Journal of
Environmental Science
and Technology

Research Article
Evaluation of Heavy Metals Accumulation in *Corbicula fluminea* in Manadas Creek, Laredo, Texas

Addo-Mensah Alfred, Vaughan Thomas and Anaya Eduardo

Department of Biology and Chemistry, Texas A&M International University, Laredo, 78041, Texas, United States of America

Abstract

Background and Objective: The Manadas Creek in Laredo, Texas is a tributary of the Rio Grande which is the source of drinking water for the city of Laredo as well as many communities downstream. Portions of the creek were sunk to runoff from a decommissioned antimony smelter plant operated by Anzon America Inc. This project was designed to study the bioavailability of trace metals to flora and fauna in the creek. **Materials and Methods:** *Corbicula fluminea* (Asiatic clam) was used as a biomarker to determine bioaccumulation. Clams were placed in cages on three sites along Manadas Creek in Laredo, Texas. Concentrations of heavy metals (As, Cd, Ni, Pb and Sb) in the soft tissues of the Asiatic clam. *Corbicula fluminea* (*C. fluminea*) and water samples from Manadas Creek were determined to assess the bioaccumulation of metals. **Results:** This study shows aqueous Ni (7.90-16.57 µg L^{-1}) and Cd (1.04-2.52 µg L^{-1}) concentration levels below the maximum contaminant level throughout Manadas Creek, whereas portions of the creek, close to the Anzon smelting plant recorded as 47.99 µg L^{-1}, Pb (53.86 µg L^{-1}) and Sb (22.80 µg L^{-1}) concentrations higher than background concentrations of unpolluted waters. Metal accumulation in *C. fluminea* (dry weight) by as 16.20 mg kg^{-1}, Pb (16.31 mg kg^{-1}) and Sb (16.43 mg kg^{-1}) were higher than the maximum allowable limit set by the US Food and Drug Administration. **Conclusion:** This study showed that metal concentrations in the creek are available for bio uptake.

Key words: *Corbicula fluminea*, antimony smelting, Manadas Creek, bioaccumulation factor, Rio Grande

Corresponding Author: Alfred Addo-Mensah, Department of Biology and Chemistry, Texas A&M International University, Laredo, 78041, Texas, United States of America Tel: 956-326-2414

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sources of heavy metal pollution in the environment are mostly from anthropogenic sources such as mining operations and smelting plants[1]. When metals are deposited into terrestrial environments by anthropogenic means, they accumulate in soils and consequently enter biological cycles and/or leach into aquatic ecosystems[2]. Metals discharged into aquatic environments can become available for use in the aquatic food chain and lead to accumulation and or magnification[3,4]. The presence of heavy metals in water streams is concerning because they are harmful to aquatic plants, animals and humans that depend on the water. This is because many heavy metal ions are known to be toxic or carcinogenic to humans[5]. They can induce DNA damage and cancer via oxidative stress, DNA mutation propagation and altering cellular process[5,6]. The ability of metals to bioaccumulate makes them potential poison agents. Metals are known to change the physiological processes of aquatic organisms. Metal pollution can affect certain aspects of cellular metabolism like lysosome stability and transport across membranes are affected by metal pollution[7,8]. Metals accumulate in sediments mainly from the deposition of metals adsorbed onto suspended particulate matter. Moreover, metals can also accumulate from the diffusion of dissolved metal species and, to a lesser extent, from the diffusion of dissolved metal species from the overlying water column to the sediment, where a change in redox potential, induced by microbes can lead to precipitation and/or co-precipitation reactions[9-12]. Bioaccumulation of heavy metals measures the uptake of metals through all possible routes when exposed to an ecosystem[12]. The extent to which metals bioaccumulate depends on the competing processes of chemical uptake (through diet and ambient environment) and elimination (through egestion and biotransformation). High bioaccumulation of metals in biomarkers like *Corbicula fluminea* is an indication of the increased environmental availability of metals. *Corbicula fluminea* (Asiatic clams) can be a good choice to use as a biomonitor because many bivalves and similarly structured organisms have been successful as biomonitors themselves.

The Anzon America, Inc., facility on the banks of Manadas Creek in Laredo, Texas was the site of metals refining operations since the Texas Mining and Smelting Company began operations in 1928. A blast furnace produced crude antimony oxide from antimony-bearing ore brought by rail from the Wadley Mine in San Luis Potosi Mexico. Activities of this smelting plant have led to significant heavy metal (especially antimony) contamination of water (Manadas Creek

included) and soil in the vicinity of the plant[14]. Stormwater runoff and aerial deposition from the approximately 28-acre plant facility were deposited into Manadas Creek and the surrounding area[15]. Prior geochemical studies of Manadas Creek reported high arsenic and antimony concentrations in the sediment[15]. The study concluded that antimony is not very bioavailable due to the current geochemical conditions observed[15]. It was, however, mentioned that changes in geochemical conditions can cause a significant increase in bioavailability of antimony. Metal bioavailability will result in high concentrations of the corresponding metals in biota, through bioconcentration and bioaccumulation[13]. Mining or smelting sites where waste is not managed properly are known to be point sources where hazardous metals are released into the surrounding ecosystem[2]. It is therefore imperative to study the bioavailability and bioaccumulation of heavy metals in Manadas Creek, which has received effluents from smelting activities. A typical antimony tailing contains antimony and other heavy metals such as As, Pb and Cd[16]. The objective of this study was to determine the bioaccumulation of heavy metals (As, Sb, Ni, Pb and Cd) in Manadas Creek using *Corbicula fluminea* as the biomarker. *Corbicula fluminea* placed at different sampling sites along the creek will be used to access the bioavailability of heavy metals in the sediments of Manadas Creek.

