Title: Methylmercury at the abandoned Cinnabar Mine, ID: Assessment of current conditions and potential impacts of remediation actions involving organic carbon additions.

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1. Introduction

The western region of North America contains numerous areas or mercury (Hg) enriched ore deposits, which were extensively mined starting in the mid-1800s and mostly closing by the 1970s (Rytuba, 2000). As a result, thousands of abandoned Hg mines occur throughout the western US (Eagles-Smith et al., 2016). The processing of the Hg ore typically occurred at the mine sites via heating the ore and condensing the Hg vapor. Due to an incomplete extraction process, the waste tailings materials generated at these mine sites typically contain Hg concentrations many orders of magnitude above background concentrations (Kim et al., 2000; Rytuba, 2000). For some of the larger production mines, these tailings piles can be several meters deep and spread out over many hectares (Gustin et al., 2003; Nacht et al., 2004). Mercury mobilization from these tailings has been shown to negatively impact downstream aquatic ecosystems which often result in fish consumptions advisories related to Hg pollution (Park and Curtis, 1997; Suchanek et al., 2008). Identifying effective strategies to reduce the impacts of these mines is an area of ongoing research and discussion.

The Cinnabar Mercury Mine is an abandoned mine in central Idaho and is part of the South Fork of the Salmon River catchment. Cinnabar Creek flows through the tailings pile at the mine site and has been shown to contain elevated Hg concentrations (Holloway, 2016). A USGS stream monitoring station on the South Fork of East Fork of the Salmon River, which is more than 10 km downstream of the mine site, measured total-Hg (THg) concentrations during periods of elevated discharge up to 26,000 ng/L and depending on the water-year has annual loads between 13 and 28 kg (Etheridge, 2015). Annual flow-weighted stream concentrations of dissolved arsenic were around 11 µg/L, which is just above applicable human health criteria values. These elevated concentrations, particularly for Hg, are believed to reflect ongoing releases from the Cinnabar mine(Etheridge, 2015). In general, most Hg that is released from abandoned Hg mines occurs via the erosion of tailings and other contaminated materials, particularly during periods of elevated discharge (Curtis et al., 2013; Eckley et al., 2015; Etheridge, 2015). Remediation efforts aimed at reducing erosion of high Hg content materials can decrease the impacts of Hg on downstream ecosystems (Marvin-DiPasquale, 2011).

In general, the ore and waste materials associated with Hg mines occurs in various inorganic forms (Kim et al., 2004; Lowry et al., 2004; Sladek and Gustin, 2003). However, the main concern with Hg pollution is due to methylmercury (MeHg), an organic form of Hg that has increased toxicity and efficiently bioaccumulates up the food-chain. Almost all of the Hg found in sport fish, or any upper tropic position fish species, is MeHg and it is the main form associated with human exposure. As such, a key aspect of reducing the impacts of Hg pollution in the environment, focuses on decreasing the production of MeHg. To be effective, Hg mine site remediation strategies should take into consideration the impacts on MeHg production.

Mercury is methylated by anaerobic bacteria, with sulfate reducing bacteria (SRB) being one of the main groups associated with this process (Ullrich et al., 2001); though other anaerobic bacteria have also been shown to methylate Hg as well (Warner et al., 2003). Within areas where anoxic conditions occur, there are several factors that affect the amount of MeHg produced: 1) the concentration of bioavailable inorganic Hg; 2) the concentration of labile organic carbon; and 3) the concentration of sulfate or other elector accepting compounds such as ferric iron. Bioavailable inorganic is typically a small percentage of the THg (Marvin-DiPasquale et al., 2009). In general, Hg bound to the solid-phase matrix may be less bioavailable, whereas Hg in the porewater/dissolved phase typically has a higher bioavailability (Hsu-Kim et al., 2013; Schartup et al., 2014). This is particularly relevant at abandoned Hg mine sites, where the Hg concentrations of tailings may be very elevated, but may mostly occur bound to sulfide containing minerals such as cinnabar that have low solubility and bioavailability. Similarly, the quality of organic carbon and not just the total quantity can be an important factor governing its sorption with inorganic Hg as well as its utilization by microbial communities(Burns et al., 2013; Marvin-DiPasquale et al., 2009; Schuster et al., 2008). Finally, the relationship between sulfate and MeHg production is also complicated and has been suggested to follow a bell shaped curve, with MeHg production occurring at an optimal mid-level of sulfate concentrations and decreasing at lower and higher concentrations(Benoit et al., 2003; Gilmour and Henry, 1991). Because multiple factors can effect MeHg production, it is often difficult to determine which of these variables is controlling the amount of MeHg produced at a location. It is even more difficult to predict how MeHg production may be effected in the future under changing environmental conditions, such as mine site remediation efforts. In light of these challenges, remediation efforts at Hg mine sites need to be able to predict impacts of the actions taken on MeHg production.

Remediation activities at Hg contaminated sites can take several forms, but often involve the excavation and disposal, capping, and/or armoring of stream channels near high Hg content materials to reduce erosion and aqueous transport. Other remediation options that have been applied to a lesser extent at Hg contaminated sites include: material stabilization/solidification, immobilization by adding stabilizing agents, vitrification, thermal desorption, soil washing (Wang et al., 2012). Some of the stabilization techniques such as biochar and activated carbon applications have shown the potential to reduce the bioavailability of inorganic Hg for methylation(Bussan et al., 2016; Gilmour et al., 2013).

Cinnabar Mine, like many abandoned mine sites in the western US, is located in a remote mountainous region, which affects the feasibility of applying different remediation options. One potential remediation option that could be implemented focuses on reducing the erosion of high Hg content tailings from the site by adding soil amendments that can promote the establishment of vegetation. While this would likely reduce the bulk loading of Hg as well as other heavy metals such as arsenic to streams, the addition of carbon has the potential to stimulate microbial activity and result in an increase in MeHg production. Providing information to assess the benefits of reduced mobilization of THg from the site and the potential impacts of on-site MeHg production is the focus of this study.

