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Heavy metal uptake and translocation by mangium (*Acacia mangium*) from sewage sludge contaminated soil

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Abstract

There are so many potentially harmful substances found in sludge particularly heavy metals (cadmium, chromium, lead, nickel, copper, mercury, zinc, iron and aluminum) . Many are known to cause cancer and other diseases. Certain compounds found in sludge have been identified to harm the reproductive systems of fish and other aquatic life. These contaminants need to be cleaned up for a safe environment. An experiment was conducted to evaluate the potential of *Acacia mangium* as a phytoremediator to absorb heavy metals from sewage sludge contaminated soils. *Acacia mangium* seedlings were planted on six different growth media (soil + sludge) were: T0 (100% soil, control), T1 (80% soil+20% sludge), T2(60% soil+40% sludge), T3(40% soil+60% sludge), T4(20% soil+80% sludge) and T5 (100% sludge). The highest growth performance such as height, basal diameter and number of leaves was found in 40% soil+60% sludge. Cadmium (Cd) and lead (Pb) were highly concentrated in the stems, chromium (Cr) and copper (Cu) in the roots, while zinc (Zn) was concentrated in both leaves and stems. *A. mangium* seems to have a high potential to absorb high amounts of Zn, Cd, Pb, Cr and Cu in the leaves, stems and roots. *A. mangium* showed high translocation factor and low bioconcentration factor values in the sludge contaminated soil as well as it was able to tolerate and accumulate high concentrations of heavy metals. The roots of *A. mangium* were found to be suitable for the absorption of heavy metals in contaminated soils. Therefore, this species can be a good phtyoremediator for sewage sludge contaminated soil and to mitigate soil pollution.

Keywords: *Acacia mangium,* Heavy metal accumulation, sewage sludge, phytoremediation.

Abbreviation: ANOVA- Analysis of variance, BCF- Bioconcentration factor, C- Celsius, Cd- Cadmium, CEC- Cation exchange capacity, Cr- Chromium, Cu - Copper, DMRT-Duncan's Multiple Range Test, HCl - Hydrochloric acid, HNO_3 - Nitric acid, Pb - Lead, TF- Translocation factor, USDA- United State Depatment of Agriculture and Zn – Zinc.

Introduction

Improper disposal of sewage sludge, compost, residential mining and industrial wastes cause serious environmental problems. For example, these wastes transport to natural vegetation and cultivated crops, where the increasingly widespread pollution has caused vast areas of land to become non-arable and hazardous for both wildlife and human population (Alloway et al., 1995). The accumulation of heavy metals in agricultural lands has been remaining many years in the soil (Alloway et al., 1991). Rapid urbanization, a consequence of economic development and increased population has led to production of huge quantities of sewage sludge in Malaysia and has posed serious environmental problems for their disposal. In Malaysia, sewage sludge is mainly produced from domestic and light industrial areas. It has been estimated that about 6 million metric tons (wet basis) of sewage sludge is produced annually. The total cost of managing sewage sludge is estimated at UD$ 0.33 billion per year (Kadir and Mohd, 1998). However, sewage sludge enters into soils due to illegal damping, disposal in landfill, septic tanks, leaking sewer lines and land application. Sewage sludge contains heavy metals which cause threats to soil quality and human health and high concentrations of heavy metal are harmful to plants, animals and humans. The environmental problems are related to plant productivity, food quality and human health (Alloway, 1990). Their potential accumulation in human tissues and bio-magnification through the food-chain can cause DNA damage and carcinogenic effects. These concerns continue to drive the need for the development and application of remediation techniques (Colleran, 1997). In the past, chemical pollution in the soil has been treated using physical and chemical processes that have proven to be expensive. The relatively recent development of phytoremediation has added itself to the existing cleanup strategies currently available for the restoration and rehabilitation of contaminated sites and can be conducted either *in situ* or *ex situ*. This method has good efficiency, environmentally friendly and comparatively low cost (Garbisu and Alkorta, 2001). A number of green plants, trees, herbs, grasses and shrubs, both aquatic and terrestrial, have been discovered to have been endowed with the wonderful properties of environmental restoration, such as decontamination of polluted soil and water. *Acacia mangium* (family Leguminosae) was selected for this study because it has rapid early growth interms of height and diamerter than the other species (Majid, 1998), and can attain a height of 30 m and a diameter of over 60 cm (MacDicken and Brewbaker, 1984). *Acacia mangium* is a fast-growing exotic tree which can thrive easily in the tropics. It has been observed that its invasive success is due to abundant seed production, widespread dispersal efficiency and the ability to remain viable in the soil for a long time. Burnt sites are quickly occupied by Acacias because they grow well in conditions with high temperatures, high light intensity and low relative humidity (MacDicken and Brewbaker, 1984). Due to ease of drilling and turning, it is a popular wood for furniture, agricultural implements, crates, particle board, and wood chips. This species is also suitable for manufacturing charcoal briquettes and activated carbon. Due to its fast growth, dense foliage, high biomass, high absorption and tolerance of heavy metals, *A. mangium* can be a potential phytoremediator (Veronica et al., 2011). Phytoremediation with *A. magium* on sewage sludge contaminated soils has not been reported. Therefore, this study was initiated with the following objectives: (1) to determine Cd, Cr, Pb, Cu and Zn uptake and translocation in *A. mangium* plant parts. (2) To quantify Cd, Cr, Pb, Cu and Zn concentrations in the growth medium before planting and after harvest.

