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Remediation of PAHs-contaminated water and sand by tropical plant (*Eleocharis ochrostachys*) through sub-surface flow system

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) removal by the tropical plant, *Eleocharis ochrostachys*, from contaminated water and sand using sub-surface flow system (SSF) was conducted for 80 days. *E. ochrostachys* was exposed to various concentrations of diesel (0.5, 1, 2 and 3% v/v). Treatments with and without the plant were conducted to analyse the effect of plants in removing PAHs. A liquid–liquid extraction method was used to extract the PAHs from the wastewater. The PAH removal with plants was significantly better than without plants ($p<0.05$). At diesel concentrations of 0.5, 1, 2 and 3%, the PAH removal percentages with plants were 89.1, 91.3, 73.0 and 71.6% from water and 95.3, 97.2, 97 and 86.2% from the sand, respectively, while the removals without plants were only 81.8, 81.0, 63 and 63.9% from water and 93.6, 93.9, 95.7 and 81.8% from the sand, respectively. Increasing colony-forming units (CFUs) surrounding the plant roots indicated enhanced growth of rhizobacteria, which could assist the removal of pollutants. Additionally, the gravel layers had removed the total suspended solid (TSS) from 87 to 98%.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of the organic pollutants, that can be spread by storm water runoff from contaminated sites and later into surface water sources (Reddy et al., 2013). Organic pollutants are common threats to terrestrial and aquatic ecosystems due to their variable toxicities, strong odour emission, high persistence in the environment with carcinogenic and mutagenic properties that can pollute receiving water bodies (Rashed, 2013), all of

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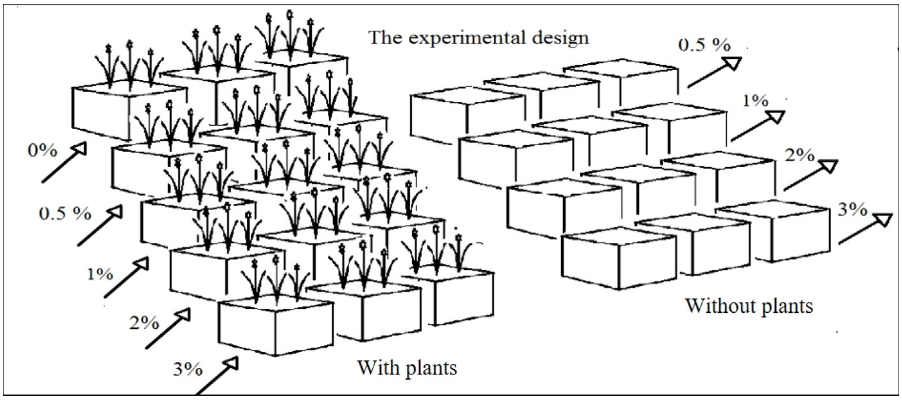


Fig. 1. Experimental design for phytoremediation of PAHs-contaminated water.

which can then adversely leave impacts on human health. According to Andreolli et al. (2013), the main source of PAHs is from petroleum and diesel spills, releases from chemical processes, combustion of fossil fuels and even tobacco smoke.

A risk assessment of personnel exposure to PAHs was carried out by Wu et al. (2019) and found that the risk percentage of having cancer were very high (74.9–99.7%). In addition, chronic or long-term exposure to PAHs may also cause jaundice, kidney and liver damage and cataracts and may encourage redness and skin inflammation (Azhari, 2012). They do not only badly affect human health but also plants and soil microorganisms' population and activities (Salehi-Lisar and Deljoo, 2015; Szczepaniak et al., 2016).

Therefore, the removal of toxic organic compounds from wastewater has been a rapid development in diverse remediation strategies to resolve this problem. There are numerous initiatives ongoing to treat and enhance the degradation efficiency of the organic pollutants especially PAHs (Lamichhane et al., 2016; Pang et al., 2011). The conventional removal methods for PAHs such as coagulation, flocculation, sedimentation, filtration or ozonation (Eeshwarasinghe et al., 2018; Haan et al., 2018). The costs of treatment methods, the toxicity of chemical materials used during treatment and the increasing concern and awareness of people on the health and environmental impacts caused by industrial sources have put pressure on the remediation industry to develop more cost-effective and environmentally friendly methods, such as phytoremediation (Lamichhane et al., 2016).