The overall objective of our study is to identify if the addition of organic material to mine tailings will increase MeHg production. The variables affecting Hg methylation rates are numerous, complex, and not entirely understood (Benoit et al., 1999; Graham et al., 2012; Hsu-Kim et al., 2013). As such, predicting future changes in Hg methylation as a result of remediation actions contain a fair degree of uncertainty. To address this uncertainty, will be using multiple lines of evidence to support our predictions. These include: 1) an assessment of current MeHg concentrations in stream water, porewater, and sediment at the site and the relationship to several ancillary parameters; 2) the measurement of Hg methylation rates at the site in response to different aqueous carbon amendments;

and 3) mesocosm experiments where carbon amendments are applied to tailings materials and MeHg production monitored. The results from these three distinct experimental approaches will be used to provide a robust assessment of the potential for increased MeHg production in response to site remediation efforts that involve carbon amendments.

2. Methods

2.1. Current MeHg concentrations at Cinnabar Mine.

Macro-Rhizon samplers (Rhizosphere Research Products, 0.15 µm, 9 cm porous part) were used for porewater collection(Seeberg-Elverfeldt et al., 2005). The Macro-Rhizons were inserted into stream sediment at the water line. Porewater was collected under vacuum into new 50 ml syringes that were luer locked to the Macro-Rhizons and subsequently transferred to separate sample bottles for THg/MeHg, DOC, sulfide, and sulfate analysis. All samples were preserved in the field following collection: THg/MeHg (ultra-pure HCl); DOC (sulfuric acid); sulfide (zinc acetate); sulfate (4°C).

Porewater samples were co-located with surface and sediment water samples at the following locations: upstream Sugar Creek (BG02), downstream Sugar Creek (SC01), Cinnabar Creek (CC02 and CC04), West Fork Cinnabar Creek (WF01-05), and the Cinnabar wetlands (WT01-3) (See Figure 3-1 in the parent document).

Collection of sediment and surface water is described in the parent document.

2.2 Stable Hg isotope addition experiments.

Solutions consisting of four different types of dissolved organic carbon were created to identify the influence of carbon quantify and quality on methylation rates. Four types of organic material were tested: 1) Proganics™ soil media, 2) Biotic Earth™ soil media, 3) biochar; and 4) natural organic matter (NOM). The Proganics™ and Biotic Earth™ are both engineered soil media designed to improve the agronomic potential of soils/substrates so that vegetation can be established and erosion reduced. The Progancis™ material is a combination of recycled thermally refined bark and wood fibers with a proprietary blend of biopolymers, biochar, and other minor constituents. The Biotic Earth™ is manufactured from thermally and mechanically processed straw, flax and peat moss, with other minor constituents. The biochar used in our study was created from a 50:50 mixture of pyrolized wood chips and poultry litter. The natural organic matter material used in our study consisted of soil litter material, which was mostly pine needles, collected at a background location near the mine site.

Approximately 100 grams of each of these organic materials were placed in separate bottles containing 1 L of DI water. After 3 days, the water from each of these mixtures was filtered (0.45 µm) to create four different organic carbon extracts. In the field, an inorganic enriched stable Hg isotope (Hg^{201}) tracer was added to each of the organic carbon extracts, plus a control solution that consisted of water collected from an on-site seep. The concentration of Hg isotope tracer in each of the solutions was 5 mg/L. Each of these solutions was purged with ultra-high purity nitrogen gas to remove all oxygen from the solutions for 1 hour prior to injecting into the soil cores.

Twenty soil core samples were collected for stable Hg isotope addition methylation assays from a small meadow created by a seep that was ~30m from the historical tailings pile at the site. The samples were

collected to a depth of ~10 cm in small diameter (5 cm) polycarbonate core tubes and capped. All cores were collected from the same meadow (~75 m^2) in the hopes of representing similar biogeochemical conditions.

Within a few hours after collecting the soil cores, the top 4 cm of each core was spiked with 1 ml of Hg^{201} isotope solution which were applied using a syringe through small holes in the core tube that were drilled 1 cm apart and sealed with silicone. Each 1 cm section of the core received 5 µg of Hg^{201} isotope for a total of 20 µg added within the top 4 cm of the core. Each of the 5 organic carbon/Hg isotope solutions was injected into 4 replicate soil cores. The soil cores were incubated for 24 hours at ambient outside temperatures (5 to 15 °C). The incubations were terminated by extruding the soil from the core tubes and placing it in 4 oz glass jars that were immediately frozen by placing in a cooler with dry ice. The methylation rates are presented as the % of added inorganic Hg tracer that was methylated per unit time.

2.3 Laboratory mesocosm study.

Three full 5-gallon buckets of tailings materials were collected from the yellow flotation tailings piles at Cinnabar Mine during the sampling in August 2016. In addition, 30L of water from Cinnabar Creek upstream of the mine was collected in new plastic carboys. The tailings and water were transported to the EPA's Manchester Environmental Laboratory (MEL). The tailings were sequentially combined into a large plastic tub and mixed by hand with a shovel. The homogenized material was then fractionally shoveled into six 3 gallon buckets (hereafter referred to as mesocosms) by scooping an approximately equal amount of material in each mesocosm back and forth until all sample material has been distributed across all buckets. The depth of material in each mesocosm was 15 cm (~50% full). The water sample was filtered (0.45 µm) and mixed 50:50 with DI water to create a working solution for use in the experiment.

Three of the mesocosms contained un-amended tailings and three were amended with 600 grams of ProGanics™ Biotic Soil Media. This addition of ProGanics™ material represented an 5% increase in the mass of the mesocosm material. This may be a higher rate of application than would typically occur during a remediation project and so the results likely overestimate the effect observed during an actual field application.

