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Adaptation Selection of Plants for Utilization in Phytoremediation of Soil Contaminated by Crude Oil

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ABSTRACT

The research on adaptation selection for utilization in phytoremediation of soil contaminated by crude oil using four type plants, such as *Helianthus annuus, Paspalum conjugatum, Sorghum bicolor,* and *Tagetes erecta* were conducted. The adaptability of four types of plants on crude oil contaminated soil at total petroleum hydrocarbon (TPH) level at 0, 3, and 6% were observed and evaluated to their morphological and anatomical responses. Parameters observed were vegetative growth of plants including growth precentage, plant height, number of leaves, root length, root dry weight, shoot dry weight, root/shoot ratio, total dry weight, and stomatal density for 9 weeks cultivation in screen house. The results show that increasing in TPH level caused in significant reductions on morphological of four plants, such as percentage of plant growth, plant height, number of leaves, root length, root dry weight, shoot dry weight, and total dry weight. In contrast, the increasing in TPH level caused to increasing in root/shoot ratio. The four types of plants studied were effective to be used as plants for phytoremediation of petroleum contaminated soil. The plants of *P. conjugatum* and *S. bicolor* are recommended as phytoremediators for further studies.

1. Introduction

Mining companies that have locations in forest areas are obliged to reforest the area (Kemenhut 2011). Reforestation of petroleum contaminated soil can not be directly planted by tree species. Planting in forest areas can not be directly planted if the total petroleum hydrocarbon (TPH) >3%, because it can cause plant death at seedling level (Setiadi 2012). Petroleum is a source of energy that has many benefits, but if wasted into the environment, would be a hazardous waste. Petroleum contamination can occur due to spills or discharges during oil mining activities, such as exploration, exploitation, processing, and transportation (Singh and Jain 2003; Eapen and D'Souza 2005).

Environmental pollution caused by oil spills may cause ecosystems in the environment to be disturbed. Hydrocarbon spills to soil and water can poison the flora and fauna that live around contaminated soil, including into the human body (Escalante-Espinosa *et al.* 2005; Merkl *et al.* 2005). Therefore, the action to overcome petroleum contaminated soil needs to be done so that pollution is not widespread.

There are three ways that can be carried out to overcome the oil spill, i.e. physically, chemically, and biologically. Physical handling is only able to cope with oil on the surface only, while chemical technique has side effects because it can cause new pollution. Nowadays, biological engineering is usually accomplished through bioremediation technique (Biswas *et al.* 2015). The bioremediation technique using plants is called phytoremediation. Phytoremediation uses certain plants and works with microorganisms to convert pollutants to be less harmful. It is a low-cost and environmentally friendly technique to reduce contaminated soils (Pilon-Smits 2005). Phytoremediation technology is usually used in conjunction with bioremediation methods for remediating contaminated soil as a polishing step, so that polluted areas can be recovered if they meet the quality standard (Yani *et al.* 2003).

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Little research in Indonesia was done to investigate potential plants for hydrocarbon phytoremediation. Not all plants will be adaptive to petroleum contamination. Studies on phytoremediation have shown that plants could help degrade petroleum hydrocarbon in soil, especially in the rhyzosphere (Macek *et al.* 2000; Gerhardt *et al.* 2009; Peng *et al.* 2009; Glick 2010). April and Sims (1990) also reported that a variety of plants have been known to have the potential to be used for phytoremediation. In contrast, some plant species could not reduce petroleum hydrocarbon in soil (Lalande *et al.* 2003; Euliss *et al.* 2008). Grasses and legumes have been used by researchers for phytoremediation studies (Merkl *et al.* 2005; Kaimi *et al.* 2006; Kirkpatrick *et al.* 2006).

Phytoremediation is a function of the root, so the plants used must have a strong root system and good root spreading (Setiadi *et al.* 1992). The selection of plant species that can be utilized for phytoremediation of a contaminants is a very important stage. According to Jones *et al.* (2004), the quality of rhizodeposition (the factor that drives the presence of microbes to the rhizosphere) depends on the type of plants. The purpose of this study was to obtain several types of plants which could adapt to petroleum contaminated soil by evaluating the decrease of TPH based on their morphological and anatomical responses to some concentrations of petroleum hydrocarbons.

2. Materials and Methods

2.1. Location of Study

The research was conducted at the Forest Biotechnology Laboratory-Biotechnology Resource Research Center-IPB University for plant adaptation preparation and plant morphological measurement. Stomatal measurement and root cross section were done at the Microtechnique Laboratory-Department of Agronomy and Horticulture-Faculty of Agriculture-IPB University. TPH analysis was carried out at the Analytical Laboratory-The Institute of Environmental Technology-*Badan Pengkajian dan Penerapan Teknologi* (BPPT). Finally, conducting plant cultivation was acomplished at the screen house of Silviculture Department of Faculty of Forestry-IPB University.

2.2. Materials

The materials used were the seeds of four types of plants, i.e. *Helianthus annuus, Paspalum conjugatum, Sorghum bicolor*, and *Tagetes erecta*. Planting media used for plant adaptation experiment was the soil collected from a depth of 0-30 cm from Serpong area, Banten Province, Indonesia, and compost. Crude oil given to the soil was derived from community crude oil wells in Bojonegoro area, East Java, Indonesia.

2.3. Research Procedure

2.3.1. Plant Adaptation Preparation

The soil used was sieved through a 2 mm screen. Soil as a growing medium (90%) was mixed with compost (10%), then poured crude oil at 0, 3, and 6% TPH. The contaminated soil were stirred manually, so that the soil was conditioned to have TPH-0, TPH-3, and TPH-6. The soil mixture was filled into plastic trays (31 x 3.5 x 23.5 cm^3 and 34 x 5 x 26.5 cm^3). Each experimental unit used 1.65 kg of mixed soil per tray.

S. bicolor seeds were first surface sterilized for 5 minutes in 5 (v/v) commercial NaOCl, then washed in running tap water and soaked with water for 30 minutes. Seeds of *P. conjugatum* were first seeded directly in the tray filled with sands for about 10 days. The seeds of *H. annuus* and *T. erecta* were grown in the tray that have been filled with planting medium. All plants were grown with a spacing of 5 x 5 cm^2.

The plants were cultivated in the screen house. A total of 72 plastic trays were maintained in the screen house (36 trays for 4 types of plants, 3 replication, 3 TPH concentrations and 36 trays for no plant treatments). The average temperature in the screen house was 27.1-31.9°C from minimum 24.9°C to maximum 44.1°C. The average relative humidity was 45.7-52.9% from minimum 29% to maximum 82%. Death plants was replaced within 7 days. Soil samples were taken before planting and after planting for TPH analysis. The same treatments were conducted in 36 trays for no plant treatments.