MATERIALS AND METHODS

Study area: This study was carried out from August, 2009 to February, 2010. Manadas Creek is a semi-arid creek and urban tributary of the Rio Grande located in Laredo, Texas. The Creek, which has a 10,122-acre total drainage area, is a major watershed within Laredo and is in an industrialized area surrounded by warehouses, a major railway line and a cement ready-mix plant. Manadas Creek also carries wastewater effluent from the North Laredo Wastewater Treatment Plant to the Rio Grande. The Manadas Creek passes through North Central Park, a recreational park (suitable for fishing) that serves families in Northern Laredo. In addition, an antimony smelter that operated from 1928 until decommissioned in 1999 is located on the creek bank approximately 2 km upstream from its confluence with the Rio Grande. Previous studies conducted on the creek found metal contamination of both sediment and groundwater. Groundwater samples collected from the creek which is very close to the decommissioned smelter plant (Site 2 in this study) contained antimony concentrations as high as 2.5 mg L^{-1} [15]. Soils collected in undeveloped portions of the smelter site were found to have antimony concentrations of up to 231 mg kg^{-1}

at depths from zero to two feet below the surface[15]. Presently, two slag sites containing antimony byproducts remain on the site for remediation. This study was performed at three sites along the main Manadas Creek. The three sites chosen represent the differences in antimony and arsenic concentration as recorded in the Baeza *et al.*[15]. Site 1 is upstream of the decommissioned smelter plant. Site 2 is downstream of the smelter but very close to the decommissioned plant. Site 3 is further down the smelter site.

Sample collection

Corbicula fluminea: Approximately 800 *Corbicula fluminea* (Fig. 1) were collected from the mainstem of the Rio Grande upstream of the confluence with Manadas Creek (Control) in August, 2009. In the lab, clams were rinsed with deionized water to remove any particles from the shell. They were housed in aquariums with filtered river water to depurate and then put in cages out in the field. At each of the sampling sites, clams were placed in stainless steel wire mesh cages (15×15×17 cm) with gravel and rock substrate suitable for clam survival. Each of the mesh cages was stocked with about 150 *C. fluminea*. Cages were placed at three locations, one upstream of the antimony smelter and processing plant (Site 1, I-35), a second just downstream from the antimony smelter plant (Site 2, Mines Road) and a third farther downstream from the plant (Site 3, Lower Manadas Creek). The lower Manadas Creek cage was lost because of flooding and was later replaced with a new cage and clams. Cages were initially placed in September, 2009. The Site 3 cage was replaced in November, 2009. The cages were checked weekly to ensure that they were still in place and underwater. They were also retrieved monthly and checked on the field for clam survival, after which they were returned to the creek. The cages were left in the creek for 6 months and then retrieved in February, 2010. About 15 clams were taken randomly from all the cages, cleaned, air-dried and measured for their length (24-36 mm) and total weight (6.3-10.4 g). All the clams were cleaned with deionized water and depurated in deionized water for 24 hrs after which they were held in sterile plastic bags and stored in a -80°C freezer until analysis. The length was measured to the nearest 1 mm with a Vernier caliper and mass to the nearest 0.1 g accuracy using an electronic balance.

Water: Water samples were collected following TCEQ recommended water sampling procedures (TCEQ 2003)[15]. Water samples were collected in plastic bottles that were triply rinsed with Milli-Q water after being pre-soaked for at least 24 hrs in Triton-X 100 wash solution (5% HNO_3 and 0.1% Triton®X-100) before use. At the collection site, the collection bottle was triply rinsed with the site water and then filled with water. Water samples were acidified immediately with HNO_3

to preserve them, labeled appropriately and transported to the laboratory for analysis. Three samples were collected at each site. Blank samples comprising Milli-Q water acidified with nitric acid were treated in the same way.

Chemicals and instrumentation: Ultrapure water (Millipore, United States) was used to prepare the standards, blanks and dilution of acid-digested samples. Nitric acid (HNO_3, 70%), Perchloric acid ($HClO_4$, 70%) and Hydrofluoric acid (HF, 51%) were purchased from Sigma-Aldrich (St. Louis, Missouri, United States). Standard reference material (SRM) 1566b oyster tissue and 1643f -trace elements in water were purchased from the National Institute of Science and Technology, Gaithersburg, Maryland, United States. Standards were prepared for instrument calibration by serial dilution of analytical grade stock solution (100 mg L^{-1}) purchased from Millipore, Germany. A calibration curve method was used to quantify the heavy metal concentrations. Acid digestion of trace metals in clams and water was done with a CEM MARS 6 Microwave Digestion System (CEM Corporation, Matthews, North Carolina, United States). Trace metal analysis was done with an Agilent ICP-720 Inductively coupled –plasma-optical emission spectrometer (Agilent Technologies, Santa Clara, California, United States).

Metal analysis

Water: Preserved water samples were analyzed using EPA standard operating procedure for metal analysis in waters[15]. Briefly, the water samples were centrifuged and filtered through a 0.45 μm polycarbonate filter and analyzed by Inductively Coupled-Plasma-Optical Emission Spectrometer (ICP-OES) for metal concentrations against a standard calibration curve. Replicate samples, field blanks and laboratory spikes were treated in the same way.

Fig. 1: *Corbicula fluminea*

Corbicula fluminea: The clams were taken from the freezer and allowed to thaw. From each sampling site, 5-6 clams were taken, rinsed with Milli-Q water and the soft tissue was taken out and placed into a precleaned crucible to create a pooled sample (ten pooled samples were created at each sampling site). The pooled sample was dried in an oven at 80°C for 24 hrs and grinded into a fine powder to evenly mix the samples before digestion. Approximately 200 mg of dried powder sample was weighed into a digestion liner and digested using 7 mL of HNO_3 and 3 mL HF in a CEM MARS 6 microwave digester for 30 min following EPA method 3051A. The digested tissue samples were then diluted to 100 mL with deionized water and filtered through a 0.45 μm polycarbonate filter and analyzed by ICP-OES[17]. Original samples collected from the Rio Grande that were not placed in the creek were treated the same way as the control.

