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Spatial variability of metal bioaccumulation in estuarine killifish (*Fundulus heteroclitus*) at the Callahan Mine Superfund site, Brooksville, ME

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Abstract

The former Callahan Mine Site in Brooksville, ME is an open-pit, hardrock mine site in an intertidal system, providing a unique opportunity to evaluate how metal-enriched sediments and overlying water impact estuarine food webs. Cu, Zn, Cd, and Pb concentrations in sediment, whole water, and Atlantic killifish (*Fundulus heteroclitus*) were evaluated at sites in Goose Pond (the Callahan Mine Site) and at reference sites. The metal concentrations of sediment, water, and fish were spatially distinct and significantly higher at the Mine Site than in the reference estuary. Sediment concentrations were particularly elevated and were above probable effects levels (PEL) for all four metals adjacent to the tailings pile. Even in this well-mixed system, water metal concentrations were significantly elevated adjacent to the tailings pile and the concentrations of Cu and Zn were above ambient water quality criteria (AWQC) for chronic marine exposure. Neither organic matter in the sediment nor salinity or pH of the water explained the metal concentrations. Adjacent to the tailings pile, killifish body burdens were elevated and were significantly related to both sediment and aqueous concentrations. In conclusion, (1) the contaminated sediment and seepage from the tailings impoundment and waste rock pile 3 create a continual flux of metals into the water column, (2) the metals are bioavailable and are bioconcentrating as evident in the killifish tissue concentrations, and (3) Callahan Mine is directly affecting metal bioaccumulation in fauna residing in the Goose Pond estuary and, potentially, in Penobscot Bay via the 'trophic nekton relay.'

Keywords

Copper; Zinc; Cadmium; Lead; Bioavailability; Bioaccumulation; *Fundulus heteroclitus*; Estuarine food web

Introduction

Mining activity and abandoned mine sites are a major source of metal contamination globally and in the United States. In 2008, the US Government Accountability Office (GAO) determined that there were at least 161,000 abandoned hardrock mine sites in the 12 western states and Alaska where most U.S. mining takes place. At least 33,000 of these sites had degraded the environment by contaminating surface water and groundwater (Mittal 2011). Waste rock and tailings from the operations are often stored in large piles that can result in persistent acid and toxic metal drainage or dust contamination to the surrounding

ecosystem (Schneider et al. 2007). The negative impacts of wet and dry deposition from mine waste piles on local human communities as well as aquatic and terrestrial wildlife are numerous and well-documented (Beyer et al. 2004; Hu et al. 2007; Kiser et al. 2010; Malcoe et al. 2002).

The United States Environmental Protection Agency (US EPA) currently has 120 mine sites listed on the National Priorities List or being remediated using the Superfund Alternative Approach (EPA 2012). This includes the Tar Creek site in Oklahoma, the Clark Fork River site and associated Milltown Reservoir in Montana, Eagle Mine in Colorado, and Bunker Hill in Idaho. All of these are contaminant sources to freshwater systems. Studies have been conducted on the effects of highly urbanized areas (Bryan and Langston 1992; Hall and Pulliam 1995; McKinley et al. 2011), landfills (Pope et al. 2011), and upstream mining (Rainbow et al. 2011) on estuarine environments. However, there is a paucity of research available on sources of metals to estuaries or marine systems and to our knowledge there are no studies on the effects of an open pit mine in an estuarine environment.

The state of Maine has a number of former hard-rock mines; the most well known and the second largest operation is the former Callahan Mine. The mine was in operation initially from 1881 until 1887. It was worked more intensely again from 1968 to 1972, during which 800,000 tons of copper (Cu) and zinc (Zn) ore were extracted. The remedial investigation (RI) for the Callahan Mine Site was conducted from 2004 through 2008 and found elevated levels of Cu, Zn, cadmium (Cd), arsenic (As), and lead (Pb) in surface soils throughout the Site. Polychlorinated biphenyls (PCBs) contamination was found in the area of the Site where the ore processing facilities were located, also in surface soils. The specific source of the PCB contamination is not known, however some of the PCB contamination is believed to be related to former transformers located near the south portion of the Primary and Secondary Crusher buildings (MACTEC 2009c). The concentrations of As, Pb and PCBs were above levels considered acceptable for residential or recreational use. The seepage from the waste rock piles and tailings impoundment to Goose Pond was found, in cases, to exceed chronic ambient water quality criteria (AWQC) concentrations for Cu, Zn, Cd, and Pb, anywhere from just above the AWQC to 2123 times higher in the case of Zn and 3581 times higher in the case of Cu for concentrations recorded in 2006 from a seep adjacent to Waste Rock Pile 3 (sample SP-509) (MACTEC 2009b). Cu, Zn, Cd, and Pb were also found to be elevated in sediments and biota tissue, particularly in hot spot locations in Goose Pond and the salt marsh adjacent to the waste rock piles and the tailings impoundment with Cu and Zn detected at 110 and 150 times higher than the Effects-Range Medium benchmark developed by the National Oceanic and Atmospheric Administration (NOAA) at location GP-24 in the salt marsh sediment (EPA 2009; MACTEC 2009a). In light of these contamination sources, we analyzed the spatial gradient of Cu, Zn, Cd, and Pb metal concentrations in the estuary sediment and whole water and the relationship to bioaccumulation in the resident forage fish, the Atlantic killifish or mummichog (*Fundulus heteroclitus*) as based on water chemistry readings, known killifish life history, and a consideration of landscape factors.