2.3.2. Data Measurement and Laboratory Analysis

2.3.2.1. Morphological and Anatomical Responses

There were three stages of harvesting: 3, 6, and 9 weeks. In each harvest, the variables measured were the precentage of plant growth (%), plant height (cm), number of leaves, root length (cm), root dry weight (g), shoot dry weight (g), root/shoot ratio, total dry weight (g), stomatal density (stomata/mm^2), and cross section of roots. Roots were separated from shoots and rinsed. Both shoots and roots were dried at 65°C for several days and weighed. Number of stomata measurement was performed under the Olympus CX 23 binocular microscope. Stomatal density is the number of stomata divided by the wide field of view. Cross section of roots was conducted by cutting the roots about 1 cm long, then placed on a glass object, put enough water to keep the sample from drying out, and placed the sample on Carton SCW-E binocular fixed magnification microscope. The fresh samples were cut as thin as possible using a razor, then it observed under the microscope.

2.3.2.2 Total Petroleum Hydrocarbon (TPH) Measurement

TPH was measured gravimetrically using Method 3540C (Soxhlet Extraction, part of Test Methods for Evaluating Solid Waste) by US EPA (1996). Each treatment had 3 replications.

2.3.3. Experimental Design and Data Analysis

This research was conducted with Completely Randomized Design. The data were analyzed using analysis of variance (ANOVA) and Duncan Multiple Range (Gomez and Gomez 1984). Data processing was done using Statistical Analysis System (SAS) program 9.4. Each plant used was a separate research unit.

3. Results

3.1. Plant Morphological and Anatomical Responses

Treatments with 3 levels of TPH was resulted in significant reductions of the four plants morphologically after 9 week planting (Table 1). The plant morphological observed by the percentage of plant growth (%), plant height (cm), number of leaves, root length (cm), root dry weight (g), shoot dry weight (g), root/shoot ratio, and total dry weight (g).

Figure 1 shows the growth percentage of the four plants selected with 3 levels of TPH treatment after 3, 6, and 9 weeks of planting. The highest growth percentage was achieved by *P. conjugatum* (100%)

Table 1. The growth performance of plant height, root length, root dry weight, shoot dry weight, root/shoot ratio, total dry weight, and stomatal density of *H. annuus, P. conjugatum*, *S. bicolor*, and *T. erecta* at 3 level of TPH (0, 3, 6%) after 9 weeks planting

Parameter and TPH level	*H. annuus*	*P. conjugatum*	*S. bicolor*	*T. erecta*
Plant height (cm)				
TPH-0	27.76±9.42 A	14.60±4.62 A	14.20±3.75 A	17.06±4.71 A
TPH-3	15.62±4.45 AB	7.26±0.96 B	9.43±0.23 B	5.37±1.07 B
TPH-6	8.83±2.46 B	2.41±0.79 B	5.07±1.10 B	2.47±0.87 B
Leaf number				
TPH-0	11.00±4.85 A	15.73±1.72 A	5.00±0.69 A	17.26±1.81 A
TPH-3	6.40±3.48 A	5.93±0.41 B	5.67±0.30 AB	10.13±0.80 B
TPH-6	4.27±3.71 A	5.27±1.22 B	4.13±0.23 B	4.53±0.61 C
Root length (cm)				
TPH-0	4.77±2.31 A	25.88±0.98 A	12.03±3.49 A	14.30±4.85 A
TPH-3	3.10±1.53 A	17.96±2.31 B	9.70±1.21 AB	8.93±1.27 AB
TPH-6	2.07±2.30 A	6.70±3.46 C	5.67±0.84 B	5.70±0.55 B
Root DW (g)				
TPH-0	0.26±0.13 A	1.33±0.53 A	0.95±0.52 A	0.47±0.40 A
TPH-3	0.59±0.27 A	1.07±0.15 A	0.61±0.34 A	0.20±0.15 A
TPH-6	0.41±0.53 A	0.30±0.31 B	0.92±0.07 A	0.26±0.18 A
Shoot DW (g)				
TPH-0	2.78±0.87 A	3.52±0.81 A	2.73±0.42 A	2.32±0.79 A
TPH-3	0.78±0.66 B	1.02±0.45 B	0.96±0.28 B	0.20±0.09 B
TPH-6	0.16±0.05 B	0.61±0.27 B	0.57±0.29 B	0.04±0.02 B
Root/shoot ratio				
TPH-0	0.09±0.04 A	0.38±0.15 A	0.35±0.40 A	0.20±0.19 B
TPH-3	0.76±0.33 A	1.05±0.34 B	0.63±0.15 B	1.00±0.10 B
TPH-6	2.56±0.12 A	0.49±0.36 B	1.61±0.27 B	6.50±0.18 A
Total DW (g)				
TPH-0	3.03±1.00 A	4.85±0.97 A	3.68±0.68 A	2.90±0.73 A
TPH-3	1.37±0.65 B	2.08±0.46 B	1.56±0.36 B	0.33±0.13 B
TPH-6	0.57±0.59 B	0.91±0.30 B	1.48±0.36 B	0.26±0.16 B
Stomatal density (stomata/mm^2)				
TPH-0	10.60±2.30 B	4.33±0.57A	15.60±2.30A	25.60±9.29 A
TPH-3	12.00±2.00 AB	5.00±1.73 A	13.30±2.51 A	23.00±1.00 A
TPH-6	15.30±0.57 A	4.00±0.00 A	11.60±2.51A	21.60±5.50 A

