TOXICITY OF VANADIUM TO *HYALELLA AZTECA* IN FRESHWATER SEDIMENT

by

Brittany Bennett

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Thesis Committee:
  Professor Allen Burton, Chair
  Professor Mike Wiley

Abstract

Lake Catherine is a reservoir located downstream of a historic mine near Hot Springs, Arkansas. The reservoir has received inputs of a range of metals associated with mining, including vanadium (V). The toxicity of V in freshwater sediments is poorly understood. In order to evaluate the success and potential need for continuing remediation efforts in Lake Catherine, a toxicity threshold for V needs to be established. This study evaluated the toxicity of V to laboratory cultured *Hyalella azteca*, a species commonly used in sediment toxicity tests. *H. azteca* were exposed to V spiked sediments and site sediments from Lake Catherine, with whole sediment V concentrations ranging from 0 ug/g dw to over 2000 ug/g dw. Mortality and growth were assessed following a 28-day chronic toxicity test to establish lethal and effect concentrations.

Mortality was the only endpoint with significant results in the spiked sediment tests, resulting in a sediment V LC_{50} of 742 ug/g and a pore water V LC_{50} of 1870 ug/L. Pore water was the best predictor of total V and V^{+5} bioaccumulation in *H. azteca* for spiked sediments (r^2 = 0.8949 and 0.8934, respectively). Significant toxicity was not observed with site sediment exposure to *H. azteca*. These results suggest that V toxicity occurs due to pore water exposure. Further, the results suggest that Lake Catherine is not toxic to *H. azteca*, despite the sediments high V concentrations.

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1. Introduction

Vanadium (V) naturally occurs in freshwater systems through rock weathering, wet and dry deposition, and soil leaching (ATSDR, 2012). Industrial activities can increase V concentrations in water and sediment well beyond background levels (Goldberg et al., 1979; Hope, 1997; ATSDR, 2012). Industrial demand for V will possibly increase as vanadium end-use is increasing in the aerospace and battery industries (USGS, 2014).

The majority of global anthropogenic V is released as emissions through fossil fuel combustion. Atmospheric deposition of anthropogenic V compounds in freshwater systems is generally considered to be less than natural sources (ATSDR, 2012). However, water bodies are susceptible to direct inputs of anthropogenic V through mining tailings and waste rock (Hope, 1997). Mining activities can also introduce additional stressors to aquatic life, such as changes in total dissolved solids, pH, nutrients, other metals, and overall habitat quality (Kelly et al., 1984). Vanadium is primarily extracted from uranium-vanadium ores, ferrophosphorous slag, iron slag, and petroleum residues (USGS, 2014).

The concentration and bioavailability of vanadium in freshwater systems is affected by a suite of water and sediment quality characteristics, including pH, iron and manganese oxyhydroxide concentrations (Harita et al., 2005), redox reactions (Wang and Sañudo-Wilhelmy, 2009), hydrogen sulfide (Wanty and Goldhaber, 1992), and organic content (Szalay and Szilágyi, 1967; Shiller and Mao, 2000). As do other metals and metalloids, V tends to sorb into suspended particles and sediment (Hamilton-Taylor and Willis, 1984; Santschi, 1984 Johannesson et al., 2000; Environment Canada, 2010). Despite the likelihood of V to contaminate benthic habitats in mining areas, few studies have evaluated the toxicity of V in freshwater sediments.

Vanadium can exist in -1 to +5 oxidation states (Environment Canada, 2010). Pentavalent V is prevalent in oxidized and alkaline environments, typically as vanadate oxyanions $H_2VO_4^-$ and HVO_4^{2-}. In reducing or anoxic waters, V^{+4} is dominant as vanadyl cations VO^{2+} and $VO(OH)^+$ (Wanty and Goldhaber, 1992). The pentavalent form is more likely toxic because it mimics phosphate and binds on enzyme sites in the cell (Cantley et al., 1978; Environment Canada, 2000). Fontaine et al. (2013) found that V^{+5} as well as other forms of V, are increasingly bioaccumulated by *H. azteca* as overlying water concentrations increase. However, there is little information on how exposure in sediments is correlated to bioaccumulation by benthic organisms.

Sediment quality guidelines (SQGs) have been developed by federal and state agencies in the United States to establish thresholds above which adverse affects to human or aquatic health might occur (U.S. EPA, 2004). A number of popular SQGs are described in the National Sediment Quality Survey (NSQS) Screening Values and NOAA's Screening Quick Reference Tables (Buchman, 2008).

Guidelines for aquatic health are generally based on existing toxicity data and partitioning coefficients (MacDonald et al., 2000). Additionally, the EPA assesses the likelihood of sediment toxicity from metals using the theoretical Equilibrium Partitioning Approach that uses acid-volatile sulfides (AVS) and organic carbon binding with simultaneously extracted metals (SEM) (U.S. EPA, 2004). In short, this metric assumes that divalent metals will bind with available sulfides and organic carbon in bedded sediments (Di Toro et al., 1992; Ankley et al., 1996; Berry et al., 1996). If sulfide concentrations exceed metal concentrations, then toxicity is not expected (U.S. EPA, 2004).

Sediment quality guidelines do not exist for V. Neither NOAA nor EPA provide standards for evaluating aquatic life effects (e.g. lethal effect, no effect) in their respective SQGs' (U.S. EPA, 2004; Buchman, 2008). This is due to a lack of quantitative data on the toxicity of V to benthic macroinvertebrates in sediments. Larsson et al. (2013) suggested that higher soil sorption capacity resulted in lower V toxicity (EC50) concentrations. This suggests pore water V concentrations may be a determining factor for toxicity in soils, which is a conclusion that may extend to sediments. Borgmann et al. (2005) found that the 7-day 50% lethal concentration (LC_{50}) for V in water ranges from under 400 ug/L to over 1000 ug/L, depending on overlying water hardness, establishing that carbonates are an important ligand of V

The objective of this study was threefold: first, to identify lethal and effect concentrations for V in sediments with the epibenthic organism *H. azteca*; second, to determine whether bioaccumulation occurred in *H. azteca*; and third, to evaluate the toxicity to *H. azteca* at a site highly contaminated with V. *H. azteca* are commonly used in freshwater sediment toxicity tests because they are benthic, sensitive to metals (Macek et al., 1976a; Macek et al., 1976b; U.S. EPA, 2000; Borgmann et al., 2005), easily cultured, and tolerate a range of physiochemical sediment conditions (U.S. EPA, 2000). Further, *H. azteca* are widely distributed and abundant across North America (Bousfield, 1958).

