

Cadmium and Zinc Content in Oil Palm Seedlings and their Phase Associations in Jawa Series Soil Applied with Phosphate Rock and Amended with Palm Oil Mill Effluent Sludge and Lime

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ABSTRACT

This study was conducted to determine the mobility and availability of Cd, Zn and P to oil palm seedlings and their soil phase association (water soluble, exchangeable, carbonate, Fe-Mn oxide, organic and residual fractions) after the soil was applied with a phosphate rock fertiliser and amended with palm oil mill effluent (POME) or lime. The potential of these amendments in reducing Cd uptake by oil palm seedlings from phosphate rock fertiliser application was also investigated. Four rates of POME (0, 5, 10 and 20 t/ha) and lime (0, 2, 4 and 8 t/ha) were applied on a Jawa Series soil (Sulfic Endoaquepts) planted with 3-month-old oil palm seedlings until the 9th month. In lime amended soil, 65% of Cd in the soil was in the immobile phase and Cd content in the root decreased with increasing rates of lime application. Meanwhile, application of POME sludge amendment resulted in 44% of Cd being in the mobile fraction and 56% in the immobile phase. The mobile fractions, which comprised the exchangeable and water soluble fractions, were found to increase with increasing POME sludge rates. However, there was no influence on the Cd content in plant parts. Application of POME sludge also increased Zn content in roots and leaves as exchangeable Zn fraction also increased with increasing application rates. As for P, the content increased in all plant parts as a result of POME sludge application. Cadmium in the soil was dominant in the mobile fraction (exchangeable), while Zn and P were dominant in the immobile fractions (residual and organic, respectively) after being amended with POME and lime. Thus, lime was found to be a better amendment in reducing Cd uptake from PR fertiliser application in comparison to POME.

Keyword: Sequential fractionation study, potential acid sulfate soil, phosphate rock fertiliser, heavy metals, alkaline mineral and biosolid amendments.

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INTRODUCTION

High rates of phosphate fertilisers (PF) are required at the early stage of oil palm growth to build P stock in soils and also to overcome high P-fixing capacity of tropical soils (Corley and Tinker 2003). However, phosphate rocks (PR) that are commonly used as PF contain Cd as an impurity which could be taken up by oil palm seedlings and consequently might enter the human body through the edible parts. It is not a common practice to lime the soil at the nursery stage or while the plant is growing in the field. However, soil amendments such as alkaline minerals (liming materials) and biosolid byproduct of palm oil mill (POME sludge) is speculated to be effective in reducing heavy metal availability and phytotoxicity.

Soil organic matter has been of particular interest in studies of heavy metal sorption by soils because of its significant contribution towards CEC and more importantly, the tendency of transition metal cations to form stable complexes with organic ligands. Organic matter of biosolids with suitable reactive groups such as hydroxyl, phenoxyl and carboxyl can effectively control the adsorption and complex formation of heavy metals with soil, and hence affect the activity of metals in soils (Lee *et al.* 2004). Therefore, high organic matter content or addition of organic matter decreases solution concentration of Cd due to its high CEC that enhances chelation of organic colloids.

Liming the contaminated soil to reduce the bioavailability of heavy metals is the most widely used remediation treatment. Lime may increase soil pH because of the release of hydroxyl ions by the hydrolysis reaction of calcium carbonate, and thus, significantly decreases the exchangeable and water soluble fraction of metals in contaminated soil; it also induces and increases the residual metal form as well as heavy metal precipitation as metal-carbonates (Lombi *et al.* 2002; Santona *et al.* 2006; Garau *et al.* 2007 and Lee *et al.* 2004).

Knowledge of both the total concentration and chemical forms or phase association of metal is necessary to understand their behaviour in the soil system (Zauyah *et al.* 2008). However, it has long been accepted that the total soil metal content alone is not a good measure of bioavailability and not a very useful tool in determining the potential risks associated with soil contamination (Prokop *et al.* 2003). Khan and Frankland (1983) state that the total concentration may indicate the potential toxicity, but the proportion of such metals in the various chemical forms will determine the actual toxicity in the soil-plant system at a particular point in time. Sequential chemical extraction techniques have been widely used to examine physico-chemical forms to better understand the processes that influence an element's potential mobility and availability. Speciation or fractionation refers to both the process and the quantification of the different defined species, forms and phases of a trace metal. This process in soils is related to the soil biogeochemical reactivity and several physico-chemical conditions of the soil (Kabata- Pendias 2004). To more fully understand the dynamics of the heavy metal in agriculture, it is important to identify the forms/fractions of the heavy metal in the soil.

