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SDG 6 in Practice: Demonstrating a Scalable Nature-Based Wastewater Treatment System for Pakistan's Textile Industry

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Abstract

Industrial wastewater management remains a critical barrier to achieving Sustainable Development Goal 6 (SDG 6) in many developing countries, where regulatory frameworks exist but affordable and scalable treatment solutions are lacking. In Pakistan, the textile sector is a leading polluter, with untreated effluents routinely discharged into rivers and agricultural lands despite stringent National Environmental Quality Standards (NEQS). This study presents a pilot-scale case from Faisalabad's Khurrianwala industrial zone, where a decentralized, nature-based bioreactor was piloted to bridge the gap between policy and practice. The system integrates four treatment stages—anaerobic digestion (AD), floating treatment wetland (FTW), constructed wetland (CW), and sand filtration (SF)—and was further intensified via nutrient amendment, aeration, and bioaugmentation with three locally isolated bacterial strains (*Acinetobacter junii* NT-15, *Pseudomonas indoloxydans* NT-38, and *Rhodococcus* sp. NT-39). The fully intensified configuration achieved substantial reductions in total dissolved solids (TDS) (46%), total suspended solids (TSS) (51%), chemical oxygen demand (COD) (91%), biochemical oxygen demand (BOD) (94%), nutrients, nitrogen (N), and phosphorus (P) (86%), sulfate (26%), and chloride (41%). It also removed 95% iron (Fe), 87% cadmium (Cd), 57% lead (Pb), and 50% copper (Cu) from the effluent. The bacterial inoculants persist in the system and colonize the plant roots, contributing to stable bioremediation. The treated effluent met the national environmental quality standards (NEQS) discharge limits, confirming the system's regulatory and ecological viability. This case study demonstrates how nature-based systems, when scientifically intensified, can deliver high-performance wastewater treatment in industrial zones with limited infrastructure—offering a replicable model for sustainable, SDG-aligned pollution control in the Global South.

Keywords: textile wastewater; wetlands; anaerobic digestion; integrated wastewater treatment system; intensification

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1. Introduction

Global progress toward Sustainable Development Goal 6 (SDG 6), ensuring water and sanitation for all by 2030, remains sluggish, especially in the developing world. As of 2023, approximately 2 billion people lack access to safe drinking water and 3.6 billion people without safely managed sanitation, nearly half the world's population [1]. This service

gap directly contributes to the unchecked release of wastewater. Untreated wastewater is a persistent challenge: more than 80% of all wastewater globally is discharged into the environment without any treatment or reuse [2]. The problem is most acute in low-income countries, where on average, only approximately 8% of industrial and municipal wastewater undergoes any form of treatment [3]. The remaining water flows untreated into rivers, lakes, and groundwater, contaminating vital water resources and posing major risks to public health and ecosystems. Consequently, water pollution has become endemic in many developing regions, undermining aquatic biodiversity and the safe water supplies needed for human well-being [4]. These conditions indicate that, without accelerated interventions, many developing countries will fall short of the SDG 6 targets for water quality and wastewater management by 2030.

Developing countries face interrelated challenges in managing water and sanitation. Rapid urbanization and population growth have often outpaced the expansion of sewage infrastructure, resulting in large volumes of domestic and industrial wastewater being dumped untreated [5]. Financial and technical constraints limit the construction of modern treatment plants, and existing facilities are often overloaded or poorly maintained [6]. Inadequate governance and enforcement exacerbate the issue as environmental regulations, where they exist, are frequently not rigorously implemented. As a result, polluted waters are common in these regions and contain pathogens from sewage, nutrients from agricultural runoff, and toxic chemicals from industries, causing waterborne disease outbreaks and environmental degradation. Recognizing these gaps, the global community has emphasized Target 6.3 of SDG 6, which calls for halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse by 2030 [7]. This ambition has spurred interest in affordable and context-appropriate solutions to manage wastewater in developing countries.

Pakistan, as a developing country with a large population and growing industrial base, exemplifies the struggle to achieve SDG 6. While access to improved water sources in Pakistan expanded under the Millennium Development Goals, with official reports citing 90%+ access to basic drinking water [8], the quality and sustainability of the water supply remain problematic. Many water sources in Pakistan are contaminated by biological and chemical pollutants, and safely managed water services, continuous, safe, and on-premises, are available to only approximately half of the population [9]. Sanitation access is similarly limited; a significant fraction of the population lacks modern sewerage, leading to direct discharge of sewage into waterways or its use in agriculture. Pakistan's wastewater treatment capacity is extremely low relative to its needs. Recent estimates suggest that only 38% of domestic wastewater is safely treated nationwide [9]. However, when considering all wastewater sources, including industrial effluents, the treated fraction may be as low as 1% [5]. In effect, the vast majority of Pakistan's wastewater is released into the environment untreated, polluting surface water and groundwater. This has led to heavy contamination of water bodies, as untreated sewage and industrial effluents are commonly diverted to rivers or used to irrigate crops, introducing pathogens and toxic substances into the food chain [5]. Consequently, waterborne diseases and toxic exposures are public health concerns, and aquatic ecosystems, including the Indus River and its tributaries, have experienced severe degradation.

The government of Pakistan has acknowledged these challenges and has taken initial steps to align with SDG 6. A National Water Policy was approved in 2018, which stresses the need for improved water quality and wastewater management. Notably, the policy mandates that industries must perform in-house treatment of their effluent to meet National Environmental Quality Standards (NEQS) before discharge to municipal sewers, with strict enforcement of the "polluter pays" principle. It also promotes the development of

centralized treatment facilities in industrial estates and encourages wastewater recycling and reuse given the country's water scarcity [10]. Additionally, Pakistan's National SDG Framework and provincial development plans have set targets for increasing wastewater treatment coverage and aiming to treat up to 50% of wastewater by 2030. Despite these policy commitments, implementation on the ground remains limited. Pakistan still has only a handful of functional municipal wastewater treatment plants, which serve a small fraction of its cities. Most urban centers rely on open drains and oxidation ponds that provide little effective treatment, and industrial effluents are often discharged without adequate oversight.

The treatment of textile effluent is achieved by using different physical [11], chemical [12], electrochemical [13], and membrane filtration methods [14]. However, these methodologies require high energy, skills, and chemicals [15]. A variety of polluted water, including storm water, agricultural runoff, and domestic and industrial wastewater, has been treated efficiently by nature-based systems called treatment wetland (TW) [16,17]. Among TW, floating treatment wetland (FTW) and constructed wetland (CW) are very economical, effective, user-friendly and sustainable technologies for the remediation of different types of effluents [16–19].

In Asia and globally, the application of nature-based technologies, floating treatment wetland (FTW), and constructed wetland is growing to treat the wastewater [20–22]. Recently, FTW has been successfully applied at several sites in Pakistan on a large scale to treat the wastewater [23]. In China, CW has been applied to treat the river and canal wastewater [24–27]. Although FTW, CW, anaerobic digester (AD), and sand filtration (SF) were extensively used to treat different types of polluted water; however, their intensified integrated system has not been evaluated for the remediation of textile industry wastewater.

Case Study: While Pakistan's water policy framework reflects a growing commitment to wastewater regulation, the gap between ambition and implementation remains stark—particularly in pollution-intensive sectors such as textiles. In Faisalabad's Khurrianwala industrial zone alone, over 200 textile units discharge chemically complex effluents containing dyes, salts, heavy metals, and surfactants—often directly into open channels—despite national discharge limits set under the National Environmental Quality Standards (NEQS) and commitments to SDG 6. Compared with the 150 mg/L NEQS threshold, typical COD levels from these effluents exceed 900 mg/L, underscoring the need for high-strength treatment solutions that are both effective and affordable. This environmental and regulatory vacuum formed the basis for piloting a decentralized, nature-based treatment system specifically designed for such conditions. Installed at a local facility, the system combines an AD, FTW, horizontal subsurface CW, and an SF unit—each selected to target a different pollutant group through natural mechanisms such as sedimentation, microbial degradation, and phytoremediation. To increase system performance, nutrient supplementation, aeration, and bioaugmentation with indigenous dye-degrading bacterial strains, *Acinetobacter junii* NT-15, *Pseudomonas indoloxydans* NT-38, and *Rhodococcus* sp. NT-39 were implemented. The result is a hybrid ecological system that addresses the complex pollutant load of textile effluent while maintaining low energy and operational costs. The Khurrianwala pilot, thus, exemplifies how intensified nature-based solutions can operationalize regulatory intent, providing a scalable model for industrial wastewater treatment in resource-constrained settings.

