COMPARATIVE PHYSICAL PHYTOTOXICITY OF *Azolla pinnata* AND *Lemna minor* IN TREATED PALM OIL MILL EFFLUENT

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ABSTRACT

The physical condition of two native small water ferns, Azolla pinnata and Lemna minor were evaluated when exposed to various concentrations of treated palm oil mill effluent (POME) in terms of their growth survival and tolerance. The experiment on the preliminary phytotoxicity was conducted in an open laboratory in Universiti Kebangsaan Malaysia for the period of 5 days. Thirty-four of 100 mL glass containers were prepared, with three replicates for each concentration, including control containers without plants and two other containers for plant control with tap water. 50 mL of treated POME was added into each glass container with different concentrations (100%, 75%, 50% and 25%) and exposed to 3 g of Azolla pinnata or Lemna minor. Observations were made on a daily basis to observe plant growth. After two days of exposure, 40% of Azolla pinnata died in 100% concentration. The dead Azolla pinnata increased to 70% on the third day and finally 100% died after 5 days for all concentrations. In contrast for Lemna minor, only 5% mortality was observed for a concentration of 100% on the fourth day and this condition remained until the end of five days. The higher capacity of Lemna minor that can grow and be sustained in treated POME indicated that the plant has higher resistance and tolerance compared to Azolla pinnata. Therefore, it is suggested for future research phytotoxicity, the treated POME can be diluted up to 10% concentration to study the durability of Azolla pinnata in POME and Lemna minor will be maintained at 100% concentration to analyse its life span in POME.

Keywords: *Azolla pinnata*, phytotoxicity, palm oil mill effluent (POME), *Lemna minor*.

INTRODUCTION

Malaysia is one of the major producers of crude palm oil, besides Indonesia, in the world which accounted for 31.9% in 2016 [1]. Total revenue of the world's palm oil is expected to rise again in 2017 over the previous years due to a recovery in the palms of the effects of El Nino, which occurs in 2016 [2]. The highest production statistics of selected food crops in Malaysia is also on the production of palm oil compared to rice, natural rubber, cocoa and kenaf, to an increased production of palm oil by 2.4% in 2015 [3]. The increase of oil palm products in Malaysia will also increase the production of wastewater from palm oil mills each year. Palm oil production process requires large volume of water. Approximately, 5 to 7.5 tons of water needed to produce 1 ton of palm oil and 50% of that water will contribute to the generation of wastewater [4] which is commonly known as palm oil mill effluent (POME)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003)(Ahmad et al. 2003). Basically, palm oil processing will produce wastewater during sterilization, separation of sludge and also during cyclone process. Typically, oil palm wastewater is treated in several stages involving few processes of cooling pone, anaerobic, aerobic and final discharge. Many POME treatment schemes currently used by the Malaysian palm oil industry including membrane bioreactor and biological method were discussed in detail by [5-7]. However, the application of these technologies is often still difficult to comply with the stringent environmental regulation in Malaysia. Phytoremediation, a quite new technology in tropical country like Malaysia, is proposed to further polish treated POME which is still rich with nutrients and most of the time hardly meets the stringent environmental regulation, using native ferns that are later to be used as livestock feeds.

Phytopremediation is one of the green technology processes utilizing plants to reduce the concentration of pollutants in contaminated soil, water or air by using herbs that attempt to control, degrade, or remove metals, pesticides, solvents, cracking materials, crude oil, and various contamination materials [8]. It is low cost, easy to handle and environmental friendly that make use of plants together with microorganism to remove or decrease contaminations in wastewater [9-12]. More than 500 species of plants are listed as a potential plant for phytoremediation [13], most of them are temperate plants. one of this research output is to identify tropical and native plants in Malaysia that can be used to further polish palm oil mill effluent as well as later be used as livestock feeds.

