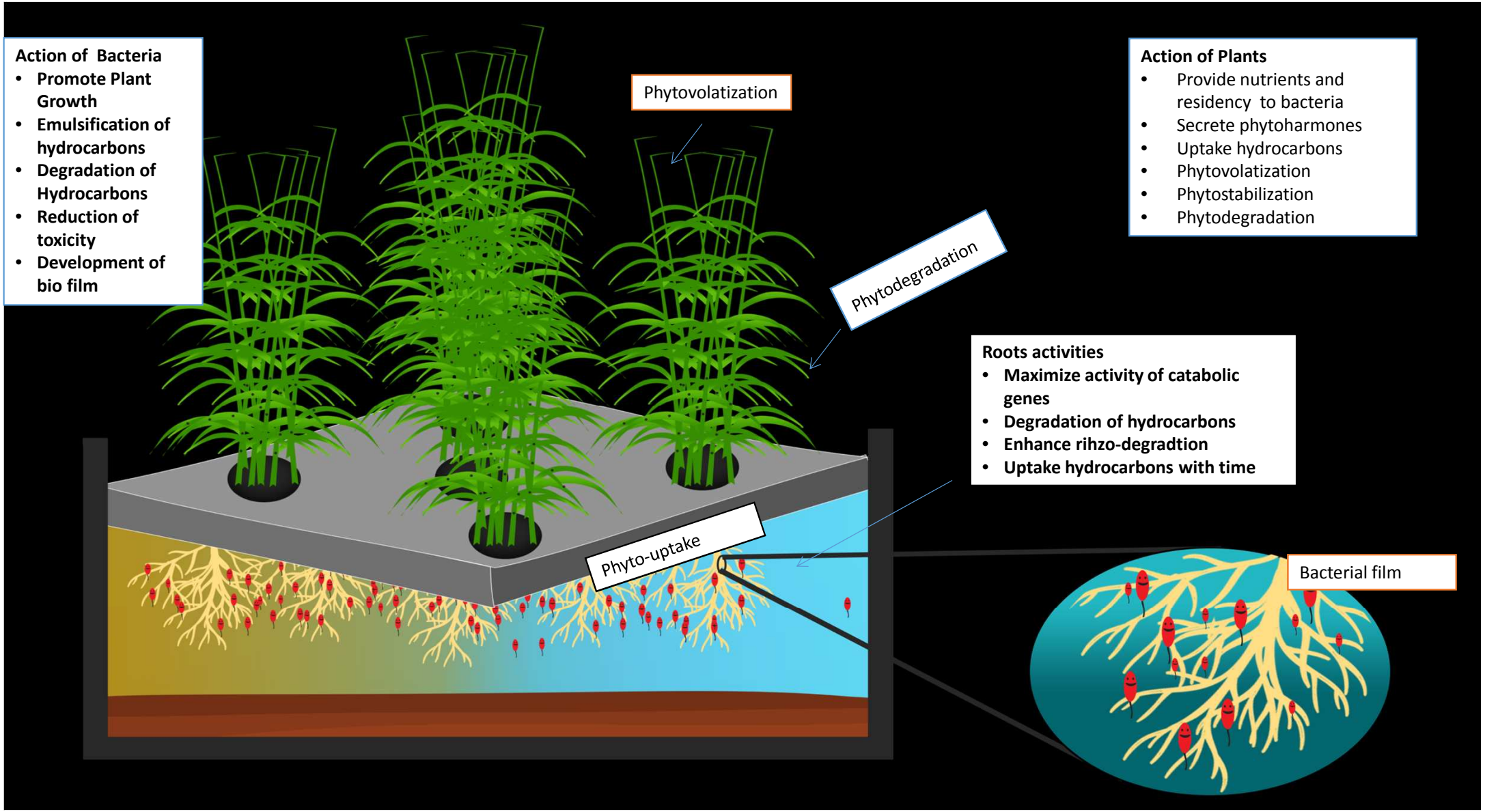
Action of Bacteria
Promote Plant Growth
Emulsification of hydrocarbons
Degradation of Hydrocarbons
Reduction of toxicity
Development of bio film
Phytovolatization
Phytodegradation
Action of Plants
Provide nutrients and residency to bacteria
Secrete phytoharmones
Uptake hydrocarbons
Phytovolatization
Phytostabilization
Phytodegradation
Roots activities
Maximize activity of catabolic genes
Degradation of hydrocarbons
Enhance rihzo-degradtion
Uptake hydrocarbons with time
Phyto-uptake
Bacterial film

# *Phragmites australis* in combination with hydrocarbons degrading bacteria is a suitable option for remediation of diesel-contaminated water in floating wetlands

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**Running title**

Bacterially assisted floating wetlands for phytoremediation of diesel oil

**Abstract**

The presence of diesel in the water could reduce the growth of plant and thus phytoremediation efficacy. The toxicity of diesel to plant is commonly explained; because of hydrocarbons in diesel accumulate in various parts of plants, where they disrupt the plant cell especially, the epidemis, leaves, stem and roots of the plant. This study investigated the effect of bacterial augmentation in floating treatment wetlands (FTWs) on remediation of diesel oil contaminated water. A helophytic plant, *Phragmites australis* (*P. australis*), was vegetated on a floating mat to establish FTWs for the remediation of diesel (1%, w/v) contaminated water. The FTWs was inoculated with three bacterial strains (*Acinetobacter* sp. BRRH61, *Bacillus megaterium* RGR14 and *Acinetobacter iwoffii* AKR1), possessing hydrocarbon degradation and plant growth-enhancing capabilities. It was observed that the FTWs efficiently removed hydrocarbons from water, and bacterial inoculation further enhanced its hydrocarbons degradation efficacy. Diesel contaminated water samples collected after fifteen days of time interval for three months and were analyzed for pollution parameters. The maximum reduction in hydrocarbons (95.8%), chemical oxygen demand (98.6%), biochemical oxygen demand (97.7%), total organic carbon (95.2%), phenol (98.9%) and toxicity was examined when both plant and bacteria were employed in combination. Likewise, an increase in plant growth was seen in the presence of bacteria. The inoculated bacteria showed persistence in the water, root and shoot of *P. australis*. The study concluded that the augmentation of hydrocarbons degrading bacteria in FTWs is a better option for treatment of diesel polluted water.

