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Propagation and Phytoremediation Preliminary Test of *Ludwigia ectovolvis* (L.) and *Scirpus mucronatus* (L.) in Gasoline Contaminated Soil

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ABSTRACT
In this study both plants *Ludwigia ectovolvis* and *Scirpus mucronatus* have been shown to phytoremediate by toxicity testing using various concentrations of gasoline. Plants grow in different ratios comprising of garden soil and sand before executing the toxicity tests. Soil mixture as garden soil to sand ratios (25:75, 50: 50, 75:25, 100% sand and 100% garden soil) to propagation the plants for 56 days. The results shown that *L. ectovolvis* could grow and survive in mixture of garden soil to sand ratio of 50:50, while for *S. mucronatus* the best favorable growth was using 75: 25 ratio of garden soil to sand. *S. mucronatus* survived the gasoline concentrations of 10, 20, 30, 40, 50, 60, 70, 90 and 100 g gasoline/kg in comparison to the control. However *L. ectovolvis* died after one day of exposure at similar concentrations, therefore the plants were tested for lower concentrations of 1, 2, 3, 5 and 7 g kg^{-1}. During 14 days of exposure, *S. mucronatus* can only survive at concentrations of 10, 20, 30 and 40 g gasoline/kg. But *L. ectovolvis* withered under concentration of 1, 2, 3, 5 g gasoline/kg at the end of exposure period. As a conclusion, *S. mucronatus* has the potential to remediate hydrocarbon contaminated soil as compared to *L. aectovolvis*.

Key words: Propagation, phytoremediation preliminary test, gasoline, *Ludwigia ectovolvis*, *Scirpus mucronatus*

INTRODUCTION
Phytoremediation is an emerging technology direct use of living plants to remediation or degrade, extract, stabilize, reduce or immobilize environmental toxins from soil, water, ground water, sludge and sediments (Agamuthu *et al*., 2010). Soil contamination is an important environmental problem. Phytoremediation use of green plants including grasses, forbs and woody species to remove harmless environmental contaminants as organic compounds in soil, this definition includes all plant-influenced biological, chemical and physical processes that aid in the uptake to degradation and metabolism of contaminants, either by plants rhizosphere, plant root system capable to translocation, bioaccumulation and contaminant storage/degradation abilities of the entire plant body (Ramos *et al*., 2010). In phytoremediation can provide ideal conditions by the fibrous root system form a continuous, dense rhizosphere (Sanusi *et al*., 2012). Phytoremediation is an efficient technology for erosion control stimulation of biodegradation and/or phytoextraction

of pollutants in soil. Many studies investigated decrease of low and high-molecular-weight petroleum hydrocarbon content in soils undergoing phytoremediation when compared to unplanted soil controls (Hamdi *et al.*, 2012).

One common contaminant is gasoline which is commonly released into the environment from refinery process. Gasoline is complex mixtures of gaseous and liquid organic compounds composed mainly of mono aromatics compounds such as benzene, xylene and toluene, these toxic aromatic have low aqueous solubility or more hydrophilic than polycyclic aromatics and high toxicity levels (Freitas *et al.*, 2011). Gasoline contains, on average approximately 80% paraffins, 14% aromatics and 6% olefins and often small amounts of alcohols and detergents ethers (Keenan *et al.*, 2010). Petroleum hydrocarbons are specific concern in pollution studies due to their structural complexity, bio-magnification and slow biodegradability, potential and above all it can cause serious health hazards associated with the environment (Kathi and Khan, 2011). Objective this study series of testing was initially designed to examine gasoline concentrations in different plant and limited levels for a variety of concentrations gasoline contaminated in soil to remove by phytoremediation process. This study led to indicate that different concentration can plant used in phytoremediation if a concentration is established testing can be used as a simple in-green house treatment and will be done to analyze the in another phytotoxicity test.

