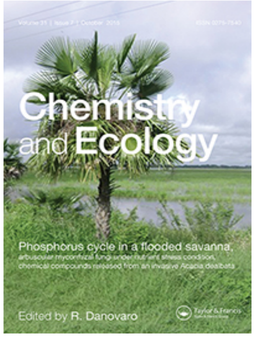

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The effects of application of agricultural wastes to firing range soil on metal accumulation in *Ipomoea aquatica* and soil metal bioavailability

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The immobilisation of heavy metals in the soil of a 25-year-old active firing range using durian (*Durio zibethinus* L.) tree sawdust (DTS), coconut coir (CC) and oil palm empty fruit bunch (EFB) was investigated. The immobilisation effects were evaluated in terms of metal accumulation in water spinach (*Ipomoea aquatica*) and soil metal bioavailability. A pot experiment was conducted by amending the firing range soil with DTS, CC and EFB at application rates of 0%, 1% and 3% (w/w), respectively. All amendments increased the biomass yield and reduced the uptake of heavy metals in the plant tissue. Zn had the highest values of Bioconcentration Factor (BCF: 0.301–0.865) and Translocation Factor (TF: 1.056–1.883). Pb was the least-accumulated and transported metal in the plant tissues, with the BCF and TF values of 0.019–0.048 and 0.038–0.116, respectively. The bioavailable fraction of heavy metals in the firing range soil decreased following the application of the three agricultural wastes studied. DTS, CC and EFB did not cause toxicity symptoms in the water spinach over the pot experiment. Therefore, DTS, CC and EFB are considered promising immobilising agents for the remediation of metal-contaminated land.

Keywords: agricultural wastes; contaminated soil; heavy metals; immobilisation; bioavailability; metal uptake

1. Introduction

Soil contamination by heavy metals is a serious environmental issue worldwide. For example, approximately 20 million hectares of arable land that accounts for 20% of the total agricultural land area in China were identified as being affected with heavy metals in 2011.[1,2] The concentration of heavy metals in soil has increased tremendously due to rapid global industrialisation. Anthropogenic activities such as mining, wastewater irrigation, biosolid and manure application along with inadequate management of pesticides and chemicals in agriculture have significantly contaminated soil and groundwater.[3,4]

Firing range activities have also contributed to elevated concentrations of heavy metals in soil. Cu- and Pb-alloy jackets and slugs are the main components of a firing bullet.[5,6] During

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shooting operations, metal particulates are produced and deposited on the soil surface. These metal particulates can be transformed into mobilised compounds that can pose a threat to soil and groundwater.[7–9]

Unlike organic contaminants, heavy metals are not biodegradable. Therefore, the heavy metals will persist for a long period of time in soils. Their presence in soil may pose a great risk to the food chain and water supplies.[10] Living organisms are exposed to the risk of heavy metals toxicity if the concentration in soil exceeds the threshold values.[11] For example, a high intake of Pb by a human can cause kidney damage, anaemia and miscarriage for pregnant women.[12] Additionally, an excess of Zn can contribute to chlorosis in plant leaves as well as the inhibition of seed germination and plant growth.[13]

Several remediation strategies are available to clean-up metal-contaminated soils. However, due to logistics and high operational cost issues, many of these techniques are not practical for implementation. An *in situ* stabilisation technique is deemed a promising alternative to remediate metal-contaminated soil as the technique is cost-effective and does not generate secondary environmental problems.[14,15] Stabilisation is a conversion process for contaminants from their original form to a physically and chemically more stable form, which is likely less mobile, less soluble and less toxic.[16,17] Through this technique, a soil amendment is added to contaminated soil to enhance geochemical processes such as precipitation, sorption, ion-exchange and redox reactions.[18,19] The soil amendment will help to reduce the solubility and bioavailability of the heavy metals in the soil. Using soil amendment will, therefore, reduce the accumulation of toxic metals in the plant tissues.[15]

The effectiveness of various low-cost materials, such as chicken manure biochar,[20] phosphate fertiliser,[21] red mud,[22] sugar cane biochar [23] and zeolites,[24] to stabilise contaminated soil has been investigated. The utilisation of these amendments has exhibited several advantages including being less expensive than the chemical stabilisation technique. In addition, the materials are also abundant, renewable and eco-friendly.

This study aims to evaluate the applicability of durian tree sawdust (DTS), coconut coir (CC) and oil palm empty fruit bunch (EFB) as immobilising agents for the remediation of metal-contaminated soil. Our previous investigation showed that the agricultural wastes studied were able to reduce the concentration of Cu, Pb and Zn in soil solutions.[25] In this study, water spinach (*Ipomoea aquatica*) was used as an indicator to determine the efficacy of the amendments in immobilising heavy metals in a firing range soil. Durian DTS, CC and EFB were chosen as potential soil amendments due to their availability in large quantities in Malaysia.

2. Materials and methods

2.1. *Preparation of soil and amendments*

A 25-year-old active firing range in Selangor, Malaysia, was selected as the sampling site. Soil samples were taken at the surface layer (up to 25 cm in depth) using a stainless steel trowel. The samples were air-dried for 1 week and were thoroughly mixed and passed through a 2 mm mesh sieve. A pipetting method described by Gee and Bauder [26] was applied for the soil textural analysis. The soil consisted of sand (76%), silt (17%) and clay (7%). Soil pH was measured in deionised water at a ratio of 1:2.5 (w/v) (soil:solution) by using a pH meter (Orion 2-Star, Thermo Scientific, Massachusetts, USA). Furthermore, the electrical conductivity (EC) of the soil was measured in deionised water at a ratio of 1:5 (w/v) (soil:solution) by using an EC meter (Orion 3-Star, Thermo Scientific, Massachusetts, USA). The organic matter (OM) content (%) in the soil was calculated from measured loss-on-ignition,[27] while the total N was determined using Kjeldahl's distillation procedure.[28] The ammonium saturation and distillation method as

