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

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Heavy metals including lead (Pb) are released continually into the environment as a result of industrial, recreational, and military activities. Lead ranked number two on the 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). *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. Soil contaminated with lead bullets was collected from the surface of the berm at Savannah River Site (SRS) Small Arms Training Academy (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.

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 complexation (Lin 1996). Biosurfactants also have the potential to change the availability of natural organic matter (Strong-Gunderson 1995). Two types of bacteria, *Alcaligenes piechaudii* and *Pseudomonas putida*, were employed as amendments based on their ability to produce biosurfactants and survive in metal-contaminated soils.

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. 1998). 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). In this study, we describe the influence of combining the two microbial and three phosphate amendments on reducing lead bioavailability in shooting range soil.

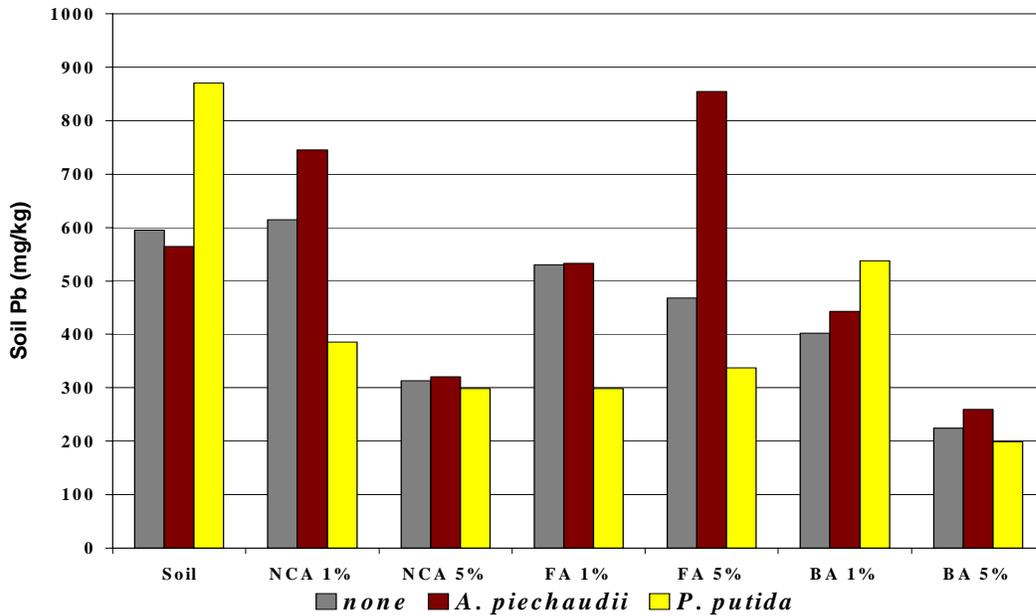
## MATERIALS AND METHODS

Soil contaminated with Pb was collected from surface soil at the SRS Small Arms Training Academy (SATA). There is concern for the possibility of Pb leaching into groundwater and nearby streams. SATA is a 50-year-old shooting range now used by SRS security personal. The soil consisted mostly of sand and the target area had sparse vegetation. Various lead bullet types and fragments were evident at the soil surface. The soil was first sieved (2mm) to remove larger lead bullet fragments and any other large particulate matter.

Combinations of the three apatites and two bacteria were added to 50 grams (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, *Pseudomonas putida* and *Alcaligenes piechaudii*, were initially cultured in peptone, tryptone, yeast, glucose (PTYG) nutrient broth containing 0.05g tryptone, 0.05g peptone, 0.05 g yeast extract, 0.05 g glucose, 0.6 g of MgSO<sub>4</sub>, and 0.07 g CaCl<sub>2</sub> in one liter 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<sup>8-9</sup> cell mL<sup>-1</sup> 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 grams of freshly sieved soil. The second control was soil autoclaved three separate times in a 72-hour time period. There were twenty-one total treatments, including untreated control soil. All soil treatments were set up in triplicates.

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 grams of soil to 45 mL of FA buffer (Difco, Detroit, MI) and vortexing 4 min. A series of dilutions were completed and 0.1 mL was aseptically inoculated onto 1% PTYG plates. Plates were counted for a seven-day period. To obtain total microbial densities, 5 grams of soil was added to 45 mL of FA buffer and vortexed four min. Dilutions were made by adding 1 mL of filtered supernatant to 9 mL of FA buffer. Ten microliters were then pipetted onto microscope slides and stained with Fluorescein Isothiocyanate (FITC).

