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Accelerated Rates of Organolead Transformation Following Nutrient Enrichment of Contaminated Ground Water

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Although no longer used for automobile fuel in the United States, alkyl leads, primarily tetraethyl lead(TEL), have been manufactured as anti-knock additives since the 1920's (Rhue et al. , 1992). Soil and ground water contamination, often including mixed alkyl leads and carrier solvent(s), have resulted from previous handling practices (Rhue et al. , 1992). From a risk standpoint, alkyl leads are generally more toxic than inorganic lead salts (Huber et al., 1978; Rhue et al. , 1992). In addition, under most environmental conditions, ionized, water soluble, alkyl lead salts form through dealkylation (Rhue et al. , 1992). Mobilization of these toxic, alkyl lead salts through ground water movement can potentially expand the area of contamination. Therefore, conversion of alkyl leads to inorganic lead(Pb^{2+}) will often reduce potential environmental and health hazards, since resulting inorganic lead salts are often less soluble and less toxic (Rhue et al. , 1992). Microbial metabolism could represent an in situ route for this conversion, although microbial alkyl lead metabolism remains controversial (Huber et al., 1978; Kozyura et al., 1961; Wood et al., 1978). Recently, both field and laboratory studies have reported evidence for biological dealkylation of alkyl leads (Macaskie and Dean, 1990; Ou et al., 1993). Here we examine the effects of nutrient stimulation of microbial populations on the rates of alkyl lead transformation to Pb^{2+} in microcosms containing contaminated ground water from two former TEL manufacturing sites.

MATERIALS and METHODS

Wells were sampled at two former TEL manufacturing sites in New Jersey (Site 1) and Texas (Site 2). Following three well volume replacements, using a steam-cleaned high volume pump, we collected samples directly into sterile sampling bottles. Samples were held on ice for shipment. In the laboratory, ground water samples were transferred to acid-cleaned, 500 mL glass separatory funnels. For the

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aerobic treatment, funnels were filled to about 70% of capacity, allowing ≈150 mL of air to remain above the sample. For other treatments funnels were completely filled. Treatments were run in triplicate. Anaerobic treatments were maintained under a nitrogen/hydrogen (95/5) atmosphere. An unamended, rather than sterilized, control was used because all known sterilization methods are likely to alter the chemical distribution of lead. Nutrient additions were made to the aerobic and anaerobic treatments. The one time mineral addition was NaH_2PO4 + NH_4Cl, both to final concentrations of 0.28%. Yeast extract, 0.02% final concentration, was added every 2-4 weeks. Anaerobic microcosms were given a single initial dose of yeast extract, 0.6% final concentration, to drive down redox conditions. Additional amendments were: (1) H_2O_2 added to aerobic microcosms whenever they received yeast extract, 0.006% final concentration, (2) sodium lactate, 0.04% final concentration, added every 2-4 weeks to anaerobic microcosms, and (3) $FeSO_4$, 0.2 mM, + Na_2SO_4, 0.003%, both final concentrations, added in a single dose to Site 2 microcosms. Reagents used for nutrient additions and sample processing contributed small amounts of lead to the analyzed samples. For Site 1 samples, total lead contributed was 14.4 µg and 7.8 µg Pb for the anaerobic and aerobic treatments, respectively. For Site 2, total lead contributed was 5.1 µg and 1.7 µg Pb for the anaerobic and aerobic treatments, respectively. This represents ≤7% of the total lead present in any one microcosm at the end of the study period.

Samples were withdrawn periodically from the microcosms to analyze lead fractions and bacterial plate counts. Three lead fractions and total lead were sampled at each time point. For total lead, 1 mL 70% nitric acid + 1 mL ICI reagent was combined with 20 mL of microcosm suspension in an acid washed polyethylene bottle. To fractionate lead species, nonpolar organolead (e.g., TEL) was extracted into ethylene dichloride (EDC), 20 mL sample/20 mL EDC, by one hour of shaking on a wrist action shaker. The extraction was repeated and both EDC extracts were filtered through a Whatmen GF/D filter at low vacuum and combined. Enough EDC to make up for evaporation losses was then used to wash the extraction glassware and filter. The wash was combined with the extract. The extracted aqueous phase was then adjusted to pH 9±0.1 and allowed to stand ≈ 1 h at room temperature. The pH adjustment caused precipitation of inorganic lead which was then filtered onto a combination Whatman GF/D+Gelman GN-6, 0.45µm filter. Filters from both fractionation steps were combined and placed in 10 mL of 2.7% nitric acid containing 1 mL of ICI reagent. From the remaining aqueous phase, 15 mL of sample was combined with 0.75 mL 70% nitric acid and 1.5 mL of ICI reagent. All fractions were stored at 4°C until analysis. ICI reagent consisted of 1778 mL 25% KI + 1778 mL concentrated HCl + 300 g KIO_3. For analysis, lead samples were first digested with nitric acid followed by colorimetric lead

analysis as the dithizone-lead complex (APHA, 1985). Bacterial numbers were followed by plate count techniques on Bacto Plate Count Agar (aerobic and unamended) and Bacto Anaerobic Agar (anaerobic) (Difco, Detroit Michigan). Anaerobic samples were processed and incubated in an anaerobic glove bag (Coy Products, Ann Arbor, MI). Sulfate reducing organisms for Site 2 were monitored by Bacto Sulfate API Broth (Difco, Detroit Michigan) following manufacturers instructions.

