Journal of Hazardous Materials xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Lead immobilization and bioavailability in microbial and root interface

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HIGHLIGHTS

- Phosphate solubilizing bacteria successfully colonized plant roots.
- Phosphate solubilizing bacteria increased water soluble P concentration in soil.
- Phosphate solubilizing bacteria and phosphate rock immobilized Pb.
- Phosphate solubilizing bacteria with phosphate rock reduced Pb uptake by plants.
- Phosphate solubilizing bacteria slowly released P to immobilize Pb without causing P leaching.

ARTICLE INFO

Article history:
Received 27 June 2012
Received in revised form 11 January 2013
Accepted 1 February 2013
Available online xxx

Keywords:
Phosphate solubilizing bacteria
Pb immobilization
Bioavailability
Phosphate rock
Microbox

ABSTRACT

A range of both soluble and insoluble phosphate (P) compounds have been used to immobilize Pb in solution and soil. However, these compounds have limitations because of low solubility or leaching of P. Phosphate solubilizing bacteria (PSB) can be used to enhance the solubility of insoluble P compounds. The effects of PSB on the immobilization of Pb in the presence of phosphate rock (PR) and subsequent reduction in Pb uptake by Indian mustard (*Brassica juncea*) in nutrient agar medium and ryegrass (*Lolium perenne*) in soil under sterile condition were tested. Root colonization of PSB was confirmed by halo formation around the root in the medium containing tricalcium phosphate. Addition of PR in the presence of PSB immobilized Pb in both agar medium and soil, and reduced Pb translocation from root to shoot. Furthermore, shoot Pb concentrations of Indian mustard in agar medium and ryegrass in soil were decreased by 58.1% and 22.8%, respectively, compared to the control. Even though soluble P compound was the most effective in the immobilization of Pb, excess P may cause eutrophication. Therefore, PSB are suggested as a co-amendment to facilitate immobilization of Pb without causing any detrimental effect on the environment.

1. Introduction

A number of technologies are available for the remediation of metal contaminated soils including soil excavation for solidification, washing, leaching, and particle size separations and in situ remediation [1]. Even though ex situ remediation involving soil excavation is the most clear-cut solution by eliminating source of contamination, it is not feasible because of large volume of excavated material and high costs. In situ remediation includes solidification, vitrification, encapsulation, attenuation, and phytoremediation. Remediation techniques by in situ immobilization of the metals using sequestering agents are preferred method for large industrial areas because immobilization reduces mobility and bioavailability of metals [2].

Immobilization of lead (Pb) by a range of amendments has been shown to reduce bioavailability of Pb, thereby reducing phytotoxicity [3]. Lead is known to form stable compounds with phosphate (P), and pyromorphite ($Pb_5(PO_4)_3Cl$) is regarded as one of the most thermodynamically stable Pb minerals under the geochemical conditions [4–6].

Nriagu [7] suggested that the interaction of Pb and P is an important mechanism immobilizing Pb in the environment, and Ma et al. [8] showed that Pb was effectively removed from aqueous solution and Pb contaminated soil solution by hydroxyapatite treatment.

Soluble P compounds such as phosphoric acid, triple super phosphate and diammonium phosphate are found to be effective in the immobilization of Pb by inducing the formation of pyromorphite.

Abbreviations: P, phosphate; PSB, phosphate solubilizing bacteria; PR, phosphate rock; TSY, trypticase soy yeast; EC, electrical conductivity; WHC, water holding capacity; ICP-OES, inductively coupled plasma optical emission spectroscopy; NBRIP, National Research Institute's phosphate.

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http://dx.doi.org/10.1016/j.jhazmat.2013.02.010

However, the addition of highly soluble P increases the risk of eutrophication and added soluble phosphate is immobilized by clay minerals in most soils, thereby becomes less soluble [9,10]. Laboratory trials using phosphate rock (PR) have been shown to be successful in immolizing Pb, but PR is not readily soluble and may not release enough P to remediate Pb in contaminated soils in field condition and require large amount of amendment application to overcome the reaction between added P and soil Ca [2,9]. Sometime soil acidification may be required since the formation of pyromorphite in soil requires the dissolution of soil Pb and added P amendments. However, soil acidification may cause the leaching of other trace elements and increase soil toxicity [11].

Phosphate solubilizing bacteria (PSB) have shown to facilitate immobilization of Pb by releasing P from insoluble P amendments [12]. Immobilization of Pb by PR in the presence of PSB reduced Pb mobility and bioavailability [13]. However, Pb immobilization and bioavailability in the microbial and root interface need to be elucidated. Most of the experiments relating to P-induced immobilization of Pb in the presence of PSB were conducted under non-sterile conditions and hence it was not possible to examine the role of inoculated PSB on the solubilization of P and subsequent immobilization of Pb [14]. To show the effect of PSB the experiments described in this study were conducted under specially designed sterile condition. The objective of this research is to evaluate the effect of PSB on Pb immobilization and Pb uptake by plant under sterile conditions. Especially, root colonization of PSB was tested to show their interaction with plant root, thereby solubilizing P and reducing Pb bioavailability.

2. Materials and methods

2.1. Phosphate solubilizing bacteria

Phosphate solubilizing bacteria (*Enterobacter cloacae*) isolated from Pb contaminated soil in Pb and Zn smelter were used as inoculants to test their effect on Pb immobilization in rhizosphere [12]. Bacteria grown in 100 mL of trypticase soy yeast (TSY) medium containing (per L) 30 g tryticase soy broth and 3 g yeast extract for 48 h were harvested by centrifugation at 4000 rpm for 20 min. The cells were resuspended in a sterilized saline solution (8.5 g NaCl/L Milli-Q water). Bacterial cells were washed twice with a saline solution and twice with sterile Milli-Q water and resuspended in 100 mL of sterile Milli-Q water.

