University of Montana
ScholarWorks at University of Montana

Graduate Student Theses, Dissertations, & Professional Papers

Graduate School

2022

Relationships Between Water, Sediment, Basal Resources, and Aquatic Insects in a Mine-Contaminated River on a Longitudinal and Habitat Patch Scale

Chelsea A. Wisotzkey
University of Montana, Missoula

Follow this and additional works at: https://scholarworks.umt.edu/etd

Part of the Natural Resources and Conservation Commons
Let us know how access to this document benefits you.

Recommended Citation

Wisotzkey, Chelsea A., "Relationships Between Water, Sediment, Basal Resources, and Aquatic Insects in a Mine-Contaminated River on a Longitudinal and Habitat Patch Scale" (2022). *Graduate Student Theses, Dissertations, & Professional Papers*. 11960.
https://scholarworks.umt.edu/etd/11960

This Thesis is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.

Relationships Between Water, Sediment, Basal Resources, and Aquatic Insects in a Mine-Contaminated River on a Longitudinal and Habitat Patch Scale

By

Chelsea A. Wisotzkey

Bachelor of Science, Colorado State University Fort Collins, Colorado, 2014

Thesis presented in partial fulfillment of the requirements for the degree of Master of Science
Systems Ecology

The University of Montana
Missoula, MT

June 2022

Approved by:

Scott Whittenburg, Dean of the Graduate School
Graduate School

Benjamin P. Colman, Chair
Department of Ecosystem and Conservation Sciences

Robert O. Hall
Division of Biological Sciences

Sean P. Sullivan
Rhithron Associates, Inc.

Wisotzkey, Chelsea, M.S., Spring 2022 Systems Ecology

Chairperson: Dr. Benjamin P. Colman

It is well established that aqueous exposure and dietary exposure both lead to the accumulation of metals and metalloids in aquatic organisms living in a contaminated environment, but the relative contribution of each remains unclear. To examine how a contamination gradient affects patterns of metal(loid)s among periphyton, BPOM (benthic particulate organic matter), water, and sediment, we collected samples from multiple sites across a 210km stretch of the mine-contaminated Upper Clark Fork River, Montana. To investigate metal(loid) accumulation patterns among basal resources and aquatic insects, we collected samples of periphyton, BPOM, and larval insects from discrete habitat types at a single site. We analyzed all samples for the metalloid arsenic (As) and the metals cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). We found that patterns in the aqueous metal and metalloid concentrations on a longitudinal scale did not show complete alignment with patterns of concentrations in BPOM and sediment and did not fully align with concentrations in periphyton. The disconnect between concentration patterns suggests that aqueous metal concentrations may not be driving metal accumulation in these environmental compartments. We also found that basal resources and aquatic insects differed in metal(loid) concentration depending on the habitat they were collected from, with wood habitats seemingly a driver of accumulation through diet. We suggest that feeding strategy, and thus diet, is partly responsible for overall accumulation patterns in aquatic insects living in a contaminated river.

Table of Contents

1 Introduction

Contamination of river water is a global problem that can threaten both human health and the environment. Activities such as mining, smelting, agriculture, urbanization, and a range of other industries have created local and regional pollution problems in nearly every country (Nriagu, 1996). Watersheds throughout the western United States are affected by drainage from historical mining operations, which can generate large quantities of waste containing metals and metalloids—hereafter referred to as metal(loid)s. When released into the environment, metal(loid)s contaminate water and sediment, which causes adverse impacts on aquatic ecosystems. Due to the ubiquity and scale of this problem, understanding the dynamics of metal behavior in freshwater environments has been a major focus of scientists ever since metal(loid)s were first recognized as threats to aquatic organisms (Carpenter, 1930; Doudoroff & Katz, 1953; Ellis, 1937). Although these early studies focused heavily on fish as the target organism, research on how metal(loid)s affect aquatic insects became more of a focus beginning in the 1960s (Warnick & Bell, 1969).

Aquatic insects are abundant in most freshwater ecosystems and perform many essential functions. Because aquatic insects are processors of organic materials, they influence nutrient cycles, primary productivity, decomposition, and translocation of materials (Wallace & Webster, 1996). Aquatic insects are a critical part of the food web, not only serving as a food source for fish and other aquatic predators (Hynes, 1970) but emerged adult insects are a source of energy for terrestrial predators inhabiting riparian zones (Collier et al., 2002). Beyond the pivotal roles of aquatic insects in ecosystems, they are commonly used as indicators to assess the health and the degree of anthropogenic disturbance to aquatic ecosystems (Rosenberg & Resh, 1993) due to their varying levels of sensitivity to chemical and physical changes in their environment.

Aquatic insects living in a contaminated environment accumulate metal(loid)s in their tissues, even if those metal(loid)s are not essential to their metabolism. Certain elements such as copper (Cu) and zinc (Zn) are essential because they are necessary for cellular function. However, at high enough concentrations, these elements can exceed metabolic needs and be toxic (Boyd, 2015). Toxicity can occur when the uptake of an element is greater than an organism’s capacity for detoxification or excretion (Hare & Campbell, 1992). Elements like the metalloid arsenic (As) and the metals cadmium (Cd) and lead (Pb) have no biological role and can be toxic even at low concentrations (Hare & Campbell, 1992). Links between metal accumulation and toxicity are well documented in the literature at many levels of biological organization, ranging from suborganismal (i.e., gene, cell, tissue); (Cain et al., 2006; Isaac et al., 2013; Mattingly et al., 2000), to organismal (Cain et al., 2006), to population (Cain et al., 2011; Schmidt et al., 2011) to community (Brix et al., 2005; Clements et al., 2000; Maret et al., 2003;) to the ecosystem (Krause et al., 2014; Mebane, 2001). Also, since insects are close to the base of the food web, metal(loid)s in insect tissue threaten higher trophic levels such as fish (Farag et al., 1999; Woodward et al., 1994). The elevated threat that metal(loid)s pose to insects and other aquatic organisms makes it necessary to understand their dynamics and what leads to their accumulation in aquatic organisms.

The behavior of metal(loid)s in aquatic ecosystems is complicated because they can exist in various physical and chemical forms, and the properties associated with these different forms ultimately influence their mobility, transport, and availability to aquatic organisms. Once metal(loid)s are introduced into the environment, they undergo biological and chemical transformations that lead to their distribution in environmental compartments such as sediments and water (Virendra et al., 1994). The biogeochemical processes that control metal(loid) mobility and bioavailability include sorption on mineral and organic surfaces, dissolution, mineralization, redox processes, and uptake by biota (Kraemer & Hering, 2004). These processes determine the metal(loid) speciation in the environment and, therefore, their bioavailability and toxicity. Dissolved phase ionic forms of metal(loid)s have traditionally been viewed as more bioavailable and potentially posing a greater toxicity and bioaccumulation risk than those in the particulate phase (Burgess & Kester, 2002), while complexed metal(loid)s have traditionally been considered as non-bioavailable and therefore, not toxic to resident biota (Calmano et al., 1993; Chapman et al., 1999; Eggleton & Thomas, 2004). Under this paradigm, efforts to predict accumulation in aquatic organisms have resulted in models such as the Free Ion Activity Model (FIAM; (Morel, 1983) and its successor, the Biotic Ligand Model (Di Toro et al., 2001), both of which define the free-metal ion as the only bioavailable species, and thus the driver of bioaccumulation and subsequent toxicity in aquatic organisms. These models predict the behavior of a free-metal ion at a biotic ligand site, typically a gill. Therefore, an aquatic exposure pathway of metal(loid) accumulation is the only route considered by these two models.

Focusing solely on aquatic exposure pathways as a source of accumulation may be insufficient, we know that diet can drive the accumulation of metal(loid)s by a variety of aquatic insects (Burgess & Kester, 2002; Cain et al., 2011; Croteau & Luoma, 2008; Irving et al., 2003; Martin et al., 2007). For example, though copper sorbed on colloidal hydrous ferric oxide is assumed to be unavailable for uptake according to the FIAM or BLM, it can be assimilated by mayflies ingesting treated periphyton (Cain et al., 2013). Based on these and other studies implicating diet as an important source of metal accumulation, "biodynamic models" have been developed which explicitly incorporate metal uptake from both food and water, allowing the relative importance of each to be quantified and compared (Croteau & Luoma, 2008).

Internal physiological parameters are integral to biodynamic models as physiology can affect how metals entering from either aquatic or dietary pathways accumulate. These parameters include uptake rates from dietary forms, uptake rates of dissolved forms (aquatic exposure), and loss rates (Croteau & Luoma, 2008; Luoma & Rainbow, 2005). Therefore, it is likely that accumulation patterns are driven by the complicated interactions between environmental parameters, and phylogenetic and physiological mechanisms (Cain et al., 2011; Croteau & Luoma, 2008). For example, influx rates from diet are determined by the concentration of metal(loid)s in the diet itself, as well as the interaction between species-specific ingestion rate and the subsequent assimilation efficiency (Luoma & Rainbow, 2005). Since taxa can differ greatly in terms of their physiology, the influence of an extrinsic parameter such as metal(loid) concentration in a given food source will be most apparent when comparing accumulation patterns among phylogenetically similar insects ingesting different food items. For example, mayflies of the same species fed different diets in a laboratory study ended up with different metal(loid) body burdens (Xie & Buchwalter, 2011).

Basal resources such as periphyton and fine particulate organic matter, both of which act as a food source for aquatic insects, likely differ in their concentrations of metal(loid)s and the bioavailability of those metal(loid)s to successive trophic levels. Periphyton is defined here as the heterogeneous yet structured mixture consisting of diatoms, algae, bacteria, detritus, and fungi, embedded within an extracellular polymeric substance (EPS) growing on submerged surfaces (Lock et al., 1984). Periphyton is a dietary source of metal exposure because it is a food source for many types of aquatic insects and can accumulate both essential and non-essential trace metal(loid)s, often reflecting dynamic fluctuations in the overlying water (Cain et al., 2011). Fine particulate organic matter (FPOM) may also be a carrier for metals, since its large surface area increases its binding potential (Schaller et al., 2011). Furthermore, FPOM is associated with microbial communities that inhabit its surface and use it as an energy source (Hieber & Gessner, 2002). Since microbes sequester a wide range of metal species (Volesky, 1994), they may control metal(loid) accumulation by aquatic invertebrates that ingest fine particulate organic matter. In contrast to periphyton, the extent of contamination and potential for metal(loid) uptake of FPOM has attracted limited attention, and thus FPOM's potential to be a dietary source of metal(loid)s to aquatic insects is unclear.

Contaminated sediment is likely a source of metal(loid)s to basal resources and the insects feeding on them. Within an aquatic environment, the proportion of metal(loid)s present as dissolved ions is usually low because most metal(loid)s either end up complexed with organic matter or suspended sediment, or deposited in the bottom sediments (Akcil et al., 2015; Fuentes-Gandara et al., 2021) via processes such as precipitation and flocculation (Bartoli et al., 2012). As much as 99% of metal(loid)s in an aquatic system can be associated with sediment due to their sorptive nature (Akcil et al., 2015). However, metal(loid)s in sediments can be re-released via changes in water conditions, such as hydrodynamics, temperature, and pH (Zoumis et al., 2001). As such, contaminated sediments act as both an important source and sink of metal(loid)s. Sediment contamination is a widespread problem in the United States, and it has been estimated that approximately 10% of sediments underlying freshwaters are contaminated (Mulligan et al., 2001). Interestingly, a complete understanding of the relationships between concentrations of metal(loid)s in water, sediment, basal resources, and aquatic insects remains unclear. Many monitoring efforts focus on water or sediment to determine the extent of metal(loid) contamination. However, understanding how concentrations in these abiotic environmental compartments relate to concentrations in basal food sources is important to estimate which compartments most reflect the potential for metal accumulation in primary consumers such as aquatic insects.

Streams are heterogeneous environments that often contain a hierarchically structured patchwork distribution of habitats (Frissell et al., 1986), with distinct biotic and abiotic conditions that may lead to differences in dietary metal exposure to aquatic insects. The definition of a "habitat patch" changes based on the scale at which it is being examined, but they are generally defined by certain combinations of velocity, depth, and substrate roughness (Brooks et al., 2005), and the size and texture of river substrates (Downes et al., 1995). These varying combinations of environmental conditions in turn influence the presence, type, and abundance of the insects, basal resources, and benthic particulate organic matter. Periphyton responds to the physical characteristics that may define a habitat patch, including light (Hill & Fanta, 2008), depth (Trbojevic et al., 2018), and substrate (Amirtharaj et al., 2022). At whatever the scale, identifying patches within a river can be a helpful way to characterize unifying physical qualities and potential niches for aquatic organisms, since conditions within a defined habitat patch have the potential to greatly influence the organisms living there. It may also be that basal resources in contrasting habitat patch types (e.g., riffles, glides, large wood) may differ in their metal(loid) concentrations due to their different environmental conditions, although few studies have explored this.

