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UPTAKE OF HEAVY METALS FROM PALM OIL MILL EFFLUENT SLUDGE AMENDED SOILS IN WATER SPINACH

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Abstract: Palm oil mill effluent (POME) sludge is generated in great amount in Malaysia and often regarded as pollutant and waste material. This study is aimed at utilizing POME sludge for growing plants. Five different treatments (POME sludge:soil ratio) were performed to evaluate the potential of POME sludge application on the growth and uptake of heavy metals, namely Cr, Cd, Cu, Zn, Fe, Mn and Ni in water spinach. Flame Atomic Absorption Spectrophotometer (FAAS) was used to determine the levels of these metals in water spinach. It was found that the concentration of heavy metals in water spinach has increased with the increasing POME sludge loadings for all metals with the exception of Cu. Elevated levels of accumulated Fe in water spinach were recorded between 200.84-660.67 mg/kg, while Cu accumulation was the least, ranging between 41.67-75.00 mg/kg. The yield of water spinach has also increased when the amount of POME sludge increased. Concentrations of Mn, Ni, Zn, Fe, Cd and Cr in water spinach have surpassed the maximum permitted levels. However, among the metals being investigated, only Mn, Fe and Ni have shown significant differences ($P<0.05$) for the mean concentration between these five treatments. No toxicity symptoms were observed on the water spinach. This may indicate the ability of water spinach to tolerate high heavy metals contents. This study suggests that an appropriate amount of POME sludge should be added onto soil to enhance the production of water spinach and to avoid unnecessary accumulation of metals, which may impose health risks to the consumers. Application of POME sludge in agricultural practices may offer a sustainable option in managing this waste material.

Keywords: Palm oil, biomass, soil, agriculture, waste.

Introduction

Agricultural residues contribute to the largest amount of biomass resources in Malaysia and one of the major contributors is the palm oil industry. Malaysia is one of the world's largest palm oil producing country other than Indonesia. According to Wu *et al.* (2009), in 2003, 3.79 million hectares were used for oil palm cultivation, which occupies more than one-third of the total cultivated areas in Malaysia. The residues of the palm oil industry include palm oil mill effluent (POME), empty fruit bunch, palm kernel shells and mesocarp fibres (Sumathi *et al*., 2008). The accumulation of oil palm biomass from palm oil industry, which is approximately 40 million tonnes per year (Mohammad *et al*., 2012), has been constantly growing with increasing global demand for crude palm oil.

The amount of wastewater sludge or better known as POME sludge generated during the production of crude palm oil is abundant. Statistics published in 2008 has shown that at least 44 million tonnes of POME was generated in Malaysia (Wu *et al*., 2010). It has been estimated that the production of 1 tonne of crude palm oil generates about 2.5-3.5 m^3 of POME (Ahmad *et al*., 2015; Jefferson *et al*., 2016). In general, utilization of sludge in agriculture is considered as one of the best waste management options as it improves the soil's aggregate stability, porosity and water infiltration rate, and supplies organic matter and major nutrients, such as N and P to the soil

(Torri & Lavado., 2008; Nwoko & Ogunyemi., 2010a; Jefferson *et al*., 2016).

Studies have been carried out to utilize POME sludge for plant growing and as amendment for agricultural soils due to its high content of nutrients and organic matter. The use of POME has been reported to improve soil productivity and increase the yield of crops (Chan *et al*., 1980). The application of POME as fertilizer has yielded the highest yield of Napier grass (*Pennisetum purpureum*), up to 3276 kg/ha due to the composition of all the major and minor elements in POME needed for its growth (Agamuthu, 1994). In addition, Teoh and Chew (1983) have also reported that a mixture of soil and POME in a 1:5 ratio has yielded vigorous growth of cocoa seedlings. Another study has indicated that the application of fermented POME to soil increased the growth and yield of maize (*Zea mays*. L) (Nwoko & Ogunyemi, 2010b).