At the beginning of the experiment the working solution was added to each mesocosm until the materials were fully saturated (un-amended: 2 Liters to reach 15% moisture content; amended: 6 Liters to reach 40% moisture content). Each mesocosm contained a sampling port 4 cm above the bottom that contained a MacroRhizon porewater sampler. Within an hour of wetting the materials, porewater samples were collected from each mesocosm for the analysis of THg, MeHg, sulfate, and DOC. In addition water was collected for analysis using a Horiba datasonde for pH, redox potential and dissolved oxygen (DO). The materials were allowed to dry over a two week period to soil moisture contents of 7% in the un-amended tailings and 28% in the amended tailings. In both types of mesocosm, this represents a 50% decrease in the water content below the initial saturated conditions. The mesocosms were then re-wetted to fully saturated conditions with the working solution and porewater collected. Fully saturated conditions were maintained over the ensuring 3 week time period by adding enough water so that there was at least 5 cm of water overlying the tailings and then sealing the mesocosms to reduce evaporation. Porewater samples were collected 1 week and 3 weeks after fully saturated conditions had been established. At the termination of the experiments, a solid-phase sample was collected from each mesocosm for THg, MeHg, TOC, and sulfide analysis.

3. Results and Discussion

3.1 Results from field measurements of water, porewater, and sediment.

3.1.1 Stream data

The dissolved MeHg (or MeHg-D) concentrations in stream samples at and downstream of Cinnabar mine are generally low (<0.15 ng/L). Only a limited number of whole water MeHg (or MeHg-W) samples were collected (n=4), however these samples show that the majority of MeHg was in the dissolved phase (mean ±SE: 83 ±7%). For context, a fairly comprehensive assessment of stream MeHg-W concentrations across the US, the mean MeHg-W concentration of 0.19 ng/L (Scudder, 2009). As such, the existing methylation potential in this area is relatively low, which is to be expected given the well oxygenated steep gradient streams with limited sediment in this area. The one exception being the water sample from the wetland area which had a MeHg concentration of 1.6 ng/L, over an order of magnitude higher than the maximum concentration measured in flowing water. These results suggest that the methylation potential at the site is not limited by the bioavailability of inorganic Hg—the amount of Hg in the tailings is so large that even a small % that is bioavailable would still be a big number. Instead methylation appears to be limited by conditions conducive to methylating microorganisms—stagnant water and fine grain sediment. The spatial extent of the wetlands in very small and does not appear to be hydrologically connected to Cinnabar Creek, at least during base flow conditions.

While all the MeHg-F stream concentrations were relatively low, the THg-F (up to 98 ng/L) and THg-W (up to 320 ng/L) concentrations are quite elevated relative to background levels for this area (THg-W: 0.67 ng/L; THg-F: 0.65 ng/L).

The THg-F concentrations were highest near the mine site and decreased with distance downstream, which is likely a result of dilution (**Figure 1**). In Sugar Creek, about 4km downstream of Cinnabar Mine, the THg-F concentrations were pretty consistent at 8.8 ±0.6 ng/L. Conversely, MeHg-F concentrations were lowest near Cinnabar Mine and increased downstream (**Figure 1**). This suggests that the conditions conducive to methylation are likely lower near the mine site and increase downstream, or that other sources of MeHg exist within other drainages of Sugar Creek. In general, these results suggest that while there is limited Hg methylation currently occurring in streams near Cinnabar Mine site, the THg exported from the mine site may become methylated downstream where conditions are more favorable. As such, efforts to reduce THg loading from the mine into area creeks may reduce downstream MeHg production.

The ratio of MeHg/THg is often used as a measure of the methylation potential in an area (Mitchell et al., 2008). Plotting the %MeHg moving downstream of the mine clearly shows that the methylation potential at the mine is low and increases with distance downstream (**Figure 2**). From other studies, the median %MeHg ratio in streams in mining areas is 2.4% (Scudder, 2009). All of the %MeHg ratios in our measurements were <2%, which indicates that the methylation potential in this area is currently low.

For the subset of stream samples where whole and filtered water samples were collected, the results show that the majority (75 ±8%) of THg was associated with particles in Cinnabar Creek. In comparison, the background stream location on Sugar Creek had only 3% of the THg associated with particles. These

results suggest that the erosion/entrainment of particles from Cinnabar mine is an important mechanism for downstream loading.

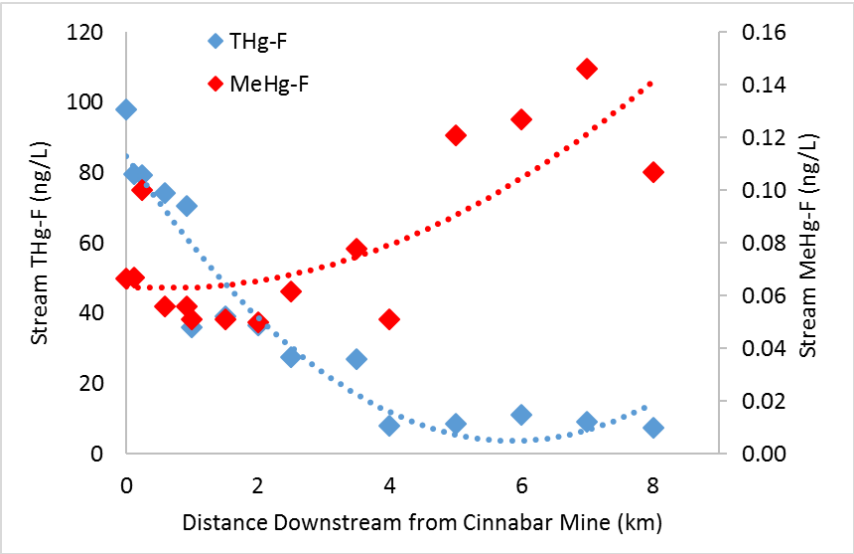

Figure 1. Graph showing filtered THg (blue diamonds) and MeHg (red diamonds) concentrations measured in the West Fork of Cinnabar Creek, Cinnabar Creek, and Sugar Creek during the August, 2016 field sampling campaign. The stream concentrations are plotted against the estimated distance downstream from the Cinnabar Mine site. A 2-order polynomial regression line was used to highlight the general downward trend in THg concentrations and upward trend in MeHg moving downstream from Cinnabar mine.

