

## **Phytoremediation of Palm Oil Mill Final Discharge Wastewater using Aquatic Macrophytes *Leersia oryzoides*, *Pistia stratoites* and *Ludwigia peploides***

*Fitoremediasi Air Buangan Akhir Kilang Kelapa Sawit menggunakan Makrofitu Akuatik *Leersia oryzoides*, *Pistia stratoites* dan *Ludwigia peploides**

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### **Abstract**

This study aimed at evaluating the performance of aquatic macrophytes to treat palm oil mill final (POM) discharge wastewater. Samples of POM final discharge wastewater were obtained from palm oil mill of KL Kepong Berhad in Changkat Asa, Tanjong Malim, Perak. Three aquatic macrophytes, *Leersia oryzoides* (rice cutgrass), *Pistia stratoites* (water lettuce) and *Ludwigia peploides* (water creeping primrose) were planted into 6L open-topped containers filled with POM final discharge wastewater. The removal efficiency (%) of BOD<sub>5</sub>, COD, NH<sub>3</sub>-N, metal content and macrophytes' mass were examined in order to evaluate the phytoremediation performance. The results showed that BOD<sub>5</sub>, COD and NH<sub>3</sub>-N removal efficiencies of 93%, 30% and 82%, were achieved for *Pistia stratoites*, 90%, 27% and 80%, for *Leersia oryzoides* and 93%, 20% and 80%, for *Ludwigia peploides*, respectively. In addition, *Pistia stratoites* and *Leersia oryzoides* were able to reduce the calcium (Ca) content by 92% and 87%, respectively while there is no significant difference in absorption of other metals like cadmium (Cd), sodium (Na), iron (Fe), and manganese (Mn). Throughout the study, increase in plant weight was observed. These results suggest that the studied aquatic macrophytes are capable in degradation of the organic and inorganic pollutants from POM final discharge wastewater.

**Keywords** phytoremediation, aquatic macrophytes, palm oil mill final discharge

### **Abstrak**

Kajian ini bertujuan menilai kebolehan tumbuhan dalam merawat air buangan akhir kilang kelapa sawit. Sampel air diperolehi dari kilang kelapa sawit syarikat KL Kepong Berhad di Changkat Asa, Tanjong Malim, Perak. Tiga makrofitu akuatik, *Leersia oryzoides*, *Pistia stratoites* dan *Ludwigia peploides*, ditanam di dalam bekas terbuka 6L yang diisi dengan air buangan akhir kilang kelapa sawit. Kecekapan penyingkiran (%) bagi BOD<sub>5</sub>, COD, NH<sub>3</sub>-N, kandungan logam dan biomas tumbuhan diperiksa dalam menilai potensi rawatan. Prestasi fitoremediasi menunjukkan kecekapan penyingkiran BOD<sub>5</sub>, COD dan NH<sub>3</sub>-N masing-masing sebanyak 93%, 30% dan 82% dicapai bagi *Pistia stratoites*; 90%, 27% dan 80% bagi *Leersia oryzoides* dan 93%, 20% dan 80% bagi *Ludwigia peploides*. Tambahan pula, *Pistia stratoites* dan *Leersia oryzoides* mampu menurunkan kandungan kalsium (Ca)

masing-masing sebanyak 92% dan 87%; sementara itu tiada perubahan signifikan dalam penyerapan logam lain seperti cadmium (Cd), natrium (Na), besi (Fe), dan manganese (Mn). Sepanjang kajian, pertambahan berat bagi tumbuhan dicerap. Keputusan ini mencadangkan bahawa makrofita akuatik mampu menguraikan pencemar organik dan inorganik dari air buangan akhir kilang kelapa sawit.

