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Evaluation of Jelutong (*Dyera cotulata*) as a phytoremediator to uptake copper (Cu) from contaminated soils

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Abstract

Soil pollutants including heavy metals are being mixed with agricultural soils and water. The potential accumulation of heavy metals in human and animal body is dangerous and causes several detrimental effects and diseases. An experiment was conducted in the glasshouse to evaluate the potential of *Dyera costulata* as a phytoremediator to absorb copper (Cu) from contaminated soils. *Dyera costulata* 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 and the highest biomass were found in T_2. The highest accumulation of Cu (89.97 ppm) was recorded in T_5. Among the plant parts, roots showed the highest concentration of copper. *Dyera costulata* showed a high translocation factor (TF) value in soil at low to medium Cu concentrations as it was able to tolerate and accumulate high concentrations of Cu. The roots of *Dyera costulata* were the main part of plant that absorbed copper in contaminated soils. This species can be an efficient phytoremediator for soils contaminated with copper.

Keywords: Contaminated soil, copper, phytoremediation, translocation.
Abbreviation: ANOVA= Analysis of variance, BCF= Bioconcentration factor, C= Celsius, CO_2 = Carbondioxide, Cd = Cadmium, CEC= Cation exchange capacity, Cr = Chromium, Cu = Copper, DMRT=Duncan's Multiple Test Range, HCl = Hydrochloric acid, HNO_3 = Nitric acid, Pb = Lead and Zn = Zinc.

Introduction

Heavy metals, such as cadmium, copper, arsenic, lead, chromium, zinc and nickel are important environmental pollutants, particularly in areas with high anthropogenic pressure. Heavy metal pollution of agricultural soils is a widespread global problem (Tandy et al., 2006), and also a major environmental concern over the past several decades. Heavy metal contamination in soil may be caused by various sources such as industrial processes, manufacturing, disposal of industrial and domestic refuse, and agricultural practices (Afshin and Farid, 2007; Koch and Rotard, 2001). Natural and anthropogenic quantities of trace metals are emitted to the atmosphere by human activities than by natural processes, which approximately are around 15 times more Cd, 100 times more Pb, 13 times more Cu and 21 times more Zn (Campbell, et al., 1983). The main sources of copper contamination in soils are pig and poultry manures, pesticides and metal finishing and microelectronics by-products. It is estimated that 390 tons of Cu and 303 tons of Zn are being excreted annually by approximately two million pigs in Malaysia (Wang, 2011). Using contaminated pig manure as a soil amendment could cause soil pollution and the accumulation of heavy metal to a toxic level throughout the food chain. Soils contamination due to the presence of toxic metals can cause serious negative consequences, such as damage of ecosystems, agricultural productivity, deterioration of food chain, contamination of water resources, economic damage and finally serious human and animal health problems (Raicevic et al., 2005). Copper is essential substance to human life, but long-term exposure to copper can cause irritation of the nose, mouth and eyes and it causes headaches, stomachaches, dizziness, vomiting and diarrhea. Moreover, high uptakes of copper may cause liver and kidney damage and even death. People with Wilson's disease are at the greater risk of health from overexposure to copper (Lenntech, 2011). Several procedures have been developed, evaluated and executed to clean polluted soils. Present available soil clean-up technologies are often high-priced, energy and time consuming, concurring with the CO_2 emissions and soil disturbing. Phytoremediation is proposed as a cost-effective, environment friendly and simple alternative for the treatment of contaminated soils (Majid et al., 2011). Using phytoremediation, the topsoils would be preserved and the amount of hazardous materials could be reduced significantly (Ensley, 2000). There are many types of plants currently used in phytoextraction, such as *Thlaspi carerulescens, Alyssum murale, A. lesbiacum, and A. tenium,* which can accumulate high levels of Zn, Cd and Cu in shoots and roots (Ariyakanon and Winaipanich, 2006). However, the remediation potential may be limited due to the slow growth and low biomass of these plants (Baker et al., 1994). Recently phytoremediation researchers have discovered that some tree species (*Acacia majium, Jatopha carcus, Dyera costulata* etc.) can accumulate high levels of metals, including Zn, Cd, Cr, Cu and Pb (Ghafoori et al., 2011). The metal accumulating ability of this plant, has been coupled with their potential to rapidly produce large quantities of shoot biomass, makes this plant ideal for phytoextraction (Veronica et al., 2011). Therefore, the present study was initiated with the following objectives: (1) to determine the Cu uptake and translocation in *Dyera costulata* plant parts and (2) to quantify Cu concentrations in the growth medium before planting and after harvest.

