Final Report

DE-SC0006847 - **Fate of Uranium During Transport Across the Groundwater-Surface Water Interface**

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(i) Background

In the DOE complex, U-238 is present in 11% of the contamination sites (Hazen and Faybishenko 2009). This is the second most common radionuclide in a survey of 144 contaminated sites, after Cs-137 (16% of the sites). Within 30 years, about one Cs-137 half-life, it would be safe to assume that U-238 ($t_{1/2} = 4.5x10^9$ yr) will be the most common radionuclide in the DOE complex, assuming no new radionuclides are reintroduced into the environment. In an earlier survey of contaminants within the DOE complex, Riley and Zachara (1992) reported that U was the most common radionuclide contaminant in groundwater and sediments (occurring at 12 sites out of 52 total sites surveyed 23%). The differences between the Hazen and Faybishenko (2009) and Riley and Zachara (1992) percentages can be attributed to neither representing complete or statistical surveys; however, all the data in the newer data set (Hazen and Faybishenko 2009) includes that from the older data set.

The fate and transport of many radionuclides, trace metals, and metalloids in porous media is closely linked to the biogeochemical reactions that occur because of organic carbon being degraded by different microorganisms using a series of terminal electron acceptors. These reactions control the redox potential in the sediments. Throughout the redoxcline that develops in such environments, radionuclides and/or trace metals can be mobilized/immobilized via processes such as reduction/oxidation, sorption/desorption, precipitation/dissolution, and/or the formation of complex ions.

The presence of wetland plants has a significant impact on the biogeochemical dynamics of the sediment-redoxcline due to the release of root exudates and root turnover, deposition of plant litter onto the surface of the sediments, and nutrient uptake. Furthermore, plant evapotranspiration affects the hydrology and concentrates chemical species in the sediments, which in turn affects reaction rates and sorption dynamics. Wetland plants have evolved specialized adaptations to supply oxygen to the roots, and to transfer oxygen from the roots into the surrounding sediment. This oxygen transfer protects roots from phytotoxins, which are common in swamps at low redox potentials, and prevents transport into the root of highly soluble Fe(II) ions by precipitating them at the root surface following oxidation to Fe(III). Because of this cycling of Fe in wetlands, from reduced to oxidized and back to reduced, iron reduction accounts for a significant fraction of carbon turnover in wetlands. Consequently, Fe and organic carbon chemistries are closely coupled. Iron cycling is an important process affecting uranium reduction, but little is known about iron cycling and iron-reducing bacterial activity in wetland sediments and its effect on uranium fate and transport.

Bioreduction of Uranium.

The bioreduction and subsequent precipitation of uranium from groundwater through the addition of an electron donor to stimulate the uranium reducing microbial population has shown potential to prevent uranium migration from contaminated sites (Lovley and Coates, 1997; Lovley and Phillips 1992; Abdelouas et al., 1998). Biological (i.e., enzymatic) U(VI) reduction to U(IV) can occur simultaneously with Fe(III) reduction (Finneran et al., 2002; Anderson et al., 2003; Istok et al., 2004) and has been shown to decrease when the system changes from iron reducing to sulfate reducing conditions, but

still occur through different processes or microbes e.g. *Desulfovibrio spp* (Finneran et al., 2002; Anderson et al., 2003).

Current evidence suggests that two major classes of microorganisms are involved in uranium reduction in the subsurface; organisms that utilize SO_4^{2-} and organisms that use Fe(III) as terminal electron acceptors. The SO_4^{2-}-reducers (SRBs) include a wide range of morphological and physiological types. Sulfate-reducing bacteria can however, be roughly separated into two physiological groups: (1) those that can completely oxidize multicarbon substrates to CO_2 and, (2) those that can incompletely oxidize these substrates to acetate. However, it is generally thought that acetate and H_2 represent the major electron donors for SO_4^{2-} reduction in anoxic sediments (Winfrey and Zeikus 1979; Lovley et al., 1982). Sulfate reducing bacteria can reduce U(VI) in cell suspensions and as bioflims; for example, the direct electron acceptor for the hydrogenase in D. vulgaris (cytochrome c3) is capable of reducing U(VI) (Lovley et al., 1993). A SO_4^{2-} reducer (*Desulfotomaculum reducens*) has been shown to grow with U as electron acceptor (Tebo and Obratsova 1998).