In aquatic ecosystems, all four metals—Cu, Zn, Cd, and Pb—have the potential to bioaccumulate in the local fauna with exposure to sources in sediments or water (Bryan and Langston 1992; Mason 2002) and transfer trophically. Benthic infauna living in contaminated sediments accumulate metals and can transfer contaminants to both pelagic and benthic organisms. Cu, Zn, Cd, and Pb are most bioavailable in their free ion form (Mason 2002). However, this form also most readily complexes, especially as salinity in the water and/or organic content in sediment increases, making the metals less bioavailable (Fernandes and Henriques 1991; Zamuda and Sunda 1982). Simultaneously, enriched sediments are potentially a source of metals to the overlying water, creating another

exposure route for pelagic species either in a dissolved, colloidal, or particulate form. Zn, with high assimilation efficiency, has been found to biomagnify in certain cases and affect higher trophic level species (Chen et al. 2000; Mathews and Fisher 2008; Reinfelder et al. 1998). Cu and Cd can either biomagnify or biodiminish depending on the position in the trophic chain (Chen et al. 2000; Rossi and Jamet 2008; Zhang and Wang 2007). Pb, on the other hand, has been found to consistently biodiminish in the food web (Chen et al. 2000; Chen and Folt 2000; Mathews and Fisher 2008; Stewart et al. 2005).

Exposure of fish to high levels of either essential (e.g. Cu and Zn) or non-essential metals (e.g. Cd and Pb) retards mental and physical development and fitness, especially in early life stages (Kearns and Atchison 1979; Lefcort et al. 1998; Maes et al. 2013; Vieira et al. 2009). Furthermore, it has been found to decrease wound healing and fin regeneration of fish (Weis and Weis 1976), alter population numbers (Poulton et al. 2010), change an ecosystem's community composition (Clements and Kiffney 1994; McKinley et al. 2011; Pascoe et al. 1994; van der Merwe et al. 2011), shift feeding patterns (Clements and Rees 1997), and decrease production of specific gene products in response to cellular overstimulation (Maes et al. 2013). In some cases, individuals can develop physiological tolerance or populations can develop genetic resistance to the metal toxicant and they can survive with sustainable numbers (Bryan and Langston 1992; Weis and Weis 1989). In particular, killifish populations are known to have a high degree of genetic variability and thus an enhanced ability to buffer stresses and accommodate to a wide-ranging pollution gradient (Whitehead et al. 2011a). Although killifish are known to be more tolerant of environmental and pollution gradients than other species, they still experience stress as a result of exposure and, by surviving, serve as vectors of contaminant transport to other fauna.

In this study, metal concentrations in Goose Pond, the estuary impacted by the former Callahan Mine in Brooksville, Maine, were evaluated. The site is notable for being one of the few, if not the only, documented open pit hard-rock mining site in an intertidal zone and provides an opportunity to research a metal contamination source in an estuarine environment. As mentioned above, risk assessment evaluations conducted by the US EPA, MACTEC, Inc., and other researchers, indicate that metal concentrations in the Mine Site are elevated above background levels (Dwyer 1973; EPA 2009; MACTEC 2009b; Rajakaruna et al. 2011). To better understand the fate of metals in the estuarine food web, specifically bioadvection, we evaluated the spatial distribution of Cu, Zn, Cd, and Pb within the Mine Site in sediments and water and the corresponding transfer of metals to fish as affected by site conditions (e.g. organic carbon, pH, salinity, flushing) and biological factors (e.g. life history factors and predictions through time). Killifish samples were collected and corresponding sediment and whole water samples were analyzed. Killifish were an ideal study species as their tissue residues represent bioavailable and thus biologically relevant metal concentrations. Killifish are ubiquitous across our study sites as well as being found along the Atlantic coast of the United States into the Gulf of Mexico. They maintain small home ranges, link lower trophic guilds with those higher, and are extremely tolerant to a range of salinities as well as a range of pollutants (Kneib and Stiven 1978; Kneib et al. 2002; McMahon et al. 2005; Valiela et al. 1977; Whitehead et al. 2011a; Whitehead et al. 2011b). Metals in whole water samples were analyzed as an indicator of total fish exposure since Cu, Zn, Cd, and Pb can be taken up by absorption over the gills or through ingestion (Schlekat et al. 2002; Xu and Wang 2002; Mathews and Fisher 2009).

The main objectives of this study were twofold: for the four metals in question, in a tidally dynamic system (1) determine if the spatial differences in sediment concentrations within the study site were reflected in the overlying surface water concentrations; and (2) evaluate the relationship of sediment and/or aqueous metal concentrations on the body burdens of

killifish. The discussion will consider whether the mine is a source of exposure to the local estuarine food web.

Methods

Study site

The Goose Pond estuary is a tidally flushed ecosystem located in Brooksville, Maine (Fig 1). The estuary has freshwater inflow from Marsh Creek in the south and empties on the north side through a reversing falls into Goose Cove and the convergence of the Bagaduce River and the Penobscot Bay. The site is primarily surrounded by private residential properties, excluding the Holbrook Island Sanctuary State Park on the east border. The mine was owned and operated by the Callahan Mining Corporation between 1964 and 1972 and was located in the lower more saline portion of the estuary (Metcalf and Eddy 2003). During the mining operation, Goose Pond was dammed both at the south end to block the Marsh Creek stream water as well as at the north end at the estuary mouth. Following the completion of the dams, Goose Pond was drained to allow for the development of an open pit mining operation. The ore deposit was a Kuroko-style volcanogenic massive sulfide body that consisted of lenses and pods of zinc, copper, lead, and iron sulfide minerals embedded in a talc and calcite mineralized zone (MACTEC 2009b). Approximately 5×10^6 tons of rock was removed from the open pit resulting in a mine pit of 125 to 300 m diameter and over 90 m in depth (Woods Hole Group 2007). When the mining operation ceased, an 8 ft section of the Goose Falls dam was opened allowing the system to flood and again be subject to tidal patterns (MACTEC 2009a). The legacy of the mining operations consists of three waste rock piles, a tailings impoundment, ore pad (location where excavated ore material was stored prior to processing), and the former mine operations area. Portions of Goose Pond were used as a settling pond resulting in the accumulation of tailing in the sediment. Dyer Cove and Goose Cove were also used to settle the sediment resulting from the de-watering of the open pit (MACTEC 2009b). An analysis of the acid producing minerals and the acid neutralizing minerals suggests that, within the waste rock piles, the ore pad area, and tailings pile, the minerals are net neutralizing. The net neutralizing condition buffers the pH of the leachate, while sufficient acid rock drainage generation does occur to create leachate with very high concentrations of metals in both the total and dissolved fractions. (MACTEC 2009b). In 2009, the US EPA selected a remediation plan to alleviate both human and ecologically based impacts. The cleanup of four residential properties with As and Pb contamination was completed in 2011. The cleanup of the PCB contamination in the former Mine Operations Area began in 2011 and is expected to be completed in September 2013. We conducted this study to establish an understanding of metal fate in the estuary prior to any remediation of the estuary.

