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METAL UPTAKE IN WATER SPINACH GROWN ON CONTAMINATED SOIL AMENDED WITH CHICKEN MANURE AND COCONUT TREE SAWDUST

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Abstract

A pot experiment was conducted to evaluate the growth and metal accumulation in water spinach (*Ipomoea aquatica*) established on a 30-year old active firing range soil amended with chicken manure (CM) and coconut tree sawdust (CTS) at application rates of 0%, 1% and 3% (w/w). Both amendments increased biomass yield and reduced plant metal uptake. The bioconcentration factor (BCF) and transportation factor (TF) values of the metals were in the order of Zn > Cu > Pb. The ammonium acetate extractable metals in soil decreased significantly ($p < 0.05$) following CM and CTS treatments. It was estimated that the off-take value of Zn could be reduced from 10.01 kg/ha (zero treatment) to 6.60 kg/ha (CM 3% w/w) and 3.17 kg/ha (CTS 3% w/w). No toxicity symptoms were observed in water spinach over the pot experiment. Therefore, chicken manure and coconut tree sawdust are two promising agents for immobilizing heavy metals in contaminated land.

Key words: chicken manure, coconut tree sawdust, contaminated soil, heavy metals, soil stabilization, water spinach

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1. Introduction

Soil contamination by heavy metals is a worldwide environmental problem. For example, in 2005 metals were the main contaminants affecting 6000 sites in Denmark (Jensen et al., 2009). Meanwhile, Sun et al. (2009) reported that about 14,000,000 ha sites in China were contaminated by heavy metals, particularly As and Cd. Besides their natural occurrence in the parent rocks, specific sources of heavy metals in soils are from anthropogenic activities such as mining, industry and agriculture (Sawidis et al., 2014; Kamari, 2011).

Activities associated with firing ranges have also contributed to elevated levels of heavy metals in soil. Typical military-grade bullets are mainly composed of Pb-alloy slugs enclosed with Cu-alloy jackets (Dermatas et al., 2004; Moon et al., 2013). Metal particulates originating from multiple impacts of bullet fragments during range operations can be oxidized and transformed into compounds that can be mobilized (Chrastný et al., 2010; Jorgensen and Willems, 1997; Landsberger et al., 1999;). Moon et al. (2013) measured 11,885 mg/kg Pb in military firing range soil collected from Busan Metropolitan City, Korea. Topsoil samples collected from a firing range in New Mexico, USA was reported to contain 9,600 mg/kg Pb (Parra et al., 2008). An analysis on metal concentrations in firing range soil of the Small Arms Training Area, Aiken, USA by Wilde et al. (2005) found 3,282 mg/kg Pb and 1,762 mg/kg Cu. Undoubtedly, this scenario may pose a great risk to

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the environment especially food chain and water supplies.

A range of remediation strategies exist for metal contaminated soils. Physical and chemical treatments such as excavation and landfill, thermal treatment, acid leaching and electro-reclamation are effective at lowering risk (EPA, 2006; Jiang et al., 2009; Kashem et al., 2010). However, these techniques are expensive and would be more ideal for relatively small contaminated sites (Basta and McGowen, 2004; Debela et al., 2012). One promising remediation technique is the *in situ* stabilization whereby an amendment is incorporated into a contaminated soil in order to immobilize heavy metals and reduce the dose(s) received by plants (receptors) without set-aside effects (Hosseini et al., 2013; Hartley and Lepp, 2008). This technique decreases the hazard potential of the contaminated soil by converting the contaminants into their least soluble, mobile or toxic form (Holm, 2001; Karbassim et al., 2014). Amendments may bind, adsorb or co-precipitate metal contaminants, reducing metal mobility and availability. Addition of organic matter to metal contaminated soil has been employed to aid their revegetation by improving fertility and decreasing the plant-availability of heavy metals (Abbott et al., 2001; Kumpiene et al., 2007; van Herwijnen et al., 2007). Organic amendments can decrease heavy metals bioavailability, shifting them from ‘plant-available’ forms, that is extractable with water or neutral salts such as calcium chloride, to fractions associated with organic matter, carbonates or metal oxides (Walker et al., 2004; Geebelen et al., 2006). In addition, the presence of functional groups on the surface of organic amendments would provide binding sites for heavy metals.

