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A Comparative Study of the Treatment Efficiency of Floating and Constructed Wetlands for the Bioremediation of Phenanthrene-Contaminated Water

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Abstract: Employing floating treatment wetlands (FTWs) and constructed wetlands (CWs) is one of the most eco-friendly strategies for the bioremediation of water contaminants. Here, the efficiency of FTWs and CWs was compared for the degradation of phenanthrene-contaminated water for the first time. The FTWs and CWs were established by vegetated *Phragmites australis* in phenanthrene (1000 mg L^{-1})-contaminated water. Both wetlands were augmented with a bacterial consortium of four bacterial strains: *Burkholderia phytofirmans* PsJN, *Pseudomonas anguiliseptica* ITRI53, *Arthrobacter oxydans* ITRH49, and *Achromobacter xylosoxidans* ITSI70. Overall, the wetlands removed 91–93% of the phenanthrene whilst the augmentation of the bacterial strains had a synergistic effect. In comparison, the CWs showed a better treatment efficiency, with a 93% reduction in phenanthrene, a 91.7% reduction in the chemical oxygen demand, an 89% reduction in the biochemical oxygen demand, and a 100% reduction in toxicity. The inoculated bacteria were found growing in the shoots, roots, and water of both wetlands, but were comparatively better adapted to the CWs when compared with the FTWs. Similarly, the plants vegetated in the CWs exhibited better growth than that observed in the FTWs. This study revealed that the FTWs and CWs vegetated with *P. australis* both had promising potential for the cost-effective bioremediation of phenanthrene-contaminated water.

Keywords: wetlands; bacterial consortium; bioremediation; resource recovery; sustainability

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1. Introduction

The petrochemical industry is one of the main sources of hydrocarbon contamination in water. The high level of hydrocarbons in the aquatic environment is mainly due to continuous advancements in the exploration and extraction of oil [1]. The wastewater generated by the petroleum industry contains high contents of one of the classes of hydrocarbons known as polyaromatic hydrocarbons (PAHs) [1–4]. Among the different PAHs, phenanthrene is a common wastewater contaminant [2,5–9]. It comprises three benzene rings and is persistent in the environment due to its high molecular weight, chemical stability, and hydrophobic nature [10,11]. It has been found to be toxic to fish, crustaceans, gastropods, mussels, and marine diatoms [12,13].

Over the years, various conventional methods such as advanced oxidation, active carbon absorption, photocatalysis, electrolytic and electro-irradiated technologies, Fenton-based advanced oxidation processes, ozonation, and pulsed dielectrics have been reported for the handling of phenanthrene-contaminated water [6,14]. However, these conventional technologies based on physicochemical methods are not sustainable due to their higher

operational cost and environmental incursions as well as the requirements for engineering skills, labor management, and operational supervision [15,16]. FTWs and CWs have shown to be a promising approach for the management of contaminated water. An FTW is a hydroponic system that provides a medium to facilitate plant growth [17–19]. CWs are products of environmental engineering skills that are built by adopting and performing processes of natural wetlands through vegetation, soil, and associated microbes in a controlled environment for the purpose of wastewater treatment [9,20]. FTWs are easily handled, imitate a natural habitat, and are a low-cost technique when compared with CWs whereas CWs provide a multi-layer medium for the remediation of contaminated water and a significant physical support for plant growth. In FTWs and CWs, plant-associated microorganisms play an important role in the remediation of water contaminated with organic pollutants [21–23]. In wetlands, vegetation plays an important role in the remediation of contaminated water. Different plant species with ornamental characteristics and an ability to withstand unfavorable and harsh conditions are used. The most commonly used flowering species are *Typha, Canna, Iris, Heliconia*, and *Zantedeschia* [24]. However, the occurrence of organic pollutants such as hydrocarbons inhibits plant growth and ultimately the population and performance of plant-associated indigenous microorganisms. To overcome this issue, the augmentation of microorganisms capable of degrading pollutants, and with plant growth-promoting (PGP) activities, has been employed in FTWs and CWs to improve the phytoremediation process [23,25,26]. Although bacterial-augmented FTWs and CWs can efficiently remove hydrocarbons from the water, their efficiency has never been compared for the remediation of PAH-contaminated water [27,28].

The present study aimed to compare the potential of FTWs and CWs vegetated with *Phragmites australis* and inoculated with a bacterial consortium to remove phenanthrene from water. Reductions in the phenanthrene concentration, COD, BOD, and toxicity level of the water were determined. The bacterial perseverance in the water, roots, and shoots of the plants as well as the growth of the plants vegetated in the FTWs and CWs were assessed.

2. Materials and Methods

2.1. In Vitro Screening of the Bacterial Strains

Nine bacterial species—*Burkholderia phytofirmans* PsJN, *Microbacteriumlacus ITRH47*, *Pseudomonas anguiliseptica* ITRI53, *Arthrobacter oxydans* ITRH49, *Achromobacter xylosoxidans* ITSI70, *Pseudomonas* sp. MixRI75, *Pantoea agglomerans* BTRH79, *Pseudomonas* sp. ITRI73, and *Pseudomonas cuatrocienegasensis* ITRH 76—were initially tested for their ability to degrade phenanthrene in shake-flask experiments. The bacterial strains were isolated from hydrocarbon-contaminated environments and had shown the potential to degrade a wide range of hydrocarbons. Moreover, the selected strains had shown plant growth-promoting (PGP) activities, including inorganic phosphorous solubilization, indole acetic acid production, amino cyclopropane carboxylic (ACC) deamination, and siderophore synthesis. Each bacterial strain was grown in Luria-Bertani (LB) broth at 30 °C in a shaking incubator. The cells were collected by centrifugation at 10,000 rpm for 5 min. The phenanthrene degradation activity of the strains was evaluated by inoculating 10 mL (10^9 cells/mL) of each bacterial strain suspension in 100 mL of M9 minimal media amended with phenanthrene (400 mg/L). The flasks were incubated in the shaking incubator for three weeks at 30 °C and 110 rpm. Among the nine bacterial strains, four bacterial strains—namely, *B. phytofirmans* PsJN, *P. anguiliseptica* ITRI53, *A. oxydans* ITRH49, and *A. xylosoxidans* ITSI70—were shown to degrade phenanthrene (data not shown). These strains were later used to augment the FTWs and CWs.

