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Remediation Effects of Different Concentration of Nano-Hydroxyapatite Level on Pb-Contaminated Soil

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Abstract

Phosphorus-containing amendments can reduce the mobility of Pb in soil. Hydroxyapatite (HAP) is one of the most commonly used phosphorus-containing amendments. With the development of nanotechnology, nano-hydroxyapatie (n-HAP) was gradually applied to remediate soil polluted by heavy metals. Considering the concentrations of HAP/n-HAP were not more than 5% in most studies, soil polluted by Pb was artificially prepared and three different concentrations of n-HAP: 5%, 7% and 10% by weight, were added into the Pb-polluted soil separately. The mixtures of soil and n-HAP were incubated for 180 d and sampled regularly. The bioaccessibility of Pb in soil was determined using simulated gastric juices of two *in-vitro* digestion tests: USPM (United States Pharmacopeia Methodology) and PBET (Physiologically-Based Extraction Test). The results showed that the immobilizing efficiency of 5% n-HAP to Pb in soil was the highest in PBET. The extractable Pb from soil by USPM was not affected by concentration of n-HAP. So, the least concentration of n-HAP, *i.e.* 5% n-HAP treatment, was the most cost-effective in USPM. Soil pH increased with concentration of n-HAP. However concentration of n-HAP had little effects on content of soil OM. According to regression analysis, more than 50% differences of the extractable Pb from soil by PBET can be explained by soil pH, while soil pH, organic matter content and incubation time together explained nearly 85% differences of extractable Pb from soil by USPM.

Keywords

Soil Pb, N-HAP, Remediation of Polluted Soil, *In Vitro* Digestion Test, Bioaccessibility

1. Introduction

Phosphorus-containing amendments can reduce the mobility of Pb in soil through ionic exchange and precipitation of pyromorphite-type minierals and are widely used for stabilization of Pb in soil (Kumpiene et al., 2008). Hydroxyapatite (HAP) is one of the most commonly used phosphorus-containing amendments. The chemical formula for HAP is $Ca_{10}(PO_4)_6(OH)_2$ (Nayak, 2010). The immobilization mechanism of HAP for soil heavy metals was summed up as cation exchange, adsorption, surface complexation, precipitation and co-precipitation (Bolan & Duraisamy, 2003). Tang et al. (2004) compared the remediation effectiveness of different phosphorus fertilizers for soil contaminated by Pb and found that HAP was the most efficient material. The absorption ability of substance is closely tied to its specific surface area. The conventional particle size of HAP used before is usually micron-size. With the development of nanotechnology, nano-hydroxyapatie (n-HAP) was gradually applied to remediate soil polluted by heavy metals (Chen et al., 2010; Zhang et al., 2010; He et al., 2013; Jin et al., 2016; Sun et al., 2018; Liang et al., 2017).

The total content of heavy metal in soil is of little use for assessment of its bioavailability, since not all heavy metals in soil are bioavailable. For example, the contents of soil Fe and Mn are relatively high but Fe/Mn deficiency in plants is a common occurrence (McBride, 1989). The potential environmental risk of heavy metals in soil is dependent on their bioavailability. The bioavailability of soil heavy metal relies more on the extractable amount of soil heavy metal under certain conditions.

In vitro digestion test was developed to determine the solubility of heavy metal from soil in simulated digestive juice. It is believed that the dissolution of heavy metal in human digestive juice is the first step for it to be absorbed. Those heavy metals which cannot be dissolved in digestive juice cannot enter circulation system either and will not be available for absorption. Thus, the solubility of heavy metal in digestive juice is referred to as bioaccessibility. The main dissolution mechanisms of digestive juice for heavy metals are the low pH value of digestive juice and the complexation of digestive enzymes, which have something in common with phytoextraction. The results of Li & Zhang (2013) indicated that the extraction result of *in vitro* digestion test can not only represent the bioaccessibility of heavy metals in soil but also the phytoavailability to a certain degree. Compared to animal test and pot experiment, *in vitro* digestion test is economical, reproducible, time-saving, environmentally friendly, easy to operate and good for batch operation (Li et al., 2015). But a universally accepted *in vitro* digestion test has not been established. The bioaccessibility results assessed by different *in vitro* digestion tests are uncomparable, as there are distinct differences in designs and procedures between different *in vitro* digestion tests.

