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Degradation of Total Petroleum Hydrocarbon in Phytoremediation Using Terrestrial Plants

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Abstract

This study focused on the total petroleum hydrocarbon (TPH) degradation in phytoremediation of spiked diesel in sand. The diesel was added to the sand that was planted with terrestrial plants. Four selected terrestrial plants used were *Paspalum vaginatum* Sw, *Paspalums crobiculatum* L. varbispicatum Hack, *Eragrotis atrovirens* (Desf.) Trin. ex Steud and *Cayratia trifolia* (L.) Domin since all the plants could survive at a hydrocarbon petroleum contaminated site in Malaysia. The samplings were carried out on Day 0, 7, 14, 28, 42 and 72. The analysis of the TPH was conducted by extracting the spiked sand using ultrasonic extraction. The determination of the TPH concentration in the sand was performed using GC-FID. The degradation of TPH depends on the plant species and time of exposure. The highest percentage degradation by *P. vaginatum, P. scrobiculatum, E. atrovirens* and *C. trifolia* were 91.9, 74.0, 68.9 and 62.9%, respectively. In conclusion, the ability to degrade TPH by plants were *P. vaginatum>P. scrobiculatum>E. atrovirens>C. trifolia*.

Keywords: phytoremediation; total petroleum hydrocarbon; degradation; terrestrial plant

1. Introduction

Hydrocarbons such as diesel fuel, crude oil and petroleum distillates are some of the world's most widely used primary energy and fuel resources, due to the energy they produce when combusted (Watanabe, 2001). Huge quantities of fuel are required to power industry, automobiles and heat homes, and with the number of times each gallon of petroleum is stored, transported, or transferred, accidents and leakages are inevitable (Surridge, 2007), making these hydrocarbons the most common global environmental contaminants. This contamination are hazardous to the health of plants and are also carcinogenic, mutagenic and potent immuno-toxicants, posing a serious threat to human and animal health (Vasudevan and Rajaram, 2001).

Aromatic hydrocarbon, asphaltene and polar fraction are more resistant to degradation. This is due to its lighter saturated hydrocarbons and is less toxic to microbes found in the rhizosphere. Rhizospheres can serve as a carbon source for microbial metabolisms. Thus, they are more likely to decompose by rhizosphere microbes and absorbed by plants roots. Schaefer and Juliane (2007) reported that the biodegradability of hydrocarbons is related to their molecular sizes. The n-alkanes of the aliphatic fraction (C10-C44) were degraded first. Heavier molecular weight compounds are more resistant to biodegradation as they are hydrophobic, with poor water solubility and bioavailability.

Biodegradation of hydrocarbons by natural populations of microorganisms allows for the conversion of hazardous substances into forms that are less or non-toxic and represents one of the primary mechanisms by which petroleum and diesel products are removed from the environment inexpensively (Lidderdale, 1993). Phytoremediation is an emerging green technology that uses plant to clean up by establishment of vegetation in soils contaminated with hazardous organic such as hydrocarbon, pesticide, and inorganic compounds such as heavy metals. In phytoremediation, grasses are the most common plants evaluated. Kuiper *et al*. (2001) suggested that contaminant-resistant grasses with highly branched deep roots systems could be used to harbour polycyclic aromatic hydrocarbons (PAH)-degrading bacteria from contaminated soils. The large surface area of their fibrous roots and their intensive penetration of soil

offer advantages for the phytoremediation of organic compound (Corgie *et al*., 2003). However, fine plant roots may be able to penetrate some of these pores, thereby increasing the contaminants available for degradation (Parrish *et al*., 2004). Some of the most complex chemical, physical, and biological interactions experienced by terrestrial plants are those that occur between roots and the surrounding soil environment (i.e., the rhizosphere). Interactions involving plants roots in the rhizosphere include root-root, root-insect, and root-microbe interactions.

The main objective of the study is to determine the degradation percentages of TPH in diesel by several local terrestrial plants species through phytoremediation. The results will be used for large scale application using phytoremediation as a green technology. To date, not much research has been done to identify potential tropical plants for phytoremediation. Thus, this aims to dig out some local terrestrial plants in Malaysia such as *E. atrovirens, P. scrobiculatum, P. vaginatum* and *C. trifolia* as potential phytoremediator plants in TPH degradation.