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

Characteristic	Soil	DTS	CC	EFB
pH	4.91	6.75	5.82	6.79
Moisture content (%)	5.2	13.6	21.5	38.9
Electrical conductivity (dS/m)	2.15	2.5	1.8	1.2
Organic matter (%)	1.3	47.4	65.6	88.7
Total nitrogen (g/kg)	0.8	65.6	26.7	9.52
Cation-exchange capacity ($cmol_c$/kg)	11.2	96.4	130.6	78
Total Pb (mg/kg)	2562	< 0.05	< 0.05	< 0.05
Total Cu (mg/kg)	771	47	12	12
Total Zn (mg/kg)	315	105	35	35
Available Pb (mg/kg)	1137	ND	ND	ND
Available Cu (mg/kg)	288	ND	ND	ND
Available Zn (mg/kg)	146	ND	ND	ND

ND, not detected.

described by Sumner and Miller [29] was used to determine the cation-exchange capacity (CEC) of the soil.

The total fraction of Cu, Pb and Zn in the soil was determined by hot aqua regia extraction. In 10 replicates, 9 mL of HCl (6.0 mol/L) and 3 mL of HNO_3 (69%) were added to 0.25 g of the soil. The soil–aqua regia mixture was left overnight for equilibration. The soil samples and blanks were digested at 125°C for 3 h. The digests were filtered and diluted up to volume (50 mL) with deionised water. In addition to the total fraction, the bioavailable fraction of Cu, Pb and Zn in the soil was also determined using ammonium acetate (1.0 mol/L, pH 7) and EDTA (0.05 mol/L, pH 7.0) at a ratio of 1:10 (w/v) (soil:extractant). Based on our previous results (data not shown for reference), the mixtures were agitated on an orbital shaker at room temperature for 1 h and were then filtered into sample containers.

In this study, three low-cost materials were tested. DTS was supplied by Jati Cemerlang Sawmill (Selangor, Malaysia), CC was purchased from APA Green Horticulture (Perak, Malaysia) and EFB was collected at Changkat Asa Oil Palm Estate (Perak, Malaysia). The amendments were rinsed with deionised water, air-dried and ground using a laboratory jar mill. The ground materials were then kept in self-sealing sample bags prior to analysis. To measure the metal content in the amendments, the materials (DTS, CC and EFB) were digested separately by using 15 mL of concentrated HNO_3(69%) at 110°C for 3 h. The characteristics of the soil and the amendments are summarised in Table 1.

2.2. *Pot experiment*

Approximately 300 g of soil sample was added to a pot with a 12.0 cm height and a 14.0 cm diameter. The soils were amended separately with the amendments at three rates of application: 0%, 1% and 3% (w/w). Each treatment was carried out in six replicates. The soils were then left to equilibrate for two weeks. During equilibration, the soils were mixed several times to maintain homogeneity and to avoid anaerobic conditions. The water spinach (*Ipomoea aquatica*) seed was then sown in each pot. The pots were placed outdoors in a nursery and arranged on a bench in a randomised block design. They were watered daily with deionised water, and the water holding capacity (70%) of the soil was monitored throughout the pot experiment. Plants were allowed to grow under natural lighting and temperature. Mean daily temperature and humidity were monitored with a digital thermometer. At the end of the 8-week pot trial, amendments were collected, immersed in deionised water and dried prior to analysis. The soil pH and ammonium acetate extractable metal content in the soil were determined, as previously described.

2.3. *Plant tissues analyses*

The plants were harvested after 8 weeks of growth. Plant shoots were cut with scissors at approximately 1.0 cm above the soil surface to avoid contamination by soil. Plant roots were washed thoroughly with deionised water to remove soil particles. The cleaned shoots and roots of the water spinach were then cut into small pieces. The plant tissues were dried in an oven at 70°C for 48 h. The dry biomass yield of each tissue was measured. The dried plant tissues were milled separately using a grinder.

In triplicate, 0.5 g of the plant tissues was ashed at 450°C in a muffle furnace for 3 h. Approximately 12 mL of concentrated HNO_3 (69%) was added to the ashed sample, and the mixture was left overnight to equilibrate. After equilibration, the samples and blanks were digested on a hot plate at 110°C for 3 h in a fume hood. The samples were left to cool at room temperature, filtered and made up to 50 mL. A Perkin–Elmer AAnalyst 400 Atomic Absorption Spectrometer was used to measure the concentration of heavy metals in the soil extracts and plant digests.

2.4. *Standard and certified reference materials*

Certified reference soil material (LGC 6135 Hackney Brick Works Soil) and standard reference plant materials (SRM 1573a Tomato Leaves and SRM 1575 Pine Needles) were used to verify the accuracy of the metal determination. The reference materials were treated and analysed using the same procedures applied for plant tissues and soil samples. The recovery rates were within 89–105% for soil and 87–96% for plant tissue, respectively.

2.5. *Statistical analysis*

A statistical analysis was performed using Minitab Software 17 (Minitab Software 17 (Minitab Inc., Pennsylvania, USA)). The experimental data were analysed by one-way analysis of variance (ANOVA). The least significant difference (LSD) between the means of the treatments was verified using Tukey's test at a significance level of $p = 0.05$, while correlation was determined by Pearson's coefficients at $p < 0.05$.

2.6. *Characterisation study*

A Hitachi SU 8020 UHR Field Emission Scanning Electron Microscope (FESEM) equipped with a Horiba Energy Dispersive X-ray (EDX) Spectrometer was used to analyse the surface morphology and the elemental composition of the amendments. To avoid electron charging, the amendments were first coated with platinum using an Automatic Platinum Sputter Coater System (Quorum Q150RS). The surface morphology was observed at different magnifications.