The relatively expensive price, especially for n-HAP, limited the application of HAP/n-HAP in remediation of soil polluted by heavy metals. The concentrations of HAP/n-HAP used in most studies were not more than 5% (Li et al., 2014; Cui et al., 2017; Liu & Guo, 2019; Baghaie & Aghilizefreei, 2021). Boisson et al. (1999) studied the effects of concentration of HAP (0.5%, 1% and 5%) on the solubility of soil metals and reported that the exchangeable metal concentrations of soils decreased with increasing HAP application. But the effects of concentration of HAP/n-HAP on mobility of heavy metals in soil were still unclear when the concentration of HAP/n-HAP was above 5%. Therefore in this study, n-HAP with different concentration (5%, 7% and 10% by weight) was separately added into prepared Pb-spiked soil to stabilize Pb in soil. Then the mixtures of n-HAP and soil were incubated for 180 d and regularly sampled. The bioaccessibility of Pb in the mixtures of n-HAP and soil were measured using two typical and commonly used *in vitro* digestion tests. They are Physiologically-Based Extraction Test (PBET) (Ruby et al., 1993, 1996) and United States Pharmacopeia Methodology (USPM) (Hamel et al., 1998). Soil pH and organic matter (OM) were measured as well. The purposes were to: 1) study the immobilization effects of n-HAP on Pb in soil at different concentration level; 2) reveal the factors influencing the bioaccessibility of Pb in soil during remediation using n-HAP; 3) compare the differences of extraction results between two *in vitro* digestion tests.

2. Material and Methods

2.1. Soil Sample

Soil was collected from a pinus massoniana forest of Guiyang city in Guizhou province of China. Collected soil was air-dried, homogenized, and stored in plastic containers. The Chemical and physical properties of soil sample were listed in **Table 1**.

2.2. Characterization of Soil Samples

Soil sample passing through a 2-mm sieve was used to prepare polluted soil, analyze pH and cation exchange capacity (CEC). Soil sample passing through a 0.15-mm sieve was used to measure OM and total contents of Pb. Soil sample passing through a 0.25-mm sieve was used to assess the soil Pb bioaccessibility.

Particle size distribution was measured using hydrometer method. Soil pH was determined by pH meter (PHS-3C) in a 1:5 ratio of soil to distilled water. Soil OM was characterized using the acid dichromate oxidation method described by Yeomans & Bremner (1988). Soil CEC was determined using the ammonium acetate method (Schollenberger & Simon, 1945). Field water holding capacity was determined using moisture equivalent method (Veihmeyer & Hendrickson, 1931). The total contents of Pb in soil were measured after microwave digestion

Table 1. Chemical and physical properties of soil sample

pH	Field Water Holding Capacity	CEC (cmol·kg^{-1})	Organic Matter (OM) (g·kg^{-1})	Particle Size Distribution (mm)			Pb (mg·kg^{-1})	
				2 - 0.02	0.02 - 0.002	<0.002	Before pollution	After pollution
4.27	31.28%	17.99	30.92	24.76%	16.99%	58.25%	12.54	1091.29

procedure (HNO_3-HF). The contents of total and extracted Pb in soil were determined by analysis using atomic absorption spectrophotometer.

2.3. Preparation, Remediation and Incubation of Pb-Contaminated Soil

The contents of Pb in soil of China were 28.6 - 25380.55 mg·kg^{-1} and averagely 1350.51 mg·kg^{-1} (Wei & Yang, 2010). Thus extra Pb (1000 mg·kg^{-1}) was added into soil, and the polluted soil was homogenized and air-dried at room temperature for a month. Then the polluted soil passing through 2-mm sieve was stored in a plastic container. The total contents of soil Pb before and after artificial pollution were listed in **Table 1**.

