Phytoremediation of Copper and Zinc in Sewage Sludge Amended Soils Using Jatropha curcas and Hibiscus cannabinus

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(Received on 8th February 2016, accepted in revised form 25th August 2016)

Summary: Phytoremediation can be potentially used to remediate heavy metal contaminated soils. A glasshouse experiment was conducted to determine the extent of Jatropha curcas and Hibiscus cannabinus efficiency to the remediation of zinc and copper contaminated soils amended with sewage sludge. An Oxisol (Munchong Series) and an Ultisol (Bungor Series) were used in this experiment, which was laid out using a randomized completely block design in six replication. The plants in pots having soil containing 0, 5 and 10% (w/w) sewage sludge were grown for six months. Phytoremediation can take place successfully as shown by the decrease of total Zn and Cu in the treated soils, where the concentrations of Zn and Cu in the tested soils were higher before planting as compared to after planting. Most of the Zn and Cu taken up by the tested plants were stored in the shoots (leaves+ stem). The fractionation of Zn and Cu in sewage sludge, untreated and treated soils was studied before and after planting. The results of the fractionation study showed that the dominant Zn and Cu in the soil were in their residual form. At harvest, the percentages of water soluble and exchangeable fraction were increased, implying that some of the residual fraction may have changed to other forms. In general, there was no significant difference between the different metal fractions in the Oxisol and Ultisol.

Keywords: Sewage sludge, Fractionation study, Oxisol, Phytoremediation, Ultisol.

Introduction

The huge quantities of sewage sludge produced worldwide and the increasing costs of chemical fertilizers have made agricultural reusing sewage sludge an attractive option. The application of sewage sludge to soil has been considered as an ecologically suitable strategy because it is rich in micronutrients, phosphorus, nitrogen and organic matter [1]. The accruing benefits from sewage sludge recycling cannot be neglected as this sewage sludge has become a subject of research in different fields in current years. The application of sewage sludge to tropical soils is one of the proposed methods of maintaining soil characteristics. However, due to its high organic matter content, N, P, Ca, K and Mg, sewage sludge can improve the properties of soil [2]. Thus, sludge application helps to improve the soil quality as a plant growth.

Based on Soil Survey Staff [3], Oxisols and Ultisols are highly weathered soils, and mainly dominated by gibbsite, kaolinite and goethite in their clay fraction. They are low in cation exchange capacity (CEC) and fix high amount of fertilizer-P, which is temporarily lock up in the soils. These, together with the low pH and low bases make the soils existing under natural conditions less productive as compared to soils in the temperate region. Thus, the infertility of these soils should be ameliorated by applying organic matter and other amendments [4, 5].

In order to rejuvenate their fertility, highly weathered soils in the tropics should be amended with appropriate soil amendments. One of the major sources of supplementary essential nutrients for the plant growth is sewage sludge, which helps improve the physico-chemical characteristics of the soils. Nevertheless, excessive use of sewage sludge should be avoided as it may cause environmental problems, like heavy metal pollution [6]. Zinc and copper in the sludge must be carefully checked because the excessive presence of these metals in the treated soils would be harmful to crops. Copper and zinc are micronutrients, but if present in high amount can be toxic and eventually retard plant growth [7]. Data on total content of heavy metals in soils can only unveil limited information about the availability and mobility of the heavy metals. Identifying their chemical forms existing in soil can help predict their potential mobility and availability to crops [8].

Increasing knowledge about the hazards of environmental pollution has led to the discovery of
new methods to reduce/prevent the contamination of the environment. Phytoremediation of heavy metal contaminated soil by the use of non-edible plant such as woody plants species offers a cost-saving and/or environment-friendly method of treating the soil. Trees are likely to be considered as the best-suited plants to improve heavy-metal accumulation based on transgenic approaches. Forest trees have several mechanisms to resist against the uptake of high amount of heavy metals.

