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THE EFFECTS OF MICRO- AND NANOHYDROXYAPATITE APPLICATION IN METAL CONTAMINATED SOIL ON METAL ACCCUMULATION IN IPOMOEA AQUATICA AND SOIL METAL BIOAVAILABILITY

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Abstract

The potential of microhydroxyapatite (MHA) and nanohydroxyapatite (NHA) to immobilise heavy metals in a 25-year old active firing range soil was investigated. The effects of immobilisation were evaluated in terms of metal accumulation in water spinach (*Ipomoea aquatica*) and soil metal bioavailability. A pot trial was conducted by amending firing range soil with MHA and NHA at application rates of 0%, 1% and 3% (w/w). Both amendments increased biomass yield and reduced plant metal uptake. The bioconcentration factor (BCF) values of the metals were in the order of Zn > Cu > Pb. The bioavailable fraction of Cu, Pb and Zn in firing range soil decreased significantly ($p < 0.05$) following MHA and NHA treatments. No toxicity symptoms were observed in water spinach over the pot trial. Therefore, MHA and NHA are two promising immobilising agents for the remediation of metal contaminated land.

Key words: Contaminated soil, heavy metal, soil stabilisation, water spinach, microhydroxyapatite, nanohydroxyapatite

INTRODUCTION

Soil contamination by heavy metals is a serious environmental problem all over the world [1]. For example, about 3.5 million sites of industrial and mine sites, landfills, energy production plants and agricultural land in Europe were reported to be contaminated by heavy metals [2]. In fact, soil contamination and strategy for soil protection were classified as two important issues for action in the European Community [2]. Meanwhile, 20 million hectares of arable land, accounting 20% of the total agricultural land area in China have been identified for heavy metal contamination [3]. In recent years, the concentration of heavy metals in soil has increased tremendously due to rapid global industrialisation. Waste emissions from industrial production, mining activities, biosolids and manure application, wastewater irrigation, and inadequate management of pesticides and chemicals in agricultural production have significantly contaminated soil and groundwater [4]. The level of heavy metals in soil has also influenced by firing range activities. Firing bullets are mainly composed of Pb-alloy slugs enclosed with Cu-alloy jackets [5,6]. Metal particulates originating from multiple impacts of bullet fragments during range operations can be oxidised and transformed into compounds that can be mobilised in soil environment [7-9]. An analysis on metal concentration in military firing range soil collected from Busan Metropolitan City, Korea by Moon et al. [6] found 11,885 mg/kg Pb. Parra et al. [10] measured 9,600 mg/kg Pb in topsoil samples collected from a firing range in New Mexico, USA. Meanwhile, firing range soil of the Small Arms Training Area, Aiken, USA was reported to contain 3,282 mg/kg Pb and 1,762 mg/kg Cu [11].

Toxic metals are not biodegradable and persist for a long period of time in soils. They are not only harmful to ecosystems and agricultural production, but also a serious threat to human wellbeing. Their presence in soil may pose a great risk to food chain and water supplies. Considerable efforts have been made to remediate metal contaminated soils. There are many techniques available for the remediation of metal contaminated soils, such as mechanical separation, solidification, soil washing, heap leaching, soil flushing and electrokinetic [12,13]. However, many of these techniques are costly and not practical to implement. Soil stabilisation is a cost-effective and promising soil remediation technique, and has been extensively used in immobilisation of heavy metals in contaminated soils [14]. This technique relies on application of the soil amendments to help retain metals in the stable solid phase by sorption, precipitation, complexation, ion exchange or redox process, thereby decreasing mobility and bioavailability of metals [14,15].

Liang et al. [14] studied the effects of biochar and phosphate application on Cd leachibility from a contaminated soil. The Cd concentration in TCLP (toxicity characteristics leaching procedure) extract was reported to reduced by 19.6% and 13.7%, respectively. They also reported that the concentration of Cd in the groundwater was reduced by up to 62.7%. The immobilisation of Pb and Zn in a contaminated soil using water treatment sludge, blast furnace slag and red mud was assessed by Zhou et al. [16]. Amending contaminated soil using the three amendments was reported to reduce $CaCl_2$, CH_3COOH, HCl and EDTA-extractable Pb and Zn significantly. The Pb and Zn uptake by Rhodes grass was found to decrease with amendments application. A significant immobilising effect was reported for 10% (w/w) treatment. Fang et al. [17] 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.

