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Full Length Research Paper

Evaluation of heavy metal uptake and translocation by *Acacia mangium* as a phytoremediator of copper contaminated soil

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Many organic and inorganic pollutants, including heavy metals are being transported and mixed with the cultivated soils and water. Heavy metals are the most dangerous pollutants as they are non-degradable and accumulate and become toxic to plants and animals. An experiment was conducted in the glasshouse to evaluate the potential of *Acacia mangium* as a phytoremediator to absorb heavy metals from contaminated soils. *A. mangium* seedlings were planted in the growth media (soil + different levels of copper). The different levels of Cu were: T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu). The highest growth performance such as basal diameter, height and number of leaves was in T_1. The highest biomass was recorded in T_1. Highest accumulation of Cu (93.55 ppm) and Zn (79.13 ppm) were recorded in T_5 while Cd (8.88 ppm) in T_3. Cu was highly concentrated in the roots, Cd was accumulated in the leaves and roots, whereas, Zn was in stems and leaves. *A. mangium* showed high translocation factor (TF) and low bioconcentration factor (BCF) values in soil at higher metal concentrations as well as it was able to tolerate and accumulate high concentrations of Cd, Cu and Zn. It may be concluded that this species can be a good efficient phtyoremediator for heavy metals (Cd, Cu and Zn) contaminated soils to mitigate soil pollution.

Key words: Heavy metals, phytoremediation, bioaccumulation capacity.

INTRODUCTION

Human, natural and anthropogenic activities concentrated some heavy elements in certain areas up to dangerous levels for living organisms (Chatterjee and Chatterjee, 2000, Kim et al., 2001). Use of sludge or urban composts, pesticides, fertilizers and emission from municipal waste incinerators, car exhausts, residues from metalliferous mining and metal smelting industry polluted extensive areas throughout the world (Zantopoulos et al., 1999, Herawati et al., 2000; Brun, 2001).

Copper an essential plant nutrient is required in small quantities. The presence of large amounts in the environment, particularly in soil, can be dangerous. Copper is strongly bound to organic matter in the soil, which permits it to be mobile (Mengel and Kirby, 1987).

Major sources of Cu pollution include release from fertilizers, fungicides, herbicides and sewage sludge (Ariyakanon and Winaipanich, 2006). Excess Cu and other metals can inhibit growth and photosynthetic activity in plants and may promote ageing of plants (Wilmer, 1983). Accumulation of Cu in the human body can cause gastroenteric problems, depression and kidney and liver disease (Kim and Lee, 2005).

Over recent decades, the annual worldwide release of heavy metals reached 939,000 t (metric ton) for copper, 783,000 t for lead and 1,350,000 t for zinc. Their accumulation can lead not only to the presence of potentially harmful residues in the cultivated crop but also to a decrease in soil fertility. Restoration of soils contaminated with potentially toxic metals and metalloids is of major global concern (Shelmerdine et al., 2009). In the past, chemical pollution in soil has been treated using physical and chemical processes that have proven to be expensive. Phytoremediation technologies have been proven successful as because low-cost, low-impact and

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environmentally sound (January, 2006).

Acacia mangium wild was selected for this study: It has rapid early growth, and can attain a height of 30 m and a diameter of over 60 cm (MacDicken and Brewbaker, 1984). Due to fast growing, dense foliage, high biomass, high absorption and tolerance of heavy metals, *A. mangium* can be a potential phytoremediator. Some studies of phytoremediation on polluted soils have been carried out but phytoremediation with *A. magium* has not been reported. Therefore, this study was initiated with the following objectives:

(i) To determine Cu, Cd and Zn uptake and translocation in *A. mangium* plant parts.
(ii) To quantify Cu, Cd and Zn concentrations in the growth medium before and after planting period.

