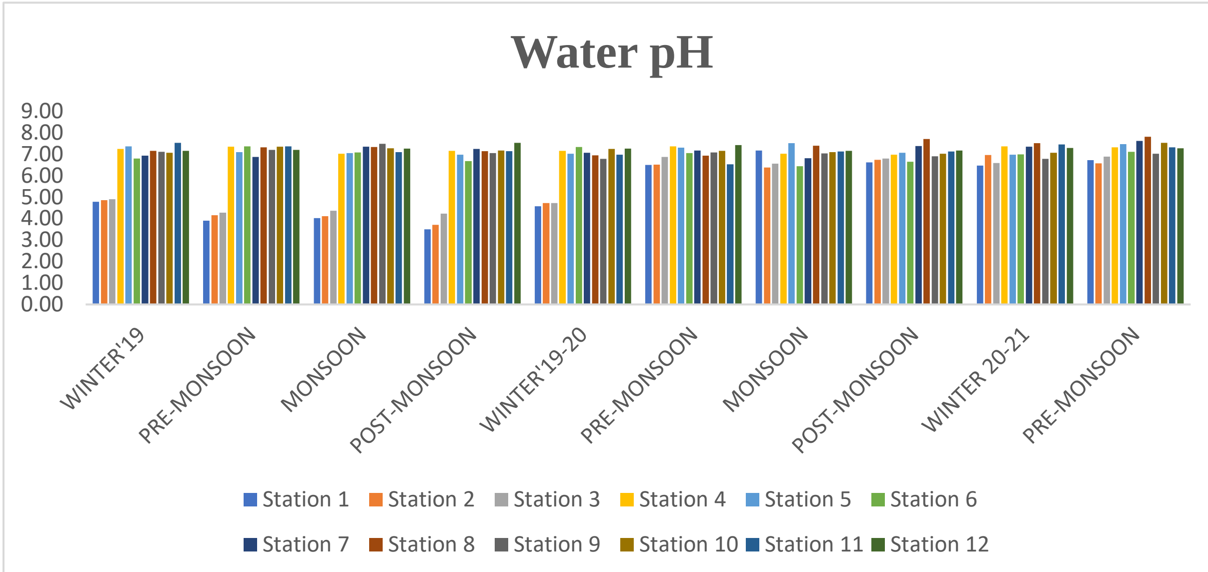


Figure: Seasonal variation of pH at station 1-12

Stations	Minimum	Maximum
1	3.50±0.71 (Post-monsoon, 2019)	7.17±0.11 (Monsoon, 2020)
2	3.71±0.56 (Post-monsoon, 2019)	6.96±0.15 (Winter, 2020-21)
3	4.23±0.53 (Post-monsoon, 2019)	6.87±0.54 (Pre-monsoon, 2020)
4	6.98±0.24 (Post-monsoon, 2020)	7.51±0.33 (Monsoon, 2020)
5	6.98±0.34 (Post-monsoon, 2019)	7.51±0.31 (Monsoon, 2020)
6	6.45±0.03 (Post-monsoon, 2020)	7.36±0.30 (Winter, 2019-20)
7	6.81±0.10 (Monsoon, 2020)	7.38±0.16 (Post-monsoon, 2020)
8	6.93±0.17 (Pre-monsoon, 2020)	7.71± 0.09(Post-monsoon, , 2020)
9	6.93±0.17 (Pre-monsoon, 2020)	7.71±0.08 (Post-monsoon, 2020)
10	7.02±0.07 (Post-monsoon, 2020)	7.36±0.04 (Premonsoon, 2019)
11	6.53±0.22 (Pre-monsoon, 2020)	7.54±0.31 (Winter, 2019)
12	7.16±0.20 (Monsoon, 2020)	7.54±0.12 (Post-monsoon, 2019)

2. Dissolved Oxygen (DO): Dissolved oxygen in water is indispensable for aquatic life for their survival. Dissolve oxygen in natural water depends on different physical, chemical and biological factors. In the present study, DO value ranged from 4.17±0.81 to 10.22±0.80 mgL^{-1}.

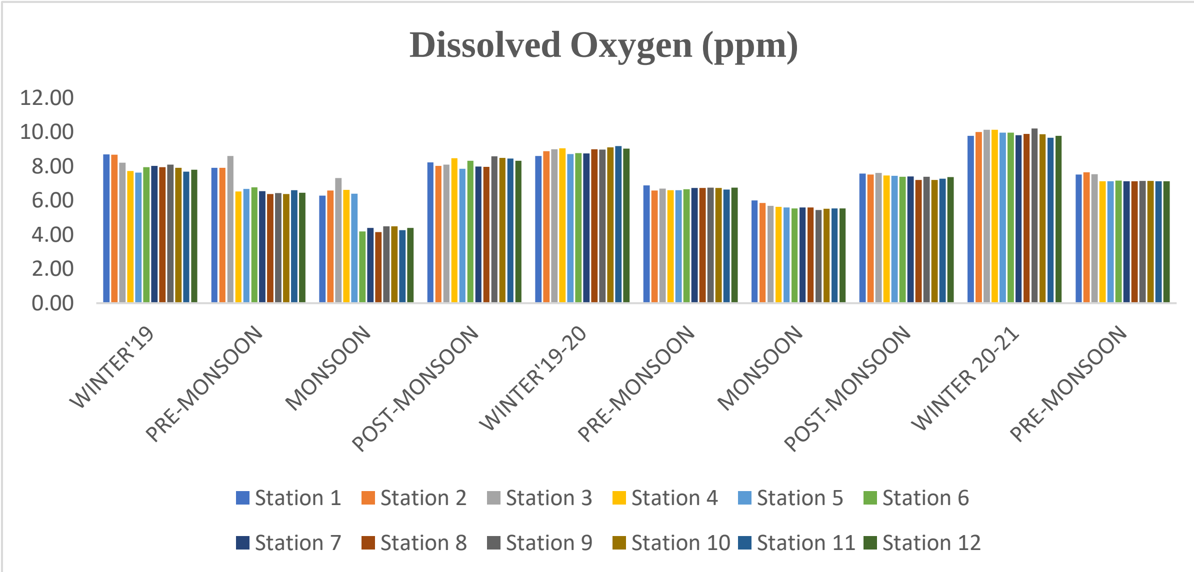


Figure: Seasonal variation of Dissolved Oxygen at station 1-12

Stations	Minimum	Maximum
1	6.02±0.80 (Monsoon, 2020)	9.79±0.42 (Winter, 2020-21)
2	5.87±0.81 (Monsoon, 2020)	10.02±0.71 (Winter, 2020-21)
3	5.70±0.65 (Monsoon, 2020)	10.15±0.62 (Winter, 2020-21)
4	5.63±0.41 (Monsoon, 2020)	10.15±0.72 (Winter, 2020-21)
5	5.60±0.04 (Monsoon, 2020)	9.48±0.50 (Winter, 2020-21)
6	4.19±0.76 (Monsoon, 2019)	9.98±0.72 (Winter, 2020-21)
7	4.41±1.14 (Monsoon, 2019)	9.83±0.52 (Winter, 2020-21)
8	4.17±0.81 (Monsoon, 2019)	9.90±0.63 (Winter, 2020-21)
9	4.50±0.93 (Monsoon, 2019)	10.22±0.80 (Winter, 2020-21)
10	4.49±0.86 (Monsoon, 2019)	9.88±0.81 (Winter, 2020-21)
11	4.28±0.78 (Monsoon, 2019)	9.68±0.49 (Winter, 2020-21)
12	4.40±0.74 (Monsoon, 2019)	9.80±0.66 (Winter, 2020-21)

3. **Total Alkalinity:** Alkalinity is the water's ability to resist changes in pH and is a measure of the total concentration of bases in pond water including carbonates, bicarbonates, hydroxides, phosphates and borates, dissolved calcium, magnesium, and other compounds in the water. Alkalinity acts as a stabilizer for pH. During the present study, the total alkalinity value was found to be lowest of 12.67±1.25 and highest of 76.00±0.82.

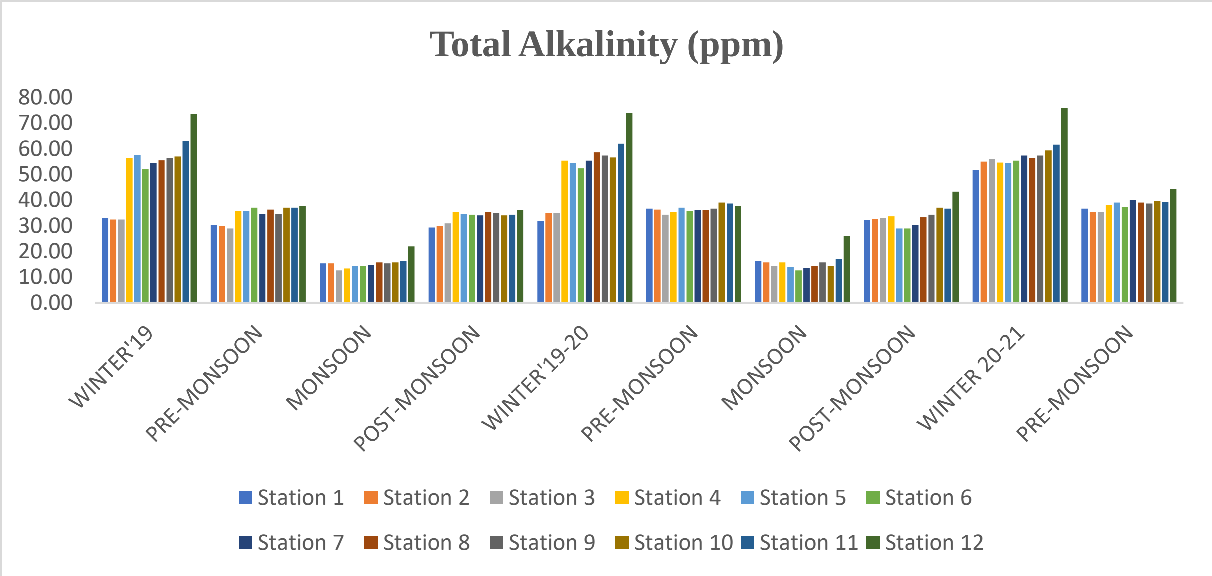


Figure: Seasonal variation of Total Alkalinity at station 1-12

Stations	Minimum	Maximum
1	15.33±3.30 (Monsoon, 2019)	51.67±1.25 (Winter, 2020-21)
2	15.33±1.88 (Monsoon, 2019)	55.00±0.82 (Winter, 2020-21)
3	12.67±0.94 (Monsoon, 2019)	56.00±2.16 (Winter, 2020-21)
4	13.33±1.70 (Monsoon, 2019)	56.50±0.50 (Winter, 2019)
5	14.33±1.70 (Monsoon, 2020)	57.50±0.50 (Winter, 2019)
6	12.67±1.25 (Monsoon, 2020)	55.33±1.25 (Winter, 2020-21)
7	13.67±1.70 (Monsoon, 2020)	57.33±2.62 (Winter, 2020-21)
8	14.33±2.62 (Monsoon, 2020)	58.67±6.16 (Winter, 2020-21)
9	15.33±3.09 (Monsoon, 2019)	57.33±7.59 (Winter, 2020-21)
10	14.33±1.70 (Monsoon, 2020)	59.33±4.99 (Winter, 2020-21)
11	16.33±2.06 (Monsoon, 2019)	63.00±2.00 (Winter, 2019)
12	22.00±2.16 (Monsoon, 2019)	76.00±0.82 (Winter, 2020-21)

4. Total Hardness: Hardness is the amount of dissolved calcium and magnesium salts in the water. Calcium and magnesium occur mainly in combination with bicarbonate, sulphate, and chloride. Total hardness values of surface water of Kopili river during the study period varied from 40.31±5.49 to 72.20±3.82.

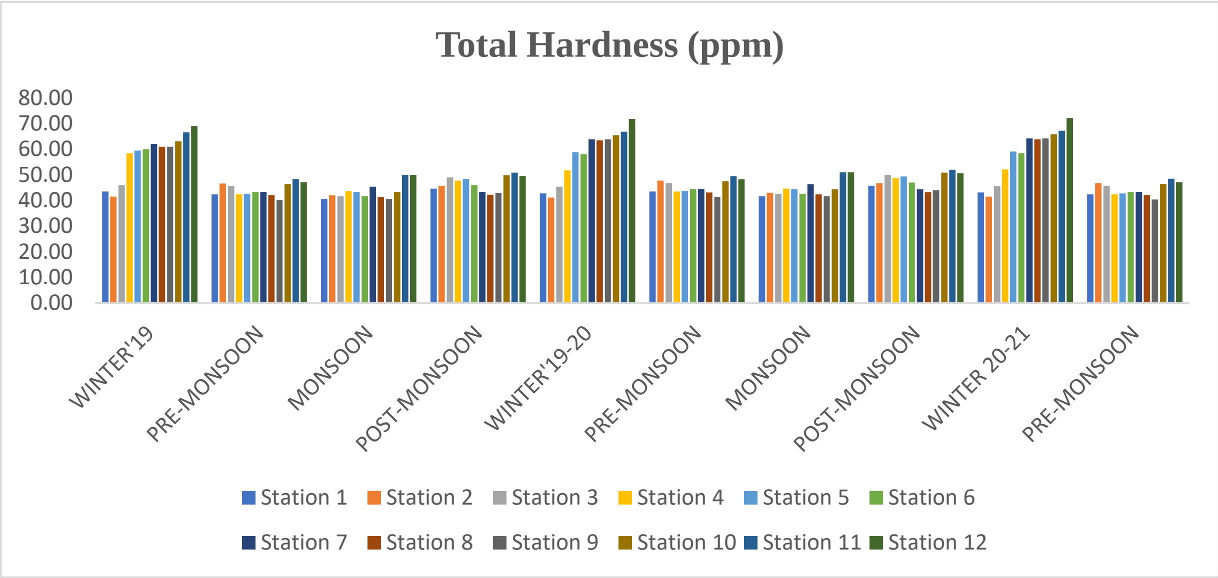


Figure: Seasonal variation of Total Hardness at Station 1-12

Stations	Minimum	Maximum
1	40.70±6.35 (Monsoon, 2019)	45.75±7.34 (Post-monsoon, 2020)
2	41.16±2.20 (Winter, 2019-20)	47.81±0.91 (Pre-monsoon, 2020)
3	41.71±1.70 (Monsoon, 2019)	50.08±8.66 (Post-monsoon, 2020)
4	42.38±2.89 (Pre-monsoon, 2019)	58.56±0.50 (Winter, 2019)
5	42.71±6.19 (Pre-monsoon, 2019)	59.56±0.55 (Winter, 2019)
6	41.71±0.47 (Monsoon, 2019)	60.06±0.61 (Winter, 2019)
7	43.37±4.50 (Pre-monsoon, 2019)	64.20±5.99 (Winter, 2020-21)
8	41.37±1.89 (Monsoon, 2019)	63.86±6.29 (Winter, 2020-21)
9	40.31±5.49 (Pre-monsoon, 2019)	64.20±4.41 (Winter, 2020-21)
10	43.37±3.03 (Monsoon, 2019)	65.86±6.29 (Winter, 2020-21)
11	48.45±0.60 (Pre-monsoon, 2019)	67.20±3.84 (Winter, 2020-21)
12	47.11±4.22 (Pre-monsoon, 2019)	72.20±3.82 (Winter, 2020-21)

5. Electrical Conductivity: Conductivity can be used as indicator of primary production (chemical richness) and thus fish production. Conductivity of water depends on its ionic concentration (Ca^{2+}, Mg^{2+}, HCO_3^-, CO_3^-, NO_3^- and PO_4^-), temperature and variations of dissolved solids. In the present study conductivity range from 49.03±6.90 (Winter, 2020-21) to 211.45± 33.39(Monsoon, 2020).

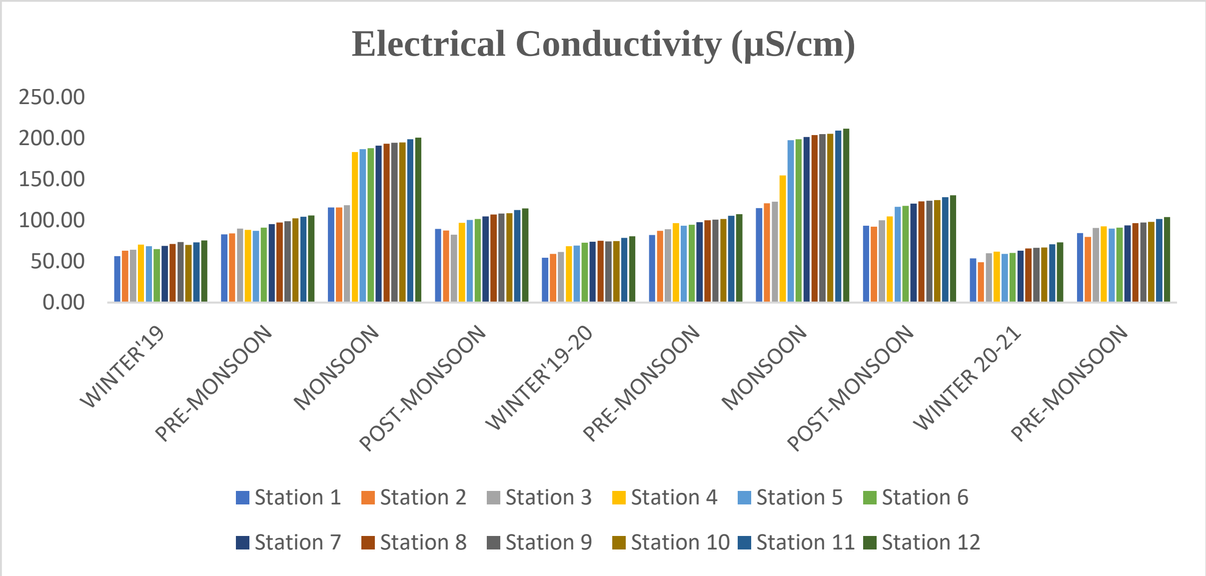


Figure: Seasonal Variation of Electrical Conductivity at Station 1-12

Stations	Minimum	Maximum
1	53.77±6.90 (Winter, 2020-21)	115.89±8.18 (Monsoon, 2020)
2	49.03±6.90 (Winter, 2020-21)	120.69±8.64 (Monsoon, 2020)
3	59.96±5.89 (Winter, 2020-21)	122.71±8.64 (Monsoon, 2020)
4	61.98±6.12 (Winter, 2020-21)	183.14±4.15 (Monsoon, 2020)
5	59.20±6.39 (Winter, 2020-21)	197.44±41.02 (Monsoon, 2020)
6	60.43±6.28 (Winter, 2020-21)	198.67±42.03(Monsoon, 2020)
7	63.30±6.17 (Winter, 2020-21)	201.54±41.12 (Monsoon, 2020)
8	65.74±6.14 (Winter, 2020-21)	203.98±41.03 (Monsoon, 2020)
9	66.74±6.09 (Winter, 2020-21)	204.98±40.39 (Monsoon, 2020)
10	67.21±6.11 (Winter, 2020-21)	205.45±42.13 (Monsoon, 2020)
11	71.10±6.18 (Winter, 2020-21)	209.34±39.89 (Monsoon, 2020)
12	73.21±6.19 (Winter, 2020-21)	211.45± 33.39(Monsoon, 2020)

6. Total Dissolved Solid: Total dissolved solid (TDS) is a measure of the total organic and inorganic substances present in a liquid. This includes anything present in water other than the pure H_2O molecules. These solids are primarily minerals, salts and organic matter that can be a general indicator of water quality. In the present investigation, the lowest value of TDS recorded was of 42.10±8.32 (Winter, 2019-20) and highest was of 160.35±26.44 (Monsoon, 2020).

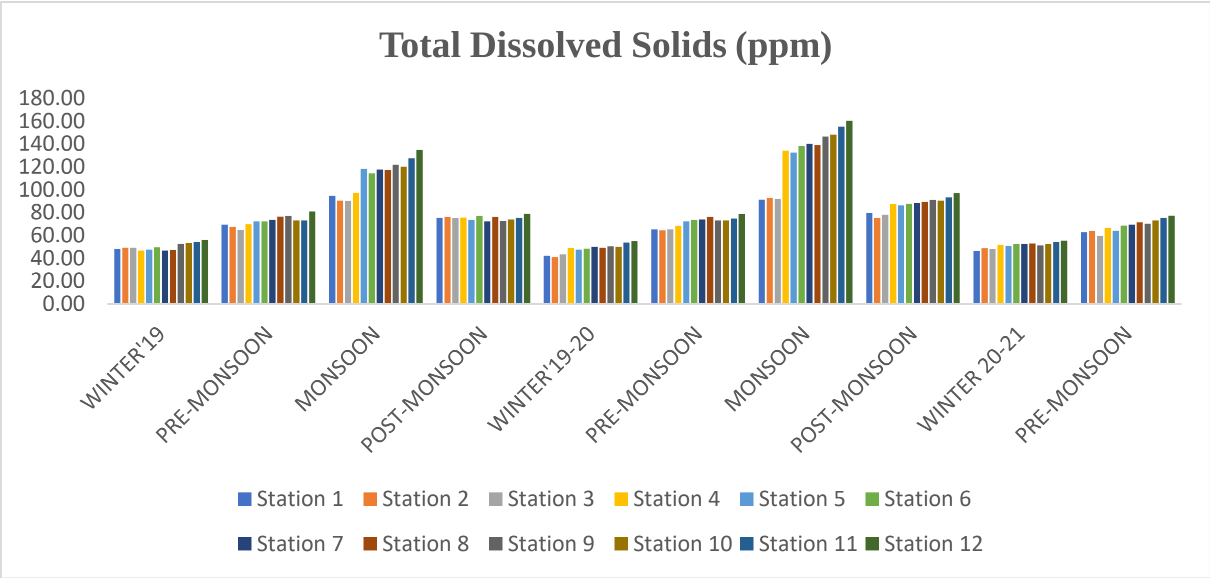


Figure: Seasonal Variation of Total Dissolved Solids at Station 1-12

Stations	Minimum	Maximum
1	42.10±8.32 (Winter, 2019-20)	94.72±3.96 (Monsoon, 2019)
2	42.76±6.84 (Winter, 2019-20)	92.62±3.04 (Monsoon, 2020)
3	43.33±8.70 (Winter, 2019-20)	91.74±7.42 (Monsoon, 2020)
4	46.58±5.43 (Winter, 2019)	134.24±22.47 (Monsoon, 2020)
5	47.41±6.49 (Winter, 2019)	132.46±20.06 (Monsoon, 2020)
6	48.39±9.52 (Winter, 2019-20)	138.12±17.41 (Monsoon, 2020)
7	46.63±5.63 (Winter, 2019)	140.18±19.81 (Monsoon, 2020)
8	47.18±5.18 (Winter, 2019)	138.93±24.82 (Monsoon, 2020)
9	50.28±5.34 (Winter, 2019-20)	146.48±27.42 (Monsoon, 2020)
10	49.92±6.02 (Winter, 2019-20)	148.13±33.37 (Monsoon, 2020)
11	53.61±4.49 (Winter, 2019-20)	155.15±27.26 (Monsoon, 2020)
12	54.86±3.93 (Winter, 2019-20)	160.35±26.44 (Monsoon, 2020)

7. Nitrate-Nitrogen: Nitrogen undergoes quick transformation in the tropical river and gets stored in the biota. In the present investigation nitrate-nitrogen value ranged in between 0.016±0.005 (Winter, 2019) and 0.637±0.070 (Monsoon, 2019) mgL^{-1}.

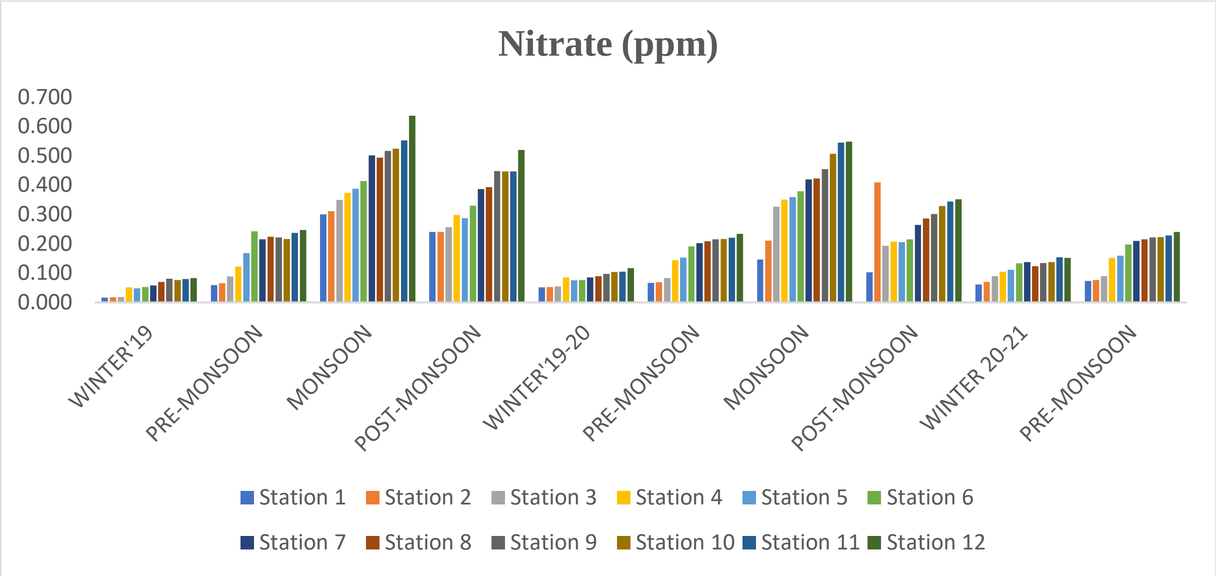


Figure: Seasonal Variation Nitrate-Nitrogen at Station 1-12

Stations	Minimum	Maximum
1	0.016±0.005 (Winter, 2019)	0.300±0.027 (Monsoon, 2019)
2	0.018±0.006 (Winter, 2019)	0.410±0.041 (Post-monsoon, 2020)
3	0.019±0.005 (Winter, 2019)	0.350±0.044 (Monsoon, 2019)
4	0.051±0.0 (Winter, 2019)	0.376±0.046 (Monsoon, 2019)
5	0.048±0.006 (Winter, 2019)	0.387±0.052 (Monsoon, 2019)
6	0.052±0.008 (Winter, 2019)	0.413±0.025 (Monsoon, 2019)
7	0.058±0.003 (Winter, 2019)	0.501±0.012 (Monsoon, 2019)
8	0.070±0.006 (Winter, 2019)	0.493±0.016 (Monsoon, 2019)
9	0.081±0.011 (Winter, 2019)	0.516±0.040 (Monsoon, 2019)
10	0.077±0.005 (Winter, 2019)	0.524±0.047 (Monsoon, 2019)
11	0.080±0.005 (Winter, 2019)	0.552±0.069 (Monsoon, 2019)
12	0.083±0.002 (Winter, 2019)	0.637±0.070 (Monsoon, 2019)

8. Nitrite – Nitrogen: Nitrite is one of the intermediate products of aerobic nitrification bacterial process, produced by the autotrophic Nitrosomonas bacteria combining oxygen and ammonia. They are unstable and depending on conditions, can be converted into nitrates or ammonia which are harmful to aquatic life. In our present investigation nitrite nitrogen lowest value was found to be 0.0016±0.0005 (Winter, 2019) $\mu g l^{-1}$ and highest to be 0.064±0.007 (Monsoon, 2019) $\mu g l^{-1}$.

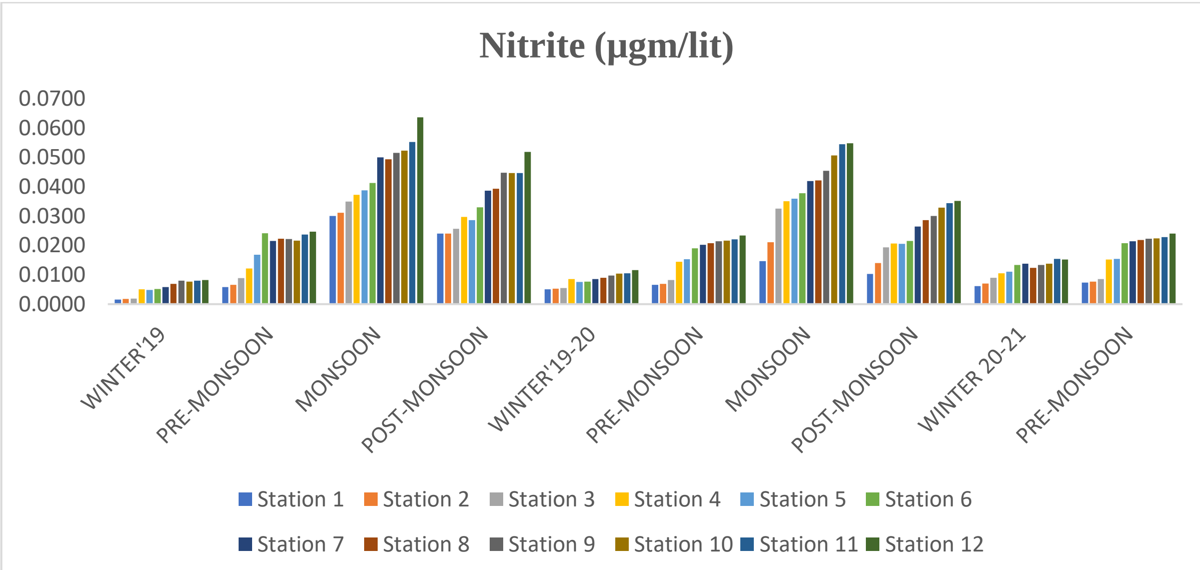


Figure: Seasonal Variation of Nitrite-Nitrogen at Station 1-12

Stations	Minimum	Maximum
1	0.0016±0.0005 (Winter, 2019)	0.036± 0.002(Monsoon, 2019)
2	0.0018±0.0006 (Winter, 2019)	0.041±0.004 (Post-monsoon, 2020)
3	0.0019±0.0005 (Winter, 2019)	0.035± 0.004(Monsoon, 2019)
4	0.0051±0.00 (Winter, 2019)	0.037±0.005 (Monsoon, 2019)
5	0.0048±0.0006 (Winter, 2019)	0.038±0.005 (Monsoon, 2019)
6	0.0052±0.0008 (Winter, 2019)	0.041±0.002 (Monsoon, 2019)
7	0.0058±0.0006 (Winter, 2019)	0.050±0.001 (Monsoon, 2019)
8	0.0070±0.0010 (Winter, 2019)	0.049±0.002 (Monsoon, 2019)
9	0.0081±0.0005 (Winter, 2019)	0.051±0.004 (Monsoon, 2019)
10	0.0077±0.0005 (Winter, 2019)	0.052±0.005 (Monsoon, 2019)
11	0.0080±0.0005 (Winter, 2019)	0.055±0.007 (Monsoon, 2019)
12	0.0083±0.0002 (Winter, 2019)	0.064±0.007 (Monsoon, 2019)

9. Total Ammonia: Ammonia is a highly toxic pollutant of the aquatic environment. The by-product of protein metabolism excreted by fish and bacterial decomposition of organic matter such as wasted food, agricultural wastes, dead planktons, sewage etc. is ammonia. The unionized form of ammonia (NH_3) is extremely toxic while the ionized form (NH_4^-) is not and both the forms are grouped together as “total ammonia”. Total ammonia values of the water samples of the Kopili River during the study period varied from 0.155± 0.35(Winter, 2019) to 2.285±0.086 (Monsoon, 2020).

