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Phytoremediation Potential of *Dipterocarpus chataceus* Planted on Sewage Sludge Contaminated Soil

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Abstract: As the population of developing countries continuously grows, the need for efficient wastewater management is vital. Wastewaters are typically channeled to wastewater treatment facilities, where it is treated and sewage sludge is produced. Sewage sludge has no value and is disposed of on land, sea or air (incineration). However, these methods can harm the environment, costly, time consuming and require expertise knowledge. An environmentally-friendly and cost effective method of disposing sewage sludge, is by using it as a soil amendment. This is because it has high organic content, which can improve plant growth. However, high levels of heavy metals are also present in sewage sludge, which can harm both plants and humans. Hence, these metals need to be removed before the sewage sludge is suitable to be used as a soil amendment. The objective of this study is to assess the potential of *Dipterocarpus chataceus* to uptake and translocate heavy metals found in sewage sludge. *D. chataceus* seedlings were planted on six different planting media; T_0 / Control (100% soil), T_1 (20% sewage sludge and 80% soil), T_2 (40% sewage sludge and 60% soil), T_3 (60% sewage sludge and 40% soil), T_4 (80% sewage sludge and 20% soil) and T_5 (100% sewage sludge) for the duration of 16 weeks. *D. chataceus* showed the best growth in the T_1 growth medium, while the lowest was in T_5. The highest concentration of heavy metal was in the roots of the *D. chataceus* plant. Cadmium (Cd), copper (Cu) and zinc (Zn) was stored primarily in the stem of the *D. chataceus* plant, followed by the least and finally the roots. Similar to iron (Fe), lead (Pb) was stored mainly in the roots of the *D. chataceus* plant. Translocation Factor (TF) was high (TF>1) for Cd, Cu, Pb and Zn. However the BCF value was below 1 for all these metals, meaning that is wasn't a suitable phytoremediator of these metals. The BCF values was high (BCF>1) for Fe, but below 1 for TF, making *D. chataceus* a potential phytostabilizer of Fe. *D. chataceus* was able to grow optimally in lower concentrations of sewage sludge (20% sewage sludge) and also able to phytostabilizer Fe in these concentrations. However, more studies need to be conducted, especially in field conditions, in order to optimize the potential of the *D. chataceus* plant as a phytoremediator.

Key words: *Dipterocarpus chataceus* · Phytoremediation · Heavy metals · Sewage sludge

INTRODUCTION

The human population is rapidly growing, especially in developing countries. These populations are also becoming more urbanized, while their economy is increasingly reliant on industries [1, 2, 3, 4]. All these conditions are causing enormous growth in the volume of wastewater. Wastewaters are highly toxic and have to be

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appropriately treated at wastewater treatment facilities. These facilities in turn produce sewage sludge, a product which has no defining value or purpose [5]. As the amount of wastewater being treated is increasing, the disposal of sewage sludge is becoming a major concern. Malaysia produces about 5 million cubic meters of sewage sludge per year and the amount is expected to reach 7 million cubic meters by 2022 [6].

Sewage sludge, sometimes called biosolids, is a solid waste product that remains after the treatment of wastewater at wastewater treatment facilities. It is usually in a very dilute suspension form, containing about 0.25 to 12% solid [6]. Sewage sludge can be unsafe to organisms, as it contains pathogens, toxic elements and high levels of heavy metals concentrations. It is commonly disposed of on land, in the sea and sometimes even incinerated [7]. Although these methods are cost effective, it can cause widespread environmental degradation. As environmental awareness are increasing among both the public and governments, these conventional sewage disposal methods are now being avoided.

One positive feature of sewage sludge is that it has potential to be used as an efficient soil amendment, as it contains high organic matter and nutrients needed by most plants [8]. Hence, plants can have improved growth when planted in sewage sludge added soils. However, there are toxic characteristics of sewage sludge that can harm plant growth. The major concern of using sewage sludge as a soil amendment is its high concentrations of heavy metals. Although heavy metals are sometimes naturally found in the soils, it is rarely in toxic levels [9]. Even essential metals such as Zn can be toxic to plants at high concentrations, as these metals are often only needed in trace amounts. Using sewage sludge as a soil amendment would result in the accumulation of high concentrations of heavy metals, as these elements do not degrade [10]. Besides that, these heavy metals have the potential to enter our food chain and even transferred to other areas through groundwater [11]. Hence, these heavy metals needs to be removed for sewage sludge to be effectively used as a soil amendment.