Bacteria able to utilize Fe(III) as electron acceptors can also be roughly separated into two groups. *Geobacter metallireducens* is representative of the first group (Lonergan, Jenter et al. 1996), which consists of strict anaerobes that completely oxidize multicarbon electron donors to CO_2 concurrent with the reduction of Fe(III). The electron donors for these organisms include a variety of aromatics and the simple C2-C4 carboxylic acids that are generated as products from the fermentation of complex organic matter. Acetate is thought to be the most significant electron donor for these types of organisms. The second group of Fe(III) -reducing organisms is typified by the facultative anaerobe *Shewanella putrefaciens*. While this organism can readily oxidize H_2 and formate, it can only partially oxidize simple organic compounds such as pyruvate. Representative organisms from both groups utilize U(VI) as an electron acceptor (Gorby and Lovley 1992) and methods are described for the bioremediation of uranium using Fe(III)-reducing organisms (Lovley and Phillips 1992).

Although both SO_4^{2-} and Fe(III) reducing microorganisms have the capability to reduce U(VI) in the subsurface, the rate of uranium reduction in many subsurface environments may be limited by the lack of availability of suitable electron donors and/or overabundance of certain electron acceptors to drive the underlying microbial respiratory processes. For example, SO_4^{2-} reduction is known to be inhibited by the presence of more electrochemically-positive electron acceptors such as NO_3^- (Sørensen 1982), while the rate of Fe(III) reduction is known to be limited by the low aqueous solubility of Fe(III). Likewise, the addition of Fe(III) is known to inhibit SO_4^{2-} reduction and methanogenesis (Lovley 1991). This latter effect is thought to occur due to the ability of Fe(III)-reducers to establish steady-state electron-donor concentrations that are too low for these less energetically favorable forms of respiration (Lovley and Phillips 1987a) (see section 3.f below for a more detailed discussion). As another example, when Fe(III) availability is not limiting, Fe(III)-reducers may outcompete SO_4^{2-} reducers for electron donors that are common to both. It is therefore important to determine which group of organisms is reducing U in mixed microbial communities, and which electron donors are most effective in stimulating their activities.

Considerable laboratory-scale research has investigated the effects of microbial activity on radionuclides (including uranium). A variety of methods have been used to obtain this information including: analysis of geochemical data (Lovley and Goodwin 1988); batch, column, and microcosm reactor studies (Wilson, McNabb et al. 1983); direct observation and culture techniques; biochemical marker techniques (Balkwill et al. 1988); molecular methods (Bowman et al. 1993); and ecological modeling (Kelly et al. 1988). The relative advantages and disadvantages of many of these methods are discussed in (Ehrlich 1996). However, it is becoming increasingly apparent that in-situ testing and analysis methods will be required to fully understand and characterize subsurface microbial metabolic activity, especially in environments that display steep biogeochemical gradients (Madsen 1991; Madsen 1998).

The results of the field stimulation at the Rifle site, CO, by Anderson et al. (2003), where U(VI) was bioreduced via the injection of acetate into the subsurface showed that after 17 days of biostimulation "*Geobacteaceae*" accounted for 89% of the microbial community. As time progressed the fraction of "*Geobacteaceae*" decreased, accounting for less than 7% by day 80, at which time the microbial community was dominated by the family "*Desulfobacteraceae*". As the growth conditions became less favorable for the *Geobacter* species the overall U(VI) removal decreased. Based on these results the authors state that "implementation of a long-term *in-situ* bioremediation strategy should optimize conditions for continued growth and/or survival of *Geobacter* species".

Reoxidation of U(IV).