Although agricultural application of POME sludge is generally considered as a valuable option in the management of waste, the toxicity effects of POME as a result of phenol and other organic acids in POME sludge which can accumulate in soils and plants, need to be addressed. Furthermore, excessive organic loads and nutrients in POME may also pollute water bodies upon direct discharge and reduce soil fertility (Mohammad *et al*., 2012). Hence, it is important to study the potential risks of POME sludge application on soil. In this study, water spinach was chosen as the model plant as it is a fast grown vegetable and largely consumed by the local people.

The objective of this study is to investigate the potential of POME sludge on the growth of water spinach by applying different POME sludge loadings.

Material and Methods

Sample Collection

Soil samples were collected from Kampung Pinang, in Kota Samarahan, Sarawak. The soil samples were collected from peat swamp areas which have not been previously fertilized or contaminated. Soil samples were randomly collected and stored in polyethylene bags. POME sludge samples were collected from a palm oil plantation in Mongkos, Serian. The plant selected for this study was water spinach (*Ipomoea reptana* Poir).

Characterizations of Soil and POME Sludge Samples

Soil and sludge samples were analysed for pH, organic matter content, particle size and texture (only for soil), total nitrogen, total phosphorus, conductivity and total heavy metals contents. Soil pH was measured using a pH meter at 1:2.5 (w/v) ratio of soil:POME sludge and water while particle size was analysed using the pipette method (USDA, 1984). Total nitrogen was determined using the Kjeldahl method as described by Bremmer and Mulvaney (1982). Loss of ignition (LOI) method (Sparks, 1996) was employed to determine the amount of organic matter while electrical conductivity was determined using a conductivity meter with the soil:solution suspension of 1:5. Total phosphorus was determined using the Molybdenum blue method (Chin, 2000). Heavy metals were analysed in the POME sludge and soil before cultivation. Aqua regia wet digestion method was employed to digest the soil and POME sludge samples (Kumar *et al*., 2009). The concentrations of Mn, Ni, Cu, Zn, Fe, Cd and Cr were measured by the Flame Atomic Absorption Spectrophotometer (FAAS).

Greenhouse Experiment

A completely randomized experiment was initiated on five regimes of POME sludge:soil composition (Table 1) and two replicates. Prior to sowing, water spinach seeds were soaked in a container-filled with tap water to allow germination. Ten plastic 3.2L pots were filled with POME sludge and soil mix. In each pot, four germinated seeds were placed on the potting mix and covered with a layer of 10mm of potting mix. During the growing period of 30 days, the plants were watered daily to aid establishment. No fertilizer was applied throughout the duration of experiment.

Table 1: Ratio of POME sludge:soil used in treatments (%)

Treatment	POME Sludge (%)	Soil (%)
1 (Control)	0	100
2	25	75
3	50	50
4	75	25
5	100	0

At 30 days after sowing, the plant height was measured from the base to the highest tip of the plant. Then, the plants were harvested and removed from the pots without damaging their roots. Following that, the herbages were dried in a force-draught oven at 70°C to achieve constant weight. The crop yield was determined using the following formula (Equation 1):

Yield = dry weight of water spinach (kg)/ pot area (hectare) (Equation 1)

The oven-dried herbages were ground into fine particles using a pestle and mortar. For each replicate, 0.1 gram of each sample was digested in 50 mL beaker by adding a mixture of 10 mL nitric acid (HNO_3) and 5 mL hydrochloric acid (HCl). The digestion was conducted on a hot plate for 4 hours. When the digestion was completed, the resulting clear solutions were filtered using Whatman No. 42 filter papers to remove any particles. Finally, the supernatants were diluted to 100 mL with deionized water. The concentrations of heavy metals in water spinach were determined by means of FAAS.

Statistical Analysis

One-way analysis of variance (ANOVA) with Tukey's test was carried out for comparison of means among the treatments. A probability of $P<0.05$ was used as the statistical significance level. Data were analyzed using the SPSS statistical program (Version 15.0).

