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Harnessing native flora and rhizobacteria in floating wetlands for sustainable water decontamination

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ABSTRACT:. Water pollution poses significant threats to ecosystems and human health. Sustainable, eco-friendly technologies for treating contaminated water using phytoremediation offer a promising solution to detoxify the polluted water. This study uses floating treatment wetlands (FTWs), a cost-effective phytoremediation technology, to treat contaminated water by employing indigenous plants and their associated plant growth-promoting rhizobacteria (PGPR). Plant samples were collected from the banks of a water channel in the sub-tropical region of Karima, Attock, Pakistan (33°39'04.2"N, 72°42'14.6"E). The plant species selected for this study were Cocklebur (*Xanthium strumarium*), Vetiver grass (*Chrysopogon zizanioides*), Parthenium (*Parthenium hystophorous*), Bermuda grass (*Cynodon dactylon*), and Hemp (*Cannabis sativa*), because of their potential for heavy metal uptake and tolerance. PGPR isolates associated with these plants underwent biochemical characterization, yielding positive results and further validated through nucleotide homology to ensure the selection of the most effective strains. The FTWs were seeded with Vetiver grass and Bermuda grass, both known for their robustness and high phytoremediation capacity. Physicochemical analyses of the contaminated water revealed significant reductions in heavy metal concentrations, including cadmium reduced (to 0.0000 mg/L), nickel (to 0.0016 mg/L), and chromium (to 0.0117 mg/L. These results underscore the effectiveness of the FTW system in improving water quality. Present work demonstrates that the integration of Vetiver grass (*Chrysopogon zizanioides*) and Bermuda grass (*Cynodon dactylon*) with their symbiotic bacteria, *Stenotrophomonas maltophilia*, in FTWs, is a viable and sustainable method for the remediation of heavy metal-contaminated water, offering a practical solution for environmental restoration and pollution control.

KEYWORDS: Contamination, Floating treatment wetland, Indigenous plants, Plant growth promoting rhizobacteria, Phytoremediation.

INTRODUCTION

Water contamination has become a global issue due to the unchecked release of hazardous heavy metals into the environment. The uncontrolled increase in the discharge of toxic heavy metals like chromium into the soil and water is primarily caused by untreated industrial waste [1]. Vegetation and aquatic food chains in running water bodies near municipal and industrial wastes are affected by heavy metal contamination. Heavy metal deposition is the leading cause of water contamination, harms soil and water flora, and negatively impacts human health [2]. Traditional degradation methods, such as thermal treatment, excavation and landfill, electro-reclamation, and acid leaching, were time and cost-demanding, resulting in the discharge of harmful chemicals [3]. Phytoremediation is a procedure that uses plants and their related microorganisms to clean up contaminants [4]. All the five selected plant species, i.e., Bermuda grass, Vetiver grass, Parthenium, Hemp, and Cocklebur, are efficient in removing contamination from the soil and water [5]. When applied to floating wetlands, this method can be incredibly efficient in treating heavy metal-contaminated water. Plant roots produce organic compounds that attract and feed microorganisms in the rhizosphere [6].

In nature, microorganisms that exist in the soil can degrade harmful metals through phytoremediation. Rhizobacteria prevent contaminated soil from becoming fertile and promote plant growth by secreting unique plant growth hormones [7]. Plant growth-promoting rhizobacteria (PGPR) release phytohormones, essential molecules in metal uptake. The in-situ and environment-friendly method of bioremediation is cost-effective and efficient. Advanced techniques such as genetic engineering have been introduced to increase the spectrum of rhizobacteria and their degrading capacity [8]. The varied microbial population in the rhizosphere frequently participates in intricate interactions with floating wetlands plants, such as symbiosis and mutualism [9]. These interactions can boost plant

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development and increase the overall performance of the heavy metal removal phytoremediation process.

