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Heavy metal uptake and translocation by *Jatropha curcas* L. in sawdust sludge contaminated soils

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Abstract

Heavy metal pollution in soil is one of the most important environmental problems throughout the world and heavy metals cause significant toxic effect on humans, animals, microorganisms and plants. An experiment was conducted in sawdust contaminated soils at glasshouse to determine the growth response, metal tolerance and phytoremediation potential of *Jatropha curcas*. The *J. curcas* seedlings were planted in the growth media: T0 (Control, 100% soil, clay), T1 (80% soil + 20% sawdust sludge), T2 (60% soil + 40% sawdust sludge), T3 (40% soil + 60% sawdust sludge), T4 (20% soil + 80% sawdust sludge) and T5 (100 % sawdust sludge). The seedlings showed the best growth performance in T2 treatment in terms of height, basal diameter and number of leaves. The highest plant biomass was recorded in T2 and the maximum reduction of copper, iron, lead and zinc was also found in this treatment. Copper, lead and zinc were highly concentrated in the roots while iron and aluminum concentrated both in roots and leaves. The *Jatropha curcas* found to have a high potential to accumulate high amounts of copper, iron, aluminum, lead and zinc in its roots, leaves and stems. Plant in control to medium contaminated soils showed maximum translocation factor. The species was able to tolerate and accumulate a high concentration of heavy metals. Being a biodiesel non-food plant, *Jatropha curcas* can be an ideal option to be grown for phytoremediation in multi-metal contaminated sites and to mitigate the soil pollution.

Keywords: Heavy metal absorption, *Jatropha curcas*, sawdust contaminated soil, phytoremediation
Abbreviations: ANOVA- Analysis of variance, Al - Aluminum, BCF- Bioconcentration factor, C- Celsius, Cd - Cadmium, CEC- Cation exchange capacity, Cr - Chromium, Cu - Copper, DMRT-Duncan's Multiple Test Range, HCl - Hydrochloric acid, Hg - Mercury, HNO_3 - Nitric acid, Pb - Lead, USDA - United State Department of Agriculture and Zn - Zinc

Introduction

Rapid industrial development and urbanization during the past two decades have increased the quantity and diversity of toxic and hazardous wastes (Abdullah, 1995). Heavy metal pollution in soil is one of the major environmental problems throughout the world. Heavy metals have a significant toxic effect on humans, animals, microorganisms and plants. Moreover, heavy metals are not subjected to degrade and therefore, remain almost indefinitely in the environment (Raskin and Ensley, 2000). The contaminations of heavy metals are released by various anthropogenic activities into the environment, such as manufacturing processes of industries, domestic refuse and waste materials particularly sawdust sludge, sewage sludge, textile industry sludge and slaughter house sludge. Sawdust originates from wood and wood based factories and gives harmful leachates into local water bodies, creating environmental hazards. However, it is used as removal of heavy metals such as Cu, Zn, Pb, Fe, Cd, Cr, As, Hg and Ni (Shukla et al., 2002). It is cheaper alternative to absorb oil or chemical spill and other pollutants (Mohamed et al., 1998). Lead and cadmium are non-essential elements, but zinc at lower concentration is an essential micronutrient for plants. Higher doses of these metals may cause metabolic disorders and growth inhibition for most of the plant species and often leading to death (Wong et al., 2003; Tripathi et al., 2007). These contaminated soils need to be cleaned up for having a safe environment. There are some existing methods such as microbial bioremediation, physical and chemical treatments, excavation and burial at a hazards waste site which are costly, time consuming and not sufficient as the treated soils remain partly contaminated. Tree plantations can be used as a method for remediation of wastelands and to restore soil fertility and productivity. For effective phytoremediation process the plant species should be non-edible and can be grown abundantly in large scale on wastelands. Plants are able to tolerate the harsh conditions such as heavy metal contaminations, low nutrient content and drought (Kumar et al., 2008). Considering all above mentioned options, among non-edible trees, *Jatropha curcas* L. (Ratanjyot) has been selected for this experiment. *J. curcas* (Linnaeus) is a bush/small tree belonging to the family of Euphorbiaceae (Jussieu, 1789). *Jatropha* is native of Central America and has become naturalized in many tropical and subtropical areas including India, Africa and North America. It grows practically all over India under a variety of agro-climatic conditions. *Jatropha curcas* is a multipurpose species with many attributes and considerable potential. The oil extracted from seeds is potentially the most valuable end-product of this species. In addition to biodiesel, by-products of *J. curcas* can be used to make a wide range of products including high quality paper, energy pellets, soap, cosmetics, toothpaste, embalming fluid, pipe joint cement, cough medicine and as a moistening agent in tobacco. The *J. curcas* (Euphorbiaceae) grows well under adverse climatic conditions because of its low moisture demands, fertility requirements, and tolerance to high temperatures (Augustus et al., 2002). Some works of phytoremediation on polluted soils have been done but phytoremediation in sawdust sludge contaminated soil with *Jatropha curcas* has not been reported yet. Therefore, the present study was initiated with the following objectives: (i) to study the growth performance of *J. curcas* in sawdust contaminated soils, (ii) to determine heavy metal concentrations in *Jatropha curcas* plant parts and (iii) to quantify the heavy metal concentrations in the growth medium before planting and after harvest.

