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Evaluation of *Ricinus communis* as a Phytoremediator of Manganese in Soil Contaminated with Sewage Sludge

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ABSTRACT

The cost to dispose of sludge is very high, and Malaysia usually treats it by composting. However, composting is not a feasible method because it is time consuming. The application of sewage sludge on cropland is economical method because it provides the plant nutrients. However, it has a serious impact on the plants, animal and environment due to high concentration of heavy metals. Therefore, phytoremediation is a green technology to remove pollutants especially heavy metals from contaminated soil. This study was conducted to determine the potential of *Ricinus communis* to remove heavy metal in soil. The seedlings were planted on six different planting media T_0 (100% soil-Control), T_2 (80% soil + 20% sewage sludge), T_3 (60% soil + 40 % sewage sludge), T_4 (40% soil + 60% sewage sludge), T_5 (20% soil + 80% sewage sludge) and T_6 (100% sewage sludge). The highest accumulation of Mn was observed in leaves. *Ricinus communis* were found to be suitable as phytoremediators to clean heavy metals especially Mn as its TF value was higher than BCF.

Keywords: Heavy metals, manganese, phytoremediation, sewage sludge

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INTRODUCTION

Population growth in Malaysia have led to accumulation of sewage sludge, an industrial and domestic by-product. According to Kadir and Mohd (1998), about 3 million metric tonnes of sewage sludge is produced yearly while Indah Water Konsortium (2003) estimated about 7 million cubic meters of sewage sludge yearly by 2022. The current method to dispose of sewage sludge is through incineration (Ødegaard, et al., 2002). Previous studies have shown that sewage sludge has its benefits as soil amendment, soil fertiliser, soil conditioner and improves soil aggregation (Sommers, et al., 1980; Mininni, & Santori, 1987; Xu et al., 2013; Xu et al., 2013). The reason is it contains a high amount of nutrient (N, P, K, Mg and Ca) and organic matter that acts as a natural fertiliser (Majid, et al., 2011). The application of sewage sludge on cropland provides the plants with nutrients and is much more economical compared with incineration, excavating soil, pumping and treating contaminated groundwater, electro kinetic systems, chemical treatment, physical barriers, soil vapour extraction, in-situ oxidation and *in-situ* vitrification.

Sewage sludge can be described as solid waste or biosolids that contain numerous heavy metals from wastewater treatments, mining residues and agricultural waste. The sewage sludge is a source of surface and ground pollution which produces bad odours (Zaini et al. 2014). Heavy metals from sewage sludge would contaminate cultivated crops and natural vegetation as they exceeds the maximum permissible limit set by the local authorities (Ahmad et al., 2015; Nasira, et al., 2015). It is concentrated in a food chain that has an adverse effect on human health and wildlife (Ahmadpour et al. 2010). According to Ahmad et al. (2015), heavy metals can harm the natural environment at low concentration through their tendency to accumulate in the food chain or because of their inherent toxicity. Accidental digestion of heavy metals may cause health problems such as skin allergies and anaemia. Thus, improper disposal of solid waste (sewage sludge) has serious repercussions to the environment. Most countries use sewage sludge sparingly as a soil amendment due to its high heavy metal concentrations.

There have been efforts to remove heavy metals from the soil by using physical and chemical processes to treat it. These have proven to be expensive (Majid et al. 2011). Phytoremediation is a low cost, low impact and environmentally sound green technology to remove heavy metals from contaminated soil using selected plants, The plant is selected based on its characteristics such as fast growing, have high biomass and natural tolerance to toxic substances (Majid et al. 2011). The objective of scientists in applying phytoremediation is to treat contamination by using the most rational and natural process.

Ricinus communis (Family Euphorbiaceae) known as castor bean was selected as a phytoremediator for this study. Previous studies have shown that this plant is a fast growing species and able to grow in polluted soil that contains heavy metals (Vara Prasad & de Oliveira Freitas, 2003; Rockwood et al., 2004; Giordani, et al., 2005; Vanaja et al. 2011). Limited information is available regarding the evaluation of *Ricinus communis* as phytoremediator of manganese. This study was initiated with the following objectives: (i) to quantify heavy metal accumulation in different plant parts such as roots, stems and leaves; and (ii) to quantify Mn concentration in growth medium before planting and after harvesting period.

MATERIALS AND METHODS

The study was conducted at the greenhouse of Universiti Putra Malaysia, Serdang, Malaysia. The duration of the experiment was six months (February 2014 to July 2014). Healthy seedlings of Ricinus communis were collected from Seri Subuh Agrofarm, Negeri Sembilan. The growing medium for Ricinus communis was soil, organic matter, river sand, in a 3:2:1 ratio. The seedlings were transplanted into suitable pots (32.0 cm height, 106.0 cm upper diameter and 69.0 lower diameters) that were filled up with the mixture of soil and sewage sludge.