Phytoremediation is a technology involving the degradation, extraction and remediation of contaminants [12, 13, 14]. It is an environmentally-friendly method of decontaminating lands as it does not damage soil structures and does not introduce other pollutants. However, only plants with certain characteristics are able to be potential phytoremediators. Some of these characteristics are fast growing, high biomass, tolerant to toxic substances and high salinity or acidity [15]. Some plants have been found to be able to accumulate high amounts of metals without showing any signs of toxicity, making them excellent phytoremediators [16].

For this study, the potential of *Dipterocarpus chataceus* as a phytoremediator of heavy metals present in sewage sludge was examined. This species belongs to the family dipterocarpaceae, as is commonly known as *keruing kertas* in Malaysia and is derived from its crisp papery dead leafy. This species is endemic to Malaysia and can be found at lowland dipterocarp forest or semi-swamp forest. Most research conducted on this species focuses on its resins [17]. Besides that, sewage sludge might not suitable to be used as soil amendment to be put on the soil for food crops. However, it shall be suitable to be added to the soil to grow industrial trees species for furniture and construction purposes. This research would be the first to test the potential of this species as a phytoremediator.

MATERIALS AND METHODS

Site Description and Seedlings: The greenhouse of University Agriculture Park, Universiti Putra Malaysia, Serdang, Selangor, Malaysia (2° 59' 18.24'' N latitude and 101° 42' 45.45'' E longitude) was selected for this study. The planting duration was for 16 weeks. The temperature at glasshouse was 27°C in the morning and 35°C in the evening while Relative humidity in the glasshouse was 65%. *D. chataceus* seedlings were germinated from cuttings of the mature stem and planted in polybags (16.0×16.0 cm) in nursery of the Faculty of Forestry nursery, Universiti Putra Malaysia. *D. chataceus* 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 Typic paleudult, fine loamy, kaolinitic and isohyperthemic type of soil and sewage sludge after one month.

Planting Medium: The planting mediums consisted of a mixture of soil and sewage sludge at different concentrations, while the control only containing soil. The planting mediums were: T_0, Control (100% soil), T_1 (20% sewage sludge and 80% soil), T_2 (60% soil and 40% sewage sludge), T_3 (40% soil and 60% sewage sludge), T_4 (80% sewage sludge and 20% soil) and T_5 (100% sewage sludge). All treatments had 6 replicates and labelled accordingly. Completely Randomized Design (CRD) was used in the arrangement of the pots in the greenhouse.

Plant and Soil Sampling: All *D. chataceus* seedlings were measure for height, diameter and number of leaves every two weeks. Soil samples were collected before and after planting, air dried and stored in plastic containers. After the planting duration, *D. chataceus* seedlings were harvested and separated into three parts: leaves, stems and roots. The plant parts were oven dried and shredded and stored in plastic containers. Soil collected before planting and after harvesting were air-dried and kept in polyethylene bags.

Soil and Plant Analyses: Acid digestion method was used to determine the concentration of heavy metals in the plant and soil samples. After digestion using aqua regia, the total concentrations of heavy metals in the samples was determined using Atomic Absorption Spectrometer [5, 18, 19]. For this study the heavy metals that were analyzed were Cd, Fe, Zn, Cu and Pb.

Plant Growth and Biomass Measurement: Plant biomass was measure separately according to leaves, stems and roots using destructive sampling method. The loss in weight upon drying is divided with the dry sample weight to obtain the percentage of moisture;

$$\%w = \frac{A - B}{B} X100 \tag{1}$$

where,

%w = Percentage of moisture in sample
A = Weight of wet sample
B = Weight of dry sample