The overall aim of this work was to evaluate the potential of microhydroxyapatite (MHA) and nanohydroxyapatite (NHA) as immobilising agents for the remediation of metal contaminated soil.

RESEARCH METHOD

In this study, the soil sample was collected from a 25-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 consists of sand (45%), clay (36%) and silt (19%). The soil pH measured in deionised water with a soil:solution ratio of 1:2.5 using a pH meter, was 4.82. The total Cu, Pb and Zn concentrations in the soil determined by aqua regia extraction were 725, 2337 and 364 mg/kg, respectively. 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 ammonium acetate extractable Cu, Pb and Zn were 318, 1066 and 135 mg/kg, respectively.

MHA and NHA (purity > 97%) were purchased from Sigma-Aldrich. Pots with a diameter of 15.0 cm and a height of 18.0 cm were filled with 400 g of soil. MHA and NHA were added 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 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. A pot experiment was carried out for 8 weeks. Water spinach (*Ipomoea aquatica*) seed was sown two weeks after addition of amendments. The pots were arranged in a randomised block design. The water content of the soils was adjusted to obtain 70% of the water holding capacity by adding deionised water daily, avoiding prolonged water logging. 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 pot experiment, the soil pH and ammonium acetate extractable metal content in the soil were determined, as previously described.

The plants were harvested at 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 washed thoroughly with deionised water and dried in an oven at 70 ºC for 48 h. After two days, the dry weight of plant tissue was measured. Dried shoots and roots were milled using a grinder. Milled samples were ashed at 450 ºC for 3 h in a furnace and digested in hot concentrated HNO_3. Metal concentrations in the plant digests and soil extracts were measured by flame atomic absorption spectrometry (AAS).

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 analysed using the same procedures applied for plant tissue and soil samples. The recovery rates were within 90-106% for soil and 86-95% for plant tissue, respectively.

All statistical analyses were performed using Minitab 15 Statistical Software (Minitab Inc., PA, USA). The data were analysed 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 $p < 0.05$.

RESULTS AND DISCUSSION

Plant Growth. The water spinach seeds germinated four days after sowing and no obvious difference in plant growth was observed up to two weeks of the pot experiment. Water spinach grown on compost (uncontaminated soil) were observed to be healthier than plants cultivated on untreated contaminated soil. MHA and NHA treatments resulted in healthy appearance on the plant leaves, whereby the leaves were greener as compared to plants grown on zero treatment (untreated) contaminated soil. Table 1 presents the dry biomass yield of water spinach after 8 weeks of growth. From Table 1, it is clear that the shoot and root yields increased with the rates of amendment application. A pronounced effect was obtained for NHA 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 3.0. MHA 3% and NHA 1% (w/w) treatments gave almost similar shoot yield, with 178-186% increment in biomass production. The highest percentage of increment in root yield was achieved with NHA 3% (w/w) treatment, followed by application of NHA at 1% (w/w). Although there was an increase in the root yield following application of the MHA, statistical analysis revealed no significant difference was obtained between the MHA 1% and MHA 3% (w/w) treatments.

Table 1. Biomass yield of water spinach.

Treatment	Dry weight (g/pot)	
	Shoot	Root
Compost*	13.26	4.51
Zero	3.38 a	1.49 a
MHA 1%	4.68 b	2.14 b
MHA 3%	6.02 c	2.77 b
NHA 1%	6.27 c	2.95 c
NHA 3%	10.16 d	3.53 d
LSD	1.01	0.59

* 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 $p < 0.05$).