Fourier Transform Infrared (FTIR) analysis was performed using a Thermo Nicolet 6700 FTIR Spectrometer. KBr discs were prepared at a ratio of 1:100 (w/w) (amendment:IR-grade KBr) in an agate mortar. The FTIR analysis was carried out in a wavenumber range of 500–4000 cm^{-1} over 30 cumulative scans and with a resolution of 4 cm^{-1}.

3. Results and discussion

3.1. *Plant growth and biomass yield*

In this study, water spinach seeds were also cultivated on compost that received no amendments in six replicates. The growth performance of the water spinach grown on untreated contaminated

Table 2. Effect of agricultural wastes application on biomass yield of water spinach.

	Dry weight (g/pot)	
Treatment	Shoot yield	Root yield
Compost	7.43 ± 0.5	3.17 ± 0.3
Control	2.33a ± 0.4	0.93a ± 0.6
DTS 1%	2.84bc ± 0.3	0.98a ± 0.2
DTS 3%	4.72c ± 0.1	1.84c ± 0.8
CC 1%	3.26b ± 0.2	1.13b ± 0.3
CC 3%	5.13c ± 0.7	1.92c ± 0.1
EFB 1%	3.37b ± 0.2	1.35bc ± 0.5
EFB 3%	6.43d ± 0.3	2.20d ± 0.2
LSD	0.91	0.25

Values represent mean of six 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 $p < 0.05$).

soil (zero treatment) and the water spinach grown on uncontaminated soil (compost) was compared. The water spinach seeds germinated five days after sowing, and no obvious difference in plant growth was observed up to three weeks of the pot experiment. Plants grown on compost exhibited a healthier appearance than plants cultivated on untreated contaminated soil.

A significant difference in plant growth was observed after three weeks of the pot experiment. Soil that received the amendment treatment produced plants with bigger and greener leaves while smaller leaves were obtained from plants that were grown on untreated contaminated soil. In addition, the plants that were cultivated on the zero treatment soil showed a slower growth progress than the plants grown on the treated contaminated soil. However, no toxicity symptoms such as reddish or burnt appearance were apparent on the leaves of water spinach that were grown on the untreated contaminated soil in the pot experiment, suggesting that water spinach is a robust plant species and has a high tolerance to elevated metal concentrations.

The dry biomass yield of the water spinach after 8 weeks of growth is presented in Table 2. As expected, the addition of DTS, CC and EFB to the contaminated soil increased the biomass yield of the water spinach. As shown in Table 2, the shoot and root yields increased with the rates of the amendment application. The application of amendments at 1% (w/w) gave almost similar shoot yield, with 140–165% increment in shoot production. The EFB treatment at 3% (w/w) resulted in a pronounced production effect, with the shoot yield for this treatment found to be higher by a factor of 2.8 than the zero treatment. The least production effect was obtained for DTS at 1% (w/w) where the root yield for this treatment showed no great difference from the root yield of the zero treatment plants. The order of treatment efficiency on the biomass production (total shoot and root) was EFB 3% (w/w) > CC 3% (w/w) > DTS 3% (w/w) > EFB 1% (w/w) > CC 1% (w/w) > DTS 1% (w/w) > zero.

Overall, EFB was the best amendment to promote biomass yield, followed by CC and DTS. This trend is closely related to the organic matter and moisture content in the amendments. For example, the organic matter content for EFB, CC and DTS was determined as 88.7%, 65.6% and 47.4%, respectively (Table 1). The three amendments studied were beneficial as growing media through the improvement of soil moisture and fertility. Amending the contaminated soils with DTS, CC and EFB increased the water holding capacity of the soils by 52%, 65% and 83%, respectively.

3.2. *Metal uptake by water spinach*

Figures 1 and 2 present the concentrations of Cu, Pb and Zn in the plant tissues after 8 weeks of the pot experiment. The water spinach accumulated more metals in its roots than in its shoots. For

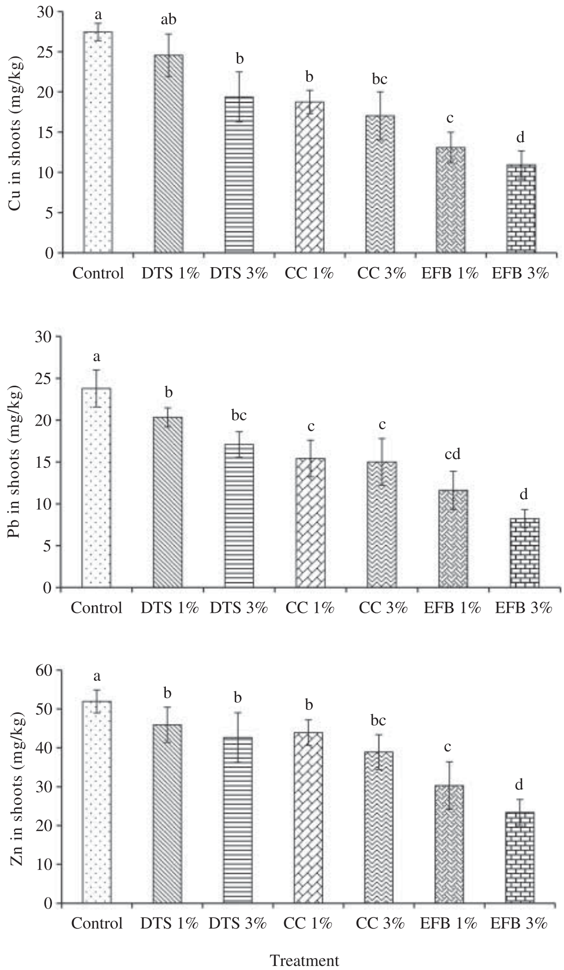

Figure 1. Metal concentration in water spinach shoots after 8 weeks of growth. Values represent mean of 18 replicates ± standard deviation. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