2. Materials and Methods

2.1. Plants preparation

Based on preliminary screening for terrestrial plants growing at a petroleum hydrocarbon contamination site in Malacca, Malaysia (data not published), four terrestrial plants were selected (*E. atrovirens, P. scrobiculatum, P.vaginatum* and *C. trifolia*) to be used in this study were. The plants were taken back and immediately planted in the greenhouse using garden soil for propagation. This is to ensure the plants grow continuously with sufficient biomass to be used in phytoremediation studies.

2.2. Experimental set-up

Diesel fuel obtained from a local gas station was dissolved in OPTIMA-grade acetone. The mixed diesel enriched sand was placed in glass tank with size of (1' x 1' x 1') filled with 10 kg sand. The spiked media were left for about two weeks prior to planting to ensure the diesel was homogenized and all acetone were removed prior to its use for planting (Banks *et al*., 2000). The spiked sand was stirred several times a day. There were two treatments, namely diesel at different concentrations (10 until 30 kg/kg) without plants or non-vegetated treatment (as control contaminant-cc) and with plants (vegetated treatment). The range of diesel concentration was based on previous study by Sanusi *et al*. (2012). Each of concentration had three replicates. Each of glass tanks was planted with 6 plants and watered with 4.6 L deionized water. The amount of water needed to water the plant is based on bulk density calculation. The moisture sand was monitored daily to ensure its consistence throughout the experiments. Harvesting was conducted on day 7, 14, 28, 42 and 72.

The physical properties of sand such as pH and temperature were carried out prior to the experiments. It indicated that the pH was in normal range (pH 6-8) at normal temperature for tropical climate (26-38^{o}C). The macronutrients (N, P, K, S, Mg, Ca, K) and a micronutrient (Cl) in sand used for grasses plantation were analyzed by a Compact IC plus 882 ion chromatography (IC) (Metrohm, Switzerland), while other micronutrients (Fe, Zn, Mn) and trace elements (Pb, As) were determined using an Optima 7300DV inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin Elmer, USA). Based on these analysis, the sand contents of the macronutrients were 29.2 mg/kg N (nitrate), 1.2 mg/kg K, 13.0 mg/kg SO_4^{2-}, 86.5 mg/kg Ca, 7.4 mg/kg Mg whereas the micronutrient contents were 6.4 mg/kg Cl^-, 5.5 mg/kg Fe, 0.04 mg/kg Zn and 1.62 mg/kg Mn. The trace elements were not detected. Based on these results, sand has been shown to contain low levels of the micro and micronutrients in comparison to normal soil (Titah, 2013). Therefore sand has been opted in the experiment.

2.3. Samples collection

One plant in each of replicates was taken out slowly from the sand. Approximately 100 g of sand were taken out using a grab method where the sample taken from one specific location, at one time. The sample was then kept in the fridge with temperature below 4^{o}C prior to analysis.

2.4. Laboratory analysis

TPH analysis was conducted with soil/sediment extraction method using ultrasonic extraction (USEPA 1996). The following steps should be performed rapidly to minimize the loss of the more volatile fractions. A 10 g sieved sludge sample was poured into a 120-mL Scotch bottle using a spatula. Immediately, approximately 100 mL methylene chloride was added to the 100 mL Scotch bottle, which was then placed at the bottom surface of the tip of the ultrasonic horn. The sample was sonicated for 30 min at a water temperature of 55–60^{o}C. The extract was filtered through 0.45-µm Whatman No. 41 filter paper and samples were then concentrated until the final volume was 2 mL. An analytical determination of the TPH concentration in the sand was performed by gas chromatography with flame

ionization (GC-FID) detection using capillary column gas chromatography (Agilent Technologies, model 7890A, GC System, UK). The percentages of TPH degradation is calculated as the following equation:

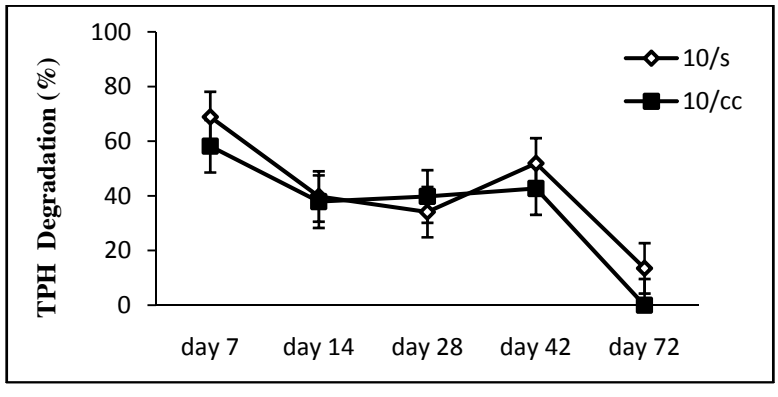
$$TPH\ Degradation\ (\%) = \frac{CTPH_{t0} - CTPH_{tn}}{CTPH_{t0}} \quad (1)$$

with, $CTPH_{t0}$ = TPH concentration at start of degradation process (Day 0)

