ACCUMULATION OF METALS AND METALLOIDS IN SEDIMENT AND AQUATIC ORGANISMS OF ECUADORIAN MANGROVE FORESTS AND IMPLICATIONS FOR HUMAN HEALTH

by

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We respectfully acknowledge the University of Arizona is on the land and territories of Indigenous peoples. Today, Arizona is home to 22 federally recognized tribes, with Tucson being home to the O'odham and the Yaqui. Committed to diversity and inclusion, the University strives to build sustainable relationships with sovereign Native Nations and Indigenous communities through education offerings, partnerships, and community service.

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Abstract

This study aims to determine the levels of toxic metals and metalloids and their ecotoxicological implications in two protected mangrove forests in Guayaquil, Ecuador: the Reserva de Producción Faunística Manglares El Salado (RPFMS) and the Refugio de Vida Silvestre Manglares El Morro (REVISMEM). Anthropogenic activities such as agriculture, urban development, and aquaculture introduce contaminants to these coastal forests, posing a risk to the ecosystems. We utilized inductively coupled plasma-mass spectrometry (ICP-MS) to quantify toxic elements.

In Chapter 1, we examined concentrations of toxic metal(loid)s in sediment, catfish (*Bagre pinnimaculatus*), Peruvian mojarra (*Diapterus peruvianus*), and mussels (*Mytella guyanensis*, *Mytella strigata*, and *Mytella trautwineana*). In addition, we conducted dietary intake surveys with residents of Puerto Hondo in RPFMS to assess potential exposure through a quantitative health risk assessment. Our analysis revealed that concentrations of Ag, As, Cd, Cr, and Pb in sediment samples exceeded the US National Oceanic and Atmospheric Administration (NOAA) "effects range low" (ERL) guidelines, while Cu, Hg, Ni, and Zn exceeded their "effects range median" (ERM) guidelines. ERL and ERM represent the concentrations above which adverse effects may begin to occur and at which effects are frequently observed, respectively. In fish samples, iAs, Cd, MeHg and Se levels surpassed the US Environmental Protection Agency (EPA) screening levels for unlimited fish consumption with higher concentrations observed in catfish for most samples. For mussel samples (*M. trautwineana*), only Zn levels exceeded the guidelines of the Food and Agriculture Administration of the United Nations (FAO). The estimated daily intake (EDI) values for inorganic As (iAs), hexavalent chromium (Cr(VI)), methylmercury (MeHg) and Pb were higher in Peruvian mojarra compared to catfish. The target hazard quotient (THQ) and the hazard index (HI) did not exceed the threshold value of 1 in both fish species and mussels. Cancer Risk (CR) values indicated a potential carcinogenic risk from iAs and Cr(VI) in Peruvian mojarra, and from iAs in catfish and mussels. For Pb, the margin of exposure (MOE) values were in the safety threshold area, concluding that there are no potential exposure risks for both children and adults.

In Chapter 2, we compared concentrations of toxic metal(loid)s in sediment and mussels (*M. strigata*) between RPFMS and REVISMEM. We found that As, Cr and Cu exceeded the ERL

and Ni exceeded the ERM for sediment in both reserves, with higher concentrations in RPFMS. Most metal(loid) showed significant differences between “within city limits” (RPFMS) and “city border area” (RPFMS), “near the city” (REVISMEM) and “far from the city” (REVISMEM). For mussels, all the elemental concentrations were below the FAO threshold values for *M. strigata*. With the exception of As, Cd, and Co, all toxic elements showed higher concentrations in “near the city” (REVISMEM) compared to the “city border area” (RPFMS).

This project aims to enhance understanding of the ecological effects of pollutants on these mangrove ecosystems, providing a scientific basis to prioritize conservation efforts and inform public policy actions. Collectively, our results show that RPFMS has more polluted sites than REVISMEM, with higher levels of toxic metal(loid)s in sediments, fish, and mussels. iAs in both mussels and fish and Cr(VI) only in Peruvian mojarra present carcinogenic risks. Pb in fish and mussel species do not pose a health risk. Catfish and Peruvian mojarra exhibit a HI lower than 1, indicating that non-carcinogenic health risks are unlikely for the Puerto Hondo community.

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List of Abbreviations

ADAFs: Age-dependent adjustment factors

ALEC: Arizona Laboratory for Emerging Contaminants

ANOVA: One-way analysis of variance

AT: Average exposure

ATSDR: Agency for Toxic Substances and Disease Registry

BMD: Benchmark-dose

BMDL: Benchmark-dose lower bound

BW: Body weight

C: Metal(loid) concentration ww

Cr (IV): Hexavalent chromium

CLIRSEN: Center for Integrated Natural Resources Remote Sensing of Ecuador

CR: Cancer risk

CSF: Cancer slope factor

DCM: Drying-cooking method

DO: Dissolved oxygen

dw: Dry weight

EDI: Estimated daily intake

ED: Exposure duration

EF: Exposure frequency

EFSA: European Food Safety Authority

EPA: United States Environmental Protection Agency

ERM: Effects range median

ERL: Effects range low

ESPOL: Escuela Politécnica Superior del Litoral

FAO: Food and Agriculture Administration of the United Nations

HI: Hazard index

HSD: Honestly Significant Difference (HSD)

ICP-MS: Inductively coupled plasma-mass spectrometry

IHD: Ischemic heart disease

IQ: Intelligence quotient

IR: Ingestion rate

iAs: Inorganic arsenic

IRIS: Integrated Risk Information System

LOD: Limit of detection

MAE: Ministerio del Ambiente, Agua y Transición Ecológica

MeHg: methylmercury

MOE: Margin of exposure

MWU: Mann-Whitney U

NDCM: Non-drying-cooking method

NRC-CNRC: National Research Council of Canada

PCA: Principal component analysis

PC: Principal component

REVISMEM: Refugio de Vida Silvestre Manglares El Morro

RfD: Reference dose

RPFMS: Reserva de Producción Faunística Manglares El Salado

THQ: Target hazard quotient

TULSMA: Texto Unificado de Legislación Secundaria de Medio Ambiente

USD: United States dollars

ww: Wet weight

Introduction

Mangrove forests, which line many tropical coasts, provide multiple benefits to people and the environment. In 2020, mangroves covered 147,359 km^2 globally, with 51% of their extant distribution in the Asian Pacific, 29% in the Americas, and 20% in Africa[1]. Mangroves sequester carbon dioxide from the atmosphere[2], promote coastal productivity and fisheries[3], harbor high biodiversity[4], serve as barriers against natural disasters[5], and act as filters, trapping contaminants and pollutants[6]. Mangrove forests are among the most productive ecosystems, offering renewable resources and essential goods to coastal communities, many of which rely on the organisms found within these habitats[4,7].

Figure 1. Ecuadorian mangrove forests in 2022[8] marked in dark green.

Ecuador, recognized as one of the world's megadiverse countries due to its high species concentration per hectare and endemism[9], is home to ~1572 km² of mangrove forests in mainland Ecuador (Fig. 1), with ~749 km² (48%) designated as protected areas[10]. The mangrove tree species are *Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia germinans, Conocarpus erectus*, *Mora oleifera*, *Pelliciera rhizophorae*, *Pterocarpus officinalis* and *Talipariti tiliaceum*, with *R. mangle* comprising most of the total cover in Ecuador[11]. Despite their many benefits, mangrove forests are disappearing globally at a rate of 1–2% each year[7]. This loss not only reduces the extent of mangrove forests, but also leads to significant carbon emissions. Mangrove deforestation accounts for about 20% of global emissions from forest loss, resulting in economic damages of ~USD $6–42 billion annually due to dimunition of ecosystem services[1]. Overall, their loss is most prevalent in emerging economies, particularly in Southeast Asia and Central and South America[4]. From 1998 to 2010, Ecuadorian mangrove cover decreased by 4.56% due to anthropogenic activities[12].

In Ecuador, the primary drivers of mangrove forest degradation include agriculture, aquaculture, and urban expansion[13]. The construction of shrimp ponds has positioned the country as one of the world’s largest shrimp exporters[14]. Although this development has contributed to national economic growth, it has also jeopardized mangroves and introduced both organic and inorganic contaminants[13]. Mangrove forests have been replaced with farmland[15], and have progressively diminished due to urbanization adjacent to cities such as Guayaquil, Machala, and Esmeraldas[16].

Since the 1970s, few policy regulations have been introduced by the Ecuadorian government to protect mangrove forests[17]. The shortage of legislative measures and the lack of effective management have allowed multiple industry sectors to contaminate these ecosystems[18]. Effluents, chemicals, and antibiotics from the shrimp industry often end up in mangrove areas[19]. The construction of shrimp ponds negatively affects mangroves by altering their hydrology, leading to excessive sedimentation[20,21], increased salinity[22], disease outbreaks[23], and the introduction of non-native species[19,21,24]. In addition, the receipt of agricultural runoff results in the accumulation of pesticide residues in mangrove waters[25], affecting both wildlife and humans, and causing adverse ecological impacts[26]. Urbanization[27] and rapid industrial development[28] also result in chemical discharges into mangrove forests, increasing the burden of pollution[29,30].

Chapter 1: Ecotoxicology in RPFMS

1.1 Introduction

Mangrove forests are among the most productive ecosystems, found in tropical and subtropical regions, and provide a vast range of environmental benefits[31]. These biologically diverse coastal forests contribute to carbon sequestration and serve as nursery habitat for numerous species[32]. As climate change accelerates and sea levels rise, mangroves help buffer coastal areas from natural disasters (e.g., floods, storms)[33,34]. Anthropogenic activities driven by the increasing demand for coastal resources are introducing contaminants into mangrove forests, disrupting their biological balance[35].

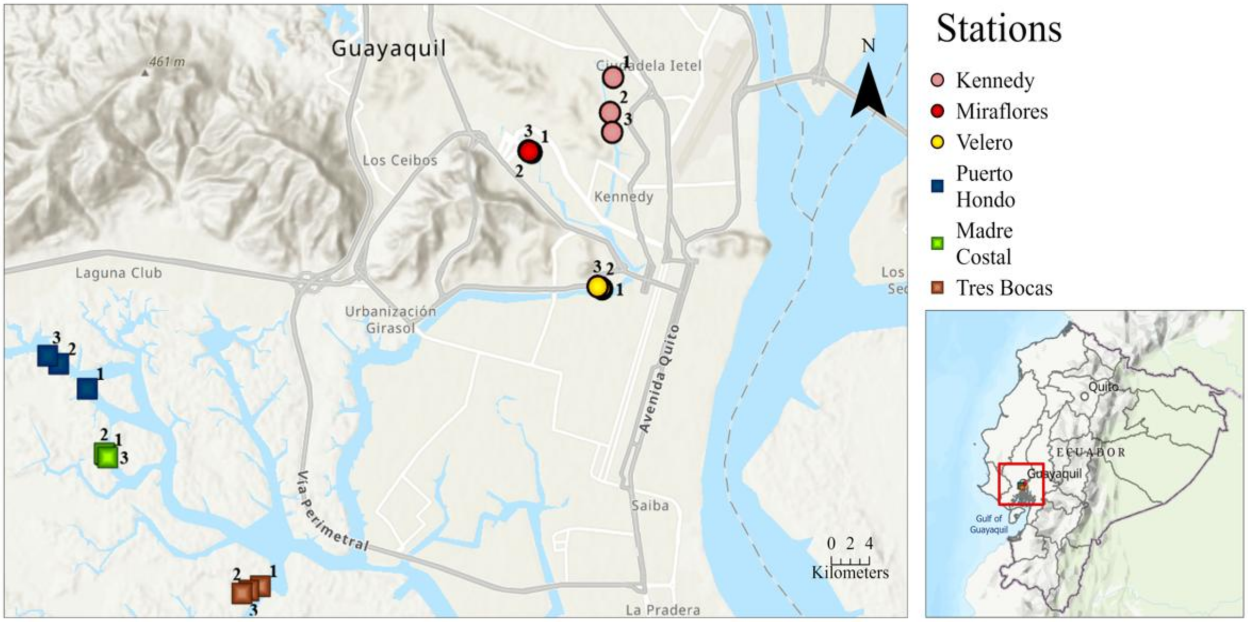


Figure 1.1 Guayaquil and locations of sampling sites: Kennedy, Miraflores, Velero ("within city limits") and Puerto Hondo, Madre Costal and Tres Bocas ("city border area"). Three substations were sampled in each station.

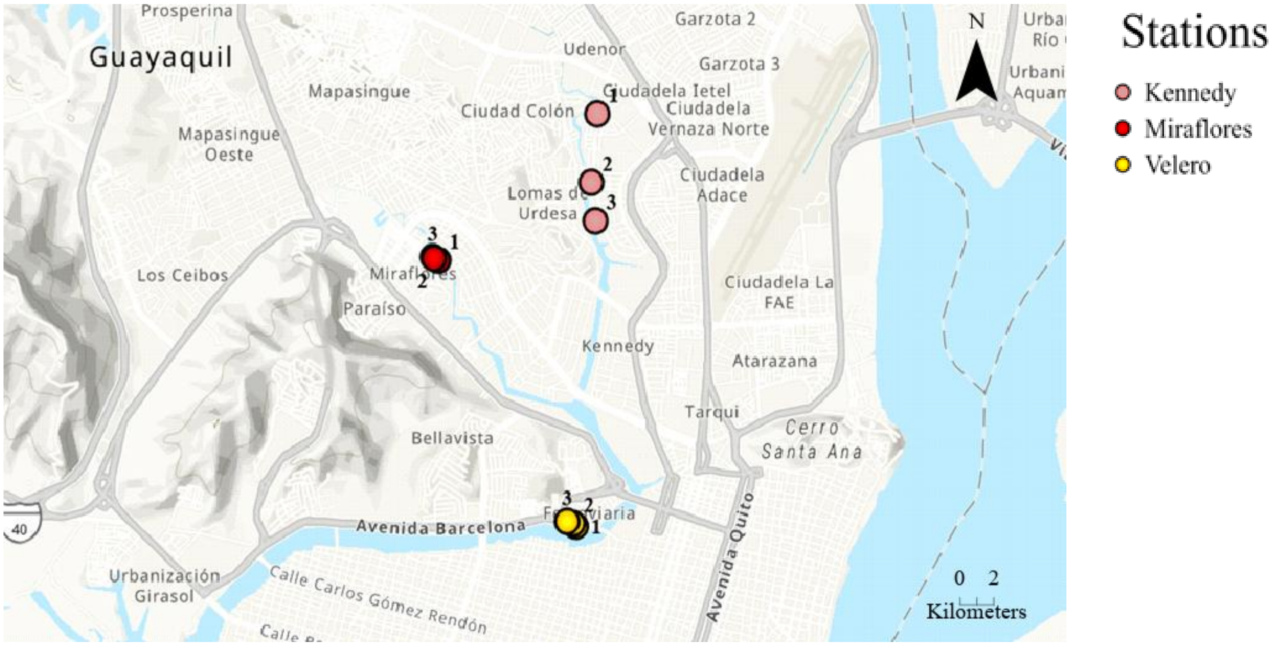


Figure 1.2 Sampling sites "within city limits": Kennedy, Miraflores, and Velero. Three substations were sampled in each station.

