Potentialities of floating wetlands for the treatment of polluted water of river Ravi, Pakistan

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ABSTRACT

River pollution has become a growing concern in developing countries. It can affect food supplies, drinking water, and environment hence impacting animal and human health. The present study was designed to explore the potential of floating wetlands (FWs) in partnership with pollutant-degrading bacteria for the clean-up of heavily contaminated water of river Ravi at microcosm scale. River Ravi receives excessive discharges of untreated sewage and industrial wastewater from the Lahore city. To this end, FWs are sustainable alternatives to treat wastewater because of their high efficiency and simplicity in the design and structure. Thus, remediation potential of FWs planted with two macrophytes namely *Phragmites australis and Brachia mutica* was evaluated in the presence of a consortium five different rhizospheric and endophytic bacteria. We found a significant reduction of organic and inorganic pollutants by the application of FWs, whose potential was further boosted by bacterial inoculation. The performance of *P. australis* was better than *B. mutica*. Briefly, plant-bacterial synergism for *P. australis* reduced COD, BOD_5, and TOC up to 85.9%, 83.3%, and 86.6% in 96 h, respectively. Total nitrogen was reduced from 37.5 to 2.07 mg l^{-1}, nitrate from 33.3 to 1.23 mg l^{-1}, and phosphorus from 2.63 to 0.53 mg l^{-1}. Trace metals were also reduced up to 79.5% for iron, 91.4% for nickel, 91.8% for manganese, 36.14% for lead, and 85.19% for chromium. The better persistence of inoculated bacteria was tracked in the root/shoot interior of *P. australis,* which suggest their potential role in improved pollutant reduction. It is thus concluded that bacterial-assisted FWs may be a suitable choice for the remediation of heavily polluted river water. Furthermore, a field-scale application of FWs for the on-site treatment of contaminated water on the Ravi river is recommended.

Keywords: Floating wetlands, hydroponic water treatment, plant-bacteria synergism, polluted river water, Ravi river

INTRODUCTION

In developing countries, rivers are crucial to human health because they provide water for drinking and irrigation purposes (Steward *et al*., 2012). On the other hand, increasing industrialization is severely deteriorating the rivers' water quality due to direct discharges of industrial effluents in rivers (Suthar *et al*., 2010; Kanu *et al*., 2011). The condition is getting worse in many parts of the world where rivers are considered as easy disposal points for effluents. River Ravi is one of them that continuously receives untreated sewage and industrial wastewater making it a heavily polluted river of Pakistan (Ahmed and Ali, 1998; Baqar *et al*., 2013). High levels of trace metals, polychlorinated biphenyls, organochlorine pesticides, and other toxic compounds have been detected from the river's water and sediments, vegetables grown on the banks, and aquatic organisms dwelling the river habitat (Baqar *et al*., 2017, 2018; Khanum *et al*., 2017; Riaz *et al*., 2018). The ecological risk assessment studies have revealed considerable risk associated during pre-monsoon and moderate risk during the post-monsoon season (Baqar *et al*., 2017).

Floating wetlands (FWs) is considered as an innovate technique for the treatment of polluted water (Headley *et al.,* 2008; Mietto *et al.,* 2013). The key factors which make FWs distinguish from other constructed wetlands are its easy installation without additional land acquisition, tolerance to water inundation, and enhancement of aesthetic value of water bodies (Chang *et al.,* 2012; Headley & Tanner 2012; Chang *et al.,* 2013; Winston *et al.* 2013). A typical FWs system comprises macrophytes that grow on a floating raft whereas the plant roots extend down in the water body leading to pollutants removal/degradation by physicochemical and biological mechanisms (Winston *et al.* 2013; Li & Guo 2017). This is achieved through efficient interactions between plants and bacteria – two main components of FWs (Afzal *et al.,* 2014a; Keizer-Vlek *et al.,* 2014; Hardoim *et al.,* 2015; Pavan *et al.,* 2015). Briefly, plant uptake nutrients for its growth and development (Chen et al., 2016), suspended particles are entrapped or settled down due to the physical effect of plant roots (Vymazal 2014); and biofilms are established on roots providing a large surface area for degradation (White & Cousins 2013). Ultimately, an increase in contact time of bacteria with contaminants leads to enhanced degradation of organic pollutants (Stewart *et al.,* 2008; Tanner & Headley 2011).

Bacterial community surviving in plant rhizo- and endosphere plays a major role in the pollutant removal scheme (Afzal *et al.,* 2014b). Rhizobacteria are known to regulate denitrification process and hence are important in the removal of nitrogen (Lin *et al.,* 2002; Tanner *et al.,* 2002); whereas endophytic bacteria play a significant role in biodegradation of organic compounds (Afzal et al., 2014; Ijaz et al., 2016). Moreover, they increase the bioavailability of trace metals and hence their removal by sorbing the metallic ion onto the bacterial cell walls or through direct uptake by the plants (Mullen *et al.*, 1989; Zhu *et al*., 2011; Khan *et al*., 2015). These bacteria also improve the plant performance due to their plant growth promoting (PGP) traits such as phytohormones production, phosphorous solubilisation, release of siderophores, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Burken 2003; Weyens *et al.,* 2009a; Weyens *et al.,* 2013; Hardoim *et al.* 2015).

Previously, FWs have been effectively implemented for the remediation of various type of polluted water (Chen *et al.,* 2014; Keizer-Vlek *et al.,* 2014; Ijaz *et al.,* 2016a; Richter *et al.,* 2016). However, the combined use of plants and bacteria for the remediation of polluted river water in FWs has been rarely investigated. It has been established that bacterial consortium performs better than the individual bacteria in pollutant degradation (Lee *et al.,* 2004, Dary et al., 2010; Srivastava et al., 2013); therefore, we also used this strategy by adding consortium of five rhizo- and endophytic bacterial strains for the enhanced remediation of polluted river

water. Furthermore, this study assesses the potential of using commercially available polystyrene board for the construction of a floating mat, which has been previously used for insulation purposes.

METHODOLOGY

Study area and water collection

River Ravi is a transboundary river that originates in India but passes across a mega metropolitan city Lahore, Pakistan. In Lahore, it receives untreated municipal and industrial wastewater at six drains outfalls, i.e., five municipal drains and one surface water drain (Baqar et al., 2014). In this study, polluted water was collected from each drain outfall during February and March 2017. The water samples were immediately transferred to National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan; where they were processed for analytical measurements and FWs establishment after homogenous mixing.

Pollution parameters assessment

Water samples were examined for various physicochemical parameters such as pH, electrical conductivity (EC), total dissolve solids (TDS), fine suspended solids (FSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total nitrogen (TN), total organic carbon (TOC), nitrate (NO_3^{-1}) and total phosphorus (TP) content using standard methods (APHA, 2012). Trace metal concentrations for iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and chromium (Cr) were monitored by using atomic absorption spectrophotometry (Spectra AA. 200, Varian Australia, Australia). Fish toxicity assay was also conducted to assess the overall water toxicity as described previously (Saleem *et al.*, 2018)

Bacterial strains

In this study, we used a consortium of five strains namely *Aeromonas salmonicida* (NCBI Accession: KF478208), *Bacillus cerus* (NCBI Accession: KF478198), *Pseudomonas indoloxydans* (NCBI Accession: MF478985), *Pseudomonas gessardii* (NCBI Accession: KF478209), and *Rhodococcus* sp. (NCBI Accession: MF326802). The strains *A. salmonicida*, and *P. indoloxydans* were endophytes because they were previously isolated from the root interior of *Polygonum aviculare* and *Typha domingensis;* whereas, *B. cerus*, *P. gessardii,* and *Rhodococcus* sp. were rhizospheric as they were isolated from the rhizosphere of *Cyperus laevigatus*, *T. domingensis* and *Poa labillardierei*, respectively (Tara et al., 2018). These bacterial strains were screened due to their abilities to degrade organic pollutants as well as promote plant development in harsh environmental conditions. Each bacterial strain was cultivated separately in Luria-Bertani (LB) broth for 24 h. The bacterial cell pellet was harvested by centrifugation followed by re-suspension in normal saline. The optical density (OD) was adjusted to 0.9 at 600 nm. This inoculum was prepared by mixing all strains together in equal proportion to prepare the bacterial consortium (10^9 CFU ml^{-1}) for inoculation of plants.

