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Heavy Metal Uptake and Translocation by *Dipterocarpus verrucosus* from Sewage Sludge Contaminated Soil

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ABSTRACT

Sewage sludge or biosolids is the solid waste that has been left after wastewater is treated in a domestic wastewater treatment facility. The most common way of sludge disposal is to dispose it on the land, sea or air (incineration). However, these methods are not environment-friendly, costly and time consuming that requires expertise and knowledge. An ideal way to manage sewage sludge is to use it as a soil amendment in agricultural land due to it's high organic matter and other base cations. Sewage sludge contains high levels of heavy metals such as lead, cadmium and zinc that can be harmful to both plants and the environment. Hence, these metals need to be removed before the sewage sludge is used as a soil amendment. The objective of this study was to assess the potential of *Dipterocarpus verrucosus* to uptake and translocate heavy metals from sewage sludge contaminated soil. The *D.verrucosus* seedlings were planted on six different planting media; Control (100% soil), T1 (80% soil and 20% sewage sludge), T2 (60% soil and 40% sewage sludge), T3 (40% soil and 60% sewage sludge), T4 (20% soil and 80% sewage sludge) and T5 (100% sewage sludge) for the duration of 16 weeks. The growth performance, of height of *D.verrucosus* was measured using diameter tape, while the basal diameter was measured using a venier caliper for every two weeks for a period of 16 weeks. The plant biomass was determined using a destructive sampling method. Plant samples were collected after harvest and soil samples were collected before and after planting. The Atomic Absorbtion Spectrophotometer (AAS) was used to determine the concentration of heavy metals in the planting media and the plant parts (leaves, stem and roots). The highest plant biomass was recorded in the T1 growth media of 20% sewage sludge and 80% soil. *D.verrucosus* plant was able to remove heavy metals of Cd, Cu, Pb, Fe and Zn effectively. The highest concentration of heavy metal in the roots of the *D.verrucosus* plant was Fe, recorded in the T5 growth media (1879.75 ppm). The highest accumulation of Zn (68.47 ppm) in *D.verrucosus* was recorded in the stem of *D.verrucosus* in the T5 growth media whereas the stem of the *D.verrucosus* in T3 recorded the highest Cd accumulation (2.85 ppm). The highest Pb uptake was recorded in the roots of *D.verrucosus* in T5 (37.3 ppm), while the lowest accumulation of Pb was noted in the stem of the *D.verrucosus* in Control (23.49 ppm). For Cd, the highest translocation factor (TF) (4.01) was recorded in T1. The lowest Bioconcentration Factor (BCF) for Cu was recorded at T5 (0.22). The highest TF for Lead was recorded in control (2.22) while the lowest was in T1 (1.64). The BCF for Zn was lowest at T3 treatment (0.16). The *D.verrucosus* plant was found to be suitable for taking up heavy metals from sewage sludge especially Cd, Zn, Cu and Pb. The roots of *D.verrucosus* are ideal in removing and storing Fe, while the stem of the *D.verrucosus* plant is ideal for the uptake and accumulation of Zn. However, more studies need to be conducted, especially in field conditions, in order to optimize the potential of the *D.verrucosus* plant as a phytoremediator.

Keywords: *Dipterocarpus Verrucosus*, Phytoremediation, Heavy Metals, Sewage Sludge, Translocation Factor, Bioconcentration FACTOR

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1. INTRODUCTION

The ever increasing human population, along with rapid industrialization and urbanization has resulted in an enormous growth in the volume of wastewater produced around the world. Wastewaters have to be treated appropriately at wastewater treatment facilities, which in turn produce solid waste products known as sewage sludge. Due to the increasing amount of wastewater needed to be treated, the amount of sewage sludge produced is constantly increasing, making proper disposal of sewage sludge a major concern. Malaysia produces about 5 million cubic meters of sewage sludge per year. The amount has been estimated to reach 7 million cubic meters per year by 2022 (Indah Water Konsortium (IWK), 1997).

Sewage sludge, also known as biosolids, is the solid waste that has been left after wastewater is treated in a domestic wastewater treatment facility. Sludge which resulted from the wastewater treatment operation is usually in a very dilute suspension form, which typically contains 0.25 to 12% of solid (Indah Water Konsortium (IWK), 1997). In addition, sludge contains pathogens, heavy metals and toxic characteristics of the untreated wastewater. It also contains high organic matter and nutrients that are essential for plant growth. Most countries very strictly regulate the usage of sewage sludge as a soil amendment because of it potentially being harmful and dangerous to humans, animals, plants and the environment, as it might contain high levels of heavy metals, organic pollutants and pathogens (Odegaard *et al*., 2002).