Results and Discussion

Properties of the growth media

Texture of soil was sandy clay having 51.71, 4.72 and 43.61% sand, silt and clay, respectively. 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. Clay soils crack excessively after drying, if they are very low in organic matters. Caly soil may lose their structure and become cloddy and compacted (Aljibury, 2011). There was a significantly difference ($P \leq 0.05$) in soil pH before planting and after harvest. Before plantation, the control soil showed the highest pH (4.74) followed by T_1 (4.72). The lowest pH observed (4.48) in the T_4, before plantation (Fig. 1a). Treatments T_2, T_3 and T_5 showed similar pH (4.51, 4.55 and 4.52, respectively). After harvest, soil pH significantly increased, in which the highest increment rate observed in T_3 (0.55) and T5 (0.55) followed by T_2 (0.44). The minimum pH change (0.2) was seen in the control (Fig. 1a). It was observed that pH was higher at harvest time which might be due to absorption of heavy metals by the plant parts (absorption of acidic elements) from the contaminated soil. Knight et al. (1997) also reported a significant increment in soil pH of contaminated soils by growing *Thlaspi caerulescens*, which is in agreement of our findings. Soil pH affects all the chemical, physical and biological characteristics of soil (Brady & Weil, 2002). Element 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 was also significantly influenced ($P \leq 0.05$) by different Cu levels, before planting and after harvest. Before planting, the highest total carbon (0.80%) was found in T_5 followed by T_2 (0.63%), T_3 (0.53%) and T_1 (0.52%) (Fig. 1b). The lowest value (0.21%) was recorded in T_4. After harvest, all growing media showed increasing trend in total carbon content. Treatment T_1 showed maximum increament (1.01%) followed by T_2 (0.81%) and the minimum was in the T_4 (0.46%) (Fig. 1b).

Growth performance

Growth parameters such as height, basal diameter and number of leaves were significantly influenced ($P \leq 0.05$) by the different treatments. The highest basal diameter increment (2.44 cm) was found in T_2 followed by T_1 (2.33 cm) and T_0 (2.21 cm) (Fig. 2a). The lowest increment recorded in the T_5 (1.66 cm), where 400 ppm Cu was incorporated in the growth media. Similar results were also observed by Majid et al. (2011) where *Acacia mangium* gave lowest basal diameter at highest Cu contaminated soils, which is in agreement with the findings of the present study. Treatment T_2 showed maximum height increment (6.34 cm) which was closely followed by T3 (6.00 cm) (Fig. 2b). Likewise its effects on basal diameter, T_4 also had the minimum influence on height increment (1.75 cm). The highest height increment of plant was observed in T_2 which might be due to lower copper concentration and higher balance in absorption of other essential elements and plant's physiological activities. The plant height decreased in T_5, which might be due to toxic effects of higher copper concentrations, causing more shoot mortality. The similar results were also reported by Kim and Lee, (2005) on degree of shoot mortality at higher concentrations of heavy metals. The number of leaves was also significantly influenced by different Cu treatments ($P \leq 0.05$). T_2 produced the maximum mean number of leaves (20) followed by T_3 (18) and T_1 (16). The minimum number of leaves (11) was found in the T_5 (Fig. 2c). Majid et al. (2011a) also reported that plant height and the number of leaves decreased or halted at media with maximum cadmium contamination, which supports the present findings. Seedlings were planted on February, 2010. Height, basal diameter and number of leaves increased in March and then decreased in April (Figs. 1a, 1b and 1c). This decrease might be due to the toxic effects of copper two months after planting (in April). The maximum increment of height, diameter and leaves were in May (three months after planting) for all treatments. Treatment T_2 showed the highest increment in height (6.34 cm) and diameter (2.44 cm) as well as maximum leaves (20) in May (Figs. 1a, 1b and 1c). This is because the soil conditions became suitable for plant growth after three months.

