On-site performance of floating treatment wetland macrocosms augmented with dye-degrading bacteria for the remediation of textile industry wastewater

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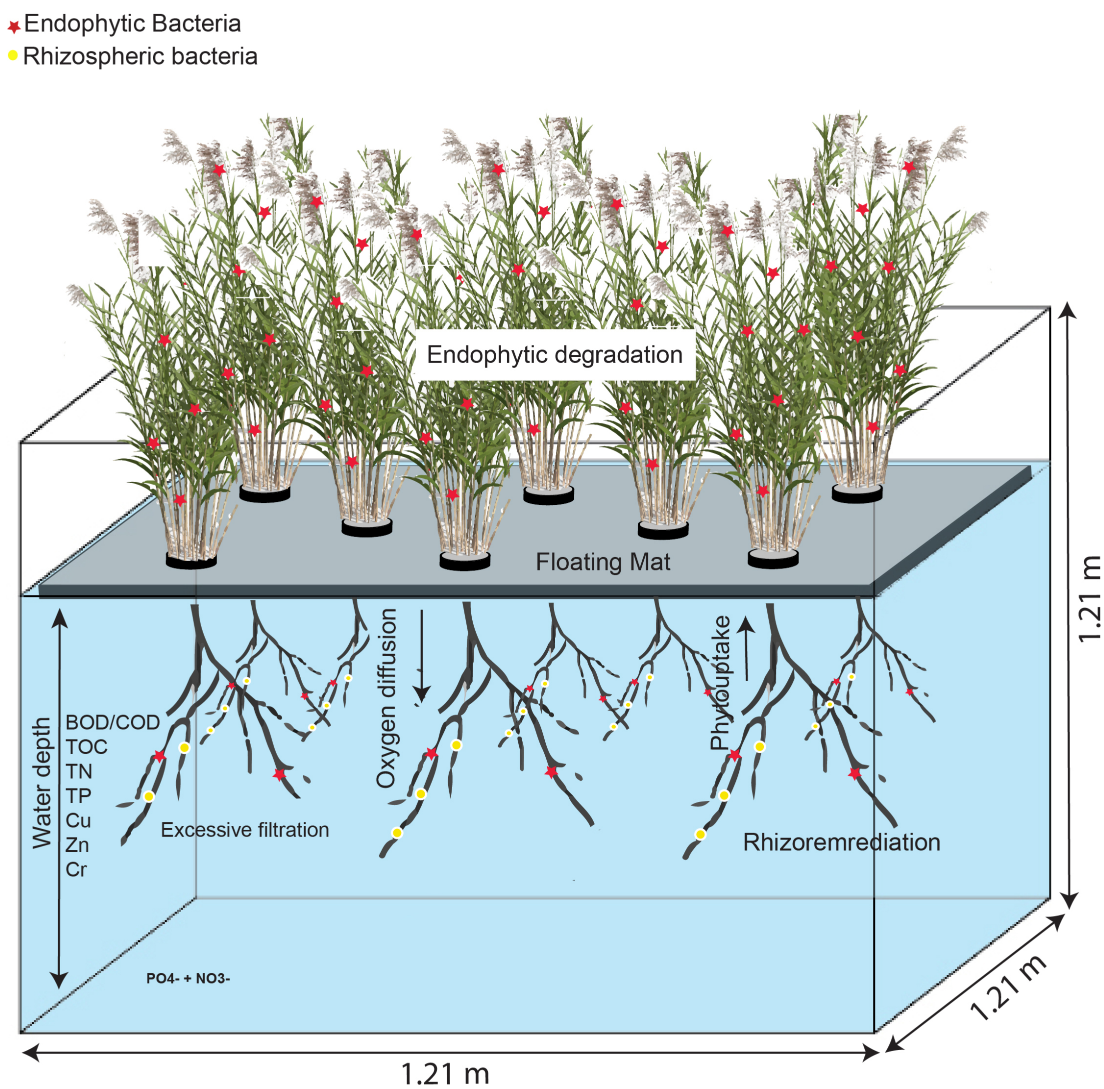
Endophytic Bacteria
Rhizospheric bacteria
Endophytic degradation
Floating Mat
1.21 m
Water depth
BOD/COD
TOC
TN
TP
Cu
Zn
Cr
Oxygen diffusion
Phytouptake
Excessive filtration
Rhizoremrediation
PO4- + NO3-
1.21 m
1.21 m

ABSTRACT

Floating treatment wetlands are considered cost-effective remediation options for various types of wastewater. Their effectiveness has been shown in several lab-scale and pilot-scale studies; however, there is a paucity of published data on pilot-scale systems treating genuine wastewater. This study aims to assess the performance of a pilot-scale system, carrying *Phragmites australis* in combination with three plant growth promoting and pollutant-degrading bacteria (*Acinetobacter junii* strain NT-15, *Rhodococcus sp.* strain NT-39, and *Pseudomonas indoloxydans* strain NT-38) for the treatment of textile industry wastewater (Interloop Limited, Faisalabad, Pakistan). Fifteen floating treatment wetlands macrocosms were established employing plants and bacteria separately or in combination. Each unit was capable to carry 1000-liter of wastewater and the system was operated in a batch-wise mode for the period of 2 years. After a year of installation, performance of all FTWs units was optimal. A high removal in organic and inorganic pollutants was observed in the vegetated tanks, whereas combined application of plants and bacteria further enhanced the removal performance, i.e., chemical oxygen demand was reduced to 92%, biochemical oxygen demand to 91%, color to 86%, and heavy metals to approximately 87% in the wastewater. The augmented bacteria displayed persistence in water as well as in the roots and shoots of *P. australis* suggesting a potential partnership with the host towards enhanced performance. Treated wastewater met the National Environmental Quality Standards of Pakistan to be discharged in the surface water without any potential risks. This pilot-scale study is a step forward towards sustainable remediation of the textile wastewater in the field.

Keywords: Textile wastewater, wastewater treatment, floating treatment wetlands, plant-bacteria partnership

INTRODUCTION

Textile industry plays a key role in the economy of many developing countries including Pakistan. However, it also causes severe water pollution due to the direct wastewater discharges in the surface water (Noreen et al. 2017). The textile industry of Faisalabad (Pakistan) represents a wide array of processes such as desizing, bleaching, mercerizing, dyeing, printing and finishing of products. This results into production of a broad range of organic and inorganic pollutants, which are discharged into the Ravi river and Chenab river through the Paharang and Madhuana drains without any pre-treatment (Azizullah et al. 2011; Imtiazuddin et al. 2012). These discharges consequently deteriorate the water quality leading to harmful effects on aquatic organisms, irrigating crops, and on human health (Ullah et al. 2017; Najam-us-Sahar et al. 2017; Daud et al. 2017). Since a few years, this condition has become worse as the level of toxic pollutants in the river's body has surged dramatically.