There are several forms of phytoremediation mechanisms. One of which is removing organics or metals from soils by absorbing and storing them in the biomass of the plants. Phytotransformation or phytodegradation refers to using plant roots to uptake, store and destroy the organic pollutants and during rhizofiltration these pollutants are removed from aqueous sources via their plant roots. Through phytostabilization the bioavailability of the pollutants is reduced by immobilizing or binding them to the soil matrix. During phytovolatilization plants take pollutants from the growth matrix, then transform and finally release them to the atmosphere. Nowadays, phytoremediation has grown popular both scientifically and commercially, and considerable attention is given to phytoextraction in which certain plant species are selected to grow on contaminated soils. In fact, during phytoextraction, pollutants are removed from contaminated soils and accumulated in plant biomass [9].

Phytoremediation has been used to develop approaches that facilitate the removal of heavy metals from soil into plants that are planted on it. Therefore, this study was undertaken to: 1) elucidate the potential of *Jatropha curcas* and *Hibiscus cannabinus* to clean up toxic heavy metals in soils treated with sewage sludge; and 2) to determine the availability and relative distribution of various forms of the metals in the sewage sludge and treated soils.

### Experimental

#### Materials

The study was conducted in a glasshouse at Universiti Putra Malaysia, Serdang Selangor, Malaysia (2° 59' 18.24"N and 101° 42' 45.45"E). Two soil types, Oxisol (*Munchong Series*) and Ultisol (*Bungor Series*), were used in this study. Soil samples were taken from the topsoil (0-20 cm depth) from two sampling sites located in the peninsular regions of Malaysia. The sewage sludge used was obtained from Indah Water Konsortium (IWK) Plant at Bandar Tun Razak Sewage Treatment, Kuala Lumpur, Malaysia. The sludge and soil samples were air-dried, passed through 2 mm mesh sieved and prepared for their physico-chemical analyses.

Two woody plant species (*Jatropha curcas* and *Hibiscus cannabinus*) were selected owing to their high biomass and high growth rate. Seedling was carefully chosen with similar size and length (± 25 cm).

a) *Jatropha curcas*: is a woody-herbaceous annual plant. It is a fast growing plant and high potential of the fiber materials. It can be cultivated in order to recover degraded soils as well as economic values of marginal lands. Taxonomy of *Jatropha curcas* according to Integrated Taxonomic Information System (ITIS) 2016 [10] as following:

- **Kingdom**: Plantae
- **Subkingdom**: Viridiplantae
- **Infrakingdom**: Streptophyta
- **Division**: Embryophyta
- **Subdivision**: Tracheophyta
- **Class**: Spermatophyta
- **Superorder**: Magnoliopsida
- **Order**: Rosaceae
- **Family**: Malpighiales
- **Genus**: Euphorbiaceae
- **Species**: *Jatropha curcas*

b) *Hibiscus cannabinus*: *Jatropha* species has currently become a new interest for researchers. It is a multipurpose species with several attributes and significant potential. It is native of Central America and has become naturalized in many tropical and subtropical zones such as North America, India and Africa, China, Brazil and other continents. It can survive tough environments of wastelands and semi-arid agro-climatic conditions. Taxonomy of *Hibiscus cannabinus* according to Integrated Taxonomic Information System (ITIS) 2016 [10]as following:

- **Kingdom**: Plantae
- **Subkingdom**: Viridiplantae
- **Infrakingdom**: Streptophyta
- **Division**: Embryophyta
- **Subdivision**: Tracheophyta
- **Class**: Spermatophyta
- **Superorder**: Magnoliopsida
- **Order**: Rosaceae
- **Family**: Malvales
- **Genus**: Malvaceae
- **Species**: *Hibiscus cannabinus*

Twenty kilograms of each soil were placed in plastic pots. Three sewage mixtures [0 (T1), 5 (T2)}
and 10 % (T3) sludge W/W] were used in this study. The plants were allowed to grow for 6 months in a glasshouse. Measurements were done every month after planting. The parameters measured were plants height, number of leaves and biomass. Heavy metals in the tested plants (Cu and Zn) were assayed every month too. The experiment was laid out using completely randomized design (CRD), replicated 6 times.

Methods

The plant heights were measured monthly during the study period. At the end the experiments period, harvested plants washed gently with deionized water to remove soil particles. Then, the plants were cut into required sections namely leaves, stems and roots. Plant biomass was measured according to the plant parts (leaves, stems and roots) separately. The fresh samples were weighed and oven dried for 48 hours at 70º C [11]. Drying done by using laboratory oven model MEMMERT UM 100. After that, the tissue samples were milled using IKA werke MF 10 Basic grinder, micro fine grinder done by sieve 1mm, then keep for analyses.