Results and Discussion

Physico-chemical Characteristics of Soil and Sludge Samples Prior Treatment

The physico-chemical characteristics of soil and POME sludge are summarized in Table 2. The soil samples from Kampung Pinang were acidic (3.5) and were also high in organic matters content (79.4%). High amount of humic materials in peat soils has been reported for causing the acidic condition (Alloway, 1995). POME sludge was slightly less acidic (pH 4.6) compared to the soil samples. The acidic condition of the sludge has resulted from organic acids produced during the fermentation process (Rupani *et al*., 2010). Compared to the soil samples, the POME sludge contained much lower total organic matters of only 18.3%. This could be due to the low degradation of the plant's fibres or palm oil residues in the POME sludge.

Particle size analysis of the soils has shown the highest percentage of silt (64.1%) followed by sand (29.0%), and clay (6.9%). This indicates that the soil is of silt loam type. Total N and total P levels were higher in the soil than in the POME sludge. Both N and P are considered as crucial plant nutrients for growth. The lower content of N in POME sludge may be due to the leaching of the N after undergoing several stages of oil extraction processes from the fibres.

Electrical conductivity is the measurement of the ability of a medium to conduct electrical current. POME sludge has demonstrated a higher value of electrical conductivity (1.79 mS/cm) compared to soil (0.22 mS/cm). Such conditions may be attributed to the presence and accumulation of various charged ions in both POME sludge and soil. Usage of chemicals during the processing of palm oil has contributed to the high amount of ions in the POME sludge (Phukan & Bhattacharyya, 2003).

Table 2: Characteristics of soil and POME sludge used as the treatment medium

Parameters	Soil	POME Sludge
pH	3.5 ± 0.01	4.6 ± 0.11
Organic matter (%)	79.4 ± 9.62	38.3 ± 0.36
Total nitrogen (N) (%)	0.6 ± 0.01	36.8 ± 0.01
Total phosphorus (P) (mg/L)	0.3 ± 0.06	12.2 ± 0.05
Conductivity (mS/cm)	0.2 ± 0.01	1.8 ± 0.01
Particle size (%)		
Clay	6.9	-
Silt	64.1	-
Sand	29.0	-

Concentration of Heavy Metals in Soil and POME Sludge Prior to Treatment

Most of the heavy metal concentrations were higher in POME sludge compared to soil, except for Fe and Cr (Figure 1) prior to treatments. Fe was found to be the most abundant element in both POME sludge and soil with the concentrations of, 471.2 mg/kg and 3311.3 mg/kg, respectively. The presence of Cr, Ni and Cd might be due to the weathering of parent materials from which the soil was formed. There is no doubt that heavy metals are naturally present in soil (Zarcinas *et al*., 2004). Concentrations of all metals, except Cu, in the soil samples were lower than the maximum permissible values for heavy metals in soil (Kabata-Pendias & Pendias, 1984). Soil properties, such as pH and organic matter also have major influences on the availability of heavy metals (Dolgen *et al*., 2007; Kanakaraju *et al*., 2007). The recorded soil acidic pH of 3.5 (Table 2) may be contributed to the high availability and concentrations of the heavy metals. The high concentrations of heavy metals, such as Fe and Zn in the soil (Figure 1), were found to be similar to the results obtained by Lai *et al*. (1999), where the concentrations of these metals had increased significantly, as the soil pH become acidic.

The concentrations of Cd, Cu, Ni, and Zn (Figure 1), in the POME sludge studied were within the USEPA standards (1993) for biosolid and maximum allowable metal concentrations. However, the concentrations of Mn, Fe, and Cr were not given in the USEPA (1993) regulations. Based on the relatively low contents of Cd, Cu, Ni, and Zn, these POME