example, for the zero treatment plants, the concentrations of Cu, Pb and Zn in the plant shoots were determined to be 27, 24 and 52 mg/kg (Figure 1), respectively. Meanwhile, 156 mg/kg of Cu, 70 mg/kg of Pb and 263 mg/kg of Zn were measured in the root tissue of the zero treatment plants (Figure 2). Amending the contaminated soil with DTS, CC and EFB reduced the metal concentrations in the shoots and roots of the water spinach. The metal concentrations of the shoots and roots decreased with the rates of the application of the amendments. Marked reductions in metal uptake were achieved following an EFB application at 3% (w/w).

As shown in Figure 1, although the DTS treatments reduced Zn shoot concentration, there was no significant difference between the 1% and the 3% (w/w) treatments. The concentration of Zn in plant shoots that received 0%, 1% and 3% (w/w) of DTS was determined as 53, 46 and 42 mg/kg, respectively. The CC application caused a significant reduction in Pb shoot concentration, which decreased from 24 to 15 mg/kg. However, no large difference was observed when CC was applied at 1% and 3% (w/w).

As presented in Figure 2, the application of DTS had reduced the concentration of Cu and Zn in the plant roots. A greater reduction was obtained when DTS was applied at 3% (w/w). A different effect was observed for Pb root concentration where the DTS treatments at 1% and

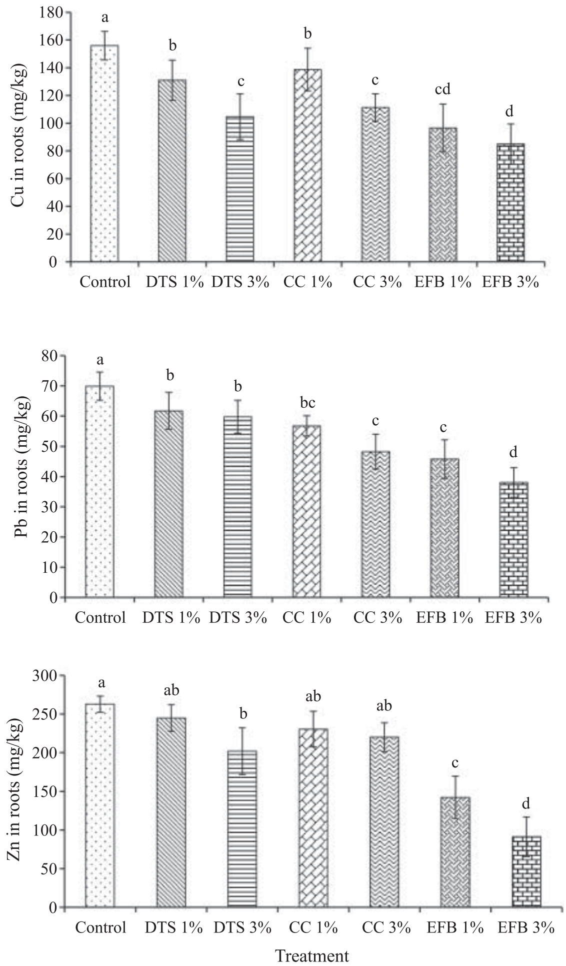

Figure 2. Metal concentration in water spinach roots after 8 weeks of growth. Values represent mean of 18 replicates ± standard deviation. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

3% (w/w) showed no significant difference in Pb reduction. Compared to the zero treatment plants, Pb root concentration was reduced from 71 to 62 mg/kg (1% DTS) and 60 mg/kg (3% DTS). A similar trend was observed for Zn root concentrations following the CC treatments. The addition of CC at 1% and 3% (w/w) reduced Zn concentration in the plant roots by 13% and 16%, respectively, compared to the plants grown on the untreated contaminated soil.

As discussed by Venegas et al. [14] and Armelin et al. [21], different amendments may cause similar effects in reducing metal accumulation in plant tissues. As observed from Figure 1, the DTS 3% (w/w) and CC 1% (w/w) treatments had similar effects in reducing Cu shoot concentrations. Meanwhile, a similar pattern of Cu accumulation in the roots of the water spinach was obtained following the DTS and CC applications (Figure 2).While there was a significant reduction in Pb root concentration after the CC 3% (w/w) and EFB 1% (w/w) treatments, the statistical analysis revealed no significant difference between these two treatments (Figure 2). In the case of Zn, the ability of the water spinach to take up Zn was similar when the contaminated soil was treated with DTS and CC at 1% (w/w) (Figures 1 and 2). Generally, of all the amendments studied, EFB showed a consistent effect in reducing metal concentrations in plant shoots and roots.

Table 3. Effect of agricultural wastes application on bioconcentration factor (BCF) values of Cu, Pb and Zn.

Treatment	Cu	Pb	Zn
Control	0.196d ± 0.2	0.062c ± 0.6	1.169d ± 0.7
DTS 1%	0.088c ± 0.4	0.048b ± 0.3	0.865cd ± 0.3
DTS 3%	0.073b ± 0.1	0.044b ± 0.4	0.616bc ± 0.5
CC 1%	0.064ab ± 0.8	0.035ab ± 0.7	0.793c ± 0.5
CC 3%	0.057ab ± 0.6	0.026a ± 0.2	0.524b ± 0.2
EFB 1%	0.042a ± 0.9	0.029a ± 0.8	0.450ab ± 0.6
EFB 3%	0.035a ± 0.7	0.019a ± 0.3	0.301a ± 0.1
LSD	0.015	0.011	0.208

Values represent mean of 18 replicates ± standard deviation. 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 $p < 0.05$).