As soils consist of a heterogeneous mixture of different organic and organo-mineral substances, clay mineral, oxides of Fe, Al, Mn and other solid components

as well as a variety of soluble substances, the binding mechanisms for metals in soils are manifold and vary with the composition of soils and their physical properties. The metal associated with different binding sites can be defined by the selective extraction method (Chen *et al.* 2000; Krishnamurti and Naidu 2003; Kim and Fergusson 1991). Metal fractions can be categorised as shown in Table 1. Water soluble and exchangeable fractions, commonly regarded as the most mobile bioavailable form of soil elements comprise free ions. This form would have a direct bearing on heavy metals uptake by plants (Table 2) (Ahnstrom and Parker 1999). Meanwhile, carbonates, Fe-Mn oxides, organic and residual fractions are considered as immobile and become mobile and phytoavailable with time.

Hence, this study was conducted to determine whether palm oil mill effluent (POME) and lime amendment can help reduce Cd uptake by oil palm seedlings as these soil amendments are commonly used in the oil palm plantations of Malaysia. The study also aimed to determine whether these amendments affect P and Zn uptake, which is highly present in PR fertilisers and serves as one of the micronutrients required by oil palm. The fractionation study was carried out as well to determine the metals and nutrients phase associations.

TABLE 1
General Cd fractions in soil

Fraction	Explanation
Water soluble	Most loosely held by soil
Exchangeable	Easily exchange
Carbonate	Bound to carbonates
Fe-Mn oxide	Bound to iron and manganese oxide
Organic	Bound to organic matter
Residual	Associated with residual matter

Sources: Kim and Fergusson (1991)

TABLE 2
Evaluation of the sequential extraction reagents for each fraction

Fraction	Reagent	Evaluation of the sequential extraction
Water soluble/exchangeable	Distilled water/1.0 MgCl	Extract most mobile, bioavailable form comprised free ions and soluble complexes
Carbonate	1.0 M NaOAc, pH=5.0	Dissolutions of soil carbonates. Solubilised specifically sorbed Cd
Fe-Mn	0.04M NH ₂ OH.HCl in 25% NaOAc	Dissolved reducible oxides
Organic	0.02M HNO ₃ , 30% H ₂ O ₂ , pH=2, 3.2 M NH ₄ OAc in 20% HNO ₃	Released substantial amounts of trace metals bound to organic matter
Residual	Aqua-regia (3:1 HNO ₃ : HCl)	Dissolution of any remaining mineral matter

Source: Kim and Fergusson(1991) and Ahnstrom and Parker (1999)

MATERIALS AND METHODS

A glasshouse study was conducted where oil palm seedlings were planted in unfertilised Jawa Series (Sulfic Endoaquepts), a potential acid sulfate soil, which represents coastal soils largely planted with oil palm in the West coast of Peninsular Malaysia. The soil was collected at Pulau Indah, Klang. Two experimental units were set-up simultaneously, with only POME biosolids being applied at different rates in one experiment and for the other, only lime at different rates being applied. The rates of POME sludge were 0, 5, 10 and 20 t/ha or 0, 66.6, 133 and 266.6 g per 20 kg soil. Meanwhile, the rates for CaCO₃ were 0, 2, 4 and 8 t/ha or 0, 13.34, 26.6 and 53.4 g per 20 kg of soil. The rate of 2 t/ha is the recommended lime requirement of highly weathered soils in Malaysia which was obtained experimentally by Shamshuddin (1989). Both amendments were replicated four times which made a total number of 32 polybags. Nitrogen, P and K fertiliser were applied based on the manuring program of Sime Darby Seed & Agriculture Services Sdn. Bhd (one of the main oil palm plantation companies in Malaysia). The amendments and fertilisers were applied once and mixed homogenously with the soil. Then, 3-month-old seedlings of the same size and height were planted in each polybag. The experimental design for this experiment was a complete randomised design (CRD). After 9 months (12-month-old oil palm seedlings), the plants were harvested. The whole plant of each treatment and replicate were sampled. The plants were separated into root, rachis and leaf portions (leaf spikelets with the mid-rib removed).