Many physicochemical techniques are effective in treating textile wastewater but they demand high operational costs, engineering skills, environmental incursions, labor administration, and several other operational processes (Liu et al. 2009; Srinivasan et al. 2014). Contrarily, advances in ecological engineering have made us develop environmental-friendly methods for the effective treatment of contaminated water (Giannetti et al., 2002; Ijaz et al., 2015; Saumya et al., 2105; Greenway, 2017). One such method is the installation of floating treatment wetlands (FTWs) in which emergent aquatic macrophytes are planted artificially on a floating raft that allows plants to grow hydroponically on the water body (Nahlik and Mitsch 2006; Wu et al., 2017; Shahid et al. 2018). Plant roots hang down to the pelagic zones in the water column offer both mechanical and biological filtering. Mechanical filtering is achieved through the physical effect of plant roots such as sedimentation, adsorption, filtration, etc. The biological filtering however is the result of bacterial degradation and plant uptake (Zhang et al. 2106; Merkhali et al. 2015). In both situations, plant roots play several important roles such as: (1) their presence reduce the water turbulence and avoid re-suspension of sediments (Smith and Kalin 2000); (2) they attach suspended matter onto the root surface which is subsequently precipitated in the systems bottom or adsorbed on to the biofilm; and (3) when adsorbed, bacterial action results into the degradation of organic pollutants (Prajapati et al. 2017). Macrophytes can hinder the algal growth by competing for nutrition and sunlight (Li et al. 2010). Lastly, these systems also act as habitate for fish harvesting (Faulwetter et al. 2011), livestock grazing (Ladislas et al. 2013), improve landscape aesthetics (Ijaz et al. 2015), and provide a significant amount of biomass to be used for bio-energy purposes (Shahid et al. 2018).

Floating wetlands have been traditionally employed to treat variety of wastewater such as stormwater (Headley et al. 2008), sewage effluents (Todd et al. 2003), municipal wastewater (Arshad et al. 2017; Sirage et al. 2017), industrial wastewater (Li et al. 2012), acid mine drainage and poultry processing wastewater (Smith and Kalin 2000; Todd et al. 2003). These systems, however, are limited in certain contexts such as poor stability and less efficient purification performance. Among them, less potential of indigenous bacteria toward the degradation of toxic organic compounds and weak metabolic capabilities of plants are prominent ones (Arslan et al. 2017). To improve the treatment efficiency of FTWs, additional approaches have been recommended among which inoculation of plant growth promoting (PGP) and pollutant-degrading bacteria is an effective method (Shehzadi et al. 2014; Saleem et al. 2018). To date, a few studies have explored the efficiency of FTWs in the presence of bacterial partnership for the remediation of different wastewaters (Kabra et al. 2013; Watharkar et al. 2015; Rehman et al. 2018). Many of them were conducted at microcosm scale for which field-scale performance is speculated. In this study, *in situ* pilot-scale FTW system was established in combination with dye degrading bacteria for the enhanced cleanup of wastewater of a textile industry, i.e., Interloop Limited, Khurrianwala, Faisalabad, Pakistan. There were enhanced pollutant removal and toxicity reduction in the bacterially assisted FTWs as a result of effective plant-bacterial partnership towards remediation of textile industry wastewater.

MATERIAL AND METHODS

Collection and characterization of textile wastewater

The untreated textile wastewater was obtained from an outlet of the wastewater equalization tank of Interloop Limited, Khurrianwala, Faisalabad, Pakistan. The wastewater was analyzed for several physicochemical parameters such as pH, color, electrical conductivity (EC), chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), total dissolved solids (TDS), total suspended solids (TSS), total nitrogen (TN), phosphate (PO_4^{-2}), Phenols, Chlorides, Sulphates, heavy metals and toxicity by using standard methods (APHA, 2005).

Bacterial strains

Three bacterial strains, i.e., *Acinetobacter junii* NT-15 (NCBI accession: MF478980), *Pseudomonas indoloxydans* NT-38 (NCBI accession: MF478985), and *Rhodococcus sp.* NT-39 (NCBI accession: MF326802), were used in the present study. The strain *A. junii* NT-15 was isolated from the activated sludge, whereas strain *P. indoloxydans* NT-38 and *Rhodococcus sp.* NT-39 were isolated from the root interior and rhizosphere of *Polygonum aviculare* and *Poa labillardierei*, respectively (Tara et al. 2018). These strains were screened because of their high dye resistance and decolorization potential. These bacterial strains were cultivated as separate cultures at 30 °C for 24 h in Luria-Bertani (LB) broth. The cells were harvested by centrifugation (×12,000 rpm) for 5 minutes at 4 °C and resuspended together at ratio of 1:1:1 in 0.9% (w/v) sterile NaCl solution. The cell suspension of each pure culture was adjusted as per the guidelines of the turbid metric method (Sutton, 2011). In the end, one liter of bacterial consortium was used as an inoculum in the FTWs as per experimental design.

Construction and implementation of FTWs for textile wastewater treatment

Floating treatment wetlands were built in the vicinity of Interloop Limited Khurrianwala, Faisalabad (Fig. 1A-D). Plastic tanks of 1000 liters of capacity (dimensions: 1.2 meter length, 1.2 meter width, and 1.2 meter height) were used to establish macrocosms. These tanks were painted from all the sides to prevent algal growth. Accordingly, floating mats of equal dimensions were prepared using polyethylene sheet as recommended earlier (Ijaz et al. 2015). Aluminum foil was used to cover all sides of the mats in order to protect them from ultraviolet radiations. Eight holes of equal diameter were drilled in each floating mat and planted with 24 healthy seedlings of *Phragmites australis*: three plants, with the height of approximately 60 cm and weight of 45 to 65 g, were inserted in each hole of the mat. *P. australis* was selected based on its successful application in phytoremediation of industrial wastewater as well as its ability to resist high concentrations of azo dyes (Todorovics et al. 2005; Stefanakis et al. 2014; Ha and Anh 2017; Rehman et al. 2018). These seedlings were previously grown in the nursery developed in the vicinity of NIBGE, Faisalabad. Soil and coconut shaving were used to support the plant seedlings. A total of 15 macrocosms were established by placing floating mats on the tank surface. The plant seedlings were then allowed to augment roots in tap water for a period of 1-month. When the plant growth was optimum, tap water was replaced with the textile wastewater. The one-liter inoculum of previously isolated bacterial strains was introduced in the FTWs as per the experimental design. Different treatments were established to study: (1) effect of textile effluent on plant growth; (2) effect of vegetation on textile effluent degradation; (3) effect of bacterial augmentation on textile effluent degradation; (4) effect of bacterial augmentation on plant growth and textile effluent degradation; and (5) plant growth in tap water (control). The sequencing fill-and-draw batch mode method was used to withdraw decontaminated water. Hydraulic retention time was set to 10 days based on lab-scale observations where a continuous decrease in organic and inorganic load was observed in initial 10-days. The system was operated for 2 years from March 2016 to March 2018. The water samples of approximately 1.5 liters were collected every two days. However, in this article, the results are reported when the system's performance was optimum/constant (after 6 months of installation). The treatment performance was established by measuring wastewater parameters such as pH, EC, TDS, TSS, COD, BOD_5, TN, PO_4^{-2}, color, phenols, chlorides, sulphates, and heavy metals (Fe, Ni, Cr, and Cd) immediately after sample collection; elsewise samples were stored until analysis according to standard methods (APHA, 2005). The experiment was performed in the ambient conditions of the industry for the period of 2 years. In this period, precipitation on average was 29 mm (Faisalabad's climate conditions); however, during rainy period, water level was manually maintained to 1000 litters by covering the macrocosms with plastic sheets.

