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UPTAKE OF HEAVY METALS BY WATER SPINACH FROM SLUDGE AMENDED SOILS

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UPTAKE OF HEAVY METALS BY WATER SPINACH FROM SLUDGE AMENDED SOILS

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DECLARATION

No portion of the work referred to this dissertation has been submitted in support of an application for another degree of qualification of this or any other university of institution of higher learning.

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Abbreviations

ANOVA - Analysis of Variance

APHA – American Public Health Association

$CaCO_3$ – Calcium Carbonate

Cd – Cadmium

Cr – Chromium

Cu – Copper

FAAS – Flame Atomic Absorption Spectrophotometer

Fe – Ferum

FSTS – Faculty of Science and Technology

HCl – Hydrochloric Acid

H_2SO_4 – Sulfuric Acid

HNO_3 – Nitric Acid

LOI – Loss of Ignition

Mg/kg – milligram per kilogram

Mg/l – milligram per liter

Mn – Manganese

NaOH – Sodium hydroxide

Ni – Nickel

OM – Organic matter

Ppm – Parts part chemistry

TKN – Total Kjeldahl Nitrogen

T/ha – Tonne per hectare

USDA – United States Department of Agriculture

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Uptake of Heavy Metals by Water Spinach from Sludge Amended Soils

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ABSTRACT

Palm oil mill sludge is generated in great amount in Malaysia and being disposed off by land application. This study aimed to determine the uptake of heavy metals namely chromium, cadmium, copper, zinc, iron, manganese and nickel by water spinach from sludge amended soils. Five different treatments were performed ranging from 0 % to 100%. The heavy metals were measured using Flame Atomic Absorption Spectrophotometer (FAAS). The yield of water spinach increased as the amount of sludge increased. The concentration of heavy metals also increased with the amount of sludge in water spinach. However, only cadmium, chromium, copper and zinc did not show any significant difference for the mean concentration between those five treatments ($P>0.05$). While manganese, iron and nickel showed significant differences ($P<0.05$). There was no toxicity symptoms observed on the water spinach. This may indicate the ability of water spinach to tolerate high heavy metals contents.

Key words: heavy metals, water spinach, palm oil sludge, soil.

ABSTRAK

Sisa pepejal kelapa sawit dihasilkan dengan banyaknya di Malaysia dan dibuang ke persekitaran. Kajian ini bertujuan untuk menentukan kandungan logam berat seperti kromium, kadmium, kuprum, zink, ferum, mangan dan nikel yang diserap oleh kangkung daripada tanah yang telah ditambahkan dengan sisa pepejal kelapa sawit. Lima rawatan berbeza yang merangkumi julat 0 hingga 100% digunakan dalam eksperimen ini. Kandungan logam berat ditentukan dengan menggunakan Spektroskopi Penyerapan Atom Nyalaan. Hasilan kangkung meningkat dengan penambahan sisa pepejal kelapa sawit. Kandungan logam berat di dalam kangkung turut meningkat dengan jumlah sisa pepejal kelapa sawit. Namun, hanya kadmium, kromium, kuprum dan zink tidak menunjukkan perbezaan yang ketara bagi purata kandungan dalam kelima-lima rawatan ($P>0.05$). Manakala mangan, ferum dan nikel pula menunjukkan perbezaan yang ketara ($P>0.05$). Tiada simptom keracunan yang ditunjukkan pada kangkung. Ini mungkin disebabkan oleh kebolehan kangkung untuk menyerap kandungan logam berat dalam kuantiti yang banyak.

Kata kunci: logam berat, kangkung, sisa pepejal kelapa sawit, tanah.

Chapter 1

1.1. Introduction

The presence of sludge which is one of the byproduct from industries has caused much problem due to the great amount of the sludge being produced. So, the sludge disposal is a major concern as there is limitation for reducing its quantity in the environment. There are two main alternative ways of sludge disposal which include incineration and land spreading (Manahan, 2005). Each of the stated way has their own disadvantages. In incineration method, the sludge is converted to ashes, however in most cases, supplementary fuel is required to burn the sludge, and thus make the method uneconomical (Dolgen *et al*., 2007) while land spreading will definitely cause no more space or area for disposal in the future. One of the suitable and effective ways for the disposal of sludge is through agricultural utilization (Parkpain *et al*., 2000).

