INTRODUCTION

Environmental hazards and the high cost of some conventional treatment processes have produced economic pressure and caused engineers to investigate for creative, cost effective and environmentally ways to control water pollution. Phyto remediation could be a sustainable remediation alternative for conventional remediation technologies [1]. It has several advantages e.g., it can be applied in situ over large areas, with low cost and the soil does not undergo significant damage [2]. This green technique is also easily accepted and often welcomed by the general public due to its significant improvement of the impacted landscape. It offers an appealing option to managers of remediation projects and is frequently listed as the best available technology to remediate contaminated sites [3]. The ability of plants to remove contaminants from the environment has been recognized and has been taken advantage of for its applications, such as land farming of waste [4]. Phytodegradation uses plants and microorganisms to break down organic pollutants in situ [5]. It includes several processes, namely, phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization [2,6]. This technology is applicable to different contaminants, including metals and radionuclides, as well as organic compounds like chlorinated solvents, polychlorobiphenyls, polycyclic aromatic hydrocarbons, pesticides/insecticides, explosives and surfactants [7]. It is used to treat contamination in industrial [8,9] and agricultural wastewater [10,11], mine drainages [11] and landfill effluent wastewater [12]. It has also been used to treat contamination with organic pollutants [13] such as petroleum. Petroleum products as energy sources around the world have caused widespread pollution by hydrocarbon, causing change in the properties of surface waters, sediments and biota [14]. There is much research that has proven the effectiveness of plants to remove contaminants from the air, soil and water [15]. Table-1 shows some previous studies on plants used to remove different contaminants.

The success of phytoremediation is dependent on a large number of factors, such as the types and concentrations of the 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 test are required before a full-scale system can be installed to ensure that the remedy with selected plants is effective. The
toxicity test of contaminants in phytoremediation is necessary to determine the ability of the plants to survive in media with different concentrations of the contaminant to determine the highest concentration of pollutants that can damage the plant and stifle its growth [25].

High contaminant concentrations may cause root damage. This has serious consequences for nutrients and the water supply for above ground plant parts [26]. In this work, we investigate the capability of Lepironia articulate to survive and grow in different concentrations of diesel in two subsurface flow and free surface systems. Constructed sub-surface flow and free surface wetlands are being increasingly implemented worldwide into wastewater treatments [27]. The difference between the sub-surface flow and free surface is the layer of water. Free surface has a thin layer of water above the media, whereas the water level of the sub-surface flow is kept just below the top of the media [28].

Diesel was chosen as the hydrocarbon contaminant due to the presence of polycyclic aromatic hydrocarbons (PAHs) such as benzene, toluene, ethyl benzene and xylene and because it is more toxic to plants than crude oil [29]. Polycyclic aromatic hydrocarbons are ubiquitous pollutants in the environment and are derived from both man-made and natural resources [30]. Polycyclic aromatic hydrocarbons are considered a great threat to the environment and human beings due to their toxicity to the higher organisms and resistance to microbial attack [31]. The hydrophobic nature of polycyclic aromatic hydrocarbons makes their clean-up extremely difficult and allows them to persist for longer periods [30]. Dskowitzy et al. [32] in study of Segara Anakan, a mangrove-fringed coastal lagoon in Indonesia, found > 50 organic contaminants in the water, sediment and macrobenthic invertebrates from the lagoon, most of which were polycyclic aromatic compounds. Total emission of polycyclic aromatic hydrocarbons has been estimated at 8,600 tons per year in the USA and about 14,100 tons/year in Europe and the present level of these compounds is about 50 times higher than in the preindustrial period. Fig. 1 shows the annual emissions of polycyclic aromatic hydrocarbons in different countries in 2002 [33].

On the Norwegian Continental Shelf (NCS) in 2012, there were a total of 122 small incidents with a total oil discharge of 16 cubic metres; acute spills of chemicals have been stable at 100-150 incidents per year and large chemical spills in 2007, 2009 and 2010 came from leakages from injection wells [34]. An extensive analysis was carried out for Al Ruwais refinery wastewater (United Arab Emirates) during the period of June 2002 to June 2003 and the analysis showed high concentrations of polycyclic aromatic hydrocarbons and phenolic compounds [35]. Kalmykova et al. [36] found organic pollutants in landfill leachates, such as alkylphenols, phthalates and polycyclic aromatic hydrocarbons at levels exceeding water quality standards.

