Template/Pro forma for Submission

NMHS-Himalayan Institutional Project Grant

NMHS-FINAL TECHNICAL REPORT (FTR)

Demand-Driven Action Research and Demonstrations

NMHS Grant Ref. No.:	NMHS/2018-19/SG49/49

Date of Submission:	2	0	1	2	2	0	2	2
	d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

PROJECT TITLE (IN CAPITAL)

DISTRIBUTION AND QUANTIFICATION OF ORGANIC CONTAMINANTS AND MICROPLASTIC CONCENTRATIONS IN LAKE SYSTEMS FROM HIMACHAL PRADESH, INDIA

Project Duration: *from* (**21.12.2018**) *to* (**20.12.2021**).

***Submitted to*:**
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GENERAL INSTRUCTIONS:

1. The Final Technical Report (FTR) has to commence from the start date of the Project (as mentioned in the Sanction Order issued by NMHS-PMU) till completion of the project duration. Each detail has to comply with the NMHS Sanction Order.
2. The FTR should be neatly typed (in Arial with font size 11 with 1.5 spacing between the lines) with all details as per the enclosed format for direct reproduction by photo-offset printing. Colored Photographs (high resolution photographs), tables and graphs should be accommodated within the report or annexed with captions. Sketches and diagrammatic illustrations may also be given detailing about the step-by-step methodology adopted for technology development/ transfer and/ or dissemination. Any correction or rewriting should be avoided. Please provide all information under each head in serial order.
3. Any supporting materials like Training/ Capacity Building Manuals (with detailed contents about training programme, technical details and techniques involved) or any such display material related to project activities along with slides, charts, photographs should be brought at the venue of the Annual Monitoring & Evaluation (M&E) Workshop and submitted to the NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora 263643, Uttarakhand. In all Knowledge Products, the Grant/ Fund support of the NMHS should be duly acknowledged.
4. The FTR Format is in sync with many other essential requirements and norms desired by the Govt. of India time-to-time, so each section of the NMHS-FTR needs to be duly filled by the proponent and verified by the Head of the Lead Implementing Organization/ Institution/ University.
5. Five (5) hard-bound copies of the Project Final Technical Report (FTR) and a soft copy of the same should be submitted to the **Nodal Officer, NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora, Uttarakhand**.

The FTR is to be submitted into following two (02) parts:

Part A – Project Summary Report

Part B –Detailed Project Report

In addition, the Financial and other necessary documents/certificates need to be submitted along with the Final Technical Report (FTR) as follows:

Annexure I	**Consolidated and Audited Utilization Certificate (UC) & Statement of Expenditure (SE)**, including the interest earned for the last Fiscal year and the duly filled GFR-19A (with year-wise break-up).
Annexure II	**Consolidated Interest Earned Certificate**
Annexure III	**Consolidated Assets Certificate** showing the cost of the equipment in Foreign/ Indian currency, Date of Purchase, etc. (with break-up as per the NMHS Sanction Order and year-wise).
Annexure IV	**List of all the equipment, assets and peripherals** purchased through the NMHS grant with the current status of use, including location of deployment.
Annexure V	**Transfer of Equipment** through Letter of Head of Institution/Department confirming the final status of equipment purchased under the Project.
Annexure VI	**Details, Declaration and Refund of any Unspent Balance transferred through Real-Time Gross System (RTGS)/ PFMS in favour of NMHS GIA General**

NMHS-Final Technical Report (FTR) *template*

Demand-Driven Action Research Project

DSL: Date of Sanction Letter

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

DPC: Date of Project Completion

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

Part A: Project Summary Report

1. Project Description

i.	Project Grant Ref. No.:	NMHS/2018-19/SG49/49						
ii.	Project Category:	Small Grant	**	Medium Grant		Large Grant		
iii.	Project Title:	Distribution and quantification of organic contaminants and microplastic concentrations in lake systems from Himachal Pradesh, India						
iv.	Project Sites (IHR States/ UTs covered) (*Location Maps attached*):	Renuka and Rewalsar Lake, Himachal Pradesh						
v.	Scale of Project Operation:	Local		Regional	**	Pan-Himalayan		
vi.	Total Budget:	38.5 (in Lakhs)						
vii.	Lead Agency:	Indian Institute of Science Education and Research Mohali (established by the Ministry of Human Resource Development (MHRD), Government of India), Knowledge City, Sector 81, Manauli, SAS Nagar, Punjab 140306 Institute website: http://www.iisermohali.ac.in/						
	Lead PI/ Proponent:	Dr. Anoop Ambili Dept. of Earth and Environmental Sciences, Indian Institute of Science Education and Research Mohali, Knowledge City, Sector 81, Manauli, SAS Nagar, Punjab 140306 Email address: anoop@iisermohali.ac.in Mobile: +919415027431						

	Co-PI/ Proponent:	Dr. Sharmila Bhattacharya Indian Institute of Science Education and Research Mohali Address for correspondence: Dept. of Earth and Environmental Sciences, Knowledge City, Sector 81, Manauli, SAS Nagar, Punjab 140306 Email address: sbhattacharya@iisermohali.ac.in Mobile/telephone/fax number: +919920642684 Dr. Arshid Jehangir Assistant Professor Department of Environmental Science, University of Kashmir, Hazratbal, Srinagar-190006, J&K. Email: arsghidj@gmail.om Mobile No: 09797070540
viii.	Implementing Partners:	Indian Institute of Science Education and Research Mohali, Sector 81, Punjab 140306, India Institute website: http://www.iisermohali.ac.in/ University of Kashmir, Hazratbal Srinagar-190006, J&K India. www.kashmiruniversity.edu.in; www.kashmiruniversity.ac.in
	Key Persons (Contact Details, Ph. No., E-mail):	Dr. Anoop Ambili Email: anoop@iisermohali.ac.in; anoop.ambili29@gmail.com Mobile: +919415027431

2. Project Outcomes

2.1. Abstract/ Summary (not more than 250-300 words)

Background: The emerging organic pollutants (e.g., PAHs, sterols) and microplastics have serious impacts on aquatic ecosystems. Many global studies have reported microplastic as one of the predominant pollutants from marine and estuarine environments, which show many harmful effects on humans (cancer and brain damage) and their lives (species loss due to starvation). The Himalayan fresh water aquatic systems are known and recognised for their endemic biodiversity, and provide economically important products and services. In this project, we have explored the occurrence, distribution, characterization and quantification of microplastics (MPs), Organic contaminants (e.g., PAHs), and phthalic acid esters (PAEs) from the freshwater aquatic environment (Renuka and Rewalsar Lake) in Indian Himalaya.

Objectives: The objectives of the present study includes investigation of the infiltrating organic contaminants into the two lake systems (Renuka and Rewalsar Lake) and to understand the ecotoxicological significance of the sedimentary persistent organic pollutants and microplastic levels by comparison with Sediment Quality Guidelines (SQGs). Further, a meticulous categorization (priority and emerging) and characterization of the organic contaminants occurring in the studied lakes to develop a deeper understanding of the compounds' source inventories and environmental fate.

Methodology: The extraction of microplastics from sediment samples has been done as per National Oceanic and Atmospheric Administration (NOAA) protocol with minor modification (Ajay et al., 2021). The visually characterized debris (micro plastics) have been analyzed under focal plane array assisted Fourier transformed infrared spectrometer (FPA-FTIR), Invia laser Raman spectrometer with a 532 nm excitation laser. The individual phthalates and other organic contaminants (e.g., PAHs, sterols) were identified and quantified using a gas chromatograph (Agilent 7890A, GC system) and mass spectrometer (MS).

Results: The MPs were detected in all water and sediment samples from Renuka Lake with abundances ranging from 02–64 particles/L and 15–632 particles/kg dw, respectively. The abundance of MPs, dominated by polyethylene and polystyrene, with the majority being fibres and fragments indicated that they were derived from plastic paints, boats or synthetic products. The concentrations of PAEs in the surface sediment samples varied from 06-357 ng/g dw. The most abundant PAEs in the Renuka surface sediments were dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP). In Rewalsar Lake, the MPs were identified in all samples with concentration of 13-238 particles/litre and 750 to 3020 particles/kg dry weight (dw) in surface water and sediments respectively. Further, the *n*-alkane distribution (n-C 10 to n-C 35), and markers of environmental pollution (fecal stanols and polycyclic aromatic hydrocarbons (PAHs)) were investigated from the surface sediments of Rewalsar Lake, Himachal Pradesh (India) to unravel natural and anthropogenic organic matter (OM) sources.