Results and discussion

Properties of the growth media

The texture of control soil was clay while the sawdust is sandy clay loam. Clay soil has high water content and nutrient retention capacities but low infiltration and aeration. Moreover, clay soils crack excessively while drying, if they are very low in organic matter. The clay soil may lose their structure and become cloddy and compacted (Aljibury, 2011). On the other hand, sandy clay loam has high infiltration and better aeration, which is good for plant growth and development (Miller and Roy, 1990). Texture is an important soil characteristic that affects soil management and crop production. Some of the treatments presented a sandy clay loam texture except control, in which T1 and T2 had a clay texture. Before planting, pH of the growth media varied from 5.05 to 6.67. Treatment T4 showed highest pH value (6.67) followed by T5 (6.64) and the lowest was in T1 (5.05). After harvest, pH was changed in the growth media and reduced in all the treatments except control and T1 (Fig 1a). T4 showed the highest pH reduction (-0.46) followed by T2 (-0.43), whereas the minimum was in the T5 (-0.15). Some treatments showed increase in soil pH having the highest increase (1.52) in the control and the lowest (0.64) in T1. The decrease of soil pH in the growth media might be due to increase in availability of heavy metals after decomposition of contaminated sawdust sludge. Soil pH affects all the chemical, physical and biological properties of soil (Brady and Weil, 2002). Chemical element accumulation in plants not only depends on their absolute content in soil but also on the level of soil acidic-alkaline and reductive oxidative conditions and content of organic matter (Lorenz et al., 1994; Golovatyj, 2002). Soil pH affects the solubility and bioavailability of elements in the soil for plant uptake. Most of the plant species survive in a relatively narrow pH range ($\geq$4.5). Before planting, the total carbon content ranged from 16 to 21.85% having the highest (21.85%) in T5 followed by T4 (20.67%) and T3 (20.00%). The lowest total carbon content (16%) was found in the control (Fig.1b). After harvest, the total carbon content was increased and T5 showed the highest increment of 4.48% followed by T4 (3.93%) and T3 (3.00%). The minimum augmentation (0.70%) was recorded in the control (Fig.1b). It was observed that carbon content increased with increase of sawdust sludge percentage in the media. Sawdust is obtained from woody materials which naturally have high carbon (Mohamed et al., 1998). The increment in soil organic matter boosts the cation exchange capacity (CEC) and the nutrient content which improves soil fertility (Rice, 2009). Organic matter also can improve water holding capacity, which increases the plants ability to withstand short droughts.

Growth performance

The three growth parameters measured in this study were height, basal diameter and number of leaves for each level of treatment. The data were taken twice a month for a total of four months. There was a significant difference ($P \leq 0.05$) in plant height, basal diameter and number of leaves among treatments in the four months of study. The maximum height increment was found in week3 and after that height increment reduced in week5. Again, the height increased in the week7 (Fig.2a). The height increment decreased in week5 and this may be due to heavy metal toxicity. The height increased again in week7 and this might be due to soil stabilization with time which favors the plant growth. Similar results were also observed by Veronica et al. (2011), where *Acacia mangium* was cultivated in cadmium contaminated soil. In week3, treatment T2 showed highest height increment (11.67 cm) followed by T3 (8.06 cm). The minimum was found in the control (4.12 cm) treatment. Similar trend was also found in the week7. The T5 and T4 treatments failed to produce maximum increase and this might be due to high content of heavy metals in sawdust contaminated soil which cause toxicity to plants and hamper its philological activity and normal growth. Majid et al. (2011) reported that higher concentration of cadmium reduced the growths of *Dyera costulata* which are in agreement with our findings. In week3, the basal diameter was also significantly influenced by the treatments T2, which showed the highest basal diameter (5.68 mm) followed by T3 (4.86 mm). The minimum diameter was found in the control soil (Fig.2b). The diameter increment augmented in every week up to week3 but after that it decreased in week5. In week7, again it began to increase. Similarly maximum leaves were found in T2 followed by T4. The control treatment showed the minimum value (Fig. 2c). The difference of number of leaves among the weeks was not significant ($p \leq 0.05$). The diameter and number of leaves increment were reduced in the week5 and this might be due to toxic effect of heavy metals. After a certain period, at week7, diameter increment and number of leaves had begun to increase again, which supposedly be due to the stable condition of soil for plant growth after a certain period of time (Veronica et al., 2011). These results showed that the plants have the ability to tolerate polymetallic sawdust sludge contaminated soils having high levels of Cu, Fe, Pb, Al and Zn which are in agreement with the findings of Qihang et al. (2011). The heavy metals toxicity and extreme infertility in the contaminated soils are the major limiting factors for the plant growth (Norwood et al., 2007). A number of studies showed that organic amendment resulted in successful re-vegetation of metal contaminated soil (Ortiz and Alcaniz, 2006; Kumar et al., 2008). So, plantation of *J. curcas* with organic amendments can be used as a suitable practice for phytoremediation in metal contaminated soils.