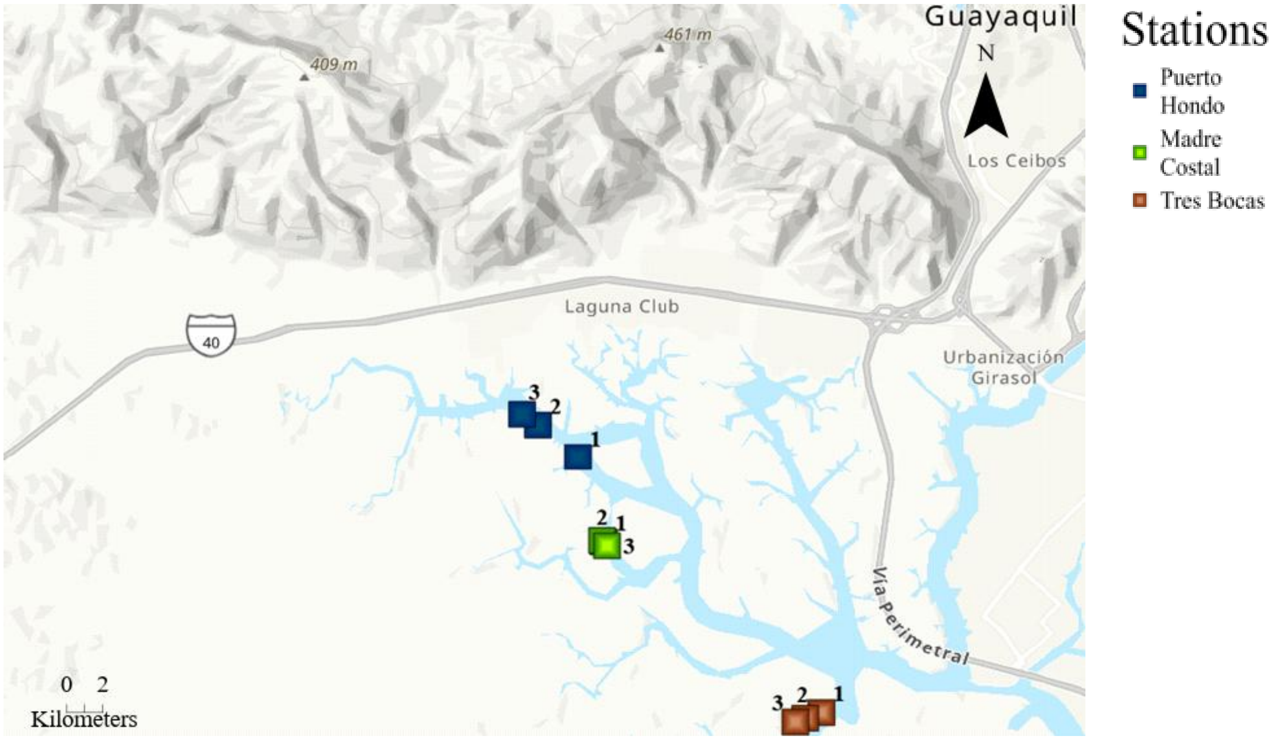


Figure 1.3 Locations of sampling sites in the "city border area": Puerto Hondo, Madre Costal and Tres Bocas. Three substations were sampled in each station.

One affected region is the Gulf of Guayaquil (Fig. 1.1), located in Ecuador's Guayas province. It is the largest estuarine ecosystem along South America's Pacific Coast[36] and it contains about 80% of the mangrove forests in the country[35]. The Reserva de Producción Faunística Manglares El Salado (RPFMS), part of this ecosystem surrounded by the city of Guayaquil (2º11′44″S, 79º53′18″W), was designated as a reserve in 2002 by Ministerial Agreement No. 142[37] according to Ministerio del Ambiente, Agua y Transición Ecológica (MAE). RPFMS covers approximately 156 km^2 [37]. In 2007, Manglares Puerto Hondo was included within the reserve limits[37].

RPFMS is habitat for many animal species including crustaceans, fishes, and birds[38]. Fishing and tourism are vital to the economy of local communities such as Puerto Hondo[37], which is home to around 1,000 people[39]. However, this coastal community faces escalating challenges from pollution associated with waste discharges[40]. Some trace elements found in aquatic environments, such as As, Cd, Hg, Pb, and Sn, are non-essential and have no known beneficial role in biological systems[41]. Various toxic metals and metalloids including As, Hg and Pb have been found in sediment in the RPFMS[35]. Due to anaerobic conditions and high organic carbon content[42], mangrove sediments accumulate toxic metal(loid)s via point source discharges, river runoff, and atmospheric deposition[43,44]. Bioindicators such as fish and bivalves, including mussels, are commonly used to monitor toxic metal(loid) contamination in mangrove ecosystems[45]. Several studies have highlighted the bioaccumulation of Hg and other organic species of metals in fish and mussels[35,46-48].

Hg is a toxic metal present in the environment from natural sources (e.g., volcanic activities and soil, rock and mineral weathering)[49]. However, human activities (e.g., logging, tillage, deforestation, coal burning, mining, industrial discharges)[49] can significantly raise the concentration of Hg in an ecosystem. Estuarine ecosystems, especially those fed by rivers with industry and urbanization within their watershed, often exhibit higher concentrations of organic and inorganic contaminants[50,51], including Hg. Once it enters the environment, microbes transform Hg into its organic form, methylmercury (MeHg), which can bioaccumulate in animal tissues and biomagnify through foodwebs[52]. Elevated MeHg levels can adversely affect the endocrine and nervous systems of birds, fish, and mammals, including humans[53], impairing the ability to obtain food, reproduce, and sustain healthy populations[54].

As, Cd, hexavalent chromium (Cr(VI)) and Pb are also toxic metal(loid)s with no biological function[55,56]. As occurs in the environment both naturally (e.g., as a result of volcanic activity) and due to anthropogenic pollution (e.g., pesticides, wood preservatives)[56]. Inorganic As (iAs) is a recognized human carcinogen capable of causing adverse health effects based on exposure time and dose concentration, targeting all organ systems[56] and causing skin lessons and multiple cancer types[56,57]. Cd, Cr(VI) and Pb also occur in the environment due to both natural and anthropogenic causes (e.g., fertilizers, electronic waste, mining)[58-60]. Pb exposure causes a variety of health effects in people, including high blood pressure and chronic heart disease for adults and impairment of the nervous system in children[61]. Previous investigations demonstrated high levels of Pb in children in Ecuador, with bivalves as a significant source of exposure[62]. Cd and Cr(VI) are particularly harmful to the kidney and liver[63,64]. Cd is classified as probable human carcinogen (B1)[65,66] while Cr(VI) is categorized as a likely human carcinogen through oral exposure[67,68].

Table 1.1 "Effects Range Low" (ERL) and "Effects Range Median" (ERM) sediment guidelines[69].

Metal(loid)	**ERL (dw) ($\frac{\mu g}{g}$)**	**ERM (dw) ($\frac{\mu g}{g}$)**
Ag	1.0	3.7
As	8.2	70.0
Cd	1.2	9.6
Cr	81	370
Cu	34	270
Hg	0.15	0.71
Ni	20.9	51.6
Pb	46.7	218
Sb	2	25
Zn	150	410

Table 1.2 Environmental Protection Agency (EPA) Unlimited Consumption Guidelines for fish[70].

Metal(loid)	**DCM ($\frac{\mu g}{g}$)**	**NDCM ($\frac{\mu g}{g}$)**
iAs	0.005	0.147
Cd	0.018	0.491
MeHg	0.002	0.049
Se	0.088	2.457

iAs: Inorganic As; MeHg:Methylmercury
DCM: Drying cooking method; NDCM: Non-drying cooking method

Table 1.3 Food and Agriculture Administration of the United Nations (FAO) consumption guidelines for shellfish[71].

Metal(loid)	**Dry weight (dw) ($\frac{\mu g}{g}$)**	**Wet weight (ww) ($\frac{\mu g}{g}$)**
Cd	12.9	2.0
Cu	194.0	30.0
Pb	3.2	0.5
Zn	194.0	30.0

Given the ecological and economical importance of RPFMS[18], this chapter focuses on analyzing concentrations of 20 toxic elements (Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, V, Zn) in the reserve's sediment. We also examine concentrations of these elements in two species of fish, catfish (*Bagre pinnimaculatus*) and Peruvian mojarra (*Diapterus peruvianus*), and in three species of mussels (*Mytella guyanensis*, *Mytella strigata*, and *Mytella trautwineana*). These data are used to perform a quantitative health risk assessment for Puerto Hondo residents based on consumption of fish and mussels, and considering exposure to iAs, Cd, Cr(VI), MeHg, and Pb.

1.2 Methodology

1.2.1 Sample collection, storage and preparation

We collected samples of sediment, mussels, and fish in 2023 in RPFMS during dry season of 2023 with the research permits No. MAATE-ARSFC-2023-3173 and No. MAATE-ARSFC-2023-0005. For sediment and mussels, a total of six substations were sampled: three within the city limits and three within the city border area. Miraflores, Kennedy, and Velero were classified as "within city limits" (Fig. 1.1). Puerto Hondo, Madre Costal and Tres Bocas were classified as "city border area" (Fig. 1.2). Fish were only collected in Puerto Hondo.

Samples were stored in labeled zip-lock bags on ice inside a cooler before being transported to the Escuela Politécnica del Litoral (ESPOL) Ecotoxicology, Quality and Environmental Health laboratory and held in -80°C freezers. Prior to analysis, fish samples were removed from -80°C and allowed to defrost. Species ID, sex, wet weight, total length, and width for each sample were recorded. A muscle biopsy was subsampled for analysis. Samples were freeze-dried and homogenized and then shipped to the University of Arizona for analysis.

1.2.2 Metal(loid) extraction and analysis

A total of 36 sediment samples, 30 fish samples (muscle), and 75 composite mussel samples of 40 individuals per sample (15 *M. guyanensis*, 30 *M. strigata* and 30 *M. trautwineana*) were analyzed at the Arizona Laboratory for Emerging Contaminants (ALEC) at the University of Arizona. The sample preparation involved microwave-assisted acid digestion using a CEM Model MARS6 system (Matthews, North Carolina) with 20 mL Teflon Xpress vessels, with digestion temperatures set to 180 °C for sediment samples (EPA Method 3051) and 200°C for fish and mussel samples (CEM Method for animal tissue). Each digestion used 0.1 g of sample treated with ultrapure concentrated HNO_3 (1mL for soils and 2mL for tissues) and 1mL of ultrapure water. Following digestion, samples were transferred to acid-washed polypropylene tubes and diluted 1:10 with diluent containing 1% HNO_3 plus 1% HCl (added to stabilize Hg in solution prior to analysis).

Concentrations of toxic metal(loid)s were determined using inductively coupled plasma-mass spectrometry (ICP-MS) with an Agilent 8900 triple quadrupole instrument (Santa Clara, CA). Laboratory-grade reagents were used as blanks, and both samples and calibration standards were prepared according to EPA Method 200.8. Analytical quality was verified using certified reference materials: National Institute of Standards and Technology (NIST) 2711 Montana soil for sediment, DORM-4 fish protein from the National Research Council of Canada (NRC-CNRC) for fish, and NIST 2976 mussel tissue for mussels. Results were reported in dry weight (dw) as $\frac{\mu g}{g}$.

1.2.3 Data standardization and regulatory thresholds

Contaminant levels of sediments were compared to the US National Oceanic and Atmospheric Administration (NOAA) “effects range low” (ERL) and “effects range median” (ERM) guidelines based on dw with a calculation of ~49% water content (Table 1.1). For fish, metal(loid) concentrations were compared to the EPA unlimited screening level for subsistence fisher consumption guidelines with a calculation of ~73% water content. The fish method of preparation defines the screening level including the non-drying cooking method (NDCM) and the drying-cooking method (smoked, dried or half-dried fish) (DCM). Therefore, the EPA's unlimited consumption guideline in wet weight (ww) and its dry weight (dw) equivalent[70] were used (Table 1.2). A conversion factor was applied with the EPA[70] assumption that 10% of As concentration is iAs[72], and that 95%[73,74] of Hg concentration is MeHg in the fish tissue. We considered the EPA’s unlimited screening level more suitable for Puerto Hondo due to the community’s subsistence-based fish consumption. Contaminant levels of mussels were compared with the consumption guidelines of the Food and Agriculture Administration of the United Nations (FAO) (Table 1.3) with a calculation of ~85% water content measured in mussel samples.

1.2.4 Statistical analysis

Statistical analyses were completed in R version 4.3.1. Samples below the detection limit (LOD) were substituted with $\frac{LOD}{\sqrt{2}}$. For sediment, fish and mussel samples, we employed both non-parametric (Mann–Whitney U (MWU) test) and parametric (Student's t-test or Welch's t-test) analyses to compare sites for each metal(loid). Normal distribution was assessed with the Shapiro-Wilk test. Homoscedasticity was examined with the Levene's test based on variances. For metal(loid)s that were initially non-normal but became normally distributed after log transformation, parametric tests were applied to the transformed data. For those that were already normal, no transformation was conducted. Metal(loid)s that remained non-normal after transformation were analyzed using the MWU test. For fish samples, we also conducted Pearson correlations among the covariates (length, weight, and width), and we conducted a linear regression with species and sex as fixed factors, and metal(loid) concentration as the dependent variable, while accounting for length as a covariate. Be was excluded from the linear regression analysis given the constant values along the data for both fish species. We conducted a principal component analysis (PCA) using log-transformed data for fish to visualize differences by species and sex, and to examine the relationship between PC1 and length. We also conducted a PCA on log-transformed data for mussels to show the differences of species per station in the reserve.

1.2.5 Human health risk assessment

Residents of Puerto Hondo (n = 290) were surveyed for their dietary intake in 2019 with the research ethical permit No. HLV-DOF-CEISH-032 as part of the 'Determinación de concentración de mercurio orgánico total en habitantes de la comuna de Puerto Hondo, Guayaquil – Ecuador' project. Of these, 193 were included in the risk assessment because they consumed fish and/or mussels in RFPMS. The following indices were calculated for iAs, Cd, Cr(VI), MeHg, and Pb (some indices include a subset of these elements): estimated daily intake (EDI), target hazard quotient (THQ), margin of exposure (MOE), hazard index (HI), and cancer risk (CR). The Spearman rank correlation was used for a sensitivity analysis.

1.2.5.1 Estimated daily intake

Following Montojo *et* al.[75] and the EPA risk assessment framework[76,77], a health risk assessment function was developed in R version 4.3.1 to determine the EDI values (eq. 1) to calculate the uptake rates of iAs, Cd, Cr(VI), MeHg, and Pb through fish and mussels. Concentrations were converted from dw basis to ww employing the water percentage for fish and mussels: ~73% of water content for catfish and Peruvian mojarra and ~85% for mussels, both previously measured (eq. 2) as described by Cresson *et* al. (2017)[78].