**Keywords:** Floating Treatment wetlands, Plant-bacteria synergism, Hydrocarbons, Bioremediation, Chemical oxygen demand, Biochemical oxygen demand.

## 1. Introduction

The demand of oil production and shipping is increasing day by day, whereas intermittent oil spillage is the leading cause of hydrocarbons contamination in soil and water. Among other fuel oils, diesel is more frequently reported cause of contamination of soil and water due to its extensive transportation and applications, i.e., automobiles and industrial sector. Alongside, diesel is extremely toxic in nature as it comprises several mutagenic and carcinogenic compounds (Arslan et al. 2014; Al-Baldawi, et al. 2015). The presence of these compounds in water bodies pertains to detrimental effects on living organisms (Moreira et al. 2011). Additionally, the compounds are deleterious to plants as these reduce the bioavailability of essential nutrients due to their hydrophobic nature (Gros et al. 2014; Arslan et al. 2014).

Remediation of water contaminated with diesel is relatively difficult due to complex nature of its components (Lin and Mendelssohn, 2009; Li et al. 2013). It contains approximately 25% of the aromatic hydrocarbons (mainly alkylbenzenes and naphthalenes) and 75% of the saturated hydrocarbons (mainly cycloparaffins and paraffins) (ATSDR, 1995). Most of these compounds are previously reported to be highly resistant to degradation in the environment (Arslan et al. 2016; Hussain et al. 2018). The conventional physiochemical remediation methods are either energy/chemical intensive or require high capital, operational and maintenance costs (World Bank, 2013; Hu et al. 2015; Younker and Walsh, 2015). Another side, phytoremediation of contaminated waters through FTWs is an effective strategy both in terms of cost and energy demands. Although the method is in practice since long time; a major bottleneck in achieving good remediation is the decreased performance of plants due to presence of toxic compounds in the wastewater (Shahid et al., 2018).

Bacterial assisted phytoremediation over the past few years has been reported as an effective method for the remediation of contaminated soil and water (Khan et al. 2013; Ijaz et al. 2016; Arslan et al. 2017). Both partners suffice the need of survival for each other. Mainly, interaction between plants and hydrocarbons to remove contaminants is important. The mechanisms of functioning behind this interaction include entrapment and, uptake of hydrocarbons, and flocculation of suspended matter by plants (Yeh et al. 2015). Moreover plants release phytohormones and enzyme such as dehalogenase, nitoductase, peroxidase and laccase from their roots that play a significant role in reduction of organic contaminants in water (Alkorta and

Garbisu 2001: Glick, 2014). Plants also have potential to eliminate organic contaminants through processes such as biodegradation, phytovolatilization, phytostabilization, metabolic transformation, extraction and stabilization (Yavari et al., 2015; Yeh et al. 2015; Chen et al. 2017).Plants also offer nutrients and shelter to their allied microbes whereas bacteria, in response, decrease the phytotoxicity by degrading xenobiotics (Weyes et al. 2009; Ijaz et al. 2015). Thus, choice of both plant and bacterial species is a crucial parameter that may improve the phytoremediation efficiency of the system (Afzal et al. 2012; Rehman et al., 2019). In case of hydrocarbons, bacteria with the potential of using hydrocarbons as carbon source along with plant growth promoting (PGP) traits were previously recommended as ideal candidates in the bacterial-assisted phytoremediation (Afzal et al. 2012; Rehman et al., 2018). Many bacteria degrade hydrocarbons into simple nutrients, which are assimilated by plants for their growth (Billore et al. 2008; Arslan et al. 2014). Likewise, *P.australis*, a helophytic grass, could transport atmospheric oxygen into the rhizosphere has been appeared as beneficial host for the inoculated bacterial communities (Saleem et al. 2018; Rehman et al. 2018). Moreover, it has the capacity to survive in the severe environmental circumstances (Davies et al. 2005; Schröder et al. 2008; Hechmi et al. 2014) particularly in waterlogged conditions.

In this study, the primary objective was to develop a better partnership between plants and their associated bacteria. Secondly, to evaluate the effect of augmentation of hydrocarbon degrading bacteria in FTWs, vegetated with *P.australis* towards phytoremediation of diesel polluted water. So a consortium of hydrocarbons degrading bacteria was inoculated in FTWs to assess the hydrocarbons degradation; toxicity reduction and the persistence of the inoculated bacteria.

## Materials and methods

### 2.1. Bacterial strains

Three pre-isolated and characterized bacterial strains, namely *Acinetobacter* sp. BRRH61, *Bacillus megaterium* RGR14, and *Acinetobacter iwoffii* AKR1, were used in this study (Fatima et al. 2015).Bacterial strains were cultured in M9 minimal salt medium containing diesel (1.0%, w/v) at 37 °C. Bacterial cells were harvested by centrifugation following their re-suspension in

NaCl solution (0.9%, w/v), optical density of each bacterial strain was made in a way to obtain $10^8$ cells $ml^{-1}$ (Sutton, 2005). Consortium of all three bacterial strains was prepared by mixing them in equal ratio (1:1:1). Fifty ml of consortium was inoculated in a FTWs, microcosm as per experimental strategy.