Soil stabilization using low-cost materials has been considered as a cost-effective technique for soil remediation. Numerous amendments have been proposed and tested for soil stabilization, including agricultural and industrial by-products. Lee et al. (2013) studied metal immobilization in a contaminated rice paddy soil using eggshell waste. The addition of eggshell wastes at 5% (w/w) was reported to reduce the toxicity characteristics leaching procedure (TCLP) of Cd and Pb by 67.9 and 93.2%, respectively. Moon et al. (2013) reported that amending a firing range soil with biochar at an application rate of 20% (w/w) reduced TCLP Pb leachability by 90%. Nwachukwu and Pulford (2009) amended a contaminated soil with green waste compost, peat and wood bark at the rates of 1%, 10% and 20% (w/w). They reported that Pb concentration in the shoots of perennial ryegrass (*Lolium perenne*) decreased significantly with amendment treatments. Li et al. (2012) treated a contaminated soil using tannery sludge char. The immobilization efficiencies of Zn, Cu and Ni by tannery sludge char amendment were reported to be 84.2, 100.0 and 40.0%. A study by Fang et al. (2012) has shown that phosphate rock tailing and triple superphosphate fertilizer were able to reduce $CaCl_2$-extractable Pb and Zn by 55.2-73.1% and 14.3-33.6%, respectively. Castaldi et al. (2009) studied metal uptake of pea and wheat cultivated on a contaminated soil treated with red mud, zeolite and lime at an application rate of 10% (w/w). The addition of amendments was found to reduce Cd, Pb and Zn accumulation in plant shoots and roots.

Intensive production of poultry meat and wood-based products has generated large amount of wastes, especially manure and sawdust. It is estimated that about 44.4 million tons of poultry manure was produced in the USA in 2008 (McDonald et al., 2009). Meanwhile, approximately 591,300 tons of broiler manure waste is generated annually in New Zealand (Bolan et al., 2010). Both low-cost materials are important sources of micronutrients for crop production and organic matter for improving physical and biological fertility of soil (Dikinya and Mufwanzala, 2010; Stirling et al., 2012).

A number of studies have reported the potential of manure as an immobilizing agent for heavy metals in soil. Walker et al. (2004) reported that amending a metal contaminated soil with cow manure increased the growth of *Chenopodium album* L. and reduced shoot concentrations of Cu, Mn and Zn. Sato et al. (2010) evaluated Cd phytoavailability in soils as affected by application of chemical fertilizer and three types of animal waste compost (AWC), derived from cattle, swine and poultry wastes. They reported that the Cd concentration in spinach grown on soil amended with AWC was 34-38% lower than in plants cultivated on chemical fertilizer-treated soil. The effectiveness of stabilization strategy depends on the nature of the contaminants, physical and chemical characteristics of the amendment, and type of soil. Han et al. (2012) studied Cd uptake by rice plants grown on three different soils treated with pig manure at the rates of 1% and 3% (w/w). Grain Cd concentrations were reported to exceed the Chinese National Food Quality Standard of 0.2 mg/kg most often on the loam, intermediate frequency on the silt loam, and least often on the sandy loam. Manure application in metal contaminated soil may not always reduce metal availability. Schwab et al. (2007) investigated heavy metal leaching from mine tailings amended with composted cattle manure, aged cattle manure and composted yard waste at an application rate of 90 tonne/ha. They found that all amendments increased the amount of heavy metal leached from the tailings, as compared to untreated mine tailings.

As discussed by Nwachukwu and Pulford (2009), Lee et al. (2013) and Fang et al. (2012), it is necessary to assess the potential of amendments for soil remediation in pot experiments, prior to full scale field studies. Such information should be considered when applying amendments on a real contaminated site, and therefore, costly future mistakes can be avoided (Lee et al., 2009; van Herwijnen et al., 2007).

The overall aim of this work was to evaluate the potential of chicken manure and coconut tree sawdust as immobilizing agents for the remediation of metal contaminated soil. The objectives of this work were: (1) to evaluate the ability of chicken manure and coconut tree sawdust to immobilize heavy metals in a contaminated soil; and (2) to study the effects of both amendments on plant growth and plant metal uptake.

2. Materials and methods

2.1. Soil and amendments

Soil sample was collected from a 30-year old active firing range in Selangor, Malaysia. The soil samples were taken at the surface layer of up to 25 cm depth using a stainless steel trowel. The samples were air-dried for 1 week, thoroughly mixed and passed through a 2 mm mesh sieve. The soil textural analysis was performed using the pipetting method as described by Gee and Bauder (1986). The soil consists of sand (79%), silt (16%) and clay (5%). The soil pH was measured in deionised water with a soil:solution ratio of 1:2.5 using a pH meter (Orion 2-Star, Thermo Scientific, USA). The electrical conductivity (EC) of soil was measured in soil and water suspension at a ratio of 1:5 (w/v) using an EC meter (Orion 3-Star, Thermo Scientific, USA). The Nelson-Sommers method (Nelson and Sommers, 1996) was used to determine the soil organic matter (OM), while the Kjeldahl distillation procedure (Mremner, 1996) was used to determine the total N. The cation exchange capacity (CEC) was determined using the ammonium-saturation and distillation method (Sumner and Miller, 1996).