Bioreduced U(IV) has been shown to reoxidize rapidly to U(VI) in the presence of oxidants such as oxygen (Gu et al., 2005; Zhou and Gu, 2005; Moon et al., 2007; Komlos et al., 2008), and nitrate (Finneran et al., 2002; Senko et al., 2002; Moon et al., 2007; Moon et al., 2009). Mn-oxides have also been shown to reoxidize U(IV) (Fredrickson et al., 2002), and Fe(III) has been shown to oxidize U(IV) under limited geochemical conditions (Ginder-Vogel et al., 2006; Sani et al., 2005). Therefore, due to the variety of oxidants that can oxidize and remobilize bioreduced U(IV), the long-term stability of bioreduced uranium is a very important issue to be considered in assessing the long-term fate of uranium in the environment. In the presence of nitrate in the influent to columns in which U(IV) had been precipitated, U(IV) was reoxidized much faster than in the presence of dissolved oxygen at the same electron acceptor equivalent (Moon et al., 2007, 2009). Since wetland sediments are usually anoxic and nitrogen limited, it is likely that U(IV) will remain stable in such sediments. Furthermore, oxygen is present only in the direct vicinity of root surfaces, where it reacts rapidly with a host of reduced species such as Fe(II).

Processes affecting radionuclides/trace metals in wetland sediments.

In aquatic sediments an oxidized surface layer of sediment is present (Gambrell and Patrick 1978, Ponnamperuma 1984; Mitsch and Gosselink 1993), which is followed by a steep redox gradient. Concentration gradients of redox species of over an order of magnitude spanning a distance of a centimeter have been observed in aquatic sediments (Brendel and Luther 1995). However, wetland sediments differ from aquatic sediments by the presence of plants, which alter sediment biogeochemistry in a variety of ways. (1) Many wetland plants have mechanisms to supply oxygen to the roots and to transfer

oxygen from the roots into the surrounding sediment (Armstrong 1979; Sand-Jensen et al., 1992; Bedford et al., 1991; Mendelssohn 1993). (2) Evapotranspiration can lower the water table inducing air or oxygenated water to enter the sediments (Hemond and Fifield 1982; Dacey and Howes 1984; Nuttle and Hemond 1988; Hussey and Odum 1992; Howes and Teal 1994). Under saturated conditions, evaporation increases the downward flow and therefore transport of chemical species from the surface to the rhizosphere and concentrates them in the pore water, which strongly affects the overall reaction kinetics (Jaffe et al., 2001; Xu and Jaffe 2006; Zazo et al., 2008). (3) Root exudation of sugars, amino acids and other readily metabolizable compounds, root production of mucilages, root sloughing of cells, and root death (Whipps 1990; Lynch and Whipps 1990; Vancura 1988) provide a major source of carbon to the microbial community responsible for redox reactions. (4) For inter-tidal sediments, aerated tidal waters mix with sediment pore waters, introducing oxygen to anoxic sediments and causing oxidation of reduced species such as Fe(II) to Fe(III) (Caetano et al., 1997).

High organic matter content in the sediment and highly variable redox and pH conditions all affect the speciation of radionuclides/trace metals and the forms in which they move through the sediment pore-water. Radionuclides/trace metals in wetland sediments may be immobilized due to sorption to different organic and inorganic soil constituents or due to precipitation. Adsorption onto iron and manganese oxides are important processes where the soil's oxidation potential is high, and adsorption to such oxides can be very important in the rhizosphere (Otte et al., 1995) due to processes such as the formation of iron oxyhydroxide plaques on roots.