The most common way of sludge management is to dispose it on the land, into the sea or to a certain extent in the air which is mainly a consequence of incineration (Odegaard *et al*., 2002). Nowadays there is an increasing environmental awareness, which is causing a switch from conventional sewage sludge disposal methods to environment-friendly methods that are very costly, time consuming and requires expertise knowledge. An ideal way for sewage sludge management is by using it as a soil amendment in agricultural land. This is because it contains high organic matter and plant nutrient (N, P, K, Ca and Mg) that makes it suitable to be used as an organic fertilizer (Singh and Argawal, 2007). However, land disposal for a long period of time may result in the accumulation of high levels of heavy metal. Sewage sludge usually contains high amounts of lead, cadmium, nickel, chromium and copper due to its industrial origin (Raymond and Felix, 2011). Even domestic sludge may contain high amount of Zinc, Copper, Cadmium, Lead (Raymond and Felix, 2011). This is especially true for sewage sludge in Malaysia, which has very high heavy metal content (Indah Water Konsortium (IWK), 1997). Heavy metals are dangerous pollutants because they are usually are non-degradable.

Certain heavy metals occur naturally in soil, but rarely at toxic level. Sometimes they are needed in small amounts by plants and animals. However, they become toxic when the concentration is too high since they are not metabolized and accumulates in the soft tissues. Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water (Kvesitadze *et al*., 2010). The heavy metals indicated in the USEPA and state regulation are trace elements that can be harmful to the environment, human, animals and plants. Hence, although sewage sludge contains essential nutrients for plants, it can also potentially be harmful to the plants due to high amounts of heavy metal such as Cd, Zn, Fe and Cu.

Phytoremediation is a technology that uses plants to degrade, extract or remediate contaminants from soil and water (Purakayastha and Chhonkar, 2010). Phytoremediation has received special attention since the last decade as this technology does not damage soil structure and just slightly changes soil microbial content (Purakayastha and Chhonkar, 2010). The assessment of the detoxification potential of the plant is determined by the rate and depth of contaminant uptake from the soil, accumulation in the plant cell and the degree of contaminant transformation to regular cell metabolites. The best plant for a particular phytoremediation task should be selected based on multiple plants characteristic (Majid *et al*., 2011). The actual phytoremediation related characteristics of the candidate plants should be fast growing, have high biomass and a natural tolerance to toxic substaces such as heavy metals and salinity (Majid *et al*., 2011).

Some plants are able to accumulate metals without showing toxicity and make excellent phytoremediators (Bennett *et al*., 2003). Hyper metal accumulating plant species have been identified in at least 45 plant families and individual species which can accumulate different metals (Reeves and Baker, 2000). These families are Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae and Scrophulariaceae. There is still a lack of information on the potential of plants from the Dipterocarpus family to act as a phytoremediator for heavy metals. Therfore, a study in this area is justified.

For this study, the plant species *Dipterocarpus verrucosus*, was selected to determine its potential to clean up toxic heavy metals present in sewage sludge.

D.verrucosus, known as "keruing merah" locally, is a species of tree in the family Dipterocarpaceae. It is endemic to Indonesia and Malaysia. The phytochemical part of these plants have been studied before (Muhtadi, 2008), but for such a broad family plants, the chemistry of Dipterocarpaceae is relatively less known. The initial research on *D.verrucosus* was mainly focused on resins such as terpenoid, sesquiterpene, triterpene and its economic value as a timber tree (Zuraida and Zain, 2011). There is a lack of research on the potential of *D.verrucosus* as a phytoremdiator. The objective of this study was to evaluate the ability of *D.verrucosus* in extracting heavy metals from sewage sludge.

2. MATERIALS AND METHODS

2.1. Site Description and Planting Materials

The study was conducted at the greenhouse of University Agriculture Park, Universiti Putra Malaysia (Serdang, Malaysia) for 16 weeks (January 2012 to April 2012). The temperature at glasshouse was 27°C in the morning and 35°C in the evening while Relative humidity in the glasshouse was 65%. The seedlings of the *D.verrucosus* tree were germinated from cuttings of the mature stem and planted in polybags (16.0×16.0 cm) in the Faculty of Forestry nursery, Universiti Putra Malaysia. The growing medium for the *D.verrucosus* seedlings were in the proportions of, soil: organic matter: river sand in a 3:2:1 ratio. The seedlings were transplanted into suitable plastic pots (32.0 cm height, 106.0 cm upper diameter and 69.0 lower diameter) that were filled up with the mixture of soil and sewage sludge after one month.

2.2. Nature of Species Planted

Dipterocarpus verrucosus, commonly known as "keruing merah", is a species of tree in the family Dipterocarpaceae. It is endemic to Indonesia (Kalimantan and Sumatra) and Malaysia. Dipterocarpaceae is the most important family of economic trees in Southeast Asia. The species of this family is one of major importance in the timber trade.