Azolla pinnata and *Lemna minor* are two native aquatic and floating ferns that can be used to treat domestic and industrial wastewater [14-17]. *Azolla pinnata* thrives without the addition of nitrogen fertilizer to sustain its growth as it has in its leaves cynobacteria as nitrogen fertilizer in rice fields of Southeast Asia [18]. For aquatic plants, duckweed is also used to recover nutrients such as nitrogen and phosphorus and heavy metals from agricultural and municipal [19-21]. Both of these plants are also able to absorb heavy metals found in wastewater [22-23]. This study aims to determine the tolerance and survival of these two plants in treated POME as to select the right concentrations of POME to be used in the next stage of phytotoxcity by these plants in POME.

MATERIAL AND METHODS

Plant collection and propagation

The two healthy and fresh plants, *Azolla pinnata* and *Lemna minor* were obtained from greenhouse at Universiti Kebangsaan Malaysia. The plant was propagated with fertilizer to ensure that plant stock is sufficient for future research. Some plant were taken and placed in a beaker filled with tap water and left for a while to remove the remnants attached to the plant roots. Then, the plants were washed with tap water, filtered and dried using tissue paper to reduce the percentage of water at the plant. Both plants' growth was observed physically in terms of their colour change. Figure 1 compares an image of healthy *Azolla pinnata* and *Lemna minor* with the dead ones. Healthy *Azolla pinnata* is a dark green, while *Lemna minor* is light green (Figure 1). *Azolla pinnata* will change their color to dark brown while *Lemna minor* will change color to white when die.

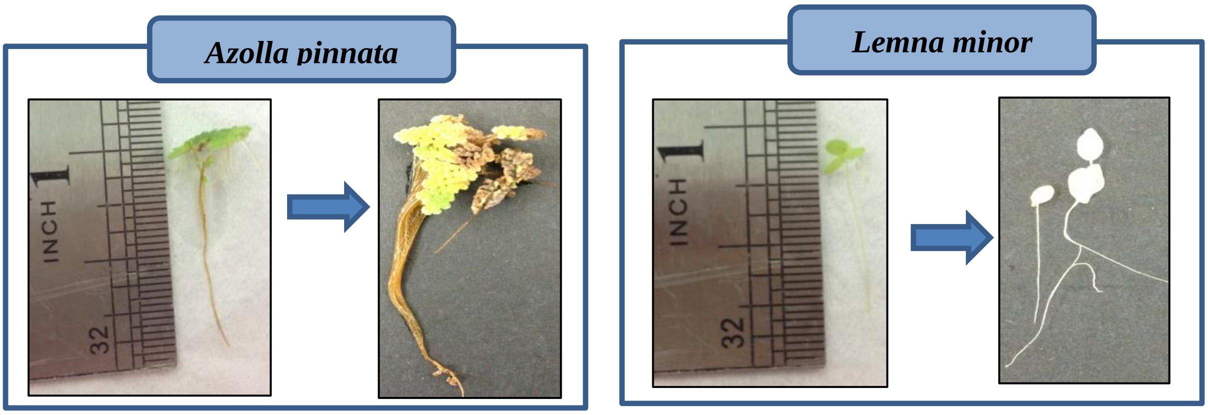


Figure 1: *Azolla pinnata* and *Lemna minor* discoloration when healthy and after death

Experimental design

A total of 34 100 mL-containers were prepared for this experiment. Sixteen containers were used for each plant (*Azolla pinnata* and *Lemna minor*). Each container contained 50 mL of treated POME obtained in Selangor with different POME concentration ($V_{treated\ POME}/V_{water}$) (100%, 75%, 50% and 25%). Table 1 lists down the characteristics of the treated POME obtained from a palm oil mill in Dengkil, Selangor. Each dilution was triplicated and control solution was also provided for each dilution without the plants. Another container acting as plant control containing plants with 50 mL tap water. Each container was loaded with 3 g of *Lemna minor* or *Azolla pinnata* as illustrated in Figure 2.

