**Recent advances in the detection of specific natural organic compounds as carriers
for radionuclides in soil and water environments, with examples of radioiodine and
plutonium**

Santschi, P.H.,^{a*} Xu, C., Zhang, S.,^a Schwehr, K.A.,^a Lin, P.,^a Yeager, C.M.,^b and
Kaplan, D.I.^c

Recent advances in the detection of specific natural organic compounds as carriers for radionuclides in soil and water environments, with examples of radioiodine and plutonium

Abstract

Among the key environmental factors influencing the fate and transport of radionuclides in the environment is natural organic matter (NOM). While this has been known for decades, there still remains great uncertainty in predicting NOM-radionuclide interactions because of lack of understanding of radionuclide interactions with the specific organic moieties within NOM. Furthermore, radionuclide-NOM studies conducted using modelled organic compounds or elevated radionuclide concentrations provide compromised information related to true environmental conditions. Thus, sensitive techniques are required not only for the detection of radionuclides, and their different species, at ambient and/or far-field concentrations, but also for potential trace organic compounds that are chemically binding these radionuclides. GC-MS and AMS techniques developed in our lab are reviewed here that aim to assess how two radionuclides, iodine and plutonium, form strong bonds with NOM by entirely different mechanisms; iodine tends to bind to aromatic functionalities, whereas plutonium binds to N-containing hydroxamate siderophores at ambient concentrations.

While low-level measurements are a prerequisite for assessing iodine and plutonium migration at nuclear waste sites and as environmental tracers, it is necessary to determine their in-situ speciation, which ultimately controls their mobility and transport in natural environments. More importantly, advanced molecular-level instrumentation (e.g., nuclear magnetic resonance (NMR) and Fourier-transform ion cyclotron resonance coupled with electrospray ionization (ESI-FTICRMS) were applied to resolve either directly or indirectly the molecular environments in which the radionuclides are associated with the NOM.

Keywords:

Iodine; Plutonium; Speciation; Natural organic matter moieties; Aromatic acids;
Hydroxamates

1. Introduction

Radionuclides that reach the environments from natural or anthropogenic sources equilibrate with different phases over time through redox, complexation, sorption, precipitation and microbial reactions with inorganic and organic phases, e.g., specific natural organic matter (NOM) compounds with clustered chelating sites. While there is evidence for natural organic matter bound radionuclides from sequential extraction experiments (e.g., Livens and Baxter, 1988; Qiao et al., 2012), such evidence is often fraught with ambiguity since the results are not very specific and can depend on the nature and order of sequential leaching agents, and prevalence for re-adsorption artefacts to different fractions (Martin et al., 1987; Nirel and Morel, 1990; Hou et al., 2007; Okoro et al., 2012). Thus, it is highly desirable to combine radioanalytical techniques with molecular level organic matter identification techniques in order to pinpoint the organic carrier molecules for specific radionuclides in the environment.

The objective of this review is to assess the recent advances in measuring natural organic matter associated radionuclides, ^{129}I and Pu, as well as molecular characteristics of these carriers. Two radionuclides that bond strongly to natural organic matter, plutonium and iodine, are used as examples to demonstrate that radionuclide binding mechanisms to organic matter can differ greatly. Furthermore, the intent of this review is to also demonstrate that it is not adequate or in some cases even important to consider total organic carbon or organic matter when attempting to understand radionuclide-NOM interactions. Instead, advances need to be made to identify the specific reactive moieties

within organic matter that are involved in the bonding. Such information is important because it helps prediction of bonding scenarios when system chemistry changes spatially or temporally. Sensitive techniques are required not only for the detection of radionuclides and their different species at low ambient concentrations prevalent in the far-field, but also for the detection of potential trace organic compounds that chemically bind to these radionuclides. Examples are discussed of studies conducted at radionuclide contaminated field sites. Particular attention is directed at measurements of organically bound radionuclides where specific organic compounds affect iodine (e.g., ^{129}I) and plutonium (e.g., $^{239,240}\text{Pu}$) speciation and environmental transport. This review does not include an exhaustive description of our understanding of radionuclide interactions with NOM, instead it focuses on recent findings related to Pu and I. Previous reviews of Pu-NOM interaction have been provided by Neu et al. (2002) and I-NOM interaction have been provided by Kaplan et al. (2014a).

2. Iodine

2.1 Analytical methods to determine ^{129}I and ^{127}I concentration and speciation

Iodine (I) exists in multiple oxidation states in the form of organic (org-I) and inorganic species (iodide, I^- , and iodate, IO_3^-) in aquatic systems, leading to complex biogeochemical cycling of stable iodine, ^{127}I , and its long-lived isotope, ^{129}I , a major by-product of nuclear fission (reviewed by Kaplan et al., 2014a). In order to assess the distribution of ^{129}I and stable ^{127}I in environmental systems on a regular basis, a sensitive and rapid method was developed, enabling us to simultaneously determine isotopic ratios ($^{129}\text{I}/^{127}\text{I}$) and I speciation via inexpensive GC-MS (Fig. 1, also see Zhang et al., 2010). Basically, iodide concentrations are quantified after being derivatized to 4-iodo-N,N-dimethylaniline. Total inorganic iodine (the sum of iodide and iodate for most environmental samples) are determined by a two-step chemical process: first iodate is reduced to iodide and followed by oxidation and derivatization steps of iodide. Thus iodate is calculated as the difference of total inorganic iodine and iodide. Total iodine, including the sum of iodide, iodate and organo-iodine, is determined by converting organo-iodine into inorganic-iodine via combustion with V_2O_5 under O_2 stream and following the way of measuring total inorganic iodine. Organo-iodine is then calculated