Determination of persistence of inoculated bacteria

The survival/persistence of inoculated bacteria in the treated wastewater and plant interior (root and shoot interior) was enumerated by viable plate count method. Samples were collected every 3 months. Shoots and roots were surface sterilized, homogenized, and the slurry was plated onto LB agar plates having 100 mg l^{-1} of azo dyes as established previously (Ijaz et al. 2015). Similarly, treated wastewater was also plated on LB agar plates and incubated at 30 °C for 24 h. Restriction fragment length polymorphism (RFLP) analysis was performed to identify the identity of inoculated strains (Afzal et al. 2012).

Plant biomass

The plant tissues were harvested every 3 months to measure shoot height, root length, shoot dry weight, and root dry weight. Briefly, shoots and roots were harvested one inch above and below the mats, respectively. The root length and shoot height were measured by using hand ruler. To measure dry biomass, plant samples were first transported to the laboratory, placed in an oven at 80 °C for 72 h, and then weighed using a digital balance. Samples were stored at -80 °C until further analysis.

Fish toxicity bioassay

Fish bioassay was performed once, during the optimal period, to test the toxicity of untreated and treated wastewater. A freshwater dwelling fish species Rohu, *Labeorohita rohita* (Family: Cyprinidae, Order: Cypriniformes) was selected for the bioassay due to the local ecological significance. Briefly, the species is present in the local streams and lakes and therefore direct discharges may lead to health issues (Khan et al. 2017). This species has previously found to accumulate heavy metals above the natural/background levels (Hamid et al. 2016). In this study, healthy specimens were obtained from Faisalabad Fish Hatchery, treated with KMnO4 solution (0.05 %) for two minutes to remove dermal contamination, and then shifted to glass aquaria containing uncontaminated water. Each fish specimen had an average body length of ~8.5 ± 0.8 cm and weight of ~4.0 ± 0.1 g. The specimens were acclimatized for 15 days prior to the effluent exposure. Subsequently, four fish groups each containing 10 individuals were placed in the aquariums (dimensions: depth 30 cm, width 30 cm, and length 45 cm). These aquariums were filled with 30 L of treated and untreated wastewater, as per the experimental design. Finally, the survival rate was determined by counting the number of alive fish every 24 h for a period of four days.

Long-term maintenance strategy

Maintenance strategy of the system involved harvesting of the plants and inoculation of the bacterial consortium in the wetland macrocosms after every 3 months during the experimental period. Accordingly, long-term system's performance was also monitored by measuring temporal response of basic water quality parameters, i.e., COD, BOD, Color, TSS, TDS, TN, Phosphates.

Statistical analysis

R-statistical language was used to compare different treatments for water quality parameters (pH, EC, COD, BOD_5, TDS, TSS, color, Phenols, Chlorides, and Sulphates), nutrients (TN, and $PO4^{-2}$), heavy metals (Fe, Cr, Ni, and Cd), plant growth parameters (root length, shoot length, fresh biomass, and dry biomass), and fish toxicity assay. The comparison was made through one-way ANOVA and Duncan's test was used after testing homogeneity of variance.

RESULTS AND DISCUSSION

Wastewater characteristics

In developing countries, unprecedented advances in textile industry have escalated the discharge of wastewater into the natural streams (Vikrant et al. 2018). In this study, physicochemical analysis of the textile industry wastewater displayed a high pollution due to COD (513 mg l^{-1}), BOD (283 mg l^{-1}), TDS (5251 mg l^{-1}), TSS (324 mg l^{-1}), Phenols (0.85 mg

l^{-1}), Chlorides (1383 mg l^{-1}), Sulphates (311 mg l^{-1}), Fe (14.4 mg l^{-1}), Ni (7.57 mg l^{-1}), Cr (9.67 mg l^{-1}), and Cd (0.88 mg l^{-1}). Their concentrations were higher than the established National Environmental Quality Standards (NEQS) of Pakistan (Table 1). This suggests an initial treatment of the wastewater of textile industry of Pakistan before discharging into the Ravi and Chenab rivers.

Performance evaluation for physicochemical parameters

The performance of FTWs in the presence of vegetation, bacteria, and vegetation and bacteria together, was evaluated by studying wastewater parameters at different time intervals (Table 2 - 4, Fig. 2). In the beginning of the second year, the system performance was relatively optimal. Based on these observations, in-depth investigations were performed. Briefly, it was found that FTWs vegetated with *P. australis* removed a high proportion of organic and inorganic pollutants as compared to the un-vegetated FTWs. This removal was further enhanced when *P. australis* and bacterial consortium were applied in combination. Briefly, in the presence of both partners, COD was reduced to 91%, BOD to 92%, and color to 86% (Fig. 2A-C). Earlier studies have also shown highest pollution reduction of textile wastewater when plants and bacteria employed synergistically (Shehzadi et al. 2014; Watharkar et al. 2015; Hussain et al. 2018a); this could be attributed to the combined enzymatic activities of both bacteria and plants to transform organic matter into simple metabolites (Kabra et al. 2013; Khandare et al. 2013). These transformed products can be taken up by plants as a part of nutrient assimilation process or eliminated in the form of gases, e.g., CO_2 and N_2. With the passage of time, this reduction in organic load was increased and maximum remediation was observed in the period of 10 days. Accordingly, a positive correlation for reduction of COD and color was seen in textile wastewater. It has been reported previously that COD determines the extent of oxidizable contaminants and hence establish positive correlation with the color of wastewater (Tara et al. 2018). The observation was consistent in this study as well. Likewise, pH of the remediated water moved from basic to neutral conditions (Table 2). This could also be associated with the combined action of dyes transformation by bacteria and plants (Watharkar et al. 2015). Accordingly, a significant amount of phenols, chlorides, and sulphates were removed from the wastewater (Figure 2D). Earlier studies have reported similar results in different types of CWs (Shehzadi et al. 2014; Hussain et al. 2018a,b).