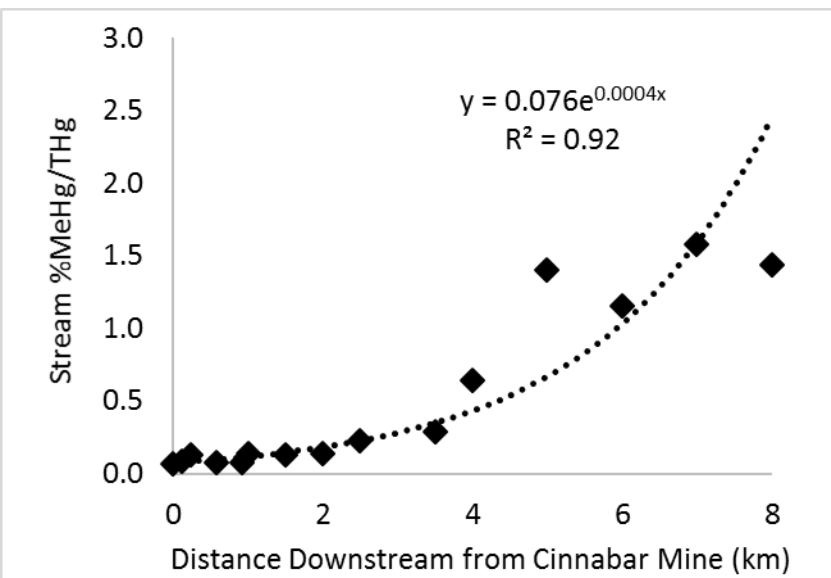

Figure 2. Graph showing the filtered %MeHg/THg downstream of Cinnabar Mine. An exponential regression line was fit to the data points.

3.1.2 Sediment and porewater data

Multiple linear regression (MLR) analysis of the sediment data showed that there was a significant relationship between TOC and MeHg (**Figure 3**; R^2=0.43, df=23, p<0.001), but that there was no significant correlation with THg (p=0.21). This is consistent with the results observed from the surface

water samples where the MeHg production appears to be related to factors other than just THg concentrations.

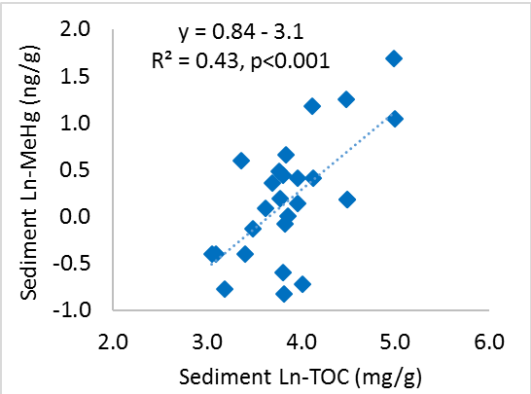

Figure 3. Stream sediment TOC versus stream sediment MeHg (on Ln-scale)

Stream sediment, porewater, and surface water THg concentrations are all significantly related (**Figure 4**). The porewater THg and DOC concentrations showed no relationship (p=0.79, df=11), suggesting that the partitioning of THg into the porewater phase is not being driven by the dissolved carbon in the system. This is in contrast to what occurred in the mesocosm experiments described later, where the addition of organic carbon to the tailings appears to have facilitate the partitioning of THg from the solid phase of tailings into the porewater phase. This difference in results may be a function of: 1) different characteristics of the natural carbon compared to the Proganics^{TM} material; 2) differences in THg sorption with the stream sediments versus the tailings; or 3) the mesocosm results representing a temporally isolated flux after new carbon is added and would be apparent under the existing conditions at the site. Regardless, under current conditions at the site, the movement of THg from the solid phase into the porewater and surface water does not appear to be controlled by DOC.

The relatively strong correlation between porewater and surface water THg-F concentrations (**Figure 4**) suggests that shallow groundwater flow may be an important source of THg during baseflow conditions.

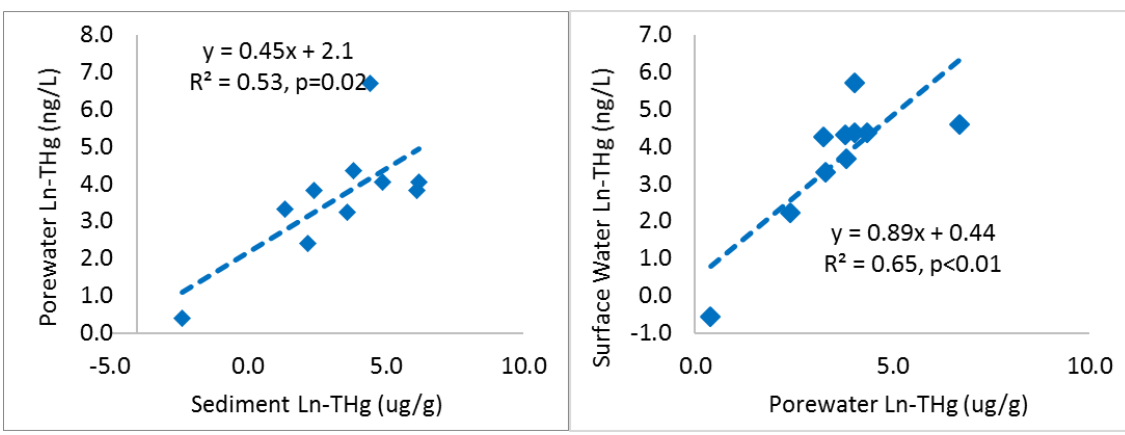

Figure 4. Left graph shows relationship between sediment and porewater THg. Right graph shows relationship between porewater and surface water THg. Both graph are plotted on natural log scales.

Unlike THg, the only parameter that porewater MeHg concentration were significantly correlated with were porewater DOC (**Figure 5**), with porewater THg and sulfate showing no significant relationship.