Many developing countries have inadequate or non-existent wastewater treatment systems. Floating treatment wetlands (FTWs) are a low-cost phytoremediation method. The buoyancy of the FTWs is caused by air-filled rhizomes and the capture of gas bubbles by the intertwined roots, which can be further supported by rafts [10]. In FTW, dense plant roots grow underneath the suspended mat and form slimy biofilms that work as physical filters for particulate pollutant removal as biosorbents for dissolved metals. In addition to the floating wetlands approach that provides buoyant to a variety of wetland plants, various other bioremediation strategies, including sedimentation basins and water treatment systems to explore the natural ability of bioremediation of plants and associated bacteria [11], are used to improve water quality and ecosystem health in ponds, lakes, rivers, and stormwater retention basins. As a result, it is indispensable to encourage the formation of bacterial communities with plant-growth-promoting ability, nitrification potential, and phosphorus solubilization. In areas with inadequate wastewater treatment infrastructure, FTWs represent a low-cost alternative. The buoyant nature of FTWs, supported by rafts and plant roots, allows for effective nutrient uptake and pollutant removal. The roots of plants in FTWs form biofilms that act as filters for specific pollutants and biosorbents for dissolved heavy metals. Several bioremediation techniques, such as sedimentation basins and constructed wetlands, can further enhance the effectiveness of FTWs by supporting microbial growth that aids in nutrient cycling and pollutant degradation [11].

In this work, the potential of indigenous plants and related bacteria as a filter system for the cost-effective removal of heavy metals from contaminated water is studied using floating wetlands as an efficient ecosystem facilitator of bioaccumulation sources of nutrients for plant roots.

MATERIALS AND METHODS

Sample Collection

Water and plant root samples were collected from a sub-tropical region of Village Karima, in District Attock in the Punjab Province of Pakistan (33°39'04.2"N, 72°42'14.6"E) (Figure 1). The selected plant species were Hemp, Cocklebur, Parthenium, Bermuda grass, and Vetiver grass (Figure 2).

Figure 1. The site of indigenous plants and water sample collection is the village of Karima, Attock.

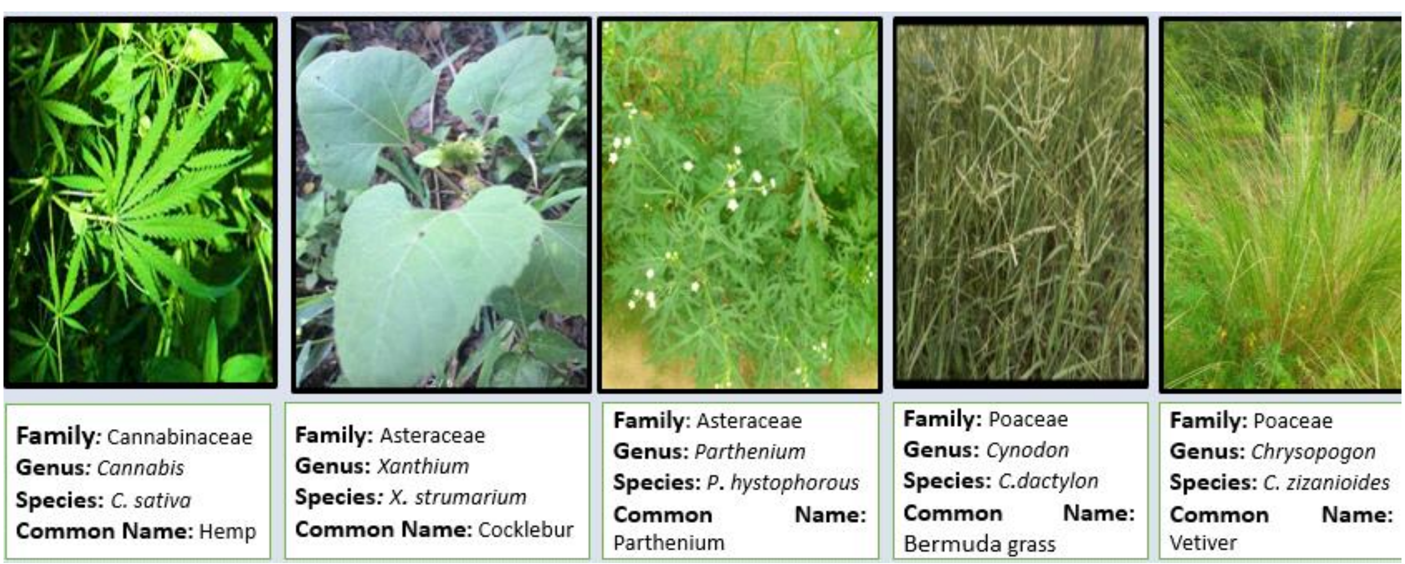

Figure 2. Indigenous plants: (i) Hemp, (ii) Cocklebur, (iii) Parthenium,(iv) Bermuda grass, (v) Vetiver grass. Selected samples were collected along with their roots.