**Kata kunci** fitoremediasi, makrofita akuatik, air buangan akhir kilang kelapa sawit

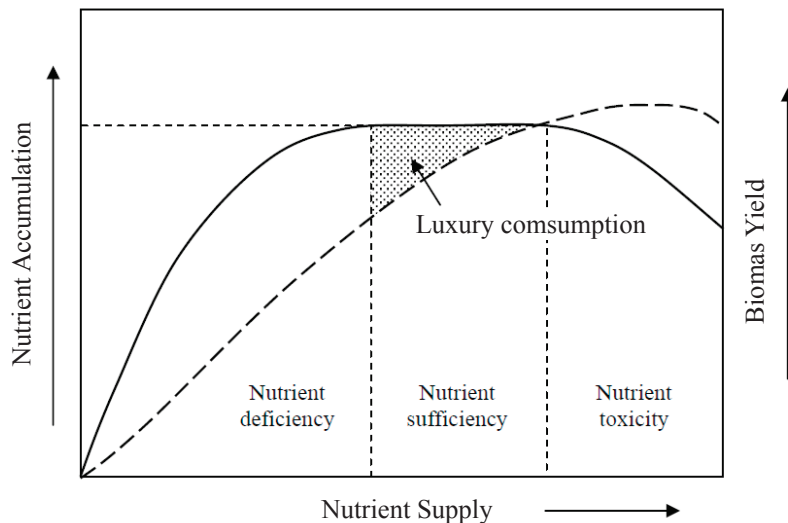
## Introduction

In phytoremediation technique, plants were used to remove, degrade, or render harmless hazardous materials present in soil and water (Vishnoi and Srivastava, 2008). The emerging technology may offer a cost effective, non-intrusive, and safe alternative as compared to conventional soil clean up techniques by using the ability of certain trees, shrubs, and grasses to remove, degrade or immobilize harmful chemical from soil or water (Vishnoi and Srivastava, 2008). Palm Oil Mill Effluent (POME) is a liquid waste produced during palm oil processing in palm oil mill. About 0.67 tonne of POME is generated for every tonne fresh fruit bunch (FFB) processed and the generated POME is then treated through biological treatment like anaerobic digestion before being discharged to the river every day (Ma, 2009). The country's Environmental Quality Act 1979 requires that all palm oil mills must treat their POME to reduce the BOD to a level of less than 100 mg/L for watercourse discharge (Environmental Quality Act 1974, 2007). Of all the natural resources, water is the most easily polluted by uncontrolled discharge of POME into watercourses (Lim *et al.*, 2009). At present not many studies have been carried out to determine the feasibility of phytoremediation of Palm Oil Mill (POM) final discharge wastewater. Therefore, this study explored the potential of three macrophytes as phytoremediators.

Aquatic macrophytes are used as they have above and below ground biomass to provide a large surface area for the growth of microbial biofilms (Brix, 1997; Greenway, 2007). These biofilms are responsible for a majority of the microbial processes in a constructed wetland system, including nitrogen reduction (Brix, 1997). Plants that have extensive root systems provide large surface area for microorganisms to colonize thus increasing the potential for decomposition of organic matter (Reddy and Sultan, 1984). There are several studies of phytoremediation to treat industrial and agricultural wastewater (Tripathi and Upadhyay, 2003; Navarro *et al.*, 2012). Tripathi and Upadhyay (2003) used *Eichornia crassipes*, *Azolla pinnata* and *Lemna minor* to treat dairy effluent and found percentage of nitrogen removal by *Eichornia crassipes*, *Azolla pinnata* and *Lemna minor* were 71.8, 62.5 and 60.13%, respectively. Navarro *et al.* (2012) found that *Eichornia crassipes* could reduce the diluted lemon industry effluents of BOD<sub>5</sub> and COD to 70 and 61%, respectively.

The rate of nutrient uptake by macrophytes is limited by its growth rate and the concentration of nutrient within the plant tissues, with nutrient storage dependent on plant tissue nutrient concentrations and potential for biomass accumulation (maximum standing crop) (Reddy and De Busk, 1987). At low to medium nutrient concentrations, plant growth (biomass) is proportional to nutrient supply (Figure 1). Increases in nutrients above this may result in the luxury uptake of nutrients by plants, but does not increase plant growth. Nutrient accumulation will eventually plateau. Beyond this point, increases in nutrient supply may cause nutrient toxicity. For this reason, macrophytes were used to remediate POM final discharge wastewater which has lower strength of pollutants as compared with

raw POME. This paper discusses the phytoremediation of POME final discharge wastewater efficiency using *Leersia oryzoides*, *Pistia stratiotes* and *Ludwigia peploides*. These macrophytes were found able to survive in POME final discharge compared to 12 other types of macrophytes during preliminary and screening tests in controlled conditions.