Soil and Plant Analyses

Soil pH was determined in a soil-water suspension using 1:2.5 soil solution ratio. Cadmium, Zn and P in plant parts were determined by the dry ashing method. All the elements in soil and plant tissue were determined using Perkin Elmer 5100 flame atomic absorption spectrophotometry except for Cd in plant parts which used PE 5100 graphite furnace atomic absorption spectrophotometry.

Sequential Extraction of Cadmium, Zinc and Phosphorus.

After the oil palm seedlings were harvested, the soil was homogenised before being air-dried. The soil was then ground to pass through a 2-mm sieve. The phase-associated forms of Cd, Zn and P (water soluble, exchangeable, carbonate, Fe-Mn oxide, organic and residual fractions) in the soil were determined through sequential extraction. The use of selective extractants to quantify the elemental content in a particular phase is described by the concept of pools in soils, of elements of different solubilities and mobilities that can be selectively sampled by extractants of different strengths. In this method, a sample is treated with a series of progressively harsher reagents to dissolve increasingly refractory forms. Ideally, the reagents are chosen to selectively attack a specific soil compartment with the minimal dissolution of a non-targeted phase (Ahnstrom and Parker 1999; Obrador *et al.* 2007). The method of sequential extraction procedure used in this study originated from Tessier *et al.* (1979), which was then modified by Yang and Kimura (1995) and Chlopecka *et al.* (1996). This method has been used to determine Cd or other heavy metal fractions in contaminated soils by several researchers (Kim and Fergusson 1991; Chowdhury *et al.* 1997; Ahnstrom and Parker 1999; Chen *et al.* 2000). For the fractionation analysis, 5 g of each soil sample was weighed into the centrifuge tube and sequential extraction was conducted as shown in Figure 1. All the elements in the different soil fractions were analysed using the PE 5100 flame atomic absorption spectrophotometry.

Statistical Analysis

Data obtained from the dry ashing and fractionation methods were subjected to ANOVA analysis using Tukey for means comparison.

RESULTS AND DISCUSSION

Cadmium and Zinc Content in Soils, Fertilisers and Amendments Used

Analyses on Jawa Series soil show that Zn was present but Cd was not detectable as shown in Table 3. Zinc was present in both amendments (POME and lime) and 9.71 mg kg⁻¹ Cd existed in lime whilst it was not detectable in POME. Urea fertiliser was free from Cd and Zn contamination while muriate of potash (MOP) contained 2.65 and 5.87 mg kg⁻¹ of Cd and Zn, respectively. Zinc in Gafsa phosphate rock (PR), used in this study, was higher (291 mg kg⁻¹) than Cd (89 mg kg⁻¹) with the Zn:Cd ratio of approximately 3:1. Thus, the main source of Cd was from the Gafsa PR fertiliser.