3.3. *Bioconcentration factor*

The ability of water spinach to accumulate metal in the shoot from a contaminated soil was further evaluated using the Bioconcentration Factor (BCF). BCF is defined as the ratio of the metal concentration in plant shoots to the metal concentration in soil.[30,31] BCF represents the availability of heavy metals in a soil that can be taken up by a plant.[30,32] In general, plants will have a BCF value of less than one. However, some plant species are able to extract and accumulate a large amount of metal in their shoots.[10] These plants species usually have a BCF value of more than 1. Therefore, they are classified as hyperaccumulator plants and are suitable for a phytoextraction remediation strategy.[31,33]

Table 3 lists the BCF values of Cu, Pb and Zn for the water spinach. The BCF of Zn determined for the zero treatment (untreated contaminated soil) was found to be higher than 1, suggesting that water spinach has a pronounced natural ability to accumulate Zn in its shoots. Therefore, the use of water spinach for the phytoextraction of Zn is feasible. The amendment treatments reduced the Cu, Pb and Zn uptake by the water spinach. For example, the application of DTS, CC and EFB at 3% (w/w) significantly ($p < 0.05$) reduced the BCF value of Zn from 1.169 (zero treatment) to 0.616, 0.524 and 0.301, respectively. Meanwhile, the BCF value of Cu decreased from 0.196 (zero treatment) to 0.073, 0.057 and 0.035 following the DTS, CC and EFB application at 3% (w/w), respectively.

From Table 3, the BCF values estimated for the treatments were in the order Zn > Cu > Pb. As defined earlier, BCF value is highly dependent on metal concentrations in plant shoots and soil. Based on the plant tissue analysis (Figure 1), Zn was considered the metal most determined in the plant shoots. As shown in Table 1, the total concentration of Zn in the soil sample (315 mg/kg) was much lower than the concentrations measured for Pb (2562 mg/kg) and Cu (771 mg/kg). Therefore, Zn had the highest BCF value. The low accumulation of Pb in the water spinach shoots can be related to the low solubility of the metal in soil, thus limiting its extraction via plant roots. Overall, the 3% EFB (w/w) treatment resulted in the best effect in reducing Cu, Pb and Zn accumulation in the water spinach shoots.

3.4. *Translocation factor*

Translocation factor (TF) refers to the ratio of metal concentration in shoots to metal concentration in roots.[30,34] TF represents the ability of a plant to translocate metals from the root to the shoot. The effects of the amendment treatments on the TF values of the metals are presented in Table 4. As shown in Table 4, the plants grown from the zero treatment have a TF value of 2.7737 for Zn. Plants with a TF value of greater than 1 are efficient in transporting

Table 4. Effect of agricultural wastes application on translocation factor (TF) values of Cu, Pb and Zn.

Treatment	Cu	Pb	Zn
Control	0.158d ± 0.2	0.129d ± 0.6	2.737d ± 0.1
DTS 1%	0.124cd ± 0.5	0.116cd ± 0.2	1.883c ± 0.3
DTS 3%	0.101c ± 0.9	0.093c ± 0.4	1.549bc ± 0.8
CC 1%	0.088bc ± 0.6	0.080bc ± 0.7	1.520b ± 0.7
CC 3%	0.063ab ± 0.4	0.077b ± 0.5	1.306ab ± 0.2
EFB 1%	0.079b ± 0.8	0.061ab ± 0.9	1.244a ± 0.5
EFB 3%	0.054a ± 0.3	0.038a ± 0.2	1.056a ± 0.9
LSD	0.023	0.025	0.425

Values represent mean of 18 replicates ± standard deviation. 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 $p < 0.05$).

metals from the root tissue to the shoot tissue.[33] This characteristic is important for *in situ* phytoextraction strategy.[10,31] The TF values of Cu, Pb and Zn decreased after the amendment treatments. The decrease in metal transport can be related to metal binding to functional groups of amendments.[6,17] Great reductions were obtained when the amendments were applied at 3% (w/w). For example, the TF value of Zn decreased from 2.737 (zero treatment) to 1.549, 1.306 and 1.056 following DTS, CC and EFB applications at 3% (w/w).

The order of TF values estimated for the treatments was found to be Zn > Cu > Pb, in agreement with the order of BCF values. Zn was the metal that was transported the most from the roots to the shoots. Both Cu and Zn are well known to be the essential micronutrients for plant growth. Cu and Zn are major components for several electron transport enzymes involved in catalysing the redox reaction in mitochondria and chloroplasts.[32] Therefore, plants tend to translocate both elements from the roots to the shoots. Low translocation of Pb might be due to its toxic effect on plants. Kanwal et al. [35] and Kim et al. [36] discussed the toxic effect of Pb on chlorophyll synthesis, photosynthetic activity and antioxidant enzymes. According to Jarvis and Leung [37] and Cabello-Conejo et al. [33], extracellular precipitation and binding of Pb to ion-exchange sites of the root cell walls are the two main mechanisms that hold Pb in plant roots. A study on metal accumulation in perennial ryegrass tissue by Kalis et al. [38] found that Pb is retained at the root surface of a plant better than Cd, Cu, Ni and Zn.

Plants have different abilities to translocate metal. For example, *Stipa barbata* was reported to translocate Zn better than Cu.[39] A similar trend was obtained by Ruiz et al. [34] for maize and sunflowers, whereby the order of TF values was reported as Zn > Cu > Pb. In contrast, Lakshmi et al. [13] reported the order of Pb > Zn for *Brachiaria ramosa*.

