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Effects of Microbial and Phosphate Amendments on the Bioavailability of Lead (Pb) in Shooting Range Soil

C. Wilson,[1] R. L. Brigmon,[2] A. Knox,[2] J. Seaman,[3] G. Smith[1]

[1] University South Carolina Aiken, 471 University Parkway, Aiken, SC 29801, USA
[2] Westinghouse Savannah River Company, Building 999W, Savannah River National Laboratory, Aiken, SC 29808, USA
[3] Savannah River Ecology Laboratory, Aiken, SC 29808, USA

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Heavy metals including lead (Pb) are released continually into the environment as a result of industrial, recreational, and military activities. Firing range soil is of particular concern due the high heavy metal concentrations, especially Pb and Cu (Landsberger et al., 1999). Lead ranked number two on the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances and was identified as a major hazardous chemical found on 47% of USEPA's National Priorities List sites (Hettiarachchi and Pierzynski 2004). Bioremediation has been demonstrated to enhance *in situ* removal and stability of contaminants in combination with physical and chemical treatments (Brigmon et al. 2002). *In-situ* remediation of lead (Pb) contaminated soils may be accomplished by changing the soil chemistry and structure with the application of microbial and phosphate amendments (Knox et al., 2005).

Biosurfactants are surface-active compounds naturally produced by soil bacteria that can bind metals. Biosurfactants have a wide variety of chemical structures that reduce interfacial surface tensions (Jennings and Tanner 2000) and have demonstrated efficient metal complexion (Lin 1996). Biosurfactants also have the potential to change the availability of natural organic matter (Strong-Gunderson 1995).

Apatites (calcium phosphate compounds) are important in the formation of Pb phosphates. Pb phosphates form rapidly when phosphate is available and are the most stable environmental form of lead in soil (Ruby et al.1994). Pyromorphites in particular remain insoluble under a wide range of environmental conditions (Zhang et al. 1998). The three apatites evaluated in the current study were North Carolina apatite (NCA), Florida apatite (FA), and biological apatite (BA). BA is ground fish bone that has few impurities such as As, Cr, or U and contains about 27% total phosphate, most of which is available. FA and NCA are two types of rock phosphates that release small amounts of phosphate over time. Total phosphate is around 30% with only 1-2% phosphate available (Knox et al. 2005). Soil contaminated with lead up to 100 parts per thousand (ppt) has been found at the Savannah River Site (SRS) Small Arms Training Academy (SATA) in Aiken, SC. There is concern for the possibility of Pb leaching into groundwater and nearby streams at the SATA. The purpose of this study is to determine the influence of combining two microbial and three phosphate amendments on reducing lead bioavailability in shooting range soil at this site.

Correspondence to: R. L. Brigmon

MATERIALS AND METHODS

Soil contaminated with lead bullets was collected with shovels from the surface of the berm at SATA in Aiken, SC. While uncontaminated soils typically have Pb levels ranging from 2 to 200 mg/kg (Berti et al. 1998), previous analysis show Pb levels of the SATA berm to reach 8,673 mg/kg. SATA is a 50-year-old shooting range used extensively by SRS security personnel. The soil consisted mostly of Blanton sand and the target area had sparse vegetation. The soil was first sieved (2mm) to remove larger lead bullet fragments and any other large particulate matter.

The experimental design was a randomized block design with a twenty-one total treatments, including untreated control soil. Each treatment had three replicates. Combinations of the three apatites (NCA, FA and BA) and two bacteria (*P. putida* and *A. piechaudii*) were added to 50 g (wet weight) of freshly sieved soil in microcosm test. The two concentrations of apatites added were 1% (0.50 g) and 5 % (2.50 g) apatite. The bacterial strains were initially cultured in peptone, tryptone, yeast, glucose nutrient broth (PTYG) containing 0.05g tryptone, 0.05g peptone, 0.05 g yeast extract, 0.05 g glucose, 0.6 g of $MgSO_4$, and 0.07 g $CaCl_2$ in 1 L water with the pH adjusted to 6.7+/-0.1. After inoculation in PTYG broth cultures were grown overnight in a shaking incubator at 25°C to a density of 10^{8-9} cell mL^{-1} and inoculated into soil (5 mL culture/50 g soil). Two types of controls were used in this project. The first control consisted of 5 mL of sterile PTYG broth added to 50 g of freshly sieved soil. The second control was soil autoclaved three separate times in a 72-hr time period.