The total Cu, Pb and Zn concentrations in the soil was determined by aqua regia extraction. In ten replicates, 9 mL of HCl (6.0 mol/L) and 3 mL of HNO_3 (69%) were added to 0.25 g of soil. The soil-aqua regia mixture was left overnight for equilibration. Soil samples and blanks were digested at 125 ºC for 3 h. The digests were filtered through Whatman No. 50 hardened filter paper into 50 mL volumetric flasks and made up to volume with deionized water. In addition to the total fraction, the bioavailable fraction of metals in soil was also determined using ammonium acetate (1.0 mol/L, pH 7) at a soil:extractant ratio of 1:10. The mixtures were agitated on an orbital shaker for 1 h at room temperature and were then filtered into sample containers.

In this study, two organic amendments namely chicken manure (CM) and coconut tree sawdust (CTS) were tested. Chicken manure was collected from a chicken farm in Klang, Selangor, while coconut tree sawdust was obtained from a saw-mill in Banting, Selangor. The chicken manure was sun dried to kill any germs available which could hinder the growth of crop. The coconut tree sawdust was air-dried for 1 week. The dried amendments were ground before analysis and use. In order to measure metal content in amendments, CM and CTS were digested separately using 15 mL of nitric acid (69%) at 110 ºC for 3 h. The characteristics of the soil and amendments are summarized in Table 1.

Table 1. Characteristics of the soil and amendments used in the study

Characteristic	*Soil*	*Chicken manure*	*Coconut tree sawdust*
pH	4.91	8.75	6.82
EC (dS/m)	2.15	7.3	1.8
OM (%)	1.3	87.6	72.9
Total-N (g/kg)	0.8	62.7	25.5
CEC ($cmol_c$/kg)	11.2	23.5	116.4
Pb (mg/kg)[a]	2544	<0.05	< 0.05
Cu (mg/kg)[a]	763	47	12
Zn (mg/kg)[a]	318	105	35
Pb (mg/kg)[b]	1137	-	-
Cu (mg/kg)[b]	292	-	-
Zn (mg/kg)[b]	135	-	-

[a] Total fraction. [b] Bioavailable fraction.

2.2. Pot experiments

Pots with a diameter of 14.0 cm and a height of 12.0 cm were filled with 400 g of soil. CM and CTS were added separately to the soil at 0%, 1% and 3% (w/w), in six replicates. The soils were left to equilibrate for two weeks. As the soil has a poor plant nutrient content, ¼ strength Hoagland's nutrient solution (Watson et al., 2003) was added to each pot thrice a week at application rate of 20 mL. The Hoagland's nutrient solution was applied to the soils for two weeks only (equilibration period). The addition of nutrient solution was discontinued when the pot experiment began.

After two weeks, each pot was tipped out and remixed to ensure homogeneity and to prevent the soil samples from becoming anaerobic. Water spinach (*Ipomoea aquatica*) seed was sown two weeks after addition of amendments. The pots were arranged in a randomized block design. The water content of the soils was adjusted to obtain 70% of the water holding capacity by adding deionized water daily, avoiding prolonged water logging. Plants were allowed to grow under natural lighting and temperature for 8 weeks. Mean daily temperature and humidity were monitored with a digital thermometer. At the end of the pot experiment, the soil pH and ammonium acetate extractable metal content in the soil were determined, as previously described.

2.3. Harvest and digestion of plant tissue

The plants were harvested after 8 weeks of growth. The aerial parts were cut at 1.0 cm above the soil surface to avoid contamination by soil using a pair of scissors, which was wiped after each use. Roots were carefully extracted from the soil and washed thoroughly with deionized water to remove soil particles. Plant tissues were dried in an oven at 70ºC for 48 h. After two days, the dry weight of plant tissue was measured. The dried shoots and roots were milled separately using a grinder.

In triplicate, 0.25 g milled plant tissue was ashed in a furnace at 450 ºC for 3 h. In a 50 mL tall form beaker, 12 mL of HNO_3 (69%) was added to the ashed sample. The plant sample was left overnight in the fume cupboard for equilibration. Samples and blanks were then digested on a hot plate at 110 ºC for 3 h, allowed to cool and then filtered through Whatman No. 50 hardened filter paper into 25 mL volumetric flasks. The solutions were made up to volume using deionized water. A Perkin-Elmer AAnalyst 400 Atomic Absorption Spectrometer was used to measure the concentration of heavy metals in plant tissue digests and soil extracts.

2.4. Standard and certified reference materials

Standard reference plant materials (SRM 1573a Tomato Leaves – National Institute of Standards & Technology, USA, and SRM 1575 Pine Needles – National Bureau of Standards, USA) and certified reference soil material (LGC 6135 Hackney Brick Works Soil – Laboratory of the Government Chemist, UK) were used to verify the accuracy of metal determination. Reference materials were treated and analyzed using the same procedures applied for plant tissue and soil samples. The recovery rates were within 92-105% for soil and 88-94% for plant tissue, respectively (Table 2).

