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Transport of Atrazine Through Large Constructed Soil Columns With and Without Switchgrass Roots

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Abstracts of Platform Presentations from the Phytoremediation Session at the 14th Annual West Coast Conference on Contaminated Soils, Sediments, and Water (March 15–18, San Diego, CA)

Session Chairs—Lee A. Newman and Jason C. White

Influence of Cultivation Conditions and Nutrient Amendments on the Phytoextraction of Weathered *p,p′*-DDE

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Presenting author—Jason C. White

Previous field studies indicate that zucchini (*Cucurbita pepo*) has a unique ability to phytoextract persistent organic pollutants from soil. It is unlikely that *C. pepo* evolved a unique mechanism favoring POP extraction and uptake, but all plants have evolved means to facilitate nutrient acquisition from soil. We have hypothesized that the exudation of organic acids as a means to acquire phosphorus could facilitate the uptake of persistent organic pollutants by increasing contaminant bioavailability to the plants. In one study, we assessed DDE uptake and organic acid exudation by zucchini (an uptaker of POPs) and cucumber (a non-uptaker of POPs) under various cultivation and nutrient conditions. Under dense planting (5 plants in a 5-kg pot of DDE-contaminated soil), zucchini accumulated significant and expected amounts of DDE but surprisingly, under these stressed conditions, cucumber phytoextracted greater amounts of DDE. The cucumber rhizosphere concentrations of organic acids were significantly higher than that of zucchini, suggesting that the increased organic acid exudation promoted DDE uptake by cucumber. Conversely, under non-stressed

conditions zucchini phytoextracted significantly greater quantities of pollutant than cucumber but no differences in organic acid content of the rhizosphere of the two species were observed. Separately, zucchini and other species were grown under field conditions and weekly amendments of different nutrients were made (nitrogen, phosphorus, nitrogen/phosphorus, aluminum sulfate to bind phosphorus in the soil). The uptake and translocation of the weathered pollutant and inorganic elements was found to vary with nutrient amendments. Lastly, data will be presented from rhizotron units constructed to facilitate not only the direct *in situ* isolation of exuded organic acids but also the isolation of xylem sap and rhizosphere soil pore water from individual plants. The role of cultivation conditions and nutrient availability in controlling root morphology, organic acid exudation, and contaminant uptake will be discussed.

Ecological Influences on PAH-Phytoremediation

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Presenting author—Clayton Rugh

Polyaromatic hydrocarbons (PAHs) are a class of persistent organic pollutants (POPs) with high cleanup priority to the US EPA. Additionally, PAH contamination represents a common hindrance to cost-effective brownfield rehabilitation efforts at numerous sites throughout the country. Our research team has partnered with Ford Motor Company to develop a phytoremediation treatment strategy for soils impacted by decades of coal processing during coke manufacture for smelting operations. We initially screened a variety of Michigan-native plant species in greenhouse pot studies to select a palette of plants for installation in a phytoremediation field trial. For evaluation of the selected species under field conditions, approximately 200 cubic yards of the coke-oven soil was collected and amended with 15% organic compost (yard litter: poultry manure::2:1), homogenized, and spread into a lined, treatment bed measuring 20 ft $\times$ 100 ft to a depth of 2 ft. The contaminated soil plot was planted with 18 different plant species each in 3 replicated sub-plots measuring $\sim$18 sq ft. Planted and unplanted soil samples were collected and analyzed 2 or 3 times per year over the course of the 3-year study. Nearly all of the planted treatments displayed superior rates of soil PAH biodegradation relative to unplanted control cells, with the most effective plant species reducing PAH content nearly 50% in the first year alone.

Other biological and environmental parameters were measured periodically or at the completion of the field trial (ending Oct. 03), including plant canopy density, phyto species competition, shoot biomass, root biomass, rooting depth, and volumetric soil moisture. The preliminary field study data was sufficiently compelling to proceed with a 1.6 acre pilot phytoremediation installation in Sept 03 in PAH-impacted soils adjacent to the coke oven facility. The relative importance of each of these factors for successful and sustained phytoremediative treatments will be discussed.