Site selection

We selected six sites in the Goose Pond (GP) estuary of the Callahan Mine Site in Brooksville, ME and two control sites in the nearby Horseshoe Cove (HC) estuary (Fig. 1). Within Goose Pond (Fig. 2), we collected samples in Goose Cove just outside the reversing falls at the mouth of the estuary (GP FALLS), on the north shore of Goose pond just before the mouth (GP MOUTH), along the north shore of Dyer Point (GP DYER), on the western shore of the estuary along side waste rock pile 3 (GP EST), on the western shore south of the former earthen dam and adjacent to the tailings impoundment (GP ADJ), and in the brackish marsh creek area upstream of the mining activity (GP BRK). Reference samples were collected in the nearby Horseshoe Cove estuary. Horseshoe Cove is on the same peninsula, but is a slightly larger estuary and opens up to the Penobscot Bay at the opposite end of the peninsula (Fig. 2). In Horseshoe Cove reference samples were collected in two locations to

pair with the Goose Pond estuarine and brackish site; within the salt marsh area (HC BRK) and further downstream in a more estuarine area (HC EST).

Sample collection and analysis

Samples were collected in August 2011 using nonmetallic sampling gear and using trace metal clean techniques. Water sampling techniques are derived from EPA Method 1669, Sampling Ambient Water for Determinations of Metals at EPA Water Quality Criteria Levels. The biota and sediment sampling techniques are described in Chen et al. 2000 and in Chen and Folt 2000. All containers, bags, and sampling materials were sequentially washed with Citronox detergent, trace metal grade HCL, and trace metal grade HNO₃, and rinsed with DDI water prior to use. While in the field, all samples were immediately frozen on dry ice and later stored at -20°C until analysis.

Sediment samples were collected during low tide using a 6 cm diameter coring tube with the top 2 cm of three cores composited into a single sample. This was repeated three times at each site for triplicate samples. Each sample was freeze-dried, homogenized, and an aliquot analyzed for total metals. Another aliquot of approximately 10 g was muffled at 550 °C for 8 hrs and allowed to cool overnight. The amount of carbon lost, as a weight, is presented as a Percent Lost on Ignition (%LOI).

Triplicate aqueous metal samples were taken at each site as whole water samples (not filtered) and were collected at high tide off of a plastic boat using trace metal clean techniques. Whole water, rather than filtered, samples were taken as they reflect a range of exposures of metals to biota including both dissolved metals as well as particulate metals. They were collected and stored in acid-cleaned Teflon® containers. Each sample was acidified in the field to pH 1 with optima nitric acid (0.4% acid volume) and was refrigerated until analyzed. Concurrent *in situ* measurements of pH, water temperature, and salinity were taken using a Hydrolab Minisonde 4A™ (Austin, TX, USA) portable system, calibrated daily.

We collected fish at mid or high tides using fish seines and minnow traps and stored these fish using trace metal clean techniques. Fish total lengths (TL) and wet weights were measured and triplicates of ~60 mm TL fish were selected (0.4 – 0.8 g DW). Whole fish were freeze dried and digested with 4 mL Optima HNO₃, and 4 mL Epure ultrapurified water in a MARSxpress microwave digestion unit (CEM, Mathews, NC) at 180 °C.

Trace metal analysis

All metal samples were analyzed by the Dartmouth Trace Element Core facility by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500cx) using both He mode (Cu, Zn, Cd) and normal mode (Pb).

Quality control for all metal results included blank, duplicate, metal spike detection, and certified reference material (SRM) analyses. The sediment had duplicate relative percent difference of 8.7, 2.8, 6.0, and 6.2 % and detection limits of 0.18, 0.18, 0.005, and 0.12 ppm for Cu, Zn, Cd, and Pb, respectively with an SRM recovery of 106%. For the water sample analyses, the SRM, NIST-1640a percent recovery were 100, 102, 98, and 92 % and detection limits were 0.23, 1.17, 0.083, and 0.12 ppb for Cu, Zn, Cd, and Pb. For the killifish analysis, the standard reference material DORM-2 was used, which is produced from dogfish muscle tissue and certified by the National Research Council of Canada. In the killifish run, DORM-2 had a recovery of 133, 107, 119, and 130 % of the published metal concentrations for Cu, Zn, Cd, and Pb, respectively, and method detection limits of 0.20, 0.40, 0.04, and 0.04 ppm for Cu, Zn, Cd, and Pb. Two of the vessels were run as blanks and

were subsequently deemed contaminated and the SRM percent recovery was reevaluated at 119, 107, 117, and 118 % for Cu, Zn, Cd, and Pb, respectively.

Data analyses

Statistical analyses of the data—general linear models—were carried out using JMP 5.01a. To evaluate the metal concentrations across the sites for sediment, water, and killifish, we applied one-way analyses of variance (ANOVA) models on untransformed data. All necessary assumptions of the ANOVA were met except equal variance; by Levene's test for Equality of Variances, significant p-values indicated that variances were unequal across sites. However, applying Box's 1954 publication on theorems on quadratic forms, moderate inequality of variance should not affect the robustness of the ANOVA test when equal sample sizes are present across the sites. With the study design of triplicates for each criterion at each site, a balanced design, the robustness of the ANOVA test is maintained. As a check, the nonparametric Welch's method was applied to the data and results were compared.

