Using Plant Species for Phytoremediation of Highly Weathered Soils Contaminated with Zinc and Copper with Application of Sewage Sludge

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The ability of woody plant species to remediate heavy metals contaminated soils was investigated with the addition of sewage sludge. *Jatropha curcas, Hibiscus cannabinus, Acacia mangium*, and *Syzygium cumini* growth was monitored on an Oxisol- and an Ultisol-treated soil with sewage sludge at a level of 0% w/w, 5% w/w, or 10% w/w. The sewage sludge was found to enhance soil fertility, as shown by an increase in soil pH, cation exchange capacity, exchangeable bases (potassium, calcium, and magnesium), available phosphorous, total carbon, and total nitrogen. However, zinc and copper accumulated in soils at toxic levels; thus, they had to be removed before being used for crop production. The concentration of the two heavy metals in *Jatropha curcas* and *Hibiscus cannabinus* at harvest were higher than those of *Acacia mangium* and *Syzygium cumini.* The high uptake of zinc and copper by the first two plant species was the result of their high translocation factor, although the bio-concentration factor was low. Thus, *Jatropha curcas* and *Hibiscus cannabinus* were considered tolerant to zinc and copper toxicity and able to remove the metals efficiently from the contaminated soils.

Keywords: Phytoremediation; Heavy metal; Oxisol; Ultisol; Translocation factor; Bio-concentration factor

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INTRODUCTION

The sources of zinc (Zn) and copper (Cu) in soil include natural occurrences related to their parent materials and those derived from human activities, such as the application of fertilizer or sewage sludge on agricultural land (Li *et al.* 2013; Nogueira *et al*. 2013; Joseph *et al*. 2014). The cumulative effect from recycling sludge generated from the treatment of municipal wastewater cannot be neglected, and it has become an important subject of research in Malaysia (Rosenani *et al.* 2004; Aishah *et al*. 2016; Nur-Nazirah *et al.* 2017). The amount of sewage sludge produced in Malaysia rapidly increases each year, with about 3.2×10^6 metric tons of domestic sewage sludge being produced annually, and it has been estimated to rise to 7×10^6 metric tons by the year 2020 (Azizi *et al.* 2013).

It is important to understand the effects of sewage sludge on soil properties; constant monitoring of the accumulation of heavy metals in soils is necessary (Bourioug *et al.* 2014). Copper and Zn are the two heavy metals with considerable human risk because they are found at high concentrations in the environment (Nogueira *et al.* 2013).

Copper is an essential trace element for living organisms. It has a significant function in some biological and physiological processes, but at high concentrations it becomes toxic (Stern 2010; Canning-Clode *et al.* 2011). Likewise, Zn is a micronutrient that becomes phytotoxic at high concentrations in soil. Usually, Zn is present in soils at low concentrations, but it can increase to a toxic level because of agricultural practices (Buccolieri *et al.* 2010; Ahmed *et al.* 2012).

Many plant species absorb and subsequently accumulate heavy metals into their biomass. The metals accumulate inside the plant cells because they cannot be easily broken down, which leads to toxicity symptoms if present above the critical levels. Moreover, the metals can cause indirect effects of toxicity (Siedlecka 2014). The use of metal absorption into the biomass of plants as a procedure for soil remediation is limited by the efficiency of the plants (Ali *et al.* 2013). Plant roots absorb and uptake metals from contaminated soils and transfer them to the shoots where the metals accumulate (Jadia and Fulekar 2009). Plants with great biomass are suitable for the remediation of contaminated soils (Kubátová *et al.* 2016).

Heavy metal tolerance in herbaceous species has been examined intensively, but woody plant species have received less attention (EPA 2000; Mahar *et al.* 2016). For phytoremediation, trees should be fast growing, easy to propagate, and capable of accumulating heavy metals (Almeida *et al.* 2007). The current study was conducted to investigate the response of weathered soils to a hypothetical increase of pollution due to the application of sewage sludge and to assess the efficiency of phytoremediation of excess Zn and Cu in Oxisol and Ultisol soil compositions. In Malaysia, these soils are considered highly weathered with low fertility (Shamshuddin *et al.* 1991; Shamshuddin and Ismail 1995). It is expected that adding sewage sludge to the soils of this nature would improve their productivity, which is good for agriculture in the long run.

The objectives of this study were to determine the effects on the chemical properties of the soils after the application of sewage sludge and to investigate the potential of four woody plant species as heavy metal accumulators for the remediation of highly weathered Malaysian soils amended with sewage sludge.

EXPERIMENTAL

Materials

Two factorial experiments were conducted in a glasshouse, one without plants and the other with plants grown in 20 kg plastic pots. For the first experiment, two soils were tested, an Oxisol soil composition (Munchong Series) in 36 pots; and an Ultisol soil composition (Bungor Series) also in 36 pots. The experiment was conducted using Complete Randomized Design (CRD), with three replications. Three sewage sludge mixtures (0% w/w, 5% w/w, and 10% w/w) were used for each soil series and were incubated for 90 days before being analyzed for their chemical properties. The sludge used in the study was obtained from the Indah Water Konsortium (IWK) Plant at the Bandar Tun Razak Sewage Treatment Plant, Kuala Lumpur, Malaysia. The sludge and soil samples were air-dried and passed through a 2 mm mesh sieve in preparation for the chemical analyses. The glasshouse was located at the Universiti Putra Malaysia, Serdang Selangor, Malaysia (2º 59´ 18.24´´N and 101 º 42´ 45.45´´E).

For the second experiment, the same experimental design was adopted. Four woody plant species (*Jatropha curcas*, *Hibiscus cannabinus*, *Acacia mangium*, and *Syzygium cumini*) were selected for this study due to their high biomass and rapid growth rate. Three sewage sludge mixtures (0% w/w, 5% w/w, and 10% w/w) were used to grow each plant. The soils treated with the sewage sludge were incubated for 90 days before planting the seedlings. The seedlings were allowed to grow for 3 months, after which their potential as phytoremediators was evaluated on the basis of their translocation factor and the bio-concentration factor values. Girotto *et al.* (2013) showed that a period of 3 months to 6 months was long enough to evaluate plant growth, determine metal uptake, and detect toxicity effects.

Soil and Tissue/Plant Analyses

Soil texture was determined *via* the pipette method (Kettler *et al.* 2001). Soil pH was measured in water at a ratio of 1:2.5 (Zhang *et al.* 2009). Basic exchangeable bases (potassium (K), calcium (Ca), and magnesium (Mg)) and the cation exchange capacity (CEC) were determined according to the methods of Ariyakanon and Winaipanich (2006). Total carbon (C) and total nitrogen (N) were analyzed using a LECO CNS analyzer (LECO Corporation, St. Joseph, MI, USA) (Moore *et al.* 2010). Available phosphorous (P) was determined as described previously (Bray and Kurtz 1945); the extracted P was measured by an auto analyzer (8000 series, Lachat Quick Chem FIA+, Hach, USA). The mineralogy of the clay fraction of the two soils was determined by X-ray diffraction analysis.

The harvested 3-month-old plants were washed gently with distilled water, and the leaves, stems, and roots were separated, oven-dried (60 °C), and dissolved in aqua-regia. The heavy metals in the solutions were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Optima 8300, PerkinElmer, USA). The plant height, leaf number, and the biomass of each plant species was determined before harvest. The measurement of the relative growth rate (RGR) was needed for the purpose of comparison between the plant species (Hadad *et al.* 2006). The relative growth rate (RGR) of the tested plants was calculated with Eq. 1 (Hadad *et al.* 2006),

$$\text{RGR} = (\ln W_2 - \ln W_1) / T \quad (1)$$

where W_2 is the initial dry weight (g), W_1 is the final dry weight (g), and T is time (months). The heavy metal uptake by the plants was calculated using Eq. 2,

$$\text{Uptake} = \text{Conc x Bio} \quad (2)$$

where Conc is the concentration of the heavy metal in the plant and Bio is the biomass of the plant.

The efficiency of a plant species to absorb and translocate heavy metals to its shoot is known as the translocation factor (TF), while its ability to accumulate heavy metals is known as the bio-concentration factor (BCF) (Marchiol *et al.* 2004; Singh 2007). TF was calculated using Eq. 3,

$$\text{TF} = \text{Conc}_{\text{ST}} / \text{Conc}_{\text{RT}} \quad (3)$$

where Conc_{S} is the metal concentration in the shoots portal plant tissue (mg kg^{-1}) and Conc_{R} is the metal concentration in the roots portal plant tissue (mg kg^{-1}).

The BCF was calculated using Eq. 4,

$$\text{BCF} = \text{Conc}_{\text{PT}} / \text{Conc}_{\text{S}} \quad (4)$$

where Conc_{PT} is the metal concentration in plant tissue (mg kg^{-1}) and Conc_{S} is the metal concentration in the soil (mg kg^{-1}).

Statistical Analysis

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

RESULTS AND DISCUSSION

Physico-chemical Characteristics of the Original Soils

Approximately 70% of the land surface area in Malaysia is occupied by highly weathered soil types classified as either Oxisol or Ultisol; these are the major soil types in the country. The pH of the Oxisol soil (Munchong Series) was 5.36 ± 0.01, while the Ultisol soil (Bungor Series) had a pH of 4.77 ± 0.02 (Table 1). In addition, these soils were a bit sandy with the topsoil texture of sandy clay loam. The pH of the Munchong soil was higher than typical soil samples found in the country; the soil was sampled at the location of a former field trial, which could have been limed by the researchers. The CEC of the Munchong and Bungor soils was 8.00 ± 0.57 $cmol_c\ kg^{-1}$ and 10.33 ± 0.01 $cmol_c\ kg^{-1}$, respectively. The average Zn and Cu concentrations in both soils were slightly higher than those reported in the Malaysian soils studied by Zarcinas *et al.* (2004), with values of 38.00 mg kg^{-1} and 34.00 mg kg^{-1}, respectively. In this study, the Zn concentration in both soils was 38.43 ± 0.03 mg kg^{-1}, while the Cu concentration was 37.90 ± 0.01 mg kg^{-1}. The concentration of both metals was higher in the Ultisol soil than in the Oxisol soil; however, the value obtained was still below the phytotoxic level.

Table 1. Physico-chemical Properties of the Soils Before Treatment

Properties	Unit	Oxisol	Ultisol
pH	-	5.36 ± 0.01	4.77 ± 0.02
CEC	$cmol_c\ kg^{-1}$	8.00 ± 0.57	10.33 ± 0.01
Exch K	$cmol_c\ kg^{-1}$	0.10 ± 0.01	0.90 ± 0.03
Exch Ca	$cmol_c\ kg^{-1}$	0.29 ± 0.01	0.83 ± 0.02
Exch Mg	$cmol_c\ kg^{-1}$	0.19 ± 0.01	0.59 ± 0.03
Avail P	mg kg^{-1}	10.70 ± 0.05	10.90 ± 0.05
Zn	mg kg^{-1}	38.30 ± 0.08	38.43 ± 0.03
Cu	mg kg^{-1}	35.50 ± 0.05	37.90 ± 0.01
Total C	%	1.75 ± 0.01	2.01 ± 0.03
Total N	%	0.13 ± 0.01	0.13 ± 0.01
Sand	%	66.48	62.69
Clay	%	28.01	28.37
Silt	%	5.44	8.89
Texture	-	Sandy clay loam	Sandy clay loam

The clay fraction of both soils was dominated by kaolinite, as shown in Fig. 1 by the prominent XRD peaks at 7.21 Å and 3.58 Å. Some gibbsite was present in the soils, as evidenced by the 4.86 Å peak. Goethite and hematite could also be present in the Munchong and Bungor soils as previously reported by Shamshuddin and Fauziah (2010). The afore-mentioned findings on the mineralogy of the clay fraction of the Oxisol soil were consistent with the findings of Shamshuddin and Anda (2012). The predominance of kaolinite and iron (Fe) and/or aluminum (Al) oxides in the clay fraction of the soils have important implications on their capacity to adsorb/desorb Zn and Cu released by the dissolution of the sewage sludge applied onto the soils.