Translocation Factor (TF) and Bioconcentration Factor (BCF): To evaluate the phytoremediation capabilities of *D. chataceus* seedlings, two indicators were used which are bioconcentration factor or bioaccumulation factor (BCF) (metal concentration ratio of plant roots to soil) and translocation factor (TF) (metal concentration ratio of plant shoots to roots). BCF is commonly used to evaluate the level of intake and storage of toxic components in plant parts and also act as a suitable index to determine whether particular tree species is ideal as hyperaccumulator species [20, 21, 22];

$$BCF = \left[\frac{\text{Metal Concentration in Roots (mg kg}^{-1}\text{)}}{\text{Metal Concentration in Soil (mg kg}^{-1}\text{)}}\right]$$

$$TF = \left[\frac{\text{Metal Concentration in Shoot (mg kg}^{-1}\text{)}}{\text{Metal Concentration in Root (mg kg}^{-1}\text{)}}\right]$$

Plants with a high BCF and TF are suitable for phytoextraction as long as the value of BCF and TF at least above one [23]. Plants with a high BCF (BCF>1) but a low TF (TF<1) are suitable for phytostabilization [23].

Statistical Analysis: Heavy metals in the soil, sludge and plant parts were analyzed using 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 [24, 25, 26].

RESULTS

General Properties of Growth Medium: The particle size analysis of the soil was silt clay. Table 1 shows that all treatments initially had lower pH (4.27 to 5.25) and the pH increased after planting for all treatments (4.41 to 5.31), the biggest increase was in T5 (4.27 to 4.42). The Total-C was highest in T_5 (13.75) and lowest in the control planting medium (0.65). All the treatments experienced a reduction in the Total-C, except for the control. The biggest reduction was observed in T_5 (13.75 to 4.63).

Table 1: The pH and total-C (%) content in growth media by different level of sewage sludge

Treat.	pH B	Total-C A	pH B	Total-C A
T_0	5.25±0.07	5.31±0.02	0.65±0.03	0.72±0.21
T_1	4.75±0.14	4.85±0.27	1.81±0.24	2.08±0.15
T_2	4.69±0.10	4.74±0.03	3.87±0.43	2.43±0.11
T_3	4.45±0.16	4.59±0.14	5.92±0.32	3.16±1.15
T_4	4.36±0.15	5.47±0.21	7.28±0.29	4.29±0.83
T_5	4.27±0.37	4.42±0.13	13.75±0.82	4.63±0.91

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

Table 2: Total height, total basal diameter and number of leaves of *D. chataceus* by 16 weeks

Treatment	Height(cm)	Basal diameter(mm)	No. leaves
T_0	55.12a	6.23a	18a
T_1	58.32a	6.56a	18a
T_2	53.84a	6.11a	16a
T_3	51.78a	6.03a	15a
T_4	49.81a	5.95a	12ab
T_5	46.86a	5.83a	7b

Note: T_0-control (100% soil), T_1 (20% sewage sludge and 80% soil), T_2 (40% sewage sludge and 60% soil), T_3 (60% sewage sludge and 40% soil), T_4 (80% sewage sludge and 20% soil) and T_5 (100% sewage sludge). Difference letters within the same column indicates significant differences at $p \leq 0.05$.

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

Treatment	Roots	Stem	Leaves
T_0	45.91ab	46.61ab	30.64ab
T_1	47.22ab	47.86ab	31.21ab
T_2	43.82a	44.20a	29.69ab
T_3	41.34a	42.87a	28.35a
T_4	40.31a	41.33a	26.02a
T_5	38.78a	39.01a	19.85a

Note: T_0-control (100% soil), T_1 (20% sewage sludge and 80% soil), T_2 (40% sewage sludge and 60% soil), T_3 (60% sewage sludge and 40% soil), T_4 (80% sewage sludge and 20% soil) and T_5 (100% sewage sludge). Difference letters within the same column indicates significant differences at $p \leq 0.05$

