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Ecological and Chemical Analysis of Heavy Metal Transduction in *Salix exigua* on the Animas and Florida Rivers

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Abstract: On August 5th, 2015, an accident from the Gold King Mine in Silverton, Colorado initiated the release of three million gallons of heavy metals into the Animas River. As heavy metals have toxic effects in high concentrations over time, it is extremely important to quantify the amounts of heavy metals in both the water itself, as well as surrounding riparian zones. This study inquired as to whether heavy metals were present in *Salix exigua*, or coyote willow, which makes up a large portion of the riparian biota in Southwest Colorado. Samples were taken from three sites, at Oxbow Park and Preserve and Trimble Lane on the Animas River, as well as from a control site on the Florida River. Six metals, including aluminum, zinc, cadmium, manganese, barium, and iron, were quantified in root and leaf samples to account for the fate and transport into riparian plants. As bioaccumulation of metals in ecosystems can have effects in many organisms, assessing the concentrations in the flora surrounding the river is essential to accounting for all aspects of river health. Metals were found to be significantly higher in roots compared to shoots, across all sites. Furthermore, the Animas River had significantly higher concentrations of heavy metals than the control site. Specifically, Oxbow Park and Preserve had the highest levels resulting from the specific geomorphology of the river section. This pilot study was essential for the quantification of heavy metal concentrations in the Animas River and will gain insight to the current ecological health post-mine spill reflecting short-term effects. It may also serve as baseline data for future studies accounting for plant health in this area that could quantify long-term effects of acid mine drainage after the Gold King mine spill.

Key words: acid mine drainage, heavy metal analysis, bioaccumulation, riparian plants, Gold King mine spill

1. Introduction

Acid mine drainage and the effects of heavy metals within both aquatic and riparian ecosystems are being brought to the attention of scientists and communities all over the United States. With the high frequency of mine spills and acid mine drainage, it is essential that we understand the short and long-term effects of heavy metals in river systems. When oxygenated water comes into contact with mine tailings containing iron sulfide, the chemical reaction causes the formation of sulfuric acid which contaminates the water. Sulfuric acid both increases the acidity of the water and leads to other heavy metals entering the solution in either a dissolved or colloidal form that would not have been present otherwise [1]. While dissolved forms of heavy metals cause a more negative impact than the precipitate form, the abundance of heavy metals such as iron, arsenic, cadmium, lead, and aluminium are all of serious environmental concern regardless of their fate and transport mechanisms, thereby representing a great challenge with regards to acid mine drainage management [2].

In light of the recent 2015 Gold King mine spill that washed three million gallons of water containing high levels of heavy metals into the Animas River, there is no better time to investigate the effects of heavy metal

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pollutants on this river and its underlying ecology [3]. By looking at heavy metal uptake in plants within multiple zones and locations of the river, we can identify what areas of the Animas River have been most affected by the spill and quantify observable differences in terms of stream gradient and morphology.

1.1 Current Status of Mines and Remediation Efforts

Acid mine drainage is a major cause of river pollution in many countries with both historic and current mining practices. The preferred treatment option is preventing the formation and migration of acid mine drainage from its source; however, this is not always a feasible method. If prevention is possible, this generally involves either flooding or sealing of mines, land-based storage of mine tailings, or underwater storage of mine tailings [4]. If prevention is not a feasible method, the next step is some form of remediation, usually involving a biological or chemical mechanism to neutralize acid mine drainage and remove heavy metals out of solution and into a solid, less harmful form to biota. Remediation can use abiotic or biotic methods, but most commonly involves the addition of a chemical-neutralizing agent (abiotic). Alkaline material added to an acidic environment acts as a buffer and accelerates the rate of metal oxidation, thereby balancing the pH of the river. This helps precipitate heavy metals out of solution, so they are not in their dissolved form and are less dangerous to both humans and the environment as a whole. Disadvantages to these techniques are that they are expensive, ineffective, and typically create a toxic chemical sludge that is difficult to remove from the ecosystem [4, 5].

1.2 Effects of Acid Mine Drainage on Ecosystems and Vegetation

As acid mine drainage gains more attention as a large environmental issue, the effects on ecosystems are being studied in more depth. Major impact areas include lakes, estuaries, coastal waters, and the particular focus of this study: rivers. The effects of acid mine drainage are sorted into four categories: metal toxicity, sedimentation, acidity, and salinization. While all are important, most studies focus on metal toxicity as it poses the most direct effects on ecosystem health and resiliency. Some of the most detrimental effects of heavy metal toxicity on an ecosystem generally include habitat modification, niche loss, bioaccumulation within a food chain, losses of food source, and ultimately elimination of sensitive species [2]. The impact of acid mine drainage is difficult to quantify and predict, particularly in continuously flowing waters such as rivers. In response, the US Agency for Toxic Substances and Disease Registry lists lead, mercury, and arsenic as the top first, second, and third most hazardous metals, respectively. As heavy metals bioaccumulate in smaller organisms such as macroinvertebrates and plants, higher organisms in the food chain such as birds, herbivores, and even humans will start to experience biomagnification of these heavy metals as well [6].