Conclusions: The distribution of the microplastic particles and the PAEs in the sediment and water samples from the Renuka and Rewalsar Lake indicated the ubiquitous contamination of plastic pollutants in fresh water aquatic systems in Indian Himalaya. The present work to best of our knowledge is the first coupled approach to evaluate the presence and distribution of microplastics and phthalates in a highland freshwater system in the Indian subcontinent. Comprehensive monitoring programs should be imposed to understand the adverse effects of the presence of these organic pollutants on the ecology of the lake. Our present work provide an important baseline for the future investigations on the hazardous impact of the organic contamination in the aquatic environments. The data about the organic contaminants in the freshwater environments are limited except for our studies in Renuka and Rewalsar Lake. More work needs to be carried out to understand the organic contaminants including microplastics occurrence, transportation and fate in the aquatic environments in Indian Himalaya.

2.2. Objective-wise Major Achievements

S#	Objectives	Major achievements (***in bullets points***)
	To develop high-resolution analytical methodology to detect the persistent organic pollutants and microplastic contaminants that have infiltrated in the Renuka and Rewalsar Lakes. To improved analytical methods will facilitate the detection of novel microplastic polymer and POPs- notably the stereoisomers and the more polar POPs.	The detailed methodology for extraction and screening of microplastics and organic compounds are provided in supplementary Information. The methodology developed during the project will be useful for undertaking the analyses of microplastics and associated contaminants in various aquatic systems in Indian Himalaya.
	To determine the partition co-efficient of the organic contaminants in order to assess the sorption of the pollutants to particulate organic matter suspended in the lake waters as well as on the sediments.	A high correlation was observed between the microplastics abundance and the PAEs concentration suggesting that the PAEs were sourced from the microplastic particles while the Rewalsar lake is characterised by absence of correlation indicating a possible leaching effect. The land use/land cover (LULC) changes and grain size analysis were used to investigate the multiple reasons and processes that govern the spatial heterogeneity of the distribution of organic contaminants in aquatic systems

	The ecotoxicological significance of the sedimentary POPs and microplastic levels will be evaluated by comparison with Sediment Quality Guidelines (SQGs).	The organic contaminants reported from Renuka and Rewalsar Lake are compared with sediment quality guidelines. Please see Annexure-2 for details.
	A meticulous categorization (priority and emerging) and characterization of the organic contaminants occurring in the studied lakes is essential in order to develop a deeper understanding of the source inventories and environmental fate of the compounds.	The various organic contaminants including microplastics were screened, quantified and reported during the project.

Note: Further details may be summarized in DPR Part-B, Section-5. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

2.3. Outputs in terms of Quantifiable Deliverables*

S#	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations, if any, & Remarks thereof:
	Development of precise and robust methodology for analysing complex organic pollutants in water and sediments Data base and knowledge base on the understanding of the source inventories and environmental fate of the compounds in the lacustrine systems. Provide an understanding of distribution levels of the organic pollutants in 2 lakes of HP. Strategy for restoration and	No. of New Database/Datasets generated on the identified dynamics (No.) No. of region-specific best practices/ models/ technologies developed/ implemented (No.) for dynamics viz., microplastic contaminants, etc.; The number of Beneficiaries(Nos.) No. of Reports/Research articles/Policy documents/Manual prepared and published (Nos.)	Detailed methodology generated for extraction and screening of organic contaminants provided in Supporting Information The key beneficiaries of the Research work will be local communities, decision-makers, project proponents, policymakers and researchers, NGOs, Health Department and all those who are directly and indirectly related to or dependent on the Himalayan fresh water ecosystem. Besides, the institutions like Ministry of Water Resources, Central Water Commission	— — — —

	maintenance of Renuka and Rewalsar Lakes		etc. will also get help from the findings of the project. The data produced from the project are published in high impact reputed journals (Annexure - 2)	

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

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

S#	Particulars	Number/ Brief Details	Remarks/ Attachment
1.	New Methodology/ Technology developed, *if any*:	Extraction of microplastics and organic contaminants	Detailed methodology provided as Supporting Information
2.	New Ground Models/ Process/ Strategy developed, *if any*:	NA	
3.	New Species identified, *if any*:		
4.	New Database established, *if any:*	Databased on microplastics and associated organic contaminants	
5.	New Patent, *if any*:	NA	
	I. Filed (Indian/ International)		
	II. Technology Transfer, *if any*:		
6.	Others, *if any*		

Note: Further details may be summarized in DPR Part-B, Section-5. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

3. New Data Generated over the Baseline Data

S#	New Data Details	Status of Existing Baseline	Addition and Utilisation New data
1	Microplastics	NA	MPs abundance and their chemical composition reported.
2	Phthalic acid esters	NA	PAEs abundance and their molecular characterisation reported.
3	Petroleum hydrocarbons, *n*-alkanes, PAHs, Sterols	NA	Data for contaminants from petroleum and sewage markers

Note: Further details may be summarized in DPR Part-B. Database files in the requisite formats (Excel) may be enclosed as annexure/ appendix separately to the soft copy of FTR.

4. Demonstrative Skill Development and Capacity Building/ Manpower Trained

S#	Type of Activities	Details with number	Activity Intended for	Participants/Trained			
				SC	**ST**	**Women**	**Total**
1.	Workshops						
2.	On-Field Trainings		To collect the surface sediment and water samples with the help of a Van-Veen grab sampler and Uwitec water sampler. Microplastic extraction from sediments	3	2	3	8
3.	Skill Development		Hands on training in extraction of organic contaminants	3	2	2	7
4.	Academic Supports		Training of BS-MS students on organic contaminants screening and extraction				
	Others (if any)						

Note: Further details may be summarized in DPR Part-B. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

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

S#	Linkages /collaborations	Detail of activities (No. of Events Held)*	No. of Beneficiaries
1.	Sustainable Development Goals (SDGs)/ Climate Change/INDC targets addressed.		
2.	Any other:		

Note: Further details may be summarized in DPR Part-B, Section-6. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

6. Project Stakeholders/ Beneficiaries and Impacts

S#	Stakeholders	Support Activities	Impacts in terms of income generated/green skills built
1.	Line Agencies/ Gram Panchayats:	The key beneficiaries of the research work will be local communities, decision-makers, project proponents, policymakers and researchers, NGOs, Health Department and all those who are directly and indirectly related to or dependent on the Himalayan fresh water ecosystem.	
2.	Govt Departments (Agriculture/ Forest/ Water):	The institutions like Ministry of Water Resources, Central Water Commission etc. Forest Departments, State pollution control boards, will also get help from the findings of the project.	
3.	Villagers/ Farmers:		
4.	SC Community:		
5.	ST Community:		
6.	Women Group:		
	Others, *if any*:		

Note: Further details may be summarized in DPR Part-B, Section-6. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

7. Financial Summary (Cumulative)

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

8. Major Equipment/ Peripherals Procured under the Project** (*if any*)

S#	Name of Equipment	Quantity	Cost (INR)	Utilisation of the Equipment after project
1.				
2.				

