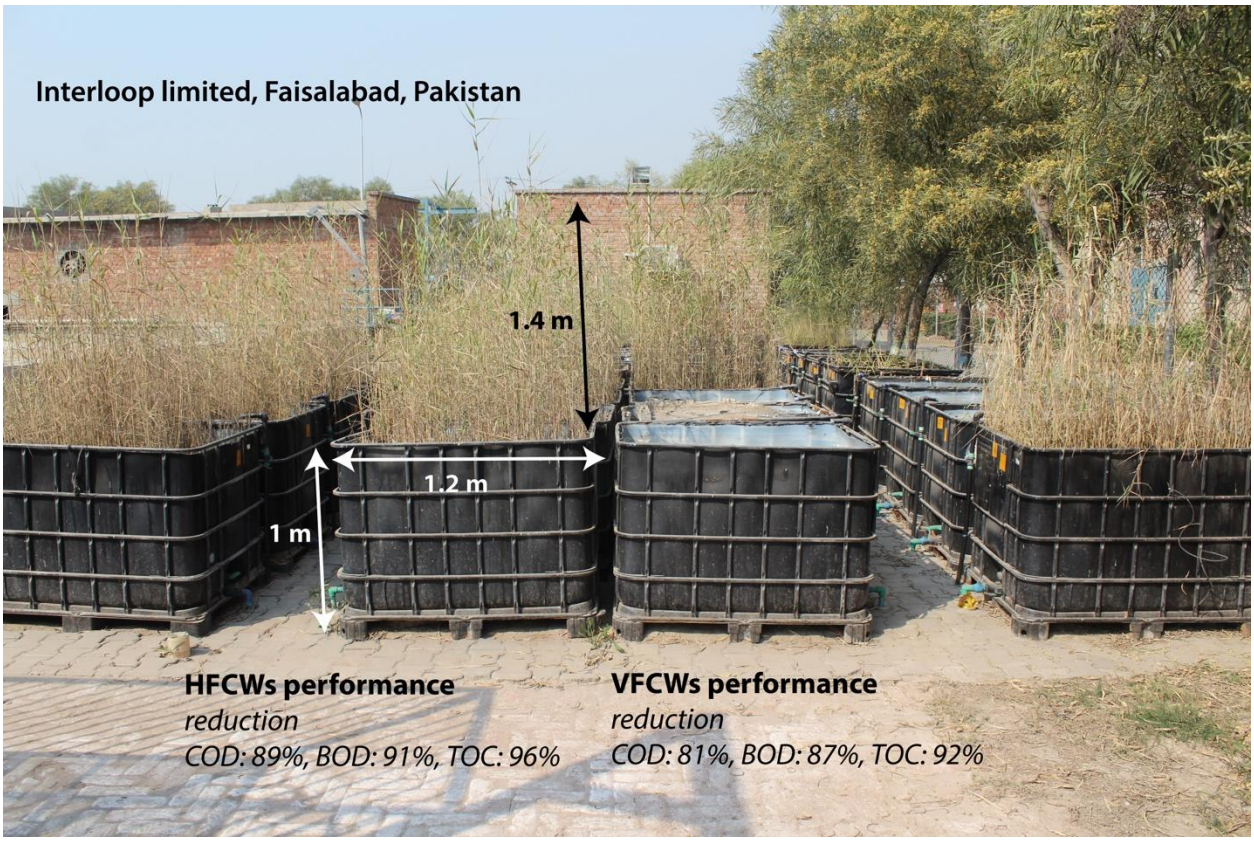
*Graphical Abstract



Highlights

Pilot-scale remediation of textile bleaching effluent was carried out via constructed wetlands.

The performance of HFCWs was better than the VFCWs.

Augmentation of bacterial endophytes further enhanced remediation in both wetland variants.

This study is step forward to the field-scale application of endophyte-assisted CWs.

Remediation of textile bleaching effluent by bacterial augmented horizontal flow and vertical flow constructed wetlands: a comparison at pilot scale

ABSTRACT

Fabric bleaching is one of the most widely used process of the textile industry that also produces a significant amount of highly polluted wastewater. Previously, expensive and chemically extensive conventional remediation systems were used to treat bleaching effluent. Despite this, the potential of constructed wetlands (CWs) as a treatment system remains un-investigated. Furthermore, most research on the use of CWs for textile effluents are conducted at laboratory scale and therefore further research at field-scale is timely. This study compares the efficacy of bacterial augmented vertical flow constructed wetlands (VFCWs) and horizontal flow constructed wetlands (HFCWs) for the remediation of textile bleaching wastewater at pilot scale. To this end, CWs macrocosms of 1000 L water capacity were planted with *Phragmites australis* and inoculated with bacterial strains possessing pollutant degradation and plant growth-promoting traits. The results showed that both variants of CWs were effective in attenuating pollutants from the wastewater; however, performance of HFCWs exceeded that of the VFCWs for almost every pollutant measure undertaken. For HFCWs, a significant reduction in COD (89%), BOD (91%), TOC (96%), and toxicity was achieved in a period of 72 h during the first month of operation. Bacterial inoculation in CWs further improved the system's performance and these bacteria also exhibited persistence in the rhizoplane (43%), root interior (56%) and shoot interior (29%) of *P. australis*. This study therefore suggests that the bacterial augmented HFCWs is a suitable approach for industrial scale textile bleach wastewater treatment.

Keywords: Textile bleaching effluent, constructed wetlands, phytoremediation, textile wastewater

1. INTRODUCTION

Textile bleaching is a crucial step in the wet processing of greige material (Eren et al., 2009). Greige material is an unfinished woven or knitted cotton fabric which is not yet bleached (Athikiat, 2013); however, the removal of natural colored impurities is essential for desired finishing or coloration (Hao et al., 2000; Sevimli et al., 2002; Narendra, 2013). During decolorization, cotton fabric is exposed to hydrogen peroxide in the presence of basic media, e.g. caustic soda, detergent, and hydrogen peroxide stabilizer. The process is therefore chemical, water, and energy intensive, which generates a large amount of wastewater. This wastewater typically has high total dissolved solids (TDS) and low organic content (Sivakumar et al., 2013); and contains salts and chlorinated organic substances (Balcioglu and Arslan, 1998). This makes the bleaching effluent to be highly toxic for discharge without any pre-treatment. In recent years, several technologies have been tested for developing cleaner and sustainable technologies for the treatment of textile industry effluents.

