Floodplain Pilot Study: Technical Briefing Paper

This briefing paper summarizes the objectives, study approach, and findings of the South River Floodplain Pilot Laboratory Study (Laboratory Pilot Study), and the plans for the South River Floodplain Field Pilot Study (Field Pilot Study). Laboratory Pilot Study findings are summarized below and plans for future Field Pilot Study are briefly discussed. The information reviewed and presented herein are not comprehensive; additional details regarding the scope of work, methods and results are documented in the Floodplain Laboratory Pilot Final Report (URS, 2014).

Introduction

Remedial options are currently being evaluated for their ability to reduce mercury uptake by South River floodplain soil biota. One option involves amending the floodplain soils with physical and chemical controls to decrease mercury bioavailability in soils. The Laboratory Pilot Study was designed to assess the viability and efficacy of a soil amendment (biochar) as a potential remedial option via its effect on soil mercury toxicity and uptake by earthworms and plants. Based on the results of the Laboratory Pilot Study, a Field Pilot Study is being planned for a field evaluation of the potential remedial option.

References

- ✓ Office of Economic Cooperation and Development (OECD). 2004. Test 222: OECD Guideline for the Testing of Chemicals. Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*). Adopted 23-November-2004.
- ✓ Office of Economic Cooperation and Development (OECD). 2006. Test 208: OECD Guideline for the Testing of Chemicals. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. Adopted 19-July-2004.
- ✓ EPA. 2013. Ecological Soil Screening Levels (Eco-SSLs). Accessed at: http://www.epa.gov/ecotox/ecossl/index.html
- ✓ URS Corporation. 2014. Floodplain Laboratory Pilot, Final Report for AOC 4, Former DuPont Waynesboro Plant, Waynesboro, Virginia. May 6, 2014.
- ✓ Berti, W.R., Guiseppi-Elie, A., Quinn, E., Jensen, R.H., Cocking, D. 2013. Evaluation of Garden Crop Mercury Uptake and Potential Exposure from Consumption of Garden Crops grown on Floodplain Soils. Human and Ecological Risk Assessment. 13(1): 215-231.
- ✓ Fagervold, S.K., Chai, Y., Davis, J.W., Wilken, M., and Ghosh, U. 2010. Bioaccumulation of Polychlorinated Dibenzo-p-Dioxins/Dibenzofurans in *E. fetida* from Floodplain Soils and the Effect of Activated Carbon Amendment. Environ. Sci. Technol. 44, 5546-5552.

Objectives

The purpose of the Laboratory Pilot Study was to test the efficacy of a soil biochar amendment in limiting the mercury bioavailability (and hence toxicity) toward biological receptors. Specific objectives were to evaluate biochar's:

- ❑ Potential to reduce mercury uptake by earthworms and plants from floodplain soil;
- ❑ Effects on mortality, weight change, and reproduction of earthworms;
- ❑ Effects on seed germination and shoot production in plants;
- ❑ Effects on potential bioavailability of mercury in soil to other ecological receptor; and
- ❑ Effects on mercury methylation in soil.

Soil Sample Collection and Characterization

Soil samples were collected on August 20, 2013 from two locations at the Augusta Forestry Center, approximately at relative river mile (RRM 11.8): one location to serve as the low total mercury (THg) "background soil" (outside of the 100-year floodplain), and the other with higher THg as the high THg test soil (within the 2-year floodplain). One composite soil sample was analyzed for a suite of chemical and physical characteristics, including metals, pesticides, herbicides soil properties [grain size; pH, total organic carbon (TOC), and cation exchange capacity (CEC) and % solids]. Three replicate samples were collected from homogenized soil and analyzed for THg to ensure that laboratory replicates had similar THg concentrations.

Laboratory Toxicity Testing

Wildlife International (Easton, MD) conducted the toxicity tests for the earthworm (*Eisenia fetida*) and three plant species, wheat (*Triticum* spp.), soybeans (*Glycine max)* and radish (*Raphanus sativus*). Procedures for both the earthworm and plant toxicity tests followed the standard protocols developed by the Office of Economic Cooperation and Development (OECD): OECD Test No. 222, Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*) (OECD, 2004) and OECD Test No. 208, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD, 2006).