Details should be provided in details **(*ref.*** Annexure III &IV).**

9. Quantification of Overall Project Progress

S. No.	Parameters	Total (Numeric)	Remarks/ Attachments/ Soft copies of documents
1.	IHR States/ UTs covered:	*Himachal Pradesh*	
2.	Project Sites/ Field Stations Developed:	*2*	*Renuka and Rewalser Lake*
3.	Scientific Manpower Developed (PhD/M.Sc./JRF/SRF/ RA):	*4*	
4.	Livelihood Options promoted		
5.	Technical/ Training Manuals prepared		
6.	Processing Units established, if any	*Organic contaminants extraction and screening*	*Detailed methodology provided as supplementary Information*
7.	No. of Species Collected, if any		
8.	No. of New Species identified, if any		
9.	New Database generated (Types):	*3*	*Microplastic and associated contaminants (e.g., PAEs, PAHs, Sterols)*
	Others (if any)		

Note: Further details may be summarized in DPR Part-B. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

11. Knowledge Products and Publications:

S#	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures
		National	International		
1.	Journal – Research Articles/ Special Issue:		2	13.7	Chemosphere Biogeochemistry
2.	Book – Chapter(s)/ Monograph/ Contributed:				
3.	Technical Reports:				
4.	Training Manual (Skill Development/ Capacity Building):				
5.	Papers presented in Conferences/Seminars:		3	NA	EGU 2022
6.	Policy Drafts/Papers:				
7.	Others, if any:				

Note: Please append the list of KPs/ publications (with impact factor, DOI, and further details) with due Acknowledgement to NMHS. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

12. Recommendation on Utility of Project Findings, Replicability and Exit Strategy

Particulars	Recommendations
Utility of the Project Findings:	POPs and microplastics in lacustrine sediments will provide the impetus for further research on the distribution and impact of the priority and emerging pollutants on the biota of many aquatic systems spread across India. Stakeholders and policymakers can use the baseline data for the sustainability measures for the aquatic systems.
Replicability of Project/ Way Forward:	The methodology developed during the project will be useful for undertaking the analyses of microplastics and associated contaminants in various aquatic systems in Indian Himalaya. The data about the organic contaminants in the freshwater environments are limited except for our studies in Renuka and Rewalsar Lake. More work need to be carried out to understand the organic contaminants including microplastics occurrence, transportation and fate in the aquatic environments in Indian Himalaya.
Exit Strategy:	The project findings will serve as a basis for management and decision-making for various central and state agencies. The key beneficiaries of the Research work will be local communities, decision-makers, project proponents, policymakers and researchers, NGOs, Health Department and all those who are directly and indirectly related to or dependent on the Himalayan fresh water ecosystem. Besides, the institutions like Ministry of Water Resources, Central Water Commission etc. will also get help from the findings of the project.

(**PROJECT PROPONENT/ COORDINATOR**)

(Signed and Stamped)

(**HEAD OF THE INSTITUTION**)

(Signed and Stamped)

Place: Mohali

Date: 20/12/22

PART B: DETAILED PROJECT REPORT

The Detailed report should include an Executive Summary and it should have separate chapters on (i) **Introduction**, (ii) **Methodologies/Strategy/Approach**, (iii) **Key Findings and Results**, (iv) **Overall Achievements**, (v) **Project's Impacts in IHR** (vi) **Exit Strategy** and Sustainability, (vii) **References**, and (viii) **Acknowledgements** (acknowledging the financial grant from the NMHS, MoEF&CC, GoI).

Other necessary details/ Supporting Documents/ Dissemination Materials (*New Products/ Manuals/ Standard Operating Procedures (SOPs)/ Technology developed/Transferred, etc., if any*) may be attached as Appendix (ces).

1 **EXECUTIVE SUMMARY** (not more than 2–3 pages)

The global annual production of plastics has increased to 330 million tons in 2015 compared to 2 million tons in 1950, and is expected to reach more than 400 million tons by 2050 (Geyer et al., 2017; Lebreton and Andrady, 2019). Among these plastic materials, approximately 45% of the total plastic is discarded to landfills while 10% are discharged into the aquatic systems (Borrelle et al., 2020). Most of the plastic materials in aquatic systems are fragmented to microplastics (1 μm–5 mm particle size as defined by the National Oceanic and Atmospheric Administration) following physical, chemical and biological disintegration processes (Barnes et al., 2009). The microplastics (MPs) are derivative of heterogeneous assemblage of particles that include diverse varieties varying in size, shape and chemical composition. The characteristics of MPs, such as morphology, composition and their density are highly dependent on its attributed sources, such as cosmetics and personal care products (primary sources) and fragmentation from heavier plastic materials (secondary sources) (Barnes et al., 2009). These primary and secondary microplastics are released into the environment either through industrial and domestic drainage discharge or by direct disposal of plastic materials into the system. The occurrence and distribution of microplastics in the freshwater environments, and how these infiltrate across the food web have been well documented (Laist, 1997). The microplastics possess two important threats to environment: (i) these particles can easily enter into the aquatic food chain which may cause blockage in digestive tract resulting in reduced growth of the organisms and, (ii) the microplastics harbor the risks of transport of adsorbed pollutants (such as persistent organic pollutants (POPs); inorganic elements) to the aquatic ecosystems.

The occurrence, distribution, characterization and quantification of microplastics (MPs) and phthalic acid esters (PAEs) from the freshwater aquatic environment are not thoroughly explored in the Indian Himalayas despite concern over their adverse effects on human health and ecosystem. In this project, we have investigated the presence of MPs and PAEs in aquatic systems (Renuka and Rewalsar Lake) from Indian Himalayas. The MPs were detected in all water and sediment samples from Renuka Lake with abundances ranging from 02–64 particles/L and 15–632 particles/kg dw, respectively. The abundance of

MPs, dominated by polyethylene and polystyrene, with the majority being fibres and fragments indicated that they were derived from plastic paints, boats or synthetic products. The concentrations of PAEs in the surface sediment samples varied from 06-357 ng/g dw. The most abundant PAEs in the sediments were dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP), since they were present in all the samples collected from the lake basin. The relatively higher abundances of MPs and higher concentrations of PAEs were generally found in the vicinity of areas impacted by anthropogenic activities. A clear correlation between the abundance of microplastics and PAEs concentration was observed suggesting that they are closely attributed to a single source. This study also provides an alternative approach to utilize the chemical additives in plastics as markers to trace the presence and distribution of MPs in the aquatic environment.

Furthermore, the knowledge of distribution and sources of organic matter (OM) in Renuka Lake is investigated to understand the biogeochemical cycling of carbon in terrestrial environments. We have evaluated the quantitative contributions of OM sources and their distribution using bulk geochemical parameters (TOC and $\delta^{13}C_{org}$), *n*-alkane indices and source specific biomarkers (C_{20} highly branched isoprenoid (HBI)) in Renuka Lake in Lesser Himalaya. The principal sources of OM in the sediments were aquatic productivity with minor input from terrestrial plants, which varied from littoral to central part of the lake. The microbial community in Renuka Lake were established using short chain *n*-alkanes and C_{20} HBI, whereas pristane/phytane (Pr/Ph) depicts depositional condition of the lake system. The land use/land cover (LULC) changes and grain size analysis were used to investigate the multiple reasons and processes that govern the spatial heterogeneity of the distribution of sedimentary OM. The results show that human activities and alterations of the aquatic landscape can significantly affect the composition and distribution of OM in aquatic systems. The present study shows that elucidating the sources and distribution of OM in an aquatic system is crucial for constraining the ecological status and aiding conservation measures.

In Rewalsar lake, our study reveals first combined report on the occurrence, distribution and concentration levels of MPs and PAEs in surface sediments from Rewalsar lake, Himachal Pradesh, India. The MPs, being ubiquitous in the study site ranged from 13-238 particles/litre and 75-302 particles/0.1Kg in water and sediment samples respectively. The MPs were dominated by polystyrene, polyethylene, polypropylene polymers. The PAEs identified includes di-isobutyl phthalate (DIBP), dibutyl phthalate (DBP), di(2-ethylhexyl) phthalate (DEHP). Interestingly, concentration of PAEs is higher towards the lake basin with maximum human intervention. The data generated from current study will help to prioritize monitoring programs and propose management strategies for lake system. Furthermore, the *n*-alkane (n-C10 to n-C35) distributions and anthropogenic markers (fecal stanols and polycyclic aromatic hydrocarbons (PAHs)) were investigated from the surface sediments of Rewalsar Lake, Himachal Pradesh (India) to disentangle natural and anthropogenic organic matter sources. The dominance of odd numbered n-alkanes (n-C_{27}, n-C_{29} and n-C_{31}) and *n*-alkanes proxy data (terrigenous/aquatic ratio (TAR), carbon preference index (CPI), Average chain length (ACL) indicate mixed input from allochthonous and autochthonous sources. Detailed examination of allochthonous sources further reflect the accumulation of pollutants (PAHs and fecal stanols) that mark the intensified toxicity in the lake system. Moreover, the occurrence of sewage contaminants particularly coprostanol and epicoprostanol suggest high anthropogenic loading due to sewage discharges. The overall accumulation of contaminants in the lake can be attributed to anthropogenic activities involving chemical and sewage overflow, agricultural and industrial discharges, land use changes and developmental activities. The uncontrollable pollution status of the Rewalsar lake is supported by low pristane/phytane (Pr/Ph) ratio that denotes anoxia. Therefore, the study provides comprehensive understanding on organic matter source apportionment as well as role of anthropogenic stressors in the wake of rapid urbanization around Rewalsar Lake. Further, the data obtained highlight the matters that need attention in order to formulate and implement the lake monitoring strategies in Indian subcontinent.

