Preliminary Test of Hydrocarbon Exposure on Azolla pinnata in Phytoremediation Process

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Abstract. The aim of this paper was to investigate the ability of small water fern of Azolla pinnata to survive when exposed to diesel contaminants. The experimental work of phytoremediation was conducted in a green house in the outdoor conditions for an observation period of 10 days. First, the small water fern, Azolla pinnata was exposed to 10, 20, 30, 40, and 50 mL/L (V_Diesel / V_Water). After one day of exposure, Azolla pinnata died for all diesel concentration due to high level of diesel concentration. Following to that, Azolla pinnata was exposed to another set of diesel concentrations of 0.5, 1, 3, and 7 mL/L. During 10 days of exposure, the fern can only survive with concentrations of 0.5, 1 and 3 mL/L but died in 7 mL/L at the end of 10 days. At the end of exposure period 20% of Azolla pinnata were withered at lower concentration of 0.5 mL/L and 100% withered at higher concentration of 7 mL/L.

Keywords: Phytoremediation , preliminary test, Diesel, Azolla pinnata

1. Introduction

Diesel is among the most dangerous substances in the environment and causes a great threat to the ecosystem and human being. The toxicity of diesel is due to the presence of aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene, which are together termed as BTEX (Lohi et al., 2008). There are many conventional methods to treat wastewater containing diesel. Phytoremediation is a green technology method for hydrocarbon remediation using plants to degrade, stabilize, and/or remove contaminants (Agamuthu et al., 2010). It is an environmental friendly, economical and low maintenance method (Peng et al., 2009). The processes of phytoremediation includes biological, chemical, and physical aid in the uptake, sequestration, degradation, and metabolism of contaminants, by the interaction between plants and organisms in the plant's rhizosphere (Edwin & Albert, 2010).

There are many species of aquatic plant having the ability to degrade hydrocarbon in their growth medium, one of them is a water fern Azolla pinnata. Azolla has common names of Mosquito fern, Water velvet, Water fern or simply Azolla, in Malaysia Azolla pinnata. Azolla is a water fern commonly found in tropical and temperate freshwater ecosystems. It is a small, free-floating fern, less than ½ inches across (Ferentinos et al., 2002). An Azolla plant is a fern frond consisting of a main stem growing at the surface of the water, with alternate leaves and adventitious roots at regular intervals along the stem (IFAS, 2001). Azolla fronds are triangular or polygonal and float on the water surface individually or in mats (Figure 1).
Azolla has several attributes that merit its consideration for widespread use as an amendment for bioaugmentation and biostimulation of contaminated sites (Cohen et al., 2004). The present study of preliminary test aims to determine the maximum concentration that will cause the total death of the aquatic plant *Azolla pinnata*. It is the first step of phytotoxicity test, to determine the diesel concentration that *Azolla* can survive and degrade. According to Stêpniewska et al., (2005) *Azolla* can bioaccumulate heavy metals and also remove organic substances from wastewater. *Azolla pinnata* has been reported to accumulate high level of arsenic from contaminated water (Rahman and Hasegawa, 2011).

2. Materials and method

20 g of *Azolla pinnata* was placed in each yellow pail containing 3 L synthetic wastewater prepared by mixing water with different diesel concentrations (Figure 2). A yellow pail without diesel acted as a plant control. Physical observation for 10 days was done to investigate the ability of the plant to survive and resist the hydrocarbon contaminant. The percentage of withered plants was determined based on approximated weight of *Azolla pinnata* withered over total weight of *Azolla pinnata* (Equation 1).

\[
\%\text{Withered plant} = \frac{\text{Weight of withered Azolla}}{\text{Total weight of Azolla}} \times 100
\]  

Figure 2. *Azolla pinnata* in pail containing synthetic wastewater

3. Results and discussion

First, a range of diesel concentrations of 10, 20, 30, 40, and 50 mL/L (\(V_{\text{Diesel}} / V_{\text{Water}}\)) were used. After one day of exposure the small water fern, *Azolla pinnata* died for all of diesel concentrations due to the toxicity of high diesel concentrations (Figure 3).
Subsequently, *Azolla pinnata* was exposed to another set of diesel concentrations (0.5, 1, 3, and 7 mL/L). During 10 days of exposure, it was observed that *Azolla pinnata* can survive with concentrations of 0.5, 1 and 3 mL/L but died in 7 mL/L at the end of 10 days (Figure 4).

Based on the results in (Figure 4) after 10 days of the diesel exposure to *Azolla pinnata*, the minimum percentage of withered plant was 20% for 0.5 mL/L, until 100% withered for 7 mL/L concentration, which means all plants died as shown in (Figure 5). A study on diesel biodegradation by derived bacteria from aquatic plants of *Azolla pinnata* as well as *Pistia stratiotes* and *Salvinia molesta* (Cohen et al., 2002) showed that all species when exposed to diesel can survive up to 0.005% diesel (v/v) (≈ 0.05 mL/L) but at a concentration of 0.05% (≈ 0.5 mL/L), *Azolla pinnata* died but for the other species they could survive. Cohen et al., (2004), mentioned that microbe growth around dead *Azolla pinnata* enhanced the biodegradation of petroleum hydrocarbon (PHC) through substantial nitrogen and phosphorous released during decomposition of dead *Azolla pinnata*.

### 4. Conclusions

Laboratory culture of preliminary experiments was conducted to assess the ability of the water fern *Azolla pinnata* to survive when exposure to different diesel concentrations. The results clearly were shown that whenever the concentration increased the withered plant also increased. At the end of 10 days, 20% of *Azolla pinnata* were withered at lower concentration of 0.5 mL/L and 50% withered at concentration of 1 mL/L. Therefore the concentration of diesel must not exceed 0.5 mL/L (v/v) in the phytotoxicity test, the next stage of phytoremediation processes, because the 50% of withered plant means the plant cannot survive and the diesel concentration was too high for this species.

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6. References