2.3. Plant and Soil Sampling and Chemical Analysis

There were six different levels of treatments used in this study, with four replicates for each treatment. The treaments consisted of a mixture of soil and dry sewage sludge and the control containing soil only: T0/Control (100% soil), T1 (80 soil and 20% sewage sludge), T2 (60 soil and 40% sewage sludge), T3 (40 soil and 60% sewage sludge), T4 (20 soil and 80% sewage sludge) and T5 (100 sewage sludge). The pots were labelled according to their compositions. The Completely Randomized Design (CRD) was used in this study. The heights, diameters and number of leaves of the *Dipterocarpus verrucosus* plants were measured every two weeks throughout the study period with diameter tape, while the basal diameter was measured using a venier caliper every two weeks. Soil samples were collected from each pot before and after planting, kept in a standard plastic container and air-dried before physico-chemical analyses. The AAS was used for analyzing the concentrations of selected heavy metals [Iron (Fe), Zinc (Zn), Cadmium (Cd), Lead (Pb) and Copper (Cu)] in the planting medium and plant parts and aqua regia was used as the extractant. Total carbon was determined using conventional method (loss on ignition), using 5 g of air dried soil which was kept in an oven for 8 h at 550°C.

2.4. Plant Growth and Biomass Measurement

The plant height and basal diameter were measured every two weeks. Plant biomass was measured separately according to leaves, stems and roots using destructive sampling method. The loss in weight upon drying is the weight originally present. The moisture content of the sample was calculated using Equation 1:

$$\%w = \frac{A - B}{B} \times 100 \tag{1}$$

Where:
%W = Percentage of moisture in the sample
A = Weight of wet sample
B = Weight of dry sample

2.5. Translocation Factor (TF) and Bioconcentration Factor (BCF)

The plant's ability to accumulate metals from soils and translocate metals from roots to shoots was estimated using the translocation factor (Equation 2) and the bioconcentration factor (Equation 3):

$$TF = \frac{\text{Metal concentration aerial parts}}{\text{Metal concentration in roots}} \tag{2}$$

$$BCF = \frac{\text{Metal concentration in roots}}{\text{Metal concentration in soil}} \tag{3}$$

2.6. Statistical Analysis

The analyses for growth and heavy metals in the soil, sludge and plant parts were done following the Analyses of Variance (ANOVA) technique and the mean values were adjusted using a post hoc test of Tukey's ($p \leq 0.05$). A comparison using an Independent Student's t-test at a 5% level was done to detect any significant differences between samples taken before planting and after harvesting. Computation and preparation of graphs were done by the use of SPSS 16.00 and Microsoft EXCEL 2003 software program.

3. RESULTS

3.1. General Properties of the Growth Media

The particle size analysis of the soil was slit clay. **Table 1** show that all treatments had lower pH (4.23 to 5.25) initially and increased after harvest (4.58 to 5.27). T2 shows the highest change in soil pH (4.23 to 4.89). Before planting, the highest total carbon was found in T5 (15.23%) and the lowest was in the T0, control (0.63%). There was a direct corelation with amount of sludge used in the growth media with the total carbon of the growth media, the higher the percentage of sludge in the growth media, the higher total carbon of the growth media. After harvest, the total carbon decreased in all the treatments except in T0, control.

The maximum total C content (5.20%) was found in T4 followed by T5 (4.57%) with the minimum content in the control of 0.91% (**Table 1**).

3.2. Growth Performance and Plant Biomass

The results showed significant difference ($p \leq 0.05$) among the treatments in terms of total height, basal diameter and number of leaves. **Table 2** shows that *D.verrucosus* planted in T1 showed the highest height (67.27 cm) which was closely followed by the T0, control (67.16 cm). Treatment 5 showed a conspicuous decrease after week 8. The basal diameter showed similar pattern with total height. After week 8, the basal diameter was constant for all treatment except for T1 and T2 which increased slightly. Treatment 5 (5.5 cm) showed the lowest basal diameter. After 16 weeks, treatment 1 produced the highest number of leaves (33). The lowest number of leaves was recorded by T5, producing only 7. The plant stems and leaves biomass showed significant difference ($p \leq 0.05$) among the treatments after 16 weeks while roots mass have no significant differences. Treatment 2 produced the highest biomass of 51.38g, 53.28g and 57.86g for roots, stem and leaves, respectively. Treatment 5 produced the lowest biomass of 23.65g and 14.46g for stem and leaves, respectively as shown in **Table 3**.

Table 1. The pH and total C (%) content in the growth media

	pH		Total-C	
Treatment	A	B	A	B
T0	4.27±0.07	4.58±0.11	0.63±0.07	0.91±0.11
T1	4.29±0.14	4.73±0.34	1.75±0.55	1.60±0.31
T2	4.23±0.10	4.89±0.33	4.09±0.68	2.10±0.28
T3	4.75±0.16	4.90±0.16	6.35±0.26	2.32±0.48
T4	4.48±0.15	4.65±0.26	7.65±0.44	5.20±1.34
T5	5.25±0.37	5.27±0.60	15.23±1.32	4.57±1.51

Note: T0-control (100% soil), T1 (80% soil and 20% sewage sludge), T2 (60% soil and 40% sewage sludge), T3 (40% soil and 60% sewage sludge), T4 (20% soil and 80% sewage sludge) and T5 (100% sewage sludge), B (before planting), A (after harvesting)

Table 2. Total height, basal diameter and number of leaves of *D. verrucosus* at 16 weeks after planting

Treatment	Height (cm)	Basal diameter (mm)	No. leaves
T0	67.16	6.6	22
T1	67.27	7.0	33
T2	66.41	5.9	16
T3	65.56	6.0	17
T4	65.31	6.0	18
T5	54.33	5.5	7