Results and discussion

Changes of properties in growth media

The control soil was clay while 100% sewage sludge was silty clay loam texture (Table 1). Texture is an important soil characteristic that plays important role in soil management and crop production. Sandy clay texture is suitable for seedling growth and development because it contains high nutrients, high CEC and water holding capacity is also high. Clay soils crack excessively while drying, if they are very low in organic matter, this soil may lose its structure and becomes cloddy and compacted (Aljibury, 2011). There was a significant difference ($P \leq 0.05$) in pH between the soil before planting and after harvest. Before planting, soil pH ranged from 4.05 to 5.04. The control showed highest pH (5.04) followed by T1 (4.61) and the lowest (4.05) was in T5 (100% sludge). After harvest, soil pH increased having the highest increment (1.12) in T4 followed by T5 (1.03) but, pH decreased in the control (Fig. 1a). The higher pH values at harvest compared to before planting might be due absorption of heavy metals from the contaminated media. Acidic elements such as Al, Fe and Mn are available at low pH and plants can subsequently absorb and remove these elements from the soils. Knight et al. (1997) also found significant increment in soil pH grown with *Thlaspi caerulescens* on contaminated soils which corroborated the findings of our studty. Soil pH affects all the chemical, physical and biological properties of soil (Brady & Weil, 2002). Elemental accumulation in plant depends not only on their absolute content in a soil, but also on soil pH (Lorenz et al., 1994; Golovatyj, 2002).

Total carbon in growth media

The carbon content (%) in the growth media was significantly different ($P \leq 0.05$) among the treatments. The maximum total carbon (3.64%) was found in T5 followed by T4 (2.78) and the minimum (1.03%) was in the control (Fig. 1b). The total carbon content increased with increasing sludge in the growth media because sewage sludge contained high organic matter compared to the control (mineral soil). At harvest, the total carbon content was also higher than before planting having the highest increment (0.88%) in T5 followed by T3 (0.62%) and the lowest was in the control (0.12%). Enrichment of organic matter in soil improves bulk density, CEC, soil fertility and water holding capacity (Rice, 2009).

Effect of sewage sludge on growth performance

Plant height, basal diameter and number of leaves were significantly (≤ 0.05) influenced by the different treatments.

Plant height

At harvest, the height increment ranged from 38.0 to 54.38 cm. Treatment T3 showed highest height (54.38 cm) followed by T5 (53.75 cm). The lowest increment (38.0 cm) was recorded in the control (Fig. 2a). The height increase in sludge treated pots compared to control might be due to sewage sludge which supplied essential nutrients for plant growth. In May (after three months) height increased sufficiently for all the treatments except control.

Basal diameter

Basal diameter increment showed a similar pattern like height (Fig. 2b). Maximum basal diameter (7.88 cm) was found in T3 followed by T2 (7.33 cm) and the minimum was in the control (6.40 cm). 100% sewage sludge failed to produce maximum basal diameter might be due to heavy metals toxicity. Liu et al. (2011) reported that application of sewage sludge at 10% ratio in mixed soils improved growth of maize seedlings, the height was increased by 44.4% compared to the control. However, at higher sewage sludge ratio, there was a trend of decreasing growth which corroborated our findings.

Number of leaves

The number of leaves increased in all treatments after three months and ranged from 20 to 31, the highest (31) was in T3 followed by T2 (30). The lowest number of leaves (20) was in the control. Treatments T4 and T5 also produced less leaves and this might be due to the toxic effect of heavy metals. The least number leaves and lowest basal diameter were in the control and this might be due to shortage of nutrients. On the other hand T3, T2 and T1 had less amount of heavy metals compared to T5 and T4 but supplied sufficient amount of nutrients. The number of leaves increased gradually every month in all treatments except the control. It appears that sewage sludge supplied sufficient nutrients for plant growth and development. This indicates that the plant is able to survive better in both control and sewage sludge contaminated soil. Similar results were also observed by Majid et al. (2012) in growing *Justicia gendarussa* grown in textile factory sludge contaminated soil.

Effect of sewage sludge on plant biomass

Dry weight of *A. Mangium* was variable among different treatments. Treatment T3 showed highest stem biomass (46.78 g) followed by T3 (42.85 g) and the lowest (35.38 g) was in the control (Table 2). The highest leaf biomass (45.46 g) was also in T3 followed by T2 (42.11 g) and the lowest (37.45 g) was in the T1. *A. Mangium* produced maximum root biomass (40.52 g) in T3 followed by T2 (38.93 g) and the minimum was in the control (35.14 g). Treatment T5 (100% sludge) which contained high nutrients did not produce the highest biomass and this might be due to the toxic effect of heavy metals. Liu et al (2011) observed similar results with sewage sludge @10% ratio mixed with soil and increased fresh weight by 62.8% compared to the control but at higher rate it decreased which is in agreement with the findings of our study.

