

## Role of Microorganisms in the Redistribution of Heavy Metals between Soil Phases: Model Study

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The effect of soil microorganisms on the redistribution of Zn and Pb compounds between solution and goethite precipitated as a coating on a sand matrix was studied. Mixed colonies of microorganisms extracted from the soil of an urban park were used to prepare cell and microbial debris suspensions. Chemical states of metal ions retained on the goethite were determined as either exchangeable (extractable by 1.0 M KNO<sub>3</sub>) and nonexchangeable (extractable by 0.3 M NH<sub>2</sub>OH-HCl in 1 M HNO<sub>3</sub>). Pure goethite without microorganisms sorbed about 99% of the added Pb and 99.4% of the Zn although 96% of the sorbed Pb and 78% of the sorbed Zn were in the nonexchangeable state. Pb concentrations decreased on the surface of the iron mineral due to living microorganisms. Sorption of Pb was inversely correlated with the concentration of organic carbon (C<sub>org</sub>) in the solution. Microorganisms clearly influenced the Zn sorption by goethite at concentrations of C<sub>org</sub> > 450 mg L<sup>-1</sup>. The amount of retained Zn was decreased primarily due to the decrease in its exchangeable fraction. Microbial debris prepared by autoclaving reduced the Pb sorption in accordance with the results for living cells. Living microorganisms accumulated more Zn than did microbial debris.

### 1. Introduction

There is a continual influx of radionuclides and heavy metals into the biosphere from both natural and anthropogenic sources. Due to their potential mobility in ecosystems and their toxicity to higher life forms, these elements have been prioritized as major inorganic contaminants. Microorganisms play an important role in the biogeochemical cycling of radionuclides and heavy metals. Microbial processes such as chelations by metabolites, oxidation-reduction, changes of solution pH, biosorption by functional groups on the cell surface, bioaccumulation, formation of stable compounds, biodegradation of organic complexes, and biomethylation may affect the behavior of these toxic elements in the environment.<sup>1</sup> Some of these processes effectively immobilize them, whereas others cause their release or transformation to different species that may be more bioavailable and/or toxic to organisms. Fundamental information on microbial-metal interactions and their implications for the environment is presented in References 2–4 and others. However, the role of microbes in the redistribution of stable and radioactive trace elements between various phases of soils has largely been neglected. The object of this study was to determine the effect of soil microorganisms on the redistribution of Zn and Pb compounds between the solution and the goethite in the model experiment.

Soil is a very complicated stochastic natural system. Numerous ecological factors influence the mineral, organic and biological components of such systems, and studying the interactions of the components at any specific moment is relatively difficult. At the present time, this justifies the use of model soil systems with a limited number of soil components for studying these interactions.

### 2. Material and Methods

Goethite-coated sand was prepared using a modification of

the procedure of Kinniburgh et al.<sup>5</sup> according to Stahl and James.<sup>6</sup> Prior to oxide preparation, the quartz sand (Carl Roth GmbH&Co, Germany) was acid washed in 1.0 M HNO<sub>3</sub> for 24 h and rinsed in deionized water. Goethite ( $\alpha$ -FeOOH) was precipitated on 500 g of sand by adding 87.5 mL of 0.17 M Fe(NO<sub>3</sub>)<sub>3</sub> and 90 mL of 0.52 M NaOH in an evaporating dish. The mixture was placed in a drying oven at 105 °C for 72 h. The mixture was stirred periodically to prevent crusting of the salts on the surface. After this procedure, goethite-coated sand was washed in deionized water to remove the residual iron mineral.

Mixed colonies of microorganisms extracted from the top horizons of soil from a public park were used to prepare the cell and microbial debris suspension. The properties of the soil from which microorganisms were cultivated are shown in Table 1.