MATERIALS AND METHODS

The experiment was conducted at the glasshouse, University Putra Malaysia. The average temperature in the greenhouse was 27, 36 and 32°C for morning, afternoon and evening, respectively. *A. mangium* was used as the test species. A completely randomized design was followed with six treatments replicated six times. The growth media prepared using soil mixing with different levels of Cu was: T_0 (control, soil), T_1 (soil + 50 ppm Cu), T_2 (soil + 100 ppm Cu), T_3 (soil + 200 ppm Cu), T_4 (soil + 300 ppm Cu) and T_5 (soil + 400 ppm Cu). After filling the pots with the growth media, 36 seedlings of uniform age and size were transplanted into the pots. Intercultural operations (weeding and watering) were done when necessary to ensure normal growth of the plant. The parameters monitored and analyzed in this study were (i) growth performance (basal diameter, height and number of leaves), (ii) soil characteristics (texture, pH and total carbon) as well as heavy metal concentrations in the growth medium and plant parts (roots, stems and leaves) were studied. Basal diameter was measured using caliper and the height was measured using diameter tape. The plant samples were collected after harvest and soil samples were collected before planting and after harvest and prepared for chemical analysis. Soil pH and organic carbon was determined by using glass electrode pH meter (Jackson, 1973) and wet oxidation method (Walkley and Black, 1935). AAS (Atomic Absorption Spectrophotometry) was used to determine the concentrations of heavy metals. Particle size distribution was analyzed by pipette gravimetric method and the texture was determined using USDA textural triangle. Plant biomass was measured separately according to leaves, stems, and roots calculated. The loss in weight upon drying is the weight originally present. The moisture content of the sample was calculated using the following equation:

$$\% W = \frac{A - B}{B} \times 100 \quad \ldots\ldots \quad (1)$$

Where, %W is the percentage of moisture in the sample; A is the weight of wet sample and B is the weight of dry sample.

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 can be calculated as follows:

$$\mathrm{BCF} = \frac{C_{root}}{C_{soil}} \quad \ldots\ldots \quad (2)$$

Where, C_{root} and C_{soil} is the metal concentration in the root and soil, respectively.

$$\mathrm{TF} = \frac{C_{aerial}}{C_{root}} \quad \ldots\ldots \quad (3)$$

Where, C_{aerial} is the metal concentration in the aerial parts.

The analysis of variance for growth, heavy metals in soils and plant parts were done following the ANOVA technique and the mean values were adjudged by DMRT (P = 0.05) method (Steel and Torrie, 1960). Computation and preparation of graphs were done by using Microsoft EXCEL 2003 software program.

RESULTS

Properties of the growth media

Soil was sandy clay. All treatments had low pH initially and slightly increased at harvest. Before planting, soil pH ranged from 4.26 to 4.70 while after harvest it varied from 4.80 to 4.86. Treatment T_5 showed the maximum change (4.26 to 4.86) in soil pH (Table 1). Knight et al. (1997) also found significant increment of soil pH after *Thlaspi caerulescens* grown in contaminated soils which corroborated the findings of our results. The total carbon content also increased at harvest having the highest (0.82 %) in the control and the lowest (0.67%) was in T_3 (Table 1).

Effect of copper on growth performance of *A. mangium*

Treatment T_1 (50 ppm Cu) produced the highest increment in basal diameter (2.70 cm) followed by T_2 (2.45 cm) (Figure1a). The lowest increment in basal diameter (2.17 cm) was noted in T_5. There was significant difference ($P \leq 0.05$) in height among all treatments. *A.mangium* planted in T_1 showed maximum increment of height (13.5 cm). The second highest increment was found in T_2 (10.1 cm) which was followed by the control (10 cm) and lowest was in T_5 (7.80 cm) (Figure1b). The maximum number of leaves (18) was also found in T_1 followed by T_2 (13). The minimum number of leaves (7) was recorded in the control (Figure 1c).

Effect of copper on plant biomass production

T_1 showed the highest biomass of 36.85 and 52.05 g for root and leaves, respectively while T_2 gave the highest stem biomass (43.72 g). The lowest leaves and stems biomass (41.02 and 38.14 g for leaves and stems, respectively) were found in T_4 treatment while T_3 exhibited lowest (28.11 g) root biomass (Table 2). Leaves produced the highest biomass than stems and roots (Table 2).

Table 1. pH and total-C (%) content in growth media as influenced by different Cu levels.