Vegetation had a prominent effect on TDS and TSS removal as compared to the unvegetated but inoculated treatments (Table 2). Briefly, vegetation reduced TDS from 5251 mg l^{-1} to 2265 mg l^{-1} (up to 57%) and TSS from 324 mg l^{-1} to 165 mg l^{-1} (up to 49%). The bacterial inoculation in vegetated treatment further increased reduction in TDS and TSS up to 1399 mg l^{-1} (73%) and 148 mg l^{-1} (54%), respectively. These results are in accordance to the previously published findings (Shehzadi et al. 2014; Tara et al. 2018; Hussain et al. 2018a,b). As of these findings, vegetated treatments also displayed better removal of nutrients (TN and PO_4^{-2}) than the un-vegetated treatments whereas highest removal efficiency was seen in the presence of plant-bacteria synergism (Table 3). The removal efficiency for TN was recorded up to 60% in the vegetated FTWs, which was increased to 87% on bacterial inoculation. In an earlier study, Sun et al. (2009) reported similar results where TN removal was enhanced up to 72% by the addition of bacteria in FTWs whereas only 50% removal was achieved in the treatments without bacteria. Similar results were obtained for PO_4^{-2} reduction as bacterial augmentation improved the removal as compared to the vegetation only. Nevertheless, percent removal for TN was higher than the PO_4^{-2}, which can be linked to the fact that nitrogen, in addition to the plant uptake, is eliminated in the form of N_2 gas whereas PO_4^{-2} stays in the system due to precipitation (Tao and Wang 2009; Vymazal, 2010).

Long-term system's performance was monitored to evaluate pollutant removal during the studied period, as shown in Figure 3. It was found that the system was relatively stable throughout the experimental period; nevertheless, performance was better in the warm months.

Reduction in heavy metals from wastewater and their bioaccumulation in plant tissues

The studied heavy metals (Fe, Ni, Cr, and Cd) were significantly removed by FTWs (Table 4). The removal efficiency was equally valid for both essential (Fe and Ni) and non-essential (Cr and Cd) heavy metals. Nevertheless, bacterial inoculation improved the bioaccumulation and phytoremediation potential of *P. australis*. The mean concentration values of heavy metals in root, shoots, and leaves were observed in the following order: Fe > Cr > Ni > Cd. Absorbed metals were partitioned among different parts of *P. australis*. Roots retained higher concentration of heavy metals than shoots and leaves with the following order: root>leaf>stem. These results are in accordance to the earlier findings demonstrating that underground part of *P. australis* shows a higher storage capacity than the aboveground organs (Štrbac et al. 2014; Kucaj and Abazi 2015; Amira and Leghouchi 2017). It has been established that roots and rhizomes of *P. australis* possess large intercellular air spaces of cortex parenchyma that allow accumulation of heavy metals far away from the metabolically active structures to avoid toxic effects (Mendonça et al. 2015). The present study confirms such a potential of *P. australis* in phytoremediation for the studied heavy metals which was further enhanced by bacterial inoculation, i.e., the system was able to remove Cr and Fe more than 90%, Ni more than 80%, and Cd more than 60%. The role of bacteria in sorbing metallic ions on their cell walls is prominent and has also been reported in terms of increasing bioavailability followed by uptake by plants (Jilani and Khan 2013; Khan et al. 2014).

Inoculated bacteria displayed persistence in FTWs

In phytoremediation, plant-associated microbial populations play a key role to mineralize organic pollutants (Khan et al. 2014). Many studies have reported that the phytoremediation potential of plants can be correlated with the proportion of bacteria in the surrounding environment (Yousaf et al. 2011; Shehzadi et al. 2014; Rehman et al. 2019). The artificial augmentation of bacteria in such a system establishes intimacy with their host depending upon the environmental conditions such as nutrient supply and their ability to colonize the host environment. Moreover, bacteria improve plant growth by reducing biotic and abiotic stress, followed by N_2 fixation, production of phytohormones, and solubilization of various essential nutrients (Glick et al. 2010; Khan et al. 2014). In this study, in order to confirm the improvement in performance of *P. australis* due to bacterial augmentation, persistence of inoculated bacteria was evaluated within the plant tissues and wastewater at the beginning of the experiment. Results showed a high persistence of inoculants in the plant interior, especially in the roots, followed by the wastewater (Fig. 4). Furthermore, RFLP analysis indicated that the relative proportion of inoculated bacterial with roots and shoots was increased significantly in the initial 3-months period, which later became stable. Although the trend was similar for both plant parts, their relative proportion was higher in the roots as compared to the shoots. This might be due to the fact that The inoculated bacteria were previously isolated from the roots and shoots of *P. australis* grown in the textile wastewater and therefore they probably have adapted necessary mechanism for proliferation in these hostile conditions (Afzal et al. 2014). By contrasts, a decline in bacterial counts was observed in the water, which might be due to the fact that contaminated water is less favorable environment for the inoculated bacteria. More specifically, rhizo- and endophytic bacteria needs a symbiotic partner such as plant roots to survive and proliferate, however, the absence can cause poor survival and growth (Arslan et al. 2014). Additionally, this might be attributed to the natural selection pressure for the newly grown community that allows bacteria to migrate from less favorable environment to the more favorable environment.

Plant biomass and growth

An important parameter in phytoremediation efficiency is the plant tolerance and survival in the presence of pollutants. The growth of *P. australis* was increased sharply in the first year, which was then sustained or remained unaffected during the remaining studied period (Figure 5). This could be attributed to the senescence effect. Nevertheless, when compared with the contaminated water, it was found that wastewater inhibited the plant biomass significantly (Table 5). This shows the toxic effects of textile wastewater on plant metabolism (Watharkar et al. 2015; Shehzadi et al. 2016; Ramya et al. 2017). In an earlier study, *Typha domingensis* exhibited similar behavior in which reduction in plant growth was seen in the presence of textile wastewater (Shehzadi et al. 2014). Nevertheless, inoculation with bacteria rehabilitated the plant growth with a prominent increase in root length (46%), shoot length (37%), root fresh weight (58%), shoot fresh weight (49%), root dry weight (55%), and shoot dry weight (46%) (Table 5). This increase in plant growth parameters could be associated with the pollutant degrading capabilities as well as ACC deaminase potential of the inoculated bacteria. Fatima et al. (2016) reported similar results in which plant growth was significantly improved by the augmented of endophytic bacteria due to their PGP activities such as indole acetic acid production, siderophores formation, and ACC deaminase activity.