2.2. Development of the FTW Setup

A sheet of polyethylene (Jumbolon-Roll) obtained from the Diamond Foam Company (a Pvt. Ltd. Company, Faisalabad, Pakistan) was used for the preparation of hydroponic mats (Figure 1). The sheet was drilled in the center for the plantation. Five healthy seedlings of *P. australis* with an even size and weight were placed in each hole. Coconut shavings

and soil were used to support the seedlings. Each vegetated mat was placed in a plastic pot with 5 L water and aided with 500 mg diammonium phosphate (DAP) to accomplish the growth requirement of the plants. The seedlings were permitted to grow. After a period of 2 months, the plants and pots were surface-sterilized using a 5% (*v*/*v*) sodium hypochloride solution and then thoroughly washed with sterilized water. The pots were filled with water spiked with phenanthrene (1000 mg/L, *w*/*v*) and augmented with the bacterial consortium according to the designed treatments. These were: C1*: microcosms containing phenanthrene-contaminated water without vegetation and the bacterial consortium; C2*: microcosms containing water (without phenanthrene) and vegetated with *P. australis*; T1*: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T2*: microcosms containing phenanthrene-contaminated water and the bacterial consortium; and T3*: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium. Each treatment had three replicates.

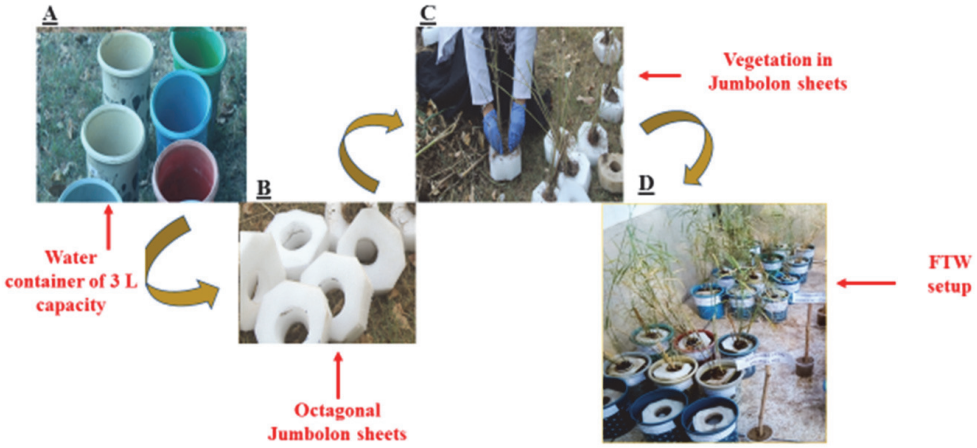

Figure 1. Development of floating treatment wetlands. Where, (**A**) shows the water containers, (**B**) shows octagonal jumbolon sheets, (**C**) shows the vegetation process, and (**D**) shows experimental setup.

2.3. Development of the CW Setup

Plastic pots containing 5 L water were used to develop the CWs (Figures 2 and 3). In each pot, gravel of different sizes was placed and five healthy seedlings of *P. australis* were vegetated. Each pot was supplemented with 5 g DAP. The seedlings were permitted to grow for a period of 2 months. The mesocosms were then sterilized by adding 5 L of a 2% (*v*/*v*) sodium hypochloride solution to each pot and then thoroughly washed with sterilized water. The water in the pots was then contaminated with phenanthrene (1000 mg/L, *w*/*v*) and augmented with a bacterial consortium according to the designed treatments. These were: C1: microcosms containing phenanthrene-contaminated water without vegetation and the bacterial consortium; C2: microcosms containing water without phenanthrene and vegetated with *P. australis*; T1: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T2: microcosms containing phenanthrene-contaminated water and the bacterial consortium; and T3: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium. All treatments of the FTWs and CWs were performed in triplicate for 60 days from 1 May to 30 June 2022.