Experimental setup and analysis

A total of eighteen FWs cells were established by using plastic tanks of capacity 15 L each. Floating mats were made from polystyrene-based board manufactured by Diamond® Jumbolon

Foam Company, Pakistan. The board was cut into 50 (length) × 36 (width) × 7 (thickness) cm and five equidistant holes were made for vegetation purpose (Fig. 1).

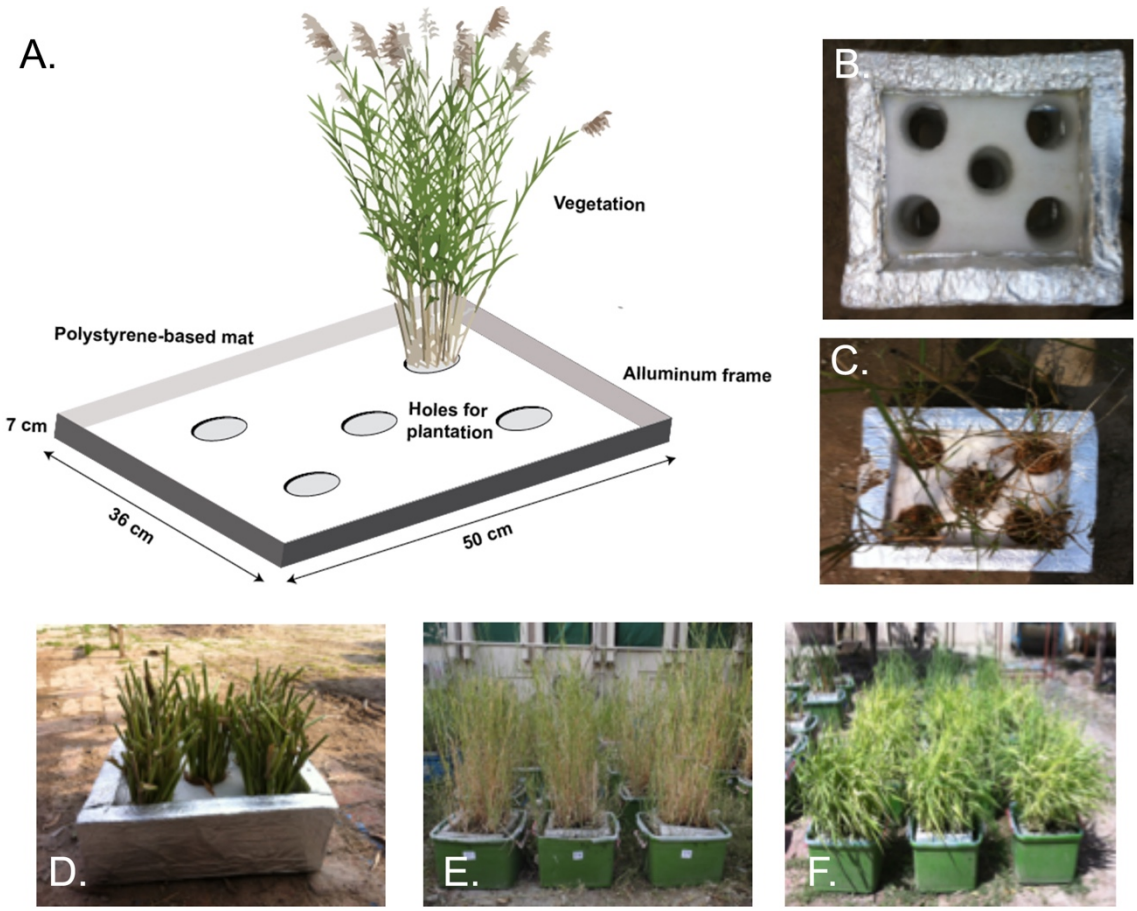

Fig. 1. Establishing the floating treatment wetlands (FTWs) microcosm experiment. (A) Schematic representation of the floating mat; (B-D) FTW reactor after vegetation installation; (E) FTWs prepared with *Phragmites australis*; and (F) FTWs prepared with *Brachia mutica.*

The borders of the floating mat were covered with aluminum foil to avoid damage caused by sunlight. Fifteen equal sized healthy seedlings of *Brachia mutica* or four of *Phragmites australis* were fixed in each hole with the help of coconut shaving. The seedlings were previously grown in the nursery of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. In the nursery, plants are grown from the cuttings in the natural environment and transported to experiment after one-month period of growth at least. The initial fresh biomass of each seedling of *P. australis* and *B. mutica* was 28.7 ± 3.4 g and 10.45 ± 2.1 g, respectively. The upper one inch of each floating mat was filled with soil and gravel to provide maximum support to vegetated plants. These vegetated mats were allowed to grow in fresh water for one month whereas Hoagland solution was supplied fortnightly to attain optimal development of roots and shoots before shifting to polluted river water tanks. The mats covered about >90 % of the water surface. The whole experiment was run in triplicates with the following treatments.

T1: Polluted water with *P. australis*

T2: Polluted water with *P. australis* and bacterial inoculation

T3: Polluted water with *B. mutica*

T4: Polluted water with *B. mutica* and bacterial inoculation

T5: Polluted water with bacterial inoculation

C: Polluted water without plant and bacterial inoculation

Treatment T2, T4, and T5 were inoculated with the previously prepared bacterial consortium (100 ml). The batch experiment was conducted in natural environmental conditions and repeated for three times. Water samples (~250 ml) were obtained with a syringe from each treatment every 24 h over the period of four days and stored in sterilized glass bottles at 4 °C for analysis.

Trace metals analysis

Trace metals analyses were performed by using atomic absorption spectrometry (AAS), Varian SpectrAA.200 (Varian Australia, Victoria, Australia). Prior to this, samples were digested with nitric acid as explained previously (method 3030 E) (APHA 2012). Briefly, 20 ml water sample was taken in digestion tubes (VELP, Scientifica) and then 5 ml of concentrated HNO_3 was added. The tubes were placed in the digester block. After 5 minutes of boiling, the digester block was turned off; upon cooling, 0.5 ml H_2O_2 was added. The tubes were put back in a digester at 25 °C for 10 minutes until a clear solution was obtained. After cooling down, the sample was transferred to measuring cylinder and the volume was raised to 50 ml. The sample was then analyzed via atomic absorption spectrometer. The chemicals used in samples and standards preparation were of analytical grade. For quality control purposes, blanks and duplicates were run in parallel. The detection limit for Fe, Ni, Mn, Pb, and Cr was 0.06, 0.01, 0.02, 0.01 and 0.06 mg l^{-1} at 98% confidence level.

Plant biomass

At the end of the experiment, plant agronomic parameters such as root and shoot length, and fresh and dry biomass were observed to see the effect of river pollution and bacterial inoculation on plant growth. For this purpose, plant shoots were harvested 1 cm above the mat surface. Root and shoot lengths were measured manually by using a measuring scale. The fresh weight of root and shoot were noted immediately. The dried biomass was evaluated by drying the roots and shoots at 60 °C in an oven for three days until a constant weight was achieved.

Persistence of inoculated bacteria

The persistence/survival of inoculated bacteria was monitored in the rhizosphere as well as in the endosphere by cultivation-dependent plate count method at the end of the experiment (Saleem *et al.*, 2018; Kämpfer *et al.,* 1996). For rhizospheric bacteria, 1 ml of rhizospheric pore water was serially diluted (10^{-6}) and then spread on to the agar plates supplemented with 1% (v/v) diesel. For endophytic bacteria, plant tissues (roots and shoots) were surface sterilized by using 70% ethanol and 2% sodium hypochlorite solution. Subsequently, 1 g of plant material was ground in a mortar and pestle to make suspension whose serial dilutions (10^{-6}) were spread on agar plates having diesel. The plates were then incubated for 48 h at 37 °C for CFU analysis, and a significant number of bacterial colonies were picked randomly for further analysis.