The application of plants and their root systems has garnered increasing interest due to their low-cost approach and also the flexibility of treatment mode either *in situ* or in place which it offers a pleasing option for managers on remediation tasks (Wuana and Okieimen, 2010). It utilizes green plants to remove, contain or render pollutant toxicity, using an integration mechanism of bioremediation involving microorganisms (Abdullah et al., 2020; Dai et al., 2020; Wolf et al., 2020). Phytoremediation has been widely applied to treat pollutants from various industrial effluents (Yusoff et al., 2019; Ahmad et al., 2017), agricultural wastewater (Said et al., 2020; Nash et al., 2019, 2020; Kadir et al., 2018), domestic wastewater (Jehawi et al., 2020), mine drainages (Arroyo et al., 2010; Lee et al., 2010), landfill wastewater (Kadlec and Zmarthie, 2010), petroleum effluent (Al-Baldawi et al., 2017, 2013b; Almansoory et al., 2017; Al-Sbani et al., 2016), wastewater contaminated with dyes (Abdulqader et al., 2019; Al-Baldawi et al., 2018) and wastewater containing heavy metals (Ismail et al., 2019; Kamaruzzaman et al., 2019; Selamat et al., 2018; Titah et al., 2018; Tangahu et al., 2013).

In contrast, very limited studies for phytoremediation of wastewater containing PAHs (Wloka et al., 2019; Petrova et al., 2017; Xiao et al., 2015) using tropical plants which are yet to be explored and tested for their potential to remove pollutants from polluted water. Therefore, the aim of this study, is to investigate the ability of a tropical grass from *Cyperaceae* family; *Eleocharis ochrostachys* to remove PAHs from diesel mixed water (which represents PAHs-contaminated water). The common name for this plant is Spike Rush and this plant can be found in wetlands, lowland rivers and shallow waters, marshes, along irrigation canals, floating islands, grasslands, paddy fields and pond margins in Malaysia, China, Cambodia, Indonesia, Japan, Laos, Myanmar and other countries (Catford and Downes, 2010). In addition, the impact of *E. ochrostachys*, on the number of bacteria in the root area (rhizobacteria), which plays a significant role in the removal of contaminant, was also examined.

2. Materials and methods

2.1. Greenhouse test of phytoremediation

About 27 aquariums, with dimensions 30×30×30 cm, were run in single exposure with four diesel concentration ranging 0.5%–3% (V_{Diesel}/V_{Water}). Each of these treatments were done in triplicate (R1, R2, and R3) and were planted with *E. ochrostachys*. Another three replicates were used as contaminant controls, i.e. without plants, as depicted in Fig. 1. Additionally, three replicates acted as plant control (plants without diesel). All aquariums were exposed once and ran in a

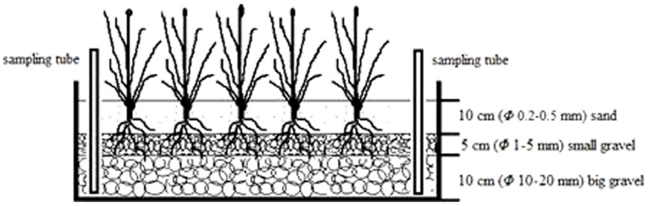


Fig. 2. Batch sub-surface flow system using aquarium.

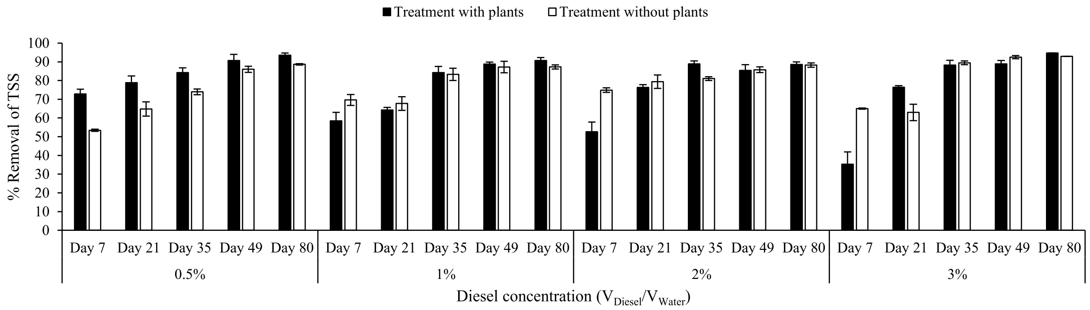


Fig. 3. TSS removal at different concentrations of diesel for treatments with and without plants.

