Remediation of polluted river water by floating treatment wetlands

Munazzam Jawad Shahid, Razia Tahseen, Muhammad Siddique, Shafaqat Ali, Samina Iqbal and Muhammad Afzal

ABSTRACT

In this study, the potential of floating treatment wetlands (FTWs), inoculated with selected bacteria, to ameliorate polluted river water was evaluated. Floating cells were prepared by vegetating plants, *Typha domingensis* and *Leptochloa fusca*, on a floating mat. The plants were inoculated with three different pollutant-degrading rhizospheric and endophytic bacterial strains. Significantly greater decrease in chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) and total organic carbon (TOC) was observed in inoculated FTWs than in the wetlands without bacterial inoculation. However, a slight decrease in pH and EC was seen in most of the treatments. The total nitrogen (TN), nitrate and total phosphorus (TP) contents decreased to 1.77 mg/L, 0.80 mg/L and 0.60 mg/L, respectively. Additionally, the concentration of iron (Fe), nickel (Ni), manganese (Mn), lead (Pb), and chromium (Cr) in the water lowered to 0.41, 0.16, 0.10, 0.25, and 0.08 mg/L, respectively. Overall the performance of *T. domingensis* was significantly better than *L. fusca*. The treated effluents meet the water quality guidelines for irrigation and aquatic life. This study revealed that FTWs supplemented with selective bacteria are a promising approach for the restoration and management of polluted river water.

Key words | bacteria, floating treatment wetlands, polluted river water, river Ravi

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INTRODUCTION

Rivers, originated through the evolutionary process of the earth, are closely related to human civilization. Rivers play an important role in human life by providing myriads of benefits such as shipping, electricity generation, water supply, food and agricultural production (Allan & Castillo 2007; Pan *et al.* 2012). However, rapid industrialization, agriculture and urbanization have intensified the exploitation of rivers leading to degradation of water quality (Pan *et al.* 2016). This situation has made it imperative to devise strategies to mitigate such river water degradation. Conventional technologies can be used for remediation of polluted river water but these methods are not perfectly efficient because of economic reasons and technological complexities (Zhao *et al.* 2012).

Floating treatment wetland (FTW), an innovative approach, offers a practical solution for treatment of polluted river water (Faulwetter *et al.* 2011; Zhao *et al.* 2012; Ijaz *et al.* 2015). A FTW is composed of vegetative plants on a floating mat through which the roots grow and hang within the water column. It depicts properties of both pond and conventional wetlands as it is an inventive combination of these technologies (Headley & Tanner 2008a; Kadlec & Wallace 2008). It can be installed in any pond or river without digging, earth moving or additional land acquisition and does not reduce the storage volume of the water body (Headley & Tanner 2012; Winston *et al.* 2013). The ability of FTW to withstand water fluctuation and inundation makes it perfect for its application in pond and river systems (Headley & Tanner 2008b). In addition to that, installation of FTW in water bodies also provides habitat for wildlife such as fish, invertebrates and birds (Mietto *et al.* 2013; White & Cousins 2013; Keizer-Vlek *et al.* 2014).

In FTW, organic matter, nitrogen and phosphorus are removed by plant–bacteria interactions through decomposition, assimilation, denitrification, sorption, entrapment in roots and finally sedimentation (Wang & Sample 2014; Ijaz *et al.* 2016a). The roots of plants absorb nutrients directly from the surrounding water and provide support for the attached microbial community (Zhang *et al.* 2016; Ashraf *et al.* 2018a). Biofilm on roots and endophytes within the aerial tissue of plants boosts up the decontamination process of wastewater (Weyens *et al.* 2013; Shehzadi *et al.* 2014; Ijaz *et al.* 2015). Bacteria degrade complex organic pollutants, assimilate nitrogen and phosphorus and reduce concentrations of nitrate, phosphate and heavy metals in polluted water by their metabolic and non-metabolic processes (Afzal *et al.* 2014; Ijaz *et al.* 2016b; Arslan *et al.* 2017). Rhizospheric and endophytic bacteria also promote plant growth and surge the resistance to biotic and abiotic stresses (Afzal *et al.* 2012; Fatima *et al.* 2015).

Although FTWs have been successfully used in mitigating the pollution load of river waters (Zhao *et al.* 2012; Fang *et al.* 2016; Pan *et al.* 2016), the plant–bacteria synergism in FTWs has not been evaluated for the maximum remediation of polluted river water. The objective of our study was to evaluate the effect of inoculation of bacteria in FTWs on the restoration of polluted river water quality. The water was analysed to meet national irrigation water quality standards. Bacterial persistence was monitored in the water and in the roots and shoots of the plants.

MATERIALS AND METHODS

River water collection

The Ravi River is 725 km long with annual average flow rate of 1,813 million m^3. In Lahore district a 72 km stretch of the Ravi River is located. This part of the river has been changed into a sludge drain due to indiscriminate disposal of untreated municipal and industrial effluent (Azfar *et al.* 2018). To prepare a true mixture, water samples were collected from various points of the river during the month of March 2017. The water samples were analysed for various physico-chemical parameters and heavy metals (Table 1) by using the standard methods (APHA 2012).