2. Materials and Methods

2.1. Sampling and Analysis of Textile Effluent

The wastewater was obtained from the effluent discharge point of a textile factory located in Khurrianwala, Faisalabad, Pakistan. The wastewater was collected at two-

hour intervals from 8 AM to 4 PM and subsequently combined to make a composite sample, which was promptly transported to the laboratory and analyzed for various physicochemical parameters, including color, TDS, BOD, COD, TSS, sulfate, P and N, via standard methods [28]. An atomic absorption spectrophotometer (AAS) (Spectra AA. 200, Varian Australia, Australia) was utilized to determine the concentrations of trace metals such as Cu, Pb, Fe, and Cd (Table 1).

Table 1. Physicochemical characteristics of textile effluent collected from the textile industry in Khurrianwala, Faisalabad, Pakistan.

Parameter	Unit	Value	NEQS
Temperature	°C	38	40
pH	--	8.69	6–9
Electrical conductivity (EC)	mS cm^{-1}	7.24	NG
Color	(m^{-1})	75	NG
Chemical oxygen demand (COD)	mg L^{-1}	913	150
Biochemical oxygen demand (BOD)	mg L^{-1}	437	80
Chloride	mg L^{-1}	1600	1000
Sulfate	mg L^{-1}	890	600
Nitrogen (N)	mg L^{-1}	1.38	NG
Phosphorous (P)	mg L^{-1}	0.56	NG
Total dissolved solids (TDS)	mg L^{-1}	3900	3500
Total suspended solids (TSS)	mg L^{-1}	260	200
Iron (Fe)	mg L^{-1}	12.54	8
Cadmium (Cd)	mg L^{-1}	0.35	0.1
Lead (Pb)	mg L^{-1}	0.67	0.5
Copper (Cu)	mg L^{-1}	1.38	1.0

NG = Not given in the NEQS list; NEQS = National Environmental Quality Standards, set by the Government of Pakistan.

2.2. Bacterial Selection and Inoculum Preparation

Seven different previously isolated bacterial strains, *Pantoea gaviniae* NT-3, *Enterobacter xiangfangensis* NT-14, *Acinetobacter junii* NT-15, *Bacillus subtilis* NT-23, *Pseudomonas monteilii* NT-20, *Rhodococcus* sp. NT-39, and *Pseudomonas indoloxydans* NT-38, were evaluated for their potential to remediate textile wastewater, as explained previously [11]. These strains were isolated from the natural ecosystems such as the polluted soil, sludge, and the rhizosphere and endosphere of different plants. Moreover, these are well characterized, and there is no genetic modification. Briefly, these bacterial strains were cultivated individually in a Luria–Bertani (LB) broth for 24 h at 30 °C, and through centrifugation (12,000 rpm) for 5 min at 4 °C, the cell pellets were collected and resuspended in a sterile 0.9% (*w*/*v*) sodium chloride (NaCl) solution. In accordance with the turbidimetric technique, the cell suspension of each pure culture was modified to obtain equal numbers of cells [29]. Afterward, 10 mL of the suspension, containing 10^9 mL^{-1} of colony forming unit (CFU), was inoculated into 200 mL of filter-sterilized wastewater and incubated at 30 °C for 10 days. The remediation potential of each strain was evaluated by checking the reduction in color, BOD, and COD of the wastewater every two days [16].

All seven bacterial strains demonstrated the ability to remediate textile effluent. A prominent greater reduction in color, BOD and COD was noted with bacterial inoculation than with the control treatment (Table 2). The performance of three bacterial strains, specifically *A. junii* NT-15, *P. indoloxydans* NT-38, and *Rhodococcus* sp. NT-39, was exceptional, leading to their selection for inoculum preparation. The cells of these three strains were harvested and resuspended as described above, and their optical density was set to 10^9 CFU mL^{-1} and combined (1:1:1) for the development of the bacterial consortium. A volume of

100 mL of this consortium was utilized as an inoculum in each unit of the FW and CW in accordance with the experimental design.

Table 2. Textile effluent degradation efficiency of different bacterial strains.

Treatment	pH	Color (m^{-1})	COD (mg L^{-1})	BOD (mg L^{-1})
Control	8.09 ^{a} ± 0.75	75 ^{a} ± 6.2	913 ^{a} ± 32.8	437 ^{a} ± 20.4
Pantoea gaviniae NT-3	7.92 ^{a} ± 0.71	25 ^{b} ± 3.4	470 ^{b} ± 19.2	204 ^{b} ± 16.4
Enterobacter xiangfangensis NT-14	7.95 ^{a} ± 0.70	26 ^{b} ± 2.8	390 ^{c} ± 17.6	225 ^{b} ± 17.1
Acinetobacter junii NT-15	7.48 ^{a} ± 0.46	20 ^{c} ± 1.5	310 ^{e} ± 12.8	175 ^{c} ± 11.2
Pseudomonas monteilii NT-20	8.01 ^{a} ± 0.74	28 ^{b} ± 3.1	400 ^{c} ± 18.2	207 ^{b} ± 14.6
Bacillus subtilis NT-23	7.80 ^{a} ± 0.68	27 ^{b} ± 3.0	360 ^{d} ± 16.0	217 ^{b} ± 16.4
Pseudomonas indoloxydans NT-38	7.58 ^{a} ± 0.55	21 ^{c} ± 2.7	290 ^{e} ± 11.2	185 ^{bc} ± 13.2
Rhodococcus sp. NT-39	7.52 ^{a} ± 0.50	22 ^{bc} ± 2.8	307 ^{e} ± 12.1	178 ^{c} ± 11.4

Each value is the mean of three replicates, ± indicates standard deviation. The incubation period was 10 days. Chemical oxygen demand (COD), and biochemical oxygen demand (BOD). Letters indicate statistically significant differences between treatments at the 5% level of significance.

2.3. Vegetation

Phragmites australis has been utilized to create FTW and CW because of its efficacy in the phytoremediation of industrial wastewaters, particularly in the presence of hazardous organic compounds [18,30,31]. Furthermore, the plant was previously shown to have a successful symbiotic relationship with the augmented bacteria [18,30]. The *P. australis* seedlings were previously cultivated at the nursery located at NIBGE, Faisalabad.

2.4. Development of an Integrated Bioreactor

A pilot-scale integrated bioreactor was developed by combining AD, FTW, CW, and a SF in series (Figure 1a,b). A total of six integrated bioreactors were developed and operated in three repeated-batch treatments in the vicinity of the NIBGE, Faisalabad (31°25′0″ N 73°5′28″ E). Twenty-four identical blue high-density polyethylene (HDPE) open-top plastic tanks, each with a functional capacity of 60 L, an internal diameter of 56 cm, and a depth of 64 cm, were utilized. A small tap was installed 5 cm above the bottom of each tank to enable sediment-free sample collection. The tanks were systematically positioned to facilitate the gravitational flow of wastewater, commencing with the AD, followed by the FTW, then the CW, and finally the SF. The wastewater samples were collected at 0 h (untreated textile wastewater), 48 h (outlet of AD), 96 h (outlet of FTW), 144 h (outlet of CW), and 145 h (outlet of SF).

2.4.1. Anaerobic Digester (AD)

For the development of six units of AD, six plastic tanks containing 10 L of tap water were inoculated with 1 kg of sludge collected from anaerobic wastewater stabilization ponds of Faisalabad city. An adaptation period of one month was provided to the microbes before the start of wastewater treatment [32].

2.4.2. Floating Treatment Wetlands (FTW)

Six units of FTW were developed as explained earlier [33]. Briefly, a total of six floating mats were created from polystyrene-based sheets with dimensions of 33 cm (length), 28 cm (width), and 7.6 cm (thickness). Five equidistant holes, each with a diameter of 5 cm, were bored into each mat and planted with *P. australis*. The plants were permitted to grow their roots for one month in tap water containing Hoagland solution. At the corners of each floating mat, an upper border of 2.5 cm height was constructed to retain soil and gravel for

the support and growth of the plant, while the edges of the floating mats were protected from UV rays by wrapping them with aluminum foil.