Toxicities were tested on the two soils with three biochar amendments: 0% (control), 5% and 10% biochar in soils (on dry weight basis). Cowboy charcoal, a biochar derived from hardwood, was used to amend the soils. Tests were conducted in soils sieved to < 2 mm and amended with biochar sieved to < 1.25 cm.

Earthworm Test

Adult earthworms were exposed to six groups of soils (two soils each at 0, 5, and 10% biochar). Four replicates for each group were tested. Test duration was eight (8) weeks. Adult mortality and growth (weight change) were measured after 4 weeks. The adults were then removed from the soils and reproductive effects (number of offspring) were evaluated following another 4 weeks. Earthworm tissue and soil samples were collected and analyzed for THg and MeHg.

Terrestrial Plant Test

Seeds for each plant species (wheat, soybeans, and radish) were planted in the six soil groups and grown for 14 to 21 days, after which seedling emergence and plant growth endpoints (shoot weight and height) were measured. Following endpoint determination, two composite samples from each species were prepared from the six replicate samples and analyzed for THg and MeHg.

Soil Characterization Results

The mean THg concentration (± standard deviation) was 57.4 ± 0.3 mg/kg in the high THg soil and 0.05 ± 0.01 mg/kg in the background soils. The low standard deviations indicate that the soils were thoroughly homogenized and that exposure conditions in the toxicity test cells would be similar among replicates. Other conclusions include:

- Soil properties (pH, TOC, % solids, and CEC) were generally similar between the two soils.

- Concentrations of several metals (cobalt, copper, manganese, selenium, and zinc) in the high THg soil exceeded EPA's Ecological Soil Screening Levels (Eco-SSLs) for plants and/or invertebrates (EPA, 2013), suggesting that these metals in the high THg soil may contribute to potential toxicity in the soil samples.
- The two soils had similar characteristics in other respects and they are not impacted by herbicides, pesticides, or polychlorinated biphenyls.

Earthworm Test Results

Overall results indicate that biochar amendment is not expected to cause any adverse effects on invertebrates. The 4-week earthworm adult exposure was repeated (Experiment I and Experiment II) to collect sufficient adult tissues for mercury analysis because several adult worms from Experiment I escaped during depuration. The results of both Experiments I and II are discussed below.

- ***Mean Mortality:*** Results indicate that biochar amendment reduced the mortality in the high THg soil to levels observed in the background soil. Mean adult mortalities in Experiment I and II were greater than 30% in the untreated high THg soil, 5% in untreated background soil, and less than or equal to 5% in both soils (background and high THg soils) treated with biochar at 5% and 10% (Figure 1).

- ***Growth and Deformities:*** All surviving worms were normal in appearance (no deformities) in the background soil (all treatments) and the high THg soil at 10% biochar. In the high THg soil, deformities in survivors were generally reduced at 5% biochar compared with the control and deformities were altogether absent from the surviving worms in the 10% biochar treatment. Mean body weights (initial and final) and mean changes in the body weights in each treatment were not statistically different from the respective controls or between high THg or background soil.

- ***Reproduction:*** No statistical differences were observed between treatments and respective controls and between the high THg soil (all treatments including control) and control background soil.

- ***Mercury Uptake:*** Uptake of MeHg by the adult and juvenile worms is generally greater in the high THg soils than in the background soil. Uptake of MeHg by juveniles, but not adults, is statistically lower ($p<0.001$) in the soils treated with biochar (for both soils) (Figure 2).

Plant Test Results

Overall results indicate the biochar amendment (at 5% and 10% levels) do not cause any appreciable adverse effects on plants grown in the high THg soil. Beneficial effects were apparent (compared to the corresponding controls) as demonstrated by the growth of wheat and radish seedlings grown in biochar-treated high THg soils.

- ***Seedling Emergence and Survival***: No adverse effects of biochar amendments (compared to corresponding controls) were apparent on seedling emergence or survival, except for soybean seedlings grown in background soil at 5% amendment. Soybean seedling emergence was statistically different at all three measurement times in the 5% amendment level compared to the control.