Despite knowing that a metal(loid)-contaminated diet causes accumulation in aquatic insects, few field-based studies attempt to discern accumulation patterns based on differences in the basal resources available to insects. This study set in the mine-waste contaminated Upper Clark Fork River in Montana had two objectives: 1.) to characterize the relationship between river water, sediment, and basal resources along a contamination gradient to see how well we could extrapolate from commonly measured environmental pools like water and sediment to less commonly examined pools like basal resources, and 2.) to examine patterns in metal(loid) concentrations in basal resources and aquatic insects living in different habitat patch types. For the first objective, we expected metal(loid) concentrations in water to be closely related to those in sediment and basal resources. For the second objective, we expected to see different metal(loid) concentrations in basal resources from different habitat patches, and in turn, we expected differences in concentrations in aquatic insects by habitat patch type due to dietary accumulation. Examining differences in metal concentrations in insects exposed to the same water but different basal resources in a field setting moves us towards understanding how factors other than aquatic exposure affect metal accumulation in organisms living in a contaminated river.

2 Materials and Methods

2.1 Description of study river

The Clark Fork River is in western Montana in the Columbia River basin. The basin's hydrology is primarily snowmelt driven, although spates can result from precipitation events. The Clark Fork River originates near Anaconda, MT, at the confluence of Warm Springs and Silver Bow Creeks. Silver Bow Creek originates in Butte, MT, and flows for approximately 40 km before it reaches Warm Springs Creek to become the headwaters of the Clark Fork. The Upper Clark Fork River (UCFR) is the approximately 210 km stretch of river that flows from the headwaters near Anaconda to where the Clark Fork meets the Blackfoot River near Missoula.

2.2 Clark Fork River mining history

Over a century of mining and smelting operations have left the UCFR contaminated with toxic trace metals, which continue to be a target for ongoing remediation efforts. Mining for gold and then silver began in the 1860s in Butte, but ultimately it was copper that became the target for mining operations (Freeman, 1900), given the abundant ore and its utility for the transmission of electricity during the industrialization of America. Ore deposits were extensively mined, milled, and smelted in the drainage of Silver Bow Creek. In 1886, smelters were also built in the nearby city of Anaconda to keep up with increasing demand. Tailings derived from these mining and ore processing operations, along with atmospheric pollution from the smelter stacks, were directly deposited in both streams and floodplains in the headwaters of the Clark Fork River near Butte and Anaconda (Moore & Luoma, 1990; Nimick & Moore, 1991). These tailings contained large quantities of toxic trace elements, including the metalloid arsenic (As) and the metals cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). A large flood in 1908 caused millions of tons of these toxic elements contained in tailings to mix with stream sediment and be washed downstream, resulting in contamination of the river and floodplain for over 500 km (Nimick & Moore, 1991). In addition to the sediment distributed in the river channel and surrounding floodplains, contaminated sediment was also captured behind the then newly built Milltown Dam just upstream of Missoula.

The extensive contamination was not formally addressed until 1983 when As groundwater contamination from the sediment behind the Milltown dam prompted the Montana Department of Environmental Quality and the Environmental Protection Agency (EPA) to develop a plan to address the contamination. The remediation plan has resulted in a series of Superfund designations, and to this day, the UCFR is still the largest complex of Superfund sites in America. Remediation efforts have been underway for decades, but extensive improvements in the environmental conditions throughout the watershed are still needed, and federal, state, and local agencies continue to contribute to ongoing cleanup and monitoring efforts.

2.3 Experimental design

To understand the patterns along the UCFR in the concentrations of metal(loid)s in basal resources and abiotic environmental compartments, we conducted a longitudinal survey of seven sites along the 210 km of the UCFR. To understand the extent to which metal(loid) concentrations varied in basal resources and invertebrates from different habitat patch types, we conducted a habitat patch survey across different habitat patch types at just one of the sites included in the longitudinal survey. The seven sampling locations for the longitudinal survey were selected along the UCFR to encompass a range of contamination levels (Table 1, Figure 1), with higher contaminant concentrations in general near the headwaters of the Clark Fork River (at Warm Springs) and lower concentrations downstream of the relatively uncontaminated Blackfoot River (Hornberger et al., 2009). The sampling location for the habitat patch survey was selected based on its high level of habitat heterogeneity.

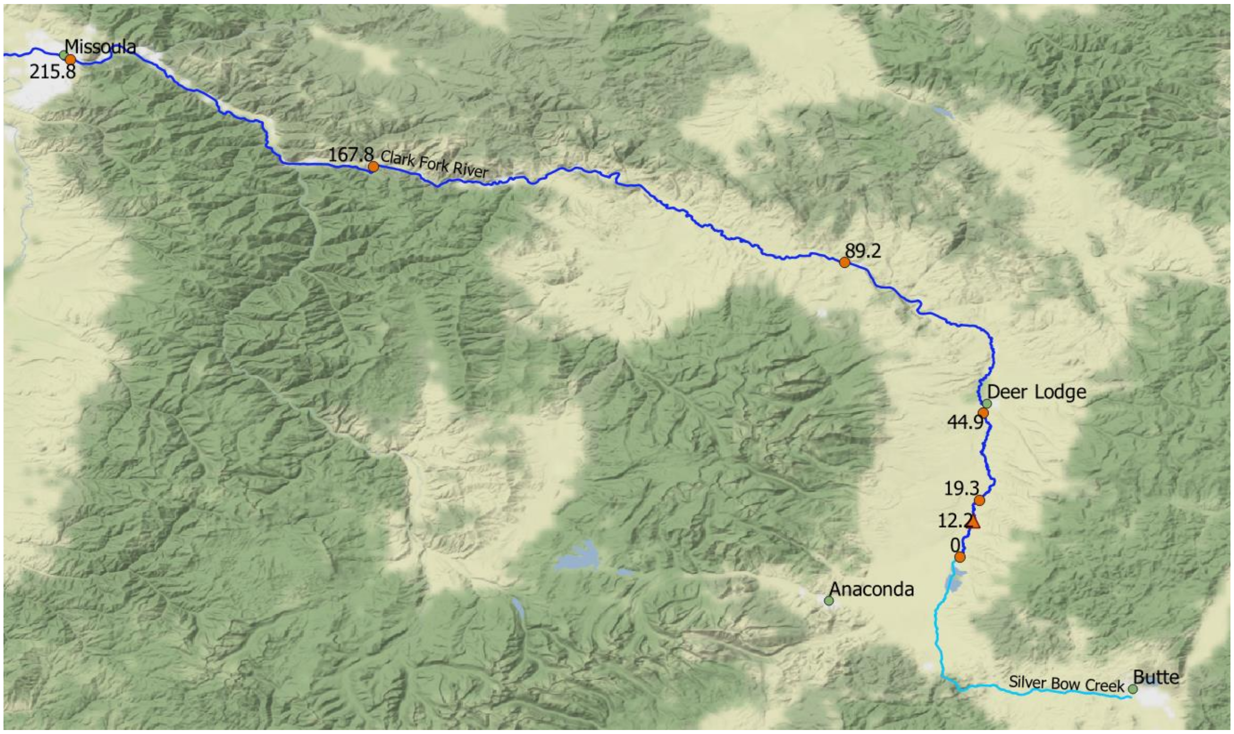

Figure 1 Map of the Upper Clark Fork River showing the location of seven sampling sites included in this study. River distances downstream in kilometers are indicated. The site marked with a triangle symbol is the site of the habitat patch survey.

Table 1 Name, distance downstream in kilometers, and coordinates of the seven sites included in the project.

Site Name	Distance downstream (km)	Latitude	Longitude
Warm Springs	0	46°11'15.1"N	112°46'15.3"W
Galen	12.2	46°14'13.7"N	112°45'11.3"W
Racetrack	19.3	46°15'55.5"N	112°44'40.5"W
Deer Lodge	44.9	46°23'01.2"N	112°44'20.9"W
Gold Creek	89.2	46°35'24.0"N	112°55'43.0"W
Bonita	167.8	46°43'17.8"N	113°34'21.7"W
Missoula	215.8	46°52'01.1"N	113°59'17.4"W

In addition to spanning the contamination gradient, we selected sites that overlapped with ongoing sampling efforts and for suitability based on physical and hydrologic conditions. The selected sites are a subset of a larger set of sites that are sampled annually by the USGS for the EPA for metal(oid)s analysis of tissues and sediments (Clark, 2020), quarterly by the Montana Department of Environmental Quality (Naughton et al., 2020), and monthly to biweekly by researchers at the University of Montana for metal(oid)s analysis of water and sediment (unpublished). Additionally, we controlled for physical and hydrological characteristics that have been shown to affect the community composition and relative abundance of periphyton (Fisher

& Dunbar, 2007; Horner & Welch, 1981; Roberts et al., 2004; Steinman & McIntire, 1987; Winter & Duthie, 2000).

For both surveys, we sampled based on habitat types. Although no consistent definition exists that includes both the geomorphologic and the ecological perspective of what defines a habitat patch, for the purposes of the habitat patch survey, the patches were defined not only by their physical and hydrologic characteristics but also by how these differences were hypothesized to alter the food resources available to aquatic organisms inhabiting the patch. We established three habitat patch types, with glides and riffles included for both surveys and large wood included for just the habitat patch survey. We sampled two habitat types at each longitudinal site, and five replicate patches for each habitat type at the site chosen for the patch survey. Initially, we had also sought to include submerged macrophytes as a fourth habitat patch type, but we later excluded it from the study due to an absence of the types of insects that were ubiquitous in other patches.

The characteristics that defined habitat patches for the patch survey were depth, velocity, and substrate. Riffle patches had a shallow depth (0.22±0.11 m; mean±SD), high flow velocity (0.90 ±0.20 m/s; mean±SD), and large cobbles covered 100% (visual estimation) of the sampled area. Glide patches were deeper (0.47±0.13 m), had lower flow velocity (0.67±0.12 m/s), and mixed substrate composed primarily of gravel to large cobble that covered approximately 90% of the sampled area, with the remaining 10% consisting of sand. Some submerged aquatic vegetation was present in the glide patches we sampled, although never in more than approximately 5% of the patch. Wood patches were the deepest patch type (0.93±0.44 m), had the lowest velocity (0.12±0.05 f/s), and primarily had sand that covered ~60% of the sampled area. No submerged aquatic vegetation was present in any of the wood patches we sampled.

We collected a variety of sample types for both surveys, including periphyton, benthic particulate organic matter (BPOM), surficial sediment, and water; aquatic invertebrates were only collected for the habitat patch survey. Periphyton and BPOM were collected from discrete habitat patches, while water and sediment were not keyed to a specific habitat patch type, instead representing site-wide conditions. Sampling occurred between September 12-16, 2019, for the longitudinal survey and August 13-21, 2019, for the habitat patch survey. Sampling occurred during annual base flow when the most downriver sites became wadable and within as short a timeframe as possible to capture a snapshot of site and habitat conditions and exclude temporal patterns.

2.4 Field sampling and processing

We collected periphyton from rock surfaces from all sites, and additionally from submerged wood at the site chosen for the habitat patch survey. The field protocol for sampling periphyton was modified from USGS sampling methods (Moulton, 2002). Epilithic periphyton was collected by scrubbing rock surfaces with an acid-washed nylon toothbrush and rinsing all dislodged material into an acid-washed 250 mL HDPE plastic bottle (VWR Laboratories, Radnor, PA) using stream water filtered through glass fiber filters (0.7 µm nominal pore size; Merck Millipore, Burlington, MA).

For wood habitats in the patch survey, we selected pieces of submerged wood, removed them from the river, and gently scraped and brushed them using an acid-washed nylon toothbrush to dislodge the periphyton growing on the wood pieces. Samples were stored in field sampling bottles and transported in a cooler with ice. At the end of each field day, samples were centrifuged at 2500 RPM for 20 min. The resulting supernatant was decanted, and the remaining periphyton sample was placed in a -80°C freezer until further processing and elemental analysis could be completed.

We sampled BPOM from both riffle and glide habitats at all sites and wood habitats from the site chosen for the habitat patch survey. A modified stovepipe benthic corer device was created by removing the bottom of a tower of two five-gallon buckets. The device was planted firmly on the stream bottom by removing all rocks from the perimeter while pushing the device into the top layer of substrate. We removed rocks around the perimeter to create a tight seal, and then we removed the surface rocks from inside the device. We agitated the water inside of the device with vigorous mixing by hand to suspend any particulate matter contained inside, making sure to focus on suspending any material on the riverbed. This water was pumped out using a small handheld boat bilge pump (Attwood, Lowell, Mi) and sieved through a 1000 µm nitex net into a catch bucket. All material larger than 1000 µm was discarded, and the water in the catch bucket was filtered (0.7 µm; Merck Millipore, Burlington, MA). The filters contained a layer of BPOM which we collected and carefully preserved by folding the filters in half and placing them in a sealed polyethylene bag (Whirl-pak, Madison, WI). Samples were stored and transported in a cooler with ice. At the end of each field day, samples were placed in a -80°C freezer until further processing and element analysis could be completed.