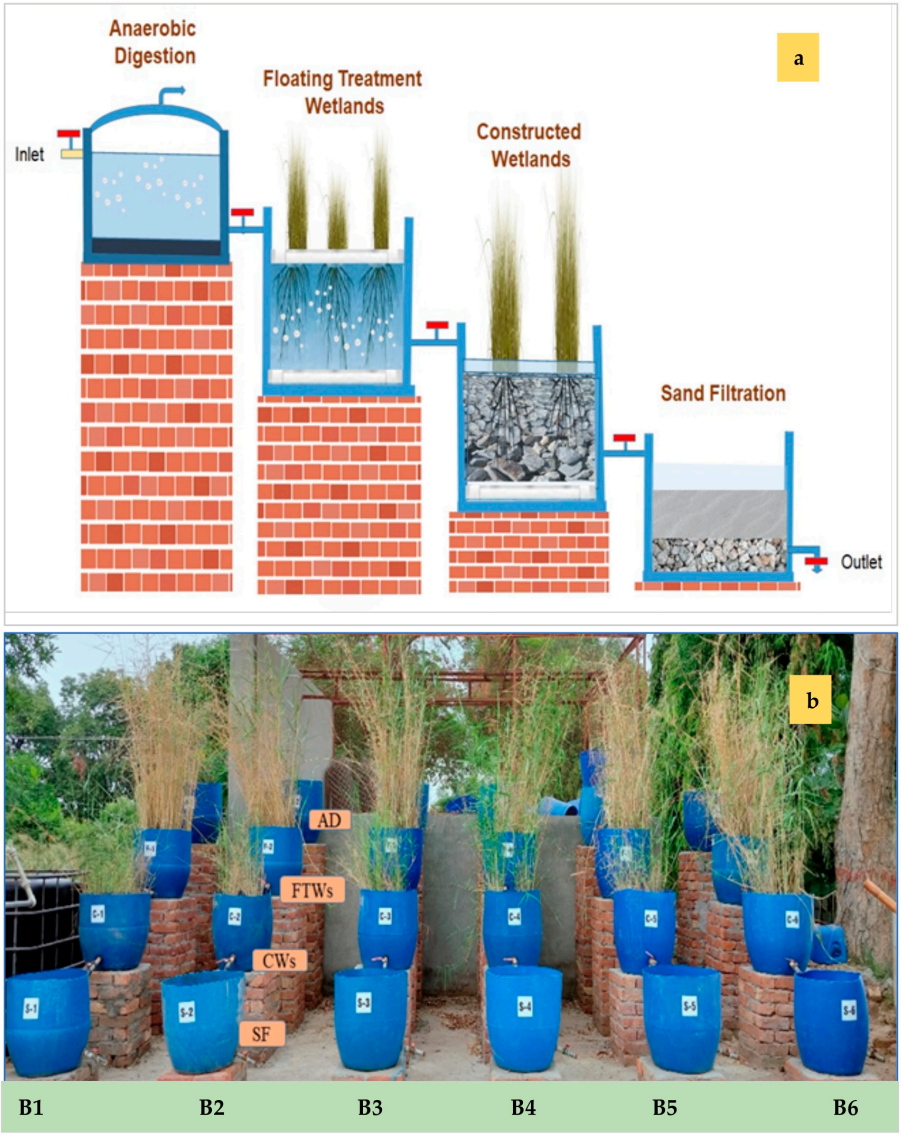


Figure 1. Schematic representation of an integrated bioreactor (**a**) and six integrated pilot-scale bioreactors (B1–B6) developed in the vicinity of the NIBGE (**b**).

2.4.3. Constructed Wetlands (CW)

Six CW units with vertical flow were constructed via a previously reported method [16]. A supporting layer (25 cm thick) consisting of coarse gravel 3–5 cm in diameter was established at the base of the tank, followed by a primary substrate layer 15 cm thick composed of fine gravel 2–3 cm in diameter and finally covered by a top layer of 10 cm of washed river sand with a diameter of 1–2 mm. The sand layer helps with the dispersion of wastewater and supports the growth of plants. Five seedlings of *P. australis* were planted in each unit and permitted to develop roots for one month in tap water supplemented with Hoagland solution [33].

2.4.4. Sand Filter (SF)

The SF was developed as explained previously [34]. It consists of sand and several layers of gravel of different sizes at the bottom. The larger gravel was at the bottom, and

the smaller gravel was at the upper side. At the top, there was a layer of sand. The upper sand layer was washed after every batch of wastewater treatment.

2.5. Intensification of Bioreactors

The units of FTW and CW were intensified with aeration and bacteria, whereas AD was intensified with nutrients, N and P, at a COD:N:P ratio of 100:5:1, as explained earlier [35]. The 100:5:1 ratio is a recommended dose for microorganisms in wastewater treatment. The specifications of the integrated bioreactors (B2–B6) are shown in Table 3.

Table 3. Different integrated bioreactors (B1–B6).

Bioreactor		Description
B1	A1	Anaerobic digester (AD)
	F1	FTW vegetated with *P. australis*
	C1	CW vegetated with *P. australis*
	S1	Sand filter (SF)
B2	A2	AD + NP
	F2	FTW vegetated with *P. australis*
	C2	CW vegetated with *P. australis*
	S2	SF
B3	A3	AD
	F3	FTW vegetated with *P. australis* + aeration
	C3	CW vegetated with *P. australis* + aeration
	S3	SF
B4	A4	AD
	F4	FTW vegetated with *P. australis* + bacterial inoculation
	C4	CW vegetated with *P. australis* + bacterial inoculation
	S4	SF
B5	A5	AD + NP
	F5	FTW vegetated with *P. australis* + bacterial inoculation
	C5	CW vegetated with *P. australis* + bacterial inoculation
	S5	SF
B6	A6	AD + NP
	F6	FTW vegetated with *P. australis* + aeration + bacterial inoculation
	C6	CW vegetated with *P. australis* + aeration + bacterial inoculation
	S6	SF

NP (nitrogen and phosphorus), FTW (floating treatment wetland), and CW (constructed wetland).

The experiment was performed under ambient conditions, and the persistence of the inoculated bacterial strains in the shoots and roots was assessed via culture-dependent plate counting [36]. The identity of the isolated bacteria was verified through the restriction fragment length polymorphism technique [30,37].

2.6. Trace Metals Analysis

An AAS, Varian SpectrAA.200 (Varian Australia, Victoria, Australia), was utilized to measure the concentration of metals in the water as described previously [38]. The trace metal concentrations in the shoots and roots were analyzed by digesting 0.5 g of their biomass in 67% nitric acid. AAS was employed to obtain the concentrations of Fe, Cd, Cu, and Pb, while quality control was maintained by complying with established standards, blanks and duplicate samples [28].

2.7. Plant Biomass and Growth Analysis

The shoot and root lengths were measured after the completion of the experiment. The plants were harvested one inch below and one inch above the mat surface. The whole plant biomass was removed from the gravel of the CW units. The weights (fresh) of the shoots and roots were recorded. Furthermore, the biomass (dried) of the plants was assessed after the shoots and roots were dried in an oven at 60 °C until a constant weight was attained [18].

2.8. Survival of Inoculated Bacterial Strains

The persistence and survival of the inoculated bacterial strains in the roots and shoots of the plants in the FTW and CW were assessed via the plate count method [6,39,40]. Briefly, plant tissues (roots and shoots) were sterilized with 70% ethanol for 10 min and 1% sodium hypochlorite (NaOCl) solution for one min. Then, the plant tissues were washed with sterilized water three times. After surface sterilization, the shoots and roots were ground with a mortar and pestle in the presence of 10 mL NaCl solution (0.9%, *w*/*v*). The slurry and water samples were plated on M9 medium containing filter-sterilized textile wastewater, 100 mL L^{-1} medium. The CFUs were counted after the plates were incubated for 48 h at 37 °C.

2.9. Toxicity Analysis

The effect of treated water on the germination of wheat (*Triticum aestivum*) seeds was determined via a previously described method [40]. Five wheat seeds were placed in a disposable plastic Petri dish containing filter paper saturated with five mL of treated and untreated textile wastewater. Seeds were permitted to germinate at 25 °C in the absence of light. The test was performed in triplicate. Germinated seeds were noted after five days. Seeds were considered to have germinated when both the plumule and radicle exceeded two mm in length. Plantlet growth was assessed by measuring root length and total length.