- ***Seedling Growth:*** Adverse effects of biochar treatments on mean seedling heights were not apparent, except for soybean grown in biochar-treated background soil, which were statistically shorter compared to the corresponding control. Apparent beneficial effects demonstrated dose-dependence in wheat seedlings in both the background and the high THg soils and radish seedling in only background soil. Mean seedling biomass results were similar to the mean seedling height results.
- ***Mercury Uptake:*** Consistent with other findings of plant uptake of mercury in the South River (Berti et al., 2013), seedlings of all three species had low THg concentrations. THg concentrations in seedlings among all treatments were low with only two exceptions, but unrelated to the concentration of biochar. In general, THg and MeHg concentrations were higher in seedling grown in the high THg soils. Addition of 5% or 10% biochar did not have any effect on MeHg concentrations (Figure 3).

Test Soils

In high THg soils, MeHg concentrations were reduced over the course of the *E. fetida* experiments (i.e., 28 days and 56 days) by 50% in the 10% biochar treatment in Experiment II. In contrast, MeHg concentrations increased by 2 to 18-fold in background soils. The higher increases in the background soil were mostly due to the low starting MeHg concentrations. However, these changes were observed in a single replicate, indicating a relatively high degree of uncertainty (Figure 4).

Overall Findings of the Pilot Study

The Pilot Study found that biochar amendment of soils produced no apparent negative effects on earthworms or plants, and may have reduced mercury or metal-related toxicity on earthworms. In addition, MeHg uptake may be reduced by biochar in juvenile earthworms. Specific findings include the following:

- Based on mortality, growth, or reproduction in earthworms, biochar-related adverse effects are not apparent in soils amended with biochar. Biochar appeared to reduce mortality of adult earthworms in the high-THg soils from RRM 11.8, which was >30% in 0% biochar controls, but absent in 5% or 10% biochar treatments.
- In addition, there were apparent biochar-related increases in growth of worms in RRM 11.8 soils. There were no effects of biochar on plant emergence, growth, or biomass.
- Methylmercury concentrations in juvenile *E. fetida* were lower in biochar treatments in both high-THg and background soils. MeHg concentrations in adult *E. fetida* were reduced in biochar amended background soil, but not in biochar-amended high THg soil.
- It is unclear whether the elevated adult mortality and apparent sub-lethal effects (e.g., deformities) observed in the control high THg soil is due to mercury (at 57.4

mg/kg dw) or due to one or more metals exceeding the corresponding Eco-SSLs for soil invertebrates.

- Biochar amendment did not affect mercury concentrations in plants. In test soils, MeHg concentrations decreased by 5% to 50% over the course of the experiments, suggesting that biochar decreased mercury methylation in soil. The opposite pattern was observed in background soils, with increases of 50% to 95%.

Planning for the Field Pilot Study

Based on the results of the Laboratory Pilot Study, a Field Pilot Study is being planned to provide further information on the efficacy of carbon amendments to reduce mercury bioavailability in the floodplain soils. The objectives are similar to those of the Laboratory Pilot, but the overall objective is to demonstrate effectiveness and safety of using carbon amendments on a field scale. Many aspects of the Field Pilot are in development, but the following provides a general description of the study design.

The Field Pilot Study will be performed in the 0.3 to 2-year floodplain of the South River. The Field Pilot will likely be conducted at the August Forestry Center. Two different types of carbon amendments will be tested: cowboy charcoal and activated carbon. The amendments will be sieved to less than 0.25 mm and added to final concentrations of 5% dry weight in soil. The amendments will be tilled into test plots in the fall of 2014 and allowed to age.

Sampling will be conducted the following spring, and will involve two types of sampling: caged earthworms and native invertebrates (e.g., earthworm, predatory mite, collembolans, enchytraeid worms, spiders). Caged earthworms will be employed to reduce the ability for earthworms to travel below the zone where biochar is applied. Earthworms will be collected from an area outside the 100-year floodplain, measured and weighed and then added to earthworm cages made from PVC pipe with screened openings. Soil from the 0.3 to 2-year floodplain will be mixed with a carbon amendment and added to the cages which will be driven into the soil for a 28-day exposure. In addition, native invertebrates will be collected from the test plots; for soil-dwelling invertebrates (e.g., earthworms) a co-located soil sample will be collected. Invertebrates and soil will be sampled for the following analytes:

- THg and MeHg (Tissue, soil)
- % lipids (invertebrate tissue; f_{lipid})
- TOC (soil; f_{oc})

