COMPARATIVE TOLERANCE AND SURVIVAL OF *Scirpus grossus* AND *Lepironia articulata* IN REAL CRUDE OIL SLUDGE

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ABSTRACT

Phytoremediation, a green technology that utilises plants to degrade and remove contaminants from environment, has become a promising treatment for removing many types of contaminants including hydrocarbon pollutants. This technology is potentially efficient, much cheaper and more environmentally friendly than the conventional mechanical clean-up methods. The main objectives of the current study were to evaluate the phytoremediation potential of two native plant species in Malaysia, Scirpus grossus and Lepironia articulata by comparing their tolerance and survival of both plants in real crude oil sludge. A preliminary test was conducted to observe whether these two plants could survive in the oily sludge. The growth characteristic of both plants was evaluated by observing the visible symptoms of withering and death of plants throughout a 30-day exposure. S. grossus and L. articulata were exposed to 3 kg of crude oil sludge in 3 L pails under greenhouse conditions. After 30 days of exposure, it was observed that 100% S. grossus could grow and survive in the sludge compared to L. articulate with only 55% survival. As a comparison, S. grossus has more potential to be used in the phytoremediation process of the real crude oil sludge.

Keywords: *Lepironia articulata*, Oily sludge, Petroleum hydrocarbons, Phytoremediation, *Scirpus grossus*.

INTRODUCTION

Oily sludge is polluted solid waste produced from petroleum industry mainly during its crude oil exploration, refining process, transportation and storage [1,2]. Disposed oily sludge normally contains toxic substances such as aromatic hydrocarbons (benzene, toluene, ethyl benzene and xylene) and poly-aromatic hydrocarbons [3]. In general, oil sludge is a complex water-in-oil (W/O) emulsion, usually includes 30–50% of water, 10–12% of solids by mass and 30–50% of oil or total petroleum hydrocarbon (TPH) [4]. Soil contamination with oily sludge or hydrocarbon can give a significant damage to environment since accumulation of pollutants in animals and plant tissue may cause death and mutations [5]. During the past years, a variety of treatment for oily sludge contaminant using physical, chemical and biological processes have been developed such as incineration, landfill, burning, solidification/stabilization, pyrolysis, photocatalysis, solvent extraction, chemical treatment and biodegradation [1]. However, most of the treatment methods have limited effectiveness, are expensive and also generate incomplete decomposition of contaminants [5]. Therefore, research and application of phytoremediation for treatment of petroleum hydrocarbon or crude oil sludge can provide useful information to treat this contaminant. Phytoremediation process by definition is a biological technology that utilizes plants to stabilize, extract, accumulate, degrade or transform contaminants in sediments, soils or aquatic environments [6,7]. This process utilizes the plant's metabolic system such as phytodegradation, phytostabilization or phytoextraction to remove nutrients and contaminants from their surrounding and store in their biomass [8]. Many plants species like bean and wheat are sensitive to petroleum contaminants and was reported having reducing growth rate in the presence of petroleum hydrocarbon [9]. Inhibition of plant growth parameters like plant length, germination and biomass due to toxic compound of petroleum hydrocarbons can be observed [10]. Therefore, employing native plant species that are tolerant and can survive with high concentration of petroleum hydrocarbon in crude oil sludge can be a key factor in the success of phytoremediation process. Although a large number of research discussing on phytoremediation of organic pollutants like petroleum hydrocarbon reported in the literature, only a few application of phytoremediation using two different native plant species like *Scirpus grossus* and *Lepironia articulata* on real crude oil sludge to represent petroleum hydrocarbon pollutant have been carried out. Both *S. grossus* and *L. articulata* are emergent plants. *S. grossus* is perennial aquatic plant with common name giant bulrush, greater club-rush, rumput menderong (Malaysia), mensiang and walingi (Indonesia) [11, 12] with a high growth rate and has the ability to degrade contaminants. It has been used to treat bauxite [11], lead [13], gasoline [14], hydrocarbon [15] and dye [16]. While, *L. articulata* commonly called as blue rash, grey sedge and tube sedge [11] also has been used to reduce contamination in water for bauxite [11] and PAHs [17]. The main objective of the present study was to determine the phytoremediation potential of both *S. grossus* and *L. articulata* as well as their growth behavior in real crude oil sludge. The most commonly observable or visual stress symptoms for both plants in this study are growth abnormalities such as wilting and death of the whole plant.

METHODOLOGY

Propagation of plants and Source of Real Crude Oil Sludge

In this study, both the native plants, *S. grossus* and *L. articulata* are taken from its natural habitat (wetlands) from Tasik Chini, Pahang. Both plants were propagated under greenhouse condition using garden soil containing top soil, organic fertilizer and sand with a ratio of 3:2:1. During plant propagation, physical parameters were measured to determine plant growth characteristics in normal condition. All of the plants used in the research were 3 months old before being used in the ranged finding test. Hence, plants with the same height and same age were used in this experiment [12]. The raw crude oil sludge in this study was obtained from a contaminated site at surrounding temperature (37℃) from a petroleum industry in Malaysia. The parameters analyzed from the extracted crude oil sludge were listed in Table 1.0.

