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Response and capability of *Scirpus mucronatus* (L.) in phytotreating petrol-contaminated soil

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HIGHLIGHTS

- *Scirpus mucronatus* (L.) showed capability in tolerating petroleum contamination.
- *Scirpus mucronatus* (L.) removed TPH from contaminated soil.
- High removal up to 82.1% in 10 g/kg concentration of TPH was obtained.
- Concentration of 30 g/kg of TPH negatively affect the structure of plant.

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ABSTRACT

The greenhouse phytotoxicity experiment was conducted to analyse and assess the capability of *Scirpus mucronatus* (L.) in tolerating and removing petrol in contaminated soil. This research was conducted for 72 days by using 5, 10 and 30 g/kg petrol as soil contaminants. Results showed that the system planted with *S. mucronatus* (L.) had high potential to treat the 10 g/kg petrol-contaminated soil and had an average Total Petroleum Hydrocarbon (TPH) removal of 82.1%. At 5 and 30 g/kg petrol, the planted system removed 74.9% and 75.8% TPH, respectively. The petrol (10 g/kg) affected the plant growth positively, which was indicated by the increase in dry and wet weights throughout the research period. The removal of the TPH in the system was performed because of the interaction of plants and rhizobacteria. SEM showed that a high concentration of petrol (30 g/kg) affected the plant tissue negatively, as indicated by the altered structures of the root and stem cells. EDX results also confirmed that petrol was absorbed by the plant, as shown by the increased carbon content in the plant's root and stem after the treatment.

1. Introduction

The petrol is a type of hydrocarbon from crude oil refinery processes that is commonly released into the environment as a contaminant (Sunar et al., 2013). Hydrocarbons possess several toxic effects to living organisms, including aquatic, soil biota and even humans (Imron et al., 2019b; Qurratu et al., 2018). The soil, water and air pollution caused by hydrocarbons may result in toxic and acute effects to the living biota (Morales Terrés et al., 2010; Titah et al., 2018). The toxic effects of the petrol contamination to the human health includes skin irritation, throat disease, bronchitis and lung cancer (Imron et al., 2020).

The phytoremediation is an aesthetically pleasant technological approach to treat petrol-contaminated soil (Imron et al., 2019a; Yahya et al., 2020). Phytoremediation processes include phytoextraction, phytodegradation, phytovolatilisation, phytostabilisation and rhizodegradation (Tangahu et al., 2011; Zhang et al., 2010) to remove contaminants from the environment. Plants play an important role during the treatment (Abdullah et al., 2020; Al-

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Ajalin et al., 2020a). Native plants are proven to have remarkable capability in treating contaminants from local sources (Said et al., 2020; Sharuddin et al., 2018). Several tropical plant species, including *Scirpus grossus* (Purwanti et al., 2019b; Tangahu et al., 2019), *Scirpus mucronatus* (Purwanti et al., 2015), *Typha angustifolia* (Purwanti et al., 2019b; Wulandari et al., 2019), *Lepironia articulata* (Ismail et al., 2015; Sharuddin et al., 2018) and *Phragmites australis* (Abed et al., 2019), have shown great capability in removing hydrocarbons from the contaminated soil and water media.

Before performing the phytoremediation, the initial analysis of the plant's capability to treat contaminants must be carried out. The phytotoxicity test is an initial test that can be conducted before performing the phytoremediation (Chandanshive et al., 2017; Kabra et al., 2012; Titah et al., 2014). Many phytotoxicity test results have already been demonstrated by researchers to assess the capability of plants in tolerating hydrocarbon contaminants (Al-Mansoory et al., 2017; Chandanshive et al., 2017; Kabra et al., 2012; Tangahu et al., 2013; Titah et al., 2014). However, the current number of studies on the phytotoxicity of the petrol-contaminated soil to Malaysian native plant species of *S. mucronatus* (L.) that have already been conducted is limited. This research is conducted to initially analyse the tolerance limit of *S. mucronatus* (L.) to the petrol-contaminated soil and assess its potential for remediation. The presented results may highlight the potential of *S. mucronatus* (L.) for use as a phytoremediator of hydrocarbon-contaminated soil especially petrol-contaminated ones.