All ANOVA tests were significant so the post hoc Tukey-Kramer HSD test was then applied with $\alpha = 0.05$. Regression analyses were used to evaluate the effect of organic content in sediment and salinity and pH readings in the water on the resulting metal concentration. Regression analyses were also applied with sediment and water as predictors of water and killifish metal concentrations.

The NOAA Screening Quick Reference Tables (SQuiRTs) and published metal concentrations present in other sites were used as comparisons to the levels present in this study. The NOAA SquiRTs provide a series of screening values of suggested toxicological benchmarks of inorganics and organics in sediments, soils, and waters of coastal hazardous waste sites (Buchman 2008). From these tables Probably Effect Level (PEL), Effect Range – Medium (ERM), and chronic Ambient Water Quality Criteria (AWQC) were applied. PEL is a threshold effect concentration referring to the chemical level above which adverse effects are expected to occur frequently. ERM represents the concentration above which adverse effect would frequently be observed. Chronic AWQC is the U.S. EPA Ambient Water Quality Criteria as experienced as a long-term exposure of a contaminant. It is considered the preferred surface water and groundwater benchmark.

For each site and for each metal, a bioaccumulation factor (BAF) was calculated by dividing the mean tissue metal concentration by that of the mean water concentration and then $\log_{10}$ transforming the results. Where water or tissue concentration values were below the analysis detection limit (BDL) of the mass spectrometer, the Trace Element Analysis Core did not provide the lower estimate, as they are not accurate. Thus, when values were BDL, the detection limit (DL) was applied to the calculation (0.236, 1.776, 0.083, and 0.118 ppb for aqueous Cu, Zn, Cd, and Pb, respectively and 40 ppb for Cd tissue concentrations). Applying the aqueous DL, a high estimate, to the BAF calculation results in a lowered calculation of BAF. This should not matter, as uptake rates should be similar at high or low metal concentrations under the assumption that there are no toxicological effects at high metal concentrations.

Ratios of the metal concentrations of GP ADJ as compared to the mean values present at Horseshoe Cove ($[\text{metal}_{\text{GP ADJ}}]/[\text{metal}_{\text{HC mean}}]$) were calculated for each metal in each compartment—sediment, water, and killifish. Again, where water concentration values were BDL, this cut-off was applied to the calculation. In this case, applying the DL in all calculations is a more conservative approach than would be if applying the estimated below detection values, which are lower; the method reduces the inter- as well as intra-site differences.

Results

For all four metals, sediment (Fig. 3), water (Fig. 4), and fish (Fig. 5) varied significantly across all sites (Table 1). This remained true when run as a non-parametric test. By Welch's Test (1) sediment Cu ($p<0.0002$), Zn ($p<0.0002$), Cd ($p<0.006$), and Pb ($p<0.180$), (2) water Zn and Cd (both $p<0.0001$), and (3) fish Cu, Zn, Cd, and Pb (all with $p<0.0003$) varied significantly across sites. Significance could not be determined for water Cu and Pb as concentrations in Horseshoe Cove were below the detection limits of the analysis. The samples collected adjacent to the tailings pile (GP ADJ) showed concentrations that were significantly higher than that of the reference sites in Horseshoe Cove. The mean and standard deviation for each bar shown in figures 4 – 5 are provided in Online Resource 1. There was 92x more Cu, 107x more Zn, 98x more Cd, and 34x more Pb recorded in the sediment from GP ADJ than in Horseshoe Cove. For all four metals, concentrations at GP ADJ and GP EST exceeded those denoted as probably effect limits (PELs) (Fig. 3) and for all metals but Pb, they also exceeded the medium effects range (ERM) (Buchman 2008). Water samples collected at GP ADJ had Cu and Zn concentrations that were above chronic Ambient Water Quality Criteria (AWQC) for unfiltered water samples (Fig. 4). GP EST and GP BRK water Cu concentrations also were above chronic AWQC (Buchman 2008). Similar to sediment concentrations, overlying water showed elevated concentrations adjacent to the tailings pile and in the case of Cu and Cd also just upstream. Killifish tissue concentrations were elevated adjacent to the tailings pile as well as just upstream and just downstream (Fig. 5). Sediment and water had higher across-site than within-site variability (larger F-statistic) than did the killifish samples and the ANOVA model had a higher goodness of fit (R^2) for sediment and water samples as compared to that of the killifish (Table 1). At the outlet of the Mine Site (GP MOUTH and GP FALLS), all three compartments—sediment, water, and fish tissue—showed levels that were not significantly different from those in the reference site.

As possible biogeochemical factors affecting the bioavailability of the metals, we considered sediment organic matter and salinity and pH in the water. Within the Goose Pond sites, percent organic matter, as measured by percent loss-on-ignition (%LOI) varied from just under 4% to almost 17% (Table 2) and %LOI was not related to the sediment metal concentration or overlying water concentrations (Table 3). Salinity and pH both varied very slightly across the sites (Table 2), but did not predict metal concentration in the water or in the fish (Table 3). There was no evidence of salinity stratification.

The metals differed in their relationships across sites and between biotic and abiotic compartments. Across all metals, concentrations in the sediments were strongly correlated to those in the water and in the biota at the same site (Table 3). By regression analyses, the sediment to biota relationship was strongest in Cd and Pb. Water metal concentrations as a predictor of biota concentrations were strongest for Pb and Zn. Bioaccumulation factors based on water concentrations indicate that bioavailability of metals from water were greatest for Cu and Zn (Table 4). At GP ADJ, sediment concentrations of Cu, Zn, and Cd were the most elevated above those recorded at local reference sites while aqueous Cu and Zn were the most elevated above those present in the reference site. Cu and Zn were also the least elevated in the killifish tissues (Table 5).