The identity of the isolated bacteria was compared with the inoculant strains via restriction fragment length polymorphism (RFLP) analysis (Rehman *et al.,* 2018; Afzal *et al.*, 2012; Liu

et al., 1997). For this purpose, colony PCR for the Inter Gene Sequence (IGS) was set up with reverse primer (5' – GGCTGCTTCTAAGCCAAC- 3') and a forward primer (5' – TGCGGCTGGATCACCTCCT- 3') in the presence of 1 µl of each bacterial colony previously suspended in PCR water. Amplified IGS product was used to set up an RFLP experiment. Each RFLP reaction was incubated at 37 °C for 3 h, and each 1X reaction constituted 7 µl IGS product, 1.5 µl R-buffer, 1 µl HindIII enzyme, and 5.5 µl deionized water (15 µl reaction). RFLP product was validated by electrophoresing DNA in 2% agarose gel on 70V and viewing in Gel Documentation system.

Toxicity reduction

Reduction in the toxicity of treated river water was measured by fish toxicity test (Afzal *et al*., 2008; Ijaz *et al.,* 2016b). Approximately 10 liters of the treated water was poured in the aquarium (depth, width, length: 30 × 30 × 45 cm; with 40 L capacity) specified for fish culture. Fish species *Labeo rohita* (Rohu) was selected for the toxicity assay due to two reasons: (1) the species is abundantly present in the local streams of Pakistan, which would depict toxicity scenario of Ravi river contamination (Khan et al. 2017); and (2) it has previously reported that the species can accumulate trace metals above the natural levels (Hamid et al. 2016). Ten fish (*Labeo rohita*) of equal weight (~ 10.5 ± 1.1 g) and length (8.3 ± 0.9 cm) were put in the aquarium. Finally, the survival rate of fish was noted every 24 h for the period of 4 days.

Statistical analysis

Results of pollution parameters, plant biomass, and bacterial persistence were subjected to statistical analysis using Statistix 8.1. Factorial Analysis of Variance (ANOVA) was applied to compare means across two or more independent variables. Further, least significant difference test (LSD) ($\alpha = 0.05$) was used to make all pairwise comparison between treatments into time. The alphabets labeled on values represent the significant/non-significant difference between/within treatments. The values sharing same alphabets are significantly not different from each other and vice versa, e.g., value labeled with "abc" is significantly not different from values labeled with a, b, or c but significantly different from value labelled by "def"/ d, e or f.

RESULT AND DISCUSSION

Pollution load assessment in the water of Ravi river

Physicochemical analysis of Ravi river water revealed a high level of contamination when compared to the Water Quality Guidelines as proposed by World Wide Fund (WWF) for Nature, Pakistan (WWF, 2007) and wastewater discharge standards as established under National Environmental Quality Standards (NEQS, 1999) of Pakistan (Table 1). More precisely, river's water was found to be slightly alkaline (pH = 8.5) with high concentrations of EC, BOD, Fe, Ni, Mn, Pb and Cr making it unfit for irrigation and aquatic life; whereas comparison with NEQs displayed even a high level of pollution than the wastewater discharge standards particularly described for TSS, COD, and BOD_5. These results are in accordance to the other studies who also reported high pollution in the river Ravi due to direct sewage and industrial discharges (Baqar et al., 2014, 2017, 2018; Khanum *et al.*, 2017; Riaz *et al*., 2018).

Changes in physical parameters of the river water after treatment

Table 2 displays the changes in physical parameters of the river's water in the period of 96 h of treatment. The FWs had a positive effect on the reduction of pH, EC, TDS, and TSS in all the treatments (T1-T5). The pH decreased significantly in all treatments with a sharp reduction in vegetated treatments (T1-T4) as compare to non-vegetated treatment (T5) and control. A decrease in pH could be due to the release of acidic root exudates or organic acids during microbial degradation of organic matter (Lynch *et al.,* 2015; Abed *et al.,* 2017). The reduction in EC might be associated with the nutrient uptake by plants and physicochemical/biological binding of pollutants. Whereas lowering of TDS and TSS load can be attributed to the physical effect of plants, sedimentation processes, and hanging roots as attachment sites for suspended particles leading to subsequent degradation (Morrison *et al.,* 2001; Huang *et al.,* 2007; Borne 2014). The total dissolved solids (TDS) contents decreased significantly in all treatments, with a sharp decrease in vegetated treatments (T1-T4) as compare to non-vegetated and control. It emphasizes the role of vegetation and associated roots in the removal of TDS from polluted water. The decrease in TDS in the control might be due to the natural sedimentation effect that results in a decrease of TSS (Borne *et al.,* 2014). However, in the presence of vegetation, plant roots might have further supported the sedimentation process due to the lesser turbulence as explained earlier (Huang *et al.,* 2007; Li *et al.*, 2016; Shahid et al., 2018). Moving on, TDS contents reduced sharply in the bacterial augmented vegetated treatments which are in agreement to the role of root-associated bacterial communities towards better removal of the suspended solids (Shahid *et al*., 2018). Thus, vegetation and bacteria together speed up the stabilization of the pollutants, reducing the water turbulence, trapping the suspended particles, increasing sedimentation, and degrading the organic contaminants (Borne *et al.,* 2014; Shahid *et al*., 2018; Rehman *et al*., 2019). Likewise, fine solids may also adhere to the bacterial biofilms and boost the removal process of dissolved and suspended particles (Liu *et al.,* 2016). In this study, the performance of *P. australis* was better than *B. mutica,* which could be attributed to the intensive root network of *P. australis,* and ability to thrive in a variety of pollutants (Saleem *et al*., 2018). Likewise, the species is found to develop a strong partnership with the artificially augmented bacterial communities (Fig. 3).

Changes in chemical parameters of the river water after treatment

In this study, COD, BOD_5 and TOC level decreased sharply up to 85% within 96 h (Fig. 2). These parameters are important indicators of organic pollution in water (Ijaz *et al.*, 2015). The presence of vegetation (T1-T4) displayed a significant effect when compared with the non-vegetated treatments; however, better reduction potential was observed in the vegetated treatments augmented with bacteria (T2 and T4). Once again, the overall performance of *P. australis* was better than *B. mutica* with and without bacterial inoculation. This enhanced removal of COD, BOD_5 and TOC load by bacterially inoculated vegetated treatments could be attributed to the ability of rhizo- and endophytic bacteria that help degrading organic pollutants into simple nutrients (Afzal *et al.,* 2014a; Ijaz *et al.,* 2016a). Accordingly, sedimentation and entrapping of suspended particles by hanging root structure may also provide support in the reduction of COD (Fonder & Headley 2010; Van de Moortel *et al.,* 2011). Similar results have been reported in earlier studies where bacterial-assisted FWs displayed a high reduction of organic pollution from industrial wastewaters (Sun *et al.*, 2009). Thus, it is a validation that plants in partnership with rhizo- and endophytic bacteria could also be employed for the treatment of organic pollution in the river water.

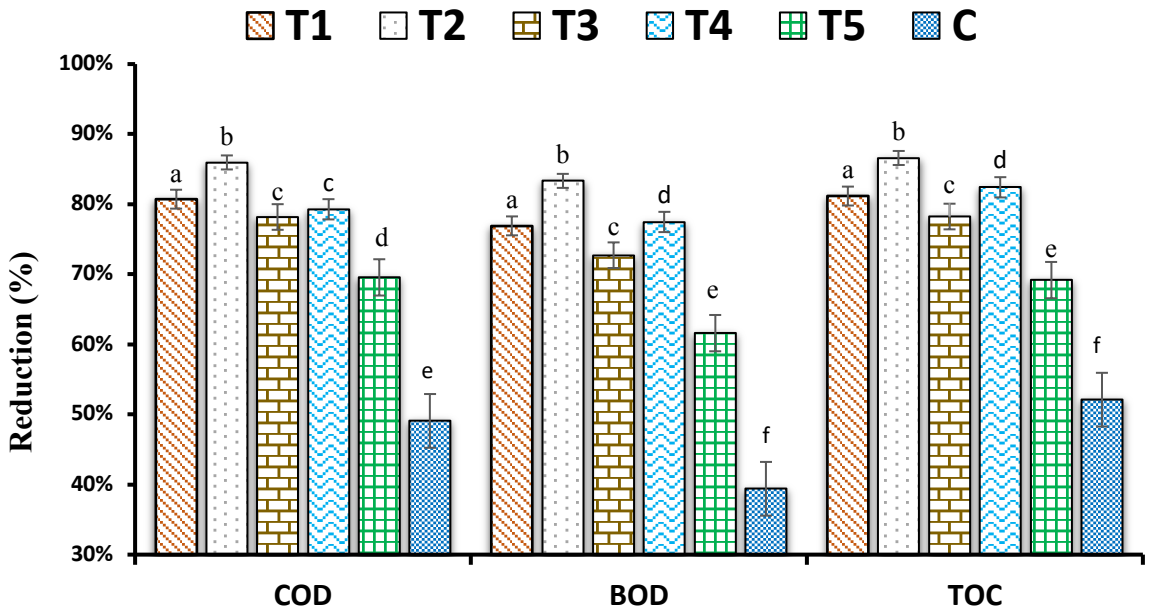

Fig. 2. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) and total organic carbon (TOC) reduction after 96 h by application of FTWs. T1: *Phragmites australis*, T2: *P. australis* and bacteria, T3: *Brachia mutica*, T4: *B. mutica* and bacteria, T5: Bacteria only, C: Floating mat only. Each value is mean of three replicates and alphabets labels represent significant differences between treatments. The bars represent the standard error.