2. Materials and methods

2.1. Plant propagation and experimental layout for plant toxicity

The experiment was conducted to evaluate the plant response and the capability of *S. mucronatus* (L.) towards petrol in soils in a greenhouse located in Universiti Kebangsaan Malaysia. Thirteen glass reactors (60 cm × 30 cm × 30 cm) were used in this experiment. Each reactor was filled with 3 kg soil mixture (75% garden soil + 25% sand). Garden soil and sand were previously screened through a 4.75 mm mesh before use (Titah et al., 2014). A spiked method with 75%:25% ratio of petrol obtained from a local petrol station in Bangi, Selangor, Malaysia and acetone (R&M Chemicals, UK) was used to contaminate each kg of soil with 5, 10 and 30 g petrol. All reactors were operated under the batch subsurface flow system.

The *S. mucronatus* (L.) plants used in this study were obtained from a greenhouse culture in the Universiti Kebangsaan Malaysia. Twenty plants of *S. mucronatus* (L.) were planted in each pot reactor. All plants (age = 2 months old) had almost similar heights. Plants were watered with previously measured volume of deionised water to reach the saturated volume of the soil bulk density (25 mL/100 g soil mixture).

The planted and the control (unplanted) contaminant systems were triplicated, and another reactor represented the control plant in normal soil and sand without petrol (Fig. 1). Each reactor underwent 72 days of exposure, and soil sampling was carried out on days 0, 7, 14, 28, 42 and 72. Three reactors were labelled as R1, R2 and R3, and an unplanted control reactor was labelled as CC. One reactor without petrol contaminant was used as control plant and labelled as PC.

2.2. Analysis of physical and chemical parameters

The physical and the chemical analyses were conducted on the sample obtained on days 0, 7, 14, 28, 42 and 72. The parameter measurement included the dissolved oxygen (DO, mg/L) by using the DO meter (GLI International Model 63, USA), temperature (°C) and pH by using a multi sample sensor IQ 150 (IQ Scientific Instruments, UK). The oxidation reduction potential (ORP, mV) was measured using the ORP metre (Cyberscan pH-300, Singapore), and the chemical oxygen demand (COD) was analysed using a UV–VIS spectrophotometer (HACH DR 6000, USA). All laboratory analyses were performed by following the standard methods (APHA, 2005).

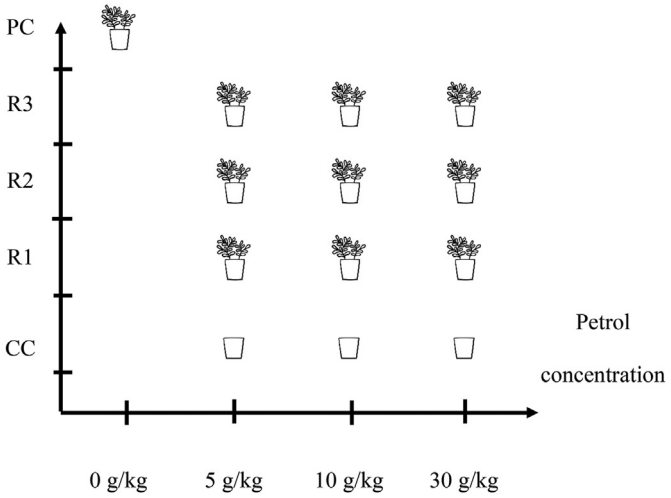

Fig. 1. Experimental layout for the plant toxicity test of *S. mucronatus* (CC = control contaminant, PC = pcontrol plant).

2.3. Observation of the plant growth

The growth response of *S. mucronatus* (L.) was observed at varying concentrations of petrol (0, 5, 10 and 30 g/kg) for 72 days. One plant was collected from each reactor on each sampling day. Plants were rinsed with tap water, and their height was determined by measuring from the stem (buried in soil) to the longest rootlet (the lower root layer) (Tangahu, 2016). All stems (upper layer) were weighed gravimetrically to obtain the wet and the dry weights for biomass quantification (Al-Baldawi et al., 2018; Imron et al., 2019a; Ogbo et al., 2010). The obtained plants were dried in an oven maintained at 70 °C (Memmert, Germany) for 72 h until a constant weight was reached. Scanning electron microscopy (SEM) with EDX spectra Model Supra 55VP (Zeiss, Germany) was also performed to analyse the effect of petrol to the plant's tissue (Ismail et al., 2020). The carbon content index was calculated on the basis of the percentage of the carbon weight from the EDX analysis.