Table 1 | Characteristics of polluted river water collected from Ravi River, Lahore, Pakistan

Parameter	Value	Water quality guidelines*	
		Irrigation	Fish/aquatic life
pH	8.5 (0.1)	6.5–8.4	6.5–8.5
EC (mS/cm)	2.3 (0.04)	15	15
TSS (mg/L)	290 (4.5)	NG	NG
COD (mg/L)	405 (6.2)	NG	NG
BOD (mg/L)	190.33 (5.5)	NG	8
TOC (mg/L)	110.00 (2.0)	NG	NG
TN (mg/L)	37.47 (1.1)	NG	NG
NO_3^{-1} (mg/L)	33.33 (1.5)	NG	NG
TP (mg/L)	2.63 (0.1)	NG	NG
Fe (mg/L)	1.53 (0.1)	5.0	0.3
Ni (mg/L)	0.54 (0.01)	0.20	0.05
Mn (mg/L)	0.85 (0.02)	0.20	0.1
Pb (mg/L)	0.83 (0.06)	0.1	0.01
Cr (mg/L)	0.36 (0.02)	0.01	0.05

*Water Quality Guidelines for Pakistan proposed by World Wide Fund for Nature, Pakistan (WWF 2007). Standard deviations are presented in parentheses. NG: not given in the list.

Bacterial strains

Previously isolated three bacterial strains were used in this study. The bacterial strain, *Bacillus cerus*, was isolated from the rhizosphere of *Cyperus laevigatus*. The other two bacterial strains, *Aeromonas salmonicida* and *Pseudomonas gessardii*, were isolated from root interiors and the rhizosphere of *T. domingensis*, respectively (Fatima *et al.* 2015). These bacterial strains were cultivated in Luria–Bertani (LB) broth at 37 °C, 120 rpm, for 24 h. Bacterial cells were harvested by centrifugation at 4 °C, 10,000 rpm for 10 minutes. The harvested cells were re-suspended in 0.9% (w/v) NaCl solution and their optical density (OD) was adjusted to 0.7 at 600 nm. These suspended cells of the strains were mixed together to get a bacterial consortium.

Plants

Two macrophtyes, *Typha domingensis* and *Leptochloa fusca*, were selected for this study. Both plants have worldwide distribution and are capable of coping with harsh environmental conditions. *Typha domingensis*, commonly known as southern cattail, is a tall, perennial herbaceous

plant widely distributed throughout temperate and tropical regions. This plant is highly adapted to extensively fluctuating water levels (Hegazy et al. 2011). *Leptocloa fusca*, commonly known as kallar grass, is a tufted perennial grass with spongy stems. It is frequently found in wetlands, and brackish and salty marshes. It is also best known for its ability to withstand the stress conditions of saline and sodic soils (Akhter et al. 2004).

Formation of FTWs and experimental design

To prepare the FTW cells, polyethylene tanks ($35.5 \times 30.5 \times 21.5$ cm) were used to store water. A floating mat ($33 \times 28 \times 7.6$ cm) was prepared from the closed cell polyethylene foam. Five holes of 5 cm diameter were made in each floating mat to provide anchorage to macrophytes. The borders of the floating mats were properly covered with aluminium foil (Figure 1).

One seedling of *T. domingensis* or ten cuttings of *L. fusca* were placed in each hole of the mat supported with coconut shaving and the remaining spaces were filled with soil, sand and gravel. The floating mats were transferred to a polyethylene tank filled with fresh water for 4 weeks to let the plants grow to appropriate size. After 30 d, the vegetated mats were shifted to tanks filled with 15 L of polluted water of the Ravi River, and inoculated with 100 mL bacterial consortium. The water was treated for 96 h. Each treatment had three replicates (e.g. T1R1,
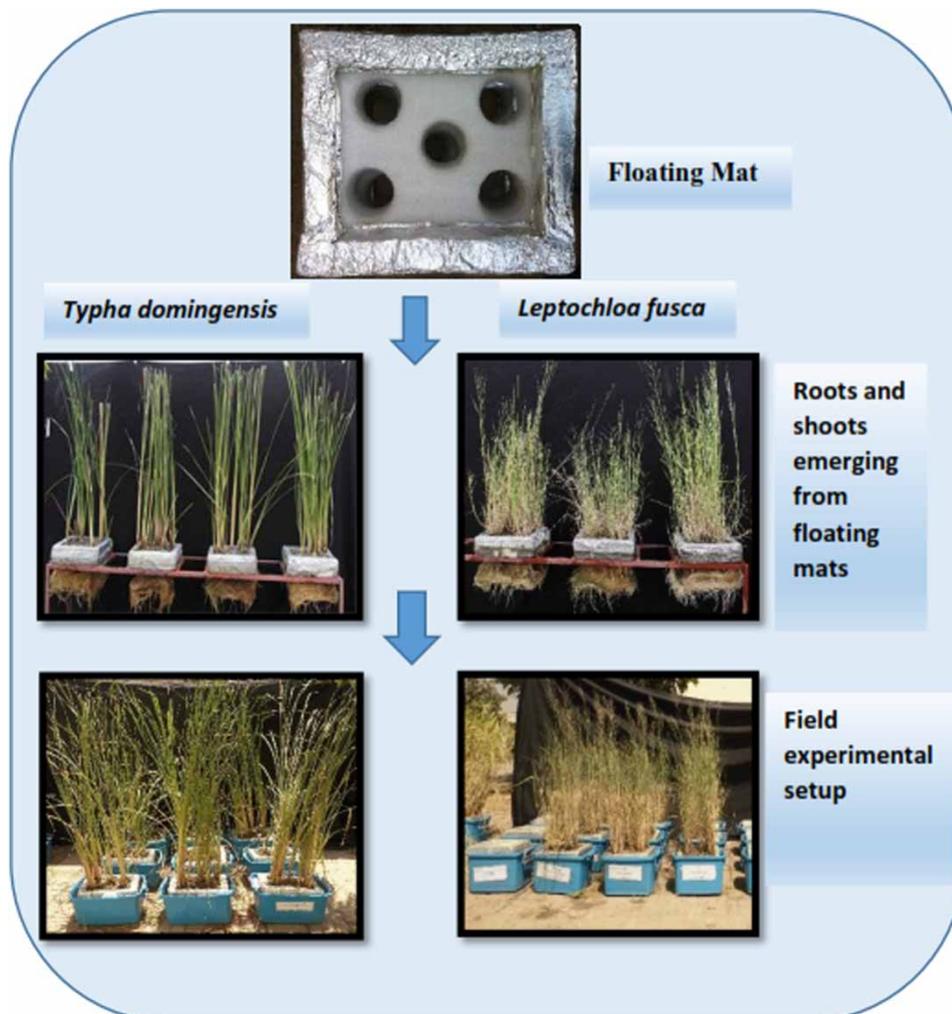
Figure 1 | Formation of floating treatment wetlands and experimental setup.

