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Influence of biogeochemistry on the availability of toxic metals in iron-replete New Jersey sediment: Development of a point-of-use trace metal sensor with integrated sediment microbial community and geochemical measurements

Summary Report

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1.0 Purpose and Focus

- Outline sediment sequential extraction and inductively coupled plasma optical emission spectroscopy (ICP-OES)
- Describe scientific methods used for Pb concentrations in chemical fractions of sediment from Raritan River, New Jersey (NJ)
- Provide total Pb and Pb sediment concentration data from sequential extractions and analyses from Raritan River, New Jersey (NJ)
- Report sediment standard reference material recoveries and quality criterion
- Assess the microbial diversity in sediments associated

2.0 Materials and Methods:

2.1 Sequential Extraction – Sediment Metal Fractions

Sequential extraction procedures are used to locate the occurrence of heavy metals and utilize 3 or 4-step sequential extractions to measure the metal of interest in the exchangeable, carbonate, iron (Fe) and manganese (Mn) oxide, organic (oxidizable), and strong acid-extractable (residual) phases (Okoro et al. 2012). The "Community Bureau of Reference" (BCR) has produced recent sequential extraction methods that use a 3-step extraction method (Elass et al. 2004, Guevara-Riba et al. 2004, Yuan et al. 2004). Traditional sequential extraction protocols (Tessier et al. 1979) and subsequent modifications (Rauret et al. 1989) require slow digestion times and with consistent heating. Accelerated approaches to sequential extraction methods were explored to decrease digestion times, lower equipment and reagent cost, and increase capacity of sediment metal analysis (Table 1). Microwave heating (Gulmini et al. 1994, Perez-Cid et al. 1999a) and ultrasound (Vaisanen and Kiljunen 2005) techniques have been used to accelerate sequential extraction. Metal yields of ultrasound techniques have been compared to accepted the Tessier protocol (Perez-Cid et al. 1999b, c) with significant differences between metals in the third (Fe and Mn fraction) and fourth (oxidizable) extracts. However, good agreement in metal concentrations were found between ultrasound accelerated and original BCR methods in each of the three chemical fractions (carbonate, Fe and Mn, and oxidizable) (Perez-Cid et al. 1998), including lead (Pb), our main toxic metal of interest.

2.2 Sequential, Residual, and Total Metal Sediment Acid Digestion Procedures

Multiple versions of a total metal sediment digestion (EPA 1996) were performed to measure residual and total concentrations of three additional metals (As, Hg, and Fe) in sediment. An ultrasound-aqua regia digest step (16N Nitric acid, 12N Hydrochloric acid) was added to a 3-step sequential extraction protocol (Perez-Cid et al. 1998) to measure the residual fraction of these heavy metals in sediment and produce a total concentration from four fractions of metals in sediment (Table 1). Total metal extractions of sample and standard reference material New York/New Jersey Waterway Sediment (SRM 1944) were used to verify total concentrations of As, Hg, and Fe and measured values of these heavy metals in the residual phase (sulfide bound) produced from our 4-step sequential metal extraction technique. We have also chosen to use SRM 1944 to assess total metal yields from total metal digests and develop "in-house" reference values of metal yields from each stage of our ultrasound, sequential extraction technique.

- 50Watt Sonic Dismembrator 110V (Fisher Scientific; Model 50)
- Analytical Balance accurate weights at 0.001 g
- Centrifuge 50 mL capacity
- Teflon spatula or spoon
- Freeze dryer (lyophilization)
- 50 mL Falcon centrifuge tubes, polypropylene
- Centrifuge tube racks 50 mL
- Macropipette and tips (1 5mL)
- Glass digestion vessels 250 mL
- Glass beakers (tops) 100 mL

2.3 Reagents – Sequential Extraction and Total Metal Acid Digestion

Organic or inorganic reagents used in digestion shall be of reagent grade (trace metal grade (TMG) or high performance liquid chromatography (HPLC) grade) chemicals and all acid cleaning of digestion surfaces with certified (ACS Plus) hydrochloric acid in dilute concentrations (35% and 10%).