$CTPH_{tn}$ = TPH concentration at specified time (Day n)

2.5. Statistical analysis

All statistical tests were performed using SPSS Version 17.0 (IBM, USA). Two-way analysis of variance (ANOVA) at the 95% confidence level ($p<0.05$) was used to evaluate significant changes



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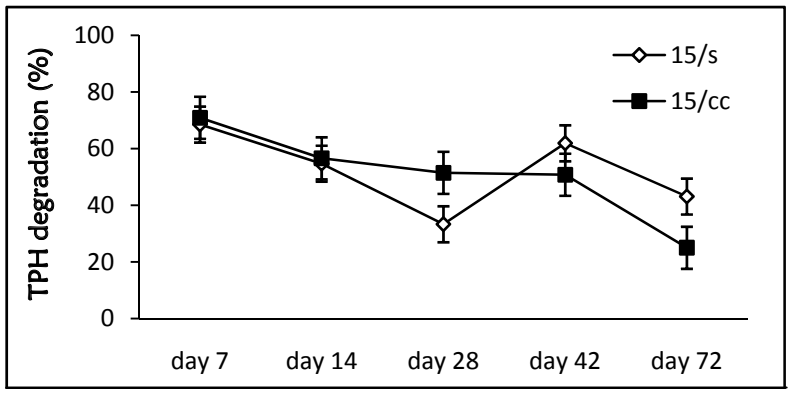

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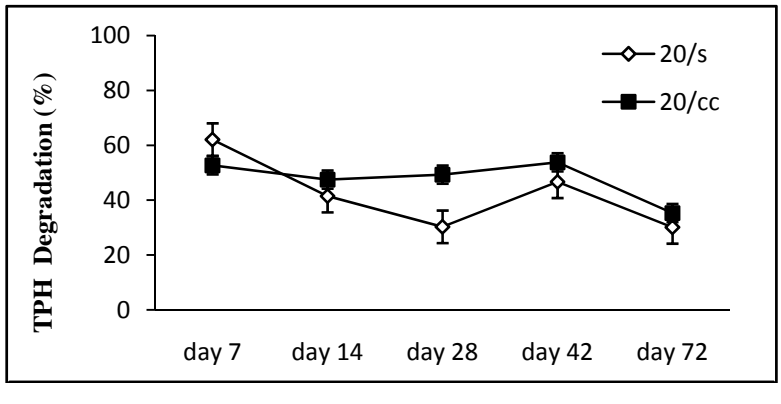


(c)

Figure 1. TPH degradation by *E. atrovirens* for sample and control starting Day 7 until Day 72 for concentration (a) 10 g/kg, (b) 15 g/kg and (c) 20 g/kg. Legend, vegetated (concentration/s) and non-vegetated/control contaminant (concentration/cc).

in TPH degradation between several diesel concentrations.

3. Results and Discussion

Fig. 1 shows the percentage degradation of TPH was high in day 7 for both vegetated (*E. atrovirens*) and non-vegetated treatment (control contaminant). The percentages degradation of TPH on day 7 were 68.9, 68.5 and 62.1% in 10, 15 and 20 g/kg of diesel respectively. The percentage degradation of vegetated treatment indicated higher percentage degradation compared to non vegetated treatment (control contaminant) especially for lowest concentration contaminants (10 g/kg) in Fig. 1(a). However, it showed reverse condition in highest concentration (20 g/kg) (Fig. 1(c)).

Phytoremediation processes are most effective where contaminants are present at low to medium levels, as high contaminant levels can inhibit plant and microbial growth and activity (USEPA 2000). However, based on ANOVA analysis, the degradation of TPH showed no significant different ($p>0.05$) in treatment with *E. atrovirens* at diesel concentration of 10, 15 and 20 g/kg. The presence of vegetation can have various effects on contaminants in soil or water. USEPA (2001) reported some studies indicate that vegetated soils are capable of more effective degradation, removal and mineralization of TPH than nonvegetated soils. Certain plant roots are capable of metabolizing or accumulating organic and nutrient contaminants.