**Figure 1** Relationship between nutrient supply and nutrient accumulation (----) and biomass yield (—) (Reddy and DeBusk, 1987)

## Methodology

### Plant Species

Aquatic macrophytes were obtained from the abandoned tin mine lake at Kalumpang, Hulu Selangor. *Leersia oryzoides*, *Pistia stratiotes* and *Ludwigia peploides* were cultured in dechlorinated water prior to experiment for acclimatization process for about 2 to 4 weeks. New adventative emergent shoots or new growing rosettes were used from the plants to get almost similar initial weight.

### POME Final Discharge

POME final discharge was procured from KL Kepong Berhad final retention pond from Changkat Asa near Tanjong Malim, Perak. Characteristics of POME final discharge wastewater were analyzed in laboratory. Details of the characteristics were described in Table 1.

### Experimental Set Up

The experiments were conducted at laboratory scale using 33 x 20 x 10 cm<sup>3</sup> containers. Six litres of POME final discharge was used in every treatment with four replicates. All treatments used macrophytes; *Leersia oryzoides*, *Pistia stratiotes* and *Ludwigia peploides*,

**Table 1** Characteristics of POM final discharge wastewater from KL Kepong Berhad Mill at Changkat Asa

Parameter	Range (mg/L)	Mean $\pm$ SD (mg/L)
BOD <sub>5</sub>	490-612	549.33 $\pm$ 61.07
COD	1025-1073	1049 $\pm$ 33.94
Ammonia (NH <sub>3</sub> -N)	43-45	44 $\pm$ 1
Total Solid	4860-5351	5150 $\pm$ 212.33
Total Suspended Solid	3506-3713	3600 $\pm$ 150.42
Total Volatile Solid	1200-1480	1333.33 $\pm$ 140.48
Total Alkalinity (as CaCO <sub>3</sub> )	13401-13511	13457 $\pm$ 50.68
Pb	0.02-0.05	0.04 $\pm$ 0.02
Mn	0.19-0.23	0.22 $\pm$ 0.01
Mg	10.21-10.66	10.41 $\pm$ 0.23
Ca	61-98	76 $\pm$ 19.47
K	bdl*	bdl
Fe	0.65-1.56	1.03 $\pm$ 0.47
Cd	0.026-0.35	0.14 $\pm$ 0.19
Na	bdl	bdl

\*bdl: below detection limit

that weighed ranging from 25g to 35 g. Containers with no vegetation were also set up as controls. Containers were placed in shady area near the laboratory to control light intensity factor. Measurements for NH<sub>3</sub>-N were conducted daily, BOD<sub>5</sub> and COD for every three days, and metal content before and after the treatment. Plant weight was monitored weekly. All experiments were repeated three times for data accuracy.

### Chemical Analyses

All chemical analyses were determined according to the Standard Method (APHA, 2005). Biochemical Oxygen Demand (BOD<sub>5</sub>) was measured based on five day period of oxygen consumption at 20 °C and Chemical Oxygen Demand (COD) was determined by closed reflux method using HACH COD reactor and HACH DR 4000 spectrophotometer. Ammoniacal Nitrogen (NH<sub>3</sub>-N) was analysed using Nessler method. Metals content of sodium (Na), ferum (Fe), calcium (Ca), cadmium (Cd), magnesium (Mg) and manganese (Mn) were analyzed with Atomic Absorption Spectrophotometer (AAS) model Analyst 220 with 50 ml sample for every metal.