Table 2. Metal concentrations in reference materials

Reference material		**Pb**	**Cu**	**Zn**
LGC 6135	Certified	391 ± 16	316 ± 41	105 ± 5
	Measured	358 ± 6	300 ± 12	110 ± 7
	Recovery (%)	91.6	94.9	104.8
SRM 1573a	Certified	-	30.9 ± 0.7	4.7 ± 0.1
	Measured	-	27.6 ± 1.3	4.4 ± 0.6
	Recovery (%)	-	89.3	93.6
SRM 1575	Certified	10.8 ± 0.5	-	3.0 ± 0.3
	Measured	9.5 ± 0.9	-	2.7 ± 0.8
	Recovery (%)	88.0	-	90.0

Values represent mean of three replicates ± standard deviation.

2.5. Statistical analysis

All statistical analyses were performed using Minitab 15 Statistical Software (Minitab Inc., PA, USA). The data were analyzed using the general linear model of one-way analysis of variance (ANOVA), followed by Tukey's test at a significance level of p = 0.05 to determine least significant difference (LSD) for the comparison of means. Correlation was by Pearson's coefficients at $p < 0.05$.

3. Results and discussion

3.1. Plant growth and biomass yield

In this study, water spinach seeds were also planted on compost and received no amendment, in six replicates. This enabled a direct comparison in terms of growth performance of water spinach grown on untreated contaminated soil (zero treatment) and uncontaminated soil (compost). The water spinach seeds germinated three days after sowing and no obvious difference in plant growth was observed up to two weeks of the pot experiment. Plants grown on compost were observed to be healthier than plants cultivated on untreated contaminated soil.

CM and CTS treatments have resulted in healthy appearance on the water spinach leaves, whereby the leaves were both longer and greener as compared to plants grown on untreated contaminated soil. The growth progress of plants grown on zero treatment soil was found to be slower than plants grown on soil received amendments. However, no toxicity symptom such as a reddish or burnt appearance was apparent on leaves of untreated water spinach. This suggests that water spinach is a robust plant species and has great tolerance to high metal concentrations. Table 3 presents the dry biomass yield of water spinach after 8 weeks of growth.

Table 3. Biomass yield of water spinach

Treatment	***Dry weight (g/pot)***	
	Shoot yield	***Root yield***
Compost*	14.88	4.06
Zero	3.41 a	1.36 a
CM 1%	6.39 c	2.92 c
CM 3%	10.75 d	3.47 d
CTS 1%	4.56 b	2.05 b
CTS 3%	6.12 c	2.69 b
LSD	1.05	0.68

*Plants grown on compost only (uncontaminated soil). Values represent mean of 6 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

As expected addition of CM and CTS to contaminated soil increased biomass yield. From Table 3, it is clear that the shoot and root yields increased with the rates of amendment application. A pronounced effect was obtained for CM treatment at 3% (w/w), of which the shoot yield for this treatment was found to be higher than zero treatment by a factor of 3.2. CM 1% and CTS 3% (w/w) treatments gave almost similar shoot yield, with 179-187% increment in biomass production. The highest percentage of increment in root yield was achieved with CM 3% (w/w) treatment, followed by application of CM at 1% (w/w). Although there was an increase in the root yield following application of the CTS, statistical analysis revealed no significant difference was obtained between the CTS 1% and CTS 3% (w/w) treatments.

CM and CTS were beneficial as growing media through improvement of soil fertility and provision of plant nutrient. CM amendment resulted in higher biomass yield than CTS. This can be explained by the fact that CM contains higher nutrient and organic matter than CTS. As presented in Table 1, the nitrogen content measured in CM was 62.7 g/kg meanwhile 25.5 g/kg nitrogen was determined in CTS. The organic matter content in CM and CTS was estimated as 87.6% and 72.9%, respectively. Lower yields of shoot and root obtained for zero treatment plants can be attributed to metal toxicity.

In many cases biomass yield has significant linear response to application rates of amendment. Walker et al. (2004) obtained a significant linear response for dry matter production of *C. album* to incorporation rates of cow manure added to metal contaminated soil. Li et al. (2008) reported that amending a metal contaminated paddy soil with a higher application rate of pig manure resulted in a greater yield of rice grain. A different observation was reported by Chiu et al. (2006), who studied the effect of pig manure compost and sewage sludge amendment on the growth of common reed plant cultivated on Pb/Zn mine tailings. They noted that application of manure compost at 5% and 10% (w/w) did not significantly affect the shoot yield of common reed, whereby the dry matter production was found to be rather similar to that of plants grown on untreated mine tailings.