However, if the sample point from the wetlands area is removed, then this correlation is no longer significant (p=0.22, df=9). Given the fairly elevated THg concentrations in the water, porewater and sediment in the Cinnabar area, it is not surprising that THg concentrations are not limiting MeHg production. Instead, MeHg production appears to be limited by carbon availability, which is generally low in the stream porewater samples (2.5 ±0.5 mg/L) and only relatively high in the wetlands (6.5 ±1.7 mg/L). Because we only see a significant relationship between porewater DOC and MeHg when including the wetlands sample, this suggests that the conditions in the wetland responsible for methylation (such as low dissolved oxygen) were a more important factor affecting methylation than just the amount of carbon present.

Relative to surface water, there is a less robust national average value for porewater since this is a less common measurement. However, one paper that included data from several streams throughout the US found that the average concentration was 0.34 ng/L (Marvin-DiPasquale et al., 2009). All but one porewater measurements in our study were below this average value, which indicates that the magnitude of the MeHg concentrations in porewater in our study are similar to streams in regions un-impacted by a Hg mine.

The stream porewater sample locations that occurred downstream of the mine site were forested/vegetated. In the context of the question this study is addressing—will organic carbon additions and the establishment of vegetation increase MeHg production—it is noteworthy that the porewater DOC concentrations from the forested stream sections were all low (2.3 ±0.4 mg/L). This suggests that establishing native vegetation on the tailings may not significantly increased porewater DOC concentrations and stimulate MeHg production.

An import message from these field measurements is that: the vast majority of the watershed area of Sugar and Cinnabar Creek are forested. Even at the Cinnabar Mine site, there are patches of vegetation. While the THg concentrations in these streams were quite elevated above background level, the methylation potential and MeHg concentrations measured within this watershed are all lower than mean and median concentrations measured in other streams throughout the US (Scudder, 2009). These results suggest, that at a watershed level, a remediation action that resulted in vegetation covering the tailings would likely have a negligible impact on organic carbon in the stream and may not result in increased MeHg production.

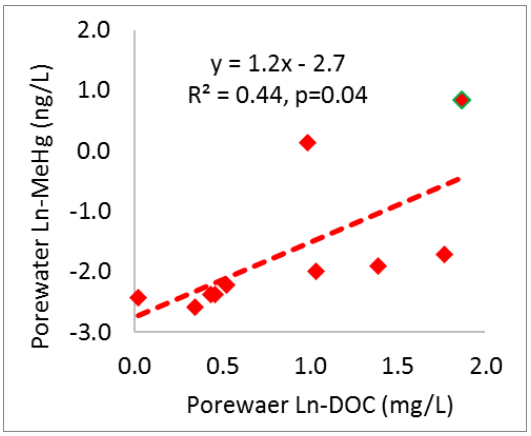

Figure 5. Porewater MeHg versus DOC concentrations. Note, the sample with the green outline is from the wetland area, whereas all other samples are from streams.

3.2 Results of the stable isotope addition methylation assays

There was a large range in DOC concentrations in the Hg isotope spike solutions (1-1,500 mg/L; **Figure 6)**. The solutions with the highest DOC concentrations were the Proganics and natural organic matter (NOM) solutions. The methylation rates in the soil cores that were spiked with these two solutions were higher than the other amendments and the control. The soil organic matter content (measured as %LOI) showed some variability between the soil cores used in the assays (68± 10 to 80± 1%--note that in Figure 6 these values are divided by 200 so that they plot on the same scale as methylation rates); however the trends in MeHg production were much more clearly affected by the dissolved carbon additions in the spike solutions than the solid organic matter content of the soils.

The low DOC concentrations in the Bio-Earth and biochar amendments suggest that not all types of soil amendments have the same propensity to generate DOC. However, it is important to note that DOC is just a measure of the total amount of carbon in the sample, but does not tell us about differences in the quality of that organic carbon. Excitation-Emission Matrixes (EEMs) are a method that has been used to identify differences in the quality of organic carbon (**Figure 7**). The results provide values for different EEMs peaks which are associated with different components of organic material. Comparing the isotopic methylation rates with each of the EEMs peaks, showed that the T and N peaks have the best correlation (R^2 value of 0.73 and 0.80 respectively). Correlations with all of the other EEMs peaks and methylation rates resulted in lower R^2 values than obtained when comparing directly with DOC (R^2=0.72). The T and N peaks are associated with relatively labile humic acid components of DOC, which may be preferred by the microbial communities involved in methylation. These results help explain why the biochar solution had a low total DOC concentration (similar to the control and Bio-Earth) values, but a higher methylation rate, similar to the NOM and Proganics solutions (**Figure 8**).

Overall, these results highlight the important role that organic carbon quality has on stimulating Hg methylation, which may not be captured by just looking at the total amount of organic carbon in a system. Furthermore, these results also suggest that different types of soil amendments (i.e. Bio-Earth, Proganics, etc) contain different qualities of carbon and may be selected to maximize the revegetation potential of a site, while minimizing an increase in Hg methylation. While these findings are compelling because they suggest that using a soil amendment such Bio-Earth instead of Proganics could be applied without stimulating MeHg production, these results are based on a single sample of the carbon quality from each spike solution and only four methylation assay samples. Additional samples may need to be collected to confirm these findings.