The plant-induced rates of oxygen transfer into the sediments can be substantial and highly variable (Armstrong 1979; Bedford et al., 1991; Brix 1993; Sorrel 1994). These rates are dependent on the type of vegetation and root size, as well as the chemical composition and oxygen demand of the sediments (Brix et al., 1996). Oxygen release rates measured for *E. sphacelata* ranged from non detection for roots placed in a deoxygenated solution, to 55 □mol h^{-1} g^{-1} dry weight when placed in a solution with a redox potential of -200 mV (Sorrell et al., 1993). Both, rooted aquatic macrophytes and wetland trees have been found to increase sediment Eh by > 200 mv, and to alter Fe^{2+} and P concentrations (Jaynes and Carpenter 1986; Sand-Jensen et al. 1982; Carpenter et al., 1983; Grosse 1997). Choi et al. (2006a), showed a significant reoxidation of sulfides to sulfate in vegetated riparian sediments in the early growing season, while no such oxidation was noted in adjacent non-vegetated plots nor in vegetated plots during the late summer or dormant seasons. These sediments contained significant amounts of trace metals immobilized with sulfide, and simulations (Choi et al., 2006b) showed that due to the excess of FeS phases, these metals remained stable in the sediments during this period of increased plant mediated oxygen transfer to the sediments.

As oxygen diffuses from the plant root into the sediments, it reacts rapidly with many reduced species including iron, forming iron plaques, oxy(hydroxi)des that precipitate on the surface of the roots. The degree of iron plaque accumulation on roots is highly variable, and depends on the concentration of ferrous iron in solution as well as its complexation with organic ligands and the availability of other exchange sites on different soil mineral and organic fractions (Mendelssohn 1993). Given the importance of biological iron reduction for the simultaneous biological reduction of U(VI), the

constant formation of such iron plaques may be key in the immobilization of uranium in wetland sediments. Little is known about the spatial distribution of iron reducer activity in wetland sediments, and if that activity is concentrated near these iron plaques as well as the precipitation of U(IV).

From these discussions it is clear that due to the complexity and diversity of the many chemical, biological, and physical processes occurring simultaneously in wetland sediments, there is a lack of fundamental knowledge that would allow for an assessment of the fate of radionuclides/trace metals, especially uranium, in such sediments. We propose to conduct carefully designed laboratory experiments to gain new insights into these processes and test the hypotheses outlined below.

(ii) Project Objectives and Hypotheses

The objective of this research were to gain fundamental new scientific understandings of the coupled physical, chemical and biological processes affecting uranium in wetland sediments. This is required for decision making for environmental remediation and long-term stewardship at many DOE sites where uranium contaminated groundwater discharges to surface waters via a groundwater-surface water interface where the sediment biogeochemistry may be affected by the presence of plants.

Specifically our goal was to gain new insights on how plant-induced alterations to the sediment biogeochemical processes affect the key uranium reducing microorganisms, the uranium reduction, its spatial distribution, the speciation of the immobilized uranium, and the long-term stability of biogenic U(IV). For this purpose we did formulated the following hypotheses:

(I) U(VI) discharged from groundwater to surface waters can be immobilized effectively as U(IV) in the sediments at the groundwater-surface water interface, if an appropriate electron donor is available in these sediments. The electron donor required to stimulate the microorganisms capable of reducing U(VI) is provided by wetland plants via their root exudates and root turnover.

(II) Oxygen released into the sediments by plants reoxidizes Fe(II), forming iron oxy(hydroxi)des, which provide the bioavailable Fe(III) for long-term iron-reducing bacteria activity, which is key for a sustained biological uranium reduction.

(III) Since wetland sediments are dynamic systems that are commonly anaerobic and wetlands are usually nitrogen limiting, uranium immobilized as U(IV) (which is readily oxidized in the presence of nitrates) will remain stable in sediments.

To test these hypotheses we did conduct a series of continuous-flow, greenhouse mesocosm studies in which we will simulate the discharge of uranium contaminated groundwater into surface water through vegetated and non-vegetated sediments. These mesocosm studies were augmented with field measurements to characterize iron forma on root surfaces, to track U in wetland pore-water throughout a full year, and to characterize the organic matter in wetland rhizosphere and its link to the microbial community.

(iii) Findings

For this report, we will summarize the specific objectives and findings of each experiment and provide the link of a peer-reviewed journal paper that describes the methodology and outcome in detail.

1. Our first goal was to demonstrate that natural organic matter and plant roots in the wetland rhizosphere can immobilize U(VI). We specifically focused on in SRS acidic sediments, which has significant implication for the long-term stewardship of U-contaminated wetlands.