Recycling industrial sludge for agricultural purposes can be beneficial. Sludge from wastewater treatment plant contains nutrients such as nitrogen (5%), phosphorus (3%) and potassium (0.5 %), and can be used to condition the soil and also act as conventional fertilizer. The humic material in the sludge improves the physical properties of the soil as well as the cation exchange capacity (Manahan, 2005). But, according to Dolgen *et al.* (2007), the main type of industry and the application rate influenced whether the sludge could be beneficial in term of effectiveness or harmful to the environment.

Plantations and palm oil mills are widely spread throughout Malaysia. This type of crop is suitable and versatile for cultivation on the various kind of Malaysian soil. It was estimated that the area covered by oil palm plantation in 1997 was 2.82 million hectares (Fuad *et al*., 1999). Despite that, palm oil industry can cause problem to the environment because it contribute organic pollutants to the surface water bodies from its effluents. The output sides or residues from the generation of oil palm include fibers (0.6 million ton per year), shells (0.2 million ton per year), and empty fruit bunches (0.9 million ton per year) (Chavalparit *et al*., 2006).

In this case, industrial sludge and wastewater sludge have considerable potential due to the availability of nutrients which can fulfill agricultural use (Spinosa and Vesilind, 2001). The usage of industrial and wastewater sludge for the growth of plant need to be demonstrated in order to understand the potential risk poses. Crops and soils will benefit from the application of industrial and wastewater sludge. The purpose of this study was to determine the effect of different palm oil sludge loadings on the heavy metals uptake by plant.

1.2. Research Problem

Recycling and applying sludge for agriculture purpose have many benefits and this has been proven by various studies. The benefits of sludge application include providing nutrients for example nitrogen, phosphorus and micronutrients to the crops; improving soil properties and also increasing the soil organic matter content. Toxic metals in sludge are another vital issue which needs to be taken into consideration. Palm oil sludge can be obtained abundantly in Malaysia and palm oil is one of the main commodities of this country. Utilization of sludge in agriculture could result in soil contamination, accumulation of heavy metals in crops and risk to human health and also environment. Thus, it is important to conduct studies to better understand the potential risk. Although there are number of studies pertaining to beneficial use of sludge, however the utilization of palm oil sludge is lacking.

1.3. Objectives

The objectives of this study are to:-

a. characterize soil and palm oil sludge.

b. investigate the potential of palm oil sludge on the water spinach growth by applying different sludge loadings.

c. determine the heavy metals uptake by water spinach after the 1 month period.

d. compare the heavy metals uptake between different treatments by employing One-Way ANOVA analysis.

Chapter 2

Literature Review

2.1. Heavy Metals

Heavy metals can be defined as elements that can form positive ions when in solution and its oxides will be converted to hydroxides rather than forming to acids when combined with water. Metals that have specific gravity greater than 5 g cm^{-3} are referred to as "heavy metals" (Liew, 2003). These metallic chemical elements also have high density and can be highly toxic or poisonous even at low concentrations (Tam and Wong, 2000). The primary source of naturally occurring heavy metals in soil is from geochemical materials while other sources are caused by human activities such as mining and industrial sectors (Martin and Coughtrey, 1982).

Various types of metals that are needed by most plants can be grouped into several classes. These include macronutrients, micronutrients, metals with uncertain role and mostly toxic metals. Under macronutrients group, metals such as potassium, calcium and magnesium are always taken up by plants together with the non-metal macronutrients such as nitrogen, phosphorus and sulfur (in the form of anions). Metals such as iron, manganese, zinc, copper, cobalt, molybdenum, selenium, sodium, silicon and chlorine (the only halogen which is essential for photosynthesis of higher plants) are classified as micronutrients. Mostly toxic group comprises arsenic, cadmium and lead. While metals with uncertain role consist of vanadium, nickel, strontium and aluminium (Csuros *et al.*, 2002).