We collected sediment samples from each longitudinal site and any low or no velocity habitat at the site chosen for the habitat patch survey. In suitable depositional areas clear of detritus and/or other debris, the top 2-4 cm of sediment was collected with a clean melamine serving spoon and placed in a field sieve with a 63 µm nitex mesh. A minimum of five grabs were taken at each sampling location, sieved, and collected into a clean 500 mL HDPE plastic bottle (VWR Laboratories, Radnor, PA). Finally, the sediment collected from the three different sampling locations within each site was homogenized by mixing the bottle contents to ensure a representative sample. Samples were stored in field bottles and transported in a cooler with ice. At the end of each field day, samples were centrifuged at 2500 RPM for 20 minutes in the same collection bottles that were used in the field. The resulting supernatant was decanted, and the remaining sediment was desiccated in a drying oven at 105°C for 24 hours and stored until further processing and element analysis could be completed.

Water sample data was obtained from the Consortium for Research on Environmental Water Systems, which sampled from all longitudinal sites. Water samples were collected bimonthly from April to August. We chose to include approximately five months of water samples to avoid attributing any potential disparities between metal(loid) patterns in the water and other sample types, as water samples from an individual date only provide an indication of metal(loid) concentrations on a relatively short time scale, while sediment (Kevin et al., 2008; Owens et al., 2001; Sharley et al., 2016) and periphyton (Bradac et al., 2009) concentrations represent longer time scales. Both filtered and whole surface water samples were collected. All filtered samples (250 mL) were obtained by filtering a portion of a larger (500 mL) mid-channel grab sample through a 0.7 µm filter (Whatman, grade GF/F, UK) into an acid-washed plastic bottle (250 mL HDPE, VWR Laboratories, Radnor, PA) and both samples were stored and transported in a cooler with ice. At the end of each day, both water samples were acidified to 1% concentrated nitric acid (HNO_3; VWR, Aristar Plus) for preservation until further processing and element analysis could be completed.

We sampled insects for the habitat patch survey using modified USGS protocols (Moulton, 2002). Insects were collected using a 500 µm canvas and mesh dip net. The substrate in approximately 1 m^2 of each habitat patch was agitated by shuffling and disrupting the substrate with boots, and the dislodged macroinvertebrates were collected in a net downstream. Rocks and wood within the 1 m^2 were picked up and scrubbed to further dislodge any clinging insects. Contents of the net were emptied into a streamside collecting tray. Three samples were collected from different, randomly selected 1 m^2 areas within each habitat patch. Insects were immediately placed in chilled, oxygenated buckets of river water and transported back to the lab at the end of each field day. At the lab, insects were depurated by being held alive in chilled water that was oxygenated using aquarium bubblers (Marine Metal, Clearwater, FL). At the end of 24 hours, they were collected and immediately placed in a -80°C freezer until further processing.

The different objectives for the longitudinal and habitat patch surveys dictated different sampling intensities for periphyton, BPOM, and sediment, although the sample collection methods were the same for both surveys. We collected more replicates for all sample types from each habitat patch type at the site chosen for the habitat patch survey since samples represented each habitat patch type rather than average site characteristics. For the longitudinal survey, epilithic periphyton was collected from 3-5 submerged rocks and composited from two areas of both riffle and glide habitats for a total of 4 replicates per site. For the habitat patch survey, a composite sample of three rocks was repeated three times for each of our three habitat patch types (i.e., glide, riffle, and wood) for a total of fifteen replicates per sample type. Samples of BPOM for the longitudinal survey were collected from two areas of both glide and riffle habitats for a total of four replicates per site. For the habitat patch survey, three replicates of BPOM were sampled from each habitat patch, for a total of fifteen replicates per patch type. For sediment, three depositional areas were sampled at each of the longitudinal sites and combined into one representative sample per site, while at the habitat patch survey site, sediment was collected from any low-velocity habitat patches where an area of clear deposition was observed. This typically occurred in the large woody debris or macrophyte patches, but not all of these habitat types were sampled for sediment due to the presence of debris and or a detrital layer that prevented an area of clear sediment deposition.

2.5 Laboratory procedures

Periphyton samples needed to be sorted and homogenized before being dried and weighed before acid digestion. Samples were processed at the University of Montana Environmental

Biogeochemistry Lab (UM EBL) before elemental analysis. We removed samples from the freezer and placed them on a clean petri dish. Each subsample was sorted by using plastic forceps to pick out any material that was obviously not part of the periphyton sample, which primarily included caddis fly cases and large pebbles. Sorted samples were transferred to a clean ceramic mortar (porcelain, unglazed) and cryogenically ground using a pestle and liquid nitrogen. The frozen periphyton was ground until small homogeneous pieces were achieved. Next, the contents of the mortar were placed in a 25 mL borosilicate glass vial (VWR Laboratories, Radnor, PA) and transferred to a drying oven at 50 °C. After 24 hours, the dried samples underwent a final homogenization step by being broken up and further ground using a glass rod to a powder-like consistency. Some samples contained fibrous material that would not easily grind down; in this case, the samples were cut up into fine pieces using a small pair of scissors. Finally, 100 mg of the dried homogenized sample was weighed into a metal-free plastic digestion tube (Digitube, SCP Science, Quebec Canada; hereafter just “digestion tube”) in preparation for acid digestion.

The BPOM samples needed to be removed from the filter they had been collected on before being dried and weighed in preparation for acid digestion. Samples were removed from the freezer and thawed out enough to unfold the filter, exposing the layer of BPOM on the filter. We scraped the BPOM from the filter into a 25 mL glass vial (VWR Laboratories, Radnor, PA) using a clean plastic straw that had been modified by part of the straw being cut away to create a scraping surface, then the filters were rinsed to remove any remaining materials with a small amount of Type I deionized water (hereafter DI; RODI-C-12BL, Aqua Solutions, Jasper, Georgia). The vials were placed in a drying oven at 50 °C for 24 hours, after which the entire contents of the vial were transferred to a pre-weighed digestion tube.

Sediment samples were ground in the same bottle they had been collected using a clean glass rod to break up all clumps, and 500 mg of the resulting ground sediment was weighed into a digestion tube in preparation for acid digestion.

Water samples were digested using an open vessel microwave digestion method. Water samples were brought to 10% V/V ultra-pure HNO_3 (VWR Aristar Ultra) in the collection tubes (VWR metal-free polypropylene centrifuge tubes). Samples were heated to 100°C over 30 minutes and held at that temperature for 10 minutes using a CEM MARS Xpress microwave digestion system

(Matthews, NC) with fiber optic temperature control. Following digestion, samples were brought to volume with 18 M Ohm DI water resulting in 5% V/V HNO_3.

From the larger assemblage of aquatic insects, we chose two genera based on their ubiquity among the habitat types, specifically *Baetis* and *Hydropsyche*. We did not identify larvae to species, given that pooling by these species does not make a difference in metal(oid)s analysis (Cain et al., 2004). As such, each genus was represented by up to three species. Insect larvae were removed from the freezer and transferred to a sorting dish. Insects were identified using descriptions from Merritt and Cummins (2008) and pictures of voucher specimens provided by a taxonomic expert (Rhithron, Missoula, MT). Younger instars that could not be easily identified and insects missing body segments were discarded. Insects were placed in a drying oven at 50 °C for 24 hours, after which a minimum 10 mg dry weight of larvae were composited in a digestion tube to achieve a minimum of three replicates for each taxon from each habitat patch.

Following sample preparation, we acid digested all samples using protocols specific to sample type, all of which were modified from USEPA Method 3050B (USEPA, 1996). For periphyton and BPOM samples, 1:1 HNO_3 (VWR Aristar Plus) was added to each digestion tube and heated until the volume was reduced. Insect larvae were similarly digested with 1:1 HNO_3 but with an additional sequential addition following reflux of H_2O_2 (Honeywell Puranal, 30%), which was added until effervescence subsided. Sediment was initially digested using 1:1 HCl (ACS Plus, Fisher Chemical), followed by 1:1 HNO_3. The resulting digest was cooled, brought to an appropriate volume with DI water, and filtered (SCP Science DigiFilter 0.45 µm Teflon Membrane).

We analyzed digest aliquots for As, Cd, Cu, Pb, and Zn using either inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectrometry (ICP-OES). Metal(oid)s in digests of all basal resources were analyzed using ICP-MS (Elan DRCII, PerkinElmer, Waltham, MA) or ICP-OES (Optima 5300, PerkinElmer, Waltham, MA) in the UM EBL. Metal(oid)s in streambed sediment were also analyzed at the UM EBL by ICP-OES. Metal(oid)s in insect tissue were analyzed by ICP-MS (iCap Q, Thermo Fisher, Waltham, MA) at the Montana Bureau of Mines and Geology (MBMG) lab in Butte, MT. Metal(oid)s in water samples were analyzed by ICP-MS (7500cx, Agilent, Santa Clara, CA) at the Plant and Soil Sciences nutrient analysis laboratory at the University of Kentucky.

Quality control was maintained for all analyses. Instrument calibration was verified by analyzing certified calibration solutions during each instrumental run. Certified internal standards were run throughout the analysis to allow for corrections due to any instrument drift. Procedural blanks, metal(loid)-spiked blanks and samples, and standard reference materials (SRM 8704 and STSD-2) were analyzed for quality control. Concentrations were corrected for instrumental drift when necessary.

2.6 Data processing and statistical analyses

To explore longitudinal patterns in the concentration of elements in basal resources, sediment, and water samples, we used generalized additive models (GAMs). All GAMs were fitted using longitudinal distance as a smooth function and resource type as a linear predictor using a Gamma distribution and a log link function, using the mgcv package (V1.8.40; Wood, 2017).

To quantify differences in metal(loid) concentrations among habitat patch types, resource types, or invertebrate taxa, generalized linear models (GLMs) were fit for the concentration of each element. We tested different data transformations (untransformed and logged) and "links" (log, identity) to explore how these variations fit the data and highlighted patterns across habitat patch types or resource types. While the marginal means and 95% confidence intervals shifted slightly between models, the overarching patterns remained largely unchanged. In the end, we opted for a Gamma distribution with a log link as it seemed to best fit our data. All GLM analyses were done using the built-in 'glm' function in base R.

For consistency in model structure, we retained all interaction effects for all elements. We calculated the estimated marginal means for each model using the emmeans package (V1.7.2; Lenth 2022), followed by post-hoc comparisons using Tukey's Honestly Significant Difference (HSD) test. We used a threshold value of $\alpha = 0.05$ for visualizing differences as implemented through the cld function in the multcomp package (V1.4.19; Hothorn et al., 2008). To allow low-value concentrations to be included in models, we censored our data before statistical analyses by replacing below detection limit concentrations with ½ Method Detection Limit (Clarke, 1998). Values that were calculated to be greater than three times the standard deviation in either direction were considered outliers and excluded from analysis, which resulted in the removal of five insect concentration values and six periphyton values.

To examine patterns in the concentration data among the different elements for the basal resources and aquatic invertebrates, we performed principal component analysis (PCA). The built-in prcomp function was used to center logged data on the means of each variable and transform the original variables (elements) to principal components (PCs). Loading arrows were included to show how much each variable contributed to a particular principal component. Statistical analyses were all performed with open source R statistical computing software (v4.2.0; R Core Team 2022).

3 Results

3.1 Longitudinal patterns

The overarching longitudinal patterns in metal(loid) concentrations differed between basal resources and sediment compared to water (Figure 2), with concentrations in basal resources and sediment peaking near the headwaters and concentrations in water peaking much further downstream for most elements. Among the basal resources and sediment, BPOM had the highest concentration for all elements, periphyton had the lowest, and sediment was intermediate between the others for Cu, Pb, and Zn and was still intermediate but closer to periphyton concentrations for As and Cd. For all elements, there was an increase followed by a decrease in metal(loid) concentration from upstream to downstream. However, the location of the maximum concentration did not always align between water and the other environmental compartments. Concentrations in basal resources typically peaked by 19.3 km, except for Cu and Pb in periphyton, which peaked at 44.9 km. In contrast, peaks in water concentrations were more variable, but with most maxima occurring after river km 19.3. Concentrations in As were highest at 12.2 km for both size fractions (whole and unfiltered). Peak concentration in Cd, Cu, and Pb occurred at km 44.9 for whole and filtered water samples. Finally, Zn peaked at km 167.8. A filtered size fraction for Zn was not included due to contamination in those samples. By 215.8 km, at Missoula, all concentrations had greatly decreased from 0 km for the basal resources and sediment. Generally, it appears that sediment and BPOM were mirroring each other well, and the filtered and unfiltered water were mirroring each other well. Periphyton did not consistently mirror either pattern. Overall, there was a disconnect between the benthos and the water column.