batch wise setup. Each aquarium was filled with three layers of different medium. The bottom layer was filled with 10 cm gravel (Φ 10–20 mm), followed with 5 cm gravel (Φ 1–5 mm), and the upper layer was filled with 10 cm fine sand (Φ 0.2–0.5 mm). No additional nutrients or fertilizer were applied to the sand. There were two tubes placed in each aquarium for sample acquisition and ventilation of the root area, as illustrated in Fig. 2. Each aquarium was planted with 12 healthy plants of one-month old *E. ochrostachys*. Ten litres of wastewater containing PAHs was prepared synthetically and was poured slowly from the upper sand layer of each aquarium. Synthetic wastewater for every aquarium was prepared by mixing tap water with diesel in different percentages concentrations of 0.5, 1, 2, and 3% (V_{Diesel}/V_{Water}) in which diesel was used as a source of PAHs pollutants. Diesel was mixed continuously with water and slowly being poured into aquariums to ensure it was homogeneously distributed. The water level was maintained at the upper sand layer to simulate a sub-surface flow (SSF) system (Al-Baldawi et al., 2013a). Water was periodically added (0.5–1.0 L) once every two days to the aquarium throughout the exposure to ensure that the plants had enough water to grow (Ismail et al., 2017).

2.2. Water sampling and monitoring

Sampling of water was conducted on day 0, 7, 21, 35, 49 and 80. Water samples were collected in clean dark glass bottles. The physicochemical parameters measured were temperature (°C), pH, Oxidation Reduction Potential (ORP) (mV), dissolved oxygen (DO) and total suspended solids (TSS). An IQ 150 multiprobe (IQ Scientific Instruments, U.S.A) and dissolved oxygen sensor (GLI International, U.S.A) were used to measure ORP and DO, respectively. TSS in water was analysed according to APHA (1998).

2.3. Rhizobacteria population observation

The colony-forming units (CFUs) of the rhizosphere was assessed at various diesel concentrations using sterile nutrient agar plate (Tryptic Soya Agar, TSA) (Al-Baldawi et al., 2017, 2013a; Ismail et al., 2020; Zhang et al., 2012). Individual plant root (10 g) were mixed with 99 mL sterile distilled water in incubator shaker (Protech, Model SI- 100D, Malaysia) at 37 °C and 150 rpm for 90 min. Next, 1 mL of sample was pipetted into 9 mL sterile saline water to create afterwards dilutions up to 10–4 (serial dilutions). Then, 0.1 mL of the serial dilutions was moved to nutrient agar plates and was spread uniformly. The bacterial colonies on each plate were counted within 16-24 h after plating and the mean number was expressed as CFU/mL water. Similar procedure was carried to access the CFUs from sand where 4 g (wet weight) of sand were mixed with 99 mL sterile distilled water. Rhizobacteria were obtained from the roots of plants harvested on each sampling day and compared with the bacteria isolated from the sand in the aquarium containing plants and from the sand adjacent to the rhizosphere area in the aquarium without plants to investigate the effect of the plants on the CFUs.

Table 1
The variation of physical parameters of wastewater used in the treatment.

Parameters	Unit	With plant	Without plant
pH	–	7.95 ± 0.65	7.95 ± 0.75
T (C°)	C°	25.65 ± 0.95	25.35 ± 1.45
ORP	mV	−58.3 ± (−30)	−66.5 ± (−36.5)
DO	mg/L	2 ± 1	1.2 ± 0.1

2.4. Biomass monitoring

The plants of *E. ochrostachys* were propagated in a greenhouse. One-month old bulrush plants, with lengths of 25–30 cm, were selected for this experiment. The plant biomass through wet and dry weight was monitored in this experiment as a growth indicator. Shoots and roots of *E. ochrostachys* were collected at 0, 7, 21, 35, 49 and 80 days of exposure. Three plants were sampled from each concentration (one bulrush from each aquarium). The length and wet weight of the plants were recorded for the roots and stems. Dry weight was obtained after 3 days of drying process to ensure constant weight using an oven (Memmert, Germany) at 50 °C (Peng et al., 2009).