Significantly different for alphabetical code

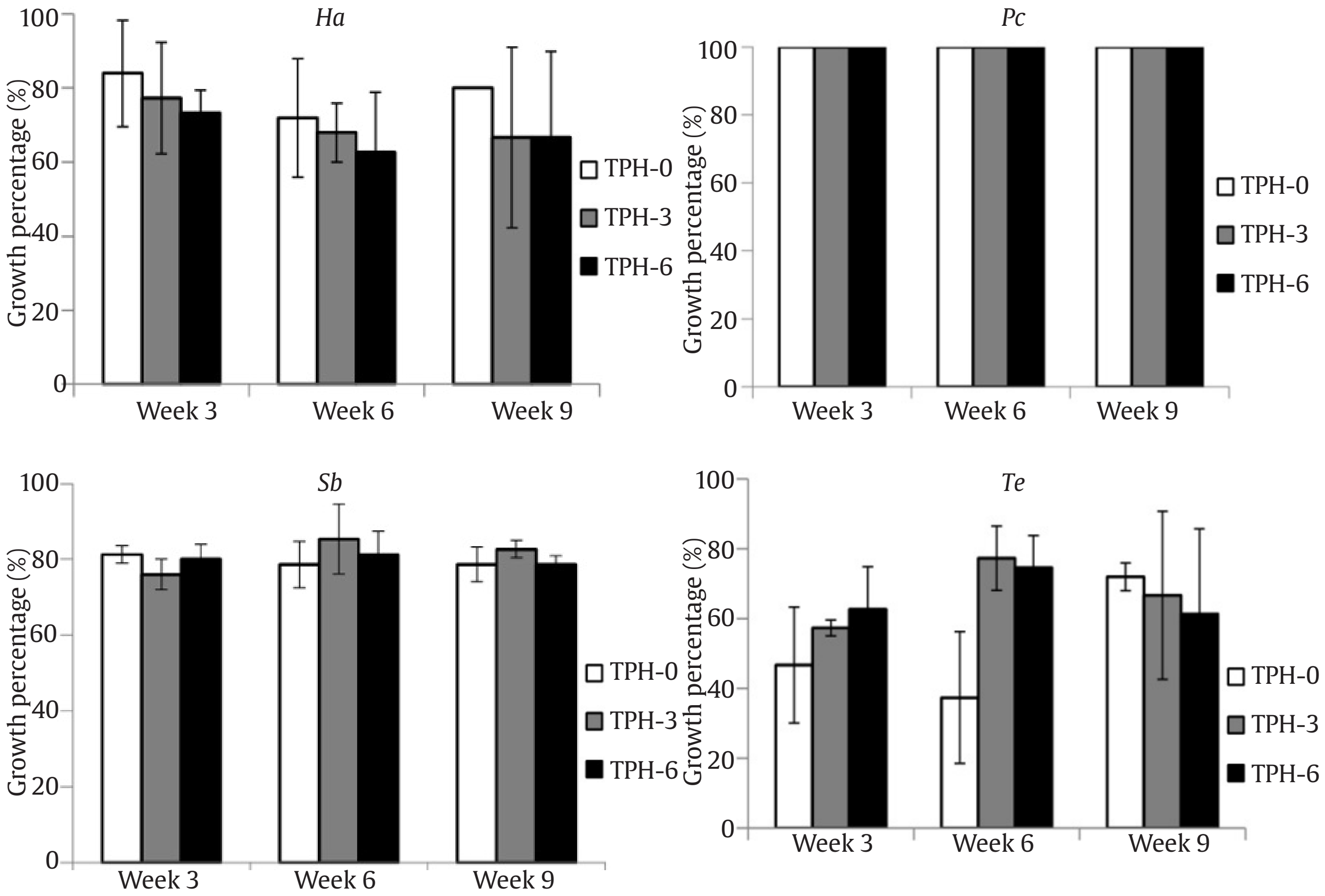

Figure 1. Growth percentage of *H. annuus* (Ha), *P. conjugatum* (Pc), *S. bicolor* (Sb), and *T. erecta* (Te) after 3 weeks, 6 weeks, and 9 weeks of planting

at TPH-0, TPH-3, and TPH-6 treatments. The better percentages after *P. Conjugatum* were S.bicolor (80%), *H. annuus* (71%), and lastly *T. erecta* (66%).

Plant heights were affected by treatments. TPH-0 (control) was the highest among other treatments (Figure 2). Treatments with the addition of crude oil of TPH-3 and TPH-6 were not significantly different, eventhough plant height of the four plants investigated at TPH-3 was higher than plant height in TPH-6. The concentration of crude oil increased, resulted in a decrease in plant height at week 3, week 6, and week 9 for all plants observed. Similar to plant height, number of leaves for each plant tended to decrease at higher TPH (Figure 3). Table 1 shows the higher of the concentration of crude oil in the soil, the less the root length of the four plants observed, eventhough there was no significant different for *H. annuus*.

The increasing in TPH level in soil caused the increasing in the root/shoot ratio for four plants observed (Figure 4), eventhough there was no significant different for *H. annuus*. Root DW of TPH treatment was not significantly different among treatments, except for TPH-3 and TPH-6 of *P. conjugatum*. There was a significantly different between TPH-3 and TPH-6 of *P. conjugatum*. The root DW and the shoot DW decreased with the increasing in TPH level (Table 1), and then the root/shoot ratio increase with the increasing in TPH level (Table 1 and Figure 4) for all plants. The root DW of *H. annuus* and *P. conjugatum* were higher at TPH-3 than at TPH-6 (Table 1). Root/shoot ratio of *P. conjugatum* at TPH-3 was higher than at TPH-6 (Figure 4).

Total DW of four plants investigated as a function of time and treatment (Figure 5). The number of leaves, root DW, shoot DW, and total DW of *H. annuus* tended to decrease with the increasing levels of TPH, although it was not significantly different for leaf number, root length, and root DW. There was a significant different of total DW between control soil (TPH-0) and treated soil (TPH-3 and TPH-6). However, there was no significantly different of total DW between TPH-3 and TPH-6.