Table 1. Physicochemical properties of the control soil and contaminated sewage sludge.

Growth media	Texture	pH	Total carbon (%)	Total N (%)	Total P (%)	Total K (%)	Heavy metal concentration				
							Cd (ppm)	Cr (ppm)	Pb (ppm)	Cu (ppm)	Zinc (ppm)
Control soil	Clay	5.04	1.03	0.79	0.02	0.03	0.89	10.7	5.25	13.9	28.5
Contaminated sewage sludge	Silty clay loam	4.05	3.64	3.20	0.40	0.15	3.52	76.0	19.2	65.2	551

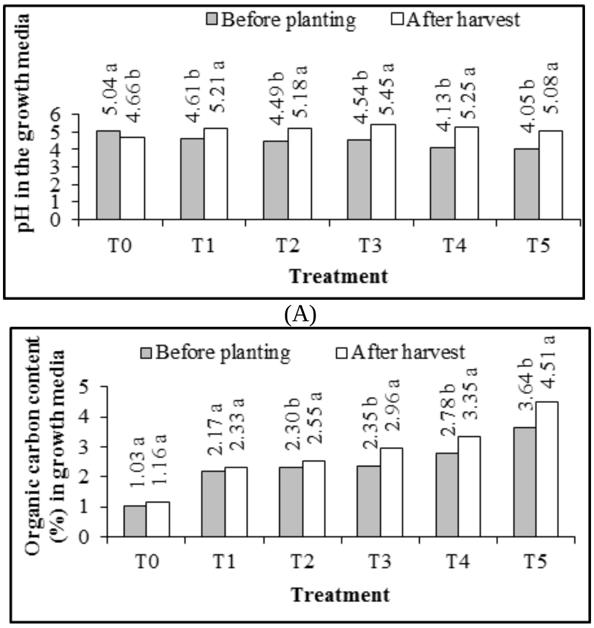
Table 2. Dry biomass of stems, leaves and roots of *Acacia mangium* at harvest as influenced by different treatments ($p \leq 0.05$).

Treatment	Stems biomass plant^{-1} (g)	Leaves biomass plant^{-1} (g)	Roots biomass plant^{-1} (g)	Total biomass plant^{-1} (g)
T0	46.78±2.12[1]	38.97±1.18	36.47±1.45	122.22±2.45
T1	40.77±1.53	37.45±1.56	35.14±1.62	113.36±2.31
T2	41.20±1.72	42.11±1.57	37.83±1.54	121.14±2.62
T3	42.85±2.51	45.46±1.64	40.52±1.07	128.83±2.53
T4	39.05±1.64	41.77±1.27	36.63±1.54	117.45±2.26
T5	35.38±1.95	40.94±1.45	39.07±1.70	115.39±1.88

Notes: T0 = 100% soil, T1 = 80% soil+20% sludge, T2= 60% soil+40% sludge, T3 = 40% soil+60% sludge, T4 = 20% soil+80% sludge and T5 =100% sludge. [1]Values of standard error of mean are given after ±.

Heavy metal concentrations in the growth media

Heavy metal concentrations in the growth media were significantly ($P \leq 0.05$) influenced by different treatments. Cadmium concentration decreased after harvest compared to before planting in all treatments (Fig. 3a). Treatment T5 showed the highest reduction (1.22 ppm) followed by T4 (1.07 ppm). The lowest reduction was in the control (0.39 ppm). It was also observed that maximum Cd concentration decreased in 100% sewage sludge whereas the other treatments showed less reduction after planting. Consequently, the growth of plant was also better in T5 (100% sewage sludge) and it is believed that this plant is able to absorb more Cd from T5 compared to the other treatments. There was a significant difference in Cr concentrations in the growth media between before planting and after harvest ($P \leq 0.05$). Before planting, Cr concentrations in the growth media varied from 10.70 to 76.05 ppm (Fig. 3b). T5 showed the highest Cr concentration (76.05 ppm) followed by T4 (37.92 ppm) and the lowest (10.70 ppm) was in the control. After harvest, Cr concentration decreased having the highest reduction (16.00 ppm) in T5 followed by T4 (12.50 ppm). The lowest Cr reduction (4.37 ppm) was in the control (Fig. 3b). Lead concentrations in the growth media were also significantly influenced by the different treatments ($P \leq 0.05$). Before planting, maximum Pd concentration (19.23 ppm) was in T5 followed by T4 (13.79 ppm). The control showed minimum concentration (5.25 ppm) (Fig. 3c). It was observed that Pb concentration increased with increase in sludge content. After harvest, Pb concentration decreased with the highest reduction (5.76 ppm) in T4 followed by T5 (5.28 ppm). The control showed the lowest reduction (0.99 ppm) (Fig. 3c). Copper concentrations in the growth media are presented in Figure 3d. There was a significant difference among the treatments as well as before planting and after harvest ($P \leq 0.05$). Before planting, Cu concentration ranged from 13.9 to 65.25 ppm, the highest (65.25 ppm) was in T5 followed by T4 (36.94 ppm) and the lowest (13.9 ppm) was in the control. After harvest, Cu concentration was significantly decreased. The highest reduction (14.80 ppm) was in T5 followed by T4 (13.00 ppm). The control showed the lowest reduction (5.61). High metal concentrations in the soil restricted germination as well as reduced root and shoot growth for many species (Shanker et al., 2005). Zn concentration was significantly variable ($P \leq 0.05$) among the different treatments (Fig. 3e). The highest Zn concentration was in T5 followed by T4 (298.44 ppm) and the lowest was in the control. After harvest, Zn concentration significantly decreased having the highest reduction (190.59 ppm) in T5 followed by T4 (187.53 ppm). The control showed lowest reduction (12.42 ppm). Sewage sludge has higher Zn compared to the control. However, Zn concentrations increased with increase in sludge (Fig. 3e), but there was no significant impact of Zn on plant growth. Plant can absorb Zn