A Micro-Oxymax respirometer (Columbus Instruments, Columbus, OH) was used to analyze the metabolic rates (oxygen consumption and carbon dioxide production) of the soil treatments. Soil microcosms were prepared with the 21 treatments and analyzed for two weeks. After the two-week period the soil was processed for further biological and chemical analysis. Spread plate technique was used for the enumeration of viable aerobic soil bacteria (Brigmon et al. 1998). The medium used was 1% PTYG agar. For enumeration, samples were prepared by adding 5 g of soil to 45 mL of FA buffer (Difco, Detroit, MI) and vortexing for 4 min. A series of dilutions were completed and 0.1 mL was aseptically inoculated onto 1% PTYG plates. The plates were counted for a seven-day period. To obtain total microbial densities, 5 g of soil was added to 45 mL of FA buffer and vortexed for 4 min. Dilutions were made by adding 1 mL of filtered supernatant to 9 mL of FA buffer. Ten μL were then pipetted onto microscope slides and stained with Fluorescein Isothiocyanate (FITC).

For determination of the pH of treated soils, samples were air-dried and 10 g added to 25 mL of distilled water. The pH was measured after the mixture was stirred and left standing for one hour. Anion and cation concentrations on the treated soils and apatites were measured with a Dionex QIC-2 ion chromatograph equipped with a conductivity detector, and a 250-mm Dionex IonPac Fast Anion

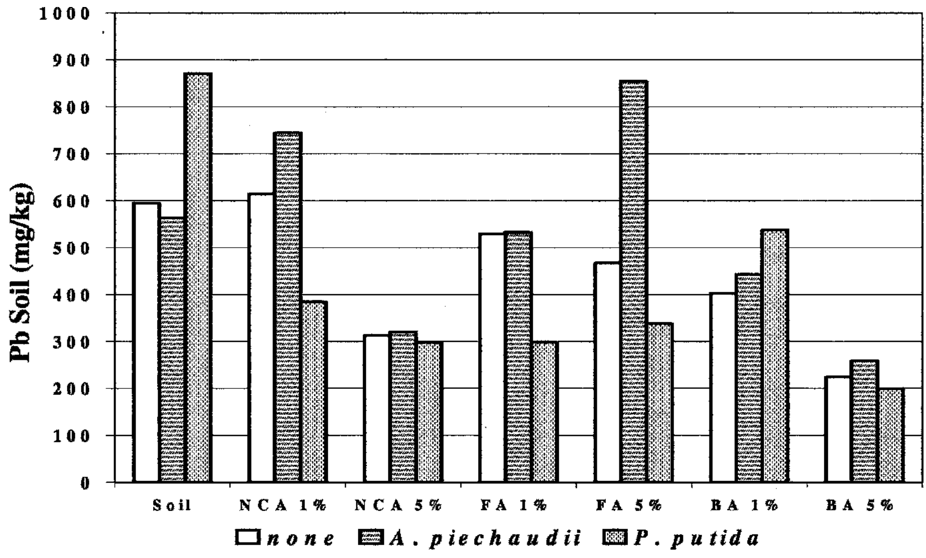

Figure 1. Soil lead availability (mg/kg) two weeks after treatment.

Analytical column (4-mm ID, 16-µm bead; Dionex Corp., Sunnyvale, CA), operated at ambient temperatures, as previously described (Brigmon et al.1998) Loss-on-ignition is a procedure that gives a percentage of the amount of organic matter present in soil (Allen 1989). Soils were slowly heated to 375°C and incubated at the temperature for an additional 2 hr. The loss-on-ignition percentage was calculated from the soil weight lost during combustion.