Soil texture was determined using pipette method [12]. Soil pH was measured in soil solution of 1:2.5 [13]. Basic exchangeable bases and cation exchange capacity of the soil was determined according to the method of Ariyakanon and Winaipanich [14]. Total carbon and total nitrogen were analyzed using LECO CNS analyzer [15] and available P was extracted by Bray and Kurts [16] method and measured by autoanalyzer (8000series, Lachat Quick Chem FIA+USA). Total Zn and Cu were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8300, PerkinElmer, USA).

Zinc and copper in soil exist in the forms of water soluble, exchangeable, carbonate bound, Fe-Mn oxides bound, organically bound and residual form. The sequential extraction method of Tessier et al. [17] which was modified by Yang and Kimura [18], Chlopecka et al. [19] and Salas et al. [20] was used to determine the various forms of the metals. Heavy metals concentrations in the extracts were determined using (ICP-OES) (Optima 8300, PerkinElmer, USA).

Heavy metals plants uptake was calculated using the formula:

\[
\text{Uptake} = \frac{\text{Heavy metals in plant (mg kg}^{-1}\text{)}}{\text{Biomass of plant (g)}}
\]

Bio-concentration factor and transfer factor were determined according to Ghosh and Singh [21] and Marchiol et al. [22] methods:

The BCF factor was calculated using the formula:

\[
\text{BCF} = \frac{\text{Metal concentration in plant tissue (mg kg}^{-1}\text{)}}{\text{Metal concentration in soil (mg kg}^{-1}\text{)}}
\]

The translocation factor was calculated using the formula:

\[
\text{TF} = \frac{\text{Metal concentration in shoots portal plant tissue (mg kg}^{-1}\text{)}}{\text{Metal concentration in roots portal plant tissue (mg kg}^{-1}\text{)}}
\]

Statistical Analysis

Data collected from this study were analyzed by analysis of variances and Tukey for mean comparison using SAS version 9.4 (SAS Institute, Inc., Cary, N.C., USA).

Results and Discussion

Characterization of the Soils at Harvest

Chemical properties of the soils as affected by treatments are given in Table-1. The pH of the Oxisol (Munchong soil) decreased from 5.36 to 5.16 in T1, from 5.66 to 5.20 in T2 and from 5.84 to 5.30 in T3. Likewise, the pH of the Ultisol (Bungor soil) decreased with the treatment, shown by the decrease from 4.77 to 4.60, 5.01 to 4.71 and 5.37 to 4.85, respectively. Both soils had low CEC which was due to the dominant presence of kaolinite and gibbsite in their clay fraction [2]. The CEC, exchangeable bases, available P, TN and TC at harvest were lower compared to those at the onset of the experiment due the effects of the plants growing in the treated soils.

