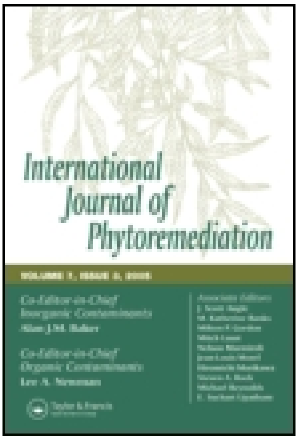
This article was downloaded by: [37.254.67.121]
On: 27 October 2013, At: 12:52
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Phytoremediation
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bijp20

Evaluation of Copper Bioaccumulation and Translocation in Jatropha curcas Grown in a Contaminated Soil

Parisa Ahmadpour [a] [b] , Mohsen Soleimani [c] , Fatemeh Ahmadpour [d] & Arifin Abdu [b]

[a] Ports and Maritime Organization (PMO) , Boushehr Maritime Rescue and Environmental Protection Department , Boushehr , Iran

[b] Department of Forest Production, Faculty of Forestry , Universiti Putra Malaysia , UPM Serdang , Selangor , Malaysia

[c] Department of Natural Resources , Isfahan University of Technology , Isfahan , Iran

[d] Pars Special Economic Energy Zone, Pseez, National Iranian Oil Co, NIOC , Boushehr , Iran

Accepted author version posted online: 03 Oct 2013.

To cite this article: Parisa Ahmadpour , Mohsen Soleimani , Fatemeh Ahmadpour & Arifin Abdu (2014) Evaluation of Copper Bioaccumulation and Translocation in Jatropha curcas Grown in a Contaminated Soil, International Journal of Phytoremediation, 16:5, 454-468, DOI: 10.1080/15226514.2013.798614

To link to this article: http://dx.doi.org/10.1080/15226514.2013.798614

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing,

International Journal of Phytoremediation, 16:454–468, 2014

ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/15226514.2013.798614

EVALUATION OF COPPER BIOACCUMULATION AND TRANSLOCATION IN *JATROPHA CURCAS* GROWN IN A CONTAMINATED SOIL

Parisa Ahmadpour,[1,2] Mohsen Soleimani,[3]
Fatemeh Ahmadpour,[4] and Arifin Abdu[2]
[1]Ports and Maritime Organization (PMO), Boushehr Maritime Rescue and Environmental Protection Department, Boushehr, Iran
[2]Department of Forest Production, Faculty of Forestry, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia
[3]Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran
[4]Pars Special Economic Energy Zone, Pseez, National Iranian Oil Co, NIOC, Boushehr, Iran

Contamination of soils with copper (Cu) has become a serious problem in the environment. Phytoremediation is an emerging green technology that uses green plants to remediate heavy metal contaminated areas. This study was conducted to evaluate the potential of* Jatropha curcas *for remediation of soils contaminated with Cu. Seedlings were planted in soils spiked with Cu in amount of 0, 50, 100, 200, 300, and 400 mg kg^{-1} (Cu$_0$, Cu$_{50}$, Cu$_{100}$, Cu$_{200}$, Cu$_{300}$, and Cu$_{400}$) for a period of five months. The maximum height and number of leaves were recorded in control (Cu$_0$) whereas the highest basal stem diameter was found in seedlings exposed to Cu$_{50}$. Copper concentrations among plant parts were in the following trend: roots > stems > leaves. The highest total Cu concentration (665 ± 1 mg kg^{-1}) and total Cu removal (1.2 ± 0.2%) based on total plant dry biomass were found in Cu$_{400}$ and Cu$_{50}$ treatments, respectively.* J. curcas *exhibited high root concentration factor (RCF > 1) and low translocation factor (TF < 1). Although Cu accumulation by the plant didn't reach the criteria of Cu hyperaccumulators, this species showed a potential to be used in phytostabilization of mildly Cu contaminated areas. However, the plant cannot be used for phytoextraction of Cu-contaminated soils.

KEY WORDS: phytoremediation, *Jatropha curcas*, heavy metals, soil pollution, removal efficiency

INTRODUCTION

Since the beginning of the industrial revolution, heavy metal contamination of the biosphere has increased considerably and became a serious environmental concern. Large areas of land are contaminated with heavy metals, such as copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), mercury (Hg), and cadmium (Cd). Heavy metals namely Cu, Fe, Zn, Mo, and Mn are micronutrients and are considered to be essential for maintaining life in biological systems (Khellaf and Zerdaoui 2010). However, at higher concentrations, these

Address correspondence to Parisa Ahmadpour, Department of Forest Production, Faculty of Forestry, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. E-mail: parisaahmadpour78@gmail.com

metals become highly toxic and threaten the health of animals and humans by influencing the quality of crops, water, and atmosphere. Unlike organic pollutant, heavy metals such as Cu cannot be degraded by microorganisms (Ma, Rajkumar, and Freitas 2008). The heavy metals Cd, Cu, Ni, and Hg create greater phytotoxicity than Zn and Pb (Raskin et al. 1994).

Contamination of soil with heavy metals such as Cu has become a serious problem all over the world which causes the reduction of agricultural yield and harmful effects on human health by entering the food chain (Jadia and Fulekar 2009). The two different ways that heavy metals enter the environment are from natural and anthropogenic sources (Jadia and Fulekar 2009). Natural sources of heavy metals contamination usually result from the weathering of mines, which are themselves created anthropogenically (Wei *et al.* 2008). There are various sources of copper in the soil resulting from human activities including pesticides, fertilizers, municipal compost, sludge and car exhaust, emissions from municipal wastes incinerators, smelting industries, mining and residues from metalliferous mine (Wei *et al.* 2008).

Cu occupies 0.1% of the Earth's crust and is an essential element for plants. It occurs in two oxidation states: Cu^+ (cuprous ion) and Cu^{2+} (cupric ion). The normal concentration of Cu in plant material ranged from 5 to 25 mg kg^{-1} (Ariyakanon and Winaipanich 2006). However, the critical concentrations of Cu in plant tissue at 10% reduction of dry weight production were found in the range of 5 to 30 mg kg^{-1}, depending on different crop species (Yang *et al.* 2002). As an essential metal in many enzymatic reactions, Cu acts as an electron donor or acceptor (Gambling, Dunford, and McArdle 2004). It acts as a co-factor in many enzymes, including superoxide dismutase, alcohol dehydrogenase, peroxidases, phosphatases, and catalases (MacPherson and Murphy 2007). In spite of being an essential element and acts as a cofactor in numerous enzymes, at higher concentrations, copper is highly toxic to plants, microorganisms and invertebrates. It creates disorders in physiological process including unexpected leaf fall, creating chlorotic spots and interfering with root growth as roots are the first plant parts to be damaged when exposed to toxic concentrations of copper (Ke *et al.* 2007). The annual widespread release of heavy metals has reached 22,000 t for Cd, 939,000 t for Cu, 1,350,000 t for Zn and 738,000 t for Pb over recent decades (Singh *et al.* 2003).