Detection and Quantification of Bacterial Dioxygenase Genes Responsible for Biodegradation of PCBs and PAHs in the Plant Rhizosphere

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Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are ubiquitous pollutants in soils and industrial brownfields. Many bacterial species can degrade these chemicals, but the genes encoding enzymes for their cometabolic degradation must be induced by a natural substrate. Previous work by our group has shown that plant monoterpenes and salicylic acid can stimulate the cometabolic degradation of PCBs, and new results suggest that other compounds such as coumarin may function for enhancing cometabolic degradation of PAHs. However, there is little information on the enzymes that are stimulated by these substances. One of the best studied genes for degradation of PAHs is the *nahAc* gene, which encodes a component of multimeric PAH dioxygenases. Because the *nahAc* gene is highly conserved, this gene may serve as a general marker for the PAH degradation potential in the rhizosphere of different plant species. Research reported here examines the relationship between the rate of degradation of PAHs and the level of the *nahAc* gene in soils using both conventional and real time PCR. Four sets of degenerate primers and probes for real-time PCR were designed based on the *nahAc* DNA sequences of 33 different bacterial species. The primer sets were tested with DNA extracted from several PAH contaminated and noncontaminated soils with different chemical and physical characteristics. The results suggest that the population size of PAH degrading organisms that contain the *nahAc* gene is normally low in both contaminated and noncontaminated soils, but is also highly dynamic. Thus, time dependence assays that quantify the time required for gene detection following exposure to naphthalene or other low molecular weight PAHs, along with real time PCR methods for quantification of the *nahAc* gene, may provide an indication of the responsiveness of the PAH degrading bacterial population in contaminated soils and their activity in soil.

Phyto-Polishing of Land-Treated Manufactured Gas Plant (MGP) Soil

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Presenting author—Zakia D. Parrish

Polycyclic aromatic hydrocarbons (PAHs) are potentially carcinogenic and found in high concentrations in manufactured gas plant (MGP) residues. However, PAHs in soils contaminated with these residues are often highly resistant to degradation, particularly after the soils have been remediated with conventional techniques such as composting or land treatment. Phytoremediation holds promise as a finishing procedure for traditional approaches when significant PAH concentrations remain.

A 12-month greenhouse study investigated the fate of the 16 EPA priority pollutant PAHs in MGP soil using phytoremediation as a secondary treatment. The contaminated soil used in this study had been land treated previously and then planted with tall fescue (*Festuca arundinacea*) and yellow sweet clover (*Melilotus officinalis*). To evaluate the impact of root decay on contaminant dissipation, plants were killed in selected vegetated pots to induce root turnover.

The tall fescue treatments had the highest root and shoot biomass and root surface area; however, this species did not induce the highest degradation rates. Significant differences were noted between treatments for seven PAHs, with unkilled yellow sweet clover resulting in 60–75% degradation of these compounds. PAH degrader populations in the vegetated treatments were more than 100 times greater than that of the unvegetated control. The phospholipid fatty acid, PLFA, structural group profile shifted over time, indicating a change in the community structure. Phytoremediation was an effective polishing tool in this experiment for PAH-impacted soil previously subjected to a biological treatment method.

Currently a greenhouse study is being conducted to investigate the phytoextraction of high molecular weight PAHs from a weathered soil. Two cultivar varieties of *Cucurbita pepo*; a zucchini (cv. Black Beauty) and a summer squash (cv. Zephyr), and a cucumber (*Cucumis sativus* cv. Marketmore) were grown in a MPG soil. Preliminary results indicate that the Black Beauty variety of phytoextracted significantly higher amounts of PAHs, particularly the larger, more recalcitrant five- and six-ring compounds.

Microbial Contribution to Polycyclic Aromatic Hydrocarbon Degradation in the Rhizosphere

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Due to the complexity of the root zone, it is yet to be ascertained how the success of phytoremediation is governed by influences of the host plant, root-associated bacteria, mycorrhizae, and eukaryotic microbes. Our research examines how microbial activity in the rhizosphere contributes to the removal of polycyclic aromatic hydrocarbons (PAHs) from soils and how this activity is controlled by the release of root exudates. Four types of plants (wheat *Triticum aestivum*, lettuce *Lactuca sativa,* zucchini *Cucurbita pepo*, and pumpkin *Cucurbita pepo*) were grown for 90 days in different concentrations of a PAH-contaminated soil obtained from a former manufactured gas plant site. After harvesting of the plants, anthracene- and chrysene-degrading strains were isolated from the root zone of all plants by selective growth on solid plates with the respective PAH as the sole carbon and energy source. 370 isolates, thus obtained, were grouped in 88 clusters based on whole genome BOX-PCR fingerprints, and representatives from each cluster were subjected to 16s rDNA sequencing. Based on preliminary analyses, isolates belong chiefly to the β- and γ-subclass of the Proteobacteria with the most common species identification as *Pseudomonas*, *Achromobacter*, *Burkholderia*, *Comamonas*, *Alcaligenes*, and *Bordetella* spp. These strains are presently being screened for degradation of a range of substrates including additional PAHs, presumed pathway intermediates, and aromatic root exudates. In addition, we are using a genomic probing approach to determine the type of ring-hydroxilating dioxygenases in all strains. Ultimately, we hope to elucidate the relationship between host plant, aromatic root exudates, and the presence and type of PAH-degrading strains in the rhizosphere.