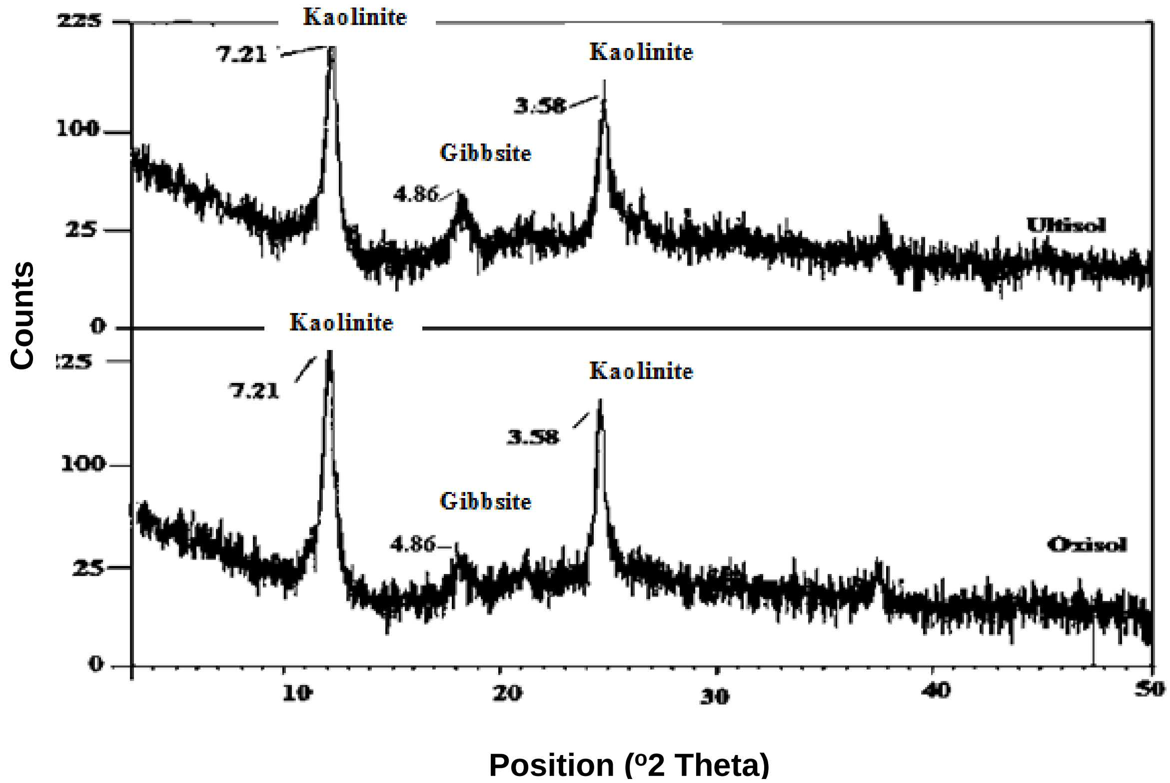


Fig. 1. X-ray diffractograms of the clay fraction of the soils under study

Table 2. Chemical Properties of the Sewage Sludge Used in the Current Study

Properties	Values
pH	6.04 ± 0.05
CEC (cmol$_c$ kg^{-1})	26.28 ± 0.16
Exch K (cmol$_c$ kg^{-1})	1.12 ± 0.02
Exch Ca (cmol$_c$ kg^{-1})	2.26 ± 0.03
Exch Mg (cmol$_c$ kg^{-1})	2.65 ± 0.06
Avail P (mg kg^{-1})	55.00 ± 1.01
Total Zn (mg kg^{-1})	454.95 ± 2.12
Total Cu (mg kg^{-1})	86.70 ± 0.51
Total C (%)	34.36 ± 0.01
Total N (%)	2.90 ± 0.02
Water content (%)	3.29

Chemical Properties of the Sewage Sludge

The pH of the sewage sludge was 6.04 ± 0.05 (Table 2), which was comparable to previous results (Serna and Pomeras 1992; Rosenani *et al.* 2004). The high pH was probably due to the addition of calcium carbonate and calcium oxide when the sewage sludge was treated at the plant. The total nitrogen content in the sludge was 2.90 ± 0.02%, which was higher than previously reported (Lindemann and Cardenas 1984; Indah Water Konsortium Sdn Bhd 1997); however, it was similar to the data reported by Rosenani *et al.* (2004). The concentrations of Zn and Cu were within the normal ranges found in sewage sludge everywhere else in the world (Alloway 1990), with the respective values of 153 mg kg^{-1} to 7012 mg kg^{-1} and 63 mg kg^{-1} to 696 mg kg^{-1}.

Table 3. Effects of Sewage Sludge Application on Soil Properties

Properties	Soil Composition					
	Oxisol			Ultisol		
Treatments	0%	5%	10%	0%	5%	10%
pH	5.36^{c} ± 0.01	5.66 ^{b} ± 0.01	5.84^{a} ± 0.02	4.77^{c} ± 0.02	5.01 ^{b} ± 0.03	5.37 ^{a} ± 0.01
CEC (cmol$_c$ kg^{-1})	8.00^{c} ± 0.57	9.11 ^{b} ± 0.01	9.39 ^{a} ± 0.01	10.33 ^{c} ± 0.01	10.67 ^{b} ± 0.05	11.22 ^{a} ± 0.06
Exch K (cmol$_c$ kg^{-1})	0.10 ^{c} ± 0.03	0.27 ^{b} ± 0.01	0.34 ^{a} ± 0.01	0.90 ^{c} ± 0.03	1.15 ^{b} ± 0.01	1.25 ^{a} ± 0.01
Exch Ca (cmol$_c$ kg^{-1})	0.29 ^{c} ± 0.01	0.80 ^{b} ± 0.04	0.98 ^{a} ± 0.01	0.83 ^{c} ± 0.02	1.55 ^{b} ± 0.05	1.79 ^{a} ± 0.01
Exch Mg (cmol$_c$ kg^{-1})	0.19 ^{c} ± 0.01	0.64 ^{b} ± 0.08	0.71 ^{a} ± 0.08	0.59 ^{c} ± 0.03	1.05 ^{b} ± 0.01	1.47 a ± 0.01
Avail P (mg kg^{-1})	10.70 ^{c} ± 0.05	14.50 ^{b} ± 0.01	24.00 ^{a} ± 0.02	10.90 ^{c} ± 0.05	15.50 ^{b} ± 0.01	26.80 ^{a} ± 0.10
Zn (mg kg^{-1})	38.30 ^{c} ± 0.08	39.28 b ± 0.05	58.45 ^{a} ± 0.02	38.43 ^{c} ± 0.11	50.37 ^{b} ± 0.05	58.70 ^{a} ± 0.05
Cu (mg kg^{-1})	35.50 ^{c} ± 0.05	39.50 ^{b} ± 0.05	48.10 ^{a} ± 0.08	37.90 ^{c} ± 0.05	40.80 ^{b} ± 0.05	49.70 ^{a} ± 0.05
Total C (%)	1.75^{c} ± 0.01	2.33 ^{b} ± 0.01	2.53 ^{a} ± 0.01	2.01 ^{c} ± 0.03	4.19 ^{b} ± 0.01	5.23 ^{a} ± 0.01
Total N (%)	0.13 ^{c} ± 0.01	0.24^{b} ± 0.01	0.31 ^{a} ± 0.03	0.13 ^{c} ± 0.01	0.46^{b} ± 0.02	0.68 ^{a} ± 0.01

Means ± standard error of the mean in the same column for the same soil with the same letter were not significantly different at p less than 0.05

Chemical Characteristics of the Soils due to Treatment

The chemical properties of the soils were significantly ($p<0.05$) affected by the sewage sludge treatments (Table 3). Generally, the soil pH, CEC, and exchangeable bases (K, Ca, and Mg) increased with an increase in the rate of sewage sludge application. The available P in the sludge treated soils was significantly higher than those of the untreated ones. For the Oxisol soil compositions, sewage sludge application increased the available P from 10.70 ± 0.05 mg kg^{-1} to 24.00 ± 0.02 mg kg^{-1}, while for the Ultisol soil compositions, it was increased from 10.90 ± 0.05 mg kg^{-1} to 26.80 ± 0.01 mg kg^{-1}. The highest value was found in the 10% sewage sludge treatment. Angin and Yaghanoglu (2011) also reported an increase in soil pH and CEC due to sewage sludge treatments. In addition, the total C and total N increased due to the application of sewage sludge. For the Oxisol soil composition, the total C increase was from 1.75 ± 0.01% to 2.53 ± 0.01 %, while the Ultisol soil composition increased from 2.01 ± 0.03% to 5.23 ± 0.01%. The increase in total organic matter content, as reflected by the total C increase, was consistent with the increase of total N from 0.13 ± 0.01% to 0.31 ± 0.03% in the Oxisol soil compositions and from 0.13 ± 0.01% to 0.68 ± 0.01% in the Ultisol soil compositions. These results were in line with the findings of Usman *et al.* (2012) and clearly indicated an improvement in soil fertility resulting from the application of sewage sludge.

The application of sewage sludge affected the Zn and Cu concentrations in both soils under investigation. The Zn concentration increased from 38.30 ± 0.08 mg kg^{-1} to 58.45 ± 0.05 mg kg^{-1} in the Oxisol soil compositions and from 38.43 ± 0.11 mg kg^{-1} to 58.70 ± 0.05 mg kg^{-1} in the Ultisol soil compositions. The Cu concentrations in the Oxisol soil compositions increased from 35.50 ± 0.05 mg kg^{-1} to 48.10 ± 0.08 mg kg^{-1}, while the Ultisol soil compositions increased from 37.90 ± 0.05 mg kg^{-1} to 49.7 ± 0.05

mg kg^{-1}. Similar trends in the results were found by Bettiol and Ghini (2011).

Chemical Properties of the Treated Soils as Affected by Plant Species

The results of the experiment after harvest of the 4 woody plant species (*J. curcas*, *H. cannabinus*, *A. mangium*, and *S. cumini*) planted in the sludge treated Oxisol and Ultisol soil compositions are presented in Tables 4 and 5, respectively.