2.10. Assessment of Contaminants in Water and Plant Samples

Water samples were collected from each unit of the bioreactor in acid-washed polyethylene bottles and analyzed for various physicochemical parameters, including COD, BOD, pH, EC, chlorides, sulfates, Na, and K, as explained previously [28].

2.11. Statistical Analysis

The statistical analysis of pollutants, plant growth, and bacterial survival was conducted via Statistix 8.1. For the comparison of means across various independent components, factor analysis of variance (ANOVA) was employed. The evaluation of significant changes among various bioreactors was achieved via the least significant difference (LSD) ($\alpha = 0.05$) test [41]. After testing homogeneity of variance, Duncan's test was applied for analysis of variance. The values were assigned letters to denote significant or nonsignificant changes between bioreactors.

3. Results and Discussion

3.1. Textile Wastewater Characteristics

The textile effluent presented higher values of several pollution indicators, such as COD (913 mg L^{-1}), BOD (437 mg L^{-1}), TDS (3820 mg L^{-1}), TSS (240 mg L^{-1}), chlorides (1600 mg L^{-1}), sulfate (875 mg L^{-1}), Fe (12 mg L^{-1}), Cd (0.35 mg L^{-1}), Pb (0.67 mg L^{-1}), and Cu (1.3 mg L^{-1}), than the national permissible limits (Table 2). This indicates the necessity for pretreatment of textile sector effluent prior to its discharge into surface water.

3.2. Biodegradation of Textile Wastewater

Among the tested strains, three, *Acinetobacter junii* NT-15, *Pseudomonas monteilii* NT-20, and *Rhodococcus* sp. NT-39, exhibited greater efficiency in removing color, COD, and BOD from wastewater (Table 2).

3.3. Performance of the Intensified Integrated Bioreactors

(a) Basic Water Quality Parameters

The variations in the physical and chemical parameters of textile wastewater over 145 h were monitored in six integrated bioreactors, each progressing through four subunits, AD, FTW, CW, and SF, at 48 h intervals for AD, FTW, and CW and 1 h for SF. All six integrated bioreactors caused significant reductions in pH, EC, TDS, and TSS (Table 4). There was a gradual decrease in the pH of the wastewater from AD to SF. The organic acids released during the microbial degradation of organic matter could be one of the causes of a reduction in pH [42,43]. EC was reduced in all the bioreactors, including the control (B1). The sedimentation, nutrient uptake by plants and physicochemical or biological binding of contaminants to plant roots can be attributed to a decrease in EC. The reduction in the TSS and TDS loads can also be associated with the attachment of suspended and dissolved particles with hanging roots as well as with sedimentation processes [44,45].

Bioreactor B1 reduced TDS from 3900 mg L^{-1} to 3100 mg L^{-1} (20% reduction), TSS from 261 mg L^{-1} to 188 mg L^{-1} (28% reduction), and the addition of NP in bioreactor B2 reduced TDS from 3960 mg L^{-1} to 3008 mg L^{-1} (24% reduction) and TSS from 252 mg L^{-1} to 172 mg L^{-1} (31% reduction). The bioreactor supported by aeration (B3) reduced the TDS from 3900 mg L^{-1} to 2825 mg L^{-1} (27% reduction) and the TSS from 246 mg L^{-1} to 168 mg L^{-1} (31% reduction), whereas the inoculation of bacteria in the bioreactor (B4) decreased the TDS from 3900 mg L^{-1} to 2344 mg L^{-1} (40% reduction) and the TSS from 256 mg L^{-1} to 163 mg L^{-1} (36% reduction). The bioreactor supported with bacterial inoculation and N and P (B5) reduced the TDS from 3965 mg L^{-1} to 2267 mg L^{-1} (42% reduction) and the TSS from 260 mg L^{-1} to 133 mg L^{-1} (48% reduction), whereas the bioreactor augmented with bacterial inoculation and supplemented with N and P and aeration (B6) showed the maximum reduction in TDS from 3963 mg L^{-1} to 2110 mg L^{-1} (46% reduction) and in the TSS from 262 mg L^{-1} to 128 mg L^{-1} (51% reduction). These results are supported by previously published findings [16,46–48]. In an earlier study, a CW system intensified with bacteria removed TDS by 32% from textile wastewater [16]. In another earlier study, FTW augmented with bacterial strains removed TDS by 35% from textile wastewater [46]. Similar results were seen in another investigation in which an FTW system supported by bacteria reduced TSD and TSS by 35% and 36%, respectively, after six-day treatment [47]. In a hybrid system consisting of different oxidation processes, it was possible to remove TDS by 21% from textile wastewater [48].

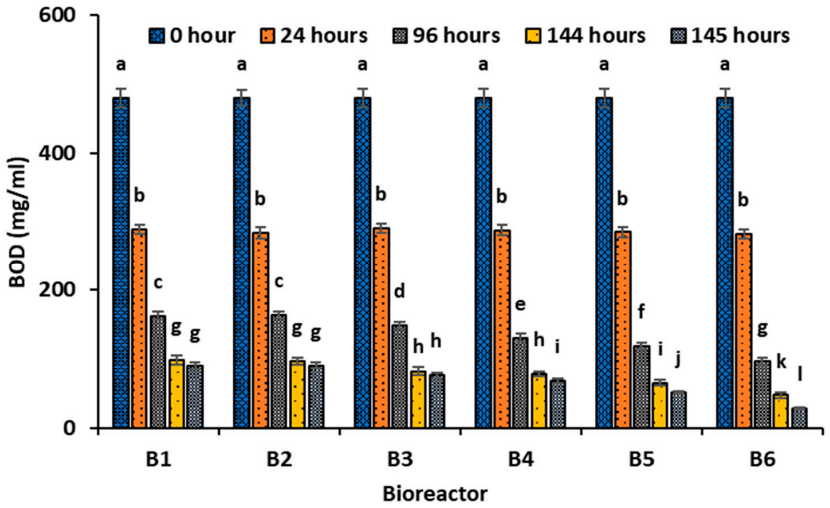
Moreover, a sharp reduction in TDS contents in the bioreactors augmented with bacteria might be due to the effective role of microbes in removing dissolved solids [38]. Consequently, the combination of vegetation and bacteria accelerates pollutant stabilization by diminishing the turbulence of water, capturing suspended solids, enhancing sedimentation, and mineralizing organic pollutants [30,38,45]. Similarly, fine solids may adhere to bacterial biofilms, thereby improving the elimination of dissolved and suspended particles [49].

Table 4. Effects on the pH, electrical conductivity (EC), total dissolved solids (TDS), and total suspended solids (TSS) of textile wastewater treated by bioreactors (B1–B6).