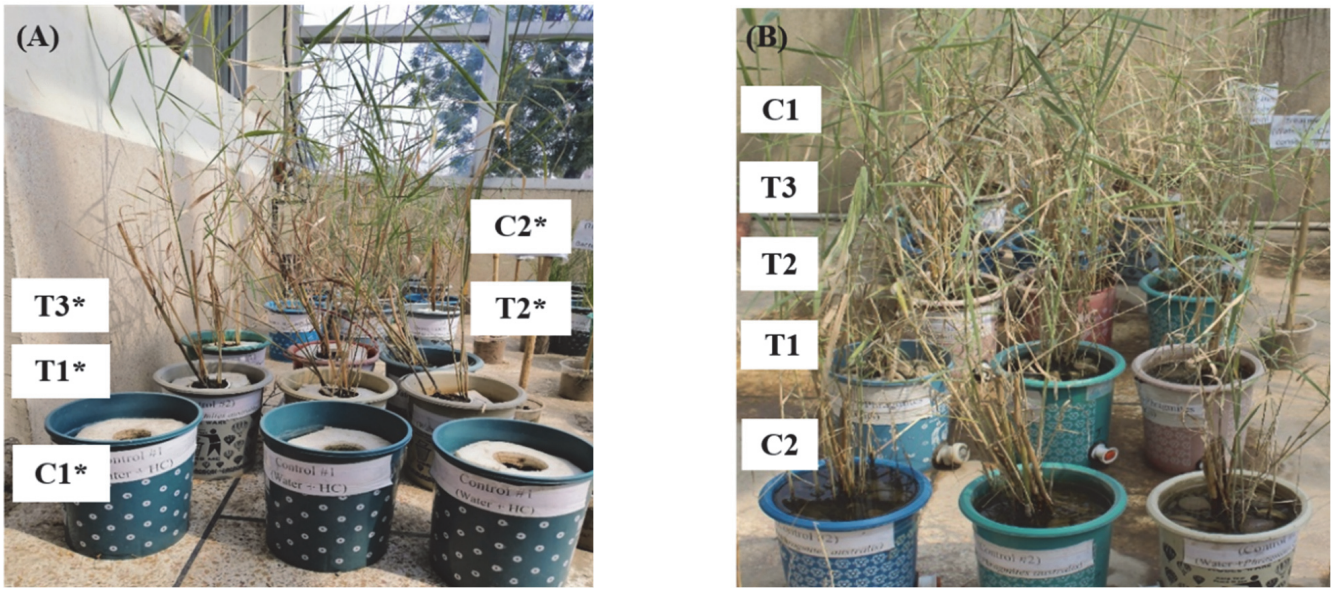

Figure 2. Development of floating treatment wetlands (**A**) and constructed wetlands (**B**) for the remediation of phenanthrene-contaminated water.

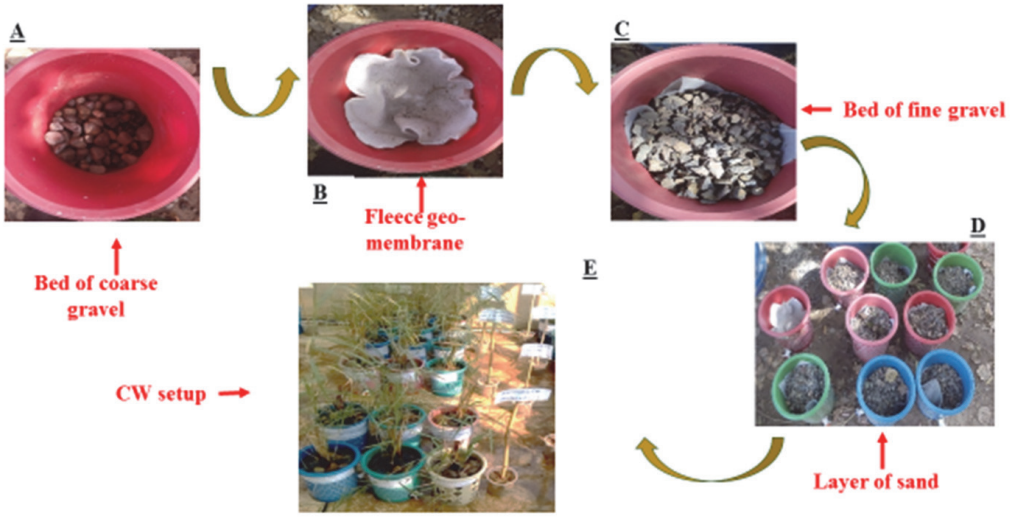

Figure 3. Development of constructed wetlands. Where, (**A**) shows the bed of coarse gravel, (**B**) shows fleece membrane, (**C**) shows bed of fine grave, (**D**) shows layer of sand, and (**E**) shows experimental setup.

2.4. Water Analyses

The water samples from the FTWs and CWs microcosms were collected with a 10 day interval up to 60 days and analyzed for different important physiochemical parameters (including COD, BOD, pH, electrical conductivity (EC), and colony-forming unit (CFU)) as per the methods previously described [29]. For the phenanthrene extraction, 10 mL of the sample was taken in a glass tube with 3 mL of pentane; it was thoroughly mixed using a vortex for 1 min. The sample was transferred into a separating funnel and given 2 min for the separation of the phenanthrene in the pentane layer. The upper pentane layer containing the phenanthrene was collected in a glass vial. The same procedure was repeated 3 times for each sample. This extracted sample was used for the quantitative analysis of the phenanthrene using a PerkinElmer Spectrum 2 analyzer.

2.5. Plant Root/Shoot Length and Biomass

The plant growth parameters, including the root and shoot length with their fresh and dry weights, were recorded upon the completion of the experiment. The root and shoot length were determined using a measuring tape. The fresh weight was measured using a balance and the dry weight was measured after drying the plant tissues in an oven at 60 °C for 3 days.

2.6. Determination and Persistence of the Inoculated Bacteria

Persistent in the water, rhizosphere, and endosphere of the plant, the inoculated bacteria were observed using the plate count method, as described previously [22]. Concisely, the water samples were coated with an M9 agar medium containing 150 mg/L phenanthrene. The surfaces of the roots and shoots were washed with 70% ethanol, followed by washing with 2% bleach. They were then rinsed 3 times with sterilized distilled water. The surface-purified roots and shoots were ground and homogenized in 10 mL of a NaCl solution (0.9% w/v). The suspension of the roots and shoots was plated on M9 agar plates containing 150 mg L^{-1} phenanthrene. These plates were incubated at 37 °C for 48 h for the colony-forming unit (CFU) counting. With the purpose of confirming the presence of augmented bacterial strains in the isolated colonies, a restriction fragment length polymorphism (RFLP) analysis was carried out.

2.7. Phytotoxicity Test

A phytotoxicity test was executed to evaluate the toxicity of the treated water. Seeds of monocotyledon, wheat (*Triticum aestivum*), dicotyledon, and Gobhisarson (*Brassica napus*) were used to assess the toxicity of the treated and untreated phenanthrene-contaminated water. Before sowing, the seeds were surface-sanitized in a 2% bleach solution followed by manifold rinses with purified distilled water. Petri plates were used to sow the seeds with 3 mL of the distilled water as the control and 3 mL of the treated water. The germination and growth of the seedlings were observed for seven days and the germination (%) was measured using Equation (1):

$$\text{Germination (\%)} = \text{number of germinated seeds} \div \text{total number of seeds sown} \times 100 \quad (1)$$

2.8. Data Analysis

The data analysis was performed using the SPSS software package. A one-way analysis of variance (ANOVA) was used to carry out the comparison among the treatments.