Based on the physical observation of the plants, starting on day 28 onwards, the colour of the plants exposed to the contaminants changed from green to pale yellow. The appearance of the control plants, did not show any changes until the last sampling day. These observation indicated that the plants changing in color due to the contaminants and not due to lack of nutrients. However, there was a changing in the plants physical properties, but still new shoots appeared from the old roots. The new shoots grow rapidly and and it continuously grew until the end of sampling day.

After one month period of the contaminants exposure, some of the plant stems degraded and left to decay in the tank for natural decomposition. The old plant roots also started to decompose underneath the sand surfaces. This seems to be another way that plants can potentially influence contaminated biodegradation, which is by the introduction of decomposing root material as concluded by Miya and Firestone (2001). Decaying root debris contributes significantly to increased soil carbon availability in rhizosphere soil. Increased microbial biomass and activity are characteristic of the commonly observed rhizosphere effect in planted soil independent of contaminant biodegradation. The degradation process involves in the contaminant sand even without the presence of *E. atrovirens* (non-vegetated treatment).

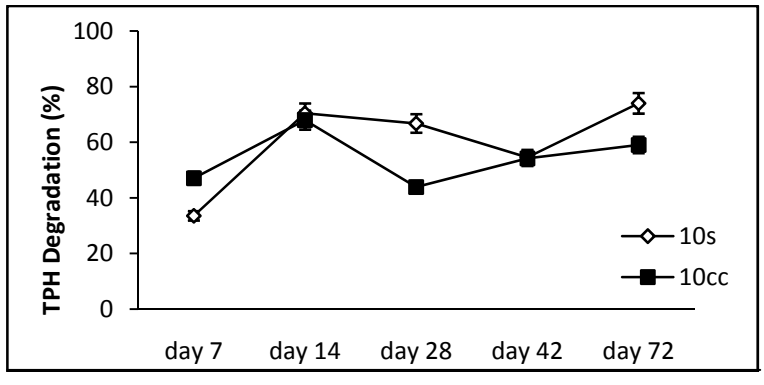
Based on Fig. 2(a), the highest degradation using *P. scrobiculatum* occurred on the last sampling time (Day 72) at the concentration of 10 g/kg. The most optimum concentration also occurred at the lowest concentration (same as with *E. atrovirens*). The percentage degradation at Day 72 in *P. scrobiculatum* was higher than at non-vegetated (control contaminant). The results indicated that the presence of plants could affect on the degradation process. Similar results were also obtained at the concentration of 30 g/kg on day 42 and 72 (Fig. 2(c)). It suggested that plants are able to supply carbon in soil and root-associated microbes with soluble exudates that increase microbial numbers and activity (Curl and Truelove, 1986). Root exudation includes the secretion of ions, free oxygen and water, enzymes, mucilage, and a diverse array of carbon-containing primary and secondary metabolites (Bertin *et al*., 2003; Uren, 2000). Root exudates potentially supply microbes with C, N, P, and/or other micronutrients required for growth of contaminant degraders. Root exudates may also supply primary carbon substrates for organisms that carry out cometabolic biotransformation (Haby and Crowley, 1996). Some studies also indicated that root exudates may also increase the bioavailability of the hydrocarbon contaminants resulting in enhanced biodegradation rates. Interactions between hydrocarbon contaminants and dissolved organic compounds present in exudates may enhance the aqueous phase concentration and/or desorption of hydrophobic contaminants (Kordel *et al*., 1997).

Fig. 2(b) shows the low degradation percentages between day 14 and 28 for vegetated treatment at diesel concentration of 20 g/kg. However, the percentage degradation by *P. scrobiculatum* increased on day 42 and 72, indicating that the degradation process is a continuous process until the end of sampling day. The percentages of degradation of TPH on day 72 were 74.0, 33.5 and 52.7% in diesel concentration of 10, 20 and 30 g/kg respectively. However, the optimum day of TPH degradation was reached on day 14 in diesel concentration of 20 and 30 g/kg. Based on ANOVA analysis, the degradation of TPH showed significantly different ($p<0.05$) in treatment with *P. scorbiculatum* at diesel concentration of 10, 20 and 30 g/kg.

