Annexure II

Results Based on Project Activities

1. Site Visit and Sample Collection

Extensive field visits were conducted across several oil exploration and production-impacted districts in Assam—namely Cachar, Tinsukia, Golaghat, Bongaigaon and Sibsagar. These regions are known for high oil drilling activity and consequent discharge of produced water (PW), which poses severe ecological threats. During the visits, soil and PW samples were systematically collected from multiple locations. These samples were analysed for physical, chemical, and hydrocarbon csontamination characteristics using GC-MS and other analytical tools.

Photoplate 1: Collection of samples from Cachar district, Assam.

Photoplate 2: Collection of samples from Sibsagar and Golaghat, Assam.

Compound	Cachar	Tinsukia	Golaghat	Bongaigaon	Sibsagar
Benzene	0.67	0.9	0.78	0.76	0.38
Toluene	0.94	0.24	0.86	0.82	0.96
Ethylbenzene	0.21	0.49	0.55	0.22	0.22
Xylene	0.73	0.65	0.85	0.59	0.97
Naphthalene	0.14	0.05	0.05	0.12	0.13
Phenanthrene	0.14	0.09	0.07	0.06	0.11
Fluoranthene	0.04	0.01	0.04	0.01	0.01
Chrysene	0.01	0.02	0.01	0.02	0.03
Total Petroleum Hydrocarbons (TPH)	153.42	203.58	178.84	196.91	284.71
Phenol	0.6	0.42	0.47	0.5	0.58

Compound	Cachar	Tinsukia	Golaghat	Bongaigaon	Sibsagar
Benzene	0.84	1.37	1.24	1.06	0.47
Toluene	1.13	0.51	1.72	1.17	1.66
Ethylbenzene	0.29	0.75	0.97	0.39	0.35
Xylene	0.9	0.8	1.67	0.89	1.37
Naphthalene	0.24	0.07	0.09	0.18	0.19
Phenanthrene	0.18	0.15	0.12	0.09	0.13
Fluoranthene	0.08	0.03	0.06	0.02	0.03
Chrysene	0.01	0.03	0.02	0.03	0.04
Total Petroleum Hydrocarbons (TPH)	340.31	358.02	326.69	360.67	395.2
Phenol	0.69	0.71	0.74	0.76	0.79

Figure 1: Concentration of toxic compounds in (a) soil samples and (b) produced water samples from various districts of Assam.

Contaminant analysis across Assam's produced water-impacted sites showed significantly higher pollutant concentrations in produced water (max: 395.2 mg/L) than in soil (max: 284.71 mg/kg). Elevated values in rows 1–3 suggest persistence of hydrocarbons and associated compounds in both matrices, with site 5 consistently showing peak contamination. Soil values remained moderate (153.42–284.71 mg/kg), while produced water showed sharp increases (340.31–395.2 mg/L), indicating greater contaminant mobility and bioavailability in the aqueous phase.

2. Isolation and Screening of Bacteria

From the contaminated soil and PW samples, 10 bacterial strains were successfully isolated. Additionally, 6 biosurfactant-producing strains with prior crude oil-degrading abilities were sourced from the Soil and Environmental Microbiology Laboratory (SEML), Assam University, Silchar. All isolates were tested for their ability to degrade PW hydrocarbons across varying concentrations (0–100%).

Table 1: Morphological and biochemical characterization of bacterial isolates.

Isolates	Cell Shape	Gram Stain	Motility	Catalase Test	Oxidase Test	Spore Formation	Hemolysis on Blood Agar	Glucose Fermentation	Nitrate Reduction	Urease Test	Indole Test	Colony Morphology
PS12	Rod	Positive	Motile	Positive	Positive	No	Beta	Positive	Positive	Negative	Positive	Circular, Smooth, Creamy
PS13	Cocci	Negative	Non-motile	Positive	Negative	Yes	Gamma	Negative	Positive	Positive	Negative	Punctiform, Rough, White
PS21	Rod	Positive	Motile	Positive	Negative	No	Alpha	Positive	Negative	Negative	Positive	Filamentous, Rough, Yellow
PS22	Cocci	Positive	Non-motile	Negative	Positive	Yes	Beta	Positive	Positive	Positive	Negative	Irregular, Smooth, White
PS24	Rod	Negative	Motile	Positive	Positive	No	Gamma	Positive	Negative	Positive	Negative	Circular, Smooth, Yellow
PS32	Rod	Positive	Motile	Positive	Positive	Yes	Alpha	Negative	Positive	Negative	Positive	Irregular, Smooth, Creamy
PS41	Cocci	Negative	Non-motile	Negative	Negative	No	Beta	Positive	Negative	Negative	Positive	Circular, Rough, White
PS52	Rod	Positive	Motile	Positive	Negative	Yes	Gamma	Negative	Positive	Positive	Negative	Filamentous, Smooth, Creamy
PS53	Cocci	Positive	Non-motile	Positive	Positive	No	Alpha	Positive	Negative	Negative	Positive	Punctiform, Smooth, Yellow
PS54	Rod	Negative	Motile	Positive	Positive	Yes	Beta	Negative	Positive	Positive	Negative	Circular, Smooth, White

16 bacterial isolates screened; all showed measurable degradation potential.

