Effect of phosphate-induced immobilization of lead on its mobility and bioavailability

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Abstract
Lead (Pb) can be immobilized using insoluble phosphate compounds in the presence of phosphate solubilizing bacteria (PSB) which facilitate phosphorus (P) solubilization from insoluble P sources. The mobility and bioavailability of Pb as affected by P-induced immobilization was examined using leaching and plant growth experiments. Phosphate solubilizing bacteria promoted the solubilization of rock phosphate, thereby leading to the immobilization of Pb without causing any significant leaching of P in soils. Phosphate solubilizing bacteria reduced Pb in pore water, and rock phosphate and soluble P amendments significantly reduced Pb accumulation by sunflower.

Key Words
Lead, phosphate solubilizing bacteria, immobilization, phosphorus, rock phosphate.

Introduction
Lead is one of the major metal contaminants attracting particular attention because of its widespread use in mining, industry and agricultural activity and distribution in the earth's crust (Wong 1985). It is generally recognized that the mobility and bioavailability of Pb in soil are more important than total Pb concentration (Tongtavee 2005). Therefore, remediation options for Pb include amelioration of soils to minimize Pb bioavailability. Phosphorus compounds are used to immobilize heavy metal contaminated environments by phosphate-heavy metal precipitation (Cotter-Howells 1996). Insoluble P compounds can be solubilized by phosphate solubilizing bacteria (PSB) by means of phosphatase enzyme activity and organic acid production (Chen 2006). Therefore, PSB can facilitate Pb-P precipitation and increase Pb immobilization with the amendment of insoluble P compounds. The objective of this research was to examine the immobilization of Pb in soils using soluble and insoluble P compounds in the presence of PSB, thereby reducing Pb mobility and bioavailability.

Methods
Incubation and leaching of Pb in spiked soil
A Pb spiked soil (2000 mg Pb/kg) was treated with phosphate sources and PSB as indicated in Table 1. The levels of P addition were 200, 800 and 1600 mg P/kg for rock phosphate and 200 and 800 mg P/kg for soluble P as KH_2PO_4. After 2 weeks of incubation, NH_4NO_3 extractable Pb was analyzed by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES, Agilent).

Table 1. Soil treatment.

Symbol	Treatment (Moisture 60 % of Water holding capacity)
S	Pb contaminated soil
SB	Pb contaminated soil + PSB bacterial solution (ca.5.5X10^8 CFU/mL)
SRP	Pb contaminated soil + rock phosphate
SRPB	Pb contaminated soil + rock phosphate + PSB bacterial solution (ca.5.5X10^8 CFU/mL)
SP	Pb contaminated soil + KH_2PO_4

A column experiment with the Pb spiked soil was conducted to examine P and Pb leaching potential in P amended soil. The Pb spiked soil samples (45 g) pre-amended with various treatments (Table 1) were placed in a 60 mL syringe, leached with 1 mM $CaCl_2$ solution and analyzed for P and Pb concentration using Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS, Agilent).

Pot experiment
The Pb spiked soil samples (400 g, <2 mm) were mixed with rock phosphate (Table 1) and placed into 600 mL opaque plastic pots. Rhizon samplers (one per pot) were horizontally placed at 2 cm from the bottom of

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the pot. Sunflower seeds (5 seeds per pot) were sown directly into the soils and the number of seedlings was thinned to 2 per pot one week after germination. The pots were watered daily and plants were harvested 6 weeks after germination. After harvesting plants, shoots and roots were separated, washed with Milli Q water, oven dried and ground. The ground plant material (0.1000 – 0.5000 g) was digested using a temperature controlled digestion block (AI Scientific Block Digestion System AIM 500). The digested plant extracts (along with blanks and spiked samples) were analyzed for metals by ICP-OES.

Results

Leaching of P and Pb

The addition of phosphate compounds immobilized Pb as shown by a decrease in NH_4NO_3 extractable Pb in soil. Phosphate solubilizing bacterial inoculation decreased NH_4NO_3 extractable Pb concentration significantly at all levels of rock phosphate amendment, and the decrease in Pb concentration increased with increasing levels of P addition. In the case of soluble P addition, the decrease in Pb concentration was not significant at 200 mg/kg of level, but Pb concentration was decreased by 57 % at 800 mg/kg of soluble P level (Figure 1) compared to the control (0 mg/kg P concentration). Rock phosphate alone treatment immobilized 0.48 %, 7.8 % and 18.8 % of Pb at 200, 400, 800 and 1600 mg P/kg, respectively, after 2 weeks of incubation compared to the control (0 mg/kg P concentration). Phosphate solubilizing bacteria in the presence of rock phosphate treatment immobilized 6.98 %, 25.6 % and 32.0 % of Pb in soil at 200, 400, 800 and 1600 mg P/kg, respectively compared to the control (0 mg/kg P concentration).

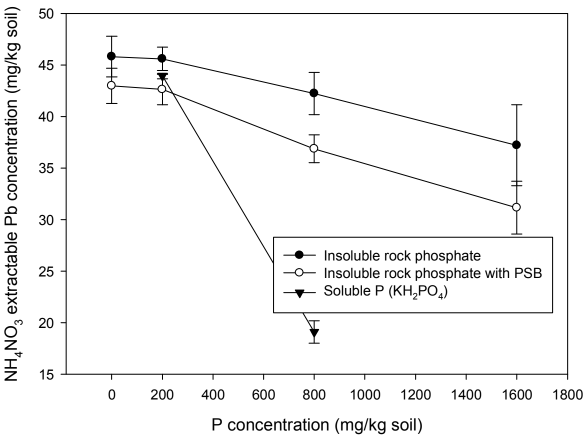

Figure 1. Ammonium nitrate extractable Pb concentration in Pb spiked soil treated with various levels of soluble P and rock phosphate and in the presence of PSB for rock phosphate.