For *P. vaginatum*, at low diesel concentration of 10 and 20 g/kg (Fig. 3 (a) and (b)), the optimum day for highest degradation were on day 28, which was almost one month after exposure. In Fig. 3(b), the degradation process were slightly slow especially on the last three sampling time (day 28, 42 and 72) at diesel

concentration of 20 g/kg. However, the highest percentage of TPH degradation (57.6%) at diesel concentration of 20 g/kg was recorded on day 14. Different result was obtained at diesel concentration of 30 g/kg (Fig. 3(c)). The optimum degradation occurs on the highest concentration (30g/kg diesel) in vegetated treatment with *P. vaginatum* (Fig. 3(c)) on day 42, in which 91.9 % of TPH was removed. Based on ANOVA analysis, the degradation of TPH showed significantly different ($p<0.05$) in treatment with *P. vaginatum* at diesel concentrations of 10, 20 and 30 g/kg.

The percentage of TPH degradation by *C. trifolia* was excellent when applied in low diesel concentration of 10g/kg (Fig. 4(a)) with the highest percentages of TPH degradation was 62.9% on day 72. The degradation continued starting from the first sampling point (day 0) until the end of sampling time (Day 72). However, the roots of the *C. trifolia* were short and only grow at the top layer of the sand, but the growth of plants



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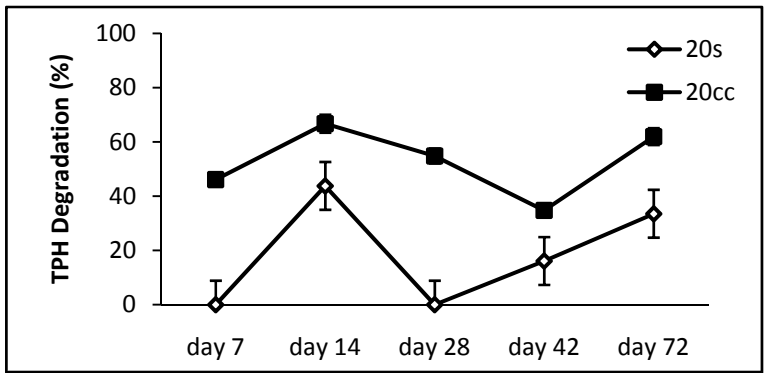

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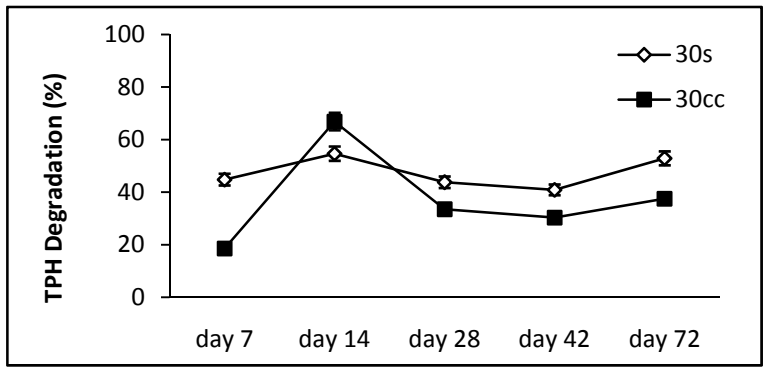
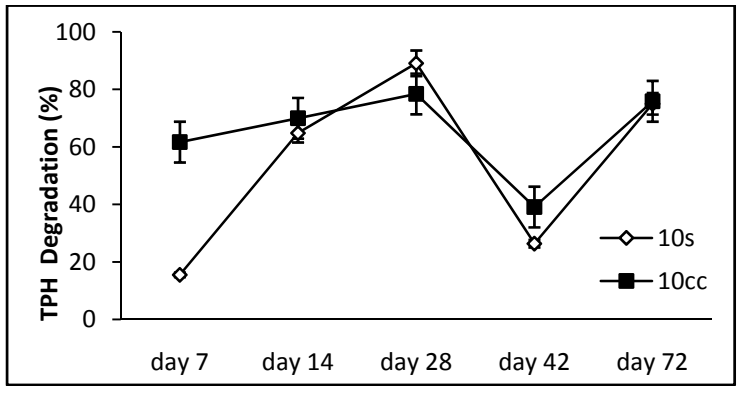


(c)

Figure 2.TPH degradation by *P. scrobiculatum* for sample and control starting Day 7 until Day 72 for concentration (a) 10 g/kg, (b) 20 g/kg and (c) 30 g/kg. Legend, vegetated (concentration/s) and non-vegetated/control contaminant (concentration/cc)