MATERIALS AND METHODS

Experimental design to propagation plant in different soil mixtures: The clean garden soil and sand were collected from the Malaysia. Plant seeds were germinated and grown for 3 months in the pots before transferring to the experiment. The plants were grown are carried out in the greenhouse in Universiti Kebangsaan Malaysia in polyethylene bags at June on 2012. Plants species were chosen to propagated (*L. ectovolvis* and *S. mucronatus*.) in different ratios from soil mixtures. Garden soil: sand mixed was as follows: 25:75, 50:50, 75:25, garden soil 100% and sand 100%. Once the selected plant species were at the average height of 15-20 cm, 15 plants was transferred into each of the 10 pots. Each pot was filled with 3 kg of the mixed ratios garden soil to sand. To ensure that the test plants of *L. ectovolvis* and *S. mucronatus* are grown in similar soil environment as in the spiked soil experiment, mixed soil ratios have been kept similar throughout the initial growing period and the tested experiments.

Sampling and analysis: Three plants from each pot with the various mix soil rations were sampled every 7 days. All the plant parts were harvested, rinsed with tap water and distilled water and measured. Both shoots and roots were weighed for the determination of wet and dry weight; the plants were dried at 75°C for 3 days to determine dry weight. Each experiment was conducted using 3 replications.

Preparation of spiked gasoline: The garden soil and sand were sieved with a 4-mm sieve to ensure uniformity and coarse fragment were removed. For best ratio to growth plant choice only mixed garden soil: sand in the ratio 75:25 was with *S. mucronatus* but as 50:50 to *L. ectovolvis* as 3 kg into each pot. Nine different concentrations of gasoline which were 10, 20, 30, 40, 50, 60, 70, 90 and 100 g gasoline/kg for every plant including another pot with no contaminant were used as control. The gasoline was obtained from a local petrol station. It was mixed with acetone as a solvent (Xu *et al.*, 2006). The ratio mixture of gasoline: acetone as 1:1 was poured into the garden soil and sand. After pouring into the soil and sand mix, it was stirred to ensure that the soil mix is homogeneous. The plants were then planted in all pots after contain the varying gasoline concentrations once the acetone has evaporated. During planting, the plants were watered to ensure continual plant growth using tap water after study bulking density result appear 25 mL per 100 g. Pots with the ratio 75:25 soil: sand and 50:50 soil: sand without gasoline were also planted as the control.

Plant growth observation: This preliminary test was conducted to physically observe at what level of gasoline contaminant can the plants grow and survive. The observation was conducted three times a week for 14 days. The observation on the number of withered plants was done in every 3 days. The percentage of withered plants in each concentration was determined relative to the total number of plants in the pot.

RESULTS

Propagation plant in different soil types: One soil ratio mixture will be chosen for best soil for plant growth. The different soil mixture is used to ensure that the mix reaches a high growth in biomass and height of plant in different ratios from garden soil and sand. Results showed that the plant grows well in the soil ratio 50: 50, soil: sand for *L. ectovolvis* in comparison to other soil mix (Fig. 1). While mix soil ratio 75: 25, soil: sand is the best soil mix for the growth of S. *mucronatus* in comparison to the other soil mix ratios (Fig. 2). Plant development was positively associated also with soil water content. The observation and measurements to the plant in wet weight and dry weight increased in different times harvested plants per percent had apparent relationship between wet weight and dry weight yield (Fig. 3 a, b).

The lowest number of plants per pot at the end of the preliminary test process in ratio 25:75 soil: sand. It was observed that plant biomass was highest in 50:50 soils: sand for plant *L. ectovolvis*

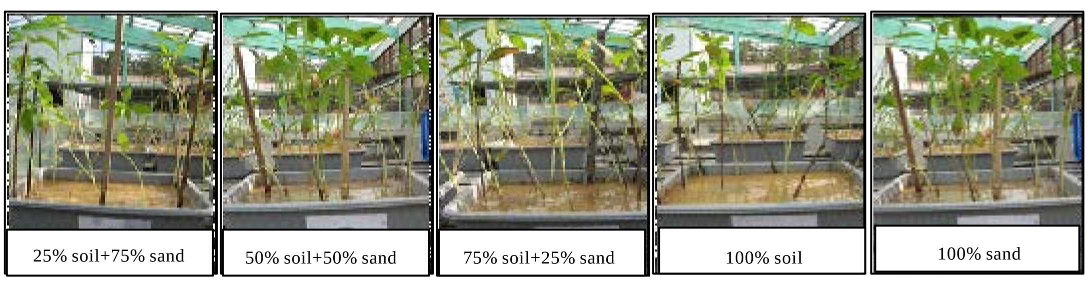

Fig. 1: Garden soil and sand mix in different ratios to propagate plant *L. ectovolvis*