2.4. Analysis of the rhizobacterial population

The rhizobacterial population was calculated using the sequence dilution method. Initially, 10 g rhizosphere soil was collected and mixed with 100 mL sterilised distilled water for the 10^{-1} dilution (Ismail et al., 2020; Purwanti et al., 2020). The mixture was then shaken at 150 rpm for 1 h to transfer the bacteria from the soil into the water. Subsequently, the sample (1 mL) was transferred into 9 mL physiological solution (8.5% NaCl) until the desired dilution was reached (Kurniawan et al., 2018). A total of 100 μL from the last three dilutions was transferred into plates that contained sterile nutrient agar (Oxoid, USA). The plates were then incubated in an oven maintained 37 °C for 24 h. The good counting was stated as the number of bacteria between the range of 30–300 cells (Purwanti et al., 2018). The number of counted colonies was

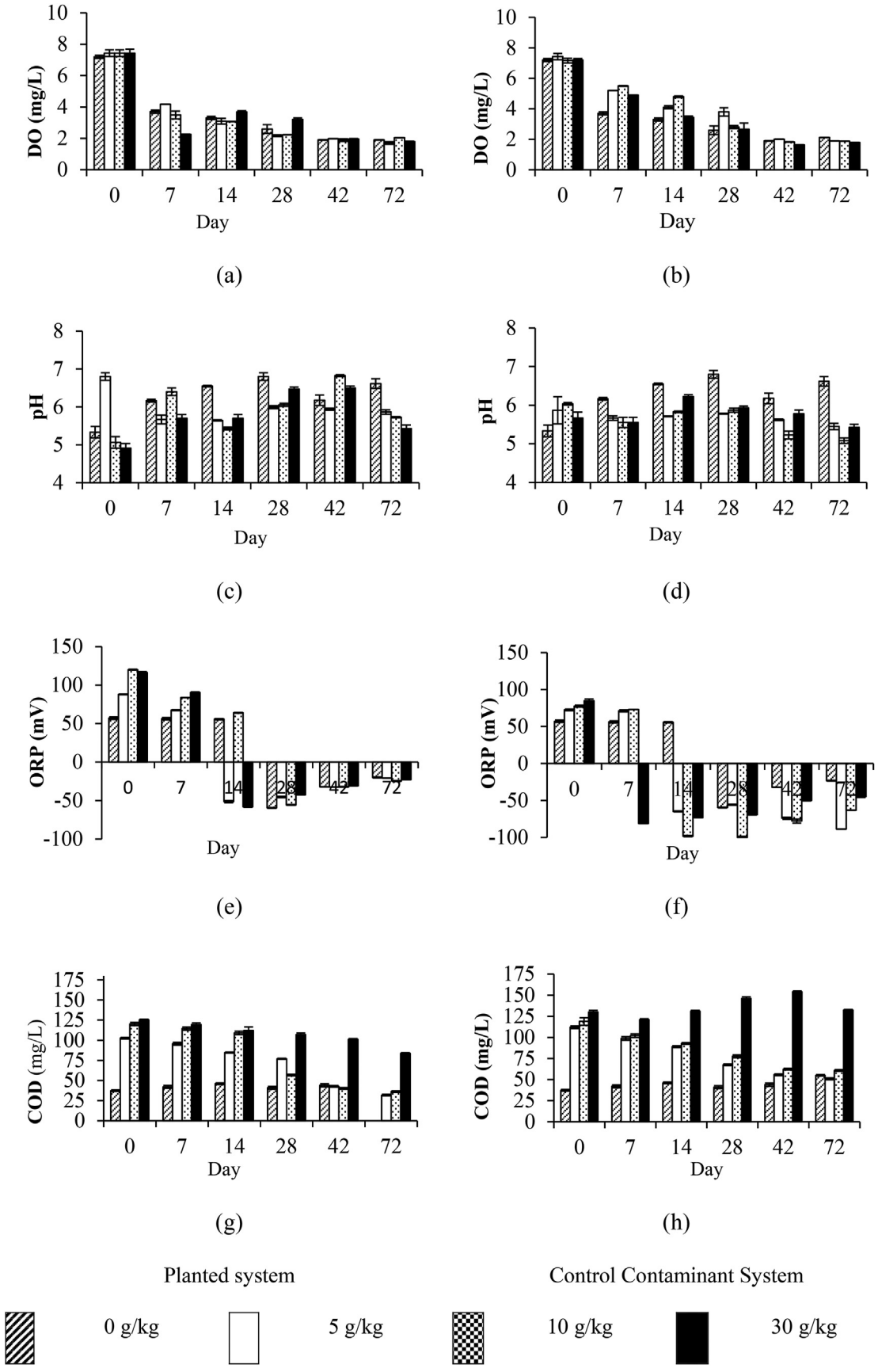

Fig. 2. Physical and chemical parameter analyses on the planted system for (a) DO, (c) pH, (e) ORP, (g) COD and control contaminant for (b) DO, (d) pH, (f) ORP, (h) COD.