T1R2, and T1R3) and the experiment was conducted in triplicates with three batches of polluted water. Six different treatments were designed as below:

T1: Vegetation of *Typha domingensis* in polluted river water

T2: Vegetation of *Typha domingensis* in polluted river water with bacterial inoculation

T3: Vegetation of *Leptochloa fusca* in polluted river water

T4: Vegetation of *Leptochloa fusca* in polluted river water with bacterial inoculation

T5: Only bacterial inoculation in polluted river water

C: Polluted river water without vegetation and bacterial inoculation.

Water sampling and analysis

During the experiment, water samples were collected from each floating wetland cell every 24 h and stored in sterilized polyethylene bottles at 4 °C. Total suspended solid (TSS), TN, nitrate (NO_3^-), and TP were analysed by the standard methods (APHA 2012). Chemical oxygen demand, BOD_5 and TOC were analysed by method 5220D (Closed Reflux, Colorimetric Method), 5210B (5-Day BOD Test), and 5310D (Wet-Oxidation Method), respectively (APHA 2012). Samples for heavy metals analysis were prepared by nitric acid digestion (method 3030 E) (APHA 2012) and analysed by atomic absorption spectroscopy. To assess the extent of improvement in the quality of polluted river water after treatment by FTWs, a fish toxicity test was performed at the end of the experiment as described earlier (Afzal et al. 2008; Rehman et al. 2018).

Determination of inoculated bacteria

In order to analyse the survival of inoculated bacterial strains, the colony forming units were determined in the root, shoot and water samples as described earlier (Ijaz et al. 2015). The colonies were analysed by restriction fragment length polymorphism (RFLP) and their identities were compared with the inoculated strains.

Plant dry biomass

The roost and shoots of the macrophytes were harvested at the end of experiment and were gently washed with tap water and dried by absorbent paper, followed by chopping into small pieces to accelerate the drying process. The chopped samples were oven-dried at 70 °C for 48 h and their dry weight was determined (Wang et al. 2014).

Statistical analysis

Data were analysed by using Statistics 8.1. Analysis of variance (ANOVA) was used to appraise the data collected from all three replicates of each treatment. Mean values were compared by using the least significant difference test (LSD).

RESULT

River water characteristics

The values of water quality parameters are given in Table 1 which indicate the pollution status of the river water. Parameters of COD, BOD_5, and TSS signify that the Ravi River water is not as required by wastewater discharge standards of Pakistan (WWF 2007).

Water remediation by FTWs

A reduction in the pH of all treatments was observed during the whole experiment. In vegetated treatments, pH decreased from 8.5 to 7.26, with maximum reduction in T4. Overall decreases in pH in T1, T2, T3 and T4 were on par with each other but significantly differed from nonvegetated and only bacterial augmented treatments (Table 2). Similarly, EC declined in all treatments, and its maximum reduction (2.27 to 1.7 mS/cm) was observed in T2.

The maximum decrease in TSS was observed during the first 24 h of the study (Table 2). Statistically TSS reduction in vegetated treatments (T1, T2, T3, and T4) was significantly more than that in nonvegetated treatments (T5 and C). In T2, the COD, BOD_5 and TOC decreased to 47, 21 and 15 mg/L from 405, 190 and 110 mg/L, respectively (Figure 2). The treatments having plants along with bacterial