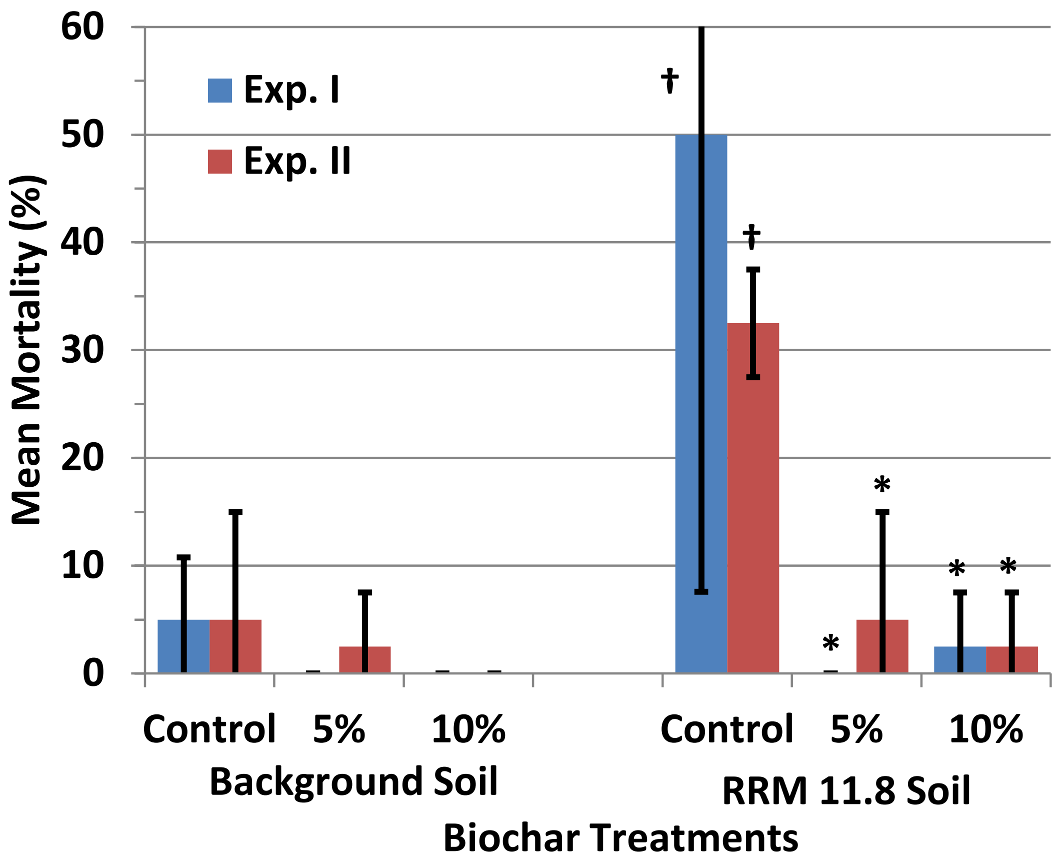
This analyte list will allow calculation of biota-soil accumulation factors (BSAF), which will control for the variability in the soil concentrations between controls and amended plots:

$$\frac{C_{worm}/f_{lipid}}{C_{soil}/f_{oc}}$$

Where C_{worm} is the concentration of mercury in invertebrates and C_{soil} is the concentration of mercury in soil (Fagervold et al., 2010). Invertebrates will be measured and weighed following collection. In addition, test soils will be collected for analysis of ancillary parameters (black carbon and nutrients).