2 INTRODUCTION

2.1. Background (max. 500 words)

Anthropogenic contamination of aqueous systems has been a crucial and longstanding environmental issue with far-reaching effects on ecosystems. Limnic systems are vulnerable to a large array of contaminants due to intense anthropogenic activities and are introduced into the freshwaters through atmospheric and terrestrial interactions. The lacustrine aqueous systems shelter a plethora of organic contaminants which include both naturally occurring pollutants (organic metabolites and byproducts) as well as synthetic chemicals that include industrial discharges, agricultural run-off, combustion-related polyaromatic hydrocarbons, municipal sewer wastes and discharges from waste water (Baldwin et al., 2016). The discharge of these organic contaminants into aqueous systems affects surface water which in turn compromises the drinking water sources; unfortunately, a large portion of the micropollutants cannot be removed through the standard purification processes from water (Conn et al., 2006). The removal mechanism and efficiency are guided by the properties of the pollutant compounds (Qu et al., 2018 and references therein).

Persistent organic contaminants (POPs) and microplastic (MP, particle size less than 5 mm) have been identified as environmentally recalcitrant and complex contaminants of growing societal concern. The POPs and microplastics have deleterious impacts to aquatic environments due to their documented ubiquity in aquatic ecosystems, long residence times, and ingestion by aquatic biota. These compounds resist mechanical, chemical and biological degradation and can migrate through long distances. The persistent organic pollutants comprise compounds such as polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons and phenols. The human populations in lacustrine regions dispose large amounts of POPs and plastic contaminants and have been identified as hotspots for accumulation of these organic contaminants. The available data on POPs and MP concentrations have been overwhelmingly focused on the marine realm (Andrady, 2011). At the same time, a dearth of studies has quantified POPs and microplastics in lacustrine ecosystems. We have chosen two lake systems in Himachal Pradesh - Renuka and Rewalsar lakes, which are major catchment of potable water and offers employment, to investigate the organic pollutants that primarily accumulate over time through anthropogenic activities. Understanding the distribution and accumulation of POPs and plastic contaminants from multiple lake systems is crucial for gauging the source, fate in water and sediments and environmental risks of these compounds. The research is focused on the occurrence, distribution, structural characterization and categorization of POPs and microplastics in water and sediments from the anthropogenically influenced lake systems from the Indian subcontinent. Efforts were made to screen new or potential POPs and microplastic constituents through the research work. The results provide the first record of the detailed spatial distribution of POPs and plastic constituents from the lake water and sediments in the Indian subcontinent.

2.2. Overview of the major issues addressed (max. 500 words)

The intensification of water pollution in a developing country like India, sheltering a population of billions, poses gigantic challenges to expanding the economy and directly affecting human health and livelihood. The Renuka and Rewalsar Lake in Himachal Pradesh are important natural resources contributing directly to the lives of a huge population as it is the main source of drinking water and employment such as agriculture, fisheries and tourism. These lakes are also considered of immense religious importance. The unrestrained pollution phenomenon thus will affect a large echelon of society with respect to basic needs and survival and hence needs immediate attention. Despite substantial advances in organic contaminants over the past two decades, several issues have been left unaddressed. A reliable estimate of spatial distribution and temporal fluctuations of the pollutant levels in lacustrine systems needs to be ascertained. This might be able to offer glimpses into the transport and fate of the components in the aqueous medium, which largely remains unclear for the POPs and microplastic (however, some are known to undergo biodegradation pathways or accumulate in the aquatic organisms). Biodegradation may lead to excess consumption of oxygen in the water, causing depletion of the aquatic life and disruption of the ecological behaviour of the lake.

The lakes have been examined and monitored for various water quality parameters like BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), total acidity, alkalinity, hardness, Ph, carbon dioxide, turbidity and ionic and elemental concentrations. However, there is a paucity of information and understanding and a lacuna in the database of the organic constituents, particularly the POPs and microplastic, present in the lakes, and needs to be investigated qualitatively and quantitatively. The POPs and microplastic are a complex and diverse range of chemicals with respect to structural characterizations and physicochemical properties. The traditional investigative approaches may not suffice to comprehensively understand this class of compounds that have started thriving in aqueous environments. A proficient methodology is thus of utmost necessity to examine these pollutants, which would allow these compounds to be systematically identified and examine the occurrence and impact on the environment.

Few peer-reviewed scientific articles globally have documented quantitative data on the spatial and temporal distribution of the diverse range of organic pollutants in aqueous ecosystems. This is vital for setting the benchmark levels for individual compounds wherein the pollutants start causing adverse environmental effects.

2.3. Baseline Data and Project Scope (max. 500 words)

The occurrence and distribution of microplastics pollutants in the densely populated Indian subcontinent have received wide attention in the recent decades. However, the available studies are mostly concentrated in the coastal regions and estuarine sediments from peninsular India (Laskar and Kumar, 2019; Reddy et al., 2006; Sruthy and Ramasamy, 2017). Few studies are conducted on the lacustrine (Gopinath et al., 2020; Manikanda Bharath et al., 2021) and riverine (Amrutha and Warrier, 2020) systems from peninsular India. The MPs pollution on freshwater ecosystems from Indian subcontinent remains largely unexplored (Vanapalli et al., 2020). To date, no data are available about the microplastics and PAEs levels in the water and sediment of the inland aquatic system in northern India and specifically Indian Himalaya. In this study, we provide an assessment of the MPs and PAEs in sediment samples from Renuka Lake. The Renuka Lake is a Ramsar site (Ramsar Handbook, 2016; Ramsar List, 2016) and an important water body that influences the ecological integrity and services of the entire region.

Moreover, in freshwater lakes, the source and composition of sedimentary organic matter (SOM) is closely related to issues such as the reduction of macrophytes (Zhang et al. 2016), distribution/diversity of fauna (Omesová & Helešic 2010), eutrophication (Xu et al. 2015; Ankit et al. 2022), and distribution of contaminants (Karickoff et al. 1979). Likewise, the SOM distribution in aquatic systems is influenced by a variety of components including runoffs, hydrodynamic conditions, lake bathymetry and grain size (Wang et al. 2012; Dong et al. 2014; Zhang et al. 2018; Zhang et al. 2020). In addition, human alterations of the aquatic landscape can strongly influence the distribution and composition of SOM (e.g., Tranvik et al. 2009; Ankit et al., 2021; Bulbul et al. 2021). The detailed information of the content, distribution and sources of OM is necessary to understand the local biogeochemical cycles for carbon, and the environmental and biological variables that regulate them. The aquatic systems in the Indian Himalaya provide critical ecosystem services for sustainable development regionally (Singh 2006; Tiwari and Joshi 2012). Renuka Lake is a RAMSAR site (wetland site designated to be of international importance under the Ramsar Convention from 8th November 2005) in the Lesser Himalaya sustaining substantial biodiversity. The quantity and content of lipid biomarkers (n-alkanes and HBI) as well as other geochemical proxies (TOC and δ^{13}Corg) in the surface sediments of Renuka Lake were investigated in order to understand the OM sources and their distribution within the lake basin. Likewise, the lipid biomarker analyses were also carried out in Rewalsar Lake to disentangle the natural versus anthropogenic organic matter sources. Further, the role of land−use/land−cover (LULC) (e.g., deforestation, intensive growth in settlements etc.) and sediment grain size on the OM abundance and distribution have also been evaluated. Both LULC and sediment particle size can affect the composition and distribution of OM in aquatic systems (Pradhan et al. 2020; Bulbul et al. 2021).

