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Application of Lherzolite on the Plant Growth and on Chemical Fractionation of Lead in Metal Contaminated Soil.

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ABSTRACT

A greenhouse pot experiment was conducted to investigate the effect of 5% application of lherzolite to a contaminated soil on the growth of radish (Raphanus sativus L.) and Japanese mustard spinach (Brassica rapa L. var. perviridis) and on chemical fractionation of lead (Pb). Both plants were grown in a sandy loam soil contaminated with Cd (4.1 mg kg^{-1}), Zn (440 mg kg^{-1}) and Pb (312 mg kg^{-1}) . The source of these heavy metals contamination in soil was surface water which was previously mixed with contaminants discharged from industrial and mining operations located in Tohoku district, Japan. Plants were grown consecutively three times in the same pots. After the third harvest, soil samples were collected and analysed by sequential extraction procedure into five operationally defined fractions (F1- exchangeable, F2-carbonate bound, F3-oxides bound, F4-organically bound and F5-residual). Application of lherzolite to soil increased plant growth three folds over the control. Lherzolite addition to soil decreased 75% of exchangeable (F1), 50% carbonate bound (F2), but it increased in the oxide bound (F3) fractions of Pb. This result indicates that the reduction of Pb in exchangeable and carbonate fractions by addition of lherzolite to the soil resulted in higher growth and decreased concentrations of Pb (82 to 94%) in plant tissues.

Keywords: Lherzolite, plant growth, lead, contaminated soil

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INTRODUCTION

Heavy metal contamination of soils is a serious problem all over the world for human health and it is getting more attention in Japan [1]. Humans, other higher animals, plants and microorganisms may be exposed to contaminants through the direct ingestion of contaminated soils, consumption of crops and vegetables grown on contaminated soils or drinking water that has percolated through such soils [2]; Lead (Pb) is a major anthropogenic pollutant, which is not biodegrade or decay. Lead is highly toxic to humans and other forms of life. Kashem *et al*. [3] investigated some contaminated and non-contaminated soils of Japan, and found that total total Cd, Zn and Pb concentrations were in the range of 0.7 to 12.5, and 90-704 and19 - 359 mg kg^{-1} , respectively. Higher amount of toxic metals in the contaminated soils compare to those in non-contaminated soils indicated a large anthropogenic input of metals in soils.

Heavy metals are present in various forms in soil. These metals are redistributed into different chemical forms with varying bioavailability, mobility and toxicity. Various forms of heavy metals in soils influence their solubility, which may directly influence their mobility in soils and phytoavailability [4, 5]. In the last decade, many wokers have attempted to assess the phytoavailability of heavy metals in contaminated soils using sequential extraction methods [6, 7] or plant assays [8, 9].

Various techniques have been used/proposed for the restoration of soils contaminated with heavy metals. Current technologies for soil remediation are time consuming and expensive. Therefore, it is important to develop techniques that can treat and stabilize heavy metals in situ in an effective and cost effective manner [10]. Lime, phosphates or organic matter residues are commonly employed for metal immobilization in soils [11].

Lherzolite [12], which is powder form and is commercially available, is being mined in Toono, Iwate Prefecture, Japan by the Miyamori Saiseki Co. Ltd, Japan. This material is mostly used as a raw material of concrete for making road in Japan; in addition, this product is also used as a raw material of Mg-fertilizer. Industrial process is essential to make effective fertilizer from the material because of its low availability of Mg for plant uptake. Lherzolite is a mixture of SiO_2 (38.5%), CaO (2.6%), MgO (36%), Fe_2O_3 (5.9 %), Al_2O_3 (1.9%), and Ni (0.17%) and had a pH 9.0. Consideration the composition of lherzolite, we assume that this material may be useful to reduce the mobility and bioavailability of Pb in contaminated soil. Furthermore, the application of lherzolite may be economically feasible as no cost is being proposed by the company at this stage. However, the effectiveness of lherzolite on Pb extractability and bioavailability has not been investigated as yet. Therefore, the aim of this study was to assess the effect of lherzolite on the solubility of Pb fractions associated with different components in contaminated soil and the uptake of the metal by radish and Japanese mustard spinach.