Note: T0-control (100% soil), T1 (80% soil and 20% sewage sludge), T2 (60% soil and 40% sewage sludge), T3 (40% soil and 60% sewage sludge), T4 (20% soil and 80% sewage sludge) and T5 (100% sewage sludge)

Table 3. Average dry weight biomass (g) for roots, stem and leaves

Treatment	Roots	Stem	Leaves
T0	48.07	51.60	32.02
T1	36.26	52.60	42.29
T2	51.38	53.28	57.86
T3	45.50	44.23	53.11
T4	49.28	40.33	55.36
T5	46.62	23.65	14.46

Note: T0- control (100% soil), T1 (80% soil and 20% sewage sludge), T2 (60% soil and 40% sewage sludge), T3 (40% soil and 60% sewage sludge), T4 (20% soil and 80% sewage sludge) and T5 (100% sewage sludge)

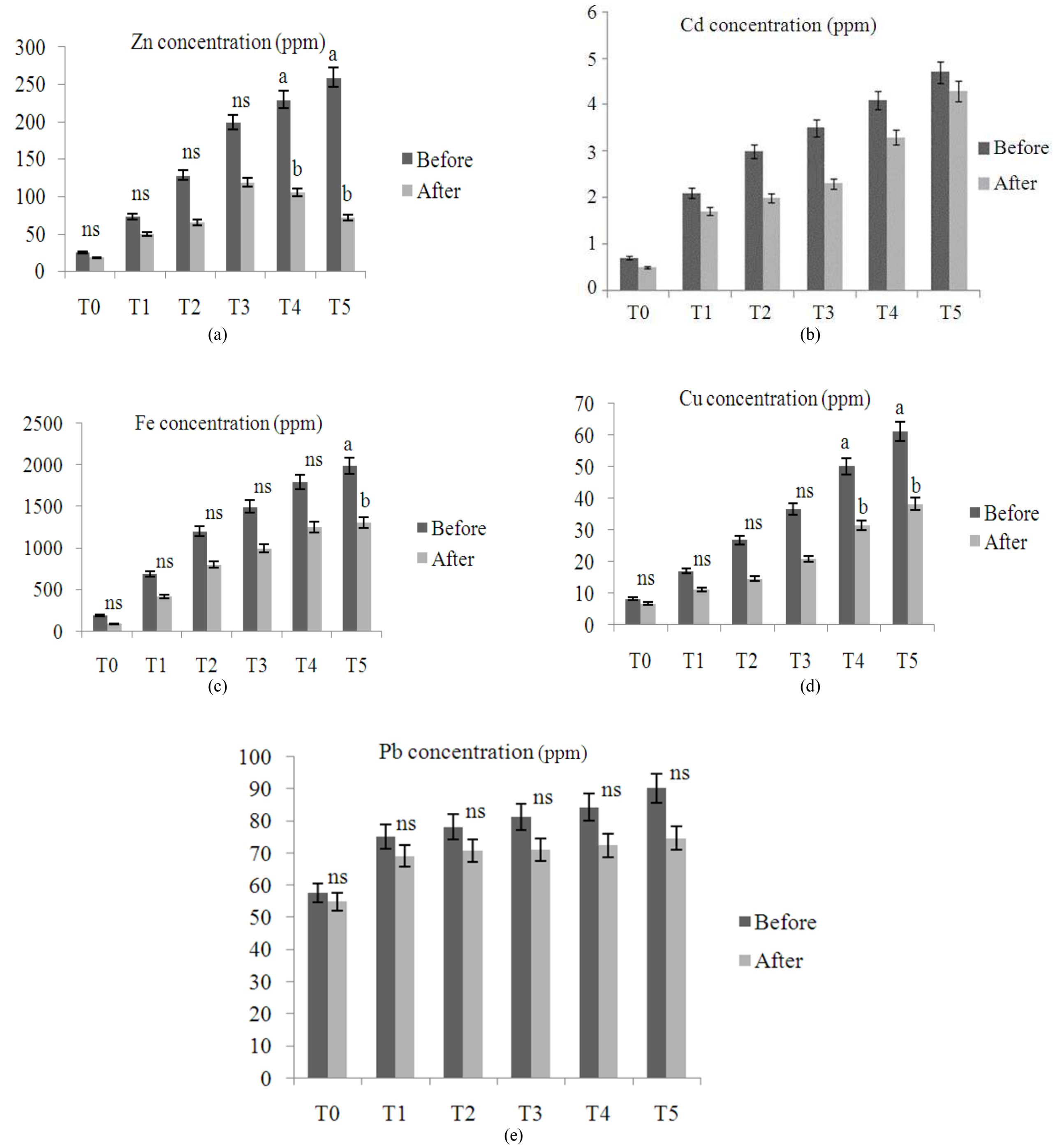

Fig. 1. The Concentrations of Zn (a), Cd (b), Fe (c), Cu (d) and Pb (e) in growth media before planting and after harvesting. * indicate significant difference between means at each treatment before planting and after harvesting according to a Student's t-test ($p \leq 0.05$), ns indicates no significant difference. T0, (100% soil-control); T1, (80% soil and 20% sewage sludge); T2, (60% soil and 40% sewage sludge); T3, (40% soil and 60% sewage sludge); T4, (20% soil and 80% sewage sludge); T5, (100% sewage sludge)