After equilibration for two weeks, sub-samples of pooled soils from each treatment were extracted using the EPA standardized Toxicity Characteristic Leaching Procedure (TCLP; 40 CFR , Chap. 1, Part 268, Appendix 1). The TCLP extraction is used by EPA as an operationally-defined measure of the leachability of sediment contaminants in response to mildly acidic ($pH \approx 4.93$), weak ligand extraction (acetate), conditions thought to be analogous to the leaching environment of municipal landfills (Davis et al. 1990). The extracting solution was 0.1M glacial acetic acid and 0.0643 M NaOH, with a final pH of 4.93. In the current study, 30 mL of leaching solution was added to 1.5 g of treated soil, the mixture was agitated on a reciprocating shaker for 18 h at 25 °C, and then centrifuged at 10,000 rpm (11,952 g) for 30 min using a Sorvall RC2-B supercentrifuge. After centrifugation, supernatants were filtered through 0.22 μm polycarbonate filters, acidified to 2% HNO_3, and analyzed for using ICP-MS.

RESULTS AND DISCUSSION

Results (Figure 1) confirmed that the combination of microbial and phosphate amendments, especially BA, lowered the Pb availability of contaminated soil in

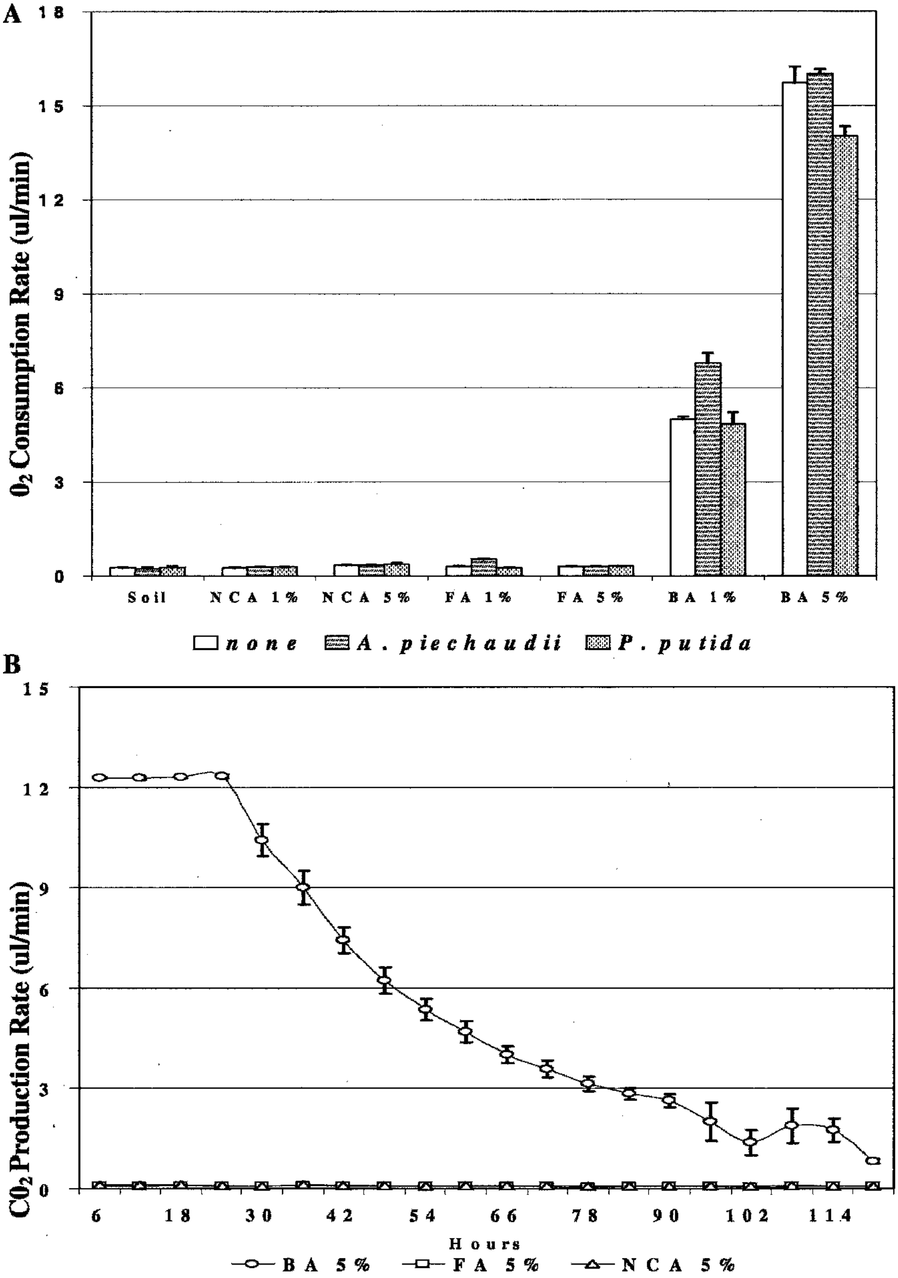

Figure 2. The average rate of O_2 consumed by the treated soils over two weeks (A). The average rate of CO_2 production of treated soils with *P. putida* and 5% phosphate apatites over five days (B). Data (n=3) are expressed as means$\pm$ S.D.

BA = Biological Apatite FA = Florida Apatite NCA = North Carolina Apatite most cases. The soil Pb availability of the autoclaved control soil was 613 mg/kg. In five of the seven treatments, the *P. putida* amendment combined with the apatites demonstrated results for lowering Pb in the contaminated soils, but more experiments are necessary to determine the benefits of microbial amendments. The *P. putida* combined with 5% BA decreased Pb to a minimum of 200 mg/kg. Microbial amendments enhanced immobilization of metals in phosphate treated soils very likely due to the kinetic of apatite (phosphate minerals) dissolution. Margolis and Moreno (1992) reported that the kinetics of apatite dissolution is enhanced in the presence of organic acids.