The toxicity of heavy metals depends mainly on their bioavailability, and also on their chemical forms in both organic and inorganic matrices. It is found that through the addition of organic matter in soil, the solubility of zinc increases by the formation of organometallic complexes. When heavy metals have been released from sewage sludge, these metals will chemically react with soil and the toxicity of the metals declines with time (Usman *et al.*, 2005). According to Kim *et al.* (2007), metals such as copper (Cu) that have a particularly strong affinity for organics, tend to become more soluble and mobile after the addition of dissolved organic matter due to the application of sludges to the soils. Furthermore, an increase of heavy metals mobility and solubility is also caused by the decline of soil pH after the addition of sewage sludge. It is known that the influence of pH on metal solubility occurs by the extent of metal complexation with organic carbon-based ligands (Torri and Lavado, 2008).

The toxicity and the mobility of heavy metals in soils not only depend on the specific chemical form and its bioavailability, but also on their total concentration, their binding state, the heavy metal properties, environmental factors and soil properties such as pH, the content and type of organic matter, redox conditions and root exudates acting as chelates. The accumulation of heavy metals in soil takes place in various geochemical forms which include water-soluble, exchangeable, carbonate associated, iron–manganese oxide-associated, organic-associated and also residual forms. Water-soluble and exchangeable fractions are considered to be bioavailable; carbonate-, oxide- and organic matter- bound fractions may be potentially bioavailable; while the mineral fraction is mainly not available to the plants or microorganisms (Rodríguez *et al*., 2009). Apart from that, periodic soil drying and low moisture content may also affect the heavy metal availability (Fuentes *et al*., 2007).

2.1.1. Heavy Metals in Plants

The potential uptake of heavy metals by plants leads to major concern regarding the crops grown on land that is amended with sewage sludge (Madyiwa *et al*., 2002). Plants take up several important metals necessary for metabolism as well as several metals that are not necessary (mainly heavy metals). Humans consumed toxic substances such as lead and cadmium that accumulated in plants food, both directly by eating contaminated plants and indirectly by eating the products of animals fed on contaminated plants. Optimal concentrations of metals that are important for plants growth depend on the plant genotype. Excessive quantities of metals uptake may cause metabolic disorders. Metals in plants physiology are grouped based on required quantities used by plants and the effects on plants (Csuros *et al*., 2002).

Sewage sludge especially from industrial areas that contains heavy metals has limited application to agricultural soil. Copper and zinc are essential heavy metals required for plants growth (Madyiwa *et al*., 2002), but the concentration of heavy metals such as copper, zinc and nickel becomes phyto-toxic when above certain threshold. Cadmium can reach deleterious level to consumer when accumulates in certain species of crops (Nyamangara and Mzezewa, 2001).

Cadmium is known as the most hazardous element based on its concentration in sewage sludge, the effect to soil chemistry, and the ability for this metal to accumulate in plant tissue (Logan and Chaney, 1983). Cadmium toxicity that can lead to physiological effects on plants include major reductions in growth rates, inhibition of seed germination, photosynthetic deficiency, respiration and transpiration alterations in nutrient homeostasis, iron (Fe) deficiency and also changes in manganese, potassium, magnesium and calcium uptake rates (Lòpez-Millàn *et al*., 2008). Crops grown on higher dosage of sludge-amendment soil contain toxic concentration of heavy metals compared to those grown on lower dose of sludge-amendment and also unamended ones (Singh and Agrawal, 2008).

The accumulation of heavy metals and their availability to plants depend on the composition of the sludge, the application rate, agricultural practice, environmental condition, soil physical-chemical properties (especially pH) and the crop species or plants genotype (Dolgen *et al*., 2007; Kanakaraju *et al*., 2007; Alexander *et al*., 2006). Through natural leaching processes and plant uptake, metals in the soil can be mobilized and moved easily. The metals availability and mobility depend on the plant species present and their ability to form organic metals complex (Parkpain *et al*., 2000).