A conversion factor was applied based on the EPA[70] assumption that 10% of As concentration is iAs[72]. The EPA does not specify the proportion of Cr(VI) relative to Cr in fish and shellfish tissue, as its concentration varies depending on site-specific conditions. Cr in protein-rich seafood is mostly trivalent given their binding proteins[79]. Most of the ingested Cr(VI) is converted to its trivalent form[80]. Given the lack of guidelines for Cr(VI) in fish and shellfish tissue, we used the conservative assumption that 2% of Cr is Cr(VI). In addition, we assumed that 95%[73,74] and 89%[81] of Hg concentration in the fish and mussel tissue is MeHg, respectively.

$$EDI = \frac{C\ \times IR\ \times ED \times EF}{BW\ \times\ AT}\ [Equation\ 1]$$

Equation 1: C ($\frac{mg}{kg}\ ww$) is the metal(loid) concentration in the fish and mussels of edible tissue, obtained from the analysis; IR $\left(\frac{kg}{person\ day}\right)$ is the ingestion rate of fish and mussels from Puerto Hondo per day, obtained from the dietary survey; ED is the duration of exposure (72.9 to 74 $years$, this accounts for the introduction of solid food at 6 months of age[82] and is based on a life expectancy in Ecuador of 73.4 to 74.5 years[83]); EF is the exposure frequency (with a range from 52 days for those who eat fish or mussels once a week to 365 days for those who eat fish or mussels daily); BW is the average adult body weight according to surveys in Puerto Hondo conducted in 2019 (Table 1.4); and AT is the mean exposure (for non-carcinogens and carcinogens for this study it is calculated as 365 $\frac{days}{year}$ multiplied by the ED, based on a lifetime exposure of 72.9 to 74 $years$).

$$C(ww) = C(dw) \times (\frac{100 - \%WC}{100})\ [Equation\ 2]$$

Equation 2: C (ww) ($\frac{mg}{kg}$) and C (dw) ($\frac{mg}{kg}$) is the metal(loid) concentration in wet and dry edible tissue respectively and $\%WC$ is the water content in wet tissue.

A Monte Carlo simulation with 10,000 iterations was performed to capture variability and uncertainty in exposure estimates. For each iteration, a potential exposure scenario was generated by randomly sampling values for C, IR, ED, EF, BW, and AT in fish and mussels, based on previously validated distributions for each variable (Table 1.4). Distributions were employed using truncated values, applying realistic minimum and maximum limits based on the data (Table 1.4). These stochastic values were then used in the exposure calculations.

Table 1.4 Parameters for the Monte Carlo simulation for catfish, Peruvian mojarra and mussels.

Parameter		**Units**	**Distribution**	**Reference**
$C_{Catfish}$	iAs	$\frac{mg}{kg}\ ww$	Normal $\bar{x}$ = 0.057, s = 0.022 min = 0.023, max = 0.108	This study
	Cd		Exponential λ = 0.003 min = 0.0005, max = 0.009	
	Cr(VI)		Normal $\bar{x}$ = 0.012, s = 0.007 min = 0.003, max = 0.029	
	Pb		Log-normal log($\bar{x}$) = -3.70, log(s) = 0.34 min = 0.012, max = 0.053	
	MeHg		Normal $\bar{x}$ = 0.03, s = 0.004	

			min = 0.024, max = 0.036	
$C_{Peruvian\ Mojarra}$	iAs		Normal $\bar{x}$ = 0.046, s = 0.023 min = 0.016, max = 0.09	
	Cd		Log-normal log($\bar{x}$) = -7.08, log(s) = 1.16 min = 0.0005, max = 0.021	
	Cr(VI)		Normal $\bar{x}$ = 0.028, s = 0.014 min = 0.012, max = 0.056	
	Pb		Log-normal log($\bar{x}$) = -3.97, log(s) = 0.48 min = 0.012, max = 0.081	
	MeHg		Normal $\bar{x}$ = 0.014, s = 0.007 min = 0.006, max = 0.027	
$C_{Mussels}$	iAs		Log-normal log($\bar{x}$) = -2.83, log(s) = 0.18 min = 0.47, max = 1.03	
	Cd		Log-normal log($\bar{x}$) = -3.78, log(s) = 0.46 min = 0.013, max = 0.056	
	Cr(VI)		Log-normal log($\bar{x}$) = -7.47, log(s) = 0.37 min = 0.002, max = 0.008	
	Pb		Gamma α = 25.25, β = 208.28 min = 0.09, max = 0.2	
	MeHg		Log-normal log($\bar{x}$) = -4.34, log(s) = 0.53 min = 0.007, max = 0.06	
$IR_{Catfish}$		$\frac{kg}{person\ day}$	Log-normal $IR_{Catfish_{log(\bar{x})}}$ = -3.71 $IR_{Catfish_{log(s)}}$ = 1.17 min = 0.003, max = 0.48	This study

$\boldsymbol{IR_{Peruvian\ Mojarra}}$		Exponential $IR_{Peruvian\ Mojarra_{\lambda}}$= 10.93 min = 0.002, max = 0.48	
$\boldsymbol{IR_{Mussels}}$		Log-normal $IR_{Mussels_{log(\bar{x})}}$= -3.76 $IR_{Mussels_{log(s)}}$= 0.43 min = 0.015, max = 0.09	
$\boldsymbol{ED}$	$years$	Uniform ED_{min}= 72.90, ED_{max}= 74.00	(WHO, 2021)[83]
$\boldsymbol{EF_{Catfish}}$	$days$	Log-normal $EF_{Catfish_{log(\bar{x})}}$= 3.70 $EF_{Catfish_{log(s)}}$= 0.92 min = 0, max = 364	This study
$\boldsymbol{EF_{Peruvian\ Mojarra}}$		Weibull $EF_{PeruvianMojarra_{\alpha}}$= 1.35 $EF_{PeruvianMojarra_{\beta}}$= 117.60 min = 0, max = 208	
$\boldsymbol{EF_{Mussels}}$		Log-normal $EF_{Mussels_{log(\bar{x})}}$= 4.01 $EF_{Mussels_{log(s)}}$= 0.23 min = 0, max = 156	
$\boldsymbol{BW}$	kg	Normal BW_{mean}= 63.89, BW_{sd}= 11.20 min = 40, max = 89	This study
$\boldsymbol{AT}$	$days$	Uniform AT_{min}= 26608.50, AT_{max}= 27010.00	(WHO, 2021)[83]

$\bar{x}$: mean; s: standard deviation; min: minimum value; max: maximum value; λ= rate (Exponential); α= shape (Gamma and Weibull); β= scale parameter (Gamma, Weibull)

1.2.4.2 Target hazard quotient

$$THQ = \frac{EDI}{RfD}\ [Equation\ 3]$$

THQ (eq. 3) for fish and mussels was determined following Montojo *et* al.[75], Sharif *et* al.[84] and Mohiuddin *et* al.[85] according to EPA guidelines. The THQ is a non-carcinogenic risk assessment provided by EPA and calculated as the ratio of EDI ($\frac{mg}{kg\ day}$) and RfD (the oral reference dose) ($\frac{mg}{kg\ day}$), which is the safe limit intake of toxic metal(loid)s. The RfD for iAs is $6.00 \times 10^{-5}\ \frac{mg}{kg\ day}$[86], for Cd is $1.00 \times 10^{-3}\ \frac{mg}{kg\ day}$[65], for Cr(VI) is $9.00 \times 10^{-4}\ \frac{mg}{kg\ day}$[67], and for MeHg is $1.00 \times 10^{-4}\ \frac{mg}{kg\ day}$[87]. A THQ < 1 means no negative health effects, while a value >1 suggests that there is likely a non-carcinogenic risk[88].

1.2.4.3 Margin of exposure

$$MOE = \frac{BMDL}{EDI}\ [Equation\ 4]$$

Given the lack of an updated RfD for Pb from EPA, a margin of exposure (MOE) for chronic effect was estimated (eq. 4) with a Monte Carlo simulation with 10,000 iterations in R (Table 1.4). The benchmark-dose lower bound (BMDL) is the lower end of the 95% one-sided confidence interval for the benchmark dose (BMD) which indicates the lowest exposure level at which a defined increase in the occurrence of an adverse effect is observed within the data range ($\frac{mg}{kg\ day}$)[89]. The European Food Safety Authority (EFSA) identified adverse health effects associated with lead exposure. For children, developmental neurotoxicity (reduction in intelligence quotient (IQ)) was observed at a BMDL of 0.5 $\frac{mg}{kg\ day}$. For adults, increased risks of cardiovascular effects (elevated blood pressure) and kidney disease were associated with BMDLs of 0.63 $\frac{mg}{kg\ day}$ and 1.5 $\frac{mg}{kg\ day}$, respectively[89]. MOE > 100 means low concern for public health[90].

1.2.4.2 Hazard index

$$HI = \sum_{i=k}^{n} THQ\ [Equation\ 5]$$

The HI is calculated by the sum of the individual THQ of metal(loid) intake through food consumption. HI was determined for iAs, Cd, Cr(VI) and MeHg (eq. 5). A HI > 1 signifies non-carcinogenic health effects[91].

1.2.4.3 Cancer Risk

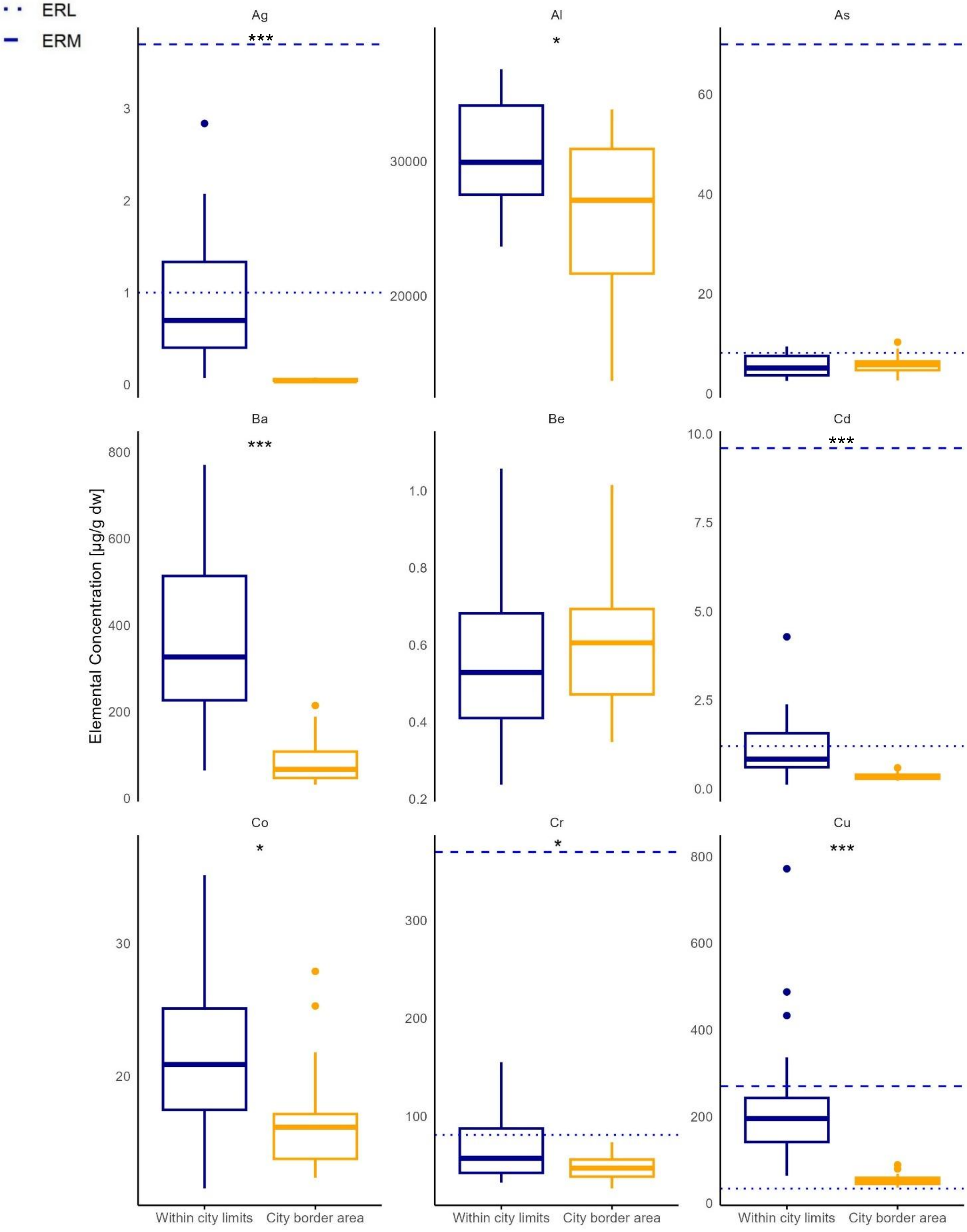
$$CR = EDI \times CSF \; [Equation\ 6]$$