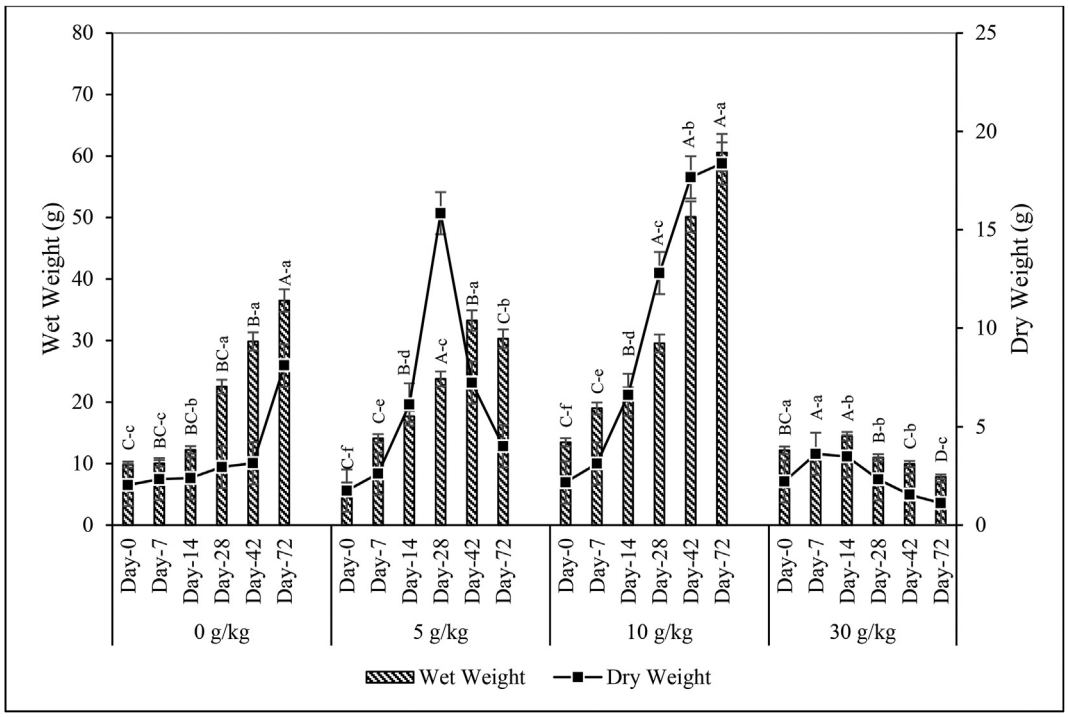

Fig. 3. Wet and dry weights of *S. mucronatus* in different petrol concentrations. Values are presented as mean ± SD ($n = 3$). Different letters (a–e) indicate the significant differences between the measured weight in the same petrol exposure.

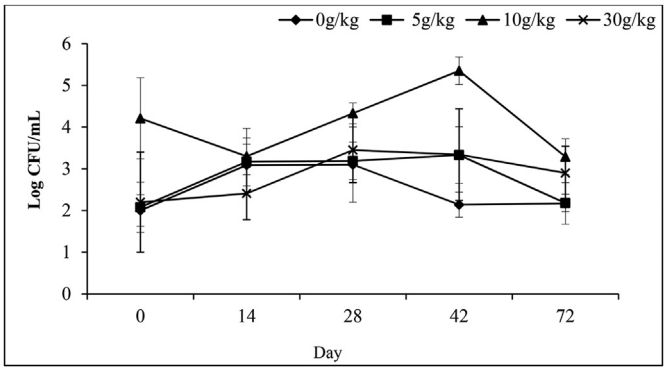

Fig. 4. Rhizobacterial population on each treatment reactor. Values are presented as mean ± SD ($n = 3$).

multiplied with the corresponding dilution and expressed as log CFU/mL (Moreira et al., 2011; Titah et al., 2019).

