Preliminary Test of Hydrocarbon Exposure on *Lepironia articulate* in Phytoremediation Process

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Abstract. The preliminary tests is physically and visually observed to estimate the concentrations of contaminants that can give negative effects on plants growth and at which concentration the plants can tolerate and survive. In this study, bulrush of *Lepironia articulate* was subjected to diesel to assess its ability to survive when exposed to diesel contaminants with different concentrations (0, 5, 10, 20, 30, 35 and 40 mL diesel/L water). The investigation was conducted in 30 days with two flow systems, free surface (FSF) and sub-surface flow (SSF), were adopted to observe in which system the plant can tolerate to diesel better. These findings will be applied in future study of phytotoxicity test to assess its ability to phytoremediate polycyclic aromatic hydrocarbon (PAHs) contamination in wastewater. At the end of diesel exposure, the result indicated that the plant growth in SFS was better compared to FSF system. The plant had shown that it could only grow and survive in concentrations of 5, 10, 20, 30, 35 and 40 mL/L water in SSF and in concentrations of 5, 10 and 20 mL/L in FSF system. The results of this study will be a basis for future study that aims to study PAHs removal from water using *L. articulate* plants.

Introduction

Environmentally hazardous and the high cost of some conventional treatment processes has produced economic pressures and caused engineers to search for creative, cost effective and environmentally ways to control water pollution. Phytoremediation could be a sustainable remediation alternative for conventional remediation technologies [1]. It has several advantages: it can be applied in situ over large areas, with low cost, and the soil does not undergo significant damages [2]. This green technique is also easily accepted and often welcomed by the general public due to its significant improvement of the impacted landscape. The ability of plants to remove contaminants from the environment has been recognized and taken advantage of its applications such as land farming of waste [3]. Phytodegradation uses plants and microorganisms to break down organic pollutants in situ [4]. It includes several processes namely, phytoextraction, phytodegradation, hizofiltration, phytostabilization and phytovolatilization [5,2]. This technology is applicable to different contaminants, including metals and radionuclides, as well as organic compounds like chlorinated solvents, polychlori- biphenyls, polycyclic aromatic hydrocarbons, pesticides/insecticides, explosivesand surfactants [6]. There are many researchers who had proven the effectiveness of plants to remove contaminants from the soil, water and air. *Abelmoschus moschatus*, *Eleocharis quiesetina* J. et C.Presl, *Acacia confusa* wil., and *Eucalyptus saligna*, *Ludwigia octovalvis* and *Scirpus grossus* are particularly effective in translocating heavy metals to easily harvestable leaves and shoots [7,8]. The *bulrush* (*Scirpus grossus*) also can be effectively applied to phytoremediation of water contaminated with total petroleum hydrocarbon TPH [9].
The success of phytoremediation is dependent on a large number of factors, such as types and concentrations of contaminant, selection of appropriate vegetation, plant growth and survival, type of system (free surface (FSF) or sub-surface flow (SSF)) and site climate. So, a greenhouse study and pilot testing (preliminary test) is required before a full-scale system can be installed to ensure that the remedy is effectively performed by selected plants. A preliminary test in phytoremediation is necessary to determine the ability of plant to survive in media with different concentrations of contaminant. It is conducted to determine the maximum concentration of the contaminant that is harmful to plant and suppress its growth. High contaminant concentration may cause root damage. This has serious consequences for nutrient and water supply to aboveground plant parts [10]. In this work, we investigate the capability of *Lepironia articulate* to survive and grow in different concentration of diesel in two systems, sub surface flow (SSF) and free surface flow (FSF). Constructed SSF and FSF wetlands are being increasingly implemented worldwide into wastewater treatments [11]. The different between the SSF and FSF is the layer of water. FSF has a thin layer of water above the media whereas the water level of the SSF is kept just below the top of the media [12].

The diesel was chosen as hydrocarbon contaminants due to the presence of aromatic hydrocarbons (PAHs) such as benzene, toluene, ethyl benzene and xylene and it is more toxic to plants than crude oil [13]. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in the environment and are derived from both man-made and natural resources [14]. PAHs is considered great threat to the environment and human beings due to their toxicity to the higher organisms and resistance to microbial attack [15]. The hydrophobic nature of PAHs makes their clean up extremely difficult and allows them to persist for longer periods [14].