3. Results and Discussion

3.1. Phenanthrene Degradation Potential of the Bacterial Strains

The phenanthrene degradation capability of the already-isolated nine bacterial strains was analyzed under in vitro conditions. All the tested bacterial strains showed an ability to degrade phenanthrene (Figure 4). This was recognized as the fact that these strains might have had specific metabolic genes (alk *B*/cyp153) that were able to degrade hydrocarbons, as reported previously [30]. Among the nine tested strains, *B. phytofirmans* PsJN, *P. anguiliseptica* ITRI53, *A. oxydans* ITRH49, and *A. xylosoxidans* ITSI70 performed much better when compared with the other strains (data not shown).

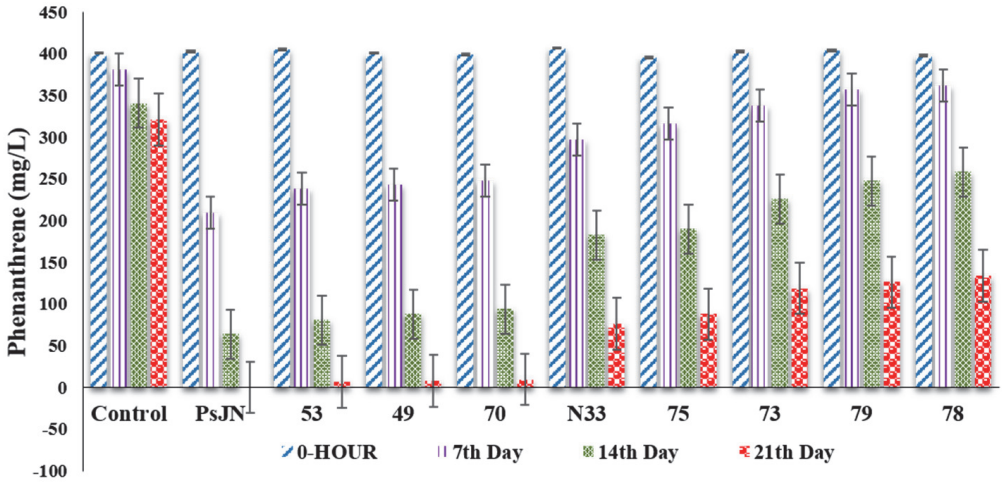

Figure 4. Hydrocarbon (phenanthrene) removal by nine different bacterial strains.

3.2. Phenanthrene Degradation Efficiency of the FTWs and CWs

The comparative efficiency of the FTWs and CWs for the biotreatment of phenanthrene-contaminated water was assessed by analyzing the treated water for different physico-chemical parameters. There was a significantly higher reduction in the COD, BOD, and phenanthrene concentration in the water treated by the FTWs and CWs when compared with the controls (C1 and C1*) (Figures 5–7). There was an 81% reduction in the COD and a 78.5% reduction in the BOD of the water treated by the CWs (T1); there was a 79.7% reduction in the COD and a 76% reduction in the BOD of the water treated by the FTWs (T1*). The treatments that contained only bacterial strains (T2 and T2*) also showed a higher reduction in the water quality parameters (COD and BOD) when compared with the controls (C1 and C1*), but their efficiency was lower than that observed in the water treated by FTWs and CWs augmented with the bacterial consortium. The performance of the CWs was greater (non-significant) than the FTWs in the occurrence and absence of bacterial augmentations. In this study, the decrease in the BOD, COD, and phenanthrene concentration in the water treated by CWs (T1 and T3) was greater (non-significant) than that of the water treated by the FTWs (T1* and T3*). Approximately, a 92% reduction in the COD, an 89% reduction in the BOD, and a 93% reduction in the phenanthrene concentration were observed in the CWs augmented with the bacterial strains (T3); similarly, a 91% reduction in the COD, an 88% reduction in the BOD, and a 92% reduction in the phenanthrene concentration were observed in the FTWs augmented with the bacterial strains (T3*) (Figures 6 and 7). In an earlier study, a 99.9% removal of phenanthrene was observed in water treated by a vertical-flow CW [10]. In another study, a comparatively smaller (85.5%) removal of phenanthrene was observed in CWs than was observed in this study [31]. Similarly, in another study, a comparatively smaller (79.91%) removal of phenanthrene was observed [32] than was obtained in this study. Usually, plants possess natural mechanisms to detoxify pollutants present in their surrounding environment [4,7,20,27,28,33,34]. Plants degrade complex organic compounds by processes known as phytodegradation [3,35–37]. This could be the basis that even the unaided vegetation considerably reduced the phenanthrene concentration and other pollution parameters in the polluted water. *Phragmites australis* allowed the bacterial strains to proliferate in the endo- and rhizosphere, which generally upsurged the plant health and treatment proficiency of the wetland systems [38].

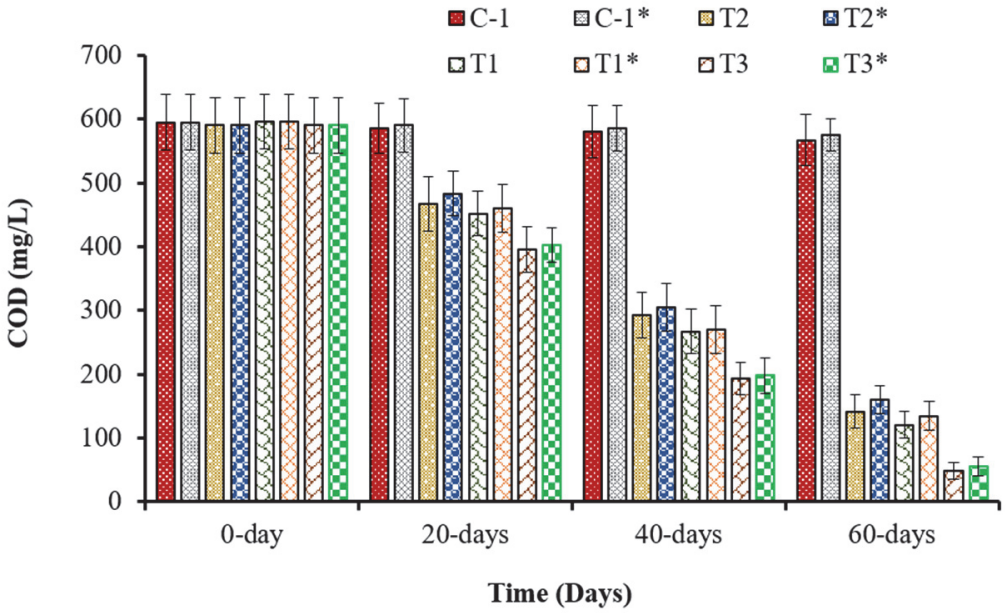

Figure 5. COD reduction in CW and FTW vegetated with *Phragmites australis*.