2.4. Project Objectives and Target Deliverables (as per the NMHS-Sanction Order)

Our primary objective focussed on developing a high-resolution analytical methodology to detect the persistent organic pollutants and microplastic contaminants that have infiltrated the Renuka and Rewalsar lakes since many of these hazardous chemicals are below the detection limit of the conventional instruments and therefore go unnoticed (Detailed methodology provided as supplementary information). Also, improved analytical methods will facilitate the detection of novel microplastic polymers and POPs- notably the stereoisomers and the more polar POPs.

- A qualitative and quantitative investigation of the infiltrating chemicals into the two lake systems will be undertaken. The study will particularly focus on the persistent organic pollutants (POPs) and microplastic concentrations, which have very large residence times in the different matrices of lacustrine ecosystems, including the water, sediments and aquatic organisms (tend to bio-accumulate).

- Determine the partition coefficient of the organic contaminants to assess the sorption of the pollutants to particulate organic matter suspended in the lake waters and on the sediments.

- The ecotoxicological significance of the sedimentary POPs and microplastic levels will be evaluated by comparison with Sediment Quality Guidelines (SQGs).

- A meticulous categorization (priority and emerging) and characterization of the organic contaminants occurring in the studied lakes is essential to develop a deeper understanding of the compounds' source inventories and environmental fate.

3 METHODOLOGIES/STRATEGY/ APPROACH

3.1. Methodologies used (max. 500 words)

All the water samples (n = 50) were homogenized by shaking the glass container and 1000 ml of sample was measured using graduated glass cylinder . Measured water samples were filtered using glass fibre filter paper having a diameter of 47 mm and pore size of 0.2 µm (Whatman CAT no. 1820-047). Further, wet sediment samples (n = 50) were dried at a temperature of 30 °C (Memmert, Model 30-1060), since an increase in temperature can transform polymers (Carr et al., 2016). For all the sediment samples, we have preferred to use sodium chloride (NaCl) for density separation and not the other salts (Zinc Chloride: ZnCl2, Zinc Bromide: ZnBr2 and Sodium Iodide: NaI), citing different factors. The salts (ZnCl2, ZnBr2 and NaI) are comparatively 10 times more expensive than NaCl and are hazardous in nature (Campanale et al., 2020). The use of NaCl provides a safe and accountable process to assess the microplastic contamination in sediment samples. The health hazard information for the respective salt is retrieved from Hazardous Material Identification System (HMIS®).

Thereafter, the dried samples were weighed (ca. 300 g) and wet peroxide oxidation (WPO) was performed using Fenton's reagent to remove the organic matter from the samples. The WPO solution was filtered through a sieve having mesh size of 4.75 mm. Subsequently, the solution was transferred to saturated sodium chloride (NaCl) solution (3 L) for density separation of microplastics from the sediment samples (Claessens et al., 2011). The solution was left to settle down and supernatant was filtered once the sediment debris is settled in the separating funnel. Separating funnel was further rinsed with sodium chloride solution to transfer all the solids to the collection cup of the filtration unit (Merck-millipore XI1504700). Further, microplastics in sediments samples (n = 5) from different locations were also extracted at varying density of ZnCl2 solution. The abundances of microplastics for these replicate analyses were found to have a relative error of no more than 5%.

The MPs from the water and sediment samples were examined under a dissecting fluorescent microscope for the size characterization. To characterize the MPs on the basis of its chemical composition, Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) and Raman spectroscopy methods were performed on selected microplastic particles (n = 120) from the water and sediment samples. For every MP particle, the analysis was performed at a spectral range of 500–3000 cm−1 with a resolution of 4 cm−1. Prior to the analysis, the slab was cleaned with acetone and the background scan was performed. Additionally, the extracted MPs from the water and sediment samples were also analyzed using the Renishaw InVia Raman microscope spectrometer coupled with three different lasers having wavelengths of 514, 633 and 785 nm respectively. The microplastics were sequentially observed and analyzed under the 785 nm laser with 1200 grating and an exposure time of 10 s. The intensity of the laser was 0.1%, scanning over a spectral range of 500–3200 cm−1. The spectra were corrected for the baseline and the characteristic wave-number was compared to the reported polymer spectra in published literature and OPUS library (DEMOLIB.S01).

3.2. Data collected and Equipment utilised (max. 500 words)

The water samples (n = 50) and surface sediment samples (n = 50) were collected from different locations covering the entire basin of the Renuka and Rewalsar Lake using a UWITEC water sampler and a Van-Veen grab sampler, respectively (Fig S1, S2 and S3). Approximately 2 kg of sediment samples and 3 L of water samples were collected from each sampling location. Leaf samples from the dominant terrestrial and aquatic vegetation (n = 16) and floating microbial mats (n = 4) were also collected during this field survey. The collected samples were wrapped with aluminium foil, sealed in zip-lock bags, transferred to the lab, and were dried at ≤ 30 °C for 3 to 4 days. The bathymetric survey was conducted using a portable GARMIN echo-sounder (model no. GPSMAP 585 plus). The sediment samples were dried and stored in aluminium foils. The water and the surface sediment samples were analysed for microplastic abundance, whereas PAEs measurements were performed on the lake surface sediments. The MPs from the water and sediment samples were examined under a dissecting fluorescent microscope (Nikon SMZ 18) coupled with an illuminator (Nikon INTENSILIGHT Epi-fluorescence illuminator) and visually observed under stereo microscope (Radical RSMr-3, Software- Radical ProCAM) for the size characterization. To characterize the MPs on the basis of its chemical composition, Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) and Raman spectroscopy methods were performed on selected microplastic particles (n = 120) from the water and sediment samples

For lipid extraction, approximately 10 g of the dried sediment samples were extracted with the help of pressurized solvent extractor (BUCHI E-914) using dichloromethane as solvent (extraction time: 45 min; extraction temperature: 100 °C; extraction pressure: 100 bar). The total extract was concentrated to 1 ml using a multi evaporation system (Multivap P-6, BUCHI). The polar fraction was separated from total extract through silica gel column chromatography (DCM: methanol (1:1)). The solution was further concentrated to 0.5 ml and transferred to GC vials. Total 1 µl of aliquot was injected to a gas chromatograph coupled to mass detector (GC-MS, 7890B GC, 5977C MSD) at PRISM Laboratory, IISER Mohali. The injection was made to a multimode inlet maintained at 320 °C with an automated liquid sampler (GC-ALS). Analyte separation was achieved with an HP-5MS capillary column. Helium was used as the carrier gas having a flow rate of 1 ml/min and the GC oven was programmed from 40° to 320 °C.

3.3. Details of Field Survey conducted if any (max 500 words)

The surface sediment samples (n=50) and water samples (n=50) were collected from Renuka and Rewalsar lake using a Van Veen grab sampler. The sedimentation rate reported from Renuka Lake is about 0.64 cm/year (Diwate et al. 2020). Thus, the surface sediments (top 5 cm of sediment) used in the present study typically represent the last 10 years. Bathymetric measurements of the lake were recorded with the help of portable GARMIN echo-sounder (model no. GPSMAP 585 plus). Leaf samples from the dominant terrestrial and aquatic vegetation (n=16) and floating microbial mats (n=4) were also collected during this field survey. The collected samples were wrapped with aluminum foil, sealed in zip-lock bags, transferred to the lab, and were dried at ≤ 40°C for 3 to 4 days.