Detoxification of wastewater

Fish toxicity bioassay suggested a successful reduction in wastewater's toxicity after treatment with FTWs. This reduction was partially effective when plant and bacteria were applied separately, i.e., death of three and five fish out of 10 specimens. Whereas, bacterial inoculation in vegetated FTWs resulted in complete detoxification as no fish died even after 72 h of exposure (Table 6). These results strengthen the earlier observations in which high reduction was observed for COD, BOD_5, TDS, TSS, color, and heavy metals in the presence of vegetation and bacteria. Earlier studies also reported similar findings in which use of plants in combination with bacteria was an efficient strategy to remove wastewater toxicity (Tanner and Headley 2011; Ijaz et al. 2016; Rehman et al. 2018).

CONCLUSIONS

This study investigated the performance of FTWs in the presence of three bacterial strains to remediate textile wastewater at pilot-scale. FTWs vegetated with *P. australis* and inoculated bacteria displayed highest pollutants removal. Bacterial persistence was prominent in the root and shoot interior suggesting optimal partnership in pollutant degradation. The system attenuated both organic and inorganic contaminants and wastewater was found to be completely detoxified. The system was operated for two years suggesting long-term potential for the on-site remediation practices. It is concluded that bacterially assisted FTWs can be exploited to treat industrial wastewaters in countries with more economic constraints like Pakistan, where capital and operational cost are of prime importance.

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Table 1
Quality parameters of textile industrial wastewater collected from Interloop, Khurrianwala located in Faisalabad, compared to the National Environmental Quality Standards (NEQS), Pakistan (Hussain et al., 2018b)

Parameter	**Unit**	**Wastewater**	**NEQS**
Temperature	°C	38 (2.63)	40
pH	--	8.8 (0.82)	6-10
Electrical conductivity (EC)	mS cm^{-1}	8.2 (0.64)	NG
Color	m^{-1}	66 (4.4)	NG
Chemical oxygen demand (COD)	**mg l^{-1}**	**513 (37.6)**	**150**
Total organic carbon (TOC)	mg l^{-1}	201 (15.9)	NG
Biochemical oxygen demand (BOD_5)	**mg l^{-1}**	**283 (17.9)**	**80**
Phenol	**mg l^{-1}**	**0.85 (0.06)**	**0.1**
Chloride	**mg l^{-1}**	**1383 (61.8)**	**1000**
Sulphate	mg l^{-1}	311 (21.9)	600
Nitrogen	mg l^{-1}	28.7 (6.0)	40
Phosphate	mg l^{-1}	16.4 (3.6)	NG
Total dissolved solids (TDS)	**mg l^{-1}**	**5251 (404)**	**3500**
Total solids (TS)	mg l^{-1}	5420 (395)	NG
Total settleable solids (TSeS)	mg l^{-1}	19.9 (1.1)	NG
Total suspended solids (TSS)	**mg l^{-1}**	**324 (29.7)**	**150**
Iron (Fe)	**mg l^{-1}**	**14.4 (0.64)**	**2.0**
Nickel (Ni)	**mg l^{-1}**	**7.57 (0.38)**	**1.0**
Chromium (Cr)	**mg l^{-1}**	**9.67 (0.26)**	**0.1**
Cadmium (Cd)	**mg l^{-1}**	**0.88 (0.02)**	**0.1**
Toxicity	--	Highly toxic	NG

Each value is the mean of three replicates; standard error among three replicates is presented in parenthesis. NG, not given in National Environmental Quality Standards (NEQS) list.

Table 2

Remediation of the textile industrial wastewater by floating treatment wetlands vegetated with *Phragmites australis* and inoculated with bacterial consortium

Days	**Control**				**T1**				**T2**				**T3**			
	pH	EC	TDS	TSS	pH	EC	TDS	TSS	pH	EC	TDS	TSS	pH	EC	TDS	TSS
0	8.73 (0.20)	8.22 (0.64)	5251 (404)	324 (30)	8.74 (0.16)	8.34 (0.66)	5252 (404)	325 (31)	8.75 (0.19)	8.27 (0.61)	5251 (404)	325 (29)	8.74 (0.20)	8.25 (0.61)	5252 (404)	324 (29)
2	8.49 (0.18)	7.72 (0.64)	4934 (411)	319 (29)	8.43 (0.17)	7.27 (0.55)	4635 (354)	275 (19)	8.19 (0.12)	6.64 (0.55)	4250 (355)	256 (18)	8.55 (0.08)	7.34 (0.67)	4699 (430)	310 (28)
4	8.38 (0.17)	7.36 (0.69)	4677 (442)	306 (28)	8.14 (0.13)	6.42 (0.43)	4103 (279)	241 (18)	7.83 (0.13)	5.73 (0.43)	3632 (277)	225 (12)	8.35 (0.13)	6.84 (0.61)	4356 (389)	296 (29)
6	8.58 (0.09)	6.7 (0.72)	4295 (462)	300 (29)	7.67 (0.10)	5.35 (0.53)	3397 (339)	224 (16)	7.46 (0.10)	4.35 (0.49)	2735 (314)	206 (11)	8.58 (0.09)	6.37 (0.69)	4016 (441)	290 (27)
8	8.88 (0.08)	6.2 (0.70)	3974 (447)	294 (29)	7.41 (0.10)	4.42 (0.43)	2843 (278)	199 (16)	7.14 (0.10)	3.49 (0.46)	2183 (295)	171 (12)	8.81 (0.11)	5.67 (0.62)	3632 (394)	286 (26)
10	9.04 (0.04)	5.7 (0.61)	3655 (389)	285 (28)	7.35 (0.06)	3.57 (0.35)	2265 (222)	165 (12)	7.12 (0.09)	2.26 (0.30)	1399 (185)	148 (10)	8.87 (0.09)	5.13 (0.46)	3292 (296)	271 (24)

Floating treatment wetlands vegetated with *P. australis* (T1), *P. australis* and bacteria (T2), and bacteria only (T3). Each value is the mean of three replicates; the standard error of three replicates is presented in parenthesis. TDS and TSS are mentioned in mg l^{-1}, whereas EC in mS cm^{-1}.