One of the advantages of using sewage sludge in soil is improving its chemical fertility. The two types of soils under study were analyzed before and after growing the plants for their chemical properties (Table-1). Differences were noted between the different treatments with regard to pH, CEC, exchangeable bases (K, Ca, and Mg), available P, total carbon and total nitrogen. The increase in CEC means that the soils were able to hold extra exchangeable cations on their newly negatively-charged surfaces and subsequently minimized the loss of these macronutrients via leaching. The foregoing discussion leads to the conclusion that addition of sewage sludge definitely improves the fertility of Oxisol and Ultisol which are considered extremely essential in sustaining the productivity of the two soil types.
Table 1: Properties of the sewage sludge and soils before planting and at harvest.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Sewage sludge</th>
<th>Treatments (%)</th>
<th>Before planting</th>
<th>At harvest</th>
<th>Before planting</th>
<th>At harvest</th>
<th>Oxisol cultivated with</th>
<th>J. curcas</th>
<th>H. cannabinus</th>
<th>Ultisol</th>
<th>Ultisol cultivated with</th>
<th>J. curcas</th>
<th>H. cannabinus</th>
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<tbody>
<tr>
<td>Oxisol</td>
<td>0</td>
<td>5.36</td>
<td>5.17</td>
<td>5.16</td>
<td>4.77</td>
<td>4.60</td>
<td>4.51</td>
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<tr>
<td>Oxisol</td>
<td>5</td>
<td>5.66</td>
<td>5.21</td>
<td>5.20</td>
<td>5.01</td>
<td>4.81</td>
<td>4.71</td>
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<tr>
<td>Ultisol</td>
<td>0</td>
<td>5.84</td>
<td>5.30</td>
<td>5.30</td>
<td>5.37</td>
<td>4.92</td>
<td>4.85</td>
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<tr>
<td>Ultisol</td>
<td>10</td>
<td>8.00</td>
<td>8.01</td>
<td>8.00</td>
<td>10.33</td>
<td>9.73</td>
<td>9.69</td>
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<tr>
<td>pH</td>
<td>26.28</td>
<td>9.11</td>
<td>8.35</td>
<td>8.33</td>
<td>10.67</td>
<td>9.75</td>
<td>9.71</td>
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<tr>
<td>CEC (emol, kg⁻¹)</td>
<td>1.12</td>
<td>0.10</td>
<td>0.08</td>
<td>0.07</td>
<td>0.90</td>
<td>0.79</td>
<td>0.79</td>
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<tr>
<td>Exch K (emol, kg⁻¹)</td>
<td>2.26</td>
<td>0.29</td>
<td>0.24</td>
<td>0.23</td>
<td>0.83</td>
<td>0.77</td>
<td>0.75</td>
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<tr>
<td>Exch Ca (emol, kg⁻¹)</td>
<td>55.00</td>
<td>0.84</td>
<td>0.64</td>
<td>0.63</td>
<td>1.55</td>
<td>0.99</td>
<td>1.09</td>
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<tr>
<td>Exch Mg (emol, kg⁻¹)</td>
<td>2.65</td>
<td>0.98</td>
<td>0.73</td>
<td>0.72</td>
<td>1.79</td>
<td>1.03</td>
<td>1.48</td>
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<td>Av. P (mg kg⁻¹)</td>
<td>34.36</td>
<td>14.50</td>
<td>10.89</td>
<td>10.28</td>
<td>15.50</td>
<td>11.90</td>
<td>10.83</td>
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<tr>
<td>TC (%)</td>
<td>39.23</td>
<td>39.50</td>
<td>10.89</td>
<td>10.28</td>
<td>15.50</td>
<td>11.90</td>
<td>10.83</td>
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<tr>
<td>TC (%)</td>
<td>39.50</td>
<td>10.89</td>
<td>10.28</td>
<td>15.50</td>
<td>11.90</td>
<td>10.83</td>
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<tr>
<td>TC (%)</td>
<td>39.23</td>
<td>11.70</td>
<td>7.80</td>
<td>7.71</td>
<td>10.90</td>
<td>6.76</td>
<td>6.87</td>
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<tr>
<td>Total Zn (mg kg⁻¹)</td>
<td>454.95</td>
<td>38.30</td>
<td>5.88</td>
<td>8.60</td>
<td>38.43</td>
<td>17.9</td>
<td>16.83</td>
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<tr>
<td>Total Cu (mg kg⁻¹)</td>
<td>86.7</td>
<td>39.50</td>
<td>10.89</td>
<td>6.93</td>
<td>40.80</td>
<td>6.40</td>
<td>7.27</td>
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</table>

A slight tendency to boost the acidification of treated soils was observed after 6 months due to the release of H⁺ when the plants were growing in the pots, showing that the soils remained acidic throughout the experimental period. Within the same period of study, the CEC, exchangeable bases (K, Ca, and Mg), available P, TC and TN were lower compared to those at day 1 as they were being utilized by plants as a source of nutrients for their vegetative growth. The CEC was decreased because of the root penetration that enhanced aeration in the soils; thus, accelerating the process of organic matter decomposition [23]. These results revealed that the growth of the tested plants had profound effects on the soil chemical properties.

Changes in Total Zn and Cu Concentration in the Soils

Changes in the concentration of Zn and Cu in the soils are shown in Fig. 1. The results had clearly shown the increase in Zn and Cu concentration in the soils due to sludge application. This study showed that the growth of the two plants had profound effects on the concentration of Zn and Cu in the treated soils. It was found that Zn and Cu concentration in the treated soils had decreased significantly due to their uptake by J. curcas and H. cannabinus.