Table 4. Changes in the Chemical Properties of Oxisol Soil Compositions with Plants due to Sludge Treatment

Plant Species		*J. curcas*	*H. cannabinus*	*A. mangium*	*S. cumini*
	Treat (%)				
pH	0	5.34^{b} ± 0.18	5.33^{b} ± 0.04	5.34^{b} ± 0.14	5.33^{b} ± 0.04
	5	5.56^{a} ± 0.05	5.55^{a} ± 0.05	5.55^{a} ±0.04	5.53^{a} ± 0.0
	10	5.67^{a} ± 0.05	5.64^{a} ± 0.04	5.65^{a} ± 0.08	5.63^{a} ± 0.07
CEC (cmol$_c$ kg^{-1})	0	7.98^{b} ± 0.05	7.97^{b} ± 0.01	7.98^{b} ± 0.04	7.98^{a} ± 0.06
	5	8.37^{b} ± 0.05	8.36^{b} ± 0.04	8.38^{a} ± 0.24	8.40^{a} ± 0.04
	10	8.38^{a} ± 0.06	8.37^{a} ± 0.03	8.39^{a} ± 0.09	8.41^{a} ± 0.05
Exch Mg (cmol$_c$ kg^{-1})	0	0.15^{b} ± 0.01	0.16^{b} ± 0.04	0.14^{b} ± 0.04	0.15^{b} ± 0.01
	5	0.49^{a} ± 0.01	0.47^{a} ± 0.03	0.43^{ab} ± 0.04	0.45^{a} ± 0.01
	10	0.59^{a} ± 0.05	0.57^{a} ± 0.05	0.55^{a} ± 0.01	0.56^{a} ± 0.04
Exch Ca (cmol$_c$ kg^{-1})	0	0.25^{b} ± 0.05	0.24^{b} ± 0.01	0.26^{b} ± 0.01	0.27^{b} ± 0.02
	5	0.65^{a} ± 0.05	0.67^{a} ± 0.02	0.76^{a} ± 0.03	0.79^{a} ± 0.01
	10	0.74^{a} ± 0.02	0.75^{a} ± 0.04	0.80^{a} ± 0.01	0.81^{a} ± 0.04
Exch K (cmol$_c$ kg^{-1})	0	0.09^{b}± 0.04	0.08^{b} ± 0.01	0.08^{b} ± 0.02	0.09^{b} ± 0.02
	5	0.21^{a} ± 0.05	0.20^{a} ± 0.04	0.21^{a} ± 0.01	0.20^{a} ± 0.01
	10	0.21^{a} ± 0.04	0.21^{a} ± 0.02	0.21^{a} ± 0.01	0.21^{a} ± 0.03
Avail P (mg kg^{-1})	0	7.90^{c} ± 0.03	7.80^{c} ± 0.04	7.90^{c} ± 0.14	7.60^{c} ± 0.04
	5	13.51^{b}± 0.04	13.61^{b} ± 0.05	13.75^{b} ± 0.04	13.77^{b} ± 0.24
	10	22.00^{a} ± 0.15	21.20^{a} ± 0.04	21.50^{a} ± 0.14	22.40^{a} ± 0.06
Total C (%)	0	1.72^{b} ± 0.05	1.30^{b} ± 0.05	1.49^{b} ± 0.04	1.45^{b} ± 0.05
	5	2.15^{ab}± 0.05	2.17^{ab} ± 0.04	2.23^{ab} ± 0.06	2.21^{ab} ± 0.04
	10	2.34^{a} ± 0.04	2.32^{a} ± 0.05	2.27^{a} ± 0.04	2.27^{a} ± 0.05
Total N (%)	0	0.06^{b} ± 0.05	0.07^{c} ± 0.04	12.34^{b} ± 0.03	13.30^{a} ± 0.04
	5	0.17^{ab}± 0.06	0.10^{b} ± 0.01	9.01^{c} ± 0.05	7.34^{b} ± 0.04
	10	0.27^{a} ± 0.05	0.24^{a} ± 0.01	14.50^{a} ± 0.04	7.02^{b} ± 0.12
Zn (mg kg^{-1})	0	9.10^{b}± 0.05	0.10^{b} ± 0.02	22.34^{b} ± 0.24	4.44^{c} ± 0.06
	5	8.13^{b} ± 0.05	0.15^{ab} ± 0.01	14.88^{c} ± 0.05	6.26^{b} ± 0.04
	10	17.08^{a}± 0.06	0.29^{a} ± 0.01	28.02^{a} ± 0.04	10.18^{a} ± 0.94
Cu (mg kg^{-1})	0	8.18^{c} ± 0.04	0.10^{b} ± 0.01	25.20^{b} ± 0.05	9.60^{ab} ± 0.05
	5	11.32^{b} ± 0.05	0.13^{ab} ± 0.02	15.14^{c} ± 0.15	10.03^{ab} ± 0.04
	10	19.01^{a} ± 0.05	0.21^{a} ± 0.01	27.01^{a} ± 0.04	11.58^{a} ± 0.03

Means ± standard error of the mean in the same column for the same plant with the same letter were not significantly different at p less than 0.05

Overall, the soil pH, CEC, exchangeable bases (K, Ca, and Mg), and available P, Zn, and Cu increased with an increasing rate of sludge application. The following discussion is based on the data in comparison to the soil compositions without growing plants (shown in Table 3). For the Oxisol soil composition (Table 4), the soil pH decreased from 5.84 ± 0.02 to 5.65 ± 0.05, while the soil pH of the Ultisol soil composition (Table 5), decreased from 5.37 ± 0.01 to 4.88 ± 0.18 due to the presence of the plants (when compared to the soil without plants presented in Table 3). The decrease in pH was attributed to the release of H^+ when the plants were growing in the sludge treated soils, as discussed by Ali *et al.* (2013). The CEC was decreased from 9.39 ± 0.01 $cmol_c$ kg^{-1} to 8.37 ± 0.03 $cmol_c$ kg^{-1} in the Oxisol soil compositions, while the Ultisol soil compositions decreased from 11.22 ± 0.06 $cmol_c$ kg^{-1} to 9.76 ± 0.11 $cmol_c$ kg^{-1}. The results were consistent with the findings of Saikh *et al.* (1998) and Lambers *et al.* (2008), who concluded that the CEC could be decreased by plants growing in the pots. Available P in the soils decreased due to plant uptake. In the Oxisol soil compositions, the available P decreased from 24.00 ± 0.02 mg kg^{-1} to 21.20 ± 0.02 mg kg^{-1}, while the Ultisol soil compositions decreased from 26.80 ± 0.10 mg kg^{-1} to 23.00 ± 0.10 mg kg^{-1}.

Table 5. Changes in the Chemical Properties of Ultisol Soil Compositions with Plants due to Sludge Treatment

Plant Species	Treat (%)	*J. curcas*	*H. cannabinus*	*A. mangium*	*S. cumini*
pH	0	4.56 ^{ab} ± 0.20	4.57 ^{ab} ± 0.19	4.55 ^{ab} ± 0.08	4.56 ^{ab} ± 0.11
	5	4.80 ^{a} ± 0.17	4.80 ^{a} ± 0.15	4.81 ^{a} ± 0.18	4.82 ^{a} ± 0.08
	10	4.89 ^{a} ± 0.10	4.88 ^{a} ± 0.18	4.90 ^{a} ± 0.20	4.89 ^{a} ± 0.22
CEC ($cmol_c$ kg^{-1})	0	9.74 ^{a} ± 0.08	9.73 ^{a} ± 0.08	9.75 ^{a} ± 0.07	9.76 ^{a} ± 0.13
	5	9.75 ^{a} ± 0.05	9.74 ^{a} ± 0.04	9.76 ^{a} ± 0.03	9.77 ^{a} ± 0.05
	10	9.76 ^{a} ± 0.11	9.75 ^{a} ± 0.05	9.77 ^{a} ± 0.28	9.78 ^{a} ± 0.08
Exch Mg ($cmol_c$ kg^{-1})	0	0.49 ^{b} ± 0.08	0.48 ^{b} ± 0.01	0.45 ^{b} ± 0.03	0.44 ^{b} ± 0.11
	5	0.95 ^{ab} ± 0.01	0.94 ^{ab} ± 0.01	0.98 ^{a} ± 0.01	0.97 ^{a}± 0.18
	10	1.03 ^{a} ± 0.05	1.04 ^{a} ± 0.02	1.02 ^{a} ± 0.08	1.01 ^{a} ± 0.20
Exch Ca ($cmol_c$ kg^{-1})	0	0.79 ^{b} ± 0.01	0.78 ^{b} ± 0.11	0.79 ^{b} ± 0.05	0.79 ^{b} ± 0.04
	5	1.01 ^{ab} ± 0.03	1.13 ^{ab} ± 0.01	1.25 ^{ab} ± 0.12	1.27 ^{ab} ± 0.05
	10	1.50 ^{a} ± 0.18	1.52 ^{a} ± 0.15	1.55 ^{a} ± 0.09	1.54 ^{a} ± 0.13
Exch K ($cmol_c$ kg^{-1})	0	0.80 ^{ab} ± 0.04	0.81 ^{ab} ± 0.01	0.82 ^{ab} ± 0.11	0.83 ^{ab} ± 0.02
	5	0.98 ^{a} ± 0.08	0.97 ^{a} ± 0.04	0.96 ^{a} ± 0.14	0.99 ^{a} ± 0.12
	10	1.02 ^{a} ± 0.05	0.99 ^{a} ± 0.05	1.00 ^{a} ± 0.01	1.02 ^{a} ± 0.15
Avail P (mg kg^{-1})	0	6.79 ^{c} ± 0.18	7.08 ^{c} ± 0.11	6.90 ^{c} ± 0.12	6.60 ^{c} ± 0.05
	5	14.21 ^{b} ± 0.20	14.30 ^{b} ± 0.05	14.25 ^{b} ± 0.09	14.19 ^{b} ± 0.09
	10	23.00 ^{a} ± 0.19	23.20 ^{a} ± 0.04	23.50 ^{a} ± 0.15	23.42 ^{a} ± 0.10
Total C (%)	0	1.01 ^{c} ± 0.01	1.96 ^{c} ± 0.05	0.82 ^{a} ± 0.01	1.01 ^{c} ± 0.01
	5	3.19 ^{b} ± 0.18	3.02 ^{b} ± 0.13	0.96 ^{a} ± 0.03	3.19 ^{b} ± 0.06
	10	4.23 ^{a} ± 0.08	4.38 ^{a} ± 0.09	1.00 ^{a} ± 0.01	4.23 ^{a} ± 0.05
Total N (%)	0	0.07 ^{c} ± 0.01	0.07 ^{c} ± 0.03	0.09 ^{b} ± 0.01	0.08 ^{b} ± 0.01
	5	0.28 ^{b} ± 0.08	0.27 ^{b} ± 0.01	0.30 ^{ab} ± 0.11	0.29 ^{ab} ± 0.01
	10	0.48 ^{a} ± 0.04	0.43 ^{a} ± 0.05	0.53 ^{a} ± 0.04	0.52 ^{a} ± 0.11
Zn (mg kg^{-1})	0	18.92 ^{c} ± 0.15	17.38 ^{c} ± 0.12	26.06 ^{c} ± 0.10	24.80 ^{b} ± 0.11
	5	24.89 ^{b} ± 0.05	25.02 ^{b} ± 0.10	31.66 ^{b} ± 0.19	25.45 ^{b} ± 0.05
	10	26.93 ^{a} ± 0.19	28.38 ^{a} ± 0.09	36.68 ^{a} ± 0.07	29.05 ^{a} ± 0.04
Cu (mg kg^{-1})	0	13.17^{a} ± 0.10	7.67 ^{a} ± 0.11	22.34 ^{b} ± 0.18	25.20 ^{ab} ± 0.10
	5	6.09 ^{b} ± 0.12	6.64 ^{ab} ± 0.09	14.88 ^{c} ± 0.21	15.14 ^{b} ± 0.11
	10	5.70 ^{c} ± 0.08	6.20 ^{ab} ± 0.11	28.02 ^{a} ± 0.25	27.01 ^{a} ± 0.21

Means ± standard error of the mean in the same column for the same plant with the same letter were not significantly different at p less than 0.05

The concentrations of Zn and Cu in the soils was affected by the plants growing in both soil compositions, with the rate dependent on the species. For the Oxisol soil compositions, the Zn concentration decreased from 58.45 ± 0.02 mg kg^{-1} to 14.50 ± 0.04 mg kg^{-1}, while the Zn concentration in the Ultisol soil compositions decreased from 58.70 ± 0.05 mg kg^{-1} to 26.93 ± 0.19 mg kg^{-1}. Following the trend for Zn, the Cu concentrations in the Oxisol soil compositions decreased from 48.10 ± 0.08 mg kg^{-1} to 7.02 ± 0.12 mg kg^{-1} and in the Ultisol soil compositions the Cu concentration decreased from 49.70 ± 0.05 mg kg^{-1} to 5.70 ± 0.08 mg kg^{-1}. The decrease in both metals in the soils was due to the uptake by *H. cannabinus* (in Oxisol soil compositions) and *J. curcas* and *H. cannabinus* (in Ultisol soil compositions).

