PHYTOREMEDIATION OF COPPER-CONTAMINATED SEWAGE SLUDGE BY TROPICAL PLANTS

G Maryam[1], NM Majid[2], MM Islam[1, 3, *], OH Ahmed[2, 4] & A Abdu[1]

[1]*Faculty of Forestry, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[2]*Institute of Tropical Forestry and Forest Product, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[3]*Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
[4]*Faculty of Agriculture and Food Science, Universiti Putra Malaysia, Bintulu Campus Sarawak, 97008 Bintulu, Malaysia*

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MARYAM G, MAJID NM, ISLAM MM, AHMED OH & ABDU A. 2015. Phytoremediation of copper-contaminated sewage sludge by tropical plants. Heavy metals are serious environmental pollutants, particularly in areas with anthropogenic activities. Heavy metal pollution in soil is a global environmental problem. An experiment was conducted to evaluate the potential of *Jatropha curcas*, *Acacia mangium* and *Hopea odorata* as phytoremediators capable of absorbing Cu from soil contaminated with sewage sludge. Seedlings were planted on six growth media as follows: 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). The highest Cu accumulation (27.6 ppm) was recorded in 100% sewage sludge. Roots showed the highest Cu concentration. Cu was highest in *J. curcas*. *Acacia mangium* and *H. odorata* showed the highest bioconcentration factor (BCF) in the control, whereas *J. curcas* showed the highest BCF in T1 (20% sludge). The highest translocation factor (TF) for *J. curcas* and *A. mangium* were in T5 (100% sludge), whereas T2 (40% sludge) showed the highest TF for *H. odorata*. *Jatropha curcas* and *A. mangium* had high TF and low BCF in soil with higher Cu concentrations. Therefore, *J. curcas* and *A. mangium* could be used to decontaminate Cu-polluted soil.

Keywords: Polluted soil, heavy metal accumulation, reclamation, translocation

INTRODUCTION

Chemical pollution in the soil and water bodies has become a major source of concern and has posed serious health problem in many countries (Majid et al. 2012). Environmental pollution by heavy metals, even at low concentrations, and long-term cumulative health effects are of major concerns (Bradl 2005). Their bioaccumulation in the human body interferes with proper functioning of the mitochondria, thereby impairing respiration as well as causing constipation, swelling of the brain, paralysis and eventually death (Opaluwa et al. 2012). The pollutants also severely affect productivity of agricultural lands and stability of natural ecosystems (Bridge 2004).

Rapid urbanisation, economic development and increased population have led to the production of huge quantities of sewage sludge in Malaysia, which poses serious environmental problems (Anonymous 2011). Sewage sludge in this country mainly originates from domestic and light industrial areas.

About 5 mil m^3 of sewage sludge are produced annually and it has been estimated to rise to 7 mil m^3 in the year 2022 (Rajoo et al. 2013). The total cost of managing the waste is estimated at USD0.33 bil $year^{-1}$ (Roslan et al. 2013). Sewage sludge from municipal wastewater treatment plants contains high concentrations of hazardous heavy metals, mainly, Pb, Cr, Cu, Zn and Ni (Rosenani et al. 2004). Due to these contaminants, recycling of sludge in agriculture is limited. The common problems associated with heavy metals are plant productivity, food quality and human health (Alloway 1990). Heavy metal accumulation

*monirupm.my@gmail.com

in human tissues and biomagnification through the food chain can cause DNA damage and carcinogenic effects (Ghafoori et al. 2011).

Cu is essential for plant growth. The normal range in tissues is 5 to 20 ppm but in most plants phytotoxicity occurs when its concentration reaches 25 to 40 ppm in dry foliage (Chaney & Giordano 1977). Permitted levels of Cu in Malaysian soil are in the range of 0.37 to 47.3 ppm, while sewage sludge contains 122 ppm (Zarcinas et al. 2003).

It is, therefore, necessary to remove these metals from sewage sludge/contaminated soil in order to minimise potential health risk (Lasheen et al. 2014). Physical, chemical and biological methods are used to remediate contaminated soil. The existing physical and chemical methods used in decontaminating soil are expensive and time consuming (Cao et al. 2002). Phytoremediation is a biological method which uses living plants to remediate organic and inorganic pollutants from contaminated soil and liquid substrates (Salt et al. 1998, Pilon-Smits 2005). It is cost effective and environmentally friendly (Kramer 2005).

In the present study, three woody species, namely, *Jatropha curcas, Acacia mangium* and *Hopea odorata* were selected due to their fast growth, dense foliage, high biomass as well as high absorption and tolerance to heavy metals (Veronica et al. 2011). There is a dearth of information on the use of woody species to remediate Cu-contaminated soil. The objectives of this study were to (1) determine Cu uptake and translocation in different plant parts, (2) compare Cu concentrations of the growth media used before planting and after harvest and (3) evaluate which of the three woody species were most efficient in receiving Cu from contaminated soil.