Four treatments were designed: 0% (Control), 5%, 7% and 10% n-HAP. Different amounts of n-HAP (20 nm, 1306-06-5, content of Pb ≤ 0.001%) were separately added into the polluted soil (480 g) and mixed thoroughly. Then about 40 g mixture of soil and n-HAP was weighed into a 100 ml plastic beaker (4.5 cm in bottom diameter and 6.7 cm in height). There were 12 cups of mixtures for each n-HAp treatment. The mixtures in cups were incubated in a thermostat incubator (HWS-1000) at 25˚C and the soil moisture was kept at 70% of field water holding capacity. Two cups of mixture from each n-HAp treatment were respectively sampled at 1, 7, 14, 30, 60 and 180 d of incubation, freeze-dried, sieved, homogenized, and stored in plastic bags for later analysis.

2.4. *In Vitro* Digestion Tests

A complete digestion process includes the sequential digestions of mouth, stomach and intestine. However the digestion procedure was usually simplified in application. Considering the solubility of metal was higher in acid environment and for the purpose of precaution, some *in vitro* digestion tests would omit the neutral digestion of mouth and/or intestine (Juhasz et al., 2007). PBET and USPM both only included the gastric digestion in this study.

Soil and simulate gastric juice were shaken in a water bath (100 r·min^{-1}, 37˚C) at a solid-liquid ratio of 1:100. After gastric incubation, 20 ml digested gastric juice were collected, centrifuged (4000 r·min^{-1}, 10 min), then filtered through 0.45 μm membrane filter for analysis. The composition and extraction procedure of simulate gastric solution of PBET and USPM were listed in **Table 2**.

Table 2. Composition and extraction procedure of simulated gastric solution of PBET and USPM.

Method	Composition of 1 L simulate gastric solution	pH	Incubation time
PBET	1.25 g pepsin, 0.5 g citrate, 0.5 g malate, 0.42 ml lactic acid and 0.5 ml acetic acid	adjusted to 2.5 with 1:1 HCl	1 h
USPM	3.20 g pepsin, 7.0 ml concentrated HCl and 2.0 g NaCl	not strictly controlled, the theoretical pH was 1.08 (Morrison & Gulson, 2007)	2 h

2.5. Quality Control

The determination of soil physiochemical properties, PBET and USPM extraction were conducted in triplicate. Standard reference material (GBW 07405) was used for quality assurance of total soil Pb measurement. The recovery of Pb in standard reference material was between 90% and 110%. The content of total and extracted soil Pb was determined by analysis using flame atomic absorption spectrophotometer (novAA350, Jena). The detection limits of flame atomic absorption spectrophotometer for Pb is 15 $\mu g \cdot L^{-1}$. The quality control of the measurement was done by measuring the standard solution every ten samples. All reagents used are analytical reagents. The water used was distilled water.

2.6. Statistical Analysis

The statistical analysis of all data was processed by Microsoft Office Excel 2003 and SPSS 16.0 for windows. The differences between data were analyzed by one-way analysis of variance. The significance criteria were $p < 0.05$ or $p < 0.01$. $p \geq 0.05$ indicates no significant differences. The backward multiple linear regression analysis was used to select variables and to establish the regression equations for the extraction results of PBET and USPM. The Pearson correlation coefficient was used for correlation analysis, and two-sided test analysis was performed.