(A)

(B)

Fig1. pH (a) and total carbon (%) (b) in the growth media before planting and after harvest of *Acacia mangium* as influenced by different treatments. Notes: Growth media indicates different proportion of sewage sludge and soil such as 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 and T5 =100% sewage sludge. Figure(s) between columns in a treatment having common letter(s) do not differ significantly at 5% level of Tukey's test.

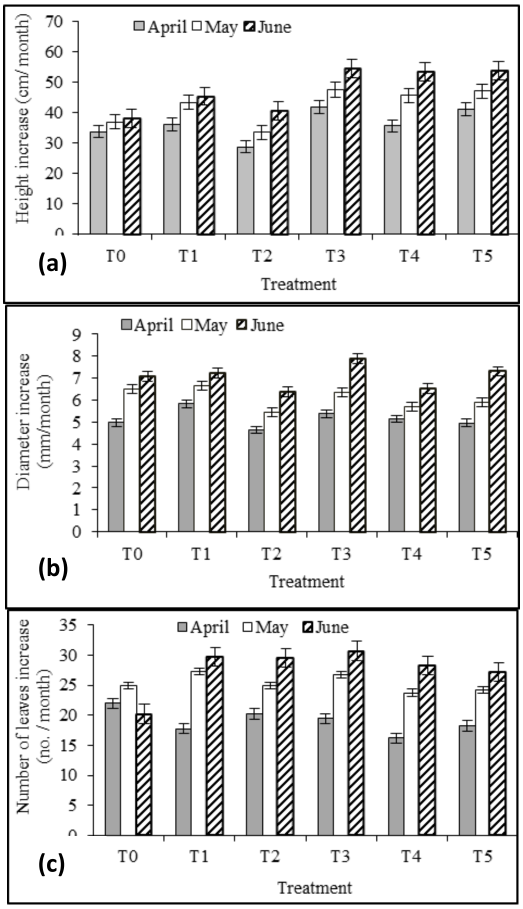


Fig 2. Plant height (a), basal diameter (b) and number of leaves (c) of Acacia Mangium at different months after planting as influenced by different treatments (Increment per month). Notes: Growth media indicates different proportion of sewage sludge and soil such as T0 = 100% soil, T1 = 80% soil+20% sewage sludge, T2= 60% soil+40% sewage sludge, T3 = 40% soil+60% sewage sludge, T4 = 20% soil+80% sewage sludge and T5 =100% sewage sludge. Means ±SE are shown in error bar (P≤0.05).

as an essential nutrient for growth but at higher concentrations it causes toxicity to plants. From previous study, it was found that several plant species, grasses and herbs as well as tree species such as *Betula pubescens* and *B. pendula* are capable of tolerating high Zn, but not necessarily with other heavy metals. Sensitive species or genotypes may, however, show toxicity symptoms at relatively low Zn levels (Anna, 1989).

Heavy metal concentrations in plant tissue

The highest Cd concentration was found in the stems followed by roots and leaves. T5 showed highest Cd concentration (1.48 ppm) followed by T4 (1.35 ppm) and T3 (1.11 ppm) in the stem. The lowest Cd concentration was in the control (Fig. 4a). In case of roots and leaves, the highest Cd concentration (1.10 and 1.00 ppm) was also in T5 followed by T4 and the lowest was in the control (0.44 and 0.34 ppm for roots and leaves, respectively).