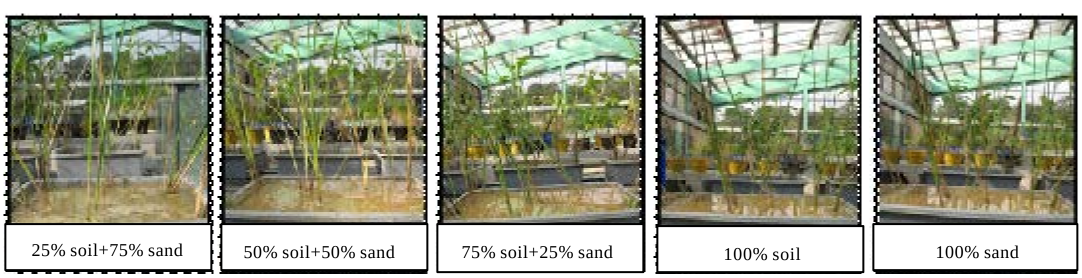

Fig. 2: Garden soil and sand mix in different ratios to propagate plant *S. mucronatus*

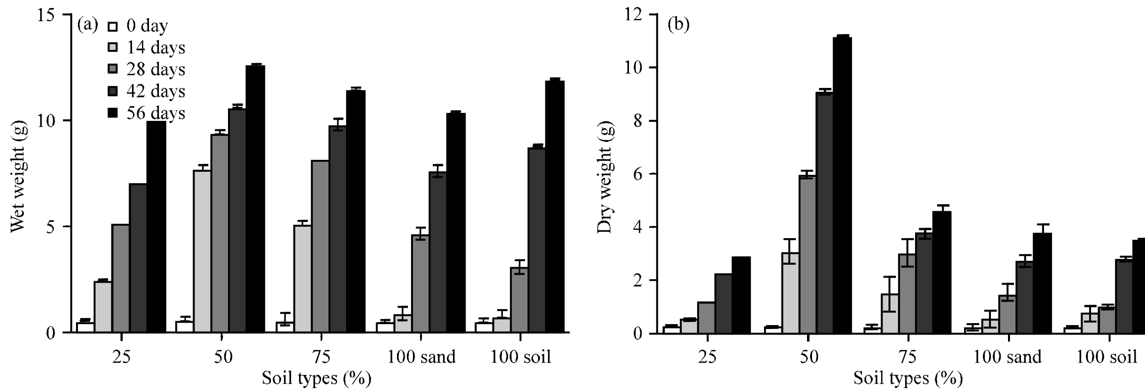

Fig. 3(a-b): (a) Wet weight and (b) Dry weight for plant *L. ectovolvis*

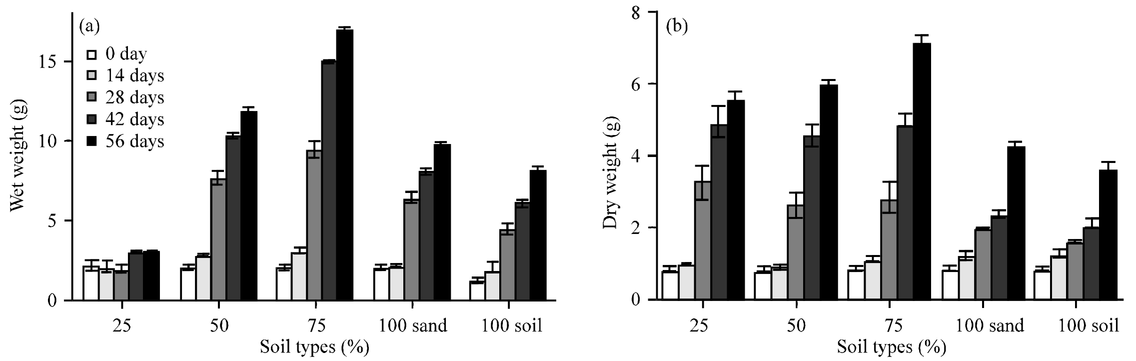

Fig. 4(a-b): (a) Wet weight and (b) Dry weight for plant *S. mucronatus*

Table 1: Soil types properties in different ratio sampling in green house soil: sand in mg L^{-1}