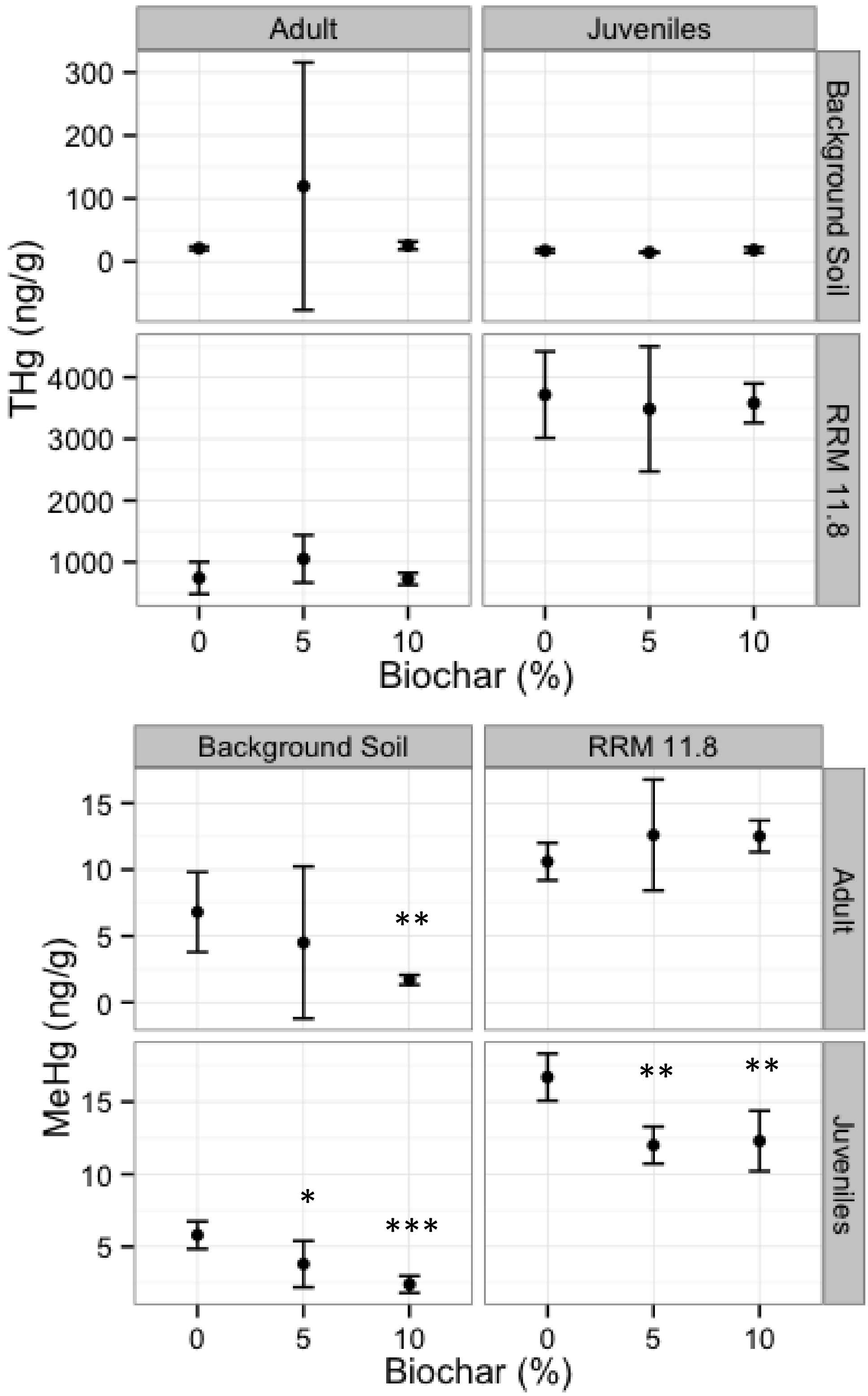
Sampling will be conducted for two successive years to test the efficacy period in soil. Because the life span of earthworms is typically one or two years, and the Laboratory Pilot Study only observed effects on uptake in juvenile earthworms, it will be important to sample earthworms that have been exposed to carbon amendment throughout their life.

Figure 1
Mortality in Adult Earthworms
Floodplain Laboratory Pilot for AOC 4
Former DuPont Waynesboro Plant, Waynesboro, Virginia



Notes:
Mean Standard Deviation shown; Exp. I - Experiment I; Exp.II – Experiment II;
*Statistically different (at $p < 0.05$) from corresponding control;
† Statistically different (at $p < 0.05$) from background control.