2.5. TPH extraction from soil samples

All samples were collected in Schott bottles and stored at below 4 °C until analysis. For extraction, 10 g soil mixture was placed into 100 mL Schott bottle, dried by mixing with sodium sulphate, added with 50 mL dichloromethane (R&M Chemicals, UK) (Al-Mansoory et al., 2017; Imron and Titah, 2018) at a temperature of 50 °C and placed in an ultrasonic cleaner (Thermo-10D, USA) for 30 min. The obtained supernatant was then filtered with glass wool. Extracts were condensed to allow the solvent to fully evaporate and left for 3–4 days in the fume hood. The final extracted sample was stored in vials for gas chromatography (Tang and Ngu, 2011), as explained in the following section.

2.6. Determination of the TPH content in soil samples

The extracted sample was analysed using the HP-5 5% phenyl methyl siloxane column (30 m × 0.32 mm i.d × 0.25 μm) with helium as the carrier gas by using a gas chromatography–flame ionisation detector (Agilent Technologies Model 7890A, UK). The temperature of the column was configured to remain for 1 min at 50 °C and then ramped for 10 min at 15 °C/min to reach 320 °C. On each sampling day, the percentage of the TPH degradation was determined using the difference of the TPH reading on sampling day 0 (initial TPH), and the TPH was measured on each sampling day compared with the initial TPH measurement.

2.7. Statistical analysis

The SPSS Statistics 17.0 (IBM, U.S.A.) was used to determine the significance of the results. The TPH degradation in each sampling period was analysed using one-way ANOVA with confidence interval of 95% (Purwanti et al., 2019a). The $p \leq 0.05$ indicates the significant difference of the results.

3. Results and discussion

3.1. Physicochemical parameter analyses

The physicochemical parameters for planted and control systems at petrol concentrations of 5, 10 and 30 g/kg are depicted in Fig. 2. Generally, the average DO and pH over the study period changed gradually (Fig. 2[a]–[d]). The observed pH was between 4 and 7.6 for the planted system and between 3.6 and 6.5 for the control system. The addition of petrol had created an acidic pH at the beginning of the exposure and then gradually increased throughout the 72-day exposure. The gradual increase in pH was due to the plant's exudates and microbial activity in the rhizosphere (Li et al., 2020).

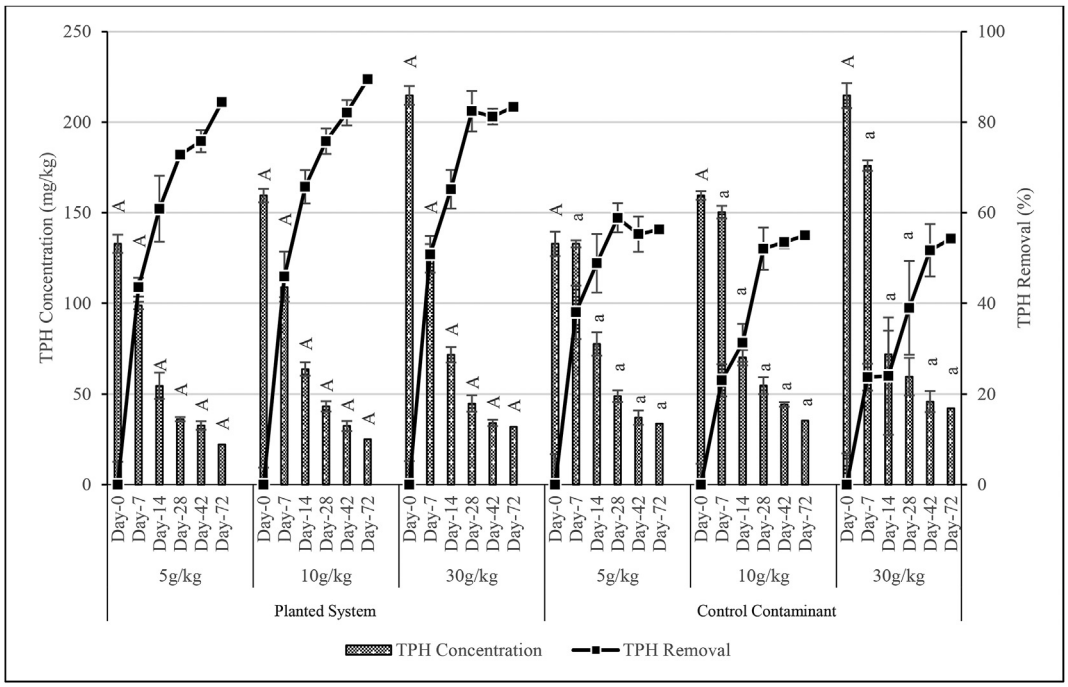

Fig. 5. TPH removal percentage for each petrol concentration. Values are presented as mean ± SD ($n = 3$). Different letters (A-a) indicate significant differences in TPH concentration and TPH removal between the planted and the control systems.