Table 3

Nutrients (TN and PO_4^{2-}) removal from textile industrial wastewater by floating treatment wetlands vegetated with *Phragmites australis* and inoculated with bacterial consortium

Days	**TN (mg l^{-1})**				**PO_4^{2-} (mg l^{-1})**			
	Control	**T1**	**T2**	**T3**	**Control**	**T1**	**T2**	**T3**
0	28.7 (6.0)	28.7 (6.0)	28.9 (5.6)	28.9 (5.7)	16.4 (3.6)	16.4 (3.6)	16.5 (3.6)	16.5 (3.6)
2	27.0 (5.4)	22.6 (4.5)	19.3 (4.1)	25.9 (5.1)	16.1 (3.6)	15.7 (3.4)	15.1 (3.4)	15.9 (3.3)
4	25.9 (5.1)	20.3 (3.9)	15.7 (2.9)	23.6 (4.7)	15.9 (3.4)	14.5 (3.1)	13.2 (2.8)	15.6 (3.5)
6	24.5 (4.9)	17.4 (3.2)	12.0 (1.9)	22.2 (4.4)	15.8 (3.4)	13.1 (2.8)	11.8 (2.7)	15.2 (3.2)
8	23.2 (4.6)	14.8 (2.7)	8.7 (1.8)	20.1 (3.7)	15.6 (3.4)	11.3 (2.3)	10.0 (2.3)	14.8 (3.0)
10	22.3 (4.5)	11.3 (2.0)	3.7 (0.6)	19.0 (3.5)	15.3 (3.3)	10.1 (2.1)	8.2 (1.9)	14.2 (2.9)

Floating treatment wetlands vegetated with *P. australis* (T1), *P. australis* and bacteria (T2), and bacteria only (T3). Each value is the mean of three replicates; the standard error of three replicates is presented in parenthesis.

Table 4
Heavy metals removal from textile industrial wastewater and their accumulation in different parts of *Phragmites australis*

Metal	Control	T1				T2				T3
	Initial conc. (mg l^{-1})	Water (mg l^{-1})	Root (mg kg^{-1})	Shoot (mg kg^{-1})	Leaves (mg kg^{-1})	Water (mg l^{-1})	Root (mg kg^{-1})	Shoot (mg kg^{-1})	Leaves (mg kg^{-1})	Water (mg l^{-1})
Fe	14.4 (0.64)	2.63 (0.27)	5.90 (0.45)	1.97 (0.15)	3.94 (0.30)	1.05 (0.11)	6.69 (0.37)	2.23 (0.12)	4.46 (0.25)	9.0 (0.47)
Ni	7.57 (0.38)	1.72 (0.12)	2.92 (0.19)	0.97 (0.06)	1.95 (0.13)	1.08 (0.09)	3.25 (0.18)	1.08 (0.06)	2.16 (0.12)	5.43 (0.35)
Cr	9.67 (0.26)	1.50 (0.14)	4.08 (0.09)	1.36 (0.03)	2.72 (0.06)	0.40 (0.10)	4.63 (0.16)	1.54 (0.05)	3.09 (0.10)	6.53 (0.38)
Cd	0.88 (0.06)	0.29 (0.06)	0.24 (0.03)	0.08 (0.01)	0.16 (0.02)	0.26 (0.03)	0.31 (0.01)	0.10 (0.01)	0.21 (0.01)	0.72 (0.04)

Floating treatment wetlands vegetated with *P. australis* (T1), *P. australis* and bacteria (T2), and bacteria only (T3). Each value is the mean of three replicates; the standard error of three replicates is presented in parenthesis.

Table 5
Effect of bacterial inoculation on biomass and root and shoot length of *Phragmites australis* vegetated in floating treatment wetlands

Treatment	**Length (cm)**		**Fresh biomass (g)**		**Dry biomass (g)**	
	Root	Shoot	Root	Shoot	Root	Shoot
Tap water	67.8 (2.6)	407 (19.1)	1189 (52.1)	2194 (62.0)	152 (6.1)	510 (13.9)
Wastewater	43.6 (2.5)	294 (14.8)	936 (64.2)	1803 (48.9)	84 (7.9)	370 (13.9)
Wastewater and bacteria	54.8 (4.9)	336 (6.9)	1083 (48.9)	1994 (94.4)	122 (7.5)	434 (26.5)

Each value is the mean of three replicates; the standard error of three replicates is presented in parenthesis.

Table 6
Fish toxicity assay of textile industrial wastewater detoxified by floating treatment wetlands vegetated with *Phragmites australis* and inoculated with bacterial consortium (BC)

Treatment	Death over time										Total deaths	Detoxification status
	0 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h		
P. australis (T1)	0	0	0	0	0	0	0	0	1	2	3	Partial
P. australis + BC (T2)	0	0	0	0	0	0	0	0	0	0	0	Complete
BC (T3)	0	0	0	0	0	0	2	1	1	1	5	Partial
Control	0	2	0	1	1	1	4	0	0	0	9	Negligible

Fig. 1. Development and implementation of floating treatment wetlands (FTWs) macrocosms for the remediation of wastewater at Interloop Limited Khurrianwala, Faisalabad. Wastewater equalization tank (A), floating mat for the plantation of *Phragmites australis* seedlings (B), nursery of P. australis in the vicinity of NIBGE, Faisalabad (C), and growth of *P. australis* in FTWs macrocosms at the end of experimentation (D).