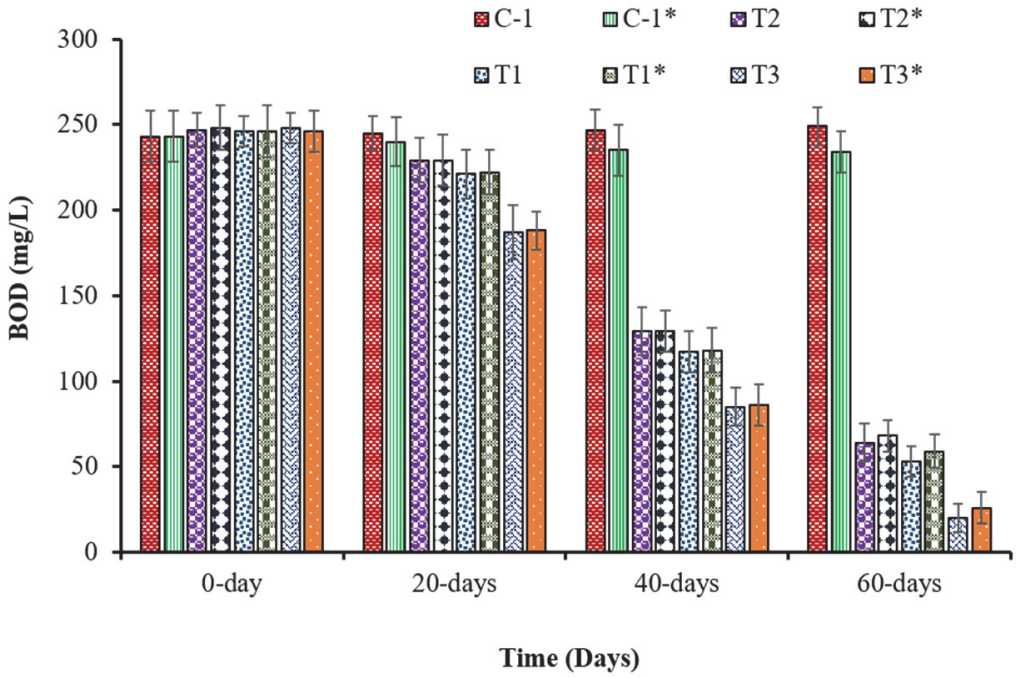

Figure 6. BOD reduction in CWs and FTWs vegetated with *Phragmites australis*.

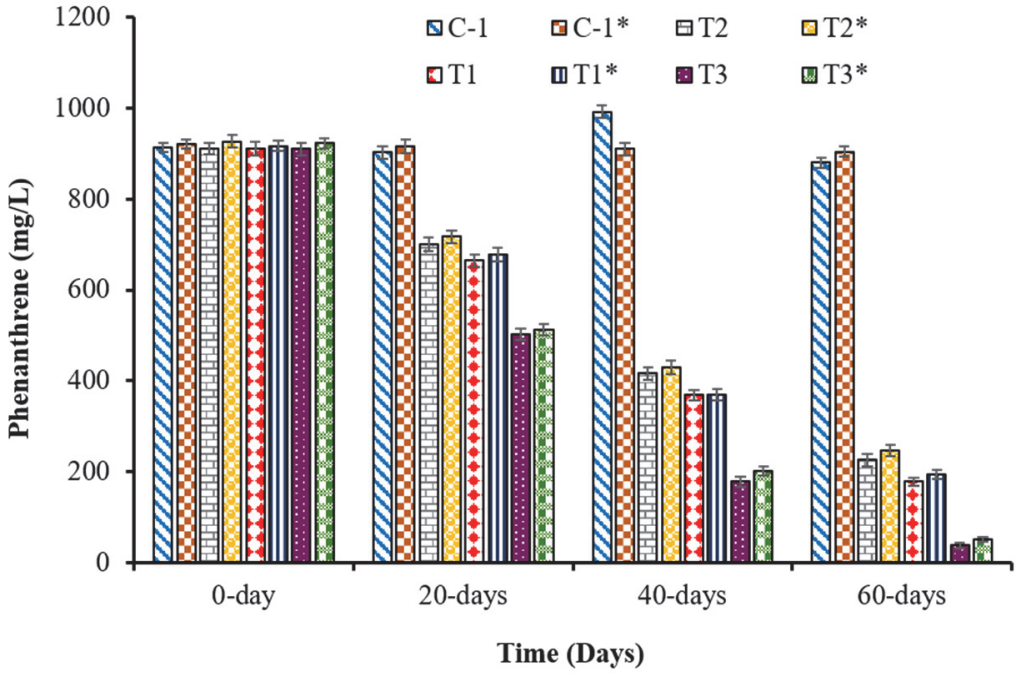

Figure 7. Phenanthrene reduction in CWs and FTWs vegetated with *Phragmites australis*.

In this study, the bacterial species inoculated in the FTWs and CWs possessed the genes responsible for hydrocarbon degradation and PGP activities, which helped the plants to improve their growth in the presence of contaminants [26]. The decrease in the COD and BOD could be associated with enzymatic activities of the bacteria that take part in the breakdown and transformation of organics into simpler substances that are used by the plants as nutrients [39]. Both parameters are vital water quality indicators, and their decline is an indication of the cleaning of contaminated water. Lastly, the gravel and sand in the CWs also acted as a filter and reduced the pollutants from the water through absorption and adsorption [28,40,41], which might also have contributed to the treatment process.