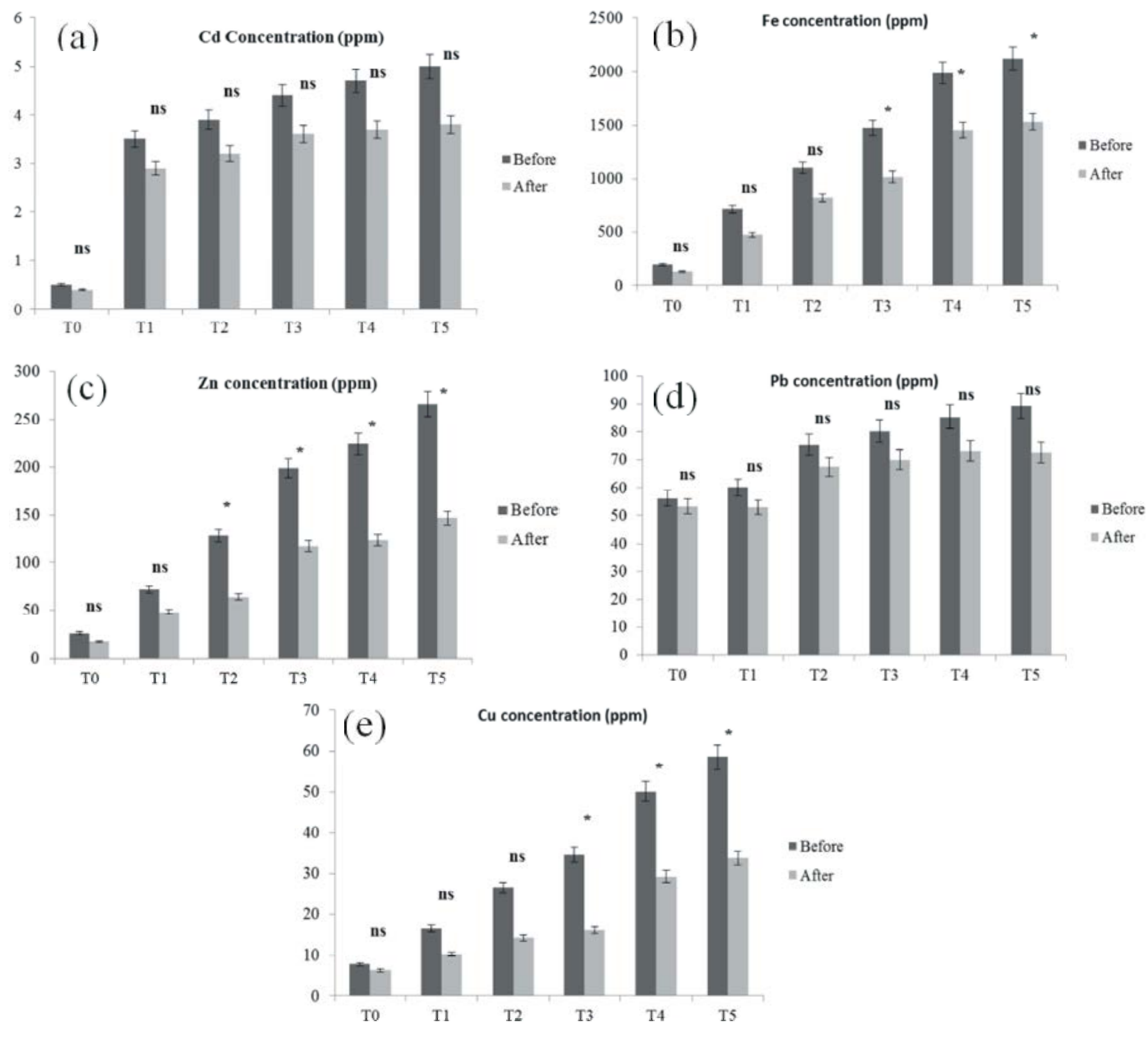

Fig. 1: The Concentrations of Zn (a), Cd (b), Fe (c), Pb (d) and Cu (e) in growth media before planting and after harvesting. * indicate significant difference between means at each treatment before planting and after harvesting according to a Student's t-test (p=0.05), ns indicates no significant difference.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