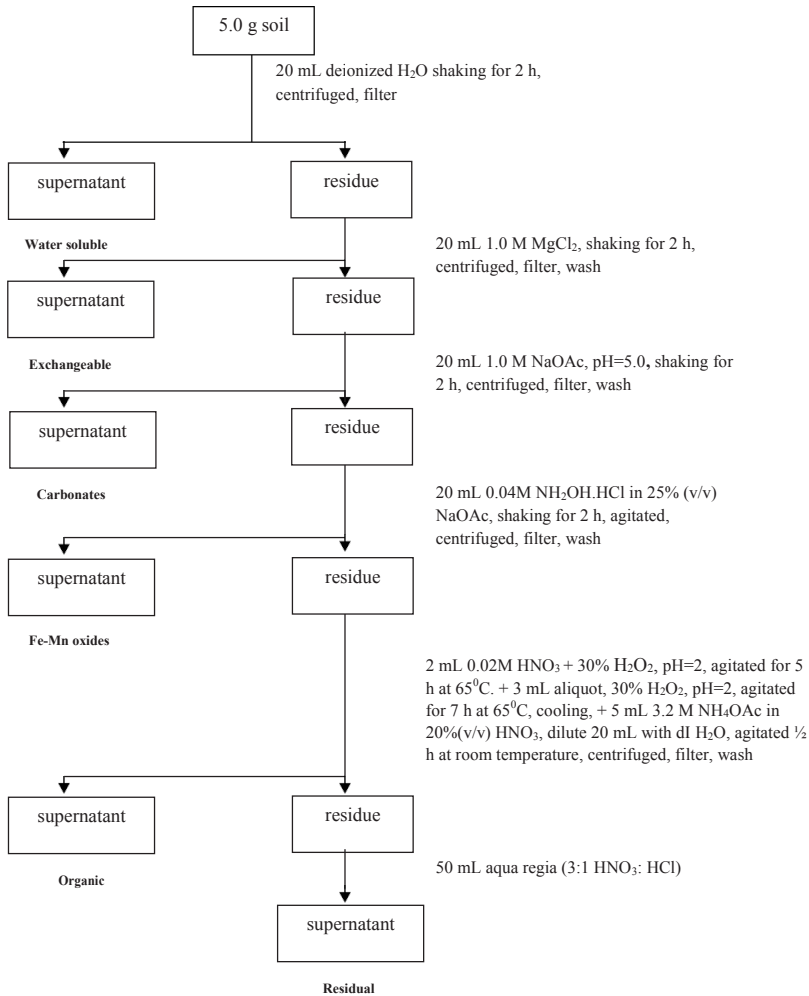


Figure 1. Sequential extraction schemes for cadmium in soil. Method of Tessier et al. (1979) and followed by the modified version of Yang and Kimura (1995) and Chlopecka et al. (1996)

TABLE 3
Cadmium and Zn concentrations (mg/kg) in soil, fertilisers and amendment used

	Jawa	PR	POME	Lime	Urea	MOP
Series Soil	(Gafsa)					
Cd	*nd	89	nd	9.71	nd	2.65
Zn	3.59	291	50	7.62	nd	5.87

*Not detectable (nd)

Cadmium, Zinc and Phosphorus Content in Plant Parts Amended with POME

There was no significant difference ($p>0.05$) in Cd content of root, leaf and rachis of plants grown in Jawa Series among the four rates of POME as shown in Figure 2(a). Meanwhile, there were significant differences ($p<0.05$) for Zn content in root and leaf between the treatment rates T2 to T4. However, the rachis showed no significant difference ($p>0.05$) among the rates, but the content generally increased from T1 to T3 as shown in Figure 2(b). Phosphorus content significantly increased ($p<0.05$) throughout the rates in all plant parts (root, leaf and rachis) as shown in Figure 2 (c).

Cadmium, Zinc and Phosphorus Content in Plant Parts Amended with Lime.

Soil amended with lime showed significant difference ($p<0.05$) in Cd content in root among four rates of amendments in which Cd concentration obviously decreased with increased lime rates from T1 to T4. Meanwhile, there was no significant difference ($p>0.05$) in Cd content of other plant parts (leaf and rachis) as shown in Figure 2(d). Zinc showed no significant difference in all plant parts among the four rates of lime as shown in Figure 2(e). Also, Figure 2(f) showed no significant difference ($p>0.05$) in P content of all plant parts.

Soil pH Amended with POME and Lime.

Soil pH amended with POME showed no significant difference ($p>0.05$) among the four rates of POME amendment as shown in Figure 3(a). However, soil pH was highest in rates T3 (4.76) and decreased slightly in rates T4 (4.71). Meanwhile, soil pH amended with lime showed a significant difference ($p<0.05$) with the soil pH increasing from rates T2 to T4 as shown in Figure 3(b). Increasing soil pH generally decreases metal availability through a precipitation reaction.

Cadmium, Zinc and Phosphorus Fractionation Study in Soil Amended with POME

The distribution of Cd in six different types of extractants for four rates of POME treatments is shown in Figure 4(a). The exchangeable Cd (mobile) and carbonates fraction (immobile) were the dominant fractions (average of 0.55 mg/kg and 0.54 mg/kg, respectively). The decreasing order of Cd fraction was exchangeable = carbonate > water soluble > residual > organic > Fe-Mn form.