Plant Biomass and the Growth Rate

The results showed that the biomass (leaves, stems, and roots) varied significantly among the plant species and that it increased with the rate of sludge application (Fig. 2).

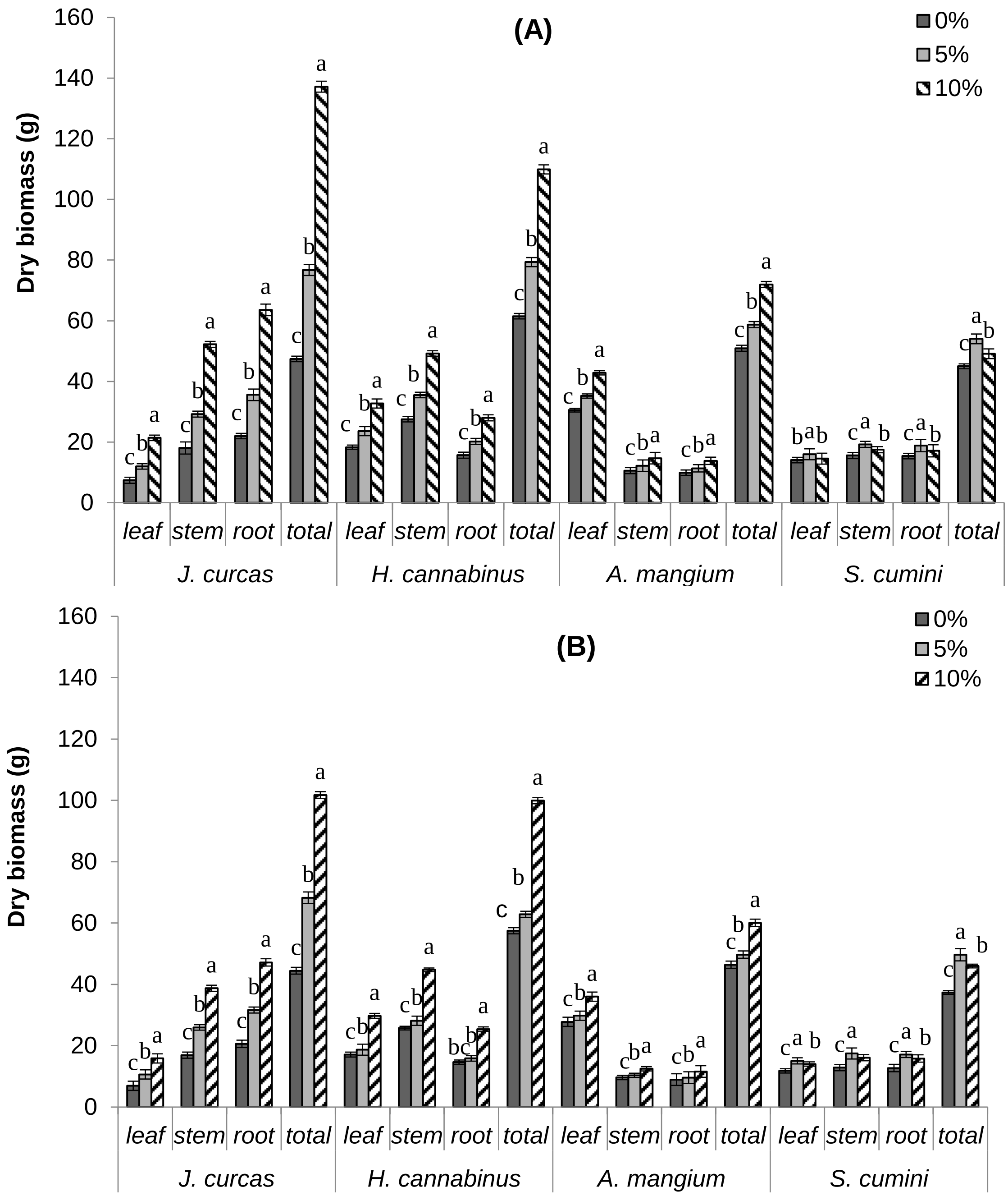


Fig. 2. Effects of the application of sewage sludge on plant biomass: (A) Grown on Oxisol soil compositions; and (B) grown on Ultisol soil compositions. Means with the same letters are not significantly different p less than 0.05.

J. curcas had the largest root biomass in comparison to its stem and leaf, while the largest biomass for *H. cannabinus* was its stem. The leaves of *A. mangium* recorded a larger biomass in comparison to its stems and roots. The growth of the 4 plants was in general enhanced by the sewage sludge treatments, which was especially shown in *J. curcas*, *H. cannabinus*, and *A. mangium*. The results showed a difference among the tested plants in terms of plant biomass. The total plant biomass was 87.13 ± 0.58 g for *J. curcas*, 83.58 ± 0.60 g for H. *cannabinus*, 60.38 ± 0.64 g for *A. mangium*, and 49.37 ± 0.05 g for *S. cumini* when grown in the Oxisol soil compositions (as shown in Table 6). For the Ultisol soil compositions, the total plant biomass was 75.73 ± 0.50 g for *H. cannabinus*, 71.48 ± 0.33 g for *J. curcas*, 52.09 ± 0.58 g for *A. mangium*, and 44.37 ± 0.05 g for *S. cumini*.

Table 6. Total Biomass of Plant Species Grown on Oxisol and Ultisol Soil Compositions

Plant Species	Mean of Plant Biomass (g)	
	Oxisol	Ultisol
J. curcas	87.13 ^{a} ± 0.58	71.48 ^{b} ± 0.33
H. cannabinus	83.58 ^{b} ± 0.60	75.73 ^{a} ± 0.50
A. mangium	60.38 ^{c} ± 0.64	52.09 ^{c} ± 0.58
S. cumini	49.37 ^{d} ± 0.05	44.37 ^{d} ± 0.05

Means ± standard error of the mean in the same column for the same soil with the same letter were not significantly different at p less than 0.05

The growth parameters and the relative growth rate of the tested plants in response to the sewage sludge treatments are shown in Table 7. The application of sewage sludge on both soil compositions resulted in a significant increase in plant height, leaf number, and growth rate, as noted previously (Anten 2004). In this study, the largest value for plant height, leaf number, or growth rate were obtained from the highest rate of sewage sludge application, except for *S. cumini.*

The increase of biomass per unit time is termed as the relative growth rate, which can be applied to discuss the results of the current study (Pulford and Watson 2003). The RGR obtained can be compared among plant species that differed widely in size. In agreement with the results of Houghton *et al.* (2013), the plants in the current study appeared to have a different relative growth rate (Table 7).

The results of this study indicated a variance for the growth rate of the plant species (Table 7). These differences in the RGR were thought to reflect the variation in physiology of the plant species (Anten 2004). The RGR of the tested plants increased with an increase in the rate of sewage sludge application, except for *S. cumini* with a 10% sludge treatment. This difference was due to the physiological characteristics of the plant species. The reason for a reduced RGR for *S. cumini* with the 10% sewage treatment could have been a reduction in some enzymes in the cotyledon and endosperms. *S. cumini* can accumulate metals in their roots (Pant and Tripathi 2012; Khatri and Pathak 2013).

J. curcas and *H. cannabinus* were the species with the highest RGR (Table 8). The maximum RGR of the plants grown on the Oxisol soil composition were as follows; *J. curcas* (0.63 ± 0.67 gDW month^{-1}), *H. cannabinus* (0.37 ± 0.33 gDW month^{-1}), *A. mangium* (0.30 ± 0.67 gDW month^{-1}), and *S. cumini* (0.21 ± 0.15 gDW month^{-1}). The same trend was observed for the plant growth in Ultisol soil compositions with *J. curcas* (0.50 ± 0.03gDW month^{-1}), *H. cannabinus* (0.33 ± 0.03gDW month^{-1}), *A. mangium* (0.24 ± 0.13gDW month^{-1}), and *S. cumini* (0.15 ± 0.05 gDW month^{-1}). The findings of this study showed that *J. curcas* and *H. cannabinus* both grew faster than *A. mangium* or *S. cumini*, which means that they have the desired characteristics as suitable candidates for phytoremediation purposes.

Table 7. Effects of Sewage Sludge Treatment on Growth of Plant Species

Soil type	Plant species	Treatments (%)	Plant height (cm)	Leaf number	Mean of RGR (gDW month^{-1})
Oxisol	*J. curcas*	0	64.00 ^{c} ± 1.09	56.00 ^{c} ± 1.20	0.20 ^{c} ± 0.01
		5	73.00 ^{b} ± 0.53	68.00 ^{b} ± 1.30	0.35 ^{b} ± 0.03
		10	80.20 ^{a} ± 1.10	75.00 ^{a} ± 2.01	0.62 ^{a} ± 0.02
	H. cannabinus	0	140.00 ^{c} ± 0.67	47.00 ^{c} ± 0.99	0.18 ^{c} ± 0.02
		5	155.00 ^{b} ± 0.39	55.00 ^{b} ± 1.20	0.24 ^{b} ± 0.01
		10	158.00 ^{a} ± 0.67	67.00 ^{a} ± 1.18	0.36 ^{a} ± 0.02
	A. mangium	0	76.00 ^{c} ± 0.34	37.00 ^{c} ± 0.20	0.12 ^{c} ± 0.01
		5	123.00 ^{b}± 0.56	39.00 ^{b} ± 0.60	0.21 ^{b} ± 0.03
		10	125.00 ^{a} ± 1.20	48.00 ^{a} ± 0.68	0.30 ^{a} ± 0.05
	H. cannabinus	0	95.30 ^{c} ± 0.53	48.00 ^{b} ± 0.64	0.12 ^{c} ± 0.04
		5	124.20 ^{a} ± 0.25	54.00 ^{a} ± 0.70	0.21 ^{a} ± 0.02
		10	99.00 ^{b} ± 1.10	50.00 ^{b} ± 1.20	0.18 ^{b} ± 0.01
Ultisol	*J. curcas*	0	62.20 ^{c} ± 0.90	55 .00^{c} ± 1.30	0.17 ^{c} ± 0.01
		5	76.30 ^{b} ± 1.02	65.00 ^{b} ± 1.70	0.27 ^{b} ± 0.01
		10	79.00 ^{a} ± 2.16	73.00 ^{a} ± 0.99	0.50 ^{a} ± 0.03
	H. cannabinus	0	130.10 ^{c} ± 1.11	34.00 ^{b} ± 0.56	0.16 ^{c} ± 0.01
		5	153.5 ^{b} ± 0.53	36.00 ^{b} ± 0.24	0.22 ^{b} ± 0.01
		10	156 ^{a} ± 1.16	46.00 ^{a} ± 0.75	0.33 ^{a} ± 0.02
	A. mangium	0	78.50 ^{c} ± 0.09	34.00 ^{b} ± 0.44	0.11 ^{c} ± 0.01
		5	130.00 ^{b} ± 2.17	36.00 ^{b} ± 0.46	0.18 ^{b} ± 0.01
		10	155.50 ^{a} ± 1.12	46.00 ^{a} ± 0.53	0.25 ^{a} ± 0.01
	H. cannabinus	0	92.80 ^{c} ± 0.67	44.00 ^{b} ± 0.84	0.10 ^{c} ± 0.01
		5	119.50 ^{a} ± 0.53	53.00 ^{a} ± 0.68	0.22 ^{a} ± 0.03
		10	97.50 ^{b} ± 0.39	47.00 ^{b} ± 0.34	0.15 ^{b} ± 0.02

RGR= Relative growth rate; (gDW month^{-1}) = dry weight gram month^{-1}.
Means ± standard error of the mean within the column that have the same letter were not significantly different (P less than 0.05).