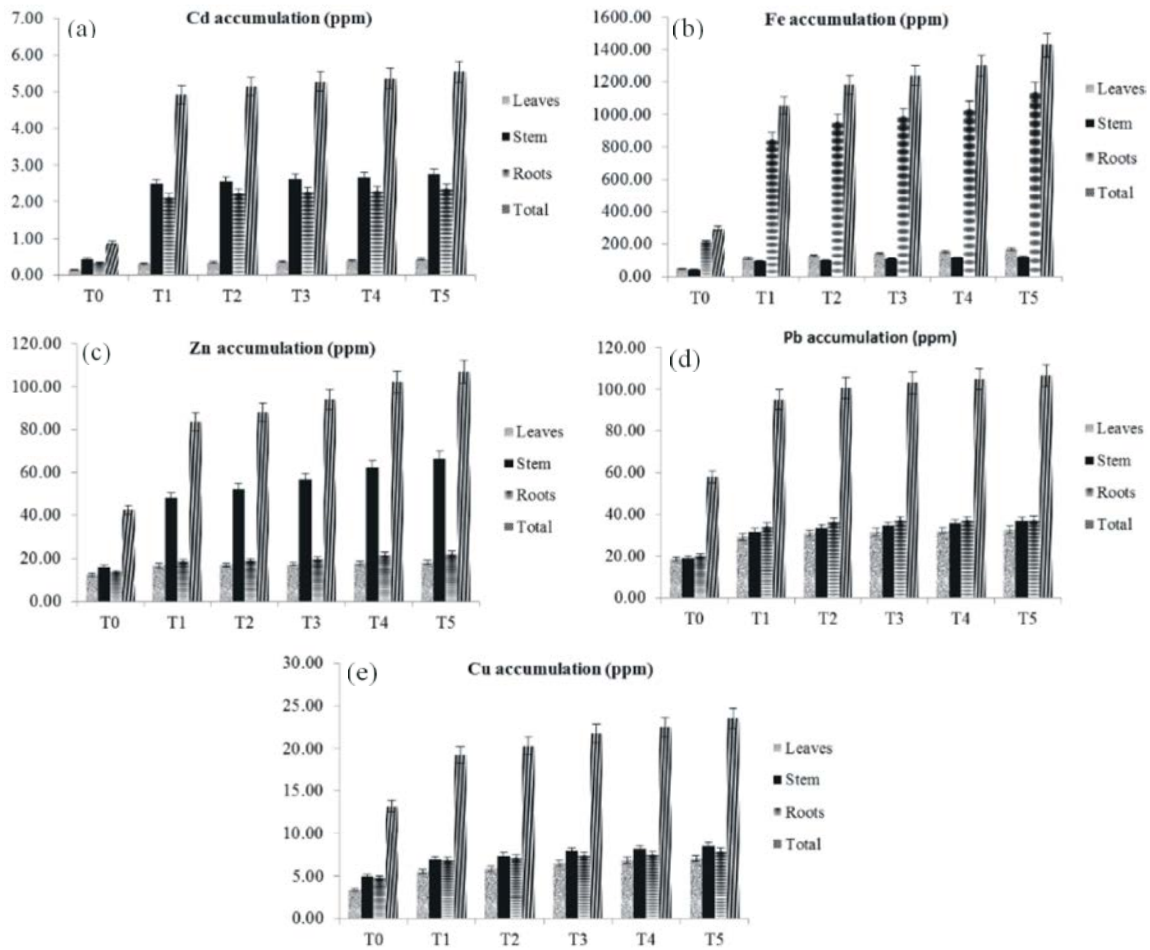

Fig. 2: Accumulation of Cd (a), Fe (b), Zn (c) Pb (d) and Zn (e) concentrations in the plant parts after harvesting of *D. chataceus* plant as influenced by different treatments. T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2 = 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4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

Growth Performance and Biomass: There was significant difference ($p \leq 0.05$) in the *D. chataceus* seedlings in terms of and number of leaves, while total height and basal diameter of *D. chataceus* do not show much difference. As shown in Table 2, *D. chataceus* seedlings planted in T_1 recorded the best growth parameters (total height, basal diameters and number of leaves) while T_5 had the lowest growth (total height, basal diameters and number of leaves). The height recorded in T_1 (58.32cm), was followed by the *D. chataceus* seedlings is control (55.21cm). Both *D. chataceus* seedlings planted in T_1 and control recorded the same number of leaves [18].

The plant's stem, roots and leaves showed significant difference ($p \leq 0.05$) among the treatments. As shown in Table 3, the highest biomass for all plant parts was recorded in T1; the biomass of *D. chataceus* seedlings in T1 was 47.22g for roots, the stem was 47.86g and the leaves was 31.21g. T5 produced the lowest biomass for all plant parts, 38.78g, 39.01g and 19.85g for roots, stem and leaves respectively.