This study used sediment from Lake Catherine, AR, which is a reservoir downstream of a former V mine. The site is undergoing reclamation. Sediment at sites within Lake Catherine exhibited high concentrations of V and other metals, yet the ecological significance of this contamination is unknown. *H. azteca* were exposed to site sediment and V-spiked sediments for multiple 28-day chronic toxicity tests.

2. Methods

2.1 Study Site

The study site, Lake Catherine, is a public-use reservoir located in the Ouachita River watershed near Hot Springs, Arkansas. Lake Catherine has 19 active discharge permits, including a mining reclamation operation (Arkansas Department of Environmental Quality, 2013). Lake Catherine public uses include drinking water, fishing, and general recreation. The Arkansas Department of Environmental Quality (2013) reported that the lake was safe for public use. Sediment quality was not evaluated in the study, but the report found that vanadium (V) concentrations ranged from 39.8 to 103.6 *u*g/L in surface waters.

In April, 2015, 22 sites were sampled for sediment and water quality parameters. These sites were chosen based on previous data collected by FTN & Associates. Samples were processed at the University of Michigan for V concentration and total metals via hot block analyses and inductively couple plasma mass spectroscopy (ICP-MS). Study sites were selected to include a V gradient. Selected study sites (Figure 1) were located in Spencer Bay and Wilson Cove, which are fed by Indian Springs Creek and Wilson Creek, respectively. A reference site, dubbed "Reference," was selected which had less V than Spencer and Wilson. The reservoir is drawn down between three and five feet each year in winter and restored in spring. A redox study was simultaneously conducted to assess the impacts of water level fluctuations on V.

2.2 Experimental Design

Several 28-day sediment toxicity tests with *H. azteca* were conducted to assess site and amended (spiked) sediment. The tests were conducted in three phases (Table 1). Phase 1 was conducted with sediment collected in June to characterize Lake Catherine's whole sediment

chronic toxicity. Phase 2 characterized sediment toxicity for site sediment collected in October. Additionally, phase 2 established a lethal concentration threshold for V. Phase 3 evaluated a gradient of V concentrations to establish an effects threshold.

Toxicity tests were modeled on EPA (2000) methods. In 300 mL high-form beakers (henceforth called test chambers), 100 mL of sediment was added. Approximately 200 mL of overlying culture water was added to each test chamber. Water was renewed every 12 hours for the duration of the test. Each test chamber had a mesh-covered water outlet at the 300 mL mark to prevent organisms from overflowing.

Ten 7-14 day old *H. azteca* were added to each test chamber and fed 1.0 mL of YCT daily. All organisms were cultured on site following EPA guidelines (U.S. EPA, 2000). Toxicity endpoints were survival and growth. Five replicates of each sediment treatment were evaluated. Five replicates of ten organisms each were collected on day 0 of each test to establish initial weights.

2.3 Sediment V Spiking

Sediments were spiked with V as $NaVO_3$ using the indirect spiking method' (Hutchins, et al., 2009; Brumbaugh, et al., 2013). Spiked sediment was composed of homogenized Lake Catherine reference sediment. The V salt was dissolved in water, then added to sediment to create a 3200 mg/kg "super spike". The super spike was gently rolled for an hour twice daily and buffered with HCl to match the pH within 0.2 of the original sediment for ten days. Super spike was then diluted with the original reference sediment to achieve a spectrum of V concentrations that encompassed known concentrations in Lake Catherine (Table 1). Diluted sediments were rolled for an hour twice daily and equilibrated for an additional 14 days.

2.4 Physiochemical Analyses

2.4.1 Sediment

Sediment samples were analyzed for total metals, V speciation, AVS and SEM, organic carbon content (%OC), percent dry weight, particle size, and amorphous and oxidized Fe associated V. Sediment samples were collected in plastic bottles. The headspace was deoxygenated with N_2 and the lid was sealed with tape to reduce potential oxygenation. Sediment samples were homogenized prior to analysis and again purged with N_2.

The University of Wisconsin State Laboratory of Hygiene analyzed total metals and V speciation via inductively coupled plasma magnetic sector (ICP-MS). AVS and SEM were measured by acid volatization with 1N HCl (Allen, et al., 1993). Sulfide was measured colorimetrically and SEM was measured through ICP-MS following filtration (0.4 um). Organic content was measured via loss on ignition (LOI) and converted to %OC via the Redfield ratio (0.36) (Redfield, 1934).

Sediments were analyzed from ponar grabs from a December sampling trip (SI 1), as well. No sediment toxicity test was conducted for this sediment, but data is provided in the appendix for reference.

2.4.2 Water

Water samples were collected in the field and lab toxicity experiments. Field samples were analyzed for basic water quality parameters (pH, temperature, dissolved oxygen (DO), conductivity), total dissolved solids (TDS), total suspended solids (TSS), hardness, and

alkalinity. June field samples were analyzed for total metals and V speciation by ICP-MS. Lab samples were analyzed for water quality and pore water chemistry.

TDS and TSS were determined following ASTM Standard Test Measure D5907 (ASTM, 2010). Pore water samples were collected using the peeper method (Brumbaugh, 2007). Peepers were deoxygenated for 48 hours prior to inserting them approximately one inch below surface level of two replicates per sediment treatment. One pore water replicate was taken down on both Day 1 and Day 28, respectively. Pore water chemistry was analyzed for total metals and V speciation via ICP-MS by the University of Wisconsin State Laboratory of Hygiene.

2.4.3 Tissues

On day 28 of each test, surviving organisms were counted and depurated for 24 hours in EDTA. Organisms were recounted, dried in a dessicator for 72 hours, and then weighed to the nearest microgram to establish growth. In the spiked toxicity test, an additional three replicates were added. Each week, one replicate was assessed for survival, but not growth. Weights were analyzed as a relative growth rate, calculated average growth per organism per day. Organisms were then analyzed for body chemistry (body burden) by the University of Wisconsin State Laboratory of Hygiene via ICP-MS.