Conventional treatment technologies are effective in the remediation of textile effluent, including the wastewater generated after the bleach bath (Balcioglu and Arslan, 1998). Nevertheless, conventional technologies have the drawbacks of greater use of sulphuric acid, cationic polymers and toxic sludge generation. Moreover, in developing countries, these technologies are usually not affordable due to high operational and maintenance costs (Zhang et al. 2014). In this regard, constructed wetlands (CWs) are an ecological alternative to conventional traditional methods, and they have shown immense potential in the remediation of a

variety of effluents generated from the industry (Arias and Brix 2005; Vymazal, 2013; Zhang et al., 2014). Principally, CWs incorporate rock or gravel matrix through which effluent is passed either horizontally or vertically depending upon the design and type of treatment. During this passage, it comes into contact with biofilms and roots and rhizomes. Here, the interaction between the microbial community, plant roots, and granular medium results in adsorption, degradation, and/or uptake of toxic compounds ultimately improving the water quality (Shehzadi et al., 2014). However, the presence of toxic chemicals in industrial wastewater often inhibits the performance of microbial community and plants which affects efficacy of the wastewater treatment system (Pandey et al., 2009). To cope with these constraints, endophytic bacteria which are capable of degrading pollutants and promoting plant health have been being extensively used in CWs for effective phytoremediation potential (Shehzadi et al. 2014; Ijaz et al., 2015; Rehman et al., 2018). Previous research has shown that, by utilizing this process, it is possible to artificially develop a biofilm on the roots and gravel of CWs that improves degradation of organic pollutants and facilitate plant metabolic growth (Afzal et al. 2014a; Fatima et al. 2016; Mitter et al. 2013; Hussain et al., 2018a,b).

The successful use of CWs has been demonstrated for remediation of dye-rich textile effluents (Hussain et al., 2018a,b). However, it remains unclear how effective this might be for the treatment of textile bleaching wastewater. Therefore, this study is a follow-up to the earlier studies that reported remediation of dye-rich textile effluent using CWs but did not specifically look at the remediation of bleaching wastewater. This study is also important for those textile units in which only bleaching is carried out to produce white textile products, i.e. dying is not performed. In these units, three steps are carried out to produce white textile: the first step is bleaching in the presence of detergent, hydrogen peroxide, caustic soda and hydrogen peroxide

stabilizer (oxidative bleaching), the second step is rinsing with cold water followed by a hot wash to remove all the residues of oxidative bleaching (rinsing), the last step is the neutralization of the bleached fabric with citric acid prior to finishing in case of full white, or make the dying for coloration (neutralization). Thus, this research aims to assess and compare the treatability of bleaching wastewater in two variants of CWs, i.e., horizontal flow CWs (HFCWs) and vertical flow CWs (VFCWs), at pilot scale which were additionally augmented with plant growth promoting and pollutant degrading endophytic bacteria. The performance of both systems was further compared in the presence and absence of inoculated bacteria via plant performance, bacterial survival, and improvement in water quality parameters including toxicity reduction. To the best of the authors knowledge, this is the first study at pilot-scale describing the phytoremediation of textile bleaching effluent in CWs.

2. MATERIALS AND METHODS

2.1. Bleaching wastewater characterization

The bleaching wastewater was obtained from the outlet of the bleaching section of Interloop Limited, Faisalabad, Pakistan. The discharge of bleaching section was already neutralized in the third step of bleaching (see introduction). The sampling was undertaken for 12 h at 2 hourly intervals, which were later mixed to make a composite and representative sample for water quality parameter analysis. Briefly, effluent was tested for pH, electrical conductivity (EC), color, chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), total dissolved solids (TDS), total nitrogen (TN), total phosphorous

(TP), and heavy metals [Cd, Fe, and Ni] by using standard protocols (APHA, 2005) (Table 1). Additionally, effluent toxicity was assessed by exposing the fish population (species: *Labeo rohita*) with untreated wastewater (for details, see section 2.6) (Afzal et al., 2008).

2.2. Bacterial strains

Pure cultures of three bacterial endophytes (*Bacillus endophyticus* PISI25, *Microbacterium arborescens* TYSI04, and *Pantoea* sp. TYRI15) were used in this study. The strains chosen had previously been isolated from the plant growing in textile industry wastewater and have demonstrated successful degradation potential (studied in shaken flask experiments; data not shown). The bacterial strains were grown individually at 30 °C for 1 day in Luria-Bertani (LB) broth (Sigma-Aldrich). To prepare the inoculum, cells were harvested by centrifugation (×14,000 rpm) for 5 minutes at 4 °C. The cells were resuspended in 0.9% (w/v) sterile NaCl solution with a ratio of 1:1:1. The cell suspension was adjusted using Turbid Metric Method instructions (Sutton, 2011). Lastly, 1 L of the mixed consortium (inoculum) was inoculated into each CW macrocosm as per the experiment design.

2.3. Construction of CWs

In this study, HFCWs and VFCWs were made in the vicinity of a textile manufacturing facility at Khurrianwala, Faisalabad, Pakistan. Plastic containers of one cubic meter volume (1000 L carrying capacity) were used as macrocosms. For VFCW, plastic sheets with holes were placed

horizontally at 15 cm above the bottom. The substrate was graded in multiple directions. Briefly, a 50 cm layer of coarse gravel (3–5 cm in diameter) was made at the bottom, which also functioned as a supporting layer. The base layer was followed by a 30 cm deep layer of fine gravel (1–2 cm in diameter), which performed the function of the main substrate. Finally, above this a fine gravel layer was added which was 15 cm in depth and made of washed river sand (1–2 mm in diameter). The function of this layer was to spread the effluent and support the plant seedlings. For HFCWs, plastic sheets with drainage holes were used to separate coarse gravel layer of 50 cm, followed by a fine gravel layer of 30 cm, and then a sand layer of 15 cm. One hundred seedlings of *P. australis* were planted in each CWs macrocosm and were allowed to grow for two months in the tap water to develop root network in the porous medium (Figure 1D). We used *P. australis* based on our earlier studies reporting its successful potential in phytoremediation especially in the presence of toxic organic compounds. Moreover, the plant was found to establish a successful partnership with the inoculated bacteria (Saleem et al., 2018b; Rehman et al., 2018).

For functioning of the VFCW, the wastewater was fed to the top layer using a water pump. The textile wastewater permeated through the layers of the CWs and then collected in wastewater reservoir. The collected wastewater was recycled to the influent of each CW. The hydraulic retention time was adjusted to 72 h using fill-and-draw strategy. The various treatments for both VFCWs and HFCWs were: tap water with vegetation (T1), wastewater without vegetation or bacterial inoculum (T2); wastewater with bacterial inoculum but without vegetation (T3); wastewater with vegetation but without bacterial inoculum (T4); and wastewater with vegetation and bacterial inoculum (T5). For the inoculated treatments, the gravel media was augmented with 1 L of the inoculum before starting the experiment. The treatments were arranged in

randomized manner. The established macrocosms during operation can be seen in Figure 1. There were 6 replicates for each treatment. The system was run for 72 h and wastewater samples were obtained at 0 h, 24 h, 48 h, and 72 h intervals.