Increasing addition of POME from 0, 5, 10 to 20 t/ha resulted in significant differences ($p<0.05$) for all Cd fractions except for the organic fraction (Figure 4(a)). The mobile fraction (exchangeable) generally increased with increasing POME rates, with the higher rates of POME addition (T3 and T4) exhibiting higher Cd concentration compared to the other rates. The increase in water soluble fraction is presumably due to the formation of a soluble organic matter metal complex. However, the concentrations of mobile fractions were very low, ranging from 0.05 to 0.75 mg/kg Cd which might be the reason for the absence of a significant difference ($p>0.05$) of Cd content between T1 to T4 in all plant parts (Figure 2(a)). Cadmium in the carbonate fraction was also high in this soil treatment and generally, there was a significant decrease ($p<0.05$) with an

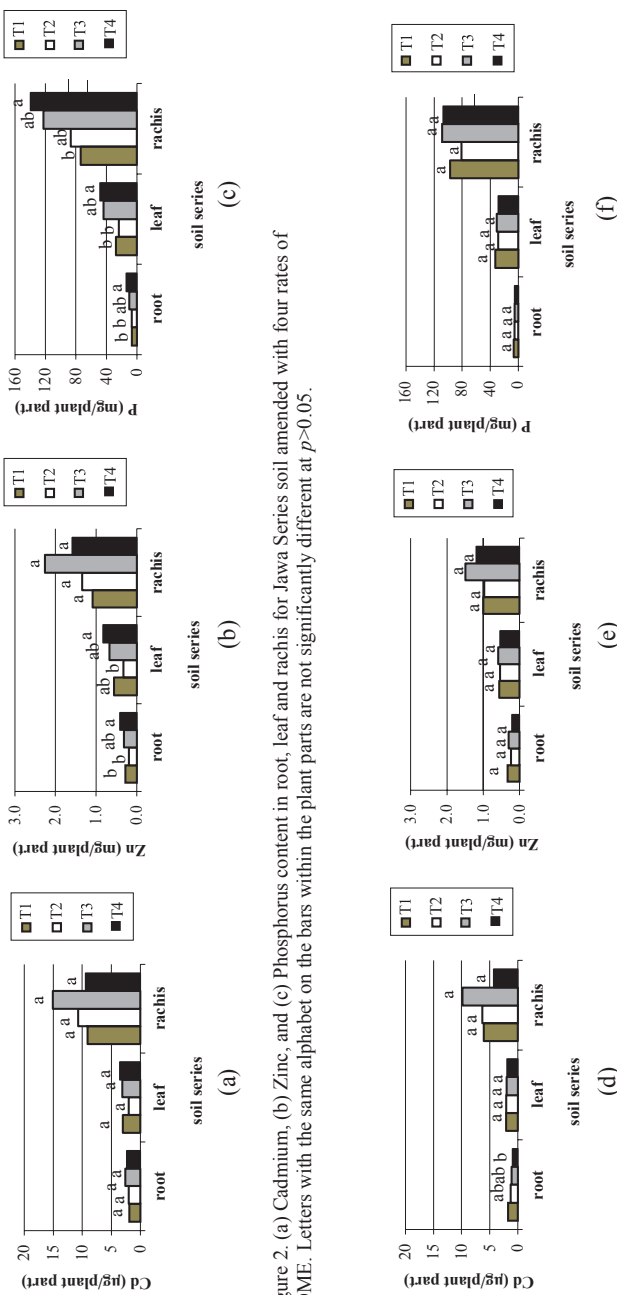


Figure 2. (a) Cadmium, (b) Zinc, and (c) Phosphorus content in root, leaf and rachis for Java Series soil amended with four rates of POME. Letters with the same alphabet on the bars within the plant parts are not significantly different at $p > 0.05$.

Figure 2. (a) Cadmium, (b) Zinc, and (c) Phosphorus content in root, leaf and rachis for Java Series soil amended with four rates of POME. Letters with the same alphabet on the bars within the plant parts are not significantly different at $p > 0.05$.