2.5. Extraction of water and sand sample for PAHs analysis

PAHs from water samples were extracted using the liquid–liquid extraction method (Tuncel and Topal, 2011). Water samples, about 100 mL, were mixed with 30 mL dichloromethane (DCM), while sand samples were dried with anhydrous sodium sulphate before being extracted ultrasonically with DCM (Al-Sbani et al., 2016). Next, PAH analysis was carried out using GC-FID (Agilent Technologies, Model 7890A, GC system, U.S.A.) in which the PAHs standard was purchased from Merck (EPA 525 PAH Mix A, Germany). The PAHs in standard solutions comprises 16 compounds which are naphthalene, acenaphthylene, acenaphthene, flourene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene. The removal percentage of PAHs was determined by comparing the PAHs concentration on a given day with the initial PAH concentrations at day 0.

2.6. Statistical data analysis

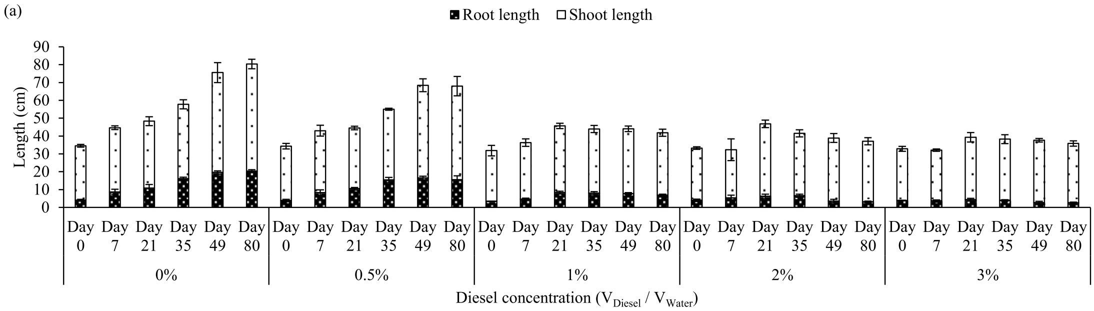
Data was analysed statistically using SPSS version 16 (SPSS, Inc., U.S.A.). Significant differences in the PAHs removal was tested between the system with plants and the system without plants. A significance level of $p<0.05$ was used for the paired-samples t-test. The interactions between factors for PAHs removal from water was evaluated using a two-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Variations of physical parameters

The monitored physical parameters of the wastewater for treatments with and without plants, at the various diesel concentration throughout the course of the experiment, are presented in Table 1. There was no significantly difference for temperature and pH among the treatments with and without plants. The temperatures ranged between 20 and 30 ° C, which is the optimum temperature for the hydrocarbon biodegradation (El-Sheikh et al., 2010). Additionally, the pH did not appear to be affected by the planted aquarium in the diesel-contaminated water treatments with and without plants. The DO ranged between 1.2 and 3, and ORP ranged between (−103) and (−28.3) mV of the wastewater for both treatments, with and without plants, indicating that the treatment environment was initially anoxic and started to change to anaerobic when diesel concentrations increased, which may have assisted the degradation of the hydrocarbons by the rhizobacteria in the root zone (Vymazal, 2010). According to Benget and Retnaningrum (2020) and Abdullah et al. (2020), rhizobacteria have the possibility to live in hydrocarbon contaminated area due to their metabolic aptitudes. They use different hydrocarbon compounds as a source of carbon and energy (Sureka and Vijayakumari, 2014) anaerobically or under anoxic condition (Meckenstock et al., 2016).

The two bottom layers of gravel (small and big size) prevented the sand from leaking to the bottom of the aquarium and were helpful in the TSS removal. The layers were used as a filter for the water from the sand, in addition to being used to create a unique environment that helped the microorganisms attach on the surfaces. The TSS decreased with time at all concentrations in both treatments, which were very similar to each other. The removal ranged between 87%–95% at the end of the period, as shown in Fig. 3. At the 0.5% diesel concentration, the TSS removal was better with plants than without plants, but there was no difference between the two treatments at 1, 2 and 3% diesel. According to Huang et al. (2013), the high viscosity has been a result of the diesel getting fixed in sand pores and adsorbed onto the surface of the sand.