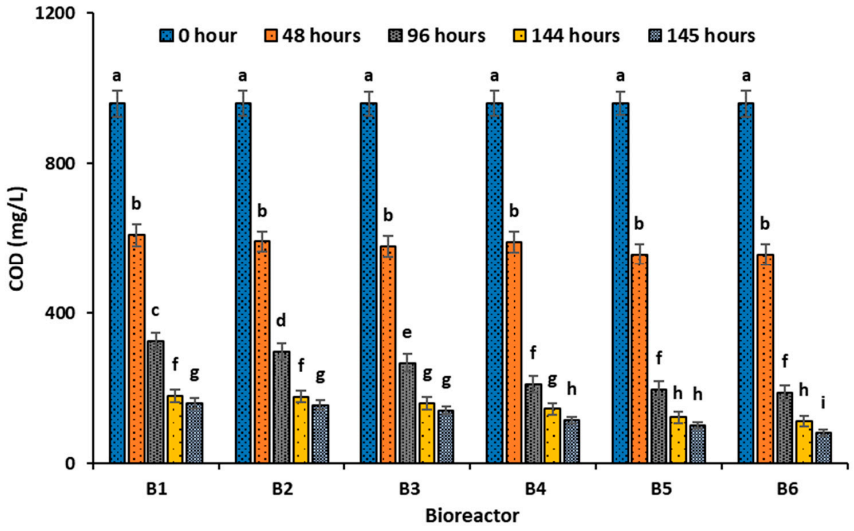
Bioreactor	pH					EC (mS cm^{-1})					TDS (mg L^{-1})					TSS (mg L^{-1})				
	Hours																			
	0 h	48 h	96 h	144 h	145 h	0 h	48 h	96 h	144 h	145 h	0 h	48 h	96 h	144 h	145 h	0 h	48 h	96 h	144 h	145 h
B1	8.92 ^{a} (0.25)	8.21 ^{a} (0.36)	8.03 ^{a} (0.41)	7.91 ^{a} (0.43)	7.70 ^{a} (0.31)	8.60 ^{a} (0.15)	6.70 ^{a} (0.22)	4.81 ^{b} (0.11)	3.11 ^{a} (0.14)	2.61 ^{a} (0.10)	3900 ^{a} (140)	3784 ^{a} (127)	3630 ^{a} (136)	3470 ^{a} (143)	3100 ^{a} (105)	261 ^{a} (16)	230 ^{a} (13)	218 ^{a} (14)	200 ^{a} (11)	188 ^{a} (12)
B2	8.90 ^{a} (0.29)	8.10 ^{a} (0.37)	7.93 ^{a} (0.41)	7.82 ^{a} (0.38)	7.48 ^{a} (0.26)	8.60 ^{a} (0.16)	6.51 ^{a} (0.17)	5.20 ^{a} (0.14)	3.10 ^{a} (0.11)	2.01 ^{b} (0.10)	3960 ^{a} (143)	3745 ^{a} (128)	3505 ^{a} (151)	3421 ^{a} (119)	3008 ^{a} (108)	252 ^{a} (17)	222 ^{a} (14)	206 ^{a} (12)	187 ^{b} (12)	172 ^{b} (10)
B3	8.93 ^{a} (0.28)	8.31 ^{a} (0.40)	7.70 ^{a} (0.41)	7.61 ^{a} (0.41)	7.42 ^{a} (0.37)	8.60 ^{a} (0.16)	6.61 ^{a} (0.15)	4.90 ^{b} (0.12)	2.80 ^{b} (0.09)	1.82 ^{c} (0.08)	3900 ^{a} (141)	3778 ^{a} (135)	3456 ^{a} (129)	3258 ^{b} (129)	2825 ^{b} (128)	246 ^{a} (16)	232 ^{a} (12)	198 ^{a} (10)	180 ^{b} (11)	168 ^{b} (13)
B4	8.90 ^{a} (0.22)	8.10 ^{a} (0.41)	7.72 ^{a} (0.37)	7.61 ^{a} (0.35)	7.61 ^{a} (0.28)	8.61 ^{a} (0.14)	6.61 ^{a} (0.12)	4.91 ^{b} (0.08)	2.60 ^{c} (0.10)	1.61 ^{d} (0.07)	3900 ^{a} (140)	3778 ^{a} (133)	3170 ^{b} (118)	2859 ^{b} (142)	2344 ^{c} (121)	256 ^{a} (15)	228 ^{a} (11)	200 ^{a} (11)	178 ^{b} (13)	163 ^{b} (11)
B5	8.91 ^{a} (0.18)	8.12 ^{a} (0.39)	7.70 ^{a} (0.39)	7.52 ^{a} (0.36)	7.51 ^{a} (0.32)	8.61 ^{a} (0.16)	6.80 ^{a} (0.11)	4.21 ^{d} (0.08)	1.80 ^{d} (0.08)	1.11 ^{e} (0.05)	3965 ^{a} (144)	3710 ^{a} (136)	3010 ^{c} (124)	2760 ^{b} (128)	2267 ^{c} (130)	260 ^{a} (15)	236 ^{a} (12)	198 ^{a} (14)	168 ^{b} (10)	133 ^{c} (12)
B6	8.97 ^{a} (0.12)	8.21 ^{a} (0.40)	7.71 ^{a} (0.27)	7.60 ^{a} (0.35)	7.40 ^{a} (0.32)	8.60 ^{a} (0.14)	6.10 ^{b} (0.08)	4.40 ^{c} (0.05)	1.60 ^{e} (0.02)	1.00 ^{f} (0.01)	3963 ^{a} (142)	3700 ^{a} (133)	2945 ^{c} (129)	2610 ^{c} (127)	2110^{d} (120)	262 ^{a} (14)	225 ^{a} (11)	188 ^{a} (13)	152 ^{c} (8.8)	128 ^{d} (9.3)

B1 (bioreactor), B2 (bioreactor intensified with nitrogen and phosphorus), B3 (bioreactor intensified with aeration), B4 (bioreactor intensified with bacteria), B5 (bioreactor intensified with nitrogen, phosphorus, and bacteria), and B6 (bioreactor intensified with nitrogen, phosphorus, aeration and bacteria). The wastewater samples were collected at 0 h (untreated textile wastewater), 48 h (outlet of anaerobic digester), 96 h (outlet of floating treatment wetlands), 144 h (outlet of constructed wetlands), and 145 h (outlet of sand filter). The error bars indicate the standard errors of three repeated-batch treatments. Letters indicate statistically significant differences between bioreactors at the 5% level of significance. Each value is the mean of three repeated-batch treatments; the standard deviation of three repeated-batch treatments is presented in parenthesis, and the alphabets (^{a}–^{f}) represent the significant/nonsignificant differences among the bioreactors.

All the bioreactors demonstrated a significant decline in the COD and BOD values over a period of six days (Figure 2). An average reduction of 39% and 40% in COD and BOD, respectively, was observed at 48 h when the wastewater samples were collected from the outlet of AD. A prominent reduction in BOD and COD was observed at the outlets of the FTW and CW after 96 and 144 h, with average reductions in COD of 73% and 84%, respectively, and 71% and 83%, respectively, in BOD. Microbes associated with plants degrade the organic matter present in wastewater and ultimately reduce the BOD and COD (16–18). There was no significant reduction in COD or BOD after the wastewater from the outlet of CW was passed through the SF.



(**a**)



(**b**)

Figure 2. Performance evaluation of bioreactors for the removal of biochemical oxygen demand (BOD) (**a**) and chemical oxygen demand (COD) (**b**). B1 (bioreactor), B2 (bioreactor intensified with nitrogen and phosphorus), B3 (bioreactor intensified with aeration), B4 (bioreactor intensified with bacteria), B5 (bioreactor intensified with nitrogen, phosphorus, and bacteria), and B6 (bioreactor intensified with nitrogen, phosphorus, aeration and bacteria). The wastewater samples were collected at 0 h (untreated textile wastewater), 48 h (outlet of anaerobic digester), 96 h (outlet of floating treatment wetlands), 144 h (outlet of constructed wetlands), and 145 h (outlet of sand filter). The error bars indicate the standard errors of three repeated-batch treatments. Letters (a–l) indicate statistically significant differences between bioreactors at the 5% level of significance.

These findings can be correlated with the results of a previous study, in which an integrated system consisting of FTW and CW showed reductions in COD and BOD of urban wastewater by 71.4% and 55.1%, respectively [50]. In another closely related study, the FTW system intensified with biochar, nutrients, aeration, and antibiotic-degrading bacteria, reducing the BOD and COD by 89 and 88%, respectively [51]. In comparison to other conventional treatment studies, nano-mat-based oxidation method showed reductions in COD and BOD of 70.2% and 54.9%, respectively [48]. These comparison studies show the enhanced performance of the current integrated systems in order to remediate textile wastewater.

(b) Removal of Nutrients

Bioreactors B1–B3 caused 55 to 59% removal of N and P from the wastewater, and B4 removed approximately 65% of both N and P (Table 5). In contrast, B5 resulted in 80% removal of both N and P, and maximum (86%) N and P removal was observed in the wastewater treated with B6. Similarly, maximum reductions in chlorides and sulfates of 41 and 26%, respectively, were observed in B6, which was restored with bacterial inoculation, and intensification with NP and aeration. Previous investigations have reported similar findings in various types of intensified FTW and CW.

In earlier studies, the FTW system intensified with bacterial inoculation reduced N and P by 70% and 40%, respectively, and the CW system supported with bacterial inoculation decreased the N and P level by 84% and 79%, respectively [16,18]. Meanwhile, a study based on a hybrid wetland treatment system reinforced by zeolite for the treatment of industrial wastewater showed N and P reductions of 60.1% and 52.6%, respectively [52]. In a different investigation, CW was utilized to treat hospital wastewater in India, which showed reductions in N and P by 33% and 58%, respectively [53]. In another earlier study, a hybrid system (anaerobic biofilter + FTW + microbial cells) similar to the current study poorly removed N (8.4%) and P (11.4%) [50]. Plants and microbes take up nutrients for their growth and metabolic activities [31,54,55]. Moreover, nutrients are also removed by sedimentation, adsorption, and absorption mechanisms associated with plants and microbes [56].