In fact, one of the most complicated issues for environmental engineering is detoxifying soils polluted with copper and other heavy metals due to their toxicity and high persistence in the environment (Jiang, Yang, and He 2004). Current conventional methods to remediate heavy metal contaminated soil and water including ex situ excavation, landfill of the surface contaminated soils, soil washing/flushing, electrokinetics etc. are expensive, time consuming, labor exhaustive and destroying the biotic and structure of the soil. These remediation techniques are not technically and financially suitable for large contaminated areas (Danh *et al.* 2009; Soleimani *et al.* 2010). Hence, it is necessary to spread out new technologies to remediate the polluted soils economically (Alkorta and Garbisu 2001).

Phytoremediation is a promising new technology that uses plants to remediate contaminated areas and is known as a low cost, environmentally and aesthetically friendly that is applied to immobilize/stabilize, degrade, transfer, remove, or detoxify contaminants including metals, pesticides, hydrocarbons, and chlorinated solvents (Jadia and Fulekar 2009; Sarma 2011). Phytoremediation can be classified into different applications, such as (i) phytofiltration or rhizofiltration, (ii) phytostabilization, (iii) phytovolatilization, (iv) phytodegradation, and (v) phytoextraction (Jadia and Fulekar 2009).

Liu, Jiang, and Hou (2001) found that copper content in roots of *Zea mays* exposed to different concentrations (10^{-5} to 10^{-3} mol L^{-1}) of copper sulfate increased with the increase in the concentration of Cu^{2+} in solution; however, no significant difference ($p > 0.05$) was found in Cu accumulation of shoots. Jiang *et al.* (2004) reported a high tolerance ability of *Elsholtzia splendens* to copper toxicity and usual growth in the presence of 80 mg kg^{-1} of available copper. There are some plants called metal hyperaccumulators which can accumulate 10 to 500 time's higher levels of metals than crops (Garbisu and Alkorta 2001; Sarma 2011). Meanwhile, some plants with low ability of Cu bioaccumulation and translocation from root to shoot may not be used for phytoextraction, but can be good candidates for phytostabilization (Testiati *et al.* 2013) which can reduce environmental and health risks arising from contaminated soils (Evangelou *et al.* 2012). Perennials *Globularia alypum* and *Rosmarinus officinalis* (Testiati *et al.* 2013), *Sesbania virgate* (Branzini, González and Zubillaga 2012), as well as poplars and willows (Evangelou *et al.* 2012) have been used for stabilization of Cu contaminated soils. Wu *et al.* (2011) reported that *Jatropha curcas* L. could be used as a suitable plant for phytostabilization in acid mine tailings containing Cu, Pb, and Al.

Therefore, *Jatropha curcas* L. (local name in Malaysia: Jarak pagar, from Euphorbiceae family) was selected for this study because of the following reasons. i) The plant can grow in heavy metal contaminated soils and also can tolerate their toxicity effects (Wu *et al.* 2011). ii) It can be simply established and grow well in poor soil due to its sturdy shrub (Mangkoedihardjo and Surahmaida 2008). iii) *Jatropha* is increasingly being used in the context of biofuel production (Rasmussen, Rasmussen and Bruun 2012) and, therefore, it may arguably be interesting to evaluate it in the context of remediation of metal polluted soils.

Although a lot of studies on phytoremediation of contaminated soils using weeds and leafy wild-vegetables and ornamentals have been carried out, however, there is a lack of information regarding the potential of tropic plant species such as *J. curcas* for phytoremediation of Cu-contaminated soils. Therefore, the main objective of this study was to evaluate the phytoremediation potential of this plant to remediate Cu-contaminated soils. Furthermore, the other goal was to assess the growth performance of *J. curcas grown in* Cu-contaminated soils.

MATERIALS AND METHODS

Soil Samples, Contamination, and Characteristics

Soil used in this study was sandy clay of Munchong series. It was taken from the field, Faculty of Agriculture, Universiti Putra Malaysia. Soil samples used as growth media were air-dried until could be crushed to pass through a 4 mm-seive. Stainless sieve was used to supply a homogenous soil composite. Copper sulphate ($CuSO_4.5H_2O$) was used and mixed with soil as a source of copper. The different levels of growth media (soil + different levels of Cu) were: Cu_0 (control soil), Cu_{50} (soil + 50 mg kg^{-1} Cu), Cu_{100} (soil + 100 mg kg^{-1} Cu), Cu_{200} (soil + 200 mg kg^{-1} Cu, Cu_{300} (soil + 300 mg kg^{-1} Cu) and Cu_{400} (soil + 400 mg kg^{-1} Cu). The Cu salts were dissolved in distilled water and added to the soil. The treated soils were saturated, mixed thoroughly and air-dried. The wetting-drying cycles was continued for 1 month for equilibration of added metals between solid phase and solution in the soil.

Soil used for analyses of physicochemical properties in laboratory passed through a 2 mm-sieve. Soil texture was determined using the Pipette gravimetric method (Tan 2005). The pH of the soil was measured in the suspension of a 1:2.5 soil: deionized water before planting and after harvest. Cation exchange capacity and exchangeable cations (i.e., Ca, Mg, and K) were determined by leaching method using 1 M ammonium acetate at pH 7.0. Available P was extracted using Bray II with a mixture including 0.03 M ammonium fluoride and 0.1 M hydrochloric acid (Akbar *et al.* 2010).

Plant Growth and Analyses

This study was conducted at the greenhouse of Faculty of Forestry, Universiti Putra Malaysia (20° 59′ 18.24″ N latitude and 101° 42′ 45.45″ E longitude). The average temperature in green house was 27, 36 and 32°C in the morning, afternoon and evening, respectively. Relative humidity was 65%. The period of study was 5 months from February to June 2010. Healthy seedlings of *Jatropha curcas* with uniform size were collected from Malaysia agriculture research institute (Mardi), Serdang, Selangor. The plant seedlings were grown in plastic pots (32.0 cm height, 106.0 cm upper diameter and 69.0 cm lower diameters) containing 10 kg soil contaminated with 0, 50, 100, 200, 300, and 400 mg kg^{-1} Cu. After filling the pots with soil, the seedlings of *J. curcas* with uniform size (one seedling for each pot) were transplanted gently without damaging the root system. The basal diameter, height and number of leaves were measured every month.