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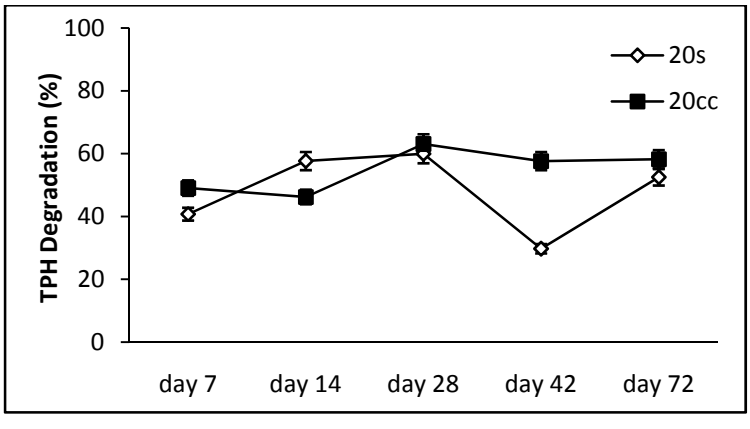

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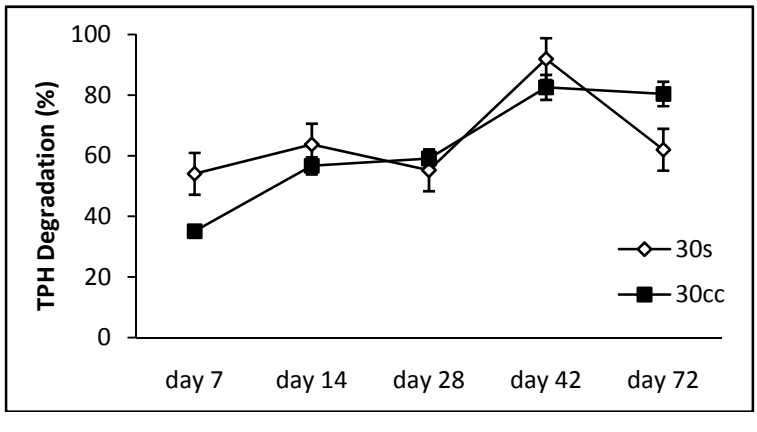

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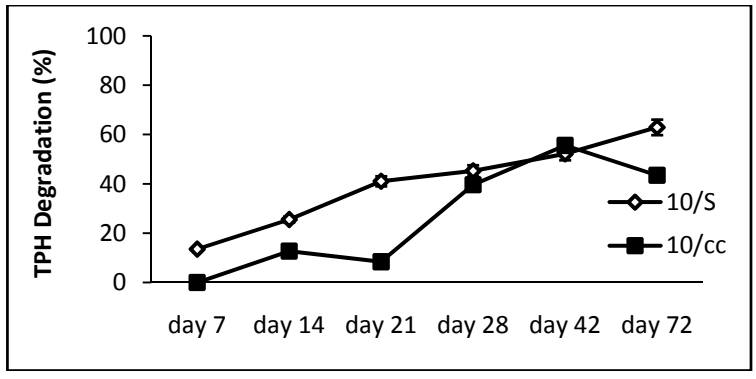
Figure 3. TPH degradation by *P. vaginatum* for sample and control starting Day 7 until Day 72 for concentration (a) 10 g/kg, (b) 20 g/kg and (c) 30 g/kg. Legend, vegetated (concentration/s) and non-vegetated/control contaminant (concentration/cc).

was rapid and the whole tank was fully covered. The highest concentration of diesel was spiked to plants has shown that the plants starting to lose the ability to give the significant role in the degradation process. The degradation of TPH also occurred at the control contaminant. However, the percentage of TPH degradation was significantly higher compared to vegetated treatment (Fig. 4). This results shows that *C. trifolia* is effective to degrade TPH in diesel at a lower level of contaminants. Based on ANOVA analysis, the degradation of TPH showed significantly different ($p<0.05$) in treatment with *C. trifolia* at diesel concentration of 10, 20 and 30 g/kg.