Heavy Metal Concentrations in Growth Medium Before and after Planting: The heavy metal concentrations before and after planting are shown in Figure 1a-e. The largest reduction in Cd level (33.33%) was in control (Fig. 1a). As shown in Fig. 1b, the largest reduction in Fe was also in control (35.61%. For Zn, the largest reduction (50.2%) was in T2 (Fig. 1c). As shown in Fig. 1d, *D. chataceus* seedlings planted in T5 recorded the highest reduction in Pb concentrations (20.07%). T3 recorded the lowest reduction in Cu (56.86%).

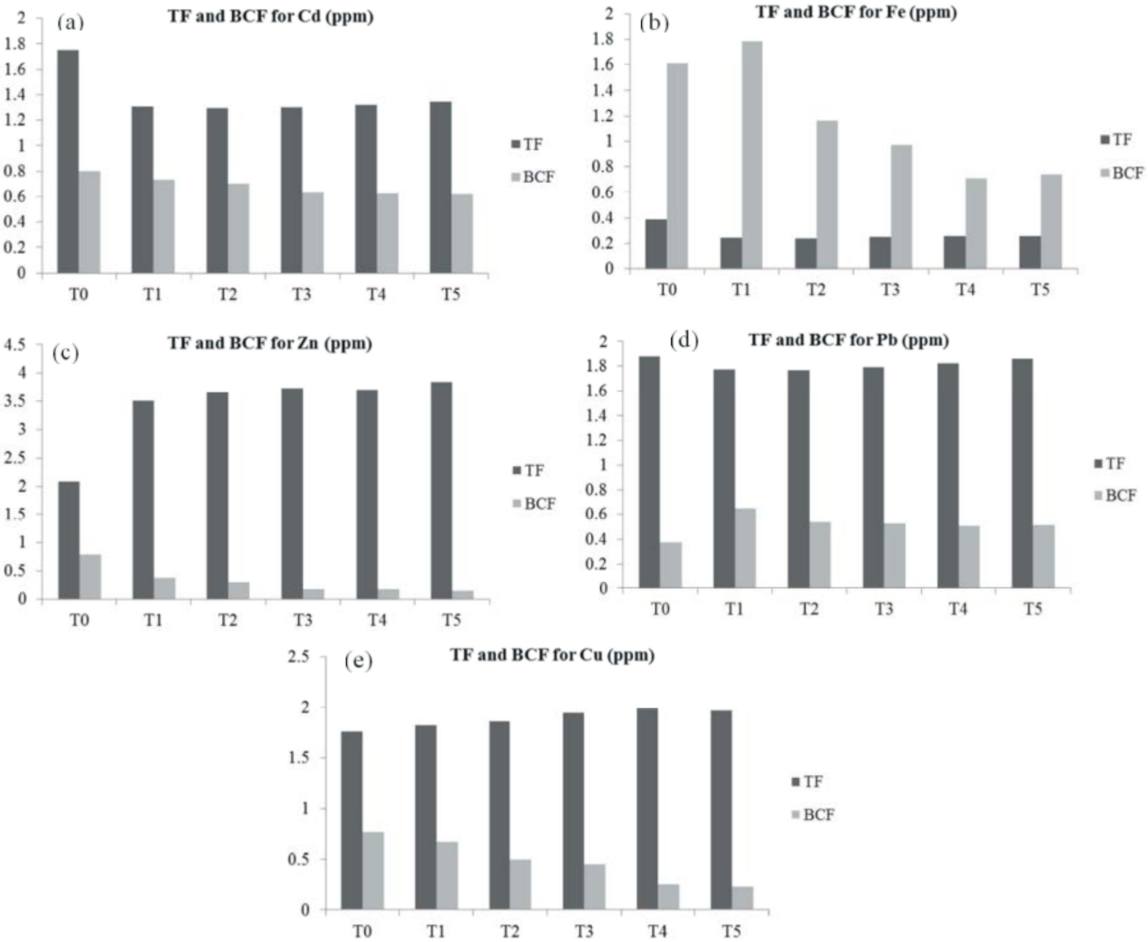

Fig. 3: Translocation factor and bioconcentration factor Cd (a), Fe (b), Zn (c) Pb (d) and Cu (e) of *D. chataceus* plant as influenced by different treatments. T_0 = 100% soil, T_1 = 80% soil + 20% sewage sludge, T_2 = 60% soil + 40% sewage sludge, T_3 = 40% soil + 60% sewage sludge, T_4 = 20% soil + 80% sewage sludge, T_5 = 100% sewage sludge.