Phytoextraction of Arsenic from CCA Contaminated Soils

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More than 70% of United States arsenic consumption, representing approximately 37 million pounds of arsenic per year, is used to produce chromated copper arsenate (CCA), a wood preservative. Weathered lumber in decks, docks, playground

equipment and garden construction can leach significant amounts of arsenic into soil and water, where it poses health risks to humans and animals. Although the use of CCA to treat wood for use in residential areas is being reduced, the existing stock of CCA-treated wood products will continue to leach arsenic for years to come. At present there is no cost-effective method to clean arsenic-contaminated soils. This research seeks to demonstrate the feasibility of using a recently discovered arsenic-hyperaccumulating fern to provide a cost-effective remediation alternative for CCA-contaminated soils in residential and industrial settings. Preliminary data demonstrate that when grown on arsenic-contaminated soils, this fern accumulates sufficient arsenic and biomass in its fronds to effectively reduce soil arsenic concentrations.

Growth chamber studies were conducted to evaluate arsenic removal by the fern from six different CCA-contaminated site soils, assessing the effects of soil pH and light intensity on the efficiency of arsenic phytoextraction. A small field demonstration was also conducted concurrently at a CCA-contaminated field site to demonstrate arsenic uptake and biomass production. The potential to further concentrate and refining recovered arsenic for storage and future recycling, as well as the ability of the fern to reduce chromium (VI) in the CCA soils to chromium (III) was also evaluated.

The anticipated result of this research is a demonstrated arsenic phytoextraction technique, accessible to homeowners as well as to environmental professionals, that uses commercially-available plants to provide cost-effective remediation of contaminated soils associated with the use of CCA-treated wood products. The results of these and other studies with CCA contaminated soils will be presented.

Transport of Atrazine Through Large Constructed Soil Columns With and Without Switchgrass Roots

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Fate and transport of atrazine in the presence of plant roots is not adequately described in literature. Our objectives were to evaluate the effects of switchgrass roots on the transport of atrazine under constant pore water velocity. Two agricultural soils, Emporia (fine loamy, siliceous, thermic Typic Hapludult) and Cullen (clayey, mixed, thermic Typic Hapludult) were used. The soils were taken from an area that has no history of pesticide application. Twelve columns (six for Emporia and six for Cullen) were used. Six columns, three of each soil type, were planted with warm season switchgrass (*Panicum virgatum* L.), and six other columns, three of each soil type, were left fallow. When the plants passed the tillering stage, the above-ground biomass was hand clipped from each column. A 505.6 mg Br/column tracer and 5.30 mg/column atrazine (3.0 kg a.i./ha) were mixed with 100 g soil and uniformly applied on the surface of each column and left for 24 h to permit adsorption of atrazine on to switchgrass roots and soil. The presence of switchgrass roots decreased infiltration of water and created surface ponding. Leaching patterns were dissimilar for columns with and without switchgrass roots. Early breakthrough and long tails in the effluent curve were observed for bromide and atrazine in the presence of switchgrass roots indicating the presence of nonequilibrium behavior. The deterministic two-site/two region nonequilibrium model provided an excellent fit to all bromide and atrazine breakthrough curves. Switchgrass likely favors the creation of macropores, and hence contribute to accelerate the transport of the front through the unsaturated zone, thus potentially increasing groundwater pollution. The breakthrough curves for both soils were similar, however, the early breakthrough and tailing of atrazine indicated the presence of nonequilibrium sorption.