Table1 Characteristics of crude oil sludge use for the survival and tolerance study

Parameters	Value (mg/kg)
Total Petroleum Hydrocarbon (TPH)	56,132
Benzene, Toluene, Ethylbenzene, xylene (BTEX)	<0.001
Ammonia (**NH_3-N**)	25.3
Nitrate (**NO_2^{-}N**)	0.005
Nitrite (**NO_3-N**)	8.2
Phosphate (**PO_4^{3-}**)	2.94
pH (no unit)	6.21

Tolerance and Survival Studies

Scirpus grossus and *Lepironia articulata* were cultivated and exposed in 100% concentration of real crude oil sludge over 30 days in a greenhouse. For each pail, three plants of each species were planted (Table 2.0) resulting in a total of 12 plants for each species required, to be exposed 3 kg of real crude oil sludge in a 3 L pail. The pails were placed inside the greenhouse under sunlight and were watered every day to maintain a constant and sufficient moisture level for both plant. There were three replicates (R1, R2, and R3) for the pails with plants, and another pail (PC) containing plants with sands only without the crude oil sludge.

Table 2 Experimental set-up of plant exposure for each plant

Condition	R1	R2	R3	PC
100% concentration of crude oil sludge	**xxx**	**xxx**	**xxx**	**xxx**

R1, R2, and R3 = Replicates; PC = Plant control without sludge; **x**= Plant

Monitoring of plant growth was carried out for 30 days on each sampling day (0, 7, 14, 21 and 30) from the three replicates of each plant. Their condition were described as healthy or dead. According to Ismail et al. [11], at the end of the observation day, the percentage of plants which has survived throughout the study period was calculated using Eq. (1):

$$\text{Plant survival (\%)} = \frac{\text{No of Healthy Plants}}{\text{Total Number of Plants}} \times 100 \qquad (1)$$

RESULTS

Physical Growth of *Scirpus grossus* and *Lepironia articulata*

The physical growth observation for each plant in pail was conducted on the dedicated sampling days. Each plant in each pail was observed based on condition of being healthy, withered or dead. Table 3 depicts the images of plant growth of *S. grossus* and *L. articulata* on day 0 until day 30 exposure.

Based on the observation, after 7 days of exposure, *S. grossus* plants were still healthy but for *L. articulate*, two plants had turned its colors from green to yellow and finally died on the first week of exposure. One of the 12 *S. grossus* in the four pails and four *L. articulata* plants were dead on the following week of exposure. Plant death continued for both *S. grossus* and *L. articulata* until day 21 with one additional sapling grew from *S. grossus* plants as shown in Figure 1. On the last week of exposure, all *L. articulata* plants were dead except for *S. grossus* plants that were still have four healthy plants including two new saplings appearance.

Figure 2 illustrates the survival percentage for *S. grossus* and *L. articulata* at the end of the observation period. Both plants show different survival abilities at the 100% concentration of real crude oil sludge. Both plants in the PC pails survived until day 21 of observation with a 100% survival rate without the addition of real crude oil sludge in the sand medium. The ability of *S. grossus* to survive can reach up to 30 days of exposure, while *L. articulata* can survive only up to day 21. The survival rates for the two plants at day 21 were 77.8% for *S. grossus* and 22.0 % for *L. articulata*. It was observed that *S. grossus* could survive in 100% concentration of real crude oils sludge until day 30 with more than 50% survival rate compared to *L. articulata* that cannot survive at all on the last week of exposure.

Table 3 Plant growth observation of *Scirpus grossus* and *Lepironia articulata* on day 0 until day 30

Days	Plant Control (PC)	*Scirpus grossus*			Plant Control (PC)	*Lepironia articulata*		
		R1	R2	R3		R1	R2	R3
0	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy
7	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy	 All plants were healthy	 2 plants healthy 1 dead	 2 plants healthy 1 dead
14	 All plants were healthy	 All plants healthy 1 sapling	 All plants were healthy	 2 plant healthy 1 dead	 All plants were healthy	 1plant healthy 2 dead	 2 plant healthy 1 dead	 2 plant healthy 1 dead
21	 All plants were healthy	 All plants healthy 1 sapling	 2 plants healthy 1 dead	 2 plants healthy 1 dead	 All plants were healthy	 All plants dead	 1 plant healthy 2 dead	 1 plant healthy 2 dead
30	 All plants were healthy	 2 plants healthy, 1 dead 1 sapling	 2 plants healthy,1 dead	 All plant dead 1 sapling	 All plant were healthy	 All plant dead	 All plants dead	 All plants dead