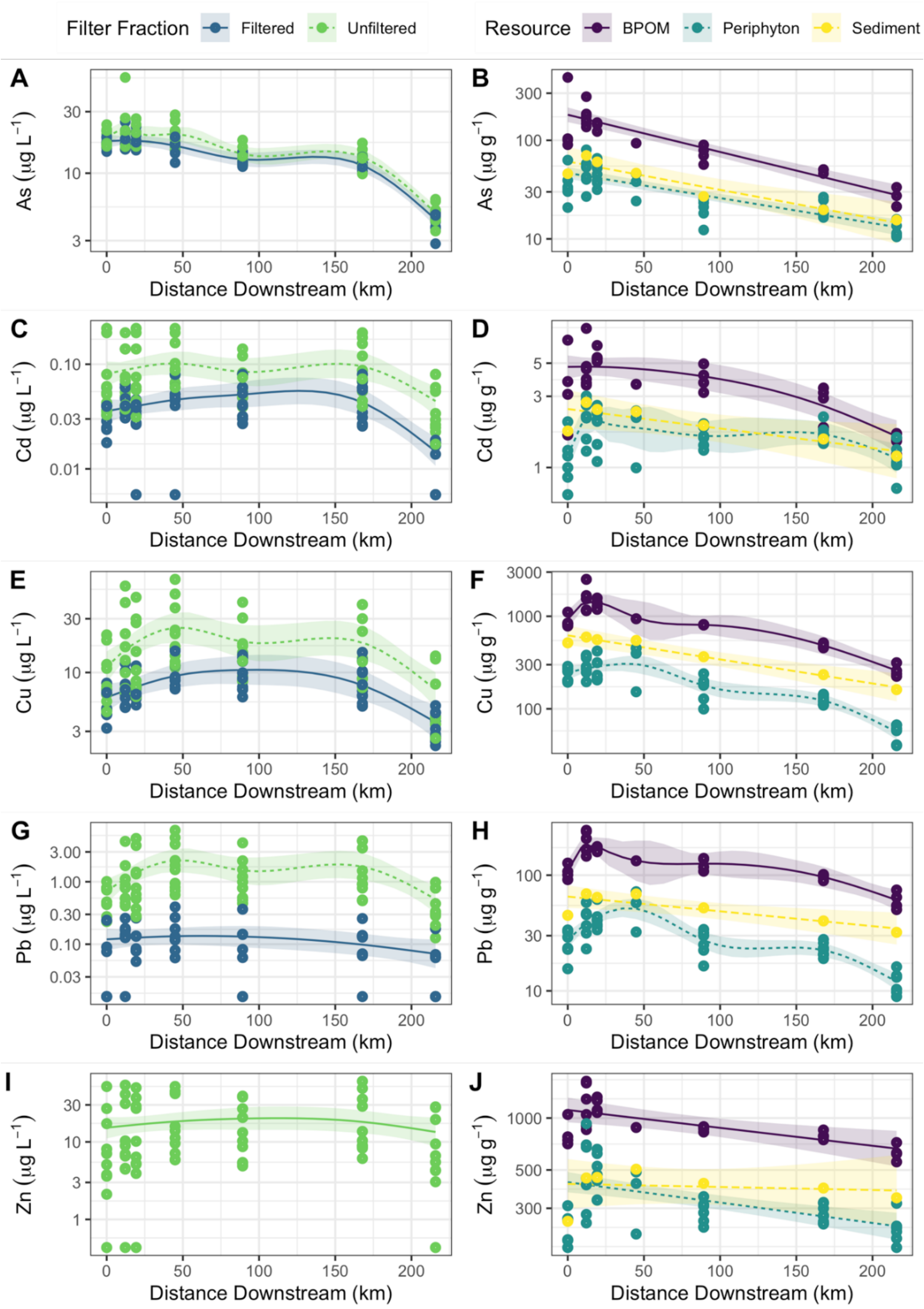

Figure 2: Plots of GAM (Generalized Additive Model) for As (A,B), Cd (C,D), Cu (E,F), Pb (G,H), and Zn (I,J). The solid lines represent loess (locally weighted polynomial) curves smoothed with a GAM fit, ribbons around the lines represent 95% confidence intervals, and each point represents a sample. Distance is represented on the x-axis in kilometers.

3.2 Basal resources across habitat patches

Much like in the longitudinal survey, BPOM from the patch survey always had higher concentrations for all elements than periphyton ($P < 0.001$ Table S2). Averaging across habitat types, the magnitude of those differences varied by element such that BPOM was 2.2-fold higher for As, 1.4-fold higher for Cd, 2.4-fold higher for Cu, 5-fold higher for Pb, and 1.8-fold higher for Zn when compared to periphyton (Figure 3).

Basal resources collected from wood habitats had the highest metal(loid) concentration for all elements, except for Zn in periphyton. Samples from wood habitats were higher for the majority of element/resource/habitat combinations (Figure 3 and Table S3). Only Zn in periphyton diverged from this pattern, with concentrations that were highest in riffle habitats.

The mean concentrations of metal(loid)s in basal resources in glide and riffle habitats showed few differences between basal resources, with BPOM and periphyton both typically having similar concentrations in both habitat types. There was little evidence of differences in sample means for BPOM collected from riffle and glide habitats (Figure 3 and Table S3). Furthermore, BPOM samples exhibited less dispersion (Figure 3). Periphyton samples showed differences in metal(loid) concentrations between glide and riffle habitats for only Pb and Zn, with periphyton from riffles having higher mean concentrations than periphyton from glides for these two elements (Figure 3).

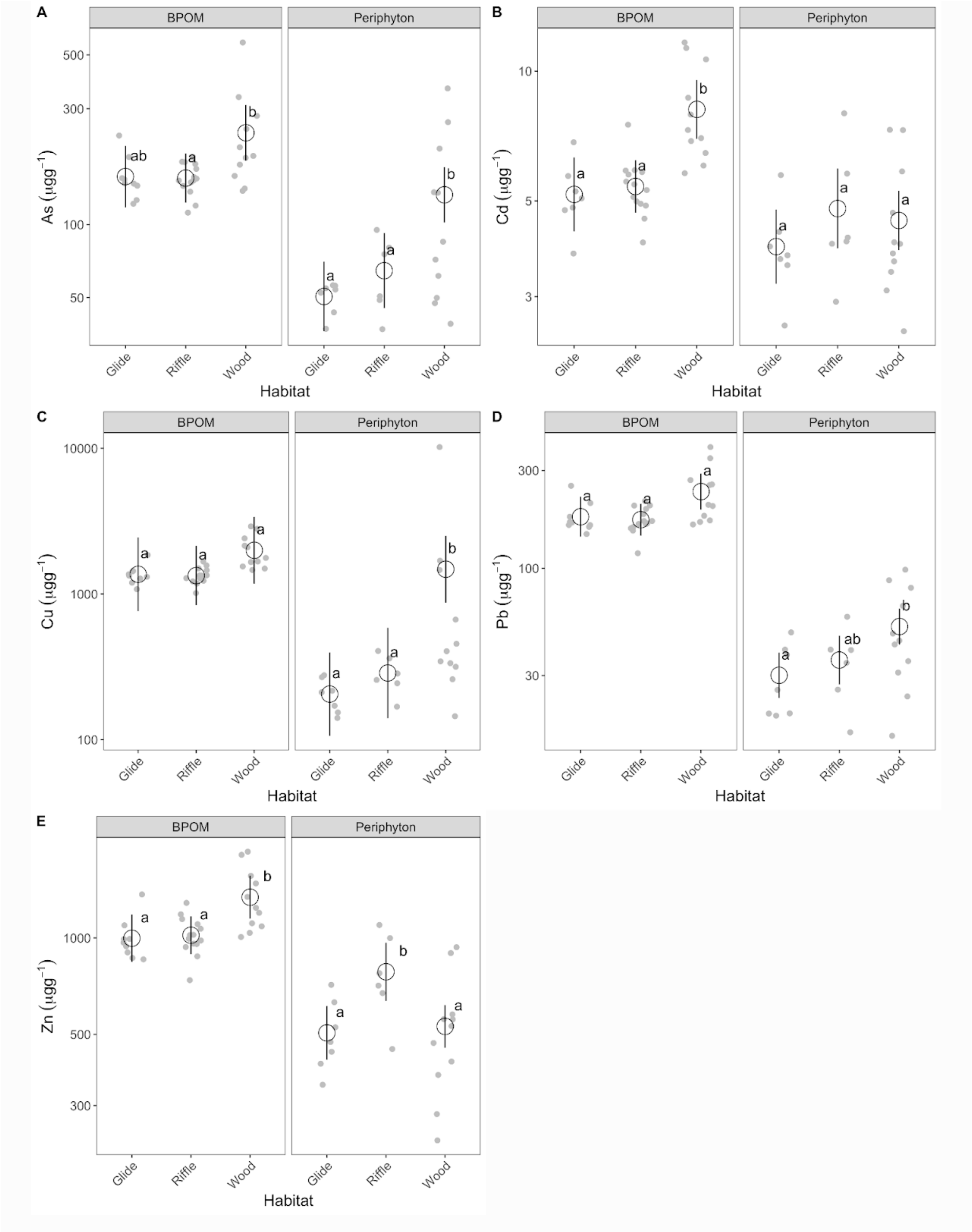

Figure 3: Metal(oid) concentrations in basal resources by habitat type for arsenic (A), cadmium (B), copper (C), lead (D), and zinc (E). Small points represent individual analyzed samples, open circles represent the means, and bars show 95% confidence intervals. Values that share a letter are not significantly different at $P < 0.05$ according to pairwise comparison using Tukey HSD tests.

3.3 Insects

Metal concentrations varied between insect taxa idiosyncratically, with *Baetis* having higher concentrations for Cd and Zn and *Hydropsyche* having higher concentrations of As, Cu, and Pb (Figure 4 and Table S5). *Hydropsyche* had As, Cu, and Pb concentrations that were 2.6, 1.5, and 2.9-fold higher, respectively, than *Baetis* when averaged across all habitat types. The lowest mean concentration in *Hydropsyche* is higher than the highest concentration in *Baetis* for all of these elements. For example, *Baetis* from wood habitats had the highest concentration of As at 34 [28 to 42] µg g^{-1} (mean [lower 95% CI to upper 95% CI]), and *Hydropsyche* from riffle habitats had the lowest concentration of As at 57 [47 to 68] µg g^{-1} (Table S5). In contrast, *Baetis* had higher concentrations of Cd (5.6-fold) and Zn (2.9-fold) than *Hydropsyche* when averaged across all habitat types. The lowest mean concentration of metal(loid) found in *Baetis* was still higher than the highest mean concentration of metal(loid) found in *Hydropsyche* for these elements. For example, *Baetis* from riffle habitats had the lowest mean concentration of Zn at 3500 [2900 to 4300] µg g^{-1}, and *Hydropsyche* from wood habitats had the highest mean concentration of Zn at 1870 [1500 to 2300] µg g^{-1} (Table S5).

In general, metal(loid) concentrations in both insect taxa varied by habitat type, with concentrations being the highest in wood and lowest in riffles. These patterns are especially clear for Cd, Cu, and Zn, for which both *Baetis* and *Hydropsyche* had mean concentrations that were highest in wood, intermediate in insects found in glides, and lowest in insects from riffle habitats. These patterns were more subtle for As and were different for Pb. For As, there was no evidence supporting that *Hydropsyche* and *Baetis* were different ($P = 0.995$). The pattern for Pb was similar for both taxa, although the pattern based on habitat type differs from the other elements in that insects collected from riffles had the highest concentrations, those from wood had intermediate concentrations, and those from glides had the lowest concentrations. Overall, the patterns by habitat type were clearer for *Baetis* than for *Hydropsyche*, with pairwise differences at $P < 0.05$ between habitat types for all elements for *Baetis*, while *Hydropsyche* only had pairwise differences at $P < 0.05$ for Pb and Cu (Figure 4 and Table S5).