3. Results

3.1. Effects of Concentration of N-HAP on the Bioaccessibility of Pb in Soil

Extractable Pb from soil by PBET were significantly decreased compared with control (Figure 1 and Table 3). It was suggested that n-HAP was effective in stabilizing Pb in soil. The 5% n-HAP treatment was especially obvious. The immobilization effects of 5% n-HAP on Pb in soil were fast and lasting in comparison with 7% and 10% n-HAP treatment. After 1 d of incubation, extracted Pb from soil by PBET in 5% n-HAp treatment was 216.76 $mg \cdot kg^{-1}$, which was already much lower than those of 7% n-HAP treatment (335.52 $mg \cdot kg^{-1}$) and 10% n-HAP treatment (320.32 $mg \cdot kg^{-1}$). The final (180 d of incubation) and average

Table 3. Statistical characteristics of bioaccessible soil Pb in PBET and USPM.

n-HAP concentration	PBET ($mg \cdot kg^{-1}$)			USPM ($mg \cdot kg^{-1}$)		
	Range	Average	CV	Range	Average	CV
0%	411 - 586	491 a	12.5%	731 - 972	893 a	9.32%
5%	217 - 308	274 b	17.5%	534 - 973	821 a	19.0%
7%	335 - 409	363 c	7.45%	567 - 876	810 a	14.8%
10%	291 - 334	304 b	6.12%	551 - 873	806 a	15.5%

CV: coefficient of variation; Different lower-case letters in a line under the same method mean there were significant differences between these two data, and it means no significant differences existed between them when the lower-case letters were the same.