Heavy Metal Concentrations in Plant Parts: The concentrations of heavy metals in *D. chataceus* plant parts (stem, roots and leaves) is shown in Figure 2a-e. Cd concentration in the *D. chataceus* plant ranged from 0.14 ppm to 0.42 ppm in the leaves, 0.42 ppm to 2.76 ppm in the stem and 0.32 ppm to 2.36 ppm in the roots (Figure 2a). Concentration of Cd in the plant parts was in the order of stem > roots > leaves (Figure 2a). The highest concentration of Cd in all plant parts was in T3 (Figure 2a).

Fe concentration in *D. chataceus* ranged from 43.26 ppm to 169.24 ppm in the leaves, 39.12 ppm to 121.33 ppm in the stem and 212.71 ppm to 1137.35 ppm in the roots (Figure 2b). Concentration of this heavy metal in the plant part of *D. chataceus* was in the order of roots > leaves > stem (Figure 2b).

Zn concentration in *D. chataceus* ranged from 12.51 ppm to 18.35 ppm in the leaves, 16.21 ppm to 66.42 ppm in the stem and 13.77 ppm to 22.04 ppm in the roots (Figure 2c). The highest concentration of Zn in all plant parts was in T5 (Figure 2c). Concentration of Zn in the plant part of *D. chataceus* was in the order of stem > roots > leaves (Figure 4.17).

Pb concentration in *D. chataceus* ranged from 18.81 ppm to 32.74 ppm in the leaves, 19.08 ppm to 36.63 ppm in the stem and 20.17 ppm to 37.31 ppm in the roots (Figure 2d). Concentration of Pb in the plant part of *D. chataceus* was in the order of roots > stem > leaves (Figure 2d).

Cu concentration in *D. chataceus* ranged from 3.41 ppm to 7.02 ppm in the leaves, 5.00 ppm to 8.56 ppm in the stem and 4.78 ppm to 7.91 ppm in the roots

(Figure 2e). Concentration of Cu in the plant part of *D. chataceus* was in the order of stem > roots > leaves (Figure 2e).

Translocation Factor (TF) and Bioconcentration Factor (BCF) of Heavy Metals: The TF and BCF of *D. chataceus* seedlings are shown in Figure 3a-e. None of the treatments had Cd BCF values of above 1, ranging from 0.62 (T_5) to 0.80 (control) (Figure 3a) while all the TF values were above 1, ranging from 1.31 (T_1) to 1.75 (T_0) (Figure 3a).

The Fe BCF values were ranging from 0.71 (T_4) to 1.79 (T_1) (Figure 3b), while all the TF values were below 1, ranging from 0.24 (T_1) to 0.39 (control) (Figure 3b).

None of the *D. chataceus* seedlings had Zn BCF values of above 1, ranging from 0.15 (T_5) to 0.79 (T_0) (Figure 3c), while all the TF values were above 1, ranging from 2.09 (control) to 3.85 (T_5) (Figure 3c).

The Pb BCF values were ranging from 0.51 (T_5) to 0.65 (T_1), hence none of the treatments had BCF values of above 1 (Figure 3d), while all the TF values were above 1, ranging from 1.77 (T_1) to 1.88 (control) (Figure 3d).

None of the *D. chataceus* seedlings had Cu BCF values of above 1, ranging from 0.23 (T_5) to 0.77 (control) (Figure 3e), while all the TF values were above 1, ranging from 1.76 (T_0) to 1.99 (T_4) (Figure 3e).

DISCUSSION

Changes in Soil Properties of Growth Medium Before Planting and after Harvesting: Soil pH was lower (more acidic) in planting mediums that had higher concentrations of sewage sludge. Applications of sewage sludge as a soil amendment have been found to increase soil pH [27]. However, the pH increased (became less acidic) after the planting of *D. chataceus*, the biggest increase being in T_5 (4.27 to 4.42). This increase in pH is due to *D. chataceus* seedlings being able to uptake acidic elements in the growth medium [28].