RESULTS and DISCUSSION

At both sites, treatments to which nutrients were added developed higher bacterial concentrations than the unamended treatments. Generally, nutrient enriched treatments maintained 10^8-10^9 colony forming units (CFU)/mL over the course of the incubation, while unamended controls remained in the 10^5-10^6 CFU/mL range. Although good microbial growth occurred in amended samples, organolead levels were relatively low, probably minimizing toxicity for the adapted populations. This may not always be the case. All microcosm samples had total lead concentrations of <15 ppm and preliminary studies showed that toxicity problems may arise in more heavily contaminated aquifers. For example, sulfate reducers were undetected in the wells with total lead >20 mg/L . Similar observations were made at both sites using turbidity and/or tetrazolium reduction as indicators of microbial growth. Absence of growth was more often associated with samples showing higher total lead concentrations. Toxicity is suggested because in most cases , better microbial growth occurred as samples were diluted to higher levels. Alkyl lead salts have been shown to be inhibitory to some microbes at $\leq$100 mg/L (Macaskie and Dean, 1990; Rhue et al. 1992). Also, other toxic compounds may have contributed to observations in the current study. Clearly, the potential for inhibition of biological alkyl lead transformation must be considered when reviewing a site for biotransformation of alkyl leads.

Lead fractionation data for the ground water samples from Site 1 generally showed the pattern expected if nutrient additions were promoting transformation of organolead species to inorganic lead (Fig. 1, Table 1). In both the aerobic and anaerobic treatments, the fraction of lead contributed by nonpolar organoleads (e.g.,tetraethyllead) declined more than two fold. The unamended control did not show such a strong trend . As a percent of total lead, inorganic lead increased by three-fold or more in the two nutrient amended treatments, while there was less than a two fold increase in the unamended treatment. Polar organolead (e.g., triethyl lead chloride) declined in all three treatments. Again, however, on a relative basis, these declines were greater in the nutrient amended systems. Mass balances for the sum of

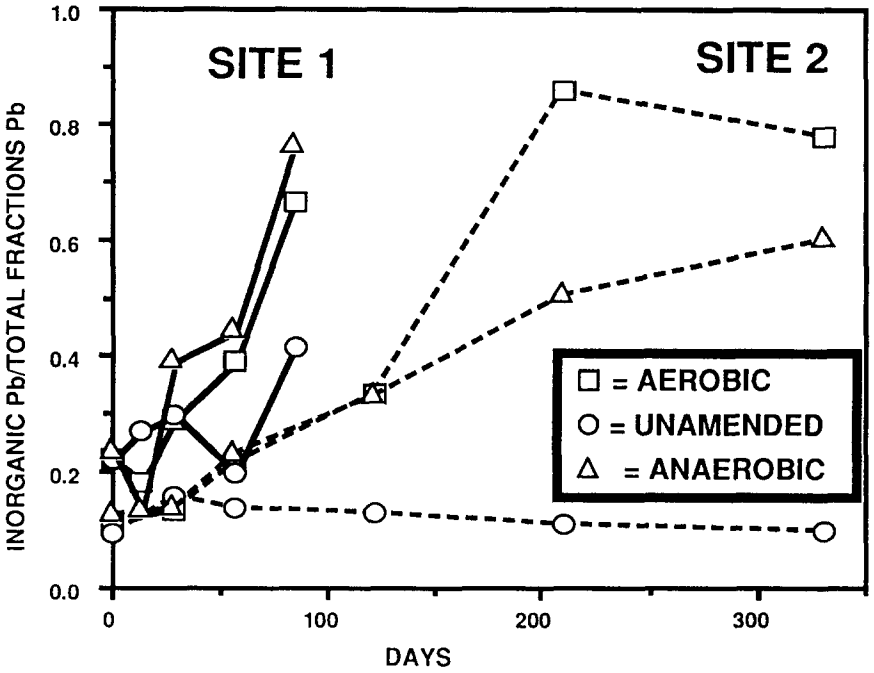

Figure 1. Inorganic lead(Pb) as a fraction of total lead over the course of the incubations.

fractions data (Day 84/Day 0) were 0.898, 0.785 and 1.32 for aerobic, unamended, and anaerobic treatments, respectively.

Observations suggested that Site 2 water chemistry was much different than that at Site 1. Rusty colored precipitates were absent in Site 2 samples, which also showed lower water conductivity and, initially, failed to develop a dark coloration in anaerobic treatments. Because of our desire to promote sulfate reduction under anaerobic conditions, iron and sulfate additions were made in order to stimulate sulfate reducing activity in the Site 2 anaerobic systems. Increased iron levels also served to allow observation of sulfide precipitates and protect microorganisms from sulfide toxicity. A dark coloration, typical of sulfide precipitates was seen within one week of the iron sulfate addition. Also, sulfate reducing organisms were detected on five out of seven sampling dates following iron sulfate addition.