The removal of heavy metals from soils is the key factor in the remediation of contaminated soils. Zinc and copper in the soils under study were decreased mainly due to their uptake by the J. curcas and H. cannabinus. Hence, phytoremediation can take place successfully as shown by the decrease of Zn and Cu in the tested soils (Fig. 1).

Fractionation of Zinc and Copper and their Availability in Sewage Sludge and Soils

In an attempt to investigate the partitioning of Cu and Zn in the sewage sludge and soils sequential extraction were employed to fractionate them into six operationally defined groups: water soluble, exchangeable, carbonate, Fe-Mn oxide, organic, and residual. Fig. 2 presents the different Zn and Cu fractions in the sewage sludge. This sewage sludge had only 8% of total Zn as water soluble fraction, while in the exchangeable form t was 5%. On the other hand, 15% of its Zn was associated with carbonate, which was probably related to addition of calcium carbonate (CaCO₃) and/or calcium oxide (CaO) coming from the sewage sludge. Another 11% of the Zn was associated with Fe-Mn Oxides, which was due to its co-precipitation in a two month sewage sludge.
treatment [24]. The highest percentage of Zn was present as the residual form, followed by the organic form. This sewage sludge had only 5% Cu existing as water soluble fraction, while 3% as the exchangeable form. Nearly 10% of the Cu was associated with carbonate and 15% in Fe-Mn Oxides form. Most of the Cu existed in the residual form (47%).

As shown in Fig. 3, the untreated Oxisol has only 7% Zn existed as water soluble fraction and 5% as exchangeable fraction, implying that most of the Zn was not easily available for plant uptake. Additionally, only 4% of the Zn was associated with soil carbonate, while nearly 29% was associated with Fe-Mn Oxides and about 43% of the Zn existed as residual form.

About 6% of Cu in the untreated Oxisol existed in water soluble fraction and only 5% as the exchangeable fraction, while 4% in carbonate form (Fig. 4). Another 28% of the Cu was in Fe-Mn form and 13% was present in organic matter. Most of the Cu occurred in the residual form (47%). The same trend was observed in the untreated Ultisol.

For the Oxisol, application of sewage sludge application increased Zn in the exchangeable form from 5% to 7%. In the carbonate fraction it increased from 4% to 9%, while that in the Fe-Mn Oxides fraction slightly increased from 29% to 31%, suggesting that Zn released by the sewage sludge could have been adsorbed by the oxides or Fe and/or Mn. Organic Zn fraction increased from 12% to 15% due to the application of sewage sludge. In contrast, sewage sludge application resulted in the decrease of Zn in the residual fraction from 43% to 31%.

The exchangeable form of Cu had slightly increased from 5% to 7% as well as carbonate fraction increased from 5% to 6%, while the carbonate fraction increased 4% to 9%. Cu in Fe-Mn oxides fraction increased from 28% to 30% due to application of sewage sludge. The organic fraction of Cu increased from 13% to 20%, suggesting that some Cu may be gradually being released upon the oxidation of organic matter, whereas the residual form of Cu had decreased from 44% to 29%. This implies that some of the residual Cu fraction may have changed to other forms.

The same trend was observed in the treated Ultisol. The application of sewage sludge had increased the exchangeable fraction of Zn and Cu, whereas the residual form had decreased. This means that the residual fraction of the Zn and Cu in the soil might have been more easily available for uptake by the two plants.

At harvest, the results showed a shift from residual form to non-residual form for the soils treated with sludge. Thus, the distribution of Zn and Cu in the untreated and treated soils varied and this influenced their behavior at harvest (Fig. 3 and 4).

For the untreated soil, the water soluble and exchangeable fraction of the metals increased, while those associated with Fe-Mn oxides and organic matter as well as in the residual fraction decreased; there was no change in the carbonate fraction. However, in the treated soil, the water soluble and exchangeable fraction increased, while Fe-Mn oxides, organic, carbonate and residual fraction decreased; the changes occurred in the two soils were due to Zn and Cu uptake by the plants. It was observed that water-soluble Zn in the soils had increased after 6 months. Lindsay [25] reported that the solubility of Zn in soil solutions increased 100-fold for each unit decrease in pH. Therefore, a unit decrease in pH due to the presence of plants could be responsible for the increase in water soluble Zn.