			Soil types		
Soil properties	Soil (%)	Sand (%)	25:75	50: 50	75:25
Na	1.18	2.76	1.98	2.48	0.813
Mg	0.457	0.309	0.231	0.519	0.427
K	3.73	1.918	1.672	4.6	3.36
Ca	1.31	3.01	2.66	2.5	1.28
Al	0.55	0.651	0.492	0.692	0.421
Mn	0.09	0.064	0.11	0.124	0.069
Cl	10.9	3.99	9.55	11.12	1.48
NO_3	0	15.5	15.11	6.08	1.42
PO_3	0.98	0	0.347	0.597	0.621
SO_4	6.96	9.414	0.347	13.8	5.85

(Fig. 3 a, b). And the best wet and dry weight per pot 75:25 soil: sand in plant *S. mucronatus* (Fig. 4 a, b). They concluded that *L. ectovolvis* propagation for 50:50 ratios had a modest potential to tolerate salts and could attain optimum growth under stress of nutrient availability in soil properties (Table 1). For 75:25 ratio of soil the nutrients were lowest as compared to other ratio of soil conditions due to the *S. mucronatus* highest the growth with the lowest value from salts and nutrient. Based on the physical observation of the plant growth for different time and measurement in Fig. 5 it illustrates the 50:50 ratio higher than other soil ratios. The result show the whole

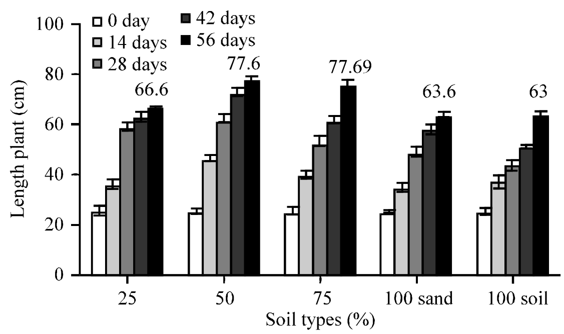

Fig. 5: Length of plant *L. ectovovis* in different soil mix

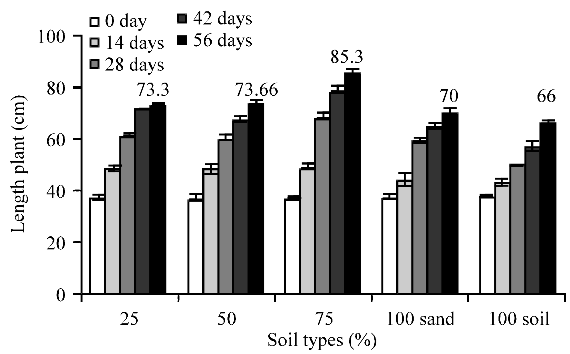

Fig. 6: Length of plant *S. mucronatus* in different soil mix

length of plant was highest 77.6 cm during this mixture on each pot on day 56 but lower show 63.6 cm in 100% sand for *L. ectovolvis* (Fig. 5). On the other hand the obtained results reported *Scirpus mucronatus* could grow successfully. Results showed the lowest length of plants per pot at the end of the preliminary test process at 66 cm but increase the plant length in 75: 25 soil: sand ratio at 85.3 cm (Fig. 6).

Physical growth of *L. ectovolvis* in different gasoline concentrations: This preliminary test was conducted to observe at what level of gasoline contaminant, the physical growth observation was conducted every 3 days on the first week of study for two plants. The range of gasoline concentrations for plant *L. ectovolvis* of 10, 20, 30, 40, 50, 60 and 100 g kg^{-1} were used after one day of exposure the plants died for all of pots in concentrations from 10 g kg^{-1} to 100 g kg^{-1}, respectively. Subsequently, plant *L. ectovolvis* was exposed to (1, 2, 3, 5 and 7 g kg^{-1}) during 14 days (Fig. 7). In conclusion, *L. ectovolvis* can only survive with concentrations of gasoline <5 g kg^{-1} at the end of 10 days (Fig. 8). Results test *S. mucronatus* in different levels of gasoline 10, 20, 30, 40, 50, 60, 90 and 100 g gasoline/kg higher concentration of gasoline affected greater on the withered percentage (Fig. 9). After one day of exposure plants died in concentrations of 90 and 100 g gasoline/kg and turned its colors from green to yellow. While the concentration 40 g gasolines/kg to 70 g gasoline/kg plants died after 7 days. But plants could grow successfully in different levels of gasoline 10 to 30 g gasoline/kg (Fig.10).