The pot soil was fertilized using 10 g NPK Blue fertilizer (Shandong, China) having 15:15:18, N:P:K during transplanting and at 60 days after planting to supply nutrients for optimum growth and development. Intercultural operations, such as watering were accomplished when necessary to ensure normal growth of the plants. The pots were watered based on 75% of the soil field capacity to prevent leaching. Plants were harvested after 5 months for evaluation of plant growth, dry biomass and heavy metal analyses. The soil samples were taken from the rhizosphere and only the soils surrounding by plant roots were considered as the rhizospheric soil.

Aqua Regia method was used for digestion of plant and soil samples as described by Ahmadpour *et al.* (2010). Plant samples were cleaned 3 times using tap and deionized water, respectively before digestion. Cu concentration in plant parts and soil samples was determined using atomic absorption spectrometry. Total C and N were determined by dry combustion using CNS 2000 analyzer. In order to evaluate the potential of plant species for phytoremediation of Cu-contaminated soil, three parameters were used including root concentration factor (RCF), i.e., the ratio of root Cu to soil Cu concentration, translocation factor (TF), i.e., the ratio of shoot Cu to root Cu concentration, and removal efficiency (RE), i.e., percentage of total Cu accumulated in plant tissues to soil Cu content (Yoon *et al.* 2006).

Statistical Analyses

This study was conducted in a completely randomized design (CRD) with 5 Soil Cd treatments and a control (soil with no Cd amendment) and 4 replications. Analysis of variance (one way ANOVA) for growth, heavy metal concentration both in soils and plant parts were implemented. Duncan multiple range test was used to reveal the significant differences of mean data of growth parameters and biomass between various treatments. Correlation analysis was also performed to relate total Cu concentrations in soil with dry biomass production and total Cu concentration in plant species. All data obtained in terms of growth, biomass and heavy metals in soil and plants were analyzed using the SAS program (Release 9.2) (Fayiga *et al.* 2004).

RESULTS AND DISCUSSION

Physico-chemical Properties of the Soil

The soil texture was sandy clay with 36.9 ± 1.9% clay content, 57.9 ± 2% sand and 5.3 ± 0.4% silt. Total N, C, P and K were 300 ± 30, 7400 ± 500, 300 ± 20 and 1000 ± 30 mg kg^{-1}, respectively. Soil was acidic with pH 4.6 ± 0.2. Adding copper sulphate showed no significant effect on soil pH. It increased at harvest compared to before planting (data not shown) up to 0.1 to 0.3 unit, however, there was no significant difference between treatments. The soil contained 9.2± 1 mg kg^{-1} available phosphorous with 14 ± 1.8 cmol(+)kg^{-1} cation exchange capacity (CEC) and 0.3 ± 0.1 dS m^{-1} electrical conductivity (EC). Considering the content of total C and N, and CEC, the plant nutrient status in the soil was relatively low. The concentration of Cd, Cu, Zn, Fe and Mn were 2.6 ± 0.2, 9.9 ± 0.3, 46.8 ± 4.6, 479.4 ± 22.9 and 30.6 ± 0.3 mg kg^{-1}, respectively.

Growth Performance of Plants

The results of basal stem diameter growth, height and number of leaves during 5 months growth are given in Figs. 1a, b, c. Based on analysis of variance, there was a significant difference ($p \leq 0.05$) in basal stem diameter, number of leaves and seedlings height between Cu_{400} and other treatments after 5 months of plant growth. However, no significant difference ($p > 0.05$) was observed during first 3 months of growth. The basal stem diameter ranged from 14.8 ± 0.8 to 17.9 ± 0.2 mm. The highest basal stem diameter was observed in seedlings grown in Cu_{100} and Cu_{50} as compared to other treatments, while the plants grown in soil treated with Cu_{400} showed the lowest basal stem diameter (14.8 ± 0.8 mm). It might be due to the toxicity effect of Cu on plant growth in soils having a high content of this metal.

The plant height ranged from 33.8 ± 1.3 to 48.9 ± 7.3 cm. The maximum plant height was found in seedlings grown in control media which was followed by Cu_{50} compared to the minimum height (33.8 ± 1.3 cm) which was found in seedlings treated with Cu_{400}. However, there was no significant difference ($p \leq 0.05$) of seedling height among various treatments.

The number of leaves ranged from 18 ± 1 to 30 ± 1.8 under different Cu concentrations which showed significant difference ($p \leq 0.05$) between treatments. Seedlings grown in control media showed the highest number of leaves compared to Cu_{400} which showed the lowest number of leaves. It was observed that the number of leaves showed similar trend with height growth. The leaves number decreased with the increase in the concentration of Cu applied to the soil.

Khatun *et al.* (2008) reported the significant growth inhibition of *Withania somnifera* exposed to various concentration of $CuSO_4$ (0, 10, 25, 50, 100, and 200 μM). It was indicated that higher concentration of Cu has a toxic effect on plant growth. Dengyi and Youbao (2002) also reported that the growth of wheat improves under low Cu concentration ($\leq$80 mg L^{-1}), however, higher Cu concentration (>80 mg L^{-1}) interfered with the seedling growth and germination which may occur due to plant characteristic and heavy metals.

Dry Biomass of Leaves, Stems and Roots

The ideal plant species for phytoremediation should tolerate and accumulate a high amount of heavy metals and produce a high yield of biomass (Sereno *et al.* 2007). The effect