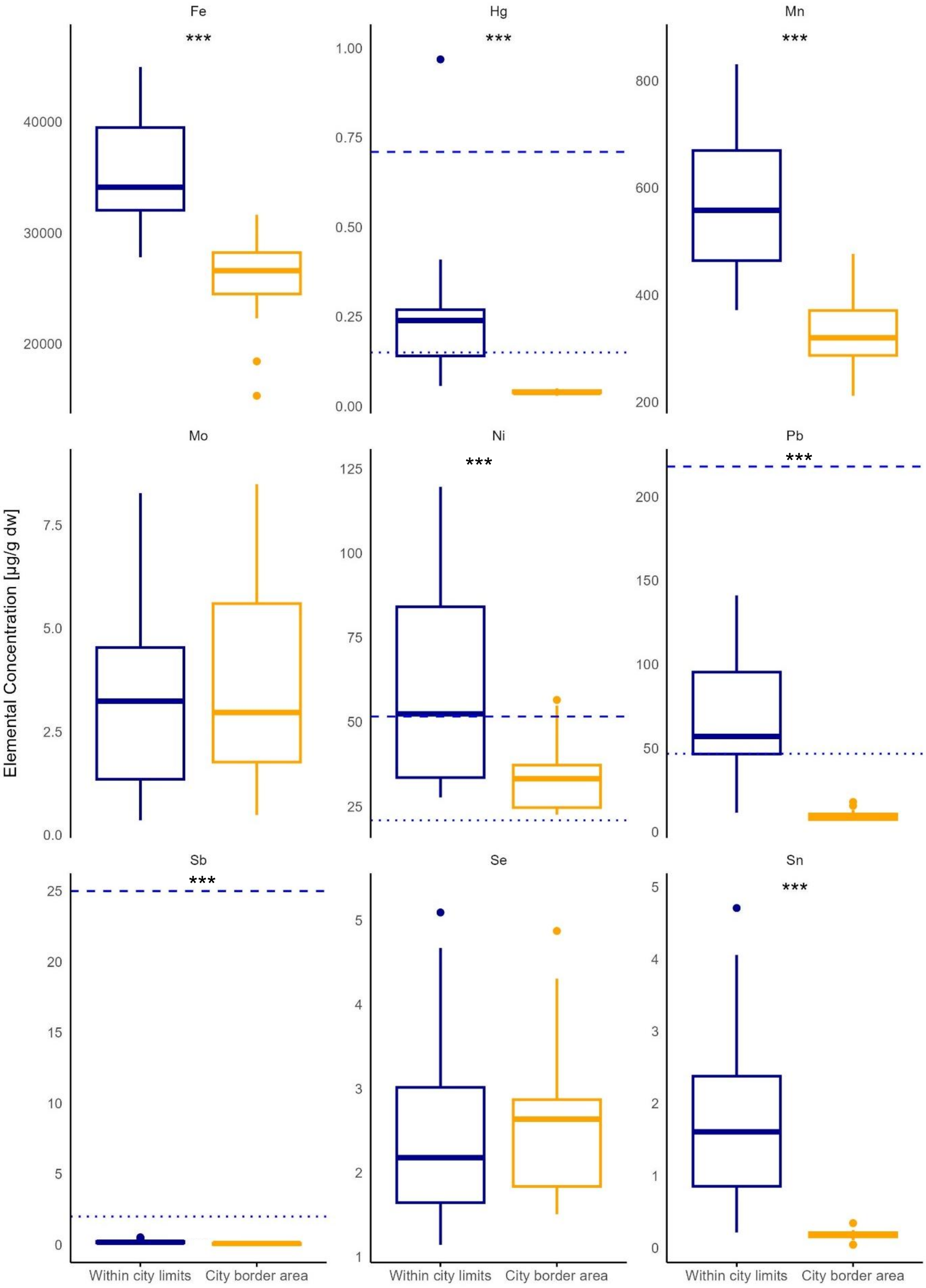
The CR was calculated by multiplying EDI by the cancer slope factor (CSF) ($\frac{mg}{kg\ day}$) (eq. 6). The CR assesses the adverse impact of a carcinogenic contaminant over a lifetime. The CSF for iAs is 32 $\frac{mg}{kg\ day}$ [86], and Cr(VI) is 0.27 $\frac{mg}{kg\ day}$[67], employing an application of age-dependent adjustment factors (ADAFs) considering a lifetime exposure. A CR ≥ 10^{-4} indicates there is likelihood of cancer risk hazard with an unacceptable risk level that would drive a management decision, whereas a CR < 10^{-6} is considered an acceptable risk level. Values between $10^{-6}$and 10^{-4} fall within a risk management range, where acceptability is determined based on site-specific considerations[88,92].

1.3 Results

1.3.1 Sediment

We compared metal(loid) concentrations in sediment samples with sediment quality guidelines (SQGs) and found that concentrations of Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn exceeded ERL guidelines (Fig.1.4). Among these, Cu, Hg, Ni, and Zn also exceeded ERM values. Samples from the “city border area” generally remained below SQGs. Among the 20 metal(loid)s analyzed, 80% showed statistically significant differences between the two zones (Fig. 1.4). Significant differences between the “within city limits” and “city border area” were observed for Ag, Al, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sn, V, Zn (Fig. 1.4). All metal(loid)s, except for As, Be, Mo and Se, exhibited higher mean concentrations “within city limits” compared to the “city border area” (Table 1.5, Fig. 1.4).


ERL
ERM
Elemental Concentration [µg/g dw]
Ag
***
0
1
2
3
Al
*
20000
30000
As
0
20
40
60
Ba
***
0
200
400
600
800
Be
0.2
0.4
0.6
0.8
1.0
Cd
***
0.0
2.5
5.0
7.5
10.0
Co
*
20
30
Cr
*
100
200
300
Cu
***
0
200
400
600
800
Within city limits
City border area


Fe
***
40000
30000
20000
Hg
***
1.00
0.75
0.50
0.25
0.00
Mn
***
800
600
400
200
Mo
7.5
5.0
2.5
0.0
Ni
***
125
100
75
50
25
Pb
***
200
150
100
50
0
Sb
***
25
20
15
10
5
0
Se
5
4
3
2
1
Sn
***
5
4
3
2
1
0
Elemental Concentration [µg/g dw]
Within city limits
City border area
Within city limits
City border area
Within city limits
City border area

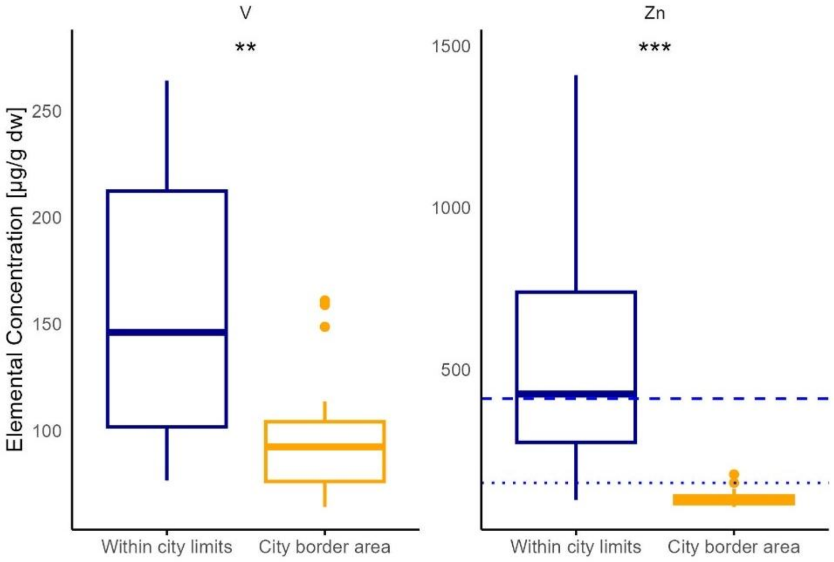


*Figure 1.4 Metal(loid) concentrations in sediment in Reserva de Producción Faunística Manglares El Salado (RPFMS). The dashed horizontal line indicates the "Effect Range Median" (ERM) guidelines. The dotted horizontal line indicates the "Effect Range Low" (ERL) guidelines. *<.05 **<.01 ***<.001.*

Table 1.5 Sediment metal(loid) concentrations ($\frac{\mu g}{g}$ dw) in Reserva de Producción Faunística Manglares El Salado (RPFMS).

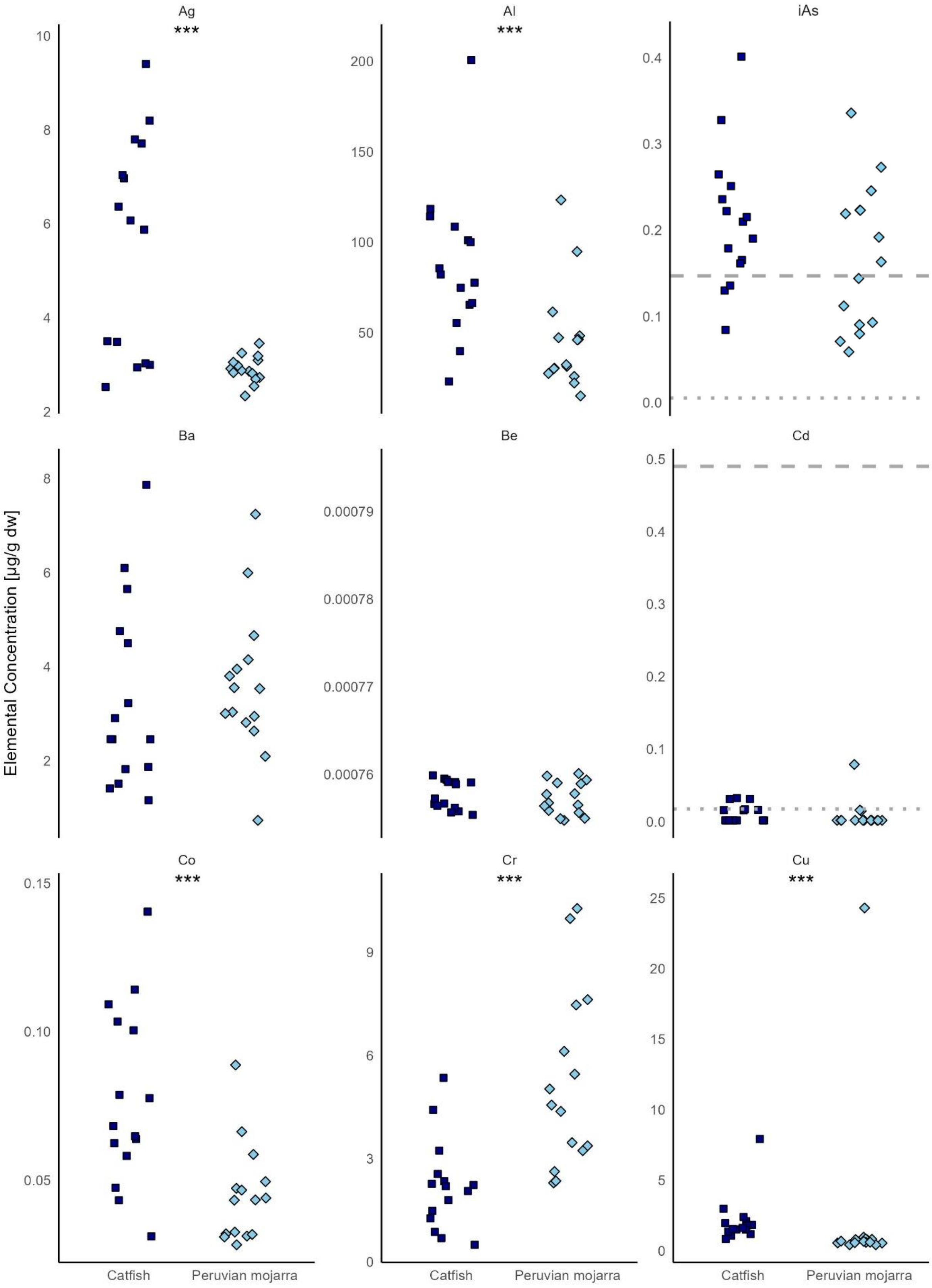
Reserve	**RPFMS** **N= 36**				
	mean ± sd **range**				
	within city limits	**city border area**	**combined**	**test**	***p*-value**
Metal(loid)					
Ag	0.96 ± 0.77 (0.07, 2.84)	0.05 ± 0.01 (0.04, 0.08)	0.51±0.71 (0.04, 2.84)	MWU	**< 0.001**
Al	30566.87 ± 4573.05 (23682.47, 36860.96)	25957.93 ± 5751.53 (13698.72, 33859.40)	28262.40 ± 5629.16 (13698.72, 36860.96)	Student's	**< 0.05**
As	5.70 ± 2.28 (2.59, 9.49)	5.89 ± 1.95 (2.67, 10.34)	5.79 ± 2.09 (2.59, 10.34)	Student's	NS

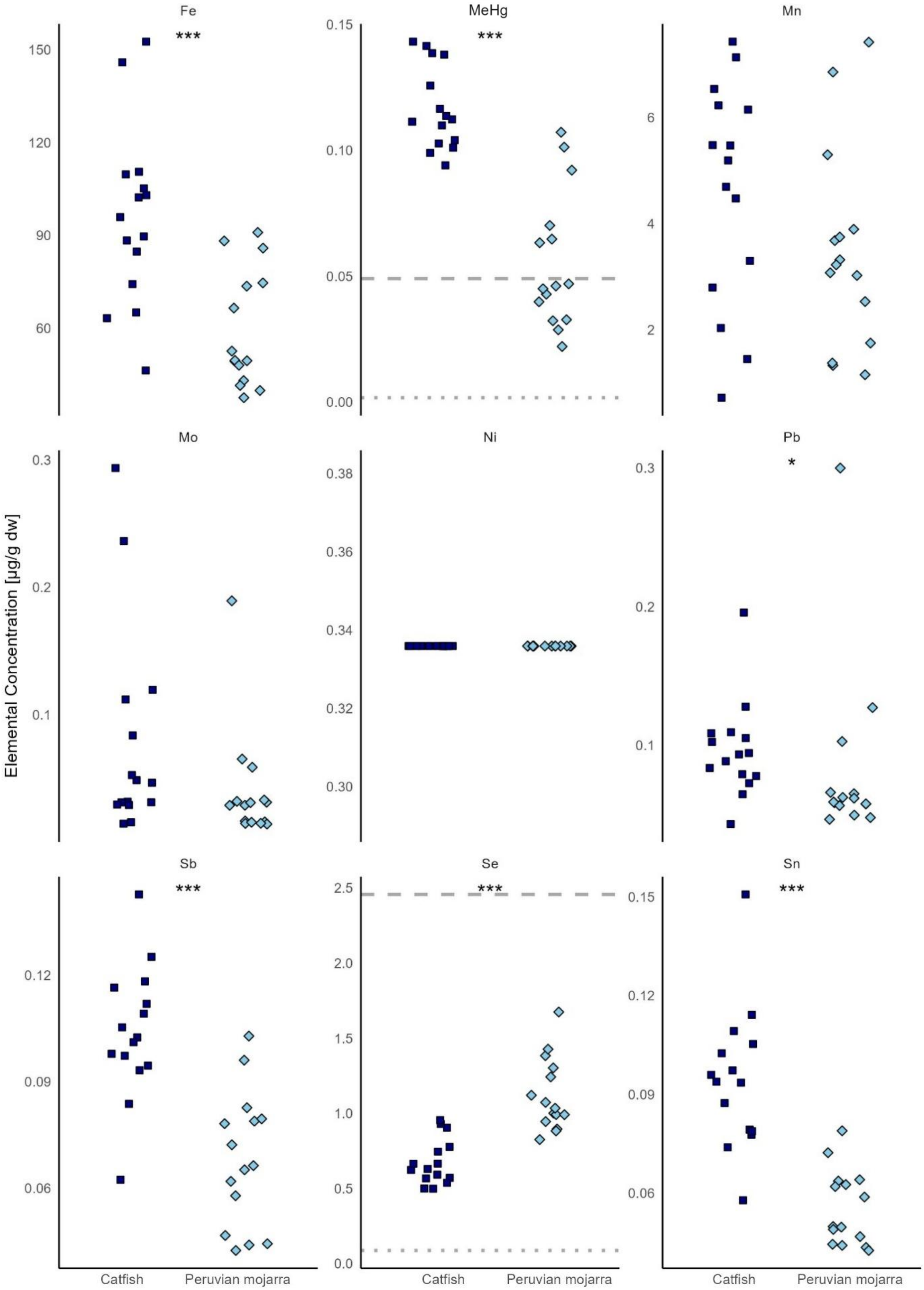
Ba	370.49 ± 215.01 (64.56, 770.69)	86.25 ± 55.33 (31.64, 214.54)	228.37 ± 211.46 (31.64, 770.69)	MWU	**< 0.001**
Be	0.57 ± 0.22 (0.24, 1.06)	0.63 ± 0.20 (0.35, 1.02)	0.60 ± 0.21 (0.24, 1.06)	Student's	NS
Cd	1.19 ± 0.98 (0.11, 4.28)	0.34 ± 0.10 (0.23, 0.59)	0.77 ± 0.81 (0.11, 4.28)	Welch's	**< 0.001**
Co	21.62 ± 6.18 (11.52, 35.15)	16.81 ± 4.29 (12.34, 27.91)	19.21 ± 5.78 (11.52, 35.15)	MWU	**< 0.05**
Cr	69.72 ± 36.03 (32.16, 155.29)	48.51 ± 13.47 (26.17, 73.58)	59.12 ± 28.88 (26.17, 155.29)	Welch's	**< 0.05**
Cu	241.86 ± 172.73 (63.59, 771.82)	54.48 ± 13.87 (36.31, 88.71)	148.17 ± 153.67 (36.31, 771.82)	Welch's	**< 0.001**
Fe	35673.13 ± 5060.75 (27819.59, 44940.81)	25659.17 ± 3990.33 (15332.62, 31629.05)	30666.15 ± 6779.37 (15332.62, 44940.81)	Student's	**< 0.001**
Hg	0.24 ± 0.20 (0.06, 0.97)	0.04 ± 0.01 (0.03, 0.05)	0.14 ± 0.18 (0.03, 0.97)	Welch's	**< 0.001**
Mn	578.65 ± 140.39 (372.12, 830.69)	326.31 ± 79.41 (212.12, 476.56)	452.48 ± 170.32 (212.12, 830.69)	Welch's	**< 0.001**
Mo	3.43 ± 2.42 (0.35, 8.28)	3.54 ± 2.34 (0.48, 8.50)	3.48 ± 2.35 (0.35, 8.50)	Student's	NS
Ni	59.12 ± 28.32 (27.69, 119.65)	33.72 ± 10.30 (22.59, 56.53)	46.42 ± 24.64 (22.59, 119.65)	Welch's	**< 0.001**
Pb	67.88 ± 34.59 (11.49, 141.12)	9.99 ± 3.08 (7.01,17.91)	38.94 ± 38.04 (7.01, 141.12)	MWU	**< 0.001**
Sb	0.21 ± 0.14 (0.03, 0.53)	0.08 ± 0.05 (0.00, 0.14)	0.15 ± 0.12 (0.00, 0.53)	MWU	**< 0.001**
Se	2.52 ± 1.23 (1.14, 5.10)	2.67 ± 0.95 (1.51, 4.88)	2.59 ± 1.09 (1.14, 5.10)	Student's	NS
Sn	1.86 ± 1.25 (0.21, 4.71)	0.18 ± 0.07 (0.04, 0.34)	1.02 ± 1.22 (0.04, 4.71)	Welch's	**< 0.001**
V	158.30 ± 62.69 (76.69, 264.19)	97.86 ± 30.49 (64.27, 161.08)	128.08 ± 57.44 (64.27, 264.19)	MWU	**< 0.01**
Zn	538.43 ± 327.47 (97.01, 1409.25)	104.58 ± 26.99 (75.50, 176.19)	321.51 ± 317.55 (75.50, 1409.25)	MWU	**< 0.001**