MHA and NHA were beneficial as growing media through improvement of soil fertility and provision of plant nutrient. Hydroxyapatite (HA) is a naturally occuring mineral form of calcium apatite with the formula $Ca_{10}(PO_4)_6(OH)_2$. It is an important material in the manufacture of fertiliser, as a source of phosphorus [18]. Due to its role as a plant nutrient provider, HA has been regarded as one of the key materials in agrochemicals formulations. In general, NHA amendment has resulted in higher biomass yield than MHA. This scenario can be related to the particle size of the HA used. The particles sizes of MHA and NHA are 3 μm and 40 nm, respectively. The smaller size of NHA accelerates the rate of degradation process. And therefore, release the phosphorus to soil-plant environment much faster than MHA. Lower yield of biomass obtained for zero treatment plants can be attributed to metal toxicity. No toxicity symptoms were observed on the plant leaves over the pot experiment. This suggests that water spinach is a robust plant species and has great tolerance to high metal concentrations.

Metal Concentration in Plant Tissue. The concentrations of Cu, Pb and Zn in plant shoots after 8 weeks of growth are given in Table 2. It is apparent that MHA and NHA treatments reduced metal concentrations in the shoot tissue of water spinach. From Table 2, metal concentrations in shoots decreased with the rates of amendments application. Marked reductions in metal concentrations were obtained for NHA treatment at 3% (w/w).

Amending contaminated soil with MHA and NHA increased soil pH from 4.82 to 7.72, therefore reducing metal availability for plant uptake. The pH values of MHA and NHA were determined as 7.53 and 7.24, respectively. The reduction in metal concentrations can also be related to the presence of functional groups (PO_4^{3-} and OH^-) on its surface. These functional groups are able to bind or complex heavy metals [15]. Fourier Transform Infrared (FTIR) analysis has confirmed the presence of functional groups on the surface of amendments, as well as the interaction between functional groups and metals (data not shown). It is clear that the accumulation of heavy metals in plant tissues is greatly affected by several factors such as the nature of the amendment, application rate of amendment, the nature of the metal contaminant, plant species and soil pH.

Table 2. Metal concentration in plant shoots.

Treatment	Concentration (mg/kg)		
	Cu	Pb	Zn
Zero	954 d	125 d	1623 c
MHA 1%	827 c	109 c	1485 c
MHA 3%	575 bc	90 b	1296 bc
NHA 1%	513 b	98 b	1144 b
NHA 3%	418 a	75 a	605 a
LSD	84	15	429

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 $p < 0.05$).

Correlations between metal concentrations in soil and metal concentrations in plant shoot were assessed using two extractants, namely EDTA and ammonium acetate (Table 3). It was found that ammonium acetate gave significant correlation between metal concentrations in soil and metal concentration in plant shoot. 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 [4,6].

Table 3. Correlations between metal concentrations in soil and metal concentrations in plant shoot.

Extractant	Metal	Shoot tissue	
		Correlation coefficient	*p*-value
EDTA	Cu	0.029	NS
	Pb	0.013	NS
	Zn	0.042	NS
Ammonium acetate	Cu	0.633	0.000*
	Pb	0.145	0.001*
	Zn	0.826	0.003*

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

Bioconcentration Factor. The bioconcentration factor (BCF) is defined as the ratio of metal concentration in plant shoots to metal concentration in soil [19]. As discussed by Yoon et al. [20], BCF is a measure of the ability of a plant to accumulate metals from soils. In this study, the influence of MHA and NHA treatments on BCF values of the metals was determined, and the values are given in Table 4. The BCF value of Zn for plants grown on zero treatment soil was calculated as 1.24, suggesting that water spinach has great potential for phytoextraction of Zn from contaminated soil. The BCF values suggest that the ability of water spinach to take up Cu, Pb and Zn from soil decreased significantly with the addition of amendments. This can be attributed to metal binding to functional groups of amendments and reduction in metal availability for plant uptake, as discussed in the preceding section.

Table 4. BCF values for Cu, Pb and Zn.

Treatment	BCF		
	Cu	Pb	Zn
Zero	0.33 d	0.22 d	1.24 c
MHA 1%	0.25 c	0.17 d	0.62 b
MHA 3%	0.24 c	0.14 c	0.53 ab
NHA 1%	0.20 b	0.09 ab	0.42 b
NHA 3%	0.14 a	0.06 a	0.26 a
LSD	0.04	0.03	0.13

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 $p < 0.05$).