Moving on, TN, NO_3^{-1}, and TP concentration was also decreased in all the vegetated treatments, and further enhanced in bacterial inoculated vegetated treatments (Table 3). The maximum reduction was seen in the treatments grown with *P. australis* and inoculated with bacterial consortia (T2). In T2, the TN, NO_3^{-1} and TP contents were reduced from 37.50 mg/l to 2.07 mg/l, 33.33 mg/l to 1.23 mg/l and 2.63 mg/l to 0.53 mg/l respectively after 96 h retention time. This could be due to the fact that *P. australis* is a helophytic grass that establishes optimal oxidation conditions in the plant rhizosphere leading to an effective partnership with denitrifying and other pollutant-degrading bacteria (Stewart *et al.,* 2008; Saleem *et al.*, 2018). The root biofilm provides a base for the prompt breakdown of pollutants and nitrogen removal (Hu *et al.,* 2010; Zhu *et al.,* 2011); while denitrification could be the likely mechanism for the observed decrease of nitrate which is widely reported process in FWs system (Fang *et al.,* 2016; Strosnider *et al.,* 2017). Additionally, plant roots can also uptake nitrogen directly for metabolic purposes. It is reported that several plant species are able to remove nitrogen ranging between 25 to 47% (Zimmo *et al.,* 2004; Zhao *et al.,* 2012). On the other hand, TP removal could be due to the adsorbing of phosphorous on to the clay particles, metal ions, or via plant uptake. However, the creation of oxic zones within the rhizosphere may boost the phosphorus removal process (Borne 2014); and plant uptake or entrapment in biofilm on roots can also facilitate the overall removal process (Stewart *et al.,* 2008; Tanner & Headley 2011).

Trace metals removal

Overall, all vegetated treatments (T1-T4) resulted in a significantly better elimination of trace metals from the river water as compared to non-vegetated treatment (T5 and C) (Table 4). This observation is in accordance with the earlier studies reporting that plant tissues can accumulate

a significant amount of trace metals (Chang *et al*., 2013). Although remove of metals takes place uptake, it is not a prime process in the FW system (Wood and Shelley 1999; Kadlec and Wallace 2008; Karathanasis *et al*., 2003; Sekomo *et al*., 2011; Chang *et al*., 2013). Next, the performance of bacterial inoculated vegetated treatments (T2, and T4) was far better than non-inoculated vegetated treatments (T1, and T3). In treatment T2, metals content was removed more efficiently than all other treatments, i.e. Fe, Ni, Mn, Pb, and Cr were removed by 94.34 %, 91.36 %, 87.06 %, 70.28 % and 89.81 % after 96 h retention time. The better oxidation of metals by *P. australis* might be the reason for the effective removal of the trace (Stewart *et al.,* 2008; Saleem *et al.*, 2018). The better performance of bacterially augmented FWs emphasized the key role of rhizo- and endophytic bacteria in the remediation of trace metals from the river's water. In earlier studies, inoculated bacteria enhanced the uptake of trace metals by improving bioavailability, by sorbing metallic ion on the bacterial cell walls, or uptake of bioavailable trace metals by plant roots (Khan *et al.,* 2015). The bacteria can also eliminate trace metals by entrapment in biofilms on roots followed by metals sulfide formation (Kadlec & Wallace 2008; Balkhair & Ashraf 2016). Another process such as binding of metal particles with the fine particle, sequestration of metals ion, iron plaque formation and oxidation by bacteria also play key roles in metals removal form polluted water (Li *et al.,* 2011; Shehzadi *et al.,* 2014; Ijaz *et al.,* 2016a). The Fe and Mn plague formation on roots of wetlands plants by microbial activity is also reported to bind metals such as Cu and Zn (Gill *et al.,* 2017). This emphasizes the combined role of macrophytes and bacteria in metals removal mechanism.

Bacterial survival

The detection of inoculated bacteria in roots, shoots, and water confirmed a high survival of inoculated bacteria during the treatment period (Fig. 3-4). The population of inoculated bacteria was high in the vegetated treatments than the non-vegetated treatments. This could be attributed to the provision of nutrition and habitat for bacterial growth by plants (Weyens *et al.,* 2009b; Afzal *et al.,* 2014a). Moreover, since these bacteria were isolated from the plant rhizo- and endosphere, they may have adopted mechanisms to proliferate in the presence of host only (Fatima et al., 2015). This observation is consistent with earlier studies treating various kinds of soil and contaminated waters (Arslan *et al.*, 2014, Saleem *et al.*, 2017; Rehman *et al*., 2018). An increased clean-up was found in the treatment with high bacterial survival that suggests the active role of bacteria in combination with the plant for efficient pollutant removal. Moving on, the high population was observed in the rhizospheric water as compared to the population found within roots and shoots. This could be due to the fact that three of the inoculated bacteria were of rhizospheric nature and therefore their proliferation would have been successful in the presence of plant roots. Previous studies demonstrated similar results where rhizospheric bacteria exhibited better colonization in the contaminated soil and water (Khan *et al.*, 2015; Arslan *et al*., 2014).

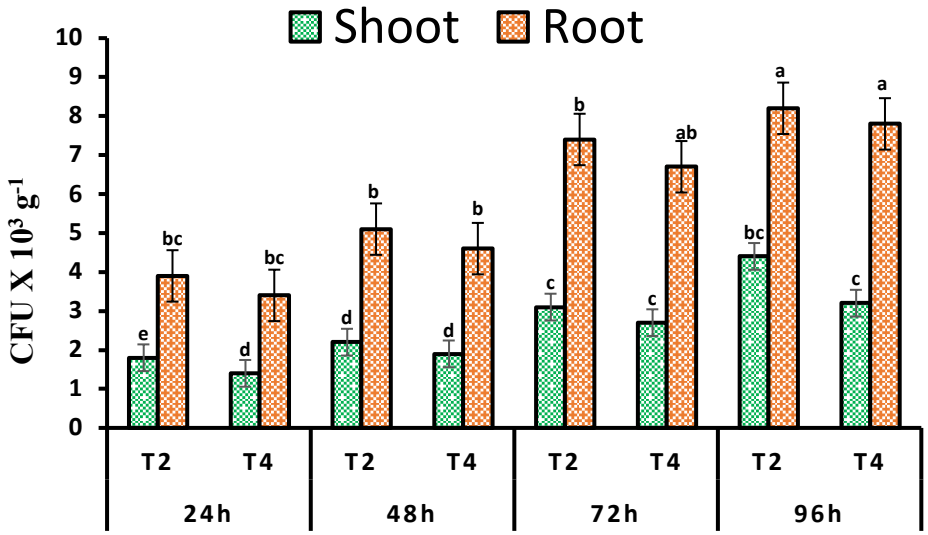

Fig. 3. Survival of inoculated bacteria in roots and shoots of inoculated treatments. T2: *Phragmites australis* and bacteria, T4: *Brachia mutica* and bacteria. Each value is mean of three replicates with standard error bar.