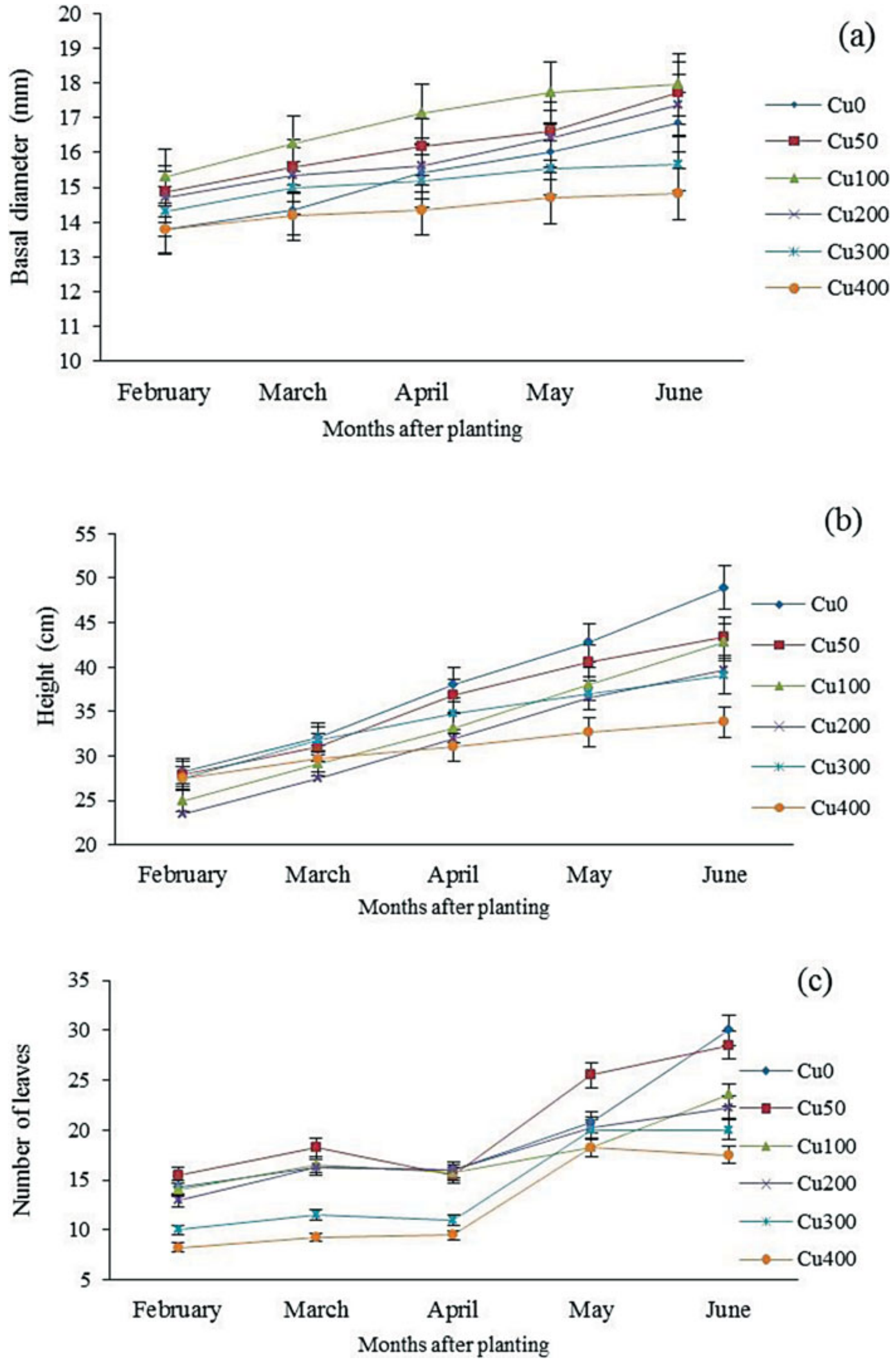


Figure 1 Plant basal diameter (a), height (b) and number of leaves (c) of *J. curcas* at different months after planting influenced by different Cu treatments including 0, 50, 100, 200, 300, and 400 mg Cu kg^{-1} (Cu_0, Cu_{50}, Cu_{100}, Cu_{200}, Cu_{300}, and Cu_{400}) (color figure available online).

Table 1 Leaves, stems and roots dry biomass (g) of *J. curcas* after 5 months growth at different Cu concentrations including 0, 50, 100, 200, 300, and 400 mg Cu kg^{-1} (Cu_0, Cu_{50}, Cu_{100}, Cu_{200}, Cu_{300}, and Cu_{400})

Treatments	Plant Parts			Total
	Leaf	Stem	Root	
Cu_0	21.9 ± 1.2^a	24.5 ± 1.9^{ab}	12.3 ± 0.8^b	58.7
Cu_{50}	19.2 ± 0.4^b	26.7 ± 1.1^a	18.5 ± 1.2^a	63.7
Cu_{100}	15.9 ± 0.5^c	22.3 ± 1.2^{bc}	16.9 ± 0.6^a	55.0
Cu_{200}	14.2 ± 0.5^c	21.4 ± 0.5^{bc}	16.7 ± 0.4^a	52.2
Cu_{300}	9.5 ± 0.6^d	19.0 ± 1.3^{cd}	11.1 ± 0.1^{bc}	39.6
Cu_{400}	8 ± 0.5^d	15.9 ± 0.2^d	10.2 ± 0.2^c	34.0

Similar letters in each column represent insignificant difference among means at a 5% level following Duncan multiple range test ($p > 0.05$). Data are means ± standard errors.

of Cu concentrations on dry biomass of plant parts are presented in Table 1. Amendment of Cu into the soil showed a negative effect on dry biomass of leaves, stems and roots. Seedlings grown in control media showed the highest leaves dry biomass compared to the other treatments. Increase in the Cu concentration applied to the soil significantly decreased the dry biomass of leaves where seedlings in Cu_{400} treatments showed up to 36% reduction in comparison to the control.

The stem dry biomass ranged from 16 to 26.7 g. Seedlings exposed to Cu_{50} showed the highest stem dry biomass compared to the lowest content which was found in seedlings exposed to Cu_{400} (Table 1). The dry biomass of stems increased from 24.5 ± 1.8 to 26.7 ± 1.1 g in seedlings grown in control media and in soils treated with Cu_{50}, respectively. The added Cu to the soil (Cu_{100} to Cu_{400}) significantly decreased the stem dry biomass indicating the toxic effect of Cu at high concentrations (Table 1). Copper is an essential nutrient for metabolism of plants and animals in low concentrations and is considered as a component in many enzymes, cofactors and proteins. However, this essential nutrient can be highly toxic and cause destructive effect in higher concentrations (Khellaf and Zerdaoui 2010; Ye *et al.* 2003). The reduction in biomass production could be attributed to the interference of cell elongation and division by heavy metals (Khatun *et al.* 2008).

The roots dry biomass ranged from 10.1 ± 0.1 to 18.4 ± 1.2 g. The roots dry biomass significantly increased in Cu_{50} treatments in comparison to the control. The roots dry biomass showed a reduction from 16.8 ± 0.5 to 10.1 ± 0.1 g in seedlings exposed to Cu_{100} and Cu_{400}, respectively. The dry biomass of different plant parts at the highest concentration of Cu applied to the soil (Cu_{400}) followed the order of stems > leaves > roots (Table 1).

Total dry biomass was varied under different Cu concentration in the soil ranging from 34 to 64.4 g. A significant difference ($p \leq 0.05$) was observed among Cu treatments in total dry biomass production. The highest total dry biomass was recorded in Cu_{50} followed by control media as compared to Cu_{400} which gave the lowest total dry biomass (Table 1). The production of total dry biomass decreased as the Cu concentration in the soil increased indicating the adverse effect of Cu on dry biomass production specifically at higher Cu concentrations. Similar results obtained by Liu *et al.* (2009) indicating copper toxicity effects on biomass of various crops including Elephant grass (*Pennisetum purpureum Schumach*), Vetiver grass (*Vetiveria zizanioides*), and the upland reed (*Phragmites australis*).