Figure 1 Saplings from *Scirpus grossus*

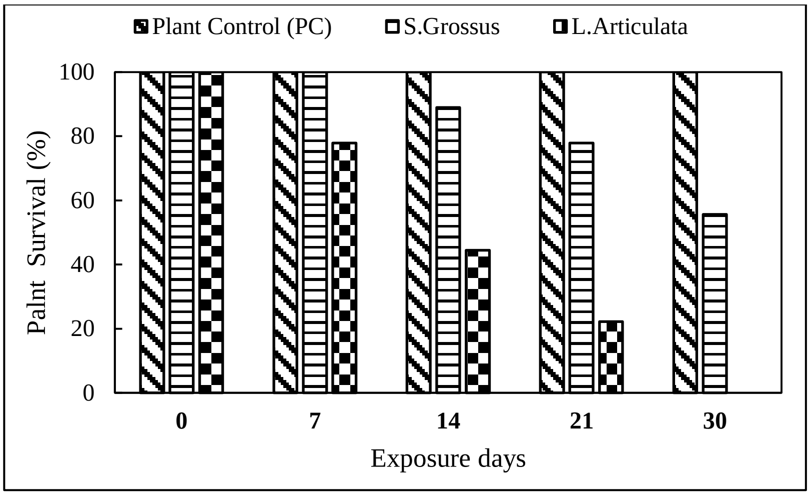

Figure 2 Percentage survival of *Scirpus grossus* and *Lepironia articulate* compared to the plant control

DISCUSSION

Generally, phytoremediation of organic contaminant by plants involve one of five main mechanisms which are phytotransformation, rhizosphere bioremediation, phytostabilization, phytoextraction and rhizofiltrationn [18]. According to Truu et al. [19], factors that contribute to the uptake, distribution and transformation of organic compounds like petroleum hydrocarbon are mainly related to physical and chemical properties of compound (e.g. water solubility, molecular weight, octanol-water partition coefficient), as well as environmental conditions (e.g. temperature, pH, organic matter, and soil moisture content) and also plant characteristics (e.g. root system, enzymes. Other than that, plants also are known to have the ability to respond and adapt to a variety of biotic and abiotic stresses [20]. Therefore, in soils polluted with organic chemicals, plants will experience a combined stress from nutritional deficiency and chemical toxicity. In this case, it is due to the 100% concentration of oily sludge used to test the performance of both *S. grossus* and *L. articulata*. *S. grossus* have shown the capability to withstand to relatively high concentration of Total Petroleum Hydrocarbon (TPH) (88,955 mg/L) and somehow can uptake and convert chemical to less toxic metabolites. In this experiment, the percentage survival of both *S. grossus* and *L. articulata* decreased from day 0 to day 30. This is because the presence of high content of Total Petroleum Hydrocarbon (TPH) in the oily sludge had affected the growth of plant when compared with control plants.

The results on the growth response obtained in this experiment, indicated that *S. grossus* plant is a good choice to develop remediation assays, although its growth was slightly affected, the plants appeared healthy and continued their normal development and even produced saplings. In contrast to the performance of *S. grossus*, *L. articulata* was unable to survive due to high concentrations of Total Petroleum Hydrocarbon causing root damage to this plant species. This inhibitory effect on plant growth may be attributed to the physical constraints induced by maximum concentration of contaminant. Total Petroleum Hydrocarbon (TPH) would cause a film of oil to form around the plant root which would act as a physical barrier, preventing or reducing both water and oxygen transfer to the plants [21]. However, a study by Ndimele et al. [22], shows that several species of plants have been shown to have the ability to grow in contaminated soils and basically can also extract the pollutant from the growth medium, The.r results are consistent with the results obtained giving evidence that *S. grossus* plant can survive even though being exposed in the maximum concentration of pollutant. According to Zand et al. [10], this kind of plant can convert the pollutants to less toxic compound and also some of plant roots can filter contaminant from the medium. In addition, as shown by another previous study [7], grass root systems have the maximum root surface area (per $m^3$of soil) of any plant type and may penetrate the soil to a depth of up to 3 m in which *S. grossus* also belongs to the grass types plant as well.

CONCLUSION

Based on the obtained results, as the days of exposure increased, the withering symptoms and plant death of *S. grossus* and *L. articulata* also increased, thus reducing the survival of these two plants due to toxicity of petroleum hydrocarbon. However, *S. grossus* is a promising species for phytoremediation process since the percentage survival of *S. grossus* in 100% concentration of oily sludge at day 30 was more than 50%. In contrast, *L. articulata* could not survive at all (0% survival) with the plants started to die after one week of exposure and all totally died at the end of 30-day exposure. Further research will be explored to increase the potential of phytoremediation process in remediation of petroleum hydrocarbon through utilization of associated microbes isolated from the rizosphere of plants as phytoremediation of petroleum-hydrocarbons is presumed to be based on the stimulation of microbial degradation in the rhizosphere.

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