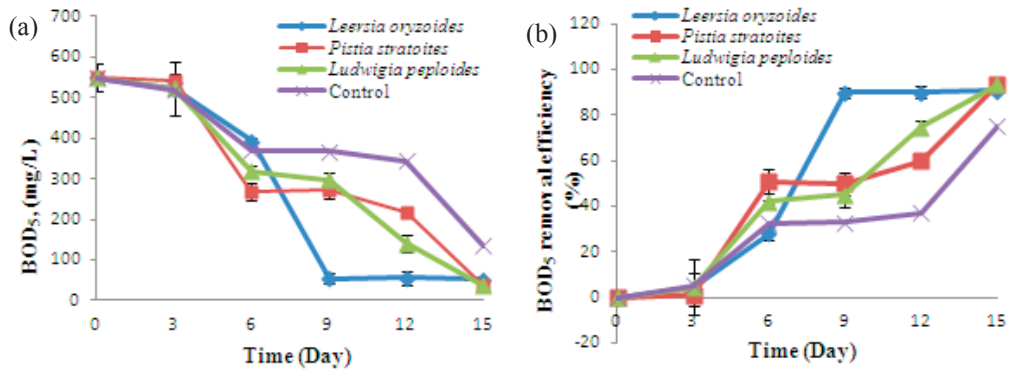
### Plant Weight

Means of plant weight for every species was monitored every week by measuring the wet weight of plant gravimetrically. Plant morphological changes such as physical appearance of the leaves for wilting signs were also observed as prescribed by Soltan and Rashed (2003).

## Results and Discussion

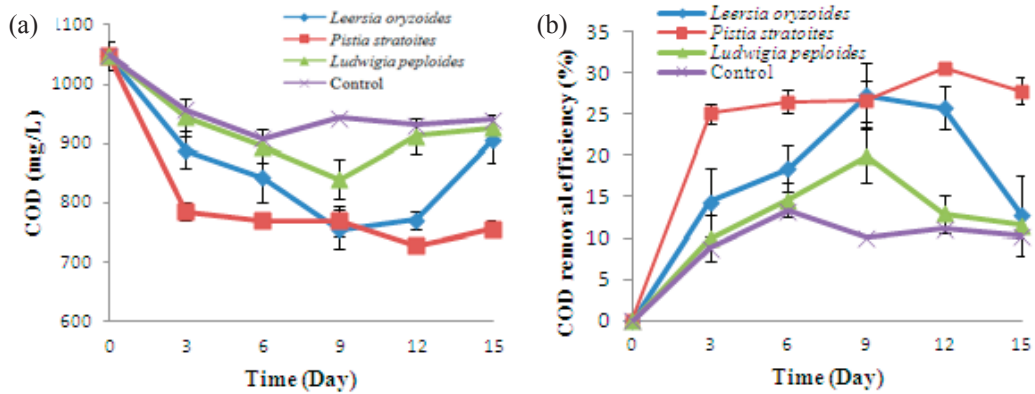
### Phytoremediation Performance

Figures 2 to 6 represent phytoremediation performances of POM final discharge. The removal efficiency of organic pollutants was determined by measuring BOD<sub>5</sub> and COD concentration throughout the study. Figures 2(a) and (b) show that plant species used were able to remove biodegradable organic compounds in POM final discharge wastewater. The BOD<sub>5</sub> removal efficiency for *Leersia oryzoides*, *Pistia stratoites* and *Ludwigia peploides* were 90.6%, 93.3% and 93.3%, respectively with final effluent concentration in the range of 52 mg/L, 36.5 mg/L and 36.5 mg/L. A reduction of BOD<sub>5</sub> concentration was also observed in control containers and this might be due to the presence of natural microorganisms in POM final discharge used. The findings demonstrate *Leersia oryzoides* as the most efficient species in removing BOD<sub>5</sub> compared to the other two species [Figure 2(a)]. Greenway and Wolley (1999) reported that, BOD<sub>5</sub> was principally removed by microbiological activity associated with plants and litter by providing surfaces for biofilm growth in wetland system.



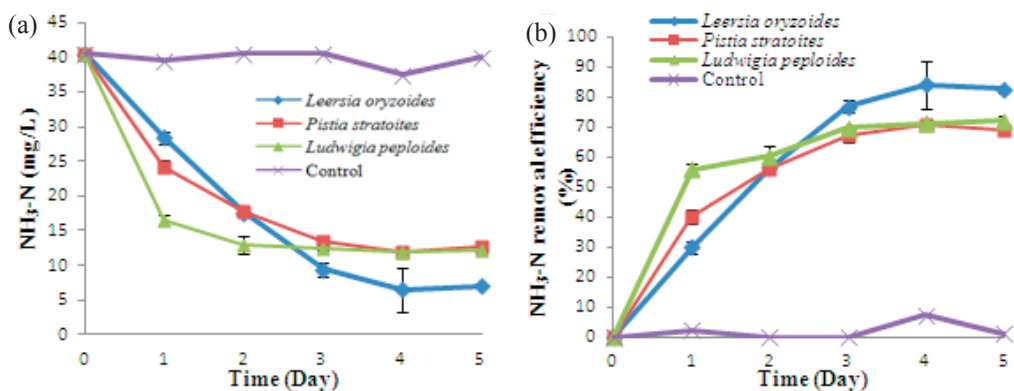
**Figure 2** (a) Changes in BOD<sub>5</sub> and (b) BOD<sub>5</sub> removal efficiency of POM final discharge wastewater treated with three macrophytes species