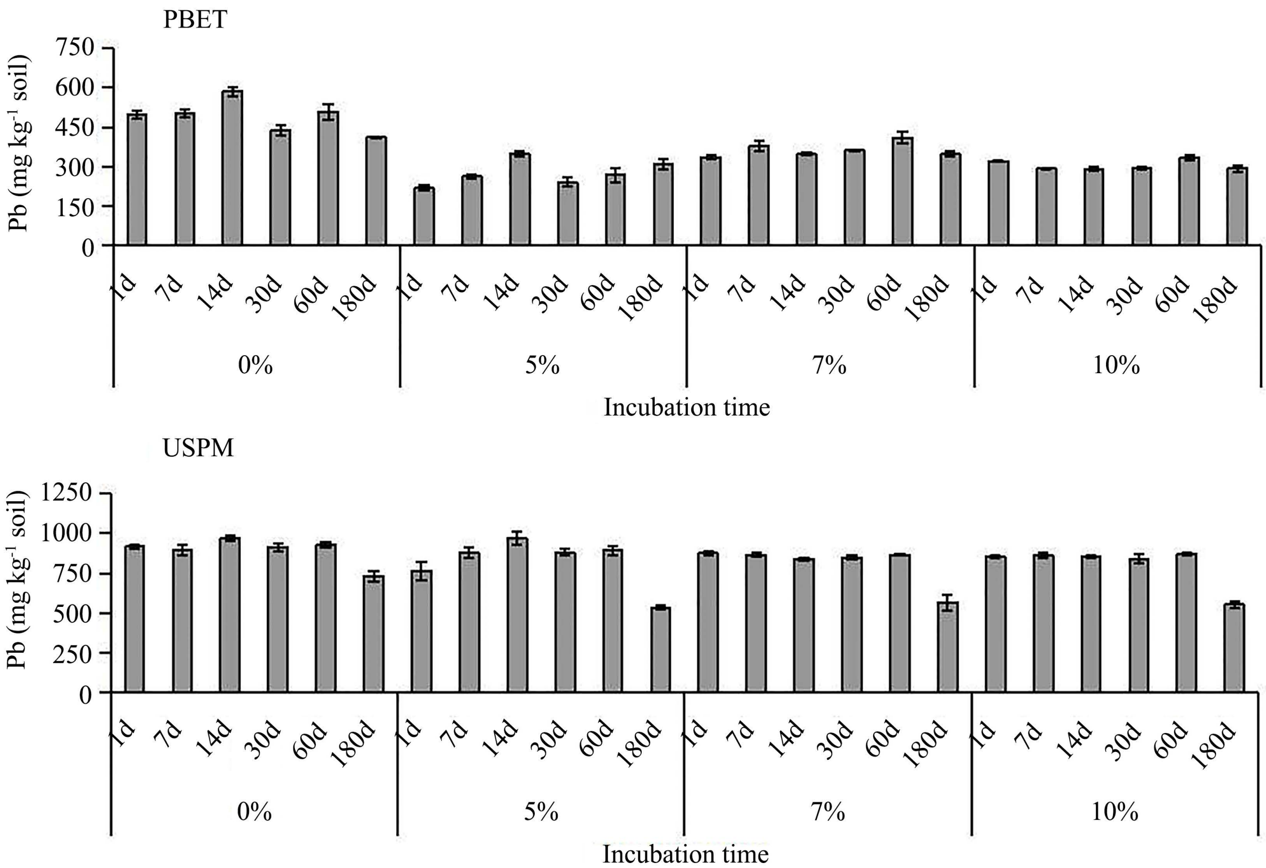

Figure 1. Bioaccessible soil Pb in PBET and USPM. 0%, 5%, 7% and 10%: the concentrations of n-HAP in soil.

extraction results by PBET between 5% and 10% n-HAP treatment had no distinct differences, and both were apparently less than that of 7% n-HAP treatment.

The extracted Pb from soil by USPM was similar to that by PBET: n-HAP treatment obviously decreased the solubility of Pb in soil in the gastric juice of USPM compared to control. After 1 d of incubation, the extracted Pb from soil by USPM in 5% n-HAP treatment was 764.23 mg·kg^{-1} and far lower than those extracted by USPM in the rest treatments. However there were some differences between the extraction results of PBET and USPM. Firstly, the extractable Pb from soil by USPM in all 4 treatments (0%, 5%, 7% and 10% n-HAP addition) showed a sharp drop on the 180th d of incubation compared to earlier days of incubation. Secondly, after 180 d of incubation, the extractable Pb from soil by USPM in 5%, 7% and 10% n-HAP treatments were respectively 533.72, 567.14 and 551.49 mg·kg^{-1} showing no significant differences. Lastly, average extraction results by USPM in 4 treatments were respectively 893.13, 820.83, 810.17 and 806.00 mg·kg^{-1} and had no significant differences.

On the basis of above analysis, the immobilizing efficiency of 5% n-HAP to Pb in soil was the highest in PBET and the 5% n-HAP treatment was the most cost-effective in USPM.

3.2. Effects of Concentration of N-HAP on Soil pH and OM

Soil pH increased with the concentration of n-HAP (Table 4). Soil pH in control was 4.05. Soil pH in 5%, 7% and 10% n-HAP treatments were 5.64, 5.90 and 5.99 respectively. Soil pH in 5%, 7% and 10% n-HAP treatments averagely increased by 1.59, 1.85 and 1.94 units compared to control. Soil pH between 7% and 10% n-HAP treatments had no significant differences and were higher than that of 5% n-HAP treatment. According to correlation analysis (Table 5), the correlation coefficient between soil pH and concentration of n-HAP was 0.914. They were positively correlated.

The average contents of soil OM in 0%, 5%, 7% and 10% treatments were respectively 19.33, 18.09, 17.57 and 17.76 $g \cdot kg^{-1}$. They were not significantly different. Content of soil OM showed little correlated relationship with concentration of n-HAP. But there were highly significant negative correlations between content of soil OM and incubation time. Their correlation coefficient was −0.554.

3.3. Influencing Factors of the Bioaccessibility of Pb in Soil

The extraction results by PBET were greatly affected by concentration of n-HAP in soil and soil pH. The bioaccessibility in PBET had significant negative correlations with concentration of n-HAP and soil pH, and their correlation coefficients

Table 4. Statistical characteristics of soil pH and OM.

n-HAP concentration	pH			OM ($g \cdot kg^{-1}$)		
	Range	Average	CV	Range	Average	CV
0%	3.97 - 4.20	4.05 a	2.08%	17.8 - 21.7	19.3 a	7.55%
5%	5.25 - 6.00	5.64 b	4.32%	16.2 - 21.9	18.1 a	12.1%
7%	5.82 - 6.03	5.90 c	1.24%	14.4 - 23.7	18.6 a	18.5%
10%	5.92 - 6.13	5.99 c	1.25%	16.2 - 20.2	17.8 a	9.85%

Different lower-case letters in a line under the same soil characteristic mean there were significant differences between these two data, and it means no significant differences existed between them when the lower-case letters were the same.