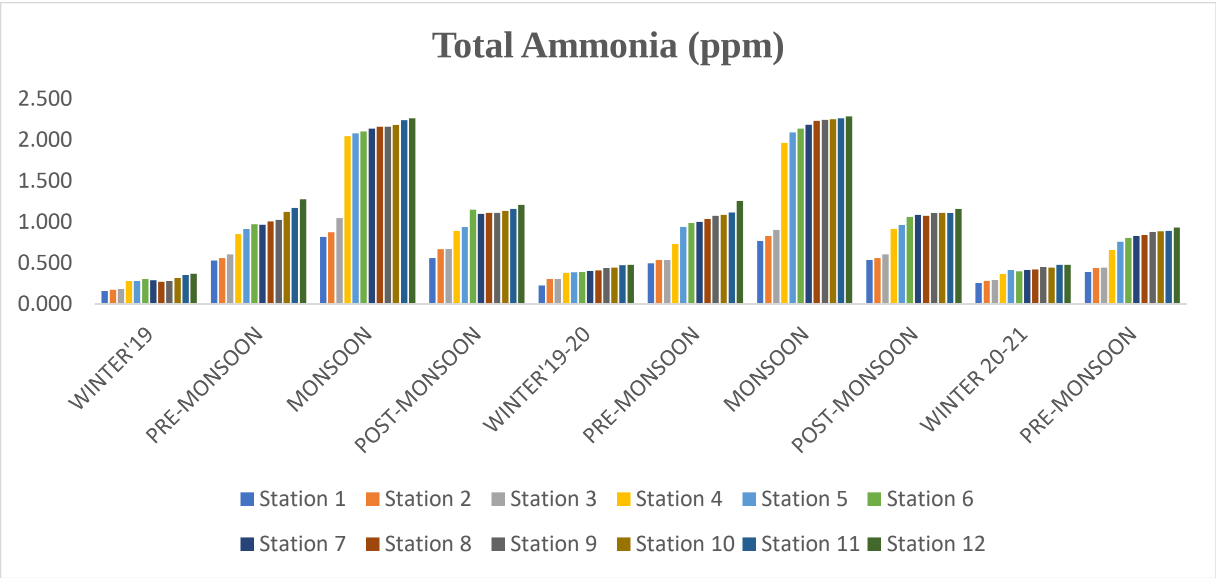


Figure: Seasonal Variation of Total Ammonia at Station 1-12

Stations	Minimum	Maximum
1	0.155± 0.35(Winter, 2019)	0.819±0.043 (Monsoon, 2019)
2	0.175±0.025 (Winter, 2019)	0.875±0.039 (Monsoon, 2019)
3	0.185±0.005 (Winter, 2019)	1.044±0.150 (Monsoon, 2019)
4	0.280±0.030 (Winter, 2019)	2.044±0.053 (Monsoon, 2019)
5	0.280±0.010 (Winter, 2019)	2.090±0.071 (Monsoon, 2020)
6	0.305±0.015 (Winter, 2019)	2.137±0.178 (Monsoon, 2020)
7	0.290±0.010 (Winter, 2019)	2.183±0.085 (Monsoon, 2020)
8	0.275±0.015 (Winter, 2019)	2.230±0.099 (Monsoon, 2020)
9	0.282±0.012 (Winter, 2019)	2.243±0.095 (Monsoon, 2020)
10	0.320±0.020 (Winter, 2019)	2.250±0.099 (Monsoon, 2020)
11	0.350±0.030 (Winter, 2019)	2.260±0.097 (Monsoon, 2020)
12	0.370±0.020 (Winter, 2019)	2.285±0.086 (Monsoon, 2020)

10. Soluble Inorganic Phosphate:

Phosphorous is an important parameter to assess the water quality since it is the limiting nutrient for plant growth in the freshwater system (Stickney, 2005) which regulates the phytoplankton production in presence of nitrogen. The availability of phosphate in water depends on the organic matter content of bottom and type of microorganisms present in the system. The release of phosphate is dependent on soil reaction. The slightly acidic condition of the medium favors the release and availability of phosphate into the water. Soluble inorganic phosphate values of the present investigation ranged from 1.21± 0.04(Winter, 2019) to 3.34±0.46 (Monsoon, 2019).

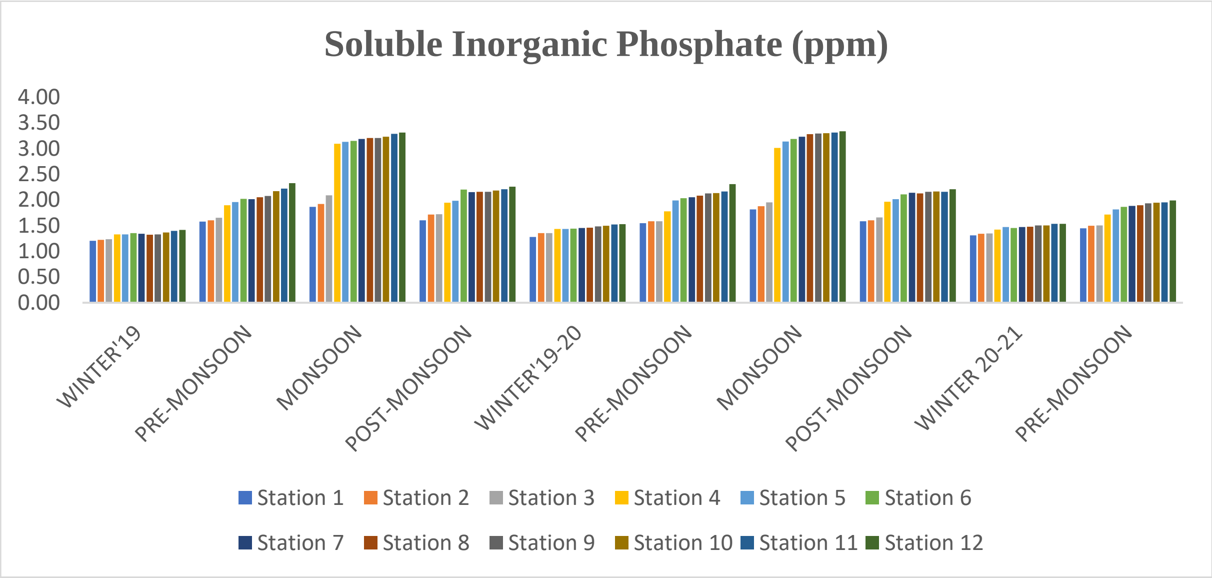


Figure: Seasonal Variation of Soluble Inorganic Phosphate at Station 1-12

Stations	Minimum	Maximum
1	1.21± 0.04(Winter, 2019)	1.87±0.04 (Monsoon, 2019)
2	1.23±0.03 (Winter, 2019)	1.93±0.04 (Monsoon, 2019)
3	1.24±0.01 (Winter, 2019)	2.09±0.15 (Monsoon, 2019)
4	1.33±0.03 (Winter, 2019)	3.09±0.05 (Monsoon, 2019)
5	1.33±0.01 (Winter, 2019)	3.14±0.10 (Monsoon, 2019)
6	1.36±0.02 (Winter, 2019)	3.19±0.12 (Monsoon, 2019)
7	1.34±0.01 (Winter, 2019)	3.23±0.14 (Monsoon, 2019)
8	1.33±0.03 (Winter, 2019)	3.28±0.11 (Monsoon, 2019)
9	1.33±0.01 (Winter, 2019)	3.29±0.08 (Monsoon, 2019)
10	1.37±0.02 (Winter, 2019)	3.30±0.09 (Monsoon, 2019)
11	1.40±0.01 (Winter, 2019)	3.31±0.22 (Monsoon, 2019)
12	1.42±0.03 (Winter, 2019)	3.34±0.46 (Monsoon, 2019)

ANNEXURE- VII
Sediment Parameters of River Kopili

1. Sediment pH: Sediment pH measures the acidic and alkaline condition of the river bed which has a direct or indirect influence on water pH and nutrient circulation. The findings of present study indicate that sediment pH varied between 6.02 (Monsoon, 2019) to 7.72 (post-monsoon, 2020).

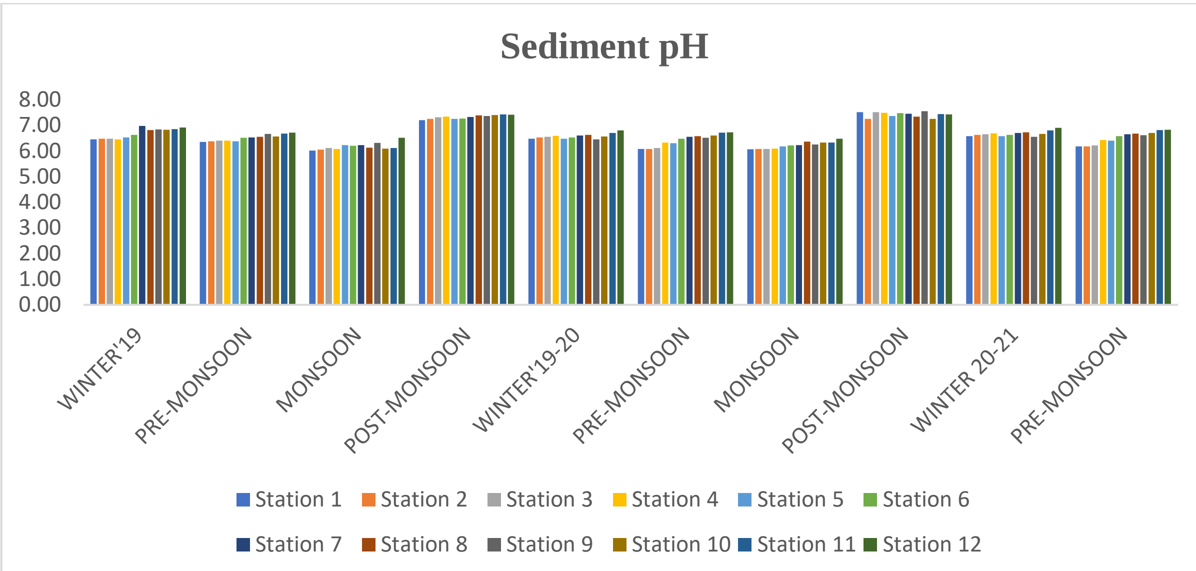


Figure: Seasonal Variation of Sediment pH at Station 1-12

Stations	Minimum	Maximum
1	6.02 (Monsoon, 2019)	7.51 (Post-monsoon, 2020)
2	6.05 (Monsoon, 2019)	7.25 (Post-monsoon, 2020)
3	6.08 (Monsoon, 2020)	7.51 (Post-monsoon, 2020)
4	6.08 (Monsoon, 2019)	7.48 (Post-monsoon, 2020)
5	6.18 (Monsoon, 2020)	7.36 (Post-monsoon, 2020)
6	6.20 (Monsoon, 2019)	7.47 (Post-monsoon, 2020)
7	6.23 (Monsoon, 2019)	7.45 (Post-monsoon, 2020)
8	6.13 (Monsoon, 2019)	7.38 (Post-monsoon, 2020)
9	6.25 (Monsoon, 2020)	7.55 (Post-monsoon, 2020)
10	6.09 (Monsoon, 2019)	7.40 (Post-monsoon, 2019)
11	6.12 (Monsoon, 2019)	7.44 (Post-monsoon, 2020)
12	6.48 (Monsoon, 2020)	7.72 (Post-monsoon, 2020)

2. **Sediment Organic Carbon:** In present investigation Sediment Organic Carbon percentages were found within the range of 0.29-2.63%, minimum during winter and maximum during post-monsoon season.

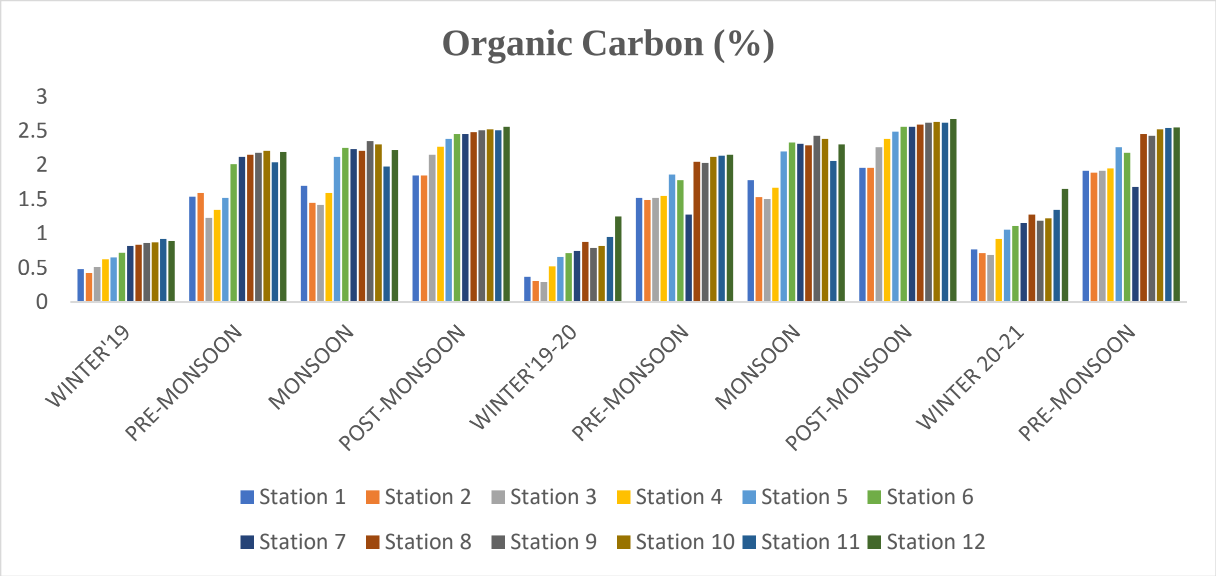


Figure: Seasonal Variation of Sediment Organic Carbon at Station 1-12

Stations	Minimum	Maximum
1	0.37 (Winter, 2020)	1.96 (Post-monsoon, 2020)
2	0.31 (Winter, 2020)	1.96 (Post-monsoon, 2020)
3	0.29 (Winter, 2020)	2.26 (Post-monsoon, 2020)
4	0.52 (Winter, 2020)	2.38 (Post-monsoon, 2020)
5	0.65 (Winter, 2020)	2.49 (Post-monsoon, 2020)
6	0.71 (Winter, 2020)	2.56 (Post-monsoon, 2020)
7	0.75 (Winter, 2020)	2.56 (Post-monsoon, 2020)
8	0.84 (Winter, 2020)	2.59 (Post-monsoon, 2020)
9	0.79 (Winter, 2020)	2.62 (Post-monsoon, 2020)
10	0.82 (Winter, 2020)	2.63 (Post-monsoon, 2020)
11	0.92 (Winter, 2019)	2.62 (Post-monsoon, 2020)
12	0.89 (Winter, 2019)	2.36 (Post-monsoon, 2020)

3. Sediment Organic Matter: Sediment organic matter of the present investigation ranged from 0.50 to 4.60 %.

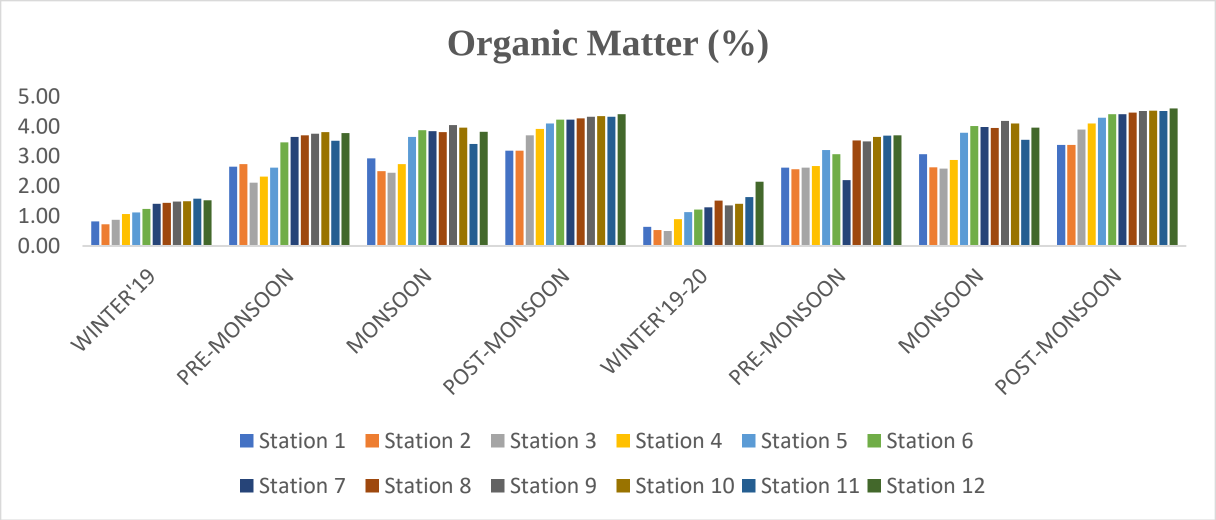


Figure: Seasonal Variation of Sediment Organic Matter at Station 1-12

Stations	Minimum	Maximum
1	0.64 (Winter, 2020)	3.38 (Post-monsoon, 2020)
2	0.53(Winter, 2020)	3.38 (Post-monsoon, 2020)
3	0.50(Winter, 2020)	3.90 (Post-monsoon, 2020)
4	0.90(Winter, 2020)	4.10 (Post-monsoon, 2020)
5	1.12(Winter, 2020)	4.29 (Post-monsoon, 2020)
6	1.22(Winter, 2020)	4.41 (Post-monsoon, 2020)
7	1.29(Winter, 2020)	4.41 (Post-monsoon, 2020)
8	1.45(Winter, 2020)	4.47 (Post-monsoon, 2020)
9	1.36(Winter, 2020)	4.52 (Post-monsoon, 2020)
10	1.41(Winter, 2020)	4.53 (Post-monsoon, 2020)
11	1.59(Winter, 2019)	4.52 (Post-monsoon, 2020)
12	1.53(Winter, 2019)	4.60 (Post-monsoon, 2020)

4. Sediment Available Nitrogen: Sediment total nitrogen of the present investigation ranged from 359.00 kgha^{-1} to 425.00 kgha^{-1}.

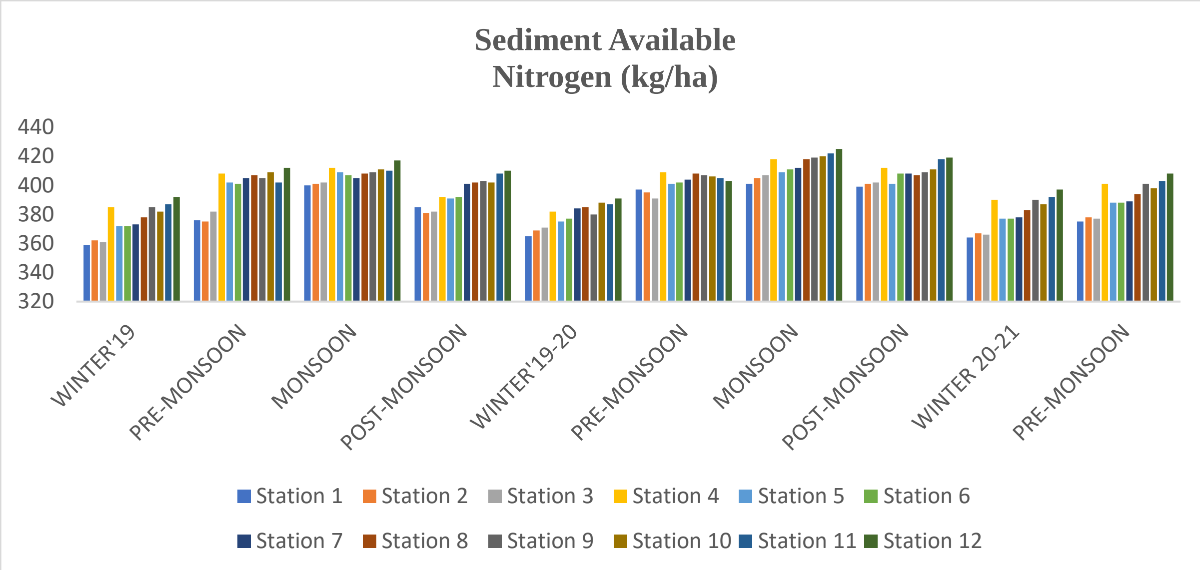


Figure: Seasonal Variation of Sediment Available Nitrogen at Station 1-12

Stations	Minimum	Maximum
1	359.00 (Winter, 2019)	401.00 (Monsoon, 2020)
2	362.00 (Winter, 2019)	405.00 (Monsoon, 2020)
3	361.00 (Winter, 2019)	407.00 (Monsoon, 2020)
4	382.00 (Winter, 2020)	418.00 (Monsoon, 2020)
5	372.00 (Winter, 2019)	409.00 (Monsoon, 2020)
6	372.00 (Winter, 2019)	411.00 (Monsoon, 2020)
7	373.00 (Winter, 2019)	412.00 (Monsoon, 2020)
8	378.00 (Winter, 2019)	418.00 (Monsoon, 2020)
9	380.00 (Winter, 2020)	419.00 (Monsoon, 2020)
10	382.00 (Winter, 2019)	420.00 (Monsoon, 2020)
11	387.00 (Winter, 2019)	422.00 (Monsoon, 2020)
12	391.00 (Winter, 2020)	425.00 (Monsoon, 2020)

5. Sediment Phosphorus: Sediment phosphorus of the present investigation ranged from 4.90 $kgha^{-1}$ to 9.28 $kgha^{-1}$.

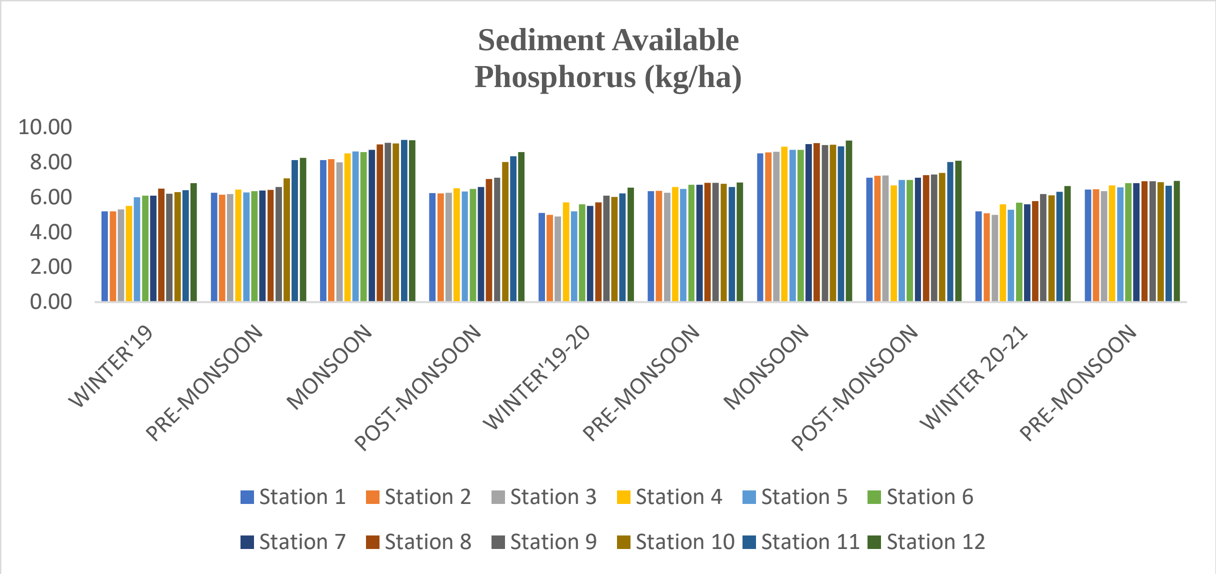


Figure: Seasonal Variation of Sediment Available Phosphorus at Station 1-12

Stations	Minimum	Maximum
1	5.10 (Winter, 2019-20)	8.50 (Monsoon, 2020)
2	5.00 (Winter, 2019-20)	8.56 (Monsoon, 2020)
3	4.90 (Winter, 2019-20)	8.61 (Monsoon, 2020)
4	5.50 (Winter, 2019)	8.89 (Monsoon, 2020)
5	5.20 (Winter, 2019-20)	8.72 (Monsoon, 2020)
6	5.60 (Winter, 2019-20)	8.71 (Monsoon, 2020)
7	5.50 (Winter, 2019-20)	9.05 (Monsoon, 2020)
8	5.70 (Winter, 2019-20)	9.10 (Monsoon, 2020)
9	6.10 (Winter, 2019-20)	9.12 (Monsoon, 2019)
10	6.02 (Winter, 2019-20)	9.08 (Monsoon, 2019)
11	6.23 (Winter, 2019-20)	9.28 (Monsoon, 2019)
12	6.56 (Winter, 2019-20)	9.27 (Monsoon, 2019)

6. Sediment Potassium: Sediment potassium of the present investigation was ranges from 71.25 $kgha^{-1}$ to 345.12 $kgha^{-1}$.

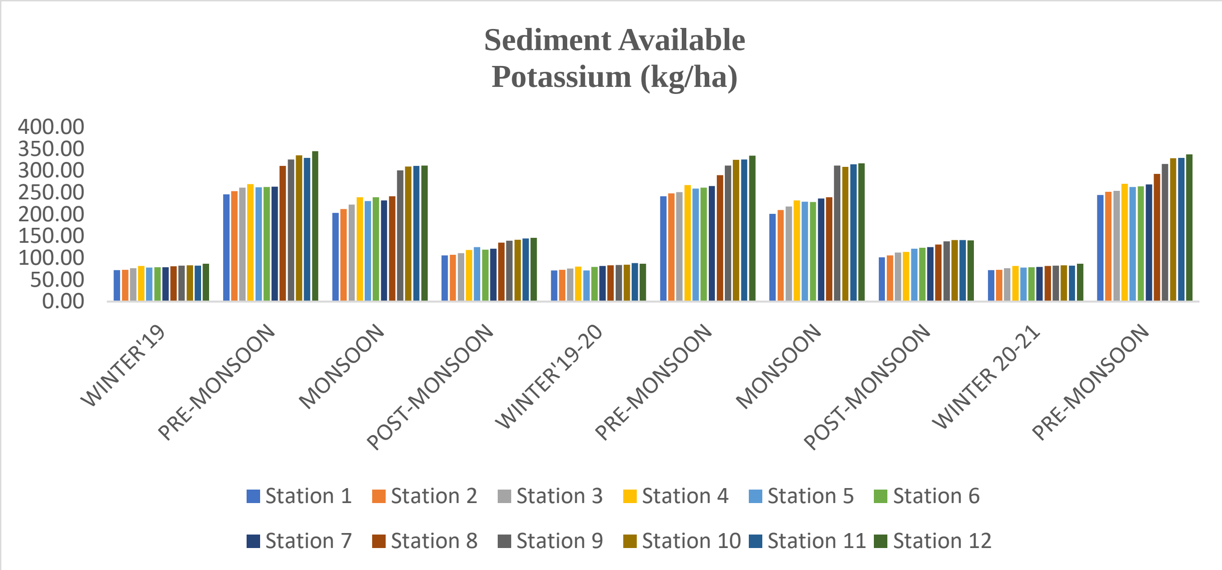


Figure: Seasonal Variation of Sediment Available Potassium at Station 1-12

Stations	Minimum	Maximum
1	71.25 (Winter, 2019-20)	245.80 (Monsoon, 2019)
2	72.91 (Winter, 2019-20)	253.40 (Monsoon, 2019)
3	75.60 (Winter, 2019-20)	261.30 (Monsoon, 2019)
4	80.00 (Winter, 2019-20)	269.05 (Monsoon, 2019)
5	71.28 (Winter, 2019-20)	261.80 (Monsoon, 2019)
6	78.92 (Winter, 2019-20)	262.80 (Monsoon, 2019)
7	79.11 (Winter, 2019)	265.25 (Pre-monsoon, 2020)
8	81.25 (Winter, 2019)	311.25 (Monsoon, 2019)
9	82.35 (Winter, 2019)	325.89 (Monsoon, 2019)
10	83.15 (Winter, 2019)	335.45 (Monsoon, 2019)
11	82.56 (Winter, 2019)	329.23 (Monsoon, 2019)
12	86.48 (Winter, 2019)	345.12 (Monsoon, 2019)

ANNEXURE VII

Photographs of Collected & Identified Fish Species

1. *Tor putitora*

2. *Neolissochilus hexagonolepis*

3. *Garra annandalei*

4. *Garra gotyla gotyla*

5. *Garra nasuta*

6. *Garra lamta*

7. *Garra lissorhynchus*

8. *Garra kempi*

9. *Opsarius bendelisis*

10. *Opsarius barna*

11. Barilius barila

12. Pethia stoliczkanus

13. Puntius chola

14. Pethia ticto

15. Pethia conchonius

16. Puntius sophore

17. Systomus sarana

18. Chagunius chagunio

19. Osteobrama cunma

20. Tariqilabeo latius

21. Labeo bata

22. Labeo calbasu

23. Labeo gonius

24. Labeo dyocheilus

25. Labeo pangusia

26. Labeo boga

27. Labeo fimbriatus

28. Labeo rohita

29. Labeo catla

30. Cirrhinus reba

31. Cirrhinus mrigala

32. Cyprinious carpio

33. Bengala elanga

34. Cabdio morar

35. Esomus danricus

36. Danio rerio

37. Devario devario

38. Devario aequipinnatus

39. Danio dangila

40. Salmostoma bacaila

41. Salmostoma phulo

42. Amblypharyngodon mola

43. Psilorhynchus homaloptera

44. Psilorhynchus balitora

45. Botia rostrata

46. Botia derio

47. Paracanthocobitis botia

48. Schistura fasciata

49. Schistura khugae

50. Schistura reticulata

	
51. Lepidocephalichthys guntea	*52. Lepidocephalichthys annandalei*
	
53. Notopterus synurus	*54. Chitala chitala*
	
55. Badis assamensis	*56. Badis badis*
	
57. Channa marulius	*58. Channa stewartii*
	
59. Channa gachua	*60. Channa punctata*

	
61. Nandus nandus	*62. Trichogaster fasciata*
	
63. Trichogaster lalius	*64. Trichogaster labiosa*
	
65. Channa striata	*66. Anabas testudineus*
	
67. Glossogobius giuris	*68. Chanda nama*
	
69. Parambassis baculis	*70. Parambassis ranga*

71. Laubuka laubuca

72. Mystus cavasius

73. Mystus tengara

74. Mystus bleekeri

75. Mystus vittatus

76. Rita rita

77. Sperata aor

78. Sperata seenghala

79. Olyra kempi

80. Tetradon cutcutia

81. *Heteropneustes fossilis*

82. *Wallago attu*

83. *Ompok bimaculatus*

84. *Ompok pabo*

85. *Ompok pabda*

86. *Glyptothorax striatus*

87. *Glyptothorax telchitta*

88. *Bagarius bagarius*

89. *Clupisoma garua*

90. *Gagata cenia*

91. *Gagata gagata*

92. *Ailia coilia*

93. *Erethistes hara*

94. *Setipinna phasa*

95. *Eutropiichthys vacha*

96. *Pachypterus atherinoides*

97. *Amblyceps apangi*

98. *Chaca chaca*

99. *Xenentodon cancila*

100. *Mastacembelus armatus*

	
101. *Macrognathus aral*	102. *Macrognathus aculeatus*
	
103. *Monopterus cuchia*	

ANNEXURE- VIII

Plankton Diversity and Biomass of River Kopili

The most sensitive component of aquatic ecosystem is the plankton which gives the signal about the environmental disturbances. Phytoplankton plays an important role in food chain as they are the key of primary productivity and also acts as a biological indicator of water quality in relation to pollution studies. Zooplankton provides fish with nutrients as they require protein, fats, carbohydrates, mineral salts and water in right proportion (Jabeen and Barbhuya, 2018). Plankton studies and monitoring are useful for assessment of the physico-chemical and biological conditions of the water in any purpose.