Heavy metals are not simply stored in the sediment of rivers, nor do they merely continue flowing forever through the water. Plants that are present within a riparian zone experience bioaccumulation of heavy metals through root contact with sediment, soil, and water over time. This is an important concept to highlight, as not only aquatic organisms experience effects, but many organisms within the adjacent riparian zones as well [7]. One recent study correlated increased levels of certain heavy metals, such as zinc, copper, and lead, to a decrease in root biomass in the genus *Brassica.* Other general effects that heavy metals have on plants include a decreased rate of photosynthesis, decrease in overall water content, and stunted growth of an entire plant, especially within the roots [8]. As vegetation is an important source of food, shelter, and detritus in a riparian and aquatic ecosystem, if plants are being affected by heavy metal uptake, this will reflect upon many organisms within an ecosystem.

Many plants transduce heavy metals from their roots into both their shoots and leaves, which is important in understanding the mechanisms by which acid mine drainage moves out of the water and into the riparian zone [1].

The amount of heavy metals present within an aquatic or riparian plant is not solely determined by the amount of that metal in the water, sediment, or soil. The limiting factor, instead, is the metal tolerance of the plants themselves [8]. Plants have different mechanisms and genes that allow for increased solubility of metals into the roots. Furthermore, some riparian plants have transport proteins that move metals into root cells, and eventually the vascular system and ultimately leaf cells [8]. This diversity within plant genetics explains why many plants may have high levels of a particular metal and the ability to translocate these metals into shoots and leaves, while other plants cannot tolerate high levels whatsoever. For example, pine pennycress (*Thlaspi)* thrives on soils with high levels of cadmium and zinc. After observing the molecular physiology, researchers found that key gene sites for zinc transport were very stimulated in this plant in comparison with other species [9].

Knowing that plants have different tolerances and mechanisms for taking up metals can help increase our understanding of variation within the community and assess the overall health of the ecosystem.

1.3 Salix spp. and Heavy Metal Transduction

Because different species have varying abilities in both uptake and transduction of metals, it would be most interesting to focus on a plant with potential to uptake relatively high amounts of heavy metals considering the acid mine drainage problem in this region. The willow family, *Salicaceae*, have been extensively studied regarding their unique ability to transduce more heavy metals in comparison to other riparian species. In one study, *Salix exigua*, or coyote willow, appeared to have the highest capacity of four species to uptake, translocate and accumulate contaminants from a river [10]. Another study found that *Salix* species in general were much more likely to absorb heavy metals from the environment because they are fast growing, have better evapotranspirational abilities, and are deep-rooting phreatic trees, meaning they have the capability to reach contaminated groundwater [11].

In addition to this research, many studies are now focusing on the relationship between bacteria in the soil and the ability for willows to translocate more heavy metals than other plants in the same community. There is increasing evidence that rhizosphere bacteria may contribute to the metal extraction process in plants, although the mechanism behind this relationship is not well understood. This may, in part, explain why willows have a higher transduction ability, particularly with certain heavy metals such as cadmium and zinc [11].

With regards to this evidence for heavy metal transduction, as well as the fact that *Salix* species are abundant in the San Juan region, coyote willow was the plant of interest of this study to better understand the riparian communities of the southwest, as well as their responses to unnaturally high heavy metal concentrations. Implementing a heavy metal study on coyote willows in different zones and locations of the Animas River in response to the Gold King Mine spill allowed us to gather baseline data needed for determining the health of the river and will serve as a template for future studies on abandoned mines and their long-term effects on plant and river ecology.

Since plants serve as bioindicators for entire ecosystems, quantifying their heavy metal uptake in a metal polluted area may help scientists reflect on what areas of the water table are under the most distress [12]. Looking at three spatially unique riparian zones and using multiple study sites helped us characterize the fate and transport of heavy metals, whether they are harming riparian plants, and what future responsibilities we have to contribute to the resiliency of the Animas river.

2. Methods

2.1 Study Sites

Three study sites were chosen for the collection of *S. exigua* within the San Juan watershed. The first two sites were on the Animas River, a 126-mile long river, which flows from headwaters at Silverton, Colorado, eventually converging with the San Juan River in Northern New Mexico (Fig. 1). The first study site was located 5.3 miles south of Baker's Bridge, accessed via Trimble Lane, as the highest amounts of heavy metals were found close to Baker's Bridge post-mine spill. Based on limited presence of willows at the Baker's Bridge site, this was the closest accessible area with presence of *S. exigua*. The second study site was located 7.1 miles south of this site, at Oxbow Park and Preserve. This stretch of the river is characterized with meandering bends and less gradient than that of Baker's Bridge, attributing to the heavy sediment build-up at this position of the river. Because these two reaches of the Animas River have unique geomorphology, they were chosen to observe the spatial differences in heavy metal concentrations on the Animas River.