Treatment	pH		Total-C (%)	
	Before planting	After harvest	Before planting	After harvest
T0	4.62±4.62[1]	4.82±o.16	0.35±0.35	0.71±0.07
T1	4.70±5.01	4.81±0.10	0.32±0.32	0.76±0.03
T2	4.65±4.71	5.33±0.16	0.40±0.40	0.72±0.02
T3	4.32±4.42	4.63±0.22	0.34±0.34	0.67±0.05
T4	4.35±4.35	4.80±0.03	0.41±0.41	0.76±0.03
T5	4.26±4.26	4.81±0.04	0.39±0.38	0.82±0.01

T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu). [1]SE value after ±.

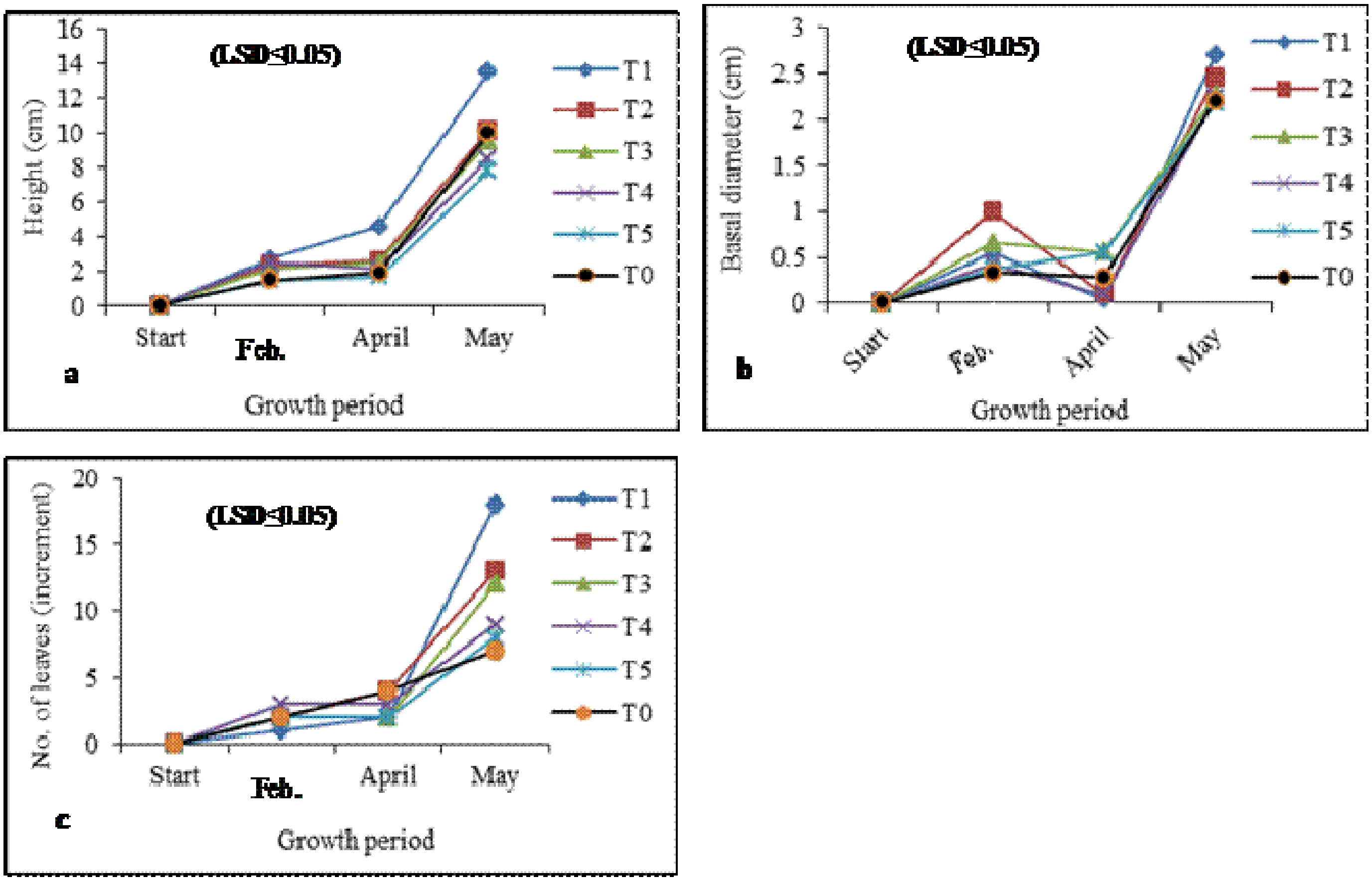

Figure 1. Increment of basal diameter (a), height (b) and number of leaves (c) at different Cu levels. T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu).

Heavy metal concentrations in growth media

There was a significant difference ($P \leq 0.05$) in Cu concentration among the treatments before planting and after harvest. Heavy metals concentration decreased after harvest than before planting among the growth media. Treatment T_4 showed the highest reduction of Cu (71.63 ppm) whereas T_2 and T_3 exhibited increased concentration in the growth media after harvest (Figure 2a). Cd concentration was significantly ($P \leq 0.05$) reduced in the growth media among the treatments (Figure 2b). Treatment T_3 showed the highest reduction of Cd (1.295 ppm) followed by the control (1.175 ppm) and the lowest reduction (0.145 ppm) was in T_1 (Figure 2b). After harvest, Zn concentration also decreased among the treatments in the growth media having the highest reduction (59.83 ppm) in T_1 followed by T_3 (33.72 ppm) and the lowest (13.60 ppm) was in the control

Table 2. Dry weight of roots, stems and leaves of *A. mangium* as influenced by different Cu levels.

Treatment	Plant biomass (g)			
	Root	Stem	Leave	Total
T0	32.30±5.30[1]	42.69±0.86	46.03±3.65	121.00
T1	36.85±6.38	39.98±2.51	52.05±1.77	128.88
T2	28.27±5.42	43.72±2.26	50.49±1.80	122.48
T3	28.11±4.70	39.50±3.39	50.50±1.58	118.11
T4	33.16±0.33	38.14±5.61	41.02±5.19	112.32
T5	32.00±6.46	38.42±5.13	41.31±4.86	111.73

T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu). [1]SE value after ±.