The average rate of oxygen consumed increased significantly with the addition of 5% BA to the soil (Figure 2A). Addition of FA and NCA did not show a significant increase in the average rate of oxygen consumption. The highest increase of oxygen consumption was produced with the addition of 5% BA and *A. piechaudii*. This was not significantly different from the 5% BA alone. The only significant increase in carbon dioxide production was with the addition of *P. putida* and 5% BA (Soil- 0.0597 ul/min, 5% BA & *P. putida*- 5.818 ul/min). The increase of the metabolic rates of the soil with the addition of 5% BA took place in the first thirty-six hours after the addition. After thirty-six hours the metabolic rates of soil with BA begin to slow eventually leveling off with the metabolic rates of soil with FA and NCA amendments (Figure 2B).

Table 1. Microbial densities, loss-on-ignition percent and pH value of test soils two weeks after addition.

	Density (cfu/g dry wt)	Loss-on-ignition %	pH value
Control Soil	2.61E+05	0.48	7.18
NCA 1%	4.49E+05	0.66	7.02
NCA 5%	6.36E+05	0.41	6.95
FA 1%	5.81E+05	0.20	7.16
FA 5%	6.52E+05	0.10	7.16
BA 1%	1.45E+06	0.60	8.55
BA 5%	1.84E+07	0.80	8.88

BA = Biological Apatite FA = Florida Apatite NCA = North Carolina Apatite

The untreated control site soil was found to have a low microbial density (Table 1). The BA amendment showed the highest microbial density in all treatments. Soil with the addition of 5% BA increased the microbial density from $2.61X10^5$ cells/gram to $1.84X10^7$cells/gram. The addition of 5% NCA and 5% FA increased the soil microbial densities to 6.36-6.52 $X10^5$ cells/gram, not as extensively as BA. There were no significant changes in the loss-on-ignition percentage with the addition of microbial or phosphate amendments to the soil. However, the loss-on-ignition percentage for pure BA was 33%, while FA was 2% and North Carolina was 5%. The pH range for the pure apatites was 6.7-6.8. With the addition of the

BA, the soil became more basic (8.55-8.88). The addition of microbial amendments to the soil did not affect the pH level.