Plant biomass

The plant biomass was significantly influenced by the different treatments ($p \leq 0.05$). Among the plant parts, stem produced the highest biomass (228 g) followed by root (86.12 g). The lowest biomass (12.36 g) was recorded in the

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

Growth media	Texture	pH	Total carbon (%)	Heavy metal concentration				
				Copper (ppm)	Iron (ppm)	Lead (ppm)	Aluminum (ppm)	Zinc (ppm)
Control soil	Clay	5.6	16	56	779	4.0	3505	41
Contaminated sawdust sludge	Sandy clay loam	6.64	21.85	13538	1041	4104	5113	14058

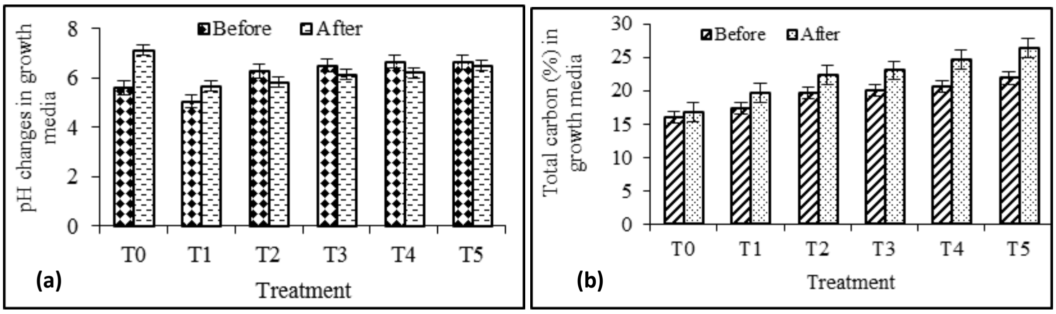


Fig 1. Change in pH (a) and total carbon (%) (b) in the growth media at harvest time of *Jatropha curcas* as influenced by different treatments. Growth media indicates different proportion of sawdust sludge and soil, i.e. T0 = 100% soil, T1 = 80% soil + 20% contaminated sawdust, T2 = 60% soil + 40% contaminated sawdust, T3 = 40% soil + 60% contaminated sawdust, T4 = 20% soil + 80% contaminated sawdust, T5 = 100% contaminated sawdust. Means ± SE are shown in error bar (p = 0.05).

leaves (Table 2). In stem, highest biomass was found in T2 (48.63 g) followed by T3 (41.08 g) and T1 (40.65 g). The lowest biomass was observed in the control (23.43 g) (Table 2). The T2 also showed highest biomass (18.42 g) in the roots followed by T3 (16.35 g), where as the lowest was in the control (11.03 g). The T4 and T5 treatments failed to produce maximum biomass and this might be due to the increment of heavy metal concentrations in these two media, leading to toxicity. Juwarkar et al. (2008) reported that higher concentrations of As, Cr and Zn in growth media reduced the growth and biomass of *J. curcas* which are in agreement with our results. Sawdust (20-60%) in combination with soil produced the highest stems and roots biomass, so this plant can be grown for phytostabilization of sawdust contaminated soils.