3. Efficiency Testing and Enzymatic Evaluation

The degradation efficiency of the bacterial isolates was assessed based on hydrocarbon removal rates. Enzymatic profiling included spectrometric analysis of biosurfactant production and other hydrocarbon-degrading enzymes. Most isolates showed high tolerance and degradation activity, even at high salinity and oil concentrations, which mimics the nature of PW. The conditions were as follows:

T1 - PS54 + BHB
T2 - BHB + 10% Produced Water + PS53
T3 - BHB + 20% Produced Water + PS53
T4 - BHB + 30% Produced Water + PS53
T5 - BHB + 40% Produced Water + PS53
T6 - BHB + 50% Produced Water + PS53
T7 - 100% Produced Water + PS53

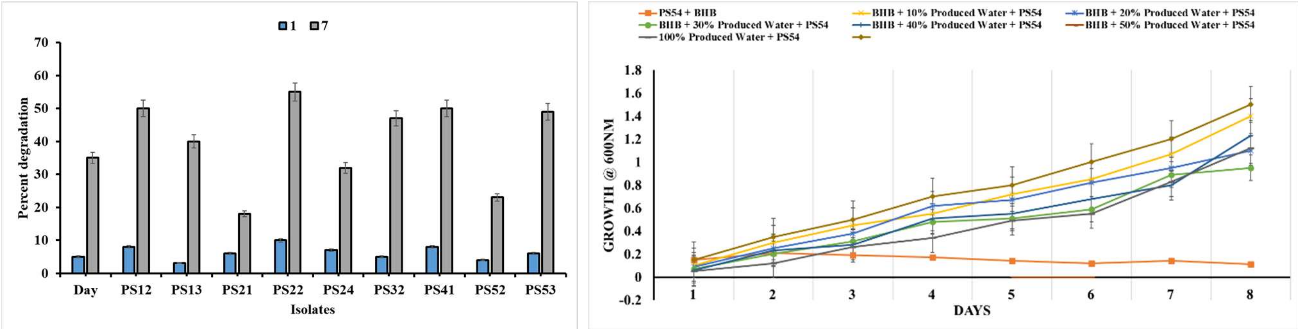


Figure 2: Percent degradation of PW by bacterial isolates (a) and growth of bacterial isolates in PW (b).

All bacterial isolates exhibited a significant increase in produced water degradation from day 1 to day 7. Among the strains, PS22 showed the highest degradation (~55%) by day 7, followed by PS41, PS12, and PS53 (each achieving over 45% degradation). In contrast, initial degradation on day 1 was minimal across all isolates (<10%), indicating time-dependent metabolic activity. These findings highlight the potential of specific isolates, particularly PS22, for effective bioremediation of produced water.

Bacterial growth increased progressively with time across all produced water concentrations up to 50%, with the highest growth observed at 30% and 40% PW. Growth was severely inhibited at 100% PW, indicating toxicity. Moderate PW levels support microbial activity, suggesting potential for bioremediation at sub-lethal concentrations.

