Chemosphere 171 (2017) 302–307

Contents lists available at ScienceDirect

Chemosphere

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

Solubilization of Pb-bearing apatite $Pb_5(PO_4)_3Cl$ by bacteria isolated from polluted environment

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HIGHLIGHTS

- Pseudomonas enhanced the solubility of pyromorphite.
- Extremely insoluble lead apatite can serve as a source of P for bacterial metabolism.
- Microbe-CPY interaction in polluted soils lower the efficiency of cleanup efforts.

ARTICLE INFO

Article history:
Received 2 October 2016
Received in revised form
4 December 2016
Accepted 11 December 2016
Available online 21 December 2016

Handling Editor: T. Cutright

Keywords:
Lead
Pyromorphite
Pseudomonas sp.
Siderophores
Biosolubilization

ABSTRACT

The main purpose of this study was to test if microorganisms isolated from heavily polluted environments can enhance dissolution of Pb-apatite (pyromorphite) resulting in remobilization of lead. Three bacterial strains belonging to the genus *Pseudomonas* isolated from underground mines in SW Poland were used in batch experiments of pyromorphite solubilization carried out in phosphate reach and phosphate poor media. Bacteria growth and evolution of Pb and phosphate concentrations as well as pH were determined. Additionally the concentration of bacterial siderophores in leaching solution was assayed. All bacterial strains were able to grow in both media in the presence of pyromorphite. The number of bacterial cells was from one to two orders of magnitude higher in the phosphate rich media. In the phosphate poor media the only source of P was the dissolving lead apatite. Bacteria enhanced the solubility of pyromorphite resulting in elevated Pb concentrations, up to 853 $\mu g\ L^{-1}$ in phosphate-rich medium and 6112 $\mu g\ L^{-1}$ in phosphate-poor medium, compared to less than 100 $\mu g\ L^{-1}$ in an abiotic control sample. Production of siderophores was characteristic for each culture and was much lower (10–1000 fold) in the phosphate-poor medium. This study demonstrates for the first time that indigenous bacteria can directly and indirectly promote the mobilization of lead from pyromorphite. This phenomenon should be considered in long term risk assessment of Pb contaminated soils after reclamation processes because bacteria can play a significant role in the efficiency of clean-up efforts and overall geochemical cycling of Pb.

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1. Introduction

Throughout the world there is growing concern that the heavy metal content of soils is increasing as the result of industrial, mining, agricultural and domestic activities. In particular, waste products from the mining and metal industries, sewage sludge, and residues from power station and waste incineration plants may contain heavy metals at high concentrations. Among heavy metals, lead is one of the most toxic elements and ingestion of contaminated food and drinking water is the most common source of lead exposure in humans (Cheikh et al., 2010; Ren et al., 2009).

Lead may exist in both inorganic forms and in organic forms. Mainly inorganic lead is cycled globally (Kabata-Pendias and Pendias, 2001). Lead may be emitted directly into air, water, and soil. The distance and geographical distribution of lead in relation to the point of emission is largely dependent on character of chemical compound and particle size. Lead in soils results mainly from dry and wet deposition of atmospheric compounds. Lead in soils is relatively immobile compared to other environments (Hoffman et al., 2003). Biological remediation of lead contaminated

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http://dx.doi.org/10.1016/j.chemosphere.2016.12.056
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soils and wastes is difficult and generally removal of Pb from soils and wastes requires chemical treatment (Cheikh et al., 2010; Ren et al., 2009).

One of the low-cost, long-term, environmentally safe methods of remediation of large areas of soil contaminated with Pb is *in situ* lead immobilization by phosphate amendments. The principle of this method a change in the speciation of Pb. Speciation is a critical factor influencing the impact of Pb on the environment including its bioavailability. Geochemically stable lead apatite precipitates by the reaction of labile soil-Pb species with the added phosphate amendments. The presence of chloride ions in natural systems allows for the formation of pyromorphite $Pb_5(PO_4)_3Cl$ (chlorpyromorphite – CPY), which has the lowest solubility, and thus is the most stable Pb-containing mineral phase under most surface environmental conditions (Lindsay, 1979; Nriagu, 1974, 1984; Topolska et al., 2016). The mechanism of CPY formation in soil solutions is through dissolution of phosphate source (often Ca-phosphate) followed by precipitation of lead apatite (Manecki et al., 2000a, 2000b).

In situ immobilization of Pb by phosphate induced formation of extremely insoluble CPY ($\log K_{sp} = -79$, Xie and Giammar, 2007) has received much attention (see for example Davis et al., 1993; Ma et al., 1993, 1994; Cotter-Howells et al., 1994; Ruby et al., 1994; Ma et al., 1995; Cotter-Howells and Capron, 1996; Laperche et al., 1997; Traina and Laperche, 1999; Cotter-Howells et al., 1999; Hettiarachchi et al., 2000; Ryan et al., 2001; Stanforth and Qiu, 2001; Cao et al., 2003; Melamed et al., 2003; Miretzky and Fernandez-Cirelli, 2008). It is commonly agreed that *in situ* sequestration of soil lead in the insoluble form of CPY prevents or at least reduces its uptake by living organisms. The long-term stability of pyromorphite was addressed by Xie and Giammar (2007). They concluded that the long-term stability of lead immobilized by precipitation of CPY will depend on its solubility and dissolution rate. Among the factors affecting solubility and dissolution rates they considered pH, saturation with Pb, the presence of organic acids and the presence of reactive mineral surfaces potentially adsorbing Pb or phosphates.