3.4. Strategic Planning for each activity with a time frame (max. 200 words)

The research work encompass a systematic approach beginning with field visits and well-rounded sampling from the proposed study areas- Renuka and Rewalsar lakes. Sampling was at different times of the year which is also essential to understand seasonal variation of the pollutants in the lakes. The samples were collected from different matrices of the lake environment including water and sediments, packaged carefully so as to avoid any overprinting and immediately transferred to laboratory. The representative surface sediment samples were collected using a Van Veen grab sampler and the deeper sediments retrieved using UWITEC gravity corer. The soluble organic contaminants (constituents that are soluble in organic solvents) was extracted from water using filter and Soxhlet technique and from the sediments using speed extractors (detailed methodologies given in supplementary Information). Analysis of the soluble compounds was carried out using Gas Chromatography Mass Spectrometry (GC-MS).

Quantitative analysis was carried out using internal standard. The compounds are identified and categorized based on the source and severity of damaging properties.

Activities	Time line (Months)					
	1-6	7-12	13-18	19-24	25-30	31-36
Literature survey						
Collection of water samples and Recovering surface sediments from Renuka and Rewalsar Lake						
Sample processing and analytical methods						
Data acquisition and interpretation						
Presentation and publication of results						

4 KEY FINDINGS AND RESULTS –

4.1. Major Activities/ Findings (max. 500 words)

The present project provided insights into the occurrence, distribution, characterization and quantification of microplastics and phthalates ∑5PAEs in water samples and surface sediments from freshwater aquatic systems (Renuka and Rewalsar Lake) in the Indian Himalaya. The distribution of the microplastic particles and the PAEs in the sediment and water samples from the Renuka and Rewalsar Lake indicated the ubiquitous contamination of plastic pollutants in a conserved aquatic sites. The western part of the studied Renuka lake system showed the highest abundance of microplastics and maximum concentration of the PAEs. Fragments and fibers were most abundant microplastic particle types, whereas DiBP and DEHP were the most dominant PAEs present in the lake. A high correlation was observed between the microplastics abundance and the PAEs concentration suggesting that the PAEs were sourced from the microplastic particles. On the other hand, lack of correlation was observed between the microplastics abundance and the PAEs concentration in Rewalsar Lake. The spatial variance indicated that these pollutants were majorly derived from anthropogenic activities (especially boating, laundering and bathing activities) in the proximity of the ancient temple. The non-anthropogenic factors, such as sediment grain size played a very limited role in the distribution and occurrence of the MPs and PAEs in the Renuka and Rewalsar Lake. Comprehensive monitoring programs should be imposed to understand the adverse effects of the presence of these organic pollutants on the ecology of the lake. Our present work provide an important baseline for the future investigations on the hazardous impact of the microplastics and PAEs contamination in the aquatic environments.

Further, the bulk geochemical parameters (TOC and $\delta^{13}C_{org}$) and saturated hydrocarbon biomarkers from the surface sediments of Renuka Lake (Lesser Himalaya) were analyzed in order to determine the distribution and origin of organic matter (OM) in the sediments. The results indicate that OM in the lake sediments is mostly derived from microbial and aquatic plants, while the contribution from terrestrial plants is minor. The abundance of periphyton communities, signified by the abundance of C20 HBI, is more pronounced in macrophyte-dominated sites with stable water column and shallow conditions. The western margin of the lake is characterized by maximum concentration of OM and may reflect the effects of anthropogenic activities therein. Moreover, the absence of correlation of TOC with sediment grain size and bathymetry suggests limited influence of hydrodynamics on the accumulation of SOM. The results of this study help to understand how anthropogenic environmental change in the region is affecting OM dynamics and carbon cycles. Further, our study also shows that use of single organic matter source indicators may lead to ambiguous outcomes, and therefore multiple proxies should be applied. In Rewalsar Lake, the spread of pollutants namely PAHs along with fecal sterol compounds particularly coprostanol, epi-coprostanol highlight input from other sources such as petrogenic and pyrolytic derivatives, sewage effluents and industrial discharges. The understanding on the OM source and its spatial variability in a lake system provides an important contribution to our understanding of the terrestrial carbon cycle, trophic status destiny, and evaluating effective conservation strategies.

4.2. Key Results (max. 500 words in bullets covering all activities)

- Microplastics (MPs) were identified in all the water and surface sediment samples of Renuka Lake having an average concentration of 21 ± 13 particles/L in water and 180 ± 143 particles/kg dw in sediment samples (Fig. S5). The concentration of MPs in water samples varied from 02 to 64 particles/L (Fig. S5), while surface sediments had a tenfold concentration of MPs ranging from 15 to 632 particles/kg dw (Fig. S5).

- Microplastic particles were analysed with a Raman spectrometer and ATR-FTIR to characterize the polymeric backbone of the MP particles. The identified microplastics were characterized as polyethylene (PE), polystyrene (PS) and polypropylene (PP) based on the characteristic wave number reported in literature (Fig. S6).

- PAEs were identified in the sediment samples including dibutyl phthalate (DnBP), di-isobutyl phthalate (DiBP), bis (2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DiNP) (Fig. 6). The concentration of the ∑5PAEs ranged from 06 to 357 ng/g dry weight (dw) in the sediment samples (Fig. S7 and S8).

- *n*-alkanes were distributed all over the Renuka Lake, but shows maximum concentration in the western periphery of the lake basin. The short chain n-alkanes in surface sediments exhibit similar spatial pattern as total n-alkanes (ΣC_{15-33}). The mid-chain showed maximum abundance close to the periphery in the central-western part of the basin.

- The Total organic carbon (TOC) content in the surface sediments of the Renuka Lake was 8.7% to 28.9%, with an average of 14.0%. The highest value observed in the western extremity of the study area, and the lowest values in the central and eastern part of the lake basin.

- The results indicate that OM in the lake sediments is mostly derived from microbial and aquatic plants, while the contribution from terrestrial plants is minor.

- The abundance of MPs in Rewalser lake surface water ranged from 13-238 particles/L, while the concentration in sediments samples varied from 750-3020 particles/kg (dry weight: d.w.). The average concentrations are registered higher for sediments (1449 particles/kg dw) as compared to water samples (130 particles/L).

- Polymers identified in sediment and water samples from Rewalser Lake were polypropylene, polyethylene and polystyrene.

- di-isobutyl phthalate (DIBP), dibutyl phthalate (DBP), DBP, DEHP were detected in surface sediments from Rewalsar Lake. The concentration of PAEs range from 1.69 μg/g to 4.03 μg/g in Rewalsar surface sediments.

- The distribution of poly aromatic hydrocarbons (PAHs) and fecal stanols were determined for the first time in sediments of the Rewalsar lake (Table S1, S2 and S3).

- The spread of pollutants namely PAHs along with fecal sterol compounds particularly coprostanol, epi-coprostanol highlight input from other sources such as petrogenic and pyrolytic derivatives, sewage effluents and industrial discharges.

- Moreover, the presence of anthropogenic compounds reflect greater anthropogenic disturbances are caused due to proximity to the settlements and lack of proper waste disposal in the region (Fig. S9-S14).

4.3. Conclusion of the study (max. 500 words in bullets)

- The project provide insights into the occurrence, distribution, characterisation and quantification of microplastics and phthalates (∑5PAEs) in water samples and surface sediments from a freshwater aquatic systems in the Indian Himalayas.

- The present work to best of our knowledge is the first coupled approach to evaluate the presence and distribution of microplastics and phthalates in a highland freshwater system in the Indian subcontinent.

- The distribution of the microplastic particles and the PAEs in the sediment and water samples from the Renuka and Rewalsar Lake indicated the ubiquitous contamination of plastic pollutants in fresh water aquatic systems in Indian Himalaya.

- The western part of the studied lake system showed the highest abundance of microplastics and maximum concentration of the PAEs in Renuka Lake. Likewise, the microplastic concentration was different among sampling sites, being more abundant to sites in the proximity of intense anthropogenic activities.

- Fragments and fibres were the most abundant microplastic particle types in Renuka Lake, while pellets and fragments form the dominant fraction in Rewalsar Lake.

- The DiBP and DEHP were the most dominant PAEs present in the Renuka Lake, whereas di-isobutyl phthalate, dibutyl phthalate (DBP) were present in Rewalsar Lake.