MATERIALS AND METHODS

Site

The experiment was conducted in a glasshouse at Universiti Putra Malaysia (4° 6′ N latitude and 101° 16′ E longitude). The average temperature of the glasshouse was 26 °C in the morning, 36 °C at noon and 30 °C in the evening.

Test plants

Jatropha curcas is a semi-evergreen shrub belonging to the family Euphorbiaceae. It is cultivated in the tropics and subtropics. It can grow on almost all types of soil, thrives even on the poorest stony soil and rock crevices. *Jatropha curcas* oil is an environmentally-safe, renewable source of non-conventional energy as well as promising substitute for diesel (Saxena 2012). *Acacia mangium* belongs to the family Leguminosae. It is a fast-growing timber species which thrives well in the tropics (MacDicken & Brewbaker 1984). 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 temperature, high light intensity and low relative humidity (MacDicken & Brewbaker 1984). *Hopea odorata* is a hardwood tree native to lowland forests of South-East Asia. It is a medium- to large-sized evergreen tree with large crown growing to 45 m tall. It is a riparian species usually occurring on deep rich soil most commonly along the banks of streams and in damp areas up to 600 m altitude.

Experimental design and treatments

A completely randomised design was followed with four replications. The growth media were prepared using soil that was mixed with different levels of sewage sludge. The treatments evaluated 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).

Seedling collection and planting

Healthy seedlings of *J. curcas, A. mangium* and *H. odorata* were collected from the Forestry Department's nursery, Negeri Sembilan, Malaysia. After filling the pots (28.2 cm × 34.2 cm) with growth media as per treatment, similar-sized 3-month-old seedlings were transplanted into the pots (one seedling pot^{-1}). Weeding and watering were done when necessary to ensure normal growth of seedlings.

Growth parameter measurement

Plant height was measured every 2 weeks using diameter tape.

Plant and soil samplings and chemical analysis

Plant samples were collected at harvest. Soil samples collected from each pot before planting and after harvest were air dried and kept in plastic containers for physico-chemical analysis. For Cu determination, 1 g of dried and ground plant sample was digested in 20 ml Aqua Regia solution (mixture of concentrated HNO_3 and HCl in ratio of 3:1) using the method. The digest was analysed for Cu using inductively couple plasma mass spectrometry (Sahoo et al. 2009). Soil particle size distribution was analysed using the pipette gravimetric method (Day 1965) and the textural class was determined using USDA textural triangle. Soil pH was determined using pH meter (Jackson 1973) while carbon, by loss on ignition (Nelson & Sommers 1996).

Plant biomass measurement

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

$$\text{Moisture} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Dry weight}} \times 100 \qquad (1)$$

Determination of bioconcentration factor (BCF) and translocation factor (TF)

BCF and TF factors were calculated as follows:

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

$$\text{TF} = \frac{\text{Metal concentration in aerial part}}{\text{Metal concentration in root}} \qquad (3)$$

Statistical analysis

The analysis for growth and heavy metal concentrations (soil and plant parts) were done using analysis of variance and the mean values differentiated using Duncan's multiple range test ($p = 0.05$) (Steel et al. 1996). Comparison using t-test was done to detect any significant difference in soil pH and carbon content in the growth media between before planting and after harvest. SAS software was used.

RESULTS AND DISCUSSION

Properties of growth media

The texture of control (100% soil) and T1 (80% soil + 20% sludge) was sandy clay, while 100% sludge treatment was clay. The percentages of clay were 30.6, 33.8, 43.0, 45.8, 46.7 and 63.1% for T0, T1, T2, T3, T4 and T5 respectively (result not shown). Clay content increased with increasing sludge content. Clay content correlated with concentration of heavy metals. Clay is one of the important factors that increase retention of heavy metals in soil because of its high cation exchange capacity. Other elements such as N, P and Zn increased with increase in sludge content but the reverse was true for S (Table 1). The highest Pb content was found in T5 (69.8 ppm) and the lowest, in control (6.18 ppm). Maximum S content was noted in the control and minimum, in T5.

Changes of soil properties in growth media

Soil pH was significantly ($p \leq 0.05$) changed between before planting and after harvest. Soil pH ranged from 4.10 to 4.95 before planting (Table 2). Soil without sludge treatment (100% soil) showed the highest pH (4.95), followed by 20% sludge-treated soil (4.35) and the minimum (4.10) was in 100% sludge. Soil pH increased after harvest; T1 showed the highest increase (+1.44), followed by T3 (+1.35). The lowest increase (+0.89) was in the control. The higher pH values after harvest might be due to absorption of heavy metals from the sewage sludge-contaminated soil. Acidic elements such as Al, Fe and Mn are available at low pH and