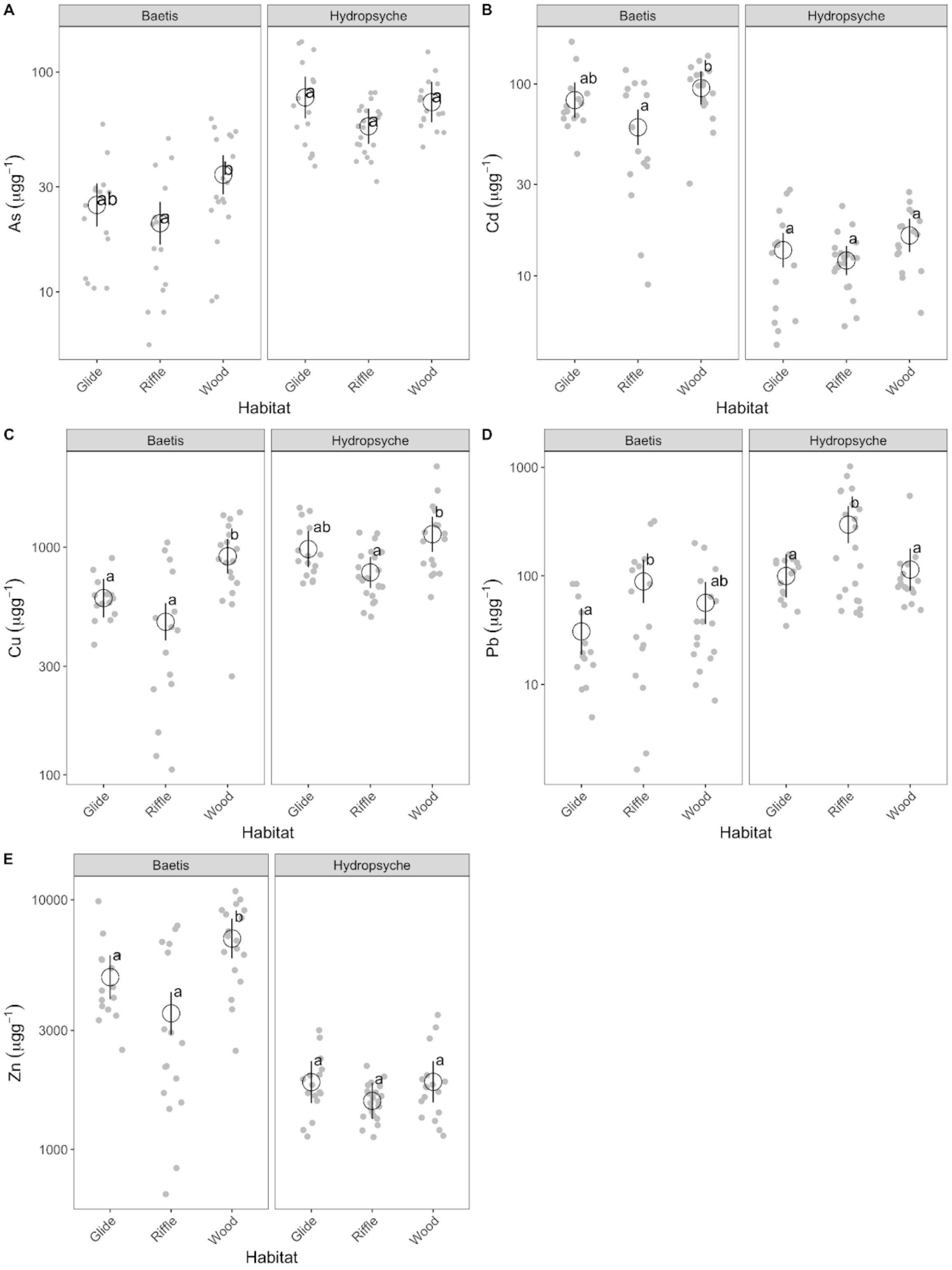

Figure 4: Metal(oid) concentrations in insects by habitat type for arsenic (A), cadmium (B), copper (C), lead (D), and zinc (E). Small points represent individual analyzed samples, open circles represent the means, and bars show 95% confidence intervals. Values that share a letter are not significantly different at $P < 0.05$ according to pairwise comparison using Tukey HSD tests.

3.4 Principal component analysis

Clusters of groups on the PCA indicated groups of samples that had similar or different characteristics. The concentrations of all elements in *Hydropsyche* more closely resembled those in the basal resources than did the concentrations in *Baetis* (Figure 5), as indicated by the overlapping clusters and associated loading arrows. The individual scores of each insect sample on the biplot showed there was no overlap between clusters of *Baetis* and *Hydropsyche*, consistent with the single element comparisons. The individual scores of each basal resource sample on the biplot showed some overlap between clusters of BPOM and periphyton. There was also some degree of overlap between the basal resources and *Hydropsyche*, although clusters did not show a complete overlap. Associations with loading arrows indicated which elements were more correlated with the corresponding group. For example, the loading arrows most associated with *Baetis* were Cd and Zn, while the loading arrows associated with *Hydropsyche* were Cu and Pb. These were consistent with the observations of higher Cu and Pb concentrations in *Hydropsyche,* and higher Cd and Zn concentrations in *Baetis* observed in our comparisons for single elements.

The first two principal components (PCs) explained 53.0% and 38.3% of the total variation of the data set, respectively, for a total of 91.3%. The PCA biplot (Figure 5) and the eigenvector matrix (Table S6) showed that PC1 described a dimension mainly associated with As, and to a lesser extent, with Pb and Zn. PC2 described a dimension mainly associated with Cd and Cu.

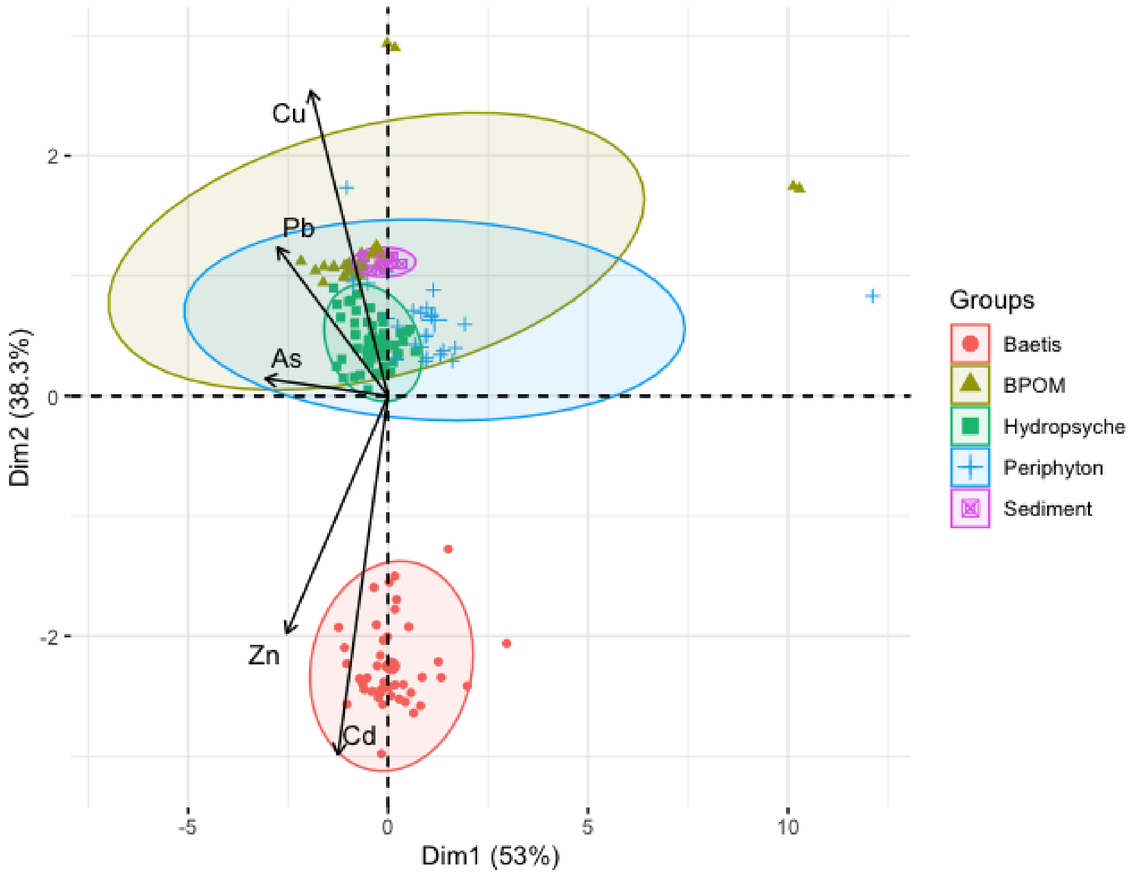

Figure 5. Principal component analysis of metal(loid) concentrations in periphyton, BPOM, Hydropsyche, Baetis and sediment. Arrows represent the loading of each element on the principal component axes in relationship to the data points. Ellipses represent the 95% CI for the data.

4.0 Discussion

4.1 Concentration in water differed from those in sediment and basal resources longitudinally

Although longitudinal patterns in basal resources and sediment tended to align with one another, these patterns were often distinct from concentrations in water. Water concentrations almost always peaked further downstream than concentrations in BPOM or sediment. Periphyton seemed to behave intermediately between water and BPOM/sediment. Periphyton had maxima that aligned with water for the elements As, Cu, and Pb and had a longitudinal pattern similar to that of BPOM and sediment.

The misaligned patterns between water and the other sample types we collected suggest that the metal(loid) concentrations in filtered and unfiltered water may not accurately indicate exposure and accumulation in sediment and basal resources. Two divergent explanations exist for why water may not be driving concentrations in other compartments. The first is that the metal(loid)s captured in our water samples are likely not just in the form of free-metal ions, and therefore their ability to drive accumulation is limited. According to the FIAM and BLM, metal(loid) accumulation an toxicity are not related to the total but to the free metal ion concentration in water. Although there are ways to model or directly measure free metal ions, modeling chemical speciation in natural waters is complicated because the nature of the complexing agents and their concentrations is often unknown (Kalis et al., 2006), and direct measurement requires special techniques that we did not use. Additionally, the concentration of free metal ions is often below detection limits (Kalis et al., 2006). Since the patterns we see in the whole and filtered water samples do not necessarily represent the patterns of free metal ions in the Clark Fork River, patterns between water and basal resources/sediment should not necessarily match. Another explanation for the misaligned patterns is that aqueous metals simply may not be driving accumulation in the samples we collected. Although the applicability of the FIAM and BLM to metal(loid) accumulation in periphyton or algae has been successfully demonstrated for certain metals in laboratory experiments (Kola & Wilkinson, 2005; Meylan et al., 2004; Vigneault & Campbell, 2005), there are also documented exceptions to the bioavailability and accumulation predictions these models provide (Errécalde & Campbell, 2000; Meylan et al., 2004; Phinney & Bruland, 1994). If something other than water (e.g., sediment) is a better indicator for accumulation in basal resources, it would explain why the patterns in water do not match. The misalignment of concentration patterns between water on the one hand and sediment and basal resources on the other illustrates further research opportunities by sampling across these contrasting gradients to better understand the contributions of dietary vs. aquatic exposure in driving metal(loid) accumulation in insects.

4.2 Metal(loid) concentrations in basal resources differed by habitat type

Samples collected from wood habitats tended to have higher metal(loid) concentrations than those collected from glides or riffles, possibly due partly to the lower stream velocity, which encourages the deposition of fine-grain sediment in wood habitat patches. One of the many factors determining sediment and metal(loid) associations is sediment grain size, though the chemical and physical relationships between sediment and metal(loid)s are extremely complex (Chapman et al., 1999). While particle size does not always predict the concentration of associated metal(loid)s, particularly in heavily contaminated rivers (Moore et al., 1989), the concentration of metal(loid)s in sediment generally increases with decreasing grain size because of the affinity of metal(loid)s to bind with fine-grained (<63 μm) particles (Oliver, 1973). Our observations of a higher percentage of smaller sediment size fractions in the wood patches we sampled are consistent with a potential role for sediment deposition driving patterns in basal resources in wood patches.

There is both a direct pathway and indirect pathway by which enhanced deposition of fine sediment may lead to elevated concentrations of metal(loid)s in BPOM and periphyton in wood habitat patches. The direct pathway is that periphyton accumulates previously suspended organic and inorganic materials, which may become entrapped in the EPS matrix of the algae, fungi, and bacteria that make up these communities (Weitzel, 1979). Studies seeking to isolate accumulated metal(loid)s address this by washing samples to rid them of extracellular material (e.g. Behra et al., 2002) to focus on intracellular metal(loid) concentrations. Since we did not take this step during sample processing, it stands to reason that a portion of the samples we processed are inorganic material, including fine sediment. Similar mechanisms may explain the patterns we observed for BPOM. The indirect pathway is that there may be accumulation via contact with contaminated sediment, which has been documented in macroinvertebrates (Fan et al., 2014; Hamidian et al., 2016), diatoms (Absil & Van Scheppingen, 1996; Sbihi et al., 2014), and soft algae (Hamidian et al., 2016). Metal(loid) contaminated sediment may also lead to accumulation in bacterial communities since microbes sequester a wide range of metal(loid) species (Fereidouni et al., 2009; Hamidian et al., 2016; Volesky, 1994). The indirect pathway is largely driven by complex geochemical processes at the sediment-water interface that result in the release of sediment-associated metal(loid)s into pore water (Shaw et al., 1990). Aqueous metals associated with pore water, either alone or in combination with direct sediment incorporation, lead to uptake via passive or active diffusion in organisms living in close proximity to this source of contamination (Pandey, 2020).

4.3 Insect metal(loid) concentrations differed by habitat type and taxa but mirrored basal resources

Insect metal(loid) concentrations differed by habitat type and largely mirrored the patterns of basal resources, with the highest concentrations for most elements occurring in insects from wood habitats. Insects in part accumulate metal(loid)s via contaminated food sources, so it stands to reason that insects living in a habitat with elevated metal(loid) concentrations in their food sources would also have assimilated elevated concentrations of metal(loid)s into their tissues. Metal(loid)s available to an insect through ingestion of a contaminated food source may be bioavailable whether associated with extracellular sediment particles or from intracellular accumulation. We know that the elevated concentrations in insects from wood habitats represent assimilated metal(loid)s because the insects were cleared of their gut contents prior to processing. Given that the differences we observed in the insects based on habitat type generally align with higher concentrations in both basal resources, our data support the importance of dietary exposure as a driver of accumulation.