Lead fractionation data from Site 2 also showed the trends expected if nutrient additions were stimulating organolead transformation (Fig. 1, Table 1). The percent of lead as nonpolar oganolead decreased approximately six fold in the aerobic treatment and two fold in the anaerobic treatment. The unamended treatment showed a slight increase in this fraction. Complementary to the decrease in nonpolar

Table 1. Lead fractions at the start and end of incubations in mg/L, mean (standard deviation of the mean)

Treatment Site/Day		Nonpolar Lead	Inorganic Lead	Polar Lead	Sum of Fractions
Aerobic					
Site 1	0	5.0 (0.25)	2.2 (1.15)	2.6 (0.72)	9.8 (0.40)
	84	2.1 (0.42)	5.9 (1.80)	0.8 (0.53)	8.8 (2.25)
Site 2	0	2.8 (0.15)	0.6 (0.06)	2.5 (1.17)	5.9 (0.98)
	330	0.08 (0.06)	0.84 (0.40)	0.16 (0.11)	1.1 (0.23)
Unamended					
Site 1	0	6.4 (0.17)	3.0 (0.96)	4.0 (4.16)	13.4 (4.94)
	84	4.2 (0.51)	4.3 (0.99)	1.7 (0.58)	10.2 (1.56)
Site 2	0	3.8 (0.51)	0.87 (0.15)	4.2 (0.00)	8.9 (0.70)
	330	4.2 (0.00)	0.64 (0.10)	1.8 (0.23)	6.6 (0.13)
Anaerobic					
Site 1	0	4.5 (0.00)	1.9 (0.12)	1.5 (0.12)	7.9 (0.12)
	84	1.8 (0.7)	8 (4.12)	.6 (0.35)	10.4 (3.4)
Site 2	0	1.7 (0.17)	0.4 (0.17)	1.0 (0.00)	3.1 (0.35)
	330	0.93 (0.23)	1.9 (0.09)	0.31 (0.03)	3.2 (0.29)

organolead, inorganic lead, as a percent of total lead, increased more than seven fold in the aerobic treatment and almost five fold in the anaerobic treatment, while the unamended treatment showed little change. On a relative basis, polar organolead also showed greater decrease in the two nutrient amended treatments than in the unamended treatment.

Mass balances for the Site 2 study were good for both the anaerobic and unamended treatments with a Day 333/Day 0 ratios for the sum of fractions equal to 1.02 and 0.742, respectively. However, for the aerobic treatment the Day 333 sum of fractions was only 18.6% of Day 0, indicating a great deal of unaccounted for lead. This imbalance was a trend that became evident over the last 200 days of the incubation. Additional washes of the microcosm glassware showed large amounts of acid soluble lead, but not EDC soluble lead, only in the aerobic treatment. This suggests that inorganic lead adsorption to the glass may have been responsible for the poor mass balance. Considering that the acid rinse was under relatively mild conditions (2.7% nitric acid, room temperature), the data suggest that lead adsorption to the glassware was an important phenomenon in the Site 2, aerobic treatment. The lead recovered by the glassware extractions approximately doubled the amount of lead recovered at the end of the study, leaving unaccounted for approximately 60% of the starting material .

We observed slow rates of organolead transformation. Calculated using a zero order loss model for comparison purposes , on a percent

organolead conversion per day basis, we obtain values for Site 1 of 0.54, 0.15, & 0.66 %/day and for Site 2 of 0.24, 0.00, & 0.15 %/day for aerobic, unamended, and anaerobic microcosms, respectively. With starting concentrations in the organolead fractions of $\leq$10 mg/l, on a weight basis this equals a transformation rate of $\leq$66 µg/L/day. In spite of the high cell numbers in our nutrient amended microcosms, on a per volume basis, this rate of organolead transformation is almost a thousand-fold slower than rates reported for TEL transformations in soil by Ou et al (1993).

Both direct and cometabolic attack have been suggested as mechanisms for organolead transformation (Macaskie and Dean, 1990). We did not try to induce a specific aerobic population in this study where yeast extract was our main carbon and energy source. Our observations suggest that simply increasing the level of microbial activity accelerated organolead transformation, an observation consistent with cometabolic attack. Similar mechanisms may be active in the anaerobic systems. In addition, the sulfur containing metabolites from microbial sulfate reduction (which we specifically encouraged in anaerobic microcosms) may also accelerate transformation to inorganic lead since they are known to react with some organolead species (Jarvie et al., 1983). In general, our observations support the evidence for a microbial role in environmental transformations of organolead . However, there is wide variation in reported transformation rates from environmental samples. Demonstrating high rates in pure laboratory culture has also not been possible. Therefore, a much better understanding of biological mechanisms is needed before such transformations can be optimized for practical use.

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