MATERIALS AND METHODS

Soil and its characteristics

A surface (0-20 cm) sandy loam soil, contaminated with heavy metals, collected from Miyagi prefecture of northeastern of Japan was used in this experiment. The sources of Pb contamination in this soil was the surface water, which was mixed previously with polluted contaminants discharged from different industrial and mining operations located at Tohoku district. Metals concentration in this soil was raised after receiving such water during 1911 to 1987. Rice and other agricultural crops are commonly grown in that area. The collected soil in moist stage was ground through a 4 mm sieve for using in the green house experiment. For laboratory analysis, a sub sample was air dried and passed through a 2 mm sieve and stored. The initial value of soil pH (1:2 soil/water suspension) was 5.1. The organic carbon content (Walkey and Black [13]) was 1.9% and CEC (extraction with 1 M NH_4OAc (pH 7.0) [14] was 25.5 cmol kg^{-1}. This soil had 58% sand and 10% clay. Total concentrations (Nitric-perchloric digest) of Cd, Zn and Pb in this soil were 4.1, 440 and 312 mg kg^{-1}, respectively.

Pot experiment and plant analysis

The experiment was conducted under natural light condition in a green house in the Iwate University, Japan. Moist soil equivalent to 1 kg dry mass was placed in plastic pot (14 cm height and 15 cm diameter) after

mixing it with lherzolite at the rates of 0 and 5% (W/W). Each pot received 1 g of N-P_2O_5-K_2O (10-10-10) mineral fertilizer (equivalent to 100 mg kg^{-1} soil of N, P_2O_5 and K_2O). After mixing the soil with amendments and fertilizers, the pots were divided into two sets. In one set, five seeds of radish (*Raphanus sativus* L.) and the other set, six seeds of Japanese mustard spinach (JM spinach) (*Brassica rapa* L. var. *perviridis*) were sown. Six days after sowing, radish plants were thinned to two per pot and JM spinach to three per pot. Each treatment was replicated three times. Water was added daily as required during the growth period. After 35 days of sowing, plants were harvested, separated into root and shoot, and washed with water. These samples were oven dried at 65 °C for 3 days, then ground, and digested with nitric-perchloric acid (3:1).

After the first harvest (cultivation 1), the same soil was used continuously (cultivation 2 and 3) to grow radish or JM spinach. Similarly to the first cultivation, 1 g of N-P_2O_5-K_2O (10-10-10) mineral fertilizer was added to the soil before the second and third cultivation. The rest of the experimental procedure for the second and third cultivation was the same as the first. The digested solutions with nitric-perchloric acid were analyzed for Pb using atomic absorption spectrophotometer (AAS) (170-30 Hitachi, Tokyo, Japan). Reagent blanks were also processed. All results are presented on dry weight (DW) basis.

Soil analysis by sequential extraction procedure

After the third harvest, soil samples were collected from each pot, air dried and ground to pass 2 mm sieve. Soil pH was measured by the same way as mentioned above. The sequential extraction procedure of Kashem and Singh [6], which was modified from Tessier *et al*. ([15], was employed to measure the metals in the fractions. The methods partitions the heavy metals into five operationally defined chemical fractions. The following steps were performed sequentially:

(F1): Air dried soil (equivalent to 2 g oven-dry) extracted with 20 mL of 1 *M* NH_4OAc, pH 7 for 2 h at 20 ºC on a rolling table (exchangeable).

(F2): Residue from the F1 extracted with 20 mL of 1 *M* NH_4OAc, pH 5 for 2 h at 20 ºC on a rolling table (specifically sorbed and carbonate bound).

(F3): Residue from F2 extracted with 20 mL of 0.04 *M* hydroxylamine hydrochloride ($NH_2OH.HCl$) in 25 % acetic acid (v/v) at pH 3, reaction time 6 h in a water bath at 80 ºC with occasional manual shaking (metals associated [sorbed or occluded] mainly on iron and manganese oxides).