A total of 46 genera of plankton were recorded from River Kopili during the study period. Population of phytoplankton was represented by 35 genera belonging to Chlorophyceae (17 genera), Bacillariophyceae (10 genera), Cyanophyceae (7 genera) and Euglenophyceae (1genera). Zooplankton population was represented by Rotifera (5 genera), Cladocera (3 genera) and Copepoda (3 genera).

The population density of plankton varied from season to season. The average minimum plankton density was found to be 21.33±3.68 units/L in monsoon 2019 in station 1 and maximum in winter 2019-20 in station 12 (626.67±13.10 units/L).

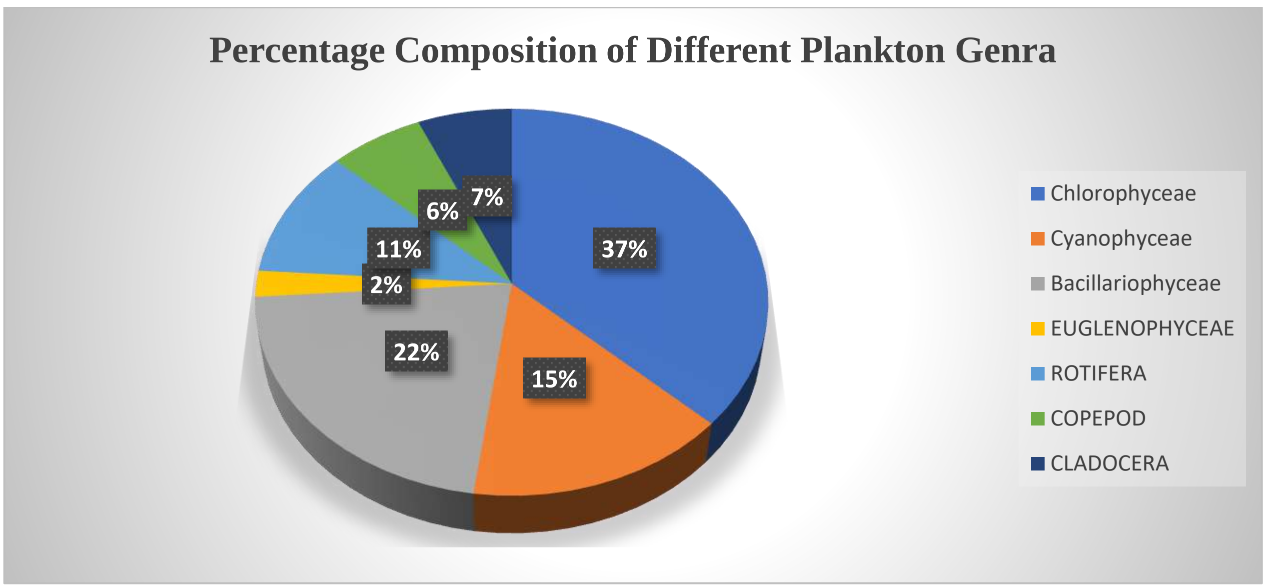


Figure: Percentage contribution of different plankton genera in river Kopili recorded during the study period.

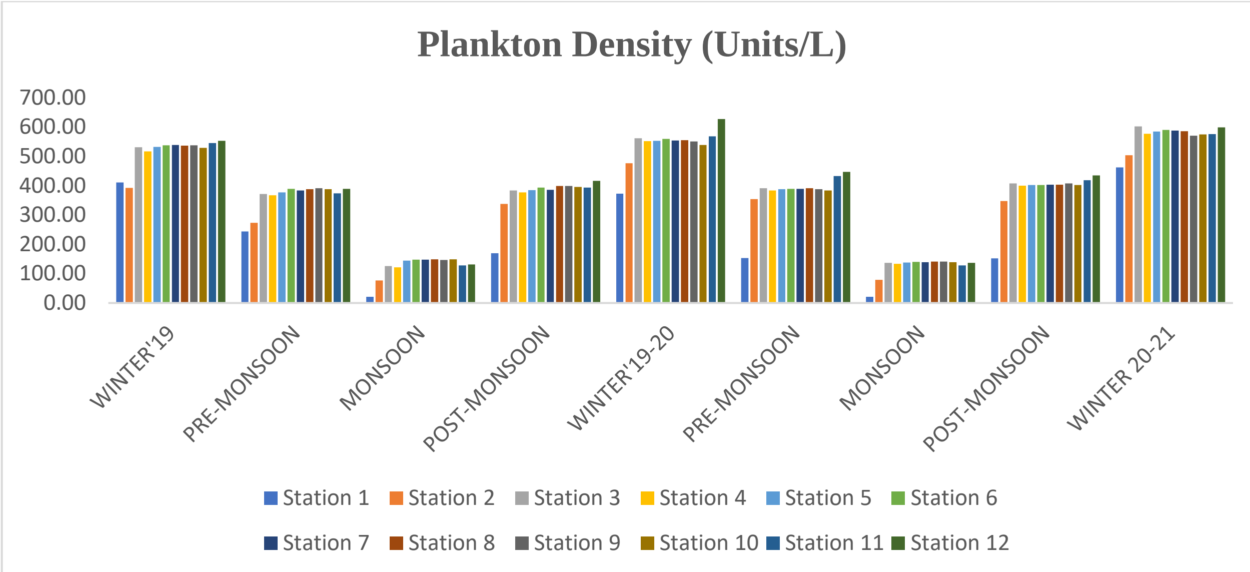


Figure: Seasonal variation of plankton density at stations 1 to 12 during the study period.

Phytoplankton:

The phytoplankton community of the study area constituted 76.09 % out of the total plankton collected throughout the studied period. Out of the 35 genera of phytoplankton recorded, Chlorophyceae comprises of 36.96 %, Bacillariophyceae 21.74 %, Cyanophyceae 15.22 % and Euglenophyceae 2.17 % of the total plankton composition. Phytoplankton density of the studied Kopili river ranges from 16 cells/L to 508 cells/L being the maximum in winter 2021 and minimum during monsoon, 2020.

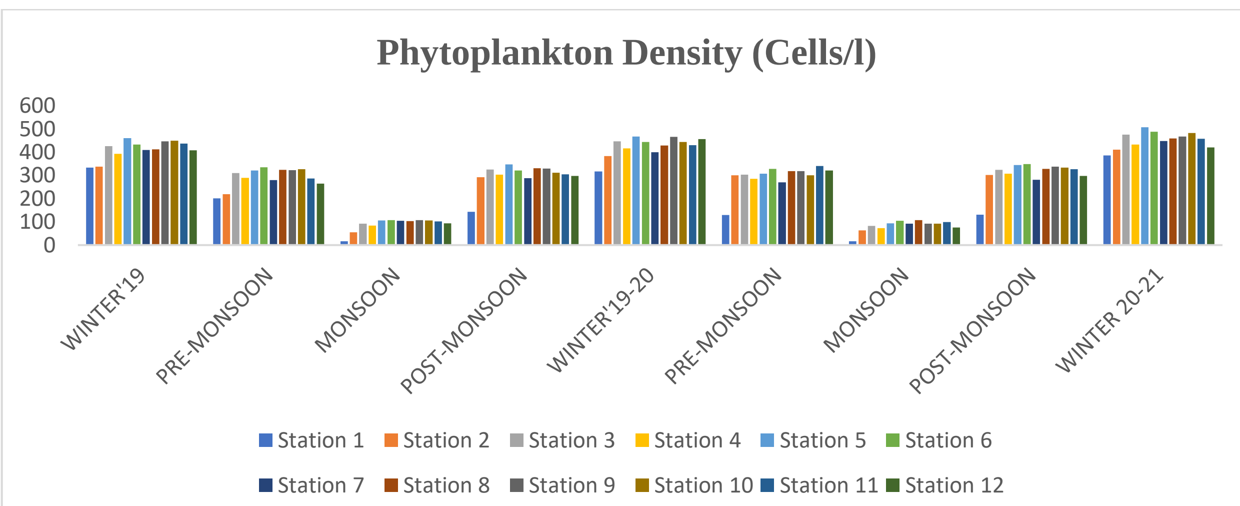


Figure: Seasonal variation of phytoplankton density at stations 1 to 12 during the study period.

Table: Phytoplankton composition of the 12 stations observed during the present study

Genus	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	Station 10	Station 11	Station 12
Chlorophyceae												
Closterium	-	+	+	-	+	+	+	+	+	+	+	+
Cosmarium	+	+	-	-	-	+	-	-	+	+	+	-
Staurastrum	+	-	+	+	+	+	+	+	+	+	+	+
Penium	+	+	+	+	+	-	+	+	+	+	+	+
Zygnema	+	+	+	+	+	+	+	+	+	+	+	+
Pediastrum	-	-	-	+	+	+	+	+	-	+	+	+
Pandorina	-	-	-	-	+	+	+	+	-	+	+	+
Oedogonium	+	+	+	-	+	+	+	+	+	+	+	+
Eudorina	+	+	+	+	+	+	+	+	+	+	+	+
Microspora	+	+	+	-	-	+	+	+	+	+	+	+
Scenedesmus	+	-	+	+	-	+	-	+	+	+	+	+
Oocystis	+	+	+	+	+	-	+	-	+	+	+	-
Cladophora	+	+	+	+	+	+	+	+	+	+	-	+
Ulothrix	-	+	-	+	+	+	+	+	+	+	+	+
Volvox	-	-	-	+	+	+	-	+	+	-	+	+
Spirogyra	+	+	+	+	+	+	+	+	+	-	+	+
Chlorella	+	+	+	-	-	+	+	+	+	-	+	+
Bacillariophyceae												
Tabellaria	+	+	+	+	+	+	+	-	+	+	+	+
Fragilaria	-	+	+	+	+	+	+	+	+	+	+	+
Navicula	-	+	-	+	+	+	+	+	+	+	+	+
Nitzschia	-	-	+	+	+	+	+	+	+	+	+	+
Amphora	+	+	+	-	+	+	+	+	+	-	-	-
Gomphonema	-	+	+	-	+	-	+	+	+	-	+	+
Cocconeis	+	+	+	-	+	+	+	+	+	+	+	+
Melosira	-	+	+	+	+	+	+	+	-	+	+	+

Cyclotella	+	+	+	+	+	+	+	+	+	+	+	+
Frustulia	+	+	-	+	+	+	-	+	-	+	+	+
Cyanophyceae												
Synedra	-	+	0	+	+	+	+	-	+	+	+	+
Chroococcus	+	+	+	-	+	+	+	+	+	+	-	-
Oscillatoria	-	-	+	+	+	+	+	+	+	+	-	+
Anabena	-	+	+	+	-	+	+	+	+	+	+	+
Merismopedia	+	-	+	+	+	-	+	+	+	+	+	+
Spirulina	+	+	-	+	+	+	-	+	+	+	+	-
Nostoc	+	+	-	+	+	+	+	+	+	+	+	+
Euglenophyceae												
Phacus	+	+	+	+	+	+	-	+	+	+	+	+

Table: Zooplankton composition of the 12 stations observed during the present study

Genus	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	Station 10	Station 11	Station 12
Rotifera												
Brachionus	-	+	+	+	+	+	+	+	+	+	+	+
Keratella	-	-	+	+	+	+	+	+	+	+	-	+
Lecane	+	-	+	-	+	+	+	+	+	+	+	+
Monostyla	-	+	-	+	+	+	+	+	+	+	+	+
Polyarthra	-	+	-	+	-	+	+	+	+	+	+	+
Copepod												
Cyclops	+	-	+	+	+	+	+	+	+	+	+	+
Diaptomus	-	+	+	+	+	+	+	+	+	+	+	+
Mesocyclops	-	+	+	+	+	+	+	+	+	+	+	+
Cladocera												
Daphinia	+	+	+	+	+	+	+	+	+	+	+	+
Moina	+	+	+	+	+	+	+	+	+	+	+	+
Bosmina	+	-	+	+	+	+	+	+	+	+	+	+

Zooplankton:

Zooplankton community constituted only 23.91 % of the total plankton hauled. A total of 11 genera zooplankton were recorded and being the highest in rotifera (10.87 %). Zooplankton density of the studied Kopili river ranges from 4 nos/m^3 to 178 nos/m^3 being the maximum in winter 2019 and minimum during monsoon, 2019.

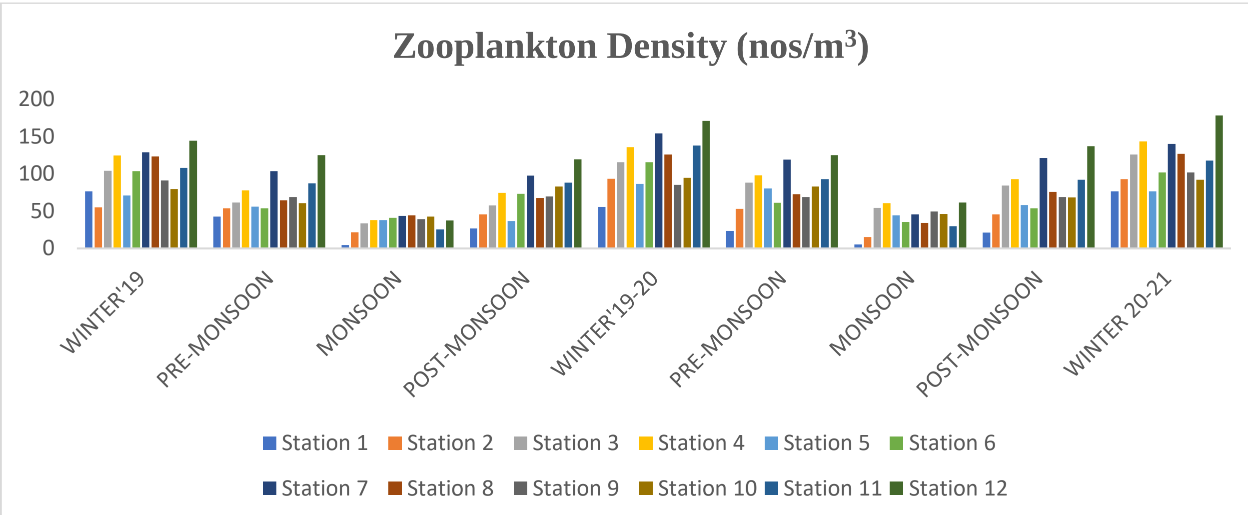


Figure: Seasonal variation of Zooplankton density at stations 1 to 12 during the study period.

Palmer's Index:

Palmer (1969) first made the list of algae genera and species which indicate organic pollution. According to Palmer, scores of 20 or more are indication of high organic pollution. By using Palmer's index of pollution for rating of water samples as lack of organic pollution, moderate and high organic polluted at all the stations were tested. The total score of Agal Genus Pollution Index (AGPI) of the sites S1<S2<S4<S5<S3<S11<S7<S10<S8<S6<S12 were calculated to be 8, 9, 15, 18, 19, 20, 21, 21, 23, 24, 24 and 25 respectively. It was observed that the total score of S1 and S2 showed below 10 which indicates lack of organic pollution. Sharpe increase in total score of 18 in station 4 indicating high organic pollution due to tourist influx according to Palmer (1969). Navicula, Nitzcha and Synedra were recorded repeatedly in lower stations of Kopili river and consider as indicators of pollution in view of results of Palmer's index.

Table: Algal genus pollution index (Palmer, 1969).

Genus	Pollution Index	Genus	Pollution Index
Anacystis	1	Micractinium	1
Ankistrodesmus	2	Navicula	3
Chlamydomonas	4	Nitzschia	3
Chlorella	3	Oscillatoria	5
Closterium	1	Pandorina	1
Cyclotella	1	Phacus	2
Euglena	5	Phormidium	1
Gomphonema	1	Scenedesmus	4
Lepocinclis	1	Stigeoclonium	2
Melosira	1	Synedra	2

Following numerical values for pollution classification of Palmer (1969), 0-10= Lack of organic pollution 10-15= Moderate pollution 15-20= Probable high organic pollution 20 or more = Confirms high organic pollution.

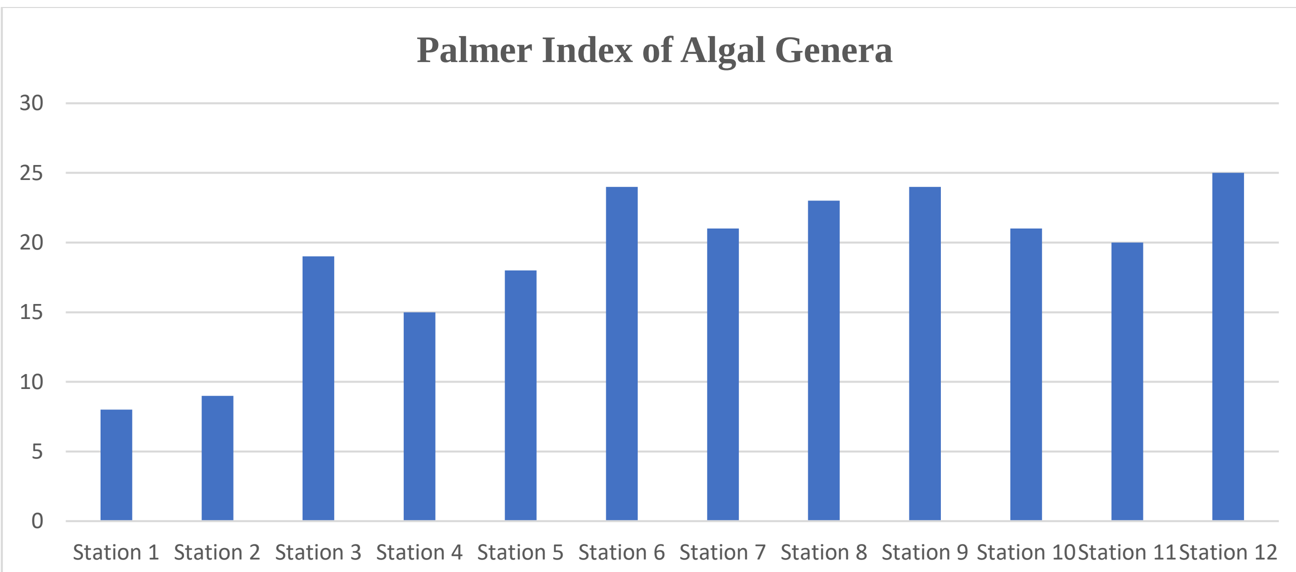


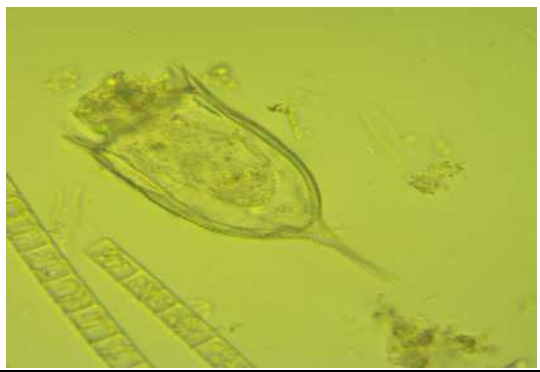
Fig:

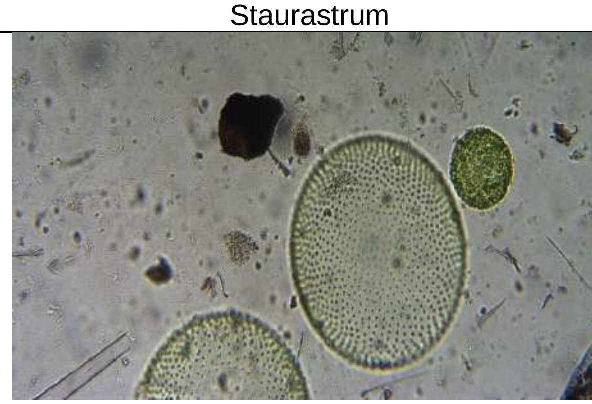
Table: Pollution index of Algal genera according to Palmer, (1969) at 12 stations of Kopili River

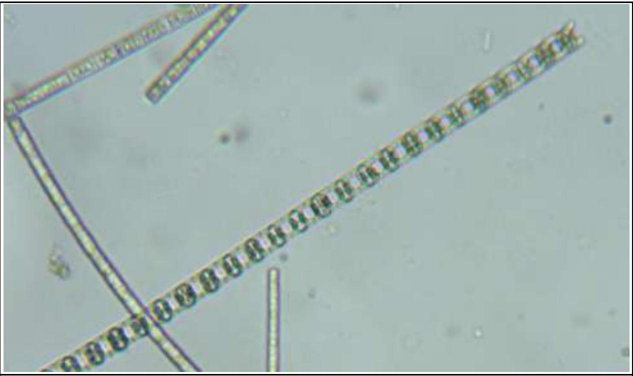
Genus	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	Station 10	Station 11	Station 12
Chlorophyceae												
Closterium	0	1	1	0	1	1	1	1	1	1	1	1
Cosmarium	+	+	-	-	-	+	-	-	+	+	+	-
Staurastrum	+	-	+	+	+	+	+	+	+	+	+	+
Penium	+	+	+	+	+	-	+	+	+	+	+	+
Zygnema	+	+	+	+	+	+	+	+	+	+	+	+
Pediastrum	-	-	-	+	+	+	+	+	-	+	+	+
Pandorina	0	0	0	0	1	1	1	1	0	1	1	1
Oedogonium	+	+	+	-	+	+	+	+	+	+	+	+
Eudorina	+	+	+	+	+	+	+	+	+	+	+	+
Microspora	+	+	+	-	-	+	+	+	+	+	+	+
Scenedesmus	4	0	4	0	0	4	0	4	4	4	4	4
Oocystis	+	+	+	+	+	-	+	-	+	+	+	-
Cladophora	+	+	+	+	+	+	+	+	+	+	-	+
Ulothrix	-	+	-	+	+	+	+	+	+	+	+	+
Volvox	-	-	-	+	+	+	-	+	+	-	+	+
Spirogyra	+	+	+	+	+	+	+	+	+	-	+	+
Chlorella	3	3	3	0	0	3	3	3	3	0	3	3
Bacillariophyceae												
Tabellaria	+	+	+	+	+	+	+	-	+	+	+	+
Fragilaria	-	+	+	+	+	+	+	+	+	+	+	+
Navicula	0	0	0	3	3	3	3	3	3	3	3	3
Nitzschia	0	0	3	3	3	3	3	3	3	3	3	3
Amphora	+	-	+	-	+	+	+	+	+	-	-	-
Gomphonema	0	1	1	0	1	0	1	1	1	0	1	1
Cocconeis	+	+	+	-	+	+	+	+	+	+	+	+
Melosira	0	1	1	1	1	1	1	1	0	1	1	1
Cyclotella	1	1	1	1	1	1	1	1	1	1	1	1
Frustulia	+	+	-	+	+	+	-	+	-	+	+	+
Cyanophyceae												
Synedra	0	2	0	2	2	2	2	0	2	2	2	2

Chroococcus	+	+	+	-	+	+	+	+	+	+	-	-
Oscillatoria	0	0	5	5	5	5	5	5	5	5	0	5
Anabena	-	+	+	+	-	+	+	+	+	+	+	+
Merismopedia	+	-	+	+	+	-	+	+	+	+	+	+
Spirulina	+	+	-	+	+	+	-	+	+	+	+	-
Nostoc	+	+	-	+	+	+	+	+	+	+	+	+
Euglenophyceae												
Phacus	+	+	+	+	+	+	-	+	+	+	+	+
Total	8	9	19	15	18	24	21	23	24	21	20	25

Some of the Identified Plankton:

	
Anabaena	Closterium
	
Cosmarium	Fragilaria
	
Eudorina	Keratella
	
Melosira	Microspora

	
Navicula	Nitzchia
	
Pediastrum	Scenedesmus
	
Spirogyra	Staurastrum
	
Ulothrix	Volvox

	
Zygnema	Brachionus
	
Copepod	Cyclotella
	
Diaptomus	Moina
	
Tabellaria	Synedra

ANNEXURE- IX

Anthropogenic factors encountered during the study period

A. Impact of Coal Mining:

The Kopili river flows down to the Brahmaputra from the Meghalaya plateau in the south – and is now infamous for carrying coal slurry and acids used in rathole mining in Meghalaya. This practice, of creating narrow holes of about a meter in diameter in which only one person can enter, has been banned by the National Green Tribunal, India's top green court, but continues illegally. Kopili and its tributaries – Kharkor, Myntriang, Dinar, Longsom, Amring, Umrong, Longku and Langkri – are known to be heavily affected by rathole mining for coal that is rampant in Meghalaya, especially in the Jaintia Hills in the eastern part of the state. The rivers run reddish due to a phenomenon called Acid Mine Drainage (AMD), caused by active and abandoned mines, coal storage sites and overburdened rocks. Leaching of heavy metals and the washing down of the soil removed to reach the coal seams add to the pollution in the rivers. Due to this AMD the river water of Kopili became highly acidic during 2019-19; due to which not a single fish species was retrieved from NC hill areas during that period.

B. Impact of Dam:

Dams can impact fish biodiversity, fish stocks and fisheries indirectly by modifying and/or degrading upstream and downstream aquatic environments, including: thermal stratification; downstream flow alteration; release f trapped sediments from reservoir to the river etc. There are two dams present on Kopili river of which one dam is under construction. Hazardous situation was occurred during November, 2019 when pipeline of dam busted a havoc situation was created; which damaged not only the river ecosystem but also its riparian zone.

C. Impact of Sand Mining:

For the construction of industry and house hold purpose people are collecting sand from different parts of Kopili river bed as the texture of the sand is very fine and it is of high demand. The mining of sand from river bed as well as from river bank causes different types of hydrobiological changes within the river system (Kondolf 1993, 1998; Savior, 2012). Sometimes, sand mining from river bed is considered as a good practice because it reduces the sedimentation problems of river. But in the present study it creates further more problems to the river ecosystem because the sediments are quarried randomly from the river bed starting from its origin upto its confluence point, which makes the river bed irregular and fragile and may generate further sedimentation to the lower reaches of the river. Sometimes they use pump machines also for lifting sand from the river.

D. Impact of Bridge Piers on Kopili River:

Construction of bridge piers have some morphological impacts over river ecosystem (Lane, 1955). Pier scouring happens when discharge of water is unexpectedly increased, washing away large volumes of soil material next to bridge piers (Ashmore and Parker, 1983; Heidarjed et al., 2010). The majority of soil particles removed are surrounded by turbidity currents and deposited as bars immediately downstream of the bridge. Further these sediments free water is started eroding the downstream banks of river (Biswas, 2010; Mani and Patowary, 2000; Naik et al., 1999). There are several bridges located along the Kopili River.

E. Turbidity:

Owing to deforestation and soil erosion, several rivers and streams remaining in a turbid condition for several months, more so during monsoon and post monsoon seasons; which includes Kopili river also. During the study period it was observed that the river water became turbid in lower stretches during monsoon and post monsoon season compared to the other seasons. The turbidity of the water hinders the condition conductive for laying eggs by which fish to reproduce. This is one of the most disadvantageous environmental conditions during the breeding season.

Mitigation Measures:

1. Mining of sand should be strictly prohibited from the river bed.
2. Illegal and unscientific coal mining should be ban in the NC hill areas
3. Alternative of hydroelectric should be applied to minimize the threat to the river ecosystem.
4. Development of sport fishery: In linkage with tourism department involving creation of angling facilities and ranching of mahseer and trout in Kopili River.
5. Breeding grounds should be declared as sanctuaries at least during the breeding season.

Different anthropogenic factors encountered during the study period

ANNEXURE- X

Pollution Status of Kopili River

1. Biochemical Oxygen Demand (BOD_3): Biochemical oxygen demand (BOD_3) is a measure of the amount of oxygen required by the aerobic micro-organisms to stabilize the biochemically degradable organic matter to a stable inorganic form present in any water bodies. Municipal sewage treatment plants, agricultural wastes, raw sewages, industrial wastage are the major sources of BOD_3. During the present investigation, BOD_3 values were found to vary from 0.33±0.02 (Winter, 2019) to 19.61±0.51 (Monsoon, 2019).

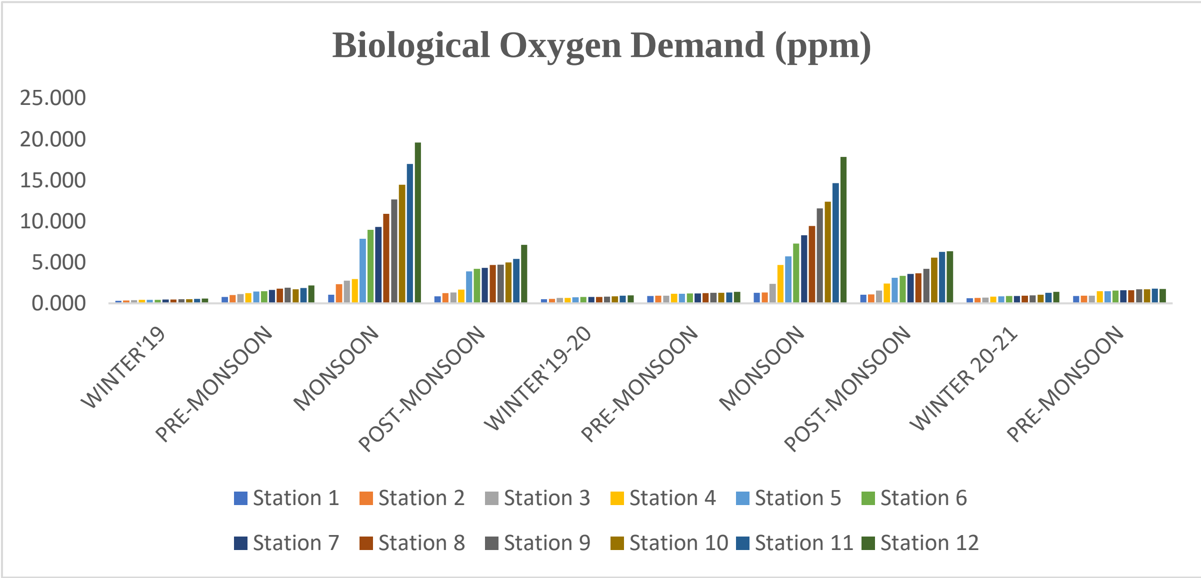


Figure: Seasonal Variation of Biological Oxygen Demand$_3$ at Station 1-12

Stations	Minimum	Maximum
1	0.33±0.02 (Winter, 2019)	1.30±0.14 (Monsoon, 2020)
2	0.34±0.02 (Winter, 2019)	2.32±0.36 (Monsoon, 2019)
3	0.37±0.19 (Winter, 2019)	2.77±0.22 (Monsoon, 2019)
4	0.44±0.19 (Winter, 2019)	4.68±0.57 (Monsoon, 2020)
5	0.44±0.02 (Winter, 2019)	7.88±0.31 (Monsoon, 2019)
6	0.44±0.02 (Winter, 2019)	8.96±0.61 (Monsoon, 2019)
7	0.47±0.02 (Winter, 2019)	9.31±0.17 (Monsoon, 2019)
8	0.48±0.02 (Winter, 2019)	10.93±0.41 (Monsoon, 2019)
9	0.49±0.02 (Winter, 2019)	12.67±1.70 (Monsoon, 2019)
10	0.50±0.02 (Winter, 2019)	14.47±0.19 (Monsoon, 2019)
11	0.53±0.02 (Winter, 2019)	16.98±1.14 (Monsoon, 2019)
12	0.60±0.02 (Winter, 2019)	19.61±0.51 (Monsoon, 2019)

2. Chemical Oxygen Demand (COD): Chemical Oxygen Demand (COD) test determines the oxygen requirement equivalent of organic matter that is susceptible to oxidation with the help of a strong chemical oxidant. During the present investigation, the minimum and maximum chemical oxygen demand values of the stations were found to be 0.53 (Winter, 2019) and 32.82 (Monsoon, 2019) respectively.