(c) Trace Metal Removal

The average concentrations of all the tested heavy metals exceeded the NEQS prior to the treatment. However, after the textile wastewater passed through the bioreactors, their levels decreased significantly (Figure 3). Bioreactor B1 removed the heavy metals Cd, Fe, Cu, and Pb by 72%, 32%, 34% and 15%, respectively. The supplementation of AD with NP (B2) improved the metal removal performance of the bioreactor by reducing the levels of Cd, Fe, Cu and Pb by 77%, 35%, 35%, and 16%, respectively. Furthermore, the inclusion of aeration in the FTW and CW (B3) increased the metal uptake efficiency by reducing the levels of Cd, Fe, Cu and Pb by 78%, 38%, 40%, and 28%, respectively.

Similar findings were observed in an earlier study in which industrial wastewater treated by FTW intensified with bacterial inoculation reduced the Fe, Cd, Pb, and Cu concentration in a similar pattern (92%, 75%, 72%, and 58%, respectively) [57]. In some other earlier studies, similar kinds of intensified FTW systems, supported by bacterial augmentation, expressed similar kind of trends in removing trace metals from wastewater [18,47,58].

Table 5. Nitrogen (N), phosphorus (P), chloride, and sulfate contents were measured at the outlets of different units of bioreactors during the remediation of textile effluent.

Bioreactor	Nitrogen (mg L^{-1})					Phosphorus (mg L^{-1})					Chlorides (mg L^{-1})					Sulfate (mg L^{-1})				
	Hours																			
	0	**48**	**96**	**144**	**145**	**0**	**48**	**96**	**144**	**145**	**0**	**48**	**96**	**144**	**145**	**0**	**48**	**96**	**144**	**145**
B1	1.38 ^{b} (0.16)	1.05 ^{c} (0.06)	0.75 ^{d} (0.05)	0.67 ^{d} (0.05)	0.62 ^{d} (0.05)	0.56 ^{b} (0.09)	0.41 ^{d} (0.08)	0.30 ^{d} (0.05)	0.26 ^{d} (0.02)	0.25 ^{d} (0.03)	1600 ^{a} (85)	1520 ^{a} (81)	1342 ^{a} (79)	1240 ^{a} (68)	1200 ^{de} (55)	890 ^{a} (35)	810 ^{a} (31)	764 ^{a} (28)	684 ^{a} (25)	660 ^{a} (24)
B2	50.0 ^{a} (0.80)	41.1 ^{a} (2.60)	33.0 ^{a} (1.90)	23.1 ^{a} (1.50)	21.0 ^{a} (2.02)	10 ^{a} (0.30)	7.85 ^{a} (0.80)	5.20 ^{a} (0.65)	4.75 ^{a} (0.34)	4.3 ^{a} (0.16)	1600 ^{a} (88)	1518 ^{a} (82)	1354 ^{a} (74)	1237 ^{a} (72)	1195 ^{e} (60)	890 ^{a} (34)	812 ^{a} (29)	780 ^{a} (29)	680 ^{a} (25)	656 ^{a} (21)
B3	1.38 ^{b} (0.17)	0.95 ^{c} (0.12)	0.73 ^{d} (0.07)	0.61 ^{d} (0.05)	0.56 ^{d} (0.04)	0.56 ^{b} (0.06)	0.40 ^{d} (0.05)	0.29 ^{d} (0.06)	0.25 ^{d} (0.02)	0.23 ^{d} (0.01)	1600 ^{a} (88)	1520 ^{b} (85)	1298 ^{a} (71)	1190 ^{a} (67)	1001 ^{f} (53)	890 ^{a} (35)	810 ^{a} (31)	747 ^{b} (31)	635 ^{b} (23)	615 ^{b} (21)
B4	1.38 ^{b} (0.16)	1.08 ^{c} (1.70)	0.70 ^{d} (1.50)	0.54 ^{d} (1.30)	0.48 ^{d} (1.28)	0.56 ^{b} (0.12)	0.43 ^{d} (0.08)	0.30 ^{d} (0.06)	0.23 ^{d} (0.08)	0.19 ^{d} (0.05)	1600 ^{a} (86)	1525 ^{a} (81)	1145 ^{b} (65)	1101 ^{b} (57)	1174 ^{e} (50)	890 ^{a} (36)	812 ^{a} (29)	737 ^{b} (26)	610 ^{c} (21)	590 ^{c} (21)
B5	50 ^{a} (0.80)	38.0 ^{b} (2.80)	24.1 ^{b} (1.50)	14.4 ^{b} (0.48)	9.9 ^{b} (0.20)	10 ^{a} (0.30)	6.20 ^{b} (0.75)	3.75 ^{b} (0.22)	2.24 ^{b} (0.24)	1.97 ^{b} (0.12)	1600 ^{a} (87)	1531 ^{a} (79)	1093 ^{b} (64)	1021 ^{c} (55)	974 ^{g} (43)	890 ^{a} (35)	809 ^{a} (30)	718 ^{b} (25)	600 ^{c} (21)	585 ^{c} (15)
B6	50 ^{a} (0.80)	37.0 ^{b} (2.50)	18.6 ^{c} (1.82)	8.1 ^{c} (0.31)	6.6 ^{c} (0.25)	10 ^{a} (0.3)	5.90 ^{c} (0.48)	3.08 ^{c} (0.25)	1.87 ^{c} (0.14)	1.33 ^{c} (0.05)	1600 ^{a} (89)	1495 ^{a} (81)	991 ^{c} (65)	927 ^{d} (49)	932 ^{g} (38)	890 ^{a} (34)	810 ^{a} (33)	690 ^{c} (28)	592 ^{c} (21)	561 ^{d} (14)

B1 (bioreactor), B2 (bioreactor intensified with nitrogen and phosphorus), B3 (bioreactor intensified with aeration), B4 (bioreactor intensified with bacteria), B5 (bioreactor intensified with nitrogen, phosphorus, and bacteria), and B6 (bioreactor intensified with nitrogen, phosphorus, aeration and bacteria). The wastewater samples were collected at 0 h (untreated textile wastewater), 48 h (outlet of anaerobic digester), 96 h (outlet of floating treatment wetlands), 144 h (outlet of constructed wetlands), and 145 h (outlet of sand filter). The error bars indicate the standard errors of three repeated-batch treatments. Letters indicate statistically significant differences between bioreactors at the 5% level of significance. Each value is the mean of three repeated-batch treatments; the standard deviation of three repeated-batch treatments is presented in parenthesis, and the alphabets (^{a}–^{g}) represent significant/nonsignificant differences among the bioreactors.