Each plant species and even their different parts accumulate different level of elements concentration. High contents of lead in soil will cause cadmium concentration to increase in the plant, while soil rich with cadmium metal will reduce the total lead uptake by the plants. This situation occurs due to the cation-exchange relation (Fazeli *et al*., 1998). Other than that, the availability of cadmium element in the soil as well as cadmium-zinc interaction also influences the cadmium accumulation in plant tissues (Yang *et al*., 2009). Besides that, the concentration of heavy metals in plants was found higher because the metals are chelated with the organic complex and when the biological and nutrient degradation of the organic molecules occur, the elements in the sludge become more available to the plants (Synman *et al*., 1998).

Although major studies have demonstrated beneficial effects on several plant species and soil based on proper amendment rate, however some plants were found not suitable for sludge amended soils because they tend to accumulate high concentrations of certain heavy metals. Several heavy metals such as zinc (Zn) and copper (Cu) can limit the application of sludge for agricultural purposes (Hua *et al*., 2008).

Different plants can accumulate certain level of heavy metals concentration. Higher plants that capable to accumulate more than (>) 100 mg/kg cadmium, more than (>) 1000 mg/kg copper, nickel, and lead, and more than (>) 10,000 mg/kg zinc in the dry matter of shoots are known as hyperaccumulator when growing in their natural habitats (Koopmans *et al*., 2008). According to Renoux *et al.* (2007), most of the effects of metals bioaccumulation in plants are due to the interactions between metals. Such interactions depend on the type of sludge being applied and also the sludge treatment processes.

Heavy metals may reduce the accumulation of biomass in newly grown tree seedlings, retard root growth, decrease the availability of essential elements and modify root morphology and architecture, thus prevent root capacity to explore soils. In addition, the excess or deficiency of certain essential metals may also inhibit protein and enzyme functions in plants which trigger photosynthetic electron transport impairment. Heavy metals can also affect seedling performance indirectly by reducing plant ability to access soil resources especially water (Fuentes *et al*., 2007).

The accumulation of heavy metals not only depends on genotype of plants itself, but it is also influenced by metal species and plant available forms of heavy metals (Lokeshwari and Chandrappa, 2006). Different amounts and types of exudates which are produced by plants is also one of the reasons that contribute to the variations in metal tolerance and uptake between plant species (Zheljazkov *et al*., 2006).

The availability of nutrients might promote plant growth, which could cause the number of uptake sites for metals in the plants to increase and thus, metals uptake may increase and metal concentrations in plants may be increase, decrease, or stay constant, depending on the relative responses of metal uptake and growth rate (Göthberg *et al*., 2004).

2.2. Sludge

Sewage sludge is referred as the insoluble solid residue remaining after sewage treatment and also known as biosolid, and domestic wastewater residuals. In general, sewage sludge consists of organic compounds, macronutrients, various ranges of micronutrients, non essential trace metals, organic micro-pollutants and microorganisms (Singh and Agrawal, 2008). Most of the sludge and industrial wastewater produced by different type of industries contain high concentration of heavy metals (Al Yaqout, 2003).

Every type of sludge from different industries has their own characteristics. This will reflect the suitability of the sludge in different application. For example, several industries such as tobacco industry, pulp and paper mill, food (vegetable) processing, fermentation industries, and flour production have low heavy metals content and also high contents of organic matter in the sludge (Dolgen *et al*., 2007). In the case of oil palm industry, results indicated that only 22.8 % of the raw material input which is from palm oil consists of valuable products while the rest is considered as by-product and waste which are generated in substantial amounts. These wastes include wastewater (640 m^3), ash (4.8 %), and decanter sludge (4.2 %). Other fraction of the waste such as fiber and shells are recycled and utilized as fuel to generate electricity and steam for the palm oil mill operation. However, some of the residues especially empty fruit bunches are used as organic fertilizer in the palm oil plantation and also dumped at the adjacent area of the mills (Chavalparit *et al*., 2006).

The main constituents of oil palm sludge are intact and ruptured oil cells in the mesocarp of the palm fruit, cell debris and residual oil. When oil palm sludge undergoes treatment through addition of enzyme (Celluclast), the chemical and physical properties of the treated sludge will be improved. For example, the sludge's chemical composition is enriched with respect to nitrogen and glucose contents which give its possibility for use as fertilizer (Ho *et al*., 1992). Untreated palm oil mill effluents are non-toxic and contain high concentration of starches, plant tissues, protein and free fatty acids. The solid materials in raw palm oil mill effluents provide a good source of organic matter (Ojonoma and Nnennaya, 2007).