The observed DO decreased, ranging from 1.7 mg/L to 7.4 mg/L, throughout the research period because no aeration was applied into the system (Fig. 2[c] and 2[d]), indicating that the interphase transfer of oxygen in the medium surface was not enough to fulfil the requirement for plants and bacteria in the rhizosphere (Al-Ajalin et al., 2020a, 2020b; Jehawi et al., 2020). This result was confirmed by the ORP analysis that showed the anoxic to the anaerobic ranges of oxidation state, which varied between 57 and 116.3 mV on day 72 (Fig. 2[e] and 2[f]). The results of the ORP proved that the soil was became anoxic and anaerobic with high amount of petrol, thereby increasing the redox potential as the petrol concentration decreased (Almaamary et al., 2019). Al-Baldawi et al. (2013) also demonstrate that the decrease in ORP shows that the condition is likely anaerobic due to the high concentration of hydrocarbon. The amount of COD decreased significantly during the 72 days of exposure in the planted and the control reactors (Fig. 2[g] and 2[h]). These results indicated that the degradation of petrol occurred inside the reactor because of the plants and the rhizobacteria in the planted system and the bacteria in the control reactor. Petrol is a type of hydrocarbon that contributes to the COD value and can be removed biologically by using plants or microorganisms (Sunar et al., 2013). For COD in leachates, the planted system with 10 g/kg petrol-contaminated soil showed the highest removal value.

3.2. Plant growth analysis

The plant survival over the 72 days of petrol exposure represented as wet and dry weights is shown in Fig. 3. In general, after seven days of treatment at all concentrations, the plant biomass showed a slight reduction in dry weight, but its physical appearance was still relatively the same with that of the control plant. This decrease was due to the plant's adaptation to the petrol-contaminated soil (Ali and Ajbar, 2013).

The plant grew normally on the control plant reactor, whereas 30 g/kg petrol negatively affected the plant's survival, as demonstrated by the decrease in the dry and the wet weights over the 72 days of research period (Fig. 3). The 10 g/kg petrol positively affected the plants, as indicated by the increasing dry and wet weights of plants throughout the 72 days of exposure. The plants exposed to 5 g/kg petrol grew normally until day 28 but then significantly decreased until the end of the research period. This result implied that the addition of 5 g/kg petrol as main carbon source did not support plant growth enough for 72 days (Nottingham et al., 2018). This result suggested that the carbon source in the 5 g/kg reactor was only enough until day 28. The addition of petrol up to 10 g/kg was also proven to increase the wet and the dry weights of the plant, indicating that the petrol provided additional nutrient that can boost the plant growth up to a certain level.

3.3. Rhizobacterial analysis

The rhizobacterial population in the *S. mucronatus* (L.) rhizosphere area was analysed at different concentrations of petrol and depicted in Fig. 4. The results in the control plant showed that the rhizobacterial population was lower than those in the presence of petrol in soil. The rhizobacterial population in the control plant was 2.14 log CFU/mL at day 42, which was lower compared with the 2.3 and 2.2 log CFU/mL observed in plants grown in soil contaminated with 5 and 30 g/kg petrol, respectively. A total of 2.4 log CFU/mL, which was higher than any other treatment system, was observed in 10 g/kg petrol-contaminated soil. This result was in accordance with the plant growth measurement, in which the reactor with 10 g/kg petrol exhibited better result compared with any other reactor. The good growth of plants may support the rhizobacterial population (Abdullah et al., 2020; Asif et al., 2019; Ismail et al., 2020; Mohan and Tippa, 2019). A slight decline in the rhizobacterial number was observed on day 72, suggesting that the given carbon source was limited up to day 42 for bacteria to continuously grow. The resistance of soil microorganisms to petroleum hydrocarbons could be supported by plant roots, and several compounds