Discussion

Sediment-bound metals

The present study shows that Goose Pond had sediment metal concentrations elevated above those present in the reference site as well as Gulf of Maine background levels; this is supported by past findings (Dwyer 1973; EPA 2009; MACTEC 2009b, a; LeBlanc et al.

2011). Sediment concentrations within Goose Pond were significantly elevated adjacent to the tailings pile (GP ADJ) where they were above possible effects level (PEL) and effects range-medium (ERM) thresholds (Buchman 2008), suggesting that the concentrations present in these sediments are harmful to aquatic organisms. Colvin et al. (unpublished data) found that polychaetes (*Neanthes arenaceodentata*) experienced a complete die-off when exposed to GP ADJ and GP EST sediments in a 28-day toxicity test. In a subsequent study with a 96-hour sediment-water interface exposure to sea urchin embryos (*Strongylocentrotus purpuratus*), all embryos showed abnormal development. Only one out of three sample had a positive calculation of the study sediments' Simultaneously Extracted Metals divided by the Acid Volatile Sulfides (Σ SEM-AVS) value; this result suggests non-toxic conditions. AVS is a measure of sulfide that has the potential to react with free metals. SEM is a measure of reactive metals as measured by the cold acid extraction method. For divalent metals (e.g. Cu, Cd, Pb, Zn, and nickel) the AVS present in the sediment binds with the SEM present forming metal sulfides and rendering the metals unavailable to biota (McGrath et al. 2002). Positive values of Σ SEM-AVS in sediment have been related to toxicity in benthic toxicity tests suggesting that the metals are bioavailable (Berry et al. 1996). According to Colvin et al., even with negative values, however, the sediments in Goose Pond proved toxic. In contrast to what would be expected, Σ SEM-AVS was not found to be an accurate predictor for toxicity in these sediments.

The substrate underlying GP ADJ as well as GP EST is composed of mine waste, which might explain the high sediment metal concentrations in these areas. GP EST is located in an area that historically was used as an effluent-settling pond for the processing of water from the tailings impoundment. As a result, the benthic substrate in this area is primarily comprised of tailings. Similarly, the benthic substrate of GP ADJ is also comprised of tailings due to erosion and overflow directly from the tailings impoundment. Further, there are aboveground seeps flowing off of the waste rock piles and the tailings pile into the estuary, which may be contributing to the metal enrichment found in both the water and in the sediments. The most visible and highest concentration seeps (Cu: 0.02 – 10 mg/L; Zn: 2 – 10 mg/L; Cd: 0.007 – 0.5 mg/L; and Pb: 0.002 – 0.2 mg/L) are flowing from the bank of Goose Pond between GP ADJ and GP EST (MACTEC 2009b). Precipitates from the seeps are observable with red-orange phases indicating iron hydroxides and white to blue precipitants consisting of calcium sulfate and aluminum and copper sulfates (MACTEC 2009b).

While lower than that of GP ADJ, GP BRK also contains levels of metals in the sediment that are enriched significantly above the levels present in our reference site, Horseshoe Cove. When Callahan Mine was in operation, there was an earthen dam between GP ADJ and GP EST. Historically, Marsh Creek (the freshwater stream) pooled at this dam, and was forced to drain south, away from the Mine Site. At the time of operation, GP BRK was likely not heavily exposed to mine waste or tailings. The sediment enrichment evident at GP BRK is likely, then, the result of the long-term deposition of particulates transported by tidal inflow following the release of the earthen dam.

The metals in Goose Pond are enriched on a fine spatial scale. GP ADJ had sediment concentrations that were significantly different from those found at GP BRK or at GP EST. In all metals except Pb, GP EST was significantly different than GP DYER. These sites are in close proximity to one another with only 300 to 500 meters of separation. Sediment concentrations just north of GP EST, starting at GP DYER and including all other sites toward the outlet of Goose Pond, had metal levels not significantly different from background levels. Ayuso et al. (2013) found that, beyond GP FALLS in Goose Cove, the sediment concentrations quickly diminished to background levels, but with a spike in sediment metal concentration just beyond GP FALLS. This is likely from historical use

when there was a sump pump releasing effluent from the mine pit into this area, which is consistent with our observations of the direct presence of mine waste having the greatest observable impact on metal levels.

The sediment data from the present study suggest that historic deposition of tailing in the bottom substrate of GP ADJ and GP EST as well as erosion from the tailings pile and waste rock pile 3 are the most pronounced sources of enrichment to the sediment. There are likely additional contributions from terrestrial seeps coming off of the waste rock piles. By applying Pb isotopes as means of identifying the source of metals, Ayuso et al. (2013) came to the same conclusion. The present study found that the metal signal in sediment is relatively low adjacent to the other waste rock piles, in the historic settling pond (Dyers Cove), and alongside the ore pad (location where excavated ore material was stored prior to processing). The highest groundwater concentrations are found beneath the ore pad (MACTEC 2009b) indicating that it has high source potential as has been found in other study areas (Velleux et al. 2006). Here, however, it appears that the soil is filtering the impact prior to discharge into Dyer Cove (GP DYER).

Sediment organic content is known to bind metals, influencing their availability and rate of flux to overlying water (Doyle and Otte 1997; Hung and Chmura 2006; Otte et al. 1993). With ranges of 6–20 % LOI, Otte et al. (1993) found significant correlations with sediment metal concentrations. In Goose Pond, even with a comparatively large range of %LOI (4–17 %), %LOI did not relate to concentrations in either sediment or water. Organic matter, therefore, may not be significantly controlling the sediment metal gradient. It may, however, help to explain the difference in concentrations between GP EST and GP ADJ. The organic content in GP EST was higher than that of GP ADJ suggesting that, over time, the tailings substrate beneath GP EST is maturing and the metals present are slowly being sequestered by the deposits of lower concentration particulates transported either from Marsh Creek or by the tides.