Biochemical characterization

The bacterial isolates identified as *Bacillus altidunis* (CY; Cocklebur), *Acinetobacter schindleri* (HK. Y; Hemp), *Bacillus cereus* (PW; Parthenium), *Bacillus cereus* (PY; Parthenium), *Stenotrophomonas maltophilia* (GY; Vetiver grass), *Stenotrophomonas maltophilia* (GW; Bermuda grass) were isolated. *In vitro* biochemical processes were carried out to investigate the properties of rhizospheric bacteria. Gram-staining [12], catalase [13], amylase activity [14], phosphorous and zinc solubilization [15], and indole-3-acetic acid (IAA) production analysis [16] were performed.

DNA extraction and PCR amplification of 16S rRNA

A high-quality genomic DNA was extracted using the Phenol-Chloroform method [17] and electrophoresed on 1% agarose gel to ensure an enormous quantity of DNA and to confirm the quality by visualization on Nanodrop (Colibri-e-2013-10, Germany). The 16S rDNA gene sequence was amplified using colony PCR, and the PCR reaction mixture (25µl) using template DNA of plant growth-promoting rhizobacteria (Figure 3). The PCR reaction mixture consisted of a DNA template: 3 µl, Forward primer (27F): 1.5 µl, Reverse primer (1492R): 1.5µl, PCR water: 6.5 µl, DreamTaq Green PCR master mix (2X): 12.5µl. Universal primer sequences used are 27F (-AGAGTTTGATCMTGGCTCAG-) and 1492R (-GGTTACCTTGTTACGACTT-) [18].

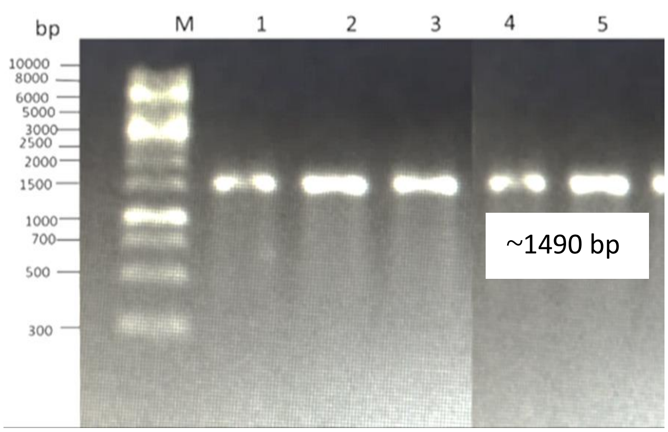

Figure 3. Amplicons of ~1490 bp conserved 16S rDNA gene sequence of plant growth-promoting rhizobacteria visualized on 1% agarose gel electrophoresis. **M:** DNA Marker**, 1:** *Stenotrophomonas maltophilia* (Vetiver grass) **2:** *Bacillus cereus* (Parthenium), **3**: *Bacillus altidunis* (Cocklebur), **4:** *Acinetobacter schindleri* (Hemp), **5:** *Stenotrophomonas maltophilia* (Bermuda grass).

Rapid molecular techniques, such as 16S rRNA gene sequencing, have been used to assess phylogenetic relatedness in bacterial communities. This work used and identified six genomic DNA samples from isolated bacteria. The sequences obtained were aligned using CLUSTALW, available in MEGA 11 [19] were used for generating phylogenetic trees based on 16S rRNA gene sequence using the Neighbour-Joining tree. Value 1000 bootstrap trees were used to estimate the 16S rRNA tree. The sequences of the rhizospheric bacteria were deposited in the National Center for Biotechnology Information (NCBI), as shown in (Table 1).

This analysis involved 11 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were 1521 positions in the final dataset (Figure 4).

Table 1. Description of GeneBank Submission

Isolate	GeneBank Strain Similarity	Accession number
PY	*Bacillus cereus*	OQ121827
GY	*Stenotrophomonas maltophilia*	OQ122145
GW	*Stenotrophomonas maltophilia*	OQ123493
PW	*Bacillus cereus*	OQ123796
HK. Y	*Acinetobacter schindleri*	OQ124198
CY	*Bacillus altidunis*	OQ127265

[1]The GeneBank similarity percentages are based on sequence alignment with the NCBI database.