Table 2 | Reduction in pollutants by FTWs from polluted river water

Parameter	Treatment	Time					LSD: $\alpha = 5\%$
		0 h	24 h	48 h	72 h	96 h	
pH	T1	8.5^{BC} (0.10)	8.26^{EFG} (0.0)	8.16^{GHI} (0.1)	7.63^{KL} (0.1)	7.25^{M} (0.1)	0.10
	T2	8.5^{BC} (0.1)	8.25^{FG} (0.0)	8.06^{I} (0.1)	7.54^{L} (0.1)	7.2^{M} (0.1)	
	T3	8.5^{BC} (0.1)	8.37^{DE} (0.1)	8.2^{GH} (0.0)	7.82^{J} (0.0)	7.26^{M} (0.1)	
	T4	8.5^{BC} (0.1)	8.31^{EF} (0.0)	8.21^{GH} (0.0)	7.67^{K} (0.0)	7.16^{M} (0.0)	
	T5	8.5^{BC} (0.1)	8.31^{EF} (0.0)	8.33^{DEF} (0.10)	8.2^{GH} (0.1)	8.13^{HI} (0.1)	
	C	8.5^{BC} (0.1)	8.42^{CD} (0.0)	8.56^{B} (0.1)	8.56^{B} (0.1)	8.77^{A} (0.0)	
EC (mS/cm)	T1	2.3^{DEF} (0.0)	2.88^{B} (0.0)	2.4^{CD} (0.1)	2.16^{GH} (0.0)	1.97^{J} (0.0)	0.10
	T2	2.3^{DEF} (0.0)	3.11^{A} (0.0)	2.3^{DEF} (0.2)	2.03^{IJ} (0.0)	1.73^{K} (0.1)	
	T3	2.3^{DEF} (0.0)	2.1^{HI} (0.1)	2.25^{FG} (0.0)	2.03^{IJ} (0.1)	2.00^{IJ} (0.0)	
	T4	2.3^{DEF} (0.0)	3.06^{A} (0.1)	2.28^{EF} (0.0)	2.16^{GH} (0.2)	1.97^{J} (0.0)	
	T5	2.3^{DEF} (0.0)	2.48^{C} (0.0)	2.38^{CDE} (0.0)	2.04^{IJ} (0.0)	2.00^{IJ} (0.0)	
	C	2.3^{DEF} (0.0)	2.35^{DEF} (0.0)	2.36^{DE} (0.0)	2.02^{IJ} (0.0)	2.00^{IJ} (0.0)	
TSS (mg/L)	T1	289.66^{A} (4.51	76.00^{B} (2.0)	33.67^{GHI} (4.7)	19.0^{K} (1.0)	18.3^{L} (1.2)	9.05
	T2	289.67^{A} (4.5)	78.33^{B} (3.1)	36.00^{FG} (3.5)	19.0^{K} (1.7)	15.7^{KL} (1.5)	
	T3	289.67^{A} (4.5)	64.00^{CD} (2.4)	40.6^{F} (1.2)	24.7^{IJK} (3.1)	17.0^{K} (2.0)	
	T4	289.67^{A} (4.5)	72.67^{BC} (6.4)	34.3^{FGH} (3.8)	24.3^{JK} (2.5)	16.67^{K} (2.1)	
	T5	289.67^{A} (4.5)	73.00^{BC} (3.6)	60.0^{DE} (3.6)	39.33^{F} (1.2)	36.0^{FG} (1.7)	
	C	289.66^{A} (4.5)	77.33^{B} (6.7)	54.0^{E} (2.7)	37.3^{FG} (0.9)	36.0^{FG} (1.0)	
TN (mg/L)	T1	37.47^{A} (1.1)	28.53^{DE} (0.2)	15.37^{K} (0.5)	6.77^{P} (0.1)	2.03^{Q} (0.1)	0.96
	T2	37.47^{A} (1.1)	25.90^{F} (0.5)	13.20^{N} (0.2)	7.73^{O} (0.2)	1.77^{Q} (0.1)	
	T3	37.47^{A} (1.1)	29.40^{D} (0.3)	22.27^{G} (0.2)	14.2^{LM} (0.3)	7.03^{OP} (0.3)	
	T4	37.47^{A} (1.1)	28.43^{E} (0.4)	20.17^{I} (0.3)	13.2^{MN} (0.2)	6.23^{P} (0.2)	
	T5	37.47^{A} (1.1)	30.73^{C} (0.5)	25.33^{F} (0.3)	19.0^{J} (0.5)	14.83^{KL} (0.8)	
	C	37.47^{A} (1.1)	32.23^{B} (0.4)	28.57^{DE} (0.7)	25.37^{F} (0.4)	21.23^{H} (0.3)	
NO_3^{-1} (mg/L)	T1	33.33^{A} (1.5)	25.47^{C} (0.5)	12.33^{I} (0.6)	3.02^{L} (0.1)	1.17^{MN} (0.2)	1.19
	T2	33.33^{A} (1.5)	23.07^{E} (0.4)	10.23^{K} (0.2)	3.50^{L} (0.1)	0.80^{N} (0.1)	
	T3	33.33^{A} (1.5)	25.00^{CD} (1.0)	18.67^{G} (0.3)	10.7^{JK} (0.6)	3.27^{L} (0.2)	
	T4	33.33^{A} (1.5)	24.90^{CD} (0.9)	16.27^{H} (0.2)	10.5^{JK} (0.5)	2.33^{LM} (0.2)	
	T5	33.33^{A} (1.5)	26.03^{C} (1.0)	21.33^{F} (0.6)	12.80^{I} (0.4)	11.67^{IJ} (0.6)	
	C	33.33^{A} (1.5)	30.13^{B} (0.2)	24.1^{DE} (0.2)	21.67^{F} (1.2)	16.47^{H} (0.5)	
TP (mg/L)	T1	2.63^{A} (0.1)	2.10^{C} (0.1)	1.47^{H} (0.1)	1.03^{J} (0.1)	0.83^{K} (0.1)	0.13
	T2	2.63^{A} (0.1)	1.77^{F} (0.1)	1.17^{I} (0.1)	0.80^{K} (0.1)	0.60^{L} (0.1)	
	T3	2.63^{A} (0.1)	2.10^{C} (0.1)	1.80^{F} (0.1)	1.27^{I} (0.1)	0.87^{K} (0.1)	
	T4	2.63^{A} (0.1)	1.94^{DE} (0.0)	1.50^{GH} (0.1)	1.17^{I} (0.1)	0.63^{L} (0.1)	
	T5	2.63^{A} (0.1)	2.10^{C} (0.2)	2.03^{CD} (0.1)	1.87^{EF} (0.1)	1.62^{G} (0.0)	
	C	2.63^{A} (0.1)	2.38^{B} (0.1)	2.39^{B} (0.1)	2.10^{C} (0.0)	1.99^{CDE} (0.0)	