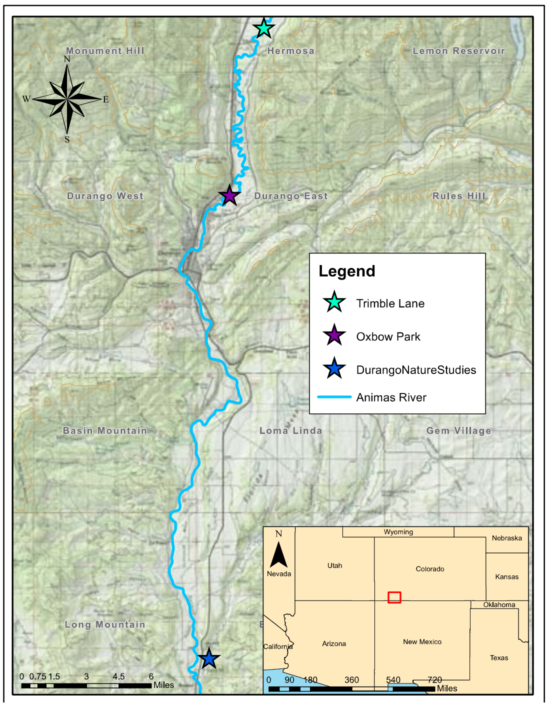


Fig. 1 Research study sites including Trimble Lane, Oxbow Park, and Durango Nature Studies.

The Florida River served as a control site, located at Durango Nature Studies, 12 miles south of Durango (Fig. 1). The Florida River is a 61-mile long tributary of the Animas River that is sourced from Lillie Lake in the Weminuche Wilderness. The Animas River had significant mining activity based in Silverton, Colorado, where the headwaters are found. In contrast, the Florida River has no past mining history. This made the Florida River an excellent control to add extra support when explaining how much of the metal concentrations in the Animas River are due to the mine spill versus natural environmental composition. Both rivers are heavily influenced by annual snowmelt higher up in the mountains during the spring and summer, as well as a monsoon season in the late summer to early fall [13].

2.2 Field Collection

The main focus of this study was to sample heavy metals in root and leaf samples of *S. exigua* to first see if there were any heavy metals present in these riparian plants. Furthermore, the question of interest was what parts of the plants had the highest concentrations amongst the different study sites and zones. At each study site, three zones were identified (Fig. 2); the first zone was classified with young willow roots, in closest proximity to the river. Leaves of the younger willows composed the second zone, and mature, established willows made up the third zone, farthest from the river. This allowed us to observe how the willows were taking up heavy metals both temporally over their life span, and spatially in relation to the river.

A preliminary test run was carried out before the main research study at Oxbow Park and Preserve to ensure that the willows had at least some form of heavy metal concentrations before complete sample collection. Two samples of the willow roots in the first zone and two samples in the second zone were collected and analyzed chemically using the MP-AES spectrometer before the initial study was thoroughly established. The preliminary test run concluded levels

Fig. 2 The three zones chosen for field sample collection, illustrating the different sample types (roots, young leaves, and old leaves) corresponding with proximity to the river (zones 1, 2 and 3, respectively). This image is from one of the three study areas, Oxbow Park and Preserve.

of six different heavy metals, so the full field collection proceeded.

Following the preliminary test runs, two 12-meter transects were set up with chaining pins at each site per zone, running parallel to the river. Four samples per zone were taken to ensure enough replication in the experiment. To account for a larger spatial distribution, and to avoid significantly damaging the willow population, three leaves or roots were clipped from each plant per meter. Each meter, a willow was sampled closest to the meter mark. This allowed for the study to account for a larger proportion of the population size by taking leaves or roots from multiple willows. Root samples were stored in Ziploc bags and leaf samples were added to brown paper bags. All samples were labelled according to the location and sample number. This process was repeated once on each transect to account for more variability. The process was repeated for each zone by moving each transect south of the original transect. The distance moved was tentative to the area sampled, as some areas were limited by topography or amount of willows present. This method allowed for four samples per zone, and twelve overall per site. The distance and elevation between each zone was measured to account for the distance and vertical elevation differences.

After harvesting the willows, all samples were stored in the drying oven in brown paper bags for at least 48 hours to ensure there was no water content in the samples before chemical analysis was performed.

2.3 Chemical Analysis

2.3.1 Plant Digestion

After all plant samples were dried for at least 48 hours, root and leaf samples were transported to the analytical laboratory for the plant digestion process. Each site's samples were digested separately, with the addition of a standard reference material, or SRM. The SRM provided was a sample of NIST tomato leaves, which included known amounts of heavy metals, and allowed for the analysis method to be validated for plant matrices. Each sample was separately ground using a mortar and pestle, and 0.5 grams of each sample were weighed and transferred into a polypropyelene digestion vial. The sample number and biomass were recorded on each tube. From there, the digestion process proceeded, in which three reagents were all used in various dilutions to digest the samples: nitric acid, hydrogen peroxide, and hydrochloric acid. Following the addition of a reagent, the samples were heated on a ModBlock at 93 degrees C in different intervals depending on which reagent was added. After the digestion process, each sample was individually filtered with a 0.45 micron syringe filter apparatus into a volumetric flask and diluted to 50 mL with 1% nitric acid in distilled water (EPA Method 3050B).