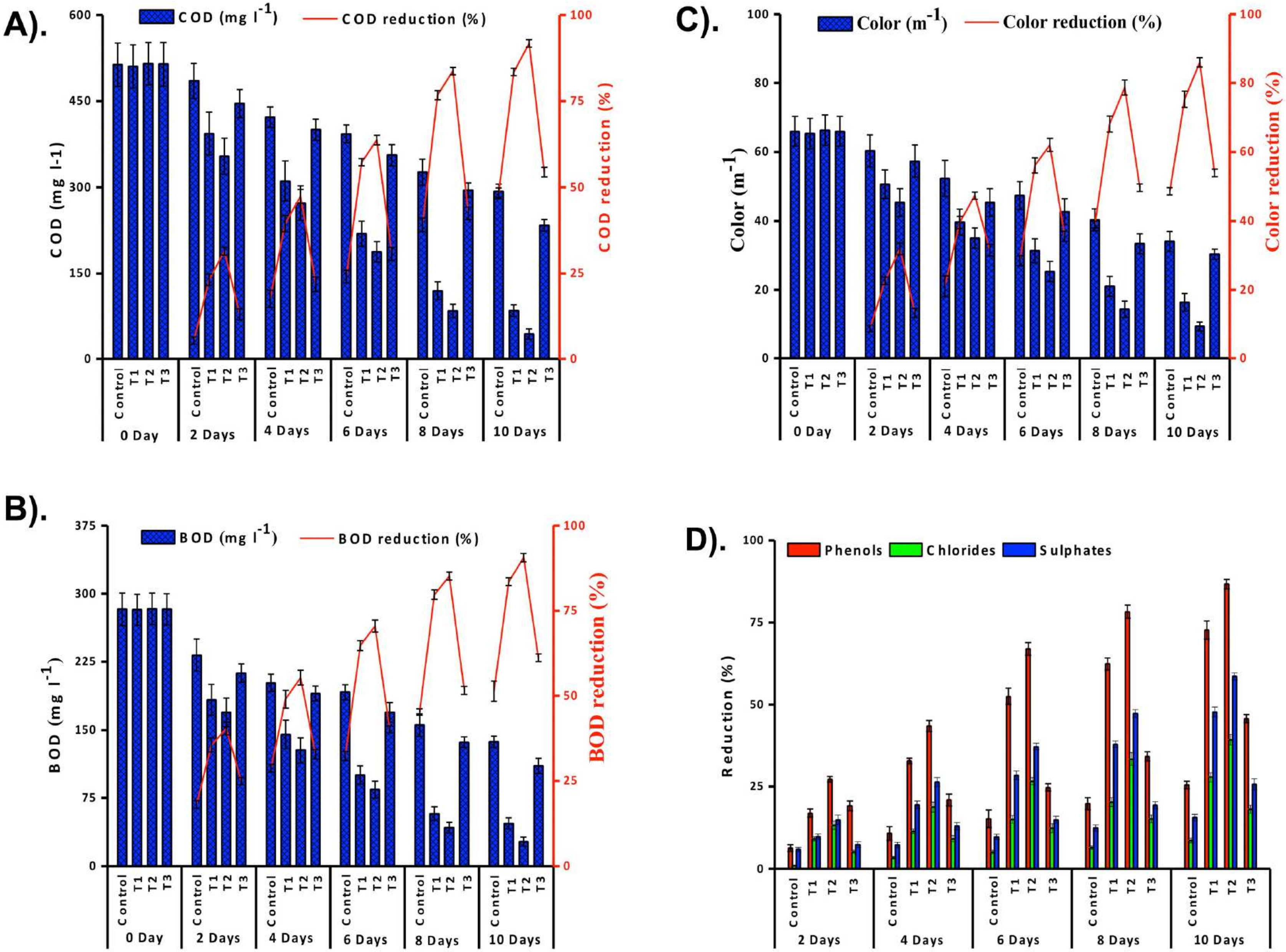

Fig. 2. Reduction of COD (A), BOD (B), Color (C), and for Phenols, Chlorides, and Sulphates (D) in the textile wastewater by floating treatment wetlands vegetated with *P. australis* (T1), *P. australis* and bacteria (T2), and bacteria only (T3). Each value is the mean of three biological replicates. Error bars indicate standard error among three replicates.

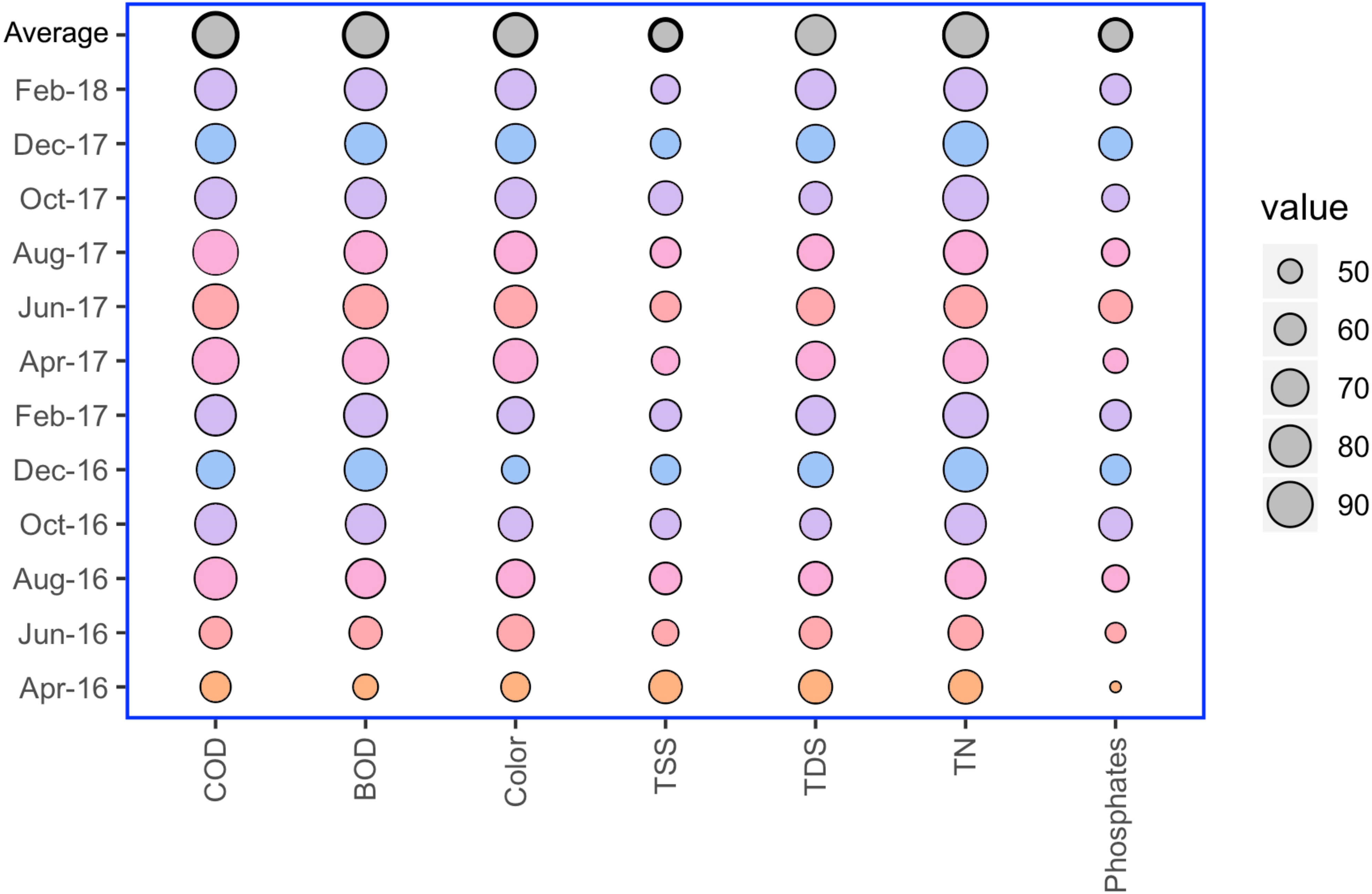

Fig. 3: Performance evaluation of FTW in terms of individual parameters, and overall response.