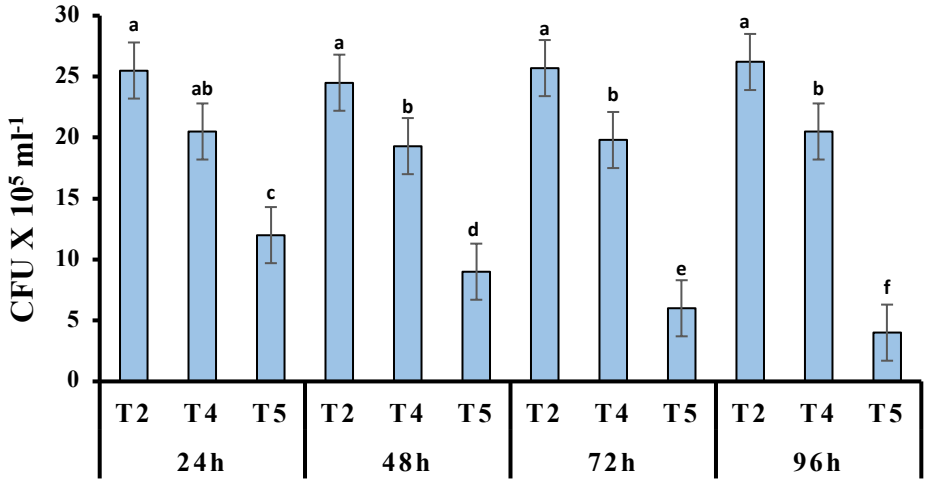

Fig. 4. Survival of bacteria in water of inoculated treatments. T2: *Phragmites australis* and bacteria, T4: *Brachia mutica* and bacteria, T5: Bacteria only. Each value is mean of three replicates with standard error bar.

Effect of bacterial inoculation on plant growth

For both plant species, bacterial inoculation significantly improved the plant biomass. This gain in plant biomass could be a direct result of reduced phytotoxicity upon successful degradation of the contaminants (Table 5) (Weyens *et al.,* 2013; Afzal *et al.,* 2014b; Wu *et al.,* 2016). Additionally, denser roots and shoots growth could have been due to the plant growth-promoting properties of the inoculated bacteria because these bacteria were previously found to have the successful potential for ACC deaminase activity, siderophore production, phosphorus solubilization, and indoleacetic acid formation (Fatima *et al.,* 2015). Previously, ACC deaminase activity is coined as a stress alleviation trait of the plant-associated bacterial communities that reduces ethylene level in the plant environment and improve its growth (Arslan et al., 2014).

Specifically, the performance of *P. australis* was better than *B. mutica*. This corresponds to its successful ability to develop a better partnership with the bacterial communities which results in getting more root biomass (Saleem et al., 2018). Also, more root biomass retains more water content which is less in the case of aboveground biomass. This observation was consistent in our findings as we also noticed more biomass for the roots as compared to the shoots of *P. australis*. However, further investigations would be interesting to see why more biomass was recorded for the shoots of *B. mutica* and not for the roots.

Detoxification of polluted river water

Fish toxicity assay revealed that the FWs treatment resulted in successful detoxification of the polluted river water (Table 6). Partial detoxification was observed for the treatment without bacterial inoculation whereas in inoculated and vegetated treatments no fish died during 96 h. This could be due to a better reduction in toxic organic compounds and trace metals (Shehzadi *et al*., 2014). Our results indicate high toxicity of the water of Ravi river for its aquatic life however wetland installation may help restoring the river habitat.

Design and durability of the mat

In this study, we used a locally available polystyrene board (Diamond Jumbolon) for the construction of a floating mat. Although, the actual purpose of the board is insulating surfaces of buildings, we found it as a suitable choice for the establishment of FWs. This is mainly because of its anti-weathering characteristics, inherent strength, temperature, and weight tolerance, and more importantly the low cost. Previously, many studies reported the use of plastic pipes, coconut fibers, polyvinyl chloride/polypropylene pipes, bamboos, etc. for the construction of FWs (Van de Moortel, 2008; Nakamura and Mueller, 2008; Hubbard et al., 2004). However, we demonstrate that commercially available polystyrene board could also be an optimal choice for the establishment of small artificial wetlands.

CONCLUSIONS

Riverine pollution has been proven to be devastating at all trophic levels in the food web. In this study, FWs efficiently remediated the polluted water of the Ravi river to meet the irrigation standards. The performance of *P. australis* was better than *B. mutica* in the presence and absence of bacteria. However, bacterial inoculation in all vegetated treatments boosted the phytoremediation efficiency of the two macrophytes. The system was highly efficient to tolerate contaminant stress and therefore it is suggested that FWs maybe an efficient and economical substitute to conventional wastewater treatment technology for the treatment of polluted river. A field-scale application is recommended for the on-site treatment of the Ravi river water in order to fully explore the presence of potential disinfection by-products and exudates released by plants, bacteria and by their mutual interactions.

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REFERENCES

Abed S. N., Almuktar S. A. and Scholz M. 2017 Remediation of synthetic greywater in mesocosm—scale floating treatment wetlands. *Ecological Engineering* **102**, 303-19.

Afzal M., Khan Q. M. and Sessitsch A. 2014a Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. *Chemosphere* **117**, 232-42.

Afzal M., Shabir G., Tahseen R., Islam E. u., Iqbal S., Khan Q. M. and Khalid Z. M. 2014b Endophytic *Burkholderia* sp. strain PsJN improves plant growth and phytoremediation of soil irrigated with textile effluent. *Clean–Soil, Air, Water* **42**(9), 1304-10.

Arslan, M., Afzal, M., Amin, I., Iqbal, S. and Khan, Q.M., 2014. Nutrients can enhance the abundance and expression of alkane hydroxylase CYP153 gene in the rhizosphere of ryegrass planted in hydrocarbon-polluted soil. *PLoS One* **9**(10), e111208.

Balkhair K. S. and Ashraf M. A. 2016 Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi Journal of Biological Sciences* **23**(1), 32-44.

Bashan Y., Holguin G. and Lifshitz R. 1993. Isolation and characterization of plant growth-promoting rhizobacteria. Methods in plant molecular biology and biotechnology. CRC Press, Boca Raton, 331-345.

Borne K. E. 2014 Floating treatment wetland influences on the fate and removal performance of phosphorus in stormwater retention ponds. *Ecological Engineering* **69**, 76-82.

Burken JG. 2003 Uptake and metabolism of organic compounds: green-liver model. In Phytoremediation: Transformation and Control of Contaminants, ed. SC McCutcheon, JL Schnoor, pp. 59–84. New York: Wiley

Chang N.-B., Islam K., Marimon Z. and Wanielista M. P. 2012 Assessing biological and chemical signatures related to nutrient removal by floating islands in stormwater mesocosms. *Chemosphere* **88**(6), 736-43.

Chang N.-B., Xuan Z., Marimon Z., Islam K. and Wanielista M. P. 2013 Exploring hydrobiogeochemical processes of floating treatment wetlands in a subtropical stormwater wet detention pond. *Ecological Engineering* **54**, 66-76.

Chen Z., Cuervo D. P., Müller J. A., Wiessner A., Köser H., Vymazal J., Kästner M. and Kuschk P. 2016 Hydroponic root mats for wastewater treatment. *Environmental Science and Pollution Research* **23**(16), 15911-28.

Chen Z., Kuschk P., Paschke H., Kästner M., Müller J. A. and Köser H. 2014 Treatment of a sulfate-rich groundwater contaminated with perchloroethene in a hydroponic plant root mat filter and a horizontal subsurface flow constructed wetland at pilot-scale. *Chemosphere* **117**, 178-84.

Fang T., Bao S., Sima X., Jiang H., Zhu W. and Tang W. 2016 Study on the application of integrated eco-engineering in purifying eutrophic river waters. *Ecological Engineering* **94**, 320-8.

Fatima K., Afzal M., Imran A. and Khan Q. M. 2015 Bacterial rhizosphere and endosphere populations associated with grasses and trees to be used for phytoremediation of crude oil contaminated soil. *Bulletin of Environmental Contamination and Toxicology* **94**(3), 314-20.

Federation W. E. and Association A. P. H. 2005 Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA*.

Fonder N. and Headley T. 2010 Systematic classification, nomenclature and reporting for constructed treatment wetlands. In: *Water and Nutrient Management in Natural and Constructed Wetlands*, Springer, pp. 191-219.

Gill L. W., Ring P., Casey B., Higgins N. M. and Johnston P. M. 2017 Long term heavy metal removal by a constructed wetland treating rainfall runoff from a motorway. *Science of the Total Environment* **601**, 32-44.