Calibration standards were prepared at various concentrations (100, 200, 500, 700, and 1000 ppb) for each heavy metal being tested, through the sequential dilution of Agilent heavy metal standards. This allowed for the external calibration of the MP-AES instrumental response to a range of heavy metal concentrations.

2.3.2 Atomic Emission Analysis

Following the acid digestion of plant samples, which solubilized the heavy metals, the samples were ready to be analyzed with the MP-AES 4200 instrument. Each sample was separately analyzed by plastic tubing that would automatically uptake each individual sample. A blank solution of 1% nitric acid in distilled water was tested in between any samples to rinse the apparatus to avoid cross-contamination in between samples.

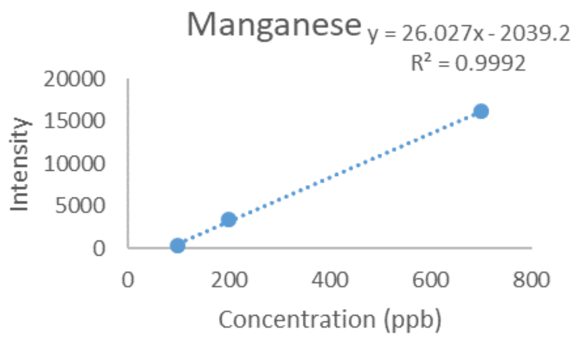
The MP-AES ran almost entirely on compressed air, coupled with a nitrogen generator, which removed all oxygen from the machine. The nitrogen allowed for a plasma that generated a high intensity emission line. Samples were passed through a nebulizer which vaporized each sample. From there, they were passed through a high heat plasma, which atomized the sample, allowing the heavy metals of the sample to emit photons at predictable wavelengths and measurable intensities. Photons with wavelengths characteristic for each element of interest were then measured by a CCD detector [14]. Each sample was tested by the instrument ten times per trial, increasing reliability of the results. The samples were tested for six heavy metals: zinc, manganese, iron, cadmium, aluminium, and barium.

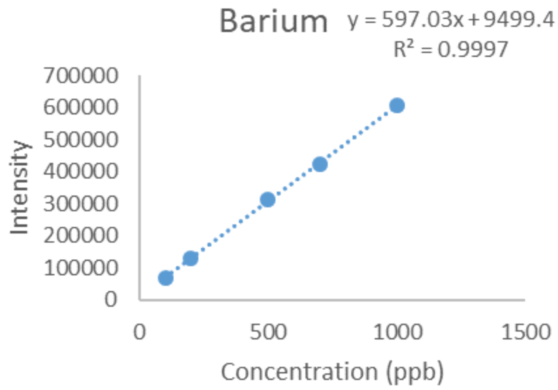
2.4 Data Analysis

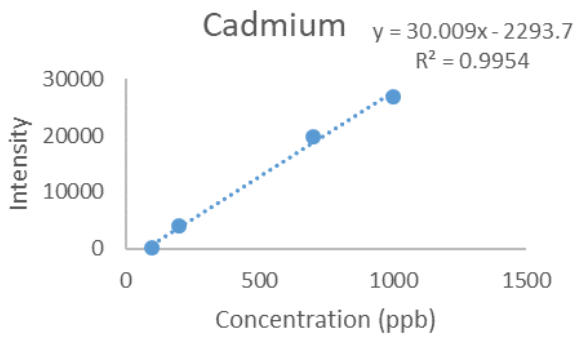
Data analysis was performed using Microsoft Office Excel after all heavy metal concentrations were recorded. Data was exported from the MP-AES 4200 software into Microsoft Excel, and actual concentrations and standard deviations were calculated. A two-way ANOVA test was performed using SPSS to account for any statistically significant data between sites, zones, as well as site-zone interactions. A p-value of 0.10 was used to conduct analyses as natural variation is typically higher in an environmental setting. In addition to this, high variability was observed between individual samples, as the metals are typically parts per billion, so even a small change can have a large effect on these small values.