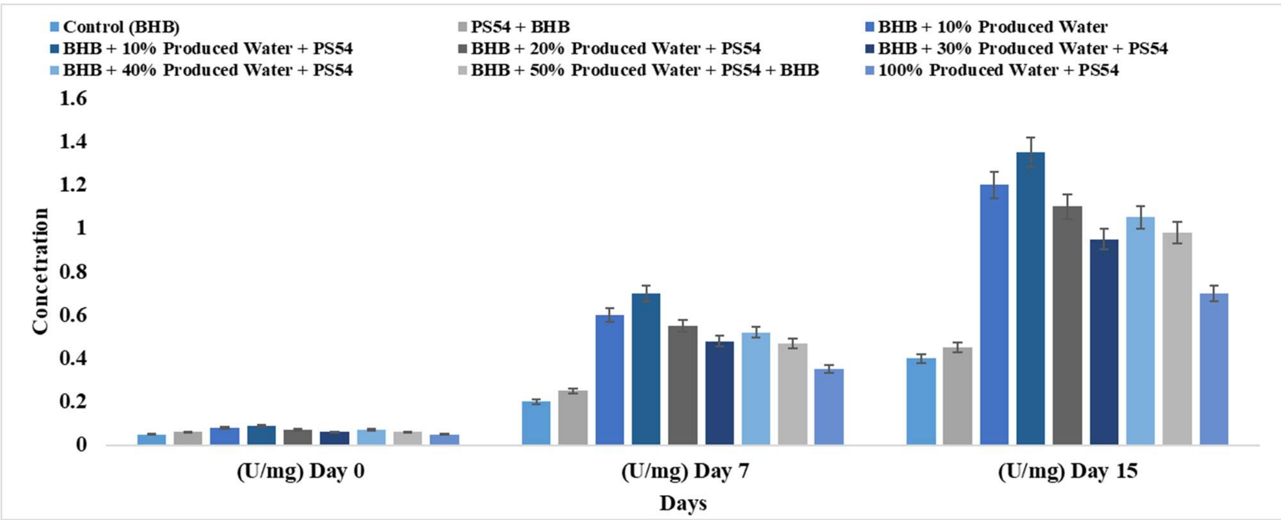


Figure 3: Catechol- 1,2-dioxygenase activity in various conditions.

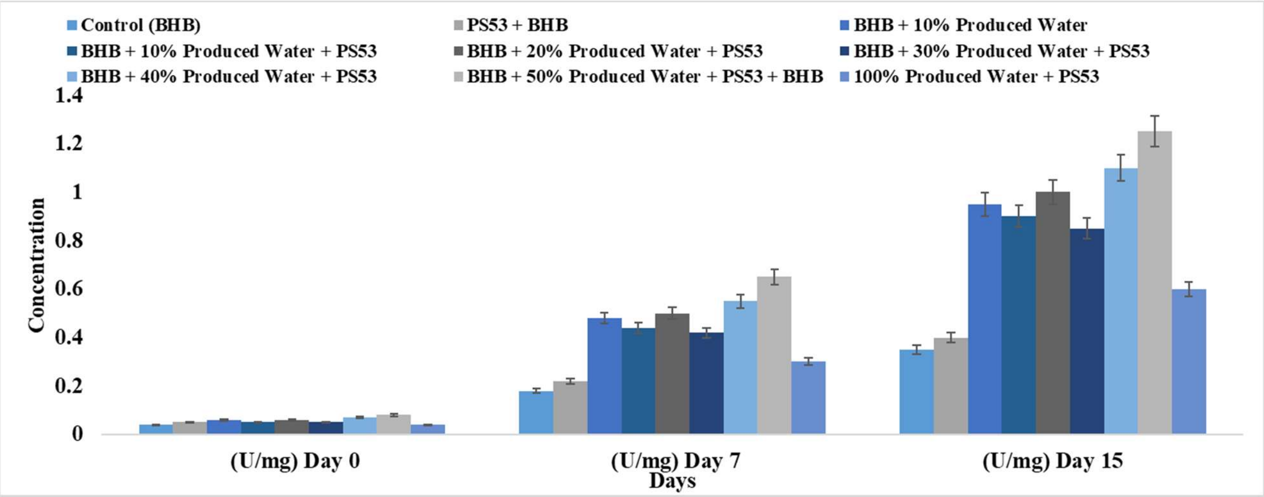


Figure 4: Catechol- 2,3-dioxygenase activity in various conditions.

Both C12D and C23D enzyme activities increased significantly from day 0 to day 15 under all treatments. The highest C12D activity (~1.45 U/mg) was observed in BHB + 10% PW + PS54, while C23D showed maximum activity (~1.25 U/mg) in BHB + 50% PW + SSL1 on day 15. Enzyme expression decreased at 100% PW, indicating concentration-dependent inhibition. Overall, PS54 favored lower PW concentrations for C12D, whereas SSL1 maintained higher C23D activity even at elevated PW levels, suggesting isolate-specific tolerance and enzyme induction potential.