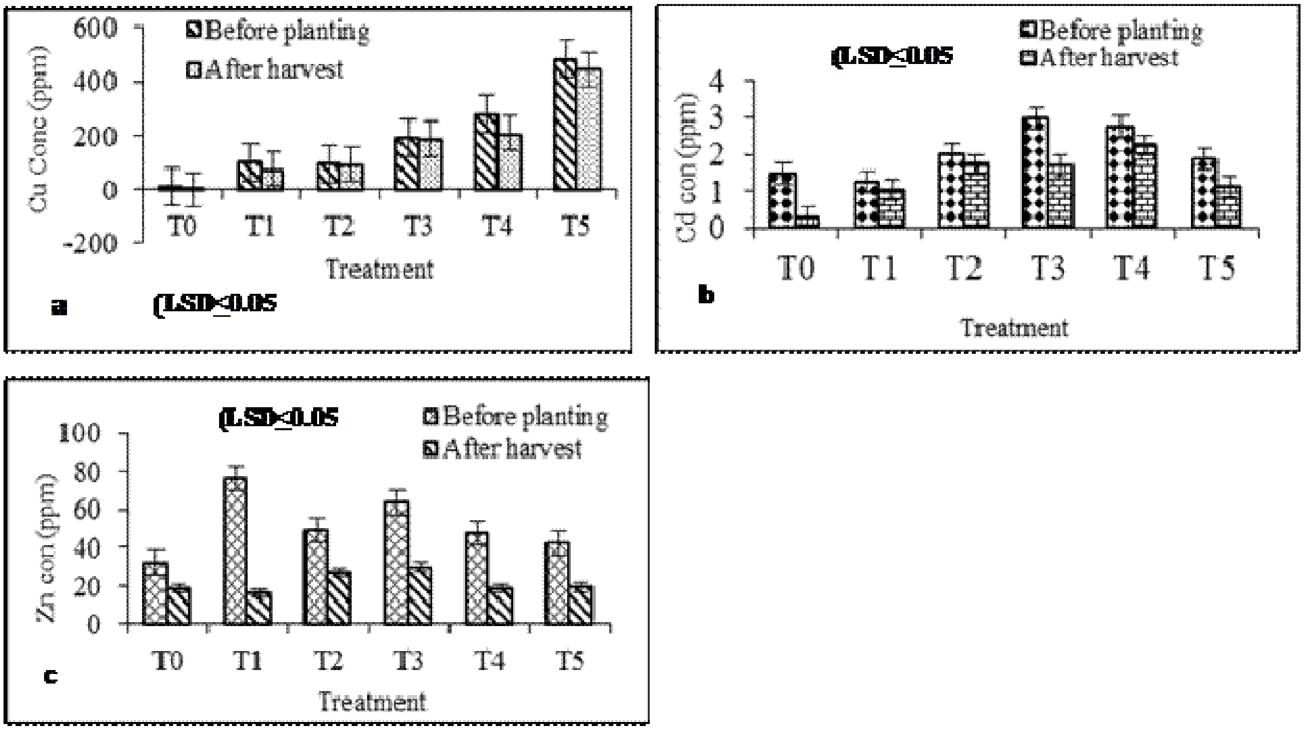

Figure 2. Concentration of Cu (a), Cd (b) and Zn (c) in growth media as affected by different treatments. T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu).

(Figure 2c).

Effect of copper on heavy metal concentrations in plant parts

Cu, Cd and Zn accumulation in the *A. mangium* were significantly ($P \leq 0.05$) influenced by the treatments. The accumulation of Cu and Cd were higher in the roots and the lowest was in the stems (Figure 3a and b). The accumulation rank was in the order of roots > leaves > stem. The highest Cu concentration (93.55 ppm) was also recorded in T_5 followed by T_4 (71.91 ppm) and T_3 (50.31 ppm). The lowest Cu absorption (12.54 ppm) was found in the control (Figure 3a). T_3 showed the highest Cd accumulation (8.88 ppm) in the plant and the lowest (1.87 ppm) was in T_1. The highest Zn accumulation (183.56 ppm) was found in T_5 and the lowest (105.1 ppm) was in T_2 treatment (Figure 3c). The heavy metal accumulation was higher in the roots than leaves and stems.

Bioconcentration and translocation of Cd and Zn

Significantly, higher BCF value of Cd (11.28) was found in the control and the minimum value (0.73) was noted in T_4 (Figure 4a). Treatment T_4 showed the highest BCF of