- A high correlation was observed between the microplastics abundance and the PAEs concentration suggesting that the PAEs were sourced from the microplastic particles while the Rewalsar lake is characterised by absence of correlation indicating a possible leaching effect.
- The sediment particle size played a very limited role in the distribution of the MPs and PAEs in the Renuka and Rewalsar Lake (Fig. S4).
- In Rewalsar Lake, the spread of pollutants namely PAHs along with fecal sterol compounds particularly coprostanol, epi-coprostanol highlight input from other sources such as petrogenic and pyrolytic derivatives, sewage effluents and industrial discharges.
- The abundance of periphyton communities, signified by the abundance of C20 HBI, is more pronounced in macrophyte-dominated sites with stable water column and shallow conditions.
- Moreover, the absence of a correlation of TOC with sediment grain size and bathymetry suggests a limited influence of hydrodynamics on the accumulation of SOM.
- Comprehensive monitoring programs should be imposed to understand the adverse effects of the presence of these organic pollutants on the ecology of the lake.
- Our present work provide an important baseline for the future investigations on the hazardous impact of the organic contamination in the aquatic environments.

5 OVERALL ACHIEVEMENTS

5.1. Achievement on Project Objectives/ Target Deliverables (max. 500 words)

The environmental chemistry of the organic contaminants is a multidisciplinary and active area of scientific enquiry having far-reaching effects to ecosystems as well as the socio-economic conditions of countries. Humans can be exposed to contaminants through dermal contact and ingestion. Direct contact through recreational activities and unintentional ingestion of polluted water can cause potential human health risk. Continuous exposure to PAHs can result in DNA damage as well as genotoxicity. Also, the presence of PAHs in uncontrolled amount in the aquatic system can lead to the bioaccumulation at higher trophic levels, that may disturb the food chain and lead to irreversible toxicity. The carcinogenicity of PAHs depends on structural characteristics (presence of fused aromatic rings). Likewise, the existing reports indicate biological effects of fecal stanols particularly coprostanol which include the formation of cataract lesions in humans and colon cancer in rats the activation of apoptosis in neurons of rats.

The present project provided insights into the occurrence, distribution, characterization and quantification of microplastics and phthalates ∑5PAEs in water samples and surface sediments from freshwater aquatic systems (Renuka and Rewalsar Lake) in the Indian Himalaya. The distribution of the microplastic particles and the PAEs in the sediment and water samples from the Renuka and Rewalsar Lake indicated the ubiquitous contamination of plastic pollutants in a conserved aquatic sites. Further, to assess the extent of pollution in a system, previous literature has evoked the effects range low (ERL) for organic contaminants such as PAHs and suggest the concentration values above ERL guideline (4.02 µg/g) mark contamination and detrimental biological effect in the ecosystem. The data recorded in the present work has values above ERL for most of the stations in Rewalsar Lake. Likewise, the studies on sewage markers have expressed that the concentration values of coprostanol above 0.5 µg/g are linked to significant sewage contamination and the obtained recorded from all the sampling stations are above the limit.

The research was directed towards improvement in the state of the knowledge of the source inventories and pathway analyses of the microplastics and POPs. The results from the proposed project are pivotal to understanding the characterization of the persistent organic pollutants and microplastic contamination in the two lakes in Himachal Pradesh; establish a baseline for future monitoring and developing effective remediation and management in the investigated lake systems.

5.2. Interventions (max. 500 words)

The physicochemical characteristics of plastics produce toxic effects on the human health and the ecosystems. The continuous discharge of microplastics into freshwater ecosystems is potentially hazardous to the niche organisms (Wagner et al., 2014). The microplastics could persist, transfer, and bio-accumulate along the trophic levels making it difficult to remove them from the aquatic environments (Arthur et al., 2009). Ingestion of the MPs with adsorbed pollutants by aquatic organisms may lead to contamination of the food web in the aquatic system. Microplastics can also act as passive samplers and adsorb hydrophobic and persistent organic pollutants (Jeong et al., 2018). Because of their low density, settling of plastic particles is hindered compared with other suspended solids and hence may travel over long distances along with the adsorbed pollutants. Due to their large surface to volume ratio and chemical composition, MPs accumulate waterborne contaminants such as heavy metals and persistent bio-accumulative and toxic (PBT) compounds.

In addition, phthalates which are widely used class of plasticizers to soften the plastic material are also a major environmental threat. The use of PAEs in daily need products including medical devices, cosmetics, personal care products (PCPs), pharmaceutical nutritional supplements, dietary supplements, children's toys etc. can lead to the higher amount of exposure for the humans (Johnson et al., 2011; Kelley et al., 2012; Gong et al., 2015). From all these sources, PAEs end up accumulating in the human body and cause various health effects including hormonal imbalance in adults and changing levels of urinary thyroid hormones in children (Boas et al., 2010; Mu et al., 2018). Given the wide distribution of MPs and PAEs, the negative and adverse effects of these environmental contaminants have drawn considerable attention of the scientific world. Some of the developed countries including European Union (EU) have banned the use of phthalates in the children's toys since 1999 (Giuilivo et al., 2016).

The variables influencing SOM distribution in terms of natural and anthropogenic stressors have the capacity to modify the nutrient threshold and change the community structure of freshwater systems. As a result, the knowledge of multiple drivers controlling the lacustrine ecosystem may be used to assess the ecological status and encourage conservation efforts. In addition, the study also shows the applicability of molecular markers for comprehensive understanding of SOM sources and can further be utilized to understand the long-term productivity changes, depositional environment and climate reconstruction.

5.3. On-field Demonstration and Value-addition of Products, if any (max. 500 words)

N-A-

5.4. Green Skills developed in State/ UT (max. 500 words)

5.5. Addressing Cross-cutting Issues (max. 200 words)

To our knowledge, this was the first integrated studies of occurrence, distribution and characterization of microplastics particles and phthalic acid esters from Indian subcontinent. The current investigation from the Indian Himalaya demonstrated the extent and the nature of organic pollutants in a freshwater lacustrine environment. Our study represented opportunities for future research to improve understanding on the partition of microplastics constituents between the sediment matrices and the water phase, the fate of these highly recalcitrant polymers and the associated pollutants in the water medium and the geosphere, and the uptake of these hazardous contaminants by organisms in a lake ecosystem. The results from the proposed project are pivotal to understanding the characterization of the persistent organic pollutants and microplastic contamination in the two lakes in Himachal Pradesh; establish a baseline for future monitoring and developing effective remediation and management in the investigated lake systems.

6 PROJECT'S IMPACTS IN IHR

6.1. Socio-Economic impact (max. 500 words)

The fresh water aquatic systems in Himalayan region offer series of ecosystem services and functions such as water for drinking and agricultural purposes, and recreation and tourism. However, in recent decades, aquatic systems in Indian Himalayas are greatly influenced by human activities such as burning of fossil fuels, sewage disposal, waste disposal and agricultural run offs. The potential contaminants produced by anthropogenic impact are hydrophobic in nature and can easily associate themselves with the existing organic matter in the system. They can affect the biota through bioaccumulation and also by incorporating into the food chain. They also have significant impact over the net organic content and geochemical processes of the system enhancing the productivity (Fielding et al., 2020). Accumulation of these compounds in an aquatic system can deteriorate the water quality.

The anthropogenic derived organic contaminants can negatively affect the habitation and increase the health risk for the organisms consuming it. For instance, introduction of non-treated plasticizers by human activity into an aquatic ecosystem can threaten the sustainability of the system by invading the trophic levels. Being impervious to degradation and hydrophobic in natures, such particulates can continue to be a part of the aquatic environment for a long period, and are difficult to discern. In order explore the anthropogenic impact in the region, we have also evaluated the spatio-temporal patterns of LULC changes in the Renuka Lake from 2000 to 2021 AD. The results show that LULC in the study area has undergone a series of changes over the past two decades. The designation of Renuka Lake as Ramsar site and ongoing restoration activities have resulted in an increase in vegetation cover (12%) and a subsequent decrease in barren land (40%). However, the built-up area in the study region have increased by 35%, with a substantial increase over the western extremity of the lake basin. As a result, it is indeed conceivable that human activities enhanced nutrients input into the lake, resulting in higher primary productivity and increase in organic contaminants in the region.