3.2. Metal concentration in plant tissue

The concentrations of Zn, Pb and Cu in plant tissues after 8 weeks of growth+ are given in Figs. 1 and 2.

It is apparent that CM and CTS treatments reduced metal concentrations in shoots and roots of water spinach. In general, shoot and root metal concentrations decreased with the rates of amendments application. Marked reductions in metal concentrations were achieved following CTS treatment at 3.0% (w/w).

From Fig. 1, although CM treatments reduced Zn shoot concentration, there was no significant difference between 1% and 3% (w/w) treatments. Application of CM at 1% (w/w) decreased Pb shoot concentration by only 9%, as compared to plants grown on untreated contaminated soil. Meanwhile, CM 3% and CTS 1% (w/w) treatments were found to have similar effects in reducing Pb shoot concentrations. CM 1% (w/w) had the least immobilizing effect whereby the concentration of Cu in water spinach shoots was reduced from 10.5 mg/kg (zero treatment) to 9.9 mg/kg. In the case of metal root concentrations (Fig. 2), no significant decrease was observed after amending contaminated soil with CM at 1% (w/w). A significant decrease ($p < 0.05$) in metal root concentrations was obtained for CM 3%, CTS 1% and CTS 3% (w/w) treatments. The addition of CM increased soil pH from 4.91 to 7.73, reducing metal availability for metal uptake in water spinach tissues. The pH value of CM was determined as 8.75 (Table 1). A similar observation in soil pH increase following CM application was also reported by Pitman and Singh (1993) and Hue (1992). The increase in soil pH can be attributed to ion exchange mechanisms which occur when terminal OH^- of Al or Fe^{2+} hydroxyl oxides are replaced by organic anions which are decomposed products of the CM such as malate, citrate and tartrate (Dikinya and Mufwanzala, 2010; Hue, 1992). In addition, the presence of basic cations in the CM released upon microbial decarboxylation can also increase soil pH (Bolan et al., 2010; Pitman and Singh, 1993). The effect of CM application on soil pH is greatly influenced by the nature of the soil.

For example, Dikinya and Mufwanzala (2010) noted that the addition of CM did not change the pH of the Luvic Calcisol, meanwhile a substantial increase was observed for Ferralic Arenosol and Vertic Luvisol. It is known that organic matter consists of humic substances, which are able to form stable metal chelates (Walker et al., 2004). This reduces the solubility of heavy metals in soil. As discussed by Kumpiene et al. (2008) and Moon et al. (2013), amendments with functional groups are particularly effective at immobilizing metal due to their ability to bind or complex metal.

In order to identify the presence of certain functional groups and chemical bonds on CTS, as well as to understand the nature of metal binding by CTS, Fourier Transform Infrared (FTIR) analysis was carried out.

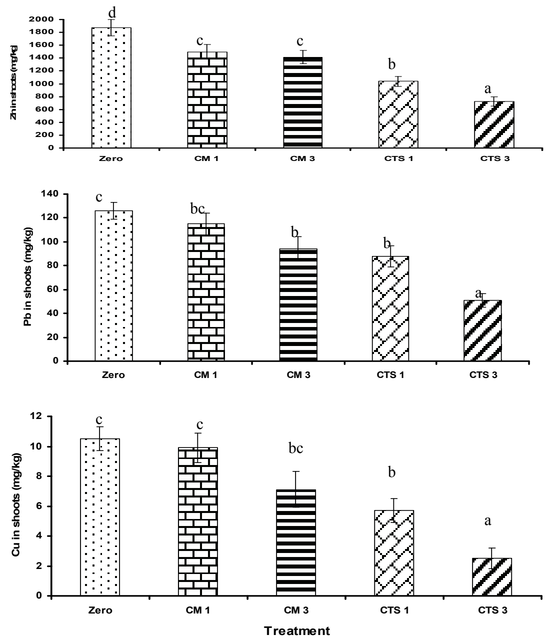

Fig. 1. Metal concentrations in water spinach shoots after 8 weeks of growth. Values represent mean of 18 replicates ± standard deviation. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$). CM 1: Chicken manure 1% (w/w), CM 3: Chicken manure 3% (w/w), CTS 1: Coconut tree sawdust 1% (w/w), and CTS 3: Coconut tree sawdust 3% (w/w).