by the difference between total iodine and total inorganic iodine. Differentiating ^{129}I from ^{127}I in each species ($^{129}\text{I}^-$, $^{129}\text{IO}_3^-$ and $^{129}\text{organo-iodine}$) is obtained by applying mass-to-charge ratio of 247 and 249 filters, and integrating the appropriate peaks in the chromatogram, for the derivative products of ^{127}I and ^{129}I iodinated N,N-dimethylaniline, respectively. This method has a detection limit of 0.04 $\mu\text{g/L}$, 0.14 $\mu\text{g/L}$ and 0.35 $\mu\text{g/L}$ for $^{127}\text{I}^-$, $^{127}\text{IO}_3^-$ and $\text{organo-}^{127}\text{I}$, respectively. The detection limit of total ^{129}I is 73 mBq/L, which is far below the ^{129}I far-field concentration in many DOE sites (e.g., Hanford Site and Savannah River Site (SRS)).

In addition to the GC-MS method, a pH-dependent solvent extraction along with combustion paired with accelerator mass spectrometry (AMS) was developed to measure ambient levels of $^{129}\text{I}/^{127}\text{I}$ isotope ratios and the speciation of ^{129}I (I^- , IO_3^- , and organo-I) (Schwehr et al., 2014). AMS enables ^{129}I speciation measurement at much lower concentrations ($<10^{-12}\text{M}$) than GC-MS (10^{-9} - 10^{-10}M). ^{129}I concentration and speciation in the contaminated groundwater collected from SRS were determined via both approaches (GC-MS vs. AMS) with consistent results.

2.2 Iodine speciation in the natural environment

Results using a novel GC-MS method demonstrated that the mobility of ^{129}I depends greatly on speciation and its concentration, pH, and sediment redox state, with equilibration times of up to 12 weeks (e.g., Xu et al., 2011a, 2015; Zhang et al., 2011, 2014; Emerson et al., 2013; Schwehr et al., 2014). For instance, at ambient concentrations ($\sim 10^{-7}\text{M}$), I^- and IO_3^- are significantly retarded by sorption onto mineral surfaces and covalent binding to NOM, while at concentrations traditionally examined in laboratory sorption studies (i.e., 10^{-4}M or higher), I^- appears to have negligible sorption to soils and to travel along with the groundwater (Zhang et al., 2011) (Fig. 2). Iodate removal from the mobile aqueous phase can also occur through incorporation into CaCO_3 crystal lattice, which was demonstrated under field conditions at the Hanford Site, in eastern Washington, USA (Xu et al., 2015; Zhang et al., 2013a, 2013b). Iodide and iodate interactions with NOM can lead to covalent binding of I to active sites of aromatic carbon moieties on the particle surface (Xu et al., 2011b, 2012, 2013). Iodine association with NOM is important in sediments, even when organic carbon concentrations are very low (e.g., $<0.2\%$ at Hanford Site) (Xu et al., 2015; Zhang et al., 2013a, 2013b).

Removal of iodine from groundwater through interaction with NOM is complicated by the release of mobile organo-I species (Xu et al., 2011a, 2011b, 2012, 2013). In a field study evaluating iodine interactions with wetland soils at the SRS, South Carolina, USA it was shown that a small fraction of NOM promoted the mobility of iodine by forming mobile organo-I species (Xu et al., 2011a, 2011b, 2012, 2013), a process that we numerically simulated using Michaelis-Menton-type coupled kinetic uptake and redox-reactions (Chang et al., 2014). Field and laboratory studies (Kaplan et al., 2011, 2014; Otosaka et al., 2011; Schwehr et al., 2014; Zhang et al., 2013a, 2013b; Xu et al., 2015) evaluated the cause for steady increases in groundwater ^{129}I concentrations (up to 37 Bq L^{-1} , three orders of magnitude greater than ^{129}I limits of 0.037 Bq L^{-1} in drinking water) emanating from radiological disposal basins at the SRS. These studies indicated that an increase of 0.7 pH units in groundwater over 17 years may explain the observed increases in groundwater ^{129}I concentrations.

Iodine speciation, as opposed to only total I, of the stemflow and throughfall rainwater was for the first time measured in two types of forests of Japan (deciduous and coniferous forests). Not only did total I get concentrated in the rainwater along its pathway from the open air, through the canopy, and along the tree stem, but also it slowly transformed from being dominated by inorganic species, to being dominated by organic species. Such I speciation data provide important information for understanding ^{129}I mobility and transport in the Fukushima Prefecture (Xu et al., 2016). Such data are necessary for conducting dose reconstruction estimates of ^{131}I , a short lived radioisotope ($t_{1/2} = 8$ days) that could not be readily and extensively measured in real time after the Fukushima Daiichi Nuclear Power Plant accident.

Species transformations were also noted in the ^{129}I plume in F-Area, SRS (Otosaka et al. 2011). The speciation of ^{129}I was primarily iodide near the source term, an unlined basin that received radioactive waste from a uranium reprocessing facility. As the ^{129}I moved along its 0.7 km path towards a wetland, the ^{129}I speciation tended to include greater percentages of iodate and organo-I. These changes were attributed to concomitant increases in groundwater pH (pH 3.2 to 6.8) and dissolved organic carbon concentrations (5 to 60 μM) (Otosaka et al. 2011). Iodine speciation did not change in a manner that could be explained by the changes in groundwater Eh (650 to 360 mV).