Quality control: The accuracy of the instrument was checked by triplicate analysis of the same samples. Method blanks, spike recovery and standard reference material (SRM) analysis were also employed. Certified SRM 1566b oyster tissue and 1643f water were analyzed in the same way as the clams and water respectively to check the accuracy of the analysis. In addition, a standard and a blank sample were run after every ten samples to check instrumental drift. Reference standards and blanks were employed at the beginning and the end of each sequence. The analytical values were within the range of certified values and the recoveries of the metals across the sampling sites varied from a low of 90% to a high of 105%.

Spiked recovery analysis was done by spiking samples with standard concentrations of the metals. The spiked samples were then digested as the other samples and the rate of recovery was calculated by using the equation[18]:

$$Recovery\ (\%) = \frac{C_{spiked\ sample} - C_{unspiked\ sample}}{C_{added}} \times 100$$

Where:
$C_{spiked\ sample}$ = Concentration of spiked sample determined from analysis
$C_{unspiked\ sample}$ = Average concentration of metal in a pooled sample
C_{added} = Concentration of known standard added

The spiked recoveries obtained (85-110%) indicated that there were minimal losses through volatilization using the digestion cocktail. Clam samples taken directly from the collection site in the Rio Grande were analyzed as controls and were found to be below detection limits.

Statistical analysis: Significant differences (p<0.05) of metal concentrations in water and *Corbicula fluminea* at the different sampling sites were defined by one-way ANOVA at a 95% confidence interval using SPSS 24.0 (SPSS Science, Chicago, Illinois, USA). The values were represented as Mean±Standard error (SE). The level of significance was set at p<0.05.

RESULTS AND DISCUSSION

Trace metal concentrations in water: Trace metal concentrations in water at each monitoring site were recorded in Table 1.

Dissolved antimony concentrations measured in Sites 1, 2 and 3 were 22.80, 22.70 and 3.64 μg L^{-1}, respectively and were all above typical antimony concentrations in natural unpolluted water bodies which is 1 μg L^{-1} [19]. Dissolved antimony concentrations in Sites 1 and 2 were not statistically different from each other but were different from Site 3. Average dissolved antimony concentration of Site 3 which is downstream of the decommissioned smelter plant (3.64 μg L^{-1}) below the maximum contaminant level (MCL) of Sb (6 μg L^{-1})[20]. Dissolved antimony concentrations measured at Site 2 which is near the plant (22.80 μg L^{-1}) can be explained by the runoff from the containment ponds at the decommissioned plant. However, sampling Site 1, which is upstream of the plant had an extremely high antimony concentration (22.71 μg L^{-1}), well above the MCL. This is unusual considering that it is upstream from the decommissioned plant. This can be attributed to the widespread atmospheric deposition of Sb from the metals during the purification of antimony which is done under controlled volatilization[21]. This process leads to the release of dust which contains Sb and co-metalloids like as, Pb, Cu and Fe. The higher-than-expected Sb in Site 1 can also be attributed to industrial discharge/runoff from industries around the location like the Ready-mix concrete company nearby. Amiri *et al.*[22] in a case study found evidence of heavy metal pollution on topsoil of surrounding areas and parking lots of cement-based factories. Runoff from this ready-mix factory is a probable source of heavy metal pollution in the creek.

Average dissolved arsenic concentrations determined at Sites 1, 2 and 3 were 31.17, 6.31 and 47.99 μg L^{-1}, respectively. These were all statistically different from each other. Arsenic toxicity is a health concern and is classified as a human carcinogen Class A, hence regulated in drinking water[23]. Site 2, which is downstream of the decommissioned smelter plant and closest to the plant, surprisingly recorded

the lowest concentration of as in the creek (2.75 µg L^{-1} with an average of 6.31 µg L^{-1}) out of the three sampling sites. This is below the MCL for arsenic (10 µg L^{-1}) and comparable to as concentrations measured during prior studies in the Manadas Creek (6.08 µg L^{-1})[15]. However, arsenic concentrations measured at Site 1 and Site 3 were all about 3 to 5-fold times the MCL. Site 3 is further downstream of the smelter plant, so runoff from the retention ponds at the site in the incidence of heavy rainfall is most likely the cause of the heavy arsenic concentrations observed. The aerial transport of as from the smelter plant during purification could also account for the high As concentrations measured in the water. The higher-than-usual average arsenic concentration (31.17 µg L^{-1}) recorded at Site 1 which is upstream of the plant could be from atmospheric deposition or activities of the cement ready-mix plant near the sampling site. Overall, the concentrations of as recorded at the three sites sampled were higher than the average arsenic concentration in rivers and streams in the United States which is less than 1 µg L^{-1} [24]. The high concentration of as in Sites 1 and 3 are comparable to rivers considered to be arsenic-polluted (Red River, 39 µg L^{-1}, Missouri River 19 µg L^{-1})[25,26].

Cadmium concentrations at Sites 1 and 2 were not statistically different but were different from Site 3. Average dissolved cadmium concentrations measured in the water sampled at Sites 1, 2 and 3 were 1.18, 1.04 and 2.52 µg L^{-1}, respectively and were all below the MCL which is set at 5 µg L^{-1} for cadmium[20]. Dissolved cadmium in the water samples at the sites analyzed was determined to be of no environmental concern. Cadmium is known to adversely affect the physiology of animals. These adverse effects include cellular apoptosis, nephrotoxicity and osteotoxicity[27-30]. Considering the toxicity of Cd and its ability to bioaccumulate in various organs, the low concentrations observed in the creek are reassuring.