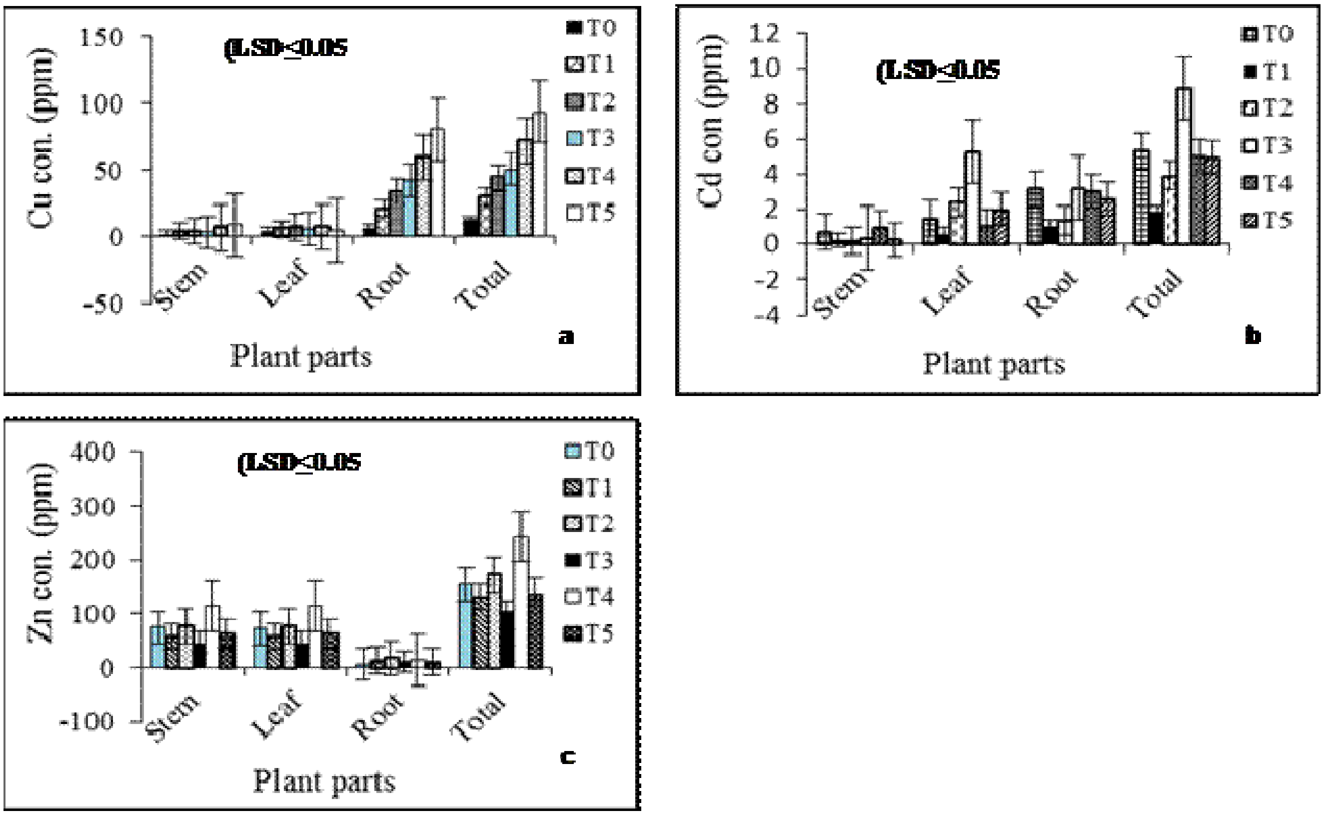

Figure 3. Accumulation of Cd (a), Cu (b) and Zn (c) into *A.mangium* as influenced by different treatments. T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu).

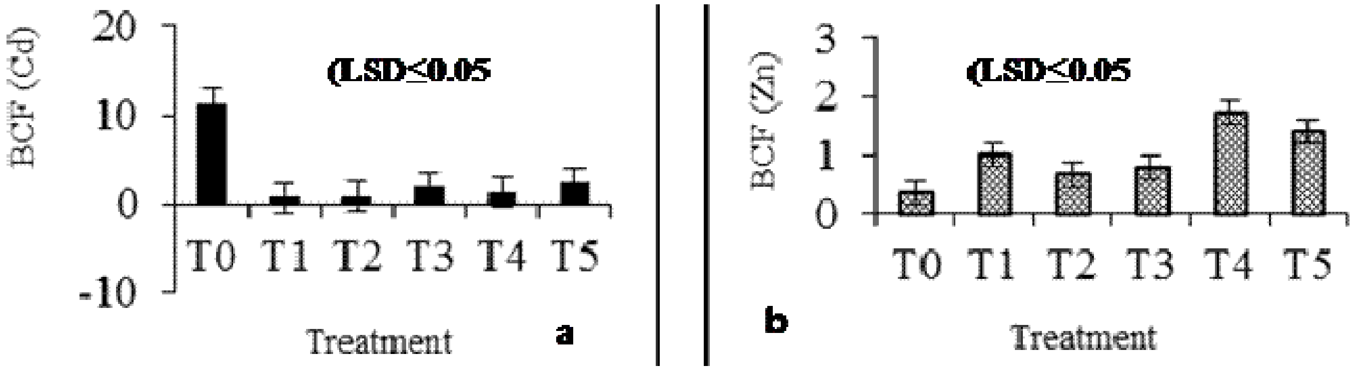

Figure 4. Bioconcentration factor of Cd (a) and Zn (b) in *A. mangium* at different Cu levels. T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu).