Table 2 Cu concentrations (mg kg^{-1}) in various parts of *J. curcas* in different treatments including 0, 50, 100, 200, 300, and 400 mg Cu kg^{-1} (Cu_0, Cu_{50}, Cu_{100}, Cu_{200}, Cu_{300}, and Cu_{400}).

Treatments	Plant Parts		
	Leaf	Stem	Root
Cu_0	17.6 ± 0.2^e	12.5 ± 0.2^e	24.6 ± 0.6^e
Cu_{50}	36.3 ± 1.5^d	43.7 ± 3.7^d	190.5 ± 9.3^d
Cu_{100}	52.2 ± 3.2^b	48.4 ± 0.2^d	208.1 ± 0.2^d
Cu_{200}	73.7 ± 0.2^a	54.0 ± 1.5^c	262.7 ± 4.3^c
Cu_{300}	45.6 ± 1.1^c	75.3 ± 1.7^b	374.3 ± 11.6^b
Cu_{400}	32.8 ± 1.8^d	81.3 ± 0.7^a	551 ± 17.4^a

Similar letters in each column represent insignificant difference among means at a 5% level following Duncan multiple range test ($p > 0.05$). Data are Means ± standard errors.

Copper Concentration in Various Plant Parts (Leaves, Stems, and Roots)

The Cu concentrations in different plant parts are presented in Table 2. The Cu concentration in leaves, stems and roots was significantly different ($p \leq 0.05$) under various levels of Cu added to the soil. The concentration of Cu in leaves ranged from 17.63 to 73.74 mg kg^{-1}. The Cu concentration in leaves was not consistent under different Cu levels added to the soil. The highest Cu concentration in leaves (73.7 ± 0.2 mg kg^{-1}) was found in seedlings exposed to Cu_{200} compared to the lowest content (17.6 ± 0.1 mg kg^{-1}) which was recorded in seedling grown in control media. The concentration of Cu in leaves significantly increased from control to soil treated with Cu_{200}. However, the Cu concentration in leaves significantly decreased when seedlings exposed to Cu_{300} and Cu_{400} indicating the toxic effect of Cu on accumulation of this element in plant.

In the case of Cu concentration in stem, seedlings showed a consistent trend when exposed to the different Cu levels (Table 2). The maximum Cu concentration in stems (81.2 ± 0.7 mg kg^{-1}) was recorded in seedlings exposed to Cu_{400} compared to the lowest content (12.5 ± 0.1 mg kg^{-1}) which was observed in seedling grown in control media. The Cu concentration in stem significantly increased ($p \leq 0.05$) with the increase in the Cu concentration applied in the soil. It indicated that the stem of *J. curcas* could tolerate Cu toxicity since the concentration of Cu in stem increased at higher concentration of Cu in the soil. However, there was no significant difference ($p > 0.05$) among seedlings exposed to Cu_{50}, Cu_{100} and Cu_{200} in Cu concentration in stem. Jadia and Fulekar (2008) reported that the uptake of heavy metals (Cd, Ni, Pb, Cu, and Zn) by Alfalfa (*Medicago sativa*) increased with the increase of metal contents in the contaminated soils.

The concentration of Cu in roots ranged from 24.5 ± 0.5 to 551 ± 17.4 mg kg^{-1}. The Cu concentration in roots showed similar trend with stem Cu concentration (Table 2). Based on the results given in Table 2, it was observed that the concentration of Cu in roots increased with the increase in the Cu levels added to the soil where the highest Cu concentration in roots was achieved by seedlings exposed to Cu_{400} compared to the seedlings grown in control media which revealed the lowest Cu concentration (Table 2). It could be attributed to the Cu adsorption on surface of plant roots or accumulation into the root cells even at high concentrations of Cu in soil. Cu concentration among different plant parts was in the following trend: roots > stems > leaves.

Generally, most of the soil Cu is unavailable to uptake by plant because of strong binding with organic matter and other colloids in soils, however at higher concentrations, this metal becomes toxic and interfere with the plant growth, root elongation and destroy the root cell membrane and epidermal cells (Lin, Jiang, and Liu 2003). Normal concentration of copper in plant tissue is reported to be 5 to 25 mg kg^{-1}. The concentration of copper in plants rarely reaches more than 100 mg kg^{-1} even in plants grown in highly contaminated soils. Copper uptake and accumulation by plants may be attributed to the soil properties such as pH and moisture, the season of the year and various levels of soil pollution (Ariyakanon and Winaipanich 2006). Lorestani, Chehregani, and Yousefi (2011) reported that *Euphorbia macroclada* was the most suitable species to be used for phytostabilization of Cu and Fe in some contaminated soils of Iran.

Total plant Cu concentration was varied under different treatment levels. It ranged from 54.7 to 665.0 mg Cu kg^{-1}. There was a significant difference ($p \leq 0.05$) among treatments in total Cu concentrations. The maximum total Cu concentration was found in Cu_{400} while the minimum was recorded in control media. It was observed that the total Cu concentration in *J. curcas* increased with the increase in Cu concentration applied to the soil which was in line with the results of Lin *et al.* (2008) for *Zea mays* L. Macnair (1987) reported that *J. curcas* has the potential to grow well in the soil contaminated with high concentrations of heavy metals. The uptake of heavy metals by plants happens in two ways including passive uptake through the mass flow of water towards the roots or by active transport within the plasma membrane of root epidermal cells (Yoon *et al.* 2006). While Cu is a usual pollutant of metal contaminated habitats, hyper-accumulation of this metal in plants occurs rarely (Rajakaruna, Tompkins, and Pavicevic 2006). Since Cu hyperaccumulators usually accumulate more than 1000 mg kg^{-1} Cu in their biomass (Liu *et al.* 2001), it should be considered that Cu accumulation by *J. curcas* is below the criteria of hyperaccumulator species.

Copper Removal by Total Plant Biomass

Removal efficiency based on plant biomass is defined as the total concentrations of metal and dry biomass of plants to total loaded metal in growth media (Soleimani *et al.* 2010). Cu removal was varied among different Cu concentration in soil (Figure 2). A significant difference ($p \leq 0.05$) was observed among treatment levels in Cu removal. The highest Cu removal (up to 1.1%) was observed in Cu_{50} followed by control treatment as compared to Cu_{400} which gave the lowest Cu removal (Figure 2). The Cu removal decreased with the increase in the Cu concentration added to the soil which may be attributed to the reduction of plant dry biomass at higher Cu concentrations.