Sewage sludge in general contains valuable plant nutrients, organic matter that can improve soil fertility (Madyiwa *et al*., 2002) and also significant amount of nitrogen, phosphorus, sulfur and calcium. During the application period, phosphorus availability can reach as high as 50%. Sewage sludge with high organic matter can improve the physical environment of the amended soil. Sewage sludge can cause risk to humans, animals and plants health due to the pathogenic microorganism content in it. However, through irradiation which is one of modern sewage sludge treatments, the number of pathogens can be reduced to low level (Nyamangara and Mzezewa, 2001).

Sewage sludge from the treatment domestic wastewater which is composted with organic fraction of domestic refuse contains high percentage of organic matter, relatively high content of nitrogen, potassium, phosphorus, calcium and magnesium, balanced level of oligoelements and lower content of non-essential heavy metals. Thus, the sludge can be a good organic fertilizer for agriculture (Jiménez *et al*., 1986). In contrast to fly ash, sewage sludge contains more major plant nutrients especially nitrogen and phosphorus, and is enriched with organic matter (Sajwan *et al*., 2003).

Sludge has both fertilizer and humus value and if supplied as liquid product, it could provide water to the plant which contains dissolved nutrients. Other trace essential elements such as selenium, sulphur and magnesium may also be valuable. The general pH value of sewage sludge is neutral which is at pH 7 (Spinosa and Vesilind, 2001). The lime content or calcium carbonate ($CaCO_3$) in paper mill sludge can have positive effects which act as neutralizing agent of acidic soil (Monte *et al*., 2008). Through the implication of wastewater or ‘reclaimed water sludge’ and municipal solid waste on land, soil fertility improves and benefits the crop production (Sajwan *et al*., 2003).

Some of the industry mills produce sludge that comprise of potential components for plants growth such as organic matter, silicate, cellulose, hemicellulose, lignin, and other components of wood furnish, and it may contain process chemicals (such as filing chemicals) and barks. Meanwhile, components in secondary sludge comprise of cellulose fibers, biomass, and wood-derived slowly biodegradable substances (lignin) (Jokela *et al*., 1997).

Sludge has a capability of contaminating portable water sources such as wells, streams, lakes and rivers when deposited very close to the water resources. Palm oil sludge can also affects soil microbial activities because the sludge is capable of causing impeded aeration in soil and resulting negative effects on soil microbial activities. However, there are some benefits or advantages of sludge application on soil. This can be seen where adequate amount of organic matter and nutrient elements are contained in most of the oil palm sludge which can be released in the process of degradation of the oil palm sludge (Ekwuribe *et al*., 2008).

2.3. Previous Studies of Metals Uptake by Plants from Sludge Amendment

Various studies have been carried out to utilize sludge for plant growing or agricultural purpose. According to Singh and Agrawal (2008), sludge amendment at the rate of 0, 80, 160, 320 t/ha dry weight in soil increased the average dry weight of the sunflower plants (*Helianthus annus L.*). The rose in plant productivity due to the sludge amendment was attributed to the increase in nutrient availability to the plants. Through the existence of macronutrients in sewage sludge, plants obtain good source of nutrients and the soil conditioning properties improve with the presence of organic constituents.

Experiments conducted by Wood *et al.* (1979) showed that raw palm oil mill effluent can be applied to the growth of crops including oil palms. Under three year's duration, soil structure and nutrients status improved which then led to the extra yield of crops and fertilizer replacement. Results also showed that, there was no substantial percolation of oxygen-demanding or other polluting elements, nor even excessive run-off on the surface during raining. According to Ojonoma and Nnennaya (2007), palm oil mill effluent that had not been treated before discharge was more polluting than domestic sewage sludge. Another study examined the effects of palm oil sludge and urea treatment on oil palm seedling using greenhouse experiment (Lal and Nor, 1994). In this study, the highest rate of net assimilation was resulted from the treatment of 0.01% urea-15% palm oil sludge-soil mixture which also led to the enhanced development and growth of the oil palm seedlings. However, the application of high (>0.01%) urea and 15% palm oil sludge to the soil caused detrimental effect in the shoot and root growth of six-month-old oil palm seedlings.