### 2.2. FTWs structure and experimental design

Fifteen FTWs microcosms were formed using polyethylene tanks, floating mats, and plants. The floating mat was prepared by using polystyrene role as elucidated earlier (Shahid et al. 2019). Briefly, each mat had 51 cm length, 38 cm width and 7.62 cm thickness. Five holes were created at equal distance in each mat for plantation of healthy seedlings of *P. australis*, i.e. one seedlings was inserted in each hole. The floating mat was placed over polyethylene tank containing 20 liters of tap water (Figure 1). In the first month of experiment, Hoagland's solution was applied to grow the plants. After one month, the tap water was put in the tanks instead Hoagland's solution, and spiked with 1% (w/v) diesel. The experimental treatments were as follows:

Control 1: Microcosms containing diesel contaminated water
Control 2: Microcosms containing water (without diesel) and *P. australis*
T1: Microcosms containing diesel contaminated water and bacterial consortium
T2: Microcosms containing diesel contaminated water and *P. australis*
T3: Microcosms containing diesel contaminated water, *P. australis* and bacterial consortium.

The experiment was run during April to June 2018 at National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. The water samples were collected every 10 days and analyzed for various physio-chemical parameters.

### 2.3. Growth of plant

To assess the effect of bacterial inoculation as well as toxicity of diesel, plant growth was studied in terms of root and shoot length and weight at the end of experimental period, i.e. 3-months. The roots and shoots were harvested 2 cm above the mat surface. Dry biomass was determined by placing roots and shoots in an oven for 48 h at 60 °C.

### 2.4. Assessment of hydrocarbons

Residual hydrocarbons in water were assessed using Fourier transform infrared spectrophotometer (FTIR). Hydrocarbons extraction was performed using dichloromethane. For

this purpose, 25 ml of treated water sample was extracted using dichloromethane (15 ml) as extracting solvent and hydrocarbon contents were determined using FTIR as described earlier (Rehman et al. 2019).

### 2.5. Analyses of water quality parameters

The collected water samples were analyzed for pH, total solids (TS), electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), total suspended solids (TSS), Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), total organic carbon (TOC), and phenols. The analyses were performed using established standard protocols (APHA, 2005).

### 2.6. Determination of persistence of inoculated bacteria

In the water, rhizosphere and endosphere of the plant, number of the inoculated bacteria was determined at different time intervals. To isolate bacteria from roots and shoots interior, their surface sterilization was performed as mentioned earlier (Afzal et al. 2012). For this purpose, plant tissues were washed thrice with sterilized distilled water, then treated with 70% ethanol for 10 minutes, and 1% NaOCl solution, modified with Tween 20 (0.01%), for 1 min. The final rinse was performed with sterilized distilled water for 2 minutes. After surface sterilization, in the presence of 10 ml NaCl solution (0.9% w/v), plant roots and shoots (5 g) were ground with mortar and pestle. The slurry suspension (100 µl) was plated on M9 media containing diesel oil (100 mg $l^{-1}$). The plates were incubated at 37 °C for 48 hours, and colony forming units (CFUs) were counted.

### 2.7. Toxicity testing

Treated water was evaluated for toxicity reduction using fish toxicity assay. The water was put in the glass tanks and aerated with an electric pump. A total of ten fish specimens of about equal weight and size were put in each tank. The survival rate for fish population was recorded for the period of 90 h (Afzal et al. 2008).

### 2.8. Statistical analysis

Microsoft Excel software methodology was used to analyze data statistically for mean and standard deviation calculation. All the experiments were conducted in triplicates.

## 3. Results and discussion

### 3.1. Hydrocarbons reduction

In this study, concentration of hydrocarbons in all microcosms was gradually decreased during the experimental period (Figure 2). The bacterial consortium augmented FTWs (T3) exhibited maximum (97.5%) hydrocarbons reduction. A number of previous studies have described that the augmentation of bacteria in wetlands enhanced the degradation of organic pollutants (Al-Baldawi, et al. 2013; Adeboye et al. 2014; Zhang et al. 2014; Rehman et al., 2018). Minimum hydrocarbon degradation was observed in the treatment with bacterial consortium only (T1) where hydrocarbons were reduced to 39.5%. On the other hand, treatment (T2) containing only vegetation showed more hydrocarbons removal (68.7%) than T1. Overall 28.8% higher reduction of hydrocarbons was achieved by T3 treatment augmented with bacterial strains and plants besides T2 treatment vegetated only with plants. It was well established that besides the degradation of hydrocarbons by microorganisms, plants may also uptake the organic pollutants and convert them into less toxic compounds (Oyedeji et al. 2013; Darajeh et al. 2014; Kosesakal et al. 2016). Nevertheless, in all treatments, removal of hydrocarbons was faster during initial 30 days. This might be due to the presence of short-chain hydrocarbons and/or nutrients in initial days. In control (C1), hydrocarbon contents were declined to 10.3% which might be accredited to the evaporation of volatile hydrocarbons, photodegradation, and/or degradation by indigenous bacterial communities (Kosesakal et al. 2016).

In T3 treatment, plant and hydrocarbons degrading bacterial strains proved to be more effective in remediation of hydrocarbons in FTWs. It was due to the reason that; in principle the active zone of hydrocarbon degradation in FTWs is the extensive roots system in rhizosphere and rhizoplane. The rhizoplane is mainly involved in plant-microbe interactions. Plants roots release compounds that can act as inducer for microbial genes involve in hydrocarbon degradation and act as co-metabolites to assist microbial degradation (Xie et al., 2012). In return, plant associated bacteria support their host plant to overcome contaminant-induced stress responses. In addition, plants can further benefit from their associated-bacteria possessing pollutant-degradation potential, leading to enhanced pollutant mineralization and lessening of phytotoxicity (Khan et al., 2013).