2.4. Bacterial persistence

It is a well-established fact that persistence of inoculated bacteria in CWs can contribute to the phytoremediation ability of the host plants (Ijaz et al., 2015; Saleem et al., 2018). In this study, we also observed the persistence and survival of endophytic bacteria in the rhizoplane (the microenvironment in the close vicinity of roots surface), and roots and shoots interior using a plate count method (Afzal et al., 2012). For this purpose, 100 µL of treated water from macrocosms and rhizoplane were spread on to LB plates, which were then incubated at 37 °C for 48 h to measure the colony forming units (CFUs). Likewise, bacterial persistence within plant roots and shoots was studied by plating ground slurry of plant tissues on LB agar plates. The slurry was prepared after surface sterilization, followed by grinding in the presence of 0.9% NaCl solution in a pestle and mortar. At least 20 distinct colonies were picked from the plates and subjected to polymerase chain reaction targeting intergenic spacer (IGS) region. Finally, the identity of isolates for inoculated bacteria was confirmed via restriction fragment length polymorphism (RFLP) (Saleem et al., 2018b).

2.5. Plant biomass

The plants were harvested at the end of three months of experimental period to measure their growth, specifically root length, shoot lengths, and biomass (Rehman et al., 2018). Briefly, plants were cut 10 cm above the surface of the gravel bed, and their shoot lengths were determined with

a measuring scale. Likewise, roots were collected by digging into the bed and their lengths were measured accordingly. The plant root and shoot samples were taken based on ranked-set sampling criteria to minimize the impact of ranking error (Mehmood et al., 2014).

2.6. Fish toxicity assay

Fish toxicity assay was carried out to assess the toxicity of the wastewater treated by both HFCWs and VFCWs. Briefly, healthy specimens of locally available fish species Rohu, *Labeo rohita* (Family: Cyprinidae, Order: Cypriniformes), were obtained from Faisalabad Fish Hatchery. *L. rohita* was selected due to its local ecological significance. Briefly, the species is present in the local streams and lakes (Khan *et al.* 2017) as well as previously found to accumulate heavy metals above the natural/background levels (Hamid *et al.* 2016). The fishes used in this study had an average weight of ~3.8 ± 0.11 g and body length of ~8.0 ± 0.73 cm. Prior to the experiment, each fish specimen was treated with $KMnO_4$ solution (0.05 %) for two minutes to remove any dermal contaminants. For the toxicity assay, 10 fish specimens were exposed to both treated and untreated wastewater obtained from each macrocosm at an interval of 12 h, 24 h, 48 h and 72 h. The filtered air was provided to the wastewater by air compressor. The fish mortality rate was determined by counting the number of alive fish.

2.7. Data analysis

The data of water quality parameters, roots and shoots lengths, biomass, and bacterial counts, was analyzed using one-way ANOVA. Finally, Box and Whisker plots were made in R-computing language for presentation and comparison purposes.

3. RESULTS

3.1. Characteristics of bleaching wastewater

Initial water quality parameters analysis demonstrated that bleaching wastewater was highly polluted. Most of the studied parameters were found to be higher than National Environmental Quality Standards (NEQS), of Pakistan. This included COD, BOD, phenol, chlorides, TDS, TSS, and trace metals (see Table 1 for details).

3.2. Performance evaluation

Vegetated VFCWs and HFCWs demonstrated a significant decline in COD, BOD, TOC, TSS, and TDS when compared with unvegetated treatments (i.e., only gravel bed) (Fig. 1 and 2, Table 2). The reduction in pollution was further increased by the augmentation of bacteria in both CWs (T5). Although phytoremediation potential of the both systems was efficient, the performance of HFCWs was better than the VFCWs in all treatments. For instance, in the bacterial-augmented HFCWs, COD was decreased from 690 to 89 mg l^{-1}, BOD from 250 to 22 mg l^{-1}, and TOC from 120 to 8 mg l^{-1} after 72 h (Fig. 2). This decrease in pollution parameters was significantly higher when compared with the treatments containing plants and bacteria separately, i.e., in the presence of bacteria and plants, respectively, COD was reduced to 301 mg l^{-1} and 162 mg l^{-1}; BOD was reduced to 135 mg l^{-1} and 85 mg l^{-1}; and TOC was reduced to 53 mg l^{-1} and 33 mg l^{-1}, after 72 h of the treatment. Similarly, maximum reduction in nutrients (TN and TP) as well as heavy metals (Cd, Fe, and Ni) were observed for the HFCWs augmented with the bacteria (for details, see Table 2 and 3). In contrast to this, effective but lower removal rates were seen for VFCWs in all the treatments. Briefly, COD was reduced to 128 mg l^{-1} for bacterial augmented

VFCWs, 327 mg l^{-1} in the presence of bacteria alone, and 188 mg l^{-1} in the presence of vegetation alone. Likewise, BOD was reduced to 32 mg l^{-1} in the mesocosms containing both plants and bacteria; up to 145 mg l^{-1} in the presence of bacteria alone, and up to 92 mg l^{-1} in the presence of vegetation alone. Similar observations were recorded for other pollution parameters as well (see Fig. 2 and Table 2 and 3).

3.3. Reduction in toxicity

A fish toxicity assay showed a significant toxicity reduction in the bleaching wastewater by passing it through both VFCWs and HFCWs (Table 4). However, among two wetlands, more toxicity reduction was seen in HFCWs, and the presence of vegetation and bacterial inoculum together (T4) displayed maximum toxicity reduction. No fish death was observed in the HFCWs having bacterial inoculation, whereas one fish died in the HFCWs without bacterial augmentation.

3.4. Effect on plant growth

In order to elucidate effect of bleaching wastewater and bacterial inoculation on the growth of *P. australis* vegetated in VFCWs and HFCWs, plant growth parameters such as root biomass, shoot biomass, and root length and shoot length were measured (Table 5). Bleaching wastewater significantly reduced the biomass, roots and shoots lengths of the plants vegetated in VFCWs and HFCWs. However, among both wetland systems, the growth and biomass of *P. australis* was higher in the HFCWs as compared to VFCWs. Moreover, bacterial inoculation also improved the growth of plant and plants gave maximum growth with bacterial inoculation in HFCWs.

Nevertheless, visual observations revealed that plant health status was compromised after the treatment because the shoots turned yellowish (Fig. 1E).

3.5. Persistence of bacterial endophytes

To evaluate the persistence of the endophytic bacteria, firstly, CFUs were estimated in the rhizoplane, root interior, and shoot interior of *P. australis* (Fig. 3). Briefly, fewer numbers of bacteria were found in the rhizosphere, root interior and shoot interior of *P. australis* vegetated in VFCWs as compared to that of vegetated in HFCWs. RFLP analysis confirmed that, in HFCWs, 43%, 56% and 29% of the bacterial community in the rhizoplane, root interior, and shoot interior was our inoculated bacterial community; whereas in VFCWs, they were recorded to be 37%, 49%, and 31%, respectively. In comparison, a low number of bacteria were found in the water of unvegetated VFCWs and HFCWs (data not shown).

3.6. Long term maintenance

The performance of both HFCWs and VFCWs was optimal in the initial 3-months period, while maximum remediation was seen at the beginning of experiment until the middle of 3rd month. In subsequent months, treatment performance was slightly compromised (supplementary data). The visual observations also revealed that plant health status was compromised because plant shoots started turning yellowish (Figure 1E, taken in the 5th month after exposure).