Root Concentration and Translocation Factor of Copper

Root concentration factor (RCF) is defined as the ratio of heavy metal concentration in plant roots to that in soil (Yoon *et al.* 2006) whereas translocation factor (TF) is defined as the ratio of heavy metal concentration in aerial parts of plant to that in roots (Mattina *et al.* 2003). The RCFs were varied under different Cu concentrations in the soil and it was in the range of 1.58 to 4.18. There was a significant difference ($p \leq 0.05$) among treatments in RCFs. The highest RCF (i.e., 4.1 ± 0.4) of Cu was found in Cu_{50} as compared to the other treatment levels, while Cu_{300} exhibited the lowest RCF (1.5 ± 0.05) (Figure 3). The RCFs were >1 under various treatments indicating a high potential of *J. curcas* to accumulate Cu

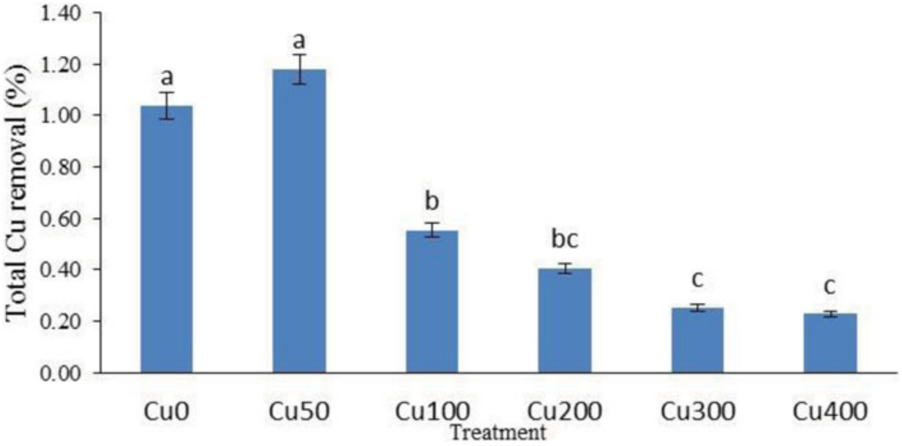


Figure 2 Total Cu removals in different treatments including 0, 50, 100, 200, 300, and 400 mg Cu kg^{-1} (Cu$_0$, Cu$_{50}$, Cu$_{100}$, Cu$_{200}$, Cu$_{300}$, and Cu$_{400}$). Similar letters above the columns indicate insignificant difference among means at a 5% level following Duncan multiple range test ($p > 0.05$). Means ± standard errors are shown in error bars (color figure available online).

in roots. Malik, Husain, and Nazir (2010) found that *Xanthium stromarium* L., *Solanum nigrum* L. and *Parthenium oleraces* L. showed the highest RCFs (14.2, 14.8, and 25.4, respectively) for Cu compared to other wild species (*Amaranthus viridis* L. and *Brachiaria reptans* L.) when grown on heavy metal contaminated soil in industrial areas in Islamabad.

TFs were also varied under different Cu concentrations in the soil and there was a significant difference ($p \leq 0.05$) among treatments in TFs (Figure 4). This index was in the range of 0.28 to 1.12. Control media showed the highest TF as compared to the other treatment levels while Cu$_{100}$ exhibited the lowest TF which may indicate the restriction of root-shoot transfer at higher metal concentration applied to the soil (Figure 4). Lorestani

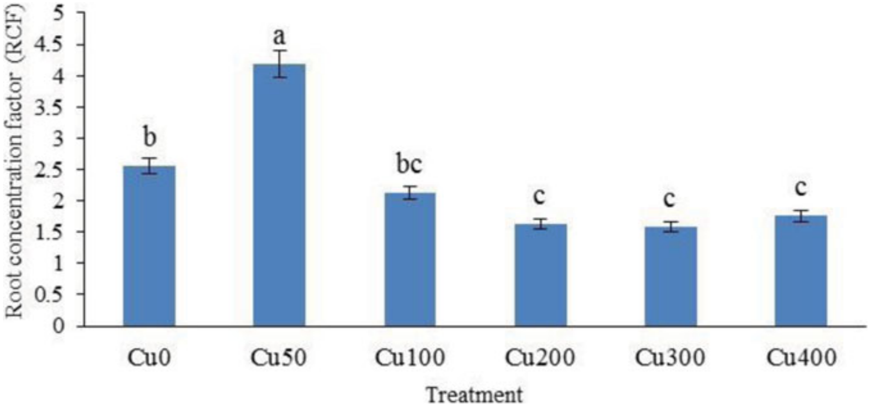


Figure 3 Root concentration factor (RCF) as influenced by different Cu treatments including 0, 50, 100, 200, 300, and 400 mg Cu kg^{-1} (Cu$_0$, Cu$_{50}$, Cu$_{100}$, Cu$_{200}$, Cu$_{300}$, and Cu$_{400}$). Similar letters indicate insignificant difference among means at a 5% level following Duncan multiple range test ($p > 0.05$). Means ± standard errors are shown in error bars (color figure available online).

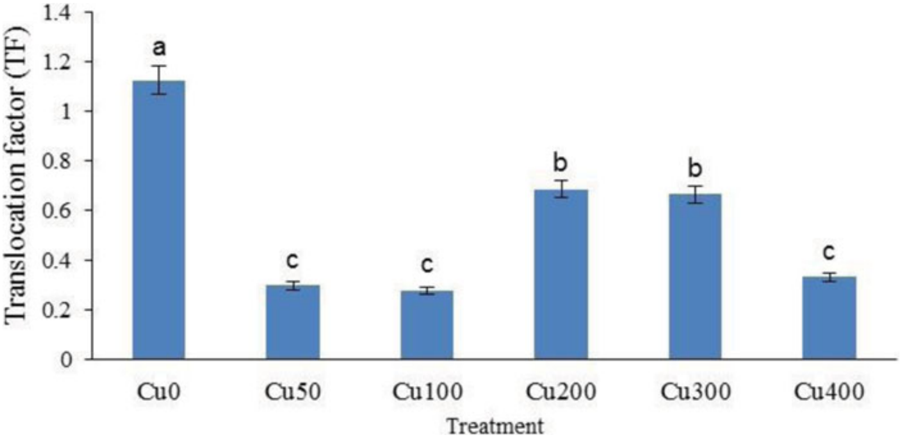


Figure 4 Translocation factor (TF) as influenced by different Cu treatments including 0, 50, 100, 200, 300, and 400 mg Cu kg^{-1} (Cu_0, Cu_{50}, Cu_{100}, Cu_{200}, Cu_{300}, and Cu_{400}). Similar letters indicate insignificant difference among means at a 5% level following Duncan multiple range test ($p > 0.05$). Means ± standard errors are shown in error bars (color figure available online).

et al. (2011) reported that *Euphorbia macroclada* which comes from the same family (Euphorbiaceae) of *J. curcas*, showed the TF<1 and RCF>1 as compared to other native plants (*Ziziphora clinopodioides*, *Cousinia* sp. and *Chenopodium botrys*) when grown in heavy metal contaminated soils in a copper mine in Iran. The result of current study was also supported with the findings of Mangkoedihardjo and Surahmaida (2008) on phytoremediation potential of *J. curcas* for Cd and Pb contaminated soil which revealed that the plant was not a metal accumulator. Generally, plants species with the root concentration factor (RCF) >1 and the translocation factor (TF) <1 are efficient to be used in phytoremediation through phytostabilization, whereas the plant species with both TF and RCF >1 are suitable to be used in phytoextraction (Yoon *et al.* 2006). Therefore, *J. curcas* may have a potential to be used in phytostabilization process in Cu contaminated soils in low level of contamination, but it's not a good choice for phytoextraction.