Heavy metal concentration in the growth media after harvest

Selected heavy metals (Cu, Fe, Pb, Al and Zn) were evaluated since they are commonly found in sawdust contaminated soil. After harvest, heavy metal concentrations were significantly changed in the growth media ($p \leq 0.05$). Copper concentration decreased in the growth media, the highest reduction observed in T2 (84%) followed by T1 (74%) and T3 (59%), respectively. The lowest reduction (21%) was recorded in the control (Fig.3a). Marcel (2006) reported that Cu becomes more soluble in acidic soils. Therefore, Cu uptake by the plant would be more in acidic soil and as a result a higher reduction in the growth will be occurred. Iron concentration decreased in all the treatments except T4 and T5. Treatment T2 showed highest reduction of iron concentration (35%) followed by T1 (34%) and T3 (21.6%). The control showed the lowest reduction (10.3%) (Fig.3b). Iron concentration decreased in the growth media, which might be due to its higher uptake by the plant. In contrast, Fe concentration increased in T4 and T5 growth media, which, might be due to more release of Fe after decomposition of sawdust. Lead (Pb) is one of the most frequently inorganic pollutants in the soils (Alkorta et al., 2004). It is potentially toxic even at low concentrations and above 400 mg Pb kg^{-1}, the soil is considered hazardous to human health (US-EPA, 2001). Lead is not an essential element and potentially is toxic to plant, animal and human. *J. Curcas* was found to remove lead (Pb) efficiently. Lead (Pb) concentration was significantly decreased in the growth media as the highest reduction recorded in T2 (96%) followed by T1 (95%) and the lowest in the control (14%) (Fig.3c). Aluminum (Al) concentration was also decreased in the growth media after harvest. The highest reduction (4585 ppm) was found in T4 followed by T5 (4141 ppm) and T3 (3372 ppm). The lowest decrease was noted in the control (1826 ppm) (Fig. 3d). It was observed that Al concentration increased with increase of sawdust content in the growth media, which might be due to decomposition of sawdust and release of more Al. Moreover, growth media was acidic, which enhanced availability of Al. It was found that Zn concentration decreased after harvest in the growth media having the highest reduction (84%) in T2 followed by T1 (82.8%) and T5 (69%), whereas Zn concentration increased in the control (Fig.3e). The decrease in Zn concentration at the growth media (except control) after harvest may be due to uptake by the plants. Zinc is an essential element for plants and animals but its excessive concentration in soil (> 30 ppm), categorizes the soil as contaminated and toxic to some animals and plants (Perk, 2006).

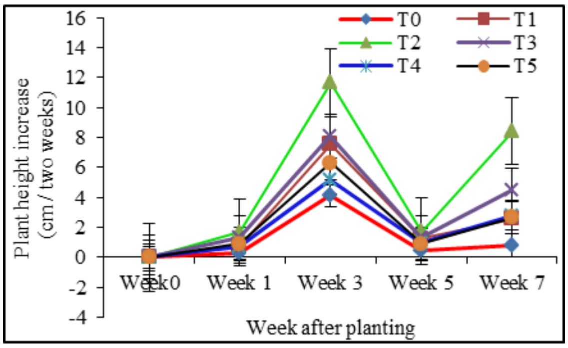
Heavy metal concentration in plant parts at harvesttime

The heavy metal accumulations in the plant parts were significantly influenced by the different treatments ($p \leq 0.05$). The highest Cu concentration (60.45 ppm) was found in the roots followed by leaves (12.05 ppm) and the lowest (2.48 ppm) was observed in the stem (Fig.4a). In root, T3 showed the highest Cu concentration (85.00 ppm) followed by T4

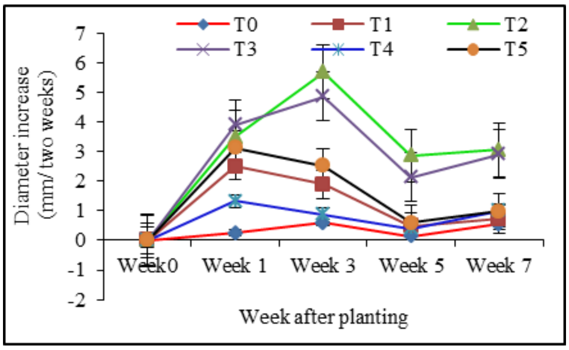
Table 2. Dry biomass of leaves, stems and roots of *Jatropha curcas* at harvest as influenced by different treatments

Treat	Dry biomass of *Jatropha curcas*			
	Leaf $plant^{-1}$ (g)	Stem $plant^{-1}$ (g)	Root $plant^{-1}$ (g)	Total $plant^{-1}$ (g)
T0	1.12 d	23.43 d	11.03 d	35.58 e
T1	2.00 bc	40.65 b	14.62 b	57.27 bc
T2	3.65 a	48.63 a	18.42 a	70.7 a
T3	2.12 b	41.08 b	16.35 b	59.55 b
T4	1.82 bc	38.62 bc	13.58 c	54.02 c
T5	1.65 c	35.58 c	12.12 d	49.35 d
SE (±)	0.043	4.60	0.63	5.38

Figure (s) in a column having common letter (s) do not differ significantly at 5% level of DMRT. Notes: T0 = 100% soil, T1 = 80% soil + 20% contaminated sawdust, T2 = 60% soil + 40% contaminated sawdust, T3 = 40% soil + 60% contaminated sawdust, T4 = 20% soil + 80% contaminated sawdust, T5 = 100% contaminated sawdust.