Soil total carbon was higher in growth media containing larger concentrations of sewage sludge. This is because sewage sludge has high organic matter, meaning that it can improve the overall soil fertility [19]. Besides that, soil organic matter can also improve the soil's water holding capacity, making plants able to survive during droughts [16]. This is an indication that sewage sludge has the potential to replace the need for organic fertilizers, having the potential to improve overall soil fertility [18, 19].

Growth Performance and Plant Biomass: The highest seedling height was recorded in T_1 (58.32cm), while the lowest was in T_5 (46.86cm). The biggest basal diameter of the *D. chataceus* plant was also in T_1 (6.56mm), while the lowest was in T_5 (5.83mm). The highest number of leaves (18) was in both T_1 and Control. The least amount of leaves (7) was shown in T_5. This clearly shows that the *D. chataceus* seedlings planted in T_1 had the best growth parameters while those planted in T_5 had the worst. The results indicates that that to obtain the best growth for *D. chataceus* plants, the growth medium needs to be 20% sewage sludge and 80% soil. In this composition, the plant is able to obtained sufficient nutrients without getting affected by the toxic elements contained in the sewage sludge [18]. This was also observed in a similar experiment for the *D. verrucosus* plant [16].

The worst growth performance was recorded in T_5 where the result shows that the *D. chataceus* seedlings are unable to tolerate soils with extremely high acidity and heavy metal concentrations.

Plant biomass was similar to the growth performance: the highest plant biomass for all plant parts was observed in T_1 while the lowest was recorded in T_5. For a plant to be a phytoremediator, it needs to have high biomass [28]. Hence, the *D. chataceus* plant has potential to act as a phytoremediator in soils which doesn't have very high concentrations of metals.

Heavy Metal Accumulations in Plant Parts: For Cd, Cu and Zn, the highest concentrations were found in the stem of the *D. chataceus* plant, followed by its roots and finally its leaves. This is a characteristic that is desirable of a phytoextractor, as the metals are stored in the stem. However, the worst growth performance was exhibited in T_5, followed by T_4 and T_3, respectively. This means that the plant was most likely experiencing toxicity from storing the metals in its stem, hence experiencing low growth [28].

The roots of *D. chataceus* seedling stored the highest concentration of Fe and Pb. This is not a trait of a phytoextractor, as a phytoextractor should store metal in the plants aerial parts [23]. However, it still has potential to be a phytoremediator; as phytostabilizers can make contaminants immobile by storing them in the plants roots [28].

Translocation Factor (TF) and Bioconcentration Factor (BCF) of *D. chataceus* Seedlings: All of the *D. chataceus* seedlings had high TF values (TF>1) for Cd, Cu, Zn and

Pb. But none of the BCF values for these metals were above 1. Based on the TF and BCF values, *D. chataceus* is not a suitable phytoremediator of Cd, Cu, Zn and Pb [23]. However, as the TF value for all these metals are high (TF>1), this species has the potential to be an indicator plant species. An indicator plant is a plant species that can be used to detect contaminants in soils. The plant needs to store the contaminants in the aerial parts, hence analyses can be conducted on these plant parts to determine the contamination condition of the soil [23].

In T1, the *D. chataceus* seedlings had high BCF values (BCF>1) but low TF values (TF<1). This means that this species is a phytostabilizer of Fe. This is especially true since the *D. chataceus* plant in T_1 had the best growth performance. However, the BCF values starts to decline with increasing concentrations of sewage sludge. This is an indication that this plant species is unable to phytostabilize high amounts of Fe concentrations in the soil.

CONCLUSION

The *D. chataceus* plant is able to grow optimally in growth mediums with lower concentrations of sewage sludge (20% sewage sludge). This means that sewage sludge can be used as a soil amendment for growing this plant species, but only with lower concentrations. Besides that, it was found that this species has the potential to be an indicator species for Cd, Cu, Pb and Zn. The most notable finding is that the *D. chataceus* plant is able to be a phytostabilizer of Fe, when Fe is present at lower concentrations. This means that *D. chataceus* is potential phytoremediator of Fe.

More studies need to be conducted to better understand the potential of this species as a phytoremediator. Firstly, the duration of the study needs to be extended. Secondly, a field study needs to be also conducted to determine if field conditions have any effects on this plant species.

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