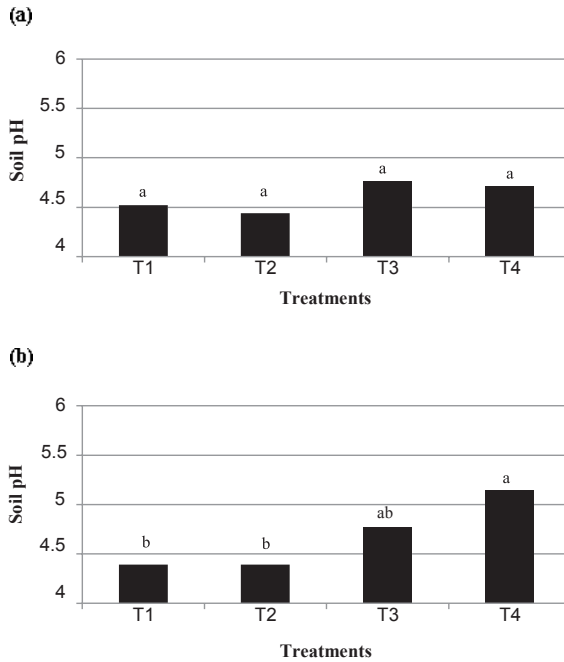


Figure 3. Soil pH of Jawa Series soil amended by (a) POME (b) lime

Letters with the same alphabet on the bars within the rates of treatment are not significantly different at $p > 0.05$.

increase in POME rates. However, POME rates had little influence on the Fe-Mn and residual fraction. This is similar to the findings of Shuman (1998) on Typic Kandudult soils amended with organic waste. Low non-significantly different Cd concentrations in the organic fraction was found for control and at all POME treated rates. Since Cd is loosely bound to organic matter (forms a weak complex with organic matter), this fraction could have been extracted at the first level of extraction. Cadmium added to soils through anthropogenic sources associates with organic matter more than mineral surfaces, but becomes available upon decomposition of the organic matter and when soils are acidified (Shuman1998). Meanwhile, the highest amount of Zn fraction was found in the residual form (immobile) as shown in Figure 4(b), probably because the soil is an Inceptisols, which is an extremely young and relatively unweathered soil. This fraction is made up of primary and secondary silicate minerals and therefore extremely inert and completely unavailable for plant uptake (Chowdhury *et al.* 1997). Next are the exchangeable and Fe-Mn fractions which are of similar values (average 3.23 and 3.2 mg/kg, respectively). The decreasing order of Zn fractions were residual > exchangeable = Fe-Mn > organic > carbonates > water soluble fractions.