Pot Experiment

Parthenium, Cocklebur, Bermuda grass, Vetiver grass, and Hemp were cultivated in containers with polluted water to evaluate plant growth, i.e., whether or not the plant would grow in the contaminated water. The plants were evaluated, and their growth was monitored for two weeks, as shown in (Figure 5). Bermuda and Vetiver grass were selected for wetland studies based on their ability to grow in contaminated water.

Floating treatment wetland experiment

A floating treatment wetland with dimensions 2″x 2″ was constructed to evaluate the potential of the selected plants and microorganisms to remove contaminants from runoff water. The raft was constructed with PVC pipes and steel net. Plastic wires were used to secure the frame to fix the selected plants. Five rafts of Vetiver and Bermuda grass, each with nine plants, were employed in the *in-situ* application of wetlands (Figure 6). The estimated cost of rafts was approximately 50 -100 $ for establishing a pilot scale FTW (Dimensions"20 × 100"), including PVC pipes and steel mesh.

Quantitative physicochemical analysis

Pollutants in the water bodies were analyzed quantitatively, and parameters, i.e., pH, electrical conductivity, temperature, total dissolved solids, dissolved oxygen, turbidity, and chemical oxygen demand, were checked.

Atomic absorption

Heavy metal detection in water samples was detected using an atomic absorption spectrometer (Model: AAS 700, Perkin Elmer USA).

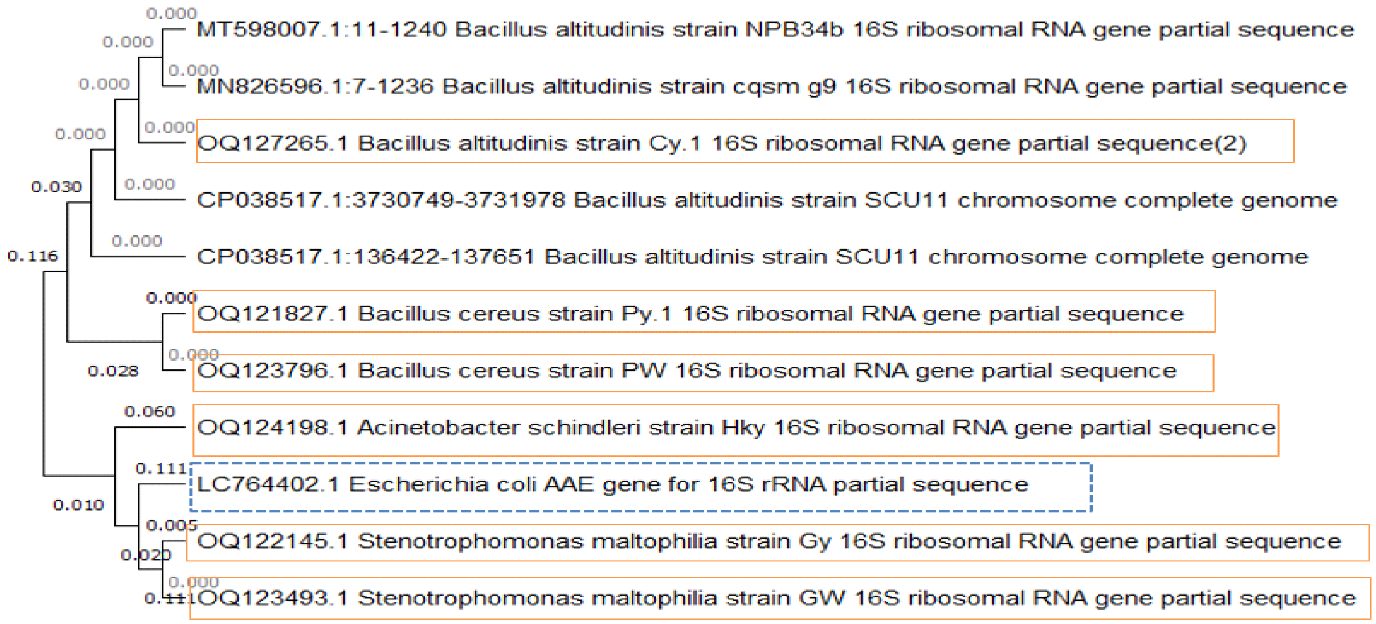

Figure 4. A cladogram showing all the six bacterial strains of *Bacillus cereus* (PY), *Stenotrophomonas maltophilia* (GY), *Stenotrophomonas maltophilia* (GW), *Bacillus cereus* (PW), *Acinetobacter schindleri* (HKY), *Bacillus altidunis* (CY) with *Escherichia coli* as an outgroup.