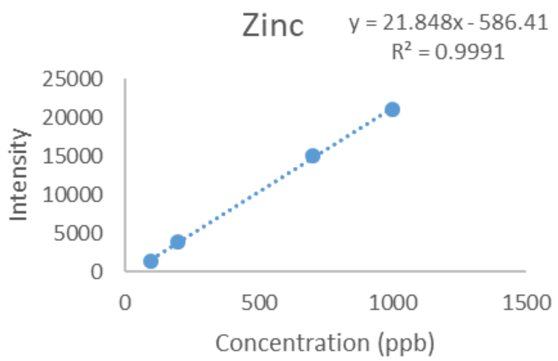
2.5 Calibrations and Corrections

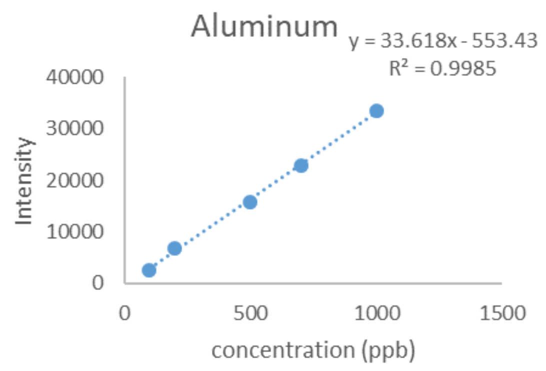
Before any of the experimental data was analysed, the calibration standards were plotted to ensure a high R-squared value. This reassured us that the MP-AES











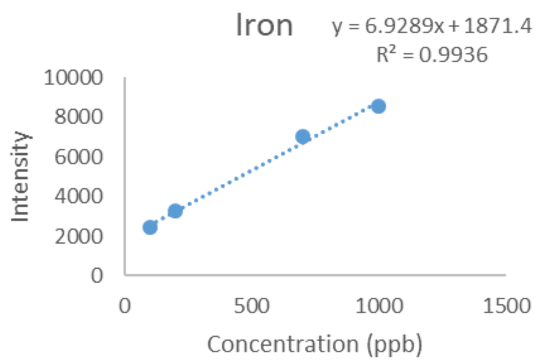


Fig. 3 Calibration curves for all six heavy metals that were analyzed. Calibration solutions were prepared for 100, 200, 500, 700, 1000 ppb, respectively using known amounts of Agilent heavy metals that were diluted using 1% nitric acid in deionized water.

Table 1 Standard reference material corrected concentration values. These values were applied to experimental plant data to correct for instrumental error. The average percent errors were used to determine if the heavy metals in experimental samples should be analyzed.

Element	Actual value	Experimental value	Average % error
Al	598	206.7	-45.3
Cd	1.5	11.8	-1.5
Ba	63	15.8	-12.0
Zn	30.9	81.5	-1.6
Fe	368	34.2	-0.2
Mn	246	133.6	-1.2

4200 was properly calibrated. All six heavy metals at this point had an R-squared value of over 99%, allowing greater assurance for accuracy within the plant sample results. The MP-AES instrument took measurements in parts per billion, which were then translated into mg/kg taking both the original dilution factor (0.05 L), as well as the mass per each sample, into account.

A correction factor was made for each plant sample as standard reference materials were digested with the experimental samples. The correction factor was determined based upon the known amounts of heavy metals in the standard reference material compared to the experimental results from the standard reference material. As the percent errors for aluminium and barium were fairly high, the experimental data for these two elements were not used to assess the heavy metal concentrations of plants on the Animas River.

Aluminium concentrations far exceeded the calibration standards prepared, as the standards could only accurately measure concentrations from 100 parts per billion to 1000 parts per billion. On the other hand, barium was also excluded from data analyses as almost all concentrations were below 100 parts per billion, of which no calibration standards were prepared. Therefore, aluminium and barium were removed from further chemical analyses of the plant samples.

3. Results

Chemical analysis revealed that there were, in fact, heavy metals present in samples at all sites, including the control river. Regardless of site or zone, there was always the same hierarchy of heavy metal concentrations observed: manganese at highest, followed by iron, then zinc, and lastly cadmium (Figs. 4-6). Site comparisons indicate that Oxbow Park and Preserve had the highest overall concentrations of heavy metals, compared with both Trimble Lane and the Durango Nature Studies sites. For example, average manganese concentrations were nearly twice as high as concentrations at the other two sites when not accounting for variation across zones. This trend was observed for cadmium and iron as well, with the exception of zinc, which did not have an apparent site effect (Figs. 4-6).

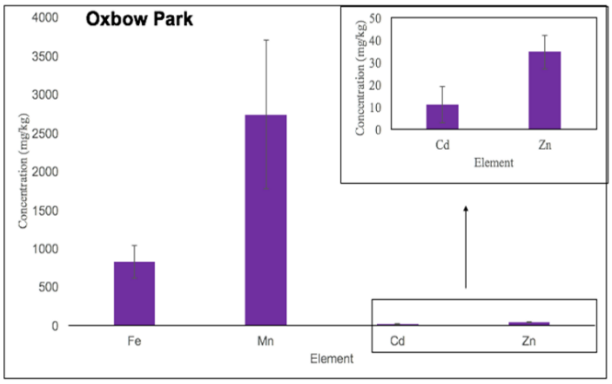


Fig. 4 Average concentrations of iron, manganese, cadmium and zinc (+/- SEM) at Oxbow Park and Preserve.