In this study, apart from a decrease in the aforementioned parameters, a similar trend of reduction was found in the other tested parameters; i.e., the pH and EC (Table 1). The decrease in pH showed that the denitrification and nitrification processes occurred well within the treatment system [33]. The pH drop from alkalinity to acidity could be because of the formation of root exudates and the degradation of organic compounds to produce organic acids [22]. The decrease in the EC might be related to the reduction in the total suspended solid (TSS) and total dissolved solid (TDS) profiles as the EC is directly related to the TDS [8]. However, this study revealed that the CWs improved the water quality more efficiently than the FTWs under the same treatment conditions.

Table 1. Comparison of the changes in pH and EC of phenanthrene-contaminated water treated by FTWs and CWs vegetated with *Phragmites australis* and amended with a bacterial consortium.

Time (Days)	C1		C1*		T1		T1*		T2		T2*		T3		T3*	
	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC
0	7.33 ± 1.4	4.78 ± 1.3	7.26 ± 1.4	4.79 ± 1.3	7.36 ± 1.8	4.8 ± 1.1	7.27 ± 1.9	4.8 ± 1.2	7.36 ± 1.9	4.79 ± 1.2	7.26 ± 1.9	4.8 ± 1.1	7.36 ± 1.7	4.79 ± 1.1	7.35 ± 1.8	4.82 ± 1.1
10	7.32 ± 1.9	4.77 ± 1.1	7.27 ± 1.9	4.79 ± 1.1	7.23 ± 2.3	4.75 ± 1.1	7.23 ± 1.7	4.79 ± 1.1	7.21 ± 1.9	4.76 ± 1.22	7.22 ± 1.9	4.79 ± 1.2	7.17 ± 2.1	4.66 ± 1.1	7.18 ± 1.9	4.78 ± 0.9
20	7.32 ± 1.9	4.78 ± 1.1	7.27 ± 1.8	4.78 ± 0.8	7.19 ± 1.6	4.61 ± 0.8	7.14 ± 1.6	4.77 ± 1.1	7.09 ± 1.7	4.63 ± 1.2	7.17 ± 2.1	4.78 ± 1.1	6.87 ± 1.1	4.61 ± 1.2	6.91 ± 2.1	4.56 ± 1.2
30	7.31 ± 1.9	4.77 ± 1.1	7.28 ± 1.7	4.78 ± 1.1	6.95 ± 1.7	4.4 ± 0.6	6.92 ± 1.5	4.74 ± 0.8	6.87 ± 0.9	4.52 ± 1.2	6.77 ± 1.9	4.76 ± 1.1	6.72 ± 1.8	4.42 ± 0.6	6.78 ± 2.1	4.37 ± 0.6
40	7.33 ± 2.1	4.76 ± 1.1	7.27 ± 1.1	4.77 ± 1.1	6.89 ± 2.3	4.36 ± 0.6	6.84 ± 1.8	4.66 ± 1.2	6.7 ± 1.9	4.41 ± 1.1	6.68 ± 1.8	4.7 ± 0.4	6.31 ± 1.8	4.27 ± 0.5	6.51 ± 2.1	4.28 ± 0.5
50	7.34 ± 1.8	4.75 ± 1.1	7.29 ± 1.4	4.75 ± 1.2	6.8 ± 2.1	4.29 ± 1.1	6.81 ± 1.8	4.5 ± 0.8	6.58 ± 1.8	4.34 ± 0.6	6.65 ± 1.8	4.59 ± 1.1	6.09 ± 1.8	3.97 ± 0.5	6.29 ± 2.1	4.17 ± 0.6
60	7.31 ± 1.9	4.74 ± 0.8	7.28 ± 1.1	4.73 ± 0.6	6.68 ± 1.8	4.21 ± 1.1	6.65 ± 1.9	4.23 ± 0.6	6.42 ± 1.7	4.27 ± 1.1	6.56 ± 0.8	4.4 ± 0.5	5.93 ± 2.1	3.69 ± 0.5	6.07 ± 2.1	3.98 ± 1.2

C1: Microcosms containing phenanthrene-contaminated water without vegetation and the bacterial consortium; C2: microcosms containing water without phenanthrene and vegetated with *P. australis*; T1: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T2: microcosms containing phenanthrene-contaminated water and the bacterial consortium; T3: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium; C1*: microcosms containing phenanthrene-contaminated water without vegetation and the bacterial consortium; C2*: microcosms containing water without phenanthrene and vegetated with *P. australis*; T1*: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T2*: microcosms containing phenanthrene-contaminated water and the bacterial consortium; T3*: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium. All treatments of FTWs and CWs were performed in triplicate for 60 days from 1 May to 30 June 2022.

3.3. Persistence of the Augmented Bacterial Strains in the Root and Shoot

Microbes are known for their capability to aid plant survival and growth in polluted environments [3,42] and also help in accelerating the phytoremediation process [43]. In this study, the persistence of the augmented bacterial strains was evaluated in the shoots and roots of *P. australis* vegetated in the CWs and FTWs. The results demonstrated a higher persistence of the microbial strains in the roots than in the shoots, which gradually amplified with the passage of time (Table 2). The greater population in the roots could be attributed to the fact that the inoculated strains were isolated from the rhizospheric and root interiors, advocating their better colonization capability in the root environment compared with the shoots [44]. It could also be due to the fact that there was a less suitable environment for the survival of the bacteria outside the host, possibly due to the toxic nature of the hydrocarbons in the water; the shoot provided a synergistic survival environment for the bacteria, and the bacteria helped the plants to obtain better nutrition from the water by degrading the phenanthrene. The inoculated bacteria exhibited a comparatively weaker survival in the microcosms without vegetation, possibly due to a shortage or limited supply of nutrients and the absence of a symbiotic partner in the phenanthrene-contaminated water, which also endorsed the aforementioned possibility. In this study, a greater number of bacteria was observed in the rhizosphere and endosphere of the *P. australis* vegetated in the CWs than in the FTWs. It was also testified that the plants hosted a variety of microorganisms as they had a broad root system that offered a large surface area for their colonization [19,45]. The metabolites produced by the plants and root exudates determined the fate of the microorganisms, including their ability, density, and diversity in the rhizosphere and endosphere [35,46].