The differences in accumulation between *Baetis* and *Hydropsyche* were likely driven in part by differences in feeding mode and therefore diet (Cain et al., 2011; Croteau & Luoma, 2008). The insects in this study have morphological-behavioral adaptations that allow them to ingest food preferentially. *Baetis* have non-specialized mouthparts that allow them to sweep periphyton and other particulate organic matter into their mouths. *Hydropsyche* uses nets to trap fine particulate organic matter from the passing water column (Cummins & Klug, 1979). Since periphyton serves as a primary food source at the base of aquatic ecosystems and is a major sink for trace metal(loid)s (Kim et al., 2012), we expected to see similar concentrations between periphyton and the insects, particularly in *Baetis* which preferentially feed on rocks with a high density of periphyton (Alvarez & Peckarsky, 2005). Conversely, *Hydropsyche* are classified as filter feeders (Cummins & Klug, 1979), thus, they are likely incorporating more suspended FPOM in their diet. Since there is not a good reason to believe suspended particulate matter would differ appreciably by patch type, we would expect fewer differences between this taxon by habitat patch type, as our data show. Differences in habitat preference may also contribute to differences in metal(loid) concentrations between *Baetis* and *Hydropsyche*. Baetis are good swimmers and have access to variable positioning in the water column or river habitats, while *Hydropsyche* are stationary filterers and are more closely associated with the benthos. The uptake of metals from dietary exposure and aquatic exposure by insects is thought to be additive, though it is difficult to determine to what extent since physiological parameters such as ingestion rate and assimilation efficiency cannot be easily measured in a field environment. Differences between the two taxa considered in this study support that feeding strategy, and thus diet, is a driver of accumulation.

Diet alone did not fully explain the patterns in metal(loid) concentrations in *Baetis* and *Hydropsyche*, as there appear to be fundamental differences in how these insects accumulate specific metal(loid)s that are likely driven by physiology. Notably, we observed that concentrations of Cd and Zn were higher in insects than in basal resources, particularly for *Baetis*. Elevated concentrations of Cd and Zn associated with *Baetis* are clearly shown in both the PCA (Figure 5) and the univariate analyses (Figure 4), where Cd and Zn are highest for *Baetis*. In a 2003 study about metal(loid) tolerance in aquatic insects (Cain et al., 2003), *Baetis* collected from the Clark Fork River had proportionately higher concentrations of Cd and Zn bound to a metal-sensitive protein, suggesting a potentially higher sensitivity to these metals. *Baetis* collected from the Arkansas River (CO, USA) exhibited accumulation of Cd, Zn *and* Cu (Kiffney & Clements, 1993), which is not what we saw in our data, underscoring the complexity introduced when considering that metal(loid) and insect-specific accumulation and tolerance may also be influenced by specific environmental parameters. Lead is another element that was notable in our study due to the aberrant patterns it exhibited compared to the behavior of the other elements included in this study. It is the only element that was not highest in either insect taxa collected from wood and instead is highest in insects collected from riffles, although the reason for this was unclear.

Some of the patterns between habitat types could also be explained by variables we did not measure, such as the developmental stages of *Baetis* and *Hydropsyche* in the different habitat types or the actual diet of the insects in the different habitat types. Development stage influences accumulation patterns, with earlier instars typically exhibiting proportionately more accumulation than later instar stages (Cadmus et al., 2020; Gintenreiter et al., 1993). In terms of the actual diet of the insects in these different habitat types, we inferred their diets based on concordance or lack thereof with basal resources that we analyzed and found strong evidence supporting the role of diet, as predicted by feeding strategies, in determining accumulation.

Taxa living in different habitat types at the same site were experiencing different levels of exposure which is driving differences in accumulation and likely toxicity. The wood habitats that produced the highest concentration of the majority of metal(loid)s measured in basal resources and insect larvae are not typically habitat types that are sampled during insect community assessments (Moulton, 2002). Interestingly, insects from riffles, which are the most common habitat type sampled during most monitoring assessments, particularly those assessing metal contamination levels, tend to have the lowest concentrations of most elements for both taxa. Traditional sampling approaches may not accurately represent an integrative view of site conditions and have the potential to underestimate the exposure and subsequent toxicity to organisms living outside of targeted habitat types or feeding on those organisms.

5 Summary

The objectives of this study were twofold: to examine patterns in the concentration of metal(loid)s in environmental compartments along a longitudinal contamination gradient and to determine whether metal(loid) concentrations differed in basal resources and aquatic insects across the habitat types they were collected from. We accomplished this by examining patterns of As, Cd, Cu, Pb, and Zn in the samples we collected from a longitudinal and separate habitat patch survey, which corresponded to our project objectives. For the longitudinal survey, we found disparate patterns when comparing water to sediment, periphyton, and BPOM. For the patch survey, we found differences in metal(loid) concentration in both insects and basal resources by habitat type. This information is complementary to a growing body of evidence that exposure to contaminated surface water is not the only driver of accumulation in organisms living in contaminated rivers. Our data also suggest that typical monitoring approaches may underestimate exposure and accumulation of metal(loids) through their focus on riffle habitats which had lower concentrations of most metal(loid)s compared with the wood patches in our study. Higher metal(loid) concentrations in basal resources and insects sampled from wood habitats support that feeding strategy, and thus diet, being partly responsible for overall accumulation patterns in aquatic insects living in a contaminated river.

6 Citations

Absil, M. C. P., & Van Scheppingen, Y. (1996). Concentrations of selected heavy metals in benthic diatoms and sediment in the Westerschelde estuary. *Environmental Contamination and Toxicology, 56*(6), 1008-1015. doi:10.1007/s001289900145

Akcil, A., Erust, C., Ozdemiroglu, S., Fonti, V., & Beolchini, F. (2015). A review of approaches and techniques used in aquatic contaminated sediments: Metal removal and stabilization by chemical and biotechnological processes. *Journal of Cleaner Production*, 86, 24-36. doi:10.1016/j.jclepro.2014.08.009

Alvarez, M., & Peckarsky, B. (2005). How do grazers affect periphyton heterogeneity in streams? *Oecologia*, 142(4), 576-587. doi:10.1007/s00442-004-1759-0

Amirtharaj, K., Ahilan, B., Rajagopalsamy, C., George, R., & Jawahar, P. (2022). Effects of different substrates on the growth and composition of periphyton in the low saline groundwater system. *Scienceasia*, *48*(1), 82-88. doi:10.2306/scienceasia1513-1874.2022.005

Bartoli, G., Papa, S., Sagnella, E., & Fioretto, A. (2012). Heavy metal content in sediments along the Calore river: Relationships with physical–chemical characteristics. *Journal of Environmental Management*, 95, S9-S14. doi:10.1016/j.jenvman.2011.02.013

Behra, R., Landwehrjohann, R., Vogal, L., Wagner, B., & Sigg, L. (2002). Copper and zinc content of periphyton from two rivers as a function of dissolved metal concentration. *Aquatic Science, 64*(3), 300-306. doi:10.1007/s00027-002-8074-9

Boyd, C. E. (2015). Micronutrients and Other Trace Elements. Cham, Switzerland: Springer International Publishing.

Bradac, P., Navarro, E., Odzak, N., Behra, R., & Sigg, L. (2009). Kinetics of cadmium accumulation in periphyton under freshwater conditions. *Environmental Toxicology and Chemistr*y, *28*(10), 2108-2116. doi:10.1897/08-511R1.1

Brix, K., DeForest, D., Burger, M., & Adams, W. (2005). Assessing the relative sensitivity of aquatic organisms to divalent metals and their representation in toxicity datasets compared to natural aquatic communities. *Human and Ecological Risk Assessment, 11*(6), 1139-1156. doi:10.1080/10807030500278628

Brooks, A., Haeusler, T., Reinfelds, I., & Williams, S. (2005). Hydraulic microhabitats and the distribution of macroinvertebrate assemblages in riffles. *Freshwater Biology*, *50*(2), 331-344. doi:10.1111/j.1365-2427.2004.01322.x

Burgess, R., & Kester, D. (2002). Release and Phase Partitioning of metals from anoxic estuarine sediments during periods of simulated resuspension. *Environmental Science & Technology, 36*(24), 5328-5334. doi:10.1021/es0115058

Cadmus, P., Kotalik, C., Jefferson, A., Wheeler, S., McMahon, A., & Clements, W. (2020). Size-dependent sensitivity of aquatic insects to metals. *Environmental Science & Technology, 54*(2), 955-964. doi:10.1021/acs.est.9b04089

Cain, D., Croteau, M., & Luoma, S. (2011). Bioaccumulation dynamics and exposure routes of Cd and Cu among species of aquatic mayflies. *Environmental Toxicology and Chemistry, 30*(11), 2532-2541. doi:10.1002/etc.663

Cain, D., Buchwalter, D., & Luoma, S. (2006). Influence of metal exposure history on the bioaccumulation and subcellular distribution of aqueous cadmium in the insect *Hydropsyche* californica. *Environmental Toxicology and Chemistry, 25*(4), 1042-1049. doi:10.1897/05-255R.1

Cain, D., Croteau, M., & Fuller, C. (2013). Dietary bioavailability of Cu adsorbed to colloidal hydrous ferric oxide. *Environmental Science & Technology, 47*(6), 2869-2876. doi:10.1021/es3044856

Cain, D., Luoma, S., & Wallace, W. (2004). Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environmental Toxicology and Chemistry, 23*(6), 1463-1473. doi:10.1897/03-291

Calmano, W., Hong, J., & Forstner, U. (1993). Binding and mobilization of heavy-metals in contaminated sediments affected by pH and redox potential. *Water Science and Technology, 28*(8-9), 223-235. doi:10.2166/wst.1993.0622

Carpenter, K. (1930). Further researches on the action of metallic salts on fishes. Journal of Experimental Zoology (56), 407-422. doi.org/10.1002/jez.1400560403

Chapman, P., Wang, F., Adams, W. J., & Green, A. (1999). Appropriate applications of sediment quality values for metals and metalloids. *Environmental Science & Technology, 33*(22), 3937-3941. doi:10.1021/es990083n

Clark, G. D. (2020). Water-quality, bed-sediment, and invertebrate tissue trace-element concentrations for tributaries in the Clark Fork Basin, Montana, October 2017- September 2018. In i. b. Geological Survey & A. United States. Environmental Protection (Eds.): Reston, Virginia : U.S. Department of the Interior, Geological Survey.

Clarke, J. U. (1998). Evaluation of censored data methods to allow statistical comparisons among very small samples with below detection limit observations. *Environmental Science & Technology, 32*(1), 177-183. doi:10.1021/es970521v

Clements, W. H., Carlisle, D. M., Lazorchak, J. M., & Johnson, P. C. (2000). Heavy metals structure benthic communities in colorado mountain streams. *Ecological Applications, 10*(2), 626-638. doi:10.1890/1051-0761(2000)010[0626:HMSBCI]2.0.CO;2

Collier, K. J., Bury, S., & Gibbs, M. (2002). A stable isotope study of linkages between stream and terrestrial food webs through spider predation. *Freshwater Biology, 47*(9), 1651-1659. doi:10.1046/j.1365-2427.2002.00903.xCroteau, M.N., & Luoma, S. N. (2008). A biodynamic understanding of dietborne metal uptake by a freshwater invertebrate. *Environmental science & technology, 42*(5), 1801-1806. doi:10.1021/es7022913

Cummins, K. W., & Klug, M. J. (1979). Feeding Ecology of Stream Invertebrates. *Annual review of ecology and systematics, 10*(1), 147-172. doi:10.1146/annurev.es.10.110179.001051

Di Toro, D. M., Allen, H. E., Bergman, H. L., Meyer, J. S., Paquin, P. R., & Santore, R. C. (2001). Biotic ligand model of the acute toxicity of metals. 1. Technical Basis. *Environmental Toxicology and Chemistry, 20*(10), 2383-2396. doi:10.1002/etc.5620201034

Doudoroff, P., & Katz, M. (1953). Critical review of literature on the toxicity of industrial wastes and their components to fish. *Sewage Industrial Wastes, 25*(7), 802-839.

Eggleton, J., & Thomas, K. V. (2004). A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environment International, 30*(7), 973-980. doi:10.1016/j.envint.2004.03.001

Ellis, M. M. (1937). Detection and measurement of stream pollution. U.S.Department of Commerce, Bureau of Fisheries.(22), 365-437.

Errécalde, O., & Campbell, P. G. C. (2000). Cadmium and zinc bioavailability to *Selenastrum capricornutum (*Chlorophyceae*)*: Accidental metal uptake and toxicity in the presence of citrate. *Journal of Phycology, 36*(3), 473-483. doi:10.1046/j.1529-8817.2000.99103.x

Fan, W., Xu, Z., & Wang, W.-X. (2014). Metal pollution in a contaminated bay: Relationship between metal geochemical fractionation in sediments and accumulation in a polychaete. *Environmental Pollution, 191*, 50-57. doi:10.1016/j.envpol.2014.04.014

Farag, A. M., Woodward, D. F., Brumbaugh, W., Goldstein, J. N., MacConnell, E., Hogstrand, C., & Barrows, F. T. (1999). Dietary Effects of metals-contaminated invertebrates from the Coeur d'Alene River, Idaho, on Cutthroat Trout. *The American Fisheries Society, 128*(4), 578-592. doi:10.1577/1548-8659(1999)128<0578:DEOMCI>2.0.CO;2

Fereidouni, M., Daneshi, A., & Younesi, H. (2009). Biosorption equilibria of binary Cd(II) and Ni(II) systems onto Saccharomyces cerevisiae and Ralstonia eutropha cells: Application of response surface methodology. *Journal of Hazardous Material, 168*(2), 1437-1448. doi:10.1016/j.jhazmat.2009.03.041

Fisher, J., & Dunbar, M. J. (2007). Towards a representative periphytic diatom sample. *Hydrology and Earth System Sciences, 11*(1), 399-407. doi:10.5194/hess-11-399-2007

Freeman, H. C. (1900). *A brief history of Butte, Montana : the world's greatest mining camp; including a story of the extraction and treatment of ores from its gigantic copper properties*. Illinois: Illinois: The Henry O. Shepard company, printers, 1900.