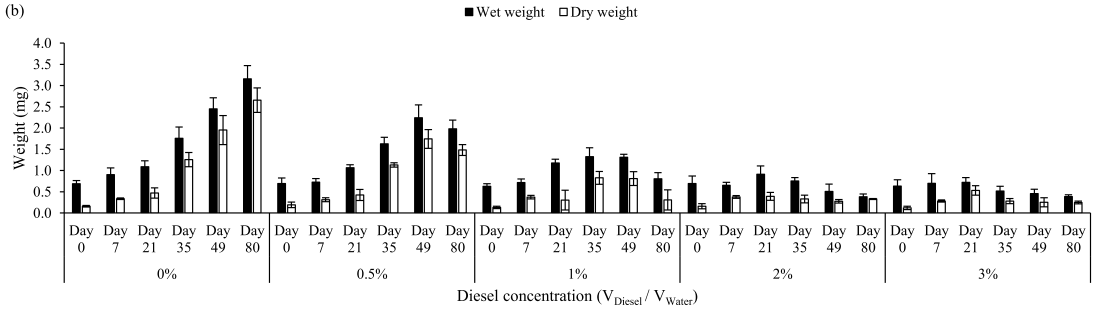


Fig. 4. Growth response parameters in the sub-surface flow system: (a) shoot and root length; (b) wet and dry weight of plants.

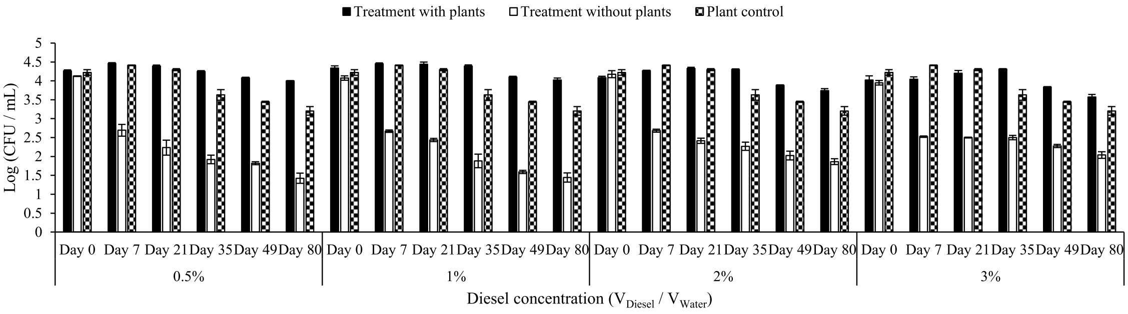


Fig. 5. Population of bacteria during treatments with and without plants in various diesel concentrations and also in plant control.

3.2. Biomass profile

Fig. 4 presents the wet and dry weights of the total content (shoot + root) of the plants grown in the contaminated water at the different diesel concentrations in the batch SSF system. The wet weights during the study period ranged from 0.7 to 3.2, 0.7 to 2.2, 0.6 to 0.8, 0.7 to 0.4 and 0.6 to 0.4 g, for 0, 0.5, 1, 2, and 3%, respectively. The plants continued growing in diesel-free sand, while all the plants died at diesel concentrations 2 and 3% after the third week of exposure. It was in agreement with the results obtained during a preliminary study in which the lethal concentration (LC50) of plant population for 15 days of exposure for *E. ochrostachys* towards diesel mixed water was in the range of 1%–2% (V_{Diesel}/V_{Water}) (Al-Sbani et al., 2014). Prolonged phytotoxicity study had caused all the plants with 1% diesel died after 35 days. In 0.5% diesel, the plants were able to resist the toxicity of the pollutants for more than 49 days. The plant in 0.5% diesel concentration grew, and the weight was 2.2 mg, the length of the root and shoot were 15.5 and 52.5 cm respectively at the end of experiment, while, with 1, 2 and 3%, the length of root was 6.8, 3.1 and 2.3 cm, and shoots were 35, 34 and 33.3 cm only at the end period of exposure, respectively. Therefore, the plant weight and length of plant significantly decreased at the higher concentrations due to diesel toxicity. Gao et al. (2006) indicated in their study that diesel substantially inhibited plant growth in soil, especially at high concentrations. The same trends were observed with the dry weights and with the lengths of the shoots and roots in this study. As shown in Fig. 4, the plant length increased with time in the control plants (0% diesel) until the end of the experiment and with 0.5% diesel until 49 days. At diesel concentrations of 1, 2 and 3%, the dry weights increased until 21 days and subsequently decreased until the end of the experiment. It can be concluded that 0.5% diesel is the minimum inhibitory concentrations for *E. ochrostachys*'s growth in which the interaction of plants with microbes has boosted its growth. According to Chauhan et al. (2015) and