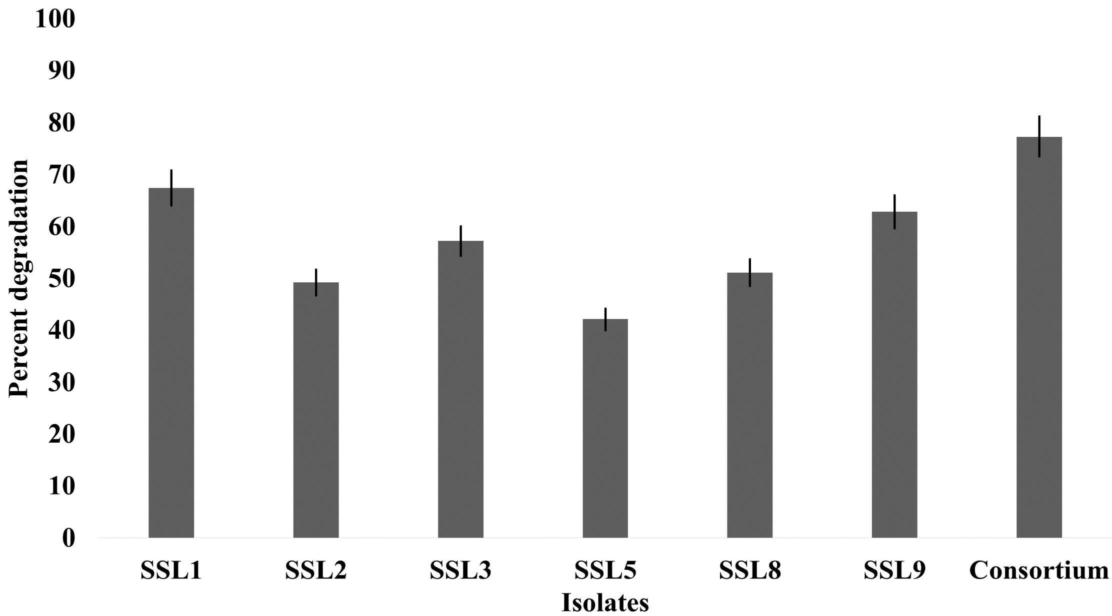


Figure 5: Degradation of PW by bacterial isolates collected from SEML, Assam University.

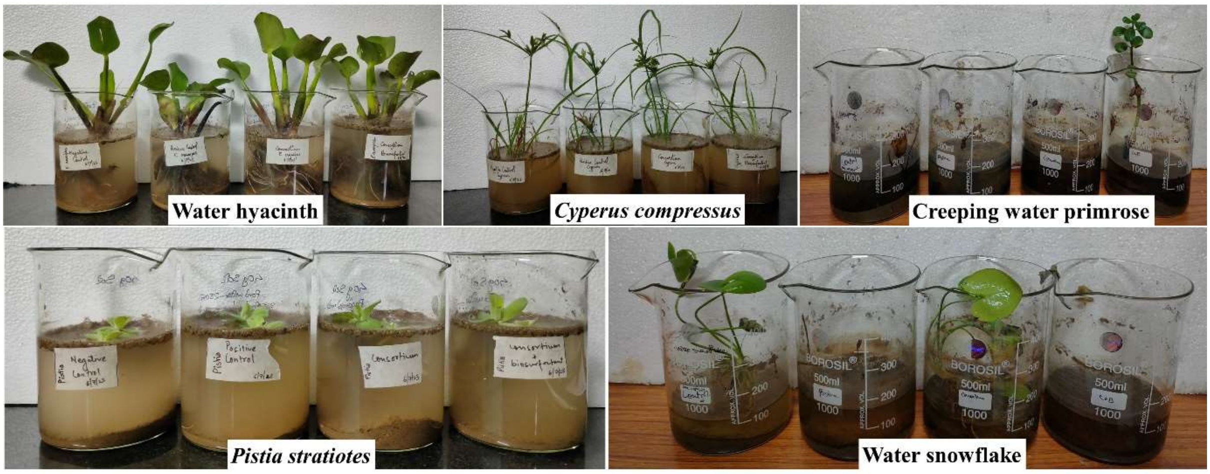
All individual bacterial isolates demonstrated varying degradation efficiencies against produced water, with SSL1 and SSL9 showing the highest performance at ~68% and ~63%, respectively. However, the bacterial consortium achieved the highest degradation rate of ~78%, indicating enhanced synergistic activity and effectiveness over single strains in produced water bioremediation.

4. Mesocosm Trials with Plant–Microbe Systems

A controlled mesocosm setup was used to simulate natural conditions and evaluate rhizoremediation potential. Six plant species were tested in combination with selected bacterial isolates. Among them, *Eichhornia crassipes* (Water hyacinth) showed the highest survival rate and synergistic removal of hydrocarbons when paired with the bacterial consortium. Other species failed under the toxic PW conditions.

For the screening of plants from degradation of Produced water. Plants name: (*Pistia Stratiotes, Cyperus compressus*, Water Hyacinth, Water Snowflake, Creeping water primrose) are set up for FTW mesocosm environmental conditions. Sixteen glass beakers of 500ml volume were used. The beakers were filled with 50 g of soil. Pond water - 270 ml and Produced water - 30 ml were used.