Zn (1.72) followed by T5 (1.40) and the minimum BCF value (0.35) was in the control (Figure 4b). The highest BF value of Cd (2.69) obtained in T_3 which was closely followed by T_4 (2.65). The control showed the lowest BF value (0.68) (Figure 5a). In case of Zn, the control showed highest TF (22.87) which was significantly higher than other treatments. The second highest TF value (7.64) was observed in T_1 followed by T_3 (5.93) and the minimum was in T_4 (3.99) treatment (Figure 5b).

DISCUSSION

Growth performance

The effect of Cu on root and shoot length, shown in Figure 1 indicated that the length generally decreased with increasing Cu concentration. The maximum height increment was recorded in T_1 and this might be due to lower concentration of metal that did not affect the

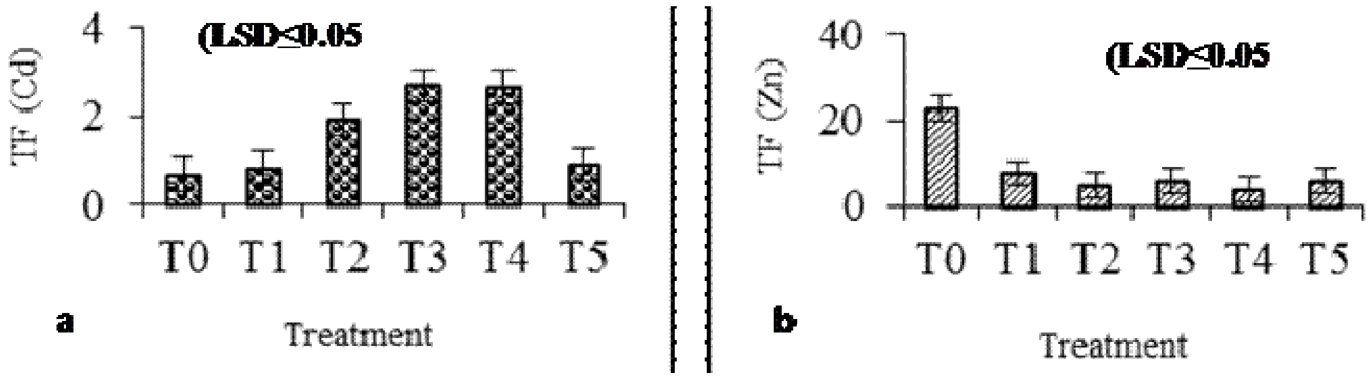

Figure 5. Translocation factor of Cd (a) and Zn (b) in *A. mangium* at different Cu levels.T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu).

absorption of other essential elements and physiological activities. Similar results were also observed by Kim and Lee (2005). All treatments showed increment in basal diameter, height and number of leaves in February and March but decreased in April. The height and basal diameter decreased in April and this might be due to Cu toxicity. In May, it increased again and showed the highest increment than the other months. The plants grew well in May and March. The height, basal diameter and number of leaves were highest in May (Figure 1). This might be due to the soil condition that has become more stable for plant growth after a certain period of time. In this circumstance, plants produced more leaves and at the same time height and basal diameter growth were much better than other months.

Plant biomass

The total biomass produced by the *A.mangium* ranged from 111.73 to 128.88 g having the highest (128.88 g) in T_1 followed by T_2 (122.48 g) and the lowest was in T_5 (111.73 g) treatments (Table 2). The highest biomass was also found in leaves followed by stems and the lowest was in the roots (Table 2). The biomass plays an important role in the absorption of heavy metals from soil and water. However, the success of phytoaccumulation depends on plant biomass and the ability of plants to absorb, accumulate and tolerate metals in their shoots. Plants used as a phytoremediator must have both high potential capacity to absorb elements from soil or water and large biomass (Ho et al., 2008).