Average dissolved Nickel (Ni) concentrations in Sites 1, 2 and 3 were 13.09, 7.90 and 12.83 µg L^{-1}, respectively. The dissolved Ni concentrations in Sites 1 and 3 were statistically different from that of Site 2. Considering that nickel concentration in Site 2 which is close to the decommissioned plant is lower than the other sites, it can be inferred that activities at the smelter plant did not release Ni into the creek. Air deposition from automobiles and other industrial activities is responsible for the nickel measured in the creek. Nickel concentrations were lower than the EPA MCL (which is set at 100 µg L^{-1})[20]. The nickel concentrations measured in the creek were however slightly higher than median nickel concentrations in surface waters (0.5-6 µg L^{-1}), in drinking water (2-4.3 µg L^{-1}) and in uncontaminated water (0.3 µg L^{-1})[31].

Lead (Pb), another toxic metal, was studied in this experiment. Average dissolved Pb concentrations in Sites 1, 2 and 3 were determined to be 3.30, 53.86 and 10.13 µg L^{-1}, respectively. These values were statistically different from each other. The average lead concentrations in Sites 1 and 3 measured were below the MCL (15 µg L^{-1}). However, the concentration of lead at Site 2 which is close to the decommissioned plant recorded the highest Pb concentration (53.86 µg L^{-1}) which is higher than the MCL but less than the EPA maximum contaminant value for acute exposure waters supporting aquatic life (65 µg L^{-1})[32]. The high Pb concentration recorded in this study can be attributed to the proximity of Site 2 to the decommissioned smelter plant. Lead is ubiquitous and is found mixed up with ores containing antimony, arsenic and bismuth[33,34]. The Pb concentration observed is comparable to high Pb concentrations observed in rivers along regions in the United States where there is lead mining[35]. Lead poisoning affects physiological processes such as inhibition of hemoglobin synthesis, kidney dysfunction and acute and chronic damage to the central nervous system and peripheral nervous systems[36].

The higher-than-usual concentration of certain metals (Sb, As and Pb) observed in water samples of the creek implies the creek can be categorized as heavily polluted in terms of antimony, lead and arsenic. This result is comparable to values obtained from studies on Meadow Creek (As = 41.6 µg L^{-1}, Sb =9.9 µg L^{-1}) which is near the Yellow Pine Mine in Valley County Idaho[37].

Trace metal concentration in the soft tissue of *Corbicula fluminea*: The results of the trace metals under study in *Corbicula fluminea* for all the sampling sites were shown in Table 2.

The mean concentration of as in pooled soft tissues of the clam was 16.20 mg kg^{-1} (Site 1), 10.22 mg kg^{-1} (Site 2) and 10.94 mg kg^{-1} (Site 3). The concentration of as at Sites 2 and 3 was different from Site 1 ($p < 0.05$). The range of as concentrations in *C. fluminea* in this study is higher than that which was observed in San Joaquin River, California 4.0-13.9, and Altamaha River, Georgia to be 3.01-6.52 mg kg^{-1} [38,39]. Bioaccumulation of As in the soft tissue of the clam at all sites did not exceed the maximum allowable limit (MAL) recommended for human consumption by setting the USFDA (86 mg kg^{-1})[40].

Table 1: Dissolved concentrations of trace metals at each study site (Mean±SE) the concentration

	Concentration of water (µg L⁻¹)				
	As	Cd	Ni	Pb	Sb
Control	-	-	-	-	-
Site 1	31.17±2.99[a]	1.18±0.71[a]	13.09±2.48[a]	3.30±1.75[a]	22.80±5.54[a]
Site 2	6.31±2.25[b]	1.04±0.32[a]	7.90±1.69[b]	53.86±9.91[b]	22.70±6.48[a]
Site 3	47.99±9.05[c]	2.52±0.75[b]	12.83±2.46[a]	10.13±2.53[c]	3.64±1.61[b]
USEPA[20]	10.00	5.00	100.00	15.00	6.00

Mean metal concentrations of sites sharing a common letter for a particular metal present no significant differences, p>0.05, Different letters represent a significant difference, p<0.05 and USEPA: Maximum contaminant level set by the US Environmental Protection Agency

Table 2: Concentration of metals in *Corbicula fluminea* at the sites under study

	Heavy metal concentration in *Corbicula* (mg kg⁻¹)				
	As	Cd	Ni	Pb	Sb
Site 1	16.20±3.30[a]	0.93±0.6[a]	3.49±1.24[a]	12.28±2.27[a]	16.43±2.80[a]
Site 2	10.22±2.03[b]	1.12±0.37[a]	6.94±1.34[b]	16.09±2.53[b]	15.56±1.87[a]
Site 3	10.94±1.57[b]	0.98±0.30[a]	4.31±0.95[b]	16.31±2.15[b]	11.48±2.35[b]
USFDA[40]	86.00	4.00	80.00	1.7	-

Mean metal concentrations of sites sharing a common letter for a particular metal present no significant differences, p>0.05, Different letters represent a significant difference, p<0.05 and USFDA: United States Food and Drug Administration Maximum allowable limits

The mean concentrations of Cd accumulated in the soft tissue of *C. fluminea* were 0.93 mg kg⁻¹ (Site 1), 1.12 mg kg⁻¹ (Site 2) and 0.98 mg kg⁻¹ (Site 3). The concentrations at all sampling sites were not different (p<0.05). The range of Cd concentration observed in *C. fluminea* is lower than the range observed in Pa Sak River (1.95 mg kg⁻¹) and Mekong River (1.72 mg kg⁻¹), but comparable to the range of Cd observed in *C. fluminea* in Lopburi River (0.88-mg kg⁻¹)[41]. The Cd concentration in *C. fluminea* at all sites was higher than that observed in Crabtree Creek, North Carolina (0.14-0.28 mg kg⁻¹)[38,39,42,43]. The average concentration of Cd in the clam did not exceed the MAL recommended by the USFDA (4.0 mg kg⁻¹)[40]. Cadmium is not a very mobile metal in aquatic environments therefore the lower cadmium concentration in the clam is as expected. Considering that chronic intake of Cd has been documented to cause health issues like urinary and cardiovascular disorders, skeletal damage and even cancer, the low levels of Cd detected in *C. fulminea* (a biomarker) in this study are good for aquatic organisms in the creek[44-46].