Hardoim P. R., Van Overbeek L. S., Berg G., Pirttilä A. M., Compant S., Campisano A., Döring M. and Sessitsch A. 2015 The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* **79**(3), 293-320.

Headley T. and Tanner C. 2012 Constructed wetlands with floating emergent macrophytes: an innovative stormwater treatment technology. *Critical Reviews in Environmental Science and Technology* **42**(21), 2261-310.

Headley T., Tanner C. C. and Council A. R. 2008 Floating treatment wetlands: an innovative option for stormwater quality applications, 11th International Conference on Wetland Systems for Water Pollution Control, Indore, India.

Hubbard, R., Gascho, G., Newton, G., 2004. Use of floating vegetation to remove nutrients from swine lagoon wastewater. Trans. ASAE 47, 1963–1972.

Hu M.-H., Yuan J.-H., Yang X.-E. and He Z.-L. 2010 Effects of temperature on purification of eutrophic water by floating eco-island system. *Acta Ecologica Sinica* **30**(6), 310-18.

Huang P., Han B. and Liu Z. 2007 Floating-leaved macrophyte (*Trapa quadrispinosa* Roxb) beds have significant effects on sediment resuspension in Lake Taihu, China. *Hydrobiologia* **581**(1), 189-93.

Hussain Z., Arslan M., Malik M. H., Mohsin M., Iqbal S. and Afzal M. 2018 Treatment of the textile industry effluent in a pilot-scale vertical flow constructed wetland system augmented with bacterial endophytes. *Science of the Total Environment* ***645***, 966-73.

Ijaz A., Imran A., ul Haq M. A., Khan Q. M. and Afzal M. 2016a Phytoremediation: recent advances in plant-endophytic synergistic interactions. *Plant and Soil* **405**(1-2), 179-95.

Ijaz A., Iqbal Z. and Afzal M. 2016b Remediation of sewage and industrial effluent using bacterially assisted floating treatment wetlands vegetated with *Typha domingensis*. *Water Science and Technology* **74**(9), 2192-201.

Ijaz A., Shabir G., Khan Q. M. and Afzal M. 2015 Enhanced remediation of sewage effluent by endophyte-assisted floating treatment wetlands. *Ecological Engineering* **84**, 58-66.

Kadlec R. H. and Wallace S. 2008 *Treatment wetlands*. CRC Press, Boca Raton, FL.

Karathanasis A., Potter C., Coyne M. 2003 Vegetation effects on fecal bacteria, BOD, and suspended solid removal in constructed wetlands treating domestic wastewater, *Ecological Engineering* ***20***(2), 157-169.

Kämpfer P., Erhart R., Beimfohr C., Böhringer J., Wagner M.,and Amann R. 1996 Characterization of bacterial communities from activated sludge: culture-dependent numerical identification versus in situ identification using group-and genus-specific rRNA-targeted oligonucleotide probes. *Microbial Ecology* ***32***(2), 101-121.

Keizer-Vlek H. E., Verdonschot P. F., Verdonschot R. C. and Dekkers D. 2014 The contribution of plant uptake to nutrient removal by floating treatment wetlands. *Ecological Engineering* **73**, 684-90.

Khan M. U., Sessitsch A., Harris M., Fatima K., Imran A., Arslan M., Shabir G., Khan Q. M. and Afzal M. 2015 Cr-resistant rhizo-and endophytic bacteria associated with *Prosopis juliflora* and their potential as phytoremediation enhancing agents in metal-degraded soils. *Frontiers in Plant Science* **5**, 755.

Li F., Pan Y., Xie Y., Chen X., Deng Z., Li X., Hou Z. and Tang Y. 2016 Different roles of three emergent macrophytes in promoting sedimentation in Dongting Lake, China. *Aquatic Sciences* **78**(1), 159-169.

Li H., Zhao H.-P., Hao H.-L., Liang J., Zhao F.-L., Xiang L.-C., Yang X.-E., He Z.-L. and Stoffella P. J. 2011 Enhancement of nutrient removal from eutrophic water by a plant–microorganisms combined system. *Environmental Engineering Science* **28**(8), 543-54.

Li X. and Guo R. 2017 Comparison of nitrogen removal in floating treatment wetlands constructed with *Phragmites australis* and *Acorus calamus* in a cold temperate zone. *Water, Air, & Soil Pollution* **228**(4), 132.

Lin Y.-F., Jing S.-R., Wang T.-W. and Lee D.-Y. 2002 Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. *Environmental Pollution* **119**(3), 413-20.

Liu J. L., Liu J. K., Anderson J. T., Zhang R. and Zhang Z. M. 2016 Potential of aquatic macrophytes and artificial floating island for removing contaminants. *Plant Biosystems* **150**(4), 702-709.

Liu W. T., Marsh T. L., Cheng H. and Forney L. J. (1997). Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology* ***63***(11), 4516-4522.

Lynch J., Fox L. J., Owen Jr J. S. and Sample D. J. 2015 Evaluation of commercial floating treatment wetland technologies for nutrient remediation of stormwater. *Ecological Engineering* **75**, 61-9.

Mietto A., Borin M., Salvato M., Ronco P. and Tadiello N. 2013 Tech-IA floating system introduced in urban wastewater treatment plants in the Veneto region–Italy. *Water Science and Technology* **68**(5), 1144-50.

Morrison G., Fatoki O., Persson L. and Ekberg A. 2001 Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River-pH, electrical conductivity, oxygen-demanding substance (COD) and nutrients. *Water Sa* **27**(4), 475-80.

Nakamura, K., Mueller, G., 2008. Review of the Performance of the Artificial Floating Island as a Restoration Tool for Aquatic Environments. World Environmental and Water Resources Congress, Honolulu, Hawaii, USA, pp. 1–10

Pavan F., Breschigliaro S. and Borin M. 2015 Screening of 18 species for digestate phytodepuration. *Environmental Science and Pollution Research* **22**(4), 2455-66.

Penrose D. M. and Glick B. R. (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiologia Plantarum*, **118**(1), 10-15.

Rehman K., Imran A., Amin I. and Afzal M. 2018 Inoculation with bacteria in floating treatment wetlands positively modulates the phytoremediation of oil field wastewater. *Journal of Hazardous Materials* **349**, 242-51.

Rehman K., Imran A., Amin I. and Afzal M. 2019 Enhancement of oil field-produced wastewater remediation by bacterially-augmented floating treatment wetlands. *Chemosphere* **217**, 576-83.

Richter J., Wiessner A., Zehnsdorf A., Müller J. A. and Kuschk P. 2016 Injection of hydrogen gas stimulates acid mine drainage treatment in laboratory-scale hydroponic root mats. *Engineering in Life Sciences* **16**(8), 769-76.

Sekomo C.B., Nkuranga E., Rousseau D.P., Lens P.N. 2011 Fate of heavy metals in an urban natural wetland: the Nyabugogo Swamp (Rwanda), *Water Air Soil Pollut*. ***214*** (1-4), 321-333.

Shehzadi M., Afzal M., Khan M. U., Islam E., Mobin A., Anwar S. and Khan Q. M. 2014 Enhanced degradation of textile effluent in constructed wetland system using *Typha*

domingensis and textile effluent-degrading endophytic bacteria. *Water Research* **58**, 152-9.

Stewart F. M., Muholland T., Cunningham A. B., Kania B. G. and Osterlund M. T. 2008 Floating islands as an alternative to constructed wetlands for treatment of excess nutrients from agricultural and municipal wastes–results of laboratory-scale tests. *Land Contamination & Reclamation* **16**(1), 25-33.

Strosnider W., Schultz S., Strosnider K. and Nairn R. 2017 Effects on the underlying water column by extensive floating treatment wetlands. *Journal of Environmental Quality* **46**(1), 201-9.

Tanner C. C. and Headley T. R. 2011 Components of floating emergent macrophyte treatment wetlands influencing removal of stormwater pollutants. *Ecological Engineering* **37**(3), 474-86.

Tanner C. C., Kadlec R. H., Gibbs M. M., Sukias J. P. and Nguyen M. L. 2002 Nitrogen processing gradients in subsurface-flow treatment wetlands—influence of wastewater characteristics. *Ecological Engineering* **18**(4), 499-520.