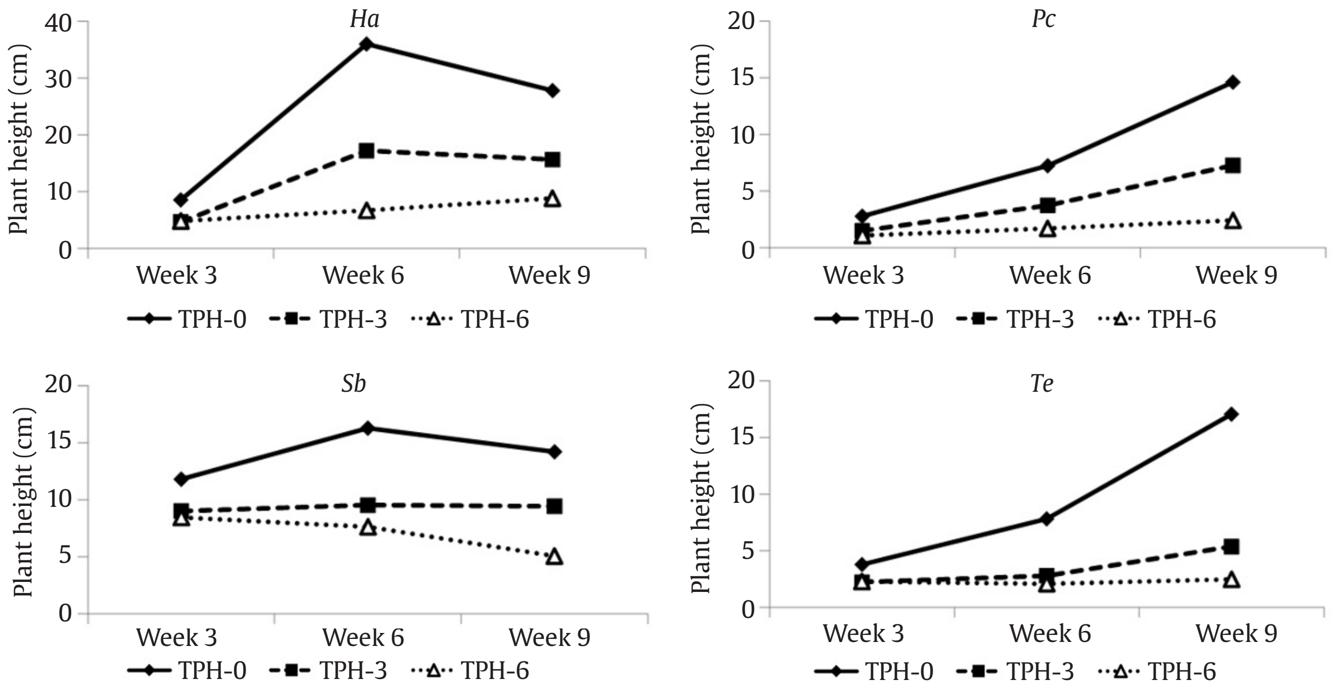

Figure 2. Plant height of *H. annuus* (Ha), *P. conjugatum* (Pc), *S. bicolor* (Sb), and *T. erecta* (Te) after 3 weeks, 6 weeks, and 9 weeks of planting

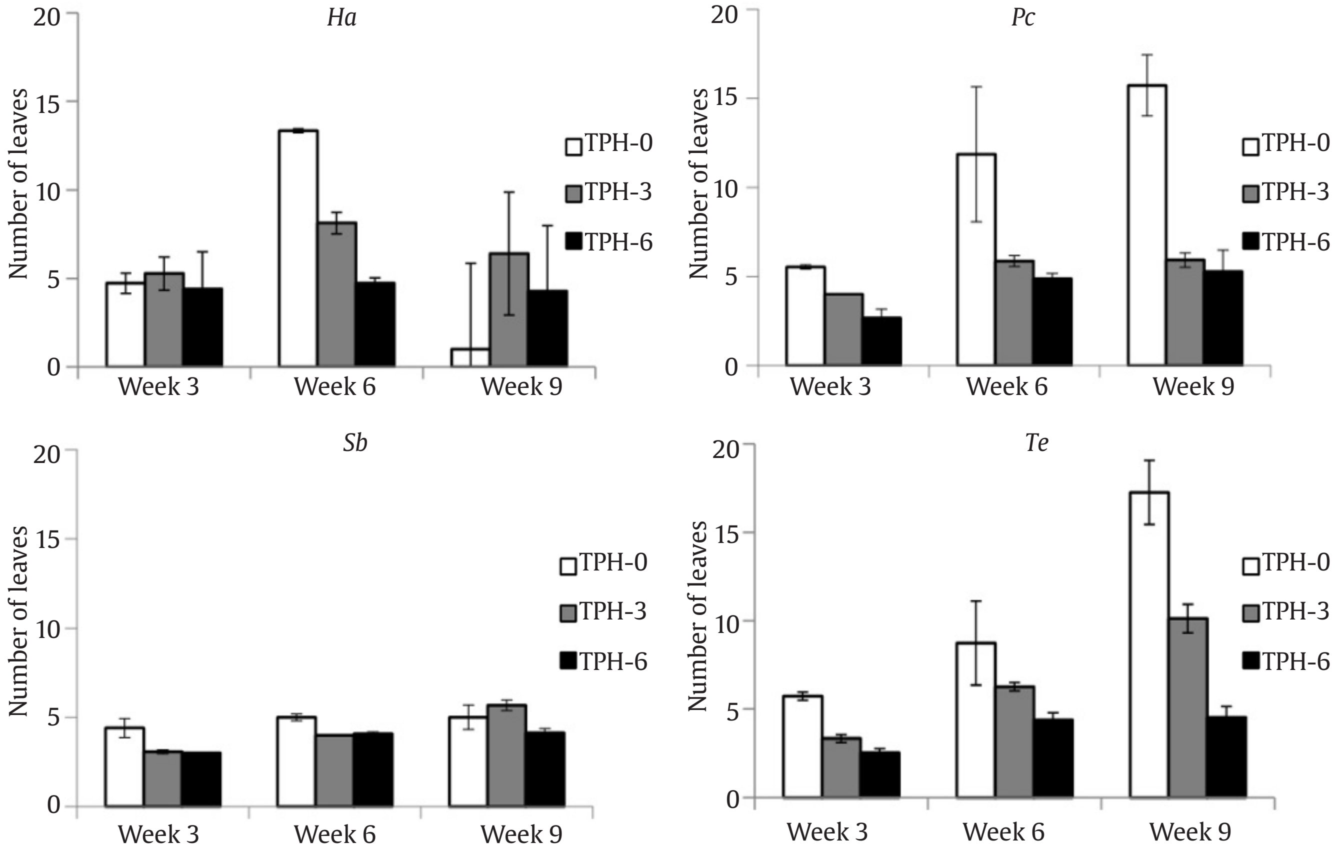

Figure 3. Number of leaves of *H. annuus* (Ha), *P. conjugatum* (Pc), *S. bicolor* (Sb), and *T. erecta* (Te) after 3 weeks, 6 weeks, and 9 weeks of planting