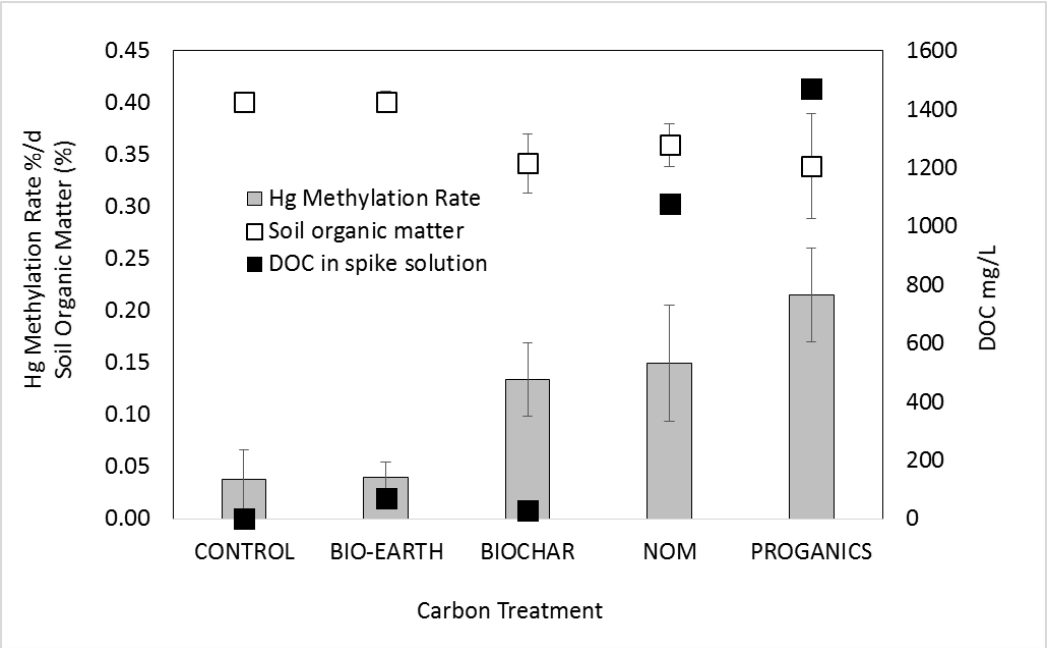

Figure 6. Graph of Hg stable isotope methylation rates and organic carbon concentrations. The columns represent the mean ±SE stable isotope Hg methylation rate from four replicate assays. The black square markers represent the DOC concentrations of the solution used to deliver the Hg isotope; and the white squares represent a measure of the mean ±SE soil organic matter content from each of the assays. The soil organic matter was measured as % loss on ignition (LOI) and in order to present that data on the scale of this graph, the values were divided by 200.

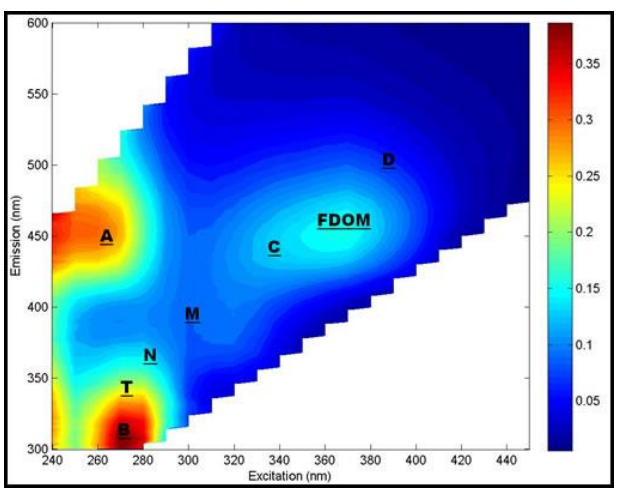

Figure 7. An example excitation-emission matrix (EEM) showing the general locations of selected fluorescence peaks. Letters represent key peaks in excitation-emission responses and are associated with different sources and components of organic matter in water. Text and Figure source (https://or.water.usgs.gov/proj/carbon/EEMS.html)

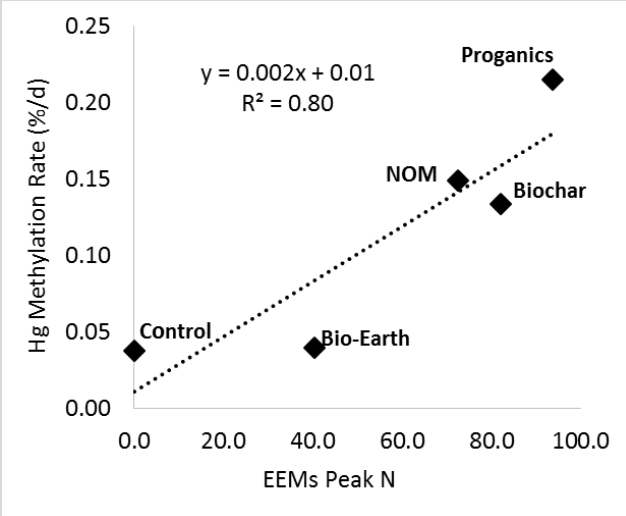

Figure 8. Regression of stable isotope Hg methylation rates versus EEMs Peak N values. The type of treatment is labeled next to each data point. The results show that the control sample and Bio-Earth both had lower methylation rates and lower Peak N values. While the NOM, biochar, and Proganics all had higher methylation rates and Peak N values.

3.3 Results of the mesocosm amendment study

The mesocosm experiments were used to test the impact of Proganics carbon amendments on tailings samples collected at the site. Both the amended and un-amended tailings showed very similar changes in DO concentrations over the duration of the experiment (**Figure 9A**). The DO concentrations quickly dropped in half after first week of the experiment, and became completely anoxic after 19 days. Whether the tailings were amended or not appeared to have no effect on the development of anoxic conditions. While both types of mesocosms showed similar impacts on DO, the amended tailings clearly had a lower redox potential (**Figure 9B**) and an increase in DOC (**Figure 9C**). Consistent with the lower redox potential in the amended tailings we also see a decrease in sulfate during the experiment (**Figure 10C**), indicating that the conditions are conducive to the activity of SRB that are often associated Hg methylation. As such, we observed MeHg concentrations to increase over the duration of the experiment in the amended tailings, but remained much lower in the un-amended tailings (**Figure 10B**). The MeHg concentrations were increasing in the amended tailings during each sampling event; however it is not clear how long and at what concentrations the MeHg concentrations would eventually plateau and steady-state conditions would develop.