### 3.2. Evaluation of water quality parameters

Reduction in COD (98.5%) and BOD (97.7%) was more prominent in the treatment (T3) containing plant and bacteria together than the treatments (T1 and T2) having plant and bacteria individually (Figure 3 and 4).In our study COD and BOD were reduced to 5.5 % and 0.7% higher than previous study of Rehman et al. (2018), who evaluated the remediation of crude oil contaminated wastewater using *P. australis* plant in FTWs. Moreover, these results are in accordance to the previous studies which described that the augmentation of bacteria in the plant rhizosphere stimulates the remediation potential of the phytoremediation system (Saleem et al. 2018; Hussain et al., 2019). Bacteria emulsify the hydrocarbons resulting in their enhanced bioavailability and degradation by microbial population (Pal et al. 2016). Comparatively, less reduction was observed in T2 than T3; and lowest reduction was observed in T1, i.e. COD reduced by 37.5% and BOD by 48.1%. In control, reduction in COD and BOD was recorded to be 11.4% and 14.1%, respectively. Similarly, TOC reduction (95.18%) was higher in T3 than other treatments (Figure 5). The lowest TOC reduction (69.75%) was found in T1 among all treatments. The maximum reduction of TOC in T3 could be due to the presence of bacteria on the roots of plants that uses organic compounds as a source of nutrients and energy (Omokeyeke et al. 2013). Likewise, more reduction (98.8%) in phenol concentration was observed in T3 than other treatments, and lowest (80.5%) was detected in T1 (table 1). Phenol removal was 2 % higher in our study than the earlier study that reported phenol degradation (96.14%) by *P. australis* in FTWs by augmentation of bacteria (Saleem et al. 2018). Similarly 71 % phenol reduction was observed in FTWs using Vetiver plant (Phenrat et al. 2017). Also, bacterially augmented treatment (T3) demonstrated maximum reduction in TS (70.19%), TSS (84.83%), TDS (70.03 %) and EC (85.23%) (Table 1). The pH value was reduced from 8.5 to 7.5 (Table 1) which is substantiated by earlier results (Ijaz et al. 2016; Rehman et al. 2018).

**Persistence of bacteria**

The effectiveness of the bacterial-assisted FTWs is associated with the augmented bacterial population in the rhizosphere and water (Khan et al., 2013; Afzal et al., 2014; Ijaz et al. 2015; Rehman et al., 2018). The inoculated bacterial numbers were enumerated in the water; rhizoplane and plant tissues (root and shoot). The results elucidated that bacterial persistence was maximum in the water of FTWs augmented with bacteria (T3) than unvegetated treatment T2 (Table 2). In different compartments of augmented FTWs , bacterial survival was observed as follows: rhizoplane ($5.1 \times 10^6$) > root interior ($4.5 \times 10^5$) > shoot interior ($1.5 \times 10^4$). Plants

supply nutrients for bacterial growth, and their population continually rises (Ijaz et al. 2016). Bacteria penetrate the subjected plants and may have an active mechanism of colonization (Compant et al., 2010). The inoculated bacteria colonize in the roots and shoots of *P. australis*, however, a decline in their numbers was observed during the period of 90 days. This might be due to decrease in the amount of biodegradable fraction of diesel (Pal et al. 2016).

### 1.3. **Plant biomass and growth**

The presence of hydrocarbons in the water could reduce the growth of plant and thus phytoremediation efficacy (Shehzadi et al. 2014; Rehman et al. 2018). The hydrocarbons are absorbed in roots and translocated to the aboveground parts of plant and ultimately affecting their growth (Tsao, 2003). In this study, biomass of shoots and roots were determined to test the effect of diesel and inoculation of bacteria on plant growth (Table 3). *P. australis* vegetated in diesel oil contaminated water (T2) exhibited less root length (51.72%), shoot length (34.32%), fresh biomass (51.18%) and dry biomass (39.43%) as compared to control plants, grown in tap water. Many previous studies have documented that hydrocarbons substantially affect the growth of plants (Barua et al. 2011; Zhou et al. 2011; Eze et al. 2013). The reduction occurred in growth of plants are due to toxicity of hydrocarbons which affects photosynthesis and causes chlorosis in vegetated plants (Barac et al. 2004; Merkl et al., 2005; Rehman et al. 2018). In this study, minimum decrease in root length (20.68%), shoot length (8.95%), fresh biomass (10.40%) and dry biomass (2.83%) of *P. australis* were observed in FTWs augmented with bacterial consortium (T3) with respect to control. Better growth of plants is credited directly to plant growth promoting bacteria, which have potential to reduce the toxicity of hydrocarbons.

### **1.4. Detoxification of diesel contaminated water**

Toxic compounds present in diesel may kill ecological receptors that are mainly fish (Robertson et al. 2007). In this study, an indication of the extent of remediation of the water was attained by exposing fish to the water of different treatments (Table 4). No toxicity was observed in water of T3 treatment (FTWs augmented with bacteria) where no fish was died after 96 h. Whereas in T1 (only bacteria augmentation) and T2 (only vegetation) treatments, death of 4 and 3 fish was occurred, respectively, however, all the fish were died in control after 24 h. Shehzadi et al. (2014) also reported a massive decline in the toxicity of water treated by constructed wetlands. High extent of fish demise in untreated water might be due to gathering of different

hydrocarbons in fish, DNA damage, cardiac dysfunction and alleviated oxidative stress (Incardona et al. 2004, Sturve et al. 2006, Pal et al. 2016).