2.3. Iodine interactions with microbes

Bacteria from a ^{129}I -contaminated aerobic aquifer at the F-area of SRS can intracellularly accumulate I^- at environmentally relevant concentrations (10^{-7} M), but account for only a minor fraction of total added iodide (0.2-2.0%) (Li et al., 2011; Schwehr et al. 2009), which can not explain the high fraction (up to 50% of total I) of organo-I in groundwater (Otosaka et al., 2011; Schwehr et al., 2014; Zhang et al., 2014). This is in contrast to marine bacteria which can accumulate up to 50% of total added iodide (Li et al., 2012a, 2012b 2014,). Additionally enzymatic oxidation of I^- may provide another pathway for iodination of NOM (Li et al., 2012a, Xu et al., 2011a, 2011b) through superoxide (Li et al., 2014), and organic acid production (Li et al., 2012b).

2.4 Spectroscopic instrumentation to characterize organo-iodine compounds

Alongside the published analytical methods to determine I concentration, speciation, and isotopic ratios, the molecular environment of I in NOM was explored using a series of advanced spectroscopic instrumentation. ^{127}I concentration in sequentially extracted fractions of two SRS soils were measured and statistically compared to various organic components determined by ^{13}C DPMAS NMR and solution state ^1H NMR. The correlative analysis indirectly revealed the molecular nature of iodine binding environment (Xu et al., 2012): radioiodine was closely associated with the aromatic groups of macromolecules containing esterified products of phenolic and formic acids or other aliphatic carboxylic acids, amide functionalities, quinone-like structures activated by electron-donating groups (e.g., NH_2), or hemicellulose-lignin-like complexes with phenyl-glycosidic linkages. Additionally, with the help of ESI-FTICRMS, (Xu et al., 2013) were not only able to capture the subtle molecular variation during the enzymatic (lactoperoxidase catalyzed iodide oxidation at pH 8-9) and non-enzymatic iodination (iodide oxidation by MnO_2 , FeCl_3 , Fe_2O_3 , H_2O_2 , NOM at acidic pH etc. and iodate reduction by MnCl_2 , FeCl_2 and NOM at acidic pH) of NOM (Fig. 3a), but also resolve the molecular characteristics of organo-I by displaying their formulas in the van Krevelen diagrams (Fig. 3b-d). Peak-to-peak comparison of the ESI-FICRMS spectra of the NOM sample before and after iodination reactions revealed the reactive sites which were involved in the reactions and those sites to which iodine was added through electrophilic

substitution. . There was direct molecular information concerning the features of organo-iodine compounds: 1) iodination of NOM is mono-substitution; 2) 91% of organo-iodine compounds were chemically-modified during the iodination process (i.e., only 9% of the cases were one iodine ‘conservatively’ replacing one hydrogen without any change of the adjacent molecular structures; 3) organo-iodine compounds were distributed and ranked in the order of abundance, in the regions of unsaturated hydrocarbons, lignins and protein; 4) O/C values were mostly lower than 0.4, and more than half (~53%) of total identified organo-iodine compounds have the O/C values less than 0.2, suggesting such organo-iodine species might have relatively low hydrophilicity and less mobility; 5) ~69% of the identified organo-iodine species contained nitrogen, which likely was present as ring-activating functionality (e.g., -NH₂ or -HNCOR) and thus enhancing the nucleophilicity of the aromatic ring for iodine to attack. ESI-FTICRMS, for the first time, directly revealed the molecular reaction mechanisms of iodine sequestration into NOM as well as the structure and nature of resulting organo-iodine compounds.

3. Plutonium

3.1 Natural Organic Matter May Attenuate or Enhance Plutonium Transport

The human and environmental risks associated with plutonium (Pu) disposal, remediation stem mainly from very long half-life of several Pu isotopes. Due to the surface-mediated reduction of Pu (V) and Pu (VI) species to Pu (IV) by many minerals (e.g., goethite, hermatite, magnetite, etc.) (Conroy et al., 2016) as well as NOM (Simpkins 2011), the low water solubility of Pu (IV) (Neck and Kim, 2001) and high affinity to mineral phases and NOM, Pu is believed to be essentially immobile. While it has been demonstrated that humic acid (Choppin, 1992) or exopolymeric substances (Roberts et al., 2008), can reduce higher oxidation states of plutonium to more stable Pu(IV), it has also been suggested that natural organic matter can stabilize sorbed Pu on goethite (Tinnacher et al., 2015) and prevent desorption. Solubility of polynuclear Pu(IV) would be enhanced, however, by humic substances (Marsac et al., 2014).

As a result, when natural organic matter is low, Pu mobility is low as well. For example, < 10% of total Pu in the low organic matter-containing groundwater (< 0.2 mg/L) was mostly associated with inorganic colloid fractions (> 20 nm) at the Nevada

National Security Site (NNSS). However, Pu seemed to be more mobile at the Rainer Mesa tunnel complex at NNSS where > 65% of the total Pu was associated with dissolved organic matter, at 15-19 mg/L DOM (Conroy et al, 2016 and references therein).