4. DISCUSSION

Recent investigations reported the successful degradation of textile effluent obtained after the dyeing operating by VFCWs and HFCWs (Hussain et al., 2018a,b). Likewise, potential of bacterial augmentation was found to boost the overall remediation efficiency. In this study, for the first time, textile bleaching wastewater was treated by bacterial augmented VFCWs and HFCWs, and their efficiency was compared. Generally, HFCWs performed better than VFCWs both in the presence and absence of bacterial inoculation and exhibited more reduction in contaminant level of the bleaching wastewater. Bacterial augmentation in both wetland systems improved plant growth, remediation of textile bleach effluent, and toxicity reduction.

Although both constructed wetlands showed efficacy to reduce pollution of the bleach effluent, HFCWs performed better than VFCWs. This might be due to the better growth of plants and a higher population of the bacteria in HFCWs than VFCWs (Hussain et al. 2018a,b). While the efficacy of both wetlands was enhanced by bacterial inoculation, this was more apparent in the HFCWs, as shown by greater reduction of COD, BOD and TDS. This result is consistent with the greater recovery of these bacteria in the HFCWs (see Fig. 3). Similarly, in earlier studies, larger decreases in COD, BOD and TDS was reported in bacterial augmented CWs than the non-inoculated CWs (Shehzadi et al., 2014; Rehman et al. 2018; Hussain et al., 2018a). Shehzadi et al. (2014) and Hussain et al. (2018a) reported significant decrease in these parameters for real textile effluent in a laboratory-scale and pilot-scale experiments, respectively; whereas Rehman et al. (2018) showed successful remediation of the crude oil wastewater. As plants are autotrophic in nature, the plat-associated microorganisms (mainly bacteria) are involved in the mineralization of organic pollutants. In phytoremediation, plant-associated

bacteria colonizing in the rhizosphere, root and shoot utilize organic pollutants as carbon and energy source (Weyens et al., 2009; Khan et al., 2013; Arslan et al., 2016). Most of the organic pollutants are degraded in the rhizosphere by rhizospheric microorganisms. A small amount of organic pollutants are taken up by plants and detoxified by conjugation with plant enzymatic system and stored in the lignin cells of plants. Latter on, these are degraded by endophytic bacteria present in the roots and shoots (Thomas and Germida, 2009; Afzal et al., 2014; Rehman et al., 2018).

It is well established fact that textile effluent is toxic in its nature owing to high usage of chemicals during its processes (Bafana et al. 2009; Khataee et al. 2010; Kadam et al., 2018). Therefore, in this study, the toxicity of bleaching effluent was also observed for different treatments. The untreated bleach effluent exhibited a high level of toxicity, whereas the toxicity level of the wastewater treated by either VFCWs or HFCWs was decreased, particularly in planted systems (Table 4). The mortality of fish might be due to the toxic nature of chemicals present in bleaching process wastewater. Similar findings have been cited by other researchers (Bafana et al., 2009; Shehzadi et al., 2014; Ijaz et al., 2016; Fatima et al., 2018), which indicated that toxicity of water and soil was decreased by bacterial inoculation in the wetlands. High toxicity for untreated textile bleach effluent reveals the possible damages to aquatic organisms owing to the discharge of non-treated bleach effluent in the environment.

In the present study, textile bleach effluent reduced the growth and development of *P. australis* vegetated in either VFCWs or HFCWs. The possible reason for this plant growth reduction might be toxic chemicals present in bleach effluent. Several researchers have reported that textile wastewater is toxic in nature, therefore, it could inhibit plant growth and biomass development (Khandare et al., 2013; Shehzadi et al., 2014, 2016; Hussain et al. 2018a,b).

Nevertheless, bacterial augmentation in both CWs improved plant biomass development. This might be due to a reduction in toxicity of the wastewater by inoculated bacteria which had traits of effluent degradation and plant growth promotion. Many researchers have reported that bacteria can reduce the toxicity of wastewater and can improve plant growth (Ijaz et al., 2015, 2016; Saleem et al., 2018a; Rehman et al., 2018, 2019; Hussain et al., 2018a,b). In this research, inoculated bacteria possessed 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase potential, which leads to decreases in stress symptoms for growing plants during phytoremediation of water and soil (Glick, 2010; Khan et al., 2013; Afzal et al., 2014). However, the lowering in the performance after the 3rd month of the experiment was most likely due to the senescence effect or chronic exposure of the pollutants. Therefore, it is recommended to harvest plant in this period and replant for better results. Nevertheless, further research is recommended to see the molecular response of the plant to the external stressors.

The persistence or survival of the inoculated bacteria in several compartments of CWs are vital for better plant growth and pollutants degradation (Ijaz et al., 2015; Arslan et al., 2017). In this study, augmented bacteria exhibited a high level of persistence in water of both CWs. Moreover, they showed persistence in the rhizoplane, root, and shoot of *P. australis*. It could be owing to the fact that these bacteria have been previously isolated from plants growing well in textile effluent and already adapted to proliferate in such an environment (Shehzadi et al., 2016). In different compartments of CWs, a high proportion of bacteria was found in the rhizoplane, then in water, root interior, and shoot interior. This could be due to plant roots being the source of nutrients for the rhizospheric bacterial community which leads to successful proliferation (Ijaz et al., 2016; Shahid et al., 2018; Hussain et al., 2018b). Among the two CWs systems, more

bacteria were found in different components of HFCWs than VFCWs. This might be due to better growth and development in HFCWs than VFCWs.

CONCLUSIONS

This study reveals that HFCWs are a better choice for the remediation of textile bleach effluent than VFCWs, and bacterial augmentation in CWs improved textile effluent remediation, plant growth, and toxicity reduction. Augmented bacteria showed persistence in the water, rhizosphere, and root and shoot interior of *P. australis*. After treatment in CWs which were inoculated with bacteria, the bleaching effluent met the NEQS of Pakistan for COD, BOD, TDS, TSS, TN, total contents (dissolved and undissolved) of studied heavy metals (Fe, Ni, and Cd); thus, substantially reducing the toxicity of the treated water as compared to the untreated effluent. Nevertheless, visual observations on plant health parameters revealed that the overall health of *P. australis* was compromised in the longer run as plant shoots started turning yellowish. More efforts are required to optimize the operational parameters of the CW_S for maximum remediation of textile bleach effluent.