- Acetic acid (concentrated), CH₃COOH.
- Sodium acetate, anhydrous (≥99.995%), CH₃COONa.
- Hydroxylamine hydrochloride (≥99.995%), NH₂0H · HCl.
- Hydrogen peroxide (30%), H₂O₂.
- Ammonium acetate (97.0%), C₂H₇NO₂.
- 12N Hydrochloric acid (Muriatic acid), HCl · H₂O.
- 16 N Nitric acid (67 to 70% w/w), HNO₃.
- 0.2N Bromine chloride (optimum 12N Hydrochloric acid, potassium bromide, potassium bromate (TMG))

Fraction	Reagents	Ultrasound Digestion	Metals Removed
F1 – Acid Soluble Fraction	10 mL of Glacial Acetic Acid (0.11 mol L ⁻¹)	50W – <u>7 min</u>	Carbonates, sulfates, hydroxide minerals
F2 – Reducible fraction	10 mL Hydroxylamine hydrochloride (0.1 mol L ⁻¹)	50W – <u>7 min</u>	Bound to Fe-Mn oxides
F3 – Oxidizable Fraction	5 mL Hydrogen Peroxide (30% w/v)	50W – <u>2 min</u>	Oxidizable organics, some humic acids
F3 – Oxidizable Fraction (stage 2)	10 mL Ammonium Acetate	50W – <u>6 min</u>	Oxidizable organics, some humic acids
F4 – Residual "Sulfide" Fraction	Aqua Regia (12N Hydrochloric acid, 16N Nitric acid)	50W – <u>7 min</u>	Dissolved, particulate fractions of Pb bound to hydrogen sulfide and sulfide

Table 1. 4-step sequential extraction method modified from Perez-Cid et al. 1999 to accommodate smaller weights of sediment samples (~0.5 g).

2.5 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) – Method Overview

Sediment sample preparation and analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES) followed a modified version of US EPA Solid Wastes (SW) - 846 method 6010c. This method is prescribed for determination of 31 elements in ground water, industrial and organic wastes, soils, sludges and sediments following the use of a toxic leaching procedure (TCLP) or extraction procedure (EP) of digestion prior to analysis. As only a single element, Pb was of primary interest, Pb analysis was performed separately due to its spectral interferences with Fe. As little spectral interference exists between elements of As, Hg, and Fe these elements were analyzed separately. Quality (calibration) and method performance were assessed with the use of calibration blanks, continuing calibration standards, and method blanks, and recoveries of standard reference material. Method blanks were less than 3 times the Pb detection limit determined by the calibration curve (calibration blanks and standards) of a given run. Continuing calibration standards were run every 8 to 10 samples and were applied to correct metal concentrations if values varied more than 10% of actual standard values. Method blanks were run for each step of 4-step sequential extraction procedure and for the total sediment digest to ensure low background Pb levels during acid digestions. Quality control measures were taken to run each step of the sequential sediment digest in duplicate to assess variability of metals within a given digest step.

2.6 Apparatus and Materials – ICP-OES Analysis

- Ultra high purity (UHP) nitrogen gas
- 15 mL Falcon centrifuge tubes, polypropylene

2.7 Reagents – ICP-OES

All analyses were performed using trace metal certified standards (*Trace*CERT, Sigma-Aldrich) diluted from concentrated (1000 mg/L) stock.

- 1 mg/L Lead nitrate (PbNO₃), in 2% nitric acid (HNO₃).
- 1 mg/L Mercury metal in 12% HNO₃
- 1 mg/L Arsenic trioxide (AsO₃) in 2% HNO₃
- 1 mg/L Iron metal in 2% HNO₃
- Ultrapure (>17 megaohm) water