Numerous studies have demonstrated the conversion of insoluble (mineral) phosphorous to an accessible form by phosphate solubilizing bacteria (see for example Sperber, 1957; Kucey et al., 1989; Taunton et al., 2000; Harris et al., 2006; Park et al., 2011; Topolska et al., 2013) and potential remobilization of Pb by low molecular weight organic acids (Debela et al., 2010; Wei et al., 2014). Still, little is known about bacteria-mediated solubilization of lead-apatite. Solubilization of lead apatite by bacteria may become particularly significant in nutrient deficient environment. Phosphorous in soils is often a limiting nutrient supplemented through the addition of fertilizers. Average soils contain about 0.05% (w/w) of phosphorous. However, usually only 0.1% of this is in the bioavailable form of orthophosphate. Plant-available phosphorous results mostly from enzymatic decomposition of organic compounds, fertilization and microbial solubilization of inorganic phosphates (Illmer and Schinner, 1995).

To date, the extent of potential remobilization of Pb by bacteria mediated dissolution of lead apatite is not fully understood and poorly quantified. We present a comparison of experimental data on the extent of CPY dissolution in the presence of three *Pseudomonas* spp., common soil bacteria strains that were isolated from the areas with elevated metal concentration. We investigate if microorganisms isolated from heavy polluted environments (containing i.a. As, Cu, Pb) can enhance dissolution of Pb-apatite resulting in remobilization of lead. We hypothesize that these microorganisms are likely to be a significant factor in potential remobilization of Pb in the long run under iron and phosphorous limiting conditions. Specific objectives are: 1) to quantify the extent of Pb-mobilization by bacteria-mediated dissolution of lead-bearing apatite; 2) to examine potential bacterial growth in the phosphate-poor environment in the presence of lead apatite; and 3) to compare the effects of three strains of bacteria isolated from the relevant environments of elevated metal concentrations.

Under limited iron conditions bacteria can produce low-molecular-weight ligands (siderophores) that bind ferric iron and then transport it to iron siderophore receptors (Dreshsel and Jung, 1998; Hersman and Huang, 2000). Siderophores are ligands that have extremely high affinity for Fe(III) and can bind other cations, e.g. Pb(II), Zn (II), Cr (III), Ga (III) or Cu (II) (Neu, 2000; Cortese et al., 2002). The role of siderophores has to be considered in different remediation strategies of metal contaminated areas as well as in the identification of pollution sources, especially in environments with low concentrations of iron, because they affect the solubilities and dissolution rate of minerals (Gadd, 2004). Abiotic siderophore promoted dissolution of CPY in the presence of deferoxamine mesylate siderophore (DFOB) has been reported previously (Manecki and Maurice, 2008). Therefore, the concentration of siderophores in the experimental solutions was also measured.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Three natural bacteria strains showing elevated tolerance to heavy metals were selected for this study. The strains of *Pseudomonas* sp. OS8 (GenBank accession No. EF491958) and OS19 (No. EF491969) were isolated from rock biofilm collected in an ancient underground gold mine located in SW Poland near Zloty Stok (Drewniak et al., 2008, 2010). Those biofilms contain high concentration of arsenic (62 mg kg^{-1}), copper (372 mg kg^{-1}), lead (10 mg kg^{-1}) and zinc (458 mg kg^{-1}). The *Pseudomonas mendocina* strain LM7 (No. EU821343) comes from organic-rich copper-bearing black shale collected from freshly exposed part of the ore at the underground copper mine in Lubin (SW Poland) (Matlakowska and Sklodowska, 2009). The black shale ores are characterized by high concentration of heavy metals: arsenic (up to 3000 mg kg^{-1}), copper (up to 110 000 mg kg^{-1}), lead (up to 20 000 mg kg^{-1}), zinc (up to 2800 mg kg^{-1}) (Matlakowska and Sklodowska, 2009). All strains were routinely grown in Luria-Bertani (LB) medium at 22 °C (Green and Sambrook, 2012). For pyromorphite dissolution experiments bacterial strains were cultivated at 22 °C in the glucose asparagine (GASN) medium (Bultreys and Gheysen, 2000) and in a modified, phosphorous-free GASN-P medium. The growth of bacteria was analyzed using an epifluorescence microscope (Nikon Eclipse 80i) after staining with 4′,6-diamidino-2-phenylindole (DAPI). Estimation of number of cells that are present in the medium was also performed by counting on LB agar plate.

2.2. Mineral synthesis

The experiments were conducted using synthetic pyromorphite prepared at ambient temperature and pressure from aqueous solutions. Portions of $K_2HPO_4 \cdot 7H_2O$, $Pb(NO_3)_2$ and KCl were weighted into separate 500 mL beakers and diluted in 300 mL of redistilled water at 22 °C. With the use of a peristaltic pump, solutions were gradually mixed into a 3 L beaker filled with 1.5 L of continuously stirred redistilled water at 22 °C. After the solutions were exhausted, the resulting suspension was left unstirred for two weeks. After aging, the precipitate was decanted, filtered, washed with water until no Cl^- was detected and air dried. The synthesis results in the formation of fine, white pyromorphite with crystals smaller than 10 μm (Fig. 1). Lead, phosphorous, chlorine and oxygen were the only elements detected by SEM/EDS elemental microanalysis (data not shown). Comparison of powder X-ray diffraction patterns with

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