2.5 Statistical Analysis

All sediment, pore water, and organism data was analyzed with Shapiro Wilk tests (a=0.05) and residual plots to test linear model assumptions. Data that was non-normal was log transformed or analyzed with a Kruskall-Wallis test. Data that was normal or normalized was analyzed using one-way ANOVAs (a=0.05) with site or treatment as factors. Tukey or Niyemi post-hoc tests were used to determine significant differences between treatments or sites. Bioaccumulation was analyzed with single linear regressions against sediment and pore water concentrations. Non-linear least squares regression was used to determine all LC values for pore water and sediment chemistry. All data was analyzed using R Studio Version 0.99.891.

3. Results
3.1 Spiked Toxicity Tests
3.1.1 Overlying Water Quality

All sediment toxicity tests were conducted with a twice-daily water renewal system using water from the same source as *H. azteca* are cultured in. Weekly overlying water quality characteristics were consistently within acceptable limits for the survival and growth of *H. azteca*, except for pH. The effect of pH on growth is discussed below. Dissolved oxygen for all treatments never fell below 5 mg/L.

3.1.2 Vanadium Recovery

Spiked sediments were analyzed for total vanadium concentrations, which differed from the nominal concentrations. Phase 3 whole sediment V recovery was significantly (p<0.05) lower than the nominal spiked concentrations for all but nominal 100 ug/g V spike (Table 2). As the nominal spiked concentration increased, the difference between the nominal and actual V concentrations increased. Recovery values for Phase 3 were not significantly different between Day 0 and Day 28. Phase 2 V recovery values were closer to the nominal concentrations (Table 3). Phase 2 and Phase 3 spiking reference sediments were collected from the same site in different months (October and November, respectively), so this is likely due to seasonal

geochemical fluctuations at the reference site. Both Fe and Mn were significantly ($p<0.05$) higher in the Phase 3 sediment as compared to the Phase 2 sediment. Phase 3 V may have bonded to Fe and/or Mn in non-labile forms during the equilibration period, causing for lower recovery in Phase 3.

3.1.3 Mortality - Sediment

H. azteca mortality was measured in order to calculate lethal concentration (LC) values. Based on measured whole sediment V concentrations and *H. azteca* mortality, survival values were fitted to a logistic curve (Figure 2). The 28-day whole sediment V LC_{50} for *H. azteca* is 742, LC_{20} of 516, and LC_{10} of 417 (*u*g/g; Table 4). The 95% confidence intervals are broad for these calculations, likely due to the limited partial mortality observed over the V gradient. The Raisin River reference had 100% (±0) *H. azteca* survival, indicating that testing procedures were adequate for *H. azteca* survival.

A significant (p < 0.05) drop in survival was observed in the 1600 treatment, with 60% (±18.17) survival corresponding to 626 ug/g V. Variance for the 1600 treatment was high compared to all other treatments. Complete mortality was observed in the 3200 treatment, corresponding with 1269 ug/g V. Mean survival ranged from 96-100% for all other treatments, including reference and control. Survival did not correlate with any other whole sediment metal concentration or overlying water quality parameters, suggesting that V controlled survival in the spiked toxicity tests.

Whole sediment V did not significantly fluctuate from day 0 to day 28, nor did total Ni, Cu, or Zn. From day 0 to day 28, V^{+5} increased in all treatments except 0, 100, and 400 spike (Figure 3), while V^{+4} increased in treatments 0, 200, 400, 1600, and 3200 (Figure 4). From day 0 to day 28, organic carbon normalized excess SEM ([SEM-AVS]/fOC; Table 5, 6) decreased due to increased organic carbon content. Sulfides fluctuated minimally across all treatments. Total Fe significantly ($p<0.05$) increased in treatment 1600 and decreased in treatment 1100, but otherwise remained static. Total Mn did not significantly fluctuate from day 0 to day 28.

3.1.4 Mortality – Pore Water

Pore water was measured for total metals and V speciation. Based on measured filtered pore water V concentrations and *H. azteca* mortality, were fitted to a logistic curve (Figure 5). The 28-day pore water V LC_{50} for *H. azteca* is 1870 (1450-2450) ug/L (Table 7). Filtered pore water V concentration increased linearly with whole sediment V concentrations. In all treatments, pore water V^{+5} was significantly ($p<0.05$) higher than V^{+4} concentrations. Treatment 1100 was excluded from all pore water analysis due to low recovery likely caused by collection error.

3.1.5 Relative Growth Rate – Sediment, Pore Water, and pH

Growth rates were measured to identify sub-lethal effects of vanadium to *H. azteca*. Relative growth rates (RGR) were calculated as the change in weight from initial per organism over 28 days. Unexpectedly, *H. azteca* RGR over 28 days did not significantly correlate to whole sediment V concentrations, pore water V concentrations, or bioaccumulation of any metal. Excluding treatment 1600, which exhibited V toxicity, all treatments displayed a slight positive trend in RGR as V concentration increased, though this relationship was not significant. Additionally, RGR did not significantly correlate with any other recovered whole sediment metals. Reference RGR was significantly ($p<0.05$) higher than all spiked treatments and the

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control. Raisin River sediment had a slightly higher pH, higher fraction of organic carbon, and sandy sediment composition.

Multiple ANOVA analyses indicated a significant (p<0.05) correlation between RGR and overlying water pH. The 28-day mean overlying water pH of treatment 0 (control) was 6.84 (±0.09). Across all spiked treatments, mean pH increased as V concentration increased, ranging from 6.92 to 7.15. The mean pH of Raisin River reference sediment was 7.33 (±0.09). Culturing water, which was used for the twice daily water exchanges, had a mean pH of 7.56 (±0.08) when not in contact with sediment.

3.1.6 Bioavailability of V

H. azteca were measured for whole body vanadium content. A series of simple linear regressions indicated that pore water concentrations are a better predictor of V bioaccumulation than sediment concentration. Bioaccumulation of total V and V^{+5} was significantly (p<0.05) related to the respective pore water concentrations (r^2 = 0.8949 and 0.8934, respectively). Bioaccumulation of V^{+4} was not explained by sediment or pore water concentration of the species. Data for treatment 1100 was omitted in pore water analyses due to low recovery likely caused by collection or processing error.