Statistical analysis: t-test (Student's or Welch's test) for normal distribution, MWU test for non-normal distribution
Significant coefficients ($p < 0.05$, $p < 0.01$, $p < 0.001$, bold numbers)

1.3.2 Fish

Analysis of metal(loid) concentrations in catfish and Peruvian mojarra revealed that iAs and MeHg exceeded both the DCM and NDCM threshold values (Fig. 1.5). Cd and Se exceeded only the NDCM thresholds in both species (Fig. 1.5). Of the 20 metal(loid)s analyzed, 65% exhibited statistically significant differences between the two species (Fig. 1.5). Significant differences were found for Ag, Al, Co, Cr, Cu, Fe, MeHg, Pb, Sb, Se, Sn, V, and Zn (Fig. 1.5). With the exception of Ba, Cr, Cu, and Se, all metal(loid)s showed higher mean concentrations in catfish (Table 1.6, Fig. 1.5).


Ag
***
Al
***
iAs
Ba
Be
Cd
Co
***
Cr
***
Cu
***
Elemental Concentration [µg/g dw]
Catfish
Peruvian mojarra
10
8
6
4
2
200
150
100
50
0.4
0.3
0.2
0.1
0.0
0.00079
0.00078
0.00077
0.00076
0.5
0.15
0.10
0.05
9
3
0
25
20
15
5


Fe
***
150
120
90
60
MeHg
***
0.15
0.10
0.05
0.00
Mn
6
4
2
Mo
0.3
0.2
0.1
Ni
0.38
0.36
0.34
0.32
0.30
Pb
*
0.3
0.2
0.1
Sb
***
0.12
0.09
0.06
Se
***
2.5
2.0
1.5
1.0
0.5
0.0
Sn
***
0.15
0.12
0.09
0.06
Elemental Concentration [µg/g dw]
Catfish
Peruvian mojarra
Catfish
Peruvian mojarra
Catfish
Peruvian mojarra

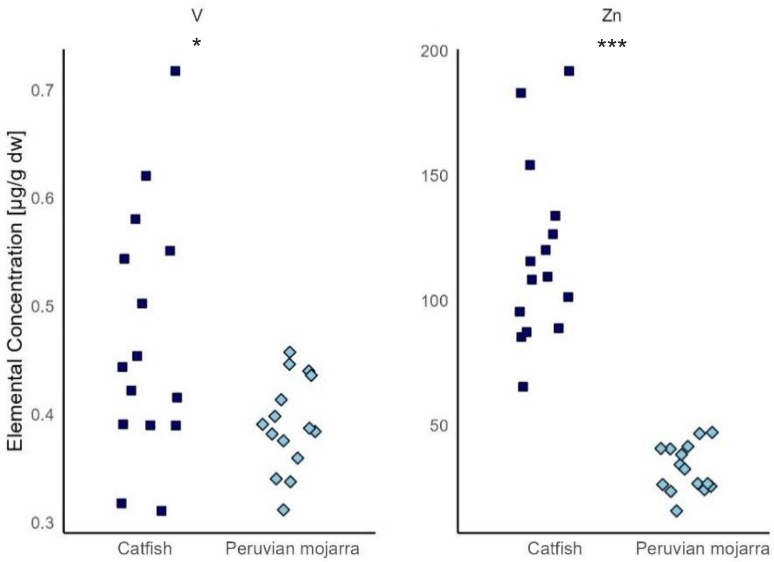


*Figure 1.5 Metal(loid) concentrations in fish from Puerto Hondo. The dashed horizontal line indicates the Environmental Protection Agency (EPA) screening level for unlimited fish consumption for subsistence fishers using the drying cooking method (DCM). The dotted horizontal line indicates EPA screening level for unlimited fish consumption for subsistence fishers using a non-drying cooking method (NDCM). *<.05 **<.01 ***<.001.*

Table 1.6 Metal(loid) concentrations ($\frac{\mu g}{g}$ *dw) in fish from Puerto Hondo.*

Species	**Catfish N= 15**	**Peruvian Mojarra N= 15**		
	mean ± sd range	**mean ± sd range**	**test**	***p*-value**
Metal(loid)				
Ag	5.32 ± 2.12 (2.53, 8.20)	2.91 ± 0.28 (2.34, 3.46)	Welch's	**< 0.001**
Al	88.54 ± 42.77 (23.11, 200.75)	45.54 ± 29.01 (15.10, 123.43)	Welch's	**< 0.001**
iAs	0.21 ± 0.08 (0.08, 0.401)	0.17 ± 0.08 (0.06, 0.34)	Student's	NS
Ba	3.50 ± 1.97 (1.42, 7.86)	3.61 ± 1.55 (0.74, 7.24)	Student's	NS
Be	0.00 ± 0.00 (0.00, 0.00)	0.00 ± 0.00 (0.00, 0.00)	MWU	NS
Cd	0.01 ± 0.01 (0.00, 0.03)	0.01 ± 0.02 (0.00, 0.08)	MWU	NS

Co	0.08 ± 0.03	0.05 ± 0.02	Welch's	**< 0.001**
	(0.03, 0.14)	(0.03, 0.09)		
Cr	2.21 ± 1.37	5.22 ± 2.61	Welch's	**< 0.001**
	(0.50, 5.35)	(2.30, 10.28)		
Cu	1.71 ± 0.55	2.25 ± 6.11	MWU	**< 0.001**
	(0.85, 3.00)	(0.44, 24.31)		
Fe	95.14 ± 29.55	56.13 ± 24.19	Welch's	**< 0.001**
	(46.37, 152.58)	(0.00, 90.97)		
MeHg	0.11 ± 0.02	0.05 ± 0.03	Student's	**< 0.001**
	(0.09, 0.14)	(0.02, 0.10)		
Mn	4.79 ± 2.05	3.45 ± 1.88	Student's	NS
	(0.72, 7.43)	(1.15, 7.42)		
Mo	0.08 ± 0.09	0.04 ± 0.04	MWU	NS
	(0.01, 0.29)	(0.01, 0.19)		
Ni	0.34 ± 0.00	0.34 ± 0.00	MWU	NS
	(0.34, 0.34)	(0.34, 0.34)		
Pb	0.10 ± 0.04	0.08 ± 0.06	MWU	**< 0.05**
	(0.04, 0.20)	(0.05, 0.30)		
Sb	0.10 ± 0.02	0.07 ± 0.02	Student's	**< 0.001**
	(0.06, 0.14)	(0.04, 0.10)		
Se	0.67 ± 0.16	1.12 ± 0.24	Student's	**< 0.001**
	(0.50, 0.95)	(0.83, 1.67)		
Sn	0.10 ± 0.02	0.06 ± 0.01	Student's	**< 0.001**
	(0.06, 0.15)	(0.04, 0.08)		
V	0.48 ± 0.11	0.39 ± 0.04	Welch's	**< 0.05**
	(0.32, 0.72)	(0.31, 0.46)		
Zn	115.26 ± 35.58	32.81 ± 9.45	Welch's	**< 0.001**
	(65.53, 191.85)	(15.83, 47.27)		

Statistical analysis: t-test (Student's or Welch's test) for normal distribution, MWU test for non-normal distribution
Significant coefficients ($p < 0.05$, $p < 0.01$, $p < 0.001$, bold numbers)

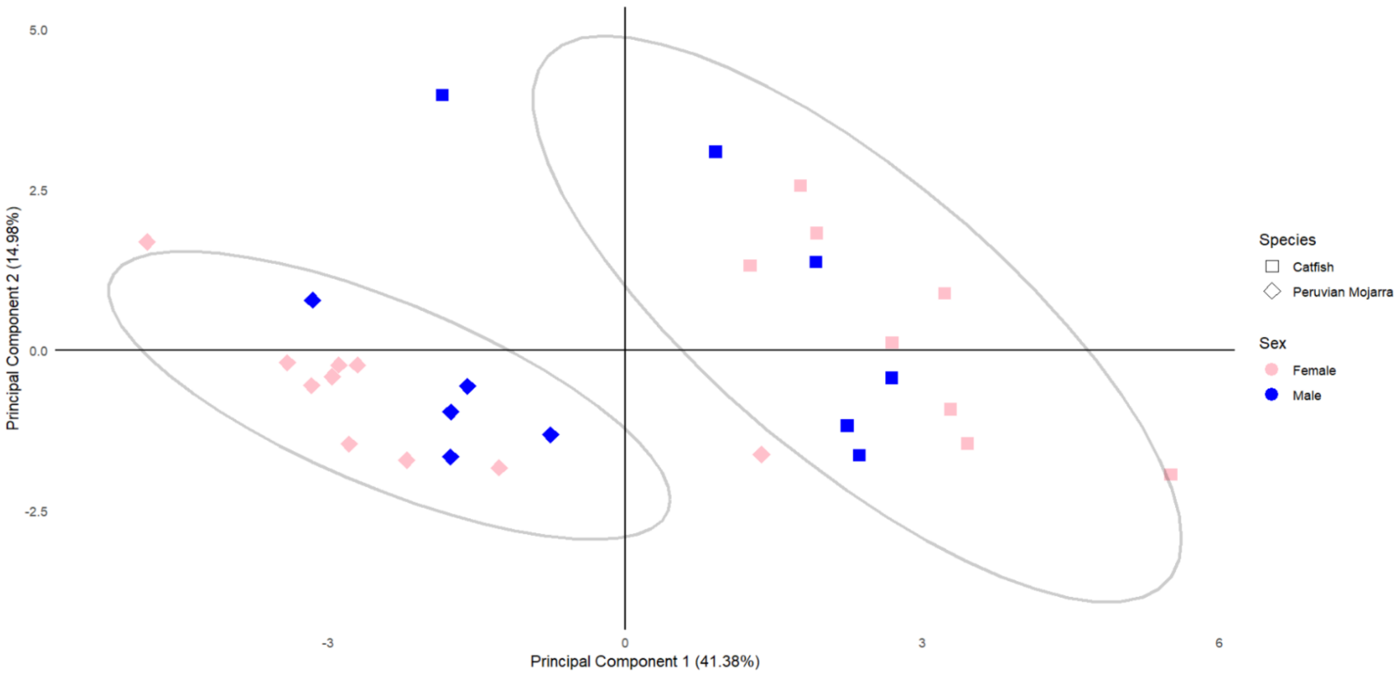


Figure 1.6 Principal component analysis (PCA) biplot: species and sex in Reserva de Producción Faunística Manglares El Salado (RPFMS).

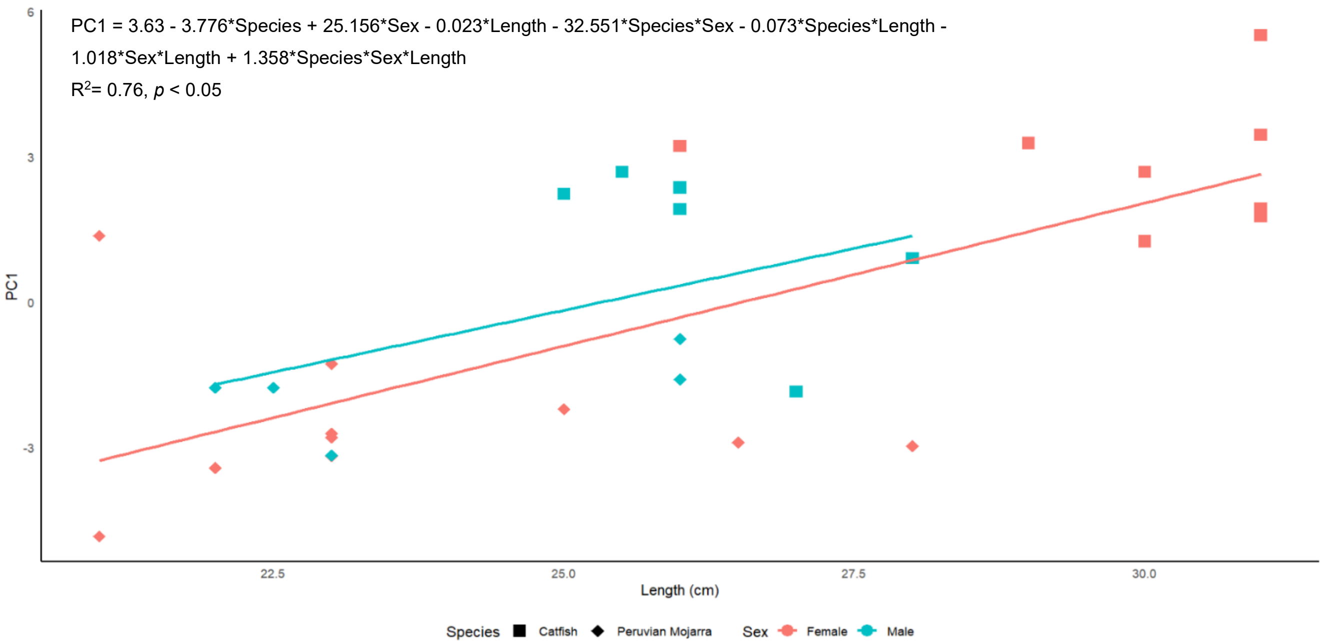
Catfish and Peruvian mojarra clustered separately for most observations on the PCA along principal component (PC) 1 (Fig. 1.6), with catfish having mostly higher values. PC1 accounted for 41.38% of the variance, with most elements loading positively, except for Se and Cr (Table 1.7). PC2 explained 14.96% of the variance, with most elements loading negatively, except for Ag, As, Hg, Zn, Al, Sn, Cu.