Table 1 Initial properties of growth media of different treatments

Treatment	%N	%P	%S	Zn (ppm)	Pb (ppm)
T0	0.92	0.81	5.10	42.6	6.18
T1	1.50	0.94	3.58	85.5	8.32
T2	1.67	1.50	3.42	164	12.4
T3	1.82	1.65	3.25	274	21.6
T4	2.23	1.80	3.24	425	45.2
T5	3.21	2.30	2.19	1202	69.8

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

Table 2 Changes in pH and total carbon (%) in the growth media at harvest (120 days after planting) as influenced by different treatments

Treatment	pH		Total carbon (%)	
	Before planting	After harvest	Before planting	After harvest
T0	4.95 a	5.84 a	1.87 e	0.79 e
T1	4.35 b	5.79 ab	2.84 de	1.67 e
T2	4.34 b	5.68 bc	4.46 d	2.94 d
T3	4.30 b	5.65 c	17.10 c	10.60 c
T4	4.20 b	5.48 d	23.50 b	16.20 b
T5	4.10 b	5.18 e	25.40 a	19.50 a

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; values in a column having common letter(s) do not differ significantly at 5% level of Duncan's multiple range test; mean values with different letter(s) at before planting and after harvest are significantly differently by t-test

plants can subsequently absorb and remove these elements from the soil. Significant increase in soil pH after cultivation of *Thlaspi caerulescens* in contaminated (Cd, Cu, Pb and Zn) soil was also reported by Knight et al. (1997). Physico-chemical and biological properties of soil are influenced by pH (Brady & Weil 2002). Elemental absorption by plants depends not only on their concentration in soil but also soil pH (Lorenz et al. 1994, Golovatyj 2002).

Total carbon in the control was significantly different between treatments before planting. Total carbon ranged from 1.87 to 25.40% (Table 2). Carbon content increased with increase in sewage sludge in the growth media. After harvest, carbon content varied from 0.79 in the control to 19.50% in T5. Carbon content decreased after harvest. Maximum reduction was in T4 (7.3%), followed by T3 (6.5%). The lowest reduction was in the control. T4 and T5 showed high levels of total carbon (organic matter) which might improve water holding capacity and soil fertility. The carbon present in the sewage sludge might be responsible for the release of negatively-charged ions that might have attracted positively-charged heavy metals and consequently resulted in higher concentration of heavy metals in the soil. Organic carbon in soil increases the binding capacity for metals (De Temmerman et al. 2003, Rodríguez Martín et al. 2007). Organic matter is the primary constituent which specifically adsorbs Cu, probably due to its cation exchange capacity (Martin & Kaplan 1998). This explains why soil applied with sewage sludge has higher concentrations of heavy metals (Clemente et al. 1991).

Biomass production

Biomass production was significantly influenced by the interaction of different levels of sludge and plant species. Dry biomass ranged from

4.5 g plant^{-1} in the control of *A. mangium* to 70.0 g plant^{-1} in T5 of *J. curcas*, which was significantly higher than the other treatment combinations (Table 3). The second highest dry biomass (55.0 g plant^{-1}) was also recorded in T5 of *A. mangium*, followed by T4 (48.6 g plant^{-1}) in *J. curcas*. *Hopea odorata* in T0, T1 and T2 as well as *A. mangium* in T1 and T2 showed statistically similar dry biomass. Weight of biomass was found to increase with increase in sludge content. Soybean was reported to produce 10.8 to 12.5 g dry biomass pot^{-1} in sludge-treated pots, while control showed 6.0 to 7.1 g dry biomass pot^{-1} (Katanda et al. 2007), which were in agreement with our findings. Among the plant species, *J. curcas* showed the highest dry matter (average 41.3 g plant^{-1}), followed by *A. mangium* (24.2 g plant^{-1}) and *H. odorata* (20.9 g plant^{-1}). T5 produced the highest biomass because of higher amounts of sewage sludge supplying more nutrients. In addition, the sludge (organic matter) increased water holding capacity and improved soil fertility (Moreno et al. 1997). It was reported that increasing the amount of sewage sludge enhanced the dry matter of maize due to increased supply of nutrient (Chitdeshwari & Savithri 2004). Biomass production is important for phytoremediation because if plants produce more biomass, more heavy metals will be absorbed from the soil.