## 2. Conclusions

We concluded from this study that performance of *P. australis* and hydrocarbons degrading bacterial strains to develop FTWs for phytoremediation of diesel contaminated water was proved to be an excellent approach. This study showed that FTWs is suitable and self-sustainable option for the remediation of diesel contamination in water and reduction of toxic effect of diesel on bacteria and plants; hence could be applicable for the remediation of diesel contaminated produced water in petroleum mining companies and oil refineries where setting up and operation of conventional wastewater treatment plants is difficult. Considering the synergism of *P. australis* with hydrocarbons degrading bacteria, bacterial augmented FTWs could be a promising approach to treat diesel oil contaminated water. Besides, studies are needed to conduct the analysis of genes transcription involved in the degradation of hydrocarbons present in diesel. Further studies are also needed to observe the abundance and expression of alkane-degrading genes in different compartments of FTWs.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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**Table 1.** Effect of bacterial inoculation on remediation of diesel oil contaminated water in floating treatment wetlands vegetated with *Phragmites australis*

| Treatment | Days | Parameter | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | pH | EC (ms/cm) | TDS (mg/l) | TS (mg/l) | TSS (mg/l) | DO (mg/l) | Phenol (mg/l) |
| Control | 0 | 8.7 (0.11) | 3.29 (0.01) | 1918 (178) | 2274 (230) | 356 (17) | 5.5 (0.11) | 0.353 (0.01) |
| | 15 | 8.5 (0.14) | 3.27 (0.01) | 1912 (194) | 2270 (218) | 350 (28) | 5.2 (0.12) | 0.343 (0.01) |
| | 30 | 8.3 (0.18) | 3.24 (0.03) | 1908 (174) | 2268 (238) | 346 (18) | 5.0 (0.14) | 0.323 (0.01) |
| | 45 | 8.2 (0.12) | 3.21 (0.09) | 1906 (187) | 2255 (198) | 336 (21) | 5.1 (0.16) | 0.321 (0.06) |
| | 60 | 8.1 (0.14) | 3.19 (0.01) | 1903 (194) | 2245 (239) | 331 (28) | 4.9 (0.12) | 0.318 (0.01) |
| | 75 | 8.0 (0.17) | 3.17 (0.05) | 1902 (128) | 2239 (264) | 329 (15) | 4.7 (0.18) | 0.317 (0.01) |
| | 90 | 8.0 (0.19) | 3.14 (0.01) | 1900 (149) | 2225 (294) | 317 (24) | 4.5 (0.15) | 0.312 (0.07) |
| T1 | 0 | 8.8 (0.12) | 3.27 (0.01) | 1921 (111) | 2276 (196) | 355 (18) | 5.3 (0.18) | 0.349 (0.01) |
| | 15 | 8.7 (0.16) | 2.32 (0.03) | 1615 (153) | 1811 (178) | 296 (15) | 5.1 (0.13) | 0.165 (0.02) |
| | 30 | 8.6 (0.10) | 2.09 (0.04) | 1312 (163) | 1509 (193) | 197 (14) | 4.8 (0.11) | 0.125 (0.005) |
| | 45 | 8.6 (0.14) | 1.23 (0.03) | 1208 (144) | 1321 (145) | 143 (19) | 4.3 (0.19) | 0.098 (0.001) |
| | 60 | 8.5 (0.18) | 1.11 (0.07) | 1129 (126) | 1217 (109) | 117 (15) | 4.0 (0.15) | 0.075 (0.004) |
| | 75 | 8.4 (0.14) | 1.09 (0.02) | 1078 (153) | 1161 (105) | 103 (16) | 3.8 (0.15) | 0.071 (0.001) |
| | 90 | 8.2 (0.17) | 1.03 (0.07) | 1011 (103) | 1117 (142) | 92 (10) | 3.5 (0.11) | 0.068 (0.004) |
| T2 | 0 | 8.7 (0.18) | 3.23 (0.04) | 1918 (251) | 2275 (198) | 359 (19) | 5.6 (0.15) | 0.351 (0.02) |
| | 15 | 8.4 (0.13) | 2.01 (0.07) | 1580 (143) | 1618 (156) | 278 (18) | 6.1 (0.17) | 0.115 (0.01) |
| | 30 | 8.2 (0.18) | 1.88 (0.08) | 1225 (134) | 1315 (145) | 167 (17) | 6.5 (0.13) | 0.089 (0.006) |
| | 45 | 8.1 (0.11) | 1.02 (0.07) | 1018 (141) | 1109 (176) | 124 (14) | 6.8 (0.12) | 0.017 (0.002) |
| | 60 | 7.8 (0.13) | 0.98 (0.08) | 987 (165) | 1098 (179) | 99 (12) | 7.0 (0.13) | 0.015 (0.001) |
| | 75 | 7.5 (0.12) | 0.87 (0.05) | 865 (154) | 1068 (154) | 63 (16) | 7.2 (0.11) | 0.012 (0.004) |
| | 90 | 7.4 (0.11) | 0.76 (0.01) | 786 (144) | 1049 (148) | 59 (11) | 7.3 (0.14) | 0.011 (0.001) |
| T3 | 0 | 8.5 (0.14) | 3.59 (0.07) | 1922 (135) | 2278 (187) | 356 (18) | 5.6 (0.14) | 0.352 (0.011) |
| | 15 | 8.4 (0.16) | 1.29 (0.06) | 1418 (125) | 1521 (195) | 233 (17) | 5.9 (0.13) | 0.092 (0.001) |
| | 30 | 8.2 (0.18) | 1.01 (0.04) | 1108 (123) | 1211 (143) | 143 (16) | 6.5 (0.17) | 0.071 (0.001) |
| | 45 | 8.0 (0.19) | 0.98 (0.03) | 925 (143) | 1038 (176) | 133 (17) | 7.4 (0.16) | 0.016 (0.001) |
| | 60 | 7.9 (0.13) | 0.84 (0.01) | 786 (132) | 838 (138) | 118 (19) | 7.5 (0.12) | 0.011 (0.001) |
| | 75 | 7.7 (0.12) | 0.63 (0.01) | 678 (143) | 708 (138) | 70 (18) | 7.5 (0.11) | 0.008 (0.001) |
| | 90 | 7.5 (0.15) | 0.53 (0.01) | 576 (145) | 679 (141) | 54 (15) | 7.6 (0.13) | 0.004 (0.001) |

**Control**: Un-vegetated microcosm containing diesel oil contaminated water; **T1**: Un-vegetated microcosm containing diesel oil contaminated water and bacterial consortium; **T2**: Vegetated microcosm containing diesel oil contaminated water; **T3**: Vegetated microcosm containing diesel oil contaminated water and bacterial consortium. Each value is a mean of three triplicates. Standard error among the replicates is presented in parenthesis. EC (electrical conductivity), TDS (total dissolved solids), TS (total solids), TSS (total suspended solids), DO (dissolved oxygen).