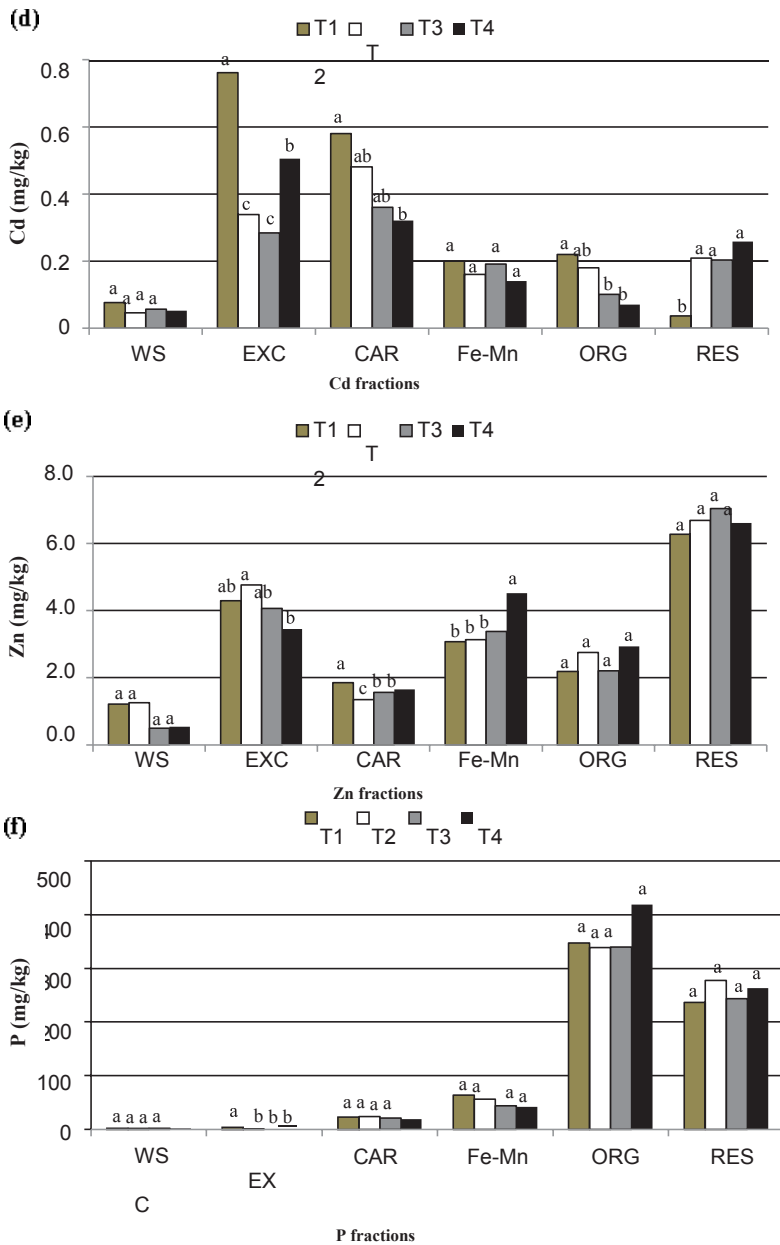


Figure 4. Concentrations of (a) Cd, (b) Zn, and (c) P fractions in Jawa Series soil amended with four rates of POME. Letters with the same alphabet on the bars within the same soil fractions are not significantly different at $p > 0.05$.

Zinc in exchangeable and Fe-Mn and organic fractions showed significant differences ($p < 0.05$) between the T4 and T2 rates of POME amendment, and no significant difference ($p > 0.05$) for the rest of the Zn fractions (water soluble, carbonates and residual) as shown in Figure 4(b). There was no significant difference of exchangeable Zn concentration between T2 and control (T1); however, the concentration significantly increased with an increase in POME rates for T2 and T4. The Zn water soluble fraction concentrations were not statistically significant. Thus, the exchangeable fraction was the mobile fraction that might have caused a significant increase ($p < 0.05$) in Zn content of roots and leaves as shown in Figure 2(b). Also, the concentration of Fe-Mn fractions was high in this soil treatment, possibly due to the soluble organic matter that led to Zn redistribution among fractions (Shuman 1998). Heavy metals in Fe-Mn oxides and organic fraction were of low solubility and high stability for biological activity and would not have a direct bearing on their uptake by plant (Ahnstrom and Parker 1999). The low Zn concentrations of organic fractions were similar to those reported by Zauyah *et al.* (2008) which indicates that the association may be relatively unstable (Kashem and Singh 1999). Kashem and Singh (1999), in their study on contaminated soils, reported that organic matter had little effect on Zn partitioning although the soils contained a higher amount of organic matter.

In this POME treated soil, P concentrations in the organic fractions were the highest, followed by residual > Fe-Mn > carbonates = exchangeable = water soluble fractions (Figure 4(c)). From this figure, P in the mobile fractions (water soluble and exchangeable) was much less than in the immobile fractions (residual, organic, Fe-Mn and carbonates). For P, only T4 residual fraction showed a significant increase ($p < 0.05$) in comparison to T2 (Figure 4(c)) whilst other fractions were not statistically different ($p > 0.05$).

Cadmium, Zinc and Phosphorus Fractionation Study in Soil Amended with Lime
Soil amended with 0, 2, 4 and 8 t/ha of lime showed dominance of Cd in the exchangeable fraction followed by carbonates > residual = Fe-Mn = organic > water soluble fraction (Figure 4(d)). Carbonates and exchangeable fractions were obviously higher than other fractions. The result obtained was similar to soil amended with POME. There were significant differences ($p < 0.05$) for all Cd fractions among the four rates of lime amendment except for water soluble and Fe-Mn as shown in Figure 4(d). The exchangeable fraction concentrations in T2 to T4 were significantly lower than in control (T1) while the residual fractions in T2 to T4 were significantly higher than in control (T1). This study revealed that increasing soil pH (Figure 3(b)) might have increased the concentration of the residual fraction which reduced Cd availability to the oil palm seedlings (Garau *et al.* 2007). The Cd concentrations in water soluble fractions of lime treated soil were lower (average 0.05 mg kg^{-1}) than in soil amended with POME (average 0.10 mg kg^{-1}). The decrease in concentrations of mobile fractions (water soluble and exchangeable) might be the reason for the significant decrease ($p < 0.05$) in Cd content of the roots (Figure 2(d)). Additionally, competition between Cd and Ca

(from lime) at root surfaces led to less Cd uptake from soils (Ramachandran and D'Souza 1998). The carbonates and organic fractions decreased with an increase in lime rates.