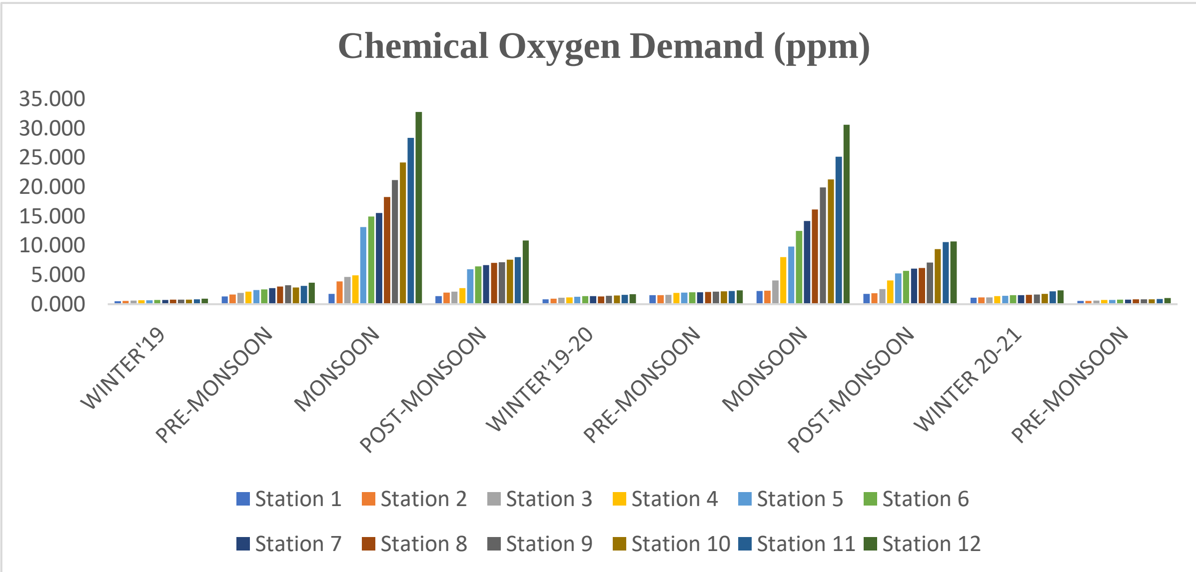


Figure: Seasonal Variation of Chemical Oxygen Demand at Station 1-12

Stations	Minimum	Maximum
1	0.53 (Winter, 2019)	2.24± (Monsoon, 2019)
2	0.54 (Winter, 2019)	3.90± (Monsoon, 2019)
3	0.60 (Winter, 2019)	4.64± (Monsoon, 2019)
4	0.70 (Winter, 2019)	8.03± (Monsoon, 2019)
5	0.70 (Winter, 2019)	13.17± (Monsoon, 2019)
6	0.71 (Winter, 2019)	14.97± (Monsoon, 2019)
7	0.74 (Winter, 2019)	15.58± (Monsoon, 2019)
8	0.76 (Winter, 2019)	18.27± (Monsoon, 2019)
9	0.79 (Winter, 2019)	21.21± (Monsoon, 2019)
10	0.80 (Winter, 2019)	24.21± (Monsoon, 2019)
11	0.85 (Winter, 2019)	28.38± (Monsoon, 2019)
12	0.96 (Winter, 2019)	32.82± (Monsoon, 2019)

3. Fecal Coliform: The fecal coliform count of Kopili river ranges from 87.50 to 1367.67 CFU/100 ml during the study period.

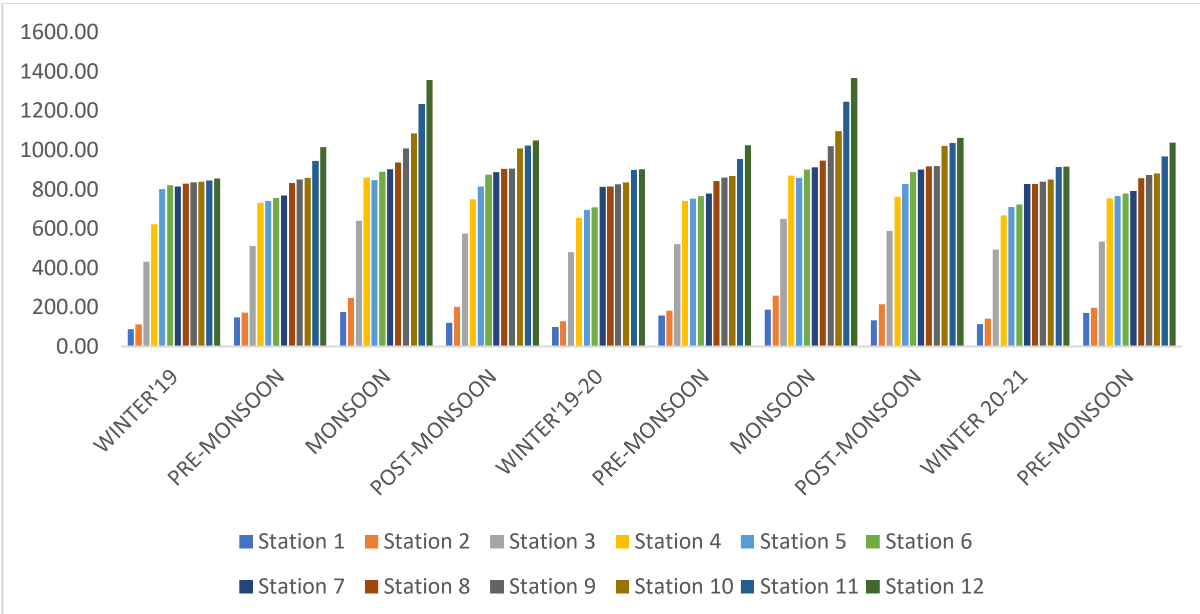


Figure: Seasonal Variation of Fecal Coliform Count at Station 1-12

Stations	Minimum (CFU/100 ml)	Maximum (CFU/100 ml)
1	87.50 (Winter, 2019)	187.67 (Monsoon, 2020)
2	112.50 (Winter, 2019)	259.33 (Monsoon, 2020)
3	432.50 (Winter, 2019)	651.00 (Monsoon, 2020)
4	622.50 (Winter, 2019)	871.00 (Monsoon, 2020)
5	696.36 (Winter, 2019-20)	858.45 (Monsoon, 2020)
6	708.89 (Winter, 2019-20)	900.96 (Monsoon, 2020)
7	769.25 (Pre-monsoon, 2019)	913.01 (Monsoon, 2020)
8	814.26 (Pre-monsoon, 2019)	947.26 (Monsoon, 2020)
9	826.00 (Winter, 2019-20)	1019.99 (Monsoon, 2020)
10	836.00 (Winter, 2019-20)	1096.00 (Monsoon, 2020)
11	845.17 (Winter, 2019)	1247.00 (Monsoon, 2020)
12	855.00 (Winter, 2019)	1367.67 (Monsoon, 2020)

The studied pollution indicating parameters (BOD, COD & fecal coliform) of Kopili river showed a similar trend of being lowest in winter seasons and highest in monsoon seasons, which may be due to the inflow of surface water as a result of rainfall which may carry both organic and inorganic load from the catchment areas.

ANNEXURE- XI

TROPHIC LEVEL STRUCTURE OF RIVER KOPILI

The estimation of trophic levels is very much essential for management of fisheries resources. We gathered all the available information regarding the feeding habits of 108 collected fish species belonging to 12 orders, 31 families and 63 genera and trophic state index values were collected from FishBase. The latter ranged from 2.0 to 4.5 and functional trophic groups were identified: (a) Pure Herbivore: Trophic Level 2.0-2.1, (b) Omnivore with a preference for vegetable material (2.1 < TROPH < 2.9), (c) Omnivore with a preference for animal material (3.01<TROPH<3.50) and (d) Carnivore (3.5<TROPH<4.0). The trophic level of the river dominated by mid-level carnivores (39.81%) followed by high level carnivores (25%), omnivores (23.15%) and herbivores (12.03%).

Sl No.	**Species**	**Trophic State Index**
1.	*Tor putitora*	2.9±0.38
2.	*Neolissochilus hexagonolepis*	3.0±0.37
3.	*Garra annandalei*	2.0±0.00
4.	*Garra gotyla gotyla*	2.0±0.00
5.	*Garra nasuta*	2.0±0.00
6.	*Garra lamta*	2.0±0.00
7.	*Garra lissorhynchus*	2.0±0.00
8.	*Garra kempi*	2.0±0.00
9.	*Opsarius bendelisis*	3.4±0.40
10.	*Opsarius barna*	3.4±0.65
11.	*Barilius barila*	3.2±0.40
12.	*Pethia stoliczkanus*	2.6±0.20
13.	*Puntius chola*	2.5±0.10
14.	*Pethia ticto*	2.2±0.00
15.	*Pethia conchonius*	2.9±0.33
16.	*Puntius sophore*	2.6±0.10
17.	*Systomus sarana*	2.9±0.20
18.	*Chagunius chagunio*	2.8±0.30
19.	*Osteobrama cunma*	2.9±0.30
20.	*Tariqilabeo latius*	2.3±0.20
21.	*Labeo bata*	2.0±0.00
22.	*Labeo calbasu*	2.0±0.00
23.	*Labeo gonius*	2.0±0.00
24.	*Labeo dyocheilus*	2.0±0.00
25.	*Labeo pangusia*	2.0±0.00
26.	*Labeo boga*	2.0±0.00
27.	*Labeo fimbriatus*	2.0±0.00
28.	*Labeo rohita*	2.2±0.12
29.	*Labeo catla*	2.8±0.22
30.	*Cirrhinus reba*	2.5±0.20
31.	*Cirrhinus mrigala*	2.3±0.20

32.	*Cyprinus carpio*	3.1±0.00
33.	*Bengala elanga*	3.4±0.40
34.	*Cabdio morar*	3.2±0.40
35.	*Amblypharyngodon mola*	3.3±0.40
36.	*Psilorhynchus homaloptera*	2.8±0.26
37.	*Psilorhynchus balitora*	2.9±0.40
38.	*Botia rostrata*	3.2±0.40
39.	*Botia dario*	3.2±0.40
40.	*Paracanthocobitis botia*	3.2±0.40
41.	*Schistura fasciata*	3.0±0.30
42.	*Schistura khugae*	3.0±0.30
43.	*Schistura reticulata*	3.0±0.30
44.	*Lepidocephalichthys guntea*	2.7±0.20
45.	*Lepidocephalichthys annandalei*	2.8±0.30
46.	*Esomus danricus*	2.4±0.10
47.	*Danio rerio*	3.1±0.10
48.	*Devario devario*	3.0±0.35
49.	*Devario aequipinnatus*	2.9±0.33
50.	*Danio dangila*	3.0±0.40
51.	*Salmostoma bacaila*	3.2±0.40
52.	*Salmostoma phulo*	3.2±0.40
53.	*Notopterus synurus*	3.5±0.00
54.	*Notopterus chitala*	3.7±0.59
55.	*Badis assamensis*	3.3±0.40
56.	*Badis badis*	3.3±0.39
57.	*Channa marulius*	4.5±0.80
58.	*Channa stewartii*	3.8±0.70
59.	*Channa gachua*	3.8±0.62
60.	*Channa punctata*	3.8±0.70
61.	*Channa striata*	3.6±0.47
62.	*Anabas testudineus*	3.0±0.40
63.	*Glossogobius giuris*	3.7±0.20
64.	*Chanda nama*	3.6±0.54
65.	*Parambassis baculis*	3.3±0.40
66.	*Parambassis ranga*	3.5±0.32
67.	*Laubuka laubuca*	3.2±0.20
68.	*Trichogaster fasciata*	2.8±0.10
69.	*Trichogaster lalius*	2.9±0.10
70.	*Trichogaster labiosa*	2.9±0.10
71.	*Mystus cavasius*	3.4±0.50
72.	*Mystus tengara*	3.2±0.40
73.	*Mystus bleekeri*	3.3±0.40
74.	*Mystus vittatus*	3.1±0.10
75.	*Rita rita*	3.7±0.57
76.	*Sperata aor*	3.6±0.53
77.	*Sperata seenghala*	3.8±0.40
78.	*Olyra kempi*	3.4±0.30
79.	*Clarias magur*	3.4±0.50
80.	*Heteropneustes fossilis*	3.6±0.30
81.	*Wallago attu*	3.7±0.56

82.	*Ompok bimaculatus*	3.9±0.40
83.	*Ompok pabo*	3.8±0.60
84.	*Ompok pabda*	3.8±0.60
85.	*Glyptothorax striatus*	3.2±0.40
86.	*Glyptothorax telchitta*	3.2±0.40
87.	*Bagarius bagarius*	3.7±0.59
88.	*Clupisoma garua*	3.7±0.59
89.	*Gagata cenia*	3.3±0.50
90.	*Gagata gagata*	3.4±0.60
91.	*Ailia coila*	3.6±0.60
92.	*Erethistes hara*	3.3±0.50
93.	*Eutropiichthys murius*	3.4±0.50
94.	*Eutropiichthys vacha*	3.9±0.63
95.	*Pachypterus atherinoides*	3.3±0.50
96.	*Amblyceps apangi*	3.3±0.40
97.	*Chaca chaca*	4.2±0.73
98.	*Xenentodon cancila*	3.9±0.62
99.	*Mastacembelus armatus*	2.8±0.27
100.	*Macrognathus aral*	3.1±0.33
101.	*Macrognathus aculeatus*	3.3±0.40
102.	*Monopterus cuchia*	3.8±0.64
103.	*Anguilla bengalensis*	3.8±0.70
104.	*Gudusia chapra*	3.1±0.30
105.	*Setipinna phasa*	3.3±0.39
106.	*Rhinomugil corsula*	2.4±0.20
107.	*Nandus nandus*	3.9±0.63
108.	*Leidon cutcutia*	3.3±0.20

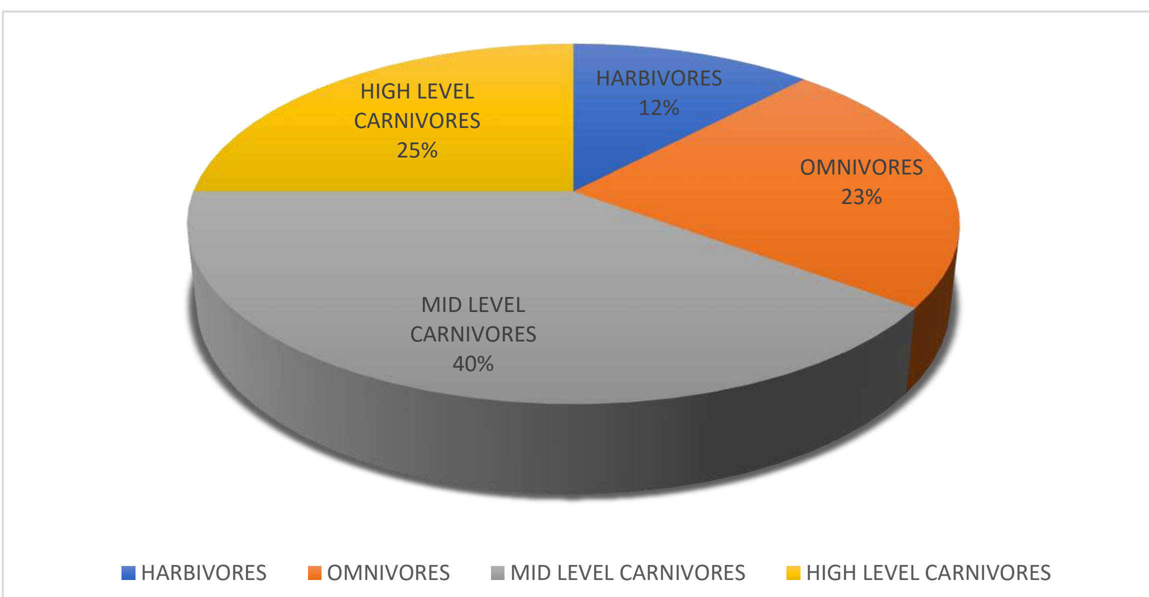


Fig: Trophic state index of fishes of River Kopili

Project ID: HSF2017-18/I-16/04

FINAL TECHNICAL REPORT

OF THE PROJECT

ON

"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

Submitted to:
Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies (NMHS)
G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal,
Almora 263643, Uttarakhand

Submitted by:

Dr. Sarada Kanta Bhagabati
Department of Aquatic Environment Management, College of Fisheries,
Assam Agricultural University, Raha, Nagaon-782 103

NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	**HSF2017-18/I-16/04**

Date of Submission:								
	d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

PROJECT TITLE
"HIMALAYAN RESEARCH FELLOWSHIP PROGRAMME"

Sanctioned Fellowship Duration: from (28/03/2018) to (28/02/2021).

Extended Fellowship Duration: from (1/03/2021) to (31/12/2021).

***Submitted to*:**

Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies, GBP NIHE HQs
Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi
E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

***Submitted by*:**
[Sarada Kanta Bhagabati]
Dept. of Aquatic Environment Management
College of Fisheries, Assam Agricultural University, Raha, Nagaon, Assam
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NMHS-Final Technical Report (FTR)

NMHS- Institutional Himalayan Fellowship Grant

DSL: Date of Sanction Letter

2	8	0	3	**2**	**0**	**2**	**2**
d	d	m	m	y	y	y	y

DFC: Date of Fellowship Completion

3	1	1	2	**2**	**0**	**2**	**2**
d	d	m	m	y	y	y	y

Part A: CUMULATIVE SUMMARY REPORT

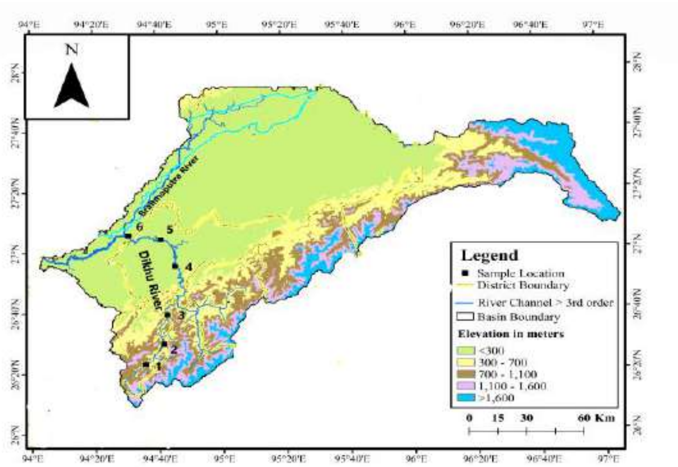
1. Details Associateship/Fellowships

1.1 Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	HSF2017-18/I-16/04
Name of the Institution/ University:	College of Fisheries, Assam Agriculture University
Name of the Coordinating PI:	1. Dr. Rajdeep Dutta Assistant Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103 2. Dr. S.K. Bhagabati, Associate Professor, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University, Raha, Nagaon-782 103
Point of Contacts (Contact Details, Ph. No., E-mail):	Email ID: drrajdeepdutta@gmail.com sskbk2002@gmail.com Phone no: +91 9854757790 +91 7896250516

1.2 Research Title and Area Details

i.	Institutional Fellowship Title:	Ecosystem Integrity and Fish Diversity of river Dikhow Assam and Nagaland.					
ii.	IHR State(s) in which Fellowship was implemented:	**Nagaland & Assam**					
iv.	Scale of Fellowship Operation	Local:		Regional:		Pan-Himalayan:	Yes

<table>
<tr><td>iii.</td><td>Study Sites covered (site/location maps to be attached)</td><td>Nagaland & Assam

</td></tr>
<tr><td>v.</td><td>Total Budget Outlay (Crore) :</td><td>INR 0.80 cr</td></tr>
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1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration	
		From	To
Research Associates			
Sr. Research Fellow			
Jr. Research Fellows	2	1/12/2018	31/12/2021
Project Fellows			

2.0 Research Outcomes

2.1. Abstract

- ***Background*:** The Eastern Himalayan region, which includes Northeast India, is regarded as one of the world's hotspots for freshwater fish variety. Assam has the most ichthyofaunal diversity of any North East state. Bhattacharjya et al., 2003 reported 217 fish species from Assam's wetlands and other waterbodies, divided into 104 genera, 37 families, and 10 orders. However, due to a variety of anthropogenic reasons, the rich and distinctive indigenous ichthyofauna of Assam are under threat. Keeping all of this in mind, an attempt has been made through this NMHS-sponsored medium grant project to examine the ichthyofaunal diversity as well as the ecosystem integrity of the Dikhow river, one of the most important South bank tributaries of the giant Brahmaputra.River which is a lifeline of millions of people of Nagaland and Assam.
- ***Aims:***

1. Baseline data of indigenous and endemic ichthyofauna of North East Himalaya
2. Pollution status and hydrobiological status of the river.

- ***Objectives:***

1. To study fish diversity of the entire stretch of the river.
2. To study physico-chemical as well as biological (plankton) water quality parameter of the entire river.
3. To study any pollutant presence in the river which may cause threat to the fish diversity.

- **Methodologies:**

Objective 1: Fish samples were taken at monthly intervals at six distinct locations on the Dikhow River, and the length and weight of the fish species were documented. Photographs of the fish specimens and their surroundings were taken. The fish samples were preserved in 10% formalin and transported to the laboratory. Standard keys were used to identify the fishes (Jayaram, 2006; Vishwanath & Nebeshwar, 2009; Kottelat, 2013).

<u>**DNA Barcoding**</u>

Fresh fish species' pectoral fins were clipped and stored in 100% ethanol for DNA barcoding. The DNA was extracted from the obtained fin clippings using the phenol: chloroform technique. The concentration of DNA samples was determined using nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). The samples were then tested for integrity using Gel Electrophoresis. Following that, a thermal cycler was used to amplify a DNA sample for the partial mitochondrial CoI gene utilizing Fish F1&R1 Primer (Eppendrof AG 22331 Hamburg). Eurofin Scientific Laboratory then sequences the PCR result. The produced barcodes were submitted to NCBI, and accession numbers for the particular fish species were acquired.

Objective 2: From January 2019 to May 2021, water and sediment samples were collected from six different locations along the Dikhow River. Some physical data, including as depth, air and surface water temperature, water velocity, TDS, and EC, were measured on the spot. Other characteristics of the water samples, such as turbidity, dissolved oxygen, pH, total alkalinity, total hardness, nitrate, nitrite, ammonia, and soluble inorganic phosphate, were measured in the laboratory in accordance with APHA guidelines (2018). The sediment samples were collected on a seasonal basis, air dried, and tested for pH, organic matter, organic carbon, using conventional techniques (Jhingran, 1992; Walky & Black, 1934). Edmondson (1959), Needham & Needham (1966), and the ICAR monograph series on algae were used to identify plankton and periphyton samples (Ramanathan, 1964; Philipose, 1967).

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Objective 3: To study the pollution status of the river, water samples from the 6 stations was collected on monthly interval and pollution status of the river was assessed in terms of Biochemical oxygen $Demand_3$ (BOD_3), Chemical Oxygen Demand (COD) using standard protocol. Palmer's pollution index was also assessed.

Results:

Objective 1: During the present investigation, a total of 60 fish species belonging to 7 orders, 18 families and 38 genera were recorded from the studied river. DNA barcodes were generated for 36 numbers of fish species from River Dikhow, submitted to NCBI and 36 numbers of accession numbers were obtained. Among the recorded fish species from River Dikhow, 1 species are assessed as endangered (2 %), 8 are near threatened (13 %), 3 are vulnerable (5%) , 43 species are least concerned (72%), 4 species not evaluated (7%) and 1 species is Data Deficit (1%) according to IUCN (2021). This is the first full record of fish species from entire stretch of river Dikhow.

Objective 2: Seasonal fluctuation analysis Data from the River Dikhow's hydrobiological characteristics show anthropogenic stress in the middle and lower portions. Only the Nazira and Sibsagar urban areas had BOD3 and COD levels that above the allowable limit, indicating a possible pollution load. Water turbidity in the Dikhow River was observed to be greater during the monsoon seasons of 2020 and 2021, perhaps owing to the influence of floods and landslides in Assam's plains and Nagaland's hills. The yearly change of the Dikhow river reveals that the sediment pH ranges from near neutral to alkaline.

During the research period, 36 different plankton genera were identified in the River Dikhow. The phytoplankton population was represented by 26 genera from the Chlorophyceae (13 genera), Bacillariophyceae (6 genera), Cyanophyceae (6 genera), and Euglenophyceae families (1 genera). Rotifera (5 genera), Cladocera (3 genera), and Copepoda (2) were all found in the zooplankton population. Plankton population density fluctuated from season to season. The average minimum plankton density was found to be 624 units/L while the highest plankton density was found to be 2178 units/L.

Objective 3: The levels of BOD3 and COD were found to be greater during the monsoon and post-monsoon seasons in Sibsagar and Nazira statins, which might be an indicator of pollution hazards during those seasons. Palmers' index followed a similar pattern. Palmer's index of pollution was used to grade water samples as having no organic pollution, moderate organic pollution, or high organic pollution at all of the stations studied. The total score of Agal Genus Pollution Index (AGPI) of the sites S3<S2<S6=S1<S4<S5 were calculated to be 7, 5, 8, 8, 11 and 31 respectively. It was discovered that the overall score of S3, S2, S6, and S1 was less than 10, indicating a lack of organic contamination. According to Palmer, a sharpe rise in overall score of 31 in station 5 indicates severe organic pollution owing to urban waste influx (1969). Navicula, Nitzcha, and Synedra were regularly detected in lower stations of the Dikhow River, particularly in Nazira and Sivsagar Town, and are regarded as pollution indicators based on Palmer's index results.

Conclusion: The river water at the upstream stretch, i.e. in the Nagaland hilly area, was discovered to be very conducive to aquatic life during 2020-21, resulting in a massive fish diversity reported at that time in that stretch. However, as it reaches the plains of Assam, urban garbage discarded from towns has become a big concern, increasing the organic load of the river water and diminishing variety. During the research period, a total of 60 fish species were recorded, several of which are being reported for the first time from the full river reach. Anthropogenic activities in the river's middle and lower segments endanger its ecosystem integrity..

Recommended: Activities that destroy habitat in rivers should be strictly restricted. In-situ protection of commercially significant and indigenous fish species is essential.

2.2. Objective-wise Major Achievements

S. No.	Objectives	Major achievements (in bullets points)
1.	To study fish diversity of the entire stretch of the river.	• 60 fish species belonging to 7 orders, 18 families and 38 genera were recorded from the studied river. Cyprinidae was the most dominant family comprising of 24 species, followed by Bagridae (7), Channidae (4), Sissoridae (4), Siluridae (4), Nemacheilidae (3), Psilorhynchidae (3), Mastacembelidae (2) species. On the other hand, rest of the families contained single species. • DNA barcodes generated: 36 fish species from River.Dikhow. • Conservation status: -1 species are assessed as endangered (2 %), 8 are near threatened (13 %), 3 are vulnerable (5%) , 43 species are least concerned (72%), 4 species not evaluated (7%) and 1 species is Data Deficit (1%) according to IUCN (2021). First full record of ichthyofaunal diversity of Dikhow river .

2.	To study physico-chemical as well as biological (plankton) water quality parameter of the entire river.	• Water and sediment samples were taken from six separate sites throughout the river's course, beginning at its headwaters and ending at the confluence point, to evaluate the river's biological state. • Turbidity was found to be greater in Nazira and Sibsagar during the research, particularly during the monsoon and post-monsoon seasons. • During the monsoon and post-monsoon seasons, electrical conductivity and total dissolved solids were found to be greater. • During the winter season, DO, total alkalinity, and total hardness were found to be greater. • The phytoplankton community of the study area constituted 72.18 % out of the total plankton collected throughout the studied period. Out of the 26 genera of phytoplankton recorded, Chlorophyceae comprises of 35.80 %, Bacillariophyceae 18.32%, Cyanophyceae 17.42 % and Euglenophyceae 0.62 % of the total plankton composition. Phytoplankton density of the studied Dikhow river ranges from 473 cells/L to 1621 cells/L being the maximum in winter 2019 and minimum during monsoon, 2020. • Zooplankton community constituted only 28.06 % of the total plankton hauled. A total of 10 genera zooplankton were recorded and being the highest in rotifera (12.46 %). Zooplankton density of the studied Dikhow river ranges from 134 nos/m3 to 712 nos/m3 being the maximum in winter 2020 and minimum during monsoon, 2020. • paper: Published 1

3.	To study any pollutant presence in the river which may cause threat to the fish diversity..	• The values of BOD_3 & COD were in higher side during the monsoon and post monsoon season in middle and lower stations, which might be the indication of pollution threats during those seasons. • The total score of Agal Genus Pollution Index (AGPI) of the sites S3<S2<S6=S1<S4<S5 were calculated to be 7, 5, 8, 8, 11 and 31 respectively. • It was observed that the total score of S2,S3,S1 and S6 showed below 10 which indicates lack of organic pollution. • Sharpe increase in total score of 11 and 31 in S4 and S5 respectively indicating high organic pollution due to urban waste influx according to Palmer (1969). *Navicula*, *Nitzcha* and *Synedra* were recorded repeatedly in lower stations of Dikhow river and consider as indicators of pollution in view of results of Palmer's index.

2.3. Outputs in terms of Quantifiable Deliverables*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved
1.	Information on the fish species of the entire river stretch based on taxonomic identification.	Taxonomic and molecular characterisation of fish fauna of the river covering its diversity, distribution,	Checklist of Fish species (New database):60 Museum specimens: 59 DNA barcodes: 36
2.	Monthly data on physical, chemical and biological parameters with statistical analysis and graphical representation		Dataset on environmental health of the river: 1 GIS Map: 1
3.	Organic as well as inorganic water pollution data must be collected on field and determine the cause of it	Palmer's index for detection of organic pollution.	BOD & COD data analysed for all stretched of the river. Lower stretches displayed higher organic load. Anthropogenic factors behind it is- • Sand Mining • Urban waste discharge. • Small industry waste disposal.