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Parameter	Unit	Value	NEQS value	APHA method used
Temperature	℃	38 (3.5)	40	2550, Temperature
pH	--	7.5 (0.58)	6-10	4500-H, Electrometric
EC	mS cm^{-1}	5.2 (0.63)	NG	2510-B, Laboratory
Color	(m^{-1})	2.5 (0.47)	NG	2120-D, Spectrophotometric
COD	mg l^{-1}	689 (48)	150	5220-D, Closed reflux colorimetric
BOD	mg l^{-1}	248 (31)	80	5210-B, Biochemical oxygen demand

Table 1. Interloop textile bleaching effluent physicochemical parameters

TOC	mg l^{-1}	307 (38)	NG	5310-B High-temperature combustion
Phenol	mg l^{-1}	0.38 (0.16)	0.1	5530-D, Phenol spectrophotometric
Chloride	mg l^{-1}	2586 (504)	1000	4500-B, Argentometric
Sulphate	mg l^{-1}	275 (18)	600	4500-E, Turbidimetric
Sodium	mg l^{-1}	6348 (530)	NG	3500-B, Flame photometric
Potassium	mg l^{-1}	95 (11)	NG	3500-B, Flame photometric
Calcium	mg l^{-1}	82 (13)	NG	3500-C, EDTA Titrimetric
Magnesium	mg l^{-1}	73 (8)	NG	3500-B, EDTA Titrimetric
Nitrogen	mg l^{-1}	22.5 (4.3)	40	3500-B, EDTA Titrimetric
Phosphorous	mg l^{-1}	13.7 (2.9)	NG	4500-N, The Kjeldahl
Total dissolved solids	mg l^{-1}	3367 (470)	3500	4500-P, Colorimetric
Total solids	mg l^{-1} $CaCO_3$	4722 (580)	NG	2540-B, Dried at 103-105 °C
Total suspended solids	mg l^{-1}	235 (8.5)	150	2540-C, Dried at 180 °C
Total settleable solids	mg l^{-1}	58 (8.3)	NG	2540-F, Settleable solids
Hardness	mg l^{-1}	504 (32)	NG	2340-C, EDTA Titrimetric
Iron	mg l^{-1}	2.35 (1.1)	2.0	3110, Atomic absorption spectrophotometric
Nickel	mg l^{-1}	1.03 (0.05)	1.0	3110, Atomic absorption spectrophotometric
Aluminum	mg l^{-1}	0.71 (0.13)	NG	3110, Atomic absorption spectrophotometric
Chromium	mg l^{-1}	0.19 (0.03)	0.1	3110, Atomic absorption spectrophotometric
Arsenic	mg l^{-1}	0.48 (0.08)	Nil	3110, Atomic absorption spectrophotometric
Cadmium	mg l^{-1}	1.05 (0.14)	0.1	3110, Atomic absorption spectrophotometric

All values are mean of 12 different samples which are collected during the interval of one week (3 months period). Values in parentheses represent standard deviation; NG = Not given in NEQS list.

Table 2. Total nitrogen (TN), total phosphorus (TP), total dissolved solids (TDS), and total suspended solids (TSS) measured in VFCWs and HFCWs during phytoremediation of bleaching effluent.

Treatment	TN (mg l^{-1})				TP (mg l^{-1})				TDS (mg l^{-1})				TSS (mg l^{-1})			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
VFCWs																
Wastewater (T2)	45.7^{a} (3.4)	40.2^{a} (3.1)	35.9^{a} (2.6)	32.8^{a} (4.2)	26.3^{a} (3.3)	23.7^{a} (2.9)	21.3^{a} (2.4)	19.5^{a} (3.4)	3860^{a} (150)	3620^{a} (165)	3582^{a} (138)	3460^{a} (125)	235^{a} (16)	210^{a} (25)	198^{a} (30)	182^{a} (23)
Wastewater & bacteria (T3)	45.7^{a} (3.4)	36.6^{a} (3.7)	31.4^{a} (3.3)	27.3^{a} (2.7)	26.3^{a} (3.3)	20.5^{a} (3.1)	17.8^{a} (2.8)	15.3^{a} (3.3)	3860^{a} (150)	3476^{a} (203)	3383^{a} (180)	3250^{a} (215)	235^{a} (16)	208^{a} (32)	173^{a} (28)	160^{a} (27)
Wastewater & plants (T4)	45.7^{a} (3.4)	32.1^{b} (3.8)	17.2^{b} (2.8)	14.6^{b} (1.7)	26.3^{a} (3.3)	15.8^{c} (2.4)	8.2^{c} (0.7)	3.1^{c} (1.1)	3860^{a} (150)^{a}	3480^{a} (170)	3040^{b} (105)	2280^{c} (160)	235^{a} (16)	175^{ab} (32)	113^{b} (19)	77^{b} (12)
Wastewater & plants + bacteria (T5)	45.7^{a} (3.4)	24.3^{c} (4.4)	11.3^{c} (1.5)	8.3^{d} (1.4)	26.3^{a} (3.3)	10.4^{d} (1.1)	3.8^{e} (0.3)	1.8^{d} (0.5)	3860^{a} (150)	3225^{b} (145)	2250^{c} (118)	1750^{d} (125)	235^{a} (16)	137^{bc} (28)	78^{c} (20)	38^{c} (10)
HFCWs																
Wastewater (T2)	45.7^{a} (3.4)	38.4^{a} (2.8)	31.0^{a} (3.2)	28.2^{a} (3.8)	26.3^{a} (3.3)	19.2^{b} (1.8)	17.5^{b} (1.8)	15.6^{b} (3.7)	3860^{a} (150)	3340^{a} (152)	3058^{b} (148)	2570^{b} (135)	235^{a} (16)	190^{a} (16)	168^{a} (21)	142^{a} (24)
Wastewater & bacteria (T3)	45.7^{a} (3.4)	35.2^{a} (3.1)	27.7^{a} (2.8)	23.4^{a} (3.1)	26.3^{a} (3.3)	17.6^{a} (2.5)	15.8^{a} (3.2)	13.7^{a} (2.7)	3860 (150)^{a}	3275^{a} (217)	2935^{a} (165)	2440^{a} (152)	235^{a} (16)	182^{a} (17)	152^{a} (18)	132^{a} (21)
Wastewater & plants (T4)	45.7^{a} (3.4)	28.7^{b} (2.7)	14.5^{b} (2.2)	9.8^{c} (1.6)	26.3^{a} (3.3)	11.7^{d} (1.9)	6.4^{d} (0.4)	3.5^{c} (0.6)	3860^{a} (150)	3245^{b} (140)	2845^{b} (175)	2240^{c} (180)	235^{a} (16)	158^{b} (24)	85^{c} (11)	46^{c} (8)
Wastewater & plants + bacteria (T5)	45.7^{a} (3.4)	21.8^{c} (3.4)	8.2^{d} (1.1)	3.6^{e} (0.5)	26.3^{a} (3.3)	8.2^{e} (0.8)	2.5^{f} (0.2)	1.2^{d} (0.3)	3860^{a} (150)	3085^{b} (105)	1960^{d} (150)	1450^{e} (172)	235^{a} (16)	108^{c} (13)	48^{d} (8)	23^{d} (7)

Each value represent means of three samples. Means in the same column followed by the same letter are not significantly different at a 5% level of significance, n = 12; values in parentheses exhibit standard deviation.