In sediments collected from a region of SRS close to a contaminated wetland and a groundwater plume, a significant correlation between Pu and sediment NOM concentrations suggested Pu immobilization by N-containing NOM compounds (Xu et al., 2014, 2015, 2016). In contrast, previous studies reported Pu can be transported several kilometers in surface waters, in the form of inorganic (Kersting et al., 1999; Novikov et al., 2006) or organic colloids (Santschi et al. 2002). For example, through field studies and laboratory experiments, Santschi et al. (2002) revealed that 10-60% of the re-mobilized Pu from contaminated soils to streams in storm runoffs, pond discharge and wind dispersion events at Rocky Flats Environmental Technology Site (Colorado USA) was associated with colloidal organic macromolecules. It has been demonstrated that NOM compounds have high affinity for metal ions and thus strongly sorb to iron oxide minerals that are usually abundantly present in the soil and sediments, and further alter the surface characteristics. NOM with different functionalities that bind Pu might interact differently with the same mineral phase, thus affecting Pu immobilization/re-mobilization. For instance, addition of desferrioxamine B (DFOB) to montmorillonite enhanced Pu immobilization (i.e., sorption to montmorillonite) as Pu-DFOB complex accumulated in the interlayer of montmorillonite. Contrary to this, when humic acid and fulvic acid were present, they strongly decreased Pu (IV) sorption to montmorillonite due to formation of organic aqueous complexes (Boggs et al., 2015). The stability constants of such Pu-NOM complexes is often much stronger than those of Pu hydrolysis products (Zimmerman et al., 2010), yet controlled by several environmental factors such as pH and ionic strength. Therefore, it is likely the integrated interactions in a binary (Pu-NOM) or ternary system (mineral phase- NOM- Pu) that ultimately determines the fate of Pu. Four mechanisms for NOM influencing the environmental mobility of Pu have been suggested: 1) formation of ternary complexes (mineral phase- NOM- Pu) on immobile surfaces; 2) formation of immobile binary complex (Pu sequestration to NOM intrinsic colloids/aggregates); 3) formation of mobile Pu-NOM complex; 4) formation of ternary

complexes on mobile inorganic colloids (Conroy et al., 2016). Thus, it is necessary to obtain detailed knowledge of various geochemical factors causing Pu fixation and re-mobilization in the presence of NOM, particularly at low Pu concentration (10^{-12} - 10^{-18} M) in the far-field of DOE sites.

3.2 Characterizing Pu-NOM Interactions at Ambient Concentrations

Unfortunately, for analytical convenience, most laboratory Pu-NOM interaction experiments are often performed at Pu concentrations that greatly exceed those observed in contaminated environments, and the results are then simply extrapolated to ambient environmental conditions. However, this assumption may be unrealistic due to the availability of limited surface-mediated oxidation-reduction reactions sites and strong Pu-binding sites, and Pu (IV) tends to form polymeric or colloidal Pu-oxide particles (i.e., intrinsic colloids) at higher concentrations (Zhao et al., 2016)

The strategy to resolve this issue is to use a suites of techniques including solvent extraction, ultrafiltration, radiolabelling and isoelectric focusing (IEF) electrophoresis, followed by alpha-counting or ICP-MS determination to obtain natural organic macromolecules that are complexing and immobilizing/remobilizing Pu (Figs. 4a and 4b, also see Xu et al., 2008, 2015b, 2016). This lengthy procedure (Fig. 4a) had to be repeated many times and the Pu-carrying macromolecules had to be accumulated to an amount sufficient for further analysis. The strategy is mainly to concentrate Pu-NOM from bulk soil or sediment to the extent that the mobile Pu fraction, which usually only accounts for a minor fraction of the total bulk sedimentary Pu and the associated organic carbon ligands (e.g., 0.07% and 0.55% of the total sedimentary Pu and OC, respectively, in the case of RFETS soil), could be detected by alpha-counting (detection limit: 5.8×10^{-5} Bq for $^{239,240}\text{Pu}$) or ICP-MS (detection limit: 0.025 pg/L for ^{239}Pu) for Pu, and the extracted and purified IEF fractions could be detected by NMR (10 mg C as desirable carbon amount for solid-state ^{13}C NMR) and ESI-FTICRMS (10 μg C needed). Thus one has to take the bulk Pu and organic carbon concentrations of the soil or sediment into consideration to test the feasibility of detecting associations of Pu with individual NOM compounds. A rule of thumb in selecting soil or sediment for such a purpose is to work

with soil OC concentration of at least 2%, and the concentration of Pu slightly contaminated (three orders of magnitude higher than global fallout values).

Purified Pu-carrying NOM can then be characterized with advanced spectroscopic instrumentation. For example, with solid-state direct-polarization magic angle spinning (DPMAS) ^{13}C NMR, ^1D ^1H NMR, 2D HSQC NMR, and 2D COSY NMR, Pu(IV) in contaminated surface soils from RFETS was demonstrated to be bound to hydroxamates embedded in cutin degradation products cross-linked to hydrophilic moieties (e.g., polysaccharides) (Xu et al., 2008).

For more detailed molecular characterization, formulas obtained via ESI-FTICRMS were assigned to those Pu-carrying macromolecules that originated from the bulk mobile colloidal fraction of soil aggregates from SRS that contained most of the added Pu (Figs. 3a and 3b). These formulas showed that 23% of them contained nitrogen (Xu et al., 2015b). A van Krevelen diagram of all formulas as well as the N-containing formulas (Fig. 4c) suggest that these macromolecules are mainly aliphatic (65% in peak number and 68% in peak intensity, respectively), with some minor fraction of aromatics. They also covered an overlapping region for all lipid-like, partially protein-like, unsaturated hydrocarbons, lignin-like, and carboxyl-containing (Fig. 4d) aliphatic and/or alicyclic molecules (CCAM). An ESI FTICR-MS spectral comparison between the IEF extract and a siderophore standard (desferrioxamine; DFO) suggested the presence of hydroxamate siderophore functionalities in the IEF extract, indicating that Pu was most likely incorporated into hydroxamate functionality-containing macromolecules by displacing Fe (III) in hydroxamates that would be facilitated by the higher stability constants between Pu (IV) and OH^- or hydroxamate (Boukhalfa et al., 2007, Almagro et al., 2008)

Another key and novel finding of this work is that hydroxamate siderophores were detected in both particulate or colloidal phases of the sediments (Xu et al., 2015b), rather than only in the low-molecular fractions (< 1000 Da). These hydroxamate siderophore moieties have previously been overlooked due to extraction and detection difficulties (Gillam et al., 1981). Moreover, hydroxamate siderophore concentrations in the soil/sediment particles or colloidal fractions seem to be a few orders of magnitude greater than those in solution (Ahmed et al., 2014; Xu et al., 2015b).