2.8 DNA extraction and sequence analysis

River sediment was collected with a 0.5m pushcore sampling device. Aliquots were subsequently stored at -80°C until DNA extraction. DNA was extracted using a Qiagen DNeasy Powersoil kit (Qiagen, Venlo, Netherlands). Preparation for sequencing of the V4 region of the 16S rRNA gene was carried out according to the Earth Microbiome Project protocol (Caporaso et al. 2011, Caporaso et al. 2012) with modifications as previously described (Case et al. 2015). Raw sequences were generated on an Illumina MiSeq platform at the Waksman Institute Genomics Core Facility (Rutgers University, Piscataway, NJ, USA). Sequence data were demultiplexed and processed using a modified version of the QIIME pipeline (Caporaso et al. 2010) as described previously (Mason et al. 2015). Prior to sample comparison, singletons and PCR contaminants were removed, and a 0.01% relative abundance threshold was applied. Additional analysis of sequence data and figure construction were performed in the R environment (RStudio v.1.1.414, R v.3.6.1) using the ggplot2 (Wickham 2009) and vegan (Oksanen et al. 2017) packages.

2.9 Sediment Porewater chemistry

Sediment pushcores were collected from four sites along the Raritan River at distances of 5.6, 12.9, 15.9, and 19.8 km from the bay. Porewater was collected from 3-4.5 cm depth intervals up to 50 cm below the sediment water interface by either centrifugation or rhizon samplers (Rhizosphere research products, Wageningen, Netherlands). Porewater anion concentrations were measured using a Thermo Scientific Dionex Aquion Ion Chromatograph (Thermo Fisher Scientific, Sunnyvale, CA, USA). Ions were eluted over 30 minutes using a 9 mM NaCO₃ eluent with a Dionex IonPac AS11 4 x 50 mm guard column and 4 x 250 mm column coupled to a Dionex AERS 500 carbonate anion suppressor. Fiver in-house standards of different concentrations bracketed sample analyses. Dissolved iron concentrations were determined spectrophotometrically using a ferrozine assay (Stookey 1970).

3.0 Results and Discussion:

3.1 Pb Partitioning in Raritan River Sediment

Seven sites were strategically sampled at key points along the Raritan River (Fig. 1) to provide a comprehensive assessment of municipal impacts (Donaldson Park, Edison Boat Dock), overlap with collaborators marsh study sites (Route 1 and Turnpike), assess impact of major tributaries (South Branch, South River Mouth), influence of a more saline environment (Crab Island). Total Pb concentrations differed by approximately a factor of seven from 14.8 to 106.2 ppm (Table 1) with highest concentrations found at RO and lowest concentrations at EB. Low recoveries of total Pb in standard reference material SRM 1944 ($51.0\pm6.0\%$, n=4) need to be considered when evaluating Pb concentrations and percentages below and indicate a need for a sequential, residual, and total metal extraction verification procedure (Section 2.2).



Figure 1. Map of sample sites along Raritan River: Donaldson Park (DP), Bridge Site- Route 1 (RO), Turnpike (TN), South Branch (SB), Edison Boat Dock (EB), South River Mouth (SR), Crab Island (CI).

Pb bound in the F1 fraction (carbonates, sulfates, hydroxide minerals) were consistently low (<2%) across seven sites sampled along the Raritan River (Table 2). However, Pb bound primarily to iron (hydrated ferric oxides (HFO, F2)) varied widely (1.5 - 31.3%) over seven sites with no apparent cross-river trend. Highest fraction of Pb bound to Fe was found at the most upstream site (DP) and at the mouth of the South (Raritan) Branch and South River indicating contributions of Fe rich sediment from major tributaries. Lowest percentage (1.5%) of Pb in Fe fraction was found at the furthest downstream site, Crab Island, indicating little contribution of Fe rich material from Raritan River-Bay. Organic bound (F3) Pb also varied (0.6 - 21.5%) over seven sites sampled. Sediment collected from neighboring RO and TN marsh sites of research collaborators (Professor Richard Lathrop, Dr. Laura Reynolds) showed very different total Pb concentrations and percentages of Pb bound in the F3 fraction. Field notes indicate dark black material at RO ("Bridge Site") and the presence of methane bubbles released from surface sediment exposed during low tide. Methane production may indicate an abundance of organic matter contributing to Pb speciation, and possibly high total Pb concentrations at RO not found at the neighboring TN site. The sulfide (F4) fraction partitioned the majority (57.7 - 88.6%) of Pb in Raritan River sediments and was found to be highest at TN (80.3%) and CI (88.6%) sites. These results illustrate the importance of sulfur biogeochemistry and its reduction to sulfide and hydrogen sulfide in Raritan River sediment. These field and lab observations indicate the spatial complexity and heterogeneity of the Raritan River ecosystem and the importance of measuring other trace metals to better understand metal biogeochemistry.