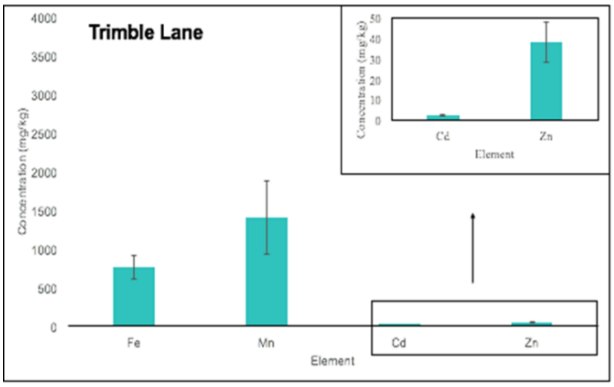


Fig. 5 Average concentrations of iron, manganese, cadmium and zinc (+/- SEM) at Trimble Lane.

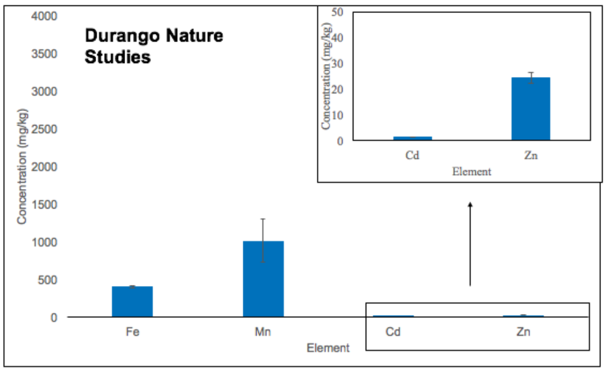


Fig. 6 Average concentrations of iron, manganese, cadmium and zinc (+/- SEM) at the Durango Nature Studies, the control site.

Again, the chemical analyses did show heavy metals for each of the four heavy metals tested, all in fluctuating quantities. Trimble Lane had higher concentrations for all heavy metals than that of the control site; however, nearly all averages at Trimble Lane were lower than at Oxbow Park and Preserve.

For both treatment sites, Oxbow and Trimble, roots always exhibited the highest concentrations of metals, followed by young leaves, then finally mature leaves, (Fig. 9). In contrast, at Durango Nature Studies, there was not always a clear trend. Most of the metals were fairly ubiquitous in concentrations across the three zones (Figs. 8-10). The only metal that exhibited the normal decreasing trend moving away from the river was manganese (Fig. 7).

Looking at synergistic effects, the site-zone interactions were significantly different for each heavy metal, including manganese, zinc, and iron, indicating that there is a correlation between each specific site, the relative zones within them and the quantity of each heavy metal ($p < 0.0001$).

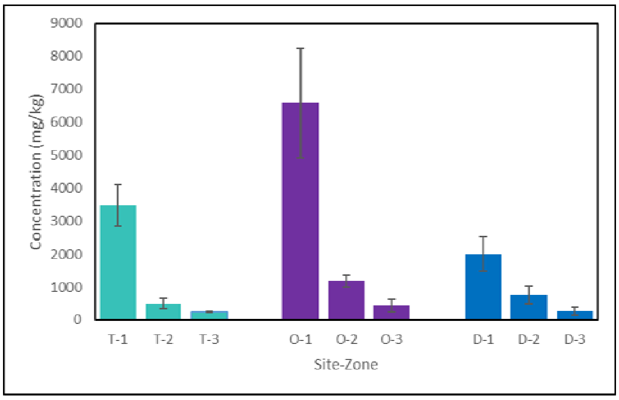


Fig. 7 Average concentrations of manganese (+/- SEM) at the three sites and separated out into the three different zones. T indicates the first site at Trimble Lane, O indicates the second site at Oxbow Park and Preserve, and D indicates the control site at Durango Nature Studies.

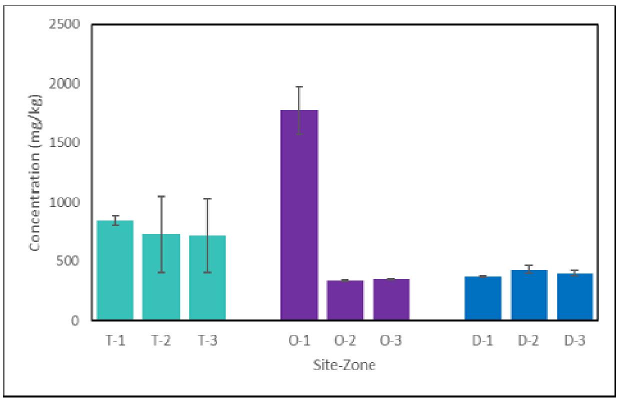


Fig. 8 Average concentrations of iron (+/- SEM) in mg/kg across the three sites and zones. For example, O-1 refers to Oxbow Park, Zone 1.