Table 8. Relative Growth Rate (RGR) of Plants Grown in Oxisol and Ultisol Soil Compositions

Plant species	Oxisol	Ultisol
	Mean of RGR (gDW month^{-1})	
J. curcas	0.63 ^{a} ± 0.67	0.50 ^{a} ± 0.03
H. cannabinus	0.37 ^{b} ± 0.33	0.33 ^{b} ± 0.03
A. mangium	0.30 ^{c} ± 0.67	0.24 ^{c} ± 0.13
S. cumini	0.21 ^{d} ± 0.15	0.15 ^{d} ± 0.05

Means ± standard error of the mean within the column that have same letter are not significantly different (P less than 0.05)

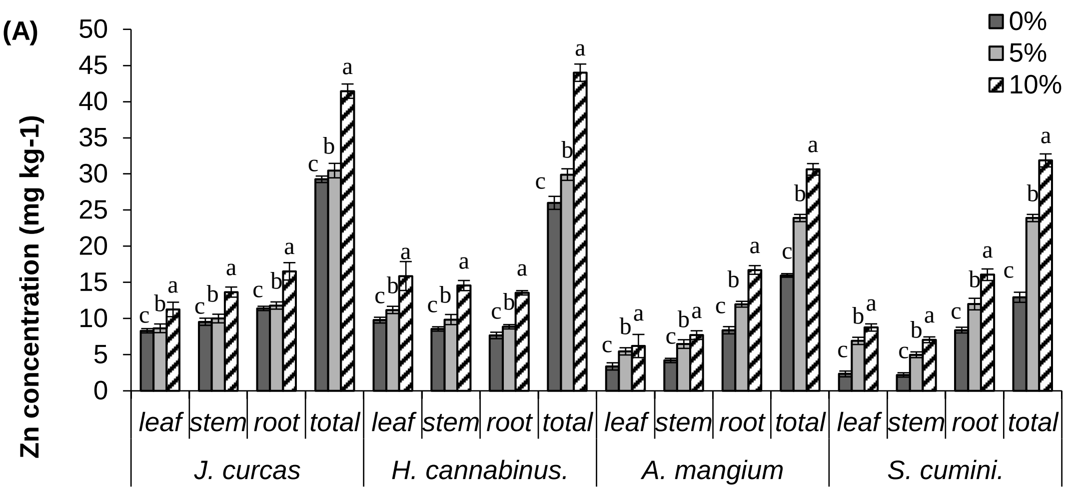
Heavy Metals in Plant Parts

The concentration of Zn in the whole tissue of the plants was as follows; *H. cannabinus* (44.00 ± 1.01 mg kg^{-1}), *J. curcas* (41.45 ± 0.05 mg kg^{-1}), *S. cumini* (31.87 ±

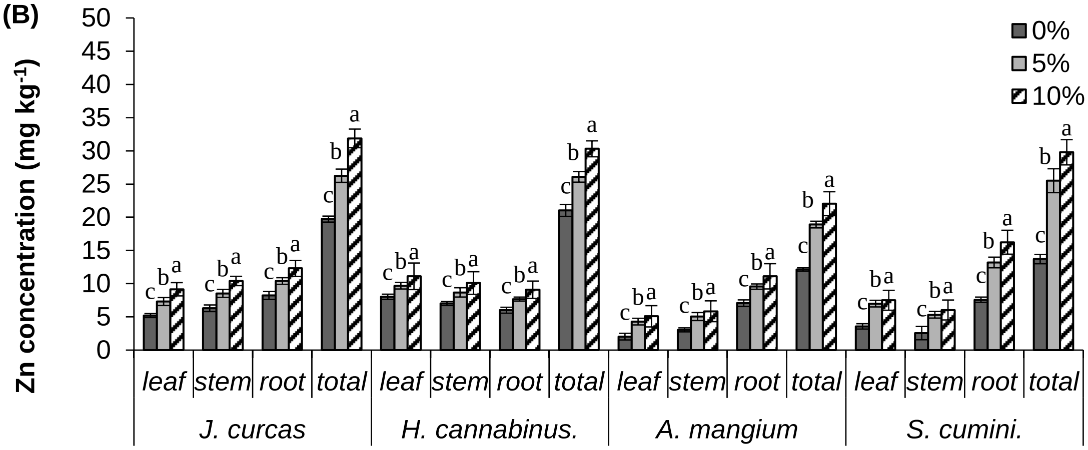
0.09 mg kg^{-1}), and *A. mangium* (30.63 ± 0.06 mg kg^{-1}), while for the case of Cu it was; *J. curcas* (44.10 ± 0.09 mg kg^{-1}), *H. cannabinus* (43.04 ± 0.04 mg kg^{-1}), *A. mangium* (35.57 ± 0.07 mg kg^{-1}), and *S. cumini* (35.36 ± 0.05 mg kg^{-1}) (Fig. 3). An earlier study by Kumari *et al.* (2016) found a similar trend in results. The difference in the mobility was probably due to the processes taking place at the soil-root interface, as explained fully by Pulford and Watson (2003), who found variation of heavy metals among various plant tissues.

The results showed that the amount of each metal in the leaf, stem, and root tissues was dependent on the tolerance of each plant species. The zinc concentration in the different plant parts varied from species to species. The plant roots absorb and uptake metals from contaminated soils then transfer them to the plant shoots where the metals accumulate (Jadia and Fulekar 2009). For the Oxisol soil compositions, the Zn content was the highest in the leaves of *H. cannabinus*, followed by *J. curcas*, *A. mangium*, and *S. cumini*, but for the Ultisol soil compositions, the stems of *J. curcas* had the highest Zn content, followed by *H. cannabinus*, *S. cumini*, and *A. mangium*. The Zn concentration in the roots of the plant grown in Oxisol soil compositions was found to be higher in *A. mangium* than *S. cumini*, *J. curcas*, and *H. cannabinus*. However, when the plants were grown in Ultisol soil compositions, the roots of *S. cumini* contained higher Zn concentrations than *J. curcas*, *A. mangium*, and *H. cannabinus* (Fig. 3).

(A)



(B)



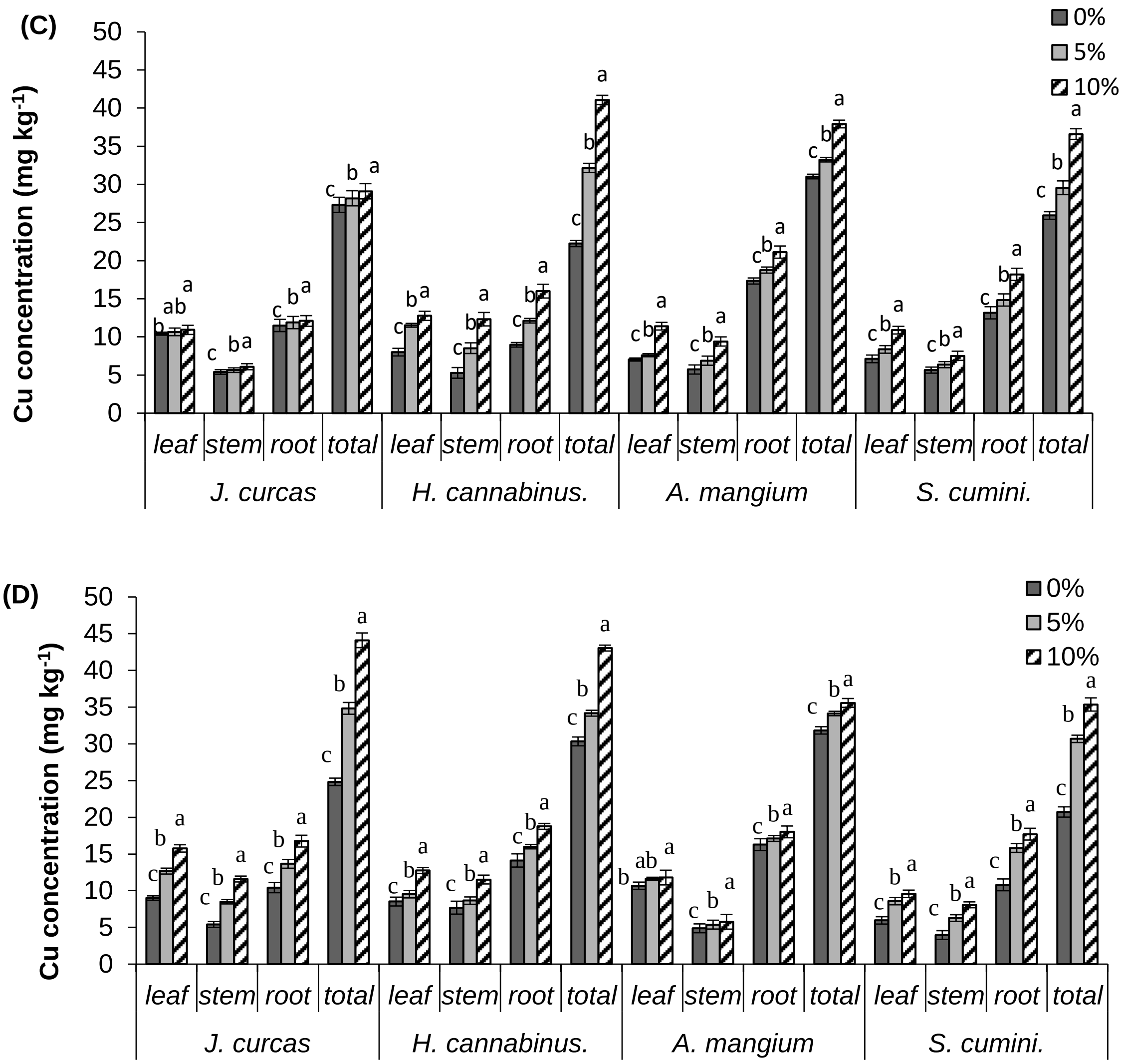


Fig. 3. Effects of sewage sludge application on Zn and Cu in plant species: (A) Zn content in the plants grown on Oxisol soil compositions; (B) Cu content in the plants grown on Oxisol soil compositions; (C) Zn content in the plants grown on Ultisol soil compositions; and (D) Cu contents in the plants grown on Ultisol soil compositions. Means with the same letters are not significantly different at p less than 0.05.