(*) As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number/ Brief Details	Remarks/ Attachment
1.	New Methodology developed	-	
2.	New Models/ Process/ Strategy developed	-	-
3.	New Species identified	-	-
4.	New Database established	6	• Total number of fish fauna • Conservation status of fish • Plankton data (Phyto and Zooplankton) • Palmer index • 12 physico-chemical water quality data. • 3 chemical sediment quality data
5.	New Patent, if any	-	-
	1. Filed (Indian/ International)	-	-
	2. Granted (Indian/ International)	-	-
	3. Technology Transfer (if any)	-	-
6.	Others (if any) DNA barcoding of fish species	36	Species specific DNA barcodes of 36 fish species from River Dikhow was generated, submitted to NCBI and accession number obtained for the first time.

3. Technological Intervention

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology		
2.	Diffusion of High-end Technology in the region		
3.	Induction of New Technology in the region		
4.	Publication of Technological / Process Manuals		

4. New Data Generated over the Baseline Data

S. No.	New Data Details	Status of Existing Baseline	Additionality and Utilisation New data
1.	Morphological identification & molecular characterisation of fish fauna of River Dikhow	No earlier record of fish fauna from River Dikhow is available	We have recorded 60 fish species and generated mitogenome sequences for 36 species from River Dikhow for the first time.. This is an first report of entire ichthyofaunal diversity from Dikhow river.
2.	Seasonal variation of hydrobiological & parameters	No earlier report on hydrobiological study of whole stretch of River Dikhow is available.	The new data will be helpful in understanding the impact anthropogenic factors on ecosystem integrity of the river. It will be also helpful in devising future fisheries development strategies in this river.
3.	Sediment characteristic of River Dikhow	No earlier report on sediment characteristic of River Dikhow is available	The new information will be helpful for future researchers working in this region
4.	Plankton diversity	No report earlier	
5.	Palmer index has been developed for the said river system	No report earlier	

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Beneficiaries
1.	Sustainable Development Goals (SDGs)	Life below water	1	
2.	Climate Change/INDC targets			
3.	International Commitments			
4.	National Policies			
5.	Other's collaborations			

6. Financial Summary (Cumulative)*

*Please attach the **consolidated and audited Utilization Certificate (UC) and Consolidated and *<u>Year-wise</u>* Statement of Expenditure (SE)** separately, *ref*. **Annexure I.**

7. Quantification of Overall Research Progress

S. No.	Parameters	Total (Numeric)	Attachments* with remarks
1.	IHR State(s) Covered:	2	
2.	Fellowship Site/ LTEM Plots developed:	6	Photographs of sampling sites and map of study area attached (Annexure- I & II)

3.	New Methods/ Model Developed:		
4.	New Database generated:		
5.	Types of Databases generated:		
6.	No. of Species Collected:	60 (DNA barcodes of 36 fish species submitted and accession number received	Accession numbers are generated (Annexure III)
7.	New Species identified:		
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	JRF:02 PhD:01	
9.	No. of SC Himalayan Researchers benefited:		
10.	No. of ST Himalayan Researchers benefited:		
11.	No. of Women Himalayan Researchers empowered:		
12.	No. of Knowledge Products developed:		
13.	No. of Workshops participated:		
14.	No. of Trainings participated:		
15.	Technical/ Training Manuals prepared:		
	Others (if any):		

***** Please attach the soft copies of supporting documents word files and data files in excel.

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)		1		Annexure X
2.	Book Chapter(s)/ Books:				
3.	Technical Reports/ Popular Articles				
4.	Training Manual (Skill Development/ Capacity Building)				
5.	Papers presented in Conferences/ Seminars		1		Annexure XI
6.	Policy Drafts (if any)				
7.	Others (specify)				

9. Recommendation on Utility of Research Findings, Replicability and Exit Strategy

9.1 Utility of the Fellowship Findings

S. No.	Research Questions Addressed	Succinct Answers (within 150–200 words)
1.	How many fish species can be found in Dikhow River?	During the study 60 fish species were recorded.
2	What are the physico-chemical parameter of river Dikhow?	The physico-chemical parameters were ambient to sustain aquatic life in all the cases but in the lower stretches due to urban discharge and infrastructure development high organic load and higher turbidity can be observed.
3	How is the trophic level structure of the fish species?	The trophic level of the river dominated by mid-level carnivores (23%), animal prefer omnivores (35%) ,plant prefer omnivore (27%) and herbivores (15%).
4	Are there any anthropogenic agents causing threat to fish species?	Unregulated fishing, destructive fishing , Road construction near river banks , urban waste discharge were observed during the study period.

9.2 Recommendations on Replicability and Exit Strategy:

Particulars	Recommendations
Replicability of Fellowship, if any	

Exit Strategy:	▯ The water of the lower stretches of Dikhow river is polluted with high organic load.This is due to high urban discharge from big towns like Sivsagar and Nazira. That's why there is low amount fish diversity recorded from these two regions. ▯ Previous reports reported that there is no proper database of fish species present in Dikhow river, but in this report a total of 60 fish species which include cold water fish species from Naga hilly areas. So, conservation efforts of indigenous ichthyofauna of the study river should be considered. ▯ Sand and pebble mining activities from river bed should be totally prohibited in order to conserve the microhabitat requirement of hill stream fishes. ▯ State fishery laws prohibiting fishing during breeding season, use of destructive fishing gears etc. should be strictly followed. ▯ Our study emphasis more to use of sustainable aquaculture as a means of rural lively hood solutions rather than depending on rivers.

(**NMHS FELLOWSHIP COORDINATOR**)

(Signed and Stamped)

(**HEAD OF THE INSTITUTION**)

(Signed and Stamped)

Place: ………………..
Date: …../……/……..

PART B: COMPREHENSIVE REPORT

EXECUTIVE SUMMARY

The Executive Summary of the fellowship should not be more than 3–5 pages, covering all essential features in precise and concise manner as stated in Part A (Cumulative Fellowship Summary Report) and Part B (Comprehensive Report).

Fellowship Report No.: ***5/5*** *(n = Sequential number; N= Total no. of fellowships granted to the Institute/ University)*

Researchers Details

Type of Fellowship (HRA/HJRF/HJPF)	Name of Himalayan Researcher	Date of Joining	Date of Resignation**	Research Title	Name of the PI & Designation
HJRF	Abhijit Choudhury	1/12/2018	28/2/19		Dr S . K Bhagabati
HJRF	Raktim Sarmah	25/10/2019	31/12/2021	Ecosystem Integrity and Fish Diversity of river Dikhow Assam and Nagaland.	Dr S . K Bhagabati

*If the appointed researcher resigned in the mid of the fellowship duration, then also mention the name of the Himalayan researcher who carried forward the fellowship.

1 INTRODUCTION

1.1 Background/ Summary of the Associateship / Fellowship Study undertaken (max. 500 words)

The river is a lotic ecosystem flowing under the influence of gravity and confluence into the sea and some into lakes. Rivers are important pathways for the flow of energy, matter and organisms through the landscape (Kagalou et al, 2002). Rivers also play a major role in assimilation or transportation of the municipal and industrial wastewater discharges continuously or occasionally or seasonally. Most of the ancient civilizations grew along the banks of the rivers. At present, most of the industries agricultural land, populated cities and towns can be found near bank of the rivers. River, a symbol of India's age-old cultural heritage and civilization, occupies a unique position in the ethos of Indian people. There are 15 major, 45 medium and more than 102 minor rivers in India with a total length of 45,000 km covering a catchment area of 3.12 million km^2. Among the major river system, the Brahmaputra is the second largest river of India traversing 900 km in the country (Handbook of Fisheries and Aquaculture). In India, river systems are traditionally classified, according to their origin - into Himalayan and Peninsular rivers, or according to the direction of flow-into East flowing and West flowing rivers (NCIWRDP 1999; Amarasinghe et al. 2005).

The North-Eastern part of India is rich in riverine resources with a total length of 19,150 km. Riverine fisheries plays an important role in the region in terms of providing livelihood and nutritional security to many fisherfolks. The state of Assam alone has 4820 km stretch of riverine resources (approx. 2,05,000 ha) mainly contributed by two main rivers basin Brahmaputra and Barak basins along with their 53 tributaries. A few reports are available on hydrobiology and fisheries of Brahmaputra and Barak basin (Dey, 1984; Jhingran, 1991; Biswas, 1998; Biswas; Baruah, 2000 & Baruah & Biswas, 2002; Bailung & Biswas 2018). Different factors relating to decline of fisheries in the Brahmaputra River basin have been discussed by Yadava and Sugunan (1992). A few assessments of surface water quality of river Brahmaputra were conducted by Saikia and Gupta (2012). Although many studies were carried out on Brahmaputra and Barak River basins, but works relating to water quality assessment on both the river basins are very scanty, especially on Brahmaputra River which is regarded as the lifeline for Assam. Irresponsible/ destructive fishing techniques, water pollution, habitat degradation are identified as some of the key threats to the indigenous fish germplasm of the state. Many indigenous fish species of the state are rapidly entering into the categories of Vulnerable, Endangered, Threatened due to these threats. Keeping all these aspects in view, through this NMHS sponsored project an attempt has been made to study ichthyofaunal diversity as well as ecosystem integrity of Dikhow river.

1.2 Baseline and Scope of the Associateship / Fellowship (max. 1000 words)

During this NMHS project first-hand information on fish fauna of River Dikhow was generated. Species specific DNA barcodes were for fish fauna of River Dikhow during the project for the first time. Morphological identification of the indigenous fish fauna of the river supported by molecular characterization will provide a complete dataset on ichthyofaunal diversity. The project has investigated the seasonal variation of physico-chemical water & sediment quality parameters, plankton composition of River Dikhow from January 2019 to May 2021. The updated new data will be helpful in understanding anthropogenic stress factors affecting ecosystem integrity of the river. This information will be very helpful in planning future fisheries development strategies of this river.

1.3 Overview of the Major Issues to be addressed (max. 1000 words)
Some of the major issues addressed through this project are:

i) Construction of roads: Even while building roads is essential for connectivity and economic growth, there is significant environmental harm, particularly to rivers, as a result of some unethical construction methods. We have seen several micro landslides into the river throughout the construction time, as well as direct dumping trash from the road construction area, both of which frequently increase the turbidity and harm aquatic life. Therefore, it is important to encourage environmentally friendly road construction methods.

ii) Water pollution: River water pollution also has an impact on the indigenous ichthyofauna. The riverine water quality is being impacted by industrial effluents, urban untreated sewage, industrial effluents, agricultural chemicals, etc., which in turn impacts the fish fauna. Effluent treatment plants are becoming necessary to curb the discharge urban sewage disposal into the river.

iii) Habitat degradation: Fish in hill streams need certain environmental conditions to thrive and survive. These fish have been modified specifically to survive in the unusual hill stream habitat. For the growth, survival, and reproduction of these fish, a swiftly moving stream current and the presence of sand, pebbles, cobbles, rocks, boulders, etc. in the stream bed are essential. But the habitat of these fish is being severely impacted by mining activity in the river bottoms. The habitat of these fish was devastated by river bed sand mining, which caused the loss of some of this priceless ichthyofauna.

iv) Public unawareness: The indigenous ichthyofauna's need for conservation is largely unknown in the state's civil society. The local communities are overfishing at an alarming rate . Precious fish fauna from various waterbodies in the state are experiencing various anthropogenic risks because people are unaware of the worth of the biodiversity of indigenous fish genetics.

v) Unavailability of alternative options: Promoting aquaculture to lessen fish's reliance on these natural resources and give fishermen other fish-focused livelihood alternatives is one of the greatest methods to conserve the native fish fauna of natural aquatic habitats. Unfortunately, the tribal inhabitants of the area are ignorant of modern scientific fish farming methods. Therefore, people rely heavily on fishing for food fish in rivers and streams.

1.4 Brief summary of the activities under taken by the researcher (max. 1000 words)

2 METHODOLOGIES, STARTEGY AND APPROACH

2.1 Methodologies used for the study (max. 1000 words)

i) **Methodology used for achieving Objective 1:** Six (6) stations were selected covering the entire stretch of the river based on elevation and fish specimens were collected during different seasons of the year. Photography of the fish specimens and their habitat were done. The morphometric measurements were recorded. The fish samples were preserved and brought to the laboratory in 10% formalin for further analysis. The fishes were identified using standard keys (Jayaram, 2006; Vishwanath & Nebeshwar, 2009; Kottelat, 2013). Species specific DNA barcodes of the fish species were generated as per the standard methodology of Ward et al. (2005). Pectoral fin clipping of fresh fish species collected in absolute ethanol for DNA Barcoding. DNA from the collected fin clipping was isolated following phenol: chloroform method. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306). Then samples were subjected to Gel Electrophoresis for checking its integrity. Followed by that amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg). The PCR product is then sequenced at Eurofin Scientific

Laboratory. The generated barcodes were submitted to NCBI and accession number were obtained for the individual fish species.

ii) **Methodology used for achieving Objective 2:** Six (6) stations were selected covering the entire stretch of the river based on elevation. Water & sediment samples were collected from these stations during different seasons of the year. Samples were collected between 10-11 am. Some of the physical parameters like depth, air & water temperature, water velocity, pH, conductivity, TDS etc. were determined *in-situ*. pH, conductivity, TDS of the river water were measured *in-situ* using a digital soil & water testing kit (Systronics India Limited/371). Other parameters like DO, alkalinity, hardness, BOD_3, COD, nitrate, nitrite, total ammonia, soluble inorganic phosphate of the water samples were carried out in the laboratory as per APHA (2018) and CPCB (2001). Soil samples were collected quarterly by Ekman's dredge separately from three sampling station for the estimation of different soil parameters (Jackson, 1973). Then the samples were dried in room temperature and pulverized to a fine size and sieved through a standard sieve and it was used for estimation of pH, organic carbon, organic matter in the laboratory. Sediment parameters like sediment pH, sediment organic carbon, sediment organic matter were estimated quarterly adopting standard procedures (Jhingran, 1992; Walky & Black, 1934).

Plankton samples were collected in duplicate by filtering 100-200 liters of river water using 28 mm mesh nylobolt plankton net as described by Santhanam *et al.* (1987). The collected plankton samples were preserved in 3-4 % formalin in separate plankton tubes. In laboratory, from the known volume plankton sample counting was done by using Sedgwick Rafter Plankton counting cell (Sharma and Saini, 2005). Plankton were identified at genera level using the identifying keys of Edmondson (1959), Needham & Needham (1966) and ICAR monograph series on algae (Ramanathan, 1964; Philipose, 1967). Plankton biomass in terms of density was determined using plankton density (Units/L) a Sedgwick Rafter Cell as per the methodology of Sharma and Saini (2005).

iii) **Methodology used for achieving Objective 3:** Water pollution studies of the river was carried out in terms of BOD_3, COD as per CPCB Guide Manual: Water and Waste Water Analysis (2011).

2.2 Details of Scientific data collected and Equipments Used (max 500 words)

a. Air & water temperatures were measured using a mercury thermometer.
b. Water velocity was measured using a current meter.
c. Parameters like pH, conductivity, TDS of the river water were measured *in-situ* using a digital soil & water testing kit (Systronics India Limited/371).
d. DO, Alkalinity & Hardness values were estimated by Titration method.
e. BOD bottles were incubated in BOD incubators.
f. For estimation of COD, water samples were digested in a KEL PLUS Automatic COD digestion system/ KES 08 L CAC.

g. Parameters like Nitrate, nitrite, total ammonia and soluble inorganic phosphate were determined using uv-visible spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202).
h. Latitude & longitude of the stations were recorded using a GPS instrument.
i. Photography of the fish specimens and stations were done using a digital camera.
j. The morphometric measurements & weight of the collected fish specimens were recorded using a vernier calliper and a pan balance respectively.
k. DNA isolation from pectoral fin clippings of the fishes was done using Phenol-Chloroform method.
l. Concentration of the DNA samples was measured with the help of nanodrop (Nabi, UV/Vis Nano Spectrophotometer, Serial No.: NB1-A-180306).
m. Integrity of DNA samples were checked using an Electrophoresis system (Biorad)
n. Amplification of DNA sample was carried out for partial mitochondrial CoI gene using Fish F1&R1 Primer with the help of a thermal cycler (Eppendrof AG 22331 Hamburg).
o. Plankton samples were collected using a plankton net.
p. Plankton & periphyton samples were observed under a Microscope.

2.3 Primary Data Collected (max 500 words)

I. The morphometric measurements & weight of the collected fish
II. Latitude & longitude of the study stations of both the rivers
III. Museum fish specimens
IV. Air & water temperatures
V. Water velocity
VI. Water pH
VII. Dissolved oxygen concentration of river water
VIII. Conductivity of river water
IX. TDS of river water
X. Total Alkalinity of river water
XI. Total Hardness of river water
XII. Biological Oxygen Demand$_3$ (BOD_3) of the river water
XIII. Chemical Oxygen Demand (COD) of the river water
XIV. Nitrogen-nitrate
XV. Nitrogen-nitrite
XVI. Total ammonia
XVII. Soluble inorganic phosphate
XVIII. Sediment pH
XIX. Sediment organic matter
XX. Sediment organic carbon

XXI. Plankton biomass

XXII. Details of Field Survey arranged (max 500 words)

Regular field survey the study river was conducted during the entire duration of the project for collection of fish specimens, water samples, sediment samples, plankton & periphyton samples. During those surveys, primary and secondary data were also collected pertaining to the objectives of the project

2.4 Strategic Planning for each Activities (max. 1000 words)

- **Fish Biodiversity Study**: Six (6) stations were selected covering the entire stretch of the river. Fish samples were collected using gill net and cast net with the help of local people. Sometimes survey was also conducted on local fish market.

- **Ecosystem Integrity Study:** Water, sediment, plankton and periphyton samples were collected from 6 different stations covering the whole stretch of Dikhow river during different seasons of the year. While selecting the stations it was ensured that every station represents different elevations. Water and plankton samples were collected from each station on monthly intervals while sediment samples were collected seasonally.

2.5 Activity-wise Timeframe followed using Gantt/ PERT Chart (max. 1000 words)

Activities	Months																									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Etc.**				**18**				**24**		**27**		**35**	**36**
Recruitment of Project Staff	■																									
Preparation & Procurements	■	■																								
Initial survey			■	■																						
Assessment of Ichthyofaunal diversity				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Evaluation of Environmental Health				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Annual Report												■									■					
Final Report Preparation & Submission																									■	■

3 KEY FINDINGS AND RESULTS

3.1 Major Research Findings

3.1.1: Water Quality & Sediment Parameters of River Dikhow:

A total of fifteen (12) water quality parameters and six (3) sediment parameters were tested at 12 different stations by covering the whole stretch of the Dikhow river for a period of 29 months from January, 2019 to May, 2021.

Data on seasonal variation of water quality parameters of River Dikhow from January, 2019 till May, 2021 is depicted on Annexure IV. Data on seasonal variation of sediment quality parameters of River Dikhow from January, 2019 till May, 2021 is depicted on Annexure V .

Comparison of water quality parameters of the study rivers with congenial values for fishes:

Sl. No	Parameter	Result (Range)	Congenial Limit	Remark
1.	Surface Water Temperature (^{0}C)	13.6-29.70		Suitable for both cold and warm water fishes.
2.	Turbidity (NTU)	3.2-121.8	20-30	Turbidity exceeds permissible limit from station 3-6.
3.	pH	7-8	7-8.5	Water pH was found to be acidic to alkaline condition during the study period.
4.	Dissolved Oxygen (ppm)	5.9-9.9	>5	Average DO values found to be within acceptable range. But during monsoon season values<5 were recorded.
5.	Total Alkalinity (ppm)	38.3-80.00	80-200	Alkalinity values found to be not congenial for fishes
6.	Total Hardness (ppm)	36.5-99.7	75-150	Hardness values found to be not congenial for fishes
7.	Electrical Conductivity (µS/cm)	118-252.00	50-1500	Found to be within acceptable range
8.	Total Dissolved Solids (ppm)	77.03-198.7	<400	Found to be within acceptable range
9				
9.	Biochemical Oxygen Demand (ppm)	1.03-12.1	<10	BOD values of station 4 & 5 were found in higher range than the congenial limit during monsoon indicating anthropogenic stress in these stations
10.	Chemical Oxygen Demand (ppm)	1.4-34.4	<20	COD values of station 4 & 5 were found in higher range than the congenial limit during monsoon indicating anthropogenic stress in these stations
11.	Nitrate-nitrogen (ppm)	0.05-0.22	0.10-3.00	Found to be within acceptable range
12.	Nitrite Nitrogen (µg/L)	0.07-0.11	0-0.50	Found to be within acceptable range
13.	Soluble Inorganic Phosphate (ppm)	0.012-0.12	0.05-0.4	Found to be more than acceptable range

14.	Total Ammonia (ppm)	0.01-0.0 3	0-1.0	Found to be more than acceptable range

3.1.2 Ichthyofaunal Diversity of Dikhow River:

This project is bringing out first ever information on ichthyofauna of River Dikhow. During the present investigation, a total of 60 fish species belonging to 7 orders, 18 families and 38 genera were recorded from the studied river. Order wise composition shows dominance of Cypriniformes (51%) followed by Siluriformes (28.00%), Perciformes (12%), Synbranchiformes (3%), Anabantiformes, Mugiliformes and Osteoglossiformes comprised of 2 % . The conservation status are 1 species are assessed as endangered (2 %), 8 are near threatened (13 %), 3 are vulnerable (5%) , 43 species are least concerned (72%), 4 species not evaluated (7%) and 1 species is Data Deficit (1%) according to IUCN (2021) Annexure III . Cyprinidae was the most dominant family comprising of 24 species, followed by Bagridae (7), Channidae (4), Sissoridae (4), Siluridae (4), Nemacheilidae (3), Psilorhynchidae (3), Mastacembelidae (2) species. On the other hand, rest of the families contained single species.

3.1.3. Plankton Biomass of River Dikhow:

During the research period, 36 different plankton genera were identified in the River Dikhow. The phytoplankton population was represented by 26 genera from the Chlorophyceae (13 genera), Bacillariophyceae (6 genera), Cyanophyceae (6 genera), and Euglenophyceae families (1 genera). Rotifera (5 genera), Cladocera (3 genera), and Copepoda (2) were all found in the zooplankton population. Plankton population density fluctuated from season to season. The average minimum plankton density was found to be 624 units/L while the highest plankton density was found to be 2178 units/L.The phytoplankton community of the study area constituted 72.18 % out of the total plankton collected throughout the studied period. Out of the 26 genera of phytoplankton recorded, Chlorophyceae comprises of 35.80 %, Bacillariophyceae 18.32%, Cyanophyceae 17.42 % and Euglenophyceae 0.62 % of the total plankton composition. Phytoplankton density of the studied Dikhow river ranges from 473 cells/L to 1621 cells/L being the maximum in winter 2019 and minimum during monsoon, 2020. Zooplankton community constituted only 28.06 % of the total plankton hauled. A total of 10 genera zooplankton were recorded and being the highest in rotifera (12.46 %). Zooplankton density of the studied Dikhow river ranges from 134 nos/m3 to 712 nos/m3 being the maximum in winter 2020 and minimum during monsoon, 2020, Annexure VII.

Palmer (1969) first made the list of algae genera and species which indicate organic pollution. According to Palmer, scores of 20 or more are indication of high organic pollution. By using Palmer's index of pollution for rating of water samples as lack of organic pollution, moderate and high organic polluted at all the stations were tested. Palmers' index also showed similar trend. The total score of Agal Genus Pollution Index (AGPI) of the sites S3<S2<S6=S1<S4<S5 were calculated to be 7, 5, 8, 8, 11 and 31 respectively. It was discovered that the overall score of S3, S2, S6, and S1 was less than 10, indicating a lack of organic contamination. According to Palmer, a sharpe rise in overall score of 31 in station 5 indicates severe organic pollution owing to urban waste influx (1969). Navicula, Nitzcha and

Synedra were recorded repeatedly in lower stations of Dikhowr river and consider as indicators of pollution in view of results of Palmer's index, Annexure VII.

3.1.4. Anthropogenic factors affecting the river ecosystem:

- Destructive fishing
- Sand mining
- Road Construction
- Waste discharge

3.2 Key Results

- During the present investigation, a total of 60 fish species belonging to 7 orders, 18 families and 38 genera were recorded from the studied river.
- Order wise composition shows dominance of Cypriniformes (51%) followed by Siluriformes (28.00%), Perciformes (12%), Synbranchiformes (3%), Anabantiformes, Mugiliformes and Osteoglossiformes comprised of 2 % .
- The conservation status are 1 species are assessed as endangered (2 %), 8 are near threatened (13 %), 3 are vulnerable (5%) , 43 species are least concerned (72%), 4 species not evaluated (7%) and 1 species is Data Deficit (1%) according to IUCN (2021).
- DNA barcodes generated for 36 fish species and 36 NCBI accession no obtained from River Dikhow for the first time.
- This is the first record of fish diversity of Dikhow River from entire stretch of the river .
- The trophic level of the river dominated by mid-level carnivores (23%), animal prefer omnivores (35%) ,plant prefer omnivore (27%) and herbivores (15%).
- Surface water temperature regime of both the rivers is congenial for both hill stream and warm water fishes.
- Turbidity of Dikhow river water found to be higher from station 3,4 and 5 maximum during monsoon season may be due to micro-landslides in the hills and also the surface run-off from catchment areas due to raining.
- pH was found to be ambient in for aquatic organism In the entire stretches of Dikhow river .
- Average BOD3 values of all the other stations than hilly areas of River Dikhow found to exceed acceptable limit (<10ppm) indicating anthropogenic stress in these stations but in the station 4 & 5 it much high due to high urban waste discharge.
- Except Station 4 &5 of River Dikhow, COD values of all other stations of River Dikhow found to exceed acceptable limit (≤20 ppm) indicating probable pollution load in these stations.
- The values of Nitrate, Nitrite, Ammonia and Phosphate was found to be congenial for fishes.

- A total of 36 different plankton genera were identified in the River Dikhow. The phytoplankton population was represented by 26 genera from the Chlorophyceae (13 genera), Bacillariophyceae (6 genera), Cyanophyceae (6 genera), and Euglenophyceae families (1 genera). Rotifera (5 genera), Cladocera (3 genera), and Copepoda (2) were all found in the zooplankton population.
- Different anthropogenic factors like urban waste discharge , sand mining , construction of roads near river bank, etc. were recorded

3.3 **Conclusion of the study undertaken**

- River Dikhow a very beautiful river starting from the hills of Nagaland to the fertile plains of Assam joining the mighty Brahmaputra.
- A 14km stretch in the upper zone of the Nagaland is protected by local community and declared as 'Green Zone' and after that stretch ichthyofaunal diversity becomes high . So, a protection zone is very important to conserve and revive biological diversity.
- As the river enters the plains of Assam crosses between urbanized towns like Nazira and Sivsagar the pollution becomes dominant leading to deterioration of water quality and declining of Ichthyofaunal diversity . But at the confluent zone pollution effect reduces and diversity increases.
- In the hilly zone of Nagaland , overfishing and unethical fishing practices are predominant . This lead to loss of indigenous fish species.
- Sand mining , waste discharge , highway construction leads to habitat destruction in the river which should be delt with rigorous policy making.
- Awareness and technological innovations should be emphasizes to reduce biodiversity losses.

- OVERALL ACHIEVEMENTS

3.4 Achievements on Objectives

1. **To study fish diversity of the entire stretch of the river.**

- During the present investigation, total of 60 fish species belonging to 7 orders, 18 families and 38 genera were recorded from the studied river. Order wise composition shows dominance of Cypriniformes (51.0%) followed by Siluriformes (28.0%), Perciformes (12.0%), Synbranchiformes (3.0%), Anabantiformes, Mugiliformes and Osteoglossiformes comprised of 2 % .
- DNA barcodes generated for 36 fish species and 36 NCBI accession no obtained from River Dikhow for the first time.
- The conservation status are 1 species are assessed as endangered (2 %), 8 are near threatened (13 %), 3 are vulnerable (5%) , 43 species are least concerned (72%), 4 species not evaluated (7%) and 1 species is Data Deficit (1%) according to IUCN (2021).
- The trophic level of the river dominated by mid-level carnivores (23%), animal prefer omnivores (35%) ,plant prefer omnivore (27%) and herbivores (15%).

2. **To study physico-chemical as well as biological (plankton) water quality parameter of the entire river.**

a) Dataset on physico-chemical parameters of water and sediment is generated of the study.

b) Dataset on physico-chemical parameters of sediment is also generated of Dikhow river.

c) Plankton diversity dataset was also generated of Dikhow river.

d) GIS Maps of the study river developed.

e) One research paper published.

3. To study any pollutant presence in the river which may cause threat to the fish diversity.

a) Pollution status of Dikhow river was studied based on the parameters viz BOD & COD.

b) Palmer's pollution index is also developed.

3.5 Establishing New Database/Appending new data over the Baseline Data (max. 1500 words, in bullet points)

- During the present investigation, a total of 60 fish species belonging to 7 orders, 18 families and 38 genera were recorded from the studied river. DNA barcodes were generated for 36 (60%) numbers of fish species from River Dikhow, submitted to NCBI and 36 numbers of accession numbers were obtained. Among the recorded fish species from River Dikhow, 1 species are assessed as endangered (2 %), 8 are near threatened (13 %), 3 are vulnerable (5%) , 43 species are least concerned (72%), 4 species not evaluated (7%) and 1 species is Data Deficit (1%) according to IUCN (2021).This is the first full record of fish species from entire stretch of river Dikhow.
- A total of 36 different plankton genera were identified in the River Dikhow
- The phytoplankton population was represented by 26 genera from the Chlorophyceae (13 genera), Bacillariophyceae (6 genera), Cyanophyceae (6 genera), and Euglenophyceae families (1 genera). Rotifera (5 genera), Cladocera (3 genera), and Copepoda (2) were all found in the zooplankton population. Plankton population density fluctuated from season to season. The average minimum plankton density was found to be 624 units/L while the highest plankton density was found to be 2178 units/L.

3.6 Generating Model Predictions for different variables
No

3.7 Technological Intervention
No

3.8 On-field Demonstration and Value-addition of Products
No

3.9 Developing Green Skills in IHR

No

3.10 Addressing Cross-cutting Issues

No

4 IMPACTS OF FELLOWSHIP IN IHR

4.1 Socio-Economic Development

The project findings can help in developing strategies for better management of fisheries resources of IHR:

- Fish based Ecotourism can be developed to uplift the standards of rural livelihood.
- Sports fisheries for mahseer and other indigenous fishes have a big potential which can be harness with proper policies.
- Ornamental indigenous fisheries have a huge scope. Standard breeding protocols should be research and developed.
- Plans should be developed to curb pollution sources on target areas.

4.2 Conservation of Biodiversity in IHR

- As the report provide habitat environmental data of the fishes, proper management plants should be developed based on such data.
- Fish species categorised as endangered, threatened, vulnerable should be prioritised and special conservation programmes should be launched.
- Pollution specific areas should be targeted based on the data and special measure should be developed.
- Existing laws based on conservations should be tightened .
- Awareness camps should be conducted on a regular basis to aware about the value of indigenous fishes.