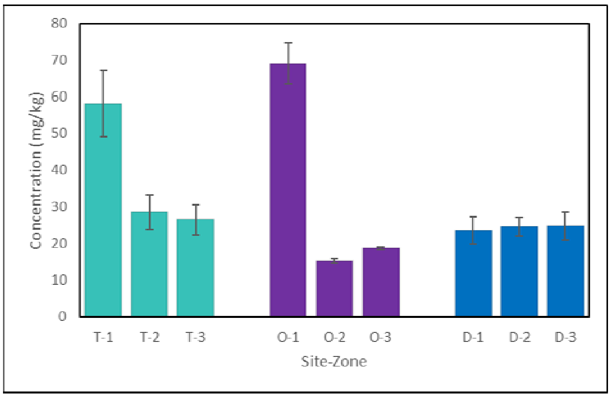


Fig. 9 Average concentrations of zinc (+/- SEM) across all study sites and zones. For example, D-1 refers to Durango Nature Studies, Zone 1.

3.1 Manganese

Looking just at the sites and excluding the zone factor, manganese concentrations were significantly different between Oxbow and the control site (p-value = 0.021), as well as Oxbow and Trimble (p-value = 0.087). Looking specifically at zones, there was no significance between zones 2 and 3 (young plants and mature plants, respectively) with a p-value at 0.689. However, all other zones did show significantly different amounts of manganese, including zones 1 and 2, and zones 1 and 3, respectively ($p < 0.0001$).

3.2 Iron

Iron showed fairly similar trends to manganese, with no significant difference in iron concentrations between Oxbow and Trimble sites ($p = 0.926$). In contrast, there was an overall observable difference between Trimble and the control site, Durango Nature Studies ($p = 0.068$). Oxbow and the control site, DNS, were also deemed significantly different ($p = 0.03$).

The amounts of iron varied significantly between the roots and young leaves, zones 1 and 2 ($p= 0.009$). Similarly, zones 1 and 3 displayed significance ($p = 0.008$). However, like the other metals, there was no significance between metal concentrations in zones 2 and 3 ($p = 0.998$).

3.3 Zinc

There were no statistically significant differences found between the Oxbow and Trimble sites for zinc ($p = 0.743$). The sites that varied significantly in their zinc load were the Trimble site and the control site, with a p-value of 0.012, as well as Oxbow and the control site, with a p-value of 0.063.

In regards to the differences in zinc across the three zones, there was no significance between young leaves and old leaves ($p = 0.984$). Like the other heavy metals, there was a significant difference in zinc between zones 1 and 2, as well as zones 1 and 3 ($p < 0.0001$).

3.4 Cadmium

Compared to the other metals found in the plant samples, cadmium was present in the lowest concentrations across each site. The root samples at Oxbow were significantly greater than any other zone or site; however, they also had extremely high standard error at this particular zone and site (Fig. 10).

Cadmium was the only metal that did not exhibit any significance across study sites, Oxbow and Trimble yielding a p-value of 0.364, Trimble and the control yielding a p-value of 0.993, and Oxbow and the control displaying a p-value of 0.307. Also different from the other metal trends, cadmium was the only heavy metal that did not exhibit any significance for site and zone interactions as well ($p = 0.294$).

The same pattern was observed for cadmium across the three zones. The roots and young leaves (zones 1 and 2), showed a p-value of 0.287, and the roots and old leaves (zones 1 and 3) had a p-value of 0.308. There was no significance, as expected, between the young and old leaves, with a p-value of 0.999.

4. Discussion

The concentrations of heavy metals in the Animas River compared with the Florida River indicate that the Gold King Mine Spill did, in fact, have a significant impact on the amounts of heavy metals being transported to the adjacent riparian zone of the Animas River. While all sites including the control contained the four heavy metals tested for, the two sites on the Animas River had significantly higher amounts of all

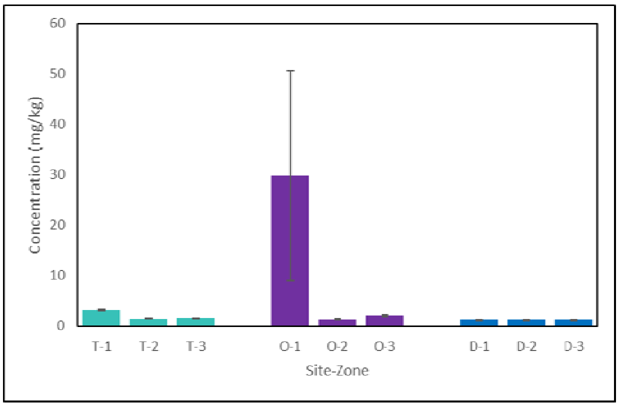


Fig. 10 Average concentrations of cadmium (+/- SEM) across all sites. Each treatment or control is subdivided into the three zones, containing roots, young leaves, and mature leaves. For example, T-1 refers to Trimble site, Zone 1.

heavy metals than the Florida River, with the exception of zinc. Because the treatments were significantly higher, this is evidence that past mining activity has had deleterious effects on the riparian organisms that rely on adjacent water sources for life.