Four different treatment were prepared as follows:
PWTNC: Soil + Pond water + Plant
PWTPC: Soil + Pond water + Produced water + Plant
PWTC: Soil + Pond water + Produced water + plant + consortium
PWTBC: Soil + pond water + Produced water + Plant + Consortium + Biosurfactant
Pond water - 270 ml
Produced water - 30 ml
Consortium: SSL1, PS22, PS53



Biosurfactant: 100 mg/L

Photoplate 3: Screening of plant for tolerance of PW and mesocosm study with the plant.

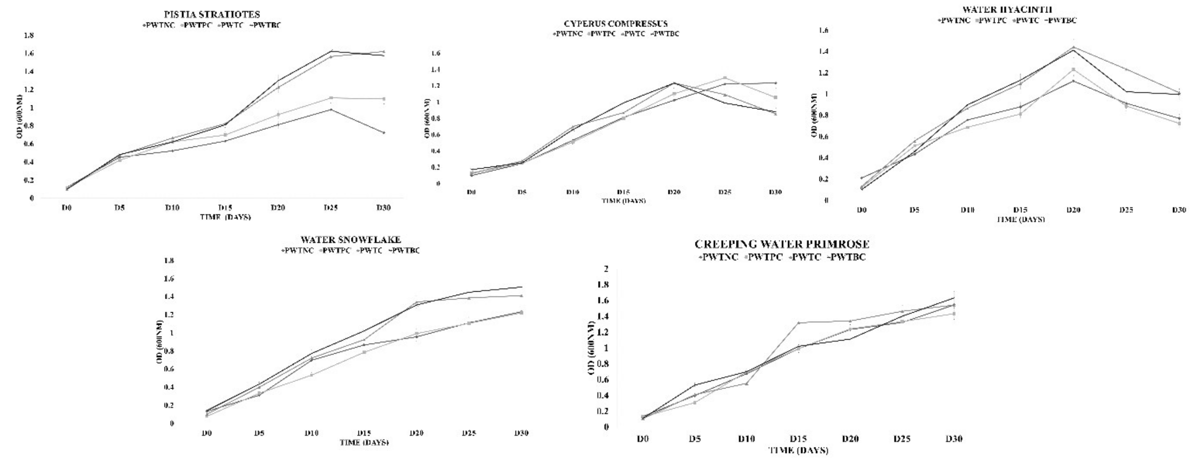


Figure 5: Growth of bacterial consortium in various treatments.

Creeping water primrose has seen the highest growth after taking OD at 600nm. Creeping water primrose growth increased from Day 1 to 30 in PWTBC also Water Snowflake growth increased from Day 1 to Day 30 in PWTBC. The lowest growth plants are Pistia Stratiotes PWTNC, Water Hyacinth PWTNC and Cyperus Compressus PWTNC

Figure 6: Growth parameters of various plants in various treatments.