4.3 Protection of Environment

- Community protected area like “Greenzone ” should be taken as example in Dikhu river where the local community protects an stretch of 14km of the river prohibits any fishing activity .More and more such areas should be promoted .
- Development of Indigenous fish hatchery should be carried out. Certain pro-active farmers have developed Mahseer hatcheries near the river to revive the Mahseer population.
- Development effluent treatment plants should carry out near urban embankments so that the urban sewer water should be treated before disposed into the river.
- Waste disposal in the river should strictly prohibited .

4.4 Developing Mountain Infrastructures

- The project has developed research infrastructure for fish biodiversity study which can be also utilised for future similar research works of this region.

4.5 Strengthening Networking in IHR

- The public will have access to every project finding. These results can be used to improve our understanding of aquatic ecosystems.
- Other IHR researchers might use the information on ichthyofauna obtained during the project for the identification and habitat ecology study of the native fish species. The study's findings may be efficiently used to create a conservation model for the IHR's indigenous fish species.
- The literatures and resources developed during the project can be utilised by other IHR regions for training, awareness, outreach activities of fisherfolks.

5 EXIT STRATEGY AND SUSTAINABILITY

5.1 How effectively the fellowship findings could be utilized for the sustainable development of IHR

- The data will be a base for planning and policy making in that IHR .
- Various future predictive models can be develop following the dataset.
- With this data species diversification in aquaculture can be studied.

5.2 Identify other important areas not covered under this study, but needs further attention .

- Climate change study in context to fish biology and river ecology should be studied.

- Aquaculture technology development for the hilly tribes.

- Development of captive breeding protocol of selected fish species. Species included in concerned categories like Endangered, Vulnerable, threatened should be studied with priority and efforts should be made to develop captive breeding protocol of these fish species.

5.3 Major recommendations for sustaining the outcomes of the fellowship in future

- Decadal monitoring should be done of the environmental parameters.

- Awareness extension program should be frequently conducted for the indigenous fauna .

- More grants related to eco-friendly technology development for sustainable lively hood should be given.

- Involvement of local communities in monitoring programs.

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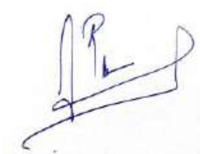


(Raktim Sarmah)

HJRF-05

(**Sarada Kanta Bhagabati /Rajdeep Dutta**)

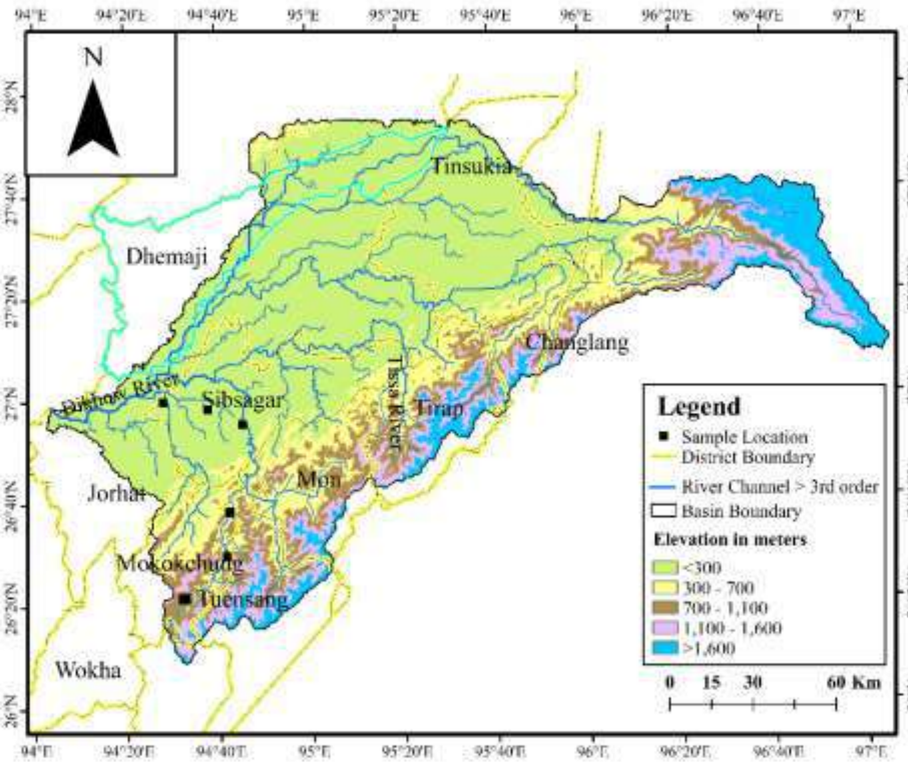
(**NMHS FELLOWSHIP COORDINATOR**)

(**HEAD OF THE INSTITUTION**)

Place: …Raha..
Date: 16./11/2022

ANNEXURE I

MAP SHOWING THE STUDY AREA OF RIVER DIKHU/DIKHOW



ANNEXURE II
DESCRIPTION OF THE STUDY AREA

Station 1: This station is situated at a latitude of 26°15′17.95"N and longitude of 94°31′42.63" E. This station is located at Longsa. This station is characterized by altitude of 667m msl. **Key Features –a)** Community based protected area in 14 km stretch path length of the river known as Green Zone. **b)** Any kind human intervention in the river is strictly prohibited in this part.

Station 2: Station two is located at a latitude of 26°17′46.33"N and longitude of 94°35′29.09" E. This station is situated at Longkong Village.

Key Features –a) Dense vegetation

b) Breeding ground of fishes.

Station 3: This station is situated at a latitude of 26°29̋51".62"N and longitude of 94°41́38.78" E. It is located at Changtongya, Mokokchung District of Nagaland. The altitude is 322 m msl. Key Features –a) Moderate gradient b) Good vegetation

Station 4: This station is located at Nazira, Sivsagar, where the river enters into the plains of Assam where The latitude is 26°55′31.64"N and longitude is 94°45′21.98" E. The elevation is 96 m msl. Key Features –a) Low gradient.b) High human settlement.

Station 5: This station is located in Sivsagar Town, Sivsagar District at a latitude of 26°58′33.08."N and longitude of 94°37′50.78" E. The elevation is 96 m msl. Key Features –a) Urban settlement nearby.b) High Anthropogenic Waste

Station 6: This station is situated at a latitude of 26°00'18''N and longitude of 92°45'34''E. It is located at Dikhow Mukh, Sivsagar district of Assam. Here the river joints Brahmaputra. The elevation is 90 m msl. Key Features –a) Rural inhabitants b) Confluent zone.

ANNEXURE- III

TROPHIC LEVEL STRUCTURE & IUCN 2021 STATUS OF FISH OF RIVER DIKHU

The estimation of trophic levels is very much essential for management of fisheries resources. We gathered all the available information regarding the feeding habits of 60 collected fish species trophic state index values were collected from FishBase. The latter ranged from 2.0 to 4.5 and functional trophic groups were identified: (a) Pure Herbivore: Trophic Level 2.0-2.1, (b) Omnivore with a preference for vegetable material (2.1 < TROPH < 2.9), (c) Omnivore with a preference for animal material (3.01<TROPH<3.50) and (d) Carnivore (3.5<TROPH<4.0).

	SPECIES	**rl category**	**Trophic level**	**Remarks**	**NCBI Accession number**
1	*Tor putitora*	EN	2.9	Omnivore(P)	OK036343
2	*Labeo pangusia*	NT	2	Herbivore	------NA----
3	*Garra naganensis*	LC	2	Herbivore	OM348524
4	*Garra nasuta*	LC	2	Herbivore	------NA----
5	*Barilius barila*	LC	3.2	Omnivore(A)	------NA----
6	*Cirrhinus mrigala*	LC	2.3	Omnivore(P)	OM348514
7	*Cirrhinus reba*	LC	2.5	Omnivore(P)	------NA----
8	*Labeo gonius*	LC	2	Herbivore	OM348515
9	*Labeo boga*	LC	2	Herbivore	------NA----
10	*Labeo dyocheilus*	LC	2	Herbivore	------NA----
11	*Labeo bata*	LC	2	Herbivore	------NA----
12	*Devario aequipinnatus*	LC	2.9	Omnivore(P)	OK036344
13	*Danio dangila*	LC	3	Omnivore(A)	------NA----
14	*Garra gotyla*	LC	2	Herbivore	OK036345
15	*Opsarius bendelinis*	LC	3.4	Omnivore(A)	OK036346
16	*Crossocheilus latius*	LC	2.3	Omnivore(P)	OK091622
17	*Esomus danrica*	LC	2.4	Omnivore(P)	OK310715
18	*Salmostoma bacaila*	LC	3.2	Omnivore(A)	OK310734
19	*Neolissochilus hexagonolepis*	NT	3.0	Omnivore(A)	OL716092
20	*Cyprinion semiplotum*	VU	2.8	Omnivore(P)	OL979442

21	*Barilius barna*	LC	3.4	Omnivore(A)	OL981360
22	*Garra lissorhynchus*	LC	2	Herbivore	OL989776
23	*Puntius sophore*	LC	2.6	Omnivore(P)	OM058030
24	*Pethia ticto*	LC	2.2	Omnivore(P)	------NA----
25	*Schistura fasciata*	LC	3	Omnivore(A)	OK103831
26	*Schistura corica*	NE	2.8	Omnivore(P)	OK103911
27	*Schistura khugae*	VU	3	Omnivore(A)	------NA----
28	*Psilorhynchus homaloptera*	LC	2.8	Omnivore(P)	OM002607
29	*Psilorhynchus khopai*	NE	*2.9*	Omnivore(P)	OM348522
30	*Psilorhynchus balitora*	NE	2.9	Omnivore(P)	OM348521
31	*Cabdio morar*	LC	3.2	Omnivore(A)	OM348523
32	*Channa auranti maculata*	DD	3.8	Carnivore	OM348525
33	*Channa marulius*	LC	4.5	Carnivore	OM348516
34	*Channa striatus*	LC	3.6	Carnivore	OM348516
35	*Nandus nandus*	LC	3.9	Carnivore	OM348517
36	*Badis badis*	LC	3.3	Omnivore(A)	OK103853
37	*Chanda nama*	LC	3.6	Carnivore	------NA----
38	*Trichogaster fasciata*	LC	2.8	Omnivore(P)	------NA----
39	*Hara hara*	NE	3.3	Omnivore(A)	------NA----
40	*Glyptothora straitus*	NT	3.2	Omnivore(A)	OM348526
41	*Gagata cenia*	LC	3.3	Omnivore(A)	OK094147
42	*Bagarius bagarius*	NT	3.7	Carnivore	------NA----
43	*Ailia coila*	NT	3.6	Carnivore	------NA----
44	*Amblyceps apangi*	LC	3.3	Omnivore(A)	OK103917
45	*Sperata seenghala*	LC	3.8	Carnivore	OK310718
46	*Sperata aor*	LC	3.6	Carnivore	OM348519
47	*Mystus cavasius*	LC	3.4	Omnivore(A)	------NA----
48	*Mystus dibrugarensis*	LC	3.3	Omnivore(A)	------NA----
49	*Mystus vittatus*	LC	3.1	Omnivore(A)	------NA----
50	*Mystus tengara*	LC	3.2	Omnivore(A)	------NA----
51	*Batasio batasio*	LC	3.3	Omnivore(A)	------NA----
52	*Ompok pabo*	NT	3.8	Carnivore	------NA----

53	*Ompok pabda*	NT	3.8	Carnivore	------NA----
54	*Ompok bimaculatus*	NT	3.9	Carnivore	------NA----
55	*Wallago attu*	VU	3.7	Carnivore	------NA----
56	*Mastacembelus armatus*	LC	2.8	Omnivore(P)	OM348520
57	*Macrognathus aral*	LC	3.1	Omnivore(A)	OM348518
58	*Channa punctata*	LC	3.8	Carnivore	OM058026
59	*Notopterus notopterus*	LC	3.5	Omnivore(A)	OK094146
60	*Rhinomugil corsula*	LC	2.4	Omnivore(P)	OK310716

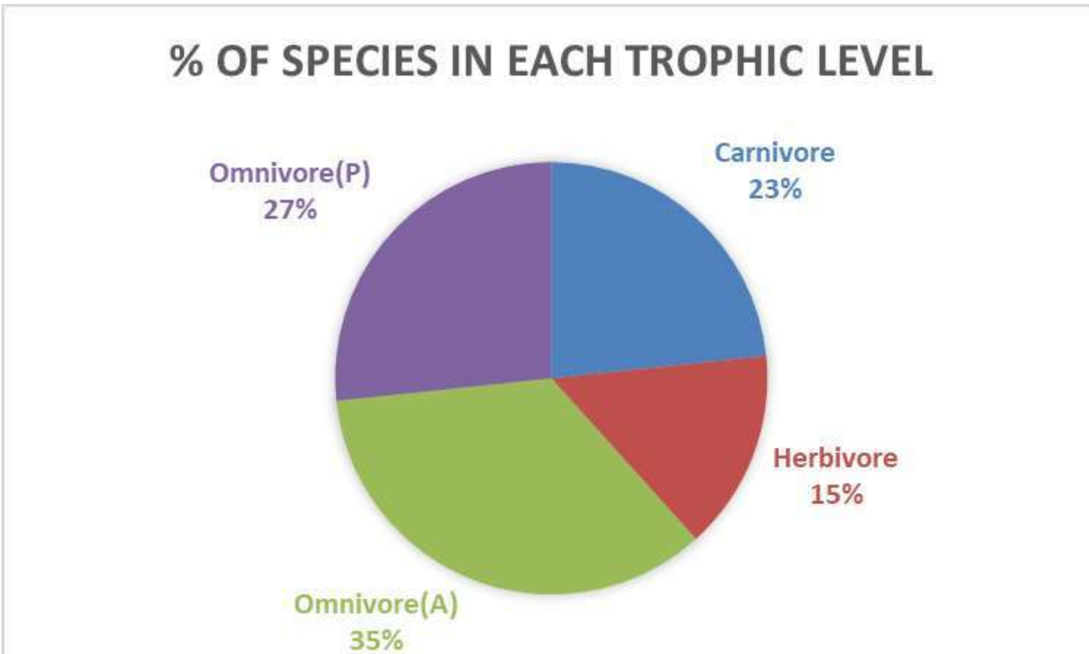


Fig 1: Trophic state index of fishes of River Dikhu

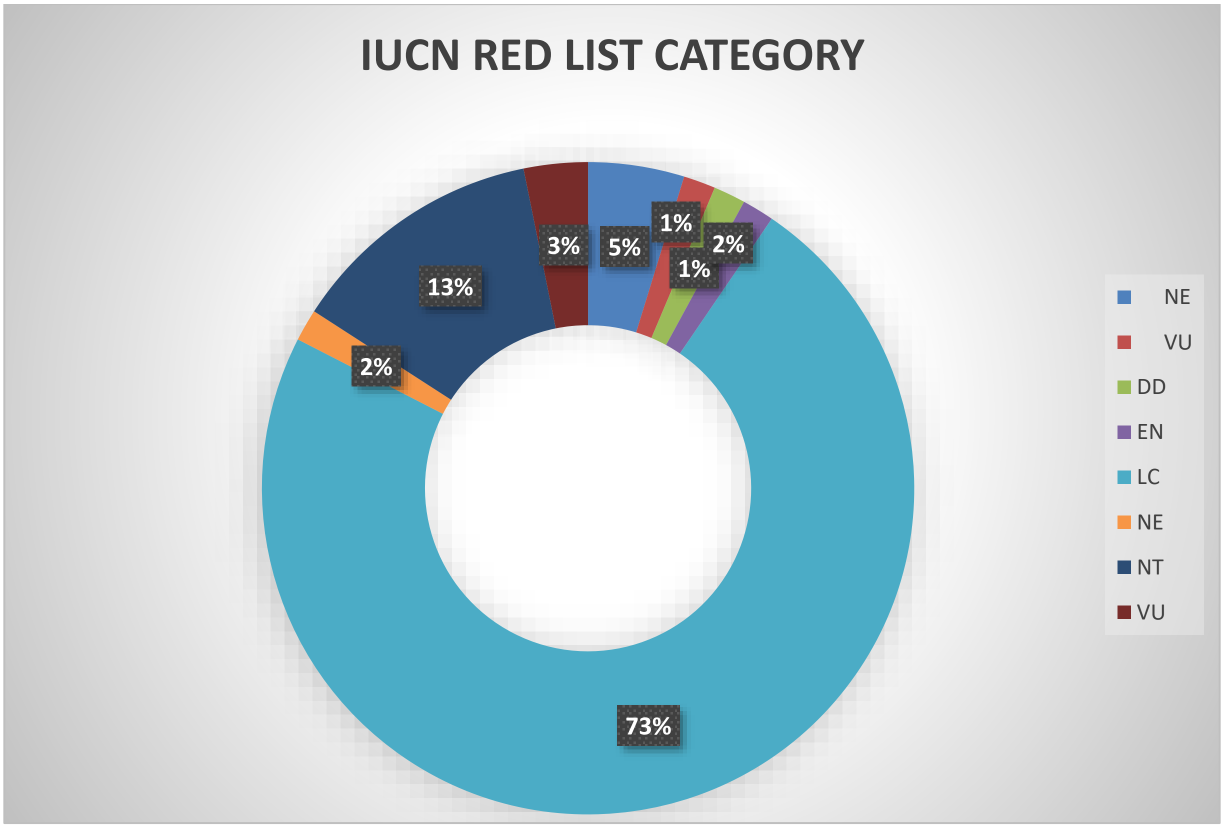


Fig 2 : 2021 IUCN RED LIST category of fishes of River Dikhu.

ANNEXURE IV

Details of the Physico-Chemical Parameters of River Dikhu

Physical parameters of water:

1. Surface Water temperature:

Water temperature is of enormous significance as it regulates various abiotic characteristics and biotic activities of an aquatic ecosystem which is recognized by many authors (Mc Combie, 1953; Hutchinson, 1957; Jana, 1973; Chari, 1980; Kataria et al., 1995; Iqbal and Katariya, 1995; Sharma and Sarang, 2004; Radhika et al., 2004. The minimum and maximum surface water temperature of Dikhu river ranges from 13.6^{o}C (winter, 2019) to 29.7 ^{o}C (Post-Monsoon, 2020).

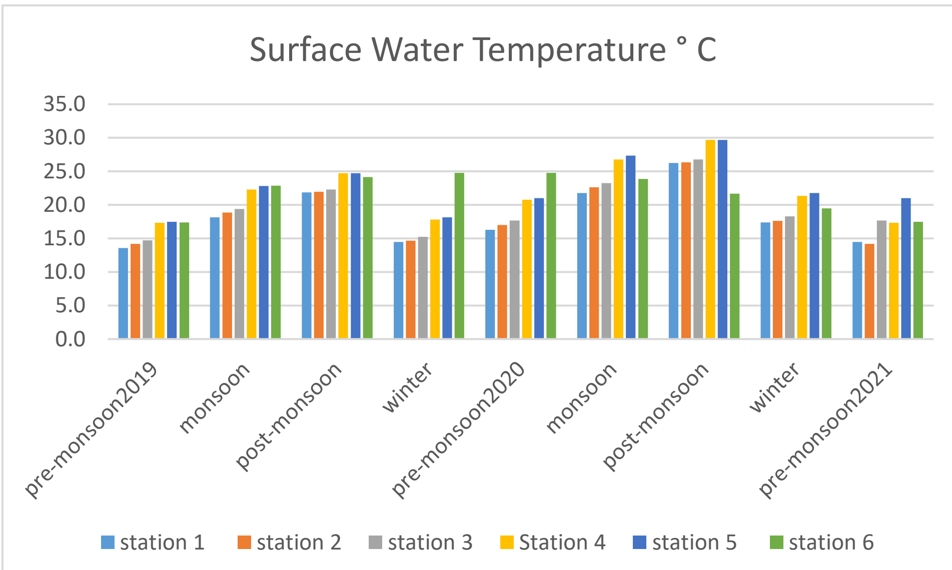


Figure 1 : Seasonal variation of surface water temperature at station 1-6

Stations	Minimum	Maximum
1	13.6 (Pre-Monsoon, 2019)	26.3 (Post-Monsoon, 2020)
2	14.2 (Pre-Monsoon, 2019)	26.3 (Post-Monsoon, 2020)
3	14.7 (Pre-Monsoon, 2019)	26.8 (Post-Monsoon, 2020)
4	17.3 (Pre-Monsoon, 2019)	29.7 (Post-Monsoon, 2020)
5	17.5 (Pre-Monsoon, 2019)	29.7 (post-Monsoon, 2020)
6	17.4 (Pre-Monsoon, 2019)	24.8 (post-Monsoon, 2020)

2. **Turbidity:** Turbidity depends on the presence or absence of clay silt, dissolved organic and inorganic matter, turbid water received from the catchment area, plankton and other microscopic organisms. (Mishra and Saksena, 1991; Singh, 1999; Kulshrestha and Sharma,

2006). Turbidity of Dikhu river water ranges between 3.2 NTU (Winter,2020) to 121.8 NTU (Monsoon 2020).

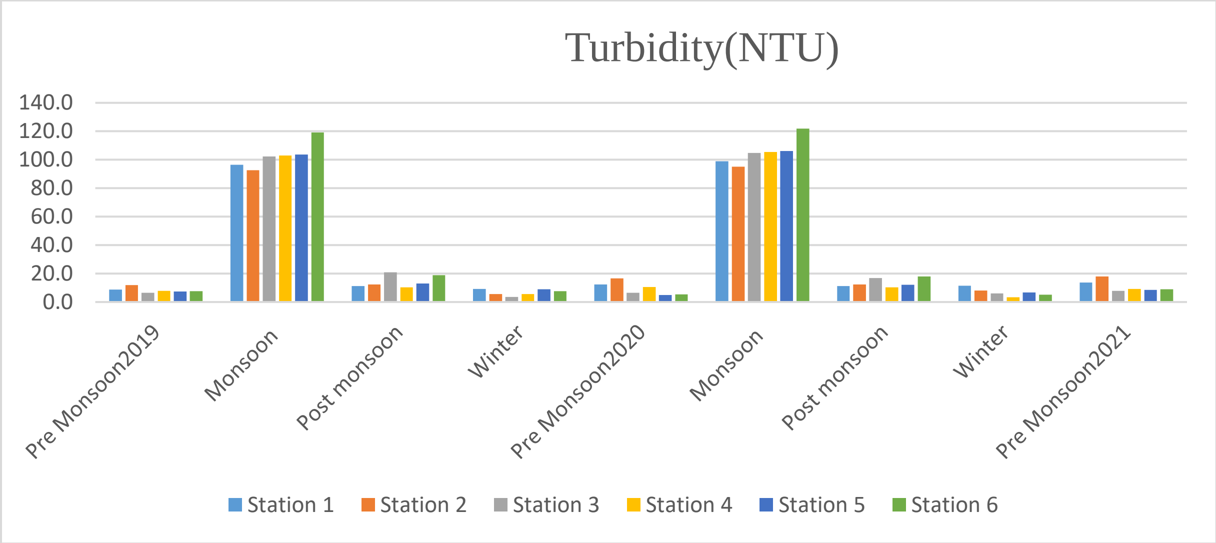


Figure 2: Seasonal variation of Turbidity at station 1-6

Stations	Minimum	Maximum
1	8.8 (Pre-Monsoon, 2019)	99.0 (Monsoon, 2019)
2	5.7 (Winter, 2019)	95.1 (Monsoon, 2020)
3	3.7 (Winter, 2019)	104.8 (Monsoon, 2020)
4	3.2 (Winter, 2019)	105.6 (Monsoon, 2020)
5	5.0 (Pre-Monsoon, 2021)	106.2 (Monsoon, 2020)
6	5.2 (Winter, 2020)	121.8 (Monsoon, 2020)

Chemical parameters of water

1. **Water pH:** pH is a measure of the acidic and alkaline condition of a water body that affects its productivity (Welch, 1952). pH of water is important because all physico-chemical reactions of water in an aquatic body take place at a definite pH which plays an important role in the productivity of river. The river water pH of Dikhu falls under neutral to alkaline conditions. Lowest pH of water was found to be 7.0 (pre-monsoon, 2019) and highest during monsoon, 2019 (8.0).

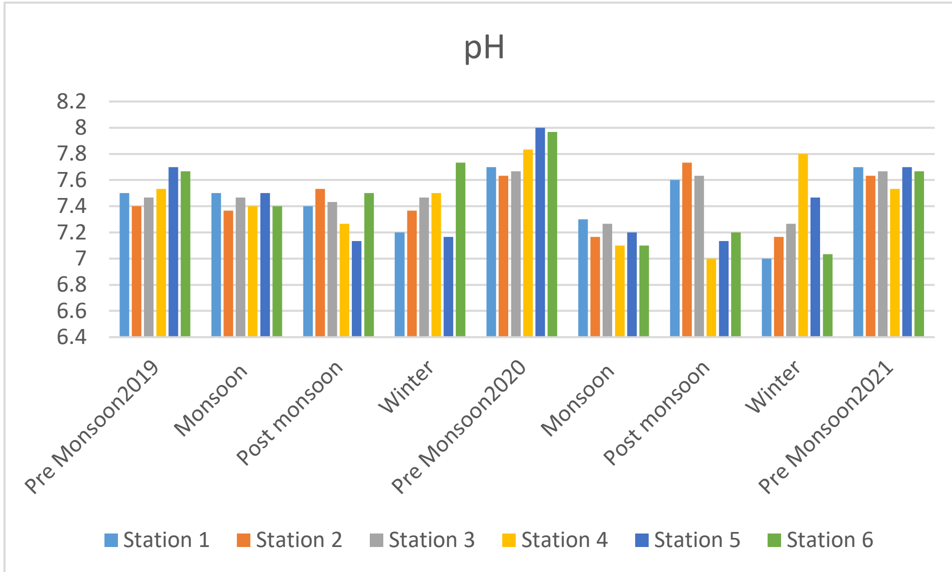


Figure 3: Seasonal variation of pH at station 1-6

Stations	Minimum	Maximum
1	7.01 (Winter, 2019)	7.72 (Pre-Monsoon, 2020)
2	7.20 (Post-monsoon, 2019)	7.76 (Winter, 2020-21)
3	7.3 (Monsoon, 2019)	7.73 (Pre-monsoon, 2020)
4	7.02 (Post-monsoon, 2020)	7.82 (Pre-Monsoon, 2020)
5	7.1 (Post-monsoon, 2019)	7.80 (Monsoon, 2020)
6	7.0 (Post-monsoon, 2020)	8.02 (Premonsoon, 2019-20)

2. Dissolved Oxygen (DO): Dissolved oxygen in water is indispensable for aquatic life for their survival. Dissolve oxygen in natural water depends on different physical, chemical and biological factors. In the present study, DO value ranged from 5.9 (Winter2019) to 9.99 (Monsoon,2020) mgL^{-1}.

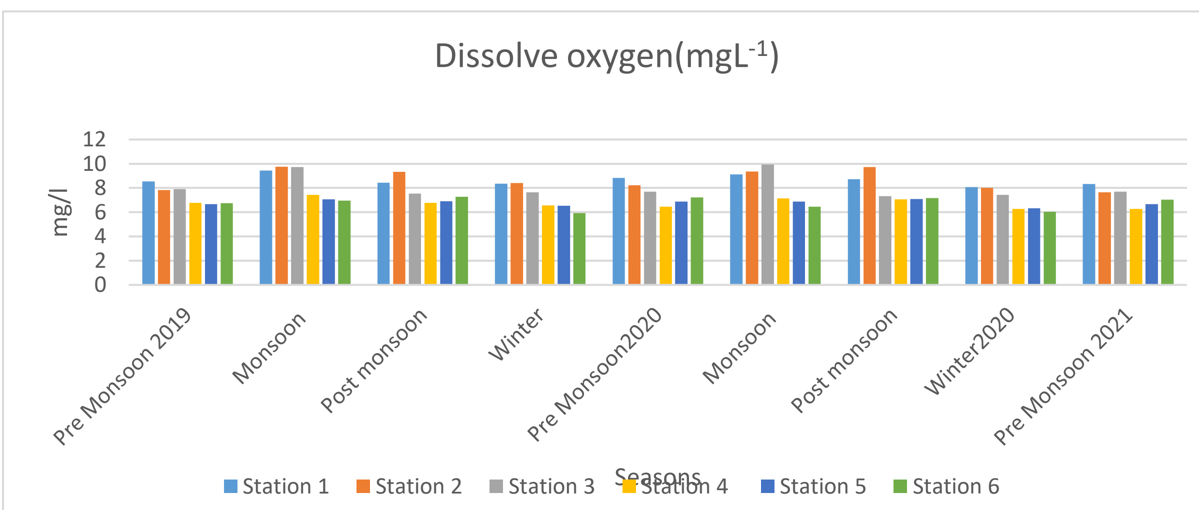


Figure 4: Seasonal variation of Dissolved Oxygen at station 1-6

Stations	Minimum	Maximum
1	8.06 (Winter, 2020)	9.43 (Monsoon, 2019)
2	7.63 (Pre-Monsoon, 2020)	9.76 (Monsoon, 2019)
3	7.33 (Monsoon, 2020)	9.93 (Winter, 2020-21)
4	6.26 (Monsoon, 2020)	7.43 (Winter, 2020-21)
5	6.33 (Monsoon, 2020)	7.11 (Winter, 2020-21)
6	5.93 (Monsoon, 2019)	7.26 (Winter, 2020-21)

3. **Total Alkalinity:** Alkalinity is the water's ability to resist changes in pH and is a measure of the total concentration of bases in pond water including carbonates, bicarbonates, hydroxides, phosphates and borates, dissolved calcium, magnesium, and other compounds in the water. Alkalinity acts as a stabilizer for pH. During the present study, the total alkalinity value was found to be in the range of 38.3 (Winter 2020) to 80.0 ppm (Post-Monsoon).

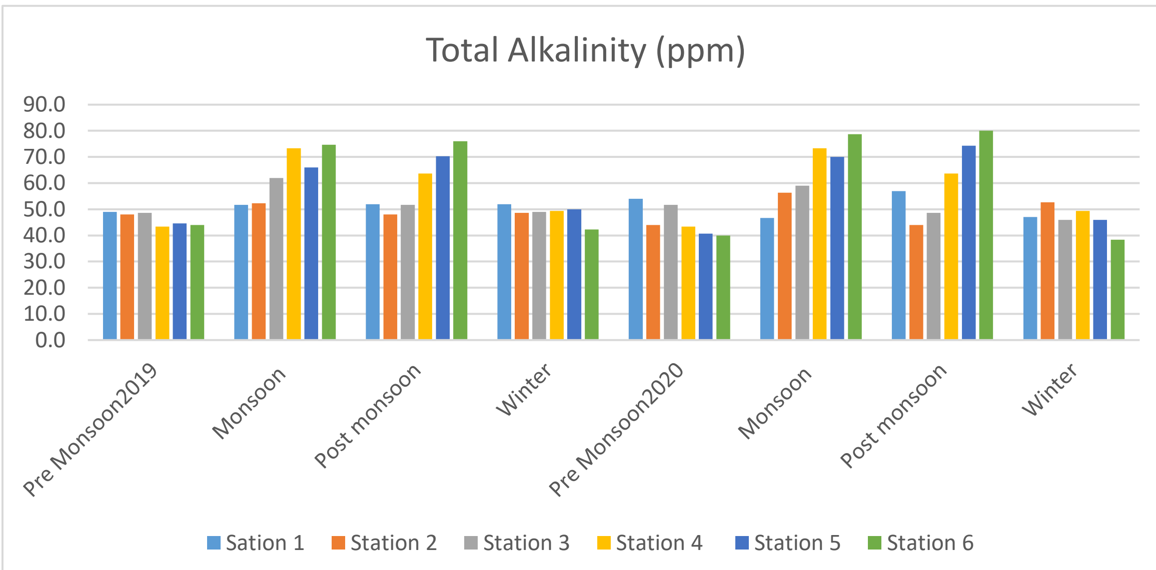


Figure 5: Seasonal variation of Total Alkalinity at station 1-6

Stations	Minimum	Maximum
1	46.7(Monsoon ,2021)	57.0 (Post Monsoon, 2021)
2	44.6 (Post-Monsoon, 2020)	52.2 (Winter, 2021)
3	46.2 (Winter, 2019)	62.6(Monsoon, 2020-21)
4	43.3 (Monsoon, 2019)	73.9 (Monson , 2019)
5	40.7 (Pre-Monsoon, 2020)	74.2 (Post-monsoon, 2020)
6	38.1 (Monsoon, 2020)	80.0 (Post Monsoon, 2021)

4. **Total Hardness:** Hardness is the amount of dissolved calcium and magnesium salts in the water. Calcium and magnesium occur mainly in combination with bicarbonate, sulphate, and chloride. Total hardness values of surface water of Dikhu river during the study period varied from 39.51 ppm (Pre-monsoon, 2021) to 99.724 ppm (Post-monsoon,2020).