Metal	Cd (mg l^{-1})				Fe (mg l^{-1})				Ni (mg l^{-1})			
	0h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
							VFCWs					
Wastewater (T2)	1.05^{a} (0.14)	0.98^{a} (0.18)	0.67^{a} (0.15)	0.55^{a} (0.08)	2.35^{a} (0.10)	2.21^{a} (0.08)	2.19^{a} (0.24)	1.72^{a} (0.32)	1.03^{a} (0.15)	1.02^{a} (0.17)	0.74^{a} (0.28)	0.38^{a} (0.12)
Wastewater and bacteria (T3)	1.05^{a} (0.14)	0.95^{a} (0.13)	0.65^{a} (0.18)	0.51^{a} (0.11)	2.38^{a} (0.10)	2.17^{a} (0.23)	1.98^{a} (0.27)	1.63^{a} (0.16)	1.03^{a} (0.15)	1.01^{a} (0.13)	0.68^{a} (0.09)	0.36^{a} (0.08)
Wastewater & plants (T4)	1.05^{a} (0.14)	0.87^{bc} (0.15)	0.58^{ab} (0.12)	0.45^{b} (0.11)	2.35^{a} (0.10)	1.56^{b} (0.05)	1.23^{b} (0.07)	1.11^{b} (0.06)	1.03^{a} (0.15)	0.68^{b} (0.11)	0.47^{b} (0.08)	0.29^{b} (0.04)
Wastewater & plants + Bacteria (T5)	1.05^{a} (0.14)	0.88^{bc} (0.18)	0.51^{b} (0.09)	0.36^{c} (0. 04)	2.35^{a} (0.10)	0.82^{d} (0.04)	0.48^{c} (0.05)	0.14^{d} (0.03)	1.03^{a} (0.15)	0.46^{c} (0.07)	0.18^{d} (0.05)	0.19^{d} (0.03)
							HFCWs					
Wastewater (T2)	1.05^{a} (0.14)	0.92^{b} (0.13)	0.59^{ab} (0.07)	0.48^{ab}(0.05)	2.35^{a} (0.11)	2.25^{a} (0.38)	2.08^{a} (0.35)	1.62^{a} (0.32)	1.03^{a} (0.15)	0.95^{a} (0.13)	0.73^{a} (0.18)	0.38^{a} (0.12)
Wastewater and bacteria (T3)	1.05^{a} (0.14)	0.90^{b} (1.14)	0.57^{ab} (0.08)	0.45^{b} (0.09)	2.35^{a} (0.11)	2.18^{a} (0.14)	1.97^{a} (0.22)	1.54^{a} (0.27)	1.03^{a} (0.15)	0.88^{a} (0.17)	0.64^{a} (0.21)	0.35^{a} (0.14)
Wastewater & plants (T4)	1.05^{a} (0.14)	0.75^{c} (0.15)	0.43^{c} (0.10)	0.28^{d} (0.06)	2.35^{a} (0.11)	1.74^{b} (0.18)	1.21^{b} (0.17)	1.28^{b} (0.17)	1.03^{a} (0.15)	0.98^{a} (0.05)	0.39^{b} (0.04)	0.23^{c} (0.01)
Wastewater & plants + Bacteria (T5)	1.05^{a} (0.14)	0.37^{d} (0.08)	0.18^{d} (0.03)	0.12^{e} (0.03)	2.35^{a} (1.11)	1.45^{c} (0.07)	1.12^{b} (0.02)	0.49^{c} (0.15)	1.03^{a} (0.15)	0.38^{d} (0.05)	0.23^{c} (0.08)	0.17^{d} (0.02)

Table 3. Removal of Cd, Fe, and Ni by VFCWs and HFCWs from textile bleaching effluent

Each value represent mean of three samples. Means in the same column followed by the same letter are not significantly different at a 5% level of significance, n = 12; values in parentheses exhibit standard deviation.

6

Treatment	12 h		24 h		48 h		72 h	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
VFCWs								

7 **Table 4.** Results of fish mortality in treated wastewater by VFCWs and HFCWs

Tap water (T1)	0	5	0	5	0	5	0	5
Wastewater (T2)	2	3	3	2	4	1	5	0
Wastewater & bacteria (T3)	1	4	2	3	2	3	3	2
Wastewater & plants (T4)	0	5	0	5	1	4	1	4
Wastewater & plants + bacteria (T5)	0	5	0	5	0	5	0	5