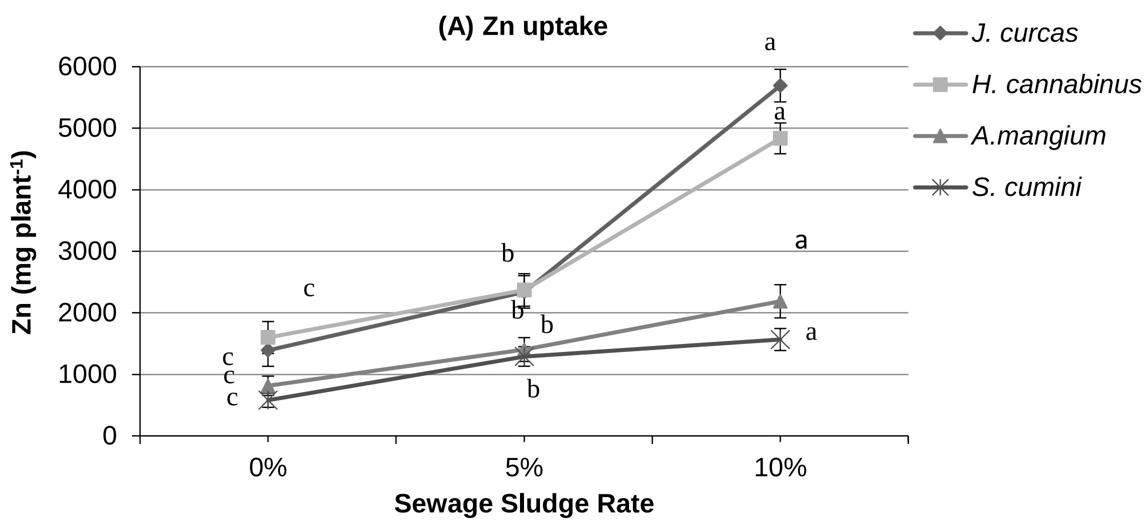
The concentration of Cu in the leaves of *H. cannabinus* grown in Oxisol soil compositions was found to be higher when compared to the leaves of *J. curcas*, *A. mangium*, and *S. cumini*. In contrast, the plants grown in Ultisol soil compositions had a higher Cu concentration in the leaves of *J. curcas* compared to that of *H. cannabinus*, *A. mangium*, and *S. cumini*. In the roots of the plants grown in Oxisol soil compositions, the Cu content in *A. mangium* was higher compared to *S. cumini*, *H. cannabinus*, and *J. curcas*. For the Ultisol soil compositions, the highest Cu concentrations were found in the roots of *H. cannabinus* (Fig. 3). These results were found to be similar to those obtained by Vaitkutė *et al.* (2010) who found that tree species grown in sludge amended soils had the highest concentration of Cu and Zn in their roots.

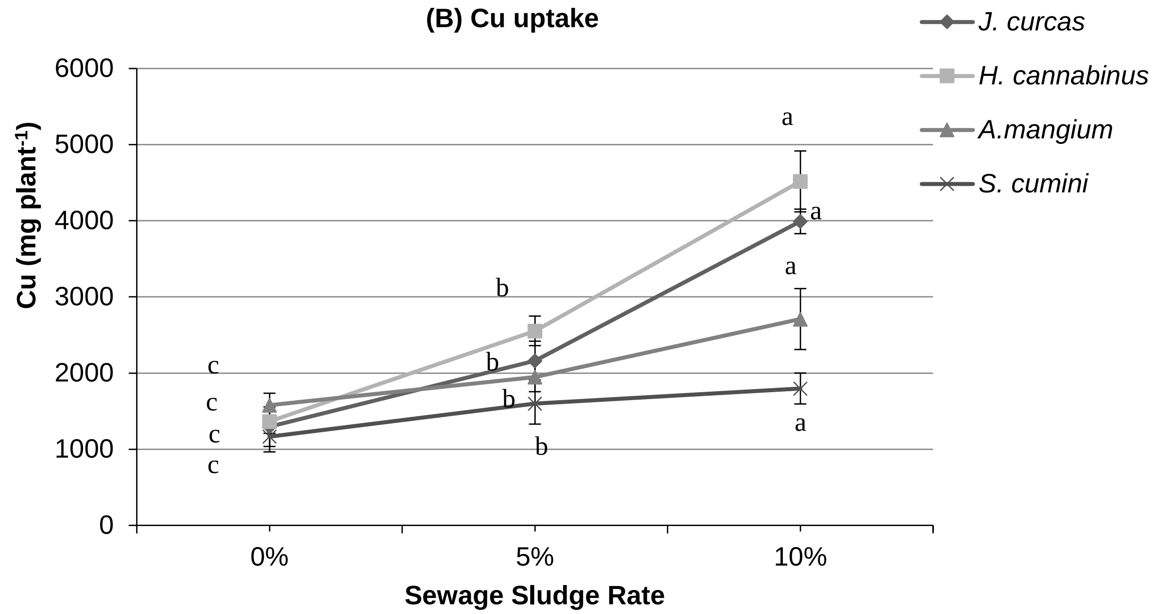
Plant species differ in their ability to uptake Zn and Cu (Fig. 4). The highest uptake values were obtained from the plants grown on soils treated with 10% sewage sludge. The results suggest that *J. curcas* and *H. cannabinus* had the potential to uptake a high amount of Zn and Cu from the sewage sludge treated soils (as shown in Table 9). A high Zn uptake was found in *J. curcas* (3141.37 ± 5.77 mg $plant^{-1}$), while a high Cu uptake was found in *H. cannabinus* (2829.00 ± 0.05 mg plant $^{-1}$). Accordingly, *J. curcas* can be cosidered a Zn hyperaccumlator and *H. cannabinus* can be considered a Cu hyperaccumulator.

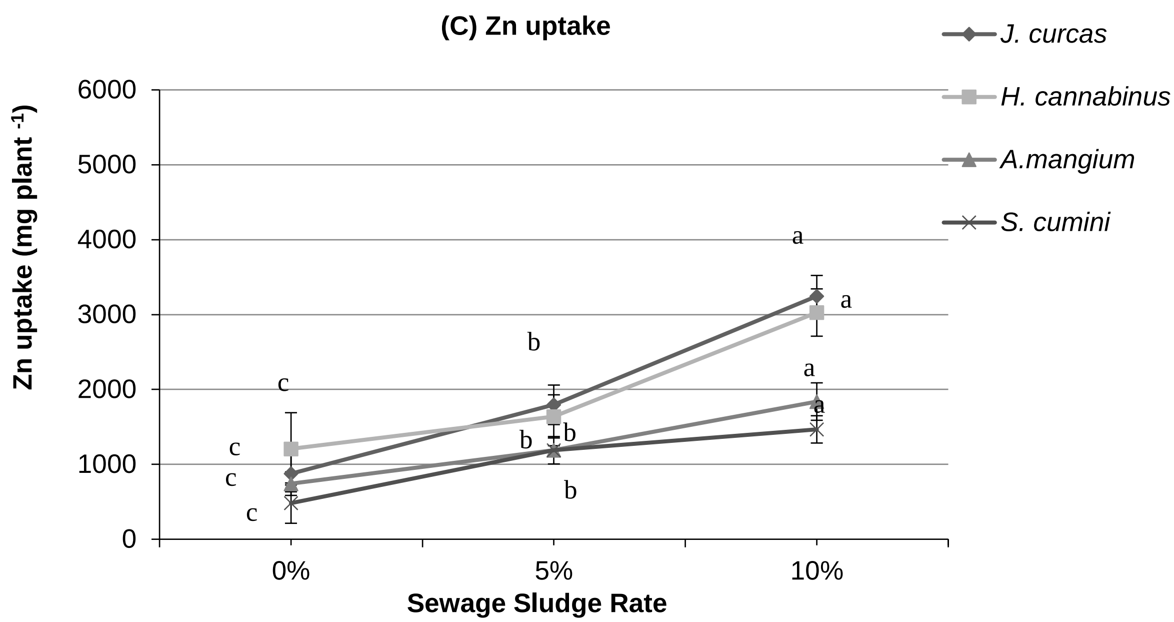
Table 9. Uptake of Zinc and Copper in Different Plant Species

Type of Soil	Plant Species	Zn Uptake (mg plant $^{-1}$)	Cu Uptake (mg plant $^{-1}$)
Oxisol	*J. curcas*	3141.37 ^{a} ± 5.77	2483.89 ^{b} ± 2.73
	H. cannabinus	2935.52 ^{b} ± 0.06	2812.21 ^{a} ± 2.72
	A. mangium	1468.57 ^{c} ± 0.06	2079.16 ^{c} ± 1.73
	S. cumini	1145.97 ^{d} ± 0.01	1520.47 ^{d} ± 0.06
Ultisol	*J. curcas*	1971.96 ^{a} ± 0.06	2654.61 ^{b} ± 0.06
	H. cannabinus	1958.13 ^{b} ± 0.06	2829.04 ^{a} ± 0.05
	A. mangium	1256.48 ^{c} ± 0.56	1769.82 ^{c} ± 0.06
	S. cumini	1045.31 ^{d} ± 0.58	1308.60 ^{d} ± 0.06

Means ± standard error of the mean in the same column for the same soil with the same letter are not significantly different at p less than 0.05.




(B) Cu uptake
J. curcas
H. cannabinus
A.mangium
S. cumini
Cu (mg plant-1)
6000
5000
4000
3000
2000
1000
0
0%
5%
10%
Sewage Sludge Rate


(C) Zn uptake
J. curcas
H. cannabinus
A.mangium
S. cumini
Zn uptake (mg plant -1)
6000
5000
4000
3000
2000
1000
0
0%
5%
10%
Sewage Sludge Rate