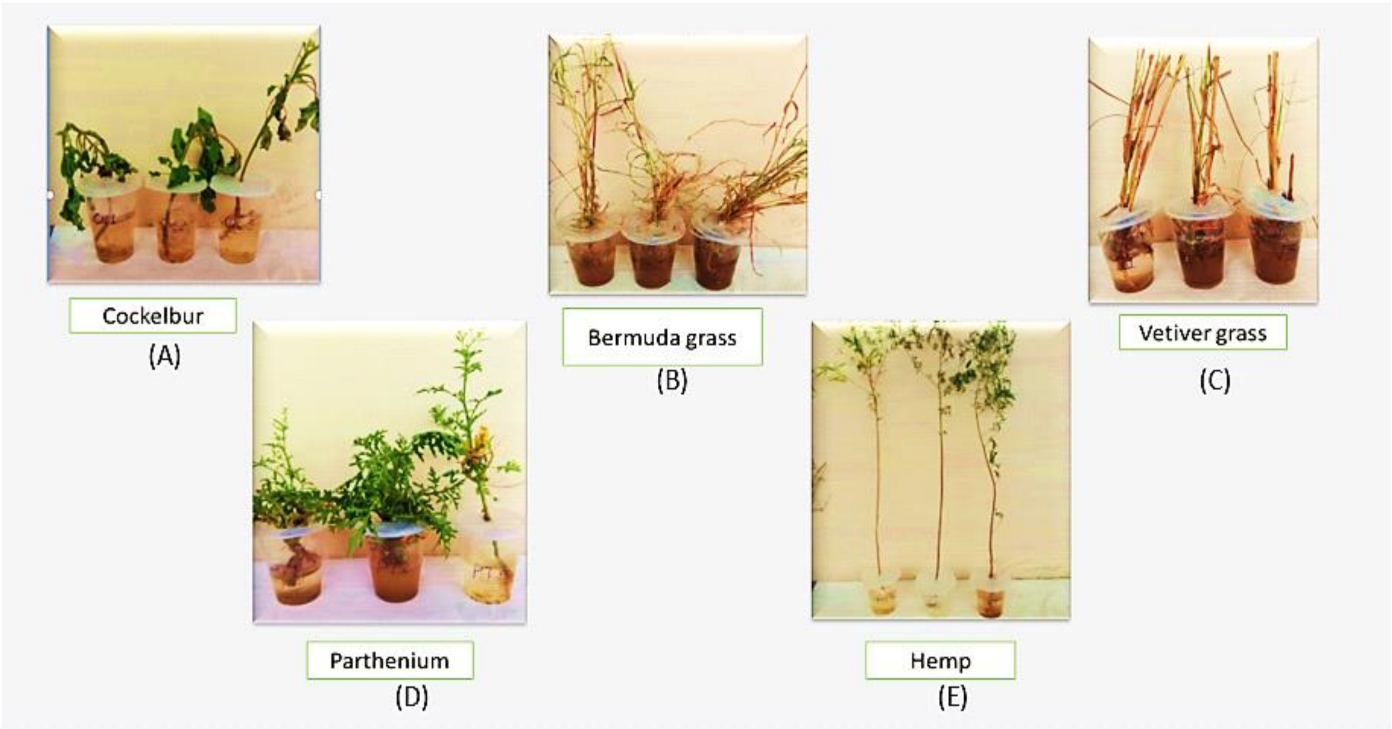

Figure 5. A pot experiment was conducted using five selected plants grown in contaminated water for two weeks: **(A)** Cocklebur, **(B)** Bermuda grass, **(C)** Vetiver grass, **(D)** Parthenium, and **(E)** Hemp.

Figure 6. *In-situ* application of Vetiver grass and Bermuda grass using a floating treatment wetland in contaminated water for 30 days.