(a)



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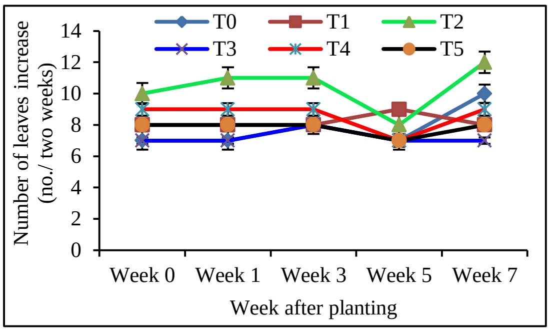

(c)

Fig 2. Plant height (a), basal diameter (b) and number of leaves (c) of *Jatropha curcas* at different weeks after planting as influenced by different treatments (increase per two weeks). Growth media indicates different proportion of sawdust sludge and soil, i.e. T0 = 100% soil, T1 = 80% soil + 20% contaminated sawdust, T2 = 60% soil + 40% contaminated sawdust, T3 = 40% soil + 60% contaminated sawdust, T4 = 20% soil + 80% contaminated sawdust, T5 = 100% contaminated sawdust. Means ± SE are shown in error bar (p = 0.05).

(79.5 ppm) and T5 (60.45 ppm). The lowest accumulation of Cu (4.52 ppm) was detected in the control. Among the treatments, T2 showed the highest concentration of Cu (12.05 ppm) in the leaves followed by T1 (10.334 ppm) and the lowest (2.68 ppm) was recorded in the control. It was observed that higher concentrations of Cu in soil caused a lwer Cu translocation in the shoot which might be due to the toxic effect of this element. The highest Fe concentration (66.71 ppm) was observed in the roots followed by leaves (57.38 ppm) and the lowest (16.35 ppm) was in the stems (Fig. 4b). Similar result was also observed by Ghavri and Singh (2010) where *Jatropha curcas* plants grown on iron rich wasteland soils. Among the treatments, T2 showed maximum Fe accumulation (66.71 ppm) in the roots which was followed by T4 (54.82 ppm). The T2 and T4 treatments also exhibited high accumulation of Fe (57.38 and 50.09 ppm, respectively) in the leaves (Fig. 4b). The lowest Fe concentration (32.33, 16.35 and 35.55 ppm for leaves, stems and roots, respectively) was detected in the control. The lead concentration in the root was significantly influenced by the different treatments ($p \leq 0.05$). Treatment T4 showed highest concentration (33.54 ppm) followed by T5 (26.51 ppm) and the lowest (6.00 ppm) was in the control (Fig.4c). Among the plant parts, root showed the highest concentration (33.54 ppm) followed by leaves (5.45 ppm), whereas the lowest (1.54 ppm) was observed in the stems. T4 also exhibited highest concentration in the leaves followed by T3, where as the lowest was found in the control. The normal Pb concentration in the plant ranges between 0.1 to 5 ppm. All the treatments exceeded the normal Pb range. So, it is wise to not use the sewage sludge for cultivation of food crops before remediation of contaminated soils. The highest Zn concentration was found in the roots (37.12 ppm) followed by stems (13.67 ppm), whereas the minimum Zn recorded in the leaves (6.86 ppm) (Fig. 4e). Among the treatments, T5 showed the highest Zn concentration in the roots (37.12 ppm) followed by T4 (28.25 ppm) and T3 (26.36 ppm). In stems, the maximum accumulation (13.67 ppm) was detected in T4