(F4): Residue from F3 extracted with 15 mL of 30 % H_2O_2 (adj. pH 2), reaction time 5.5 h in a water bath at 80 ºC with occasional manual shaking. After cooling, 5 mL of 3.2 *M* NH_4OAc in 20 % (v/v) HNO_3 was added; sample was shaken on a rolling table for 0.5 h at 20 ºC and finally diluted to 20 mL with water (complexed with organic matter).

(F5): The residue from F4 fraction, extracted with 7 M HNO_3, reaction time was 6 h in a water bath at 80 ºC with occasional shaking (residual)**.**

Following each extraction or wash, mixtures were centrifuged at 10, 000 rpm for 0.5 h. Metals concentrations in each extract or digest were determined by the AAS. Analysis of variance, t-test and Tukey's pair wise comparison were carried out by Minitab (Minitab Software). The level of significance was $p < 0.05$ if not mentioned differently in the text.

RESULTS

Plant growth and Pb concentration in plant tissue

Lherzolite amendment increased plant growth about two to three folds of control soil pots, in all repeated cultivations. In the soil without amendment of lherzolite showed reduced growth; and significantly poor shoot and root biomass (dry weight) as compared to soil amended with lherzolite (Table 1).

Table 1: Dry matter yield (gm pot^{-1}) of radish and Japanese mustard spinach of three consequative harvest grown in control and lherzolite amended soils

Treatment	Plant							
	--Radish--							
	--------------------Shoot---------------------				---------------------Root---------------------			
	Cultiv. 1	Cultiv. 2	Cultiv. 3	Total	Cultiv. 1	Cultiv. 2	Cultiv. 3	Total
Control	0.51 b	0.57 b	0.51 b	1.59 b	4.12 b	0.24 b	0.24 b	4.60
With Lherzolite	0.72 a	1.5 a	1.46 a	3.68 a	11.8 a	2.24 a	1.75 a	15.79
	-------------------------------Japanese mustard spinach-------------------------------------							
Control	3.66 b	0.67 b	0.28 b	4.61 b	0.81 b	.07 b	0.03 b	0.91 b
With Lherzolite	4.44 a	2.55 a	2.26 a	9.25 a	1.15 a	0.20 a	0.25 a	1.60 a

Means followed by same letter in each item are not significantly different at *P<0.05.*

The concentration of Pb in the plant tissue was affected by the lherzolite application in both plants regardless of different harvests. In the shoots of radish and MJ spinach, Pb concentration varied from 22 to 34 mg kg^{-1} and in the roots, it varied from 18 to 67 mg kg^{-1} when plants grown in the control soil, the corresponding values varied from 3.2 to 5.0 mg kg^{-1} in the shoots and 2.7 to 6.9 mg kg^{-1} in the roots of plants grown in the lherzolite amended soil (Table 2). Addition of lherzolite, reduced Pb concentration by 80 to 87% in the shoots and 82 to 94% in the roots over the control.

Table 2: Lead concentration (mg kg^{-1}) in radish and in JM spinach plant parts grown in control and lherzolite amended soils

Treatment	Plant							
	--Radish--							
	--------------------Shoot---------------------				----------------Root (bulb)------------------			
	Cultiv. 1	Cultiv. 2	Cultiv. 3	Average	Cultiv. 1	Cultiv. 2	Cultiv. 3	Average
Control	30 a	34 a	28 a	31 a	22 a	18 a	20 a	20 a
With Lherzolite	4.0 b	4.2 b	3.2 b	3.8 b	3.0 b	3.2 b	2.7 b	3.0 b
	-------------------------------Japanese mustard spinach-------------------------------------							
Control	25 a	22 a	27 a	25 a	45 a	42 a	46 a	44 a
With Lherzolite	3.8 b	3.2 b	4.9 b	4.0 b	5.8 b	5.3 b	6.9 b	6.0 b

Means followed by same letter in each item are not significantly different at *P<0.05.*

Chemical partitioning of Pb

Lead concentration in different fractions of soil after the final harvest amended with and without lherzolite (Control) are presented in Table 3 and the proportion (percent of total) are shown in Figure 1A. The proportion of Pb in the soil without lherzolite (Control) was 3.7% (12 mg kg^{-1}) in the exchangeable (F1), 13% in the carbonate (F2), 37% in the oxide (F3) and 11% in the organic (F4) and 35% in the residual (F5) fraction. Majority of Pb was associated in the oxide (F3) and in the residual (F5) fractions.