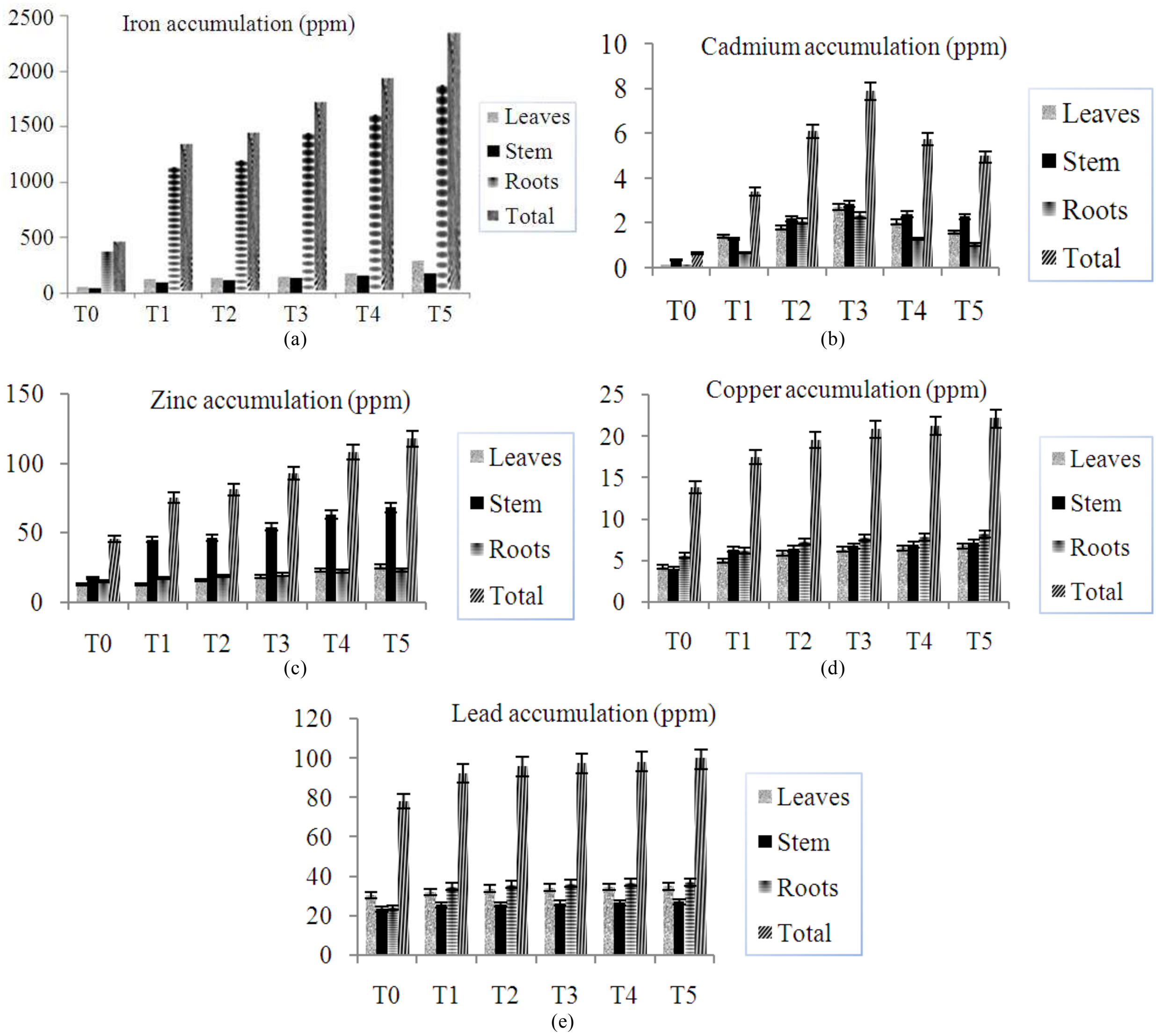

Fig. 2. Accumulation of Cd (a), Fe (b), Zn (c) Cu (d) and Pb (e) concentrations in the plant parts after harvesting of *D.verrucosus* plant as influenced by different treatments. T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2 = 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4 = 20% soil + 80% sewage sludge, T5 = 100% sewage sludge

3.3. Heavy Metal Concentrations in Growth media before Planting and after Harvesting

The concentrations of heavy metals before planting and after harvesting are shown in **Fig. 1a-e**. The *D.verrucosus* was found to be able to generally remove the heavy metals, such as Zn, Pb, Fe, Cd and Cu especially in T5 where the planting medium contained 100% sewage sludge. The Zn content of the sewage sludge was 259.42 ppm before planting in T5 and after harvesting, the Zn level decreased to 71.65 ppm (**Fig. 1a**). The highest decrease in Cd levels in the *D.verrucosus* growth media was observed in T3, where the initial level of Cd was 3.5 ppm while the level of Cd after harvesting was 2.3 ppm (**Fig. 1b**). The level of Fe in the T5 growth media decreased after harvesting (1986.9 ppm) compared to the initial Fe level (1308.1 ppm), as shown in **Fig. 1c**. The highest decrease in Cu levels in the *D.verrucosus* growth media was observed in T5, where the initial level of Cu was 61.23 ppm while the level of Cu after harvesting was 38.22 ppm (**Fig. 1d**).