**Table 2**. Persistence of inoculated bacteria in the water, root and shoot of *Phragmites australis*

| **Treatments** | **Colony forming unit (CFU) × $10^5$** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0 day** | **15 day** | **30 day** | **45 day** | **60 day** | **75 day** | **90 day** |
| Water (CFU $ml^{-1}$) | 27.84 (1.42) | 21.63 (1.32) | 19.25 (0.93) | 18.86 (1.85) | 16.76 (1.28) | 13.73 (1.45) | 11.94 (1.46) |
| Rhizoplane (CFU $g^{-1}$) | ND | 14.25 (0.83) | 22.80 (1.34) | 31.65 (2.68) | 41.22 (2.75) | 46.46 (3.69) | 51.44 (3.87) |
| Root (CFU $g^{-1}$) | ND | 7.29 (1.33) | 15.73 (1.57) | 24.73 (1.12) | 33.38 (1.75) | 39.34 (2.4) | 45.47 (3.38) |
| Shoot (CFU $g^{-1}$) | ND | 2.26 (1.65) | 5.75 (1.78) | 8.91 (0.37) | 11.03 (0.65) | 13.95 (1.52) | 15.46 (1.54) |

ND = not determined. Each value is a mean of three triplicates. Standard error among the replicates is presented in parenthesis.

| Treatments | Fresh biomass (g) | | Dry biomass (g) | | Length (cm) | |
|---|---|---|---|---|---|---|
| | **Root** | **Shoot** | **Root** | **Shoot** | **Root** | **Shoot** |
| C2 | $392^{a}$ (27) | $454^{a}$ (34) | $101^{ab}$ (15) | $216^{a}$ (19) | $88.4^{a}$ (7.4) | $76.7^{a}$ (4.9) |
| T2 | $195^{b}$ (12) | $218^{b}$ (17) | $86^{b}$ (9) | $106^{b}$ (14) | $42.7^{c}$ (3.8) | $44.4^{c}$ (3.4) |
| T3 | $360^{a}$ (31) | $398^{a}$ (24) | $115^{a}$ (16) | $193^{a}$ (21) | $70.1^{b}$ (7.3) | $58.1^{b}$ (3.7) |

**Table 3.** Effect of bacterial inoculation on biomass, root and shoot length of *Phragmites australis*

**C2**: Vegetated microcosm containing tap water; **T2**: Vegetated microcosm containing diesel contaminated water; **T3**: Vegetated microcosm containing diesel contaminated water and bacterial consortium. Each value is a mean of three triplicates. Standard error among the replicates is presented in parenthesis.

**Table 4.** Fish toxicity assay of diesel oil contaminated water detoxified by floating treatment wetlands

| **Treatment** | **Fish death over time** | | | | **Total death** | **Detoxification status** |
|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | | |
| Control | 10 | 0 | 0 | 0 | 10/10 | Negligible |
| T1 | 1 | 1 | 1 | 1 | 4/10 | Partial |
| T2 | 0 | 1 | 1 | 1 | 3/10 | Partial |
| T3 | 0 | 0 | 0 | 1 | 1/10 | Complete |

**Control**: Un-vegetated microcosm containing diesel oil contaminated water; **T1**: Un-vegetated microcosm containing diesel oil contaminated water and bacterial consortium; **T2**: Vegetated microcosm containing diesel oil contaminated water; **T3**: Vegetated microcosm containing diesel oil contaminated water and bacterial consortium. Each value is a mean of three triplicates. Standard error among the replicates is presented in parenthesis.


**Figure 1:** Development of floating treatment wetlands (FTWs) microcosms for the remediation of a diesel oil contaminated water**. Control 1:** Un- vegetated microcosm containing diesel oil contaminated water. **Control 2:** vegetated Microcosm containing tap water. **Treatment1:** unvegetated microcosm containing diesel oil contaminated water and bacterial consortium. **Treatment2:** Vegetated microcosm containing diesel oil contaminated water. **Treatment3:** Vegetated microcosm containing diesel oil contaminated water and bacterial consortium