RESULTS AND DISCUSSION

A cost-effective phytoremediation method for treating different kinds of wastewater is floating treatment wetlands (FTWs). The intertwined roots and air-filled rhizomes, which rafts can further support, capture gas bubbles, and give the FTWs their buoyancy. The full-scale system treating wastewater was assessed over a three-year study period. The pond stabilization received 60% sewage and 40% industrial wastewater from Faisalabad, Pakistan. Compared to the influent, the effluent's heavy metal concentrations decreased, and all measured water quality indices demonstrated a significant improvement by the FTWs, 79% of which was the system's maximum removal capacity [10].

Due to the treatment efficiency, FTWs have shown promising results in various parts of the world. Their generalizability in Pakistan's geographical area depends on several factors. With careful consideration of environmental, geographical, social, and economic factors, FTWs can be adapted and implemented in various regions of Pakistan. The conventional activated sludge process is energy-intensive, accounting for 50-60% of the total energy consumption in wastewater treatment plants [28]. The energy consumption of conventional activated sludge process is around 0.5-1.5 kWh/m³ [29].

The results indicate the presence of bacterial diversity of *Bacillus cereus, Bacillus altidunis, Stenotrophomonas maltophilia, Acinetobacter schindleri* as rhizobacteria, and biosorbents available in the soil. They have excellent potential for growth-promoting, zinc mobilization, nitrogen fixation, phosphate solubilization, and IAA production, as shown in (Table 2). *Stenotrophomonas maltophilia* was demonstrated as the most efficient nitrogen fixer.

Table 2. *Summary of standard biochemical tests*

Sr. No	Plants	Gram-staining	IAA Assay	Catalase test	Amylase test	Nitrogen fixation	Phosphorous solubilization	Zinc mobilization	Identified strain
1.	Parthenium	+	+	+	+	-	+	-	*Bacillus cereus*
2	Cocklebur	+	+	+	-	-	+	-	*Bacillus altidunis*
3.	Hemp	-	+	+	-	-	+	+	*Acinetobacter schindleri*
4.	Vetiver	-	+	+	+	+	+	-	*Stenotrophomonas maltophilia*
5.	Bermuda	-	+	+	+	-	+	-	*Stenotrophomonas maltophilia*

[2]**a**. IAA: Indole-3-Acetic Acid **b.** +: Positive result **c.** -: Negative result **d.** Gram's staining indicates the Gram reaction of the bacteria (+: Gram-positive, -: Gram-negative). **e.** IAA Assay tests for the production of Indole-3-Acetic Acid. **f.** Catalase test for the presence of the catalase enzyme. **g**. Amylase test determines the presence of the amylase enzyme. **h.** Nitrogen fixation evaluates the ability to fix atmospheric nitrogen. **i.** Phosphorus solubilization assesses the ability to solubilize phosphorus. **j.** Zinc mobilization tests the ability to mobilize zinc.

Plant roots and floating wetlands provide a large surface area for microorganisms to develop biofilms for nutrient intake and degradation in this cooperation between plants and bacterial communities [20]. *Acinetobacter schindleri* had a significantly high zinc mobilizing property, as shown in (Figure 7). Moreover, two bacterial strains, i.e., *Stenotrophomonas maltophilia* and *Bacillus cereus*, were phosphorous solubilizers [21]. Biocontrol activity was observed in three bacterial strains, *Bacillus cereus, Stenotrophomonas maltophilia, and Bacillus altidunis*, against fungus. They protected themselves and their surroundings from fungi.

Figure 7. *Acinetobacter schindleri* (Hemp) showing clear zone formation for zinc mobilization**.**

Traditional water purification methods require considerable energy and chemicals. In contrast, FTWs rely on natural processes and are energy-efficient. They have the advantage of being low-maintenance once established, reducing the environmental footprint of water treatment processes. However, they may be slower in removing pollutants than conventional methods, especially in high contamination levels. Despite this, FTWs are highly sustainable and can be scaled up in urban and rural settings.

The rhizobacteria associated with the plants in this study demonstrated excellent potential for metal mobilization, nitrogen fixation, and phosphate solubilization. *Stenotrophomonas maltophilia* was the most efficient nitrogen fixer, while *Acinetobacter schindleri* exhibited remarkable zinc mobilization capabilities.

The microbial biofilm formation in the rhizosphere, particularly on the roots of Vetiver and Bermuda grass, was crucial for heavy metal uptake and degradation [20].