Table 2. Presence of the bacterial strains in the water, roots, and shoots of *Phragmites australis* vegetated in CWs and FTWs.

Treatment	Time (Days)							
	0		20		40		60	
	Colony-Forming Units (10^7 CFU mL^{-1} water)							
	CWs	FTWs	CWs	FTWs	CWs	FTWs	CWs	FTWs
T2/T2*	8.21 (2.78)	8.28 (2.32)	7.62 (2.50)	7.55 (2.58)	5.95 (2.65)	6.12 (2.62)	5.08 (2.28)	5.25 (2.35)
T3/T3*	7.72 (2.52)	8.27 (2.42)	7.53 (2.84)	7.65 (2.56)	7.23 (2.74)	6.57 (2.35)	6.29 (2.35)	5.17 (1.92)
	Colony-Forming Units (10^7 CFU g^{-1} root)							
T2/T2*	–	–	3.68 (0.58)	3.65 (0.57)	4.96 (0.68)	4.93 (0.58)	5.82 (0.64)	5.79 (0.43)
	Colony-Forming Units (10^7 CFU g^{-1} shoot)							
T3/T3*	–	–	2.37 (0.68)	2.34 (0.62)	2.46 (0.37)	2.43 (0.37)	2.54 (0.61)	2.53 (0.54)

T2: Microcosms containing phenanthrene-contaminated water and the bacterial consortium; T3 and T2*: microcosms containing phenanthrene-contaminated water and the bacterial consortium; T3*: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium. All treatments of FTWs and CWs were performed in triplicate for 60 days from 1 May to 30 June 2022.

3.4. Effect of Different Treatments on the Plant Biomass and Growth

In this study, the plants vegetated in the bacterial-augmented wetlands (T3 and T3*) produced fresher and more dried biomass when compared with the plants vegetated in the treatments without a bacterial augmentation (T1 and T1*) (Table 3). This suggested that the bacteria possessed beneficial activities that might have helped the plants to grow well in the presence of contaminants. The plant growth-promoting activities of the microbes enabled the plants to amend their stress and improve their growth [26]. More shoot growth was observed in the CWs when compared with the FTWs; however, more root growth was observed in the FTWs compared with the CWs (Table 4 and Figure 8). More root growth in

the FTWs might have been due to a space factor; in the FTWs, the roots had more space to grow compared with the CWs.

Table 3. Fresh (FW) and dried biomass (DW) of *Phragmites australis* in response to phenanthrene contamination and bacterial augmentation in FTWs and CWs.

	Constructed Wetlands		Floating Treatment Wetlands	
	Fresh Biomass (g)	**Dried Biomass (g)**	**Fresh Biomass (g)**	**Dried Biomass (g)**
C2/C2*	166 (10)	123 (14)	163 (12)	118 (11)
T1/T1*	99 (11)	56 (14)	95 (13)	53 (9)
T3/T3*	158 (9)	117 (14)	155 (11)	113 (10)

C2: Microcosms containing water without phenanthrene and vegetated with *P. australis*; T1: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T3: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium; C2*: microcosms containing water without phenanthrene and vegetated with *P. australis*; T1*: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T3*: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium. All treatments of FTWs and CWs were performed in triplicate for 60 days from 1 May to 30 June 2022.

Table 4. Effect of different treatments on the root length (RL) and shoot length (SL) of *Phragmites australis* vegetated CWs and FTWs.

	CWs		FTWs	
	SL (cm)	**RL (cm)**	**SL (cm)**	**RL (cm)**
C2	70.8 (9.2)	27.6 (4.8)	67.2 (9.5)	30.6 (7.4)
T1/T1*	40.3 (8.7)	15.7 (2.4)	39.5 (8.2)	18.8 (3.7)
T3/T3*	61.5 (10.9)	20.8 (3.4)	57.6 (9.8)	23.2 (5.1)

C2: Microcosms containing water without phenanthrene and vegetated with *P. australis*; T1: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T3: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium; T1*: microcosms containing phenanthrene-contaminated water and vegetated with *P. australis*; T3*: microcosms containing phenanthrene-contaminated water vegetated with *P. australis* and augmented with the bacterial consortium. All treatments of FTWs and CWs were performed in triplicate for 60 days from 1 May to 30 June 2022.