Relationship Between Soil Cu, Dry Biomass and Cu Accumulation in Plants

Cu concentration in the soil and total dry biomass of *J. curcas* was significantly ($p \leq 0.01$) related to each other (r = –0.92). The negative correlation between these two parameters revealed that the total dry biomass of *J. curcas* decreased with the increase in the total Cu concentration in the soil. Higher concentrations of heavy metals can reduce the plant growth and biomass production by affecting the plant physiology (Grifferty and Barrington 2000). Copper has a great toxicity to normal plants, and depending on the crop plant species, critical tissue copper concentration at 10% decrease of dry mass were considered to be 5 to 30 mg kg^{-1} (Jiang *et al.* 2004).

Correlation analysis between total Cu concentration in the soil and in *J. curcas* was significantly different ($p \leq 0.01$). The total Cu concentration in the soil was significantly related to the total Cu concentration in *J .curcas* (r = 0.96). This positive correlation indicated that total concentration of Cu in *J. curcas* increased with an increase in the total

Table 3 Mass Balance of Cu in different treatments for each pot including 0, 50, 100, 200, 300, and 400 mg Cu kg^{-1} (Cu_0, Cu_{50}, Cu_{100}, Cu_{200}, Cu_{300}, and Cu_{400}). Data are means of 3 replicates.

Treatments	Soil background Cu (mg)	Cu amendment into the soil (mg)	Accumulated Cu in plant biomass (mg)	Residual Cu in soil after planting (mg)	Leached Cu (mg)#	Error## (%)
Cu_0	96.7	0	1.0	77.6	0	18.7
Cu_{50}	96.7	476	5.4	381	0	32.5
Cu_{100}	96.7	979	5.4	794	0	25.7
Cu_{200}	96.7	1621	6.6	1449	0	15.3
Cu_{300}	96.7	2366	6.0	2234	0	9.0
Cu_{400}	96.7	3133	7.2	2791	0	13.4

#There was no leaching during plant growth.

##It means the (input Cu – output Cu)*100/input Cu.

concentration of this metal in the soil. Similar results were obtained by Xiong and Wang (2005) on copper bioaccumulation in Chinese cabbage (*Brassica pekinensis Rupr.*). Some plants have an ability to tolerate, remain and survive in heavy metal contaminated soils that are toxic for other plants. The tolerance of some species to high concentration of heavy metals may be attributed to some potential mechanisms at the cellular level (Yruela 2005). Production of metallothionein (i.e., a family of cysteine-rich, low molecular weight proteins) which bonds to metals may help plants to tolerate toxic metals (Sereno *et al.* 2007).

Mass Balance of Input and Output of Soil Cu

As it's demonstrated in Table 3, up to 1% of Cu in soil has been removed by plant biomass which shows that more time for plant growth is needed to remediate soil completely. Additionally, enhancing phytoremediation efficiency using plant species which produce high biomass and with ability to uptake more metals is necessary. One of the reasons of the difference between input and output results could be due to heterogeneity of Cu concentrations in soil samples.

CONCLUSION

Planting of *J. curcas* in Cu-contaminated soils exhibited that Cu was highly accumulated in roots rather than stems and leaves. Total accumulated Cu in the plant biomass did not meet the standard value for Cu hyperaccumulators (i.e., > 1000 mg kg^{-1}). Having a RCF more than one in all Cu-amended treatments showed the potential of accumulation of copper by the plant roots, however, because of lower amount of Cu translocation factor (<1) in the plants, *J. curcas* could not be a good choice for phytoextraction of highly contaminated soils. However, it may be used through phytostabilization approach to prevent distribution of Cu in mildly contaminated areas. In-depth long-term studies to determine the physiological process such as respiration and photosynthesis as well as a field trial in the future is highly recommended for further confirmation of the results and the potential of the selected species for phytoremediation.

ACKNOWLEDGEMENTS

This research was financially supported by a Fundamental Research Grant Scheme (FRGS) from the Ministry of Higher Education of Malaysia (MOHE) through the University Putra Malaysia, Malaysia (UPM). We thank Mr. Ariffin Abu Hassan for his kind assistance during the laboratory analysis at the laboratory of Soil Science, Faculty of Forestry and Faculty of Agriculture, Universiti Putra Malaysia. The research was also supported by National Iranian Oil Company (NIOC) and Research and Technology Center of Pars Special Economic Energy Zone (PSEEZ).

REFERENCES

Ahmadpour P, Nawi AM, Abdu A, Abdul-Hamid H, Singh DK, Hassan A, Majid NM, Jusop S. 2010. Uptake of heavy metals by *Jatropha curcas* L. planted in soils containing sewage sludge. Am J Appl Sci 7(10):1291–1299.

Akbar M, Ahmed O, Jamaluddin A, Majid N, Abdul-Hamid H, Jusop S, Hassan A, Yusof KH, Abdu A. 2010. Differences in soil physical and chemical properties of rehabilitated and secondary forests. Am J Appl Sci 7(9):1200–1209.

Alkorta I, Garbisu C. 2001. Phytoremediation of organic contaminants in soils. Bioresour Technol 79(3):273–276.

Ariyakanon N, Winaipanich B. 2006. Phytoremediation of copper contaminated soil by *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var. *radiata*. J Sci Res Chula Univ 31(1):49–56.

Branzini A, González RS, Zubillaga M. 2012. Absorption and translocation of copper, zinc and chromium by *Sesbania virgata*. J Environ Manage 102:50–54.

Danh LT, Truong P, Mammucari R, Tran T, Foster N. 2009. *Vétiver grass*, *Vetiveria zizanioides*: A choice plant for phytoremediation of heavy metals and organic wastes. Int J Phytorem 11(8):664–691.

Dengyi LIU, Youbao W. 2002. Effects of Cu and As on germination and seedling growth of crops. Chin J Appl Ecol 2(13):179–182.

Evangelou MWH, Robinson BH, Günthardt-Goerg MS, Schulin R. 2012. Metal uptake and allocation in trees grown on contaminated land: Implications for biomass production. Int J Phytorem 15(1):77–90.