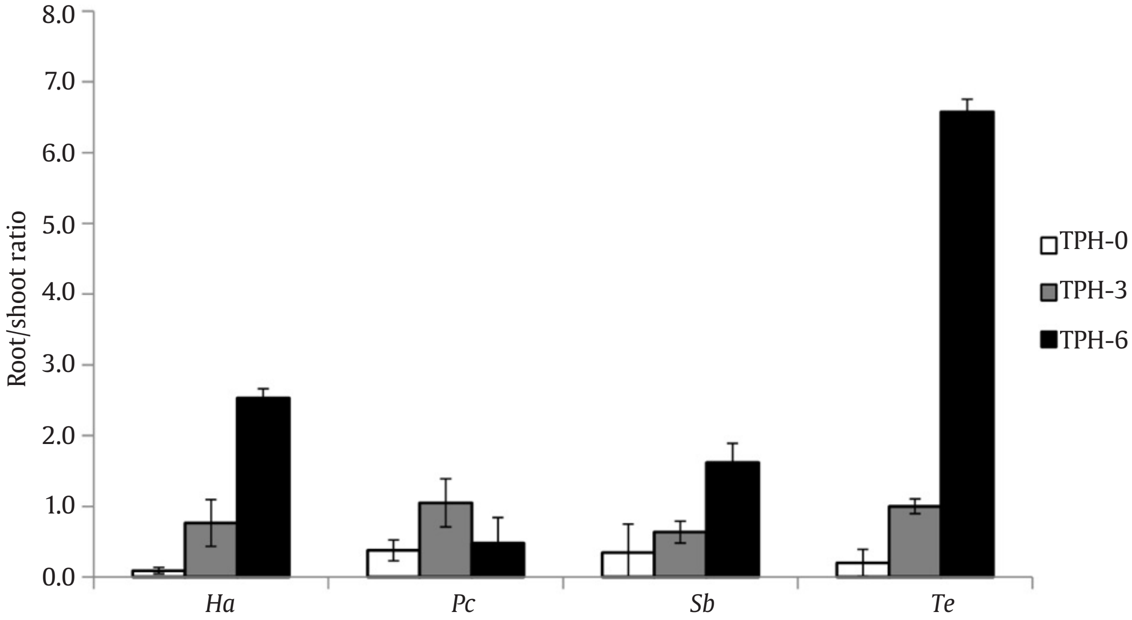

Figure 4. Root/shoot ratio of *H. annuus* (Ha), *P. conjugatum* (Pc), *S. bicolor* (Sb), and *T. erecta* (Te) after 9 weeks planting

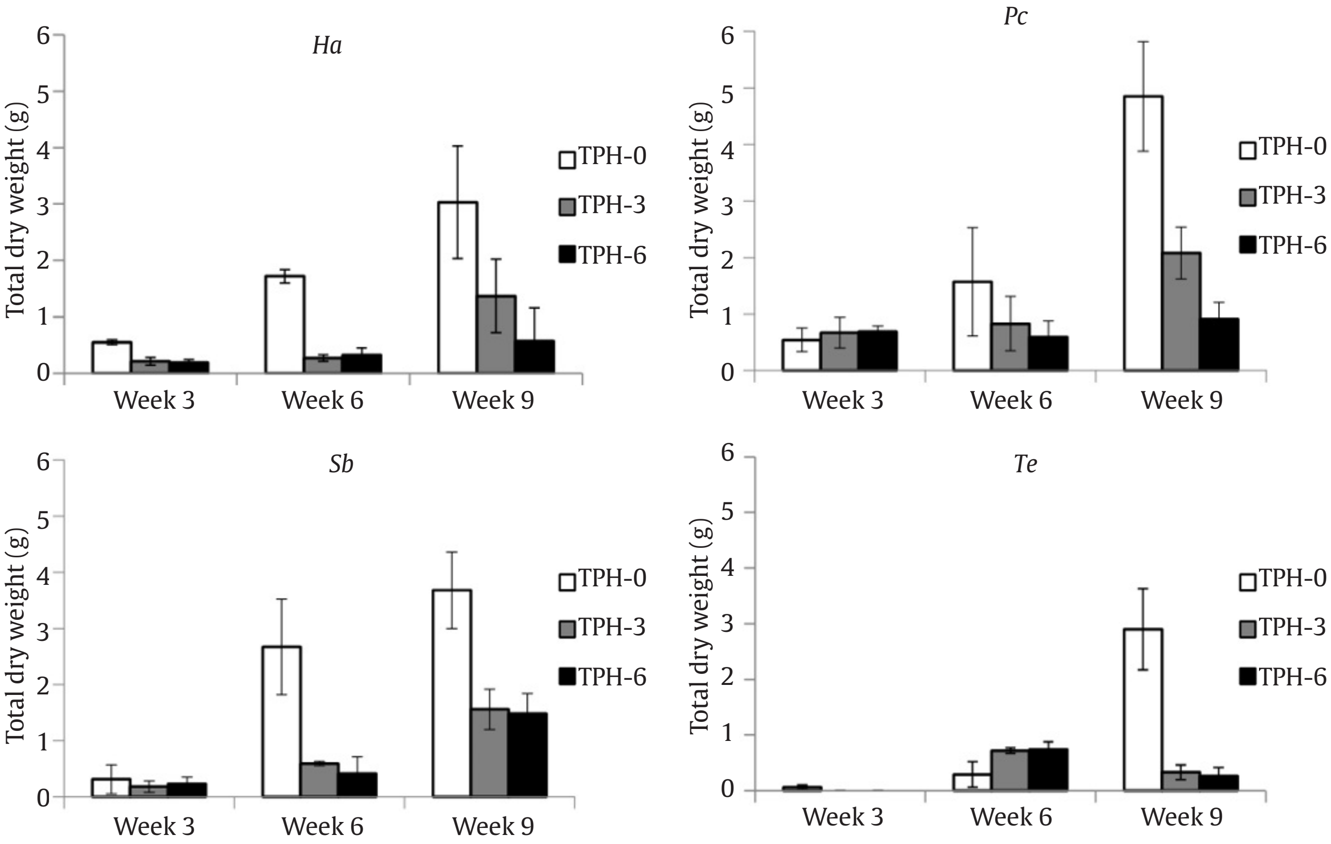

Figure 5. Total dry weight of *H. annuus* (Ha), *P. conjugatum* (Pc), *S. bicolor* (Sb), and *T. erecta* (Te) after 3 weeks, 6 weeks, and 9 weeks of planting

The results of the morphological response of *P. conjugatum* showed that each parameter observed (plant height, number of leaves, root length, root DW, shoot DW, and total DW) decreased with increasing TPH. The parameters observed for *S. bicolor* decreased with increasing TPH levels, except for the number of leaves on TPH-3, which was higher than the control (Table 1). The *T. erecta* plant as well as the three plants described above decreased the parameters measured on the morphological aspects with increasing TPH (Table 1). Differences were observed among treatments, except root DW, which did not have a significantly different.

Stomatal density of *P. conjugatum, S. bicolor,* and *T. erecta* decreased with the increase of TPH level, however no significantly different was seen for stomatal density in 3 level of TPH. The lowest stomatal density occurred at TPH-6 in *P. conjugatum,* then *S. bicolor,* and *T. erecta* (Figure 6). In contrast, enhanced stomatal density was found in *H. annuus* with the increase of TPH.

Figure 7 shows cross sections of *S. bicolor* root at TPH-0, TPH-3, and TPH-6. At TPH-0, the epidermis was seen under normal condition. At TPH-3, the epidermis have been contaminated by petroleum entering the roots, some of the oil have reached the part of the cortex and endodermis. Oil quantity was seen more at TPH-6 treatment.

3.2. Total Petroleum Hydrocarbon (TPH)

TPH decline occurred significantly in all four types of plants used within 9 weeks. Figure 8 shows that the four selected plants were able to significantly reduce TPH (79.3-91.1%) compared to TPH-0. The highest percentage decrease was achieved by *P. conjugatum* of 91% in TPH-3 and 90% in TPH-6. In the control treatment (without plant), there was also a 73% TPH reduction at TPH-3 and 67% at TPH-6.