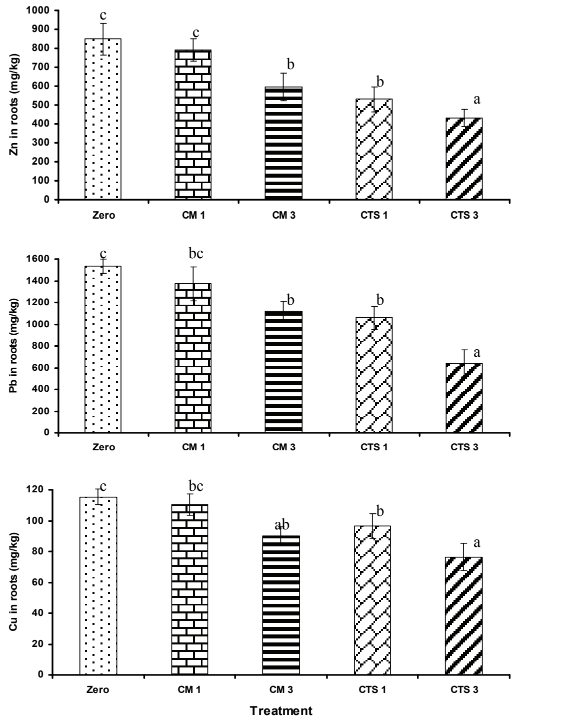

Fig. 2. Metal concentrations in water spinach roots after 8 weeks of growth. Values represent mean of 18 replicates ± standard deviation. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$). CM 1: Chicken manure 1% (w/w), CM 3: Chicken manure 3% (w/w), CTS 1: Coconut tree sawdust 1% (w/w), and CTS 3: Coconut tree sawdust 3% (w/w)

FTIR analysis was performed on a Thermo Nicolet 6700 FTIR Spectrometer using the KBr disc technique. A broad absorption band observed at 3327 cm^{-1} in Fig. 3 corresponds to the stretching vibration of O-H. The FTIR spectrum of CTS exhibits characteristic frequencies of C=O stretching vibration for carbonyl group at 1718 cm^{-1}. The NH_2 scissoring vibration was observed at 1596 and 1506 cm^{-1}. According to Crews et al. (1998), the absorption band at 1232 cm^{-1} corresponds to the C-N stretching vibration. The C-O stretching vibration for primary alcohol groups appeared at 1029 cm^{-1} (Williams and Fleming, 1995). Interaction of CTS with heavy metals led to a shift in wave number from 3327 cm^{-1} to 3271 cm^{-1} for O-H stretching vibration and from 1718 cm^{-1} to 1723 cm^{-1} for C=O stretching vibration.

A significant change in absorption intensity of C=O, NH_2, C-N and C-O stretches was observed.

It is evident from FTIR analysis that OH and NH_2 functional groups are available on the surface of CTS. FTIR spectra suggest that metal binding affects all chemical bonds associated with O and N atoms. Hence, it is reasonable to speculate that O and N atoms are indeed the main binding sites for metal on CTS, whereby both atoms can bind metal ions through complexation.

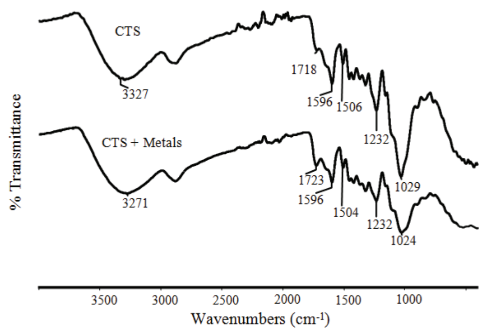

Fig. 3. FTIR spectra of CTS before and after metal binding

It is clear that application of amendments to contaminated soil may influence the behavior of plant in accumulating metals. This behavior is mainly affected by several factors such as the nature of the soil, amendment and metal contaminant, application rate of amendment and plant species. Correlations between metal concentrations in soil and metal concentrations in plant tissues were made using two extractants, namely EDTA and ammonium acetate (NH_4OAc) (Table 4).

It was found that ammonium acetate gave significant correlations between metal concentrations in soil and metal concentration in plant tissues. In contrast, EDTA exhibited poor correlations. The poor correlation between EDTA extractable metal concentrations and plant tissue metal concentrations may be because EDTA is a good extractant for metal associated with organic matter, which may not be available for uptake by plants (Nwachukwu and Pulford, 2009).

3.3. Bioconcentration factor (BCF) and translocation factor (TF)

The bioconcentration factor is defined as the ratio of metal concentration in plant shoots to metal concentration in soil (McGrath and Zhao, 2003).

Table 4. Correlations between metal concentrations in soil and metal concentrations in plant tissue of water spinach

Extractant	*Metal*	*Shoot tissue*		*Root tissue*	
		Correlation coefficient	*p-value*	*Correlation coefficient*	*p-value*
EDTA	Pb	0.011	NS	- 0.408	NS
	Cu	0.026	NS	- 0.192	NS
	Zn	0.039	NS	- 0.064	NS
NH_4OAc	Pb	0.385	0.003*	0.571	0.002*
	Cu	0.514	0.002*	0.328	0.001*
	Zn	0.746	0.000*	0.620	0.004*

n = 65, NS: Not significance, Pearson's correlation coefficient and significance at $p < 0.05$).