The highest fraction of Zn concentrations in the lime treated soil was found to be the residual form and which decreased in the order residual > exchangeable = Fe-Mn > organic > carbonates > water soluble fraction (Figure 4(d)). In this lime treated soil, the exchangeable fraction had a similar value with Fe-Mn fraction (average 4.14 and 4.13 mg kg⁻¹, respectively) but was higher than the other immobile fractions (carbonate and organic). Zinc showed a significant difference ($p < 0.05$) for exchangeable, carbonate and Fe-Mn fraction, while there was no significant difference ($p > 0.05$) for water soluble, organic and residual fractions (Figure 4(e)). The exchangeable Zn decreased with increasing lime rates from T2 to T4. Zn concentrations in water soluble fractions were not statistically different. The immobile fraction (Fe-Mn) significantly increased for the highest lime treatment of 8 t/ha compared to the other treatments. This clearly showed that lime amendment decreased the Zn mobile fraction. The pH increase induced by the lime treatment favoured heavy metal precipitation and also could have resulted in an increase in heavy metal sorption by variable charged colloids such as organic matter and Fe-Mn oxides, finally resulting in reduced concentration of available metal (Garau *et al.* 2007).

Phosphorus concentrations in organic fractions exhibited the highest levels compared to other fractions. The decreasing order of P concentration in the soil fractions for lime treated soil was organic > residual > Fe-Mn > carbonates > water soluble = exchangeable (Figure 4(f)). Immobile fractions (residual and organic fraction) were much higher than in water soluble and exchangeable fractions (mobile fractions). In summary, for the sequential fractionation study, POME amendment resulted in 44% of Cd in mobile fraction with 56% associated with the immobile fractions, while lime amendment caused 35% of Cd to be in the mobile and 65% in the immobile fractions. However, the highest Cd concentrations were in the mobile fraction (exchangeable) for POME, and the immobile fraction, carbonate (which can turn to mobile fraction when soil condition changes) with lime amendment.

About 70% of Zn was associated with immobile fractions in soil amended with POME and lime, with 30% being in the mobile fractions. Soil amended with POME and lime resulted in less than 1% of P being in the mobile fractions and the rest in the immobile fraction. Table 4 shows the summary of decreasing order of Cd, Zn and P fractions for Jawa Series soil amended with POME sludge and lime. Thus, in this study, it was found that lime was a better soil amendment in reducing Cd uptake from PR application in comparison to POME.

TABLE 4
 Distribution of Cd, Zn and P fractions in Jawa Series soil amended with POME and lime

Soil Series	Amendment	Element	Fraction
Jawa	POME sludge	Cd	Exchangeable = Carbonate > Water Soluble > Residual > Organic > Fe-Mn
		Zn	Residual > Exchangeable = Fe-Mn > Organic > Carbonate > water Soluble
		P	Organic > residual > Fe-Mn > Carbonate = Exchangeable = Water Soluble
	lime	Cd	Exchangeable > Carbonates > Residual = Fe-Mn = Organic > Water Soluble
		Zn	Residual > Exchangeable > Fe-Mn > Organic > Carbonate > water Soluble
		P	Organic > residual > Fe-Mn > Carbonate > Water Soluble = Exchangeable

CONCLUSION

Jawa Series amended with four rates of POME resulted in an increase in the uptake of Zn and P (nutrients) by plant parts. Meanwhile, Cd (heavy metal) content decreased in roots when amended with lime. Cadmium was dominant in the mobile fraction (exchangeable) in Jawa Series amended with both POME and lime. Furthermore, the Cd concentration generally increased with an increase in POME rates while it decreased for lime throughout the rates. Meanwhile, Zn and P were dominant in the immobile fractions (residual and organic, respectively) for Jawa Series amended with both POME and lime.

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