

Juliana G. de Freitas:

Challenges for Ethanol Fuels Contaminated Sites Assessment

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The use of ethanol as an additive in gasoline is increasing significantly in North America, raising concerns about the consequences of ethanol usage when monitoring the subsurface for groundwater contamination. One piece of the problem is the effect of ethanol on contaminant distribution in the source zone. Ethanol can change important properties of the system which can result in an unexpected distribution of the gasoline contaminants. Ethanol can also enhance the solubility of aromatic hydrocarbons such as benzene.

In 2D visualization experiments gasoline was injected in the top of the unsaturated zone and then ethanol was injected in the same position. It was shown that ethanol can mobilize the gasoline and alter its distribution significantly. The visualization experiments also indicate that ethanol stays mainly in the capillary fringe, being transported above the water table along with cosolubilized hydrocarbons. However, typical monitoring techniques applied at fuel contaminated sites are not intended to sample the capillary fringe. Although under some conditions a typical monitoring well may draw some ethanol contaminated water, providing an indication of contamination, the sample will be diluted and therefore the measured concentration may be significantly smaller than the actual concentration in the capillary fringe. Therefore, the horizontal transport of high concentrations of hydrocarbons and ethanol in the capillary fringe at field scale presents significant challenges for monitoring. For example, since sampling in the vadose zone requires suction, volatile losses are more likely to happen when sampling water from the vadose zone. Laboratory experiments showed that the use of ceramic suction samplers may result in loss of volatile organics of up to 30%, depending on the pressure applied and compound properties. The concentration of ethanol in the aqueous phase was also shown to interfere with the sample bias.

Olugbenga J. Owojori:

Influence of Salinity on Uptake and Toxicity of Zinc in the Earthworm *Eisenia fetida*

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Salinization of soil is a global problem especially in semi-arid and arid regions. Few studies have considered the influence of salinity on beneficial soil organisms, and their uptake of metals. Recent studies on the development of Biotic Ligand Model (BLM) suggest that cations (at low concentrations), may protect organisms against metal toxicity but effects of these cations at high concentrations remain speculative. In order to assess the influence of salinity on uptake and toxicity of zinc to earthworms, specimens of *Eisenia fetida* were exposed to zinc for 28 days using OECD artificial soil adjusted with 0, 2000 and 4000 mg/kg NaCl. Zn was added as ZnCl₂ in a range of concentrations (0, 250, 500, 750 and 1000 mg/kg Zn). The endpoints; mortality, weight change, and internal zinc concentration were assessed at Day 1, 7, 14 and 28 while cocoon production was assessed only at day 28. In the substrates, nitric acid, DTPA and CaCl₂ extractable zinc concentrations were also determined. The results indicated that NaCl increased the availability of zinc in the substrates. Weight, mortality and internal zinc concentrations in the worms were not significantly affected by NaCl and zinc as individual substances, but in combination both had significant effects on all of these parameters. In contrast, cocoon production was significantly affected by increased NaCl and ZnCl₂ as individual substances, and the effects were more severe when both substances were present. The 28- d EC₅₀ for cocoon production was 2020 mg/kg NaCl. The observed increase in toxicity with increased salinity could not be explained fully by zinc accumulation in the tissues of the worm. It is concluded that salinity as a result of increased Na⁺ has a synergistic effect with zinc in toxicity to these earthworms. The implications in the development of BLM for earthworms are discussed.

Zarath M. Summers:

Adaptive Evolution: a Strategy for Better Understanding and Optimizing Environmental Biotechnologies

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Adaptive evolution studies can provide new insights into the physiology and ecology of environmentally relevant organisms by identifying which mutations in genes and regulatory pathways are associated with adaptations producing a more beneficial phenotype. Genome-scale *in silico* modeling of the metabolism of *Geobacter* species has indicated that these organisms have a metabolic potential far beyond that previously demonstrated in culture. Some of these potential metabolic features may have practical benefit. For example, lactate is a convenient electron donor to add to the subsurface in order to promote *in situ* uranium bioremediation, but lactate is not a common electron donor in natural anaerobic sedimentary environments. *Geobacter sulfurreducens* does not naturally grow well on lactate, but with the appropriate selective pressure a strain that could rapidly grow on lactate was developed. In a similar manner, strains of *G. sulfurreducens* have been evolved that can metabolize sugars and glycerol, substrates that the wild-type strain does not utilize. This has important implications not only for bioremediation applications, but also for the conversion of various wastes to electricity in *Geobacter*-based microbial fuel cells. Effective subsurface bioremediation and electricity production with *Geobacter* also requires higher rates of extracellular electron transfer than is typical for *Geobacter* in pristine subsurface environments. In order to create a strain of *G. sulfurreducens* capable of more rapid extracellular electron transfer; cells were repeatedly transferred as rapidly as possible with Fe(III) oxide as the electron acceptor. A strain was developed which can transfer electrons to Fe(III) oxide 10 times faster than wild type. Strains are currently being adapted for other applications, such as serving as syntrophs in the conversion of wastes and hydrocarbons to methane. Whole-genome resequencing facilitates the identification of the mutations associated with these adaptations, which should aid in the further optimization of application-specific strains for carrying out complex cellular processes.