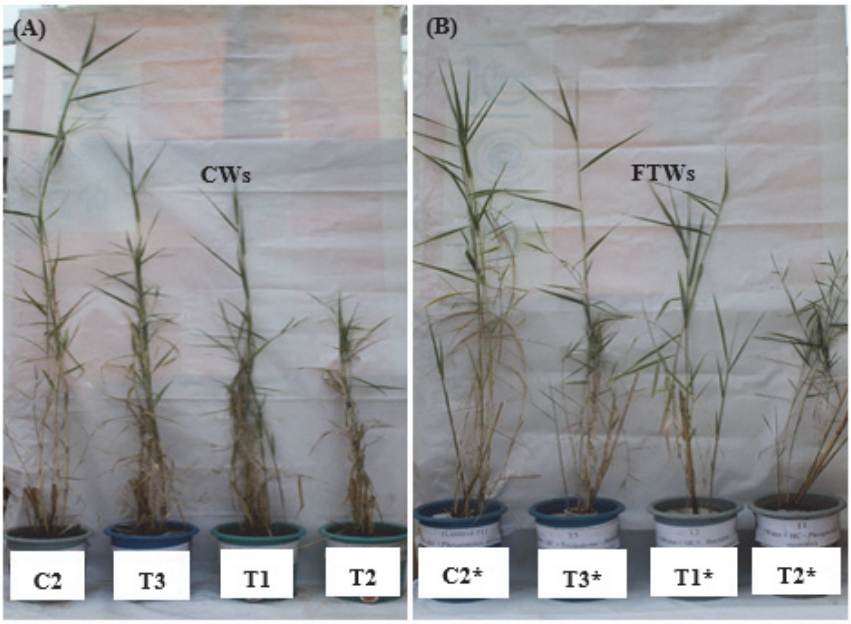

Figure 8. *Cont.*

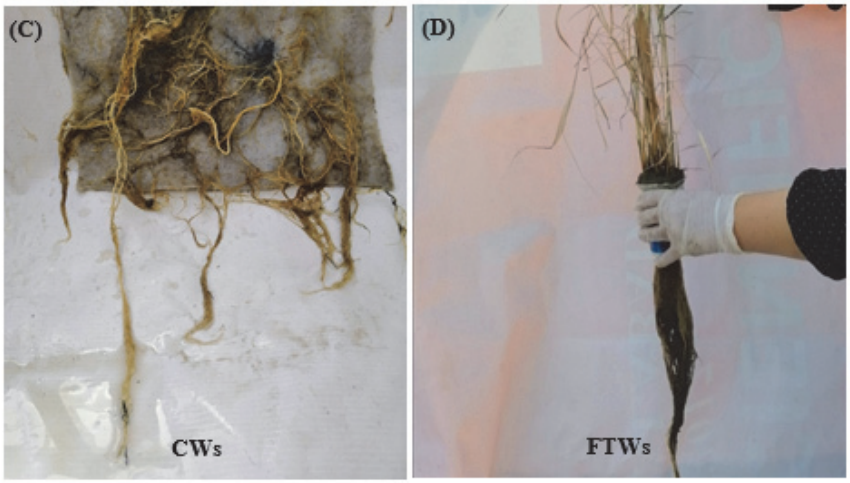

Figure 8. *Phragmites australis* growth comparison in CWs and FTWs: (**A**) *P. australis* growth in CWs; (**B**) *P. australis* growth in FTWs; (**C**) *P. australis* root growth in CWs; (**D**) *P. australis* root growth in FTWs.

3.5. Toxicity Reduction in the Water Treated by CWs and FTWs

The efficacy of the CWs and FTWs for the remediation of phenanthrene-contaminated water was assessed by exposing the seeds of *Triticum aestivum* and *Brassica napus* to the water treated by both wetlands. The germination rate of the seeds was lower when they were allowed to grow in the untreated phenanthrene-contaminated water compared with the germination rate of the seeds that were grown in the water treated by the CWs and FTWs. Hydrocarbon contamination retards the biomass production of plants due to its toxicity [2,23]. A poor seed germination rate of various other crops grown with untreated water has also been reported [47]. The highest germination rate was found in the seeds that were exposed to the water treated by the bacterial-augmented CWs (Figure 9). The maximum germination of the seeds in the presence of the water treated by the bacterial-augmented CWs and FTWs might be due to the mineralization of the phenanthrene by the augmented bacteria.

FTWs and CWs are the most cost-effective techniques compared with conventional methods. Wetlands imitate natural habitats and have a green partner and biodegraders for the remediation of contaminated water [48]. They do not use any chemicals or have a high-cost infrastructure for the construction, unlike other conventional treatments.

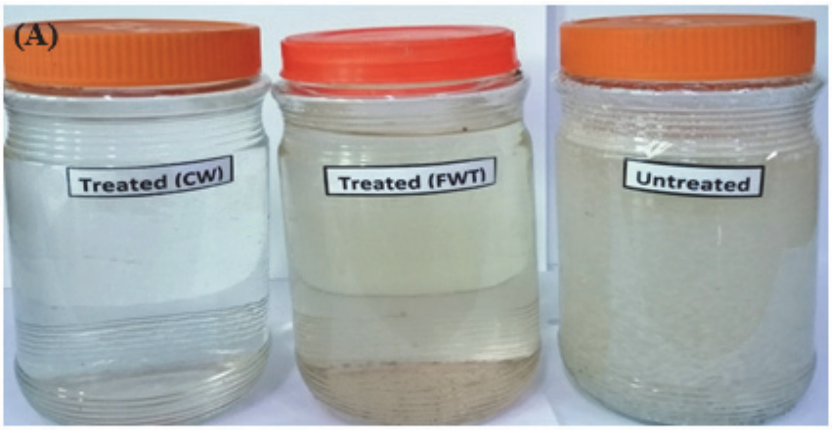

Figure 9. *Cont.*

Figure 9. Bioassay to compare the treated water of CWs and FTWs: (**A**) visual comparison of turbidity level of contaminated water treated in CTWs and FTWs; (**B**) *Brassica napus* seed germination in treated water of CWs and FTWs; (**C**) *Triticum aestivum* seed germination in treated water of CWs and FTWs.

4. Conclusions and Prospects

Both wetlands (CWs and FTWs) showed the potential for the remediation of phenanthrene-contaminated water. Between these two types of wetlands, the CWs were found to be more effective for the removal of phenanthrene from the water when compared with the FTWs. In addition, greater plant biomass and growth were observed in the CWs than in the FTWs. The augmented bacteria showed a persistence in the different components of the FTWs and CWs, which was relatively better in the CWs. However, further studies should be conducted focusing on the metabolic activities of the inoculated bacteria and the expression of their genes in different compartments of FTWs and CWs to completely understand and harness the phenanthrene degradation potential of these bacterial strains to further improve the treatment efficiency of wetlands. Moreover, different plant species are needed to develop wetlands to assess their phytoremediation potential and, most importantly, pilot-scale investigations are required. Nevertheless, the present study is a step forward to compare the efficacy of CWs and FTWs and to combine the use of plants and bacteria in wetlands for the maximum remediation of contaminated water.

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