4. Summary and Conclusions

In summary, NOM can play a significant role in controlling the fate and transport of radionuclides. This can in part be attributed to the extremely high concentration of binding sites in NOM, but also, as this review demonstrates, to the wide assortment of binding sites. Iodine can form extremely strong covalent bonds to NOM, especially to certain aromatic moieties, whereas Pu can be chelated to hydroxamate compounds. The implication of understanding how radionuclide bind to NOM is that it provides the underpinning for predicting the long-term fate and transport of radionuclides in dynamic systems undergoing temporal and spacial chemical or physical changes. For example, changes in system pH as a plume migrates from the near field to the far field would be expected to have a very different effect on I-NOM interactions, than to Pu-NOM interactions because of the greater pH-dependent role of microbial processes in the former. Taking these two radionuclides (i.e., I and Pu) as examples, NOM largely controlled their biogeochemistry, even at very low NOM levels. Finally, it is important to study these systems under environmentally relevant concentrations for both radionuclide and NOM, since key NOM-radionuclide interaction processes for immobilizing or remobilizing radionuclides were found to be extremely concentration dependent.

While low-level measurements are a prerequisite for learning more about environmental tracers as well as for nuclear forensics, we have to go beyond that if we want to learn more about the environmental behavior of radionuclides, and attempt to get their in-situ speciation, which ultimately controls their mobility and transport in natural environments.

5. Acknowledgements

This work was funded by the U.S. Department of Energy (DOE) Office of Science Subsurface Biogeochemistry Research program (ER65222-1038426-0017532), DOE SBR grant #DE-ER64567- 1031562-0014364, and DOE SBR award # DE-SC0014152. This work has also been funded through DOE contract DE-AC09-08SR22470 with Savannah River Nuclear Solutions, LLC.

6. References:

- Ahmed, E., Holmström, S.J.M., 2014. The effect of soil horizon and mineral type on the distribution of siderophores in soil. *Geochim. Cosmochim. Acta* 131(0), 184-195.
- Almagro, L., Gomez Ros, L.V., Belchi-Navarro, S., Bru, R., Ros Barcelo, A., Pedren, M. A., 2008. *J. Exp. Botany* Dec.10, 1-14, doi:10.1093/jxb/ern277.
- Boggs, M. A.; Dai, Z. R.; Kersting, A. B.; Zavarin, M., Plutonium(IV) sorption to montmorillonite in the presence of organic matter. *Journal of Environmental Radioactivity* 2015, 141, 90-96.
- Boukhalfa, H., Reilly, S.D., Neu, P., 2007. Complexation of Pu(IV) with the natural siderophore desferrioxamine B and the redox properties of Pu(IV)(siderophore) complexes. *Inorg. Chem.* 46(3), 1018-1026.
- Chang, H.-S., Xu, C., Schwehr, K.A., Zhang, S., Kaplan, D.I., Seaman, J.C., Yeager, C., Santschi, P.H., 2013. Model of radioiodine speciation and partitioning in organic-rich and organic-poor soils from the Savannah River Site. *J. Environ. Chem. Engineering* 2, 1321-1330.
- Choppin, G.R.; and Du, M., f-Element complexation in brine solutions. *Radiochim. Acta* **1992**, 58/59, 101-104.
- Conroy, N. A.; Zavarin, M.; Kersting, A. B.; Powell, B. A., Effect of Natural Organic Matter on Plutonium Sorption to Goethite. *Environmental Science & Technology* **2017**, 51, (1), 699-708.
- Denham, M., Vangelas, K.M., 2008. Biogeochemical gradients as a framework for understanding waste-site evolution. *Remediation* 19, 5-17.
- Emerson, H.P., Xu, C., Ho, Y.-F., Zhang, S., Schwehr, K.A., Lilley, M., Kaplan, D.I., Santschi, P.H., Powell, B.A., 2014. Geochemical controls of iodine transport in Savannah River Site subsurface environments. *Applied Geochem.* 45, 105–113.
- Gephart, R. E., 2003. "Hanford: A conversation about nuclear waste and cleanup," Rep. No. PNNL-SA-37974. Pacific Northwest National Laboratory (PNNL), Richland, WA (US).