The mean concentrations of lead accumulated in the soft tissue of *C. fluminea* at Sites 2 (16.09 mg kg⁻¹) and Site 3 (16.31 mg kg⁻¹) were different from Site 1 (12.28 mg kg⁻¹). The range of concentrations in *C. fluminea* measured at all sites was higher than Lopburi (3.43 mg kg⁻¹), Mekong River (3.61 mg kg⁻¹) and Pa Sak River (8.55 mg kg⁻¹)[41]. Average concentrations of Pb in the soft tissue of *C. fluminea* at all the sampling sites (14.89 mg kg⁻¹) exceeded the MAL recommended by USFDA (1.7 mg kg⁻¹) by 9-fold[40].

The mean concentrations of Ni accumulated in the soft tissue of *C. fluminea* at the sampling sites were 3.49±1.24 mg kg⁻¹ (Site 1), 6.94±1.34 mg kg⁻¹ (Site 2) and 4.31±0.95 mg kg⁻¹ (Site 3). Nickel concentrations in clam at Sites 2 and 3 were different from Site 1. The concentration ranges of Ni in *C. fluminea* at the sampling sites (3.49-6.94 mg kg⁻¹) in this study were higher than that observed in San Joaquin River, California (0.9-2.0 mg kg⁻¹) but comparable to that observed in Altamaha River, Georgia (1.45-9.23 mg kg⁻¹)[38,39]. The average Ni concentrations in the soft tissue of *C. fluminea* at all sites sampled were lower than the MAL recommended by the USFDA (80 mg kg⁻¹)[40].

The mean Sb concentrations measured in the soft tissue of *C. fluminea* at the sampling sites were Site 1 (16.43±2.80), Site 2 (15.56±1.87) and Site 3 (11.48±2.35). The Sb concentration in *C. fluminea* Sites 1 and 2 is different from Site 3. The range of Sb concentration measured in *C. fluminea* at the sampling sites in this study was higher when compared to that measured in *C. fluminea* in Odiel River, Spain (0.01-0.09 mg kg⁻¹)[47].

Across all the sampling sites, Pb, Sb and concentrations in *C. fluminea* were very high. Ranging from 7.81-20.22 mg kg⁻¹ for Pb, 7.90-18.28 mg kg⁻¹ for Sb and 7.16-20.30 mg kg⁻¹ for As. These values were higher than concentrations measured in the soft tissue of *C. fluminea* in unpolluted surface waters[38,39,43]. Site 2 recorded high metal concentration in the soft tissue of *C. fluminea* because of its proximity to the decommissioned smelter plant. Although the metal concentration in the water was lower, the high metal concentration in the sediment (As 2.1-14.2 mg kg⁻¹, Sb 0.2-470.9 mg kg⁻¹) served as a constant reservoir to be concentrated in the soft tissues of the clam[15]. Thus, metals stored in insoluble forms in the sediment of the creek are bioavailable to maintain the equilibrium of antimony in the dissolved phase and hence the higher bioconcentration.
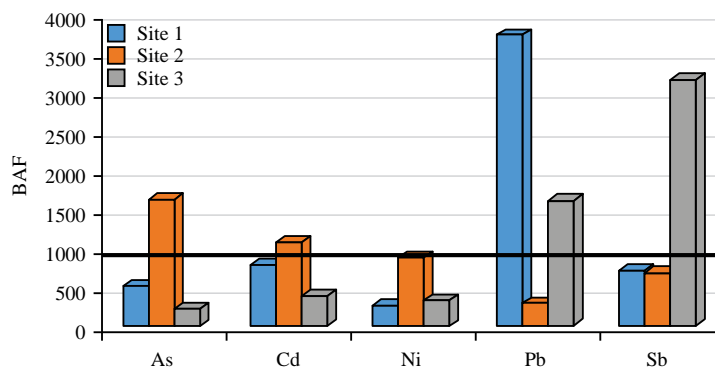
Fig. 2: Bioaccumulation factor of metals in *Corbicula fluminea*

Bioaccumulation factors: The bioaccumulation factor (BAF) was calculated using the equation[48]:

$$BAF = \frac{\text{Concentration of metal in aquatic species}}{\text{Concentration of metal in water}}$$

The BAF values are represented in Fig. 2. Arnot and Gobas[12] established BAF ranges and categorized BAF values having less than 1000 as less probability of accumulation, BAF values between 1000 and 5000 as bioaccumulative and values greater than 5000 as highly bioaccumulative[12,48]. Using this criterion, Ni and Cd at all sites studied will be categorized as having less probability to bioaccumulate in *C. fluminea* found in Manadas Creek. Arsenic did not bioaccumulate in Sites 1 and 3 and was moderately bioaccumulated in Sites 2. Antimony did not bioaccumulate at Sites 1 and 2 but bioaccumulated at Site 3. The present study shows that all metals understudy bioaccumulated in *C. fluminea* to some extent (Fig. 2). The order within which the metals studied were concentrated in the soft tissues of the clam as presented in Fig. 2 was Ni<Cd<As<Sb<Pb. This order was comparable to that observed by Shoults-Wilson *et al.*[39], who found that as accumulated the most out of the metals studied whereas Ni had the least accumulation in *C. fluminea*.