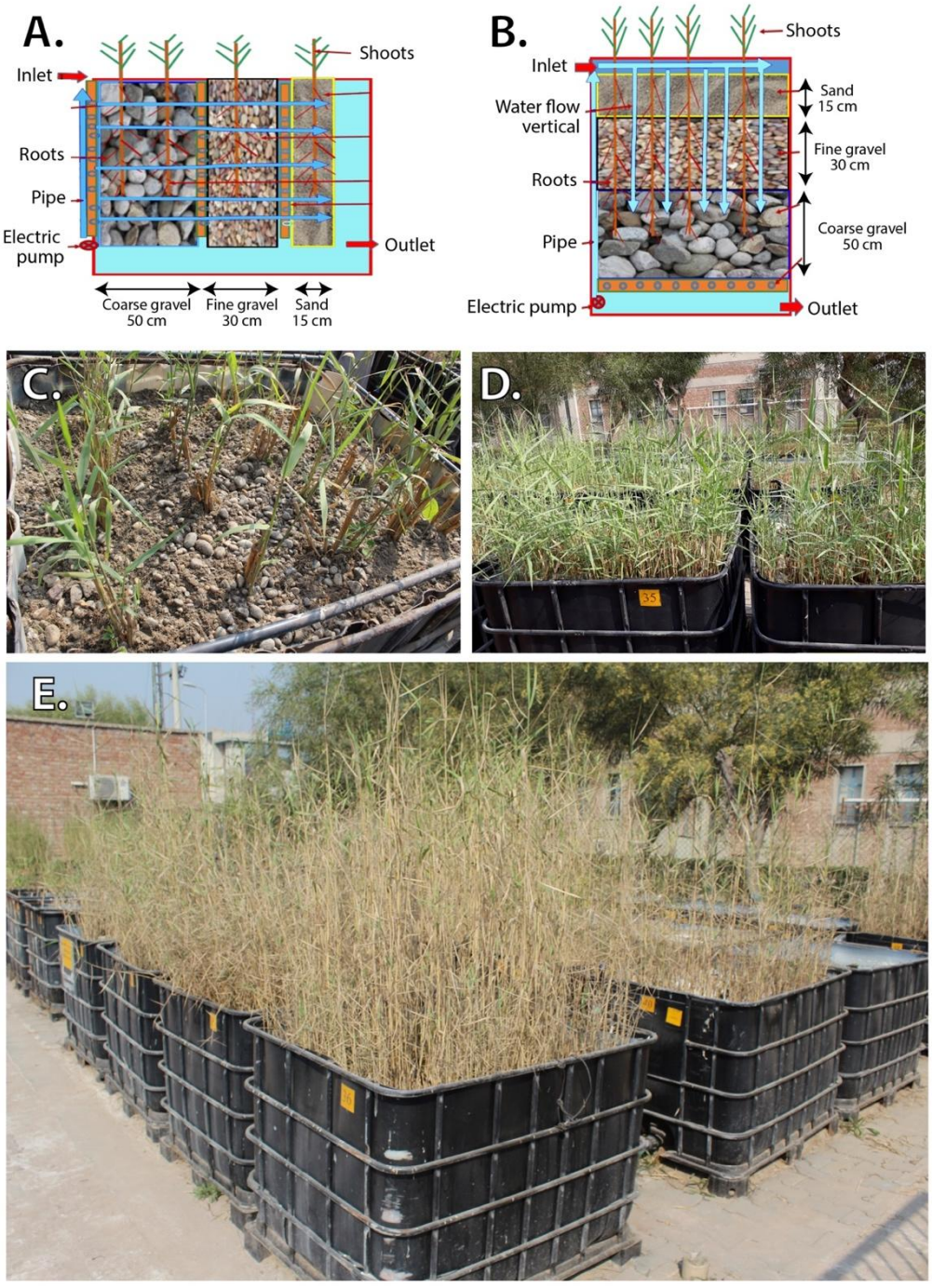
HFCWs

Tap water (T1)	0	5	0	5	0	5	0	5
Wastewater (T2)	2	3	3	2	4	1	5	0
Wastewater & bacteria (T3)	1	4	3	3	3	2	3	2
Wastewater & plants (T4)	0	5	1	4	1	4	1	4
Wastewater & plants + bacteria (T5)	0	5	0	5	0	5	0	5

Table 5: Effect of effluent toxicity and bacterial inoculation on growth of *Phargmites australis*

Treatments	Dry biomass (g)		Length (ft)	
	Root	Shoot	Root	Shoot
	VFCWs			
Tap water (T1)	585[a] (27)	3072[a] (26)	5.8[a] (0.3)	6.5[a] (0.6)
Wastewater & plants (T4)	435[c] (21)	2450[c] (23)	3.2[c] (0.2)	4.7[c] (0.4)
Wastewater & plants + bacteria (T5)	548[b] (24)	2736[b] (30)	4.3[b] (0.2)	5.4[b] (0.3)
	HFCWs			
Tap water (T1)	604[a] (27)	3153[a] (21)	6.1[a] (0.4)	6.8[a] (0.4)
Wastewater & plants (T4)	438[c] (19)	2628[c] (19)	3.4[c] (0.1)	4.9[b] (0.5)
Wastewater & plants + bacteria (T5)	554[b] (23)	1864[b] (23)	4.7[b] (0.2)	5.7[ab] (0.3)

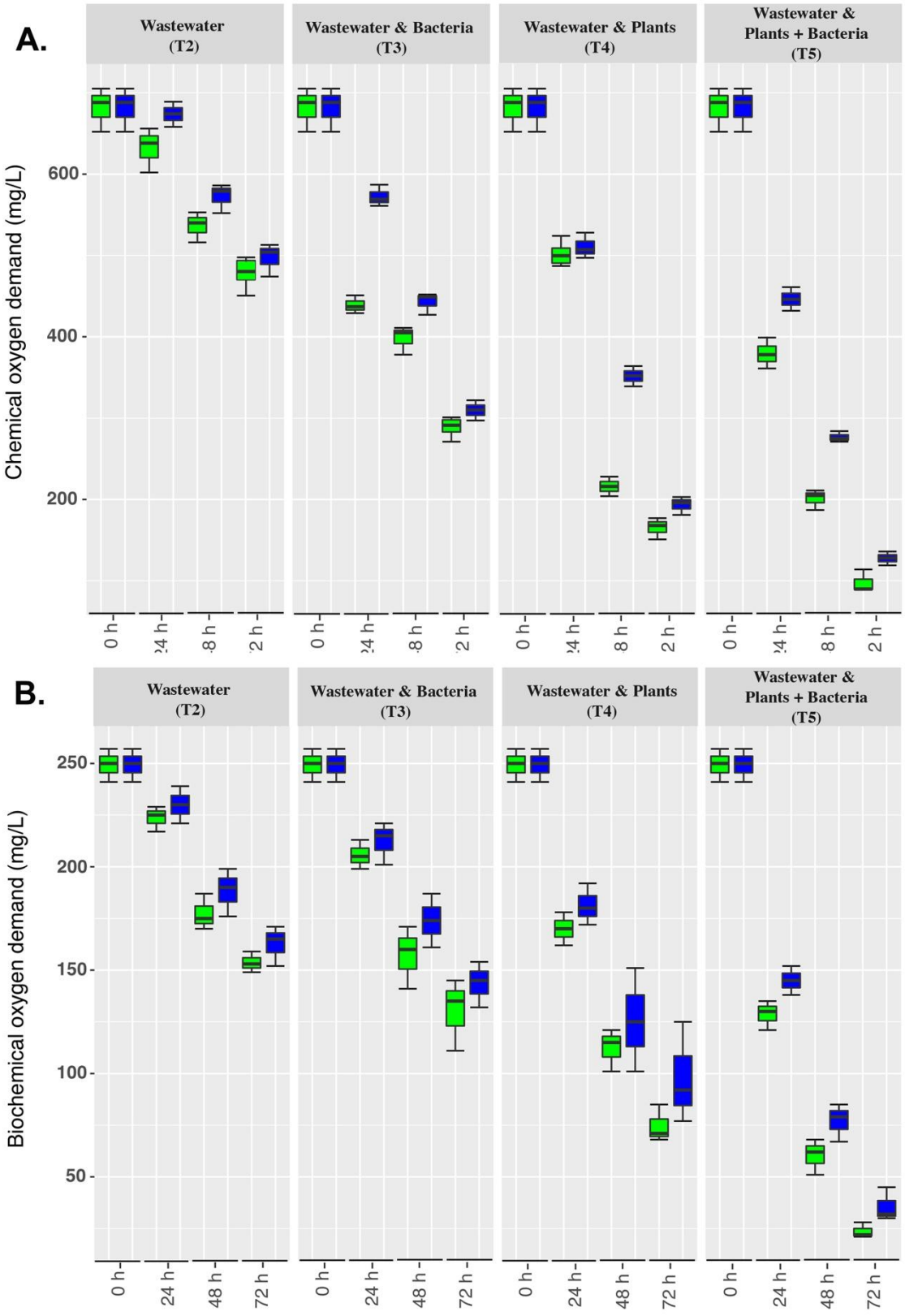
Each value represent means of three samples. Means in the same column followed by the same letter are not significantly different at a 5% level of significance, values in parentheses represent standard deviation.



28

29 **Figure 1:** The design and installation of HFCWs and VFCWs at Interloop, Khurianwala, Faisalabad,
30 Pakistan. (A-B) Schematic representation of HFCWs and VFCWS, respectively, (C-D) planting of
31 *Phragmites australis* in the macrocosms, (E) *P. australis* after the operation of CWs for treatment of
32 bleaching effluent (5^{th} month of experimental period).