Plant Biomass

There was no significant difference ($P \leq 0.05$) in plant biomass among the all treatments. Root biomass varied from 38.35 to 49.50g having the highest value in T_2 followed by T_3 (Table 1). The lowest biomass (38.35g) was noted in the control. Treatment T_2 produced highest stem biomass (56.80g) followed by T_1 (52.16g) and the lowest (45.20 g) was in the T_5. Leaf biomass varied from 42.22 to 61.70g with the highest (61.70 g) in T_2 followed by T_3 (58.75g) (Table 1). The lowest leaf biomass (42.22g) was recorded in the T_5. The total biomass ranged from 127.47 to 168.00g, in which the highest observed in T_2 followed by T_3 (154.59g) and T_1(149.00g) and the lowest (127.47g) in T_5. A strong relationship between root content and growth inhibition detected, in response to copper, when seedlings were grown in culture solution at increasing concentrations of $CuSO_4$ (0.012–5 μM). The growth was decreased due to increasing concentration of $CuSO_4$ (Arduini et al., 1996) which corroborated our results.

Concentration of copper in the growth media

Copper concentration significantly varied ($P \leq 0.05$) in the growth media by application different treatments. Before planting, T_5 showed highest Cu concentrations (584.7 ppm) followed by T_4 (Fig. 3). The lowest Cu concentration was found in the control. After harvest, Cu concentration significantly reduced in the growth media. Maximum Cu reduction (152.17 ppm) was found in T_2 followed by T4 (117.35 ppm). The control showed minimum Cu reduction (5.88 ppm) in the growth media (Fig. 3). Copper concentration in the growth media was decreased at harvest and this might be due to the uptake by Jelutong. Copper contamination of soils may cause phytotoxicity. Plant growth was affected in high Cu concentrations media and this is why the plants failed to absorb more Cu. On the other hand, Cu concentration was very low in the control, in which the

Table 1. Dry weight of roots, stems and leaves of *Dyera costulata* as influenced by different copper levels.

	Plant biomass (g)			
Treatments	Root	Stem	Leaves	Total
T_0(Control)	38.35	51.35	47.96	137.66
T_1(50 ppm Cu)	44.42	52.16	52.42	149.00
T_2(100 ppm Cu)	49.50	56.80	61.70	168.00
T_3(200 ppm Cu)	45.39	50.45	58.75	154.59
T_4(300 ppm Cu)	39.92	48.50	44.65	133.07
T_5(400 ppm Cu)	40.05	45.20	42.22	127.47
SE(±)	1.73	1.58	3.18	5.67