3.5. *Bioavailability of heavy metals in the soil*

As discussed by Naidu and Bolan,[40] the accumulation of toxic metals in plant tissues is influenced mainly by the bioavailable fraction and not the total fraction. The bioavailable fraction is defined as the mobile fraction of contaminants that are readily available for uptake by plants, animals and humans.[11] Figure 3 presents the bioavailability of Cu, Pb and Zn in the firing range soil after 8 weeks of the pot experiment. The applications of the amendments reduced the ammonium extractable metals in the soils. For example, the bioavailability of Cu was reduced by 28%, 51% and 62% following the DTS 3% (w/w), CC3% (w/w) and EFB 3% (w/w) treatments, respectively. DTS and CC caused similar effects in reducing the bioavailable fraction of Pb, while the statistical analysis revealed no significant difference between the two treatments. For example, the bioavailability of Pb decreased from 1137 mg/kg (before treatment) to 745 and 693 mg/kg following the DTS 3% and CC 3% (w/w) treatments, respectively. In the case of Zn,

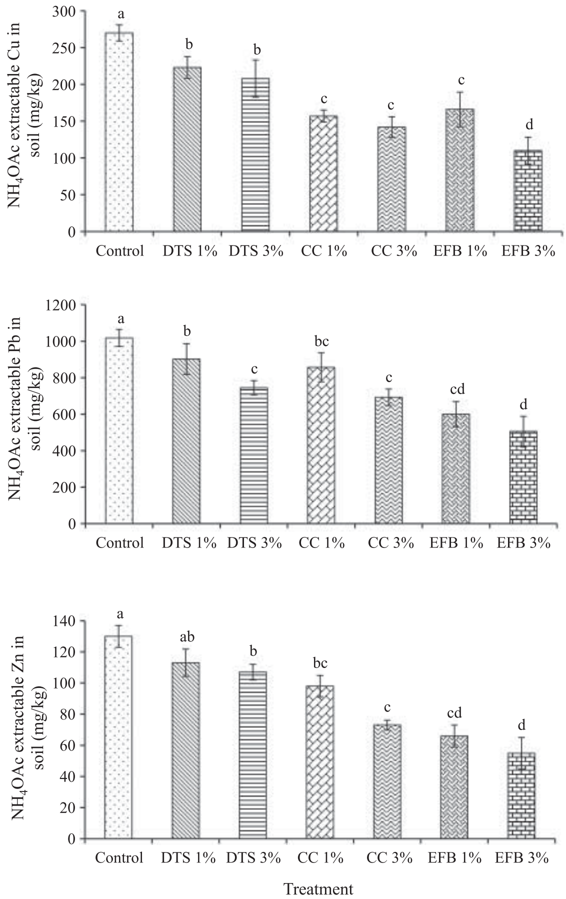

Figure 3. Ammonium acetate extractable metals in soil after 8 weeks of pot experiment. Values represent mean of 18 replicates ± standard deviation. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

the amendment treatments at 3% (w/w) reduced the ammonium acetate extractable Zn in the soil significantly. The reduction in the bioavailability of metals in soils can be related to the immobilising effect of the amendments and uptake by the plants.

The effectiveness of a stabilisation or immobilisation strategy may also rely on soil pH.[10,23] The influence of soil pH on the immobilisation of heavy metals in a contaminated soil was assessed by measuring the soil pH before and after the amendment treatments (pot experiment). The addition of DTS, CC and EFB to the contaminated soils increased the pH of the firing range soil by 0.3–0.5 units (data not shown). A slight increase in the pH value of the firing range soil suggests that soil pH is not a main factor that influences metal bioavailability in this study.

It is clear that the application of amendments to the contaminated soil may affect the behaviour of the plants in accumulating and transporting metals. The ability of plants to extract, transport and accumulate contaminants is mainly influenced by several factors such as the nature of the soil, the amendment and metal contaminant, the application rate of the amendment, and the plant species. The correlations between metal concentrations in soil and metal concentrations in plant tissues were examined using two extractants, namely, EDTA and ammonium acetate. As shown in Table 5, ammonium acetate was found to have significant correlations between metal concentrations in soil and metal concentrations in plant tissues. In contrast, poor correlations were obtained following the EDTA extraction. The poor correlation between EDTA extractable

Table 5. Correlations between metal concentrations in soil and metal concentrations in plant tissue of water spinach.

		Shoot tissue		Root tissue	
Extractant	Metal	Correlation coefficient	p-Value	Correlation coefficient	p-Value
EDTA	Pb	0.025	NS	− 0.307	NS
	Cu	0.047	NS	− 0.128	NS
	Zn	0.051	NS	− 0.085	NS
Ammonium acetate	Pb	0.214	0.005*	−0.495	0.003*
	Cu	0.549	0.002*	−0.231	0.006*
	Zn	0.833	0.000*	−0.708	0.001*

$n = 65$, NS, not significant, Pearson's correlation coefficient and significance at $p < 0.05$.

metal concentrations and plant tissue metal concentrations can be explained by the fact that EDTA is an excellent extractant for metal bound to organic matter, which may not be available for uptake by plants.[35]

3.6. *Characterisation: SEM, EDX and FTIR analyses*

The surface morphology of the amendments was examined by SEM analysis. Interaction with heavy metals resulted in significant changes on the surface of the amendments. The SEM images of CC before and after the pot experiment at 10,000 × magnification are shown in figure S1 as an example. Before the pot experiment, CC displayed a dense and smooth surface texture (figure S1(a)). Seed-like deposits were observed on the surface of CC following interaction with heavy metals (figure S1(b)).