Van de Moortel, A., 2008. Use of floating macrophyte mats for treatment of CSOs. In: Proceedings of the 11th International Conference on Urban Drainage. Edinburgh, Scotland.

Van de Moortel A. M., Du Laing G., De Pauw N. and Tack F. M. 2011 Distribution and mobilization of pollutants in the sediment of a constructed floating wetland used for treatment of combined sewer overflow events. *Water Environment Research* **83**(5), 427-39.

Vymazal J. 2014 Constructed wetlands for treatment of industrial wastewaters. *Ecological Engineering* **73**, 724-51.

Wagner M., Amann R., Lemmer H. and Schleifer K. H. 1993 Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. *Applied and Environmental Microbiology* **59**(5), 1520-25.

West M., Fenner N., Gough R. and Freeman C. 2017 Evaluation of algal bloom mitigation and nutrient removal in floating constructed wetlands with different macrophyte species. *Ecological Engineering* **108**, 581-8.

Weyens N., Schellingen K., Beckers B., Janssen J., Ceulemans R., van der Lelie D., Taghavi S., Carleer R. and Vangronsveld J. 2013 Potential of willow and its genetically engineered associated bacteria to remediate mixed Cd and toluene contamination. *Journal of Soils and Sediments* **13**(1), 176-88.

Weyens N., Taghavi S., Barac T., van der Lelie D., Boulet J., Artois T., Carleer R. and Vangronsveld J. 2009a Bacteria associated with oak and ash on a TCE-contaminated site: characterization of isolates with potential to avoid evapotranspiration of TCE. *Environmental Science and Pollution Research* **16**(7), 830-43.

Weyens N., van der Lelie D., Taghavi S. and Vangronsveld J. 2009b Phytoremediation: plant–endophyte partnerships take the challenge. *Current Opinion in Biotechnology* **20**(2), 248-54.

White S. A. and Cousins M. M. 2013 Floating treatment wetland aided remediation of nitrogen and phosphorus from simulated stormwater runoff. *Ecological Engineering* **61**, 207-15.

Winston R. J., Hunt W. F., Kennedy S. G., Merriman L. S., Chandler J. and Brown D. 2013 Evaluation of floating treatment wetlands as retrofits to existing stormwater retention ponds. *Ecological Engineering* **54**, 254-65.

Wood T.S., Shelley M.L. 1999 A dynamic model of bioavailability of metals in constructed wetland sediments, *Ecological Engineering*, ***12***(3), 231-252.

Wu H., Xu K., He X. and Wang X. 2016 Removal of nitrogen by three plant species in hydroponic culture: Plant uptake and microbial degradation. *Water, Air, & Soil Pollution* **227**(9), 324.

Zhao F., Xi S., Yang X., Yang W., Li J., Gu B. and He Z. 2012 Purifying eutrophic river waters with integrated floating island systems. *Ecological Engineering* **40**, 53-60.

Zhu L., Li Z. and Ketola T. 2011 Biomass accumulations and nutrient uptake of plants cultivated on artificial floating beds in China's rural area. *Ecological Engineering* **37**(10), 1460-6.

Zimmo O., Van der Steen N. and Gijzen H. 2004 Nitrogen mass balance across pilot-scale algae and duckweed-based wastewater stabilisation ponds. *Water Research* **38**(4), 913-20.

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

Parameter	Value	Water quality guidelines (WWF)		NEQs (Pakistan)
		Irrigation	Fish/aquatic life	Wastewater discharge
pH	8.5 (0.10)	6.5-8.4	6.5-8.5	6–9
EC (mS cm^{-1})	2.3 (0.04)	15	15	NG
TSS (mg l^{-1})	290 (38)	NG	NG	150
COD (mg l^{-1})	405 (6.24)	NG	NG	150
BOD_5 (mg l^{-1})	190.3 (5.51)	NG	8	80
TOC (mg l^{-1})	110.5 (2.00)	NG	NG	NG
TN (mg l^{-1})	37.5 (1.14)	NG	NG	NG
NO_3^{-1} (mg l^{-1})	33.3 (1.53)	NG	NG	50
TP (mg l^{-1})	2.63 (0.12)	NG	NG	NG
Fe (mg l^{-1})	1.53 (0.12)	5.0	0.3	8.0
Ni (mg l^{-1})	0.54 (0.01)	0.20	0.05	1.0
Mn (mg l^{-1})	0.85 (0.02)	0.20	0.1	1.5
Pb (mg l^{-1})	0.83 (0.06)	0.1	0.01	0.5
Cr (mg l^{-1})	0.36 (0.02)	0.01	0.05	1.0

*Water Quality Guidelines for Pakistan proposed by World Wide Fund (WWF) for Nature, Pakistan (2007). NEQs: National Environmental Quality Standards. NG: Not given in the list. Standard deviation are presented in parenthesis.

Table 2. Changes in physical parameters of polluted river water by floating treatment wetlands application

Parameter	0 h	T1		T2		T3		T4		T5		C	
		24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h
pH	8.5^{bc} (0.07)	8.37^{de} (0.0)	7.73^{m} (0.05)	8.25^{ghi} (0.05)	7.76^{m} (0.11)	8.36^{def} (0.01)	7.76^{m} (0.05)	8.36^{def} (0.02)	7.63^{n} (0.05)	8.39^{de} (0.0)	8.13^{j} (0.05)	8.43^{cd} (0.01)	8.62^{a} (0.06)
EC mS/cm	2.0^{a} (0.0)	1.78^{e} (0.01)	1.58^{l} (0.0)	1.72^{fg} (0.0)	1.54^{m} (0.01)	1.86^{d} (0.0)	1.64^{ij} (0.01)	1.79^{e} (0.01)	1.59^{kl} (0.02)	1.92^{c} (0.01)	1.79^{e} (0.01)	1.99^{a} (0.0)	1.95^{bc} (0.0)
TDS (mg/l)	1279^{a} (0.0)	1141^{l} (1.53)	1010^{v} (1.0)	1101^{n} (1.53)	983^{w} (1.0)	1188^{h} (1.0)	1047^{r} (1.0)	1147^{j} (1.0))	1015^{u} (1.0)	1231^{f} (1.15)	1145^{k} (1.15)	1271^{b} (1.15)	1248^{e} (0.58)
TSS (mg/l)	278^{a} (1.41)	68.6^{b} (0.57)	10.6^{l} 0.57)	62.3^{c} (0.57)	12.6^{kl} (0.57)	24.3^{g} (1.2)	14^{jkl} (2.05)	41.6^{d} (1.15)	18.6^{hi} (2.08)	30.3^{ef} (0.57)	13^{kl} (0.0)	70.3^{b} (0.57)	26^{g} (1.02)

T1: *Phragmites australis*, T2: *P. australis* and bacteria, T3: *Brachia mutica*, T4: *B. mutica* and bacteria, T5: Bacteria only, C: Floating mat only. Each value is mean of three replicate and alphabets labels present significant differences between treatments. Standard deviations are presented in parenthesis.