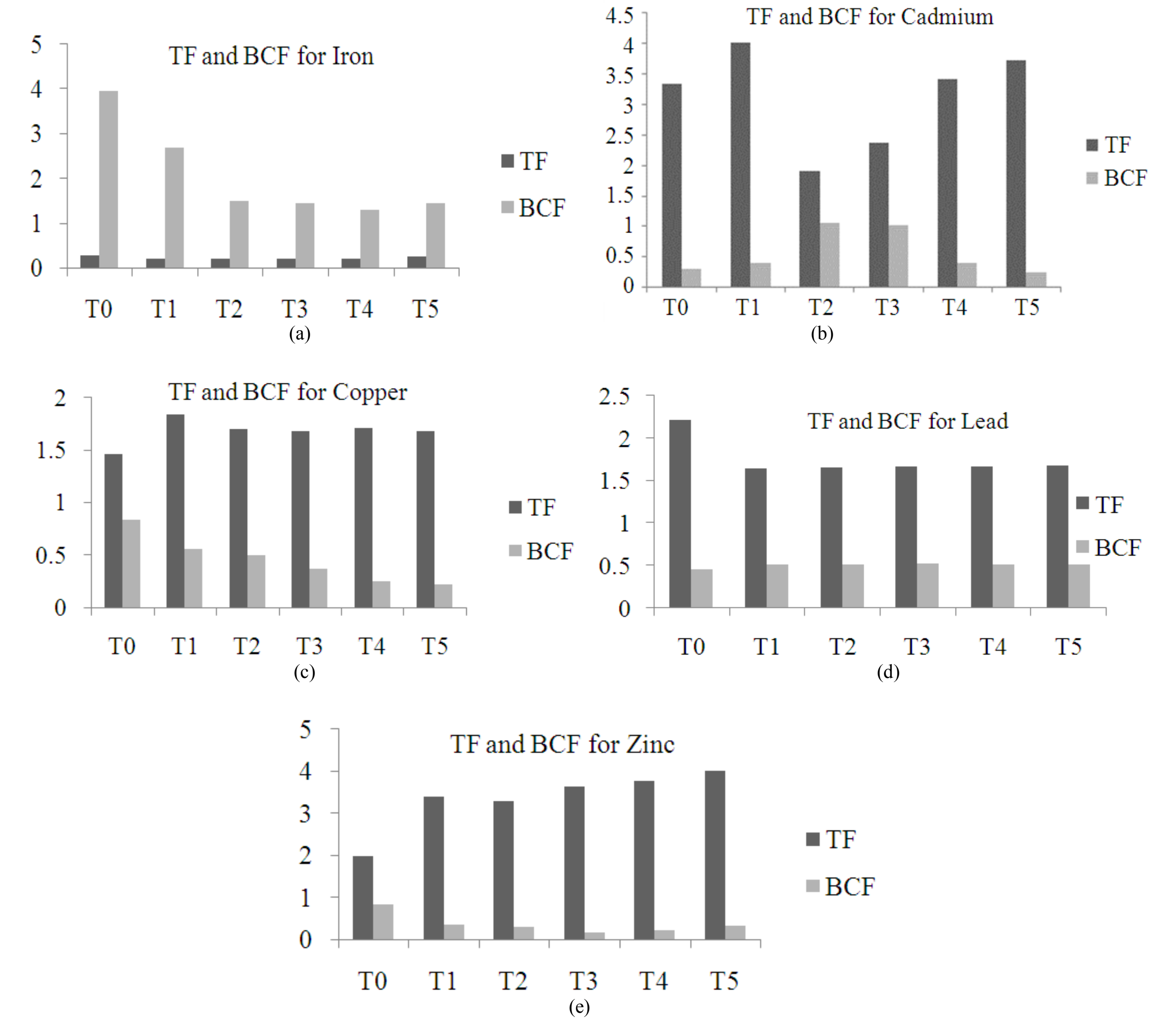

Fig. 3. Translocation factor and bioconcentration factor Fe (a), Cd (b), Cu (c) Pb (d) and Zn (e) of *D.verrucosus* plant as influenced by different treatments. T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2 = 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4 = 20% soil + 80% sewage sludge, T5 = 100% sewage sludge

The level of Pb in the T5 growth media decreased after harvesting (90.24 ppm); the initial Fe level 74.7 ppm, (**Fig. 1e**).