Figure 2
Mercury Concentrations in Earthworms
Floodplain Laboratory Pilot for AOC 4
Former DuPont Waynesboro Plant, Waynesboro, Virginia



Notes:
Mean Standard Deviation shown.
*p<0.05; ** = p<0.01; ***p<0.001

Figure 3
Mercury Concentrations in Seedlings
Floodplain Laboratory Pilot for AOC 4
Former DuPont Waynesboro Plant, Waynesboro, Virginia

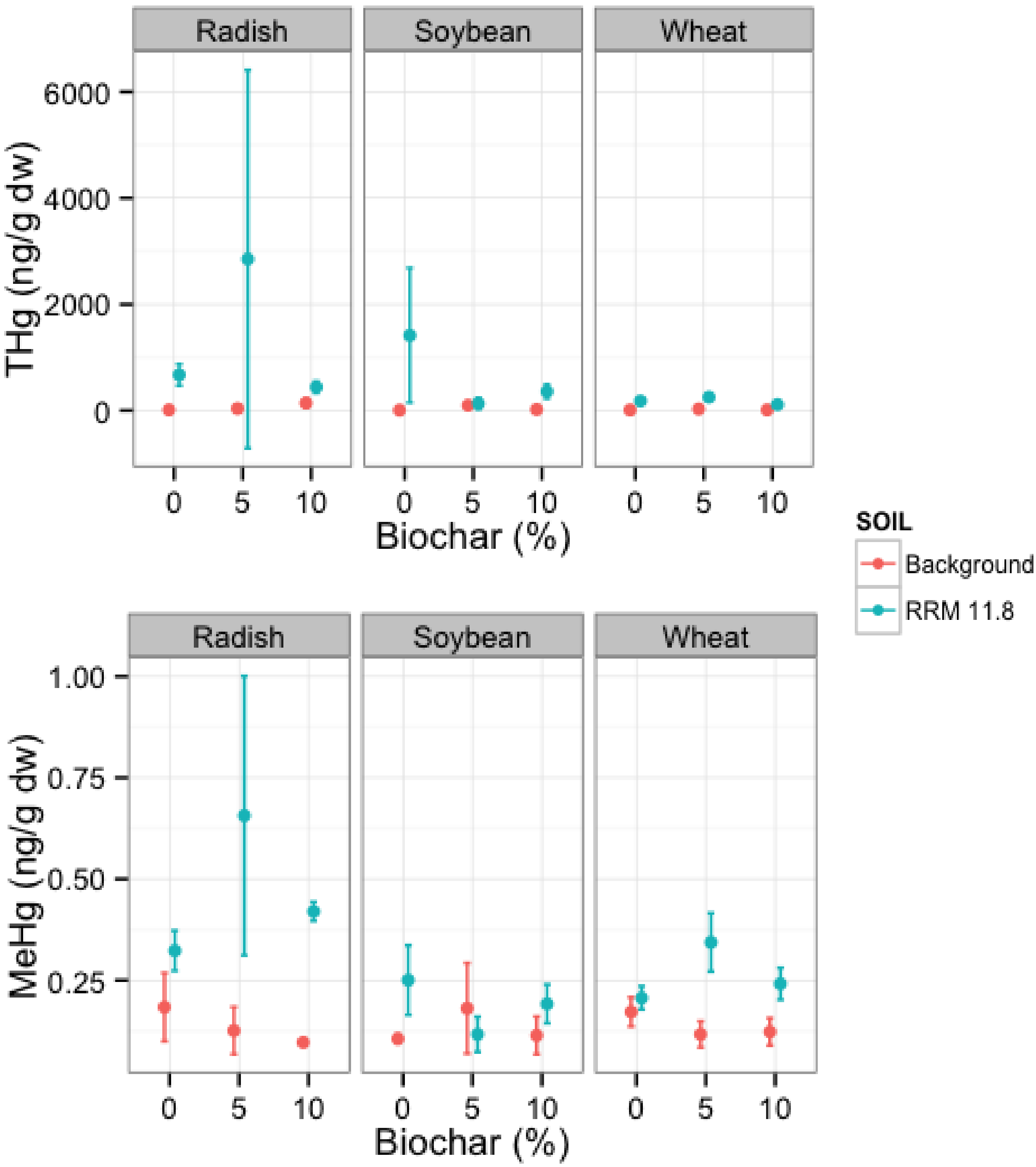


Figure 4
Mercury Concentrations in Test Soils
Floodplain Laboratory Pilot for AOC 4
Former DuPont Waynesboro Plant, Waynesboro, Virginia