T1 (*Typha domingensis*), T2 (*T. domingensis* and bacteria), T3 (*Leptochloa fusca*), T4 (*L. fusca* and bacteria), T5 (only bacteria), C (without bacteria and vegetation). Each value is the mean of three replicates and alphabetic labels present significant differences between treatments. Standard deviations are presented in parentheses. LSD: least significant difference test.

inoculation (T2 and T4) caused significantly greater decrease in COD, BOD$_5$ and TOC as compared with treatments (T1 and T3) without bacterial inoculation.

Plant and bacterial systems significantly reduced the concentration of TN and NO$_3^-$ in the polluted river water (Table 2). Floating wetlands planted with *T. domingensis* in combination with a bacterial consortium rendered maximum reduction in TN concentration in the polluted water as compared with all other treatments. Similarly the highest

decrease of nitrate (NO$_3^-$) was observed in the floating wetlands carrying plants of *T. domingensis* and a consortium of bacteria (Table 2). A combination of both plants and bacteria in FTW cells also removed maximum TP.

Removal of heavy metals

Vegetative treatments (T1, T2, T3 and T4) significantly reduced more heavy metal contents in the polluted river
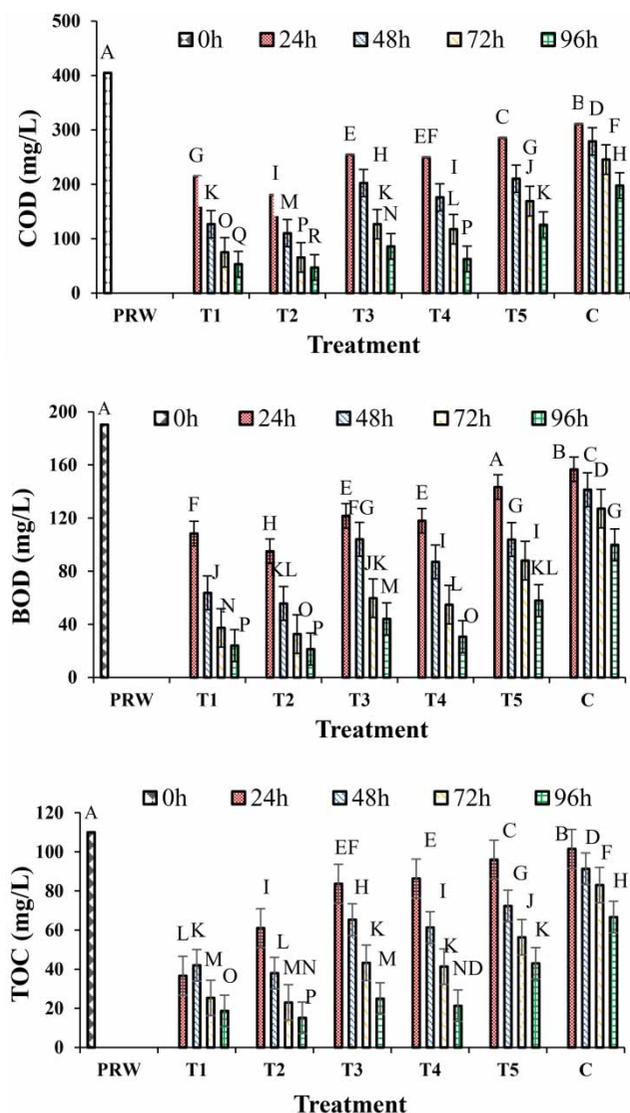
Figure 2 | Chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total organic carbon (TOC) reduction in polluted river water (PRW) by floating treatment wetlands vegetated with *Typha domingensis* (T1), *T. domingensis* and bacteria (T2), *Leptochloa fusca* (T3), *L. fusca* and bacteria (T4), only bacteria (T5), and without vegetation and bacteria (C). Each value is the mean of three replicates. Error bars indicate errors among three replicates. Means sharing the same alphabetic letters are statistically non-significant.

water as compared with nonvegetated treatments (T5 and C) (Table 3). The maximum removal of heavy metals was observed in T2 and T4.