These results clearly show that the addition of organic material to the tailings has the potential to generate high concentrations of MeHg. However, in the mesocosms the tailings were saturated with stagnant water and allowed to go anoxic. This is a critical factor for allowing methylation to occur. Under current conditions at the Cinnabar Mine site, there are only a few spatially isolated areas where the tailings are saturated with stagnant (or slow moving) water, such as the wetland area and a few seeps. It is worth noting that the MeHg concentrations measure in the un-amended tailings (all <0.4 ng/L) are of similar magnitude as porewater measurements collected along the streams and discussed previously in section 3.1. The amended tailings showed porewater MeHg concentrations by the end of the experiment that were larger than any of the values measured in the field, including the wetland areas. These results suggest that the addition of organic carbon to the tailings has the *potential* to generate high concentrations of MeHg under the right conditions (i.e. anoxic stagnant water). However,

these results likely represent a theoretical upper level of MeHg production (though we do not know how high these concentrations would have reached if the experiment was continued longer), and it is unlikely that an actual organic carbon amendment to the tailings in the field would result in saturated anoxic conditions developing.

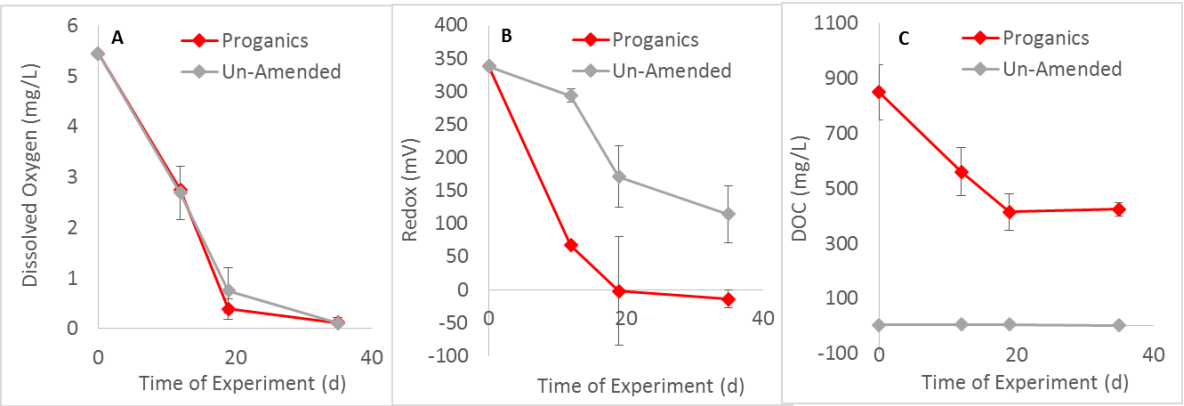

Figure 9. Graphs of several ancillary parameters (mean ±SE) measured during the duration of the mesocosm experiments: A) Dissolved Oxygen (DO); B) Redox potential; and C) DOC.

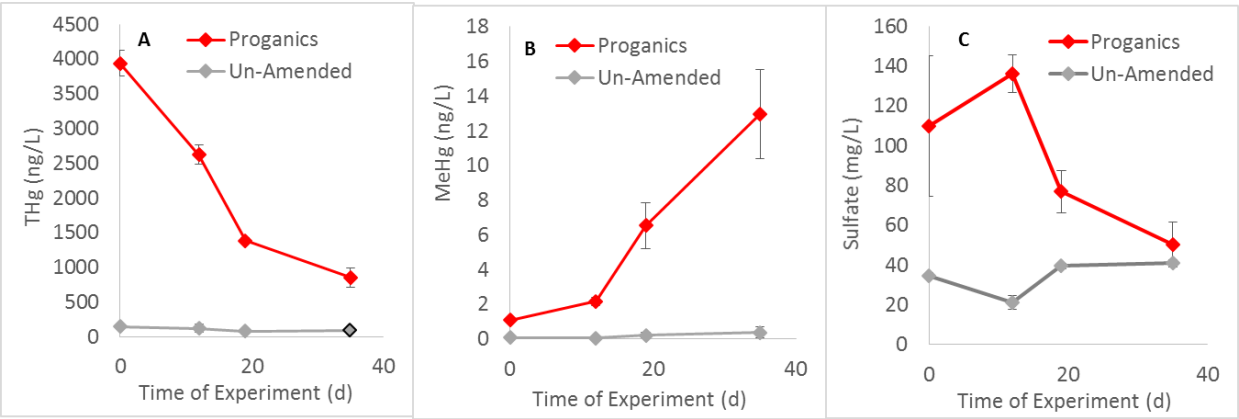

Figure 10. Graphs of measured parameters (mean ±SE) during the duration of the mesocosm experiments: A) THg (DO); B) MeHg; and C) Sulfate. All symbols represent the mean value from the three replicate mesocosms, with the exception of the THg value from the un-amended tailings collected on the 35th day of the experiment (symbol shown with black outline on graph A), which is based on the mean of two samples. The third sample had a concentration of 5,490 ng/L which was over an order of magnitude larger than the other two mesocosm, but was also an order of magnitude higher than measured from this same mesocosm during the previous three sampling events on days (0, 12 and 19). As such, it was considered an outlier and not included in the analysis.

The results showing that adding water and carbon to high Hg content tailings results in MeHg production is consistent with our expectations and a large body of scientific literature on the factors affecting Hg methylation (as summarized in Ullrich et al, 2001). The results showing much higher THg concentrations in the amended tailings were more surprising, particularly given the magnitude of the difference between the amended and un-amended tailings (amended: 3,940 ±326 ng/L; un-amended: 148 ±21 ng/L; **Figure 10A**). There are two key aspects of these results. One is that the THg concentrations were highest at the start of the experiment and dropped significantly over the duration of the study, though the study was not conducted long enough to identify when and if the THg concentrations would become similar to the un-amended tailings. However, extrapolating a regression slope from these data points indicates that a concentration of 90 ng/L (which is what the un-amended

tailings showed) would be reached after 40 days. The second key point is that the highest concentrations of THg occurred when conditions in the materials were still oxic (see **Figure 9A**). As such, these results from the mesocosm study would likely translate well to what we might see during an on-site application, with the caveat that our application rate of Proganics material was likely higher than what would occur during an actual remediation.