In Malaysia, most hazards are from the main source of energy, which is petroleum. Most of the studied locations showed a high contribution of polycyclic aromatic hydrocarbons from combusted fuel, coal, biomasess and wood materials, except for the southern part of Peninsular Malaysia, which revealed a dominance of petroleum products [37]. In Malaysian and Singaporean waters, 39 oil spill incidents from tanker accidents have been documented from 1960 to 1993 [38] and nearly 70-80 % of rivers and streams in Malaysia carried polluted water [39]. This enormous amount of pollution makes us here in Malaysia look for the native and correct plant for use in removing pollutants. In this study, we are looking for a plant that can be used to break down and remove polycyclic aromatic hydrocarbons from wastewater. This plant should be able to live and adapt and resist the pollution source of polycyclic aromatic hydrocarbons-diesel—in this research. Lepironia articulate has been selected for the study of the diesel effect on this plant.

L. articulate is commonly called grey sedge or krajoed [3]. It has a horizontally creeping and woody rhizome. Its shoot

---

**TABLE-1**

| Plant | Contamination | Contaminant media | Ref. |
|-------|---------------|------------------|------|
| Abelmoschus moschatus, Eclerocharis quissetina J. et C. Presl. | Heavy metals | Soil and water | [16] |
| Acacia confusa wild. and Eucalyptus saligna | | | |
| Pinus densiflora, Populus tomentiglandulosa and Thuja orientalis | | | |
| Ludwigsia octovalvis | Arsenic | Sand | [17] |
| Populus alba L. and Populus nigra L. | Cd, Cu, Fe, Pb and Zn | Soil | [18] |
| Helianthus annuus L | Cd and Zn | Soil | [19] |
| Juncus maritimus and Phragmites australis | Cd | Sand | [20] |
| Malva parviflora, Portulaca oleracea, Cynodon dactylon, Chhenopodium album and Amananthus albus | Nutrients and metals | Soil | [21] |
| Tomato, sunflower, soybean and alfalfa | Aged DDTs residues | Soil | [22] |
| Salix viminalis | Polycyclic aromatic hydrocarbons | Soil amended with contaminated sewage sludge | [23] |
| Scirpus grossus | Total petroleum hydrocarbon (TPH-D) | Water | [13] |
| Pinus densiflora, Populus tomentiglandulosa and Thuja orientalis | Diesel | Soil | [24] |

---

**Fig. 1.** Annual emission of polycyclic aromatic hydrocarbons in different countries [Ref. 33]
is cylindrical, greyish green, hollow and transversely septate. It belongs to the family Cyperaceae and it is on the margins of fresh water swamps and swamp forest [5]. *L. articulate* used in this study was taken from Tasik Chini, Pahang in Malaysia and Fig. 2 shows the physical appearances of this plant.

Any plant that is to be used for phytoremediation must first be tested to determine its tolerance to exposure by means of a phytotoxicity test and an extended phytotoxicity test [25]. Hence, this study aims to ascertain the highest concentration of diesel that the *L. articulate* is able to withstand and which system (sub-surface flow or free surface) is better for its growth. The results of this study will then contribute to a research project to remove and degrade hydrocarbon by the phytoremediation method.

### EXPERIMENTAL

Firstly, synthetic wastewater was prepared by mixing water with different diesel concentrations (0, 5, 10, 20, 30, 35 and 40 mL diesel/L water). It was done by mixing tap water with diesel. For example, for 5 mL/L, we added 5 mL diesel for every one liter of water and so with the rest of the concentrations. We needed a pail without diesel to act as a control to compare with diesel concentration to know if the effects were from the diesel or from other conditions. Secondly, three healthy bulrush plants of *L. articulate* were planted in six pails each filled with 3 kg of sand (sub-surface flow and free surface). Next, synthetic wastewater was poured into the sand for both sub-surface flow and free surface systems, which required 0.8 and 2 L of wastewater, respectively. Throughout the exposure, the addition of some water to the plants was done occasionally to ensure that the plants had sufficient water to grow. Also, the level of water will decrease with the time due to natural evaporation so we needed to maintain the level of water to simulate sub-surface flow and free surface conditions. Fig. 3 shows the difference between sub-surface flow and free surface systems.

The plant growth was observed physically for 30 days under greenhouse conditions in the two systems (sub-surface flow and free surface) in order 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 by using the following eqn.:

\[
\text{Withered plant (\%) = } \frac{\text{Number of withered plants}}{\text{Number of total plants}} \times 100
\]

Also, the plants were observed for 30 days and the number of plants that died in each pail was recorded to determine the LC50 values. LC50 is defined as the concentration that is lethal for 50 % 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 a different concentration exposure to the contaminant [41].