Table 2. Analysis of phosphate apatites, soil without treatment, and soil treated with phosphate apatites after two weeks.

	NH_3 mg/kg	Na mg/kg	K mg/kg	Ca mg/kg	Cl mg/kg	NO_3 mg/kg	SO_4 mg/kg	PO_4 mg/kg
NCA	2.7	18.1	3.8	55.6	0.9	3.1	178.8	1.5
FA	1.5	13.8	5.1	29.3	1819.7	1.9	148.9	1.7
BA	49.1	1070.0	371.6	26.7	2.9	<1	197.1	232.6
Control Soil	1.6	1.4	2.1	7.4	1.2	3.7	14.8	1.5
NCA 1%	1.4	1.1	2.6	9.5	0.8	4.9	24.0	<1
NCA 5%	1.4	2.9	4.2	17.7	0.9	4.9	54.3	1.2
FA 1%	1.3	0.8	2.9	9.7	0.7	4.7	17.3	1.4
FA 5%	1.3	1.7	4.1	12.6	28.0	5.2	25.2	1.5
BA 1%	50.6	34.5	10.3	9.1	148.6	<1	47.9	2.2
BA 5%	207.2	134.8	43.5	16.3	12.0	<1	89.0	1.6

BA = Biological Apatite FA = Florida Apatite NCA = North Carolina Apatite

The untreated shooting range soil (control) was found to be nutrient poor (Table 2). The anion and cation concentrations of pure BA were relatively higher than the concentrations of NCA and FA. The soil with 5% BA added was also higher in nutrient concentrations except for the availability of phosphate and nitrate. As shown in Table 2 the ammonium was much higher in the BA-treated soils. Ammonium is an important inorganic source of nitrogen for microorganisms and can contribute to enhanced biomass and organics that can bind metals including Pb (Fan et al., 2000).

The main goal of *in situ* soil remediation techniques is to reduce mobility, bioavailability and toxicity of the specific toxic/pollutant compound. By reducing Pb bioavailability in the soil, as shown in Figure 1, environmental mobility is reduced so the Pb transport from the contaminated source areas to any receptors including sediment, groundwater, or surface water is limited. To gain insight into the relationship between soil metabolites including organic matter and metal sequestration, the capability to profile biological metabolites and related materials (e.g., soil humate) is crucial (Fan et al 2000). The significant increase in soil metabolic activity by addition of BA demonstrates a stimulation of microbial activity and associated byproducts (Figure 2). These results did not demonstrate a significant impact based on the microorganisms added (Figure 2). However, more work in this area of bioaugmentation is ongoing. The combination of techniques described in this study offers many advantages to remediation including effectiveness, low energy, low cost, and ability for *in situ* applications. Numerous laboratory and field studies have clearly demonstrated the effectiveness of apatite addition in reducing the solubility and/or bioavailability of Pb in contaminated soils and sediments (Ma et al. 1993; Ma et al. 1994), as well as other metals and radionuclides (Arey et al. 1999; Seaman et al. 2001). Studies have also demonstrated the effectiveness of biosurfactants in soil bioremediation (Sandrin et al. 2000; Kosaric 2001; Bodour 2003).

This is the first report demonstrating both the use of microbial and phosphate amendments in remediation of Pb contaminated soil. Adjusting the soil chemical and biological composition in a way that decreases bioavailability would be a preferred method for stabilizing Pb contaminated soil over widespread areas such as shooting ranges. This action would decrease potential plant uptake and mobility of Pb limiting further spread of contamination. This technology could be applied to other Pb contaminated sites including landfills, brownfields, or industrial sites. The amendments may also be useful for remediation of soil containing similar inorganic metal contaminants such as cadmium, chromium, and/or zinc.