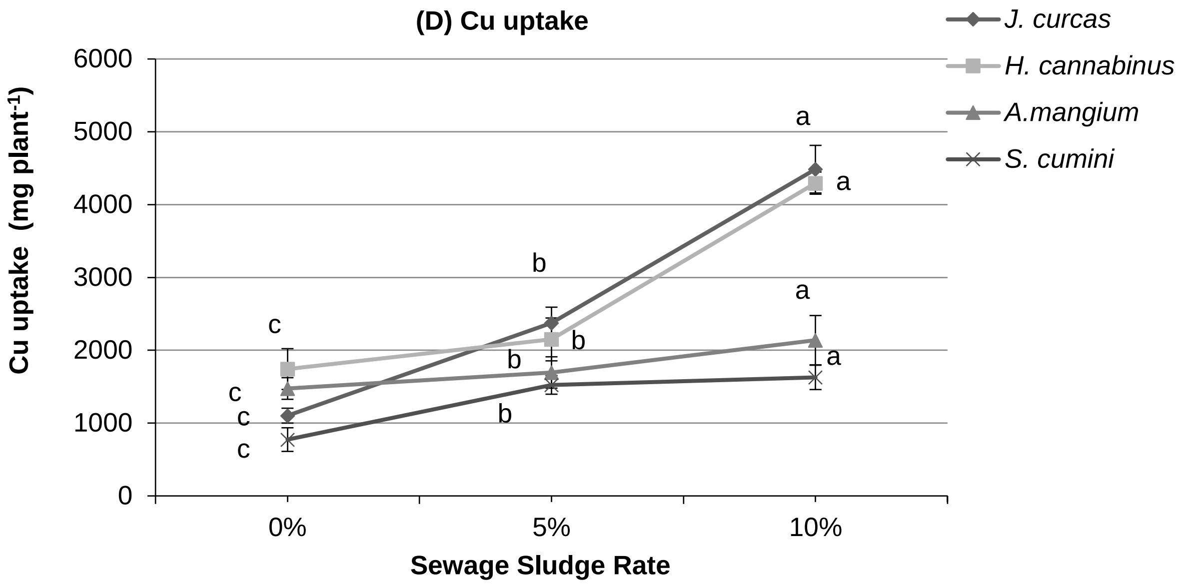
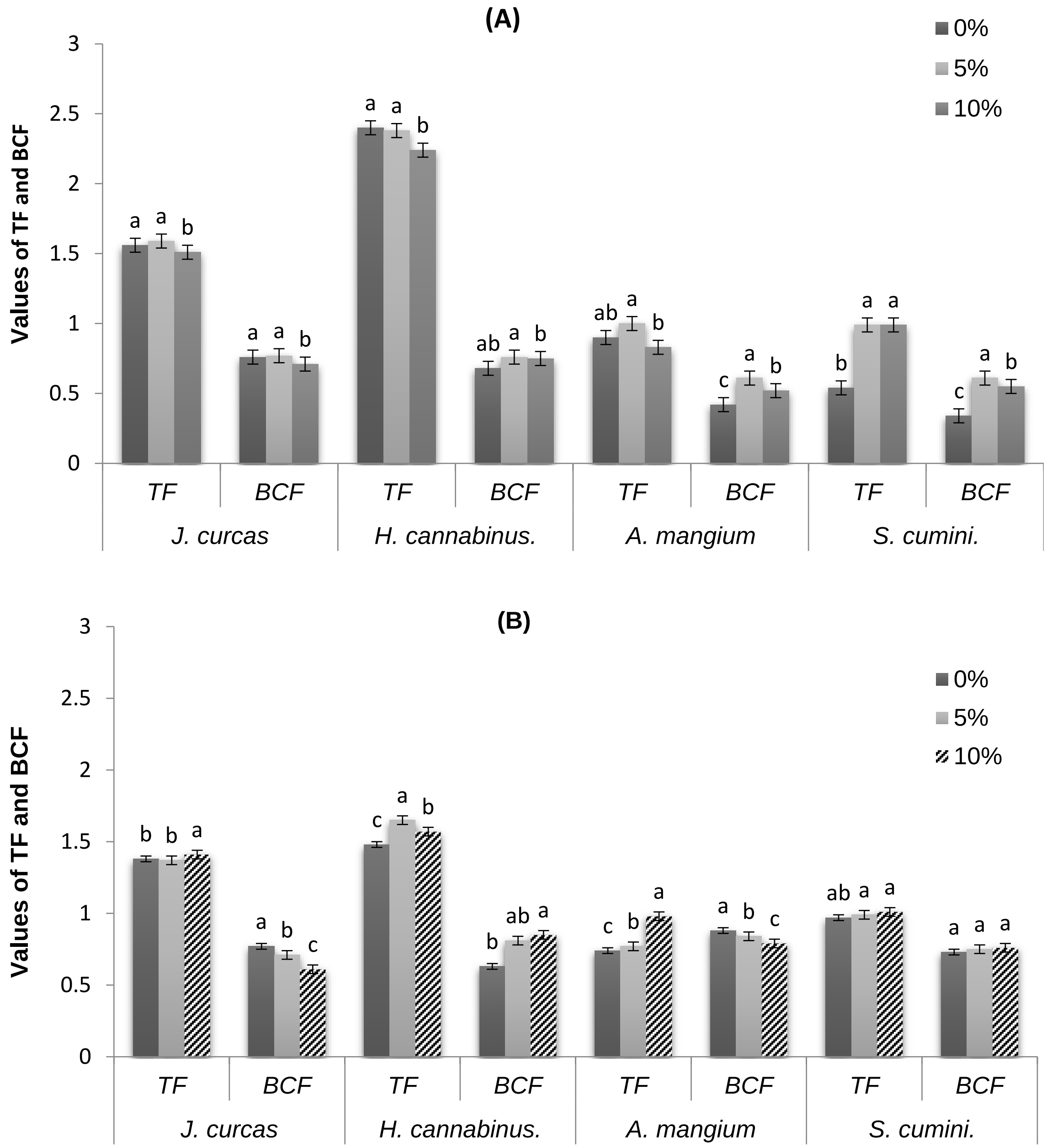


Fig. 4. Effects of the Application of Sewage Sludge on the uptake of Zn and Cu in Different Plant Species: (A) Zn uptake by the plants grown in Oxisol soil compositions; (B) Cu uptake by the plants grown in Oxisol soil compositions; (C) Zn uptake by the plants grown in Ultisol soil compositions; and (D) Cu uptake by the plants grown on Ultisol soil compositions. Means with the same letters are not significantly different at p less than 0.05.

Translocation Factor and Bio-concentration Factor

There were significant differences among the plant species in their ability to translocate Zn and Cu into the shoots and their efficiency in accumulating heavy metals (Fig. 5; Table 10).

J. curcas and *H. cannabinus* had a TF of more of than 1, while *A. mangium* and *S. cumini* had values less of than 1. Hence, it could be assumed that *J. curcas and H. cannabinus* had the potential for being phytoremediators since both had a TF greater than 1. According to Wei and Chen (2006), plant species with a translocation factor value greater than 1 had the ability to uptake heavy metals from the soil and accumulate them in their shoots.


(A)
Values of TF and BCF
0%
5%
10%
TF
BCF
J. curcas
H. cannabinus.
A. mangium
S. cumini.
(B)

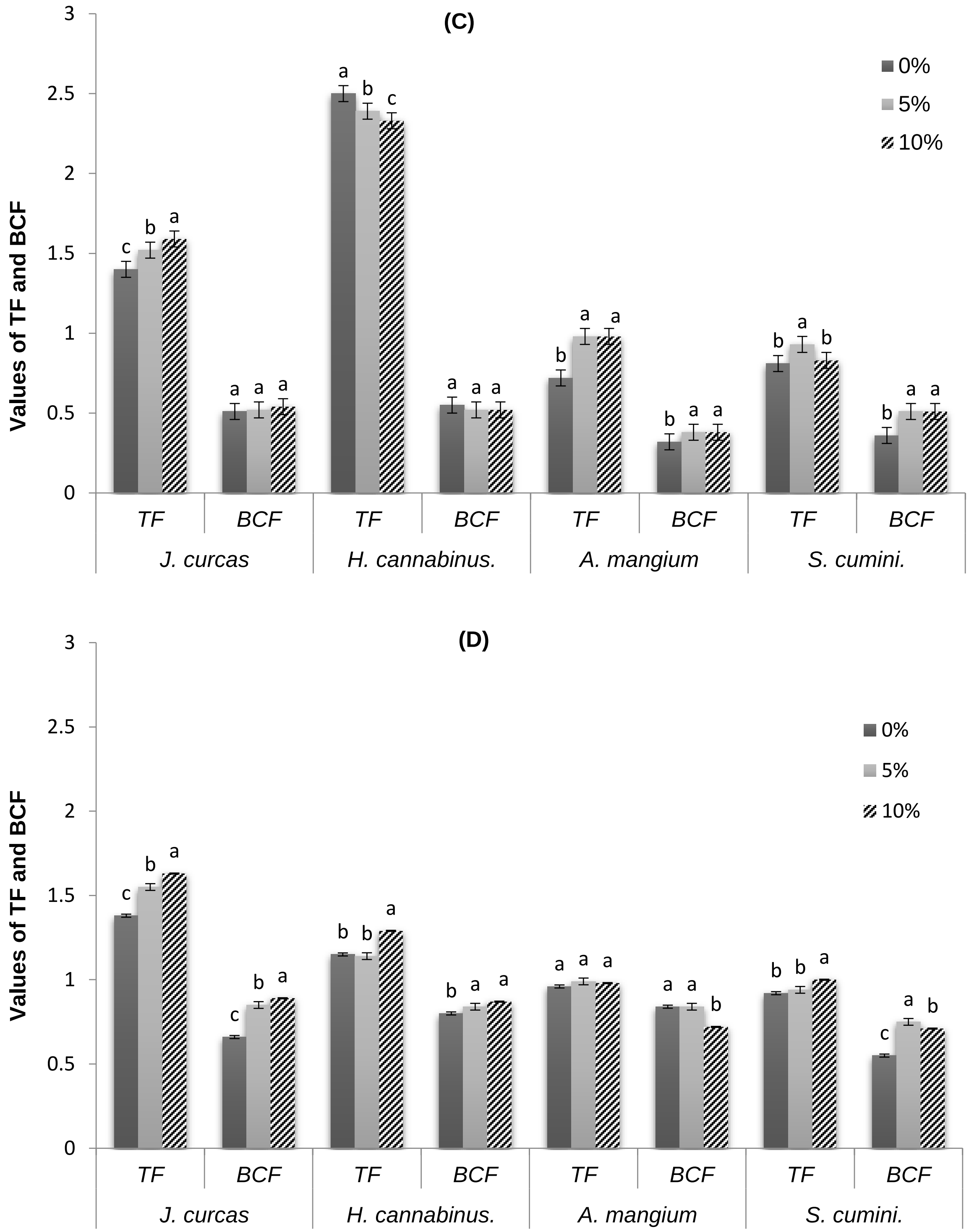


Fig. 5. Effect of the application of sewage sludge application on the TF and BCF values: (A) TF and BCF values for Zn of the plants grown in Oxisol soil compositions; (B) TF and BCF values for Cu of the plants grown in Oxisol soil compositions; (C) TF and BCF values for Zn of the plants grown in Ultisol soil compositions; and TF and BCF values for Cu of the plants grown in Ultisol soil compositions. Means with the same letters are not significantly different at p less than 0.05.

Table 10. TF and BCF for Zn and Cu of the Tested Plant Species

Type of Soil	Plant Species	TF		BCF	
		Zn	Cu	Zn	Cu
Oxisol	*J. curcas*	1.49 ^{b} ± 0.01	1.52 ^{a} ± 0.02	0.75 ^{a} ± 0.01	0.70 ^{b} ± 0.01
	H. cannabinus	2.42 ^{a} ± 0.02	1.19 ^{b}± 0.03	0.73 ^{a} ± 0.01	0.76 ^{ab} ± 0.01
	A. mangium	0.89 ^{c} ± 0.01	0.97 ^{c} ± 0.02	0.52 ^{b} ± 0.01	0.84 ^{a} ± 0.02
	S. cumini	0.86 ^{c} ± 0.02	0.96 ^{c} ± 0.01	0.50 ^{b} ± 0.01	0.75 ^{ab} ± 0.01
Ultisol	*J. curcas*	1.50 ^{b} ± 0.06	1.52 ^{a} ± 0.02	0.52 ^{a} ± 0.01	0.80 ^{ab} ± 0.02
	H. cannabinus	2.37 ^{a} ± 0.07	1.19 ^{b} ± 0.01	0.53 ^{a} ± 0.02	0.84 ^{a} ± 0.01
	A. mangium	0.89 ^{c} ± 0.01	0.96 ^{bc} ± 0.06	0.36 ^{b} ± 0.01	0.80 ^{ab} ± 0.01
	S. cumini	0.86 ^{c} ± 0.01	0.89 ^{c} ± 0.05	0.46 ^{ab} ± 0.01	0.67 ^{b} ± 0.01

TF = Translocation factor; BCF= Bio-concentration factor
Means ± standard error of the mean in the same column for the same soil with the same letter are not significantly different at p less than 0.05

H. cannabinus had the highest TF value for Zn (2.42 ± 0.02) in comparison to that of the *J. curcas, A. mangium*, and *S. cumini*. This meant that *H. cannabinus* was the best plant species to be used as a phytoaccumulator for Zn due to its ability to translocate Zn to its shoots. On the other hand, *J. curcas* produced the highest TF value for Cu (1.52 ± 0.02) in comparison to that of the other plant species. Thus, it can be assumed that *J. curcas* was the best plant species to be used as a phytoaccumulator for Cu.