Metal toxicity depends on the chemical associations in soils. For this reason, determining the chemical form of a metal in soils is important to evaluate its mobility and bioavailability. Results show there were differences among Zn and Cu in the preferential formation of bonds. All the six Zn and Cu fractions increased with increasing sewage sludge application. In sewage sludge itself, high Zn and Cu amount existed in the residual form, while the application of the sewage sludge into the soils tended to shift the forms of Zn and Cu away from residual fraction to other fractions that might be more available for plant uptake. This was probably due to the differences in binding properties between the soil components and heavy metals from the sewage sludge. Our results showed that the usage of sewage sludge had influenced on the distribution of Zn and Cu in both treated soils. The bioavailability among the elements varied, with Cu and Zn were more likely to be released into the sewage treated soils compared to the others.

Soil factors may influence Zn and Cu availability and solubility in sewage sludge amended soil, such as pH, CEC, total metals concentration and texture. Soil pH played an important role in the availability of Zn and Cu, where the solubility of these metals increased as soil pH decreased. Chlopecka et al. [19] found that the soils with pH < 5.6 contained relatively more of all metals in the exchangeable form than in soils with pH was > 5.6. Oxisol and Ultisol, tend to be characterized by low pH values, low activity clays (kaolinite), low organic matter contents and high levels of Fe oxides, which might be expected to result in relatively high availability of Zn and Cu.
Fig. 1: Changes in Zn and Cu in the soils: (A) Zn in Oxisol with J. curcas, (B) Zn in Oxisol with J. curcas, (C) Zn in Ultisol with J. curcas, (D) Cu in Ultisol with J. curcas, (E) Zn in Oxisol with H. cannabinus, (F) Cu in Oxisol with H. cannabinus, (J) Zn in Ultisol with H. cannabinus, and (H) Cu in Ultisol with H. cannabinus.
Fig. 2: Forms of Zn and Cu in the sewage sludge.

WS = Water Soluble Fraction, EXC = Exchangeable Fraction, CAR = Carbonate Fraction, Fe-Mn = Fe-Mn Oxides Fraction, ORG = Organic Fraction and RES = Residual Fraction

Fig. 3: Forms of Zn in untreated Oxisol (A and B), in treated Oxisol (C and D).

WS = Water Soluble Fraction, EXC = Exchangeable Fraction, CAR = Carbonate Fraction, Fe-Mn = Fe-Mn Oxides Fraction, ORG = Organic Fraction and RES = Residual Fraction
WS=Water Soluble Fraction, EXC=Exchangeable Fraction, CAR=Carbonate Fraction, Fe-Mn =Fe-Mn Oxides Fraction, ORG= Organic Fraction and RES= Residual Fraction

Fig. 4: Forms of Cu in untreated Oxisol (A and B), in treated Oxisol (C and D).

The trend is shown by data presented in Fig. 3 and 4. The highest amount of Zn and Cu was found in the non-residual fractions, which was consistent with the study of Zauyah et al. [26]. The amounts of non-residual fractions (water soluble fraction, exchangeable fraction, carbonate fraction, Fe-Mn oxides fraction and organic fraction) represents the amounts of active heavy metals, while those of the residual fractions may be considered to be the stable form and thus not available to plants. Increase in organic matter content due to sewage sludge application might have eluted soluble ligands, which are known to mobilize Zn and Cu. These results suggest that the availability of Zn and Cu to trees and the subsequent metal accumulation in their tissues varied with sludge treatment and soil conditions.

Changes in the Biomass of Plants

There were differences in the average of plant biomass between the tested plants grown on Oxisol and Ultisol; plant biomass grown on Oxisol was higher compared to that of the Ultisol (Fig. 5). The biomass of the two tested plants varied significantly. J. curcas and H. cannabinus had the higher biomass when grown in the soils treated 10% sludge compared to other treatments. The biomass of J. curcas grown on Oxisol was 198.91, while that grown on Ultisol was 137.97 g. As for H. cannabinus, it was 216.60 and 188.16 g, respectively.