The soil used for this study was from the Munchong Series. while sewage sludge was taken from STP Bandar Tun Razak. The soil samples were used as growth media and the soils were mixed with sewage sludge. Physical and chemical properties of soil and sewage sludge were analysed. There were six different treatments with four replicates for each treatment; T_0 - Control (100% soil), T_1 (80% soil + 20% sewage sludge), T_2 (60% soil + 40% sewage sludge), T_3 (40% soil + 60% sewage sludge), T_4 (20% soil+ 80% sewage sludge) and T_5 (100% sewage sludge). The pots were arranged in a Randomized Complete Block Design (RCBD) in a greenhouse. Soil samples were collected from each pot before planting and after harvesting. All soils and sewage sludge were air dried and sieved to <2 mm.

The aqua regia method was used to determine the concentration of Mn in soil. The aqua regia solution contained a 3:1 mixture of concentrated hydrochloric acid (HCl) and nitric acid (HNO_3). Five grammes of soil were put into 50 ml of Aqua Regia solution in a PTFE (polytetrafluoroethylene) beaker and left to react overnight. Then, the samples were heated on the hot plate to approximately 200°C for two hours. When cooled and after making up to 250 ml, the samples were filtered by Atomic absorption spectrophotometry (AAS) (Justin, et al., 2011).

The dry ashing method was used to determine the concentration of heavy metals in the plant. The plants were harvested and washed gently with distilled water to remove soil particles and dust. The leaves stems and roots were cut and the fresh samples were weighed and dried in the oven at 60°C for 24 hours (Heryati et al. 2011). After the samples had cooled, 1 g of ashed tissue was dissolved in a 10 ml dilute nitric acid- hydrochloric acid digestion in 50 millilitres beaker. Finally, the samples were filtered to remove the suspended solids .The heavy metals were determined using atomic absorption spectrophotometry (AAS) (Justin et al. 2011).

The plant height and a number of leaves were measured every month. Plant biomass was measured separately according to its leaves, stems and roots. Two indicators are used to detect the accumulation of heavy metals: a) Bioconcentration Factors (BCF) which indicate the ability of a plant species that can accumulate metals into its tissue from the soils or water; b)Translocation Factors (TF) which indicate the competence of the plant species in translocating metals from roots to shoots. The plant's ability to accumulate metals from soils and translocate metals was estimated using the TF in equation 1 and the BCF in equation 2 (Yoon, et al., 2006):

$$TF = \frac{Metal\ concentration\ aerial\ parts}{Metal\ concentration\ in\ roots} \quad [Eqn.\ 1]$$

$$BCF = \frac{Metal\ concentration\ in\ roots}{Metal\ concentration\ in\ soil} \quad [Eqn.\ 2]$$

The analysis of variance (ANOVA) was used for growth parameters, heavy metals in the soil and plant parts and the mean separation test were carried out using Tukey's test ($p \leq 0.05$). The computation and preparation of graph were done using SPSS 16.00 and Microsoft EXCEL 2003 software program.

RESULTS AND DISCUSSION

Physical and Chemical Properties of Soil and Sewage Sludge

The physical and chemical analysis of the control media are shown in Table 1.1. In this experiment, the texture in the control was sandy clay (because the topsoil contained some sand) at a depth of 0-25 cm. The proportion of sand, silt and clay was 41.24%, 7.72%, 51.04% respectively. A high percentage of clay helps to store enough water. Due to the negative charge that belongs to clay, we can determine the fertility of the soil from clay minerals. The percentages of total N and C were 0.11% and 1.62%, while the C/N ratio was 1.62%. The soil was acidic, with a pH of 5.51 (normally, Malaysian soils are very acidic). The available phosphorus was 0.92 mg kg^{-1} and available CEC was 7.38 cmolckg^{-1}. The concentrations of exchangeable bases, such as K, Mg, Ca and Na, were 0.40 cmolckg^{-1},

Table 1
Selected physical and chemical analysis of soil and sewage sludge

Analysis	Soil	Sludge
Texture	Sandy clay	-
Sand (%)	41.24	-
Silt (%)	7.72	-
Clay (%)	51.04	-
Total N (%)	0.11	1.39
Total C (%)	1.62	63.65
C/N ratio	14.73	45.79
pH (1:2.5 soil: water)	5.51	6.19
Available P (mg kg^{-1})	0.92	80.68
CEC (cmolckg$^{-1)}$	7.38	27.88
Exchangeable cations (cmolckg-1)		
K (cmolckg^{-1})	0.40	2.50
Mg (cmolckg^{-1})	0.15	1.80
Ca (cmolckg^{-1})	0.62	5.44
Na (cmolckg^{-1})	0.11	0.73
Total heavy metal (mg kg^{-1})		
Cu (mg kg^{-1})	0.008	4.93
Fe (mg kg^{-1})	30.875	1602.13
Mn (mg kg^{-1})	0.003	9.73
Pb (mg kg^{-1})	0.087	10.35
Zn (mg kg^{-1})	0.135	68.38