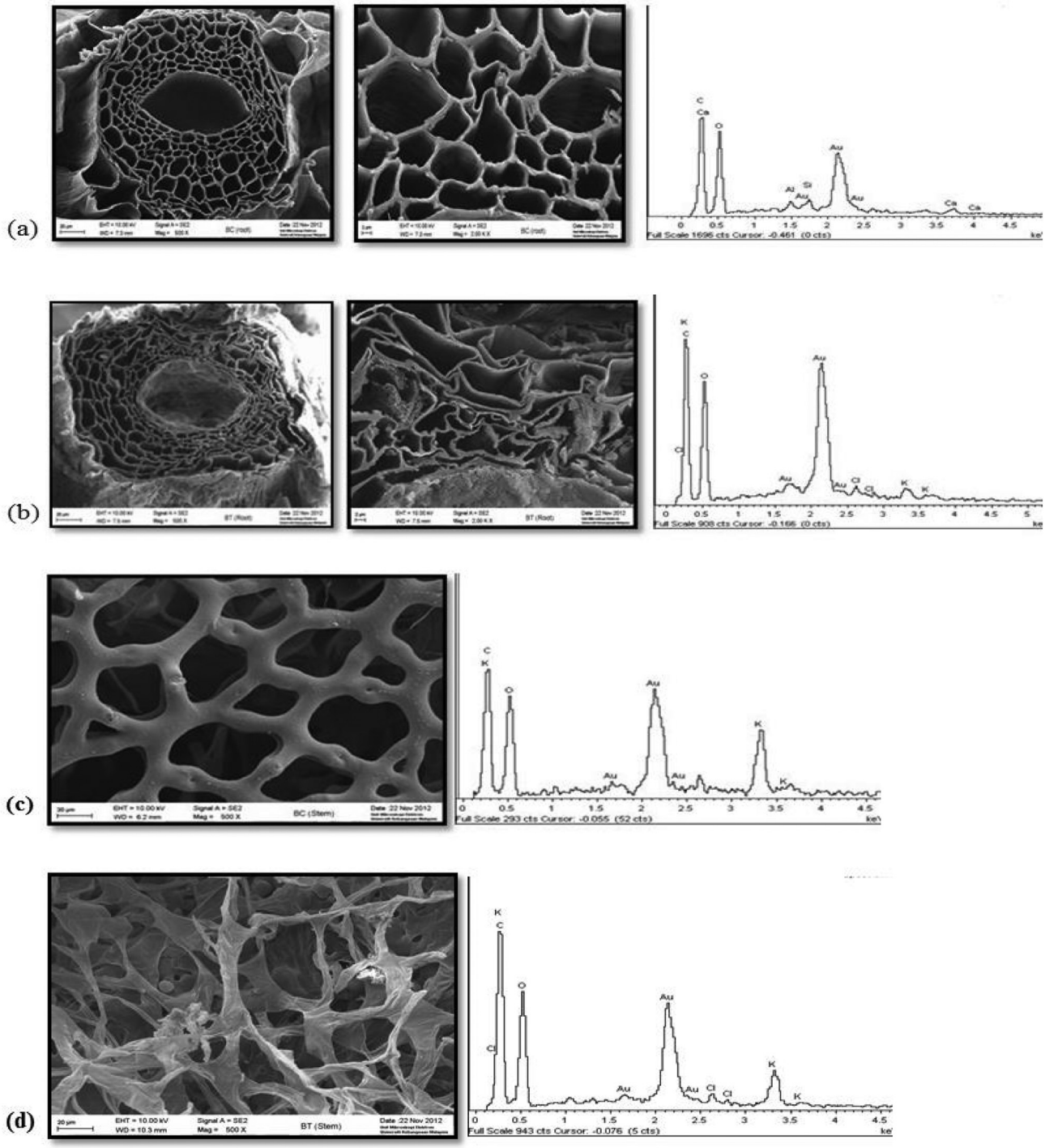

Fig. 6. SEM–EDX analysis of (a) root (control), (b) root (exposed), (c) stem (control) and (d) stem (exposed) of *S. mucronatus* after 72 days of exposure to 30 g/kg petrol.

Table 1
Carbon content in *Scirpus mucronatus* part.

Part of plants	Carbon content (% weight)	
	Control	Exposed to petrol
Root	49.3	52.6
Stem	44.8	53.2

are released from roots to promote the hydrocarbon degradation by microbes (Cao et al., 2012; Ismail et al., 2020; Jehawi et al., 2020). In addition, the ability of rhizobacteria to degrade pollutants, their survival and their metabolic activity are influenced by the composition of plant root exudates (Khan et al., 2013). This mechanism is called phytostimulation, in which plants support the growth of rhizobacteria to perform the rhizodegradation (Hawrot-Paw et al., 2019).