3.2 Site Toxicity Test
3.2.1 Toxicity Test Results

The June sediment site toxicity test was conducted for all sites sampled in Lake Catherine. No significant mortality or growth effects to *H. azteca* were observed in June. As such, the October toxicity test evaluated the two most contaminated sites in Lake Catherine (Wilson 1 and Spencer 1) and the Reference site. Again, no significant mortality or growth effects to *H. azteca* were observed (Figure 6). Similarly, there were no significant correlations between sediment V concentration and *H. azteca* mortality and growth for June or September. Mean mortality from toxicity tests using June sediment were lower in Spencer Bay sites than Wilson Cove, but those treatments had high variance.

3.2.2 Sediment and Pore Water Chemistry

Site sediment chemistry was analyzed for all sites in both June and October, though for the October site toxicity test, *H. azteca* were only exposed to Reference, Spencer 1. Sediments were collected at the end of the 28-day toxicity test for October, but V and total metal concentrations did not significantly vary from site concentrations.

Whole sediment samples from June and October were analyzed for total metals and V speciation. Sites were analyzed for sediment type, as well (Table 8). In June, whole sediment V concentrations exceeded 1000 ug/g at Spencer 1 and all Wilson sites. In October, whole sediment V concentrations exceeded 1000 ug/g at Wilson 1-3 (Figure 7). Nearly all sites exceeded threshold effect concentrations for Cu, Ni, and Zn (Table 9, 10).

Since no significant toxicity was exhibited by *H. azteca* during site sediment exposure, potential V binding factions were analyzed. Organic carbon normalized excess SEM in June (Table 11) exceeded potential toxicity thresholds (>150) at the Reference site and at the Spencer 4 site. Both sites had significantly lower *f*OC than all other June sites. Organic carbon normalized excess SEM in October (Table 10) did not exceed potential toxicity thresholds (>150) at any sites (Table 12). Total Fe concentrations were significantly (*p*<0.05) higher in all Wilson sites for both June (Table 11) and October than all other sites. Total Mn concentrations

were significantly ($p<0.05$) lower than all other sites in Reference and Spencer 2-4 sites for both June (Table 11) and October (Table 12). Iron across all sites but Reference was significantly ($p<0.05$) higher than all spiked sediments, which may explain why V was bioavailable in the spiked toxicity test but not in the site toxicity test.

October pore water samples were analyzed for total V and V speciation. In the site toxicity test, *H. azteca* were only exposed to Reference, Spencer 1, and Wilson 1. Pore water V^{+5} concentrations were below 1 ug/L for all exposure sites, which is significantly ($p<0.05$) lower than spiked sediment pore water concentrations for V^{+5}. This may explain why toxicity was not observed in site sediments, as V^{+5} is the more toxic V species. Total V concentrations in pore water were correlated to sediment total V concentrations.

4. Discussion

Little information exists in the primary literature on the toxicity of V in sediments to benthic macroinvertebrates. NOAA's sediment reference tables (Buchman, 2008) do not report any V toxicity thresholds for freshwater or marine sediment. We conducted a V toxicity study by spiking freshwater sediment from the "reference" site in Lake Catherine. This site exhibited low metal contamination compared to the study sites of Spencer Bay and Wilson Cove, which were heavily contaminated with V.

Our spiked study found a whole sediment V LC_{50} of 742 ug/g and pore water V LC_{50} of 1870 ug/L to *H. azteca*. The confidence intervals of these values are broad, due to the treatments exhibiting only one partial mortality out of eight concentrations spanning over 1200 ug/g. Our concentration gradient was expected to display multiple partial mortalities. However, total V concentrations were much lower than nominal V concentrations for the full study, which ranged from 39.6% to 103% of the nominal concentration. When comparing total metal recovery of the 1600 and 3200 treatments in phase 3 to phase 2, we found that total iron was significantly higher in phase 3. We suspect that V was bound by the iron into non-labile forms during the two-week equilibrium phase of the spiking procedure. Iron and manganese oxides are two of the dominant binding factions for V (Environment Canada, 2000).

Our study reaffirms the notion that toxicity thresholds are highly context-dependent (e.g. Allen, 1993). Our spiked study found a 28-day pore water LC_{50} of 1870 ug/L. Borgmann et al. (2005) found multiple overlying water V LC_{50} ranging from under 400 to over 1000 ug/L as water hardness increased. While the culture water used in our toxicity test had a hardness of over 100 mg/L, most sites in Lake Catherine had hardness values of under 30 mg/L. Alkalinity was also much lower in site water than in culture water. While the Borgmann et al. (2005) study indicates that toxicity should be present in Lake Catherine due to lower hardness, the alkalinity of Lake Catherine is unfavorable to the more toxic species of vanadium, V^{+5}.

Our spiked sediment toxicity test revealed toxicity at V concentrations much lower than the background V levels in our site sediment toxicity test. This suggests that site V was bound into biologically unavailable forms, possibly due to the significantly higher levels of iron and organic carbon. Our study did not identify non-labile forms of V, which would have helped explain this discrepancy in toxicity results.

Further, both Spencer Bay and Wilson Cove displayed high sediment concentrations of Cd, Cu, Ni, and Zn. In many cases, these concentrations exceeded benthic macroinvertebrate sediment toxic and probable effect thresholds (TEC and PEC) established by NOAA (Buchman, 2008). In several sites, acid volatile sulfides were insufficient to bind all of the simultaneously extracted metals. As such, we expected to observe significant mortality or growth effects to *H.*

azteca. We did not observe any significant effects, indicating that ligands were available to bind excess metals in biologically unavailable forms. Our study was not able to sufficiently explain the lack of toxicity observed in such a highly contaminated site.

A study on the relationship between water level fluctuation and vanadium speciation, as well as the resulting toxicity, using Lake Catherine site sediment was simultaneously conducted with our study. In mesocosms, Nedrich et al. (in preparation) altered water levels over 12 days and measured total metals and vanadium speciation in sediment and pore water. They found that Zn and Cd flux into pore water at Lake Catherine and may be contributing to adverse effects on *Hyalella* and benthos.