<u>Sample</u> <u>Name</u>	<u>F1 (% of</u> <u>total)</u>	<u>F2 (% of</u> <u>total)</u>	<u>F3 (% of</u> <u>total)</u>	<u>F4 (% of</u> <u>total)</u>	<u>Total [Pb]</u> ppm
RR_DP	1.0	31.3	0.8	66.9	23.7
RR_RO	1.4	11.4	21.5	65.7	106.2
RR_TN	1.5	15.6	2.6	80.3	35.7
RR_SB	1.0	28.7	0.6	69.7	21.8
RR_EB	0.5	11.0	17.9	70.5	14.8
RR_SR	1.8	24.0	16.5	57.7	43.7
RR_CI	0.9	1.5	9.0	88.6	62.1

Table 2. Total Pb concentrations [Pb] and percentage of Pb bound in four sediment fractions from seven sites in Raritan River, New Jersey.

3.2 Microbial Diversity and distribution in Raritan River Sediments

A survey of the sediment microbial diversity in four locations along the Raritan River between the Lawrence Brook and the Sayersville rail bridge revealed broad similarities in the community composition of river sediments. Sediments with lower salinity in the overlying waters contained more methanogenic archaea than sulfate reducing bacteria, while sediments with higher salinity in the overlying water tended to include more anaerobic methane oxidizing archaea (ANME). Additionally, the community contained a variety of canonical sulfate reducing, iron/metal reducing, and dechlorinating bacterial taxa. No clear pattern with respect to depth in the sediment pushcores or location along the river was observed at the level of bacterial family or order. However, there is some apparent depth and site variation in the genera distribution, which indicates some niche differentiation in microbial diversity.

The presence of sulfate reducing and iron/metal reducing bacterial taxa points to a role for a interlinked sulfur and metal cycle in the Raritan sediments. This indicates to a role for bacterial sulfide formation in sequestering iron and other metals as metal sulfides. Multiple iron and metal

oxididizing bacterial taxa are also present, which indicates a cyclic process that can lead to the resuspension of metals under particular nutrient and redox conditions.



Figure 2: Distribution of microbial metabolisms associated with iron, sulfur, and methane cycles in the Raritan River sediments between the Lawrence Brook and Sayersville sampling sites. These metabolic guilds represent between 25-60% of the sedimentary microbial population, with the other primarily containing aerobic and anaerobic heterotrophic bacteria and archaea.

3.3 Porewater chemistry of Raritan River Sediments

Sulfate concentrations of overlying water and sediment pore waters from four sites along the Raritan River showed decreases in concentration from the overlying water with increasing depth (Fig 1). These decreases are indicative of bacterial sulfate reduction. The large deficits in sulfate concentration in surface sediments compared to overlying water in Site 2 and Site RR2 are indicative of rapid oxygen depletion and sulfate reduction over a narrow depth interval. This is indicative of high organic loading at these locations relative to Site 1 and Site 3. In contrast to sulfate concentrations, dissolved iron (II) concentrations increased from 0-40 μ M in overlying waters to as high as 1.8 mM in the shallower sediment horizons of Sites 2 and RR2 and subsequently decreased with depth. The combination of rapid sulfate reduction, iron reduction, and the lack of an apparent sulfide odor is indicative of the formation of amorphous pyrite (FeS) in these sediments. Bacterial species mediating organic carbon degradation coupled to iron and sulfate reduction led to a coupled iron, sulfur, and carbon cycle that resulted in a FeS species that appears to be associated with the sorption of additional metal species including Pb.



Figure 3: Sulfate concentrations of overlying water and sediment pore waters from four sites along the Raritan River.

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