Frissell, C. A., Liss, W. J., Warren, C. E., & Hurley, M. D. (1986). A Hierarchical framework for stream habitat classification- viewing streams in a watershed context. *Environmental Management, 10*(2), 199-214. doi:10.1007/BF01867358

Fuentes-Gandara, F., Pinedo-Hernandez, J., Gutierrez, E., Marrugo-Negrete, J., & Diez, S. (2021). Heavy metal pollution and toxicity assessment in Mallorquin swamp: A natural protected heritage in the Caribbean Sea, Colombia. *Marine Pollution Bulletin, 167*, 112271. doi:10.1016/j.marpolbul.2021.112271

Gintenreiter, S., Ortel, J., & Nopp, H. J. (1993). Bioaccumulation of cadmium, lead, copper, and zinc in successive developmental stages of *Lymantria dispar L*. (Lymantriidae, Lepid): a life cycle study. *Archives of Environmental Contamination and Toxicology TOX, 25*(1), 55-61. doi:10.1007/BF00230711

Hamidian, A. H., Zareh, M., Poorbagher, H., Vaziri, L., & Ashrafi, S. (2016). Heavy metal bioaccumulation in sediment, common reed, algae, and blood worm from the Shoor river, Iran. *Toxicology and Industrial Health, 32*(3), 398-409. doi:10.1177/0748233713500835

Hare, L., Campbell, P. (1992). Temporal Variations of trace metals in aquatic insects. *Freshwater Biology, 27(1),* 13-27. doi.org/10.1111/j.1365-2427.1992.tb00519.x

Hieber, M., & Gessner, M. O. (2002). Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology, 83*(4), 1026-1038. doi:10.1890/0012-9658(2002)083[1026:COSDFA]2.0.CO;2

Hill, W. R., & Fanta, S. E. (2008). Phosphorus and light colimit periphyton growth at subsaturating irradiances. *Freshwater Biology*, *53*(2), 215-225. doi:10.1111/j.1365-2427.2007.01885.x

Hornberger, M. I., Luoma, S. N., Johnson, M. L., & Holyoak, M. (2009). Influence of Remediation in a Mine-Impacted River: Metal trends over large spatial and temporal scales. *Ecological Applications*, *19*(6), 1522-1535. doi:10.1890/08-1529.1

Horner, R. R., & Welch, E. B. (1981). Stream periphyton development in relation to current velocity and nutrients. *Canadian journal of fisheries and aquatic sciences*, *38*(4), 449-457. doi:10.1139/f81-062

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal. 50*(3), 346-363.

Hynes, H. B. N. (1970). The Ecology of Stream Insects. *Annual Review of Entomology, 15*(1), 25-42. doi:10.1146/annurev.en.15.010170.000325

Irving, E. C., Baird, D. J., & Culp, J. M. (2003). Ecotoxicological response of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: implications for toxicity testing. *Environmental toxicology and chemistry, 22*(5), 1058-1058.

Isaac, W., King, C., & Wing Shing, H. (2013). Influence of heavy metals on glyceraldehyde-3-phosphate dehydrogenase interactions in *Chironomus riparius* larvae. *Environmental toxicology and chemistry, 323*(8), 1882.

Kalis, E. J. J., Weng, Dousma, F., Temminghoff, E. J. M., & Van Riemsdijk, W. H. (2006). Measuring free metal ion concentrations in situ in natural waters using the donnan membrane technique. *Environmental Science & Technology, 40*(3), 955-961. doi:10.1021/es051435v

Kevin, G. T., Philip, N. O., Ramon, J. B., & Celso, G. (2008). Sediment and contaminant sources and transfers in river basins (Vol. 4, pp. 83-135): Elsevier B.V.

Kim, K. S., Funk, D. H., & Buchwalter, D. B. (2012). Dietary (periphyton) and aqueous Zn bioaccumulation dynamics in the mayfly *Centroptilum triangulifer*. *Ecotoxicology, 21*(8), 2288-2296. doi:10.1007/s10646-012-0985-1

Kola, H., & Wilkinson, K. J. (2005). Cadmium uptake by a green alga can be predicted by equilibrium modelling. *Environmental Science & Technology, 39*(9), 3040-3047. doi:10.1021/es048655d

Kraemer, S. M., & Hering, J. G. (2004). Biogeochemical controls on the mobility and bioavailability of metals in soils and groundwater. Aquatic sciences, *66*(1), 1-2. doi:10.1007/s00027-004-0004-6

Kraus, J., Schmidt, T., Walters, D., Wanty, R., Zuellig, R., & Wolf, R. (2014). Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. Ecological Applications, *24*(2), 235-243.

Lenth, R. (2022). emmeans: estimated marginal means, aka least-squares means. R package version 1.7.3.

Lock, M., Wallace, R., Costerton, J., Ventullo, R., & Charlton, S. (1984). River Epilithon: toward a structural-functional model. Oikos, *42*(1), 10-22.

Luoma, S. N., & Rainbow, P. S. (2005). Why Is metal bioaccumulation so variable? Biodynamics as a unifying Concept. *Environmental Science & Technology, 39*(7), 1921-1931. doi:10.1021/es048947e

Maret, T. R., Cain, D. J., MacCoy, D. E., & Short, T. M. (2003). Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *Journal of the North American Benthological Society, 22*(4), 598-620. doi:10.2307/1468356

Martin, C. A., Luoma, S. N., Cain, D. J., & Buchwalter, D. B. (2007). Cadmium ecophysiology in seven stonefly (*Plecoptera*) species: delineating sources and estimating susceptibility. *Environmental Science & Technology, 41*(20), 7171-7177. doi:10.1021/es071205b

Mattingly, K. S., Mackie, R. S., Oray, C., McGaw, M., Klimowski, L., Carlson, J. O., . . . Rayms-Keller, A. (2000). Metal-responsive genes from aquatic insects as biomarkers of metal pollution. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology, 126*, 124-124. doi:10.1016/S1095-6433(00)80246-9

Mebane, C. A. (2001). Testing bioassessment metrics : Macroinvertebrate, sculpin, and salmonid responses to stream habitat, sediment, and metals. *Environmental Monitoring and Assessment, 67*(3), 293-322. doi:10.1023/A:1006306013724

Meylan, S., Behra, R., & Sigg, L. (2004). Influence of metal speciation in natural freshwater on bioaccumulation of copper and zinc in periphyton: a microcosm study. *Environmental Science & Technology, 38*(11), 3104-3111. doi:10.1021/es034993n

Moore, J. N., Brook, E. J., & Johns, C. (1989). Grain size partitioning of metals in contaminated, coarse-grained river floodplain sediment: Clark Fork River, Montana, U.S.A. *Environmental geology and water sciences, 14*(2).

Moore, J. N., & Luoma, S. N. (1990). Hazardous wastes from large-scale metal extraction. A case study. *Environmental Science and Technology, 24*(9), 1278-1285. doi:10.1021/es00079a001

Morel, F. o. (1983). *Principles of aquatic chemistry*. New York: New York : J. Wiley.

Moulton, S. R., & Geological, S. (2002). Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program. Reston, Va.: Reston, Va. : U.S. Dept. of the Interior, U.S. Geological Survey : Information Services distributor.

Mulligan, C. N., Yong, R. N., & Gibbs, B. F. (2001). An evaluation of technologies for the heavy metal remediation of dredged sediments. *Journal of Hazardous Material, 85*(1), 145-163. doi:10.1016/S0304-3894(01)00226-6

Naughton, J. M., Burke, M., Merritt, B., Traxler, T., Kerans, B., Bollman, W., & Swant, G. (2020). *Monitoring Report for 2019 Clark Fork River Operable Unit*. Retrieved from https://deq.mt.gov/Portals/112/Land/FedSuperFund/Documents/cfr/Documentsand Reports/CFR 2019 Monitoring Report.pdf?ver=2020-10-20-145945-583.

Nimick, D. A., & Moore, J. N. (1991). Prediction of water-soluble metal concentrations in fluvially deposited tailings sediments, upper Clark Fork Valley, Montana, U.S.A. *Applied Geochemistry, 6*(6), 635-646. doi:10.1016/0883-2927(91)90074-Y

Nriagu, J. O. (1996). A History of Global Metal Pollution. *Science, 272*(5259), 223-224. doi:10.1126/science.272.5259.223

Oliver, B. G. (1973). Heavy metal levels of Ottawa and Rideau River sediments. *Environmental Science & Technology, 7*(2), 135-137. doi:10.1021/es60074a009

Owens, P. N., Walling, D. E., Carton, J., Meharg, A. A., Wright, J., & Leeks, G. J. L. (2001). Downstream changes in the transport and storage of sediment-associated contaminants (P, Cr and PCBs) in agricultural and industrialized drainage basins. *Science of the Total Environment, 266*(1), 177-186. doi:10.1016/S0048-9697(00)00729-4

Pandey, L. K. (2020). In situ assessment of metal toxicity in riverine periphytic algae as a tool for biomonitoring of fluvial ecosystems. *Environmental Technology & Innovation, 18*, 100675. doi:10.1016/j.eti.2020.100675

Phinney, J. T., & Bruland, K. W. (1994). Uptake of Lipophilic Organic Cu, Cd, and Pb Complexes in the Coastal Diatom *Thalassiosira weissflogii*. *Environmental Science & Technology, 28*(11), 1781-1790. doi:10.1021/es00060a006

Roberts, S., Sabater, S., & Beardall, J. (2004). Benthic microalgale colonization in streams of differing riparian cover and light availability. *Journal of Phycology, 40*(6), 1004-1012. doi:10.1111/j.1529-8817.2004.03333.x

Rosenberg, D. M., & Resh, V. H. (1993). *Freshwater biomonitoring and benthic macroinvertebrates*. New York: New York : Chapman & Hall.

Sbihi, K., Cherifi, O., Bertrand, M., & El Gharmali, A. (2014). Biosorption of metals (Cd, Cu and Zn) by the freshwater diatom *Planothidium lanceolatum*: a laboratory study. *Diatom Research, 29*(1), 55-63. doi:10.1080/0269249X.2013.872193

Schaller, J., Brackhage, C., Mkandawire, M., & Dudel, E. G. (2011). Metal/metalloid accumulation/remobilization during aquatic litter decomposition in freshwater: A review. *Science of the Total Environment*, 409(23), 4891-4898. doi:10.1016/j.scitotenv.2011.08.006

Sharley, D. J., Sharp, S. M., Bourgues, S., & Pettigrove, V. J. (2016). Detecting long-term temporal trends in sediment-bound trace metals from urbanised catchments. *Environmental Pollution, 219*, 705-713. doi:10.1016/j.envpol.2016.06.072

Schmidt, T., Clements, W., Zuellig, R., Mitchell, K., Church, S., Wanty, R., . . . Lamothe, P. (2011). Critical tissue residue approach linking accumulated metals in aquatic insects to population and community-level effects. Environmental Science & Technology, *45*(16), 7004-7010.

Shaw, T., Gieskes, J., & Jahnke, R. (1990). Early diagenesis in differing depositional environments: The response of transition metals in pore water. *Geochimica Et Cosmochimica Acta, 54*(5), 1233-1246.

Steinman, A. D., & McIntire, C. D. (1987). Effects of irradiance on the community structure and biomass of algal assemblages in laboratory streams. *Canadian journal of fisheries and aquatic sciences, 44*(9), 1640-1648. doi:10.1139/f87-199

Trbojevic, I., Jovanovic, J., Kostic, D., Popovic, S., Predojevic, D., Karadzic, V., & Simic, G. S. (2018). Periphyton developed on artificial substrates: effect of substrate type and incubation depth. *Russian Journal of Ecology, 49*(2), 135-142. doi:10.1134/S1067413618020145

USEPA. (1996). *Method 3050B (1996) Acid digestion of sediment, sludges and soils*.

Vigneault, B., & Campbell, P. G. C. (2005). Uptake of cadmium by freshwater green algae: effects of pH and aquatic humic substances. *Journal of Phycology, 41*(1), 55-61. doi:10.1111/j.1529-8817.2005.04068.x

Virendra, M., Jaffery, F. N., & Viswanathan, P. N. (1994). Risk assessment of water pollutants. *Environmental Monitoring and Assessment, 29*(1), 29-40. doi:10.1007/BF00546776

Volesky, B. (1994). Advances in biosorption of metals : selection of biomass types. *FEMS Microbiol Rev, 14*(4), 291-302. doi:10.1016/0168-6445(94)90046-9

Wallace, J. B., & Webster, J. R. (1996). The Role of Macroinvertebrates in Stream Ecosystem Function. *Annual Review of Entomology, 41*(1), 115-139. doi:10.1146/annurev.en.41.010196.000555

Warnick, S. L., & Bell, H. L. (1969). The acute toxicity of some heavy metals to different species of aquatic insects. *Journal of Water Pollution, 41*(2), 280-284.