In comparison to other mine sites and other anthropogenically affected sites, sediment concentrations of Zn, Cd, and Pb adjacent to the tailings site were high, equivalent to those detected in the waste rock piles of the Tar Creek Superfund site in Oklahoma (Schaidler et al. 2007) and slightly lower than the soil levels of Cu and Ni found in a copper smelting area in Ontario, Canada (Freedman and Hutchinson 1980). As compared globally to the sediment concentrations of mining sites, the levels found in Goose Pond and Goose Cove are not unusual; however, regionally the site represents a hotspot of Cu, Zn, and Pb for the Gulf of Maine surpassed only by levels in some areas of Boston Harbor (LeBlanc et al. 2011; MACTEC 2009b).

Aqueous metals

Addressing our first study aim, we found that the spatial differences in sediments are reflected in the overlying water with our sampling site adjacent to the tailings pile experiencing the highest levels of metals. While there typically is a strong correlation between sediment concentration and that in associated pore-water and overlying water (Mason 2002), it is surprising to see such a strong correlation in a tidally influenced estuary where there is a natural mixing of the water column that could result in more homogenous aqueous metal concentrations. For all sites but HC BRK, there is no indication of salinity stratification. Instead the systems appear to be relatively homogeneous with a well-mixed water column. Tidal cycles measured over a month (May 2007 – June 2007) indicate that GP BRK experiences a wide range of salinities (0 – 26 ppt) with most daily tidal fluctuations ranging from about 10 ppt to 24 ppt (MACTEC 2009b). Together these data suggest that the system experiences a relatively large exchange of fresh water to salt water and can be considered well mixed. Further, our sampling stations were in relatively close

proximity to one another separated by only 300 to 500 meters. Nonetheless, water concentrations in Goose Pond parallel those present in the sediment; water was metal-enriched adjacent to the tailings pile and farther upstream in our GP BRK site. We sampled water during high tide when the saltwater backs up into the Marsh Creek brackish area; likely that is why we saw elevated metals in the water, particularly Cu and Cd, upstream of the tailings pile. Cu and Zn (unlike Cd and Pb) stand out as being present in concentrations that exceed AWQC for whole waters (Buchman 2008).

Studies have found that water chemistry factors can affect aqueous metal concentrations. Metals can complex with carbon, sequestering them in sediment (Amiard et al. 2007) (Baumann and Fisher 2011). This is particularly true for Cu, which is almost entirely bound to organic matter in marine systems, while Cd is less strongly bound to carbon (Mason 2002). In this system, however, we found that %LOI was not predictive of the overlying aqueous concentrations for any of the evaluated metals and, as stated above, %LOI also did not predict sediment concentrations. Simultaneously, the salinity and pH of the water did not predict aqueous metal concentrations present.

The metals from the sediment appear to be continuously fluxing to the overlying water and water concentrations may also be influenced by terrestrial seeps draining from between the tailings pile and waste rock pile 3 (EPA 2009). Zn concentrations in sediments were most tightly correlated to concentrations in the overlying surface water followed by Pb and Cu suggesting that these metals are experiencing the highest rate of fluxing from the sediments to the water and thus are likely the most available for accumulation by phytoplankton, microorganisms, and algae (Pope et al. 2011). MACTEC, Inc., evaluated the distribution coefficients of concentrations in the sediment to its corresponding interstitial water (K_d) (MACTEC 2009b). Their data suggest that Cd and Pb have the highest rate of flux from sediment to pore water, and potentially from the pore water to overlying water. However, the investigation also found that pore water concentrations were relatively low suggesting that, overall, the Callahan Mine Site is experiencing low fluxing rates and seeps are potentially contributing to the water signal. Both sediment fluxing and terrestrial seeps appear to be sources, with correlating patterns and strong relationships between sediment and water concentration suggesting sediments as the dominant source of metals to the overlying water.

Abiotic to biotic transmission

Completing the second aim, it was found that the study organism, killifish, had the highest body burdens in the sites with the highest sediment and aqueous concentrations. Bioaccumulation was significantly above background levels adjacent to the tailings pile and just downstream, adjacent to waste rock pile 3. Killifish forage in a relatively constrained area and are likely integrating metal signals from only one site (McMahon et al. 2005); however, we had anticipated some overlap in killifish body burdens given the small distance between sampling sites. While more variable than the sediment and water compartments, fish tissue data show distinct spatial differences in metals in the tissue concentrations across our sampling sites. Most nonmethylated metals have a short half-life and are depurated rapidly (Mason 2002) and therefore, this strong spatial pattern of bioaccumulation suggests that the killifish in certain regions of Goose Pond estuary are constantly exposed to either dietary or aqueous metals from the Callahan Mine Site.

Body burdens depend on a number of external factors including, but not limited to, water chemistry affecting the bioavailability of the metal(s) in question, routes of uptake, accumulation and elimination efficiencies, and growth dilution. Salinity and alkalinity negatively correspond with metal aqueous bioavailability (Bryan and Langston 1992; Dutton and Fisher 2011); however, neither salinity nor pH were found to be driving factors behind

the concentrations present in our fish samples. To account for age and feeding differences, we used only killifish of the same species and of the same size class. The size class of killifish that we evaluated—60 mm total length—were likely second season matures and would have predated on amphipods, tanaids, polychaetes, and small crabs as well as grazed the sediment-water interface for detrital material and benthic algae (Kneib and Stiven 1978). They consume mainly from the benthic layer where flora and fauna are exposed to the elevated metal concentrations in the sediment.

Together, both the sediment-bound as well as the aqueous metals are likely to be a source to local fauna and the metals may be available to biota either through waterborne or dietary exposure, or both (Folt et al. 2002; McCarty and Mackay 1993; Wang et al. 1999). In the study, the metals were not only bioavailable, but were also bioconcentrating within fish tissue many times higher than were present in the water.

