

MICROBIAL AND GEOCHEMICAL PROCESSES CONTROLLING THE OXIDATION AND REDUCTION OF ARSENIC IN SOILS¹

D.C. Masur², R.E. Macur, and W.P. Inskeep

Extended Abstract: Arsenic (As) is an important environmental contaminant in mining, agricultural, geothermal and sedimentary groundwater environments. As such, a better understanding of the factors which control the speciation and transport of arsenic in soils and natural waters is vital for compliance with new United States As drinking water standards and the bioremediation of contaminated lands. The fate and toxicity of As is highly dependent on its predominant valence state and chemical form: As^V generally sorbs more strongly to soils therefore is less mobile and less bioavailable than As^{III}. The relative abundance of arsenite (As^{III}) and arsenate (As^V) in soil and natural water environments is influenced by geochemical conditions and the types of microorganisms present that may transform As via detoxification, dissimilatory reduction or chemolithotrophic oxidation pathways. Due to their structural similarities, phosphate and arsenate are analogs - both enter a microbial cell via phosphate transporters. Consequently, the presence of phosphate may inhibit microbial uptake of arsenate, thus preventing the microbial reduction of As^V to As^{III} via the *ars* operon. The goal of the present work was to evaluate the effects of As and phosphate concentrations on the fate and transport of As in soil environments.

Laboratory column experiments were conducted to evaluate the influence of As^{III, V} concentration in soils on microbial community composition, and to identify the organisms and mechanisms responsible for As transformations occurring under unsaturated (aerobic) conditions. It is hypothesized that low concentrations of As contamination will not result in large reductions in microbial diversity in soils given the ubiquity of As detoxification genes; however, high concentrations of As will select for organisms that either are very efficient in detoxifying As or utilize As in energy conservation. Indigenous microbial populations within a previously non-contaminated agricultural soil (Post Farm, Bozeman, MT) were

Additional Key Words: smelter-impacted soils, arsenate, arsenite, phosphate, inhibition, transport

¹ Paper was presented at the 2006 Billings Land Reclamation Symposium, June 4-8, 2006, Billings MT and jointly published by BLRS and ASMR, R.I. Barnhisel (ed.) 3134 Montavesta Rd., Lexington, KY 40502.

²Deanne C. Masur, M.S. Candidate; Rich Macur, Research Associate; and William P. Inskeep, Professor; Land Resources and Environmental Sciences Department, Montana State University, Bozeman, MT 59717.

exposed to As^{III} or As^V at three concentrations (2 mg L⁻¹, 20 mg L⁻¹ and 200 mg L⁻¹) dissolved in “natural” soil solution media (SSM) for approximately 30 days at room temperature. Analytical samples were obtained approximately every third day throughout the experiment and were analyzed using hydride generation atomic absorption spectrometry (HG-AAS). Molecular and cultivation samples were obtained at the conclusion of the experiment. Near complete As^{III}-oxidation was the only transformation process observed at 2 and 20 mg As L⁻¹ and values of 200 mg L⁻¹ were inhibitory. Though As^V is the thermodynamically favored As species under these (aerobic) conditions, sterile controls showed less than 15% oxidation over the 30-day period. This suggests that the abiotic oxidation rate is relatively slow and a biotic mechanism is responsible for the observed oxidation in the non-sterile treatments. DNA extracted from molecular samples was PCR-amplified to obtain 16S short fragment sequences (~300 bp), which were separated using denatured gradient gel electrophoresis (DGGE). DGGE banding patterns suggest a diverse microbial signature at the 2 and 20 mg As L⁻¹ concentrations with reduced diversity at the 200 mg L⁻¹ level. However, cultivation and long-fragment (~1380 bp) DNA sequencing of microbial isolates from the treatment columns yielded twenty-eight isolates from fourteen genera from the 2 mg As L⁻¹ treatments, twenty-one isolates from eleven genera from the 20 mg As L⁻¹ treatments, and eighteen isolates from ten genera from the 200 mg As L⁻¹ treatments. This suggests that a decrease in microbial diversity as a result of As perturbation may be minimal, which could be explained by the ubiquity of As detoxification genes across diverse groups of microorganisms allowing them to withstand As pressure. While overall diversity was relatively consistent, the most commonly encountered genus appeared to shift from *Streptomyces* in both the 2 and 20 mg As L⁻¹ treatments to *Variovorax* in the 200 mg As L⁻¹ treatments. This may suggest that *Variovorax* spp. are more efficient in detoxifying arsenic or may utilize As in energy generation.

Liquid culture experiments were conducted to determine whether increased phosphate (PO₄) levels in soil minimize biological responses to As^V or As^{III}. It is hypothesized that high concentrations of phosphate may reduce As^V uptake thus limiting reduction via detoxification or dissimilatory mechanisms. Microorganisms used in the experiments were isolated from soils obtained from two As-stressed sites including an anthropogenically stressed site impacted by aerial As contamination (Anaconda-Deer Lodge, MT) and a site impacted by naturally occurring geothermal As-rich irrigation water (Gallatin county, MT). Two arsenite-oxidizing microorganisms - *Agrobacterium tumefaciens* and *Variovorax* sp. - and three arsenate-reducers - *Agrobacterium tumefaciens*, *Arthrobacter* sp., and *Bacillus* sp. - were selected for use. Cultures were initially spiked with various As: PO₄ ratios and grown in a synthetic soil solution equivalent media (SSE) while incubated (at 30 °C) for approximately a 48-hour period on a shaker (set at 120 rpm). A total of six As:PO₄ ratio combinations were used to elucidate which might play a larger role in inhibition of As-transformations: As:PO₄ ratio or absolute PO₄ concentration (see Table 1). Samples were obtained at four points throughout the incubation period and analyzed using inductively coupled plasma spectrometry (ICP) and HG-AAS. Preliminary results support that increased phosphate levels in soil may decrease microbiological reduction of arsenate, while having little effect on As^{III}-oxidation. Current results also suggest inhibition of As^V-reduction by phosphate is dependent on both the absolute amount of PO₄ as well as the ratio of PO₄:arsenate.

Table 1. Ratios used in experiments.

Ratio of As:PO₄	[As] (μM)	[P] (μM)
1:5	10	50
1:5	100	500
1:10	5	50
1:10	100	1000
2:1	100	50
20:1	1000	50