It is generally accepted that metal uptake by benthic macroinvertebrates primarily occurs through dissolved metals in pore and/or overlying water exposure (Borgmann, 2000). Our findings agree with that conclusion, as both total V and V^{+5} bioaccumulation were strongly correlated to the respective pore water concentrations. Whole sediment V was correlated, though less strongly, to total V bioaccumulation, indicating that sediment may be an adequate measure of total V bioavailability. However, bioaccumulated V^{+5} and V^{+4} species were not explained by the respective sediment concentrations.

It is well established that trace metal speciation is key to identifying potential toxicity to aquatic life (e.g. Solomans and Förstner, 2012). The pentavalent form of V is more toxic to aquatic organisms because it mimics phosphate and binds on enzyme sites in the cell (Cantley et al., 1978; Environment Canada, 2000). Our study suggests that identifying V species in pore water is useful for assessing V bioavailability and toxicity to *H. azteca*. Our site toxicity study measured pore water for September sediment, which had V^{+5} concentrations less than 1 ug/L for all sites. These values are significantly lower than the 1870 ug/L pore water toxicity threshold found in the spike toxicity test. This may account for the lack of mortality observed in the site toxicity tests, despite the high total V sediment concentrations.

There are three standard metrics of toxicity for aquatic invertebrates: survival, growth, and reproduction. For *H. azteca*, growth and survival easier to quantify, as they do not reproduce until they are five weeks or older. While we observed a clear mortality threshold for V in our spiked study, we did not find a significant relationship between growth and V concentration.

As V concentration increased, pH increased. While pH levels were in an acceptable range for *H. azteca*, there was a significant relationship between pH and RGR. Frances and Stokes (1987) found that *H. azteca* are not resilient to sudden pH changes. In all spiked treatments and the reference, pH was lower than that of the water used for *H. azteca* culturing. Though our water renewal system used the same water as the *H. azteca* culturing water, the pH of the sediment was much lower. It is possible that pH was a co-stressor, though pH may be an indicator of increased dissolved metals. Future studies should more tightly control for co-stressors.

The first goal of this study was to establish a freshwater sediment and pore water toxicity threshold for V to *H. azteca*. Our results indicate that V toxicity is best evaluated through pore water concentrations. Bioaccumulation of the toxic state of V, V^{+5}, is linearly related to the concentration of V^{+5} in the pore water. As such, speciation studies of pore water will be a strong indicator of potential toxicity of a site. The second goal of this study was to evaluate the toxicity of a highly contaminated reservoir, Lake Catherine. Significant mortality was not observed in the lake, indicating that there are ligands available to mitigate the site's metal toxicity. While the reservoir had high V sediment concentrations, it had low V^{+5} pore water concentrations, which suggests that pore water is the dominating factor in bioavailability and toxicity of V.

Tables and Figures

Study sites: Aerial views of Spencer Bay, Wilson Cove, and Reference sites.

Spencer Bay Sample Sites

Wilson Cove Sample Sites

Reference Site

Figure 1 Locations marked represent GPS coordinates taken during sampling trips at Lake Catherine, AR.

Table 1

Phase	Site Sediment	Spiked Sediment	Controls
Phase 1 – Site toxicity test	June: Spencer 1-4 June: Wilson 1-4 June: Reference 1 October: Spencer 1 October: Wilson 1 October: Reference 1	N/A	Sand
Phase 2 – Vanadium upper threshold toxicity test		1600 ug V mg^{-1} 3200 ug V mg^{-1}	Raisin River Sediment
Phase 3 – Vanadium effects toxicity test	November: Reference 1	100 ug V mg^{-1} 200 ug V mg^{-1} 400 ug V mg^{-1} 800 ug V mg^{-1} 1100 ug V mg^{-1} 1600 ug V mg^{-1} 3200 ug V mg^{-1}	Raisin River Sediment

Table 2

Phase 3: Whole sediment V recovery for Day 0 and Day 28 of the Phase 3 toxicity test.

Nominal V (ug/g spiked)	Measured V (ug/g dry weight)			
	Day 0	±	Day 28	±
0 (Raisin River Reference)	21.4	2.1	24	2
0 (LC control)	75.4	4.4	72	6
100	103	6	106	6
200	144	8	134	11
400	216	4	204	12
800	376	22	358	29
1100	498	8	464	39
1600	626	0.2	702	58
3200	1269	72	1155	95

Table 3

Phase 2: Whole sediment recovered V for Day 28 of the phase 2 spiked toxicity test

Nominal V (ug/g spiked)	Measured V (ug/g dry weight)	
	Day 28	±
0 (Raisin River Reference)	25	2
0 (LC control)	25	4
1600	1482	6
3200	2612	11

Phase 3: Logistic regression (red line) of *H. azteca* survival (%) against whole sediment V concentrations (ug/g) with 95% confidence intervals (dashed lines).
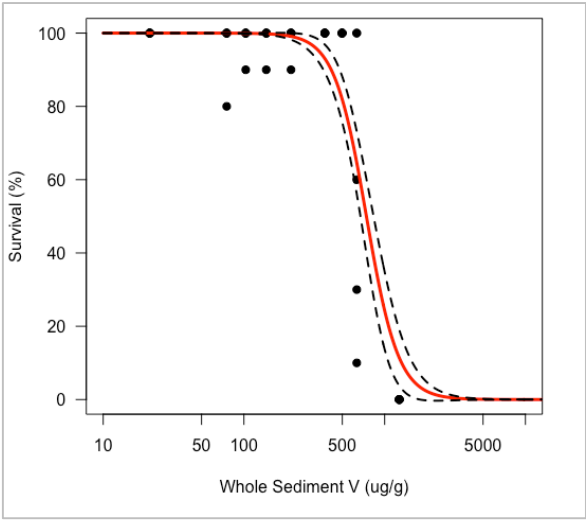

Figure 2 *Red line represents logistic regression of survival (%) against whole sediment V (ug/g); dashed lines represent upper and lower 95% confidence intervals; points represent means of survival across treatment replicates (n=5).*

Table 4

Phase 3: 28-day whole sediment LC_x for *H. azteca*

	Whole Sediment V Concentration (ug/g)	95% CI (ug/g)
LC_{50}	742	673-834
LC_{20}	516	465-576
LC_{10}	417	367-479