370 Gillam, A.H., Lewis, A.G., Andersen, R.J., 1981. Quantitative determination of
 371 hydroxamic acids. *Anal. Chem.* 53(6), 841–844.
 372 Hou, X., Aldahan, A., Possnert, G. 2007. Speciation Analysis of Radionuclides in the
 373 Environment. NKS-B Speciation project report. NKS-176; ISBN 978-87-7893-242-6.
 374 Kaplan, D.I., Denham, M. E., Zhang, S., Yeager, C., Xu, C., Schwehr, K.A., Li, H.P., Ho,
 375 Y.-F., Wellman, D., Santschi, P.H., 2014a. Radioiodine biogeochemistry and
 376 prevalence in groundwater. *Critical Rev. Environ. Sci. Technol.* 44(20), 2287-2335.
 377 Kaplan, D.I., Roberts, K.A., Schwehr, K.A., Lilley, M.S., Brinkmeyer, R., Denham,
 378 M.E., DiPrete, D., Li, H.-P., Powell, B.A., Yeager, C.M., Zhang, S.J., Santschi, P.H.,
 379 2011. Evaluation of a radioiodine plume increasing in concentration at the Savannah
 380 River Site. *Environ. Sci. Technol.* 45, 489-495.
 381 Kaplan, D.I., Xu, C., Huang, S., Lin, Y.M., Tolic, N., Roscioli-Johnson, K.M., Santschi,
 382 P.H., Jaffé, P.R., 2016. Unique organic matter and microbial properties in the
 383 rhizosphere of a wetland soil. *Environ. Sci. Technol.* 50, 4169–4177, doi:
 384 10.1021/acs.est.5b05165.
 385 Kaplan, D.I., Zhang, S., Roberts, K.A., Schwehr, K.A., Xu, C., Creeley, D., Ho, Y.-F., Li,
 386 H.-P., Yeager, C.M., Santschi, P.H., 2014b. Radioiodine concentrated in a wetland. *J.*
 387 *Environ. Radioactivity* 131, 57-61.
 388 Kersting, A.B., Efurud, D.W., Finnegan, D.L., Rokop, D.J., Smith, D.K., Thompson, J.L.,
 389 1999. Migration of plutonium in ground water at the Nevada Test Site. *Nature* 397
 390 (6714), 56–59.
 391 Li, H.-P., Brinkmeyer R., Jones W.L., Zhang, S., Xu, C., Ho, Y.-F., Schwehr, K.A.,
 392 Kaplan, D.I., Santschi, P.H., Yeager, C.M., 2012a. Iodide Oxidizing Activity of
 393 Bacteria from Subsurface Sediments of the Savannah River Site, SC, USA, in
 394 Kawaguchi, M., Misaki, K., Sato, H., Yokokawa, T., Itai, T., Nguyen, T.M., Ono, J.,
 395 Tanabe S. (Eds.), *Interdisciplinary Studies on Environmental Chemistry Vol. 6 -*
 396 *Environmental Pollution and Ecotoxicology.* Terra Scientific Publishing Company
 397 Tokyo, pp. 89-97.
 398 Li, H.-P., Brinkmeyer, R., Jones, W.L., Zhang, S.J., Xu, C., Schwehr, K.A., Santschi,
 399 P.H., Kaplan, D., Yeager, C.M., 2011. Iodide accumulation by aerobic bacteria

isolated from subsurface sediments of a ^{129}I -contaminated aquifer at the Savannah River Site, S.C. *Applied Environ. Microbiol.* 77(6), 2153–2160.

Li, H.-P., Daniel, B., Creeley, D., Grandbois, R., Zhang, S., Xu, C., Ho, Y.-F., Schwehr, K.A., Kaplan, D.I., Santschi, P.H., Hansel, C., Yeager, C.M., 2014. Superoxide production by a manganese-oxidizing bacterium facilitates iodide oxidation. *Applied Environ. Microbiol.* 80(9), 2693-2699.

Li, H.-P., Yeager, C.M., Brinkmeyer, R., Zhang, S., Ho, Y.-F., Xu, C., Jones, W.L., Schwehr, K.A., Otosaka, S., Kaplan, D.I., Santschi, P.H., 2012b. Organic acids produced by subsurface bacteria enhance iodide oxidation in the presence of hydrogen peroxide. *Environ. Sci. Technol.* 46, 4837-4844.

Livens, F.R., and Baxter, M.S. 1988. Chemical Associations of Artificial Radionuclides in Cumbrian Soils. *J. Environ. Radioactivity*, 7, 75-86.

Marsac, R., Banik, N.L., Marquardt, Kratz, J.V., 2014. Stabilization of polynuclear plutonium(IV) species by humic acid. *Geochim. Cosmochim. Acta*, 131, 290-300.

Martin, J.M., Nirel, P., and Thomas, A.J. 1987. Sequential extraction techniques: Promises and Problems. *Mar. Chem.*, 22, 313-341.

Neck, V., Kim, J.I., 2001. Solubility and hydrolysis of tetravalent actinides. *Radiochim. Acta* 89, 1-16.

Neu, M.P., 2000. In: *Chemical Interactions of Actinides in the Environment*. Los Alamos Science 26, 416.

Nirel, P., and Morel, F.M.M. 1990. Pitfalls of sequential extractions. *Wat. Res.* 24(8), 1055-1056.

Novikov, A.P., Kalmykov, S.N., Utsunomiya, S., Ewing, R.C., Horreard, F., Merkulov, A., Clark, S.B., Tkachev, V.V., Myasoedov, B.F., 2006. Colloid transport of plutonium in the far-field of the Mayak production association, Russia. *Science* 314 (5799), 638–641.

Okoro, H.K., Fatoki, O.S., Adekola, F.A., Ximba, B.J., Snyman, R.G. 2012. A Review of Sequential Extraction Procedures for Heavy Metals Speciation in Soil and Sediments. 1:181. doi:10.4172/scientificreports.181.