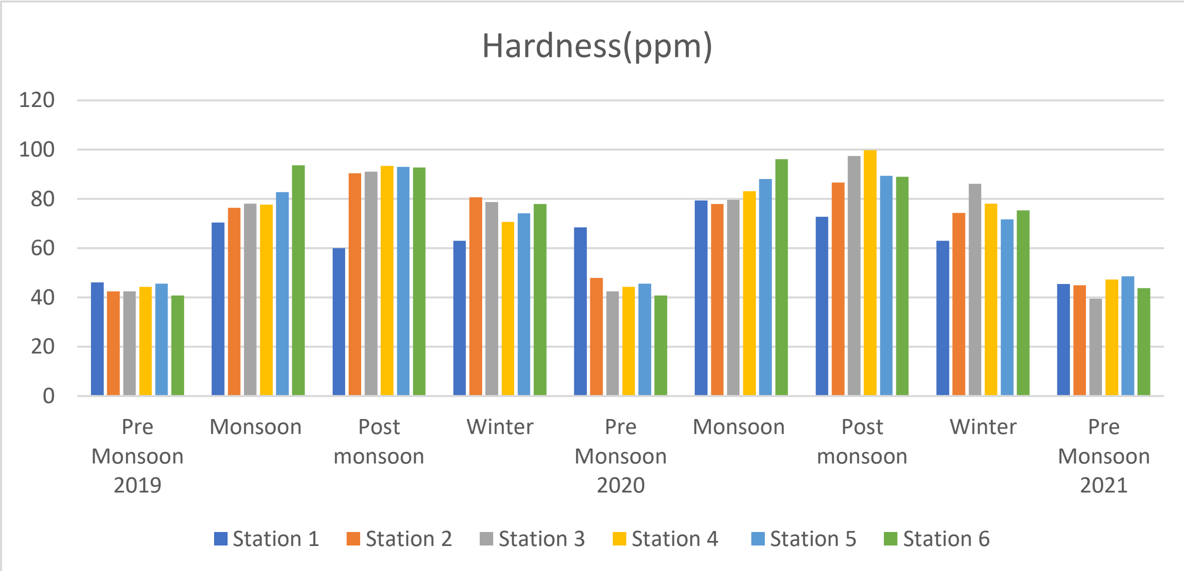


Figure 6: Seasonal variation of Total Hardness at Station 1-6

Stations	Minimum	Maximum
1	45.5 (Pre-Monsoon, 2021)	79.40 (monsoon, 2020)
2	42.5 (Pre-Monsoon, 2019)	90 (Post-monsoon, 2019)
3	39.5 (Pre-Monsoon, 2021)	97.3 (Post-monsoon, 2020)
4	44.2 (Pre-monsoon, 2020)	99.7 (Post-Monsoon, 2019)
5	45.61 (Pre-monsoon, 2020)	93.09 (Post Monsoon, 2019)
6	40.8 (Pre-Monsoon, 2020)	96.14 (Monsoon, 20)

5. Electrical Conductivity: Conductivity can be used as indicator of primary production (chemical richness) and thus fish production. Conductivity of water depends on its ionic concentration (Ca^{2+}, Mg^{2+}, HCO_3^-, CO_3^-, NO_3^- and PO_4^-), temperature and variations of dissolved solids. In the present study conductivity range from 118 to 252 **(µS/cm)**.

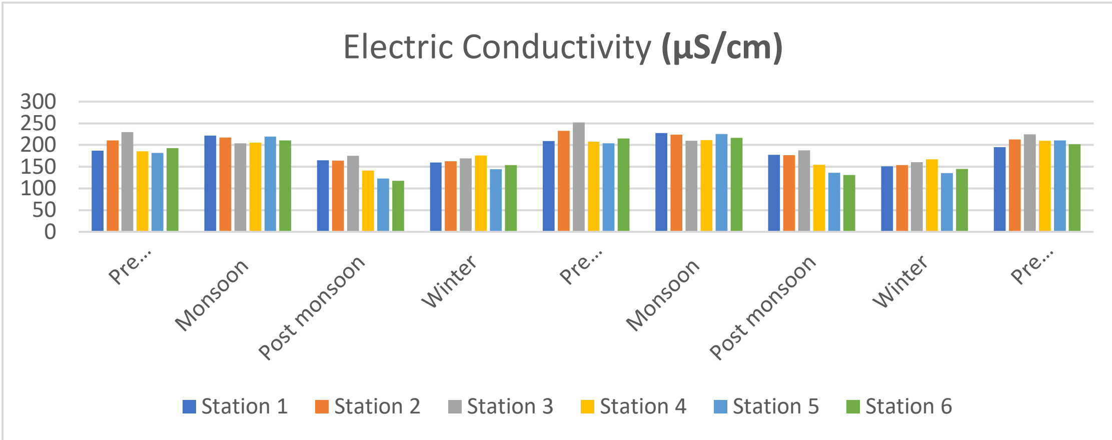


Figure 7: Seasonal Variation of Electrical Conductivity at Station 1-6

Stations	Minimum	Maximum
1	151 (Winter, 2020-21)	228 (Monsoon, 2020)
2	154 (Winter, 2020-21)	233 (Pre-Monsoon, 2020)
3	160 (Winter, 2020-21)	230 (Pre-Monsoon, 2019)
4	141 (Post-Monsoon, 2020-21)	210 (Pre-Monsoon, 2021)
5	135 (Winter, 2020)	225 (Monsoon, 2020)
6	118(Post-Monson, 2020-21)	217 (Monsoon, 2020)

6. Total Dissolved Solid: Total dissolved solid (TDS) is a measure of the total organic and inorganic substances present in a liquid. This includes anything present in water other than the pure H_2O molecules. These solids are primarily minerals, salts and organic matter that can be a general indicator of water quality. In the present investigation, the lowest value of TDS recorded was of 77.03 (Winter, 2019-20) and highest was of 198.7 (Pre-Monsoon, 2020).

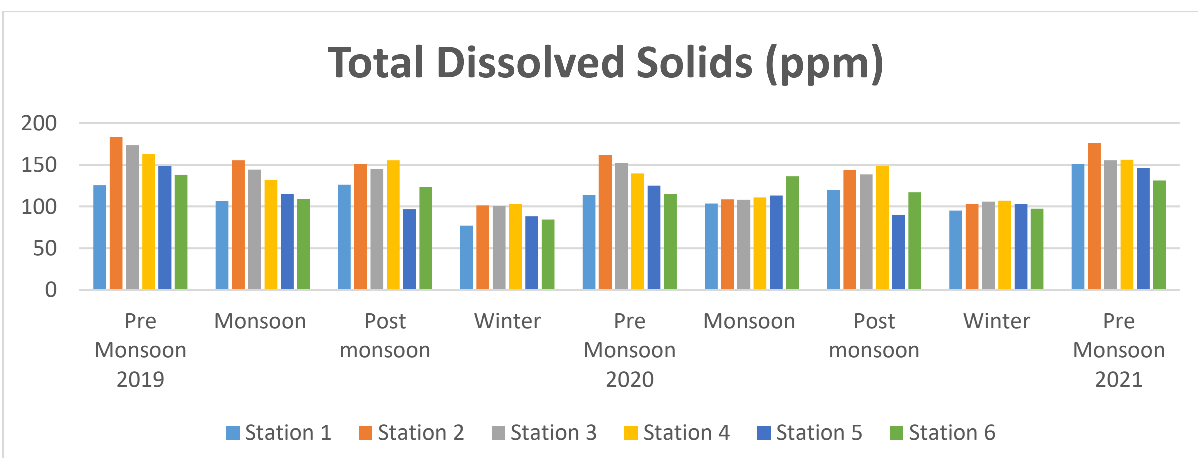


Figure 8: Seasonal Variation of Total Dissolved Solids at Station 1-6

Stations	Minimum	Maximum
1	77.03 (Winter, 2019-20)	151.4(Pre-Monsoon, 2019)
2	101.3 (Winter, 2019-20)	183.3 (Pre-Monsoon, 2019)
3	101.2 (Winter, 2019-20)	173.6 (Pre-Monsoon, 2019)
4	103.2 (Winter, 2019)	163.3 (Pre-Monsoon, 2019)
5	88.3 (Winter, 2019)	149.3 (Pre-Monsoon, 2019)
6	84.4 (Winter, 2019-20)	138.2 (Pre-Monsoon, 2019)

7. Nitrite-Nitrogen: Nitrite is one of the intermediate products of aerobic nitrification bacterial process, produced by the autotrophic Nitrosomonas bacteria combining oxygen and ammonia. They are unstable and depending on conditions, can be converted into nitrates or ammonia which are harmful to aquatic life. In the present investigation nitrite-nitrogen value ranged in between 0.079 (Winter, 2019) and 0.11 (Monsoon, 2020) mgL^{-1}.

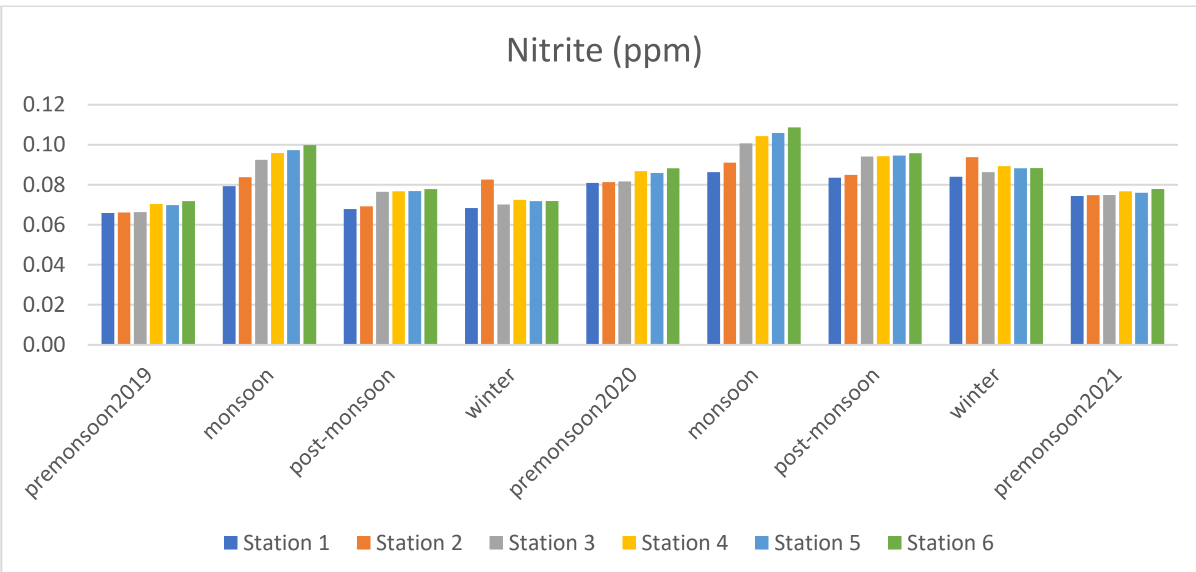


Figure 9 : Seasonal Variation Nitrite-Nitrogen at Station 1-6

Stations	Minimum	Maximum
1	0.06 (Winter, 2019)	0.09 (Monsoon, 2019)
2	0.07 (Premoonsoon,Winter, 2019)	0.13 (Monsoon, 2020)
3	0.07 (Premoonsoon,Winter, 2019)	0.15 (Monsoon, 2019)
4	0.06 (Winter, 2019)	0.11 (Monsoon, 2020)
5	0.06 (Winter, 2019)	0.11 (Monsoon, 2019)
6	0.07 (Premoonsoon,Winter, 2019)	0.12 (Monsoon, 2020)

8. Nitrate – Nitrogen: Nitrogen undergoes quick transformation in the tropical river and gets stored in the biota. In our present investigation nitrite nitrogen lowest value was found to be 0.05 µgl^{-1} (Winter, 2021) and highest to be 0.22 µgl^{-1} (Monsoon, 2020).

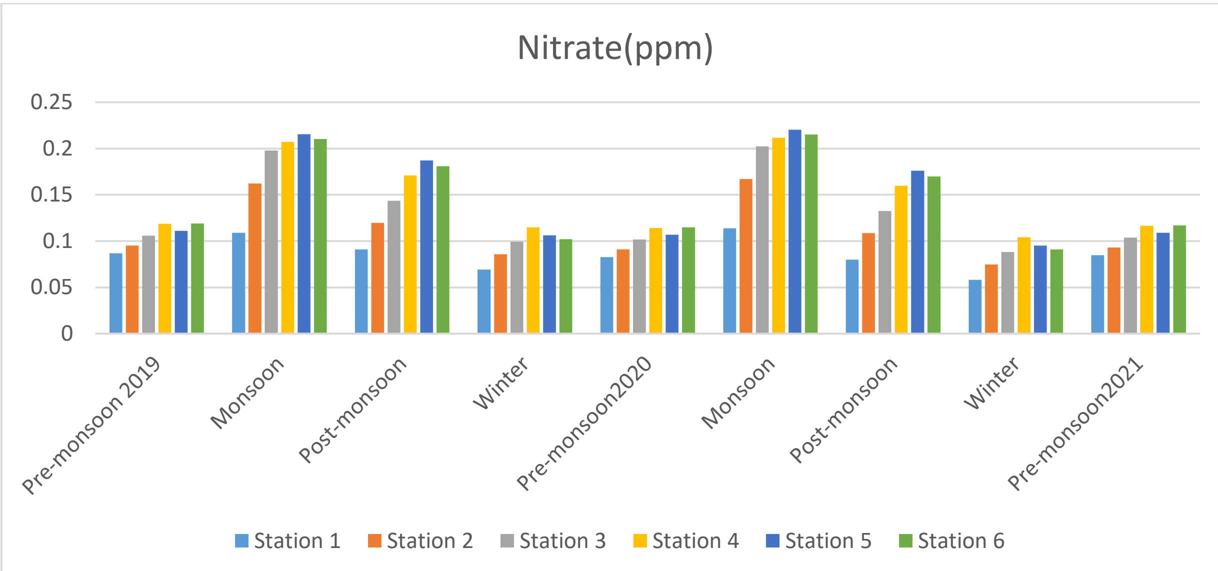


Figure 10: Seasonal Variation of Nitrite-Nitrogen at Station 1-6

Stations	Minimum	Maximum
1	0.058 (Winter, 2019)	0.11(Monsoon, 2019)
2	0.074 (Winter, 2020)	0.16 (Monsoon, 2020)
3	0.088 (Winter, 2020)	0.20(Monsoon, 2020)
4	0.103 (Winter, 2020)	0.2 (Monsoon, 2019)
5	0.095 (Winter, 2020)	0.22 (Monsoon, 2020)
6	0.090 (Winter, 2020)	0.215 (Monsoon, 2020)

9. Total Ammonia: Ammonia is a highly toxic pollutant of the aquatic environment. The by-product of protein metabolism excreted by fish and bacterial decomposition of organic matter such as wasted food, agricultural wastes, dead planktons, sewage etc. is ammonia. The unionized form of ammonia (NH_3) is extremely toxic while the ionized form (NH_4^-) is not and both the forms are grouped together as “total ammonia”. Total ammonia values of the water samples of the Dikhu River during the study period varied from 0.01 (Winter, 2019) to 0.032(Monsoon, 2020).

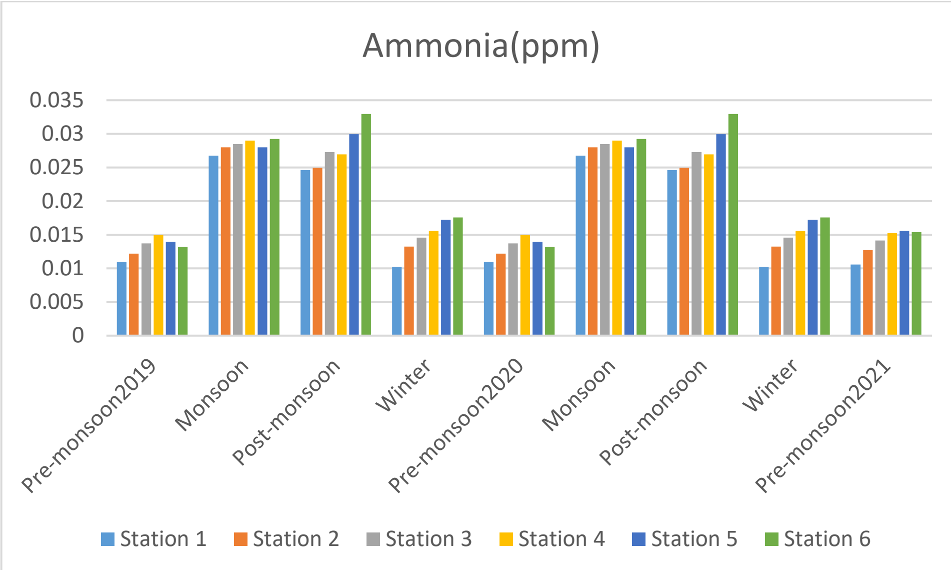


Figure 11: Seasonal Variation of Total Ammonia at Station 1-6

Stations	Minimum	Maximum
1	0.010 (Winter, 2020)	0.026 (Monsoon, 2019)
2	0.0122 (Pre-Monsoon, 2020)	0.028 (Monsoon, 2019)
3	0.013 (Pre-Monsoon, 2020)	0.0285 (Monsoon, 2019)
4	0.014 (Pre-Monsoon, 2020)	0.031 (Monsoon, 2019)
5	0.013 (Pre-Monsoon, 2020)	0.027 (Monsoon, 2020)
6	0.013 (Pre-Monsoon, 2020)	0.032 (Monsoon, 2020)

10. Soluble Inorganic Phosphate:

Phosphorous is an important parameter to assess the water quality since it is the limiting nutrient for plant growth in the freshwater system (Stickney, 2005) which regulates the phytoplankton production in presence of nitrogen. The availability of phosphate in water depends on the organic matter content of bottom and type of microorganisms present in the system. The release of phosphate is dependent on soil reaction. The slightly acidic condition of the medium favors the release and availability of phosphate into the water. Soluble inorganic phosphate values of the present investigation ranged from 0.012 (Winter, 2021) to 0.12 (Monsoon, 2019).

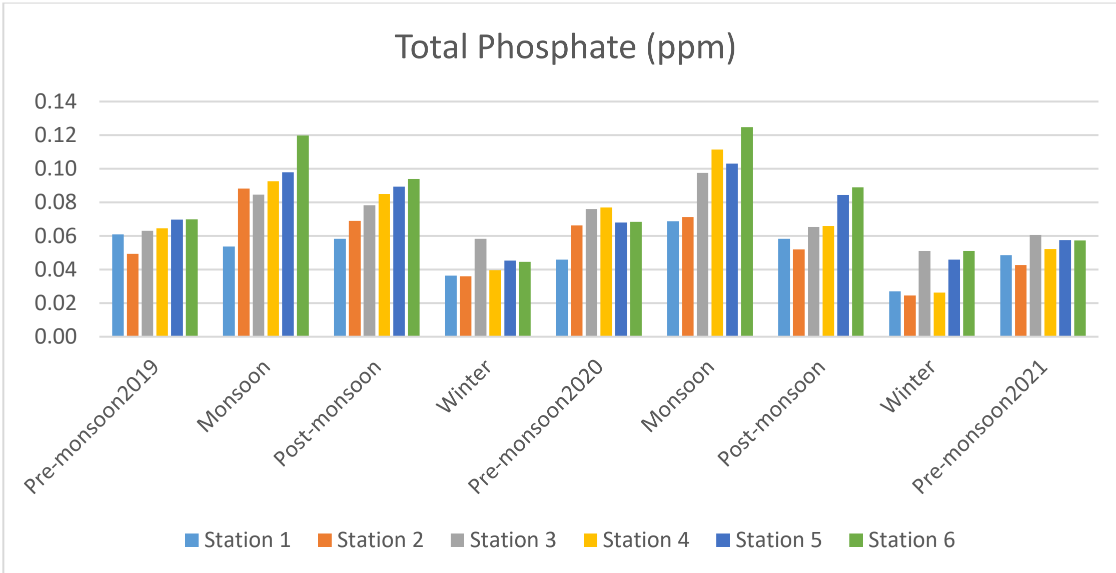


Figure 12: Seasonal Variation of Soluble Inorganic Phosphate at Station 1-6

Stations	Minimum	Maximum
1	0.03 (Winter, 2020)	0.07 (Monsoon, 2020)
2	0.02 (Winter, 2020)	0.09 (Monsoon, 2019)
3	0.05 (Winter, 2020)	0.10 (Monsoon, 2019)
4	0.03 (Winter, 2020)	0.11 (Monsoon, 2020)
5	0.05 (Winter, 2020)	0.10 (Monsoon, 2019)
6	0.04 (Winter, 2019)	0.12 (Monsoon, 2019)

ANNEXURE- V
Sediment Parameters of River Dikhu

1. Sediment pH: Sediment pH measures the acidic and alkaline condition of the river bed which has a direct or indirect influence on water pH and nutrient circulation. The findings of present study indicate that sediment pH varied between 7.9 (Monsoon, 2019) to 8.8 (Winter, 2020).

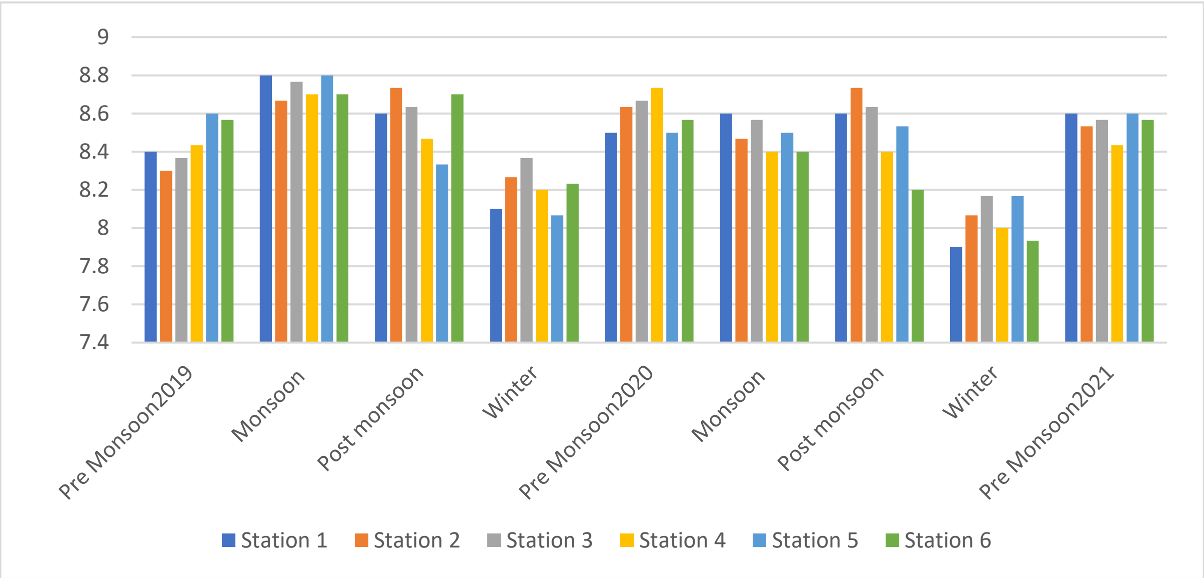


Figure 1: Seasonal Variation of Sediment pH at Station 1-6

Stations	Minimum (Average)	Maximum (Average)
1	7.9 (Winter, 2021)	8.8 (Monsoon, 2019)
2	8.06 (Winter, 2020)	8.73 (Post-monsoon, 2020)
3	8.16 (Winter, 2020)	8.76 (Monsoon, 2020)
4	8.0 (Winter, 2020)	8.7 (Pre-monsoon, 2020)
5	8.06 (Winter, 2020)	8.8 (Monsoon, 2020)
6	7.9 (Winter 2019)	8.7 (monsoon, 2020)

2. **Sediment Organic Carbon:** In present investigation Sediment Organic Carbon percentages were found within the range of 0.39-2.36 %, minimum during winter and maximum during onsoon season.

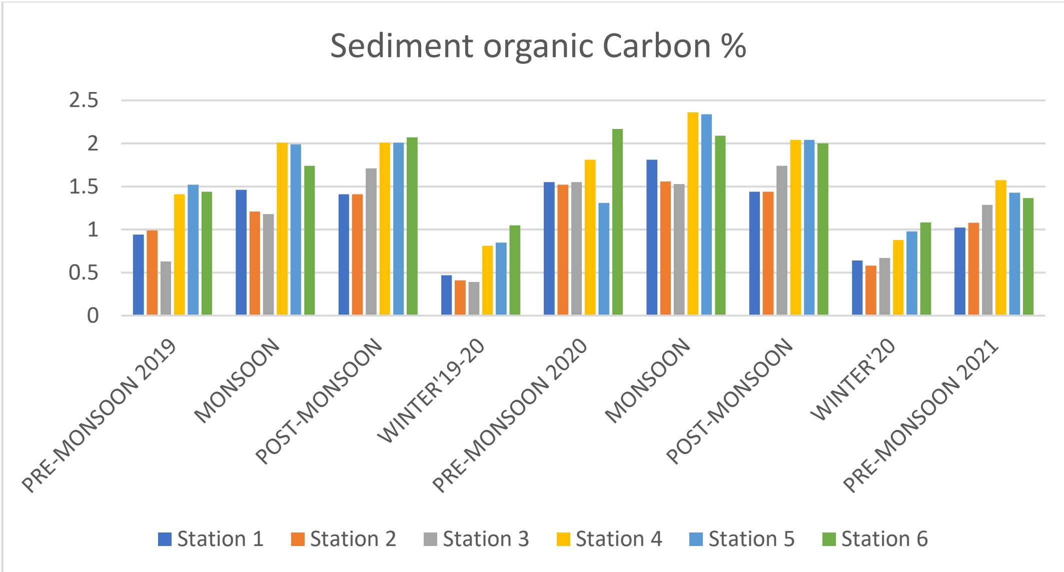


Figure 2: Seasonal Variation of Sediment Organic Carbon at Station 1-6

Stations	Minimum	Maximum
1	0.47 (Winter, 2020)	1.81 (Pre-monsoon, 2020)
2	0.41 (Winter, 2020)	1.56 (Monsoon, 2020)
3	0.39 (Winter, 2020)	1.74 (Pre-monsoon, 2020)
4	0.81 (Winter, 2020)	2.36 (Monsoon, 2020)
5	0.85 (Winter, 2020)	2.34 (Monsoon, 2020)
6	1.05 (Winter, 2020)	2.17 (Pre-monsoon, 2020)

3. Sediment Organic Matter: Sediment organic matter of the present investigation ranged from 0.49 to 5.02 %.

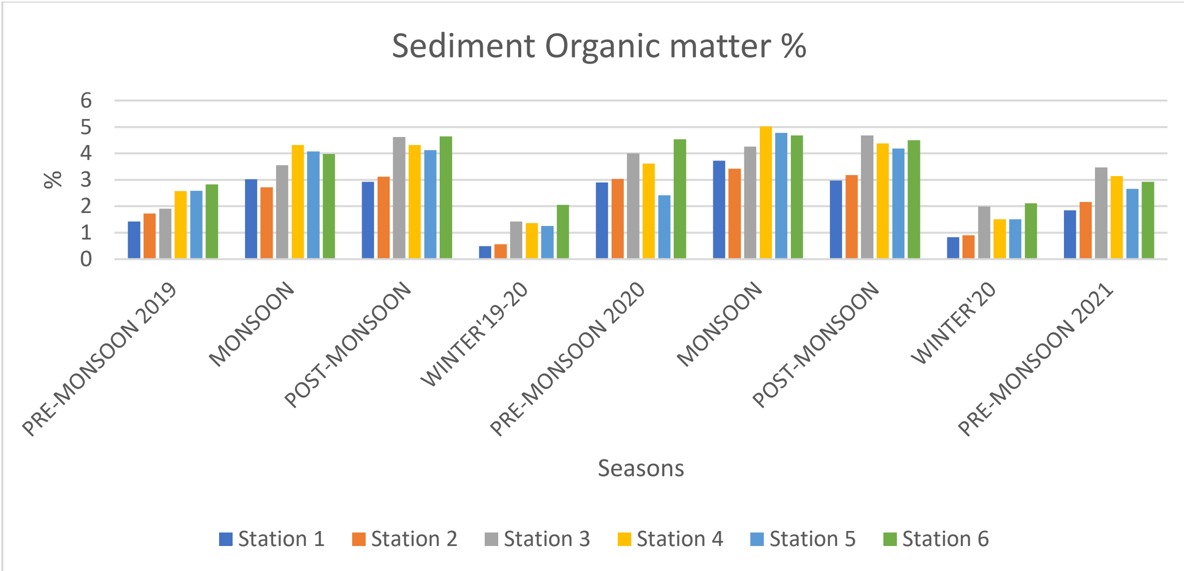


Figure 3: Seasonal Variation of Sediment Organic Matter at Station 1-6

Stations	Minimum (Average)	Maximum (Average)
1	0.49 (Winter, 2020)	3.72 (Monsoon, 2020)
2	0.57(Winter, 2020)	3.42 (Monsoon, 2020)
3	1.43(Winter, 2020)	3.68 (Post-monsoon, 2020)
4	1.37(Winter, 2020)	5.02 (Monsoon, 2020)
5	1.25(Winter, 2020)	4.78 (Monsoon, 2020)
6	2.05 (Winter, 2020)	4.68 (Monsoon, 2020)

ANNEXURE VI

Photographs of Collected & Identified Fish Species

	
1. *Tor putitora*	2. *Neolissochilus hexagonolepis*
	
3. *Devario aequipinnatus*	4. *Garra gotyla gotyla*
	
5. *Garra nasuta*	6. *Garra naganensis*
	
7. *Opsarius bendelisis*	8. *Opsarius barna*
	
9. *Barilius barila*	10. *Puntius sophore*

	
11. Trichogaster fasciata	*12. Labeo bata*

	
13. Labeo gonius	*14. Labeo dyocheilus*
	
15. Labeo pangusia	*16. Labeo boga*
	
17. Cirrhinus mrigala	*18. Cirrhinus reba*

	
19. Esomus danricus	*20. Cabdio morar*
	
21. Danio dangila	*22. Salmostoma bacaila*

	
23. Psilorhynchus homaloptera	*24. Psilorhynchus balitora*
	
25. Schistura khugae	*26. Schistura fasciata*

	
27. Notopterus synurus	*28. Badis badis*
	
29. Channa marulius	*30. Channa punctata*
	
31. Channa striata	*32. Chanda nama*
	
33. Mystus tengara	*34. Parambassis ranga*

	
35. Mystus vittatus	*36. Mystus cavasius*
	
37. Sperata aor	*38. Sperata seenghala*

	
39. Ailia coilia	*40. Wallago attu*
	
41. Ompok bimaculatus	*42. Glyptothorax striatus*

	
43. Ompok pabda	*44. Glyptothorax striatus*

	
45. Erethistes hara	*46. Macrognathus aral*
	
47. Amblyceps apangi	*48. Mastacembelus armatus*

ANNEXURE- VII

Plankton Diversity and Biomass of River Dikhu

The most sensitive component of aquatic ecosystem is the plankton which gives the signal about the environmental disturbances. Phytoplankton plays an important role in food chain as they are the key of primary productivity and also acts as a biological indicator of water quality in relation to pollution studies. Zooplankton provides fish with nutrients as they require protein, fats, carbohydrates, mineral salts and water in right proportion (Jabeen and Barbhuya, 2018). Plankton studies and monitoring are useful for assessment of the physico-chemical and biological conditions of the water in any purpose.