Biogeochemistry of uranium in wetlands plays important roles in U immobilization in storage ponds of U mining and processing facilities but has not been well understood. The objective of this work was to study molecular mechanisms responsible for high U retention by Savannah River Site (SRS) wetland sediments under varying redox and acidic (pH = 2.6–5.8) conditions using U L_3-edge X-ray absorption spectroscopy. Uranium in the SRS wetland sediments existed primarily as U(VI) bonded as a bidentate to carboxylic sites (U–C bond distance at ~2.88 Å), rather than phenolic or other sites of natural organic matter (NOM). In microcosms simulating the SRS wetland processes, U immobilization on roots was 2 orders of magnitude higher than on the adjacent brown or more distant white sands in which U was U(VI). Uranium on the roots were both U(IV) and U(VI), which were bonded as a bidentate to carbon, but the U(VI) may also form a U phosphate mineral. After 140 days of air exposure, all U(IV) was reoxidized to U(VI) but remained as a bidentate bonding to carbon. This study demonstrated NOM and plant roots can highly immobilize U(VI) in the SRS acidic sediments, which has significant implication for the long-term stewardship of U-contaminated wetlands. Details of the experiments can be found in: Li, D., D. Kaplan, H. Chang, J. Seaman, P. Jaffe, P. Koster van Groos, K. Scheckel, C. Segre, N. Chen, D.T. Jiang, M. Newville, A. Lanzirotti, "Spectroscopic Evidence of Uranium Immobilization in Acidic Wetlands by Natural Organic Matter and Plant Roots," *Environmental Science and Technology*, 2015, *49* (5), pp. 2823–2832, doi: 10.1021/es505369g, and Li, D., H.S. Chang, J.C. Seaman, P.R. Jaffe, P. Koster van Groos, D.T. Jiang, N. Chen, K.G. Scheckel and D.I. Kaplan, "Retention and Chemical Speciation of Uranium in a Wetland on the Savannah River Site," Journal of Environmental Radioactivity, 131 (2014) pp. 40-46, doi: 10.1016/j.jenvrad.2013.10.017.

2. Next, our goal was to demonstrate that we can operate long-term wetland mesocosms to immobilize U.

Small-scale continuous flow wetland mesocosms (~0.8 L) were used to evaluate how plant roots under different iron loadings affect uranium (U) mobility. When significant concentrations of ferrous iron (Fe) were present at circumneutral pH values, U concentrations in root exposed sediments were an order of magnitude greater than concentrations in root excluded sediments. Micro X-ray absorption near-edge structure (μ-XANES) spectroscopy indicated that U was associated with the plant roots primarily

as U(VI) or U(V), with limited evidence of U(IV). Micro X-ray fluorescence (μ-XRF) of plant roots suggested that for high iron loading at circumneutral pH, U was co-located with Fe, perhaps co-precipitated with root Fe plaques, while for low iron loading at a pH of ~4 the correlation between U and Fe was not significant, consistent with previous observations of U associated with organic matter. Quantitative PCR analyses indicated that the root exposed sediments also contained elevated numbers of *Geobacter* spp., which are likely associated with enhanced iron cycling, but may also reduce mobile U(VI) to less mobile U(IV) species. Details of the experiments can be found in: Koster van Groos, P.G., D.I. Kaplan, H. Chang, J.C. Seaman, D. Li, A.D. Peacock, K.G. Scheckel, and P.R. Jaffé, "Uranium fate in wetland mesocosms: Effects of plants at two iron loadings with different pH values," *Chemosphere*, 2016, Vol. 163, pp. 116-124, doi: 10.1016/j.chemosphere.2016.08.012.

3. The above two experiments have demonstrated that U can be immobilized in the wetland rhizosphere. A major question we wanted to determine was if water table fluctuations that can occur during dry or drought periods, and expose the immobilized U to oxic conditions, would result in its mobilization.