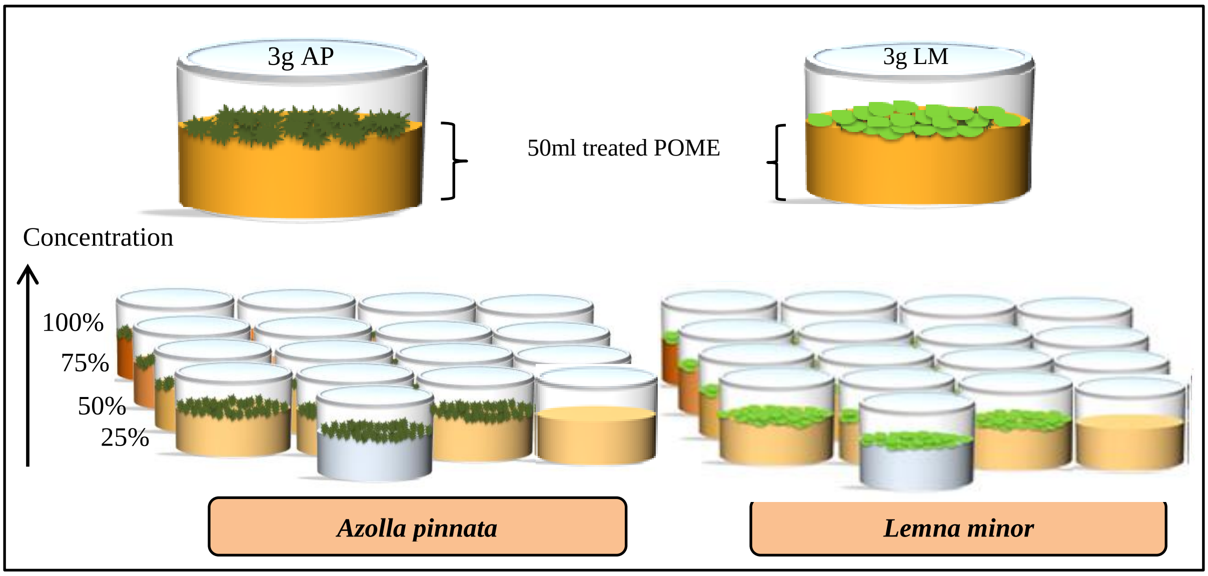


Figure 2: Design of experiment

Table 1: Characteristics of treated POME

No.	Parameter	Value

1.	COD	620 mg/L
2.	BOD	300 mg/L
3.	NH4-N	5.5 mg/L
4	DO	6.9 mg/L
5.	pH	8.2

Physical observation of plant

Observations were made every day starting from day 0 to day 5 and quantified with the percentage of plants that can survive for each concentration of treated POME. Table 2 shows the physical indicator of *Azolla pinnata* and *Lemna minor* observed for healthy and dead ones. *Azolla pinnata* will turn brown from the center of the fronds and eventually become black when it dies. For *Lemna minor*, it turned white when dead, as shown in Table 2.

The tolerance and survival of both plants are quantified based on the area of the healthy one compare to the total area covered by the whole fern on the water surface and also on the colour change.

Table 2: Indicator for healthy and dead plants for *Azolla pinnata* and *Lemna minor*

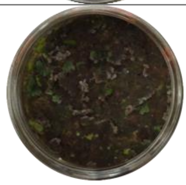
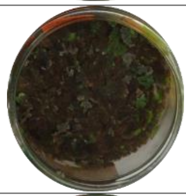
	Fresh/Healthy	Dead
Azolla pinnata		
Lemna minor		

RESULTS AND DISCUSSION

Effect of treated pome on plant growth

Azolla pinnata's ability to survive in treated POME of different dilutions were observed throughout 5 day-exposure as shown in Table 3. No colour changes were observed on the first day of exposure. Unlike on the second day, only 60% of the plants survived in 100% treated POME (no dilution), but in 75%, 50% and 25% dilution, most plants in the containers were still alive and healthy. On the third day, only 30% of the plants were still green at 100% POME, while on the other dilutions it had shown a significant decrease. Physically, it can be seen on the fourth and fifth day that all the plants turned black and died indicating that *Azolla pinnata* failed to resist the toxicity of the treated POME solution. Moreover the longer the exposure, the darker the colorization of the plant leading to invariably death of plants. Murodov et al [24] also show *Azolla* sp. plants could not withstand toxicity on remaining wastewater but Rai [25] found that it is capable of treating heavy metals (Hg and Cd) in wastewater.