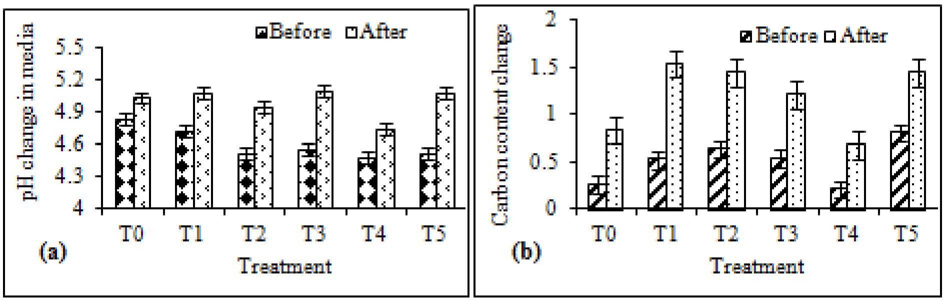


Fig 1. Change in pH (a) and total carbon (b) in the growth media at harvest of *Dyera costulata* as influenced by different copper levels. Growth media indicates soil mixing with different levels of cadmium, i.e. T_0 =Control/ soil, T_1 = 50 ppm Cu, T_2 = 100 ppm Cu, T_3 = 200 ppm Cu, T_4 = 300 ppm Cu and T_5 = 400 ppm Cu. Means ± SE are shown in error bar (p=0.05).

absorption was also low. Perk, (2006) reported that copper is highly toxic to several species which are in agreement with the findings of our results.

Copper concentration in the plant parts

Copper concentration in *D. costulata* plant parts was significantly influenced by the different treatments (P≤0.05). In the leaves, treatment T_2 showed highest Cu concentration (11.13 ppm) followed by T_1 (9.58 ppm) (Fig. 4). The lowest Cu concentration (1.29 ppm) was in the control. Cu concentrations in the stem ranged from 1.46 to 8.95 ppm having the highest in T_2 (8.95 ppm) followed by T_4 (8.35 ppm) and the lowest (1.46 ppm) was in the control (Fig. 4). Treatment T_5 showed highest Cu concentration (81.971 ppm) in the roots, which was statistically identical to T_4 and different from rest of the treatments ($P \leq 0.05$). T_4 gave the second highest concentration value in the roots (78.02 ppm) followed by T_3 (45.16 ppm). The minimum concentration of Cu observed in the control (1.32 ppm). It was observed that Cu concentration in the root was higher than the leaves or stems (Fig. 4). However, Cu concentration in the roots increased with increment of Cu in the growing media. Tang et al. (1999) reported that *E. haichowensis*, grown at higher Cu concentration soil, absorbed more Cu which is in agreement with our findings.

Translocation factor

Translocation factor (TF) was significantly influenced by the different levels of copper (P≤0.05). Translocation factor varied from 0.10 to 3.78 (Fig. 5). The highest TF (3.78) observed in T_1 which was significantly higher than other treatments (P≤0.05). Control showed the second highest TF, which was also significantly higher from the remaining treatments. The lowest TF value (0.10) observed in the T_5 (Fig. 5), which may imply the restriction in root-shoot transfer at higher metal concentrations of soil. Similar results were found by Yoon et al. (2006). Majid et al. (2011b) also observed lowest TF at higher metal concentration during cultivation of *Justicia gendarussa* in textitle-sludge contaminated soil. In our study, the translocation was more prominent in T_1. TF of metal excluder species is <1, whereas metal accumulator species has TF>1 (Baker, 1981). It was observed that *Dyera cotulata* under treatments with lower to medium Cu concentration has higher TF values (>1). In fact, *Dyera cotulata* had higher TF in soils at low to medium copper concentrations. Heavy metal tolerance with high TF value was suggested for phytoaccumulator of contaminated soils (Yoon et al., 2006); therefore, *Dyera cotulata* can be used as a potential phtyoremediator for soils contaminated with copper.