Based on the results mentioned above, it can be seen that the highest TPH reduction was *P. conjugatum*. Figure 8 shows that the concentrations of TPH-3 and TPH-6 were not significantly different in all plants. However, the concentration of TPH-0 was significantly different from TPH-3 and TPH-6.

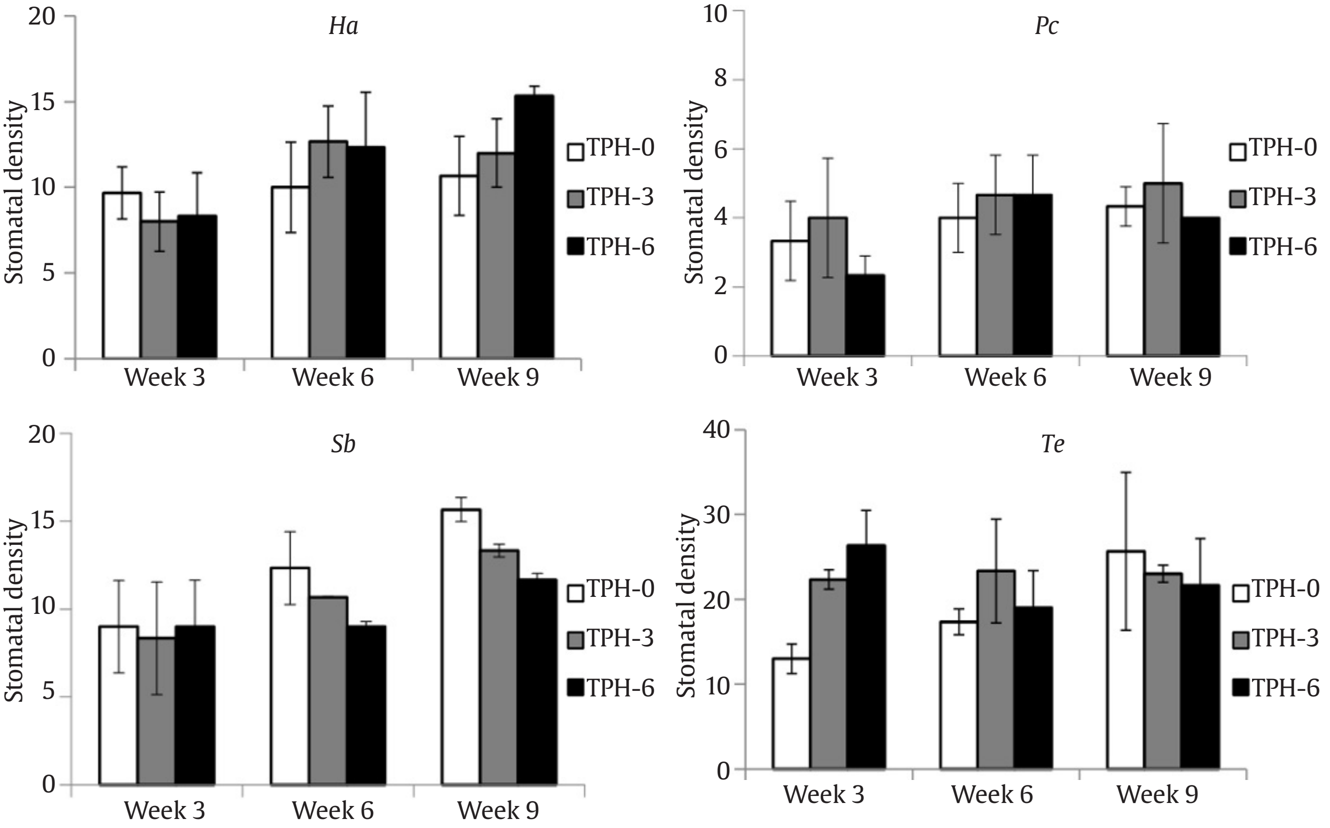

Figure 6. Stomatal density of *H. annuus* (Ha), *P. conjugatum* (Pc), *S. bicolor* (Sb), and *T. erecta* (Te) after 3 weeks, 6 weeks, and 9 weeks of planting