Growth performance

Heights of *J. curcas, A. mangium* and *H. odorata* were significantly influenced by the different levels of sewage sludge and months ($p \le 0.05$) (Figure 1). The increase in height of *J. curcas* ranged from 1.77 to 9.00 cm (Figure 1a). The highest height increase was in T5 in July which was significantly higher than the other combinations. T4 showed the second highest height (8.24 cm) in July. The lowest height increase (1.77 cm) was in the control in May. T5 also showed significantly highest height increase (9.50 cm) in *A. mangium*, followed by T4 (8.00 cm) in July (Figure 1b). The lowest increase (1.45 cm) was in the control in May. In the case of *H. odorata*, height increase ranged from 1.62 to 8.54 cm (Figure 1c). The highest height increase was in T5 (8.54 cm), followed by T4 (7.56 cm) in July. The lowest height increase (1.62 cm) was in T2 in May. The increase in height with increasing sludge content might be due to more nutrients from the sewage sludge. The sludge may also improve other physico-chemical properties such as bulk density, porosity, water holding capacity (Aggelides & Londra 2000) and nutrient availability (Debosz et al. 2002)), which are essential for plant growth and development. All species were able to tolerate high amounts of contaminated sewage sludge. All species showed highest height increase in July and lowest, in May. The height of all species significantly increased with month ($p \le 0.05$). This indicates good growth in sewage sludge-contaminated media. These results explained why the three species survived and showed normal growth (in terms of height) regardless of treatment. Similar results were also reported by Majid et al. (2012) in textile factory sludge-contaminated soil grown with *Justicia gendarussa*.

Table 3 Dry biomass production of *Jatropha curcas, Acacia mangium* and *Hopea odorata* under different levels of sludge at 120 days after planting ($p \le 0.05$) as influenced by sludge and plant species

Treatment	*Jatropha curcas* (g plant^{-1})	*Acacia mangium* (g plant^{-1})	*Hopea odorata* (g plant^{-1})
T0	19.9 j	4.5 m	11.5 l
T1	30.0 fg	12.3 l	14.5 kl
T2	34.3 e	12.9 l	16.4 k
T3	45.0 d	25.4 hi	23.9 i
T4	48.6 c	35.0 e	27.1 gh
T5	70.0 a	55.0 b	32.1 ef

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; values in column having common letter(s) do not differ significantly at 5% level of Duncan's multiple range test

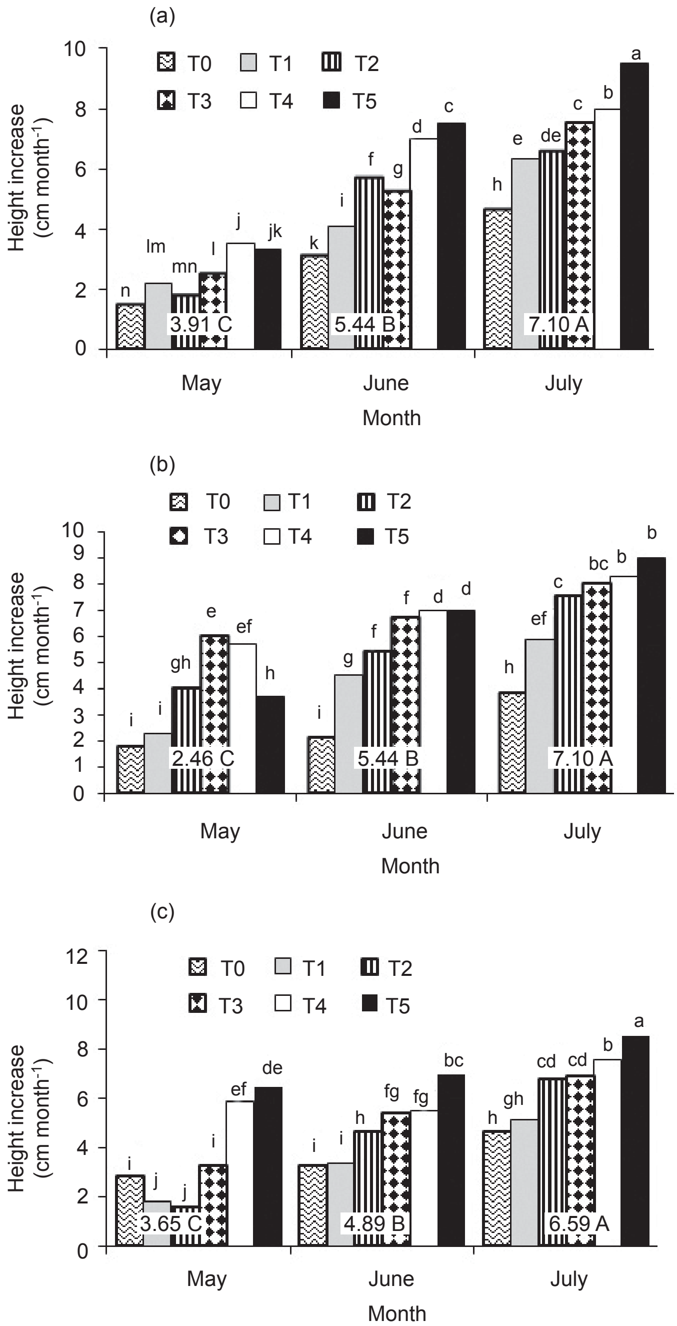

Figure 1 Monthly height increase of (a) *Jatropha curcas,* (b) *Acacia mangium* and (c) *Hopea odorata* under different levels of sludge; T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2= 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4 = 20% soil + 80% sewage sludge and T5 = 100% sewage sludge; boxed values on the bars indicate average overall sludge treatments; similar alphabets above bars indicate no significant difference at 5% level of Duncan's multiple range test