429 Okoro, H.K., Fatoki, O.S., Adekola, F.A., Ximba, B.J., Snyman, R.G. 2012. A Review of
 430 Sequential Extraction Procedures for Heavy Metals Speciation in Soil and Sediments.
 431 1:181. doi:10.4172/scientificreports.181.
 432 Otosaka, S., Schwehr, K.A., Kaplan, D.I., Roberts, K.A., Zhang, S., Xu, C., Li, S.-P., Ho,
 433 Y.-F., Brinkmeyer, R., Yeager, C.M., Santschi, P.H., 2011. Transformation and
 434 transport processes of ^{127}I and ^{129}I species in an acidic groundwater plume at the
 435 Savannah River Site. *Sci. Total Environ.* 409, 3857–3865.
 436 Qiao, J., Hansen, V., Hou, X., Aldahan, A., Possnert, G. 2012. Speciation Analysis of
 437 ^{129}I , ^{137}Cs , ^{232}Th , ^{238}U , ^{239}Pu and ^{240}Pu in Environmental Soil and Sediment.
 438 *Appl. Rad. Isotopes*, 70(8), 1698-1708
 439 Roberts, K.A., Santschi, P.H.; and Honeyman, B.D., Pu(V) reduction and enhancement of
 440 particle-water partitioning by exopolymeric substances. *Radiochim. Acta* **2008**, 96(9-
 441 11), 739-745.
 442 Santschi, P.H., Roberts, K.A., Guo, L., 2002. Organic nature of colloidal actinides
 443 transported in surface water environments. *Environ. Sci. Technol.* 36(17), 3711–3719.
 444 Schwehr, K.A., Otosaka, S., Merchel, S., Kaplan, D.I., Zhang, S., Xu, C., Li, H.-P., Ho,
 445 Y.-F., Yeager, C.M., Santschi, P.H., ASTER Team., 2014. Speciation of iodine
 446 isotopes inside and outside of a contaminant plume at the Savannah River Site. *Sci.*
 447 *Total Environ.* 497–498, 671–678.
 448 Schwehr, K.A., Santschi, P.H., Kaplan, D.I., Yeager, C.M., Brinkmeyer, R., 2009.
 449 Organo-iodine formation in aquifer sediments at ambient concentrations. *Environ. Sci.*
 450 *Technol.* 43, 7258–7264.
 451 Simpkins, L. A. Influence of natural organic matter on plutonium sorption to gibbsite.
 452 Clemson University, Ann Arbor, MI, 2011.
 453 Tinnacher, R.M.; Begg, J.D.; Mason, H.; Ranville, J.; Powell, B.A.; Wong, J.C.;
 454 Kersting, A.B.; and Zavarin, M., Effect of Fulvic Acid Surface Coatings on Plutonium
 455 Sorption and Desorption Kinetics on Goethite. *Environ. Sci. Technol.* **2015**, 49,
 456 2776–2785.
 457 Xu, C., Athon, M., Ho, Y.-F., Chang, H.-S., Zhang, S., Kaplan, D.I., Schwehr, K.A.,
 458 DiDonato, N., Hatcher, P.G., Santschi, P.H., 2014. Plutonium immobilization and re-

459 mobilization by soil mineral and organic matter in the far-field of the Savannah River
 460 Site, USA. *Environ. Sci. Technol.* 48, 3186–3195.

461 Xu, C., Chen, H.M., Sugiyama, Y., Zhang, S., Li, H.-P., Ho, Y.-F., Chuang, C.-Y.,
 462 Schwehr, K.A., Kaplan, D.I., Yeager, C., Roberts, K.A., Hatcher, P.G., Santschi, P.H.,
 463 2013. Novel molecular-level evidence of iodine binding to natural organic matter from
 464 Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Sci. Total Environ.*
 465 449, 244–252.

466 Xu, C., Miller, E.J., Zhang, S., Li, H.-S., Ho, Y.-F., Schwehr, K.A., Kaplan, D.I.,
 467 Roberts, K.A., Otosaka, S., Brinkmeyer, R., Yeager, C.M., Santschi, P.H., 2011a.
 468 Sequestration and re-mobilization of radioiodine (^{129}I) by soil organic matter and
 469 possible consequences of the remedial action at Savannah River Site. *Environ. Sci.*
 470 *Technol.* 45, 9975–9983.

471 Xu, C., Santschi, P.H., Zhong, J.Y., Hatcher, P.G., Francis, A.J., Dodge, C.J., Roberts,
 472 K.A., Hung, C.-C., Honeyman, B.D., 2008. Colloidal cutin-like substances cross-
 473 linked to siderophore decomposition products mobilizing plutonium from
 474 contaminated soils. *Environ. Sci. Technol.* 42(22), 8211–8217.

475 Xu, C., Zhang, S., Athon, M., Ho, Y.-F., Li, H.-P., Grandbois, R., Schwehr, K.A.,
 476 Kaplan, D.I., Yeager, C.M., Wellman, D., Santschi, P.H., 2015a. A re-evaluation of
 477 radioiodine transformation and migration in the subsurface of Hanford Site. *J.*
 478 *Environ. Radioactivity* 139, 43–55.

479 Xu, C., Zhang, S., Ho, Y.-F., Miller, E.J., Roberts, K.A., Li, H.-P., Schwehr K.A.,
 480 Otosaka, S., Kaplan, D.I., Brinkmeyer, R., Yeager, C.M., Santschi P.H., 2011b. Is soil
 481 natural organic matter a sink or source for radioiodine (^{129}I) at the Savannah River
 482 Site? *Geochim. Cosmochim. Acta* 75, 5716–5735.

483 Xu, C., Zhang, S., Kaplan, D.I., Ho, Y.-F., Schwehr, K.A., Roberts, K.A., Chen, H.M.,
 484 Didonato, N., Athon, M., Hatcher, P.G., Santschi, P.H., 2015b. Evidence for
 485 hydroxamate siderophores and other N-containing organic compounds controlling
 486 $^{239,240}\text{Pu}$ immobilization and remobilization in a wetland sediment. *Environ. Sci.*
 487 *Technol.* 49, 11458–11467.

488 Xu, C., Zhang, S., Sugiyama, Y., Nobuhito Ohte. N., Ho, Y.-F., Fujitake, N., Kaplan,
 489 D.I., Yeager, C.M., Kathleen Schwehr, K.A., Santschi, P.H., 2016. Factors controlling

iodine and $^{239,240}\text{Pu}$ concentrations and mobility in soils from the Northwestern Fukushima Prefecture, Japan. *J. Environ. Radioactivity* 153, 156-166.

Xu, C., Zhong, J.Y., Hatcher, P.G., Zhang, S., Li, H.-P., Ho, Y.-F., Schwehr, K.A., Kaplan, D.I., Roberts, K.A., Brinkmeyer, R., Yeager, C.M., Santschi, P.H., 2012. The molecular environment of stable iodine and radioiodine (^{129}I) in natural organic matter: evidence from NMR. *Geochim. Cosmochim. Acta* 97, 166–182.