^{137}Cs Partitioning to Wetland Sediments and Uptake by Plants

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More than 1,200 hectares of wetlands on the Savannah River Site, Aiken, SC, USA are contaminated with 21 TBq (564 Ci) of ^{137}Cs and pose a significant remediation challenge to the Department of Energy (DOE). The objective of this study was to determine the influence of the sediment flooded condition (redox status) on the tendency for plants to take up ^{137}Cs, as estimated by the concentration ratio (CR = activity per dried plant / activity per dried sediment). This data will eventually be used in human and ecological risk assessment calculations. Thirty paired plant and soil samples were collected from a wetland and an adjoining terrestrial system. Estimates of concentration ratios were often in excess of 1.0, indicating that the plants concentrated ^{137}Cs in their tissue to levels greater than existed in the sediment. CR values of plants growing on terrestrial sites were significantly greater than the CR values of the same species grown in aquatic locations. Additionally, sequential extraction of sediments indicated that between 50 and 85% of the ^{137}Cs was strongly bond and likely in a chemical form that was of limited bioavailability. Since predictions of plant ^{137}Cs concentrations based on sediment ^{137}Cs concentrations was tenuous and not captured well by any single parameter, such as the CR, it is recommended that risk assessment related to plants be based directly on plant ^{137}Cs concentrations. Relying solely on sediment ^{137}Cs concentration data, either total or some chemically defined fraction could result in large errors in risk estimations.

Effects of EDTA on the Phytoremediation of Soils Contaminated by Cd, Zn, and Pb

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The effects of phytoremediation of metal-contaminated soils can enhance by the addition of synthesized chelating agents. In this study, rainbow pink (*Dianthus chinensis*) and vetiver grass (*Vetiver zizanioides*) were growing in the soils contaminated by cadmium (20 mg/kg soil), zinc (500 mg/kg soil) and lead (1,000 mg/kg soil) to study the effects of applying Na-EDTA on the phytoremediation of metal-contaminated soils.

The results indicated that the concentrations of Cd, Zn, and Pb in the soil solution of rainbow pink increased significantly after Na-EDTA were added (5 and 10 mmol EDTA/kg soil) ($p < 0.05$). The Cd and Pb concentrations in the shoot of rainbow pink also significantly increased ($p < 0.05$), but the total uptake was only significantly increased for the Pb ($p < 0.001$). Even the concentrations of Cd, Zn, and Pb in the soil solution of vetiver grass significantly increased after Na-EDTA were added (5 and 10 mmol EDTA/kg soil) ($p < 0.05$), most of the concentrations in the shoot of vetiver grass were not significantly increased, except for Zn treated with 5 mmol EDTA/kg soil. Vetiver grass can grow well in the contaminated soils, and the growth rate was not affected by the toxicity of Cd, Zn, and Pb.

Results of this study also indicate that rainbow pink can uptake Cd and Zn in the contaminated soils, and the application of Na-EDTA can significantly increase it's uptake of Pb from contaminated soils. Vetiver grass can survive in the Cd-, Zn-, and Pb-contaminated soils. The concentrations of heavy metal in the shoot and total uptake of heavy metals by vetiver grass were not affected by the increasing toxicity of heavy metal after Na-EDTA were added. Both rainbow pink and vetiver grass are suitable for the phytoremediation of Cd-, Zn-, and Pb-contaminated soils.

Purification Effect of Areca Palm for Continuously Emitting Formaldehyde in a Real Office Environment

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Presenting author—Takashi Oyabu

Plant air-purification effect to formaldehyde in a real office environment was examined. A potted Areca palm (height: about 2 m) was adopted as a subjective plant. Plant purification effect was evaluated using a tin oxide gas sensor, and a commercial tool for formaldehyde measurement was also used. The effect of plant purification capability became higher as the atmospheric concentration was higher. The concentration was lower as the plants were installed. The effect did not greatly increase when the number of the installed pots was increased to seven from four. Concentration of carbon dioxide increased until 450 ppm by placing the pots at night. It is, however, below the regulation value (1000 ppm) of Japan building managing law. Light intensity fluctuated periodically and the carbon dioxide concentration changed periodically according to the fluctuation of light intensity from outside. There was a negative correlation between those characteristics. However, the characteristic of carbon dioxide concentration delayed 1 hour than the light intensity characteristic. The correlation coefficient was about −0.9. The exposure volume for formaldehyde became smaller as the number of potted plants was increased. In the experiment, formaldehyde was emitted continuously and the atmospheric concentration was kept at about 0.3 ppm. It was decreased to about 0.1 ppm by the potted plants. This is close to the regulated value of 0.08 ppm by WHO.