Copper concentration in growth media

Before planting, Cu concentration was significantly different between treatments (Table 4). The highest Cu concentration was in T5 (122.0 ppm), followed by T4 (84.6 ppm). The lowest (13.7 cm) was in the control. Cu concentration increased with

Table 4 Changes of Cu concentrations in the growth media under different levels of sludge 120 days after planting of *Jatropha curcas, Acacia mangium* and *Hopea odorata*

Treatment	Cu concentration before planting (ppm)	Cu concentration after harvest (ppm)		
		Jatropha curcas	*Acacia mangium*	*Hopea odorata*
T0	13.70 e	1.77 l	3.85 jk	2.25 kl
T1	22.40 d	2.24 kl	5.32 ij	13.00 g
T2	23.80 d	6.27 hi	12.80 g	13.80 fg
T3	30.60 c	8.00 h	15.50 f	17.60 e
T4	84.60 b	17.80 de	23.30 c	19.50 d
T5	122.00 a	21.70 c	58.30 a	28.70 b
Mean	49.60	9.63	19.80	15.80

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; values in column having similar letter(s) do not differ significantly at 5% level of Duncan's multiple range test

increasing sludge content in the growth media. After harvest, Cu concentration in the growth media was significantly influenced by the interaction of different levels of sludge and plant species. Cu concentration decreased in all species. Maximum Cu reduction (-100.3 ppm) was found of T5 in *J. curcas,* followed by T5 (-93.3 ppm) in *H. odorata* and T4 (-66.8 ppm) in *J. curcas* (Table 4). T0 showed lowest reduction (-9.9 ppm) in A. *mangium.* Highest Cu reduction was found in T5, followed by T4 and the lowest was in the control. The decrease at harvest might be due to plant uptake. Between the species, highest Cu reduction was in *J. curcas,* followed by *H. odorata* and *A. mangium.* Cu in soil before planting was 13.7 mg kg^{-1}, while sewage sludge contained 122.0 mg kg^{-1}. Bloemen et al. (1995) stated that the normal variation of Cu in soil was between 5 and 50 mg kg^{-1}. Sewage sludge disposed of on land often contains considerable amount of Cu (Nan et al. 2002). Malaysian sewage sludge contains high levels of Cu, i.e. 63 to 732 mg kg^{-1} (Rosenani et al. 2004). The concentration of Cu also depends on several factors such as texture, pH and total carbon content. Cu concentration is also highly influenced by the amount of organic matter in the soil and factors affecting Cu solubility particularly soil containing sewage sludge (Xiong et al. 2005).

Copper concentration in plant parts

Jatropha curcas

Cu concentration was significantly different between treatments for different plant parts of *J. curcas* (Figure 2a). In the roots, Cu concentration ranged from 3.45 in the control to 11.60 ppm in T5, which was significantly higher than the other treatments. T5 also showed the highest Cu accumulation (6.52 ppm) in the stems, followed by T4 (5.64 ppm). The lowest accumulation (1.37 ppm) was in the control. For leaves, Cu concentration varied from 1.57 in the control to 9.45 ppm in T5, followed by T4 (6.84 ppm). Similar results were also reported by Wislocka et al. (2006) whereby Cu concentration was in the range of 0.20 to 6.93 ppm in the leaves of silver birch. Between the plant parts, average Cu accumulation was in the order of root > leaf > stem.

Acacia mangium

For roots, Cu concentration ranged from 3.33 ppm in the control to 6.63 ppm in T5 (Figure 2b), which was significantly different from the other treatments. Cu concentrations of T2, T3 and T4 were not significantly different in the roots. In the case of leaf and