Table 5. Correlations analysis.

	n-HAP concentration	Incubation time	PBET	USPM	pH	OM
n-HAP concentration	1					
Incubation time	−0.054	1				
PBET	−0.658**	−0.067	1			
USPM	−0.247	−0.834**	0.358	1		
Soil pH	0.914**	0.107	−0.769**	−0.376	1	
OM	−0.155	−0.554**	0.161	0.322	−0.312	1

*$p < 0.05$; **$p < 0.01$; PBET: bioaccessible soil Pb in PBET; USPM: bioaccessible soil Pb in USPM.

were −0.658 and −0.769 respectively. The bioaccessibility in USPM only had high significant negative correlations with incubation time and their correlation coefficient was −0.834. The bioaccessibility between PBET and USPM represented no evident correlation relationship and their correlation coefficient was only 0.358.

According to the regression equations (Table 6), more than 50% differences between the amounts of Pb extracted by PBET can be explained by soil pH. More than 80% differences between the amounts of Pb extracted by USPM can be explainded by soil pH, soil OM and incubation time together.

4. Discussions

4.1. The Relationship between Concentration of N-HAP and Bioaccessibility of Pb in Soil

The immobilizing mechanisms of n-HAP for Pb in soil were to absorb Pb in soil forming pyromorphite and to increase soil pH reducing the solubility of soil Pb. Thus the extractable Pb from soil should decrease with increasing n-HAP addition. But as the results indicated, the immobilizing efficiency of n-HAP in PBET to Pb in soil was the best when the concentration of n-HAP was 5%. The average bioaccessibility in 5% n-HAP treatment was obvious less than that in 7% n-HAP treatment and showed no differences from that in 10% n-HAP treatment. It was probably because nanomaterials can easily aggregate, leading to an alteration to their surface sorption and decline of their dissolution rates (Gilbert et al., 2009; Cui et al., 2013; Li et al., 2014). It was deduced that aggregate of n-HAP increased with the concentration of n-HAP in soil because high concentration added the possibility of encounter. So compared to 5% n-HAP treatment, the average bioaccessibility in 7% n-HAP treatment was much higher. But the average bioaccessibility in 10% n-HAP treatment was lower compared to 7% n-HAP and there was no significant differences compared to 5% n-HAP. It may because the alteration of surface sorption and decline of dissolution rates when n-HAP aggregated were compensated by increasing of concentration of n-HAP in soil.

The average amounts of Pb extracted from soil by USPM were no distinctly different among 0%, 5%, 7% and 10% n-HAP treatments. This may be explained by the low pH of simulated gastric juice and long time of extraction in USPM. Low pH and long time mean high ability to extract heavy metal from soil. Due to the strong extraction ability of *in vitro* digestion tests, bioaccessible Pb were distributed among the three sequential fractions of BCR (Madrid et al., 2008). But the amounts of other heavy metals extracted from different soils by USPM should be compared in future research to test this speculation. After all, there were only

Table 6. Regression equations of bioaccessible soil Pb in PBET and USPM.

Regression equation	R^2
PBET = 67.911 (Invertase activity) − 88.650	0.618
USPM = −58.278pH − 17.535OM − 1.879 (Incubation time) + 155.161	0.849

PBET: bioaccessible soil Pb in PBET; USPM: bioaccessible soil Pb in USPM.

one soil sample and Pb involved in this study.