33


A.
Wastewater (T2)
Wastewater & Bacteria (T3)
Wastewater & Plants (T4)
Wastewater & Plants + Bacteria (T5)
Chemical oxygen demand (mg/L)
600
400
200
0 h
24 h
48 h
72 h
B.
Wastewater (T2)
Wastewater & Bacteria (T3)
Wastewater & Plants (T4)
Wastewater & Plants + Bacteria (T5)
Biochemical oxygen demand (mg/L)
250
200
150
100
50
0 h
24 h
48 h
72 h

34

35

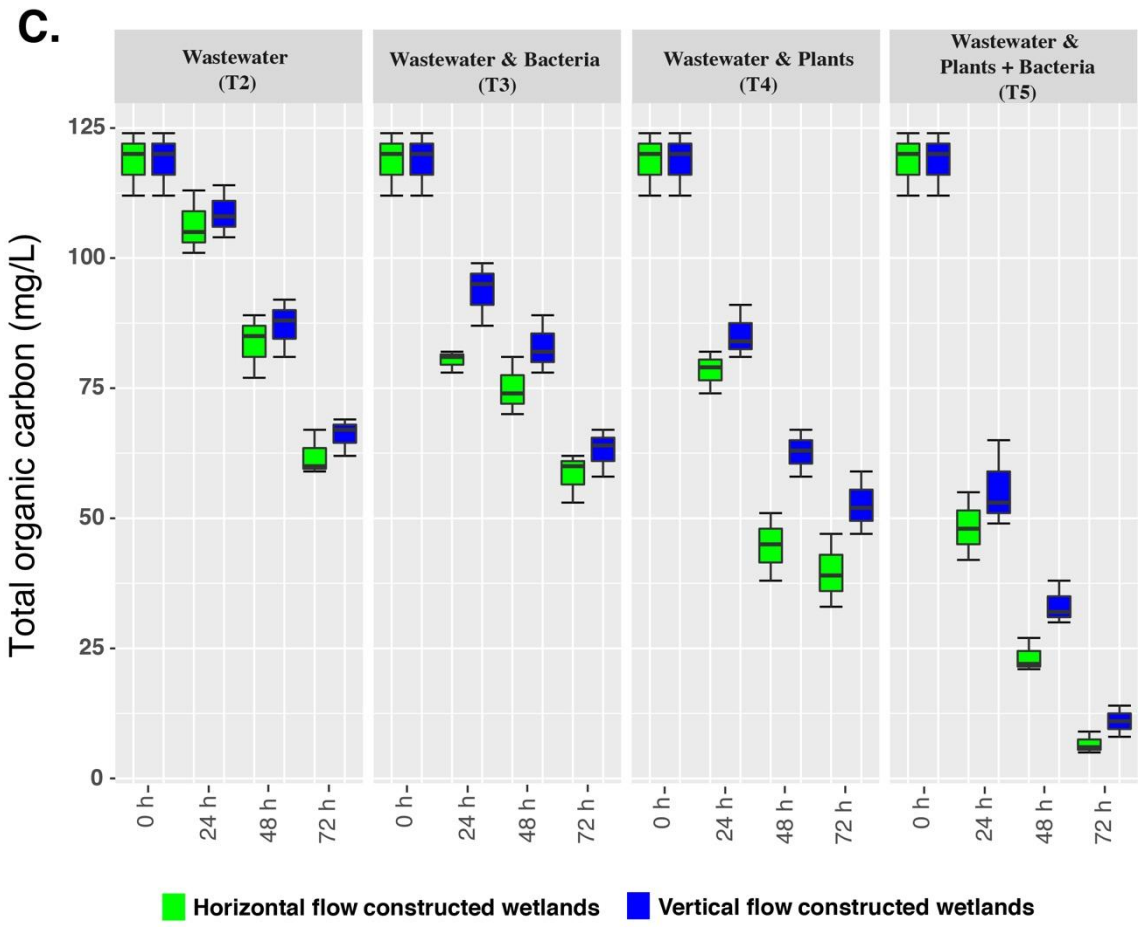


Figure 2: Concentrations of COD (A), BOD (B), and TOC (C) in the bleaching wastewater of textile industry during the operation of HFCWs and VFCWs.

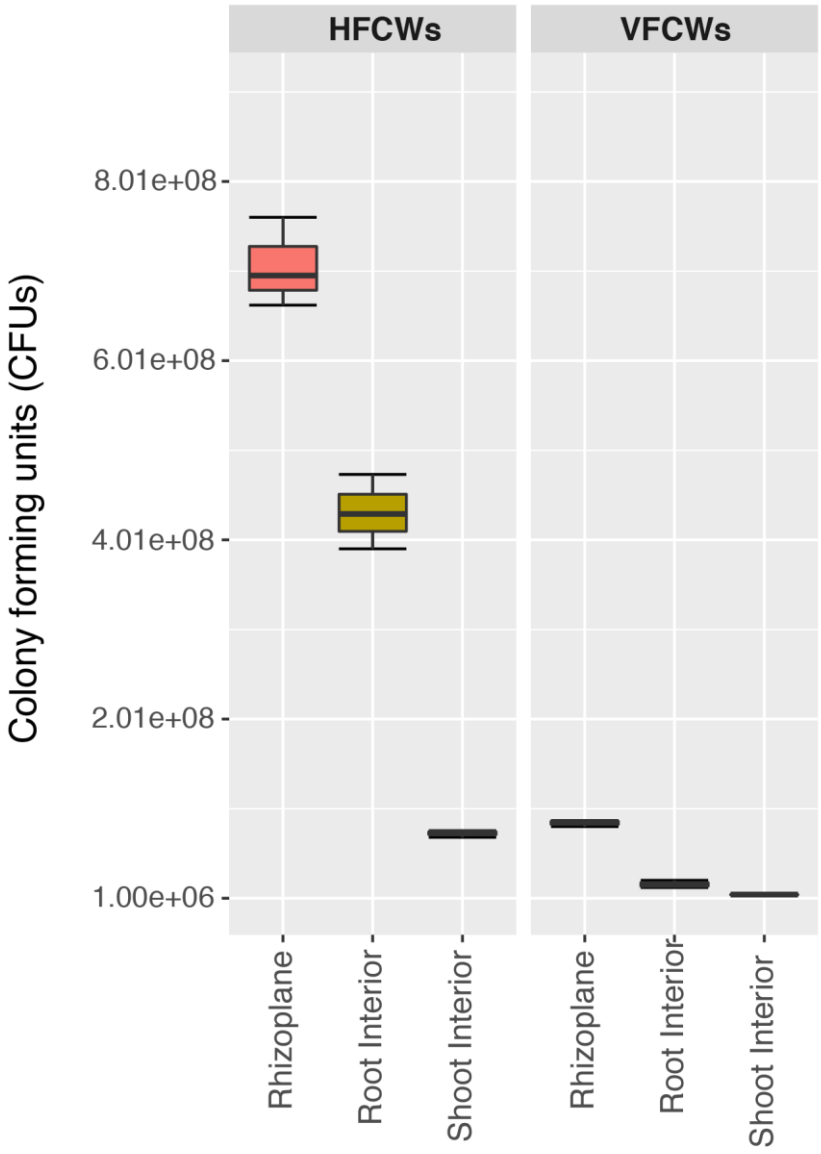


Figure 3: Inoculated bacteria population in water (CFU/ml), rhizosphere (CFU/g soil), root interior (CFU/g root) and shoot interior (CFU/g shoot) of *Phragmites australis* vegetated in VFCWs and HFCWs.