0.15 cmolckg^{-1}, 0.62 cmolckg^{-1} and 0.11 cmolckg^{-1} respectively. The concentrations of Cu, Fe, Mn, Pb and Zn were 0.008 mg kg^{-1}, 30.875 mg kg^{-1}, 0.003 mg kg^{-1}, 0.087 mg kg^{-1} and 0.135 mg kg^{-1} respectively.

Plant Height, Number of Leaves and Heavy Metal Accumulation in Plant Parts

Figure 1 shows the significance difference ($P \leq 0.05$) in plant height based on treatments and age. In these experiments, plant height ranged from 8 cm to 114 cm at 6 months of age. On average, application of sewage sludge increased plant height of *Ricinus communis*. The number of leaves increased with increasing time except for T_5 (Figure 2). It was proven that, *Ricinus communis* are not tolerant in highly contaminated soil with sewage sludge.

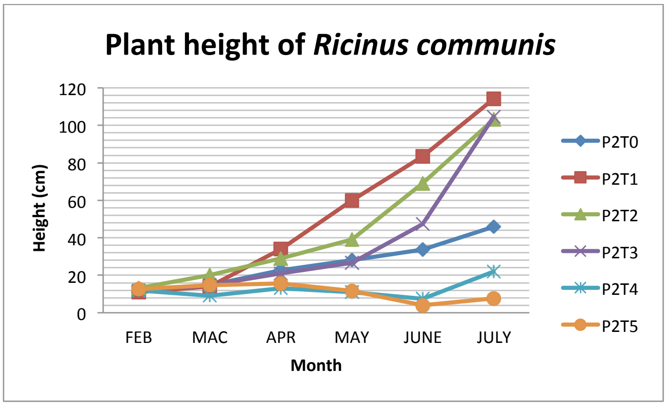

Figure 1. Plant height of *Ricinus communis* from February 2014 to July 2014

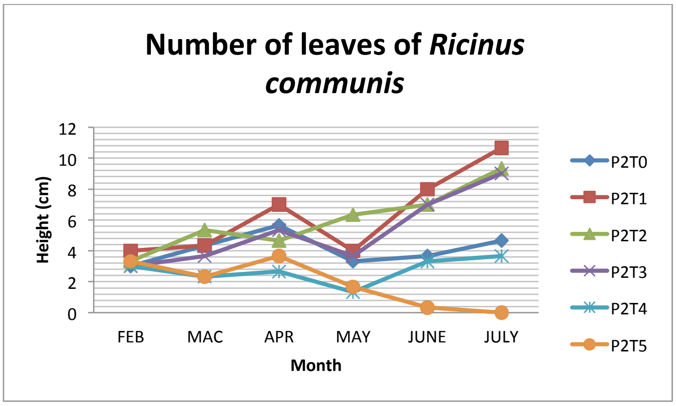

Figure 2. Number of leaves of *Ricinus communis* from February 2014 to July 2014

Mn concentration had a significant effect (P ≤0.05) on different plant parts at different levels of treatments (Figure 3). The range of Mn concentration in leaves, stems and roots were between 0.04 mg/kg and 0.60 mg/kg (T_5). The graph showed that the concentration of Mn was in the order of leaves>roots>stems. According to Sánchez,et al., (2005) and Zakir, et al., (2008), Mn concentration was high in the soil and plant parts after sewage sludge was applied in the growth medium. Besides, the increased concentration of Mn are related to the excess of Zn in plant shoots (Abdu et al. 2011). Hyperaccumulator plant species can accumulate Mn element up to 100 or 1000 times followed by non-accumulator plants (Tangahu et al. 2011).