Metal concentration in plant shoots and soil greatly affects the BCF values. When comparing to Cu and Pb, more Zn was measured in the plant shoots (Table 2). In addition, the total concentration of Zn in the soil (364 mg/kg) was lower than the 725 mg/kg measured for Cu. Therefore, Zn had a greater BCF value than Cu.

Off-take Values. The effect of MHA and NHA application on metal accumulation in plant tissues was further evaluated in terms of off-take value. The off-take value considers both metal concentration in plant tissues and biomass yield [19,20]. The amount of Cu, Pb and Zn removed by water spinach from soil is given in Table 5. It is also important to estimate the off-take value in kg/ha unit as this will provide an insight into the real effect of soil amendments if applied on a contaminated site [2]. The off-take value (kg/ha) was based on conversion factor of pot area to hectare.

From Table 5, it is observed that the removal of Cu, Pb and Zn by water spinach decreased following MHA and NHA treatments. Overall, Zn was the metal most extracted by plants, whereas Pb was the least. At the end of the pot experiment, it is estimated that 0.39 mg/pot of Cu, 0.07 mg/pot of Pb and 3.78 mg/pot of Zn were removed from the untreated contaminated soil. It is also estimated that the off-take value of Zn could be reduced from 9.45 kg/ha (zero treatment) to 5.30 kg/ha (NHA 1% w/w) and 3.00 kg/ha (NHA 3% w/w).

Table 5. Removal of Cu, Pb and Zn from soil.

Treatment	Off-take (mg/pot)			Off-take (kg/ha)*		
	Cu	Pb	Zn	Cu	Pb	Zn
Zero	0.39	0.07	3.78	0.98	0.18	9.45
MHA 1%	0.28	0.06	3.22	0.70	0.16	8.05
MHA 3%	0.21	0.06	2.49	0.53	0.16	6.23
NHA 1%	0.22	0.05	2.15	0.56	0.13	5.30
NHA 3%	0.17	0.05	1.20	0.43	0.10	3.00

* Estimation was based on conversion factor of pot area to hectare.

Bioavailable Fraction of Metals. The uptake of heavy metals by plants is mainly influenced by the bioavailable fraction of metals, not the total fraction of metals in soil [14,15]. Therefore, the effect of application of MHA and NHA on bioavailable fraction of Cu, Pb and Zn in soil was studied using ammonium acetate extraction. The ammonium acetate extractable metals in soil after 8 weeks of the pot experiment are presented in Table 6.

Table 6. Ammonium acetate extractable metal in soil.

Treatment	Concentration (mg/kg)		
	Cu	Pb	Zn
Zero	280 d	1018 d	118 d
MHA 1%	223 c	902 cd	103 c
MHA 3%	208 bc	745 b	75 b
NHA 1%	166 b	857 c	52 ab
NHA 3%	110 a	633 a	44 a
LSD	53	102	25

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 $p < 0.05$).

Amending soil with MHA and NHA decreased the bioavailability of Cu, Pb and Zn significantly, particularly at application rate of 3% (w/w). A lower reducing effect was obtained when amendments were applied at 1% (w/w). For example, the ammonium acetate extractable Pb in soil (1066 mg/kg) decreased to 1018 mg/kg (zero treatment), 857 mg/kg (NHA 1% w/w) and 633 mg/kg (NHA 3% w/w) after 8 weeks of pot experiment. The reduction in the amount of metal extracted after the pot experiment can be related to immobilisation effect of the amendments and uptake by plants.

CONCLUSION AND SUGGESTION

Results from this study highlight the potential of MHA and NHA as immobilising agents for the remediation of metal contaminated land. Due to its smaller size, larger surface area and more active sites, NHA was more effective than MHA in immobilising Cu, Pb and Zn in contaminated soil, and reducing plant metal uptake. Pot experiment however is only one aspect of such utilisation. The effectiveness of both amendments as soil amendments rely on their stability in the soil-water environment. It is necessary to study the biodegradation of MHA and NHA and their effect on metal bioavailability.

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