**TABLE 1: Properties of Urban Park Soil Used for the Extraction of Soil Microorganisms**

pH <sub>CaCl2</sub>	C <sub>org</sub> <sup>a</sup> (%)	Biomass, $\mu$ g Ninhydrin N g <sup>-1</sup>	Pb total, mg kg <sup>-1</sup>	Zn total, mg kg <sup>-1</sup>	Fe total, mg kg <sup>-1</sup>
6.8	5.3	34.1	313	483	45000

The solid medium prepared to cultivate the microorganisms comprised (in g L<sup>-1</sup>): tryptone, 5; yeast extract, 2.5; dextrose, 1; and agar, 15. Mixed colonies from urban park soil were grown at 37 °C for 24 h. After growth, 15 mL of cold deionized water was added to a Petri dish with microorganisms in order to wash colonies from the medium and to prepare the suspension. Part of the suspension was autoclaved to evaluate the effect of microbial debris on heavy metal sorption by goethite. Concentrations of biomass and microbial debris in the suspensions were determined as concentrations of organic C using a DIMATOC analyzer.

Five mL of cell suspension of soil microorganisms with concentrations of living cells of 0, 280, and 450 mg L<sup>-1</sup> in organic carbon, and 5 mL of suspension consisting of microbial debris after autoclaving of 810 mg L<sup>-1</sup> in C<sub>org</sub>, were added

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into tubes containing:

- 1) 1 g of the goethite-coated sand and 15 mL solution of  $\text{Pb}(\text{NO}_3)_2$ ;
- 2) 1 g of the goethite-coated sand and 15 mL solution of  $\text{Zn}(\text{NO}_3)_2$ ;
- 3) 1 g of the goethite-coated sand and 15 mL deionized water.

The experimental design is shown in Table 2. The concentrations of Zn and Pb in the solution were each 0.1 mM ( $6.54 \text{ mg L}^{-1}$  and  $20.7 \text{ mg L}^{-1}$ , respectively, or  $130.8$  and  $414 \text{ mg kg}^{-1}$  of coated sand). Tubes with microorganisms and corresponding controls (no inoculum) were incubated unshaken at  $22^\circ\text{C}$  for 20 days. Sorption experiments were carried out in triplicate.

**TABLE 2: Experimental Design**

GOETHITE-COATED SAND					
Zn		Pb		Without METALS	
C solution = $6.54 \text{ mg L}^{-1}$		C solution = $20.7 \text{ mg L}^{-1}$			
C sand = $130.8 \text{ mg kg}^{-1}$		C sand = $414 \text{ mg kg}^{-1}$			
Cell suspension	Suspension from microbial debris	Cell suspension	Suspension from microbial debris	Cell suspension	Suspension from microbial debris
Concentration of organic C, $\text{mg L}^{-1}$					
280		280		280	
450	810	450	810	450	810

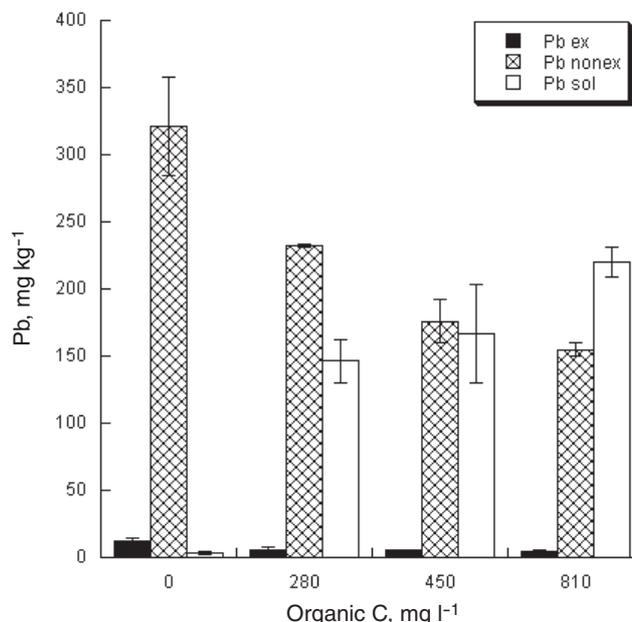
To determine the loss of metals from solution, Pb and Zn concentrations in the equilibrium solutions were measured. After the equilibrium, the solid phase was provided for the desorption experiments after decanting, filtering the metal treatments solution and weighing the tubes to account for occluded solution. The chemical states of the metals (exchangeable or non exchangeable) were determined by adding 10 mL of 1.0 M  $\text{KNO}_3$  and agitating for 1 h at  $150 \text{ cycles min}^{-1}$ . Nonexchangeable Zn and Pb were recovered by dissolving the oxide surface after decanting the  $\text{KNO}_3$  solution. Goethite was dissolved using 40 mL of 0.3 M  $\text{NH}_2\text{OH}\cdot\text{HCl}$  in 1 M  $\text{HNO}_3$ . This extraction was performed in a hot water bath at  $40^\circ\text{C}$  for 2 h.<sup>6</sup>