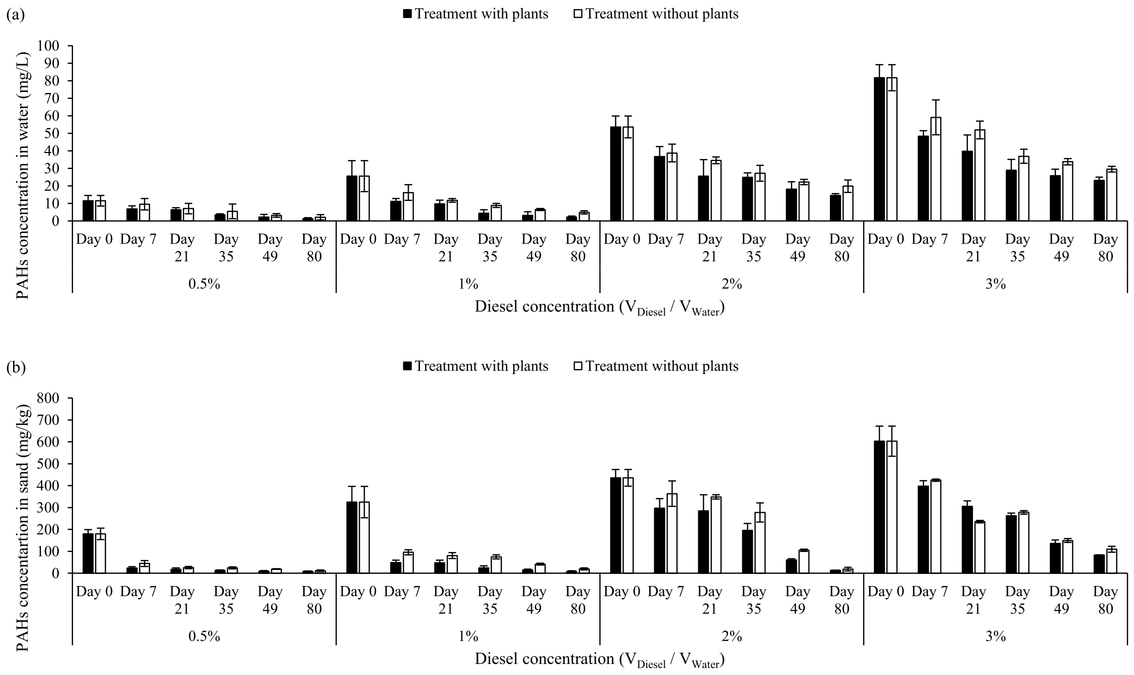


Fig. 6. Decreasing PAHs in (a) wastewater and (b) sand, with and without plants for different diesel concentrations. Bars indicate the standard error of three replicates.

Kamaruzzaman et al. (2020), rhizobacteria, specifically termed as plant growth promoting rhizobacteria (PGPR), have the potential to facilitate plant growth via several mechanisms such as indole acetic acid (IAA), cytokinins and gibberellins, but, the diesel concentration should be kept lower than the minimum inhibitory concentration to avoid toxicity that can cause inhibition on plant growth.