Table. 3 Reduction in pollutants concentration by application of FTWs

Pollutant	Treatment	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	LSD $\alpha = 0.05$
TN (mg/l)	T1	37.50^{a} (1.14)	26.37^{f} (0.37)	19.37^{j} (0.25)	10.53^{o} (0.58)	3.00^{r} (0)	
	T2	37.50^{a} (1.14)	26.47^{f} (0.11)	16.23^{k} (0.20)	8.33^{p} (0.05)	2.07^{s} (0.11)	
	T3	37.50^{a} (1.14)	30.47^{c} (1.28)	21.97^{h} (0.20)	13.43^{m} (0.40)	7.67^{p} (0.32)	0.73
	T4	37.50^{a} (1.14)	29.70^{d} (1.12)	21.33^{hi} (0.15)	11.50^{n} (0.5)	6.27^{q} (0.25)	
	T5	37.50^{a} (1.14)	30.73^{c} (0.46)	25.33^{g} (0.29)	19.0^{j} (0.5)	14.83^{l} (0.76)	
	C	37.50^{a} (1.14)	32.23^{b} (0.37)	28.57^{e} (0.73)	25.37^{g} (0.40)	21.23^{i} (0.32)	
NO_3^{-1} (mg/l)	T1	33.33^{a} (1.53)	24.13^{e} (0.15)	15.57^{j} (0.20)	8.03^{o} (0.05)	2.0^{s} (0)	
	T2	33.33^{a} (1.53)	23.47^{f} (0.50)	13.53^{k} (0.15)	6.20^{p} (0.2)	1.23^{t} (0.11)	
	T3	33.33^{a} (1.53)	27.40^{c} (0.26)	18.57^{h} (0.11)	9.40^{n} (0.1)	5.17^{q} (0.20)	0.62
	T4	33.33^{a} (1.53)	26.13^{d} (0.23)	18.30^{h} (0.26)	7.97^{o} (0.15)	3.33^{r} (0.41)	
	T5	33.33^{a} (1.53)	26.03^{d} (1.0)	21.33^{g} (0.58)	12.8^{l} (0.34)	11.67^{m} (0.57)	
	C	33.33a (1.53)	30.13^{b} (0.23)	24.10^{e} (0.17)	21.67^{g} (1.15)	16.47^{i} (0.50)	
TP (mg/l)	T1	2.63^{a} (0.12)	2.10^{cd} (0.1)	1.53^{i} (0.0)	1.13^{kl} (0.0)	0.93^{m} (0.05)	
	T2	2.63^{a} (0.12)	1.73^{gh} (0.02)	1.13^{kl} (0.05)	0.83^{m} (0.05)	0.53^{n} (0.01)	
	T3	2.63^{a} (0.12)	2.13^{c} (0.11)	1.83^{fg} (0.05)	1.33^{j} (0.20)	1.10^{l} (0.1)	0.13
	T4	2.63^{a} (0.12)	2.30^{b} (0.2)	1.63^{hi} (0.05)	1.23^{jk} (0.05)	0.90^{m} (0.1)	
	T5	2.63^{a} (0.12)	2.10^{cd} (0.17)	2.03^{cd} (0.05)	1.87^{ef} (0.05)	1.62^{hi} (0.02)	
	C	2.63^{a} (0.12)	2.38^{b} (0.07)	2.39^{b} (0.05)	2.10^{cd} (0)	1.99^{de} (0.01)	

T1: *Phragmite australis*, T2: *Phragmite australis and Bacteria*, T3: *Brachia mutica*, T4: *Brachia mutica and Bacteria*, T5: Bacteria only, C: Floating mat only. Each value is mean of three replicate and alphabets labels present significant differences between treatments. Standard deviations are presented in parenthesis.

LSD: Least significant difference: the difference between the population is significant when value is greater than 0.05

Table 4. Reduction in concentration of heavy metals by application of floating treatment wetlands

Metals	Treatment	Time			
		24 h	48 h	72 h	96 h
Fe (%)	T1	19.39^{e} (5.77)	47.71^{i} (1.00)	60.35^{l} (5.77)	86.71^{p} (5.77)
	T2	30.28^{g} (5.77)	50.98^{j} (1.00)	72.33^{n} (5.77)	94.34^{q} (5.77)
	T3	18.95^{e} (1.00)	44.01^{h} (1.53)	56.43^{k}(1.53)	79.30^{o} (1.15)
	T4	27.02^{f} (1.53)	52.94^{j} (0.00)	63.40^{m} (1.00)	86.06^{p} (5.77)
	T5	12.42^{c} (1.73)	15.03^{d} (0.00)	30.28^{g} (5.77)	46.19^{i} (2.53)
	C	7.41b^{b} (1.53)	10.89^{c} (1.15)	14.81^{d} (5.77)	18.52^{e} (5.77)
Ni (%)	T1	32.10^{h} (3.54)	62.35^{mn} (6.13)	74.07^{o} (4.83)	84.57^{p} (5.58)
	T2	42.59^{j} (6.85)	64.81^{n} (5.07)	82.72^{p} (7.58)	91.36^{q} (6.57)
	T3	16.05^{ef} (1.58)	37.65^{i} (6.58)	50.62^{k} (7.58)	56.17^{l} (4.58)
	T4	18.52^{fg} (1.85)	38.27^{i} (3.57)	53.70^{l} (3.64)	61.11^{m} (5.53)
	T5	11.73^{cd} (1.53)	16.05^{ef}(1.53)	20.99^{g} (3.58)	20.99^{g} (2.53)
	C	7.41^{b} (1.38)	9.88^{bc} (1.58)	8.64^{b} (0.98)	14.20^{de} (1.58)
Mn (%)	T1	31.37^{g} (5.15)	60.71^{j} (8.32)	79.61^{l} (7.57)	87.06^{m} (6.95)
	T2	50.98^{e} (3.58)	83.53^{d} (6.79)	90.98^{e} (10.58)	91.76^{f} (7.41)
	T3	31.37^{f} (5.58)	52.94^{h} (6.46)	76.08^{j} (5.58)	79.61^{l} (5.58)
	T4	53.33^{g} (4.58)	80.05^{k} (6.74)	87.06^{m} (4.56)	89.80^{m} (7.57)
	T5	16.86^{b} (1.52)	20.78^{c} (1.58)	25.49^{d} (3.15)	33.33^{e} (5.78)
	C	12.16^{f} (1.57)	15.29^{g} (1.06)	20.00^{i} (3.54)	24.31^{j} (3.15)
Pb (%)	T1	22.49^{de} (5.77)	29.72^{g} (7.64)	47.39^{j} (2.52)	58.63^{k} (5.77)
	T2	27.31^{fg} (5.77)	28.92^{g} (1.00)	60.24^{k} (1.00)	70.28^{l} (5.77)
	T3	23.69^{de} (1.15)	30.12^{g} (1.00)	40.16^{i} (5.77)	48.59^{j} (5.77)
	T4	25.30^{ef} (1.00)	28.51^{g} (5.77)	33.33^{h} (5.77)	60.24^{k} (5.27)
	T5	6.02^{b} (1.49)	15.26^{c} (5.77)	21.69^{d} (0.00)	24.10^{de} (0.00)
	C	3.21^{b} (1.15)	5.22^{b} (5.77)	12.85^{c} (5.77)	14.46^{c} (1.00)
Cr (%)	T1	26.85^{e} (1.15)	50.00^{hi} (1.00)	65.74^{k} (5.77)	75.93^{l} (5.77)
	T2	40.74^{g} (1.53)	64.81^{jk} (5.77)	76.85^{l} (5.77)	89.81^{m} (5.77)
	T3	20.37^{d} (5.77)	39.81^{g} (5.77)	51.85^{i} (5.27)	62.04^{j} (5.77)
	T4	36.11^{f}(0.00)	47.22^{h} (0.00)	64.81^{jk} (5.77)	75.93^{l} (5.77)
	T5	13.89^{c} (5.27)	21.30^{d} (5.77)	33.33^{f}(1.00)	34.26^{f} (1.15)
	C	5.56^{c} (5.27)	11.11^{d} (1.00)	12.96^{f} (5.77)	13.24^{f}(4.04)

T1: *Phragmites australis*, T2: *Phragmites australis* and bacteria, T3: *Brachia mutica*, T4: *Brachia mutica* and bacteria, T5: Bacteria only, C: Floating mat only. Each value is mean of three replicate and alphabets labels present significant differences between treatments. Standard deviations are presented in parenthesis.

Table 5. Fresh and dry biomass of roots and shoots of the plants

Treatment	Root (g)		Shoot (g)	
	Fresh	Dry	Fresh	Dry
T1	459^{b} (15.48)	58^{b} (6.50)	212^{d} (8.63)	106^{d} (5.34)
T2	508^{a} (26.83)	76^{a} (6.45)	235^{c} (12.78)	120^{c} (7.25)
T3	100^{d} (7.35)	12^{d} (2.39)	354^{b} (21.46)	204^{b} (5.45)
T4	119^{c} (6.38)	20^{c} (3.45)	488^{a} (18.7)	260^{a} (6.54)

T1: *Phragmites australis*, T2: *Phragmites australis* and bacteria, T3: *Brachia mutica*, T4: *Brachia mutica* and bacteria. Each value is mean of three replicates, alphabets labels present significant differences between treatments and standard deviations are presented in parenthesis.

Table 6. Fish toxicity assay of the river water detoxified by floating treatment wetlands

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

T1: *Phragmites australis*, T2: *Phragmites australis* and bacteria, T3: *Brachia mutica*, T4: *Brachia mutica* and bacteria, T5: Bacteria only, C: Floating mat only.