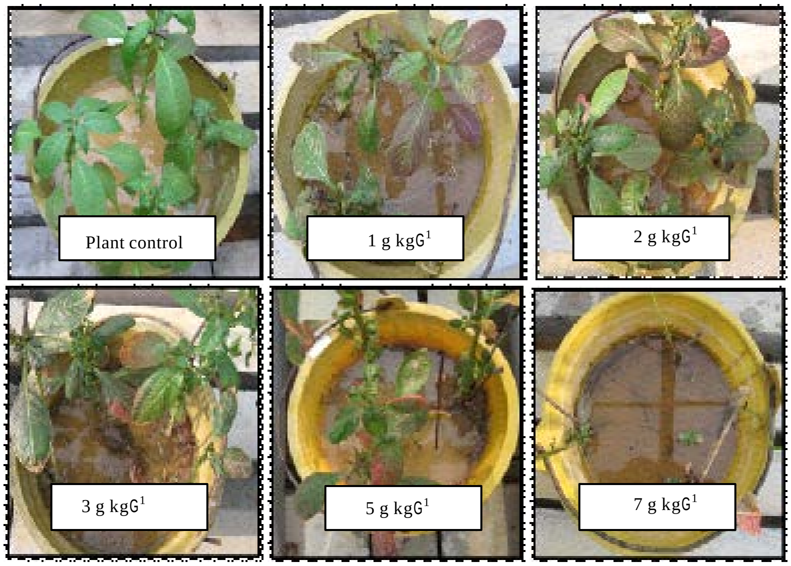

Fig. 7: Growth of *L. ectovolvis* in different gasoline concentrations on 14 days

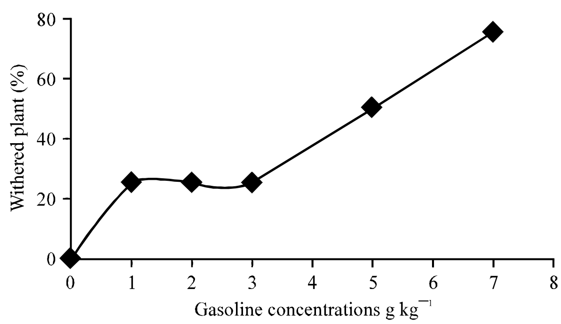

Fig. 8: Percentage of withering *L. ectovolvis* according to gasoline concentration on 14 days

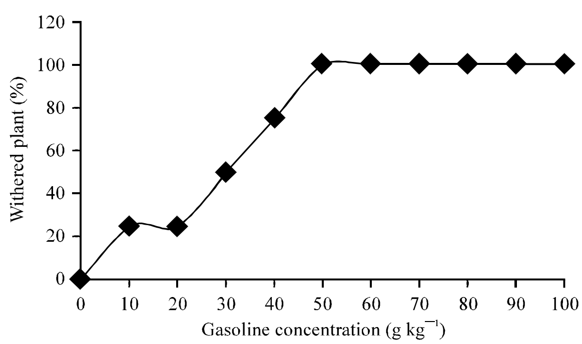

Fig. 9: Percentage of withering *S. mucronatus* according to gasoline concentration on 14 days

DISCUSSION

Propagating, multiplying, perpetuating a species and maintaining the health of plants is one of the most rewarding processes, easy and economical ways of increasing your plant stock. Most