Survival and colonization of inoculated bacteria

Significant numbers of inoculated bacteria were observed in the roots, shoots and water (Table 4). The bacteria colonized

more in the roots than shoots. The number of inoculated bacteria continuously increased in the roots and shoots. In water, the concentration of inoculated bacteria increased in the first 48 h and then decreased. However, in nonvegetated treatments (T5), the number of inoculated bacteria continuously decreased.

Plant dry biomass

The total dry biomass of roots and shoots of *T. domingensis* was higher than *L. fusca* (Table 5). The dry biomass of treatments inoculated with the bacterial consortium was higher than treatments without bacterial inoculation.

DISCUSSION

In this study, the pH of the polluted river water was gradually decreased with the passage of time. The release of organic acids by the roots of plants and their associated microbial pollutant degradation activities contribute to lowering the pH of the water (Zhai *et al.* 2013). Electrical conductivity (EC) is a handy and useful indicator of total salt content in water (Morrison *et al.* 2001; Zhao *et al.* 2013). In the present study, EC decreased with the passage of time. A slight increase in EC after 24 h may result due to release of some liquid exudates from the roots of the plants (Ijaz *et al.* 2016b). The decrease in EC with time was as a result of nutrient uptake by the plants and the binding of dissolved elements with suspended particles (Zhao *et al.* 2013).

In this study, considerable removal of TSS from the polluted river water by the application of FTWs was observed. Natural sedimentation is the key factor by which large particles settle down to the bottom of ponds. Following sedimentation, vegetation also plays an important role in TSS removal. The expanding roots of FTWs provide a large surface area for the attachment of soluble solids. Earlier studies also reported that TSS contents tend to decline in water with FTW as a consequence of the trapping of soluble solids by roots (Morrison *et al.* 2001).

In this study, vegetated FTWs, with or without bacterial inoculation, efficiently removed the organic and inorganic pollutants from polluted river water. However, the performance of FTWs inoculated with bacteria was better and faster

Table 3 | Reduction in heavy metals by FTWs from polluted river water

Metal	Treatment	Time					LSD: $\alpha = 5\%$
		0 h	24 h	48 h	72 h	96 h	
Fe (mg/L)	T1	1.53[A] (0.12)	1.14[CDEF] (0.09)	0.83[HIJ] (0.02)	0.63[KLM] (0.12)	0.47[MN] (0.06)	0.18
	T2	1.53[A] (0.12)	0.67[JKL] (0.50)	0.73[JK] (0.03)	0.57[KLMN] (0.03)	0.41[N] (0.01)	
	T3	1.53[A] (0.12)	0.97[FGHI] (0.01)	0.83[HIJ] (0.01)	0.73[JK] (0.01)	0.52[LMN] (0.02)	
	T4	1.53[A] (0.12)	1.00[FGH] (0.00)	0.82[IJ] (0.01)	0.66[JKL] (0.01)	0.42[N] (0.02)	
	T5	1.53[A] (0.12)	1.22[BCD] (0.03)	1.27[BC] (0.06)	1.07[DEFG] (0.06)	0.95[GHI] (0.00)	
	C	1.53[A] (0.12)	1.32[BC] (0.02)	1.37[AB] (0.12)	1.20[BCDE] (0.01)	1.03[EFG] (0.06)	
Ni (mg/L)	T1	0.54[A] (0.01)	0.40[G] (0.02)	0.27[K] (0.00)	0.18[N] (0.00)	0.13[O] (0.010)	0.02
	T2	0.54[A] (0.01)	0.36[H] (0.01)	0.23[L] (0.01)	0.16[N] (0.01)	0.09[P] (0.01)	
	T3	0.54[A] (0.01)	0.45[E] (0.01)	0.29[J] (0.03)	0.23[L] (0.01)	0.18[MN] (0.01)	
	T4	0.54[A] (0.01)	0.41[FG] (0.01)	0.34[I] (0.02)	0.20[M] (0.01)	0.16[N] (0.01)	
	T5	0.54[A] (0.01)	0.48[CD] (0.01)	0.45[E] (0.01)	0.43[F] (0.00)	0.43[F] (0.01)	
	C	0.54[A] (0.01)	0.50[B] (0.01)	0.49[BC] (0.00)	0.49[BC] (0.00)	0.46[DE] (0.00)	
Mn (mg/L)	T1	0.85[A] (0.02)	0.57[F] (0.01)	0.33[JK] (0.02)	0.15[MN] (0.01)	0.12[O] (0.02)	0.03
	T2	0.85[A] (0.02)	0.51[H] (0.02)	0.35[IJ] (0.01)	0.12[S] (0.00)	0.10[P] (0.01)	
	T3	0.85[A] (0.02)	0.58[F] (0.01)	0.37[I] (0.01)	0.22[L] (0.02)	0.17[M] (0.01)	
	T4	0.85[A] (0.02)	0.54[G] (0.01)	0.30[K] (0.01)	0.21[L] (0.02)	0.14[NO] (0.01)	
	T5	0.85[A] (0.02)	0.71[C] (0.02)	0.67[D] (0.01)	0.63[E] (0.01)	0.57[F] (0.06)	
	C	0.85[A] (0.02)	0.75[B] (0.01)	0.72[C] (0.01)	0.68[D] (0.00)	0.64[E] (0.01)	
Pb (mg/L)	T1	0.83[A] (0.06)	0.51[IJ] (0.02)	0.42[KL] (0.01)	0.39[LM] (0.01)	0.35[MN] (0.01)	0.04
	T2	0.83[A] (0.06)	0.45[K] (0.02)	0.34[N] (0.01)	0.29[O] (0.02)	0.25[O] (0.02)	
	T3	0.83[A] (0.06)	0.65[DEF] (0.01)	0.61[FG] (0.02)	0.57[GH] (0.01)	0.54[HI] (0.01)	
	T4	0.83[A] (0.06)	0.62[EF] (0.02)	0.57[GH] (0.01)	0.54[HI] (0.02)	0.49[J] (0.02)	
	T5	0.83[A] (0.06)	0.68[BCD] (0.00)	0.68[BD] (0.01)	0.65[CDE] (0.00)	0.65[CDE] (0.00)	
	C	0.83[A] (0.06)	0.71[B] (0.01)	0.70[B] (0.01)	0.70[B] (0.00)	0.68[BCD] (0.01)	
Cr (mg/L)	T1	0.36[A] (0.02)	0.28[D] (0.01)	0.23[EF] (0.02)	0.14[J] (0.01)	0.10[KLM] (0.01)	0.02
	T2	0.36[A] (0.02)	0.20[G] (0.01)	0.13[J] (0.01)	0.11[K] (0.01)	0.08[MN] (0.01)	
	T3	0.36[A] (0.02)	0.23[EF] (0.02)	0.18[H] (0.01)	0.14[J] (0.02)	0.11[KL] (0.02)	
	T4	0.36[A] (0.02)	0.22[EF] (0.01)	0.16[I] (0.01)	0.09[LMN] (0.01)	0.08[N] (0.01)	
	T5	0.36[A] (0.02)	0.31[C] (0.00)	0.28[D] (0.01)	0.24[E] (0.01)	0.24[E] (0.01)	
	C	0.36[A] (0.02)	0.33[B] (0.00)	0.31[C] (0.03)	0.29[D] (0.01)	0.28[D] (0.01)	