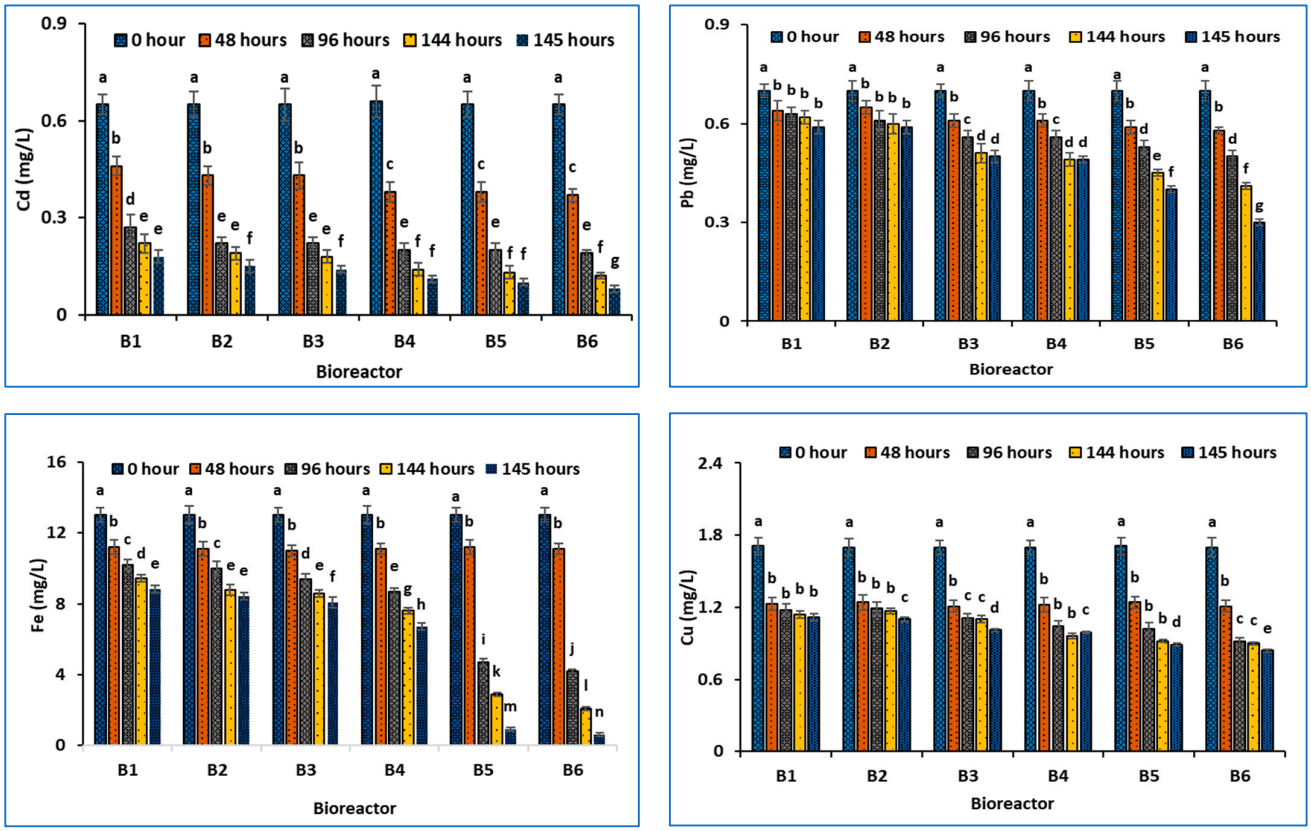


Figure 3. Heavy metal (Cd, Fe, Cu, and Pb) removal by bioreactors (B1–B6) during remediation of textile wastewater. B1 (bioreactor), B2 (bioreactor intensified with nitrogen and phosphorus), B3 (bioreactor intensified with aeration), B4 (bioreactor intensified with bacteria), B5 (bioreactor intensified with nitrogen and phosphorus and bacteria), and B6 (bioreactor intensified with nitrogen, phosphorus, aeration and bacteria). The wastewater samples were collected at 0 h (untreated textile wastewater), 48 h (outlet of anaerobic digester), 96 h (outlet of floating treatment wetlands), 144 h (outlet of constructed wetlands), and 145 h (outlet of sand filter). The error bars indicate the standard errors of three repeated-batch treatments. Letters (a–n) indicate statistically significant differences between bioreactors at the 5% level of significance.

Bioreactor B4, which was inoculated with bacteria from the FTW and CW, presented a performance similar to that of B3. In B5, supplementation with bacteria and NPs increased the metal removal efficiency by reducing the levels of Cd, Fe, Cu, and Pb by 84%, 92%, 48%, and 42%, respectively. The maximum elimination of these toxic metals was observed in B6, which was empowered with bacteria, NPs, and aeration, and here, the levels of Cd, Fe, Cu, and Pb were reduced by 87%, 95%, 50%, and 57%, respectively.

The adsorption and/or absorption of metals by plants and their associated microbes may explain the efficient removal of these metals. Previous investigations have indicated that introduced bacteria facilitate the absorption of trace metals by increasing their bioavailability, adsorbing metallic ions onto bacterial cell walls, and enhancing bioavailable metal uptake by plants [56,59]. Bacteria effectively remove trace metals through trapping in biofilms on roots, subsequently leading to the formation of metal sulfides [60]. Processes such as the binding of metals with fine particles, sequestration of metal ions, iron plaque formation, and bacterial oxidation are crucial for the removal of metals from contaminated water [56,57].

Although several studies were performed to evaluate the efficiency of FTW and CW for the remediation of different types of wastewaters [16,18]. In addition to this, there are also some studies, in which different integrated systems were developed and evaluated for

their bioremediation potential. In most of these studies limited number of water quality parameters were evaluated, and missing the analysis of key pollutants [61–64]. In another study, closely related to the current study, an integrated system consisting of an anaerobic biofilter, FTW and microbial fuel cells only moderately reduced BOD (51%) and COD (71%) also showed poor performance in removing N (8.4%) and P (11.4%) [50]. While in the current study, the integrated system consisting of AD, FTW, CW, and SF, intensified with nutrients, bacteria, and aeration, evaluated the maximum water quality parameters and key pollutants comprehensively and displayed efficient results.

(d) Persistence of the Inoculated Bacteria

In phytoremediation, pollutants undergo degradation and mineralization through the catabolic activities of microbial communities associated with plants. Consequently, overall treatment is linked to the population of microbes [30,57]. In the present study, the bacterial persistence in the roots and shoots of *P. australis* collected from different bioreactors with FTW and CW was determined (Table 6). The maximum quantities of the inoculated bacteria detected in the roots and shoots of the plants increased with increasing NP concentration and aeration (B6). A greater population of inoculated bacteria was noted in the interior of the roots than in the shoot interior. Many earlier studies also reported the survival and metabolic activities of inoculated bacteria in the roots and shoots of plants cultivated to establish CW and FTW [15,18,56].

Table 6. Persistence of inoculated bacteria in the root interior and shoot interior of *P. australis* cultivated in floating treatment wetland (FTW) and constructed wetland (CW) of the bioreactors (B4–B6).

Bioreactor	Cfu $g^{-1} \times 10^4$											
	Acinetobacter junii **15**				*Pseudomonas monteilii* **20**				*Rhodococcus* **sp. 39**			
	Root Interior		**Shoot Interior**		**Root Interior**		**Shoot Interior**		**Root Interior**		**Shoot Interior**	
	FTW	**CW**	**FTW**	**CW**	**FTW**	**CW**	**FTW**	**CW**	**FTW**	**CW**	**FTW**	**CW**
B4	2.70 ^{c} (0.50)	3.81 ^{c} (0.30)	0.70 ^{c} (0.14)	0.60 ^{c} (0.12)	2.80 ^{c} (0.40)	2.70 ^{c} (0.30)	0.91 ^{c} (0.19)	0.83 ^{c} (0.12)	3.52 ^{c} (0.27)	2.71 ^{c} (0.28)	0.74 ^{c} (0.13)	0.88 ^{c} (0.11)
B5	3.41 ^{b} (0.23)	4.10 ^{b} (0.30)	1.51 ^{b} (0.12)	1.01 ^{b} (0.11)	4.21 ^{b} (0.30)	4.61 ^{b} (0.30)	1.61 ^{b} (0.11)	1.51 ^{b} (0.10)	4.90 ^{b} (0.30)	4.70 ^{b} (0.23)	2.11 ^{b} (0.16)	1.74 ^{b} (0.15)
B6	4.80 ^{a} (0.40)	4.61 ^{a} (0.30)	1.91 ^{a} (0.13)	1.82 ^{a} (0.13)	5.20 ^{a} (0.40)	5.11 ^{a} (0.31)	2.30 ^{a} (0.20)	2.10 ^{a} (0.18)	5.80 ^{a} (0.35)	5.32 ^{a} (0.29)	2.40 ^{a} (0.17)	2.13 ^{a} (0.16)

B4 (bioreactor intensified with bacteria), B5 (bioreactor intensified with bacteria, nitrogen and phosphorus), and B6 (bioreactor intensified with bacteria, nitrogen, phosphorus, and aeration). The error bars indicate the standard errors of three repeated-batch treatments. Letters (a–c) indicate statistically significant differences between bioreactors at the 5% level of significance.

(e) Effects on Plant Growth

The effects of bacterial inoculation and various treatments (nutrients, aeration, and bacteria) on the growth of *P. australis* cultivated in the FTW and CW were investigated via measurements of plant growth parameters, including shoot biomass, root biomass, shoot length, and root length (Table 7). A comparison of the two wetland systems revealed that the growth and biomass of *P. australis* were greater in the FTW than in the CW. Among the various bioreactors, the growth of plants was maximum in B6 having bacterial inoculation, and intensification with NP and aeration, followed by this in B5, in which the growth of plants increased with bacterial inoculation and intensification with NP. Earlier investigations also reported that the intensification of wetlands with nutrients, aeration and bacteria improved plant growth [56,64].