Table 1.7 Principal component loadings of log-transformed metal(loid) concentrations (n=30) in fish, with elements organized for each PC by rank order of the absolute value of the loading. Be and Ni were excluded given their constant values.

Metal(loid)	**PC1**	**Metal(loid)**	**PC2**
Sn	0.317	Ba	-0.495
Zn	0.311	Cr	-0.428
Fe	0.299	V	-0.343
Al	0.296	Ag	0.284
Hg	0.263	Se	-0.278

Sb	0.256	Mn	-0.272
Mo	0.255	As	0.238
Pb	0.251	Mo	-0.219
V	0.249	Hg	0.171
Co	0.246	Zn	0.140
Ag	0.225	Fe	-0.129
Cu	0.215	Pb	-0.123
Se	-0.215	Al	0.112
Cd	0.205	Sb	-0.112
Mn	0.146	Cd	-0.068
Cr	-0.141	Co	-0.055
As	0.091	Sn	0.052
Ba	0.073	Cu	0.037

For catfish, neither PC1 nor PC2 differed significantly between males and females (t=-1.22, df=8.22, *p*>0.05 and t=1.21, df=11.99, *p*>0.05, respectively). Similarly for Peruvian mojarra, neither PC1 nor PC2 differed significantly between males and females (t=-0.3, df=9.99, *p*>0.05 and t=-0.15, df=5.18, *p*>0.05 respectively). The length of fish in both species accounting for sex combined was positively correlated with PC1 and PC2 ($p<0.05$) (Fig. 1.7 and Fig. 1.8).


PC1 = 3.63 - 3.776*Species + 25.156*Sex - 0.023*Length - 32.551*Species*Sex - 0.073*Species*Length - 1.018*Sex*Length + 1.358*Species*Sex*Length

R^2= 0.76, $p < 0.05$

Figure 1.7 Principal component (PC) 1 vs length (cm) of catfish and Peruvian mojarra. Coefficients shown relative to reference: Peruvian mojarra and male.

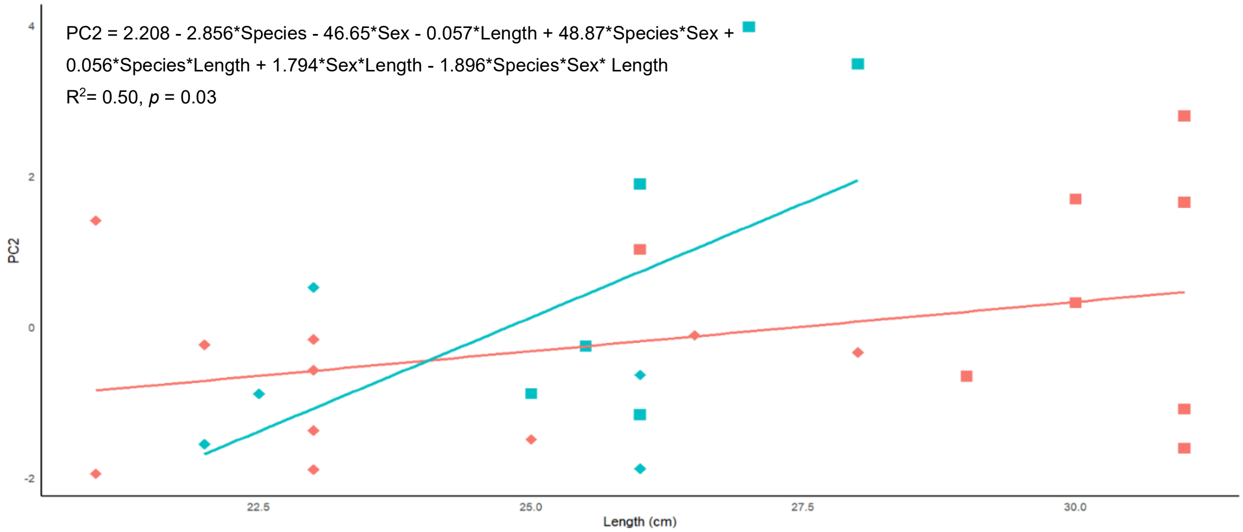


Figure 1.8 Principal component (PC) 2 vs length (cm) of catfish and Peruvian mojarra. Coefficients shown relative to reference: Peruvian mojarra and male.

In the regression model predicting metal(loid) concentration from fish species, sex, and length (Table 1.8), Ag, Co, Cr, Cu, Fe, Hg, Ni, Sb, Se, Sn, V, and Zn showed statistical significance ($p < 0.05$). Species was a significant factor in the regression for concentrations of Ag, Al, Fe, Hg, Ni, Sb, Se, Sn, and Zn. Sex was marginally significant for As, Cu, and Ni. Length was marginally significant for Cu and Ni.

Table 1.8 Regression coefficients of the multiple linear regression model for fish.

Metal(loid)	**F**	**R²**	***p*-value**
Ag	6.40	0.44	**0.002**
Species (-0.478, **0.018**)			
Sex (0.033, 0.809)			
Length (0.009, 0.761)			
Al	4.14	0.33	0.016
Species (-0.888, **0.010**)			
Sex (-0.139,0.549)			
Length (-0.037, 0.470)			
As	2.01	0.20	0.138
Species (-0.748, 0.124)			
Sex (-0.663, **0.058**)			

Length (-0.054, 0.480)			
Ba	0.36	0.04	0.781
Species (0.131, 0.682)			
Sex (0.218, 0.341)			
Length (0.009, 0.854)			
Cd	1.19	0.13	0.334
Species (-0.773, 0.281)			
Sex (-0.753, 0.143)			
Length (-0.043, 0.701)			
Co	4.96	0.38	**0.007**
Species (-0.352, 0.153)			
Sex (0.038, 0.810)			
Length (0.041, 0.259)			
Cr	5.03	0.38	**0.007**
Species (2.339, 0.074)			
Sex (0.244, 0.787)			
Length (-0.149, 0.464)			
Cu	3.88	0.32	**0.021**
Species (-0.05, 0.900)			
Sex (-0.507, 0.088)			
Length (0.122, 0.069)			
Fe	5.03	0.38	**0.007**
Species (-0.499, **0.014**)			
Sex (-0.019, 0.884)			
Length (-0.006, 0.842)			
Hg	19.00	0.69	**<0.001**
Species (-0.060, **<0.001**)			
Sex (0.0137, 0.142)			
Length (0, 0.815)			
Mn	1.61	0.16	0.2143
Species (-0.833, 0.477)			
Sex (-0.570, 0.494)			
Length (0.122, 0.515)			
Mo	1.90	0.19	0.155
Species (-0.637, 0.199)			
Sex (-0.442, 0.210)			
Length (0, 0.992)			
Ni	8.36	0.50	**<0.001**
Species (0, **0.042**)			

Sex (0, **0.052**)			
Length (0, 0.079)			
Pb	1.678	0.17	0.197
Species (-0.404, 0.110)			
Sex (-0.265, 0.140)			
Length (-0.027, 0.500)			
Sb	9.74	0.54	**<0.001**
Species (-0.027, **0.020**)			
Sex (0.009, 0.241)			
Length (0.0018, 0.307)			
Se	15.82	0.66	**<0.001**
Species (0.659, **<0.001**)			
Sex (0.108, 0.222)			
Length (0.0285, 0.152)			
Sn	15.39	0.65	**<0.001**
Species (-0.502, **0**)			
Sex (-0.061, 0.506)			
Length (0.010, 0.634)			
V	3.062	0.27	**0.047**
Species (-0.166, 0.121)			
Sex (-0.044, 0.554)			
Length (0.006, 0.705)			
Zn	40.01	0.83	**<0.001**
Species (-1.191, **<0.001**)			
Sex (0.001, 0.992)			
Length (0.014, 0.620)			

Explanatory variables (Estimate std., p-value)
Significant coefficients (p < 0.05, bold numbers)

The linear correlation coefficients indicate a strong relationship between the total length and weight for Peruvian mojarra and between total length and width for catfish (Table 1.9).

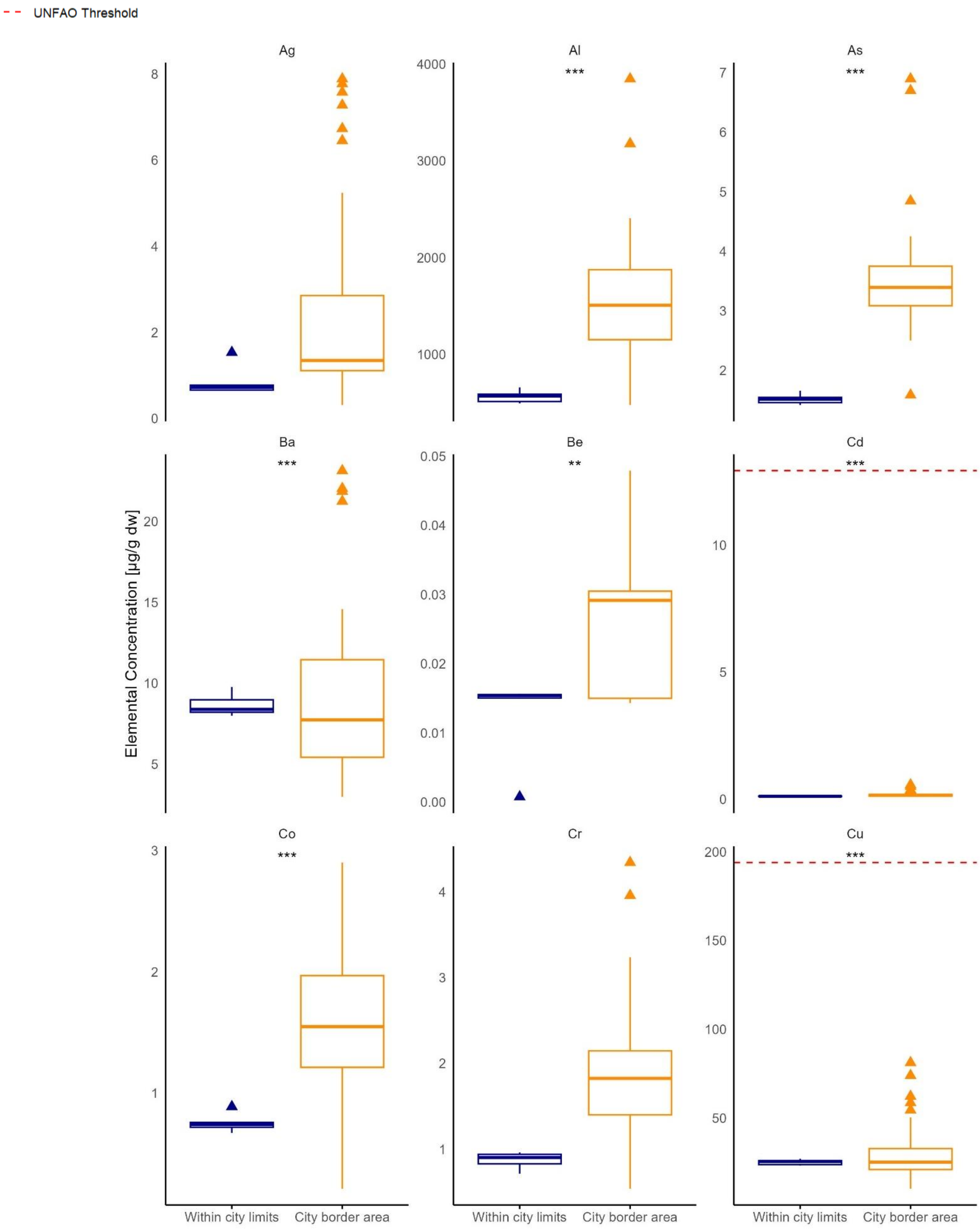
Table 1.9 Correlation matrix for length, weight, and width of fish samples with values of Pearson's correlation coefficients, r (n = 30).