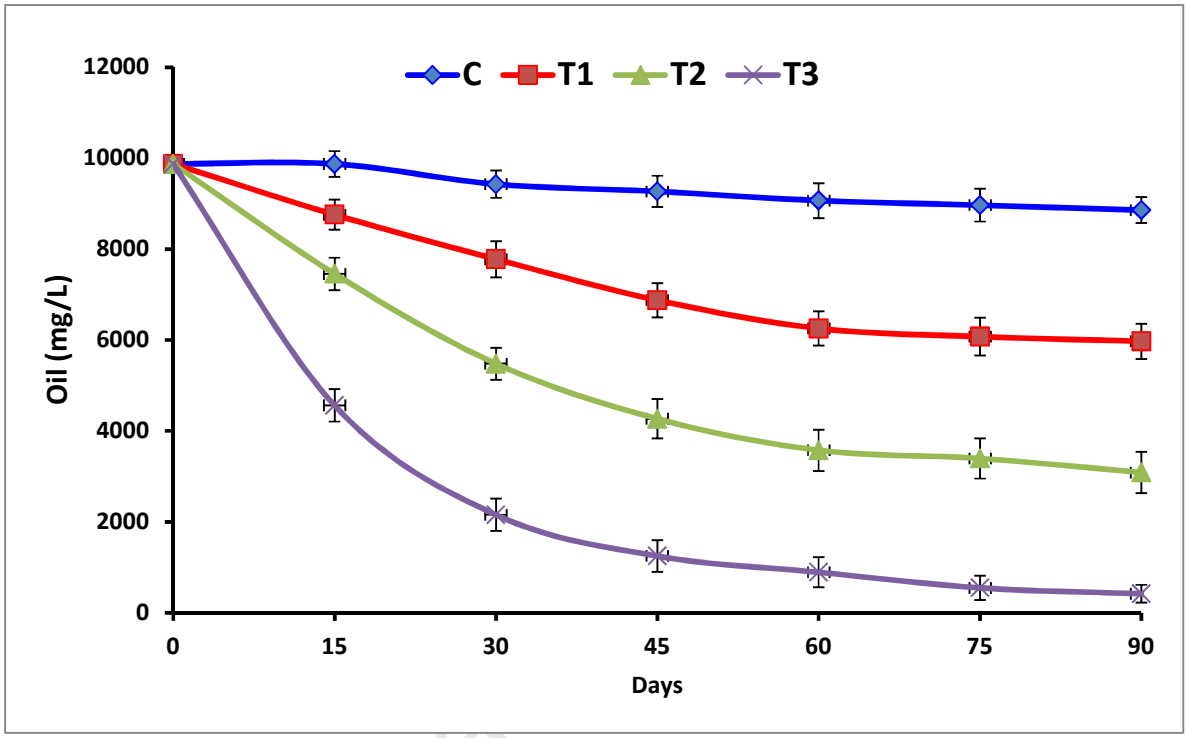


**Figure 2:** Diesel oil reduction in water by floating treatment wetlands. **C:** Un-vegetated microcosm containing diesel oil contaminated water; **T1:** Un-vegetated microcosm containing diesel oil contaminated water and bacterial consortium; **T2:** Vegetated microcosm containing diesel oil contaminated water; **T3:** Vegetated microcosm containing diesel oil contaminated water and bacterial consortium. Each value is a mean of triplicate determinations. Error bars indicate the standard error among three replicates.

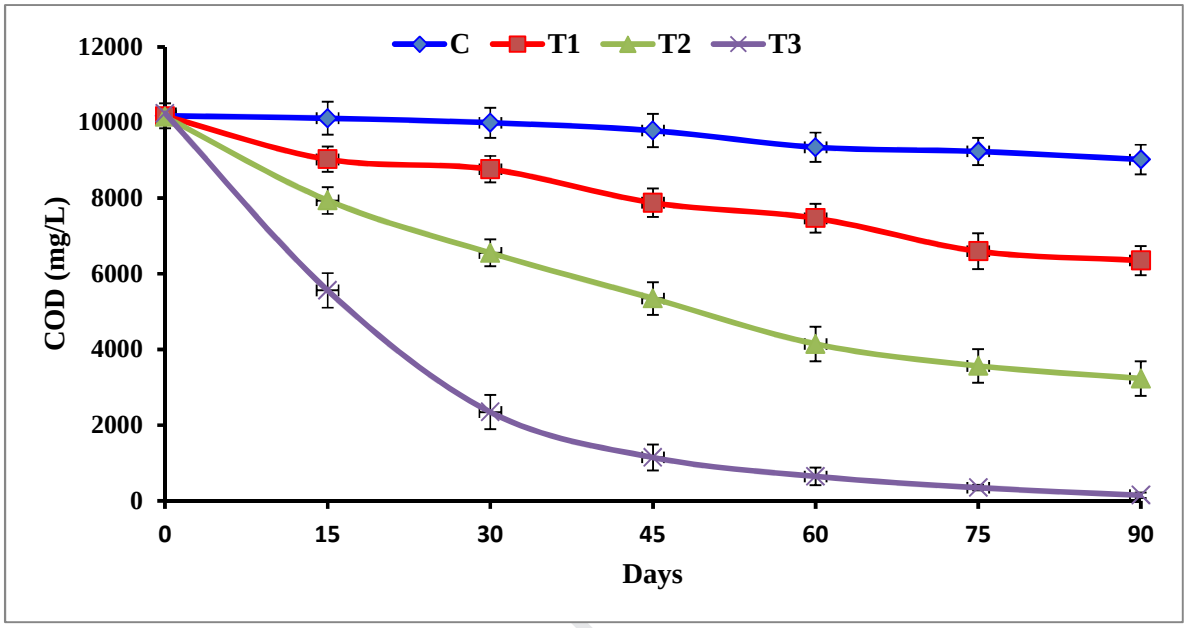


**Figure 3:** COD reduction in water by floating treatment wetlands. **C:** Un-vegetated microcosm containing diesel oil contaminated water; **T1:** Un-vegetated microcosm containing diesel oil contaminated water and bacterial consortium; **T2:** Vegetated microcosm containing diesel oil contaminated water; **T3:** Vegetated microcosm containing diesel oil contaminated water and bacterial consortium. Each value is a mean of triplicate determination. Error bars indicate the standard error among three replicates.