The biotic uptake from either water or from sediment appeared to be dependent on the metal with Pb and Cd overall showing lower body burden relative to water than Cu and Zn (Table 4). This is likely because Cu and Zn are essential metals and are biologically regulated, while Pb and Cd are not (Bryan et al. 1983; Mason 2002; McGeer et al. 2003). This difference may explain why Pb and Cd had the highest ratio of fish tissue metal concentrations from GP ADJ against background levels (Table 5). Pb and Cd BAF's were also higher than Cu and Zn across the sites.

Possible effects beyond Goose Pond proper

There is a need to consider metal concentrations in a contaminated system in a context beyond that provided by toxicity test results (Fisher and Hook 2002; Whitehead et al. 2011a). Metals can be physically transported or bioadvected from their source. The baseline ecological risk assessment for the Callahan Mine Site stated that the site poses a risk of harm to the benthic and pelagic communities, insectivorous birds (e.g. spotted sandpiper), and piscivorous birds (e.g. great blue heron), but only within the sediment hotspots (MACTEC 2009a). However, through the physical transport of the metals by the tide and freshwater input as well as ecological transport mechanisms of trophic nekton relay, both nutrients and contaminants from the Mine Site have the potential to transfer from waste rock seeps and sediment deposits to coastal food webs. The metals may biotransfer as predatory fish and piscivorous birds that use the estuary as feeding grounds or a nursery carry the contaminants out of the system in their gut and tissues. Thus, even with low metal concentrations in the sediment, water, and fish at the estuary outlet (GP MOUTH and GP FALLS), the transport of the metals from within the site may nonetheless have effects in coastal marine food webs with Goose Pond killifish as a possible vector for ecological exposure of transient species. Ayuso et al. (2013) found that Pb concentrations of lobsters and crabs were elevated beyond GP MOUTH in the channel between Holbrook Island and Cape Rosier. This may be due to the historical pollution in this area by the effluent pipe in Goose Cove or may be indicative of metals bioadvection.

Conclusion

Data from abiotic and biotic compartments indicate that there is a significant hotspot of contamination in Goose Pond estuary coinciding with the tailings impoundment, waste rock pile 3, and/or seeps coming from these two areas. Despite the tidal and dynamic nature of the system, the spatial distribution of metals is apparent on a fine scale with very little variability within a site as compared to across sites. It is clear that in this ecosystem, sediments and the adjacent tailings pile are serving as potential sources of contaminants to the estuarine food web.

Our findings suggest that metals, continually fluxing to the overlying water from the sediments and/or seeps, are bioavailable and may be transferred through the food web resulting in metal bioadvection beyond the local scale of the Goose Pond estuary. In another former mine site, Rainbow et al. (2011) found that even after thirty years, there was no change in sediment and biota metal concentrations. With this in mind, it is unlikely that the contamination and the resulting bioaccumulation will naturally attenuate given the large source of metal in contaminated sediments, tailings, and rock piles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Sampling sites in Goose Pond (the Callahan Mine site) and our reference site, Horseshoe Cove. Horseshoe Cove Brackish = HC BRK and Horseshoe Cove Estuarine = HC EST. Please refer to Fig. 2 for insert details of the Callahan Mine Site.

Fig. 2. Sampling sites in Goose Pond, the Callahan Mine Site. Goose Pond Brackish = GP BRK; Goose Pond Adjacent to the tailings pile = GP ADJ; Goose Pond Estuarine area = GP EST; Goose Pond at Dyer Cove = GP DYER; Goose Pond at the estuary mouth = GP MOUTH; and Goose Pond just outside of the reversing falls = GP FALLS.

Fig. 3. Sediment metal concentrations (triplicate mean $\pm$ SD) of (A) copper, (B) zinc, (C) cadmium, and (D) lead across six sites in Goose Pond and two sites in Horseshoe Cove. The dashed lines indicate probable effects level (PEL) and effects range-medium (ERM). Lowercase letters indicate significantly similar multiple comparisons as determined by the Tukey-Kramer HSD test

Fig. 4. Whole water metal concentrations (triplicate mean $\pm$ SD) of (A) copper, (B) zinc, (C) cadmium, and (D) lead across six sites in Goose Pond and two sites in Horseshoe Cove. The dashed line indicates chronic Ambient Water Quality Criteria (AWQC) for unfiltered marine waters with the Cd and Pb criteria above the concentrations our data shows. Lowercase letters indicate significantly similar multiple comparisons as determined by the Tukey-Kramer HSD test. Bars with 'bdl' means that the concentrations were below the detect limits of the instrument: 0.24, 1.78, 0.08, and 0.12 ppb for Cu, Zn, Cd, and Pb respectively.

Fig. 5. Killifish (0.4 – 0.8 g DW) whole body metal concentrations (triplicate mean $\pm$ SD) of (A) copper, (B) zinc, (C) cadmium, and (D) lead across six sites in Goose Pond and two sites in Horseshoe Cove. Lowercase letters indicate significantly similar multiple comparisons as determined by the Tukey-Kramer HSD test. Bars with 'bdl' means the concentrations were below the detect limits of the instrument: 0.04 for Cd.