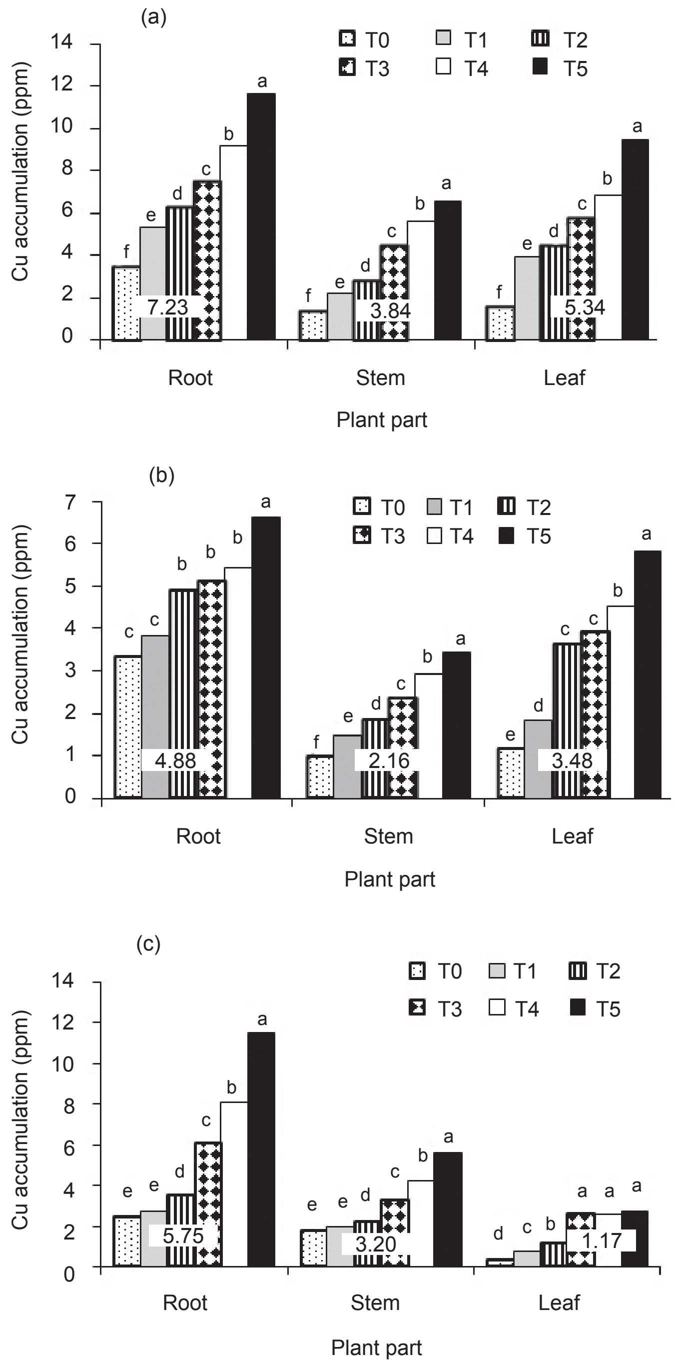

Figure 2 Copper concentrations in different parts of (a) *Jatropha curcas,* (b) *Acacia mangium* and (c) *Hopea odorata* under different levels of sludge at 120 days after planting; T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2= 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4 = 20% soil + 80% sewage sludge and T5 =100% sewage sludge; boxed values on the bars indicate average overall sludge treatments; similar alphabets above bars indicate no significant difference at 5% level of Duncan's multiple range test

stem, Cu concentrations significantly varied between treatments ($p \leq 0.05$). The highest Cu accumulation (5.82 and 3.43 ppm for leaf and stem respectively) was in T5, followed by T4 (4.52 and 2.91 ppm for leaf and stem respectively). The lowest accumulation was in the control. Between the plant parts, highest Cu concentration was in the root, followed by leaf and stem. Majid et al. (2011) also reported highest Cu absorption in roots.

Hopea odorata

Cu accumulation (Figure 2c) was also significantly influenced by the different treatments in different plant parts of *H. odorata* ($p \leq 0.05$). For root, Cu concentration ranged from 2.52 ppm in the control to 11.53 ppm in T5, which was significantly higher than the other treatments. T5 showed the highest Cu accumulation (5.60 ppm) in the stem, followed by T4 (4.23 ppm) and the lowest (1.78 ppm) was in the control. Between the plant parts, the highest Cu accumulation was in the root, followed by the stem and the lowest was in the leaf. Cu accumulation increased with increasing sludge content. Cu tends to be immobilised and is held primarily in the roots (Pulford & Watson 2003). Cu accumulation in the root was higher than in the shoot. This may be due to restricted translocation of Cu from roots to shoots and hence low accumulation in stems and leaves.

Total copper accumulation in different plant species

Total Cu absorption in the plants was significantly influenced by the interaction of different treatments and plant species ($p \leq 0.05$). The highest total accumulation (27.6 ppm) was in T5 of *J. curcas* which was significantly higher than the other treatments (Figure 3). T4 showed the second highest total absorption (21.6 ppm) which was also significantly different from other treatments. The lowest total Cu accumulation (4.7 ppm) was in the control of *H. odorata,* which was statistically similar to T1 in *H. odorata* and T0 in *A. mangium.* Between the plant species, *J. curcas* showed the highest mean accumulation (16.4 ppm), followed by *H. odorata* (10.7 ppm) and *A. mangium* (10.5 ppm). In 100% sewage sludge media, the total Cu accumulation was in the order of *J. curcas* (27.6 ppm) > *H. odorata* (19.8 ppm) > *A. mangium* (15.9 ppm). This finding indicated that *J. curcas* and *H. odorata* accumulated greater amounts of Cu than *A. mangium* from sewage sludge-contaminated media.