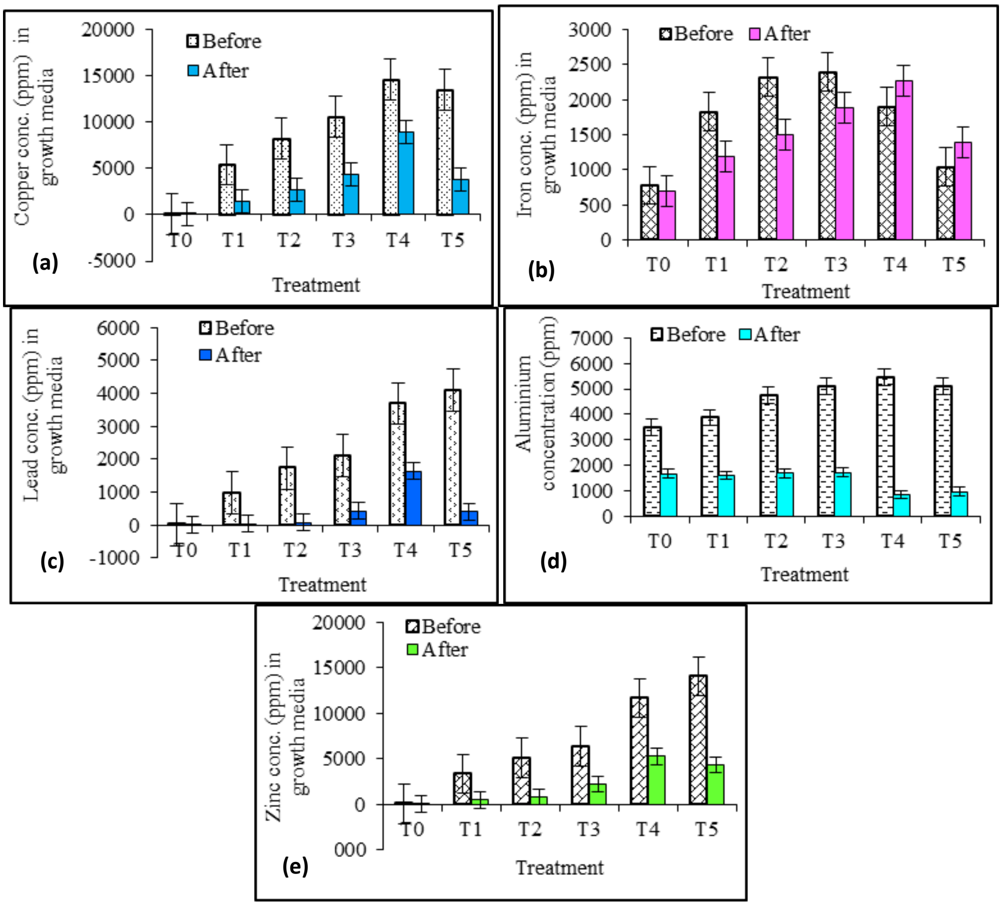

Fig 3. Change in copper (a), iron (b), lead (c) aluminium (d) and zinc (e) concentrations in the growth media after cultivation of *Jatropha curcas* as influenced by different treatments. Growth media indicates different proportion of sawdust sludge and soil, i.e. T0 = 100% soil, T1 = 80% soil + 20% contaminated sawdust, T2 = 60% soil + 40% contaminated sawdust, T3 = 40% soil + 60% contaminated sawdust, T4 = 20% soil + 80% contaminated sawdust, T5 = 100% contaminated sawdust. Means ± SE are shown in error bar (p = 0.05).

followed by T2 (12.85 ppm), whereas the minimum (7.35 ppm) was in the control. The highest Al accumulation (40.00 ppm) was observed in T5 followed by T4 (38.55 ppm) and the lowest (15.34 ppm) was recorded in the control (Fig.4d). The roots exhibited highest absorption (40.00) followed by leaves (18.25 ppm) and stems (6.42 ppm), respectively. It was observed that Al absorption increased with increase of sawdust sludge percentage in the growth media. Accumulation and distribution of heavy metals in plant tissues are important to evaluate the role of plant in remediation of heavy metals in soils (Friedland, 1989). To stabilize metal contaminated sites, a lower metal concentration in stem is preferred, in order to prevent the metals which enter into the ecosystems (Taylor and Percival, 2010).

Translocation factor

The translocation factor was significantly influenced by the different treatments ($p \leq 0.05$). The highest translocation factor (1.14, 1.24 and 1.13 for Cu, Al and Zn, respectively) was found in the control followed by T1 (0.77, 1.09 and 1.01 for Cu, Al and Zn, respectively). The lowest translocation factor was observed in the T5 (0.19, 0.59 and 0.53 for Cu, Al and Zn, respectively), which may imply the restriction in soil-root and root-shoot transfer at higher metal concentrations in the soil. Similar results were found by Yoon et al. (2006) (Fig.5). Majid et al. (2012) also reported lowest TF at higher metal concentrations on cultivation of *Justicia gendarussa* in textile sludge contaminated soil. Translocation was more prominent in the control. A low to medium metal concentration in soil showed the higher TF values in plant. *Jatropha curcas* exhibited higher TF in low to medium multi-metal contaminated soils. Heavy metal tolerance with high TF value was suggested for phytoaccumulator of contaminated soils (Yoon et al., 2006) and; therefore, *Jatropha curcas* can be used as a potential phtyoremediator for metal contaminated soils.