4.2. Correlation Analysis between Concentration of N-HAP and Content of Soil OM

Concentration of n-HAP had little effects on the content of soil OM. Their correlation coefficient was merely −0.155. The content of soil OM was significantly correlated with incubation time. Their correlation coefficient was −0.554. It suggests that the content of soil OM decrease with increment of incubation time. This may because the soil pollution by Pb was alleviated and the activities of soil enzyme were gradually recovered during the remediation. It was known that heavy metal pollution would inhibit the soil microbial activities. There was research indicating increase of activities of soil urease, phosphatase and dehydrogenase after n-HAp application (Wei et al., 2016). It was also found that n-HAP application increased the ratio of fungi to bacteria (Xu et al., 2016).

4.3. The Comparison of PBET and USPM

The extractable Pb from soil by USPM was much higher than that by PBET. That was because the composition and procedure of PBET and USPM were different. The pH of gastric juice of USPM was apparent lower (about 1.08) than that of PBET (2.5). The incubation time of USPM was also much longer (2 h) than that of PBET (1 h). Moreover, the extraction results by PBET and USPM were not significantly linearly correlated. So different *in vitro* tests were uncomparable to some extent.

To uncover the factors, especially soil characteristics, influencing the results of *in vitro* digestion tests has been an important research direction. It was known that the sequential extraction results had disagreement with the extraction results of *in vitro* digestion tests (Tang et al., 2004). Hansen et al. (2007) found no evident correlation between bioaccessibility and the soil parameters. Most researches indicated there was a significant correlation between the total concentrations and bioaccessibility of soil metals (Mercier et al., 2002; Carrizales et al., 2006; Poggio et al., 2009; Roussel et al., 2010; Sialelli et al., 2010, 2011; Luo et al., 2012a, 2012b). But it was obvious that the total concentrations of soil metals can not well explain the question: why extraction results of Pb in different soil by the same *in vitro* digestion test varied evidently. It was already known in this study that there were different soil factors controlling the solubility of soil Pb in the simulated gastric juice. More than half differences between the amounts of Pb extracted by PBET was related with soil pH. Therefore n-HAP application can efficiently decrease bioaccessible Pb in soil extracted by PBET through raise soil pH. Still there were almost 50% differences between the amounts of Pb extracted by PBET waiting for explanation by soil characteristics. Unlike PBET, soil pH, content of soil OM and incubation time together explained more than 80% differences between the amounts of Pb extracted by USPM. Especially the correlation coefficient of incubation time and the amounts of Pb extracted by USPM was as high as −0.834. The average amounts of Pb from soil extracted by USPM in 0%, 5%, 7% and 10% n-HAP treatments showed no significant differences. It might be suggested that effects of soil characteristics on bioaccessible Pb extracted by USPM were limited due to the low pH of simulated gastric juice and long time of extraction. The influences of incubation time on the bioaccessible Pb extracted by USPM were the results of soil ageing. Ageing period strongly influenced soil ecotoxicity and long-term polluted soil was less toxic (Schreck et al., 2011). Rout et al. (2016) found that there occurs a significant rearrangement of U in different fractions with ageing and no correlation was observed between the U content in different fractions and the adsorbents of respective fractions such as soil organic matter (SOM), Fe/Mn oxides (hydroxides) carbonates, soil cation exchange capacity (CEC).

5. Conclusion and Perspectives

Based on the bioaccessiblity of soil Pb assessed using PBET and USPM, it was suggested that the concentration of n-HAP should be 5% when n-HAP was applied to remediate Pb pollution in soil. The concentration of n-HAP was significantly correlated with soil pH, whose correlation coefficient was 0.914. Incubation time was significantly correlated with soil OM and the correlation coefficient was −0.554. There was no significantly linear relationship between the extraction results by PBET and USPM. According to the regression analysis, more than 50% differences of the results of PBET were explained by soil pH while nearly 85% differences of the results of USPM were explained by soil pH, content of soil OM and incubation time together. There were only one soil sample, one kind of heavy metal, two *in vitro* digestion tests and two properties of soil involved in this study. More factors influencing the results of different *in vitro* digestion tests should be explored.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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