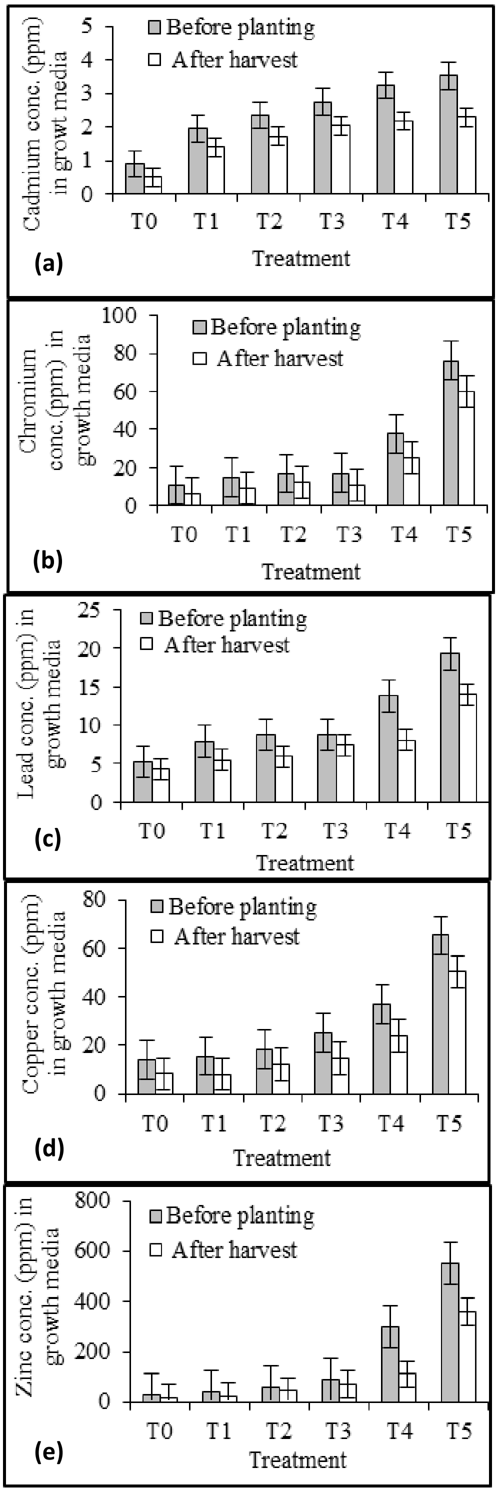


Fig 3. Change in cadmium (a), chromium (b), lead (c), copper (d) and zinc (e) concentrations in the growth media after cultivation of Acacia Mangium as influenced by different treatments. Notes: Growth media indicates different proportion of sewage sludge and soil such as T0 = 100% soil, T1 = 80% soil+20% sewage sludge, T2= 60% soil+40% sewage sludge, T3 = 40% soil+60% sewage sludge, T4 = 20% soil+80% sewage sludge and T5 =100% sewage sludge. Means ±SE are shown in error bar (P≤0.05).

Cadmium is not required for plant growth but is relatively soluble in soils. Terrestrial plants show high uptake of Cd and Zn in the root system. Certain plant species have storage organs in their rhizomes that can also store heavy metals. These plants can be used as phytoremediators. Cadmium uptake rate by crops is influenced by factors such as soil pH, salinity, humus content, species and the presence of other elements especially zinc. Increasing soil zinc is known to reduce cadmium availability to plants because Zn inhibits cadmium uptake and translocation from roots to shoots (Chaney, 1983). Cr concentrations in the plant parts are shown in Figure 4b. T5 showed maximum Cr concentration (4.49 ppm) followed by T4 (3.52 ppm) in the roots and the minimum (0.57 ppm) was in the control. In low sludge content media, T1 showed highest Cr concentration (2.55 ppm) followed by T2 (2.50 ppm) in the stems and the lowest (0.50 ppm) was in T5 (Fig. 4b). It was observed that the highest concentrations were in the roots while stems and leaves had lower concentration. Ghosh and Singh (2005) reported that maximum Cr accumulation was in the roots followed by stems and leaves in all the species of weeds which are in agreement with the findings of our study. T5 showed the lowest Cr concentration in the stems and roots and this might due to toxic effect of Cr which prevented translocation of heavy metals from roots to shoots. Similar results were also reported by Zhao et al. (2003). Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves. Hence, exposure to high level of Cr can affect total dry matter production and yield of plants. Cr also causes negative effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition. The bioavailability and toxicity of Cr in the soil depends on its speciation. Chromium mainly found in the Cr (III) and Cr (VI) oxidation states (Zayed and Terry, 2003). Chromium (III) is generally present in soil as relatively unavailable, insoluble oxides of Cr and Cr-Fe. The usual observation is that the Cr (VI) form is more toxic and more mobile than the Cr (III) form (Zou et al., 2006). Generally, plants have a low capacity to translocate Cr in high contaminated soil (Barcelo and Poschenrieder, 1997) which are inagreement with the findings of our study. Different plants vary in their ability to accumulate Cr in tissues (Zayed et al., 1998).