Table 7. Effects of bacterial inoculation on the biomass, root length, and shoot length of *Phragmites australis* cultivated in the floating treatment wetland (FTW) and constructed wetland (CW) of the bioreactors (B1–B6).

Bioreactor	Germination Rate (%)		Average Root Length (mm)		Average Total Length (mm)	
	UW	TW	UW	TW	UW	TW
B1	30 ^{a} (3.0)	75 ^{b} (5.3)	11.1 ^{e} (0.60)	23.0 ^{d} (1.30)	20.1 ^{e} (0.80)	52.1 ^{d} (2.60)
B2	31 ^{a} (2.6)	81 ^{ab} (4.8)	14.0 ^{c} (0.70)	30.1 ^{b} (1.60)	25.0 ^{b} (0.80)	51.3 ^{d} (2.50)
B3	31 ^{a} (4.3)	82 ^{a} (3.6)	13.1 ^{d} (0.50)	27.1 ^{c} (2.30)	23.1 ^{d} (0.70)	48.0 ^{e} (2.0)
B4	29 ^{a} (3.5)	80 ^{ab} (5.4)	14.0 ^{c} (0.45)	29.3 ^{b} (1.30)	24.0 ^{c} (0.80)	49.0 ^{e} (2.0)
B5	30 ^{a} (2.8)	82 ^{a} (3.2)	15.1 ^{b} (0.45)	30.0 ^{b} (1.85)	25.2 ^{b} (0.80)	51.0 ^{d} (1.95)
B6	31 ^{a} (3.5)	86 ^{a} (3.5)	16.2 ^{a} (0.70)	32.0 ^{a} (1.65)	27.1 ^{a} (0.85)	52.1 ^{d} (1.75)

B1 (bioreactor), B2 (bioreactor intensified with nitrogen and phosphorus), B3 (bioreactor intensified with aeration), B4 (bioreactor intensified with bacteria), B5 (bioreactor intensified with nitrogen, phosphorus and bacteria), and B6 (bioreactor intensified with nitrogen, phosphorus, aeration and bacteria). The wastewater samples were collected at 0 h (untreated textile wastewater), 48 h (outlet of anaerobic digester), 96 h (outlet of floating treatment wetland), 144 h (outlet of constructed wetland), and 145 h (outlet of sand filter). The error bars indicate the standard errors of three repeated-batch treatments. Letters (a–e) indicate statistically significant differences between bioreactors at the 5% level of significance.

(f) Phytotoxicity Analysis

A phytotoxicity assessment revealed that untreated textile wastewater caused 60 to 70% inhibition of seed germination and restricted the growth of the total root length (Table 8). The wastewater treated by all the bioreactors (B1–B6) improved the seed germination and overall growth of the saplings. The wastewater treated by the bioreactor with bacteria, NPs, and aeration (B6) resulted in the greatest degree of seed germination (86%), with average root and total lengths of 32 mm for the radicle and 52 mm for the plumule. Several earlier studies reported that the application of FTW and CW reduces the toxicity of wastewater, including textile wastewater [18,31,40]. This wheat's seed germination assay is not sufficient to evaluate the toxicity of the water. Therefore, more toxicity assays, such as comet, Ames, genotoxicity, and fish toxicity, should be performed to evaluate the toxicity of treated and untreated textile wastewater [65,66].

This is a bench-scale batch study performed in the ambient conditions to treat the textile wastewater; however, we should perform more studies at a larger scale in the textile industry. We should consider the flow and retention time, and pollution reduction in the wastewater. There may be a high and continuous flow of wastewater in a textile industry and the presence of high concentrations of inhibitory chemicals, such as dyes and salts, in the wastewater, which may affect plant growth and the efficiency of the integrated system. Moreover, the duration of this study is small, and in long-term operation, there may be clogging in CW, and the performance of the bioreactor may varies due to variation in weather conditions and microbial population in AD, FTW, and CW.

Table 8. Phytotoxicity assessment of textile wastewater treated with different bioreactors (B1–B6).

Bioreactor	Biomass (g)								Length (cm)			
	Root				Shoot				Root		Shoot	
	Fresh		Dry		Fresh		Dry					
	FTW	CW	FTW	CW	FTW	CW	FTW	CW	FTW	CW	FTW	CW
B1	168 ^{d} (9.0)	153 ^{b} (8.6)	32 ^{b} (2.5)	20 ^{c} (2.0)	840 ^{c} (19.0)	594 ^{c} (17.0)	116 ^{c} (7.0)	95 ^{c} (6.0)	64.9 ^{b} (2.6)	52.1 ^{b} (2.1)	221.0 ^{c} (6.7)	212.2 ^{b} (4.7)
B2	213 ^{b} (10.0)	197 ^{a} (12.0)	44 ^{a} (3.2)	33 ^{a} (2.8)	977 ^{a} (21.0)	834 ^{a} (18.0)	141 ^{a} (8.0)	133 ^{a} (7.5)	71.6 ^{a} (2.7)	57.3 ^{a} (2.4)	253.2 ^{a} (6.2)	228.3 ^{a} (7.3)
B3	180 ^{c} (9.0)	161 ^{b} (9.0)	37 ^{b} (3.1)	25 ^{b} (2.0)	861 ^{b} (20.0)	711 ^{b} (18.0)	124 ^{b} (6.5)	100 ^{b} (6.0)	66.7 ^{b} (2.4)	53.8 ^{b} (2.1)	230.8 ^{b} (6.1)	213.3 ^{b} (5.3)
B4	181 ^{c} (11.0)	163 ^{b} (9.0)	38 ^{b} (3.0)	26 ^{b} (2.5)	864 ^{b} (18.0)	714 ^{b} (16.0)	123 ^{b} (6.5)	102 ^{b} (5.0)	67.0 ^{b} (2.4)	54.0 ^{b} (2.1)	231.1 ^{b} (4.8)	213.6 ^{b} (4.3)
B5	214 ^{b} (13.0)	198 ^{a} (11.0)	46 ^{a} (3.3)	34 ^{a} (3.0)	980 ^{a} (21.0)	836 ^{a} (18.0)	145 ^{a} (8.0)	135 ^{a} (7.0)	71.8 ^{a} (2.7)	57.6 ^{a} (2.3)	253.6 ^{a} (4.6)	228.6 ^{a} (5.6)
B6	239 ^{a} (12.0)	209 ^{a} (9.5)	48 ^{a} (3.5)	37 ^{a} (3.0)	997 ^{a} (22.0)	841 ^{a} (17.0)	149 ^{a} (8.5)	137 ^{a} (7.0)	73.4 ^{a} (3.1)	57.9 ^{a} (2.5)	255.1 ^{a} (4.1)	229.0 ^{a} (5.4)

B1 (bioreactor), B2 (bioreactor intensified with nitrogen and phosphorus), B3 (bioreactor intensified with aeration), B4 (bioreactor intensified with bacteria), B5 (bioreactor intensified with nitrogen, phosphorus, and bacteria), and B6 (bioreactor intensified with nitrogen, phosphorus, aeration, and bacteria). Each value is the mean of three repeated-batch treatments; the standard deviation of three repeated-batch treatments is presented in parenthesis, and the alphabets (a–d) represent significant/nonsignificant differences among the bioreactors. UW (untreated wastewater), and TW (treated water).

4. Conclusions

This study responds to Pakistan's growing regulatory push under SDG 6 by addressing a persistent gap in industrial wastewater treatment—particularly in high-discharge textile zones such as Faisalabad, where untreated effluent continues to pollute surface and groundwater systems. The pilot-scale deployment of a four-stage, nature-based bioreactor integrating AD, FTW, CW, and SF proved effective in remediating complex textile wastewater. However, the targeted intensification—through nutrient amendment, aeration, and the introduction of locally isolated dye-degrading bacterial strains—transformed the system into a high-performance, regulation-compliant solution. The treated effluent met Pakistan's NEQS discharge limits and exhibited no residual toxicity, confirming its safe reuse potential in applications such as horticulture. Importantly, the inoculated bacteria persisted in the plant roots and shoots, suggesting that long-term microbial–plant synergy is essential for stable operation. This pilot not only validates the technical and ecological feasibility of intensified nature-based systems but also offers a replicable model for decentralized, low-cost industrial wastewater management in developing countries. Bridging the divide between policy mandates and on-ground implementation, this approach presents a scalable pathway for aligning local action with global water sustainability goals.

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