Materials and methods

Site description

The experiment was conducted at the greenhouse, Universiti Putra Malaysia, at 4°06′ N latitude and 101° 16′ E longitude from April 15 to July 30, 2009. The average temperature in the greenhouse was 27, 36 and 32°C for morning, afternoon and evening, respectively.

Preparation of growth media and treatment combination

After separating stones, pieces of brick and coarse solids, the sawdust sludge was mixed with top soil on a volume to

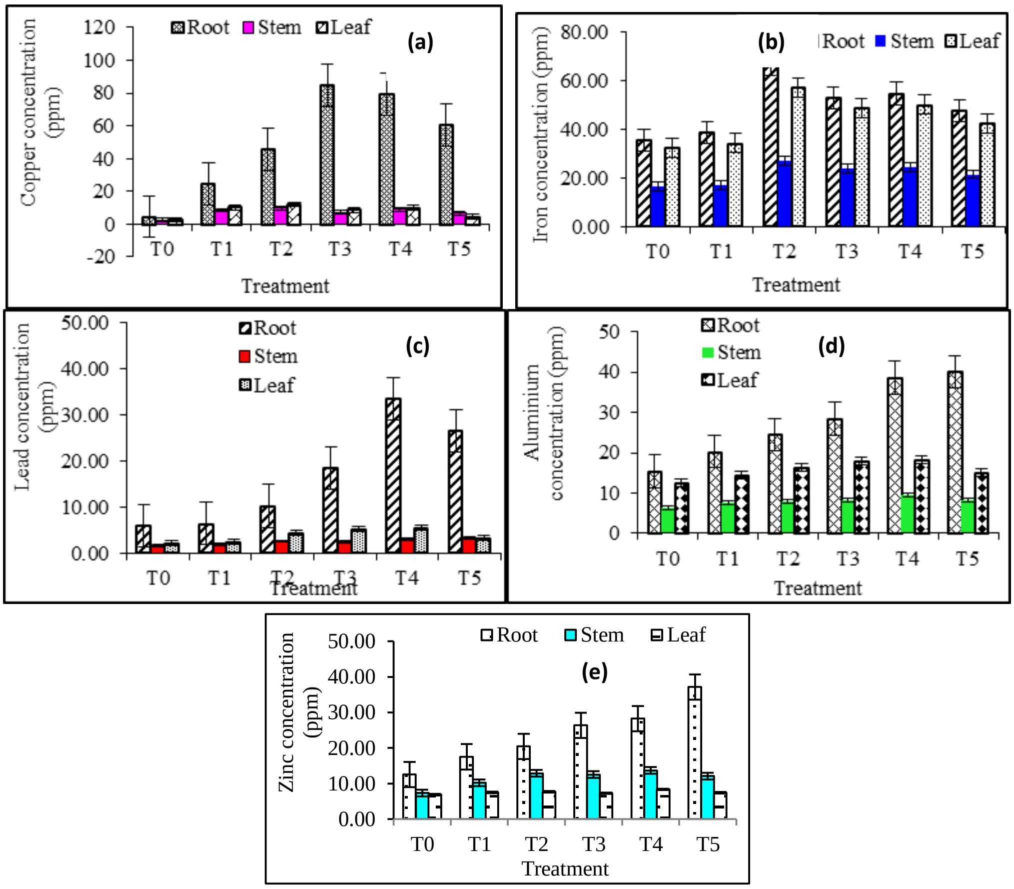

Fig 4. The Copper (a), Iron (b), Lead (c), Aluminium (d) and Zinc (e) accumulation in different parts of *Jatropha curcas* as influenced by different treatments. Growth media indicates different proportion of sawdust sludge and soil such as T0 = 100% soil, T1 = 80% soil + 20% contaminated sawdust, T2 = 60% soil + 40% contaminated sawdust, T3 = 40% soil + 60% contaminated sawdust, T4 = 20% soil + 80% contaminated sawdust, T5 = 100% contaminated sawdust. Means ± SE are shown in error bar (p = 0.05).

volume basis using mixture machine. The growth media was prepared using soils with different levels of sawdust sludge were: T0 (Control, 100% soil), T1 (80% soil + 20% sawdust), T2 (60% soil + 40% sawdust), T3 (40% soil + 60% sawdust), T4 (20% soil + 80% sawdust) and T5 (100 % sawdust). The texture of control soil is clay while the sawdust is sandy clay loam. The control soil was acidic (pH 5.6), whereas contaminated sawdust was slightly acidic (pH 6.64). The sawdust sludge is obtained from woody materials which naturally have high carbon (21.85%). Heavy metals such as Cu, Fe, Pb, Al and Zn concentrations were high in sawdust contaminated soil compared to the control (Table 1). The experiment was conducted as a Completely Randomized Design (CRD) with six replications.