Heavy metal concentrations in growth medium

After harvest, Cu concentration decreased in the growth media. The highest Cu reduction (71.63 ppm) was observed in T_4 because *A. mangium* was found to remove Cu efficiently in this treatment. The T_3 showed the highest Cd reduction (1.295 ppm) while the maximum Zn reduction (59.83 ppm) was noted in T_1 (Figure 2). Plants that grow on Cu contaminated soils can evolve mechanisms to tolerate and absorb Cu in plant cells (Ernst et al*.,* 1992). *Silene vulgaris* (Moench) has evolved extremely Cu-tolerant plants at highly contaminated sites in Germany (Schat and Ten, 1992).

Heavy metal concentrations in plants parts

The Cu accumulation ranged from 4.50 to 40.27 ppm and the ranking of the plant parts in respect of accumulation was in the order of roots > leaves > stem. Baker (1981) also reported higher Cu absorption in root than shoot which are in agreement with the findings of our results.T_5 showed the highest Cu concentration (80.37 ppm) in the roots (Figure 3). In leaf, highest Cu accumulation (7.08 ppm) was found in T_4. Treatment T_5 also showed high Cu concentration (8.66 ppm) in the stems. The leaves showed the highest Cd content followed by roots and stems. The highest Cd concentration (5.27 ppm) was also observed in T_3 at leaves. The Cd concentrations in roots and stems ranged from 1.35 to 3.22 ppm and 0.18 to 0.38 ppm, respectively. The maximum Zn concentration (79.13 ppm) was found in the leaves followed by stems (77.65 ppm Zn) and the minimum was in the roots (Figure 3c). T_5 showed the highest Zn concentrations in the stems (77.65) and leaves (79.12). The lowest Zn concentration (43.74) was recorded in T_2.

Bioconcentration and translocation Factor of Cd and Zn

The BCF value is defined as the metal concentration ratio of plant roots to soil whereas the TF value is described as the ratio of heavy metals in aerial parts to root (Yoon et al., 2006). BCF and TF values are important for estimating the plant's potential for phytoremediation. The highest BCF (11.28) of Cd was found in the control whereas the other treatments showed small values having the lowest (0.73) in T_1 (Figure 4a), which may indicate the restriction in soil-root transfer at higher metal concentrations in the soil. Gussarsson et al. (1995) reported that addition of Cu in soil decreased Cd uptake by the plants. Similar results were found by Yoon et al. (2006). Ho et al. (2008) also observed 1.92 to 3.21 BCF in Pb treated kenaf (*Hibiscus cannabinus* L.). For Zn, highest BCF value (1.72) was found in T_4 followed by T_5 (1.42) and the minimum was in the control (0.35) (Figure 4b). Luo and David (1995) also reported that additional Cu increased the amounts of plant-available Zn. Large amount of plant-available Zn would lead to luxury uptake and accumulation of Zn in the plant, leading to the toxicity which may decrease dry matter yields. In translocation factor, Zn exhibited higher TF values (>3) in all the treatments having the highest (22.87) in the control and the lowest (3.99) was in T_4. In case of Cd, treatment T_3 showed the maximum TF value (2.69) and the minimum was in the control (0.68). It was observed that translocation of Zn much higher than Cd. *A. mangium* showed high TF and low BCF in soil at higher metal concentrations. Heavy metal tolerance with high TF and low BCF value was suggested for phytoaccumulator of contaminated soils (Yoon et al., 2006). *A. mangium* can be used as a potential phytoaccumulator for contaminated soils.

Conclusion

A. manigium planted in 50 ppm Cu produced maximum number of leaves, highest height and basal diameter. The highest biomass was recorded in T_1. Cu was highly concentrated in the roots, Cd was accumulated in the leaves and roots, whereas, Zn was in stems and leaves. *A. mangium* showed high TF and low BCF in soil at higher metal concentrations as well as it was able to tolerate and accumulate high concentrations of Cu, Cd and Zn. Therefore, this species can be used as a phtyoremediator for Cd, Cu and Zn contaminated soils and to mitigate soil pollution. The plant physiological aspects such as photosynthesis and respiration rate need to be studied. A field experiment also need to be conducted to confirm the results obtained from the glass house study.

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