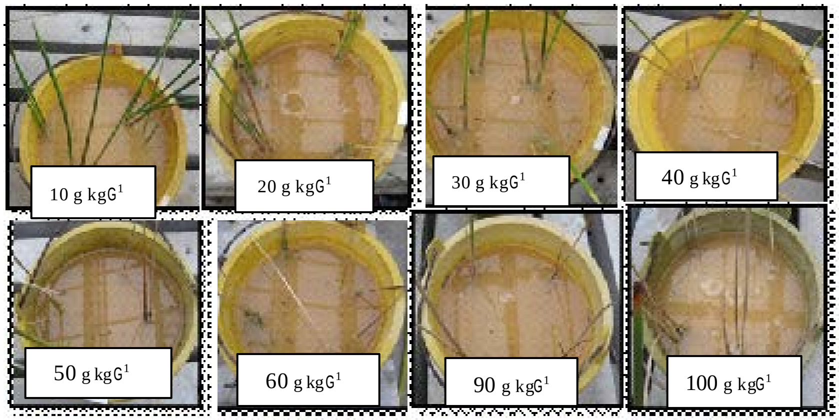

Fig. 10: Growth of *S. mucronatus* in different gasoline concentrations on 14 days

phytoremediation research has focused on specific plant to be used in phytoremediation to enhance the degradation of organic pollutants and gasoline was chosen as a model contaminant to represent petroleum hydrocarbons environment (Kathi and Khan, 2011). Exposure to gasoline can occur for workers on the manufacturing chain, from gasoline production to consumer use, gasoline at docks and gasoline fueling stations or gasoline exposure is inhalation of gasoline vapors during automobile refueling (Keenan *et al.*, 2010). The common name of *Scirpus mucronatus* is bulrush, while *L. ectovolvis* Mexican primrose-willow. Laboratory culture of preliminary experiments was conducted to assess the ability of the *L. ectovolvis* and *S. mucronatus* to survive in different soil mixture after 56 days of measurements to wet and dry weight and plant length.

Under preliminary test the physical observation for two plants in pots with concentrations of gasoline had turned its colors from green to yellow. Similar results were observed in the study (Sanusi *et al.*, 2012). The present study demonstrated that plant *S. mucronatus* can grow in higher gasoline concentrations more from *L. ectovolvis* in all treated soils at the end of 14 days. Also Al-Baldawi *et al.* (2011) demonstrated the ability of the aquatic plant *Salvinia molesta* in preliminary experiments can survive with different concentrations to diesel exposure and showed results the concentration increased, the withering of plant also increased. The continued growth of the plant in the presence of the organic contaminant and other growth in biomass this mean the plant is a potential phytoremediant (Erute *et al.*, 2009). The obtained results are almost similar to the study conducted by Al-Baldawi *et al.*, (2012) which concluded *Azolla pinnata* could not grow successfully in different levels of diesel concentrations of 10, 20, 30, 40 and 50 mL L^{-1} (V Diesel/V Water) after one day. While (Cohen *et al.*, 2002) showed when exposed to diesel all species can survive up to 0.05 mL L^{-1} diesel (v/v) but in concentration of 0.5 mL L^{-1} plant *Azolla pinnata* died due to toxicity of high diesel concentrations. Results show when *S. mucronatus* exposure in different levels of gasoline died after one day in high concentrations. While the concentration 40 g gasolines/kg to 70 g gasoline/kg plants died after 7 days. But grow successfully in low concentration of gasoline the study also agrees with the Purwanti *et al.* (2012) observed when exposure plants *S. mucronatus* to diesel fuel concentrations of 5, 10, 15, 100 and 200 g kg^{-1} had changed their colors, from green to yellow or and plant in concentrations of 100 and 200 g kg^{-1} were withered after 10 days of exposure. Erute *e t al.*, 2009) which concluded that *Paspalum scrobiculatum* can grow in different concentration of crude oil (2.5, 5, 7.5, 10.0, 12.5 and 15.0%) and the length was significantly reduced by the presence of crude oil.

CONCLUSIONS

L. ectovolvis could survive and grow successfully in soil: sand ratio of 50:50, but plant *S. mucronatus* in soil: sand ratio of 75:25. The results were clearly showed that as the gasoline concentration increased the increase in number of plants withering was observed. At the end of 14 days, 20% of *L. ectovolvis* withered at lower concentration of 1 g gasoline /kg and 50% of the plants withered at concentration of 5 g gasoline/kg. But S. *mucronatus* showed 50% withering in 30 g gasoline/kg. Therefore, these two plants is a good candidate for remediation of hydrocarbon contaminated soil. Hence, the future phytotoxicity test will be conducted on 1-5 g gasoline/kg to *L. ectovolvis* and ranging from 10-30 g gasoline/kg to *S. mucronatus*.

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