### RESULTS AND DISCUSSION

The observation was carried out on a daily basis for 7 days during the exposure period and then three times a week until the end of the period. In this experiment, observations of withering effects of diesel on the physical plant (wilted or dried) were conducted, based on the change of colour from the usual green colour to a brown colour.

After 30 days of exposure to diesel contaminants, the plant had shown that it could grow and survive in all diesel concentrations (0, 5, 10, 20, 30, 35 and 40 mL/L) in an sub-surface flow system. As shown in Fig. 5, all bulrush plants were still green in 0, 5, 10 and 20 mL/L concentrations; one bulrush withered and died in 30 and 35 mL/L diesel concentrations and two bulrushes withered in 40 mL/L.

In the free flow system, all bulrush plants could grow and survive in 0 and 5 mL/L, two bulrushes withered and their colour changed to brown and then died in 10 and 20 mL/L, but all died in 30, 35 and 40 mL/L, as shown in Fig. 4.

In the experimental set-up, there was a pail planted without diesel (acting as a control) and all the plants in this pail grew and survived. Also, all plants were able to grow and survive in low concentrations, up to 20 mL/L in the sub-surface flow system and 5 mL/L with the free surface system. So the plant in another pail died due to the high concentration of diesel.

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 eqn. 1. As shown in Fig. 5, the withered plant percentage was 0 % at 0, 10 and 20 mL/L; 33 % occurring at 30 and 35 mL/L; and 66.7 % at 40 mL/L in sub-surface flow. 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 result of this study suggested that this plant showed a high resistance to diesel concentrations and performed better compared to plants from other studies, which were offered to different concentrations of diesel. For example, in the study of *Azollapinnata* [42], the plant showed resistance to diesel concentration only up to 0.5 mL/L. Also, *Eleocharis ochrostachys* and *Scirpus grossus* up to 5 and 2.5 mL/L, respectively, with the sub-surface flow system [43,44]. However, the plant in this study can survive and tolerate a 39.2 mL/L diesel concentration. Therefore, it is expected that this plant will give impressive results if used to treat diesel contaminated soil or water, due to its high ability to adapt to it.

The dose-response curve is plotted as a cumulative number of withered plants by each diesel concentration used for sub-surface flow and free surface systems. The concentration effect of the diesel on the withered percentage of *Lepironia articulata* correlated as a linear regression ($R^2 = 0.8426$) and the range of 50 % withered plants appears approximately at 39.2 mL/L in sub-surface flow. In free surface ($R^2 = 0.7773$) 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 sub-surface flow shows a better performance compared to the free surface system due to the non-existence of a sticky layer that could prevent the arrival of oxygen to plant roots, which is needed for microbial degradation process.

Conclusion

A laboratory culture of preliminary experiments was conducted to assess the ability of Lepironia articulata to survive at different concentrations of diesel in different system exposure systems viz., sub-surface flow and free surface. The results clearly showed that whenever the concentration increased, the number of withered plants also increased. The range of 50 % withered plants was found at approximately 39.2 mL/L concentrations in sub-surface flow and at 14.16 mL/L in free surface. There-before, diesel concentrations of less than 39.2 mL/L in sub-surface flow and less than 14.16 mL/L in free surface were considered for future phytotoxicity studies to ensure that plants are able to survive, with a value of 50 % withered plants. This result indicates that this plant is a good choice to use in phytoremediation of polycyclic aromatic hydrocarbons from diesel and was better than other plants that were used in previous studies.

ACKNOWLEDGEMENTS

The authors thank the Universiti Kebangsaan Malaysia (DIP-2014-020) and Tasik Chini Research Centre for supporting this research project and the Higher Education Ministry of Libya for providing a doctoral scholarship to first author.

REFERENCES

1. N. Witters, R. Mendelsohn, S. Van Passel, S. Van Slycken, N. Weyens, E. Schreurs, E. Meers, F. Tack, B. Vanheusden and J. Vangronsveld, Biomass Bioenergy, 39, 470 (2012).
2. C. Camesselle, R.A. Chirakkara and K.R. Reddy, Chemosphere, 93, 626 (2013).
3. J. Wunbua, K. Nakhapakorn and S. Jirakajohnkool, Songklanakarin J. Sci. Technol., 34, 329 (2012).
4. E.E. Etim, Int. J. Environ. Bioenergy, 2, 120 (2012).
5. P. Domyns and S. Te-chato, J. Agric. Technol., 6, 1595 (2013).