Pb concentrations in the plant parts were influenced by the different treatments. The stems showed highest Pb concentration followed by roots and leaves (Fig. 4c). T1 showed highest Pb concentration (36.25 ppm) followed by T2 (27.90 ppm) in the stems. In roots, maximum Pb concentration (19.09 ppm) was in the control followed by T1 (17.25 ppm) and the minimum was in T4 (10.9 ppm). In case of leaves, T1 showed the highest concentration (14.75 ppm) followed by the control (11.25 ppm) and the lowest (8.25 ppm) was in T5 (Fig. 4c). It was observed that Pb absorption and translocation decreased with increase in amount of sludge in the growth media. It was also reported that the excessive aount of Pb decreases absorption of Cu and Zn by the plants while absorption of Cd may increase (Kabata-Pendias and Pendias, 1999). Accumulation of Pb is in order of stems>roots> leaves. High level of Pb also causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and adversely affects mineral nutrition (Sharma and Dubey, 2005).

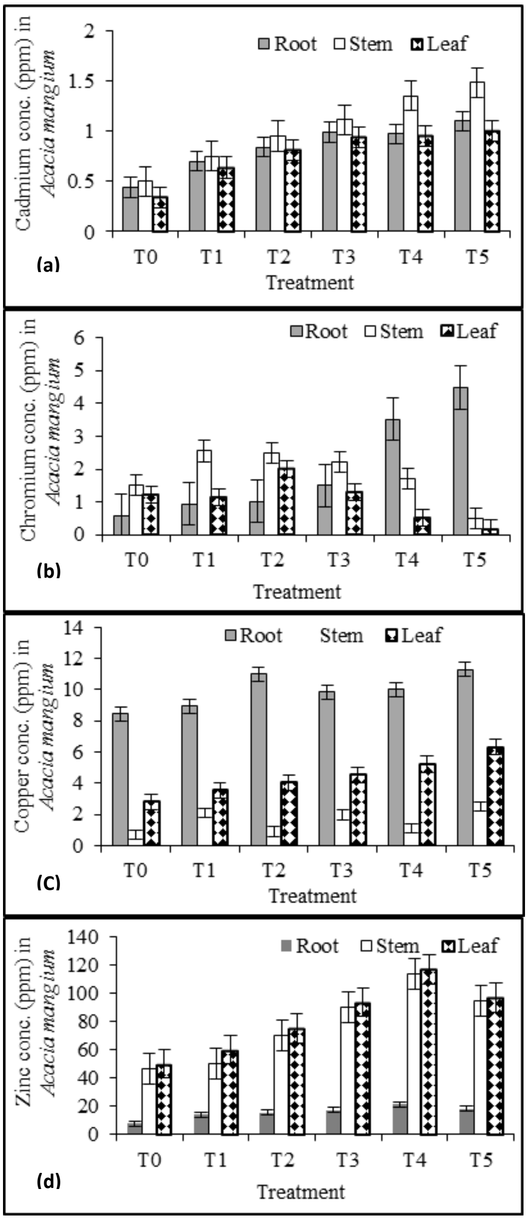


Fig 4. Cadmium (a), chromium (b), lead (c), copper (d) and zinc (e) accumulation in different parts of *Acacia mangium* as influenced by different treatments. Notes: Growth media indicates different proportion of sewage sludge and soil such as T0 = 100% soil, T1 = 80% soil+20% sewage sludge, T2= 60% soil+40% sewage sludge, T3 = 40% soil+60% sewage sludge, T4 = 20% soil+80% sewage sludge and T5 =100% sewage sludge. Means ±SE are shown in error bar ($P \leq 0.05$).

Among the plant parts, roots showed the highest concentration followed by leaves and stems (Fig. 4d). Majid et al (2011) also reported higher Cu absorption in roots than shoots which are in agreement with the findings of this present study. In roots, the highest concentration (11.3 ppm) was found in T5 followed by T4 (10.03 ppm) and the lowest (8.45 ppm) was in the control. In case of leaves, T5 also showed the highest concentration (6.30 ppm) followed by T4 (5.20 ppm) and the lowest (2.80 ppm) was in the control. It was observed that Cu concentration in the plant parts increased with increase in sludge content. Cu is an essential element but at higher concentration causes toxicity leading to chlorosis. The growth is also stunted and initiation of roots is slow. The restricted root development may result in a lowered water and nutrient uptake. Zinc concentration in the plant parts were also significantly influenced by the different treatments (Fig. 4e). Maximum zinc accumulation was in the leaves followed by stems and the minimum was in the roots. Majid et al (2011) also reported highest accumulation of Zn in the leaves followed by root which corroborated our results. In leaves, T4 showed the highest concentration (117.00 ppm) followed by T5 (97.00 ppm) and the lowest (50.00 ppm) was in the control. In case of stems, similar Zn absorption was recorded among the treatments. In roots, highest Zn accumulation (21.00 ppm) was also detected in T4 followed by T5 (18.00 ppm) and the lowest (7.45 ppm) was in the control (Fig. 4e). It was observed that Zn accumulation by the plant increased with increase in sludge content. Zn is an essential element for plat growth and development but at higher concentration it causes toxicity and limit growth of both roots and shoots (Fontes and Cox, 1998). Zn toxicity also causes chlorosis in the younger leaves which become yellow and can extend to older leaves after exposure to high soil Zn levels (Ebbs et al., 1997). Excess Zn can also give rise to manganese (Mn) and copper (Cu) deficiencies in plant shoots. Such deficiencies have been ascribed to a hindered transfer of these micronutrients from roots to shoots.