Figures 3(a) and 3(b) show the changes in COD and COD removal efficiency during phytoremediation of POM final discharge wastewater. This results show that COD removal efficiency was lower as compared to BOD<sub>5</sub>. COD removal efficiency achieved by *Pistia stratoites*, *Leersia oryzoides* and *Ludwigia peploides* were only 30.6%, 27.3% and 19.9%, respectively. Scholz and Hedmark (2009) reported that *Phragmites australis* can reduce COD of rich organic matter of urban run-off wastewater with efficiency in a range of 59.7% to 71.4 %. It is believed that COD was not significantly decreased due to macrophyte deterioration which results in increase of the organic matter concentration (Greenway, 2007).



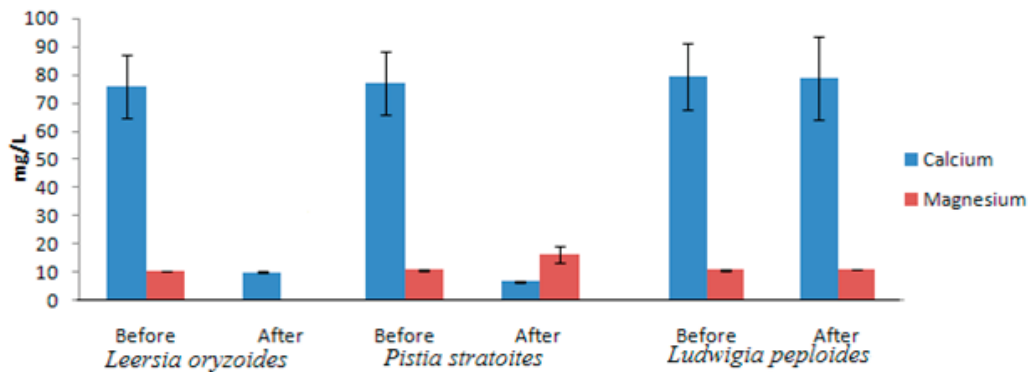
**Figure 3** (a) Changes in COD and (b) COD removal efficiency of POM final discharge wastewater treated with three different macrophytes species

Figures 4(a) and 4(b) show the changes in  $\text{NH}_3\text{-N}$  and  $\text{NH}_3\text{-N}$  removal efficiency throughout the phytoremediation study.  $\text{NH}_3\text{-N}$  removal efficiency achieved by *Leersia oryzoides*, *Pistia stratiotes* and *Ludwigia peploides* were 83.9%, 70.7% and 72.1%, respectively. Among the three species used in this study, *Leersia oryzoides* (cutgrass) was most efficient in  $\text{NH}_3\text{-N}$  removal. During phytoremediation  $\text{NH}_3\text{-N}$  is removed either through plant uptake as a nutrient or nitrification and denitrification by microbial activity (Siracusa and La Rosa, 2006). The ability of plants to remove  $\text{NH}_3\text{-N}$  was proven by Moreno *et al.* (2001) in a study using *Phragmites australis* (common reed) planted in columns with oil refinery. The mechanism of  $\text{NH}_3\text{-N}$  removal during phytoremediation was alleged to involve the aerobic zone in plant root which allow nitrification to be occurred (Lim and Polprasert, 1998). Diffusion of oxygen from the roots of emergent macrophytes maintains an oxidised sediment surface layer and microenvironment around the root zone. This modifies the sediment redox conditions facilitating aerobic microbial processes including nitrification (Greenway, 2005). At the same time, the plants also provided surface areas for microbial attachment by forming biofilms (Tousignant *et al.*, 1999). This finding shows that microorganisms play an important role to facilitate  $\text{NH}_3\text{-N}$  removal during phytoremediation.