There was a correlation between the aqueous concentration of Ni (R^2 =0.9662) and Sb (R^2 = 0.974) and the concentration of metal in *C. fluminea*. This was agreed with other field and laboratory research that has shown agreement between water and tissue concentrations of mussels[49-54]. Such studies have demonstrated that dietary pathways are responsible for metal uptake. For the other metals studied (As, Pb and Cd), there was no correlation, implying the existence of alternative sources or competing pathways as found in other studies[50]. Bioaccumulation of metals in biological tissues in an aquatic environment is very complex. It is influenced by factors such as concentration, pH, water

temperature, hardness, exposure time and feeding habits[50,55]. Exposure of *C. fluminea* to metals in this study showed no detrimental effects on the overall health or mortality of the clam as observed in other studies. The impact of time of exposure on the accumulation of the metals under study in the tissue of *C. fluminea* is not clearly defined in the results observed. This has been observed in other studies. Field studies by Bonnail *et al.*[42] over a period of 21 days found accumulation of the metals under study (As, Cd, Cr, Co, Fe, Ni, Mn, Pb, Sb and Zn) in *C. fluminea* except for Cd and As. Peltier *et al.*[55] observed a decline in Cd and Ni accumulation in *C. fluminea* after 28 days. They also found a peaked as concentration in *C. fluminea* after 28 days and then a decline afterward. In this current study, the long exposure of *C. fluminea* (minimum of four months) to contaminated sediments in Manadas Creek could be a contributing factor to the elevated metal concentrations observed in the soft tissue of *C. fluminea*.

The preliminary data obtained in this study addresses some questions in understanding the bioavailability of heavy metals in Manadas Creek. Bioaccumulation of metals in fauna is tissue and species-dependent rather than bioavailable concentration of the metal[51]. *Corbicula fluminea* which was used in this experiment is a filter feeder, feeding on algae, bacteria and fine detritus. The length of time (4-6 months) that these clams were exposed to heavy metals in the creek could also explain the high bioconcentration factors recorded. Clams in general have been successful biomarkers of highly contaminated waters[13]. *Corbicula fluminea* has demonstrated that it can be used as a biomarker to study the extent of heavy metal pollution in Manadas Creek. The dissolved concentration of metals in the creek is not a good indicator to predict the bioaccumulation of metals into the tissue of *C. fluminea* at any of the sites sampled. The result of this work has generated the need to investigate other sources of metal pollution apart from the decommissioned plant site. This is

because areas in the creek that are upstream from the point source demonstrated unusually high bioaccumulation of heavy metals. This points to a possible air deposition and runoff of heavy metals from industries around such as the ready-mix cement factory into the creek.

CONCLUSION

Results obtained from this study show that Manadas Creek is heavily polluted with heavy metals, that even at low dissolved metal concentrations, bioaccumulation can occur because metals trapped in the sediments are bioavailable. Since the creek is a tributary to the Rio Grande, the elevated metal concentrations should be concerning. Although prior studies on geochemical conditions point to the non-bioavailability of these metals, the constant equilibrium between metal concentration in the water and sediment and extended time of exposure can cause these metals to be bioaccumulated in their natural ecosystem. The creek should be thus designated for 'catch and release fishing until there is remediation and the metal levels are known to be safe. The proximity of the creek to the decommissioned antimony plant, coupled with changes in hydrogeochemical conditions, such as changes in flow rate and disturbance of sediments, makes it a suitable ecosystem to study the geochemical behavior of Sb. The bioaccumulation observed could be due to the length of exposure of the species to metals, changes in geochemical conditions during the experiment or the general metal-tolerant ability of *Corbicula fluminea*.

SIGNIFICANCE STATEMENT

An antimony smelter plant that was situated on the banks of the Manadas Creek in Laredo, Texas has exposed the water and sediment of the creek to heavy metal pollution from antimony and other heavy metals. This study evaluates the extent of metal pollution in a semi-arid creek in Laredo, Texas using *Corbicula fluminea* as a biomarker. Findings from this work showed that even at low dissolved metal concentrations, bioaccumulation can occur because metals trapped in the sediments are bioavailable. Results from this work have generated the need to investigate other sources of metal pollution apart from the decommissioned plant site. This is because areas in the creek that are upstream from the point source demonstrated unusually high bioaccumulation of metals.

ACKNOWLEDGMENT

REFERENCES

1. Xing, W., H. Liu, T. Banet, H. Wang, J.A. Ippolito and L. Li, 2020. Cadmium, copper, lead and zinc accumulation in wild plant species near a lead smelter. Ecotoxicol. Environ. Saf., Vol. 198. 10.1016/j.ecoenv.2020.110683.

2. Adnan, M., B. Xiao, P. Xiao, P. Zhao, R. Li and S. Bibi, 2022. Research progress on heavy metals pollution in the soil of smelting sites in China. Toxics, Vol. 10. 10.3390/toxics10050231.

3. Ahmed, A.S.S., M. Rahman, S. Sultana, S.M.O.F. Babu and M.S.I. Sarker, 2019. Bioaccumulation and heavy metal concentration in tissues of some commercial fishes from the Meghna River Estuary in Bangladesh and human health implications. Mar. Pollut. Bull., 145: 436-447.

4. Achary, M.S., K.K. Satpathy, S. Panigrahi, A.K. Mohanty and R.K. Padhi *et al.*, 2017. Concentration of heavy metals in the food chain components of the nearshore coastal waters of Kalpakkam, Southeast Coast of India. Food Control, 72: 232-243.

5. Briffa, J., E. Sinagra and R. Blundell, 2020. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon, Vol. 6. 10.1016/j.heliyon.2020.e04691.

6. Kocadal, K., F.B. Alkas, D. Battal and S. Saygi, 2020. Cellular pathologies and genotoxic effects arising secondary to heavy metal exposure: A review. Hum. Exp. Toxicol., 39: 3-13.

7. Mitra, S., A.J. Chakraborty, A.M. Tareq, Talha Bin Emran and F. Nainu *et al.*, 2022. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. J. King Saud Univ. Sci., Vol. 34. 10.1016/j.jksus.2022.101865.