An example of chromatogram on TPH analysis at day 0 and the end of exposure (day 72) using GC-FID is shown in Fig. 5. It depicts that high carbon chains



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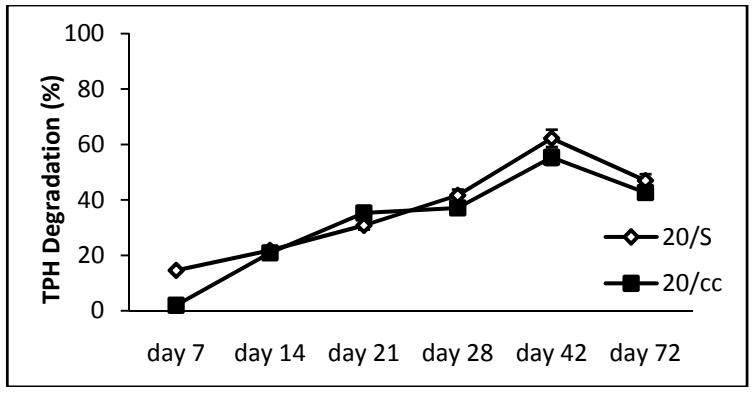

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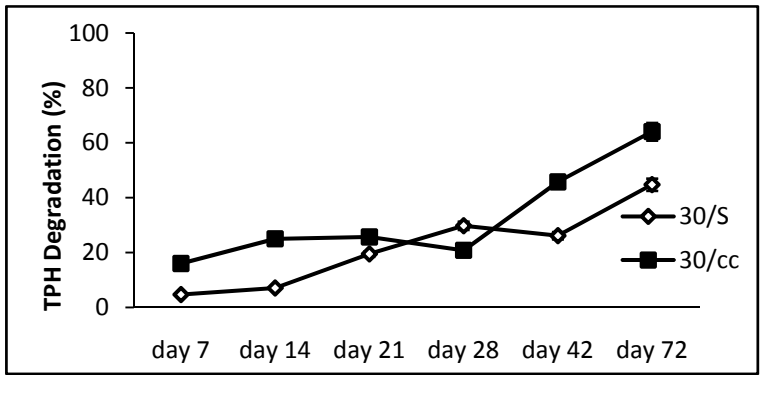

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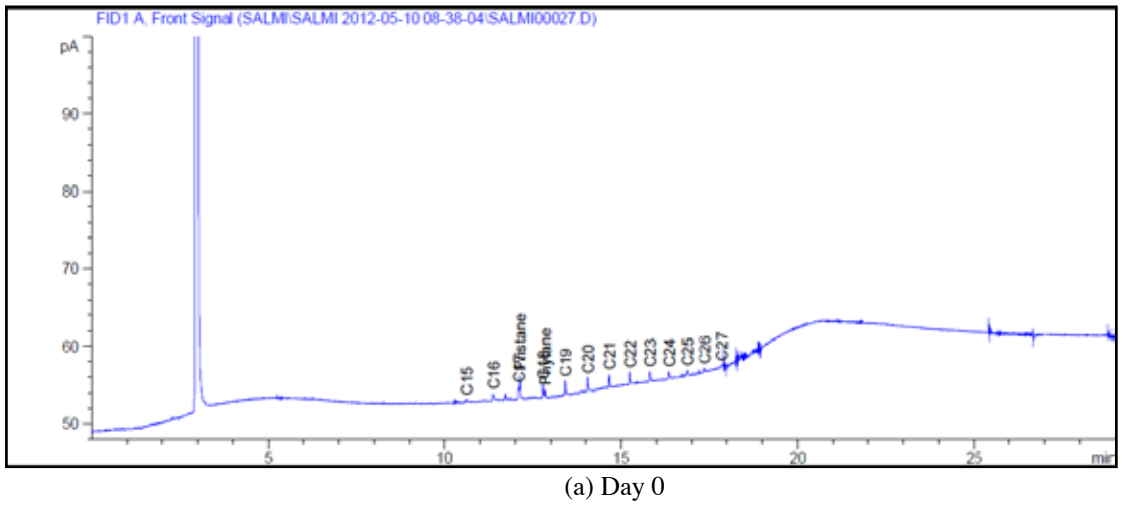
Figure 4. TPH degradation by *C. trifolia* for sample and control starting Day 7 until Day 72 for concentration (a) 10 g/kg, (b) 20 g/kg and (c) 30 g/kg. Legend, vegetated (concentration/s) and non-vegetated/control contaminant (concentration/cc).

were detected on Day 0 (Fig. 5(a)), meanwhile Fig. 5(b) showed lower carbon chains. It indicates that TPH can be degraded through phytoremediation.