Test plant

Jatropha curcas was used as the test plant. Healthy samples, similar in form, were selected for this study. Before planting, a seedling was tested for toxicity in 100% sewage sludge without soil for about one week (Majid et al., 2012). After filling the pots (28.2 cm × 34.2 cm size) with appropriate soil and sawdust sludge mix, seedlings were transplanted into the pots.

Measurement of growth variables and intercultural operations

Thirty plants were used to measure growth parameters including basal diameter, number of leaves and height at certain interval of time. The height was measured using a ruler. Basal diameter was measured using a digital caliper. The growth parameters were measured twice in a month. All intercultural operations such as weeding, irrigation and pest management were done when required.

Plant and soil sampling and chemical analysis

The plant samples were collected after harvest. The soil samples were collected from each pot before planting and after harvest. The soil samples were kept in a standard plastic container and air-dried before physico-chemical analysis. For analysis of heavy metals, 1.0 g of dried plant sample and 20 ml aqua regia solution (mixture of concentrated HNO_3 and HCl in a ratio of 3:1) was taken into the digestion tube and then digested at 80 to 120^0 C for 3 hours. After filtering of digested material into 100 ml beaker the solution was

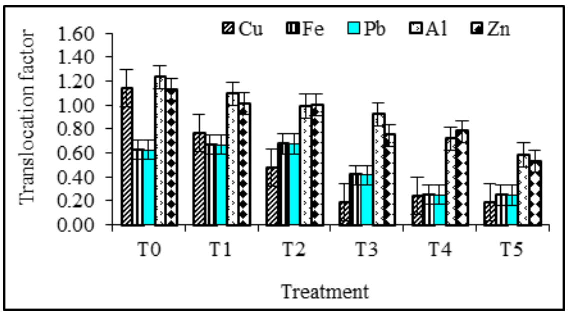

Fig 5. Translocation factor of heavy metals in *Jatropha curcas* as influenced by different treatments. T0 = 100% soil, T1 = 80% soil + 20% contaminated sawdust, T2 = 60% soil + 40% contaminated sawdust, T3 = 40% soil + 60% contaminated sawdust, T4 = 20% soil + 80% contaminated sawdust, T5 = 100% contaminated sawdust. Means ± SE are shown in error bar (p = 0.05).

ready for analysis and ICP-MS (Inductively Couple Plasma Mass Spectrometry) method (Sahoo et al., 2009) was applied for analyzing the concentrations of heavy metals. Particle size distribution was analyzed by pipette gravimetric method and the texture was determined using USDA textural triangle (Day, 1965). Soil pH and total carbon were determined using glass electrode pH meter and loss on ignition method (Jackson, 1973; Konen et al., 2002), respectively.

Plant biomass measurement

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

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

(Black, 1965)

Translocation factor

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

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

(Barman et al., 2000; Gupta et al., 2008)

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 adjusted by DMRT (P=0.05) method (Steel et al., 1996). Comparison using t-test was also done to detect any significant differences between before planting and at harvest.

Conclusion

The highest biomass and growth performance, in terms of height, basal diameter and number of leaves was found in 40% sawdust in combination with 60% soil. The maximum reduction of copper, iron, lead zinc was also observed in this combination. Cu, Pb and Zn were highly concentrated in the roots while Fe and Al were concentrated both in roots and leaves. *Jatropha curcas* found to have a high potential to accumulate high amounts of Cu, Fe, Al, Pb and Zn in the roots, leaves and stems. The zero (control) to medium contaminated soils showed maximum translocation factor of heavy metal. The species was able to tolerate and accumulate a high concentration of heavy metals. Being a biodiesel non-food plant, *Jatropha curcas* can be an ideal option for phytoremediation in multi-metal contaminated sites. Therefore, *Jatropha curcas* can be cultivated to remediate polluted soils. The plant physiological aspects such as photosynthesis and respiration should also be evaluated to study the effects of heavy metals during uptake and storage in plant tissues. A field experiment also needs to be conducted to confirm the results of this greenhouse study.

Acknowledgement

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