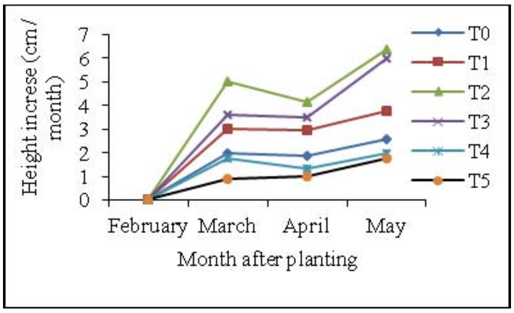
Materials and methods

Site description and growth media

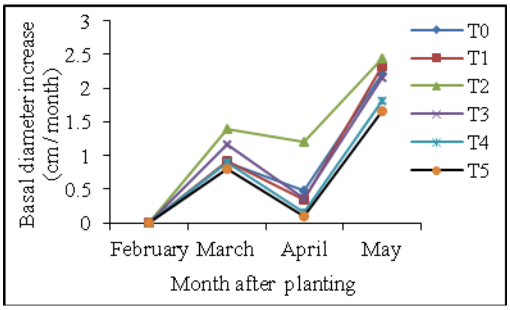
The experiment was conducted at the glasshouse, Universiti Putra Malaysia, Serdang, Selangor, Malaysia during February to May 2010. The temperature was 26° C in the morning, 36° C at noon time and reduced to 30° C in the evening. The growth media was a mixture of soil with different levels of copper, viz.: T_0 (control), 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). A Completely Randomized Design (CRD) with four replications was used in this study.

Nature of plant

Dyera costulata was the test plant. The Jelutong (*Dyera costulata*) is a large timber tree, preferring primary evergreen lowland or hill forest up to 300 m. It grows to approximately 60 m tall with diameters of 2 m and boles clear and straight for 30 m. It grows in Malaysia, Borneo, Sumatra and southern Thailand (Middleton, 2004). It is considered as low risk - least concerned plant because this species regenerates readily in logged-over forest, it coppices well and is extremely resistant to girdling.



(A)



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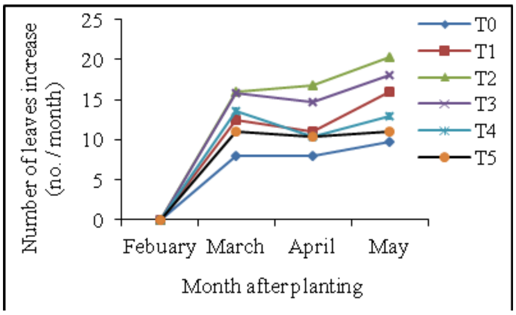

(C)

Fig 2. Plant height (a), basal diameter (b) and number of leaves (c) of *Dyera costulata* at different months after planting as influenced by different treatments (increase per month). Growth media indicates soil mixing with different levels of copper, i.e. 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. Means ± SE are shown in error bar (p=0.05).

Plantation of seedlings and growth measurement

Three-month-old Healthy seedlings, similar in form, were selected for this study. Earthen pots with 28.2 cm × 34.2 cm dimension were used for the experiment. Each pot was filled with 10 kg of soil. Seedlings of uniform age and size were transplanted at 1 seedling per pot. Intercultural operations (weeding and watering) were done when necessary to ensure normal growth of the seedlings. Twenty four plants were used to measure growth parameters including basal diameter, number of leaves, and height. The height was measured using a ruler and basal diameter by a caliper. The growth parameters were measured 15 days intervals.

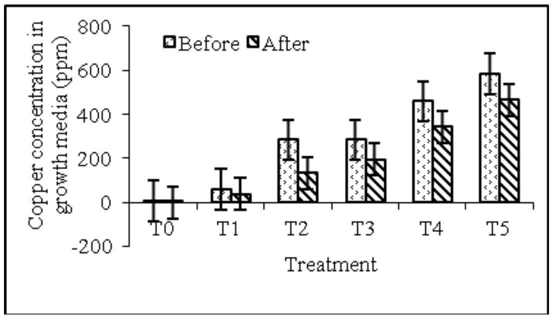

Fig 3. Copper concentrations in the growth media at harvest of *Dyera costulata* as influenced by different copper levels. Growth media indicates soil mixing with different levels of copper, i.e. T_0 =Control/ soil, T_1 = 50 ppm Cu, T_2 = 100 ppm Cu, T_3 = 200 ppm Cu, T_4 = 300 ppm Cu and T_5 = 400 ppm Cu. Means ± SE are shown in error bar (p=0.05).