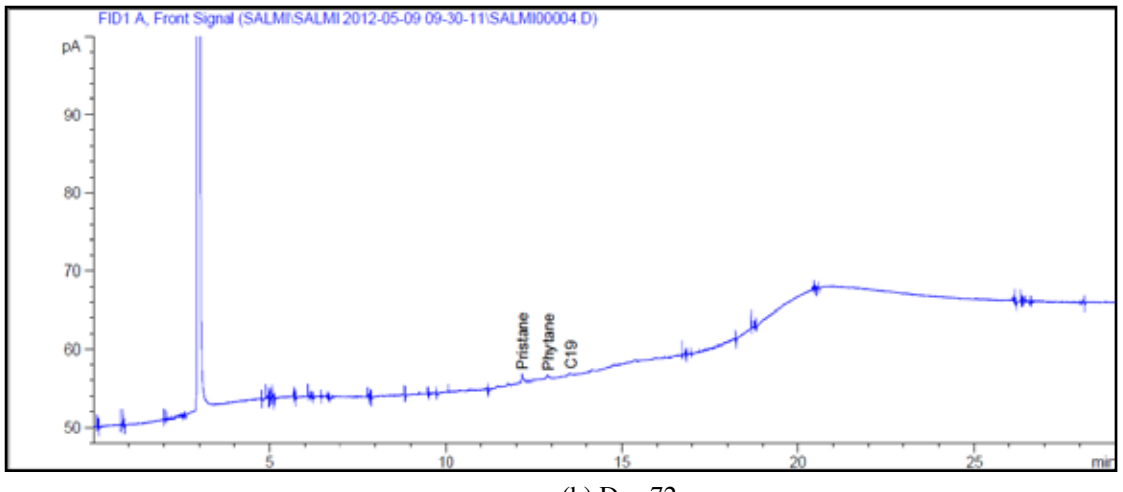
Fig. 6 shows a typical fresh-dry weight of plant biomass for *E. atrovirens*. The fresh-dry weight of plant increased at control and diesel treatment, indicating that plant can grow well at diesel spiked sand. Similar trend in fresh-dry weight occurred on other plants.

4. Conclusions

The highest percentage of TPH degradation by *E. atrovirens* reached 68.9% at diesel concentration of 10 g/kg on Day 7, meanwhile the highest percentage of TPH degradation by *P. scrobiculatum* and *C. trifolia* were 74.0 and 62.9% at diesel concentration of 10 g/kg on Day 72, respectively. The highest percentage



(a) Day 0



(b) Day 72

Figure 5. Chromatogram on analysis of TPH at day 0 and 72 using GC-FID

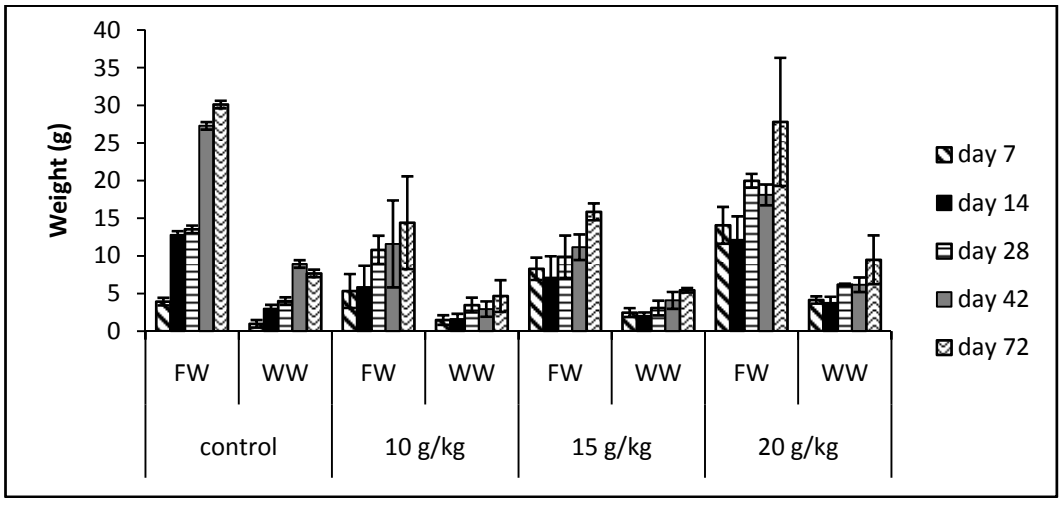


Figure 6. A typical fresh-dry weight of plant

degradation by *P. vaginatum* reached 91.9% at diesel concentration of 30 g/kg on Day 42. The degradation of TPH depends on the plant species and time of exposure. Based on the percentage of TPH degradation in diesel contaminant results for terrestrial plant, we conclude that *P. vaginatum* >*P. scrobiculatum* > *E. atrovirens* > *C. trifolia*.

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