Phase 3: Mean whole sediment V^{+5} concentrations on day 0 and day 28 with standard deviation bars against mean whole sediment V concentration on day 0 with standard deviation bars.
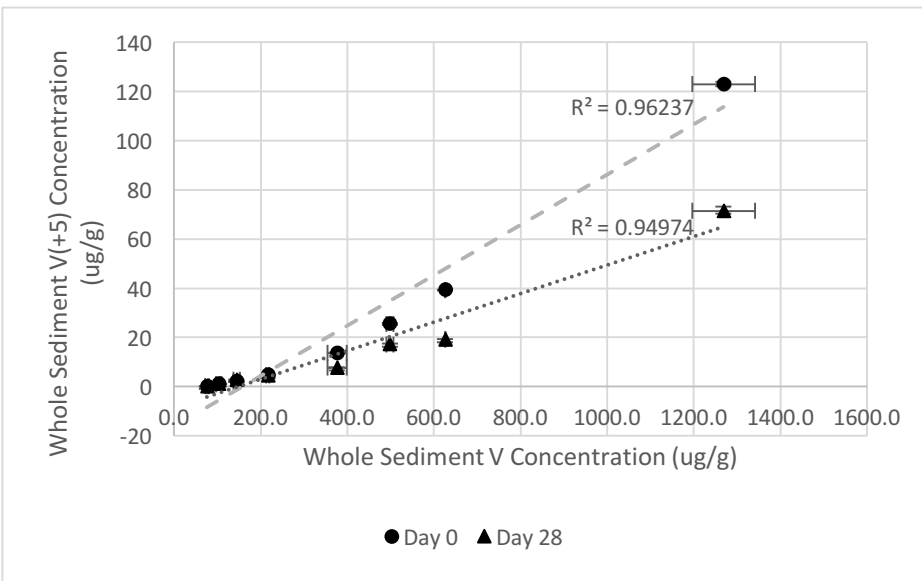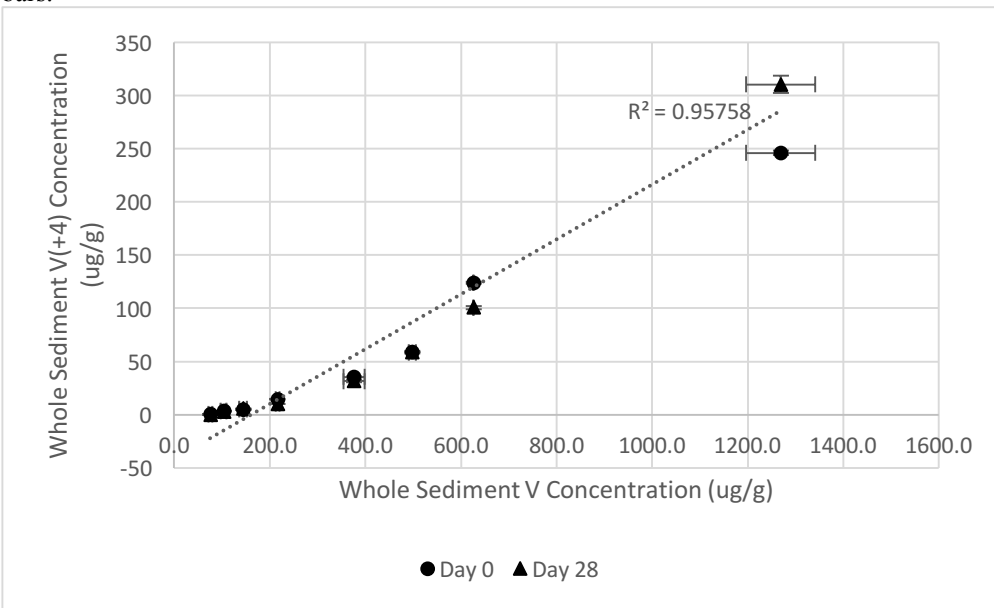

Figure 3 All points represent averages of concentrations (ug/g); Bars represent standard deviation of total V (horizontal) and V^{+5} (vertical).

Phase 3: Mean whole sediment V^{+4} concentrations on day 0 and day 28 with standard deviation bars against mean whole sediment V concentration on day 0 with standard deviation bars.

Figure 4 *All points represent averages of concentrations (ug/g); Bars represent standard deviation of total V (horizontal) and V^{+4} (vertical).*

Table 5

Phase 3: Day 0 binding faction values of spiked, control, and reference sediments.

Treatment	AVS (*u*mol/g dw)	*f*OC	SEM-AVS (fOC)	Total Fe	±	Total Mn	±
0 Spike	0.008	0.015	16.095	13429	843	324	18
100 Spike	0.011	0.022	36.374	13390	839	311	17
200 Spike	0.006	0.017	106.575	13334	430	310	0
400 Spike	0.009	0.013	209.628	13212	13	304	4
800 Spike	0.009	0.017	302.850	13211	826	313	17
1100 Spike	0.008	0.018	378.952	12982	8	297	3
1600 Spike	0.012	0.017	729.826	12269	18	286	4
3200 Spike	0.021	0.017	1305.486	12683	797	289	16
Raisin River	0.150	0.032	14.950	12069	289	351	55

AVS=Acid volatile Sulfides; *f*OC=fraction of organic carbon; SEM=simultaneously extracted metals; SEM-AVS(*f*OC)= organic carbon normalized excess SEM; ± =standard deviation.

Table 6

Phase 3: Day 28 binding faction values of spiked, control, and reference sediments.

Treatment	AVS (*u*mol/g dw)	*f*OC	SEM-AVS (fOC)	Total Fe (ug/g)	±	Total Mn (ug/g)	±
0 Spike	0.002	0.025	13.916	13021	1158	311	25
100 Spike	0.004	0.028	30.625	13570	860	334	20
200 Spike	0.003	0.025	29.422	13845	1247	340	27
400 Spike	0.012	0.024	109.061	13199	875	305	18
800 Spike	0.011	0.024	163.450	13653	1229	333	27
1100 Spike	0.010	0.027	156.044	12407	1123	291	24
1600 Spike	0.009	0.025	427.450	13929	1241	344	28
3200 Spike	0.028	0.026	800.977	12894	1164	289	23
Raisin River	0.042	0.036	-1.156	12130	1106	450	37

AVS=Acid volatile Sulfides; *f*OC=fraction of organic carbon; SEM=simultaneously extracted metals; SEM-AVS(*f*OC)= organic carbon normalized excess SEM; ± =standard deviation.