Bioconcentration and translocation factors

For *J. curcas,* the highest BCF value (2.39) was in T1, followed by the control (2.09) and the lowest was in T4 (Figure 4a). The control showed the highest BCF values of 0.86 and 1.12 for *A. mangium* and *H. odorata* respectively. BCF values decreased with increasing sludge content both for *J. curcas* and *A. mangium,* which might

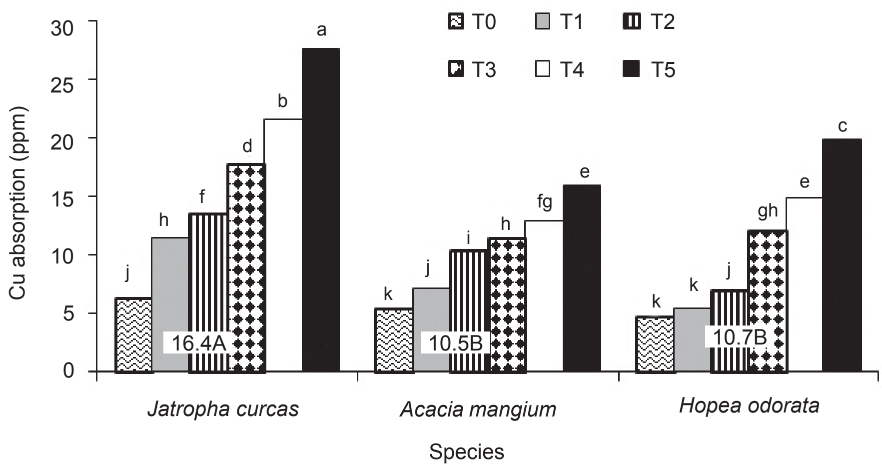

Figure 3 Total copper accumulation in *Jatropha curcas, Acacia mangium* and *Hopea odorata* as influenced by different levels of sludge and plant species 120 days after planting; T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2= 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4 = 20% soil + 80% sewage sludge and T5 =100% sewage sludge; boxed values on the bars indicate average overall sludge treatments; similar alphabets above bars indicate no significant difference at 5% level of Duncan's multiple range test

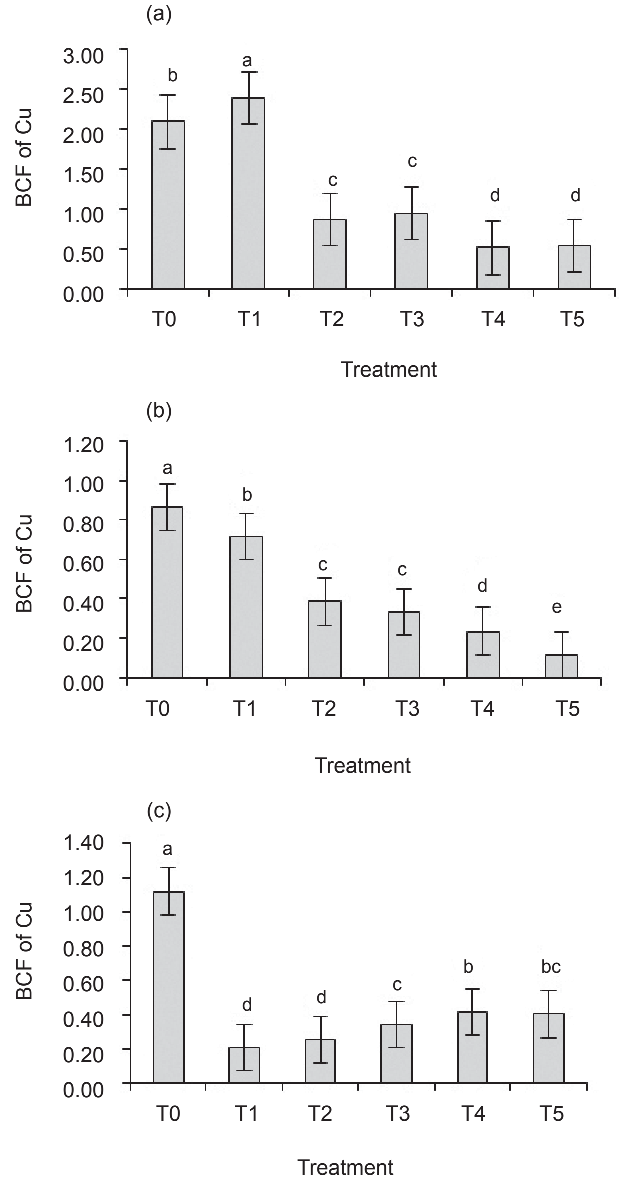

Figure 4 Bioconcentration factor (BCF) of Cu in (a) *Jatropha curcas,* (b) *Acacia mangium* and (c) *Hopea odorata* under different levels of sludge at 120 days after planting; T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2= 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4 = 20% soil + 80% sewage sludge and T5 =100% sewage sludge; bars with same letter do not differ significantly at 5% level; standard errors shown in error bars ($p \leq 0.05$)

imply the restriction in soil–root transfer at higher metal concentrations in the soil. Similar results were reported by Yoon et al. (2006).