**Figure 4** (a) Changes in  $\text{NH}_3\text{-N}$  and (b)  $\text{NH}_3\text{-N}$  removal efficiency of POM final discharge wastewater treated with three different macrophytes species

Figure 5 shows the Mg and Ca uptake by *Leersia oryzoides*, *Pistia stratoites* and *Ludwigia peploides* during the phytoremediation study. *Leersia oryzoides* was able to remove Ca up to 86.7 % and almost complete removal of Mg. Meanwhile, *Pistia stratoites* was only able to remove Ca but seems to release Mg into the solution. Uptake of Ca and Mg was not observed in *Ludwigia peploides*. Ca plays an important role in regulating various plant cell functions. One of the functions is in the regulation of the protein pump that regulates the uptake and movement of nutrients into roots and throughout cells within the plant. At the root level, Ca activates the stimulation of the protein channels that take up nutrients. Adequate availability of Ca at the root surface is required for this process to work effectively (Patterson, 2000).

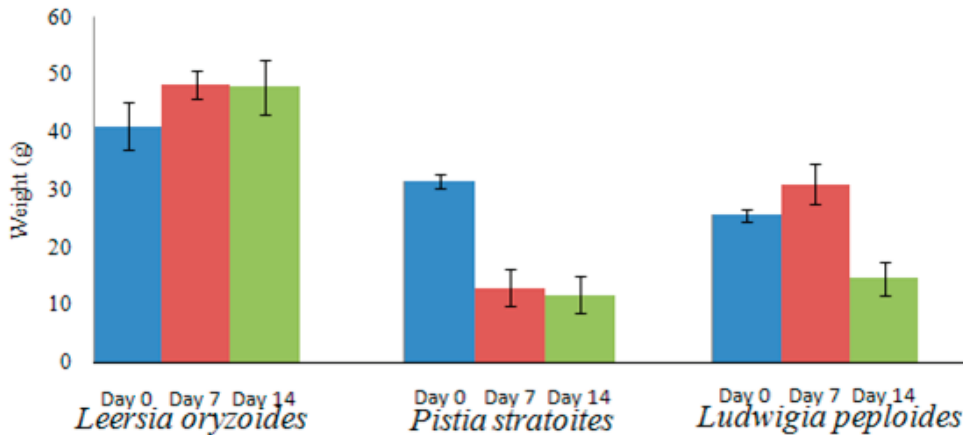


**Figure 5** Magnesium and Calcium removal by *Leersia oryzoides*, *Pistia stratoites* and *Ludwigia peploides* during the phytoremediation study

### Plant Weight

Changes in plant weight throughout the phytoremediation study are shown in Figure 6. Increased in plant weight was observed in *Leersia oryzoides* and *Ludwigia peploides*. However, *Ludwigia peploides* weight was decreased on day 14. Increased in plant weight might be related to the macronutrient uptake such as Ca and Mg as both elements are important for plant growth. In contrast, *Pistia stratoites* showed a gradual decrease in weight throughout the study. This may be due to the fragile structure and small size of *Pistia stratoites* as compared to the other two macrophytes which make it vulnerable to pollutant stress. Perhaps the release of Mg reduces the biosynthesis of chlorophyll, which might affect its growth and development. Further investigation is recommended in order to reveal the element's pathway.





**Figure 6** Changes in plant weight during phytoremediation of POM final discharge wastewater

## Conclusion

Phytoremediation of POM final discharge wastewater was feasible using *Pistia stratoites*, *Leersia oryzoides* and *Ludwigia peploides*. *Leersia oryzoides* was found to be the most efficient macrophytes for  $\text{NH}_3\text{-N}$  removal. *Pistia stratoites* and *Leersia oryzoides* were able to reduce calcium (Ca) content by 92% and 87%, respectively, while Mg was almost completely absorbed by *Leersia oryzoides*. Throughout the study, increase in plants weight was observed for *Leersia oryzoides* and *Ludwigia peploides*. These findings would be of prominent importance for subsequent scaling up treatment of POM final discharge wastewater.

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