Table 1

Analysis of variance of sediment, water, and killifish metal concentrations as predicted by site (pooled variance ANOVA with a balanced design)

Variable	Units	Metal	R ²	MS	F _{7,16}	P
Sediment	ppm	Cu	0.9815	528089	121.4358	<0.0001*
Sediment	ppm	Zn	0.9885	1344091	196.5662	<0.0001*
Sediment	ppm	Cd	0.9866	339.406	168.3555	<0.0001*
Sediment	ppm	Pb	0.9743	110423	86.518	<0.0001*
Water	ppb	Cu	0.8737	60.2081	15.5647	<0.0001*
Water	ppb	Zn	0.9952	6152.94	467.4504	<0.0001*
Water	ppb	Cd	0.9839	0.186368	137.1961	<0.0001*
Water	ppb	Pb	0.9906	5.20793	235.9185	<0.0001*
Killifish	ppm	Cu	0.7636	1945.9	7.2695	0.0002*
Killifish	ppm	Zn	0.6053	20207.6	3.45	0.0138*
Killifish	ppm	Cd	0.5435	0.332762	2.6792	0.0393*
Killifish	ppm	Pb	0.6039	88.3695	3.4308	0.0142*

Table 2

Sediment percent loss on ignition (%LOI) with corresponding standard deviation (SD) and pairwise comparison and high tidewater salinity and pH^a

Site	Mean $\pm$ SD LOI (%)	LOI Tukey-Kramer HSD	Benthic salinity (ppt)	Benthic reading depth (m)	Surface salinity (ppt)	pH log(L/mol)
GP BRK	16.96 $\pm$ 2.22	A	28.37	0.24	28.40	7.43
GP ADJ	8.96 $\pm$ 0.51	B,C,D	29.03	0.42	28.66	7.37
GP EST	14.84 $\pm$ 3.27	A,B	29.38	0.58	29.40	7.36
GP DYER	13.26 $\pm$ 4.04	A,B,C	29.72	1.61	29.54	7.31
GP MOUTH	3.99 $\pm$ 1.22	D	29.68	2.85	29.71	7.49
GP FALLS	4.04 $\pm$ 1.17	D	30.64	4.43	29.47	7.38
HC BRK	7.41 $\pm$ 1.77	C,D	29.71	0.53	27.11	6.58
HC EST	9.51 $\pm$ 1.59	B,C,D	30.99	1.96	30.82	7.39

^a salinity and pH readings represent one sample (n=1) while %LOI represents triplicate samples (n=3). pH readings were taken within the upper 10 cm of the surface

Table 3

Linear regression analyses results of biochemistry parameters and metals by compartment across all sites

Metal	Variable	by Variable	R²	F_{1,6}	P
Cu	%LOI	Sediment	0.04	0.24	0.6408
	%LOI	Water	0.16	1.16	0.3229
	Salinity	Water	0.28	2.29	0.1810
	Salinity	Killifish	0.08	0.49	0.5086
	pH	Water	0.18	1.29	0.3002
	pH	Killifish	0.08	0.52	0.4964
Zn	Sediment	Water	0.75	18.23	0.0053*
	Sediment	Killifish	0.69	13.48	0.0104*
	Water	Killifish	0.55	7.31	0.0354*
	%LOI	Sediment	0.04	0.22	0.6554
	%LOI	Water	0.22	1.66	0.2451
	Salinity	Water	0.25	1.99	0.2078
	Salinity	Killifish	0.10	0.67	0.4435
	pH	Water	0.10	0.64	0.4551
	pH	Killifish	0.12	0.83	0.3973
	Sediment	Water	0.88	44.97	0.0005*
Cd	Sediment	Killifish	0.76	18.54	0.0051*
	Water	Killifish	0.85	33.64	0.0012*
	%LOI	Sediment	0.07	0.46	0.5216
	%LOI	Water	0.37	3.50	0.1105
	Salinity	Water	0.32	2.85	0.1421
	Salinity	Killifish	0.21	1.61	0.2515
Pb	pH	Water	0.14	0.97	0.3628
	pH	Killifish	0.07	0.45	0.5256
	Sediment	Water	0.69	13.44	0.0105*
	Sediment	Killifish	0.98	393.87	<0.0001*
	Water	Killifish	0.77	21.00	0.0038*
	%LOI	Sediment	0.01	0.05	0.8304
	%LOI	Water	0.25	1.96	0.2107

Metal	Variable	by Variable	R ²	F _{1,6}	P
	Salinity	Water	0.29	2.45	0.1689
	Salinity	Killifish	0.13	0.92	0.3735
	pH	Water	0.09	0.59	0.4732
	pH	Killifish	0.06	0.39	0.5539
	Sediment	Water	0.75	18.37	0.0052*
	Sediment	Killifish	0.91	63.78	0.0002*
	Water	Killifish	0.89	49.38	0.0004*

Table 4Bioaccumulation factor ($\log_{10}[\text{biota}]/[\text{aqueous}]$) by site and metal^a

	Cu	Zn	Cd	Pb
GP BRK	3.46	3.47	2.84	3.30
GP ADJ	3.70	3.42	3.18	3.65
GP EST	4.07	3.70	3.04	3.60
GP DYER	3.89	3.70	2.48	3.65
GP MOUTH	3.41	4.20	2.67	3.41
GP FALLS	4.57	4.14	2.68	3.48
HC BRK	<i>4.37</i>	<i>4.60</i>	<i>2.68</i>	<i>2.62</i>
HC EST	<i>4.38</i>	<i>4.82</i>	<i>2.68</i>	<i>3.77</i>

^a values in italics are conservative estimates: they include water and tissue concentration values that were below the instrument detection limits and thus these cut-off were applied to the calculation (0.24, 1.78, 0.08, and 0.12 ppb for Cu, Zn, Cd, and Pb aqueous concentrations and 40 ppb for Cd tissue concentrations).

Table 5Ratio of metal enrichment to background levels [GP ADJ]/[HC mean]^a

	Cu	Zn	Cd	Pb
Sediment	92.0	107.9	97.5	34.2
Water	<i>54.0</i>	<i>50.9</i>	<i>8.2</i>	<i>32.3</i>
Killifish	11.4	2.7	25.8	45.6

^a values in italics are conservative estimates: they include water and tissue concentration values that were below the instrument detection limits and thus these cut-off values were applied to the calculation (0.24, 1.78, 0.08, and 0.12 ppb for Cu, Zn, Cd, and Pb aqueous concentrations and 40 ppb for Cd tissue concentrations).