In *J. curcas* and *A. mangium*, T5 showed the highest TF (1.37 and 1.40 for *J. curcas* and *A. mangium* respectively), followed by T4 (1.36 each for *J. curcas* and *A. mangium*) (Figures 5a and 5b). The lowest TF was in the control. In the case of *H. odorata*, BCF increased and TF decreased with increase in sludge content of the growth media except for the control. Translocation was more prominent in T5. TF of metal excluder species is < 1, whereas metal accumulator species have TF > 1 (Baker 1981). With the exception of the control, the remaining treatments exhibited higher TF values in *J. curcas* and *A. mangium* (> 1). *Jatropha curcas* and *A. mangium* had higher TF and low BCF in soil at higher Cu concentrations. Phytoaccumulators are noted for having high TF and low BCF. Therefore, *J. curcas*

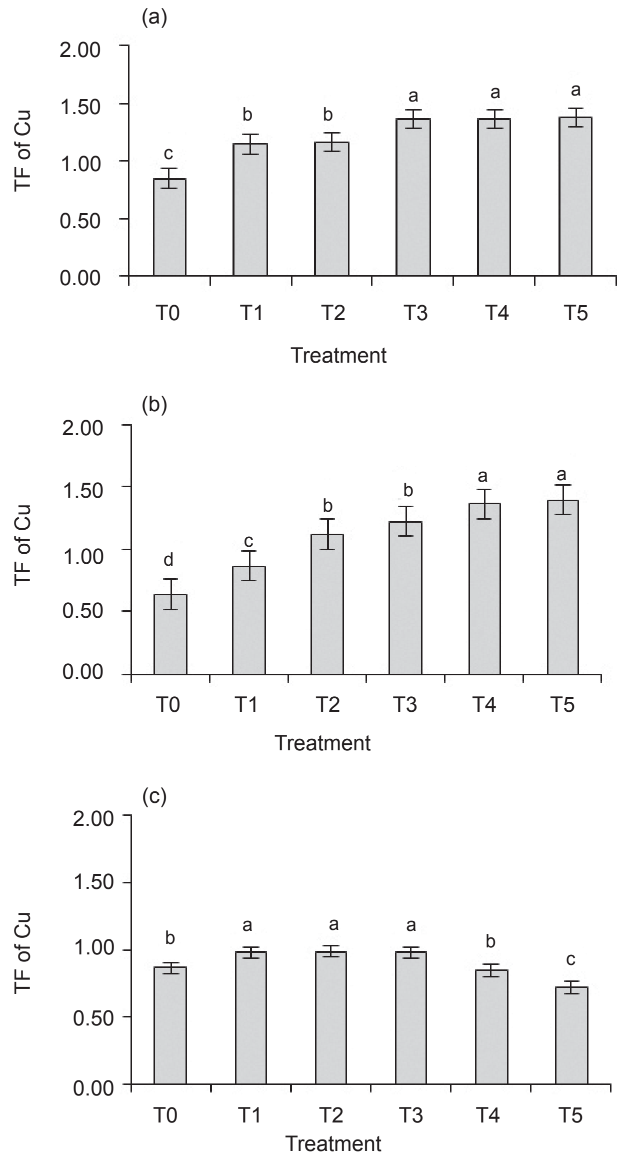

Figure 5 Translocation factor (TF) of Cu in (a) *Jatropha curcas*, (b) *Acacia mangium* and (c) *Hopea odorata* under different levels of sludge at 120 days after planting; T0 = 100% soil, T1 = 80% soil + 20% sewage sludge, T2= 60% soil + 40% sewage sludge, T3 = 40% soil + 60% sewage sludge, T4 = 20% soil + 80% sewage sludge and T5 =100% sewage sludge; bars with same letter do not differ significantly at 5% level; standard errors shown in error bars ($p \leq 0.05$)

and *A. mangium* could be used to decontaminate Cu-polluted soil.

CONCLUSIONS

Maximum Cu accumulation was found in 100% sewage sludge-treated plants. Between plant parts, root contained the highest Cu concentration and it was highest in *J. curcas* between the plant species. *Acacia mangium* and *H. odorata* showed highest BCF in the control, while *J. curcas* showed highest BCF in T1. Maximum TF for *J. curcas* and *A. mangium* was in T5, whereas T2 showed highest TF for *H. odorata*. *Jatropha curcas* and *A. mangium* had high TF and low BCF in soil at higher Cu concentrations. Heavy metal tolerance with high TF and low BCF values was suggested for phytoaccumulators of contaminated soil. Therefore, between the three species, *J. curcas* and *A. mangium* could be used as phytoremediators for contaminated soil and to mitigate Cu pollution in the soil.

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