8. Perumalsamy, N. and K. Arumugam, 2013. Enzymes activity in fish exposed to heavy metals and the electro-plating effluent at sub-lethal concentrations. Water Qual. Exposure Health, 5: 93-101.

9. Ustaoğlu, F., S. Kükrer, B. Taş and H. Topaldemir, 2022. Evaluation of metal accumulation in Terme River sediments using ecological indices and a bioindicator species. Environ. Sci. Pollut. Res., 29: 47399-47415.

10. Buffle, J., K.J. Wilkinson and H.P. van Leeuwen, 2009. Chemodynamics and bioavailability in natural waters. Environ. Sci. Technol., 43: 7170-7174.

11. Zeng, G., K. Yu, J. Li, W. Wu, H. Qiao, Y. Duan and C. Liu, 2020. Heavy metal accumulation and release risks in sediments from groundwater-river water interaction zones in a contaminated river under restoration. ACS Earth Space Chem., 4: 2391-2402.

12. Arnot, J.A. and F.A.P.C. Gobas, 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev., 14: 257-297.

13. Rainbow, P.S. and D.J.H. Phillips, 1993. Cosmopolitan biomonitors of trace metals. Mar. Pollut. Bull., 26: 593-601.

14. McNeely, J.H. and D.J. Kleiner, 1952. Antimony Smelter. TSHA, Austin, Texas.

15. Baeza, M., J. Ren, S. Krishnamurthy and T.C. Vaughan, 2010. Spatial distribution of antimony and arsenic levels in Manadas Creek, an urban tributary of the rio grande in Laredo, Texas. Arch. Environ. Contam. Toxicol., 58: 299-314.

16. Zhou, Y., B. Ren, A.S. Hursthouse and S. Zhou, 2019. Antimony ore tailings: Heavy metals, chemical speciation, and leaching characteristics. Pol. J. Environ. Stud., 28: 485-495.

17. Tamasi, G. and R. Cini, 2004. Heavy metals in drinking waters from Mount Amiata (Tuscany, Italy). Possible risks from arsenic for public health in the Provience of Siena. Sci. Total Environ., 327: 41-51.

18. Ilieva, D., A. Surleva, M. Murariu, G. Drochioiu and M.M. Al Bakri Abdullah, 2018. Evaluation of ICP-OES method for heavy metal and metalloids determination in sterile dump material. Solid State Phenom., 273: 159-166.

19. Filella, M., N. Belzile and Y.W. Chen, 2002. Antimony in the environment: A review focused on natural waters. Earth Sci. Rev., 57: 125-176.

20. Wołowicz, A. and M. Wawrzkiewicz, 2021. Screening of ion exchange resins for hazardous Ni(II) removal from aqueous solutions: Kinetic and equilibrium batch adsorption method. Processes, Vol. 9. 10.3390/pr9020285.

21. Park, S.C., M.I. Boyanov, K.M. Kemner, E.J. O'Loughlin and M.J. Kwon, 2021. Distribution and speciation of Sb and toxic metal(loid)s near an antimony refinery and their effects on indigenous microorganisms. J. Hazard. Mater., Vol. 403. 10.1016/j.jhazmat.2020.123625.

22. Amiri, H., E. Daneshvar, S. Azadi and S. Azadi, 2022. Contamination level and risk assessment of heavy metals in the topsoil around cement factory: A case study. Environ. Eng. Res., Vol. 27. 10.4491/eer.2021.313.

23. Zhou, Q. and S. Xi, 2018. A review on arsenic carcinogenesis: Epidemiology, metabolism, genotoxicity and epigenetic changes. Regul. Toxicol. Pharmacol., 99: 78-88.

24. Lettenmaier, D.P., E.R. Hooper, C. Wagoner and K.B. Faris, 1991. Trends in stream quality in the continental United States, 1978-1987. Water Resour. Res., 27: 327-339.

25. Berg, M., C. Stengel, T.K. Pham, H.V. Pham and M.L. Sampson *et al.*, 2007. Magnitude of arsenic pollution in the Mekong and Red River Deltas: Cambodia and Vietnam. Sci. Total Environ., 372: 413-425.

26. Nimick, D.A., J.N. Moore, C.E. Dalby and M.W. Savka, 1998. The fate of geothermal arsenic in the Madison and Missouri Rivers, Montana and Wyoming. Water Resour. Res., 34: 3051-3067.

27. Bhattacharyya, M.H., 2009. Cadmium osteotoxicity in experimental animals: Mechanisms and relationship to human exposures. Toxicol. Appl. Pharmacol., 238: 258-265.

28. Watkin, R.D., T. Nawrot, R.J. Potts and B.A. Hart, 2003. Mechanisms regulating the cadmium-mediated suppression of Sp1 transcription factor activity in alveolar epithelial cells. Toxicology, 184: 157-178.

29. Iwata, K., H. Saito, M. Moriyama and A. Nakano, 1991. Association between renal tubular dysfunction and mortality among residents in a cadmium-polluted area, Nagasaki, Japan. Tohoku J. Exp. Med., 164: 93-102.

30. Iwata, K., H. Saito, M. Moriyama and A. Nakano, 2008. Follow up study of renal tubular dysfunction and mortality in residents of an area polluted with cadmium. Occup. Environ. Med., 49: 736-737.

31. Schrenk, D., M. Bignami, L. Bodin, J.K. Chipman and J. del Mazo *et al.*, 2020. Update of the risk assessment of nickel in food and drinking water. Eur. Food Saf. Authority, Vol. 18. 10.2903/j.efsa.2020.6268.

32. Sharma, R.K. and S. Gulati, 2014. Water Quality Issues and Solutions in India. In: Comprehensive Water Quality and Purification, Ahuja, S. (Ed.), Elsevier, Amsterdam, Netherlands, ISBN: 978-0-12-382183-6, pp: 21-39.