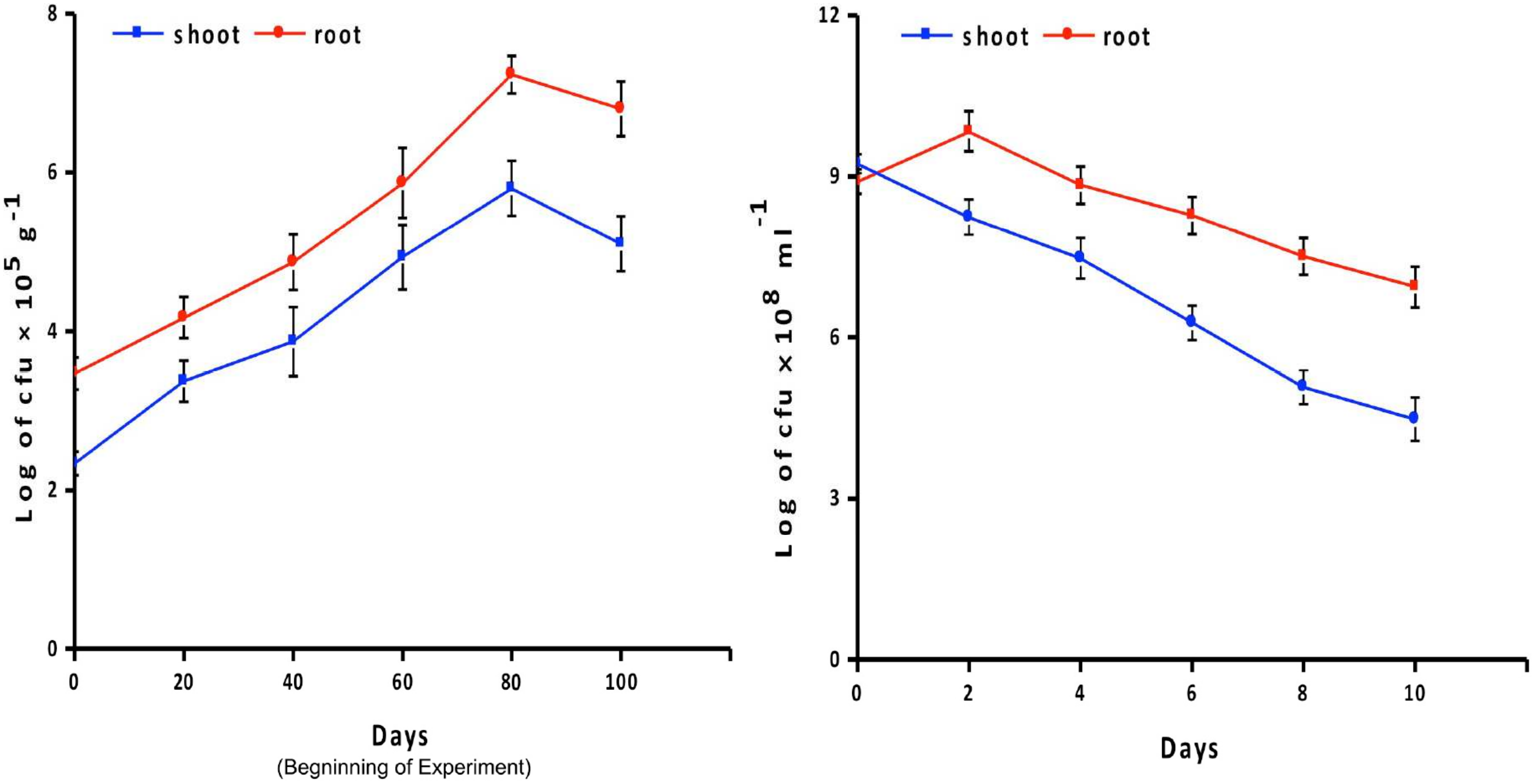

Fig. 4. Bacterial survival and colonization in water and in root and shoot interior of *Phragmites australis* for the macrocosms containing wastewater with vegetation and bacterial inoculation (T2).

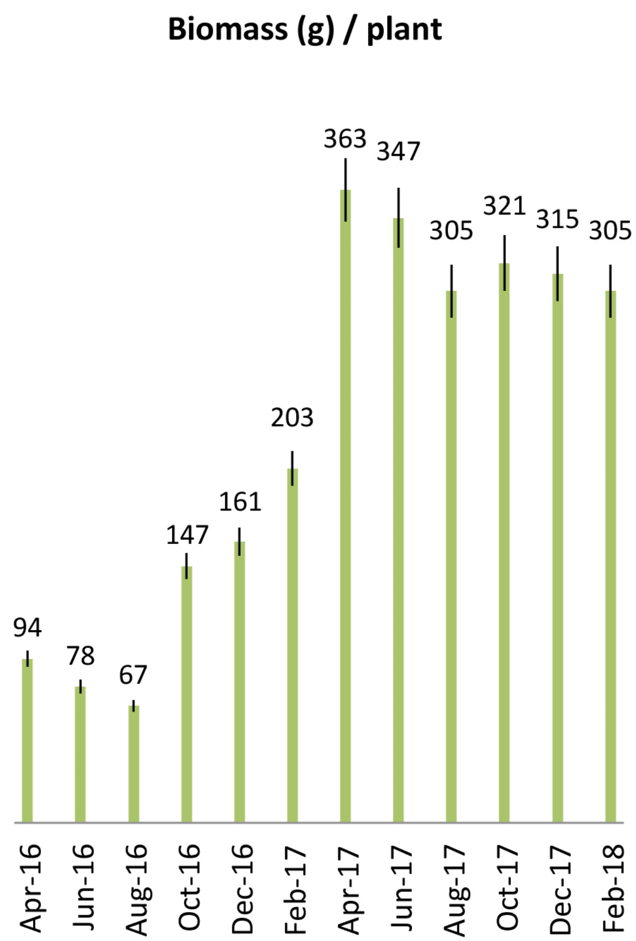

Fig. 5: Biomass of *Phragmites australis* per plant obtained after every 3-months of harvesting.

Highlights

Bacterially assisted FTWs macrocosms were engineered to treat real textile effluent

Combined application of plants and bacteria had a prominent effect on pollution reduction

Inoculated bacteria displayed persistence in different components of the FTWs

Treated wastewater met the National Environmental Quality Standards (NEQS) of Pakistan