The total leached Pb concentration was the highest in soluble P treated Pb spiked soil and the lowest in rock phosphate treatment with PSB (Figure 2 (a)). Phosphate solubilizing bacterial inoculation with rock phosphate reduced Pb leaching by 18 % compared to the control. The total leached P concentration in Pb spiked soil was 0.04% and 8.9 % from the rock phosphate and soluble P treated soils, respectively (Figure 2 (b)). Higher levels of P application increase the risk of eutrophication of ground water because increased P application increased the leaching of P from the treated soil columns (Basta and McGowen 2004).

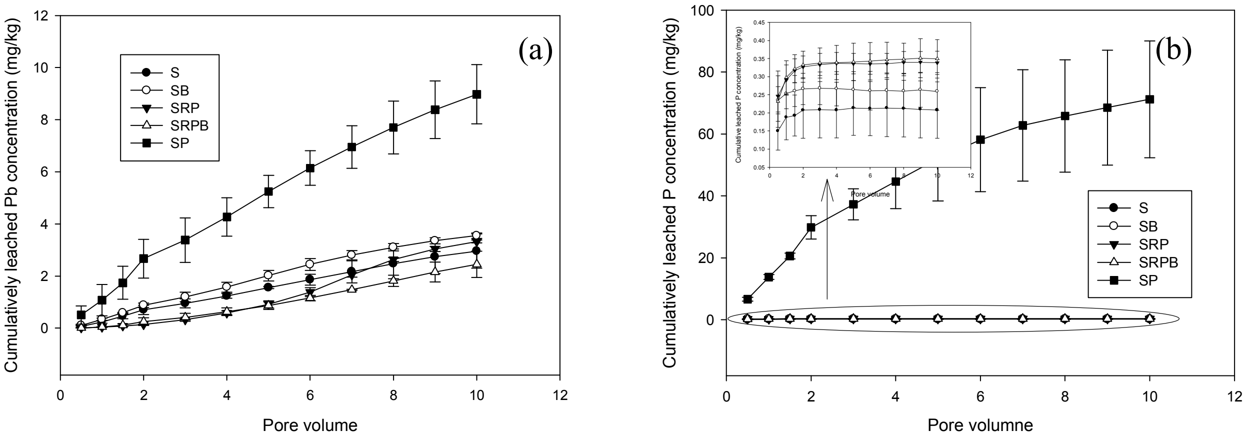

Figure 2. Cumulative concentration of Pb (a) and P (b) in the leachate.

Pot experiment
Lead concentration in pore water in soluble P amended soil was higher than other treatments. The high Pb concentration in pore water from soluble P amended soil may be attributed to the mobilization of dissolved organic carbon (DOC). There was a significant correlation between DOC and Pb concentrations in pore water indicating that DOC formed complexes with Pb, thereby increasing Pb mobility (Levonmaki *et al.* 2006). Phosphate solubilizing bacterial inoculation with rock phosphate reduced Pb concentration in soil solution up to 40 days of plant growth (Figure 3). This effect can be explained by P solubilization by PSB from rock phosphate and subsequent immobilization of Pb through Pb-P precipitation. Phosphorus amendments significantly reduced shoot and root Pb concentration (Figure 4). Rock phosphate, rock phosphate with PSB and KH_2PO_4 decreased Pb concentration of sunflower shoot by 57 %, 56 % and 76 %, respectively, compared to the control.

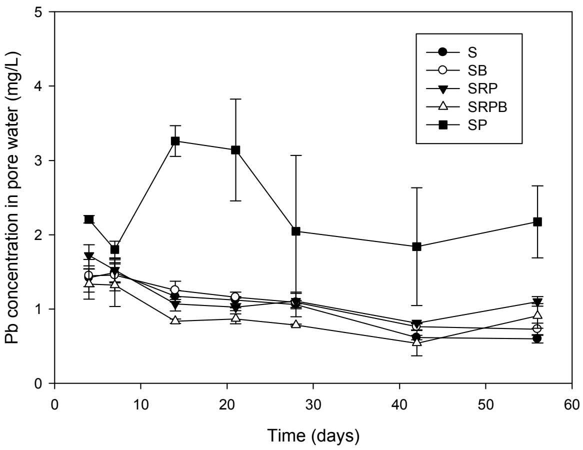

Figure 3. Lead concentration in pore water with sunflower growth.

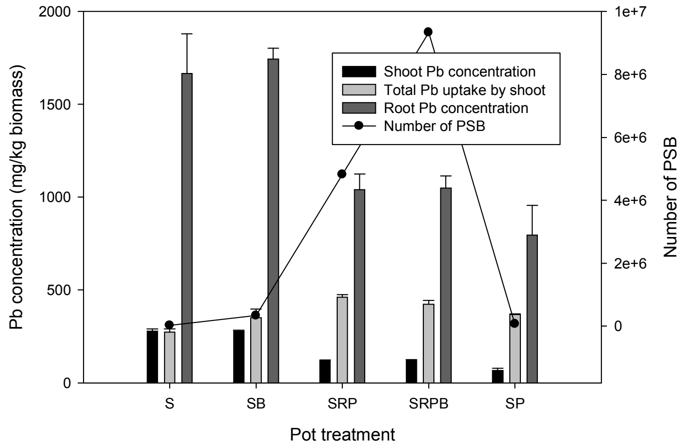

Figure 4. Lead accumulation by sunflower shoot and root.

Conclusion
Insoluble P sources in the presence of PSB showed the potential to immobilize Pb without any detrimental effects on P and Pb leaching. Phosphate solubilizing bacteria enhanced the immobilization of Pb in soil, thereby reducing the mobility and bioavailability of Pb in soil.

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