The growth of the tested plants was influenced by sewage sludge treatments. It appeared that the plants responded positively to sludge application; hence, sewage sludge can be regarded as a soil amendment if the presence of excess of Cu and Zn are not taken into consideration. This sewage sludge supplied some nutrients for the vegetative of the plants growth. J. curcas and H. cannabinus grew quite well in the soils treated with sewage sludge (Fig. 5). In this study, 10% sludge treatment gave the highest biomass followed, by 5% sludge treatment. The results highlighted the increase in the biomass production for plants gradually with time. It showed that the amounts of heavy metals added to the soils by sludge application did not affect biomass production or induce phytotoxicity symptoms in J. curcas and H. cannabinus.
Accumulation and Distribution of Zn and Cu in the Plant Tissues

It was expected that application of sewage would increase the concentration of Zn and Cu in the tested plants during experiment period. Data in Fig. 6 show that it was so. The highest content of Zn and Cu was recorded in the \textit{J. curcas} and \textit{H. cannabinus} grown in the sewage sludge treated soils.

The distribution of Zn and Cu in \textit{J. curcas} and \textit{H. cannabinus} showed consistent accumulation trend. Distribution of Zn and Cu in both of the tested plants was in the following sequence shoots (stems + leaves) > roots. This implies that \textit{J. curcas} and \textit{H. cannabinus} do not store Zn and Cu in their roots, but translocate the metals to their shoots.

Accumulation and distribution of Zn and Cu in plant tissues are important aspects of assessing the role of \textit{J. curcas} and \textit{H. cannabinus} for remediation of contaminated soils. The tissues of the leaves, stems and roots were analyzed to get a picture of Zn and Cu distribution in plant parts. The results showed that the concentrations of Zn and Cu in the plant tissues were influenced by sludge application. Zn and Cu were accumulated in \textit{J. curcas} and \textit{H. cannabinus} in various degrees, depending on soil type and the rates of sewage sludge application. Zn and Cu concentrations were evidently increased with increasing level of sludge application.

Under acidic conditions, free ionic metal species are thought to be more prevalent. The results showed that there was good relationship between treatments and heavy metals accumulated in the tested plants. The analyses of plant parts indicated that greater amounts of Zn and Cu were sequestered in the shoots compared to the other parts of the plants. So, it was believed that \textit{J. curcas} and \textit{H. cannabinus} could remove Zn and Cu from Oxisol and Ultisol by concentrating them in the shoots. The ability of the plants to hyper-accumulate Zn and Cu in their tissues might have helped their ability to tolerate the presence of the excessive amount of Zn and Cu.

The Uptake of Zinc and Copper

Fig. 7 shows the rate of Zn and Cu uptake by \textit{J. curcas} and \textit{H. cannabinus} within the period of the study. The metals found in the soils demonstrated a significant increase in their concentration in the plant tissues. The uptake of Zn and Cu depended on
the rate of sewage sludge applied, soil type and/or plant species.

Plants uptake of Zn and Cu is proportional to the metal concentration in the sewage sludge. By adding sewage sludge into the tested soils, Zn and Cu concentrations in the plants were found to be significantly increased. The reason for the increased uptake and accumulation of Zn and Cu in the tissues of *J. curcas* and *H. cannabinus* could be the low pH level. Akan et al. [27] in their study indicated that soil acidity was known to increase the mobilization of heavy metals; thus, increasing their uptake by plants. Furthermore, roots of plants increase metal bioavailability by extruding protons to acidify the soil and subsequently mobilize the metals. The increased uptake of Zn and Cu by plants could also be related to the dissolution of organic carbon supplied by the sewage sludge. The amount of Zn and Cu reduced from contaminated soils by plants is dependent on two factors: 1) The biomass produced by the plants; and 2) The ratio of metal concentration in the shoot tissue to the soil, which represents the metal bioconcentration factor [28].