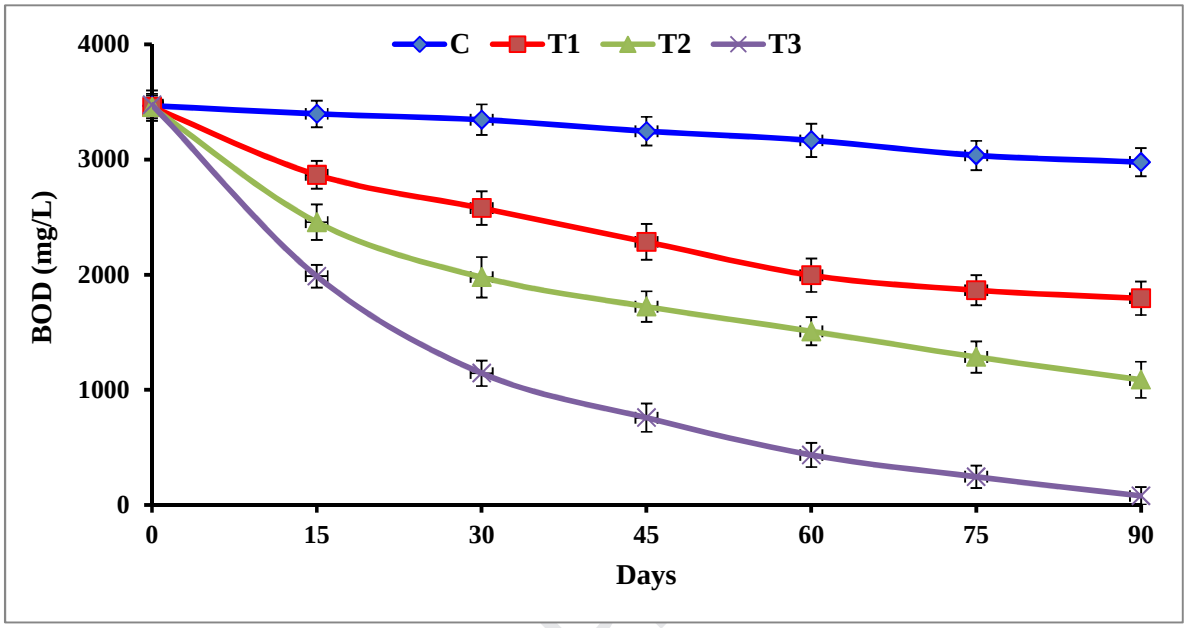


**Figure 4:** BOD reduction in water by floating treatment wetlands. **C:** Un-vegetated microcosm containing diesel oil contaminated water; **T1:** Un-vegetated microcosm containing diesel oil contaminated water and bacterial consortium; **T2:** Vegetated microcosm containing diesel oil contaminated water; **T3:** Vegetated microcosm containing diesel oil contaminated water and bacterial consortium. Each value is a mean of triplicate determination. Error bars indicate the standard error among three replicates.

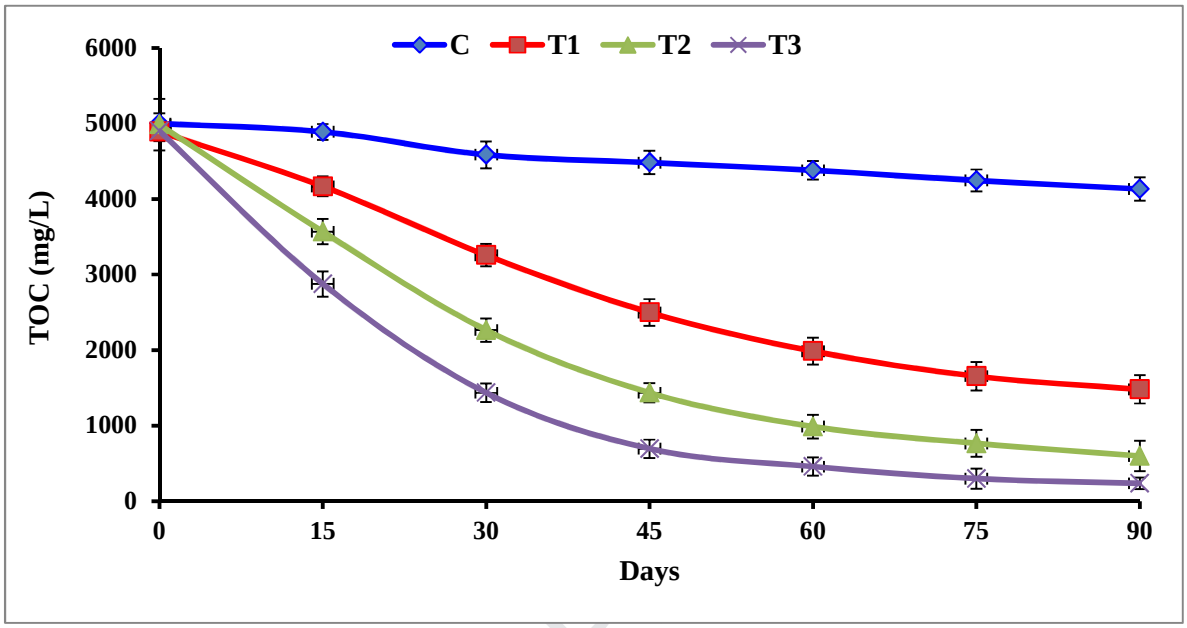


**Figure 5:** TOC reduction in water by floating treatment wetlands. **C:** Un-vegetated microcosm containing diesel oil contaminated water; **T1:** Un-vegetated microcosm containing diesel oil contaminated water and bacterial consortium; **T2:** Vegetated microcosm containing diesel oil contaminated water; **T3:** Vegetated microcosm containing diesel oil contaminated water and bacterial consortium. Each value is a mean of triplicate determination. Error bars indicate the standard error among three replicates.

► Plant–hydrocarbons degrading bacteria partnerships is an emerging hydrocarbon remediation approach.

► Plant associated microcosms can enhance hydrocarbon degradation

► *Phragmites australis* stimulates hydrocarbons degrading bacteria to degrade hydrocarbons in water

► *Phragmites australis* associated- hydrocarbons degrading bacteria can reduce phytotoxicity and evapotranspiration of hydrocarbons.