To understand better the fate and stability of immobilized uranium (U) in wetland sediments, and how intermittent dry periods affect U stability, we dosed saturated sandy wetland mesocosms planted with *Scirpus acutus* with low levels of uranyl acetate for 4 months before imposing a short drying and rewetting period. Concentrations of U in mesocosm effluent increased after drying and rewetting, but the cumulative amount of U released following the dry period constituted less than 1% of the total U immobilized in the soil during the 4 months prior. This low level of remobilization suggests, and XANES analyses confirm, that microbial reduction was not the primary means of U immobilization, as the U immobilized in mesocosms was primarily U(VI) rather than U(IV). Drying followed by rewetting caused a redistribution of U downward in the soil profile and to root surfaces. Although the U on roots before drying was primarily associated with minerals, the U that relocated to the roots during drying and rewetting was bound diffusely. Results show that short periods of drought conditions in a sandy wetland, which expose reduced sediments to air, may impact U distribution without causing large releases of soil-bound U to surface waters. Details of the experiments can be found in: Gilson, E., S. Huang, P. Koster van Groos, K. Scheckel, O. Qafoku, A. Peacock, D. Kaplan, P. Jaffe, "Uranium Redistribution due to Water Table Fluctuations in Sandy Wetland Mesocosms," *Environmental Science and Technology*, 2015, 49 (20), pp. 12214–12222, doi: 10.1021/acs.est.5b02957.

4. In parallel to the laboratory mesocosm work, we deployed dialysis samplers at SRNL to test if U behavior in natural systems tracks that what was observed in laboratory mesocosms.

Previous studies have shown that Tims Branch wetlands on the Savannah River Site in South Carolina, USA is an effective environmental sink for sequestering the 44 tons of uranium (U) released into the system. The objective of this study was to evaluate over the course of a year, the fluctuations in sediment porewater U concentrations as a function of

sediment depth, and the conditions and the extent that the contaminated wetlands acted as an environmental source for U. Sediment desorption tests indicated that U was strongly bound (K_d values were 2100–6900 L/kg), and sequential extraction experiments indicated that a majority of the U was associated with the readily oxidizable fraction (presumably, organic matter fraction). *In situ* porewater samples were collected using diffusion samplers that were placed in the contaminated wetlands and their uranium concentrations indicated that as much as 3×10^{-5} wt-% of the system U was in the mobile aqueous phase (federal maximum contaminant levels (MCL) = 0.03 μg/L U). Aqueous U concentrations were correlated to *Eh* ($r = 0.422$; $n = 113$; $p \leq 0.001$). These data also suggested that there may be a critical *Eh* at ~400 mV, above which aqueous U concentrations increased significantly ($p \leq 0.01$) by more than an order of magnitude. These results have implications on the long-term stewardship of this contaminated system; sediment organic matter concentrations and wetland hydrology and plant vegetation need to be maintained in a manner that does not permit strong reoxidation of the system. This could be achieved by minimizing land-use changes or the occurrences of forest fires and ensuring that the system's hydrology is not greatly altered. Details of the experiments can be found in: Kaplan, D.I., S.W. Buettner, D. Li, S. Huang, P.G. Koster van Groos, P.R. Jaffé, and J.C. Seaman, "*In situ* porewater uranium concentrations in a contaminated wetland: Effect of season and sediment depth," *Applied Geochemistry*, http://dx.doi.org/10.1016/j.apgeochem.2016.11.017.

5. Since much of the U immobilized in the wetland rhizosphere is associated with iron phases, we did a rigorous geochemical analysis of these phases to further investigate the nature of the iron phases in the rhizosphere that affect U immobilization.