Table 3: Concentrations of Pb (mg kg^{-1}) in the various chemical forms of soils at the end of the experiment without (-L) and with (+L) application of lherzolite (percent of total in each fraction are in parenthesis)

Plant	Treatment	F1	F2	F3	F4	F5	SumT[a]	Total[b]	%Recov[c]
Radish	Control (- L)	12 a (3.8)	46 a (14)	119 b (37)	35 a (11)	106 a (33)	318	317	100
	With Lherzolite (+ L)	3.4 b (1.1)	28 b (9.3)	136 a (45)	30 a (10)	108 a (36)	302	310	97
JM spinach	Control (- L)	11 a (3.5)	49 a (16)	117 b (37)	33a (11)	103 a (33)	313	315	99
	With Lherzolite (+ L)	2.7 b (0.8)	24 b (7.8)	145 a (46)	31 a (10)	102 a (34)	305	308	99

Means followed by same letter in each item are not significantly different at $P<0.05$.
F1-exchangeable, F2-carbonate bound, F3-oxide bound, F4- organic bound, F5-residual;
[a] Sum of F1 to F5; [b] Total metal concentration (nitric –perchloric digest);
[c] Recovery (SumT/Total × 100);

        To show the redistribution of Pb in different soil fractions after lherzolite amendment, the amount of Pb shifted from easily mobile to less mobile fractions was calculated from the difference between the percent of metal in each fraction in the control and in the lherzolite treated soil (Figure 1B). Lherzolite addition to soil decreased 75% of exchangeable (F1), 50% carbonate bound Pb (F2), but it increased in the oxide bound (F3) fractions of Pb. Lead in the other forms (F4 and F5) remained constant (Figure 1B). This result indicates that the reduction of Pb in exchangeable (F1) and carbonate fractions (F2) by addition of lherzolite to the soil resulted in higher growth and decreased concentrations of Pb in the plant tissues (Table 1 and 2).
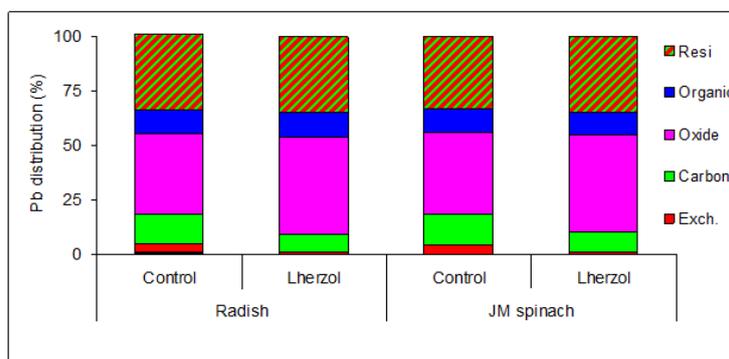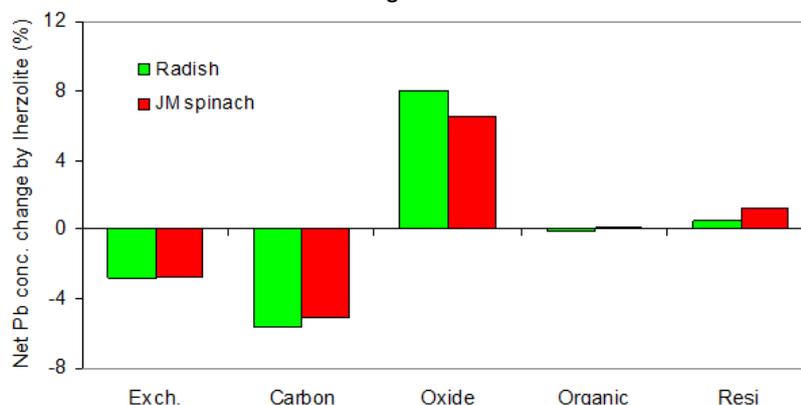
Figure 1A