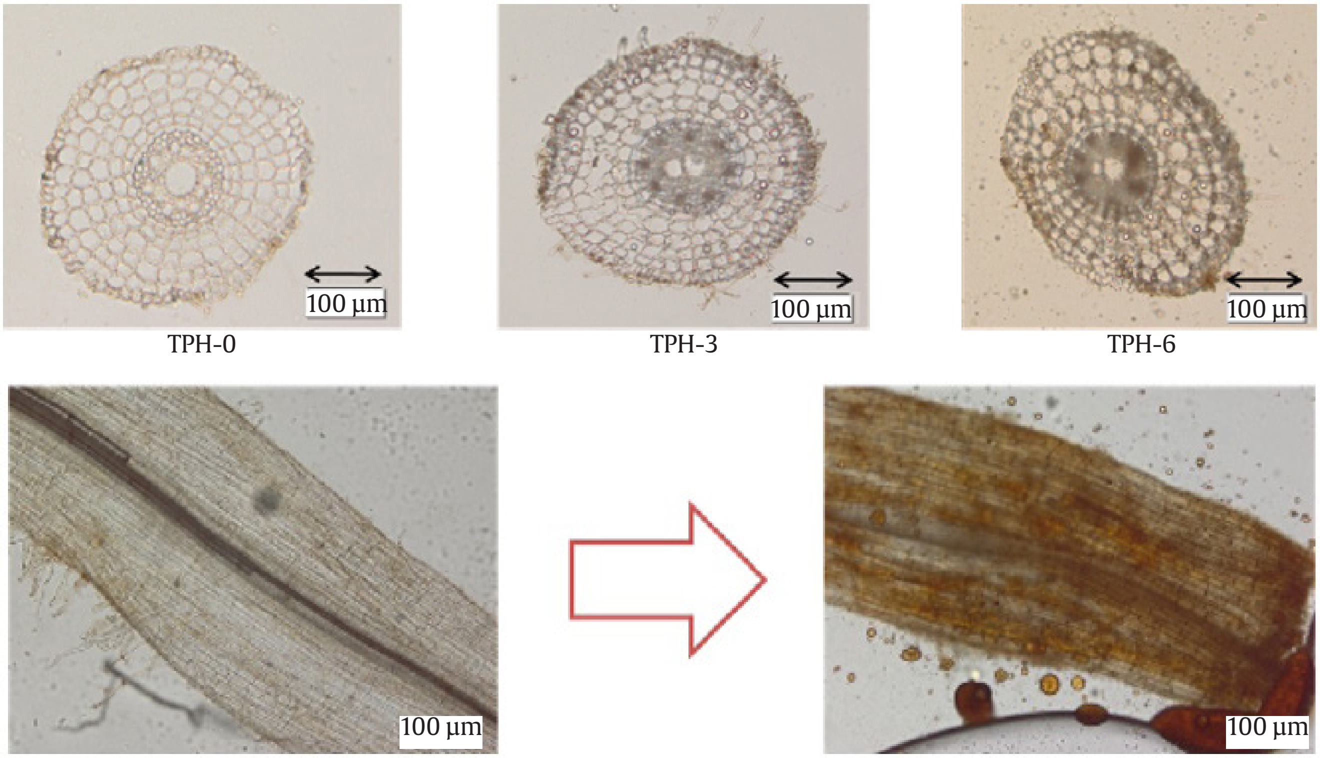

Figure 7. Section of *S. bicolor* root at TPH-0, TPH-3, and TPH-6 treatments(top). Root condition on TPH-0 and TPH-6 (below)

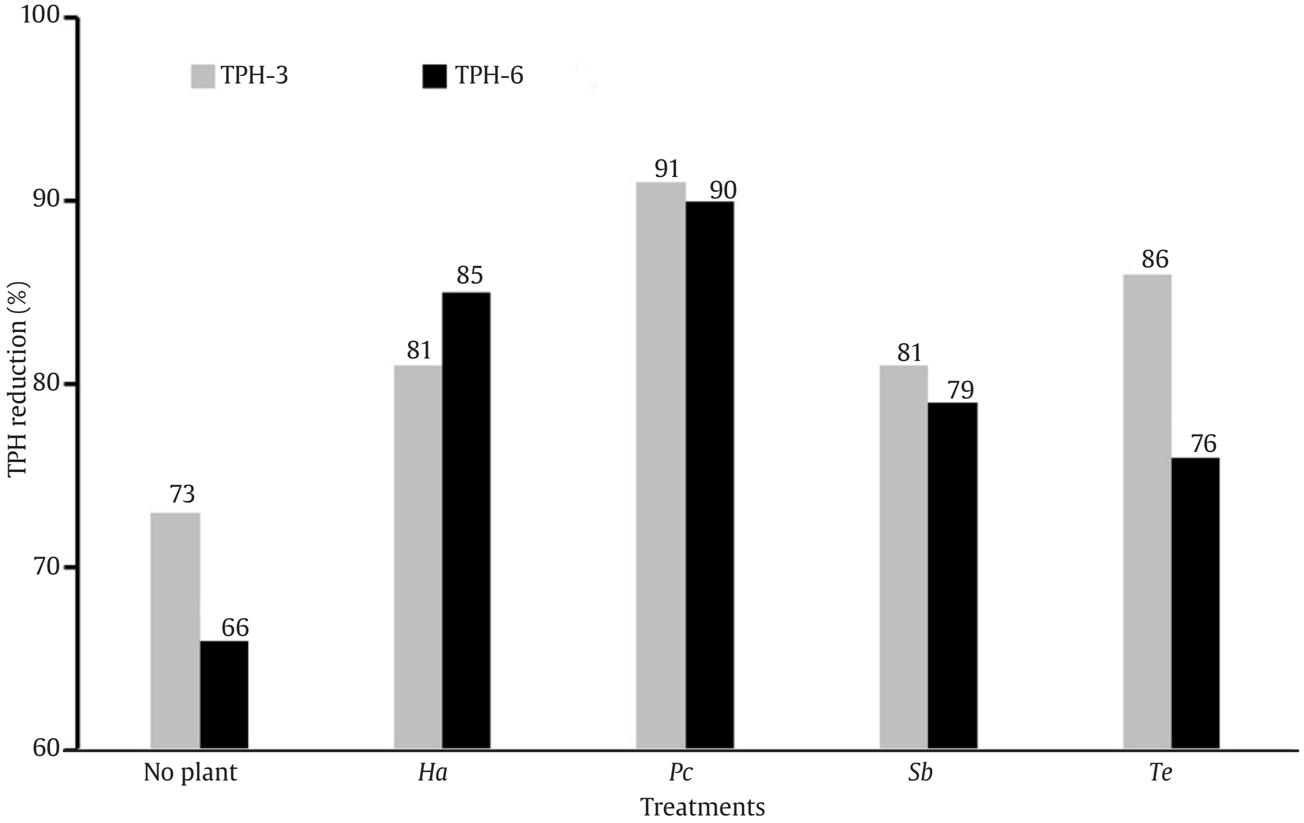

Figure 8. Percentage reduction of TPH in soil for control (no plant) and with *H. annuus* (Ha), *P. conjugatum* (Pc), *S. bicolor* (Sb), and *T. erecta* (Te) after 9 weeks planting

4. Discussion

4.1. Plant Morphological and Anatomical Responses

Contamination of petroleum in soil have adverse effects to plant morphology and anatomy. Reduction in plant height are typical response caused by petroleum hydrocarbon contamination (Lin and Mendelssohn 1998; Ikhajiagbe and Anoliefo 2011). Amadi *et al.* (1996) reported that the presence of petroleum in soil caused the blocking of oxygen and water into the plant. The presence of crude oil in soil causes some harmful elements will be more available to the plant, so that plant growth is disrupted. Similar result was obtained by Basumatary and Bordoloi (2016) that *Cynodon dactylon* grown in petroleum contaminated soil had a plant height reduction. Inhibition of nutrients and water enter the plant, also caused the number of leaves produced by plants to decrease (Osuagwu *et al.* 2013). Okoloko and Berley (1982) also reported that petroleum contaminated soil causes disruption of membrane integrity and enzyme systems, thus ultimately disrupting the plant's metabolic system.

The decrease in root length was caused by osmotic stresses due to the increase of crude oil concentration, so that cell division at the root tip was inhibited. Some plants had the ability to increase the root area under hydrocarbon stress conditions by increasing the root length and multiplying the root branches to extend the root range to deeper soil layers, so that the root had a great opportunity to absorb more nutrition and water from soil.

The petroleum hydrocarbon stress condition triggered the plant to further increase root growth and suppressed the growth of the shoot so that the root/shoot ratio became larger than the optimum condition (Table 1). The occurrence of the response is a form of plant adaptation to the conditions of petroleum hydrocarbon stress. By pushing the growth of the roots will provide a greater opportunity to absorb water by reaching a deeper layer of soil. Suppressing shoot growth reduces water loss (transpiration) through the leaves (Wu and Cosgrove 2000).