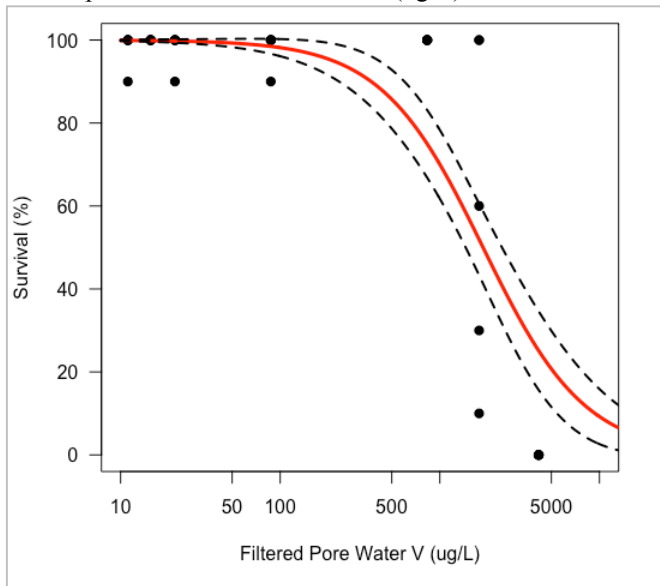Phase 3: Logistic regression of *H. azteca* survival (%) against filtered pore water V concentrations (ug/L).

Figure 5 *Red line represents logistic regression of survival (%) against filtered pore water V (ug/L); dashed lines represent upper and lower 95% confidence intervals; points represent means of survival across treatment replicates (n=5).*

Table 7

Phase 3: 28-day filtered pore water LC_x for *H. azteca*

	Filtered Pore Water V Concentration (ug/g)	95% CI (ug/g)
LC50	1870	1450-2450
LC20	674	466-941
LC10	371	234-599

Table 8

Phase 2: Texture of sediment collected in Lake Catherine

Characteristic	Mean (± SD)		
	Reference	Spencer	Wilson
Sand (>53 um)	60.875 (0.757)	29.405 (2.694)	46.35 (7.13)
Silt (2-53 um)	32.69 (0)	39.385 (8.676)	47.42 (9.22)
Clay (<2 um)	6.435 (0.757)	32.705 (3.853)	6.22 (2.10)
Sediment Texture	Sandy Loam	Clay Loam	Loam

Phase 1: *H. azteca* survival (%) following exposure to Spencer 1, Wilson 1, and Reference sediments from Lake Catherine, AR.
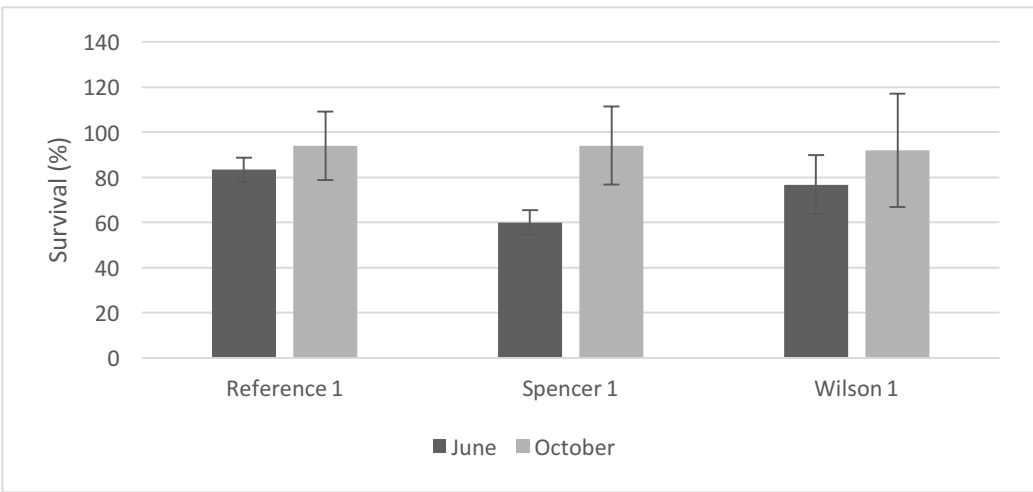
Figure 6 *Columns represent mean of survival across treatment replicates (n=5); bars represent standard deviation of means.*

Table 9

Site characterization: Concentrations (ug/g) of metals in June shallow sediment core samples in Lake Catherine

Metal	PEC	TEC	Spencer								Wilson								Reference	
			Site 1	±	Site 2	±	Site 3*	±	Site 4	±	Site 1	±	Site 2	±	Site 3	±	Site 4	±	Site 1	±
Cu	149	31.6	60.2	3.1	35.6	1.8	40.4	2.4	16.2	0.8	153.5	7.9	193.7	9.5	134.3	6.6	139.7	6.9	11.4	0.6
Ni	48.6	22.7	37.3	3.0	37.3	3.1	43.1	3.4	18.2	1.5	165.6	13.7	290.2	23.8	161.6	13.1	104.2	8.3	11.6	1.2
V	N/A	N/A	1018.6	59.9	219.3	12.6	283.0	16.2	233.1	13.6	1131	65	1670	98	1094	64	2897	166	147.5	9.0
Zn	459	121	175	19	172	18	191	21	91	10	516	55	800	86	411	44	443	50	43	5

Probable and threshold effect concentration (PEC and TEC, respectively) reported in NOAA SQuiRT tables (Buchman, 2008); ± represents respective standard deviations.