Figure 1B

Figure 1: Lead distribution (A) and phase changes (B) after lherzolite additions to soil

DISCUSSION

The results showed that addition of lherzolite to soil transformed Pb from the F1 and F2 fractions to F3 fractions (Fig. 1A). Metals in the F1 and F2 fractions would be readily bioavailable to the environment where as metals in the oxide (F3), organic (F4) and residual (F5) fractions are tightly bound and treated as immobile fractions and hence less bioavilable. In the lherzolite amended soils, pH value increased by 1 unit, due to alkaline nature of the lherzolite. This increase of pH in lherzolite amended soil might led to fixation of Pb in one hand, but, on the other hand, chemical composition of lherzolite might also led to either fixation by providing higher surface areas or to precipitation of these metals. The application of hydrous iron or manganese oxides [16]; Ca and Mg carbonates, and Ca oxides [17, 18]; zeolites, bringite, hydrous oxides of Fe, Al and Mn [19, 20] can significantly reduce metal mobility in soils and their uptake by plants.

Lombi *et al*. [18] investigated the efficiency of red mud (Fe-oxide rich material) to fix heavy metals in contaminated soils and compared with lime and beringite (modified aluminosilicate). Furthermore, a sequential extraction procedure was performed to investigate the mechanism of metal fixation by different amendments. They found soil pH was a common mechanism of action for all the amendments. The results of sequential extraction also showed that the application of red mud led to an increase in the FeMn-oxides fraction. They reported that specific chemisorption, and possibly metal diffusion into the oxide particles could also be the mechanisms responsible for the fixation of metals in the red mud. Our results of pH increase are in accordance with those of Paulose *et al*. [21], where they found 1 unit of pH rise by the application of 5% $CaCO_3$ and the influence of $CaCO_3$ on metal sorption was related to the increase in pH.

In addition, the rising of soil pH increased the pH dependent charges and also increased the metals adsorbed on soil particles ([22]. This pH dependent immobilization of Cd Zn and Pb has also been reported by Cheng and Hseu [23]. They found lower proportion of metals in the exchangeable fraction and higher in the oxide bound fraction in amended soil than in the control soil. In our study, lherzolite behaved greatly like those of red mud as well as lime and beringite to a small extent because lherzolite is a compound of multi elements (Si, Ca. Mg and Fe oxides). This may be speculated that in the case of lherzolite, fixation mechanism could be mostly due to pH increase but also ion exchange and precipitation or coprecipitaion of metals with Al, Fe and Mn oxides [24].

The exchangeable and carbonate fractions are generally considered immediate nutrient reservoir for plants [7]. It was clearly shown in this experiment that growth of radish and JM spinach were much enhanced by amendment with lherzolite, and Pb concentrations in plant tissues were greatly reduced. The reduced bioavailability of Pb by amendment with lherzolite might be result of pH rise. This increase of soil pH in the soil amended with lherzolite reduced the phytotoxicity of Pb, resulting in significantly lower Pb concentrations of shoots and roots plants (Table 2). Our results coincided well with the findings of other investigators [8, 18, 19], who reported that the decreased concentrations of metals by the crops grown in soils amended with chemicals was related to the decrease of the bioavailable fractions of metals in soil.

Improvement of growth (Table 1) by the addition of lherzolite can be partially explained by its detoxifying effect of Pb in the soil supplied with other nutrients (Si, Ca, Mg) derived from lherzolite. Besides Ca and Mg, essential nutrients, Si has also been shown to have beneficial role in plant growth and dry matter production ([24].

CONCLUSIONS

Application of lherzolite significantly reduced the F1 and F2-Pb (Mobile) in the soil, and transformed them to forms with lower availability, resulted enhance plant growth and reduced concentrations of Pb in the plant tissues. These results suggested that addition of lherzolite to the soil improved the growth of crops by reducing the bioavailability of metals in the contaminated soil.

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