33. Ren, B., Y. Zhou, A.S. Hursthouse and R. Deng, 2017. Research on the characteristics and mechanism of the cumulative release of antimony from an antimony smelting slag stacking area under rainfall leaching. J. Anal. Methods Chem., Vol. 2017. 10.1155/2017/7206876.

34. Lager, T. and K.S.E. Forssberg, 1989. Beneficiation characteristics of antimony minerals a review-Part 1. Miner. Eng., 2: 321-336.

35. Beneš, P., M. Čejchanová and B. Havlík, 1985. Migration and speciation of lead in a river system heavily polluted from a smelter. Water Res., 19: 1-6.

36. Mohammed, A.S., A. Kapri and R. Goel, 2011. Heavy Metal Pollution: Source, Impact, and Remedies. In: Environmental Pollution, Trevors, J.T. (Eds.), Springer, Dordrecht, Netherlands, ISBN: 978-94-007-1913-2, pp: 1-28.

37. Dovick, M.A., T.R. Kulp, R.S. Arkle and D.S. Pilliod, 2015. Bioaccumulation trends of arsenic and antimony in a freshwater ecosystem affected by mine drainage. Environ. Chem., 13: 149-159.

38. Leland, H.V. and B.C. Scudder, 1990. Trace elements in *Corbicula fluminea* from the San Joaquin River, California. Sci. Total Environ., 97-98: 641-672.

39. Shoults-Wilson, W.A., J.M. Unrine, J. Rickard and M.C. Black, 2010. Comparison of metal concentrations in *Corbicula fluminea* and *Elliptio hopetonensis* in the Altamaha River system, Georgia, USA. Environ. Toxicol. Chem., 29: 2026-2033.

40. Qin, L.Y., R.C. Zhang, Y.D. Liang, L.C. Wu and Y.J. Zhang *et al.*, 2021. Concentrations and health risks of heavy metals in five major marketed marine bivalves from three coastal cities in Guangxi, China. Ecotoxicol. Environ. Saf., Vol. 223. 10.1016/j.ecoenv.2021.112562.

41. Sow, A.Y., K.H. Dee, S.W. Lee, A.A.L. Eh-Rak, 2019. An assessment of heavy metals toxicity in Asian clam, *Corbicula fluminea*, from Mekong River, Pa Sak River, and Lopburi River, Thailand. Sci. World J., Vol. 2019. 10.1155/2019/1615298.

42. Bonnail, E., A.M. Sarmiento, T.A. DelValls, J.M. Nieto and I. Riba, 2016. Assessment of metal contamination, bioavailability, toxicity and bioaccumulation in extreme metallic environments (Iberian Pyrite Belt) using *Corbicula fluminea*. Sci. Total Environ., 544: 1031-1044.

43. Lewbart, G.A., L.S. Christian, C.A. Harms and A.J. van Wettere, 2010. A comparison of heavy metal concentrations and health assessment in Asian clams *Corbicula fluminea* from Florida and North Carolina. J. Aquat. Anim. Health, 22: 73-77.

44. Kumar, S. and A. Sharma, 2019. Cadmium toxicity: Effects on human reproduction and fertility. Rev. Environ. Health, 34: 327-338.

45. Fatima, G., A.M. Raza, N. Hadi, N. Nigam and A.A. Mahdi, 2019. Cadmium in human diseases: It's more than just a mere metal. Indian J. Clin. Biochem., 34: 371-378.

46. Genchi, G., M.S. Sinicropi, G. Lauria, A. Carocci and A. Catalano, 2020. The effects of cadmium toxicity. Int. J. Environ. Res. Public Health, Vol. 17. 10.3390/ijerph17113782.

47. Bonnail, E., F. Macías and V. Osta, 2019. Ecological improvement assessment of a passive remediation technology for acid mine drainage: Water quality biomonitoring using bivalves. Chemosphere, 219: 695-703.

48. Abu Noman, M., W. Feng, G. Zhu, M.B. Hossain, Y. Chen, H. Zhang and J. Sun, 2022. Bioaccumulation and potential human health risks of metals in commercially important fishes and shellfishes from Hangzhou Bay, China. Sci. Rep., Vol. 12. 10.1038/s41598-022-08471-y.

49. Li, Z., X. He and C. Feng, 2023. A review of freshwater benthic clams (*Corbicula fluminea*): Accumulation capacity, underlying physiological mechanisms and environmental applications. Sci. Total Environ., Vol. 857. 10.1016/j.scitotenv.2022.159431.

50. Luoma, S.N. and P.S. Rainbow, 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol., 39: 1921-1931.

51. Angelo, R.T., M.S. Cringan, D.L. Chamberlain, A.J. Stahl, S.G. Haslouer and C.A. Goodrich, 2007. Residual effects of lead and zinc mining on freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). Sci. Total Environ., 384: 467-496.

52. Luoma, S.N., R. Dagovitz and E. Axtmann, 1990. Temporally intensive study of trace metals in sediments and bivalves from a large river-estuarine system: Suisun Bay/Delta in San Francisco Bay. Sci. Total Environ., 97-98: 685-712.

53. Griscom, S.B., N.S. Fisher and S.N. Luoma, 2002. Kinetic modeling of Ag, Cd and Co bioaccumulation in the clam *Macoma balthica*: Quantifying dietary and dissolved sources. Mar. Ecol. Prog. Ser., 240: 127-141.

54. Deram, A., F.O. Denayer, D. Petit and C. van Haluwyn, 2006. Seasonal variations of cadmium and zinc in *Arrhenatherum elatius*, a perennial grass species from highly contaminated soils. Environ. Pollut., 140: 62-70.

55. Peltier, G.L., M.S. Wright, W.A. Hopkins and J.L. Meyer, 2009. Accumulation of trace elements and growth responses in *Corbicula uminea* downstream of a coal- red power plant. Ecotoxicol. Environ. Safety, 72: 1384-1391.