Bioconcentration factor and translocation factor

Bioconcentration factors (BCF) of heavy metals are presented in Figure 5. In case of Cd and Pb, the control showed highest BCF values (0.88 and 4.48 for Cd and Pb, respectively) followed by T1 (0.50 and 3.12 for Cd and Pb, respectively). The lowest BCF values (0.44 and 1.02 for Cd and Pb, respectively) were in T5. In Cd, T4 showed the highest translocation factor (2.37) followed by T5 (2.25). The maximum TF (2.96) for Pb was observed in T1 followed by T2 (2.82) and minimum (1.89) was in the control (Fig. 6). Translocation factor of metal excluder species is <1 whereas metal accumulator species has TF>1 (Baker 1981). The highest BCF of 0.88 and 4.48 for Cd and Pb were in the control but the other treatments showed lower values having the lowest in T5 (Fig. 5), which may imply the restriction in soil-root transfer at higher metal concentrations in the soil. Similar results were found by Yoon et al. (2006). Ho et al. (2008) also observed 1.92- 3.21 BCF in Pb-treated kenaf (*Hibiscus cannabinus* L.). In the case of Cu and Zn, the highest BCF (1.16 and 0.61 for Cu and Zn, respectively) was in the control followed by T1 (1.11 and 0.50 for Cu and Zn, respectively) and the lowest (0.19 and 0.05 for Cu and Zn, respectively) was in T5 (Fig. 5). The control also showed highest TF (4.81) for Cr followed by T2 (4.41) and the lowest was in T5 (0.16) (Fig. 6). It was observed that translocation decreased with increase in sludge content and this might be due to higher concentration of heavy metals which hampered normal physiological and metabolic activities and subsequently reduced metal translocation from roots to shoots. In case of Zn, The highest TF (12.89) was in the control followed by T4 (10.86), T3 (10.79) and T5 (10.61). The minimum TF (7.97) was in the T1 (Fig. 6). It was found that higher concentration of Zn in the media did not affect the translocation of Zn in *A. mangium*. However, it was observed that all the treatments except Cu exhibited higher TF values (>1). *A. mangium* has high TF and low BCF in soil at higher metal concentrations. Heavy metal tolerance with high TF and low BCF values was suggested for phytoaccumulators of contaminated soils (Yoon et al. 2006) and therefore *A. mangium* can be used as a potential phytoremediator for contaminated soils and to mitigate soil pollution.

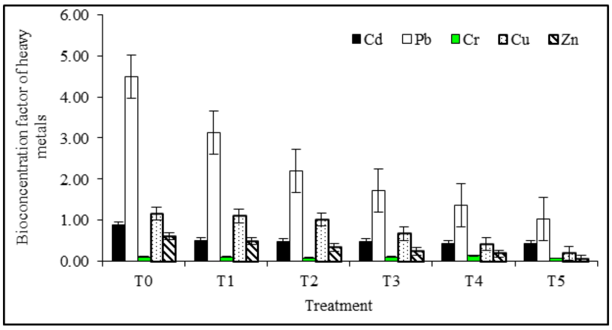


Fig 5. Bioconcentration factors of heavy metals in *Acacia mangium* as influenced by different treatments. Notes: T0 = 100% soil, T1 = 80% soil+20% sewage sludge, T2= 60% soil+40% sewage sludge, T3 = 40% soil+60% sewage sludge, T4 = 20% soil+80% sewage sludge and T5 =100% sewage sludge. Means ±SE are shown in error bar ($P \leq 0.05$).

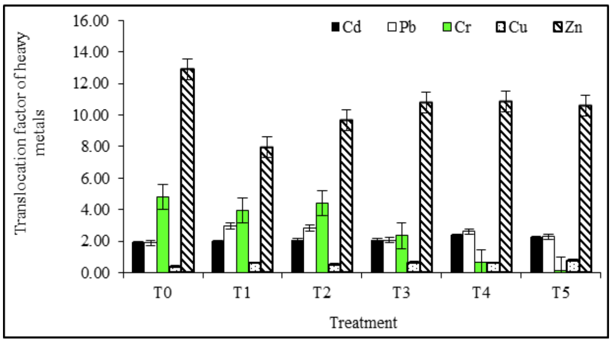


Fig 6. Translocation factors of heavy metals in *Acacia mangium* as influenced by different treatments. Notes: T0 = 100% soil, T1 = 80% soil+20% sewage sludge, T2= 60% soil+40% sewage sludge, T3 = 40% soil+60% sewage sludge, T4 = 20% soil+80% sewage sludge and T5 =100% sewage sludge. Means ±SE are shown in error bar ($P \leq 0.05$).