T1 (*Typha domingensis*), T2 (*T. domingensis* and bacteria), T3 (*Leptochloa fusca*), T4 (*L. fusca* and bacteria), T5 (only bacteria), and C (without bacteria and vegetation). Each value is the mean of three replicates and alphabetic labels present significant differences between treatments. Standard deviations are presented in parentheses. LSD: least significant difference test.

than those without inoculation. It suggests that the inoculation of plants with bacteria enhances their natural ability to utilize their metabolic mechanisms to eliminate various types of pollutants from wastewater (Vymazal 2014; Fang *et al.* 2016). In addition to this, the better performance of inoculated FTWs can be attributed to the ability of bacteria to mineralize organic pollutants (Afzal *et al.* 2014; Shehzadi *et al.* 2014; Ijaz *et al.* 2015). The bacteria on roots, hanging in the water column, contribute to the degradation of organic matter leading to reduction in COD and BOD$_5$ (Khan *et al.* 2013; Shehzadi *et al.* 2016). In this study, the rapid and higher reduction in COD, BOD$_5$ and TOC in bacterial inoculated treatment is probably due to the degradation of

organic matter by the high number of bacteria in the water and on the roots.

In rivers, the discharge of domestic wastewater and agricultural runoff are the major source of TN, leading to eutrophication (Zhao *et al.* 2012). Nitrification, denitrification, uptake by plants and microbes and volatilization are the processes responsible for removal of nitrogen from water (Tanner *et al.* 1999). However, in FTWs, roots are exposed to water only, which may enhance the nutrient uptake by plants (Stewart *et al.* 2008; Li *et al.* 2010). In this study, FTWs efficiently removed N and NO$_3^-$ from the river water. The use of *T. domingensis* in FTWs removed nitrogen and NO$_3^-$ more efficiently than *L. fusca,* which

Table 4 | Survival of inoculated bacteria in roots, shoots and water of FTWs

Treatment	CFU (10³/g root)				CFU (10³/g shoot)				CFU (10⁵/mL water)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
T2	2.57[D] (0.4)	4.90[C] (0.8)	6.27[B] (0.6)	7.17[A] (0.3)	2.17[E] (0.3)	3.10[D] (0.3)	4.50[B] (0.3)	5.10[A] (0.3)	17.4[G] (1.1)	21.9[DE] (0.6)	25.37[C] (0.8)	27.03[B] (0.7)
T4	1.40[E] (0.4)	2.37[D] (0.4)	4.17[C] (0.4)	6.13[B] (0.3)	1.17[F] (0.2)	2.73[D] (0.4)	3.83[C] (0.4)	4.27[B] (0.2)	17[G] (1.0)	19.17[F] (0.8)	21.6[E] (1.4)	23.3[D] (0.5)
T5	–	–	–	–	–	–	–	–	18.17[FG] (1.4)	28.73[A] (0.8)	19.2[F] (0.7)	14.63[H] (0.4)
LSD: $\alpha = 5\%$	0.79				0.43				1.45			

T1 (*Typha domingensis*), T2 (*T. domingensis* and bacteria), T3 (*Leptochloa fusca*), T4 (*L. fusca* and bacteria), T5 (only bacteria), and C (without bacteria and vegetation). Each value is the mean of three replicates and alphabetic labels present significant differences between treatments. Standard deviations are presented in parentheses. LSD: least significant difference test.