During the research period, 36 different plankton genera were identified in the River Dikhu. The phytoplankton population was represented by 26 genera from the Chlorophyceae (13 genera), Bacillariophyceae (6 genera), Cyanophyceae (6 genera), and Euglenophyceae families (1 genera). Rotifera (5 genera), Cladocera (3 genera), and Copepoda (2) were all found in the zooplankton population. Plankton population density fluctuated from season to season. The average minimum plankton density was found to be 624 units/L while the highest plankton density was found to be 2178 units/L.

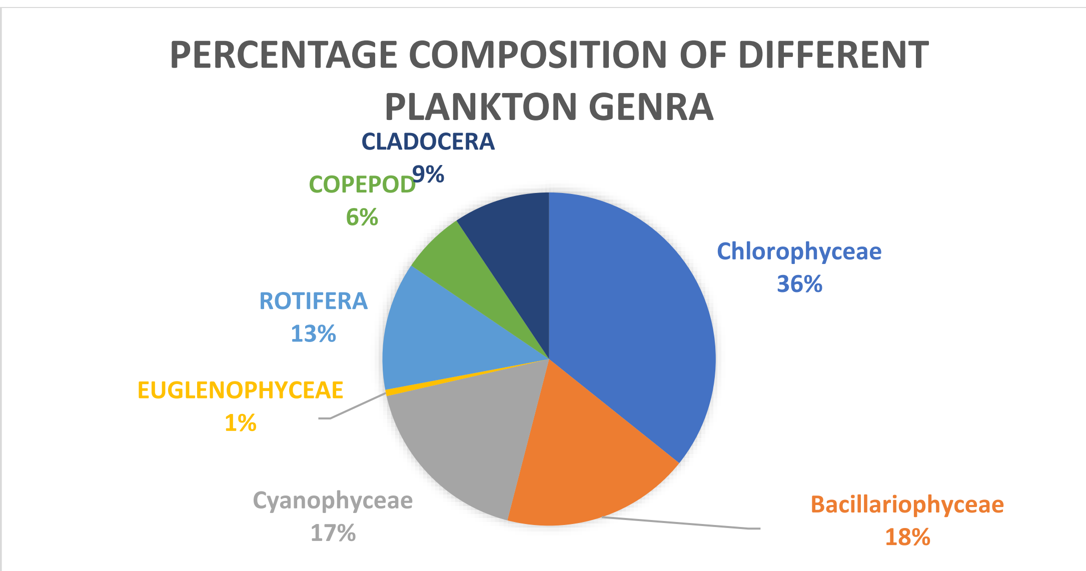


Figure 1: Percentage contribution of different plankton genera in river Dikhu recorded during the study period.

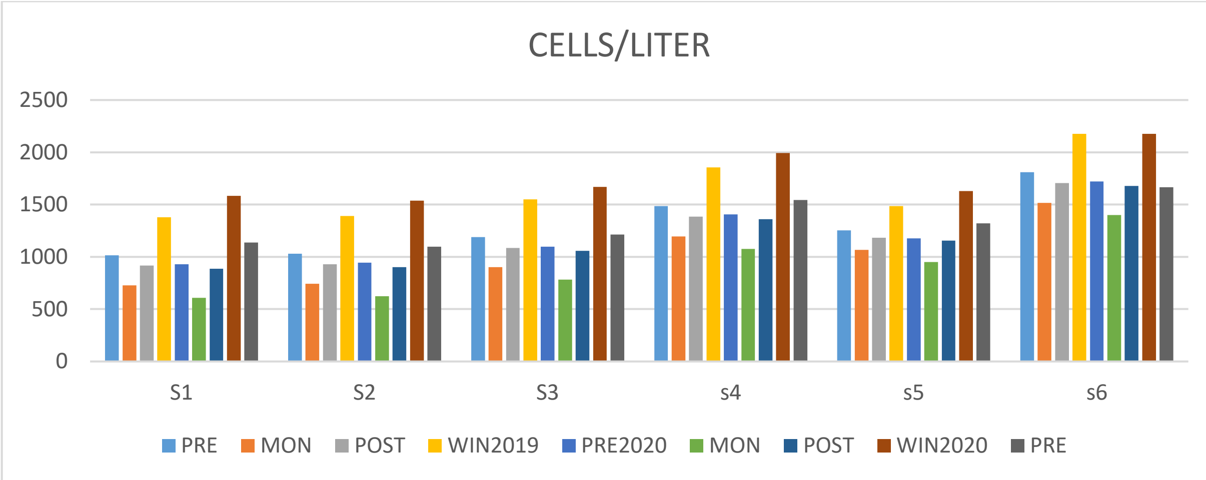


Figure 2: Seasonal variation of plankton density at stations 1 to 6 during the study period.

Phytoplankton:

The phytoplankton community of the study area constituted 72.18 % out of the total plankton collected throughout the studied period. Out of the 26 genera of phytoplankton recorded, Chlorophyceae comprises of 35.80 %, Bacillariophyceae 18.32%, Cyanophyceae 17.42 % and Euglenophyceae 0.62 % of the total plankton composition. Phytoplankton density of the studied Dikhu river ranges from 473 cells/L to 1621 cells/L being the maximum in winter 2019 and minimum during monsoon, 2020.

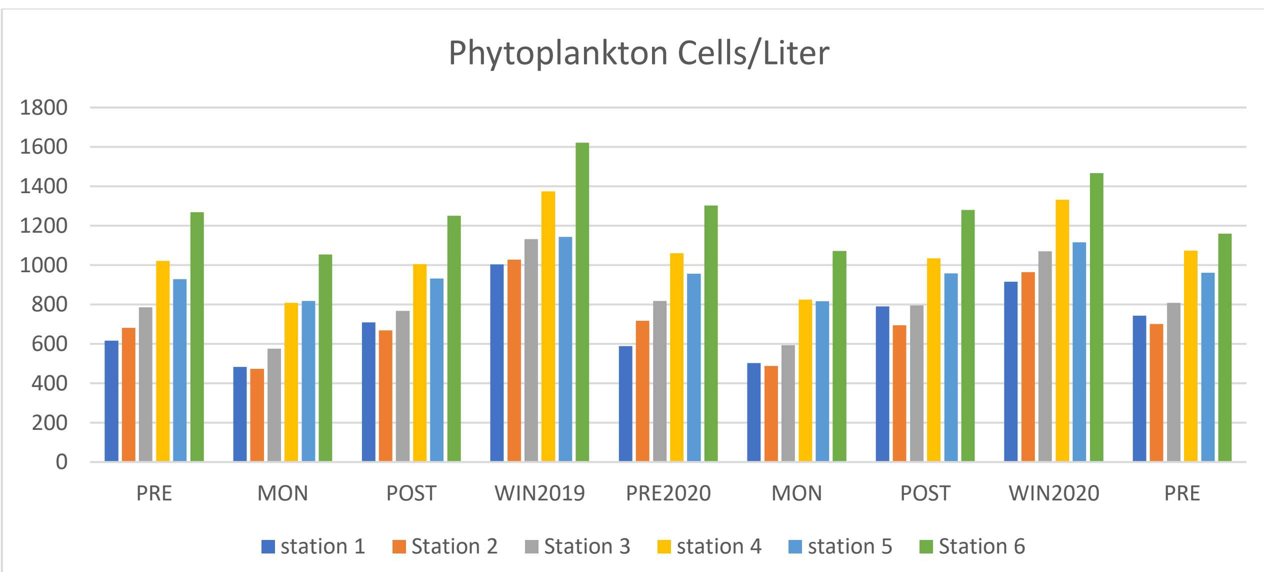


Figure 3: Seasonal variation of phytoplankton density at stations 1 to 6 during the study period.

Table 1: Phytoplankton composition of the 6 stations observed during the present study

Genus	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
Chlorophyceae						
Closterium	+	+	+	+	+	+
Cosmarium	+	+	+	+	-	+
Chlamydomonas	-	-	-	-	+	-
Zygnema	+	+	+	+	-	+
Pediastrum	+	+	+	+	+	+
Pandorina	+	+	-	+	-	+
Ocystis	+	+	+	+	+	+
Eudorina	+	+	+	+	+	+
Oocystis	+	+	+	+	+	+
Euglena	-	-	-	_	+	-
Volvox	+	+	-	+	-	+
Spirogyra	+	+	+	+	+	+
Chlorella	-	+	+	-	-	-
Bacillariophyceae						
Tabellaria	+	+	+	+	-	+
Fragilaria	+	+	+	+	+	+
Navicula	+	+	+	+	+	-
Nitzschia	-	-	-	-	+	-
Cyclotella	-	+	-	+	+	+
Frustulia	+	+		+	+	+
Cyanophyceae						
Synedra	-	-	+	+	+	+
Oscillatoria	-	-	-	+	+	-
Anabena	+	+	+	-	-	+
Merismopedia	+	+	+	+	+	+
Spirulina	+	+	+	+	-	+
Nostoc	+	+	+	+	+	+
Euglenophyceae						
Phacus	+	-	-	+	+	+

Table 2: Zooplankton composition of the 6 stations observed during the present study

Genus	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
Rotifera						
Brachionus	+	+	+	+	+	+
Keratella	+	+	+	+	+	+
Lecane	+	+	+	+	-	+
Polyarthra	+	+	+	+	+	+
Copepod						
Cyclops	+	+	+	+	+	+

Diaptomus	+	+	+	+	+	+
Cladocera	+					
Daphinia	+	+	+	+	+	+
Moina	+	+	+	+	+	+
Bosmina	+	+	+	+	+	+

Zooplankton:

Zooplankton community constituted only 28.06 % of the total plankton hauled. A total of 10 genera zooplankton were recorded and being the highest in rotifera (12.46 %). Zooplankton density of the studied Dikhu river ranges from 134 nos/m^3 to 712 nos/m^3 being the maximum in winter 2020 and minimum during monsoon, 2020.

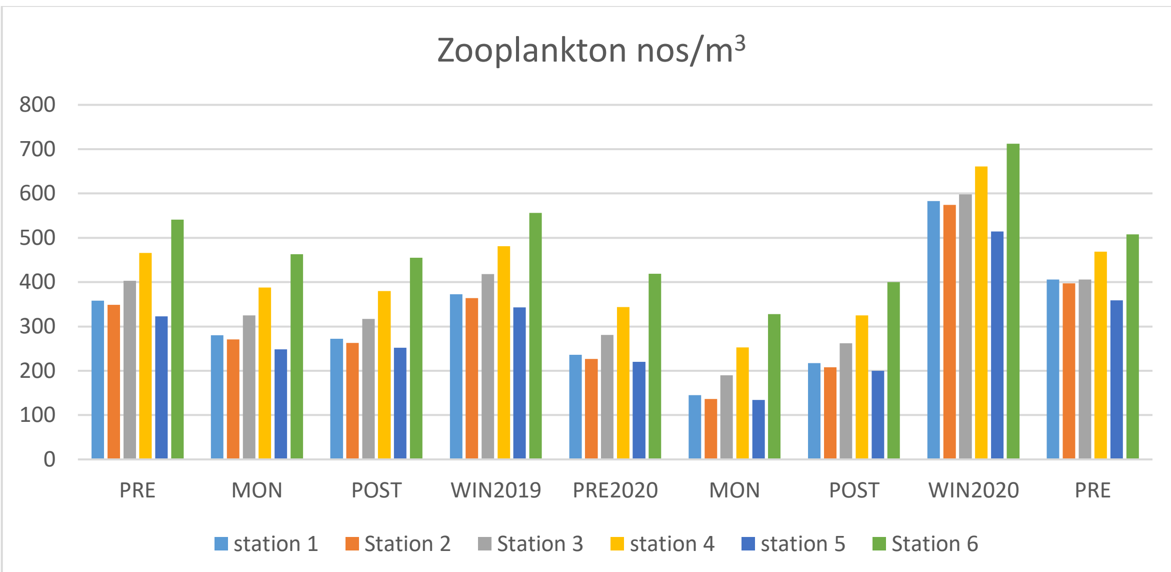


Figure 4: Seasonal variation of Zooplankton density at stations 1- 6 during the study period.

Palmer's Index:

Palmer (1969) first made the list of algae genera and species which indicate organic pollution. According to Palmer, scores of 20 or more are indication of high organic pollution. By using Palmer's index of pollution for rating of water samples as lack of organic pollution, moderate and high organic polluted at all the stations were tested. The total score of Agal Genus Pollution Index (AGPI) of the sites S3<S2<S6=S1<S4<S5 were calculated to be 7, 5, 8, 8, 11 and 31 respectively. It was discovered that the overall score of S3, S2, S6, and S1 was less than 10, indicating a lack of organic contamination. According to Palmer, a sharpe rise in overall score of 31 in station 5 indicates severe organic pollution owing to urban waste influx (1969).

Navicula, Nitzcha and Synedra were recorded repeatedly in lower stations of Dikhu river and consider as indicators of pollution in view of results of Palmer's index.

Table 3: Algal genus pollution index (Palmer, 1969).

Genus	Pollution Index	Genus	Pollution Index
Anacystis	1	Micractinium	1
Ankistrodesmus	2	Navicula	3
Chlamydomonas	4	Nitzschia	3
Chlorella	3	Oscillatoria	5
Closterium	1	Pandorina	1
Cyclotella	1	Phacus	2
Euglena	5	Phormidium	1
Gomphonema	1	Scenedesmus	4
Lepocinclis	1	Stigeoclonium	2
Melosira	1	Synedra	2

Following numerical values for pollution classification of Palmer (1969), 0-10= Lack of organic pollution 10-15= Moderate pollution 15-20= Probable high organic pollution 20 or more = Confirms high organic pollution.

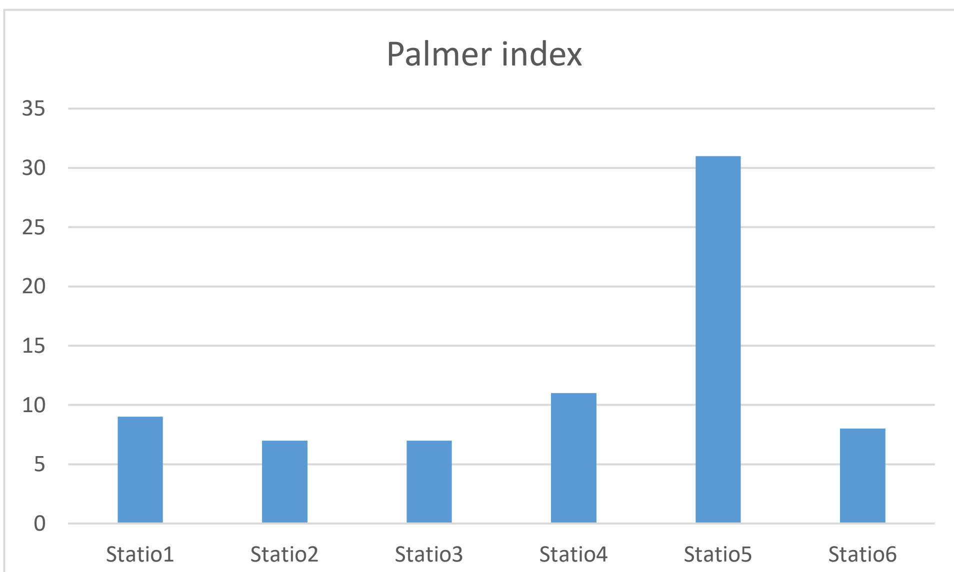


Fig 5: Palmer Index at stations 1-6 during the study period.

Table 4: Pollution index of Algal genera according to Palmer, (1969) at 6 stations of Dikhu River

Genus	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
Chlorophyceae						
Closterium	1	1	1	1	1	1
Cosmarium	+	+	-	-	-	+
Staurastrum	+	-	+	+	+	+
Penium	+	+	+	+	+	-
Zygnema	+	+	+	+	+	+
Pediastrum	+	-	-	+	+	+
Pandorina	1	1	0	1	1	1
Chlamydomonas	-				4	
Oedogonium	+	+	+	-	+	+
Eudorina	+	+	+	+	+	+
Microspora	+	+	+	-	-	+
Scenedesmus	-	0		0	0	-
Oocystis	+	+	+	+	+	-
Cladophora	+	+	+	+	+	+
Ulothrix	-	+	-	+	+	+
Volvox	+	-	-	+	+	+
Spirogyra	+	+	+	+	+	+
Chlorella	0	0		0	3	-
Euglena	-	-	-	-	5	-
Bacillariophyceae						
Tabellaria	+	+	+	+	+	+
Fragilaria	+	+	+	+	+	+
Navicula	3	3	3	3	3	0
Nitzschia	0	0			3	0
Amphora	+	-	+	-	+	0
Gomphonema	0			0		0
Cocconeis	+	+	+	-	+	+
Melosira	1	1	1	1	1	1
Cyclotella	-	1	0	1	1	1
Frustulia	+	+	-	+	+	+
Cyanophyceae						
Synedra	0	0	2	2	2	2
Chroococcus	+	+	+	-	+	+
Oscillatoria	0	0		0	5	0
Anabena	-	+	+	+	-	+
Merismopedia	+	-	+	+	+	-
Spirulina	+	+	-	+	+	+
Nostoc	+	+	-	+	+	+
Euglenophyceae						
Phacus	2	+	+	2	2	2

Total	9	7	7	11	31	8

ANNEXURE- VIII

Anthropogenic factors encountered during the study period

A. Impact of Overfishing :

The river Dikhu flows through the hills of Nagaland reaching the plains of Assam at Nazira ultimately joining the mighty Brahmaputra at Dikhowmukh. It has a high Ichthyofaunal diversity at Longkong and also at Dikhowmukh. But both of this area facing serious overfishing issues. At Longkong Fish Weir with low mesh are constructed in the river resulting growth and recruitment overfishing. This are traditional weirs with modified low mesh synthetic nests. This weirs are been operated during pre-monsoon season and the catch composed of indigenous hill stream fishes.The low mesh size may result in loss of ichthyofaunal diversity of that locality .In Dikhowmukh where the river joins the Brahmaputra there is a high number of small scale fishing fleet . Although they follow Assam state ban fishing period law and other such fisheries legislation but still overfishing is prevalent as motorized boats and high yielding gear are introduced .

B. Impact of Road construction :

For developing economy and increasing social upliftment good roads are important. But improper road construction may lead to an environmental disaster. During the study period road construction was prominent in the hilly areas of Nagaland. To widen the roads, tress and hills are been cut off resulted landslides throughout the monsoon season. This led to increase in the turbidity of the Dikhu River and also its organic load. The sudden landslides into the river cause habitat destruction of the local fauna.

C. Impact of sewage discharge :

As the urban population is increasing at an alarming rate the rate of sewage disposal is also in an increase .Non-point source of sewage discharge can be directly seen in Dikhu river specially in Nazira and Sivsagar area. This discharge of waste increase the organic load in the river which in turn decreases the fish diversity.

D. Impact of bridge piers:

Construction of bridge piers have some morphological impacts over river ecosystem (Lane, 1955). Pier scouring happens when discharge of water is unexpectedly increased, washing away large volumes of soil material next to bridge piers (Ashmore and Parker, 1983; Heidarjed et al., 2010). The majority of soil particles removed are surrounded by turbidity currents and deposited as bars immediately downstream of the bridge. Further these sediments free water is started eroding the downstream banks of river (Biswas, 2010; Mani and Patowary, 2000; Naik et al., 1999). There are several bridges located along the Dikhu River.

This is one of the most disadvantageous environmental conditions during the breeding season.

Mitigation Measures:

1. Stricter law should be enforced to curb overfishing .
2. Alternate livelihood solutions like integrated aquacultural practice should be encourage.
3. Enhancement of Community based conservational areas like ‘Green zone’.
4. Incentives ecofriendly road construction technologies.
5. Creation of more effluent treatment plants in major urban areas like Nazira and Sivsagar.

Different anthropogenic factors encountered during the study period

ANNEXURE- IX

Pollution Status of Dikhu River

1. Biochemical Oxygen Demand (BOD_3): Biochemical oxygen demand (BOD_3) is a measure of the amount of oxygen required by the aerobic micro-organisms to stabilize the biochemically degradable organic matter to a stable inorganic form present in any water bodies. Municipal sewage treatment plants, agricultural wastes, raw sewages, industrial wastage are the major sources of BOD_3. During the present investigation, BOD_3 values were found to vary from 1.03 (Winter, 2019) to 12.1 (Monsoon, 2019).

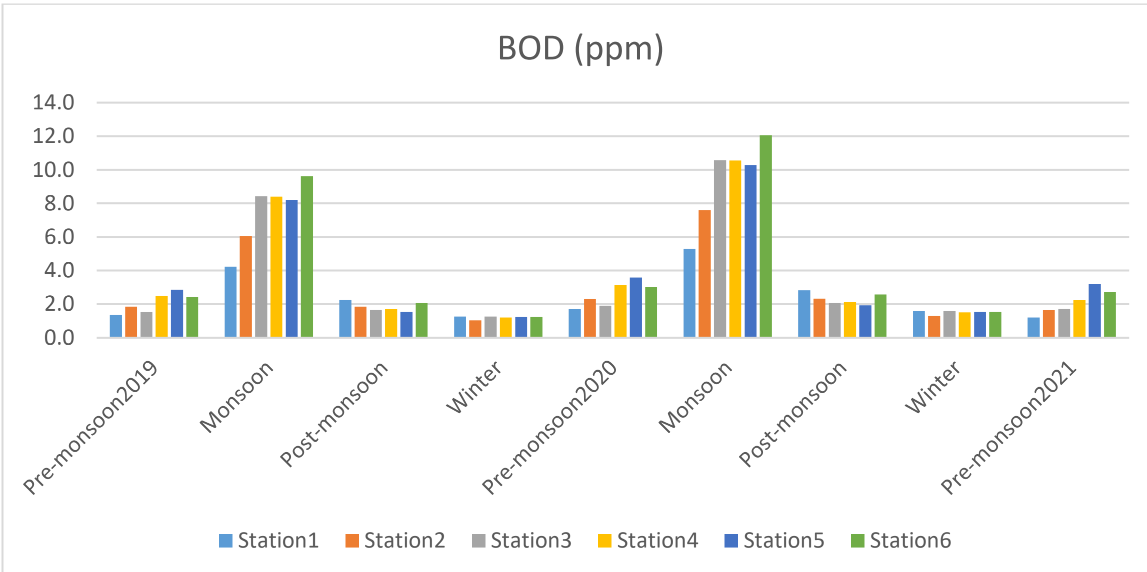


Figure1: Seasonal Variation of Biological Oxygen Demand$_3$ at Station 1-6

Stations	Minimum	Maximum
1	1.2 (Pre-Monsoon, 2021)	5.3 (Monsoon, 2020)
2	1 (Winter, 2019)	7.6 (Monsoon, 2019)
3	1.3 (Winter, 2019)	10.6 (Monsoon, 2019)
4	1.2 (Winter, 2019)	10.5 (Monsoon, 2020)
5	1.3 (Winter, 2019)	10.3 (Monsoon, 2019)
6	1.2 (Winter, 2019)	12.1 (Monsoon, 2019)

2. Chemical Oxygen Demand (COD): Chemical Oxygen Demand (COD) test determines the oxygen requirement equivalent of organic matter that is susceptible to oxidation with the help of a strong chemical oxidant. During the present investigation, the minimum and maximum chemical oxygen demand values of the stations were found to be 1.4 (Winter, 2020) and 34.4 (Monsoon, 2019) respectively.

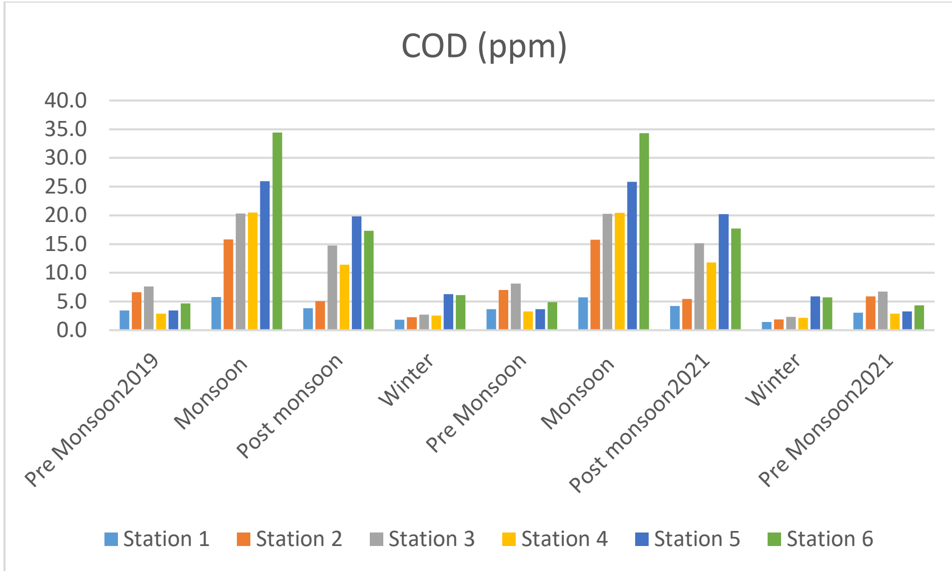


Figure 2: Seasonal Variation of Chemical Oxygen Demand at Station 1-6

Stations	Minimum (Average)	Maximum(Average)
1	1.4 (Winter, 2020)	5.8 (Monsoon, 2019)
2	1.9 (Winter, 2020)	15.8 (Monsoon, 2019)
3	2.3 (Winter, 2020)	20.3 (Monsoon, 2019)
4	2.1 (Winter, 2020)	20.5 (Monsoon, 2019)
5	3.2 (Pre-monsoon, 2021)	25.9 (Monsoon, 2019)
6	4.3 (Pre-monsoon, 2021)	34.4 (Monsoon, 2019)

ANNEXURE X

RESEARCH PAPER PUBLISHED

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Seasonal variation of water quality parameters of river Dikhow in Nagaland and Assam

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Abstract
Dikhow River is one of the largest tributaries of the Brahmaputra river basin originating from Yezami village near Zunheboto town of Nagaland. It debouches into the mighty river Brahmaputra river at Dikhowmukh, Sivasagar district, Asam covering a length of 255.8 km contributing 0.7% runoff. The geographical area of the Dikhow catchment is approximately 3100 km^2, covering 85% of Nagaland, 10% of Assam and 5% of Arunachal Pradesh. The study area of the entire river stretch was divided into 6 stations whose elevation ranged from 90.83 msl to 669.9 msl (3 in Nagaland and 3 in Assam). These stations were selected to study the hydro-biological profile of the river stretch, its pollution status and fish diversity from January 2019 to February 2020. The important physico-chemical water quality parameters of the river in these stations like Dissolved Oxygen (5.2-10.1 mg/L), pH (7.0-8.2), Turbidity (3.8-143.8 NTU), Total Hardness (24.02-121.01 mg/L), Total alkalinity (33-89 mg/L), etc. were found to be ambient for the survival of aquatic fauna in some parts in some period of the year.

Keywords: Dikhow, environmental health, physical parameter, chemical parameter

Introduction

Water is a vital natural resource, it has many fascinating properties that are essential to life, covering three-fourths of the surface of the Earth. Clean water is important for everyone's social, environmental and economic well-being. Geologically, economically, socially and spiritually, the rivers are of considerable significance. While they contain just around 0.0001 % of the world 's overall water content at any given moment, rivers are critical water and nutrients carriers of every part of the planet. They are critical components of the hydrological cycle and function as surface water drainage channels (Longchar *et al.*, 2018) [8]. The rivers of the planet drain almost 75 per cent of the land surface of the earth. The river ecology is formed through the interaction of river biota and its hydrogeochemical environment. It is defined by the continual transfer of various items, such as organic matter and nutrients, from the drainage basin to the river and downstream with the running water. River environments are adaptive to the natural hydrological regime and many components of these systems depend on flooding to share resources, nutrients, sediments and living organisms, not just with water (Acreman, 2000).

Dikhow River is one of the largest tributaries of the Brahmaputra river basin originating from Yezami village near Zunheboto town of Nagaland. It debouches into the mighty river Brahmaputra river at Dikhowmukh, Sivasagar district, Asam covering a length of 255.8 km contributing 0.7% runoff. The geographical area of the Dikhow catchment is approximately 3100 km^2, covering 85% of Nagaland, 10% of Assam and 5% of Arunachal Pradesh. River Dikhu 's major tributaries are Yangyu and Nanung in the Tuensang and Mokokchung district. The Dikhu River is not only a popular tourist attraction but also an important source of people's livelihood. The Dikhu River is a lifeline for millions of people in Assam and Nagaland.

A river's water content is the composition of many interrelated compounds, which are subject to local and temporal fluctuations and are often influenced by the water flow amount. Surface water resources are more vulnerable to contamination than groundwater (Ogubanjo and Rolajo, 2004) [11], especially in developing countries where heavy industrialisation, increasing urbanisation and adaptation to new agricultural practices play an important role in raising

ANNEXURE XI

INTERNATIONAL SYMPOSIUM ATTENDED

Participation Certificate

First International Symposium on Aquatic Biodiversity of the North Eastern Region of India

Presented to:
Raktim Sarmah

for their presentation on the study

First Report of Ichthyofaunal Diversity of an Important Tributary of Brahmaputra: Dikhow River

Authors: Raktim Sarmah, Rajdeep Dutta, and Sarada Kanta Bhagabati

at the First International Symposium on Aquatic Biodiversity of the North Eastern Region of India held on October 26th and 27th 2021. We thank you for your contribution towards conservation of Aquatic Biodiversity in the North Eastern Region of India.

Dr Uwe Scholz, Project In-Charge, GIZ NERAQ Project

Prof. Dandadhar Sarma, Department of Zoology, Gauhati University

Dr Dhriti Banerjee, Director, Zoological Survey of India