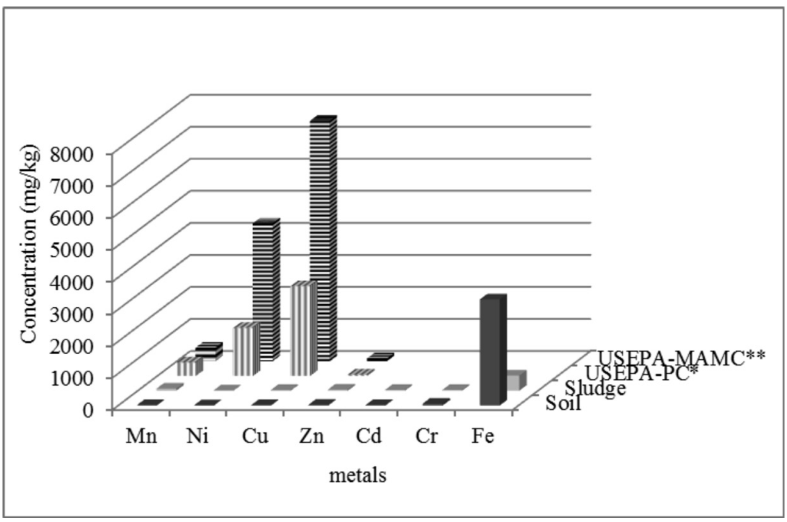

Figure 1: Levels of heavy metals in soil and POME sludge prior to treatments in comparison to the USEPA acceptable standards (USEPA, 1993) (Note: PC-Pollutant concentration biosolid; MAMC-maximum allowable metal concentrations)

sludge were tested in this study for potential application in agriculture.

Accumulation of Heavy Metals in Water Spinach

The application of POME sludge increased the uptake of Mn, Ni and Fe in water spinach (Table 3). The concentration of heavy metals increased from 75 to 219 mg/kg for Mn, 194 to 459 mg/kg for Ni and 298 to 633 mg/kg for Fe when the rate of POME sludge was doubled from 50% to 100%. The uptake of other heavy metals averaged ~56 mg/kg for Cu, ~296 mg/kg for Zn, ~130 mg/kg for Cd and ~286 mg/kg for Cr across all POME sludge treatments.

The concentration of Fe was found to be the highest, which was followed by Zn, Ni and Mn. These microelements are needed for plant growth and the levels exceeded the permissible level for vegetables, as indicated by Weigert' (1991) (Table 3). However, no sign of toxicity on plants, such as yellow leaf (chlorosis) and necrosis (leave wilting, dark green and slightly thickening) (Sun & Wu, 1998) were observed in the water spinach plants. This may be due to the great tolerance of the water spinaches towards heavy metals (Dolgen *et al*., 2007). No fertilizer was added during this study. As the POME sludge slowly degraded with time, it might have released adequate amount of nutrient elements and organic matters. Similar result of the increase in plant growth following the application of high palm oil sludge content was reported by Ekwuribe *et al.* (2008). Their study found that the number of cowpea root nodules have increased with palm oil sludge application.

The levels of Cr and Cd were compared with the limits stipulated by Weigert' (1991) and the Codex Alimentarius Commission standard (2001), respectively (Table 3). These non-essential metals were remarkably higher (multiplied by 124 in Cr and 648 in Cd) than the acceptable levels and their elevated accumulations may be posing a threat to human consumption. The high concentrations of these metals could be due to the acidic properties of the POME sludge (pH 4.6), and the soil (pH 3.5), which could have increased the solubility of these metals. Accumulation of Cd toxicity in water spinach could be due to the fact that this element is easily taken up by plants, especially in leafy vegetables (Lokeshwari & Chandrappa, 2006). Similarly, the extreme concentration of Fe uptake (633 mg/kg at 75% POME sludge application) was caused by the presence of high amounts of Fe in both the soil and sludge (Figure 1). This finding agreed with Umar *et al*. (2007) where high concentration of Fe for water spinach sampled in Nigeria was linked to the soil chemical composition.