The elemental composition of the amendments was determined by an EDX analysis. The EDX spectra of DTS before and after the pot experiment are presented in figure S2, as an example. Based on the EDX analysis, carbon and oxygen were found to be the main constituents in DTS, CC and EFB. The energy lines observed at 0.27 and 0.52 keV represent the features of carbon and oxygen, respectively (figure S2(a)). The features of platinum were observed at 2.05 and 9.44 keV. As previously described (Section 2.6), the amendments were coated with platinum to prevent electron charging effects that could interfere with the EDX analysis. Following the pot experiment, the energy lines of Zn were observed at 1.01 and 8.37 keV (figure S2(b)), confirming the ability of DTS to immobilise Zn in the contaminated soil.

The effectiveness of an amendment to immobilise heavy metals in contaminated soil also relies on the presence of functional groups.[6,17] It is imperative to understand the possible binding mechanism(s) of metal ions onto amendments. Therefore, an FTIR analysis was carried out to identify the presence of functional groups and determine the chemical bonds of the amendments. The FTIR spectra of EFB before and after the pot experiment are shown in figure S3, as an example. As shown in figure S3(a), the −OH and N−H stretches are represented by a broad and strong absorption band that appeared at 3217 cm^{-1}.[41] Meanwhile, the alkyl groups are represented by two absorption bands observed at 2916 and 2848 cm^{-1}. An absorption band observed at 1579 cm^{-1} corresponds to the N–H bending vibration associated with the amine group. The FTIR spectrum of EFB exhibits a characteristic of C–N stretch at the wavenumber of 1247 cm^{-1}.[41] A sharp absorption band observed at 1036 cm^{-1} was assigned to the C–O stretch.

The FTIR spectrum of EFB significantly changed following the interaction with heavy metals during the 8-week pot experiment (figure S3(b)). For instance, the formation of a new absorption band at 1538 cm^{-1} can be related to the interaction between the amine groups of EFB and the heavy metals. In addition, the interaction between the heavy metals and amendments has also caused the absorption intensity of the O–H stretching vibration to decrease. The absorption bands corresponding to O–H, C–N and C–O vibrations shifted to a new wavenumber following interaction with heavy metals. For example, the absorption band representing the OH group shifted from 3217 to 3288 cm^{-1}. Meanwhile, the absorption band corresponding to the C–N stretch shifted from 1247 to 1236 cm^{-1}. In the case of C–O stretching vibration, the absorption band shifted from 1036 to 1022 cm^{-1}.

An FTIR analysis revealed that the three amendments studied exhibited similar functional groups and chemical bonds, namely, hydroxyl (OH) and amine (NH_2) groups, C–H stretch of alkyl groups, C–O and C–N stretches. Following the pot experiment, several changes in the FTIR characteristics were observed including the formation of a new absorption band, the change in absorption intensity and the shift in the wavenumber of the functional groups. Presumably, these changes can be attributed to the complexation mechanism between metal ions and active sites of amendments.[17,22,24] Such a mechanism is important for the success of an immobilisation technique.[14,18]

4. Conclusions

The results of this study highlight the feasibility of three agricultural wastes, namely, DTS,CC and EFB as soil amendments to remediate contaminated soil. The application of the amendments reduced the bioavailability of Cu, Pb and Zn in the firing range soil and the uptake of heavy metals by the water spinach. The presence of hydroxyl and amine groups in the amendments favoured the immobilisation of heavy metals in the firing range soil. The pot experiment, however, determined the fundamental aspects of the soil stabilisation technique. Information is also needed on the biodegradation of the three agricultural wastes studied in metal-contaminated soil. Agricultural wastes are available in large quantities in Malaysia. Their utilisation as clean-up materials to restore contaminated soil would provide a green solution to their disposal.

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Disclosure statement

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Supplemental data

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References

[1] Wei B, Yang L. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchem J. 2010;94(2):99–107.