3.3. Bacterial populations throughout the exposure

The removal of hydrocarbons through degradation mostly depends on the capabilities of the microorganisms in the rhizosphere (Liu et al., 2011; Taccari et al., 2012). In this study, the effects of the plants on the rhizobacterial populations (CFUs) for all treatments with and without plants in 0.5, 1, 2 and 3% diesel concentrations and without diesel (plant control) were evaluated by comparing the differences between them. The CFUs of *E. ochrostachys* were evaluated on each sample day (0, 7, 21, 35, 49 and 80), as shown in Fig. 5. The results showed that the concentrations of rhizobacteria population in the treatments with 0.5 and 1% diesel concentrations with the plant increased for 7 days. They were the highest CFU in all concentrations (2.89×10^4 and 2.86×10^4 CFU/mL, respectively) and then began decreasing until the end of exposure period (9.97×10^3 and 1.633×10^4 CFU/mL, respectively). In contrast, with 2%, CFU increased until 21 days (2.78×10^4 CFU/mL), then decreased until the end period (1.06×10^4). For the 3% diesel concentration, CFU increased until day 35 (2.04×10^4 CFU/mL), then decreased until day 80 (6.42×10^3 CFU/mL). Without diesel (control plant), CFU increased on day 7 up to 1.61×10^4 and then decreased to 1.7×10^3 at the end of exposure. The CFUs were higher in the systems containing plants than in the systems without plants. The roots of *E. ochrostachys* might exude nutrients that act as food for bacteria to grow in the rhizosphere as compared to the systems without plants (Abdullah et al., 2020). At day 0, CFUs were 4.13, 4.07, 4.18 and 3.95 CFU/mL, with 0.5, 1, 2, and 3% diesel concentrations without plants. After, CFUs decreased until 1.42, 1.44, 1.86 and 2.04 CFU/mL, respectively, at the end of the experiment. Based on these findings, the importance of using plants for the treatment of pollutants is not only due to their ability to absorb pollutants; the plants also increase the number of microbes, which are an important element in breaking down pollutants. Furthermore, the CFUs were higher in the roots planted with diesel compared to the roots without diesel (control plant) due to certain bacteria that feed exclusively on hydrocarbons. Although all plants in the 1, 2 and 3% diesel concentrations eventually died, the CFUs were nearly identical among the diesel concentrations and were still higher than the CFUs in the aquariums without plants for the same concentrations. According to Stottmeister et al. (2003), the microbial degradation of dead roots causes the formation of new secondary soil pores and later provides the oxygen required by the microbial degradation process. This fact may explain the different CFU results between the systems with and without plants after the plants died in the high diesel concentrations.

Table 2
Results of two-way ANOVA analysis and their interaction of parameters of PAHs in the water and sand.

Source	PAHs in water		PAHs in sand	
	F	Sig.	F	Sig.
Concentration	6,739.844	$p<0.05$	852.732	$p>0.05$
Model	524.478	$p<0.05$	65.016	$p>0.05$
Time	99.173	$p<0.05$	384.738	$p>0.05$
Concentration * Model	186.025	0.531	5.069	$p>0.05$
Concentration * Time	0.739	$p<0.05$	33.946	$p>0.05$
Model * Time	6.198	$p<0.05$	1.891	0.103
Concentration * Model * Time	5.462	0.950	1.185	0.297

3.4. PAH content in wastewater and sand samples

Fig. 6 compares the two treatments (with and without plants) with regard to PAH removal from the wastewater and sand during 80 days of exposure at each diesel concentration. PAHs reduced at the end of the period until 8.38, 9.07, 13.08 and 82.9 mg/kg in the sand in the aquarium containing plants and until 1.25, 2.2, 14.5 and 23.2 mg/L in the water with diesel concentrations 0.5, 1, 2 and 3%, respectively; the PAHs concentrations without plants were 11.5, 19.8, 18.86 and 109.7 mg/kg in the sand and 2.08, 4.86, 20 and 29.5 in the water for the same concentrations, as presented in Fig. 6. At the end of the experiment, the PAH removal percentages with plants were 89.1, 91.3, 73.0 and 71.6% in wastewater and 95.3, 97.2, 97 and 86.25% in sand for 0.5, 1, 2 and 3% (V_{Diesel}/V_{Water}) diesel concentrations, respectively. In contrast, for the same concentrations without plants, the removal percentages were only 81.8, 81.0, 63 and 63.9% in wastewater and 93.6, 93.9, 95.7 and 81.8% in sand, respectively. These results demonstrated the importance of the role of the plants in the elimination of the PAHs.