Weitzel, R. L., & American Society for Testing and Materials. Committee, D. o. W. (1979). Methods and measurements of periphyton communities : a review. Philadelphia, Pa.: Philadelphia, Pa. : ASTM.

Winter, J. G., & Duthie, H. C. (2000). Stream epilithic, epipelic and epiphytic diatoms: habitat fidelity and use in biomonitoring. *Aquatic Ecology, 34*(4), 345-353. doi:10.1023/A:1011461727835

Wood, S.N. (2017). Generalized additive models: An Introduction with R. Chapman & Hall. 2nd Edition.

Woodward, D. F., Brumbaugh, W. G., Delonay, A. J., Little, E. E., & Smith, C. E. (1994). Effects on rainbow trout fry of a metals-contaminated diet of benthic invertebrates from the Clark Fork River, Montana. *The American Fisheries Society,, 123*(1), 51-62. doi:10.1577/1548-8659(1994)123<0051:EORTFO>2.3.CO;2

Xie, L., & Buchwalter, D. B. (2011). Cadmium exposure route affects antioxidant responses in the mayfly *Centroptilum triangulifer*. *Aquatic Toxicology, 105*(3), 199-205. doi:10.1016/j.aquatox.2011.06.009

Zoumis, T., Schmidt, A., Grigorova, L., & Calmano, W. (2001). Contaminants in sediments: remobilisation and demobilisation. *Science of the Total Environment, 266*(1), 195-202. doi:10.1016/S0048-9697(00)00740-3

7 Appendix

Table S1. GAM summary statistics

Summary statistics for the Generalized Additive Models (GAM) for basal resources and sediment. Resources are benthic particulate organic matter (BPOM), sediment and epilithic periphyton.
Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1

```
as_conc ~ resource + s(dist, by = resource, k = 7)
Approximate significance of smooth terms:
                            df               f                   p-value
s(dist):resourceBPOM        1                124.37              < 2e-16          ***
s(dist):resourceEpilithic   1                90.99               < 2e-16          ***
s(dist):resourceSED         1                17.53               7.92e-05         ***
R-sq.(adj) =  0.651         Deviance explained = 87.9%
GCV = 0.10573               Scale est. = 0.10553                 n = 78

cd_conc ~ resource + s(dist, by = resource, k = 7)
Approximate significance of smooth terms:
                            df               f                   p-value
s(dist):resourceBPOM        2.164            20.705              <2e-16           ***
s(dist):resourceEpilithic   1.000            5.292               0.0039           ***
s(dist):resourceSED         1.000            4.936               0.2974             *
R-sq.(adj) =  0.651         Deviance explained = 77.3%
GCV = 0.097198              Scale est. = 0.07155                 n = 78

cu_conc ~ resource + s(dist, by = resource, k = 7)
Approximate significance of smooth terms:
                            df               f                   p-value
s(dist):resourceBPOM        5.637            32.70               < 2e-16          ***
s(dist):resourceEpilithic   4.864            53.18               < 2e-16          ***
s(dist):resourceSED         1.000            28.35               1.65e-06         ***
R-sq.(adj) =  0.873         Deviance explained = 94.8%
GCV = 0.063981              Scale est. = 0.053102                n = 78

pb_conc ~ resource + s(dist, by = resource, k = 7)
Approximate significance of smooth terms:
                            df               f                   p-value
s(dist):resourceBPOM        5.861            15.29               < 2e-16          ***
s(dist):resourceEpilithic   5.008            36.24               < 2e-16          ***
s(dist):resourceSED         1.010            8.18                0.00569           **
R-sq.(adj) =  0.929         Deviance explained = 94.3%
GCV = 0.056075              Scale est. = 0.044279                n = 78

zn_conc ~ resource + s(dist, by = resource, k = 7)
Approximate significance of smooth terms:
                            df               f                   p-value
s(dist):resourceBPOM        1                10.18               0.0021            **
s(dist):resourceEpilithic   1                21.00               1.92e-05         ***
s(dist):resourceSED         1                0.07                0.7917
R-sq.(adj) =  0.742         Deviance explained = 75.4%
GCV = 0.10329               Scale est. = 0.098704                n = 78
```

Table S2. GLM summary statistics

Summary statistics for the generalized linear model (GLM) that includes basal resources and habitat types. Basal resources included are BPOM and periphyton and are represented by “resource”. Habitats included are glide, riffle and wood and are represented by “habitat”.
Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1

glm(formula = as_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.00605	**
resource	1	0.00000462	**
habitat:resource	2	0.93513	
glm(formula = cd_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.00242	**
resource	1	0.00000599	***
habitat:resource	2	0.00538	**
glm(formula = cu_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.0891	.
resource	1	0.0182	*
habitat:resource	2	0.7163	
glm(formula = pb_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.0715	.
resource	1	<2.2e16	***
habitat:resource	2	0.1562	.
glm(formula = zn_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.038399	*
resource	1	<3.1e12	***
habitat:resource	2	0.000329	***

Table S3. Tukey HSD summary statistics for basal resources

Results from Tukey Honestly Significantly Different (HSD) test. Resources are benthic particulate organic matter (BPOM) and periphyton. Mean represents marginal mean. SE is standard error. LCL and UCL are upper and lower 95% confidence interval values. CLD is “compact letter display” representing pairwise comparisons.

Arsenic							
Habitat	Resource	Mean	SE	df	LCL	UCL	CLD
Riffle	BPOM	155.34	18.47	52	122.37	197.19	a
Glide	BPOM	157.60	23.37	52	117.04	212.21	a
Wood	BPOM	238.78	32.03	52	182.44	3102.52	a
Glide	Periphyton	50.55	8.50	52	36.07	70.83	a
Riffle	Periphyton	64.56	11.72	52	44.84	92.94	a
Wood	Periphyton	132.59	17.78	52	101.31	173.54	b
Cadmium							
Habitat	Resource	Mean	SE	df	LCL	UCL	CLD
Glide	BPOM	5.18	0.52	50	4.23	6.33	a
Riffle	BPOM	5.40	0.38	50	4.68	6.23	a
Wood	BPOM	8.15	0.66	50	6.94	9.58	b
Glide	Periphyton	3.92	0.39	50	3.20	4.80	a
Wood	Periphyton	4.51	0.36	50	3.83	5.29	a
Riffle	Periphyton	4.80	0.52	50	3.86	5.97	a
Copper							

Habitat	Resource	Mean	SE	df	LCL	UCL	CLD
Riffle	BPOM	1338.89	318.59	50	830.57	2158.31	a
Glide	BPOM	1365.41	405.22	50	752.71	2476.85	a
Wood	BPOM	1996.30	535.90	50	1164.88	3421.14	a
Glide	Periphyton	205.85	69.27	50	104.78	404.39	a
Riffle	Periphyton	287.26	104.41	50	138.52	595.70	a
Wood	Periphyton	1480.02	397.31	50	863.62	2536.36	b
Lead							
Habitat	Resource	Mean	SE	df	LCL	UCL	CLD
Riffle	BPOM	172.61	15.76	52	143.71	207.41	a
Glide	BPOM	178.32	20.31	52	141.89	224.10	a
Wood	BPOM	236.57	24.37	52	192.40	290.90	a
Glide	Periphyton	30.05	3.88	52	23.19	38.94	a
Riffle	Periphyton	35.67	4.97	52	26.96	47.19	ab
Wood	Periphyton	51.98	5.35	52	42.27	63.91	b
Zinc							
Hab	Resource	Mean	SE	df	LCL	UCL	CLD
Glide	BPOM	997.57	86.59	52	841.51	1182.57	a
Riffle	BPOM	1019.59	70.96	52	889.59	1168.60	a
Wood	BPOM	1339.59	105.17	52	1148.52	1562.42	b
Glide	Periphyton	505.61	49.76	52	416.91	613.19	a
Wood	Periphyton	529.72	41.59	52	454.17	617.84	a
Riffle	Periphyton	784.28	83.37	52	636.77	965.95	b

Table S4. Insect GLM summary statistics

Summary statistics for the generalized linear model (GLM) that includes insects and habitat types. Insects included are *Baetis* and *Hydropsyche* and are represented by “resource”. Habitats included are glide, riffle and wood and are represented by “habitat”.

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1

glm(formula = as_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.0424	*
resource	1	<2.2e-16	***
habitat:resource	2	0.2369	
glm(formula = cd_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.0000307	***
resource	1	<2.0e-14	***
habitat:resource	2	0.0103	*
glm(formula = cu_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.00000238	***
resource	1	0.0000108	***
habitat:resource	2	0.562	
glm(formula = pb_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.000210	***
resource	1	0.000198	***
habitat:resource	2	0.071202	.
glm(formula = zn_conc ~ hab * resource, family = Gamma(link = “log”)			
	df	P-value	
habitat	2	0.0000016	***
resource	1	<2.2e16	***
habitat:resource	2	0.000259	***

Table S5. Tukey HSD summary statistics for insects

Results from Tukey Honestly Significantly Different (HSD) test. Resources are the insects *Baetis* and *Hydropsyche*. Mean represents marginal mean. SE is standard error. LCL and UCL are upper and lower 95% confidence interval values. CLD is “compact letter display” representing pairwise comparisons.

Arsenic							
Hab	Resource	Mean	SE	df	LCL	UCL	CLD
Riffle	Baetis	20.52	2.36	97	16.33	25.79	a
Glide	Baetis	24.84	2.86	97	19.76	31.22	ab
Wood	Baetis	34.13	3.59	97	27.70	42.05	b
Riffle	Hydropsyche	56.67	5.39	97	46.92	68.44	a
Wood	Hydropsyche	72.97	7.89	97	58.88	90.45	a
Glide	Hydropsyche	76.71	8.55	97	61.48	95.71	a
Cadmium							
Hab	Resource	Mean	SE	df	LCL	UCL	CLD
Riffle	Baetis	59.45	6.49	96	47.86	73.83	a
Glide	Baetis	82.39	9.00	96	66.34	102.33	ab
Wood	Baetis	95.24	9.77	96	77.70	116.74	b
Riffle	Hydropsyche	12.03	1.08	96	10.05	14.38	a
Glide	Hydropsyche	13.62	1.44	96	11.04	16.79	a
Wood	Hydropsyche	16.24	1.67	96	13.25	19.91	a
Cadmium							
Hab	Resource	Mean	SE	df	LCL	UCL	CLD
Riffle	Baetis	470.69	45.68	96	388.22	570.69	a
Glide	Baetis	597.71	60.04	96	489.65	729.61	a
Wood	Baetis	913.05	80.89	96	765.81	1088.60	b
Riffle	Hydropsyche	775.65	62.16	96	661.58	909.39	a
Glide	Hydropsyche	981.98	92.28	96	814.88	1183.35	ab
Wood	Hydropsyche	1141.61	104.07	96	952.64	1368.07	b
Lead							
Hab	Resource	Mean	SE	df	LCL	UCL	CLD
Glide	Baetis	30.73	7.71	96	18.67	50.57	a
Wood	Baetis	56.35	12.84	96	35.85	88.58	ab
Riffle	Baetis	88.67	20.82	96	55.63	141.32	b
Glide	Hydropsyche	99.64	23.40	96	62.52	158.81	a
Wood	Hydropsyche	114.08	26.00	96	72.58	179.31	a
Riffle	Hydropsyche	296.41	59.36	96	199.18	441.10	b
Zinc							
Hab	Resource	Mean	SE	df	LCL	UCL	CLD
Riffle	Baetis	3507.91	350.23	98	2877.41	4276.56	a
Glide	Baetis	4896.74	504.92	98	3990.61	6008.63	a
Wood	Baetis	6994.15	658.36	98	5802.43	8430.63	b
Riffle	Hydropsyche	1566.24	133.36	98	1322.75	1854.56	a
Glide	Hydropsyche	1861.29	185.83	98	1526.75	2269.13	a
Wood	Hydropsyche	1866.46	180.78	98	1540.07	2262.02	a

Table S6. PCA eigenvalues and eigenvectors

Principal component analysis loadings of total concentrations of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) of insects by habitat type, showing eigenvalues and percent variance explained by each component axis. “Percent” refers to the amount of total variation the different eigenvalues represents. PC: Principal component.

Numbers separated by parenthesis are cos2 of variable/percent contribution of variable to that PC. A high cos2 indicates a good representation of that variable on the PC, and is highlighted in bold if > 0.6.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalue	2.65	1.92	0.25	0.12	0.06
Percent	53.0	38.3	5.1	2.3	1.2
Cumulative Percent	53.0	91.3	96.4	98.7	1.00
Variables	**Eigenvectors**				
	PC1	PC2	PC3	PC4	PC5
Log As	**0.87/32.76**	0.00/0.10	0.09/37.26	0.04/29.88	0.00/0.00
Log Cd	0.14/5.47	**0.82/42.81**	0.00/1.40	0.00/0.52	0.03/49.81
Log Cu	0.34/12.92	**0.59/30.98**	0.01/3.78	0.04/38.92	0.01/13.40
Log Pb	**0.70/26.54**	0.14/7.34	0.15/57.56	0.01/8.54	0.00/0.03
Log Zn	**0.59/22.32**	0.36/18.77	0.00/0.00	0.03/22.12	0.02/36.76