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REFERENCES

Allen, SE (1989) Analysis of soils. In: S. E. Allen (ed) Chemical Analysis of Ecological Materials, Blackwell Publishers, Oxford, p. 7-45

Arey JS, Seaman JC, Bertsch PM (1999) Immobilization of uranium in contaminated sediments by hydroxyapatite addition. Environ Sci Technol 33: 337-342

Berti W, Cunningham S, Cooper E (1998) Case studies in the field-in-place inactivation and phytorestoration of Pb-contaminated sites. In: J. Vangrosveld, S. Cunningham (ed) Metal-Contaminated Soils: In Situ Inactivation and Phytorestoration, Landes Bioscience, TX., p. 235-248

Bodour A, Drees K, Maier R (2003) Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils. Appl Environ Microbiol 69: 3280-3287

Brigmon RL, Bell NC, Freedman DL, Berry CJ (1998) Natural attenuation of trichloroethylene in rhizosphere soils at the Savannah River Site. J Soil Contam 7: 433-453

Brigmon RL, Camper D, Stutzenberger F (2002) Bioremediation of compounds hazardous to health and the environment—An overview. In: V. P. Singh (ed) Biotransformations: Bioremediation technology for health and environmental protection, Elsevier Science Publishers, The Netherlands, p 1-28

Davis SP, Waller P, Bailou J, White P (1990) Quantitative estimates of soil ingestion in normal children between ages 2 and 7 years: Population-based estimates using aluminum, silicon, and titanium as soil elements. Arch Environ Health 45: 112-122

Fan TW-M, Higashi RM, Lane AN (2000) Chemical characterization of a chelator-treated soil humate by solution-state multinuclear two-dimensional NMR with FTIR and pyrolysis-GCMS. Environ Sci Technol 34: 1636-1646

Hettiarachchi G, Pierzynski G. (2004) Soil lead bioavailability and in situ remediation of lead-contaminated soils: A review. Environmental Progress. 23: 78-93

Jennings E, Tanner R (2000) Biosurfactant-producing bacteria found in contaminated and uncontaminated soils. Proceedings of the 2000 conference on hazardous waste research. 299-306

Kosaric N (2001) Biosurfactants and their application for soil bioremediation. Food Technol Biotechnol 39: 295-304

Knox AS, Kaplan DI, Paller MH (2005) Natural phosphate sources and their suitability for remediation of contaminated media. Sci Total Environ (In press).

Landsberger S, Iskander F, Basunia S, Barnes D, Kaminski M (1999). Lead and copper contamination of soil from industrial activities and firing ranges. Biological Trace Element Research 71-72: 387-396.

Lin S (1996) Biosurfactants: Recent reviews. J Chem Tech Biotechnol 66: 109-120

Ma QY, Traina SJ, Logan TJ (1993) In situ lead immobilization by apatite. Environ Sci Technol 27: 1803-1810

Ma QY, Traina SJ, Logan TJ, Ryan JA (1994) Effects of aqueous Al, Cd, Cu, Fe(II), Ni and Zn on Pb immobilization by hydroxyapatite. Environ Sci Technol 28: 1219-1228

Margolis HC, Moreno EC (1992) Kinetics of hydroxyapatite dissolution in acetic, lactic and phosphoric acid solutions. Calcif Tissue Int 50: 137-143.

Ruby M, Davis A, Nicholson A (1994) In situ formation of lead phosphates in soils as a method to immobilize. Environ Sci Technol 28: 646-654

Sandrin T, Chech A, Maier R (2000) A Rhamnolipid biosurfactant reduces cadmium toxicity during naphthalene biodegradation. Appl Environ Microbiol 66: 4585-4588

Seaman J, Meehan T, Bertsh P (2001) Immobilization of ^{137}Cs and U in contaminated sediments using soil amendments. J Environ Qual 30: 1206-1213

Strong-Gunderson J, Palumbo A (1995) Bioavailability enhancement by addition of surfactant and surfactant-like compounds. In: R. Hinchee, F. Brockman, C. Vogel (ed) Microbial processes for bioremediation, Battelle Press, Ohio, p. 33-50

Zhang P, Ryan J, Yang J (1998) In vitro soil Pb solubility in the presence of hydroxyapatite. Environ Sci Technol 32: 2763-2768