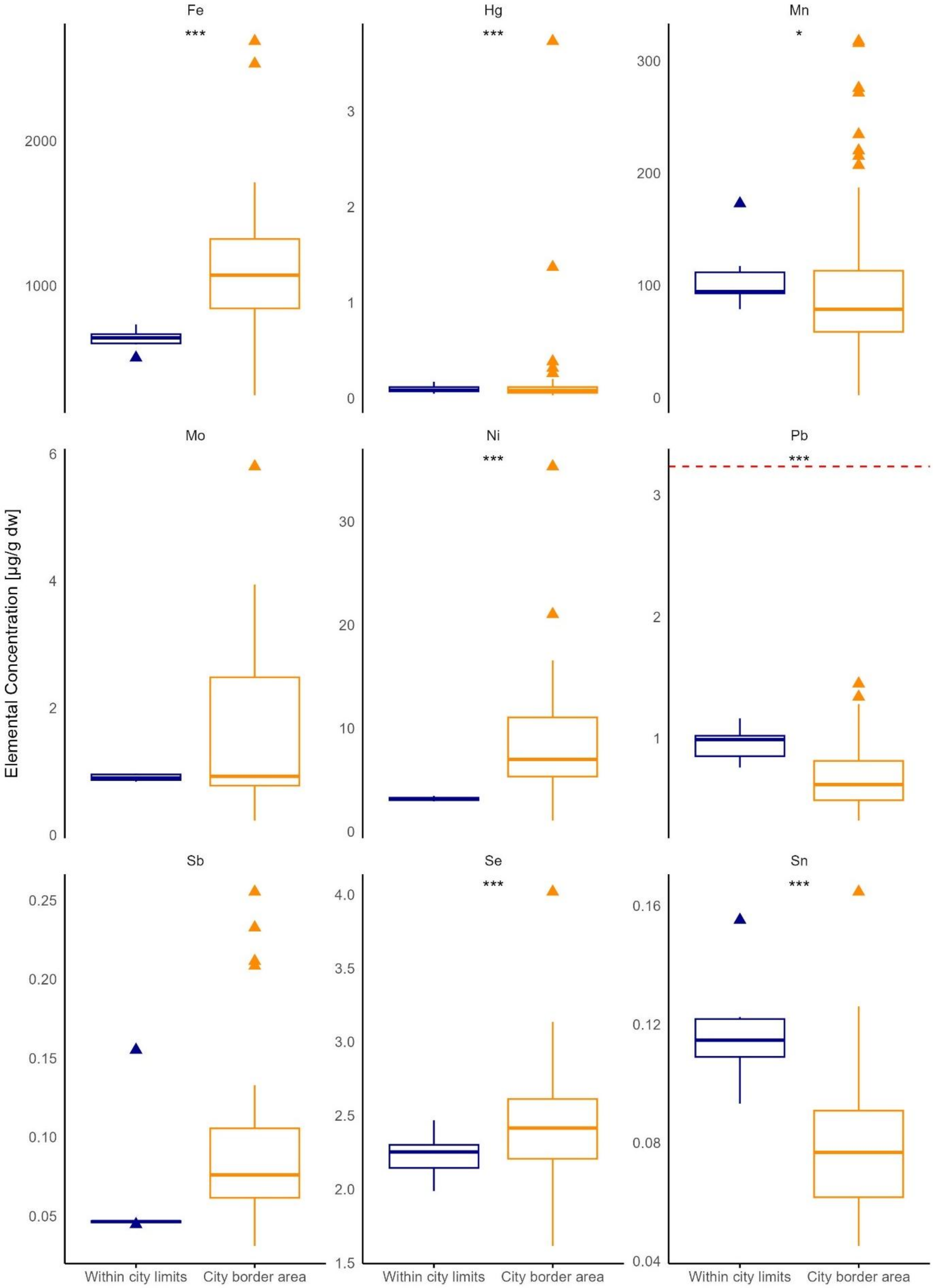
	Peruvian Mojarra			**Catfish**		
	Length	Weight	Width	Length	Weight	Width
Length	-			-		
Weight	**0.92**	-		0.38	-	
Width	0.40	0.47	-	**0.86**	0.15	-

Significant coefficients ($p < 0.05$, bold numbers)

1.3.3 Mussels

For *M. trautwineana*, out of the 20 metal(loid)s analyzed, 75% displayed statistically significant differences between the two zones ("within city limits" and "city border area"*;* Fig. 1.9). Significant differences were found for Al, As, Ba, Be, Cd, Co, Cu, Fe, Hg, Mn, Pb, Se, Sn, V and Zn (Fig. 1.9). Except for Pb and Sn, all significantly different metal(loid)s had lower mean concentrations in "within city limits" compared to the "city border area" (Table 1.10, Fig. 1.9). However, only a few samples of mussels, all of which were *M. trautwineana*, were found in "within city limits" (Fig. 1.10). Metal(loid) concentration in mussel samples of all three species (*M. guyanensis*, *M. strigata* and *M. trautwineana*) showed that only Zn exceeded the FAO guidelines, and that was for just a single sample of *M. trautwineana* in the "city border area" (Figs. 1.9 & 1.10).


UNFAO Threshold
Ag
Al
***
As
***
Ba
***
Be
**
Cd
***
Co
***
Cr
Cu
***
Elemental Concentration [µg/g dw]
0
2
4
6
8
1000
2000
3000
4000
2
3
4
5
6
7
5
10
15
20
0.00
0.01
0.02
0.03
0.04
0.05
0
5
10
1
2
3
1
2
3
4
50
100
150
200
Within city limits
City border area
Within city limits
City border area
Within city limits
City border area


Fe
***
2000
1000
Hg
***
3
2
1
0
Mn
*
300
200
100
0
Mo
6
4
2
0
Ni
***
30
20
10
0
Pb
***
3
2
1
Sb
0.25
0.20
0.15
0.10
0.05
Se
***
4.0
3.5
3.0
2.5
2.0
1.5
Sn
***
0.16
0.12
0.08
0.04
Elemental Concentration [µg/g dw]
Within city limits
City border area
Within city limits
City border area
Within city limits
City border area

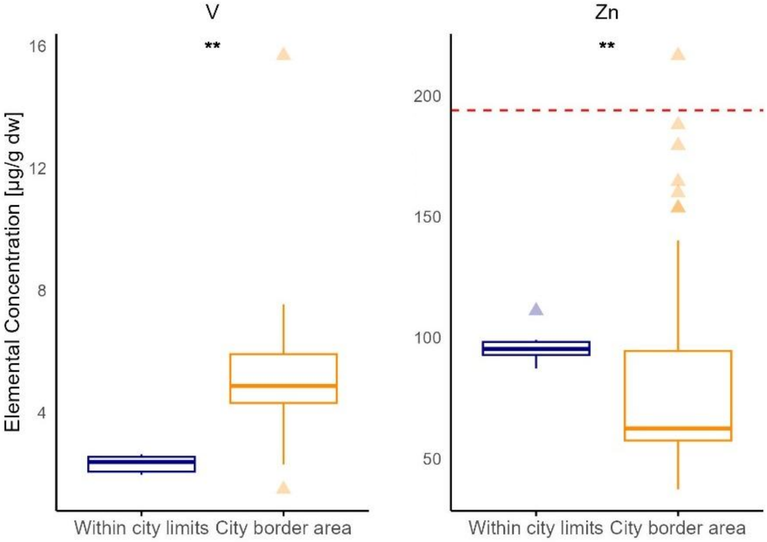


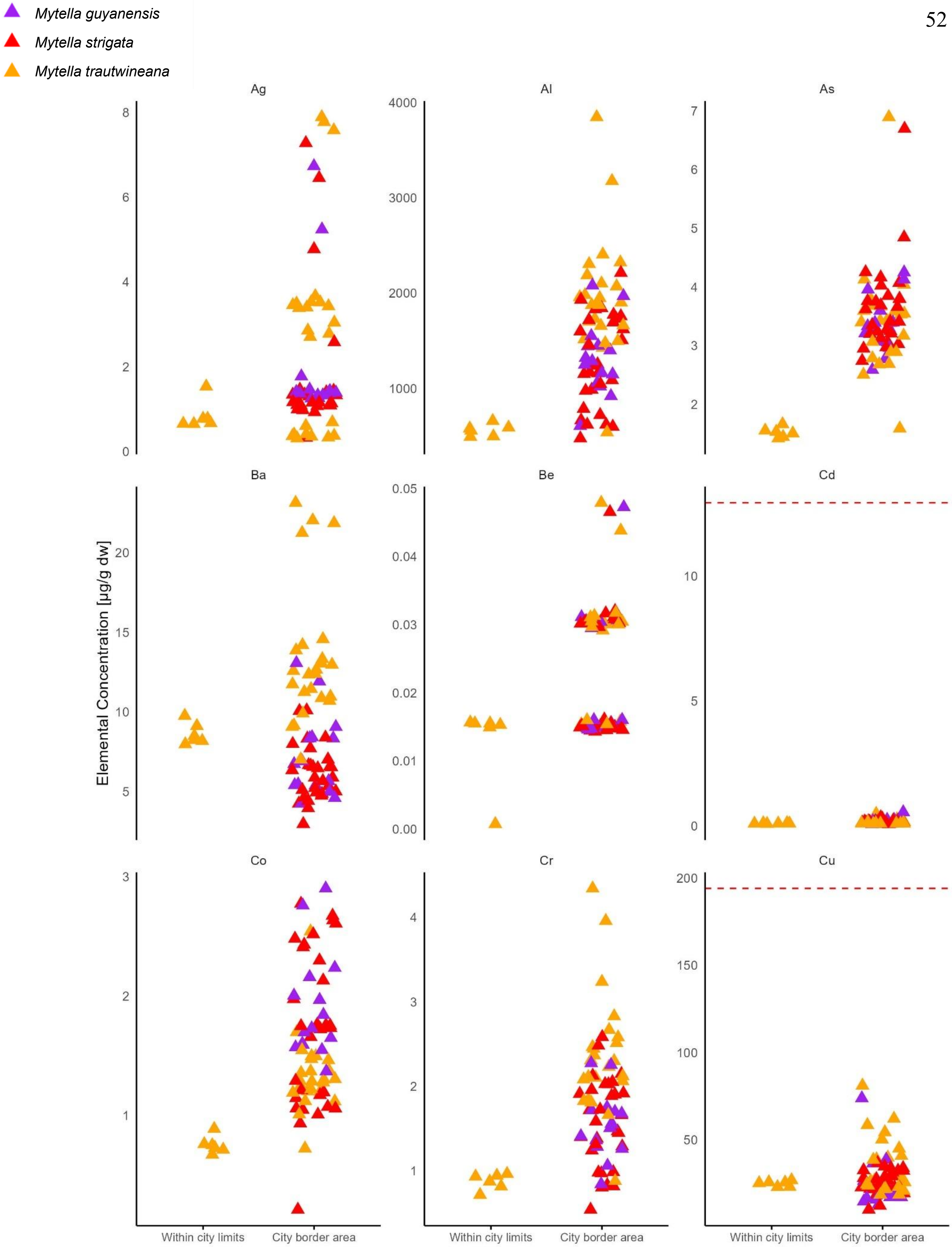
*Figure 1.9 Metal(loid) concentrations in mussels (Mytella trautwineana) in Reserva de Producción Faunística Manglares El Salado (RPFMS). *<.05 **<.01 ***<.001.*

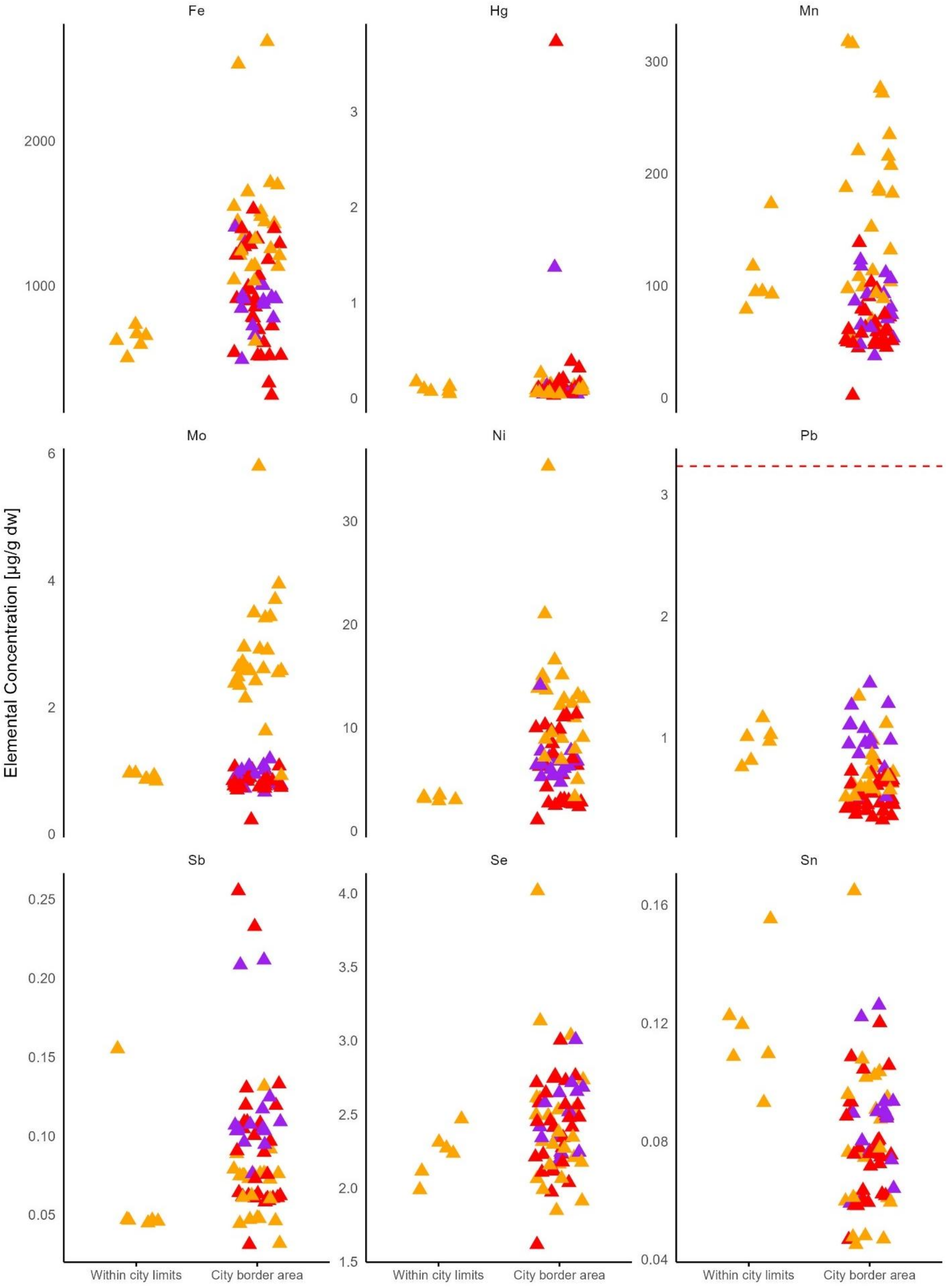
Table 1.10 Mussels (Mytella trautwineana) metal(loid) concentrations ($\frac{\mu g}{g}$ dw) in Reserva de Producción Faunística Manglares El Salado (RPFMS).