Another indication that the Gold King Mine Spill had a significant and unnatural ecological impact was the spike in heavy metal concentrations in the roots on the Animas River compared to the Florida River. This spike in metals is synchronized with the release of three million gallons of heavy metals. Juxtapose this to the relatively unchanging levels of heavy metals on the control site, indicating that the natural metals in the environment are being taken up at a more constant rate, as there is no mining activity above the Florida River. Because the levels stay ubiquitous between zones on the Florida River, this is evidence that the metals have been taken up at a constant rate and have not experienced a plume of metals, as did the Animas River.

Another profound finding was the change in heavy metal concentrations in the samples relative to various stream morphologies at a given site. As Oxbow Park is a slower river flow with a meandering morphology, sediment deposition accumulates in higher amounts on the point bars. After the plume, the high concentrations of heavy metals were deposited on these point bars where *S. exigua* tends to grow in abundance compared with other reaches of the river. This is reflected by water quality tests that were conducted right after the Gold King Mine spill, which displayed highest levels of contaminants at Baker's Bridge and Oxbow Park [15]. This study showed Oxbow Park to always have significantly higher metal loads in samples compared to the control group, while Trimble Lane was not always different from the control. Again, this may be due to the faster water velocity and streamlined features that make up the area around the Trimble site. Looking deeper into this study, the most apparent trend observed was the significantly higher concentrations of all heavy metals in the roots, compared to the leaves. This was evident, regardless of which site the plants were located at, or which metal was being tested for. When compared using a 2-way ANOVA, the differences in concentrations between the root samples (zone 1) and the leaf samples (zones 2 and 3), were almost always statistically significantly different. The only metal tested that did not show significantly different concentrations between roots and leaves was cadmium, which may be due to the relatively low concentrations observed in the plants. The levels may not have been high enough to show any trends. Furthermore, the levels of aluminium, manganese and iron may have been so much higher than cadmium that it did not allow for the cadmium to be taken up in a greater portion on the Animas River.

The overall trend of higher metal concentrations observed in the roots can be supported by past data, particularly *Salix spp.* Since roots are the first part of a plant to come into contact with the metal ions, and transport of the heavy metals to shoots is relatively low, they typically accumulate higher concentrations which are then stored in the roots [16]. This is most likely a mechanism evolved to protect the shoot, and therefore, the photosynthetic pathways of the plant from being affected [16]. There were never significant differences between the young plant leaves and the mature plant leaves, which may mean that there is not a lot of transduction occurring from the roots to the shoots, soon after the Gold King Mine Spill. In contrast, the control river did not exhibit these same patterns; this could indicate that natural metals have been taken up for many years and are ubiquitous across the roots and shoots. The overwhelming amount in the roots on the Animas River suggests that the plume has fully saturated the roots with heavy metal content and does highlight the need to keep monitoring these concentrations in the upcoming years.

Another interesting trend observed in this study was the variation of concentrations between metals. Clearly, the metals were all taken up at different levels, as the concentrations vary in the water itself. Aluminium and manganese are much higher in the water, which makes sense that these were the two highest metals observed [3]. Furthermore, metals are taken up in different amounts depending on both the tolerance and nutrient needs of the plant. Metals such as zinc and manganese are needed as secondary metabolites, so willows tend to uptake higher quantities of these metals [7]. In addition, *S. exigua* has been noted to have a high tolerance for metals such as cadmium and zinc, which may be due to their symbiotic relationship with rhizosphere bacteria which has a high affinity for these metals and allows for better extraction of these metals from the sediment into the roots [11]. This could help better explain some of the trends we see with varying concentrations of metals within the plants.

Future studies would be beneficial to obtaining more data, and thus, more evidence of elevated heavy metals on the Animas River. Because there were only twelve samples per site and four samples per zone, the variation in heavy metal concentrations was relatively high, even within each individual treatment. For a similar study in the future, perhaps eliminating one variable may help increase the replication factor. By just looking at roots versus leaves, or looking at two sites instead of three, more samples could be taken to reduce the large standard error that was observed in the results of this study. It would also be of interest to take samples from the same site over the next decade, in order to see if any transduction is occurring from the roots to the shoots. The samples on the Animas River may end up levelling out, similar to that of the Florida River if another large mine spill does not occur. Particularly regarding phytoremediaton efforts, if the roots are transporting heavy metals to the leaves in high quantities, *S. exigua* could be a potential natural resource for remediation efforts that could eliminate chemical treatment of the water.

The plants on the Animas River are undoubtedly being exposed to higher heavy metal concentrations than on similar rivers in the Southwest region. This reflects that anthropogenic effects are not only causing poor water quality; the effects are being amplified into the riparian zones, and potentially the entire ecosystem in this area. While heavy metals are more prevalent in this area considering the environmental composition, it is our duty to make a conscious effort to reduce the amount of metal pollutants that are being secreted into water sources and to continue to monitor and mitigate the amounts of metals that are getting transported into both the aquatic and riparian zones of this region.

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