Table 10

Site characterization: Concentrations (ug/g) of metals in October ponar grab samples in Lake Catherine

Metal	PEC	TEC	Spencer								Wilson								Reference	
			Site 1	±	Site 2*	±	Site 3*	±	Site 4	±	Site 1	±	Site 2	±	Site 3*	±	Site 4*	±	Site 1	±
Cu	149	31.6	49.16	4.79	53.5	2.9	55.56	0.48	13.5	1.6	166.78	3.39	148.3	8.3	124.7	13.6	24.4	1.5	28.60	2.14
Ni	48.6	22.7	45.58	6.10	47.2	4.3	48.31	1.50	17.8	2.7	221.25	3.75	197.8	15.9	86.2	10.2	30.3	2.6	30.79	3.10
V	N/A	N/A	461	28	476	35	653	6	232	27	1599	14	1229	84	1826	182	327	19	469	27
Zn	459	121	193	25	246	32	226	5	89.1	15.7	610	24	608	67	515	79	106	13	103	13

Probable and threshold effect concentration (PEC and TEC, respectively) reported in NOAA SQuiRT tables (Buchman, 2008); ± represents respective standard deviations.

Table 11

Phase 1: Binding faction values of June Lake Catherine sediments.

Site	AVS (*u*mol/g dry weight)	*f*OC	SEM-AVS	SEM:AVS (fOC)	Total Fe (ug/g)	±	Total Mn (ug/g)	±
June								
Reference	0.164	0.006	2.275	362.046	11788	783	184	10
Spencer 1	2.235	0.028	0.787	27.687	53431	3326	1004	56
Spencer 2	2.002	0.063	2.347	37.392	35564	2233	512	30
Spencer 3	9.491	0.026	-6.604	-253.253	36272	2260	431	24
Spencer 4	0.175	0.005	2.407	460.384	30709	1907	831	47
Wilson 1	6.837	0.040	3.760	94.157	91460	5676	1592	88
Wilson 2	1.347	0.101	5.866	58.174	108439	6808	5182	289
Wilson 3	8.358	0.051	3.127	61.016	56877	3621	1199	66
Wilson 4	38.398	0.049	-22.981	-473.134	85945	5378	1748	109

fOC is fraction of organic carbon, calculated by the initial dry weight (g) of sediment divided by the final weight after ashing (see methods); SEM:AVS (fOC) is the organic carbon normalized excess of simultaneous extracted metals (umol/g); ± are standard deviations of the respective metal concentrations.

Table 12

Phase 1: Binding faction values of October Lake Catherine sediments.

Site	AVS (*u*mol/g dry weight)	*f*OC	SEM:AVS (fOC)	Total Fe (ug/g)	±	Total Mn (ug/g)	±
October							
Reference	1.052	0.023	41.610	29652	1954	534	33
Spencer 1	1.968	0.061	30.102	42487	2849	833	48
Spencer 2	3.977	0.094	41.972	42912	2966	782	46
Spencer 3	3.778	0.103	36.309	41309.9	489.5	587.74	1.40
Spencer 4	1.441	0.025	57.182	33771	4054	930	104
Wilson 1	11.739	0.094	124.429	65231.3	880.4	1446.55	2.19
Wilson 2	14.379	0.167	85.885	65870	5014	1940	130
Wilson 3	6.352	0.098	63.102	64311	6415	769	74
Wilson 4	0.090	0.038	0.850	21512	1562	630	38

fOC is fraction of organic carbon, calculated by the initial dry weight (g) of sediment divided by the final weight after ashing (see methods); SEM:AVS (fOC) is the organic carbon normalized excess of simultaneous extracted metals (umol/g); ± are standard deviations of the respective metal concentrations.

Phase 1: Whole sediment total V (ug/g) for all sites sampled in June and October at Lake Catherine, AR, with spiked toxicity test LC_{50}.
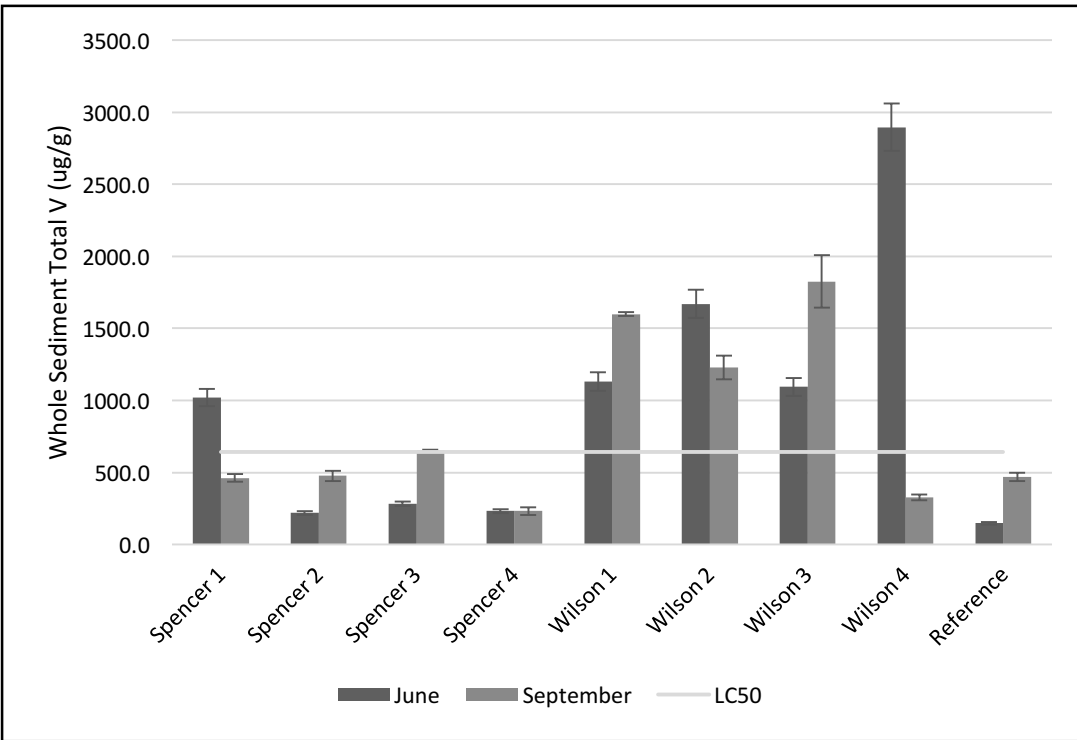

Figure 7 *Columns represent means of total V (ug/g); bars represent standard deviation of means.*

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