Additionally, we note that the highest percentage removal was with the concentration of 1% in both water and sand. These results were consistent with previous studies conducted by Fismes et al. (2002), Gao and Zhu (2004) and Oleszczuk and Baran (2005). They concluded that both the uptake and accumulation of contaminants were interrelated with their soil concentrations. The removal of PAHs in 1% diesel was greater than 0.5% diesel concentration from the water and sand. The concentration of PAHs in sand was higher in 1% diesel concentration compared to 0.5% diesel concentration, contributing to more source of carbon and energy for plant and bacteria. In contrary, the plants in 2% and 3% diesel died after two weeks of exposure. Therefore, after two weeks, the degradation of hydrocarbons was solely by the rhizobacteria, therefore decreasing the removal compared to the first two concentrations. The microbial activity in the sand was a critical factor governing the degradation of the organic micro-pollutants (Yang et al., 2011), even after the deaths of the plants, at all diesel concentrations. The complete degradation of organic pollutants is assisted by the microbial activity, in which the microbes use the organic pollutants as a source of carbon and energy (Srivastava et al., 2019).

In addition, the survival of plants and rhizobacteria is also due to the nutrient availability. Nutrient is listed as one of the factor affecting biodegradation of hydrocarbons at which cell growth requires nitrate (N), sulphur (S), magnesium (Mg), potassium (K), calcium (Ca), and iron (Fe) to develop new cells (Imron et al., 2020; Allamin et al., 2020). Referring to the sand analysis done by Titah et al. (2014), sand contained nitrate (N), potassium (K), sulphate (SO_4^{2-}), calcium (Ca), magnesium (Mg), chlorine (Cl^-), iron (Fe), zinc (Zn), and manganese (Mn). The existence of these nutrients and minerals ensure the chemical reactions involved in maintaining the living state of the plant cells and the rhizobacteria occurs normally (Imron et al., 2020). The nutrient availability was similar in all diesel concentrations and no additional nutrients was added throughout the 80 days of exposure. The plant and rhizobacteria continued using PAHs and available nutrients for their growth without any toxic conditions which can be observed by the death of plants.

For the PAH removal from water and sand, two-way ANOVA analysis results for the parameters and their interactions are presented in Table 2. The results indicate that the PAH removal differed significantly ($p<0.05$) with diesel concentration, treatment (with and without plants) and time in water and sand. Additionally, there was a statistically significant interaction between (treatment * time) and (time * concentration), but there was no significant interaction between (concentration * treatment) and (treatment * time * concentration) ($p>0.05$) in the water. In the sand, there was a statistically significant interaction between (time*concentration) and (concentration*treatment) but no significant interaction between (treatment * time) and (treatment * time * concentration).

4. Conclusions

E. ochrostachys was used to treat PAHs-contaminated water and sand with an SSF system at different diesel concentrations over the course of 80 days. The results indicated that the phytoremediation was effective in PAHs remediation from wastewater and sand. *E. ochrostachys* has removed PAHs by as much as 89.1, 91.3, 73.0 and 71.6% in wastewater and 95.3, 97.2, 96.99 and 86.25% in sand for the 0.5, 1, 2 and 3% (V_{Diesel}/V_{Water}) diesel concentrations, respectively. *E. ochrostachys* play a role in phytoremediation of PAHs from water contaminated with diesel with high efficiency in 1% diesel concentration, while with higher diesel concentrations up to 3%, the plants were inhibited and died. Also, the results highlight the importance of the plants to increase rhizobacteria population, which will help in the removal of PAHs. Using two layers of small and big gravel was helpful for reducing the TSS from the water from 87 to 98%, and it provided a space for the interactions between pollutants, roots and microbes.

CRediT authorship contribution statement

Nadya Hussin AL Sbani: Conceptualization, Investigation, Data curation, Methodology, Writing - original draft. **Siti Rozaimah Sheikh Abdullah:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Resources. **Mushrifah Idris:** Supervision. **Hassimi Abu Hasan:** Supervision. **Israa Abdulwahab Al-Baldawi:** Investigation, Data curation. **Omar Hamed Jehawi:** Data curation. **Nur 'Izzati Ismail:** Writing - review & editing, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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