Table 3: Concentrations of heavy metals (mg/kg ± standard deviation) in water spinach grown under five regimes of POME sludge:soil composition after 30 days from sowing in comparison with standards

Metals (mg/kg)	**POME sludge: soil composition (%)**					**P-value**	**Limit for vegetables****
	0:100 (control)	25:75	50:50	75:25	100:0		
Mn	65.0±2.36a	71.7±16.50a	88.3±2.35a	186.7±37.71b	251.7±7.07b	0.001	500
Ni	100.0±51.86a	219.2±30.18a	261.7±7.07a	406.7±70.71b	511.7±16.50b	0.006	67.90
Cu	48.3±30.64 a	69.2±38.89a	45.0±11.78a	41.7±2.35a	75.0±18.93a	0.697	73.30
Zn	87.5±14.25a	140.0±27.85a	366.7±55.56a	348.3±200.34a	538.3±40.07a	0.083	99.40
Fe	200.8±64.82a	335.8±52.03a	358.3±25.93a	660.0±51.86b	606.7±10.00b	0.008	425.50
Cd	112.5±36.53a	105.8±17.68a	123.3±61.28a	133.3±103.71a	173.3±22.56a	0.912	0.20***
Cr	52.5±14.18a	67.5±17.68a	98.3±44.78a	163.3±91.28a	165.0±82.49a	0.342	2.30

*Mean in the same column with the same superscript are not significantly different (P<0.05) according to Tukey test.
** Weigert'(1991)
*** FAO/WHO- Codex Alimentarius Commission

Of all the heavy metals analyzed, only Mn and Cu were found to be lower than the maximum allowed concentration as recommended by Weigert' (1991) (Table 3). The lowest concentration of Cu, compared to the other heavy metals, might be due to the metal-metal interactions at the roots. Cu has antagonistic effects with Zn, Fe and Cd, which had accumulated to higher concentrations than Cu. Similar response was observed for the effects of heavy metals in dill, peppermint, and basil plants where Cd and Cu had antagonistic effects (Zheljazkov *et al*., 2006). Essential micronutrients, i.e. Ni and Zn extracted from the water spinach were found to be seven and three times higher than the critical level as recommended by Weigert' (1991). These micronutrients must also be controlled by appropriate low POME sludge application rate or by ameliorating the soil and sludge composition with liming application (Dolgen *et al*., 2007).

In general, this study has shown high accumulations of heavy metals in water spinaches. Leafy vegetables, in general, have a high tendency to accumulate heavy metals. For example, Sharma *et al*. (2007) have found high accumulations of Cd, Cr, Zn, and Mn in sugar beet (*Beta vulgaris*), a type of leafy vegetable.

Analysis of Plant Growth

After 30 days from sowing, there was a significant increase in the plant height from ~53mm to ~ 240mm as POME sludge application was doubled from 25% to 50% (Table 4). Likewise, the crop yield also accumulated from 58.6kg/ha to 228.7kg/ha with POME sludge application up to 50%. Factors such as the existing content of organic matters and nutrients (N and P) (Table 2) could have contributed to the improvements in the plant's growth. A study by Hua *et al*. (2008) have shown similar trends in the sewage sludge amendment for two turfgrass species (ryegrass and fescue), whereby the highest sewage sludge loading has caused an increase of turfgrass biomass when compared to the control treatment.

Table 4: Plant height and yield of water spinaches grown under five regimes of POME sludge:soil composition after 30 days from sowing

POME sludge: soil composition (%)	Height ± S.E. (mm)	Yield ± S.E. (kg/ha)
0:100	49.8 ± 10.25	58.6 ± 3.51
25:75	57.0 ± 28.00	72.8 ± 3.35
50:50	223.5 ± 51.50	228.7 ± 14.65
75:25	256.0 ± 46.00	494.5 ± 325.95
100:0	196.1 ± 134.60	501.3 ± 426.23

S.E.: Standard error

Conclusion

The results obtained in this study have revealed that POME sludge application can lead to the significant increases of Mn, Ni and Fe in water spinach, which are parallel with increasing amount of POME sludge. The sequence of mean heavy metal concentrations in water spinach is; Fe>Ni>Zn>Mn>Cd>Cr>Cu. The results of this study suggest that POME sludge can be applied up to 50% in soil mixture for agriculture use. Lime should be applied to neutralize the acidic condition of the growing media, which could reduce the release of heavy metal toxicity. Therefore, similar experiment can be repeated with the application of lime to validate these results.

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