2.2. Soil and phosphate compounds

The Pb contaminated soil sampled from a shooting range was dried at room temperature and sieved through a 2-mm mesh. The soil was analyzed for soil texture, water holding capacity (WHC), pH, electrical conductivity (EC), organic matter, Olsen P, and total and NH_4NO_3-extractable Pb concentrations as described by Park et al. [12,13]. The properties of the soil used in this experiment are presented in Table 1.

Phosphate rock (Nutri-Tech Solution) used as an insoluble P compound is finely ground PR (particle size < 2.0 mm, pH: 8.4 in 1:5 water extract) containing 26% of silica, 10% of phosphorus, 2.0% of iron, 0.66% of magnesium, 0.6% of potassium, 0.16% of carbon, 0.15% of manganese, 0.012% of copper and 0.33% of zinc. Potassium dihydrogen phosphate (KH_2PO_4) was used as a soluble amendment.

Table 2
Composition of treatments in nutrient agar medium and soil.

Samples	Description
M	Nutrient agar medium containing 50 mg/L of Pb
MP	Nutrient agar medium containing 50 mg/L of Pb and KH_2PO_4 at 200 mg P/L of the medium
MR	Nutrient agar medium containing 50 mg/L of Pb and phosphate rock at 200 mg P/L of the medium
MRB	Nutrient agar medium containing 50 mg/L of Pb, phosphate rock at 200 mg P/L of medium and phosphate solubilizing bacteria (ca. 1.0×10^7 CFU/mL of the medium)
S	Pb contaminated soil
SRP	Pb contaminated soil containing phosphate rock at 800 mg P/kg of the soil
SRPB	Pb contaminated soil containing phosphate rock at 800 mg P/kg of the soil and phosphate solubilizing bacteria (ca. 2.0×10^7 CFU/g of the soil)

2.3. Growth conditions of plants

Plant growth experiments were conducted using Pb spiked nutrient agar medium and Pb contaminated soil. Hoagland nutrient solution (50%) was used for this plant growth experiment [15]. The full strength nutrient solution was composed of macronutrients and micronutrients. The macronutrients (mmol/L) were: NH_4NO_3, 5.0; K_2SO_4, 2.0; $CaCl_2$, 4.0; $MgSO_4{\cdot}7H_2O$, 1.3. The micronutrients (μmol/L) were: Fe(II)-EDTA, 50; H_3BO_4, 10; $ZnSO_4{\cdot}7H_2O$, 1.0; $CuSO_4{\cdot}5H_2O$, 1.0; $MnSO_4{\cdot}5H_2O$, 5.0; $Na_2MoO_4{\cdot}2H_2O$, 0.5; and $CoSO_4{\cdot}7H_2O$, 0.25. To support bacterial growth, 0.2% of glucose was added to the solution and 0.5% of agar was added to solidify the medium. pH was adjusted to 6.5 using 0.1 M KOH or HCl solution. The medium containing macronutrients and agar was autoclaved for 20 min at 121 °C prior to the addition of micronutrients. Solution containing micronutrients were separately sterilized by 0.22 μm syringe filter. A 250 mL of sterilized nutrient solution was added into γ-ray irradiated 1 L container (110 mm in diameter and 140 mm in height, microbox, Combiness, Belgium), mixed with 0.5 g of sterilized PR or soluble phosphate compound and filter sterilized $Pb(NO_3)_2$ (50 mg Pb/L nutrient solution), and allowed to cool down. Bacterial suspension (2.5 mL, ca. 1.0×10^9 CFU/mL) was inoculated into the nutrient solution before solidification and the same amount of sterile Milli-Q water added medium served as a control.

Indian mustard (*Brassica juncea*) seeds were surface-sterilized with a mixture of ethanol and 30% H_2O_2 (1:1) for 20 min and washed with sterile Milli-Q water [16]. Fifteen sterilized Indian mustard seeds were placed on the surface of nutrient agar medium and covered with γ-irradiated polypropylene with a filter battery to prevent microorganism infection (Combiness, Belgium). The details of treatments are described in Table 2. The experiment was conducted in triplicate except M and MP treatments. Indian mustard was allowed to grow for 3 weeks in a glass house, with natural sunlight providing a photoperiod of 12–14 h. The temperature was maintained at 28 °C. Indian mustard grown in the nutrient medium is shown in Fig. 1.

The immobilization of Pb and growth of plants were also tested in soil under sterile condition. Sterilized Pb contaminated soil (150 g) containing 1.2 g of PR was placed in γ-ray irradiated 1 L container (110 mm in diameter and 140 mm in height, microbox, Combiness, Belgium). For PSB inoculation bacterial suspension (3 mL, ca. 1.0×10^9 CFU/mL) was sprayed on the surface of the soil

Table 1
Properties of soil used for the experiment.

Soil	Soil texture	WHC (%)	pH	EC (μS/cm)	Organic matter (%)	Olsen P (mg/kg)	Total Pb (mg/kg)	NH_4NO_3– extractable Pb (mg/kg)
SR	Sandy loam	38.5	5.88	34.7	0.70	3.78	346	28.7

Please cite this article in press as: J.H. Park, N. Bolan, Lead immobilization and bioavailability in microbial and root interface, J. Hazard. Mater. (2013), http://dx.doi.org/10.1016/j.jhazmat.2013.02.010