Another study was conducted by O'Brien *et al.* (2002) using paper mill sludge to grow corn (Zea mays L.). The results showed that the production of crop is equivalent to or greater than those obtained through commercial fertilizer. Tsakou *et al.* (2002) quoted that Flax (*L.*

usitatissimum) grown on soil amended with sludge showed flowering and fruiting began 3 weeks earlier, suggesting short time of cultivation when compared to those planted with conventional fertilizer. Early reproductive cycle of plants may depend on the faster development and greater biomass production in the plants grown with sludge-amended soil.

The application of paper mill sludge to wheat plant specifically (lignin which is one of the contents inside the paper mill sludge) showed that the biomass and shoot height increased while the accumulation of several heavy metals in the wheat shoots decreased (Zhang *et al*., 2004).

In an experiment conducted by Fazeli *et al*. (1998), the levels of cadmium (1.8 ppm) and nickel (26 ppm) were found highest in the leaves of the paddy plant compared to the seeds and roots part through the irrigation of paper mill effluent. Paddy seeds showed the lowest cadmium level (0.2 ppm) which might be due to the increased soil pH of the amended area that present alkaline nature. No deformation of plant parts, unusual spot on stems and leaves, and any stunted growth of paddy shoots and roots were detected as these parameters can show the sign of toxicity for the plants. The factors that affect the accumulation of heavy metals in plants include soil composition, soil moisture, soil pH, level of elements such as manganese and iron, and also plant factors such as plant species, part of the examined plant and growth stages (Kumpiene *et al*., 2008; Kim *et al*., 2007; Göthberg *et al*., 2004).

The manganese, Mn contents in the cucumber plants seemed not increase significantly with the sludge application. Heavy metals such as cadmium, lead and chromium were not detected in the green parts of cucumber plant same also with nickel. No toxicity symptoms were noted in the plants despite higher levels of zinc and iron than the accepted level (Dolgen *et al*., 2007). On the other hand, it was found that the total metal concentrations in the soil amended with sewage sludge were very high but gave no toxicity effects symptoms to the mixed and Kikuyu grasses (Madyiwa *et al*., 2002).

A study by Ekwuribe *et al*. (2008) showed that, when the level of oil palm sludge increased, the length of cowpea cultivars roots decreased. This seemed to be caused by low wet ability and anaerobic condition that formed before the decomposition of palm oil sludge. However, in the case of nodulation, results indicated that an increase in oil palm (8000 l/ha) caused more yield of root nodules in all the tested cultivars. This work has shown that the oil palm sludge amendment can increase soil nutrients as the sludge degrades and enhance crop production.

Differences in the accumulation level of heavy metals between plant species have been widely studied. Leafy vegetables such as spinach and lettuce were found to accumulate higher concentration of heavy metals (cadmium, lead, zinc and copper) than the root and legumes type vegetables like pea, carrot, French bean and bean. The vegetables were differ distinctively in their heavy metals content where, legume types (Leguminosae) tend to be low accumulators, root types (Umbelliferae and Liliaceae) tend to be moderate accumulators while leafy vegetables (Compositae and Chenopodiaceae) being high accumulators (Alexander *et al*., 2006).

The accumulation of cadmium, lead and mercury were higher in the different parts of water spinach when the nutrients strength in the medium was lower and the metal-induced effects on the plants were found to be bigger. In nutrient-enriched environments, the metals bioavailable fraction may be reduced due to the binding of nutrient anions. Besides that, the heavy metals uptakes in water spinach may also be affected by metal-nutrient competition, since nutrient cations and metals compete for uptake sites (Göthberg *et al*., 2004).

A study by Yaser *et al.* (2007) showed that, *Cymbopogun citratus* growth was improved when planted using compost of palm oil mill sludge and sawdust due to the suitable density (about 1500 kg m-3) and water holding capacity (15%) of the media.