Table 5 | Plant dry biomass under different treatments

Treatment	Root (g)	Shoot (g)
T1	46 (4)	124.6 (9)
T2	79.3 (8.5)	162.6 (7.5)
T3	18.3 (2.1)	135 (4.4)
T4	21.6 (2.5)	164 (6)

T1 (*Typha domingensis*), T2 (*T. domingensis* and bacteria), T3 (*Leptochloa fusca*), and T4 (*L. fusca* and bacteria). Each value is a mean of three replicates with standard deviation in parentheses.

might be attributed to the vigorous growth of the roots of *T. domingensis*. Roots of plants provide a base for periphytic microorganisms which enhance the breakdown of organic pollutants and nitrogen removal from water (Hu *et al.* 2010; Zhao *et al.* 2012).

In this study, although the phosphorus concentration was low (2.5 mg/L) in the water, the average removal efficiency was about 67% to 76% in the vegetated FTWs, which is much higher than those reported previously (Stewart *et al.* 2008; Tanner & Headley 2011). In FTWs, phosphorus removal may depend upon the sorbent/substrate surrounding the roots (Yang *et al.* 2008; Lijuan *et al.* 2017), surface area and aeration (Stewart *et al.* 2008). Plants and microbial populations that colonize plant tissues and the rhizosphere are the major factors responsible for the removal of phosphorus (Stewart *et al.* 2008). In this study, FTWs inoculated with a bacterial consortium have shown a high percentage of P removal as compared with non-inoculated treatments, which may be due to the enhanced activity of bacteria. Moreover, the P removal efficiency of *T. domingensis* was better than that of *L. fusca*, which might be the result of the vigorous growth of its roots (Table 2). In addition to microbial activity in the rhizosphere, physical processes also play a key role in P removal (Zhu *et al.* 2011).

In this study, a significant amount of heavy metals was removed from the polluted river water. Adsorption, direct uptake by plants, entrapment by bacterial biofilms and metal sulphide formation can be the major processes involved in removal of heavy metals from contaminated waters (Kadlec & Wallace 2008). Other factors which may contribute in the removal of metal ions from water entail pH, redox potential, hydrated oxides and carbonates of

Table 6 | Fish toxicity assay of polluted river water detoxified by FTWs

	Fish death over time				
Treatment	24 h	48 h	72 h	96 h	Total death
T1	2	0	0	0	2/10
T2	0	1	0	0	1/10
T3	1	0	0	0	1/10
T4	1	0	1	0	2/10
T5	3	1	0	0	4/10
C	5	2	0	0	7/10

T1 (*Typha domingensis*), T2 (*T. domingensis* and bacteria), T3 (*Leptochloa fusca*), T4 (*L. fusca* and bacteria), T5 (only bacteria), and C (without bacteria and vegetation).

metals and biofilm development on the rhizoplane (Hansel *et al.* 2001). Sequestration of metal ions and iron plaque development around the roots of submerged macrophytes are common processes of heavy metal removal (King & Garey 1999; Hansel *et al.* 2001; Ashraf *et al.* 2018b). Oxidation by bacteria may also enhance iron plaque formation (Li *et al.* 2011).

In this study, heavy metal removal was increased by the inoculation of bacteria to the plants. The enhanced performance due to bacterial inoculation can be attributed to the ability of bacteria to sorb metals on their cell walls (Ijaz *et al.* 2015; Ashraf *et al.* 2018c). Bacterial inoculation also helps in the uptake of heavy metals by plants because their bioavailability is increased with microbes (Sessitsch *et al.* 2013; Khan *et al.* 2015; Ijaz *et al.* 2016b).

The survival of fish in treated water indicated detoxification and pollutant reduction in the river water (Table 6). Moreover, bacterial augmentation in FTWs further improved water quality and reduced the toxicity of the water. Many earlier studies also demonstrated that plant–bacteria synergism is a more effective approach in the detoxification of polluted water (Shehzadi *et al.* 2014; Ijaz *et al.* 2015).

CONCLUSIONS

The aim of this study was to evaluate the effect of bacterial inoculation in FTWs on the remediation of polluted river water. The developed FTW system efficiently decreased COD, BOD, TOC, TN, NO$_3$, TP and heavy metals in the polluted river water. The performance of *T. domingensis* was better than *L. fusca* in the removal of most of the pollutants. Therefore, *Typha domingensis* is a better choice for developing FTWs for the treatment of polluted river water. The inoculation of FTWs with bacteria further boosted the efficiency of the plants in removing the pollutants from the water. The FTW-treated water complies with the national standards of water for irrigation and aquatic life. These results suggest that FTWs with bacterial inoculation are a simple and efficient technology for the treatment of polluted river water and for the natural ecosystem of river to flourish. Floating treatment wetlands augmented with bacteria can be applied on wastewater collection drains to mitigate their pollutant load prior to discharging it into rivers or other water systems. Additional research is needed to explore the practical application of the developed FTW system at a commercial scale.

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