Fayiga AO, Ma LQ, Cao X, Rathinasabapathi B. 2004. Effects of heavy metals on growth and arsenic accumulation in the arsenic hyperaccumulator *Pteris vittata* L. Environ Pollut 132(2):289–296.

Gambling L, Dunford S, McArdle HJ. 2004. Iron deficiency in the pregnant rat has differential effects on maternal and fetal copper levels. J Nutr Biochem 15(6):366–372.

Garbisu C, Alkorta I. 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technol 77(3):229–236.

Grifferty A, Barrington S. 2000. Zinc uptake by young wheat plants under two transpiration regimes. J Environ Qual 29(2):443–446.

Jadia CD, Fulekar MH. 2008. Phytotoxicity and Remediation of heavy metals by Alfalfa (*Medicago sativa*) in soil-vermicompost media. Adv Nat Appl Sci 2(3):141–151.

Jadia CD, Fulekar MH. 2009. Phytoremediation of heavy metals: Recent techniques. Afr J Biotechnol 8(6):921–928.

Jiang LY, Yang XE, He ZL. 2004. Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. Chemosphere 55(9):1179–1187.

Ke W, Xiong Z, Jin Z, Ke S. 2007. Differences of Cu uptake and acid phosphatase activities of two *Elsholtzia haichowensis* Sun populations. Acta Ecol Sin 27(8):3172–3181.

Khatun S, Ali MB, Hahn EJ, Paek KY. 2008. Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of in vitro grown plants. Environ Exp Bot 64(3):279–285.

Khellaf N, Zerdaoui M. 2010. Growth response of the duckweed *Lemna gibba* L. to copper and nickel phytoaccumulation. Ecotoxicology 19(8):1363–1368.

Lin J, Jiang W, Liu D. 2003. Accumulation of copper by roots, hypocotyls, cotyledons and leaves of sunflower (*Helianthus annuus* L.). Bioresource Technol 86(2):151–155.

Lin Q, Shen KL, Zhao HM, Li WH. 2008. Growth response of *Zea mays* L. in pyrene-copper co-contaminated soil and the fate of pollutants. J Hazard Mater 150(3):515–521.

Liu DH, Jiang WS, Hou W. 2001. Uptake and accumulation of copper by roots and shoots of maize (*Zea mays* L.). J Environ Sci 13:228–232.

Liu H, Liu YJ, Yang MF, Shen SH. 2009. A comparative analysis of embryo and endosperm proteome from seeds of *Jatropha curcas*. J Integr Plant Biol 51(9):850–857.

Lorestani B, Chehregani M, Yousefi N. 2011. Phytoremediation potential of native plants growing on a heavy metals contaminated soil of copper mine in Iran. World Acad Sci Engin Technol 77:377–382.

Ma Y, Rajkumar M, Freitas H. 2008. Inoculation of plant growth promoting bacterium Achromobacter xylosoxidans strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J Environ Manage 90(2):831–837.

Macnair MR. 1987. Heavy metal tolerance in plants: a model evolutionary system. Trends Ecol Evol 2(12):354–359.

MacPherson IS, Murphy MEP. 2007. Type-2 copper-containing enzymes. Cell Mol Life Sci 64(22):2887–2899.

Malik RN, Husain SZ, Nazir I. 2010. Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pakistan J Bot 42(1):291–301.

Mangkoedihardjo S, Surahmaida. 2008. *Jatropha curcas* L. for phytoremediation of lead and cadmium polluted soil. World Appl Sci J 4(4):519–522.

Mattina MJI, Lannucci-Berger W, Musante C, White JC. 2003. Concurrent plant uptake of heavy metals and persistent organic pollutants from soil. Environ Pollut 124(3):375–378.

Rajakaruna N, Tompkins KM, Pavicevic PG. 2006. Phytoremediation: An affordable green technology for the clean-up of metal-contaminated sites in Sri Lanka. Ceylon J Sci Biol Sci 35(1):25–39.

Raskin I, Kumar PBA, Dushenkov S, Salt DE. 1994. Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5(3):285–290.

Rasmussen VL, Rasmussen K, Bruun TB. 2012. Impacts of Jatropha-based biodiesel production on above and below-ground carbon stocks: A case study from Mozambique. Energy Policy 51:728–736.

Sarma H. 2011. Metal hyperaccumulation in plants: A review focusing on phytoremediation technology. J Environ Sci Technol 4(2):118–138.

Sereno ML, Almeida RS, Nishimura DS, Figueira A. 2007. Response of sugarcane to increasing concentrations of copper and cadmium and expression of metallothionein genes. J Plant Physiol 164(11):1499–1515.

Singh OV, Labana S, Pandey G, Budhiraja R, Jain RK. 2003. Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biotechnol 61(5):405–412.

Soleimani M, Hajabbasi MA, Afyuni M, Mirlohi A, Borggaard OK, Holm PE. 2010. Effect of endophytic fungi on cadmium tolerance and bioaccumulation by *Festuca arundinacea* and *Festuca pratensis*. Int J Phytorem 12(6):535–549.

Tan KH. 2005. *Soil Sampling, Preparation, and Analysis*. 2nd ed. New York (NY): Taylor and Francis.

Testiati E, Parinet J, Massiani C, Laffont-Schwob I, Rabier J, Pfeifer HR, Lenoble V, Masotti V, Prudent P. 2013. Trace metal and metalloid contamination levels in soils and in two native plant species of a former industrial site: Evaluation of the phytostabilization potential. J Hazard Mater 248–249(1):131–141.

Wei L, Luo C, Li X, Shen Z. 2008. Copper accumulation and tolerance in *Chrysanthemum coronarium* L. and *Sorghum sudanense* L. Arch Environ Contam Toxicol 55(2):238–246.

Wu Q, Wang S, Thangavel P, Li Q, Zheng H, Bai J, Qiu R. 2011. Phytostabilization potential of *Jatropha curcas* in polymetallic acid mine tailings. Int J Phytorem 13(8):788–804.

Xiong ZT, Wang H. 2005. Copper toxicity and bioaccumulation in Chinese. Environ Toxicol 20(2):188–194.

Yang XE, Long XX, Ni WZ, Ye ZQ, He ZL, Stoffella PJ, Calvert DV. 2002. Assessing copper thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. J Environ Sci Health B 37(6):625–635.

Ye Z, Baker A, Wong M, Willis A. 2003. Copper tolerance, uptake and accumulation by *Phragmites australis*. Chemosphere 50(6):795–800.

Yoon J, Cao X, Zhou Q, Ma LQ. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368(2–3):456–464.

Yruela I. 2005. Copper in plants. Braz J Plant Physiol 17(1):145–156.