According to Salt et al., [29] Sun et al., [30] and Thakur et al. [31], accumulating capability in plants have been used to define metal hyperaccumulators including 10 mg g\(^{-1}\) in their tissue for Zn and Cu, hyper-accumulator has high tolerance capability to heavy metals, even does not show visible toxic symptoms under a certain concentration. *J. curcas* and *H. cannabinus* were accumulated Zn and Cu with high amount exceed 10 mg g\(^{-1}\) (Fig. 7); thus, it was believed that tested as hyperaccumulator plants could remove Zn and Cu from Oxisol and Ultisol by concentrating them in their tissue especially shoots.

**Removal Coefficients (Translocation and Bio-Concentration Factors)**

Fig. 8 shows the mean TF and BCF values of Zn and Cu for *J. curcas* and *H. cannabinus* during the period of the study. The TF values were obviously different among the two plant species grown in the treated soils. For example, the mean translocation factor of Zn for *J. curcas* was high for the plants grown on Oxisol (1.53) and Ultisol (2.43), while that of Cu was a bit lower, with values of 1.40 and 1.48, respectively. The TF value of Cu for *H. cannabinus* was higher in Oxisol (1.52) compared to that of Ultisol (1.17). The mean TF values were significantly different (p ≤ 0.05) between treatments. The results showed that *J. curcas* and *H. cannabinus* have BCF of < 1 for Zn and Cu. The BCF values were not significantly different (p ≤ 0.05) between treatments.
Fig. 6 Zn and Cu content in plants at harvesting: (A) Zn in *J. curcas* planted on Oxisol (B) Cu in *J. curcas* planted on Oxisol (C) Zn in *J. curcas* planted on Ultisol (D) Cu in *J. curcas* planted on Ultisol (E) Zn in *H. cannabinus* planted on Oxisol (F) Cu in *H. cannabinus* planted on Oxisol (J) Zn in *H. cannabinus* planted on Ultisol; and (H) Cu in *H. cannabinus* planted on Ultisol.
Fig. 7: The uptake of Zn and Cu: (A) Zn uptake by *J. curcas* planted on Oxisol (B) Cu uptake by *J. curcas* planted on Oxisol (C) Zn uptake by *J. curcas* planted on Ultisol (D) Cu uptake by *J. curcas* planted on Ultisol (E) Zn uptake by *H. cannabinus* planted on Oxisol (F) Cu uptake by *H. cannabinus* planted on Oxisol (J) Zn uptake by *H. cannabinus* planted on Ultisol (H) Cu uptake by *H. cannabinus* planted on Ultisol.

The results shown in Fig. 8 had proven beyond doubt that *J. curcas* and *H. cannabinus* had great ability to uptake and subsequently translocate Zn and Cu in their parts. It was believed that *J. curcas* and *H. cannabinus* varied in their tolerance to heavy metals toxicity. Furthermore, their efficiency of extracting of Zn and Cu from soils is dependent not only on their concentration in the sewage sludge, but also on the properties of the soil. Data presented in this study clearly indicated that Zn and Cu taken up by *J. curcas* and *H. cannabinus* were largely retained in the shoot of the plants. Their ability to accumulate the metals was consistent with the high TF values of more than 1 and the low BCF values of less than 1. On this basis, *J. curcas* and *H. cannabinus* can be recommended for the remediation of Zn and Cu contaminated soils in Malaysia.

Means for same soil with the same letter are not significantly different at p≤0.05 (Tukey test)

Fig. 8: Phytoremediation efficiency of *J. curcas* and *H. cannabinus* planted in Oxisol and Ultisol.
Conclusion

The application of sewage sludge into highly weathered soils in Malaysia in some ways improves their chemical fertility, except for the accumulation of the excessive amount of Zn and Cu. These metals have to be removed from the soils for sustainable crop production by a process called phytoremediation. It was shown by the present study that the excess amount of Zn and Cu could be removed from the soils by growing *J. curcas* and *H. cannabinus*. Later, the plant biomass containing the metals has to be cut and subsequently discarded somewhere else. Both metals were largely retained in the shoots of the plants. Their ability to accumulate the metals was consistent with the high TF values of more than 1 and the low BCF values of less than 1. On this basis, *J. curcas* and *H. cannabinus* can be recommended for the remediation of Zn and Cu contaminated soils, especially the Oxisols and Ultisols that are contaminated with the metals.

Acknowledgements

We would like to express our sincere gratitude to Universiti Putra Malaysia for the financial and technical supports.

References