3.4. Heavy Metal Concentration in Plant Parts

The concentration of Zn, Cd, Fe, Cu and Cd in plant parts (leaves, stem and roots) after 16 weeks of planting are shown in **Fig. 2a-e**. The highest Fe accumulation was observed in the roots of *D.verrucosus* in T5 (1879.75 ppm), as shown in **Fig. 2a**. Furthermore, it was found that the leaves of the *D.verrucosus* absorbed higher levels of Fe compared to that of stem (**Fig. 2a**).

Figure 2b shows that the stem of the *D.verrucosus* in T3 growth media accumulated the highest amount of Cd (2.85 ppm), while the lowest concentration was observed in the roots in control (0.15 ppm). **Figure 2c** shows the highest Zn uptake was noted in the leaves at the control growth media (12.61 ppm). **Figure 2d** shows that the highest concentration of Cu was stored in the roots in the T5 treatment (8.23 ppm). This was also similar for Pb, where the highest concentration of Pb was found in the roots of the *D.verrucosus* plant in T5 (37.2 ppm), as recorded in **Fig. 2e**.

3.5. Translocation Factor (TF) and Bioconcentration Factor (BCF) of Heavy Metals

The TF and BCF of heavy metals for the *D.verrucosus* plant are shown in **Fig. 3a-e**. The TF for Iron was the lowest among the TF of all the other metals (**Fig. 3a**). The highest TF for Fe was recorded in Control (0.26) and the lowest was recorded for Treatment 1 (0.19). For Iron, the TF was most prominent in Control and Treatment 5. However, Fe recorded the highest BCF among the heavy metals. The highest BCF for Iron was recorded in Control (3.96), followed by Treatment 1 (2.68). For Cd (**Fig. 3b**) and Copper (**Fig. 3c**), the highest TF (4.01 and 1.84 for Cd and Cu respectively) was recorded in Treatment 1. The lowest BCF for Cd and Cu was recorded at Treatment 5 (0.24 and 0.22 respectively). The highest TF for Lead was recorded in control (2.22) while the lowest was in treatment 1 (1.64) (**Fig. 3d**). The BCF for Lead was lowest at Control and Treatment 5 (0.44 and 0.49 respectively). The TF for Zn was most prominent in Treatment 3, Treatment 4 and Treatment 5 (3.63, 3.77 and 4.01 respectively). The highest BCF for Zn was observed at Control (0.84), while the lowest BCF for Zinc was recorded at Treatment 3 (0.17) (**Fig. 3e**).

4. DISCUSSION

4.1.Changes in General Properties of Growth media

Due to tratments, soil pH had increased. The highest change in soil pH was treatment 2 (from 4.23 to 4.89). This change in pH indicated that *D.verrucosus* was able to remove acidic elements such as Fe and other heavy metals from the growth media (Ghafoori *et al*., 2011), indicating that this plant had the potential to increase the pH of soils. It was found that the higher the percentage of sludge in the growth media, the higher total carbon of the growth media. This indicates that the application of sewage sludge to soil can improve the overall organic matter content of the growth media (Arifin *et al*., 2011). An increase in organic matter could potentially improve the overall soil fertility (Rice, 2002). Besides that, soil organic matter improves the soil water holding capacity, making plants able to withstand short periods of droughts (Rice, 2002). Hence, sewage sludge has the potential to be used as an effective soil amendment, even having the potential of replacing the need for organic fertilizers.

4.2. Growth Performance and Plant Biomass

The highest total height of the *D.verrucosus* plant (67.27cm) was recorded in the T1. Besides that, the *D.verrucosus* plant in the T1 growth media also produced the highest number of leaves (33). The basal diameter was found to be constant after 8 weeks of planting; although the T1 and T2 were found to have slightly higher basal diameter. This shows that the *D.verrucosus* plant showed normal growth in terms of basal diameter (Majid *et al*., 2011). However, the *D.verrucosus* plant in treatment 5 exhibited the lowest basal diameter (5.5 cm). Furthermore, the *D.verrucosus* plant in treatment 5 also recorded the lowest number of leaves (7). These results indicate that to attain the optimum growth of the *D.verrucosus* plant, the ideal composition of the growth media should be 20% sewage sludge and 80% soil. This is most likely due to sufficient organic matter contribution by the sewage sludge without severely increasing the pH of the soil (Parisa *et al*., 2010) content. The T5 growth media exhibited the worst growth performance, indicating that a 100% sewage sludge growth media would be the least ideal growth media for the *D.verrucosus* plant, maybe due to higher acidity and heavy metal content (Mangkoedihardjo and Surahmaida, 2008). After 16 weeks, treatment 5 produced the lowest biomass of 23.65g and 14.46g for stem and leaves, respectively. This is a clear indication that the *D.verrucosus* plant is unable to grow optimally in soil with very high amounts of sewage sludge. Treatment 2 produced the highest biomass of 51.38g, 53.28g and 57.86g for roots, stem and leaves. The plant biomass plays an important role in the absorption of heavy metal from soil and water (Majid *et al*., 2011). Plant which is used as a phytoremediator must have both high potential capacity to absorb elements from soil or water and large biomass. Treatment 2 with the combination of 60% soil and 40% sludge produced the highest biomass, indicating that this plant can be used for remediation of sludge contaminated soils (Majid *et al*., 2011).