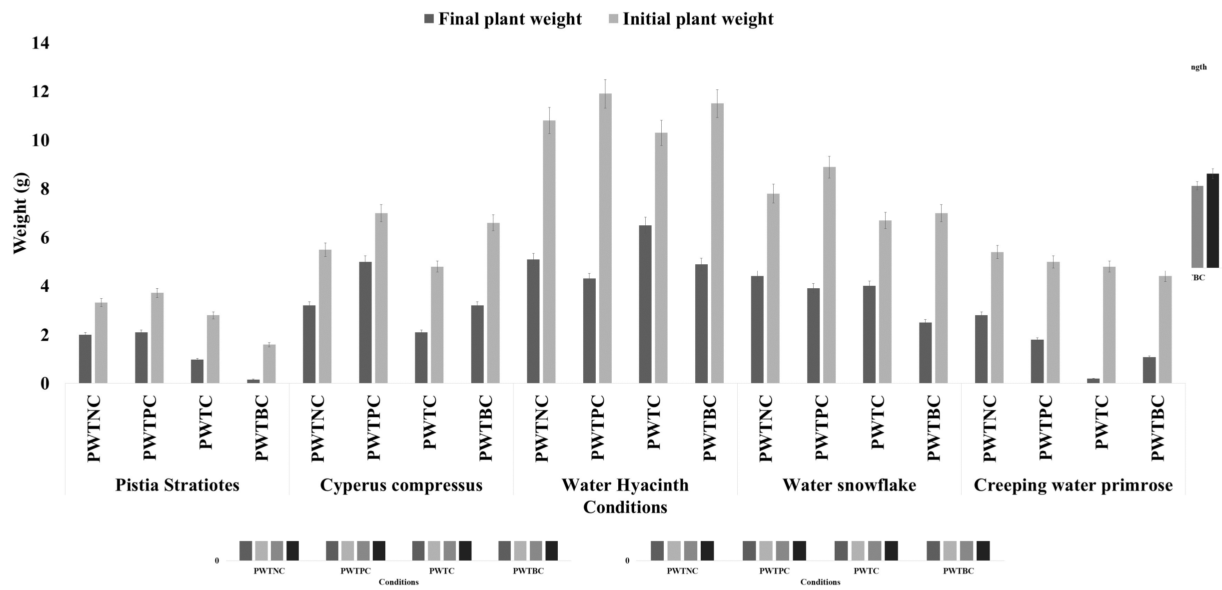


Figure 7: Weight of plants in various treatments.

Creeping Water Primrose showed optimal growth in PWTBC over 30 days. The highest shoot growth was from 11.6 cm to 11.8 cm, and root length reached 7.7 cm. Initial and final weights in PWTNC were 5.4 g and 2.8 g, respectively. The lowest shoot growth was 9.9–10.9 cm in PWTBC. *Cyperus compressus* showed optimal growth in PWTNC (1.232 cm). Initial and final shoot lengths in PWTC and PWTNC were similar (12.5–12.8 cm). The highest root length was recorded in PWTPC (6.1 cm). The lowest shoot growth was seen in PWTBC (11–11.8 cm). Water Hyacinth showed optimal growth in PWTC (1.012 cm). The highest shoot length was in PWTBC (10.3–11.6 cm), while root growth peaked in PWTC (7.5–8.6 cm). Initial weight was highest in PWTPC (11.9 g); final weight was highest in PWTC (6.5 g). Water Snowflake grew best in PWTBC (1.506 cm). Final shoot length was highest in PWTC (10.3 cm) and root length peaked in PWTPC (7.9 cm). *Pistia stratiotes* had maximum growth in PWTC (1.621 cm). Shoot length increased from 5.2 cm to 6.0 cm in PWTBC and PWTNC, while root length rose from 4.1 cm to 5.0 cm.

Overall, high produced water concentrations negatively affected plant growth, leading to the death of most plants by day 21. Only a few species showed tolerance and survived the full trial period.

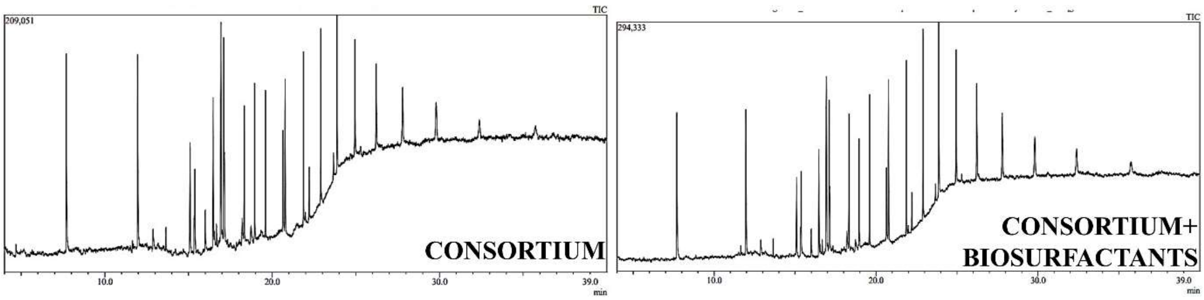


Figure 8: GC-MS analysis of PW in various treatments.