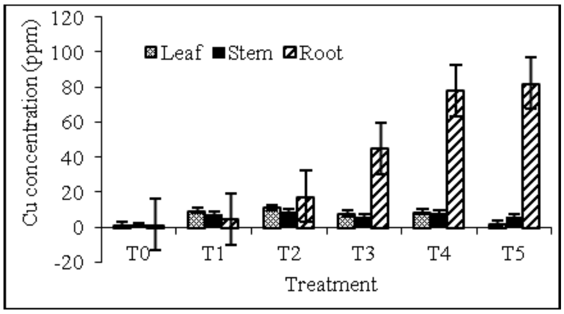

Fig 4. Copper accumulation in different parts of *Dyera costulata* as influenced by different treatments. T0 =Control/ soil, T1 = 50 ppm Cu, T2 = 100 ppm Cu, T3 = 200 ppm Cu, T4 = 300 ppm Cu and T5 = 400 ppm Cu. Means ± SE are shown in error bar (p=0.05).

Plant and soil sampling and chemical analysis

Soil samples were collected, dried and ground for chemical analysis. Particle size distribution was analyzed by pipette gravimetric method. Soil pH and total carbon were determined using glass electrode pH meter (Jackson, 1973) and loss on ignition method, respectively. Plant samples (whole plants) at harvest were collected for heavy metal analysis. 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 acid digested at 80 to 120° C for 3 hours. After digestion the solution was filled into 100 ml beaker and ready for analysis using ICP-MS (Inductively Couple Plasma Mass Spectrometry) method (Sahoo et al., 2009).

Plant biomass measurement

Plant biomass was measured separately for leaves, stems, and roots and calculated accordingly. The moisture content of the sample was calculated using the following equation:

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

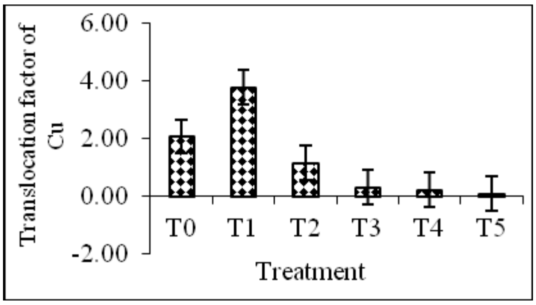

Fig 5. Translocation factor of Cu in *Dyera costulata* at different Cu levels. T_0 =Control/ soil, T_1 = 50 ppm Cu, T_2 = 100 ppm Cu, T_3 = 200 ppm Cu, T_4 = 300 ppm Cu and T_5 = 400 ppm Cu. Means ± SE are shown in error bar (p=0.05).

Determination of translocation factor (TF)

The plant's ability to translocate metals from roots to shoots was estimated using the translocation factor (TF). TF factor was calculated as follows:

$$TF = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in root}} \qquad (2)$$

Statistical analysis

Analysis of variance for growth and heavy metals concentrations (in soil and plant parts) were done following the ANOVA test and the mean values were compared by DMRT (P=0.05) method (Steel et al., 1996). Comparison using t-test was also done to detect any significant differences before planting and after harvest time.

Conclusion

The highest growth performance mainly height, basal diameter and number of leaves were found in the T_2 (100ppm Cu) treatment. The highest accumulation of copper (89.97 ppm) was found in the most contaminated media (400ppm Cu). Among the plant parts, roots showed the highest concentration of copper. *Dyera costulata* showed high translocation factor and low bioconcentration factor values in soil at low-medium copper concentrations and was also able to tolerate and accumulate high concentrations of copper. The roots of *Dyera costulata* were found to be suitable for the absorption of copper in contaminated soils. This species can be an efficient phtyoremediator for soils contaminated with copper.

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