Wetlands mitigate the migration of groundwater contaminants through a series of biogeochemical gradients that enhance multiple contaminant-binding processes. The hypothesis of this study was that wetland plant roots contribute organic carbon and release O_2 within the rhizosphere (plant-impact soil zone) that promote the formation of Fe(III)-(oxyhydr)oxides. In turn, these Fe(III)-(oxyhydr)oxides stabilize organic matter that together contribute to contaminant immobilization. Mineralogy and U binding environments of the rhizosphere were evaluated in samples collected from contaminated and non-contaminated areas of a wetland on the Savannah River Site in South Carolina. Based on Mössbauer spectroscopy, rhizosphere soil was greatly enriched with nanogoethite, ferrihydrite-like nanoparticulates, and hematite, with negligible Fe(II) present. X-ray computed tomography and various microscopy techniques showed that root plaques were tens-of-microns thick and consisted of highly oriented Fe-nanoparticles, suggesting that the roots were involved in creating the biogeochemical conditions conducive to the nanoparticle formation. XAS showed that a majority of the U in the bulk wetland soil was in the + 6 oxidation state and was not well correlated spatially to Fe concentrations. SEM/EDS confirm that U was enriched on root plaques, where it was always found in association with P. Together these findings support our hypothesis and suggest that plants can alter mineralogical conditions that may be conducive to contaminant immobilization in wetlands. Details of the experiments can be found in: Kaplan, D.I., R. Kukkadapu, J. Seaman, B. Arey, A. Dohnalkova, S. Buttner, D. Li, T. Varga, K.G. Scheckel, P.R. Jaffe, "Iron Mineralogy and Uranium-Binding

Environment in the Rhizosphere of a Wetland Soil," *Science of the Total Environment*, 2016, Vol. 569–570, pp. 53–64, doi: 10.1016/j.scitotenv.2016.06.120.

6. Organic matter in the wetland rhizosphere is unique and drives the microbial ecology that in turn affects the fate and transport of uranium and other trace metals of concern. Hence we conducted a rigorous analysis and characterization of wetland organic matter at SRNL and the corresponding microbial characterization.

Wetlands attenuate the migration of many contaminants through a wide range of biogeochemical reactions. Recent research has shown that the rhizosphere, the zone near plant roots, in wetlands is especially effective at promoting contaminant attenuation. The objective of this study was to compare the soil organic matter (OM) composition and microbial communities of a rhizosphere soil (primarily an oxidized environment) to that of the bulk wetland soil (primarily a reduced environment). The rhizosphere had elevated C, N, Mn, and Fe concentrations and total bacteria, including *Anaeromyxobacter*, counts (as identified by qPCR). Furthermore, the rhizosphere contained several organic molecules that were not identified in the nonrhizosphere soil (54% of the >2200 ESI-FTICR-MS identified compounds). The rhizosphere OM molecules generally had (1) greater overall molecular weights, (2) less aromaticity, (3) more carboxylate and N-containing COO functional groups, and (4) a greater hydrophilic character. These latter two OM properties typically promote metal binding. This study showed for the first time that not only the amount but also the molecular characteristics of OM in the rhizosphere may in part be responsible for the enhanced immobilization of contaminants in wetlands. These finding have implications on the stewardship and long-term management of contaminated wetlands. Details of the experiments can be found in: Kaplan, D.I., C. Xu, S. Huang, Y. Lin, N. Tolić, K.M. Roscioli-Johnson, P.H. Santschi, and P.R. Jaffé, "Unique Organic Matter and Microbial Properties in the Rhizosphere of a Wetland Soil," *Environmental Science and Technology*, 2016, 50, 4169−4177, doi: 10.1021/acs.est.5b05165.

7. The Feammox process, which refers to ammonium oxidation under iron reducing conditions, has been reported in many wetlands, including wetlands at SRNL. Hence, we conducted an experiment to determine if Feammox bacteria can reduce U(VI).

This study investigated the possibility of links between the biological immobilization of uranium (U) and ammonium oxidation under iron (Fe) reducing conditions. The recently-identified *Acidimicrobiaceae* bacterium A6 (ATCC, PTA-122488) derives energy from ammonium oxidation coupled with Fe reduction. This bacterium has been found in various soil and wetland environments, including U-contaminated wetland sediments. Incubations of *Acidimicrobiaceae* bacteria A6 with nontronite, an Fe(III)-rich clay, and approximately 10 µM U indicate that these bacteria can use U(VI) in addition to Fe(III) as an electron acceptor in the presence of ammonium. Measurements of Fe(II) production and ammonium oxidation support this interpretation. Concentrations of approximately 100 µM U were found to entirely inhibit *Acidimicrobiaceae* bacteria A6 activity. These results suggest that natural sites of active ammonium oxidation under Fe reducing conditions