For determination of treated soils pH, samples were air-dried and 10 grams 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 Analytical column (4-mm ID, 16-µm bead; Dionex Corp., Sunnyvale, CA),



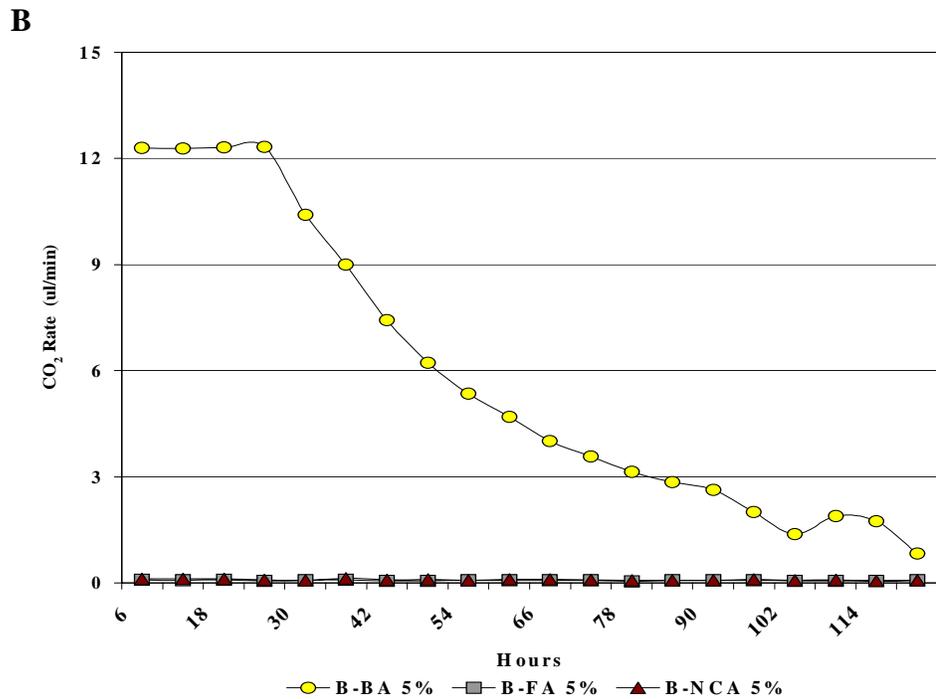
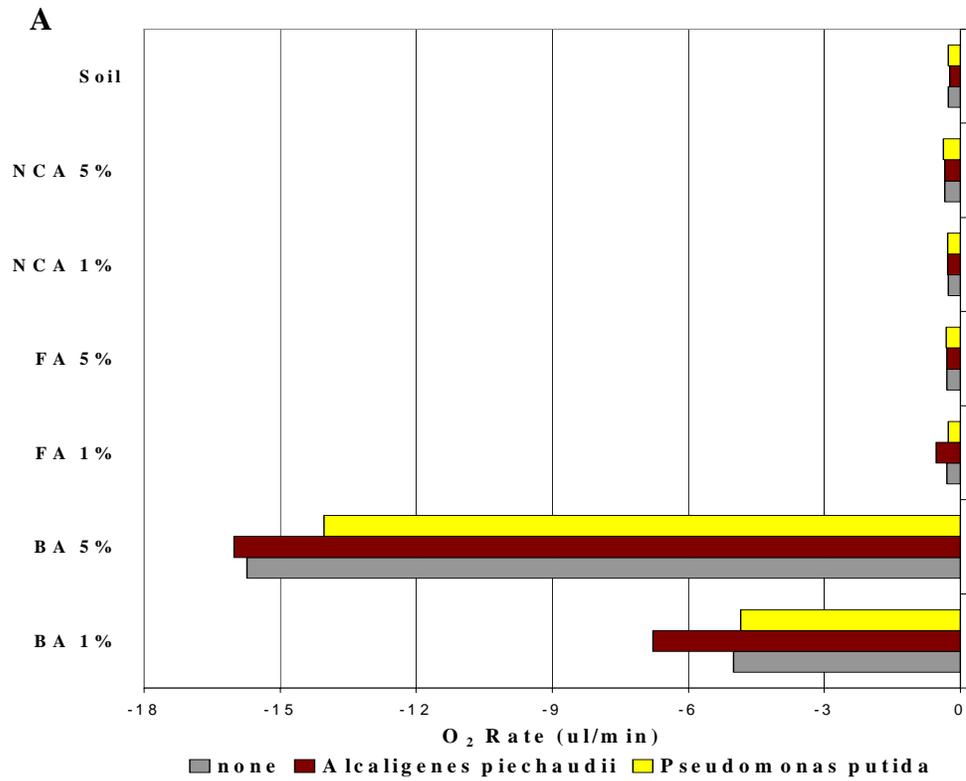
**Figure 1.** Soil Lead availability (mg/kg) two weeks after treatment