*Lepironia articulate* (Fig. 1) is commonly known as grey sedge or krajood [16]. It has rhizome horizontally creeping and woody. Its shoot is cylindrical, greyish green, hollow and transversely septate. It belongs to the family Cyperaceae and is the margins of fresh water swamps, and swamp forest [4].

![Fig. 1: An image of Lepironia articulate.](image)

**Materials and method**

First, synthetic wastewater was prepared by mixing water with different diesel concentrations (0, 5, 10, 20, 30, 35 and 40 mL diesel/L water). Secondly, three healthy bulrush plants of *Lepironia articulate* were planted in 6 pails each filled with 3 kg of sand (SSF and FSF). Next, synthetic wastewater was poured into the sand for both SSF and FSF systems which required 0.8 and 2 L of wastewater respectively. Throughout the exposure, addition of some water to the plants was done occasionally to ensure the plants having sufficient water to grow. Fig. 2 shows the different between SSF and FSF system.
The plant growth was observed physically for 30 days in the two systems (SSF and FSF) to investigate the ability of the plant to survive and resist the hydrocarbon contaminant. At the end of exposure period, the percentage of withered plants was determined using the following Eq. 1:

\[
\text{% the percentage of withered plants} = \frac{\text{number of withered plant}}{\text{number of total plant}} \times 100
\]  

(1)

Also, the number of died plants in each pail are recorded to determine the LC50 values. LC50 is defined as the concentration at which 50% lethal of the population of experimental organisms. The diesel concentration that results in 50% of the measured effect can be determined from the dose-response relationship graph. The dose-response relationship describes the change in effect on an organism caused by different concentration exposure of contaminant [17].

**Results and Discussion**

After 30 days of exposure to diesel contaminants, the plant had shown that it could grow and survive in all diesel concentration (0, 5, 10, 20, 30, 35 and 40 mL /L) in the subsurface flow system. As shown in Fig 3, all bulrush plants were still green in 0, 5, 10 and 20 mL /L, one bulrush withered in 30 and 35 mL /L and two bulrushes were withered in 40 mL /L as depicted in Fig 3.

In the free flow system, all bulrush plants could grow and survive in 0 and 5 mL/L, two bulrushes were withered in 10 and 20 mL /L but all died in 30, 35 and 40 mL /L as shown in Fig 4.
The percentage of withered plants in each concentration was determined relative to the total number of plants in the pot. The number of withered plants was recorded, and their percentage was determined using Eq (1). As shown in Fig. 5, the withered plant percentage was 0% in 0, 10 and 20 mL/L, 33% occurring in 30 and 35 mL/L, 66.7% in 40 mL/L in SFS. In the free flow system, 0% of plants were withered in 5 mL/L, 66% in 10 and 20 mL/L and 100% withered in 30, 35 and 40 mL/L.

The dose-response curve plotted as a cumulative number of withered plants by each diesel concentrations used for SSF and FSF systems. The concentration effect of the diesel on the withered percentage of *Lepironia articulate* correlated as a linear regression (R²=0.8426) and the range of 50% withered plants appears approximately at 39.2 mL/L in SSF. In FSF (R²=0.7773) and the range of 50% withered plants appears approximately at 14.16 mL/L (Fig 6).
These results and calculations have indicated that the plant growth in SFS had performed better compared to the FSF system. This may be due to no sticky layer preventing the arrival of oxygen to plant roots that is required by microbial degradation process.

**Conclusions**

Laboratory culture of preliminary experiments was conducted to assess the ability of *Lepironia articulata* to survive at different diesel concentration in two system exposure systems (SSF and FSF). The results clearly show that whenever the concentration increased the withered plant number also increased. The range of 50% withered plants was found at approximately 39.2 mL/L concentrations in SFS and at 14.16 mL/L in FSF. Therefore, diesel concentrations of less than 39.2 mL/L in SFS and less than 14.16 in the FSF system will be considered for future phytotoxicity studies to ensure that plants are able to survive, as the value of 50% withered plants means that plants cannot survive and indicates that the diesel concentration was too high for this species.

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