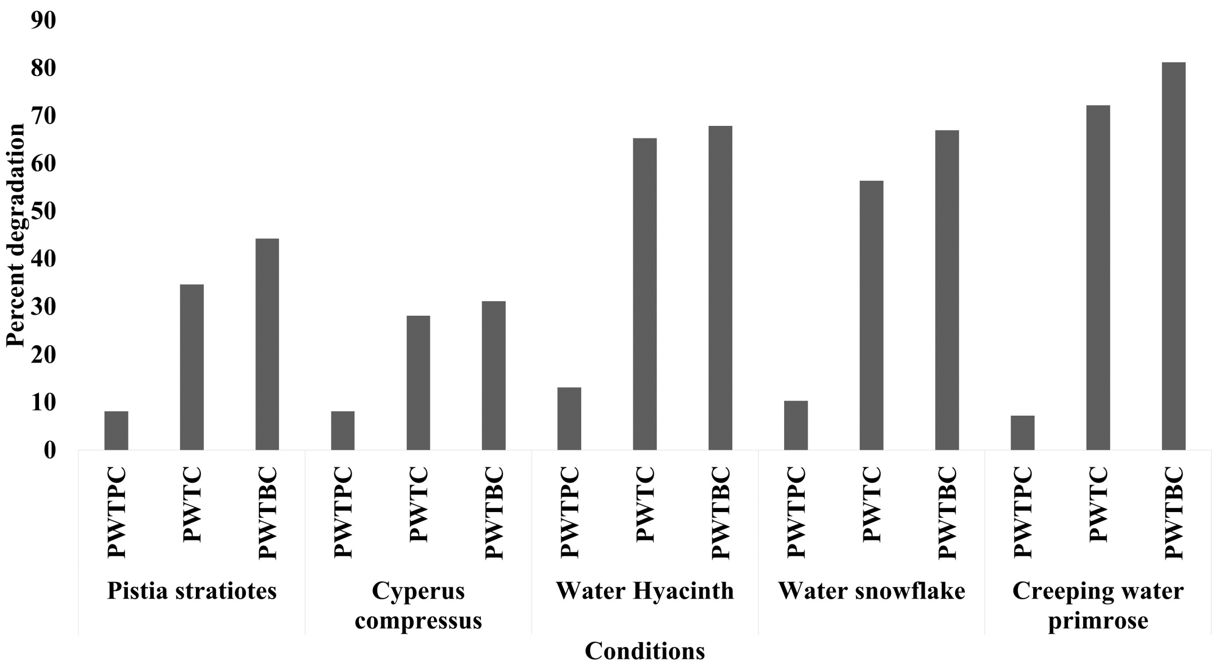


Figure 9: Percent degradation of PW iin various treatments.

Across all plant species, the PWTBC treatment exhibited the highest percent degradation, highlighting the synergistic effect of plant–microbe–biosurfactant interaction. *Creeping Water Primrose* showed the maximum degradation (~80%) under PWTBC, followed by *Water*

Snowflake and *Water Hyacinth*. In contrast, *Pistia stratiotes* and *Cyperus compressus* displayed relatively lower degradation efficiencies, with the least observed under PWTPC (plant + produced water only). Overall, a consistent enhancement in degradation was noted across all species from PWTPC to PWTBC, emphasizing the crucial role of microbial consortium and biosurfactant in boosting hydrocarbon degradation.

E. crassipes (Water Hyacinth) identified as the most effective phytoremediator; significant hydrocarbon reduction observed.

5. Community Engagement and Awareness

Surveys and awareness programs were conducted in two major oil-affected districts—Cachar and Sibsagar—as part of phase I outreach. Farmers and villagers were sensitized about the environmental and health hazards of PW contamination. Feedback was gathered to assess socio-economic impacts and local knowledge.

Photoplate 4: Community engagement and awareness under the project.

Photoplate 5: Awareness programme with various farmers and effected villagers.

Output: 50 individuals reached in phase I; workshops initiated for further stakeholder engagement.

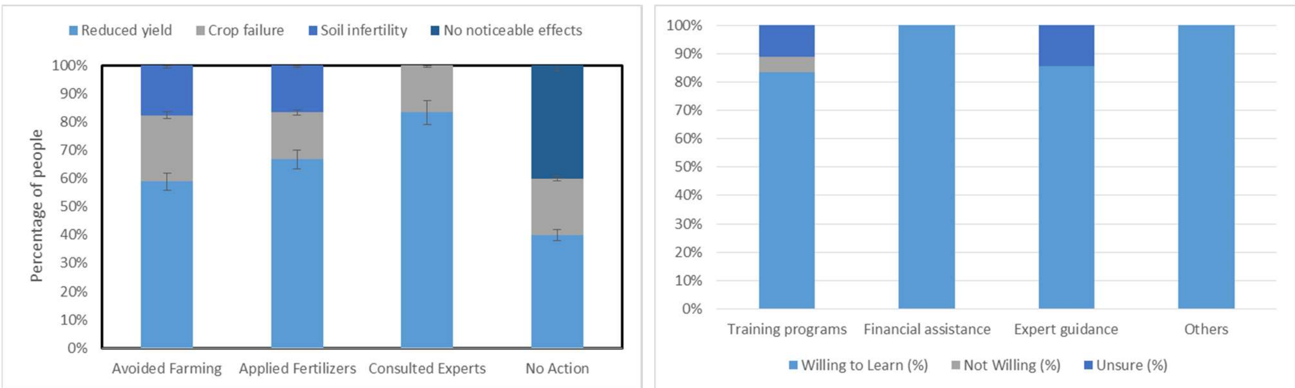
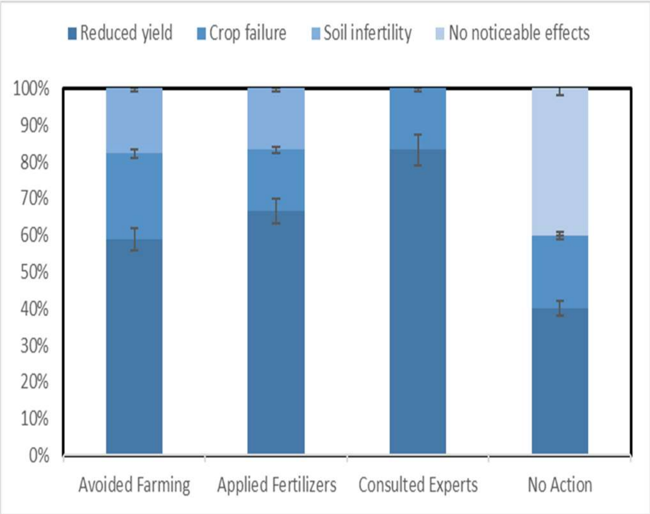