As discussed by Yoon et al. (2006), BCF is a measure of the ability of a plant to accumulate metals from soil. Plants with a BCF value of greater than 1 are suitable for phytoextraction (Ruiz et al., 2009). In this study, the influence of CM and CTS treatments on BCF values of the metals was determined and the values are given in Table 5.

Table 5. BCF values for Pb, Cu and Zn

Treatment	***BCF***		
	Pb	**Cu**	**Zn**
Zero	0.19 d	0.22 d	1.15 d
CM 1%	0.15 d	0.17 c	0.63 c
CM 3%	0.12 c	0.14 b	0.58 bc
CTS 1%	0.08 b	0.11 ab	0.44 b
CTS 3%	0.05 a	0.08 a	0.27 a
LSD	0.03	0.04	0.15

Values represent mean of 18 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

The BCF value of Zn for plants cultivated on zero treatment soil was calculated as 1.15, suggesting that water spinach has great potential for phytoextraction of Zn from contaminated soil. CM 1% and 3% (w/w) treatments had similar effects on Zn accumulation, whereby the BCF value of Zn was found to decrease from 1.15 (zero treatment) to 0.63 (CM 1%) and 0.58 (CM 3%), respectively. The application of CM at the rate of 1% (w/w) did not significantly ($p < 0.05$) reduce Pb uptake in water spinach. Overall, the ability of water spinach to take up metals from contaminated soil decreased with the addition of amendments. The decrease in metal accumulation can be attributed to metal binding to functional groups of amendments and reduction in metal availability in soil, as discussed in the preceding section.

The concentration of metal in plant shoot and soil greatly affects the BCF value. In this study, the BCF values of Zn were found to be higher than the BCF values computed for Pb and Cu (Table 5). This can be explained by the fact that more Zn was measured in the plant shoots as compared to Pb and Cu (Fig. 1). In addition, the total concentration of Zn in the contaminated soil (318 mg/kg) was lower than the 2544 mg/kg measured for Pb.

The translocation factor is defined as the ratio of metal concentration in plant shoots to metal concentration in roots (Ruiz et al., 2009), and is a measure of the ability of a plant to translocate metals from the roots to the shoots (Yoon et al., 2006). From phytoremediation viewpoint, an efficient phytoextraction strategy should have high TF values because metals must be in the aerial parts of the plants so that they can be removed at harvest (Pulford and Dickinson, 2006; Vamerali et al., 2010). The effects of CM and CTS treatments on the TF values of the metals are given in Table 6.

Water spinach grown on contaminated soil received no amendment gave a TF value of 1.09 for Zn. According to Baker and Brooks (1989) and Cunningham et al. (1995), plants with a TF value of 1 are efficient in transporting metals from the root tissue to the shoot tissue. Therefore, phytoextraction of Zn using water spinach is feasible. However, amending contaminated soil with CM and CTS reduced the translocation of metals in water spinach, especially with CTS at application rate of 3% (w/w). Following CTS 3% (w/w) treatment, the BCF value of Zn decreased significantly ($p < 0.05$) from 1.09 (zero treatment) to 0.14. There was no great difference in the TF value of Pb for plants grown soil treated with CM 3% and CTS 1 % (w/w).

Table 6. TF values for Pb, Cu and Zn

Treatment	***TF***		
	Pb	**Cu**	**Zn**
Zero	0.14 d	0.25 d	1.09 d
CM 1%	0.11 c	0.19 c	0.60 c
CM 3%	0.07 b	0.15 b	0.52 bc
CTS 1%	0.08 b	0.11 ab	0.40 b
CTS 3%	0.04 a	0.09 a	0.14 a
LSD	0.03	0.05	0.26

Values represent mean of 18 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

In general, the BCF and TF values of Pb, Cu and Zn showed a similar trend following the immobilization treatments. This suggests that both amendments had a similar effect on metal accumulation and transport in water spinach tissue. Based on the BCF and TF values, metal uptake in water spinach tissue is in the order of Zn > Cu > Pb. Zn and Cu are essential micronutrients for plant growth. Therefore, the transport and accumulation of Zn and C were found to be higher than Pb. Unlike Zn and Cu, Pb normally causes toxic effect to plants (Kashem et al., 2010; Vamerali et al., 2010). The toxic effect of Pb on chlorophyll synthesis, photosynthetic activity and antioxidant enzymes has been discussed by Kim et al. (2003). Overall, the trend obtained for BCF and TF values corroborates the trend observed for metal concentration in plant roots (Fig. 2). Pb was the metal most measured in plant roots and the least transported from the root to the shoot of water spinach.