operated at ambient temperatures, as previously described (Brigmon et al.1998) Loss-on-ignition is a procedure that gives a rough indication of the amount of organic matter present in soil (Allen 1989). The temperature was slowly raised to 375°C and soil remained in the furnace for two hours. The loss-on-ignition percentage was calculated from the weight lost during combustion.

After equilibration for two weeks, sub-samples of 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 is comprised of 0.1M glacial acetic acid and 0.0643 M NaOH, with a final pH of 4.93. In the current study, thirty mL of leaching solution were 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 minutes using a Sorvall RC2-B supercentrifuge. After centrifugation, supernatants were filtered through 0.22 $\mu$ m pore-size polycarbonate filters, acidified to 2% HNO<sub>3</sub>, and analyzed for metals using ICP-MS.

## RESULTS AND DISCUSSION

Results (Figure 1) confirmed that the combination of microbial and phosphate amendments lowered the Pb availability in contaminated soil in most cases (significant results with biological apatite). In five of the seven treatments, the *P.*



**Figure 2.** The average rate of oxygen consumed by the soil with treatments over a two week period (A). The rate of carbon dioxide production of soil treatment with *P. putida* and 5% phosphate apatites over a five day period (B)

*putida* amendment combined with the apatites demonstrated the best results for lowering Pb in the contaminated soils. 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 kinetic of apatite dissolution is enhanced in the presence of organic acids. Phosphate and microbial amendments are an economical environmentally safe approach to remediation.

The average rate of oxygen consumed increased 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*. The only significant increase in the average rate of 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).

	Density (cfu/g dry wt)	Loss-on- ignition %	pH value
NCA	---	4.67	6.82
FA	---	2.24	6.7
BA	---	33.10	6.75
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

**Table 1.** Microbial densities, loss-on-ignition percent and pH value of apatites and test soils.

The soil was found to have a low microbial density (Table 1). The BA amendment showed the highest microbial densities in all treatments. Soil with the addition of 5% BA increased the microbial density more than a full order of magnitude. The addition of 5% NCA and 5% FA raised the microbial density, but not as significantly 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%. With the addition of the BA, the soil

became more basic. The addition of microbial amendments to the soil did not affect the pH level.

	Ammonium (mg/kg)	Sodium (mg/kg)	Potassium (mg/kg)	Calcium (mg/kg)	Chloride (mg/kg)	Nitrate (mg/kg)	Sulfate (mg/kg)	Phosphate (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
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

**Table 2.** Analysis of phosphate apatites, soil without treatment, and soil treated with phosphate apatites

The shooting range soil 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 here in Figure 1, environmental mobility is reduced so the Pb transport from the contaminated source areas, as these surface soils, to any receptors including sediment, groundwater, or surface water is limited. Bioremediation has been demonstrated to enhance *in situ* removal and stability of contaminants in combination with physical and chemical treatments (Brigmon et al. 2002). Bioavailability refers to the fraction of a contaminant, including Pb, that can be taken into any biological entity, be it plant, earthworm, or human (Alexander and Alexander 2000).

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 combination of techniques described here offers many advantages to remediation including effectiveness, low energy, low cost, and ability for *in situ* applications. In many cases, the exudate chemistry that underlies the function is poorly understood,

particularly regarding complexation of inorganic pollutants such as Pb. 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 (Cd), chromium (Cr), and/or zinc (Zn).

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