In addition to their role in metal solubilization, these bacteria also helped promote plant growth by producing plant growth hormones like indole-3-acetic acid (IAA) [21]. The plant-bacterial synergism significantly enhanced the overall efficiency of the phytoremediation process.

The atomic absorption analysis of the water samples showed a significant reduction in heavy metal concentrations over the 30-day experiment period. Cd, Cr, and Ni levels were drastically reduced, especially in the FTW systems with Vetiver and Bermuda grass (Table 3), which confirms the effectiveness of FTWs in removing toxic metals from contaminated water.

Vetiver and Bermuda grass growers would be used for the FTW applications to eliminate the contaminated water on a small scale. Studies reported that the Vetiver grass, Umbrella palm, and the bacteria associated, i.e., *Stenotrophomonas maltophilia*, effectively eliminated pollutants from the water, especially Cd, Ni, and Cr, causing detrimental environmental effects [22, 23].

It is critical to evaluate the selection of appropriate wetland plant species when constructing floating wetland-based phytoremediation systems for heavy metal removal, considering their metal-accumulating abilities and compatibility with the given water body. The results of atomic absorption and several physicochemical analyses of water are mentioned in (Table 3).

The majority of the identified plant species are well-known for their phytoremediation abilities. They grow well and help eliminate pollution from both water and soil. In reported studies, the selected plants were Hemp, Cocklebur, Parthenium, Bermuda grass, and Vetiver grass efficiently eliminated pollutants using phytoremediation [24]. Heavy metals can be hyperaccumulated in the tissues of some plant species without causing harm. Metal-resistant bacteria are usually present in the rhizospheres of these hyper-accumulating plants [25]. These bacteria can mobilize and solubilize heavy metals, making them more readily uptake by hyperaccumulator plants [26].

Furthermore, enhancing microbial diversity in the rhizosphere through accurate management practices may increase heavy metal removal effectiveness and contribute to the overall ecosystem health of the water body. Regular monitoring and adaptive management are essential to ensure the long-term efficacy of floating wetland phytoremediation projects.

Table 3. Atomic absorption and physicochemical analyses of four water samples collected at ten-day intervals.

Sample No.	Days	Ph	T°C	E.C. (µs/cm)	TDS (ppm)	DO (mg/l)	Turbidity (NTU)	COD (mg /l)	Atomic Absorption (ppm)		
									Cd	Cr	Ni
1	0	5.27	24.3	710	198	5.89	2.22	322	0.0070	0.1540	0.1359
2	10	5.96	24.5	690	184	4.69	2.01	184	0.0006	0.1270	0.0430
3	20	6.76	24.9	674	176	4.26	1.98	143	0.0000	0.0760	0.0420
4	30	7.26	25.2	662	165	3.34	1.87	98	0.0000	0.0117	0.0016

[3]**a.** T□C: Temperature in degrees Celsius **b.** E.C.: Electrical Conductivity **c.** TDS: Total Dissolved Solids **d.** DO: Dissolved Oxygen **e.** Turbidity is measured in Nephelometric Turbidity Units (NTU) **f.** COD: Chemical Oxygen Demand **g.** Atomic absorption values are given in parts per million (ppm) for Cadmium (Cd), Chromium (Cr), and Nickel (Ni).

CONCLUSION

In conclusion, in designing a floating treatment wetland, it is critical to consider its long-term maintenance, the selection of plants that can live and develop in various climates, and microbial diversity as biosorbents. Hence, Vetiver grass and Bermuda grass and their associated bacteria, i.e., *Stenotrophomonas maltophilia,* proved efficient for the FTW in removing contaminants from the polluted water. Using large-scale FTWs could help remove contaminants from water and make water fit for drinking and irrigation purposes. The cost-effective analysis suggests that FTWs are a viable alternative to conventional, costly water treatment methods. The broader implications of this study support the use of FTWs for large-scale water treatment, especially in regions with limited access to advanced treatment technologies. Future research should focus on optimizing plant species selection, improving microbial interactions, and scaling up FTW applications to address more significant environmental issues.

DECLARATION

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Authorship Contributions

Conceptualization: A.J., S.H., S.N., Design and Supervision: A.J., S.N., Experimentation: A.J. Data Interpretation: A.J., S.N., S.H. Writing and Reviewing of the Manuscript: A.J., S.N.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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