A. mangium and *S. cumini* had TF values of less than 1. For these plant species, the translocation mechanism for Zn and Cu was probably controlled *via* metal transport proteins, especially phytochelatins or metalothioneins, and not passively by diffusion through cell membranes. The Zn and Cu transported through the cell membrane was restricted to avoid toxicity (Kramer *et al.* 2007). The BCF for Zn and Cu was less than 1 for all the tested plant species, indicating that they had no ability to store high amounts of Zn or Cu in their roots. However, *J. curcas* and *H. cannabinus* had a high TF, which was a characteristic of heavy metal tolerant plants.

Impact of the Application of Sewage Sludge on the Environment

This study clearly showed that the chemical properties of the soils were affected by the application of sewage sludge, shown by the increase in soil pH from 5.36 ± 0.01 to 5.84 ± 0.02 for Oxisol soil compositions and from 4.77 ± 0.02 to 5.37 ± 0.01 Ultisol soil compositions (from 4.77 ± 0.02 to 5.37 ± 0.01) (Table 3). The increase in soil pH above 5 precipitates Al^{3+} in the soil solution as inert Al-hydroxides (Shamshuddin *et al.* 1991); thus, it would no longer affect crop production. In addition, the application of sewage sludge into the soils increased their CEC, exchangeable bases (K, Ca, and Mg), available P, total C, and total N. The increase in CEC of both soils was related to the soil pH increase due to the sludge treatment. According to Shamshuddin and Ismail (1995), the CEC of soil containing variable-charged minerals (goethite and hematite, which were present in both soils) increased with an increase in soil pH. Overall, this study indicated that the environmental conditions for crop production on the Oxisol and Ultisol soil compositions under study had somewhat improved.

However, on closer examination of the results, the authors believe that the continuous application of sewage sludge in the long run would accumulate Zn and Cu to toxic levels. This notion was confirmed by the study of Bettiol and Ghini (2011).

The increase in the heavy metals was probably due to the presence of high organic matter in the sewage sludge (Clemente *et al.* 2003). The concentration of Zn and Cu in the Ultisol soil compositions was higher compared to the Oxisol soil compositions, which was related to the higher CEC of the former soil compositions. The cation exchange capacity plays an important role in the adsorption of heavy metals in soils (Shaheen *et al.* 2014). Some of the Zn and Cu released by the sewage sludge could have been adsorbed by goethite and hematite; therefore, they were retained in the soils becoming harmful to crops in the long run.

Removal of Zn and Cu *via* Phytoremediators

The Zn and Cu concentration in the soils were affected by the change in pH. The soil pH was found to decrease due to the plant species growing on the treated soils. The author's results given in Tables 4 and 5 showed that the soil pH was reduced during the growing period from 5.84 ± 0.02 to 5.65 ± 0.05 in the Oxisol and in Ultisol soil compositions it was decreased from 5.37 ± 0.01 to 4.88 ± 0.18. Therefore, the availability and uptake of heavy metals by the roots increased (Ali *et al.* 2013). The soil pH plays an important role in the increased availability and uptake of Zn and Cu by plant roots and their transportation into the shoot system. Csavina *et al.* (2012) found that the solubility of heavy metals was greater as the soil pH decreased, resulting in an increase in the concentration of Zn and Cu in the soil solution. The plant species with a high TF were able to remove the Zn and Cu from the contaminated soils and efficiently transport to their shoots.

Effects of the Application of Sewage Sludge on Plant Biomass and the Growth Rate

The application of sewage sludge stimulated the growth of the plant species, which was indicated by an increase in their biomass (as shown in Fig. 2, Tables 6 and 7). The biomass of the plants was enhanced by the availability of the extra nutrients released by the decomposing sludge, which was evident in *J. curcas*, *H. cannabinus*, and *A. mangium*.

There was a slight difference in the average plant biomass between the plants grown on Oxisol soil compositions and those grown on Ultisol soil compositions. The plants grown on Oxisol soil compositions had a higher biomass compared to those grown on Ultisol soil compositions (as shown in Table 6). The result showed a difference among the treatments in terms of plant biomass. The increase in biomass of the four tested plant species could be due to the improvement of soil fertility, resulting from the application of sewage sludge. The nutrients in the sewage sludge improved the plant growth *via* the absorption of the released micro- and macronutrients, which were able to overcome the adverse effects of the excess Zn and Cu in the soils. These findings indicated that the sewage sludge has a potential as an organic fertilizer provided that the excess Zn and Cu can be removed from the agricultural soils (Khan *et al.* 2013).

The RGR is an index of seedling growth (Pulford and Watson 2003). There was an increase in the biomass of the plants grown on the soils amended with sewage sludge. This study indicated that there was variation on the RGR among the treatment and species (as shown in Table 7). It was noted that the RGR of the plant species was increased with an increased application of sewage sludge, indicating that they were able to tolerate soils contaminated with sewage sludge (Crisóstomo *et al.* 2007).

J. curcas and *H. cannabinus* grew faster and larger than *A. mangium* and *S. cumini*, indicative of the desired characteristics for phytoremediation over the former (as shown in Table 8). According to Woodbury (1993), applying sewage sludge onto soil has some benefits, such as an improvement in plant growth rate due to the high nutrients in the sewage sludge.

Influence of Sewage Sludge on Heavy Metals in the Tested Plants

A higher content of Zn and Cu was found in the plants grown in the soils treated with sewage sludge compared to those without treatment (as shown in Fig. 3). This result indicated that the sewage sludge was very effective in increasing the mobility of the Zn and Cu into the plant roots. This means that the application of sewage sludge increased the uptake Zn and Cu by the plants. The increased uptake of Zn and Cu leading to the accumulation in the shoots and roots of the plants can be related to the change in soil pH and the dissolved organic carbon found in the soils after the treatment with sewage sludge (Bada and Kalejaiye 2010). A higher Zn concentration was found in *H. cannabinus* (44.00 mg kg $^{-1}$) and a higher Cu concentration was found in *J. curcas* (44.10 mg kg $^{-1}$) compared to the other plant species. *H. cannabinus* and *J. curcas* accumulated a high Zn and Cu concentration, respectively, without any visual phytotoxic symptoms, indicating the existence of internal detoxification mechanisms in the plant species. The present study suggested that *H. cannabinus* and *J. curcas* were suitable for the revegetation of heavy metals contaminated soils and for decontamination purposes.

Uptake of Zinc and Copper

The tested plants differed greatly in their Zn and Cu uptake levels, as well as their accumulating characteristics. The heavy metal uptake by the plants is shown in Fig. 4. It was observed that the ability of the plants to uptake Zn and Cu was directly proportional to the biomass of the plants which, in turn, was dependent on the application rate of the sewage sludge. Treating the soils with sewage sludge greatly affected the uptake of Zn and Cu by the plants; a higher uptake of Zn and Cu was found by the plants grown in the soils treated with sewage sludge when compared to soils without. From these results, it is possible to infer the capacity of the sewage sludge to supply Zn and Cu to the tested plants. These findings indicated that the application of sewage sludge stimulated the uptake of Zn and Cu due to the increased biomass of the plants.

The uptake of the metals varied greatly among the plant species (Table 9). The results suggest that *J. curcas* and *H. cannabinus* had the potential to remove Zn and Cu from the treated soils, as shown by their high uptake levels. The highest Zn uptake value was found by *J. curcas* (3141.37 ± 5.77 mg plant^{-1}), while the highest Cu uptake level was found by *H. cannabinus* (2829.00 ± 0.05 mg plant $^{-1}$). This fast and high uptake of the heavy metals would result in shorter and, therefore, less expensive remediation periods. Hence, *J. curcas* and *H. cannabinus* can be considered as Zn and Cu hyperaccumulators, which can effectively be used to remediate contaminated soils.

Plants have several transport mechanisms to uptake heavy metals from soil. Zinc and copper uptake by the plants is dependent on the nature of the metals, soil physico-chemical properties, as well as the plant species. As such, Zn and Cu bioavailability is the basic prerequisite for its uptake by plants, where the roots compete with the soil particles for the uptake of the metals (Nouri *et al.* 2009).

Phytoremediation Efficiency

This study has shown a difference in the ability of plants to translocate Zn and Cu to the shoots and the efficiency of plants to accumulate heavy metals from the soil, based upon the species (Fig. 5; Table 10). There were differences among the tested plants based on the TF values; the Zn TF value for *J. curcas* and *H. cannabinus* was higher compared to the TF values of *A. mangium* and *S. cumini.* This had demonstrated that *J. curcas and H. cannabinus* had a greater ability to translocate Zn and Cu compared to *A. mangium* and *S. cumini.* This result was similar to the result of a previous study by Kumari *et al.*

(2016).

The zinc and copper BCF value was less than 1 for all treatments (Fig. 5), which was consistent with the findings of Bech *et al.* (2012). These researchers believed that plants varied in their tolerance to heavy metals toxicity. Many factors can affect the value of BCF, such as the concentration of heavy metals in the soils, the accumulative ability, and physiology of plant species (Niu *et al.* 2007).

Among the four tested plant species, *J. curcas and H. cannabinus* had a high TF value (greater than 1) and a low BCF value (less than 1), indicating that these plants were phytoaccumulators of Zn and Cu, which agreed with the study of Yoon *et al.* (2006) who found that plants with high TF and low BCF values were good phytoaccumulators for heavy metals present in contaminated soils.

The translocation factor (TF) and bio-concentration factor (BCF) for *A. mangium and S. cumini* were almost always less than 1. This means that the metal bioaccumulation phenomenon did not take place in *A. mangium and S. cumini*. *A. mangium* and *S. cumini* were found to be inappropriate for phytoremediation purpose as shown by their low TF value (less than 1) (Ali *et al.* 2013).

Furthermore, if enough biomass is accumulated, it will decompose under a controlled environment. The biodegraded materials (compost) will be used for a glasshouse experiment to determine its effects on soil and the growth of a crop (*e.g.* corn). If the biomass really contained excessive amounts of the metals taken up from the treated sewage sludge, the growth of the crop will be affected. Such being the case, the plant biomass has to be discarded and cannot be used for agriculture.

CONCLUSIONS

1. This study showed that the application of sewage sludge has the ability to improve the fertility of the poor highly weathered soils in the tropics.
2. The enhancement of soil fertility was evident by the increase in soil pH, CEC, exchangeable bases, available P, total C, and total N, which resulted from the application of sewage sludge.
3. However, the excess availability of Zn and Cu in the soils is a negative side effect of using sewage sludge. The excess Zn and Cu in the soils with sewage sludge applied can be effectively removed *via* phytoremediators, such as *J. curcas* and *H. cannabinus*.
4. Both plant species have the potential for being suitable phytoremediators, since both have a translocation factor (TF) value greater than 1, which indicates that the species are able translocate high amount of Zn and Cu to the shoots.
5. Thus, *J. curcas* and *H. cannabinus* are recommended to be the suitable candidates for the phytoremediation of Oxisol and Ultisol soil compositions contaminated with Zn and Cu due to the application of sewage sludge. Note that a requirement for a tree species to be a candidate for suitable phytoremediation techniques is to be fast growing. Such being the case, woody species are a good choice because they grow well even under harsh conditions and produce a large amount of biomass within a short time.

ACKNOWLEDGMENTS

The authors acknowledge Universiti Putra Malaysia for financial and technical support during the conduct of the research.

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Article submitted: May 9, 2019; Peer review completed: August 16, 2019; Revised version received and accepted: August 20, 2019; Published: September 18, 2019.
DOI: 10.15376/biores.14.4.8701-8727