In general, inorganic Hg has a strong affinity for binding with sulfur compounds. Organic matter contains several functional groups that include sulfur, which inorganic Hg likes to bind to. This is why almost all dissolved Hg in aquatic systems is associated with DOC. In our mesocosm experiment, the addition of Proganics material resulted in some of this carbon dissolving into the water (becoming DOC; **Figure 9C**) and the sulfur containing functional groups on this DOC were able to "pick up" some of the more loosely bound inorganic Hg in the tailings. Presumably, the vast majority of the THg associated with the tailings if fairly tightly bound and the ability for DOC to facilitate the mobilization of Hg from the solid phase of tailings into the aqueous phase may be short lived (i.e. ~40 days) at the high rates we observed, but may continue over a longer duration at a reduced rate. Our mesocosm study did not last long enough to identify the longer term impacts of the carbon addition on THg mobilization.

Theoretically, the addition of organic material could reduce the pH, which could further facilitate the partitioning of Hg from being bound to tailings and promote it dissolving into the aqueous phase. Though in our study, we observed similar pH values in the amended and un-amended tailings (amended: 5.7 ±0.2; un-amended: 5.8 ±0.6).

3.4 Overall conceptual model assessment of results.

Our conceptual model of Hg dynamics at the site suggest that during baseflow periods some of the Hg associated with the tailings dissolves into the water as it moves towards Cinnabar Creek. This results in elevated THg-F concentrations in Cinnabar Creek. THg-P concentrations are also elevated during baseflow conditions; however these concentrations are expected to increase by several orders of magnitude during stormflow periods, which are observable several km downstream (Etheridge, 2015). Apart from the small wetlands area, the conditions conducive to MeHg production at the mine site are low. Conditions favorable for methylation increase downstream of the site, but still remain relatively low. Further downstream, the methylation potential likely further increases as the gradients decrease further and the river shifts from being dominated by erosion to deposition. As such, efforts to reduce Hg transport from the mine site may reduce Hg availability for methylation downstream.

The results from all three aspects of this study show that MeHg concentrations increase with organic carbon concentrations. This is consistent with Hg literature on this topic. On one hand, this conclusion could suggest that the addition of organic carbon in order to facilitate vegetation and reduce erosion could increase MeHg production. While this remains a possible outcome; it is unlikely that saturated and anoxic conditions would develop in much of the area of the tailings. Currently at the site, there are several small seeps, as well as the wetland area where the soil or tailings are saturated, anoxic conditions exist, and MeHg production is high. This is seen not only in the data from the wetlands, but also from the meadow/seep area where our soils samples for the methylation assay were collected (soil MeHg: 102 ±19 ng/g). For comparison, at the end of mesocosm experiments, the amended tailings only had a MeHg concentration of 7 ±2 ng/g. The areas at Cinnabar mine site that are affected by seeps/saturated conditions are already colonized by vegetation. The area of the tailings that remains un-vegetated is well-drained and contains dry materials. If organic matter were added to these areas, there would likely be an increase in moisture during precipitation events and snowmelt, but on an

annual basis would likely have water contents well below saturation and conditions not conducive to methylation.

In general, the areas at the mine site where conditions become saturated already are vegetated and are likely important zones of MeHg production. However, given the isolated spatial extent of these areas, their current influence on MeHg concentrations in the West Fork of Cinnabar Creek is minimal (see results from section 3.1). Adding organic carbon and vegetation to the drier sections of the tailings may result in a modest increase in MeHg production (similar to observed in the first few weeks of the mesocosm experiments), but the more dramatic increases in MeHg observed later in the experiments are unlikely.

Under current conditions, the methylation potential increases downstream from the site. This effect was measured within the first 8km downstream, but likely increases further downstream as the stream gradient decreases and deposition is more common. Additions in organic material at the mine site are believed to have a minimal influence on downstream sediment total organic carbon (TOC) or water dissolved organic carbon (DOC) contents because the footprint of the mine site/carbon addition would be a small percentage of the overall watershed for Cinnabar and Sugar Creeks. Because the proposed remediation of the site is expected to result in a reduction in the downstream loading of total-Hg (THg), it is assumed that this would have a larger effect of decreasing downstream MeHg production than a small increase in carbon loading. The results of the mesocosm experiments raise an important concern that the addition of organic carbon may facilitate the partitioning of Hg from the solid phase of the tailings into the aqueous phase. The increased production of aqueous THg that could be transported downstream where conditions are more conducive to methylation would be a negative outcome of an amendment to the tailings. However, our results suggest that this mobilization of THg may be confined to the first few months following an organic matter application.

4. Summary

In summary, the main conclusions from these studies are:

- Organic carbon concentrations are well correlated with MeHg concentrations at the site under existing conditions in sediment and porewater. Organic carbon additions also were shown to increase MeHg production in short-term isotope addition experiments and longer term mesocosm experiments.
- Under current conditions, MeHg production at the mine site is driven by hydrological flow paths that result in saturated conditions developing in soils and tailings. These areas are spatially isolated and do not exert at large influence on MeHg concentrations in Cinnabar Creek.
- Addition of organic material/vegetation to the well-drained sections of the tailings is expected to significantly reduce erosion of high Hg content material into the stream, particularly during high flow events where very large pulses of THg have been observed several km downstream of the mine (see Etheridge, 2015). Because these tailings would remain un-saturated during most of the year, they are not expected to become a large source of MeHg.
- The conditions favorable to Hg methylation increase downstream from the mine site. Efforts to reduce THg from being transported downstream from the mine site could help reduce downstream MeHg production.
- The potential for carbon additions to facilitate the partitioning of THg into the aqueous phase in association with DOC remains an important concern, as this Hg would be available for

methylation downstream (more so than particulate bound Hg). However, the large magnitude of this effect is likely temporally isolated to the first few months following carbon application.

- Different types of soil amendments contain different qualities of organic matter and appear to have different effects on Hg methylation rates. For example, Bio-Earth soil amendments appear to contain a carbon that does not promote methylating microorganisms to the same extent that other soil amendments do.

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