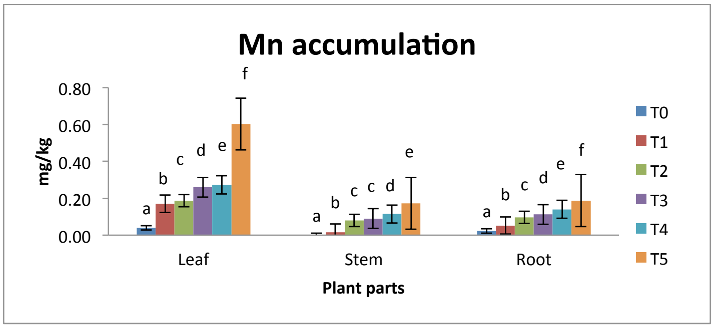

Figure 3. Concentrations of Mn in plant parts after harvesting of *Ricinus communis*. Different letters indicate difference between means at each treatment before planting and after harvesting. T_0 = Control, T_1 = 80% soil + 20% sewage sludge, T_2 = 60% soil + 40% sewage sludge. T_3 = 40% soil + 60% sewage sludge, T_4 = 20% soil + 80% sewage sludge, T_5 = 100% sludge

Concentration of Mn before planting and After Harvesting in the Growth Medium

There is a stark difference (P≤0.05) between Mn concentrations and treatments before planting and after harvesting (Figure 4). The results showed that Mn concentration was high due to the concentration of sewage sludge. However, the Mn concentration decreased after harvesting. The ranged of Mn concentration in growth medium with *Ricinus communis* was between 1.10 mg/kg (T_1) and 9.73 mg/kg (T_5). Parisa et al. (2010) stated that Mn concentration in the soil and plant tissue increased after applying the sewage sludge in the growth medium. In these experiments, Ricinus communis contained high Mn concentration in growth medium with a value of 9.73 mg/kg (T_5). High concentration of Mn in the soil restricted germination of plants as well as reduced root and shoot growth. According to Schulte and Kelling (1972), the availability of Mn was influenced by soil pH, organic matter content, moisture and soil aeration. Soil pH increased when Mn concentration in growth medium is increase due to the high content of sewage sludge. Soil pH affects plants' heavy metal uptake. The minimum soil pH in *Ricinus communis* was found in T_0 (the control), with a value of 5.26, while the maximum soil pH in *Ricinus communis* was found in T_5, with a value 6.36. Commonly, acidic soils are likely to contain high Mn and cause toxicity to the plants. However, this sewage sludge contains high calcium due to lime treated sludge. There are many symptoms of Mn toxicities to the plants such as distorted leaves, dark specks on leaves and leaf tissue dying at the leaf margins.

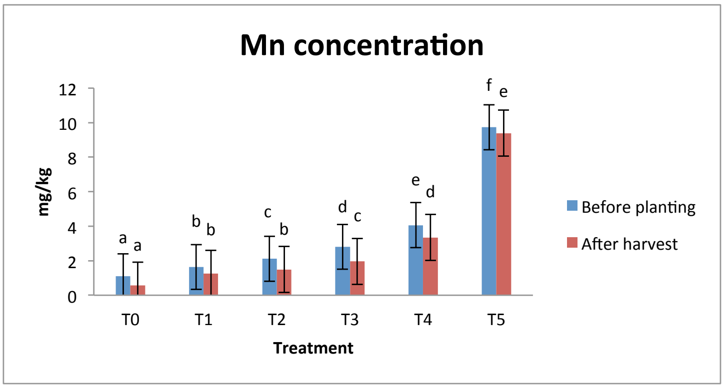

Figure 4. Concentrations of Mn in growth medium of *Ricinus communis* before planting and after harvesting. Different letters indicate difference between means at each treatment before planting and after harvesting. T_0 = Control, T_1 = 80% soil + 20% sewage sludge, T_2 = 60% soil + 40% sewage sludge. T_3 = 40% soil + 60% sewage sludge, T_4 = 20% soil + 80% sewage sludge, T_5 = 100%

Bioconcentration Factor (BCF) and Translocation Factor (TF) in *Ricinus communis*

Based on Table 1, the values of Mn BCF's was below 1 (0.02 to 0.07), while the TF values were above 1 (1.93 to 4.16). The results showed that *Ricinus communis* could be a good phytoextractor because its TF value was above 1 while BCF was low.

Table 2
Bioconcentration factor and translocation factor of Mn in different plant species

Species	BCF						TF					
	T_0	T_1	T_2	T_3	T_4	T_5	T_0	T_1	T_2	T_3	T_4	T_5
Ricinus communis	0.04[b]	0.04[b]	0.07[c]	0.06[c]	0.04[b]	0.02[a]	1.93[a]	3.62[d]	2.83[b]	3.10[c]	2.72[b]	4.16[e]

BCF = Metal concentration ratio of plant roots to soil and TF = Metal concentration ratio of plant shoot to roots

CONCLUSIONS

The present study confirmed that the *Ricinus communis* are suitable as phytoremediators to treat soil contaminated with sewage sludge with a high concentration of Mn. The plant was able absorb heavy metals and stored them in the stem based on the results that showed that the TF value was higher compared than BCF. However, the duration of the study needs to be extended. These experiments need to be conducted in the field in order to verify if this species can be used as a good accumulator for heavy metals in large areas.

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