6.2. Impact on Natural Resources/ Environment (max. 500 words)

Microplastics are emerging as one of the main pollutants in water bodies with huge impact on marine and freshwater environments. The microplastics possess two important threats to environment: (i) these particles can easily enter into the aquatic food chain which may cause blockage in digestive tract resulting in reduced growth of the organisms and, (ii) the microplastics harbor the risks of transport of adsorbed pollutants (such as persistent organic pollutants (POPs); inorganic elements) to the aquatic ecosystems. The studies show that observed plastic concentrations differ by several orders of magnitude suggesting that population density, level of urbanization and industrialization within catchment areas play a significant role in distribution of microplastics (Klein et al., 2015).

In addition to the physical threats of MPs to aquatic environments, many harmful additives (e.g., plasticizers) used during the production of plastics result in health risks and ecological imbalance (Rahman and Brazel, 2004; Halden, 2010). Plasticizers are used to enhance the flexibility of the polymers and the percentage of phthalates being blended in plastic could rise to approximately 70% with varying plasticity (Zeng et al., 2008; Josh et al., 2012; Zheng et al., 2014; Zhang et al., 2018a, 2018b). The phthalic acid esters (PAEs; Phthalates) are a family of plastic additives widely used in polymeric materials (Zhao et al., 2004; Yang et al., 2009; Chen et al., 2016; Kumar et al., 2017; Lee et al., 2020). The PAEs are known to have adverse toxic effects on the aquatic organisms and the human species that include endogenous hormonal disruption, and chronic reproductive disorders (Guo et al., 2012). The continuous accumulation of PAEs and other phthalate derivatives in the bottom sediments can pose potential ecological and environmental risks due to the developmental toxicity of these compounds (Lyche et al., 2009; Ventrice et al., 2013; Dekant, 2020; Ai et al., 2020). Despite their adverse effects on all forms of life, microplastics are less studied in Indian subcontinent as the country being among the major plastic consumers and producer of 5.6 million tons of plastic waste annually. The results from the proposed project provide baseline for organic pollutants and microplastic contamination in the two lakes in Himachal Pradesh and developing effective remediation and management in the investigated lake systems. The present study contribute considerably to the knowledge of the microplastic and organic contaminant distribution, occurrence, and will help local, state and central government authorities while developing the pollution related policies to monitor and control the pollution of the aquatic systems. It will also be helpful for the public health associations to control the emergence of plastics as well as the risk factors.

6.3. Conservation of Biodiversity/ Land Rehabilitation in IHR (max. 500 words)

Our study will help the policy makers to take note on new emergent contaminant derived from organic contaminants and plastic debris while formulating the policy guidelines on plastic derived toxins. These studies are the first integrated study of microplastic particles' occurrence, distribution and characterisation and phthalic acid esters from the Indian subcontinent. The current investigation from the Indian Himalayas demonstrated the extent and nature of pollutants in a freshwater lacustrine environment. Our study represented opportunities for future research to improve understanding on the partition of microplastic constituents between the sediment matrices and the water phase, the fate of these highly recalcitrant polymers and the associated pollutants in the water medium and the geosphere, and the uptake of these hazardous contaminants by organisms in a lake ecosystem.

The findings of the project will serve as a basis for management and decision making for various Centre and State agencies like Ministry of Water Resources, Central Water Board, Irrigation and Flood Control, Lakes and Water ways Development Authority, State Pollution control Board, Department of Ecology, Environment and Remote Sensing, Public Health Engineering, Urban Planning, Ministry of Environment and Forests, Universities and Research institutions. Besides, the institutions like Ministry of Water Resources, Central Water Commission etc. will also get help from the findings of the project. Besides, the work has been published in high quality impact journals to give the visibility of work in the field of science at regional and international level.

6.4. Developing Mountain Infrastructures (max. 200 words)
N-A

6.5. Strengthening Networking in State/ UT (max. 200 words)
N-A

7 EXIT STRATEGY AND SUSTAINABILITY

7.1. Utility of project findings (max. 500 words)
The project findings will serve as a basis for management and decision-making for various central and state agencies. The key beneficiaries of the Research work will be local communities, decision-makers, project proponents, policymakers and researchers, NGOs, Health Department and all those who are directly and indirectly related to or dependent on the Himalayan fresh water ecosystem. Besides, the institutions like Ministry of Water Resources, Central Water Commission etc. will also get help from the findings of the project. Additionally, the work has also been published in high quality impact journals to give the visibility of work in the field of science at regional and international level.

7.2. Other Gap Areas (max. 200 words)
The evaluation of organic contamination in vulnerable ecosystem such as mountainous lakes is consequential as this system responds rapidly to the natural and anthropogenic changes. The Indian Himalayan aquatic systems are overburdened due to increasing population, modernization and industrial growth that has resulted in higher level of pollution. The anthropogenic activities have led to contaminants accumulation, ecological degradation and higher sedimentation rates in Himalayan lakes. However, the microplastic and associated organic contaminants pollution on freshwater ecosystems from Indian subcontinent remains largely unexplored. To date, except for our studies in Renuka and Rewalsar, no data are available about the microplastics and PAEs levels in the water and sediment of the Indian Himalayas. Therefore, more studies generating baseline data on various organic contaminants and their concentration level in aquatic systems should be an immediate priority in Indian Himalaya.

7.3. Major Recommendations/ Way Forward (max. 200 words)

To our knowledge, our study provide the first data for occurrence, distribution and characterization of microplastics particles and phthalic acid esters from aquatic systems (Renuka and Rewalsar Lake) in Indian Himalayas. The investigations from the Indian Himalaya demonstrated the extent and the nature of pollutants in freshwater lacustrine environment. Our study represented opportunities for future research to improve understanding on the partition of microplastics constituents between the sediment matrices and the water phase, the fate of these highly recalcitrant polymers and the associated pollutants in the water medium and the geosphere, and the uptake of these hazardous contaminants by organisms in a lake ecosystem. Furthermore, comprehensive monitoring programs should be imposed to understand the adverse effects of the presence of these organic pollutants on the ecology of the lake. Our present work provide an important baseline for the future investigations on the hazardous impact of the microplastics and PAEs contamination in the aquatic environments from Indian Himalaya.

7.4. Replication/ Upscaling/ Post-Project Sustainability of Interventions (max. 500 words)

The present project involves extraction, purification, density separation, filtration, visualization, categorization, and chemical characterization of microplastics in surface waters and sediments. The characterization of microplastics had been carried out using Micro Raman spectroscopy and Fourier transformed infrared spectroscopy (FTIR). The gas chromatography-mass spectrometry had been applied to determine the polymer composition of plastic debris extracted from various aquatic environments. The Individual phthalates and other organic contaminants (PAHs and sterols) were identified and quantified using a gas chromatograph (Agilent 7890A, GC system) equipped with a non-polar capillary column (HP5-MS, 30m × 250μm × 0.25μm) and mass spectrometer (MS). Sample injection carried out in 1:1 split mode with an initial inlet temperature of 320 C. The GC oven temperature was set at 60 C (held 2 min) and then increased to 320 C at 8 C/min (12 min held). The methodology developed during the project will be useful for undertaking the analyses of microplastics and associated contaminants in various aquatic systems in Indian Himalaya (detail information provided as supplementary information). The data about the organic contaminants in the freshwater environments are limited except for our studies in Renuka and Rewalsar Lake. More work need to be carried out to understand the organic contaminants including microplastics occurrence, transportation and fate in the aquatic environments in Indian Himalaya.

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APPENDICES

Appendix 1 – Details of Technical Activities

Appendix 2 – Copies of Publications duly Acknowledging the Grant/ Fund Support of NMHS

Appendix 3 – List of Trainings/ Workshops/ Seminars with details of trained resources and dissemination material and Proceedings

Appendix 4 – List of New Products (utilizing the local resources like NTFPs, wild edibles, bamboo, etc.)

Appendix 5 – Copies of the Supporting Materials like Manual of Standard Operating Procedures (SOPs) developed under the project

Appendix 6 – Details of Technology Developed/ Patents filled, if any

Appendix 7 – Any other

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