Microbial biomass was determined by the fumigation-extraction method according to Amato and Ladd.<sup>7</sup> All heavy metal analyses were performed by atomic absorption spectrometry.

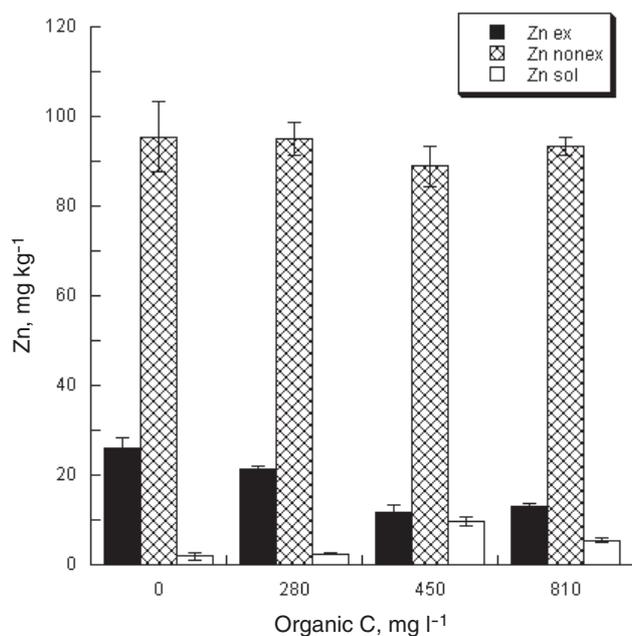
### 3. Results and Discussion

The goethite-coated sand without microorganisms or microbial debris sorbed about 99% of the added Pb and 99.4% of the Zn (Figure 1) indicating that pure goethite possesses a high zinc and lead sorption capacity. Iron oxides significantly affect heavy metal reactions in soils because they occur as coatings, concretions and as discrete particles of colloidal size; they also have a strong affinity for metal ions.<sup>8</sup> The sorption ability of iron oxides is determined by the degree of crystallinity, the surface area, reaction time and pH.<sup>9</sup> The pH of the solution changes the speciation of the metals in solution as well as the nature of the adsorbent. Our study was carried out at a weakly alkaline pH of the solution, which induced negatively charged goethite surfaces. Stahl and James<sup>6</sup> showed that goethite coatings increase sorption of Zn by fine quartz sand, principally in nonexchangeable form, at  $\text{pH} > 6$ . Our experiment also demonstrates that the nonexchangeable chemical state plays a dominant role in the adsorption of both Zn and Pb on the surface of the iron mineral in all treatments (Figures 1 and 2). The amount of Pb retained in the nonexchangeable state on the surface of the pure goethite was approximately 96.3% ( $321.4 \text{ mg kg}^{-1}$  sand). The nonexchangeable fraction of Zn was less, averaging 78.4% ( $95.5 \text{ mg kg}^{-1}$ ) of the sum adsorbed by goethite.