There are two main mechanisms holding Pb in plant roots, namely binding of Pb to ion exchange sites on the root cell walls and extracellular precipitation (Jarvis and Leung, 2002). It is known that plants have different abilities to take up and translocate heavy metals in their tissues. For example, maize and sunflower had preference of Zn > Cu > Pb (Ruiz et al., 2009). Meanwhile, Yoon et al. (2006) reported the order of Pb > Cu > Zn for

Cyperus esculentus and Cu > Zn > Pb for *Rubus fruticosus*.

3.4. Off-take values

The effect of the application of CM and CTS on metal uptake in water spinach was further evaluated in terms of off-take value. The off-take value considers both metal concentration in plant tissues and biomass yield, and has been regarded as an important parameter in evaluating the effectiveness of amendments for metal immobilization (Kamari, 2011). The off-take value was calculated per pot (mg/pot) and per hectare (kg/ha). The estimation in kg/ha was based on a conversion factor of pot area to hectare. The amount of Pb, Cu and Zn removed by water spinach from contaminated soil is given in Table 7.

From Table 7, it is observed that application of CM and CTS reduce the removal of Pb, Cu and Zn by water spinach. The influence of amendments was less apparent for Pb uptake because Pb was the metal least extracted by plants. At the end of the pot experiment, it is estimated that 0.07 mg/pot of Pb, 0.36 mg/pot of Cu and 3.85 mg/pot of Zn were removed from the untreated contaminated soil.

Amending contaminated soil with CM and CTS at the rate of 3% (w/w) decreased the off-take value of Zn from 10.01 kg/ha (zero treatment) to 6.60 and 3.17 kg/ha, respectively. The reduction in the off-take values of metals can be attributed to the immobilization effect of the chicken manure and coconut tree sawdust.

3.5. Bioavailable fraction of metals

The uptake of heavy metals by plants is mainly influenced by their bioavailable fraction rather than by the total amount in soil (Vamerali et al., 2010; Pulford and Dickinson, 2006).

Therefore, the effect of the application of CM and CTS on bioavailable fraction of Pb, Cu and Zn in contaminated soil was investigated using ammonium acetate as extractant. The ammonium acetate extractable metals in soils after 8 weeks of the pot experiment are given in Table 8. Amending contaminated soil with CM and CTS decreased the bioavailability of metals significantly ($p < 0.05$), particularly at application rate of 3% (w/w). A lesser effect in reducing the metal bioavailability was obtained when amendments were applied at 1% (w/w). The decrease in bioavailable fraction of heavy metals after the pot experiment can be attributed to immobilization effect of the amendments and uptake by plants. Since plants were watered with deionized water daily, metals may have leached from the soil to the pot saucer. Therefore, leaching may have also contributed to the decrease in metal concentration of soil. Table 8 also presents the percentage decrease in the ammonium acetate extractable metals in the soil after the pot experiment.

The untreated contaminated soils (controls) show a decrease in bioavailable fraction of heavy metals. This could be due to the changes in the chemical form of the metal that are not related to the amendments during the pot experiment. It is apparent that CTS 3% (w/w) was the best treatment in lowering bioavailable fraction of metals, meanwhile CM 1% (w/w) was the least effective treatment in immobilizing metals.

4. Conclusions

Results from this study highlight the potential of chicken manure and coconut tree sawdust as immobilizing agents for the remediation of metal contaminated land. Pot experiment however is only one aspect of such utilization.

Table 7. Removal of Pb, Cu and Zn from contaminated soil using water spinach

Treatment	***Off-take (mg/pot)***			***Off-take (kg/ha)***		
	Pb	**Cu**	**Zn**	**Pb**	**Cu**	**Zn**
Zero	0.07	0.36	3.85	0.18	0.94	10.01
CM 1%	0.06	0.27	3.29	0.16	0.70	8.55
CM 3%	0.06	0.20	2.54	0.16	0.52	6.60
CTS 1%	0.05	0.22	2.08	0.13	0.57	5.41
CTS 3%	0.04	0.18	1.22	0.10	0.47	3.17

Table 8. Ammonium acetate extractable metals in soils

Treatment	***Concentration (mg/kg)***			***Decrease (%)***		
	Pb	**Cu**	**Zn**	**Pb**	**Cu**	**Zn**
Zero	1050 c	274 d	129 d	4.1	6.2	4.4
CM 1%	1005 c	251 cd	101 c	11.6	14.0	25.2
CM 3%	821 b	187 c	80 b	27.8	35.9	40.7
CTS 1%	986 b	149 b	65 b	13.3	49.0	51.9
CTS 3%	644 a	103 a	34 a	43.4	64.7	74.8
LSD	65	46	21			

Values represent mean of 18 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

The effectiveness of chicken manure and coconut tree sawdust as soil amendments rely on their stability in the soil-water environment. It is necessary to study the biodegradation of both amendments and their effect on metal bioavailability.

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