4.3. Heavy Metal Concentrations in Plant Parts

The highest accumulation of Zn (68.47 ppm) in *D.verrucosus* was recorded in the stem at T5 growth media. In all treatments, the Zn accumulation is highest in the stem compared to the leaves and roots. Since the

D.verrucosus plant in the T5 growth media exhibited the worst growth performance, it indicates that Zn toxicity in plants limited the growth of the plant (Fontes and Cox, 1998). Besides that, excess Zn has been found to cause manganese and copper deficiencies (Ebbs and Kochian, 1997). The stem of the *D.verrucosus* in T3 recorded the highest Cd accumulation (2.85 ppm), followed by the leaves of the *D.verrucosus* in T3 (2.7 ppm). This is because cadmium is a mobile heavy metal, easily transported from the root of the plant to the stem and leaves. The roots of the *D.verrucosus* were found to absorb high levels of Fe compared to the stem and leaves. The highest accumulation of Fe was recorded in the roots of the *D.verrucosus* in T5 (1879.75 ppm). The highest Pb uptake was recorded in the roots of *D.verrucosus* in T5 (37.3 ppm), followed by the roots of the *D.verrucosus* in T4 (36.93 ppm), while the lowest accumulation of Pb was noted in the stem of the *D.verrucosus* in control (23.49 ppm). The roots of the *D.verrucosus* in T5 had the highest uptake of Cu (8.23 ppm). The lowest Cu uptake was recorded in the stem of the *D.verrucosus* in the Control (4.03 ppm). Lower levels of Pb and Cu in the stem compared to the roots of the *D.verrucosus* plant was due to Pb and Cu being less mobile heavy metals.

4.4. Relationship between Heavy Metal Concentrations in the Growth Media and the Accumulation of Heavy Metal in Plant Parts

The highest concentration of Fe, Zn, Cu and Pb in all plant parts (roots, leaves and stem) of the *D.verrucosus* plant was recorded in the T5 growth media. This shows that the higher the concentration of Fe, Zn, Cu and Pb in the growth media, the higher the uptake of the heavy metals by the *D.verrucosus* plant. This increase in accumulation of heavy metal negatively affected the growth performance of the plant (Arifin *et al*., 2011). The uptake of Cd by the *D.verrucosus* plant was highest in T3 indicating that the *D.verrucosus* plant was unable to uptake Cd when there are high concentrations of Cd present in the soil (Majid *et al*., 2011). This indicates that the *D.verrucosus* plant is only able to uptake Cd at an optimum level when the Cd concentration of the growth media is not higher than 4 ppm. The highest concentration of heavy metal in the roots of the *D.verrucosus* plant in the T5 growth media was Fe (1879.75 ppm), followed by Zn (23.46 ppm), while the minimum was Cd (1.05 ppm). This shows that the roots of the *D.verrucosus* plant are most efficient in uptaking and storing Fe. Despite Fe being highly insoluble, the presence of organic ligands can convert Fe to be in a mobile form; hence the high uptakes of Fe by the roots of the *D.verrucosus* plant (Perk, 2013). The stem of the *D.verrucosus* plant was also found to be highly efficient in storing Zn.

4.5. Differences in Translocation Factor (TF) and Bioconcentration Factor (BCF) among Treatments and Heavy Metals

The TF for iron was the lowest among all other TF (0.19 in treatment 1), while it's BCF was highest among all other BCF (2.67 in Treatment 1). This is due to the *D.verrucosus* plant storing almost its entire Fe uptake in its roots (Yoon *et al*., 2006). Low TF and high BCF shows that the *D.verrucosus* plant is not suitable to be used as phytoextractor for iron (Majid *et al*., 2011), as ideal phytoremediator plants should store heavy metals in its stem. However, the TF for Cd, Zn, Cu and Pb was above 1 while its BCF was low, indicating that the *D.verrucosus* plant could be a good phytoextractor of Cd, Zn, Cu and Pb. (Baker, 1981).

5. CONCLUSION

The *D.verrucosus* plant was found to be suitable for taking up heavy metals from soil contaminated with sewage sludge. This is especially true for Zn and Cd since the plant stores the heavy metals in its stem, which are removed from the soil when the plant is harvested for getting its resin. Fe was also stored in the *D.verrucosus* plant, but mainly in the roots. The potential of *D.verrucosus* plant to be used as a phytoextractor for Fe is unfavourable, indicated by its low TF and high BCF. Based on the TF and BCF values, the *D.verrucosus* can be recommended to be used as a phytoremediator, especially for Cd, Zn, Cu and Pb. Primarily, the duration of the study needs to be extended and the effectiveness of the *D.verrucosus* plant in accumulating heavy metals in an open environment needs to be also conducted. The *D.verrucosus* plant has the potential to be a phytoremediator plant but needs to be further evaluated to optimize its potential.

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