The heavy metal concentrations in our experimental solution



**Figure 1.** Influence of microorganisms ( $280$  and  $450 \text{ mg L}^{-1}$  of organic C) and microbial debris ( $810 \text{ mg L}^{-1}$  of organic C) on the redistribution of Pb compounds between solution and goethite after 20 days of incubation (Average values and Standard deviations). Pb ex: the concentration of exchangeable-state Pb; Pb nonex: the concentration of nonexchangeable-state Pb; Pb sol: the concentration of Pb in the solution.



**Figure 2.** Influence of microorganisms ( $280$  and  $450 \text{ mg L}^{-1}$  of organic C) and microbial debris ( $810 \text{ mg L}^{-1}$  of organic C) on the redistribution of Zn compounds between solution and goethite after 20 days of incubation (Average values and Standard deviations). Zn ex: the concentration of exchangeable-state Zn; Zn nonex: the concentration of nonexchangeable-state Zn; Zn sol: the concentration of Zn in the solution.

were no doubt below toxic levels. Mergeay et al.<sup>10</sup> reported minimal inhibitory concentrations for *E. coli*: Zn  $1.0 \text{ mM}$ , Pb  $5.0 \text{ mM}$ .

Microorganisms had a significant effect on Pb and Zn sorption by goethite coatings from solution. Pb concentrations increased in the sorption solutions and decreased on the surface of the iron mineral due to living microorganisms (Figures 1 and 2). The absolute concentrations of the nonexchangeable-state metals were reduced to a greater extent than those of the exchangeable state, whereas the relative content of

both fractions dropped by a factor of approximately two. The sorption of Pb by goethite is inversely correlated with the concentration of organic carbon in the solution (Figure 1).

Microorganisms reduced the sorption of Zn only slightly compared with Pb. They clearly influenced the Zn sorption by goethite at concentrations of  $C_{\text{org}} \geq 450 \text{ mg L}^{-1}$ . The amount of retained metal is decreased primarily due to a decrease in its concentration in the exchangeable state (Figure 2).

Most probably the main reasons for the retention of metals by microorganisms are biosorption and bioaccumulation. Biosorption of toxic metals and radionuclides is based on the use of ligands or functional groups situated on the outer surface of the cell. The process requires neither an active membrane transport mechanism nor metabolic energy in order to function and is controlled in a non-directed physicochemical reaction.<sup>11</sup> Metals interact with reactive amphoteric groups (carboxyl and phosphate) within the constituent polymers of bacterial cell walls and external sheaths and capsules. Some wall and capsular polymers react with dissolved ions as if they were an open ion exchange resin, whereas others exhibit more selectivity with a greater partitioning ability.<sup>12</sup> At pHs between 5 and 8, these polymers are ionized and naturally anionic.<sup>13</sup> Under our experimental conditions the ionized cell walls or cell debris accumulated high amounts of heavy metals and were pushed away from goethite coatings with negative charges, which reduced Pb and Zn sorption by goethite.

Both living and dead biomasses of microorganisms exhibit biosorption.<sup>14</sup> Non-viable or dead biomass can be obtained through pretreatment of biomass. Physical pretreatment methods such as heating, autoclaving, freeze-drying and boiling, and chemical pretreatments such as using acidic, alkaline and organic chemicals showed enhancement or reduction in metal bioadsorption, depending on the strains and treatment procedures used.<sup>15, 16</sup> In our experiment microbial debris prepared by autoclaving reduced Pb sorption by goethite in accordance with the results for living cells of microorganisms. An opposite reaction was detected for Zn. Living microorganisms accumulated more Zn than did microbial debris. The reduced bioadsorption capacity of the autoclaved suspension for Zn versus live cells may be explained by the loss of intracellular uptake.

The principal driving force for metal ion sorption by biotic and abiotic soil components is their net negative surface charge. So, along with soil and microbial community composition the intensive properties of soil systems such as pH and Eh have great importance for the fate of radioactive and stable trace elements. On the other hand these properties may depend

on the physiological activity of soil microorganisms. For a satisfactory understanding and prediction of the behavior of toxic elements in the environment, further investigation involving the modeling of soil systems is required.

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