Zhang, S., Chen, X., Creeley, D., Ho, Y.-F., Li, H.-P., Grandbois, R., Schwehr, K., Kaplan, D., Yeager, C., Wellman, D., Santschi, P.H., 2013b. Response to comment on “Iodine-129 and Iodine-127 speciation in groundwater at Hanford Site, U.S.: iodate incorporation into calcite”. *Environ. Sci. Technol.* 47, 13205-13206.

Zhang, S., Du, J., Xu, C., Schwehr, K.A., Ho, Y.-F., Li, H.-P., Roberts, K.A., Kaplan, D.I., Brinkmeyer, R., Yeager, C.M., Chang, H.-S., Santschi, P.H., 2011. Concentration dependent mobility, retardation and speciation of iodine in surface sediment from the Savannah River Site. *Environ. Sci. Technol.* 45, 5543-5549.

Zhang, S., Ho, Y.-F., Creeley, D., Roberts, K.A., Xu, C., Li, H.-P., Schwehr, K.A., Kaplan, D.I., Yeager, C.M., Santschi, P.H., 2014. Temporal variation of iodine concentration and speciation (^{127}I and ^{129}I) in wetland groundwater from the Savannah River Site, USA. *Environ. Sci. Technol.* 48, 11218-11226.

Zhang, S., Schwehr, K.A., Ho, Y., Xu, C., Roberts, K.A., Kaplan, D., Brinkmeyer, R., Yeager, C.M., Santschi, P.H., 2010. A novel approach for the simultaneous determination of iodide, iodate, and organo-iodide for ^{127}I and ^{129}I in Environmental Samples Using Gas Chromatography - Mass Spectrometry. *Environ. Sci. Technol.* 44, 9042-9048.

Zhang, S., Xu, C., Creeley, D., Ho, Y.-F., Li, H.-P., Grandbois, R., Schwehr, K.A., Kaplan, D.I., Yeager, C.M., Wellman, D., Santschi, P.H., 2013a. Iodine-129 and Iodine-127 speciation in groundwater at the Hanford Site, U.S.: Iodate incorporation into calcite. *Environ. Sci. Technol.* 47, 9635-9642.

Zhao, P.; Begg, J. D.; Zavarin, M.; Tumey, S. J.; Williams, R.; Dai, Z. R.; Kips, R.; Kersting, A. B., Plutonium(IV) and (V) Sorption to Goethite at Sub-Femtomolar to Micromolar Concentrations: Redox Transformations and Surface Precipitation. *Environmental Science & Technology* **2016**, 50, (13), 6948-6956.

521 Zimmerman, T. N.; Powell, B. A., Plutonium-humic acid stability constant determination
522 and subsequent surface complexation studies. *Geochimica Et Cosmochimica Acta*
523 **2010**, 74, (12), A1237-A1237.
524
525
526

Figure captions

Fig. 1. Flow chart of iodine speciation (total iodine, TI; total inorganic iodine, TII; organo-iodine, OI; iodide and iodate) and quantification by GC-MS method after derivatization to 4-iodo N,N -dimethylamine, as well as principle for simultaneous measurement of ^{127}I and ^{129}I at elevated environmental samples (detection limit: 2 pCi $^{129}\text{I}/\text{L}$ for liquid sample and 2 pCi $^{129}\text{I}/\text{g}$ for solid sample) (Zhang et al., 2010). Reprinted with permission from Environmental Science and Technology. American Chemical Society. Copyright 2010.

Fig. 2 Breakthrough curves of tritium and iodide a) and iodate b) in Savannah River Site wetland sediment, FSI-18 (relative concentration = concentration in effluent/concentration in influent solution). Iodide/iodate applied to the columns at two concentration levels, 7.87 nM and 78.8 μM . For each species, the two solutions (at high and low concentrations) were both spiked with ^{125}I and ^{127}I . Spike solutions (15-20 mL) were added and then flushed with artificial groundwater for 30 to 45 days (Zhang et al., 2011). Reprinted with permission from Environmental Science and Technology. American Chemical Society. Copyright 2011.

Fig. 3. a) Flow chart of iodination of natural organic matter (NOM) starting from iodide or iodate via enzymatic or non-enzymatic pathways, b) ESI-FTICRMS analysis in negative mode NOM extracted from surface soil of F-Area of Savannah River Site amended without (up) and with (down) I^- - through enzymatic reactions c) an example of identification of organo-iodine peak through peak-to-peak comparison between the un-iodinated and iodinated NOM spectra and molecular formula assignment; d) van Krevelen diagram of all identified organo-iodine formulas (Xu et al., 2013). Reprinted with permission from Science of the Total Environment. Copyright 2013.

Fig. 4. a) Flow chart of extraction and purification of plutonium-carrying macromolecules from soil or sediment samples; b) isoelectric focusing spectrum showing the Pu concentration distribution of the macromolecules along the pH gradient; c) van Krevelen diagram showing all 1259 formulas (blue) and the N-containing formulas (red) of the Pu enriched fraction purified via isoelectric focusing experiment; d) Kendrick mass defect (KMD) plot of FTICR-MS peaks on the trend line analyzed by COO KMD analysis (carboxylic-rich alicyclic molecules (CRAM)- double bond equivalent (DBE)/C = 0.30-0.68; DBE/H = 0.20-0.95; DBE/O = 0.77-1.75; carboxyl-containing aliphatic molecules (CCAM) = O/C 0-0.40, H/C 0.85-2.00) (Xu et al., 2015b). Environmental Science and Technology. American Chemical Society. Copyright 2015.

Fig. 2.

Fig. 3

Fig. 4