Figure 10: (a) Contamination Effects on Crops vs. Steps Taken to Address It; (b) Support Needed for Adoption vs. Willingness to Learn About Rhizoremediation



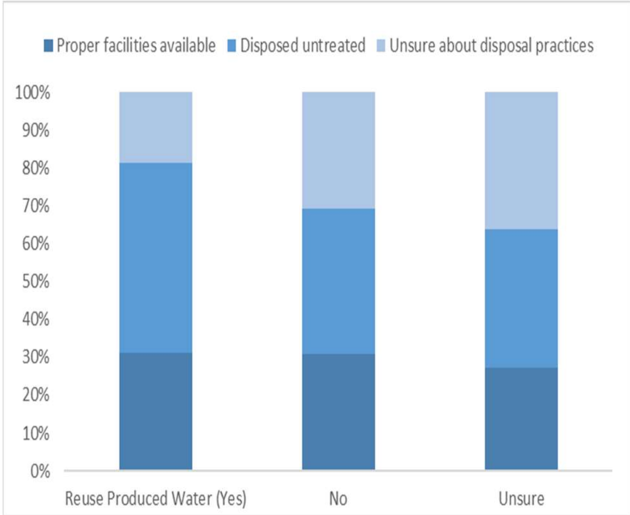


Figure 11: (a) Contamination Effects on Crops vs. Steps Taken to Address It; (b) Reuse of Produced Water vs. Awareness of Disposal Methods

Field surveys involving 40 farmers revealed widespread awareness of soil contamination caused by crude oil spills and the discharge of untreated or chemically treated produced water. Affected soils exhibited deteriorated texture and fertility, resulting in poor plant growth and significantly reduced crop yields. Although farmers attempted to restore soil health using fertilizers and organic amendments (e.g., compost, manure), these efforts yielded limited success. Inadequate support from responsible authorities further exacerbated the situation. Most farmers expressed willingness to adopt sustainable remediation strategies such as rhizoremediation, provided they receive appropriate training, expert guidance, and institutional support.

5. Conclusion:

The first-year outcomes of the project exhibit a scientifically foundation for the sustainable remediation of produced water (PW)-impacted environments in Assam. Site investigations across five oil-contaminated districts revealed significant hydrocarbon pollution, with PW exhibiting markedly higher contaminant concentrations than associated soils. From these challenging matrices, 16 bacterial isolates—10 indigenous and 6 laboratory-sourced—were screened, all of which displayed measurable degradation potential. Notably, isolates PS22, PS53, SSL1, and SSL9 exhibited high enzymatic activities and hydrocarbon degradation efficiencies, with a bacterial consortium outperforming individual strains, achieving ~78% PW degradation.

Enzyme assays confirmed time- and concentration-dependent activation of catechol 1,2- and 2,3-dioxygenases, highlighting the catabolic potential of selected strains even under saline and toxic conditions. Mesocosm trials using floating macrophytes Water Hyacinth as promising phytoremediator when coupled with the consortium and biosurfactant, achieving up to 80% hydrocarbon removal under PWTBC treatment. These findings highlight the synergistic advantage of integrated plant–microbe–biosurfactant systems for PW detoxification.

Community engagement activities confirmed local awareness of PW-induced ecological degradation and revealed strong farmer willingness to adopt rhizoremediation approaches, given adequate institutional support and capacity-building. Collectively, this year’s findings validate the feasibility of bioaugmentation-assisted rhizoremediation for PW-polluted environments and provide critical inputs for scaling up site-specific phytoremediation strategies in subsequent project phases