by *Acidimicrobiaceae* bacteria A6 could be hotspots of U immobilization by bioreduction. This is the first report of biological U reduction that is not coupled to carbon oxidation. Details of the experiments can be found in: Gilson, E.R., S. Huang, and P.R. Jaffé, "Biological Reduction of Uranium by *Acidimicrobiaceae* bacterium A6," *Biodegradation*, 2015, Volume 26, Issue 6, pp. 475-482, doi: 10.1007/s10532-015-9749-y.

(iv) Students and Postdoctoral Fellows Supported by this Project

Postdoctoral Fellows: Paul Koster (Princeton University), Shan Huang (Princeton University), HyunShik Chang (SRNL).

Graduate Students: Emily Gilson (Princeton University), S.W. Buettner (University of Georgia).

(v) Publications from this Project

v. 1. **Peer reviewed Journal Presentations (already discussed above):**

Kaplan, D.I., S.W. Buettner, D. Li, S. Huang, P.G. Koster van Groos, P.R. Jaffé, and J.C. Seaman, "*In situ* porewater uranium concentrations in a contaminated wetland: Effect of season and sediment depth," *Applied Geochemistry*, http://dx.doi.org/10.1016/j.apgeochem.2016.11.017.

Koster van Groos, P.G., D.I. Kaplan, H. Chang, J.C. Seaman, D. Li, A.D. Peacock, K.G. Scheckel, and P.R. Jaffé, "Uranium fate in wetland mesocosms: Effects of plants at two iron loadings with different pH values," *Chemosphere*, 2016, Vol. 163, pp. 116-124, doi: 10.1016/j.chemosphere.2016.08.012.

Kaplan, D.I., R. Kukkadapu, J. Seaman, B. Arey, A. Dohnalkova, S. Buttner, D. Li, T. Varga, K.G. Scheckel, P.R. Jaffe, "Iron Mineralogy and Uranium-Binding Environment in the Rhizosphere of a Wetland Soil," *Science of the Total Environment*, 2016, Vol. 569–570, pp. 53–64, doi: 10.1016/j.scitotenv.2016.06.120.

Kaplan, D.I., C. Xu, S. Huang, Y. Lin, N. Tolić, K.M. Roscioli-Johnson, P.H. Santschi, and P.R. Jaffé, "Unique Organic Matter and Microbial Properties in the Rhizosphere of a Wetland Soil," *Environmental Science and Technology*, 2016, 50, 4169−4177, doi: 10.1021/acs.est.5b05165.

Gilson, E.R., S. Huang, and P.R. Jaffé, "Biological Reduction of Uranium by *Acidimicrobiaceae* bacterium A6," *Biodegradation*, 2015, Volume 26, Issue 6, pp. 475-482, doi: 10.1007/s10532-015-9749-y.

Gilson, E., S. Huang, P. Koster van Groos, K. Scheckel, O. Qafoku, A. Peacock, D. Kaplan, P. Jaffe, "Uranium Redistribution due to Water Table Fluctuations in Sandy Wetland Mesocosms," *Environmental Science and Technology*, 2015, 49 (20), pp.

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Kaplan, D. I., J.C. Seaman, C. Xu, S. Huang, D. Li, R. Kukkadapu, P. Santschi, P., and P. Jaffe, "Organic and Mineralogical Properties of the Rhizosphere that Promote Uranium Binding in Wetland Soils," ASA, CSSA & SSSA International Annual Meeting, 2016, American Society of Agronomy, Phoenix, AZ.

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Gilson E., S. Huang, P.G Koster van Groos, K. Scheckel, A.D. Peacock, D.I. Kaplan and P.R. Jaffé, "Fate of Uranium in Wetlands: Impact of Drought followed by Re-flooding," 2015 Environmental System Science (ESS) Principal Investigators (PI) Meeting.

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