3.4. TPH analysis

TPHs are common groups of persistent organic pollutants in the environment and known to be toxic to many organisms. The TPH concentration in each contaminated soil reactor is depicted in Fig. 5. During this study, the initial TPH concentrations declined significantly. After 72 days of exposure, 82.1% of TPH was removed from the reactor with 10 g/kg petrol, whereas only 53.9% of TPH was removed from the control reactor. The total degradation of TPH was 74.9% and 75.8% in the planted systems with 5 and 30 g/kg petrol, respectively. Only 54.4% and 52.7% of TPH were degraded in the control reactors (unplanted system) with 5 and 30 g/kg petrol. The TPH removal on the unplanted system was obtained from the microbial activity in the soil (Zhang et al., 2010). The capability of plants to degrade TPH might be subtracted from the plants with the unplanted system removal percentage. The plant's roots are very effective in stimulating the rhizobacterial activity by realising exudates through the root tip and absorbing petroleum as carbon source that promote the removal of TPH in the soil (Al-Baldawi et al., 2017; Cai et al., 2010).

The statistical analysis showed that all remaining concentrations and TPH removal in the planted system were significantly higher than those of the unplanted system. The planted system showed higher removal increment starting from day 14 and continued to increase until the end of the research period. In accordance with the rhizobacterial population and the plant growth, the TPH removal was increasing throughout the research period. First, the significant removal of TPH was obtained on day 14, in which the plant growth and the rhizobacterial population also showed significant increment. The rhizobacterial population decreased, and the plant growth and the TPH removal were still observed after day 42. This phenomenon suggested that the rhizobacteria performed important mechanisms of the TPH breakdown at the initial stage of treatment (assisted by root exudates), whereas plants contributed to the TPH removal by adsorbing the transformed compound of petrol at the final stage of treatment (Alagić et al., 2015). This research finding indicated the ability of *S. mucronatus* (L.) to remove the TPH from the petrol-contaminated soil within the first 14 days of exposure.

3.5. SEM–EDX analysis

The presence of petrol as soil contaminant may inhibit plant growth (Morales Terrés et al., 2010). SEM–EDX revealed the effect of 30 g/kg petrol on the roots and stems of *S. mucronatus* (L.) after 72 days of exposure (Fig. 6). Compared with those from the control plant, two sections of plant root and stem in the contaminated soil were completely altered. Petrol damaged the root structure severely. The altered structure of the root and the stem of *S. mucronatus* (L.) exposed to petrol was due to the toxicity of the given concentration (Al-Mansoory et al., 2017; Titah et al., 2014). The high concentration of hydrocarbon may cause a significantly negative effect on the plant tissues in the root or stem (Al-Baldawi et al., 2015).

The carbon content index obtained from the EDX analysis (Table 1) showed that plants absorbed increased carbon in the contaminated soil, thereby confirming the uptake of TPH. The EDX spectra and the microscopic measurements showed that the percentage of carbon by weight in stem and root were 52.6% and 53.3%, respectively. For the control plant, the carbon values were 49.3% in stem and 44.8% in root. This result confirmed that *S. mucronatus* (L.) performed the uptake of transformed hydrocarbon compounds (after degradation by rhizobacteria) into their root and stem. Remarkable differences in the carbon content index on root were observed, suggesting that the adsorbed hydrocarbon compounds were likely to be accumulated on the roots of plants.

4. Conclusions

The phytotoxicity test of petrol-contaminated soil to *Scirpus mucronatus* (L.) showed that the 30 g/kg petrol negatively affected plants, as indicated by the lowest measured weights and removal on the COD parameter. SEM–EDX analyses confirmed that the exposure of soil to 30 g/kg petrol altered the root and the stem cell structures of plants significantly. The highest TPH removal (82.1%) was obtained on the planted system exposed to 10 g/kg petrol, whereas the control only removed 53.9% of TPH. The removal of petrol inside the system occurred due to the interaction of rhizobacteria and plants as the reactor with 10 g/kg petrol showed the highest number of rhizobacterial population amongst all reactors.

Credit author statement

Asia Fadhile Almansoory: Formal analysis, Data curation, Visualization, Investigation, Writing - original draft. Mushrifah Idris: Conceptualization, Validation, Supervision. Siti Rozaimah Sheikh Abdullah: Conceptualization, Validation, Writing - original draft, Supervision, Funding acquisition. Nurina Anuar: Conceptualization, Validation, Supervision. Setyo Budi Kurniawan: Validation, Visualization, Writing - original draft, Writing - Review & Editing.

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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