Materials and methods

Site description

The experiment was conducted at the greenhouse (4°06′ N latitude and 101° 16′ E longitude), Universiti Putra Malaysia from March to June 2009. The temperature of the green house in the morning was 26^{0} C , 36^{0} C at noon and 30^{0} C in the evening.

Growth media

Treatments including six different media were: T0 (control, 100% soil), T1 (80% soil+20% sludge), T2 (60% soil+40% sludge), T3 (40% soil+60% sludge), T4 (20% soil+80% sludge) and T5 (100% sludge). The texture of control soil was clay while the sewage sludge media was silty clay loam. The sewage sludge media was more acidic (pH 4.04) than the control (pH 5.04). The nutrient content was relatively high (3.64% C, 3.20% N, 0.40% P and 0.15% K) in sewage sludge while very low (1.03% C, 0.79% N, 0.02% P and 0.03% K) in the control (Table 1). Sewage sludge also contained 3.52 ppm Cd, 76.0 ppm Cr, 19.2 ppm Pb, 65.2 ppm Cu and 551 ppm Zn and the heavy metal composition of the control was 0.89 ppm Cd, 10.7 ppm Cr, 5.25 ppm Pb, 13.9 ppm Cu and 28.5 ppm Zn (Table 1).

Test plant and planting

Acacia mangium was used as the test plant. Five months old, thirty six healthy saplings and similar in form were selected for this study. Before planting, a seedling was tested for toxicity in 100% sewage sludge without soil for about one week (Majid et al., 2012). After filling the pots (28.2 cm × 34.2 cm size) with appropriate soil and sludge mix, seedlings were transplanted into the pots and grown for four months under the experimental condition.

Intercultural operation and growth variable measurement

Intercultural operations (weeding and watering) were done when necessary to ensure normal growth of the seedlings. The growth parameters including basal diameter, height and number of leaves were measured once every 2 weeks during the study period using diameter tape while the basal diameter was measured using a venier caliper.

Plant and soil sampling and chemical analysis

Plant samples were collected at harvest and soil samples were collected from each pot before planting and after harvest and kept in a standard plastic container and air-dried before physico-chemical analysis. For analysis of heavy metals, 1.0 g dried plant sample and 20 ml aqua regia solution (mixture of concentrated HNO_3 and HCl in a ratio of 3:1) was placed into the digestion tube and digestion completed at 80 to 120^0 C for 3 hours. The digest was filled into 100 ml beaker and the solution analyzed for heavy metal using ICP-MS (Inductively Couple Plasma Mass Spectrometry) method (Sahoo et al., 2009). Particle size distribution was analyzed by pipette gravimetric method and the texture was determined using USDA textural triangle (Day, 1965). Soil pH and total carbon were determined by glass electrode pH meter and loss on ignition method (Jackson, 1973; Konen et al., 2002), respectively.

Plant biomass measurement

Plant biomass was measured separately according to leaves, stems, and roots and calculated. The loss in weight upon drying is the weight originally present. The moisture content of the sample was calculated using the following equation

$$\%Moisture = \frac{\text{Wt.wet sample} - \text{Wt.dry sample}}{\text{Wt.dry sample}} \times 100 \quad (1)$$

(Black, 1965)

Determination of bioconcentration factor and translocation factor

The plant's ability to accumulate metals from soils and translocate metals from roots to shoots can be estimated using the bioconcentration factor (BCF) and translocation factor (TF), respectively. BCF and TF factors were calculated as follows:

$$\text{BCF} = \frac{\textbf{Metal concentration in root}}{\textbf{Metal concentration in soil}} \ldots \ (2)$$

(Yoon et al., 2006; Ho et al., 2008)

$$\text{TF} = \frac{\textbf{Metal concentration in aerial parts}}{\textbf{Metal concentration in root}} \ldots (3)$$

(Yoon et al., 2006; Ho et al., 2008)

Statistical analysis

The analysis of variance for growth and heavy metal concentrations (in soil and plant parts) was done following the ANOVA test and the mean values were adjusted by DMRT (P=0.05) method (Steel and Torrie, 1960). Comparison using Tukey's-test was also done to detect any significant difference between before planting and at harvest. Computation and preparation of graphs were done by using Microsoft EXCEL 2003 program.

Conclusion

The plant was able to remove zinc, cadmium, lead, chromium and copper from the growth media. At harvest, heavy metal concentrations (Cd, Cr, Cu and Zn) decreased in the growth media having the highest reduction in T5 while T4 showed the highest reduction in Pb. The combination of 40% soil + 60% sludge gave the best growth performance in terms of height, basal diameter and number of leaves. *A. mangium* showed high absorbing capacity of heavy metals. Cd and Pb were highly concentrated in the stems while Cr and Cu were accumulated in the roots. Zinc was however concentrated in the leaves. *A. mangium* was able to tolerate and accumulate high concentrations of heavy metals. This species therefore, can be used as a potential phytoremediator for sewage sludge contaminated soils and to mitigate soil pollution.

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