Table 3: Physical observation of the toxicity of treated POME on *Azolla pinnata*

POME Concentration (%)	Exposure duration (days)				
	1	2	3	4	5
100					
75					
50					
25					
0 Control					

Similar amount (3 g) of *Lemna minor* was also exposed to the treated POME with different dilution for 5 days. For *Lemna minor*, it was observed that healthy light green color of the plants remained unchanged until the third day of exposure (Table 4). The plants were still fresh in the all dilutions of treated POME (25% up to 100%). On day 4, it was found that only 5% of the plants turned white indicating death signs in the 100% treated POME and remained the same until the end of 5-day exposure. This is proven that *Lemna minor* is highly resistant to toxicity even in 100% treated POME. Kamyab et al [26] had also demonstrate the suitability of *Lemna minor* to treat final POME and Ozengin and Elmaci [27] also indicated the capability of this plant to treat laboratory wastewater.

Table 4: Physical observation of the toxicity of treated POME on *Lemna minor*

POME Concentration (%)	Exposure duration (days)				
	1	2	3	4	5
100					
75					

50	
25	
0 Control	

Comparative tolerance of *Azolla pinnata* AND *Lemna minor*

The physical observation for the plant tolerance and survival was quantified in terms of the number of plants that still remained fresh and green. Figure 6 clearly shows that *Lemna minor* has higher resistance (95%) to the toxicity of treated POME with only 5 % becoming white and died compared with *Azolla pinnata*. Up to day 5, *Lemna minor* was still able to survive while *Azolla pinnata* from to die starting day 3 and mostly died on day 5.

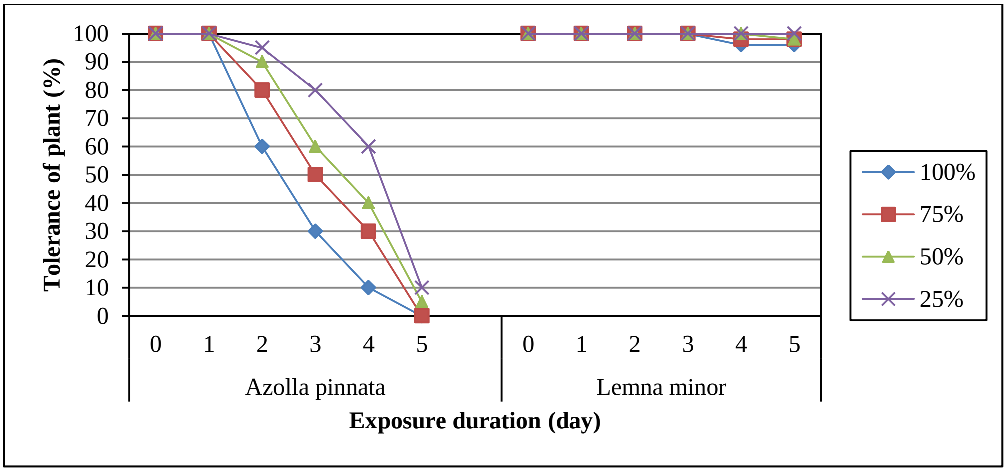


Figure 3. Comparison on the toxicity of treated POME on *Azolla pinnata* and *Leman minor*

CONCLUSIONS

Among the four different dilutions of treated POME, *Lemna minor* displayed higher tolerance and survival level compared to *Azolla pinnata*, 95% of *Lemna minor* remained fresh and green even in the 100% treated POME. However, *Azolla pinnata* mostly died at the end of 5-day exposure even in the lowest dilution (25%) of treated POME. Through this physical observation, it could be concluded that *Lemna minor* is a fern that is able to grow in treated POME containing high nutrient and this study will be roceeded to analyze in details the efficiency of *Lemna minor* to remove nutrient from treated POME and determine the characteristic of POME before and after treatment in the next prolonged exposure of phytotoxity test.

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