The presence of petroleum hydrocarbons in soil inhibits photosynthesis, so carbon translocation is disturbed for shoot and root growth (Daly *et al.* 1988). Merkl *et al.* (2004) also reported that plant DW was significantly reduced in soil contaminated by petroleum hydrocarbons. Stress due to petroleum is analogous to the drought stress. Drought stress in some plants causes stomatal size and the number of stomata decrease (Price and Courtois 1991). They argued that the decrease of stomatal number is one of plant mechanisms to adapt to harsh environment by improving the balance and status of water. The status of plant water is useful for maintaining turgor and the entire biochemical process for plant activity and growth. Haryadi and Yahya (1988) also reported that reducing the amount of stomata is useful for reducing transpiration, so plants can maintain cell turgor, therefore plants can grow normally. *P. conjugatum* in this study had a low stomatal density as a form of adaptation to oil stress.

Unlike the three plants observed, stomatal density of *H. annuus* enhanced with the increase of TPH. Similar results obtained in *Leymus chinensis* (Zhenzhu and Guangsheng 2008). Zhang *et al.* (2006) also reported that stomatal density of *Triticum aestivum* also increased because of drought stress. Yin *et al.* (2006) argued that this phenomenon might not be associated with the plant response due to drought stress.

4.2. Root Destruction

Figure 7 clearly shows the negative effect of petroleum contaminated soil to the plant. At TPH-3, some of the crude oil have reached part of the cortex and endodermis of *S. bicolor* root, therefore it disturbed nutrition and water entering the roots. Figure 7 also shows that the oil particles were clearly more visible at TPH-6 compared to the control (TPH-0) and TPH-3 treatment. The crude oil might have damaged plant roots at TPH-6. The oil has reached cortex, endodermis, and stele (the central part of the root), which contains xylem and phloem. Petroleum might have translocated to the shoot, however the adaptation mechanism are not clearly understood.

4.3. Total Petroleum Hydrocarbon (TPH) Degradation

P. conjugatum and *Tagetes erecta* were usually used as phytoremediators for heavy metals polutant (Anoc and Rom 2000; Pas-Alberto *et al.* 2007; Ccoelho *et al.* 2017; Goswami and Das 2017). Little research in Indonesia was done to investigate potential plants for hydrocarbon phytoremediation. Setiadi *et al.* (2014) reported the use of four types of plants (*P. conjugatum, Pueraria javanica, S. bicolor*, and *T. erecta*) for adaptation in petroleum contaminated soil obtained from Sumatera island at TPH concentration of 0.43, 1.41, 4.69, and 8.15% respectively. They concluded that grass type of plants (*P. conjugatum* and *S. bicolor*) had better growth potential compared to dicotyl species (*P. javanica* and *T.*

erecta). Phytoremediation on petroleum contaminated soil using four types of grasses was also conducted by Salim and Suryati (2014) in Indonesia. After 12 months of planting, the four types of grass plants which were *Eleusine indica, Paspalum notatum, Setaria splendida,* and *Stenotaphrum secundatum* could play a role in the process of remediation of polluted soil containing 3.23% TPH. The highest reduction percentage was obtained from *Paspalum notatum* (38.81%), followed by *Eleusine indica* (38.69%), *Setaria splendida* (36.34%), and *Stenotaphrum secundatum* (29.32%). Ervayenri (2015) used *Ficus* sp. seedlings for his phytoremediation study and found out that *Ficus* sp. was not able to survive more than 5% TPH without inoculation by arbuscular mycorrhizal fungi (AMF).

The results of this research showed that *P. conjugatum* and *S. bicolor* were most resistant to crude oil at TPH-3 and TPH-6 levels compared to the other two plants (*Helianthus annuus* and *Tagetes erecta*) although not significantly different. This is consistent with the opinion of April and Sims (1990) that grasses have enormous potential compared to other plants as phytoremediators, because grasses have a strong root system and good roots distribution in soil. The fibrous and highly branched grass root systems allow large volumes of soil to be explored for nutrient uptake and transport (Siddiqui *et al.* 2001; Merckl *et al.* 2005). Merkl *et al.* (2005) showed enhanced degradation of petroleum hydrocarbon using tropical grasses and legumes after a few months. Removal of 30% TPH was reported by Huang *et al.* (2005) in *Festuca arundinaceae* in 120 days. Razmjoo and Adavi (2011) found out that there was 40% TPH reduction by *Cynodon dactylon* in 6 months.

Phytoremediation of contaminated soil of petroleum hydrocarbons is dependent on highly tolerant and growing plant species in locations in the presence of peroleum (Hernández-Ortega *et al.* 2012). Plants on contaminated soil also allow the biological transformation of organic contaminants by stimulating microbial activity through root exudates. The root exudates acts as a source of nutrients for microbes degrading organic pollutants (Anderson *et al.* 1993). In addition, root exudates are also co-substrate for microbes. Roots can alter the soil porosity, so it may help the microbe in degrading petroleum hydrocarbons in soil (Yan-zheng and Li-zhong 2003).

Plants can remove toxic effects of contaminants in petroleum contaminated soil, because plants can adapt by certain physiological responses, such as improvement in nutient and water uptake and free-radical scavenging systems induction to prevent cell distruction (Misra and Gupta 2006). The occurrence of stress in plants causes a decrease in leaf osmotic potential followed by accumulation of proline (Sopandie *et al.* 1996). Hydrocarbon exposure also cause oxidative stress, which is indicated by over-expression of superoxide dismutase (SOD) (Gupta *et al.* 1993).

The function of plant roots is not only in absorbing nutrients and water in the soil, but also in overcoming the presence of contaminants in the soil. Mutualism symbiosis between microbes and plants by recycling, solubilization, and supplying vitamins, amino acids, and plant hormones by microbes, stimulates the plant growth (Escalante-Espinosa *et al.* 2005). In this study, a higher degradation of TPH in soil vegetated with the four types of plants used compared to unvegetated soil. However, there was a TPH redution at TPH-3 (73%) and at TPH-6 (67%) for the treatment without plant. This might be due to microclimate changes, such as temperature, light, dissolving to water, and composting.

5. Conclusion

According to the ability in decreasing the TPH, it can be concluded that all four types of plants that were selected could grow under TPH-3 and TPH-6 conditions. It means that the four types of plants are potential as phytoremediators, eventhough in this study, there was a TPH reduction at TPH-3 and TPH-6 at the treatment without plant. Selected grass species studied could be used for phytoremediation of petroleum hydrocarbon contaminants in soil due to their adaptability. *P. conjugatum* and *S. bicolor* are recommended for future experiments based on their growth rate and great tolerance to petroleum hydrocarbon. This is one of the first studies explaining the success of utilizing *P. conjugatum* and *T. erecta* for phytoremediation of soil contaminated by crude oil.

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