[2] Xi JF, Yu XZ, Zhou LX, Li DC, Zhang GL. Comparison of soil heavy metal pollution in suburb fields of different regions. Soils. 2011;43:769–775.
[3] Zhang J-H, Fan W-W. Metal partitioning and relationships to soil microbial properties of submerged paddy soil contaminated by electronic waste recycling. Chem Ecol. 2015;31(2):147–159.
[4] Almaroai YA, Vithanage M, Rajapaksha AU, et al. Natural and synthesized iron-rich amendments for As and Pb immobilisation in agricultural soil. Chem Ecol. 2014;30(3):267–279.
[5] Dermatas D, Menouno N, Dutko P, Dadachov M, Arienti P, Tsaneva V. Lead and copper contamination in small arms firing ranges. Global NEST J. 2004;6:141–148.
[6] Moon DH, Park J-W, Chang Y-Y, et al. Immobilization of lead in contaminated firing range soil using biochar. Environ Sci Pollut Res. 2013;20:8464–8471.
[7] Jorgensen SS, Willems M. The transformation of lead pellets in shooting range soils. AMBIO. 1997;16:11–15.
[8] Landsberger S, Iskander F, Basunia S, Barnes D, Kaminski M. Lead and copper contamination of soil from industrial activities and firing ranges. Biol Trace Element Res. 1999;71–72:387–396.
[9] Chrastný V, Komárek M, Hájek T. Lead contamination of an agricultural soil in the vicinity of a shooting range. Environ Monit Assess. 2010;162:37–46.
[10] Ahmad A, Al-Othman AAS. Remediation rates and translocation of heavy metals from contaminated soil through *Parthenium hysterophorus*. Chem Ecol. 2014;30(4):317–327.
[11] Ali Z, Malik RN, Qadir A. Heavy metals distribution and risk assessment in soils affected by tannery effluents. Chem Ecol. 2013;29(8):676–692.
[12] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicology profile for lead. Atlanta, GA: US Department of Health and Human Services; 2007.
[13] Lakshmi PM, Jaison S, Muthukumar T, Muthukumar M. Assessment of metal accumulation capacity of *Brachiaria ramosa* collected from cement waste dumping area for the remediation of metal contaminated soil. Ecol Eng. 2013;60:96–98.
[14] Venegas A, Rigol A, Vidal M. Viability of organic wastes and biochars as amendments for the remediation of heavy metal-contaminated soils. Chemosphere. 2015;119:190–198.
[15] Bian R, Joseph S, Cui L, et al. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. J Hazard Mater. 2014;272:121–128.
[16] Alvarenga P, Mourinha C, Farto M, et al. Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: benefits versus limiting factors. Waste Manage. 2015;40:44–52.
[17] Kumpiene J, Lagerkvist A, Maurice C. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments - a review. Waste Manage. 2008;28(1):215–225.
[18] Lee S-H, Park H, Koo N, Hyun S, Hwang A. Evaluation of the effectiveness of various amendments on trace metals stabilization by chemical and biological methods. J Hazard Mater. 2011;188(1):44–51.
[19] Ruttens A, Adriaensen K, Meers E, et al. Long-term sustainability of metal immobilization by soil amendments: cyclonic ashes versus lime addition. Environ Pollut. 2010;158(5):1428–1434.
[20] Park JH, Lamb D, Paneerselvam P, Choppala G, Bolan NS, Chung J-W. Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. J Hazard Mater. 2011;185(2):549–574.
[21] Armelin MJA, Trevizam AR, Muraoka T, et al. Phosphate effect on the content of selected elements in a lettuce variety grown at a contaminated soil. J Rad Nucl Chem. 2014;301(1):17–21.
[22] Lee S-H, Lee J-S, Choi YJ, Kim J-G. In situ stabilization of cadmium-, lead-, and zinc-contaminated soil using various amendments. Chemosphere. 2009;77(8):1069–1075.
[23] Abdelhafez AA, Li J, Abbas MHH. Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil. Chemosphere. 2014;117:66–71.
[24] Janoš P, Vávrová J, Herzogová L, Pilařová V. Effects of inorganic and organic amendments on the mobility (leachability) of heavy metals in contaminated soil: A sequential extraction study. Geoderma. 2010;159: 335–341.
[25] Yusoff SNM, Kamari A, Putra WP, et al. Removal of Cu (II), Pb (II) and Zn (II) ions from aqueous solutions using selected agricultural wastes: adsorption and characterisation studies. J Environ Protect. 2014;5:289–300.
[26] Gee GW, Bauder JW. Particle size analysis. In: Klute A, editor, Methods of soil analysis. part I. Physical and mineralogical methods. Madison, WI: ASA-SSAA; 1986. p. 383–411.
[27] Lu RK. Analysis methods of soil agricultural chemistry. Beijing, China: Chinese Agricultural Science Technology Press; 2000. p. 60–65.
[28] Jackson ML. Soil chemical analysis. New Delhi, India: Prentice Hall of India; 1967. p. 498–576.
[29] Sumner ME, Miller WP. Cation exchange capacity and exchange coefficients. In: Sparks DL, editor. Method of soil analysis: chemical methods. Madison, WI: American Society of Agronomy; 1996. p. 1031–1075.
[30] Serbula SM, Radojevic AA, Kalinovic JV, Kalinovic TS. Indication of airborne pollution by birch and spruce in the vicinity of copper smelter. Environ Sci Pollut Res. 2014;21:11510–11520.
[31] McGrath SP, Zhao F-J. Phytoextraction of metals and metalloids from contaminated soils. Curr Opinion Biotechnol. 2003;14(3):277–282.
[32] Yu X-Z, Wang D-Q, Zhang X-H. Chelator-induced phytoextraction of zinc and copper by rice seedlings. Ecotoxicol. 2014;23:749–756.
[33] Cabello-Conejo MI, Becerra-Castro C, Prieto-Fernández A, et al. Rhizobacterial inoculants can improve nickel phytoextraction by the hyperaccumulator *Asylum pintodasilvae*. Plant Soil. 2014;379:35–50.
[34] Ruiz E, Rodríguez L, Alonso-Azcárate J, Rinćon J. Phytoextraction of metal polluted soils around a Pb–Zn mine by crop plants. Int J Phytorem. 2009;11(4):360–384.

[35] Kanwal U, Ali S, Shakoor MB, et al. EDTA ameliorates phytoextraction of lead and plant growth by reducing morphological and biochemical injuries in *Brassica napus* L. under lead stress. Environ Sci Pollut Res. 2014;21:9899–9910.
[36] Kim IS, Kang KH, Johnson-Green P, Lee EJ. Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction. Environ Pollut. 2003;126:235–243.
[37] Jarvis MD, Leung DWM. Chelated lead transport in *Pinus radiata*: an ultrastructural study. Environ Exp Bot. 2002;48:21–32.
[38] Kalis EJJ, Temminghoff EJM, Town RM, Unsworth ER, van Riemsdijk WH. Relationship between metal speciation in soil solution and metal adsorption at the root surface of ryegrass. J Environ Qual. 2008;37:2221–2231.
[39] Nouri J, Khorasani N, Lorestani B, Karami M, Hassani AH, Yousefi N. Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. Environ Earth Sci. 2009;59(2):315–323.
[40] Naidu R, Bolan NS. Contaminant chemistry in soils: Key concepts and bioavailability. In: Naidu R, editor. Development in soil science: chemical bioavailability in terrestrial environments. Oxford: Elsevier; 2008. p. 9–38.
[41] Nakamoto K. Infrared and Raman spectra of inorganic and coordination compounds. part B: applications in coordination, organometallic, and bioinorganic chemistry. New Jersey: John Wiley & Sons; 2009. p. 20–23.