Reserve	**RPFMS** **N= 30**				
	mean ± sd **range**				
	within city limits	**city border area**	**combined**	**test**	***p*-value**
Metal(loid)					
Ag	0.85 ± 0.34	2.77 ± 2.36	2.38 ± 2.25	MWU	NS
	(0.65, 1.54)	(0.31, 7.89)	(0.31, 7.89)		
Al	563.35 ± 62.42	1961.50 ± 620.44	1681.87 ± 793.43	MWU	**< 0.001**
	(493.32, 659.62)	(539.73, 3848.70)	(493.32, 3848.70)		
As	1.52 ± 0.09	3.49 ± 0.93	3.02 ± 1.13	MWU	**< 0.001**
	(1.42, 1.66)	(1.59, 6.90)	(1.42, 6.90)		
Ba	8.64 ± 0.68	13.44 ± 4.32	12.48 ± 4.32	Welch's	**< 0.001**
	(7.98, 9.77)	(7.03, 23.14)	(7.03, 23.14)		
Be	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	MWU	**< 0.01**
	(0.00, 0.02)	(0.01, 0.05)	(0.00, 0.05)		

Cd	0.11 ± 0.01	0.15 ± 0.08	0.14 ± 0.08	MWU	**< 0.001**
	(0.09, 0.13)	(0.09, 0.50)	(0.09, 0.50)		
Co	0.75 ± 0.07	1.35 ± 0.32	1.23 ± 0.38	MWU	**< 0.001**
	(0.67, 0.89)	(0.72, 2.54)	(0.67, 2.54)		
Cr	0.87 ± 0.09	2.36 ± 0.72	2.06 ± 0.88	MWU	NS
	(0.71, 0.96)	(0.88, 4.34)	(0.71, 4.34)		
Cu	25.04 ± 1.56	34.99 ± 16.43	33.00 ± 15.19	MWU	**< 0.001**
	(23.11, 27.01)	(18.71, 81.19)	(18.71, 81.19)		
Fe	628.89 ± 77.84	1420.36 ± 439.78	1262.07 ± 508.05	Welch's	**< 0.001**
	(501.88, 732.29)	(616.65, 2687.09)	(501.87, 2687.09)		
Hg	0.10 ± 0.04	0.10 ± 0.05	0.10 ± 0.04	Welch's	**< 0.001**
	(0.05, 0.17)	(0.04, 0.26)	(0.04, 0.26)		
Mn	108.59 ± 33.95	165.67 ± 79.61	154.25 ± 75.93	Welch's	**< 0.05**
	(78.95, 173.10)	(54.84, 317.74)	(54.84, 317.74)		
Mo	0.90 ± 0.05	2.82 ± 0.90	2.44 ± 1.12	MW	NS
	(0.84, 0.96)	(0.92, 5.80)	(0.84, 5.80)		
Ni	3.18 ± 0.20	12.54 ± 6.29	10.67 ± 6.77	Student's	**< 0.001**
	(2.92, 3.46)	(3.30, 35.31)	(2.92, 35.31)		
Pb	0.96 ± 0.15	0.69 ± 0.21	0.74 ± 0.23	Student's	**< 0.001**
	(0.76, 1.16)	(0.39, 1.34)	(0.39, 1.34)		
Sb	0.06 ± 0.04	0.07 ± 0.02	0.07 ± 0.03	MWU	NS
	(0.04, 0.16)	(0.03, 0.13)	(0.03, 0.16)		
Se	2.23 ± 0.17	2.43 ± 0.46	2.39 ± 0.43	Student's	**< 0.001**
	(1.99, 2.47)	(1.85, 4.02)	(1.85, 4.02)		
Sn	0.12 ± 0.02	0.08 ± 0.03	0.09 ± 0.03	Welch's	**< 0.001**
	(0.09, 0.16)	(0.05, 0.16)	(0.05, 0.16)		
V	2.31 ± 0.29	6.40 ± 2.29	5.58 ± 2.63	MWU	**< 0.01**
	(1.96, 2.62)	(2.30, 15.69)	(1.96, 15.69)		
Zn	96.73 ± 8.10	123.88 ± 35.32	118.45 ± 33.51	Welch's	**< 0.01**
	(87.23, 111.15)	(80.77, 216.72)	(80.77, 216.72)		

Statistical analysis: t-test (Student's or Welch's test) for normal distribution, MWU test for non-normal distribution
Significant coefficients (p < 0.05, p < 0.01, p < 0.001, ***bold numbers****)*


Mytella guyanensis
Mytella strigata
Mytella trautwineana
Elemental Concentration [µg/g dw]
Ag
0
2
4
6
8
Al
1000
2000
3000
4000
As
2
3
4
5
6
7
Ba
5
10
15
20
Be
0.00
0.01
0.02
0.03
0.04
0.05
Cd
0
5
10
Co
1
2
3
Cr
1
2
3
4
Cu
50
100
150
200
Within city limits
City border area
Within city limits
City border area
Within city limits
City border area


Fe
Hg
Mn
Mo
Ni
Pb
Sb
Se
Sn
Elemental Concentration [µg/g dw]
2000
1000
3
2
1
0
300
200
100
0
6
4
2
0
30
20
10
0
3
2
1
0.25
0.20
0.15
0.10
0.05
4.0
3.5
3.0
2.5
2.0
1.5
0.16
0.12
0.08
0.04
Within city limits
City border area
Within city limits
City border area
Within city limits
City border area

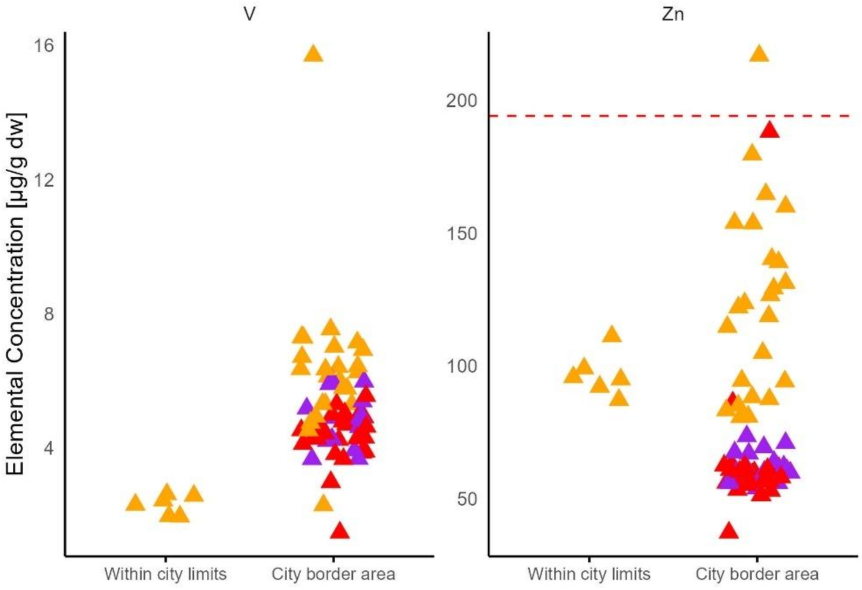


Figure 1.10 Metal(loid) concentrations in mussels (Mytella guyanensis, Mytella strigata and Mytella trautwineana) in Reserva de Producción Faunística Manglares El Salado (RPFMS).

Mytella trautwineana, shown in red in Fig. 11, was the only species found in the "within city limits" group and is primarily located on the positive side of PC1. *Mytella trautwineana* samples from the "city border area" are clustered on the negative side of PC1, while *M. guyanensis* and *M. strigata* are positioned more strongly along the positive axis of PC2 (Fig. 1.11).

PC1 is driven by negative loadings for all tested elements, with V, Fe, Cr, Mo, and Al showing the strongest negative loadings. On the other hand, PC2 shows strong positive loadings for Sb, Co, As, Ag and Cd (Table 1.11). The position of *M. trautwineana* "within city limits" lies along the positive end of PC1, suggesting that this site has lower metal(loid) concentrations than the "city border area". Furthermore, *M. trautwineana* shows generally lower PC1 values than the other two mussel species, suggesting that it has higher metal(loid) concentrations (Figs. 1.10 & 1.11).

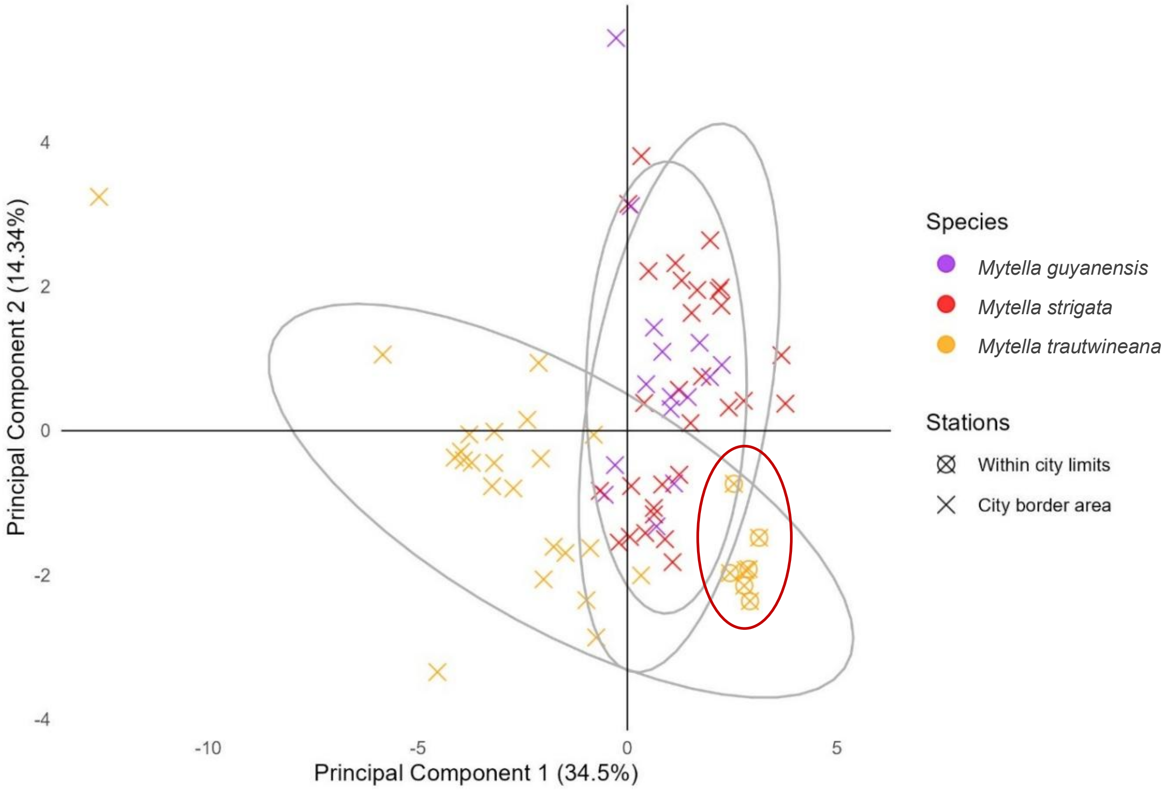


Figure 1.11 Principal component analysis (PCA) biplot of metal(loid)s in mussels at the stations "within city limits" and "city border area" in Reserva De Producción Faunística Manglares El Salado (RPFMS).

Table 1.11 Principal component loadings of log-transformed metal(loid) concentrations (n=75) in mussels, with elements organized for each PC by rank order of the absolute value of the loading.

Metal(loid)	PC1	Metal(loid)	PC2
V	-0.351	Sb	0.436
Fe	-0.333	Co	0.413
Cr	-0.332	As	0.298
Mo	-0.327	Ag	0.284
Al	-0.313	Cd	0.251
Ba	-0.290	Se	-0.187
Zn	-0.272	Pb	0.176
Ni	-0.251	Ni	-0.174
Be	-0.226	Mn	-0.150
Cu	-0.226	Zn	-0.143
Mn	-0.205	Fe	-0.127
Ag	-0.185	Ba	-0.107

Se	-0.141	Be	-0.104
As	-0.126	Mo	0.103
Cd	-0.085	V	-0.079
Co	-0.068	Al	-0.074
Sn	0.047	Cr	0.056
Pb	-0.042	Sn	0.011
Hg	0.003	Cu	0.005
Sb	0.000	Hg	0.436

1.3.4 Health risk assessment

iAs, Cd, Cr(VI) and MeHg had THQ values below 1, indicating no significant non-carcinogenic health risk in fish and mussels. iAs and Cr(VI) CR values for Peruvian mojarra and only iAs for catfish exceeded the baseline (Table 1.12). For mussels, only iAs indicated values above the CR threshold. However, none of the values for Cd, Cr(VI), or MeHg exceeded the THQ or CR limits. An HI remained below 1 for fish species and mussels.

Table 1.12 Target hazard quotient (THQ), hazard index (HI), and cancer risk (CR) results for inorganic arsenic (iAs), Cd, hexavalent chromium (Cr(VI)) and methylmercury (MeHg) in fish and mussels.

Species	**Metal(loid)**	**EDI** ($\frac{mg}{kg\ day}$)	**RfD** ($\frac{mg}{kg\ day}$)	**CSF** ($\frac{mg}{kg\ day}$)	**THQ mean**	**HI**	**CR mean**
Catfish	iAs	6.91×10^{-6}	6.00×10^{-5}	32	12.0×10^{-2}*	0.15*	**2.21×10^{-4}**
	Cd	1.04×10^{-6}	1.00×10^{-3}	-	1.04×10^{-3}*		-
	Cr(VI)	1.35×10^{-6}	9.00×10^{-4}	0.27	1.50×10^{-3}*		3.64×10^{-7}**
	MeHg	3.33×10^{-6}	1.00×10^{-4}	-	3.30×10^{-2}*		-
Peruvian Mojarra	iAs	1.64×10^{-5}	6.00×10^{-5}	32	27.0×10^{-2}*	0.34*	**5.23×10^{-4}**
	Cd	7.86×10^{-7}	1.00×10^{-3}	-	7.86×10^{-4}*		-
	Cr(VI)	1.02×10^{-5}	9.00×10^{-4}	0.27	1.14×10^{-2}*		**2.77×10^{-6}**
	MeHg	5.14×10^{-6}	1.00×10^{-4}	-	5.15×10^{-2}*		-
Mussels	iAs	3.01×10^{-6}	6.00×10^{-5}	32	6.08×10^{-2}*	0.06*	**9.64×10^{-5}**
	Cd	1.28×10^{-6}	1.00×10^{-3}	-	1.29×10^{-3}*		-
	Cr(VI)	9.12×10^{-8}	9.00×10^{-4}	0.27	1.01×10^{-4}*		2.46×10^{-8}**
	MeHg	7.66×10^{-7}	1.00×10^{-4}	-	7.66×10^{-3}*		-

EDI: Estimated daily intake; RfD: Reference dose; THQ: Target hazard quotient; HI: Hazard index; CR: Cancer risk
*Below acceptable baseline risk for THQ, <1, HI, <1
**Below acceptable baseline risk for CR, $<10^{-6}$
Bold, above acceptable baseline

The sensitivity analysis revealed that IR and EF had significant positive effects on the fish and mussels for THQ and CR, followed by C, while BW showed a negative influence; ED and AT showed negligible sensitivity for all species in fish and mussels (Table 1.13, Fig. 1.12). The concentration of Cd in catfish was treated as a constant because the observed values exhibited almost no variability. As a result, it was not modeled as a stochastic variable.

Table 1.13 Spearman correlation values for inorganic As (iAs), Cd, hexavalent Cr (Cr(VI)) and methylmercury (MeHg) in catfish, Peruvian mojarra and mussels for concentration (C), ingestion rate (IR), exposure frequency (EF), exposure duration (ED),body weight (BW) and average time (AT).

Species	**Metal(loid)**	**C**	**IR**	**EF**	**ED**	**BW**	**AT**
Catfish	iAs	0.267	0.706	0.604	-0.008	-0.107	-0.003
	Cd	-	0.774	0.593	-0.004	-0.140	0.003
	Cr(VI)	0.372	0.694	0.554	-0.012	-0.102	-0.010
	MeHg	0.066	0.765	0.602	-0.010	-0.109	-0.008
Peruvian Mojarra	iAs	0.321	0.718	0.540	0.014	-0.113	-0.007
	Cd	0.470	0.660	0.492	0.015	-0.098	-0.013
	Cr(VI)	0.298	0.727	0.546	0.014	-0.114	-0.007
	MeHg	0.301	0.724	0.550	0.014	-0.114	-0.007
Mussels	iAs	0.326	0.757	0.508	-0.006	-0.012	-0.013
	Cd	0.637	0.612	0.413	0.001	-0.017	-0.008
	Cr(VI)	0.209	0.783	0.535	0.016	-0.025	-0.009
	MeHg	0.733	0.527	0.357	-0.009	-0.014	-0.011

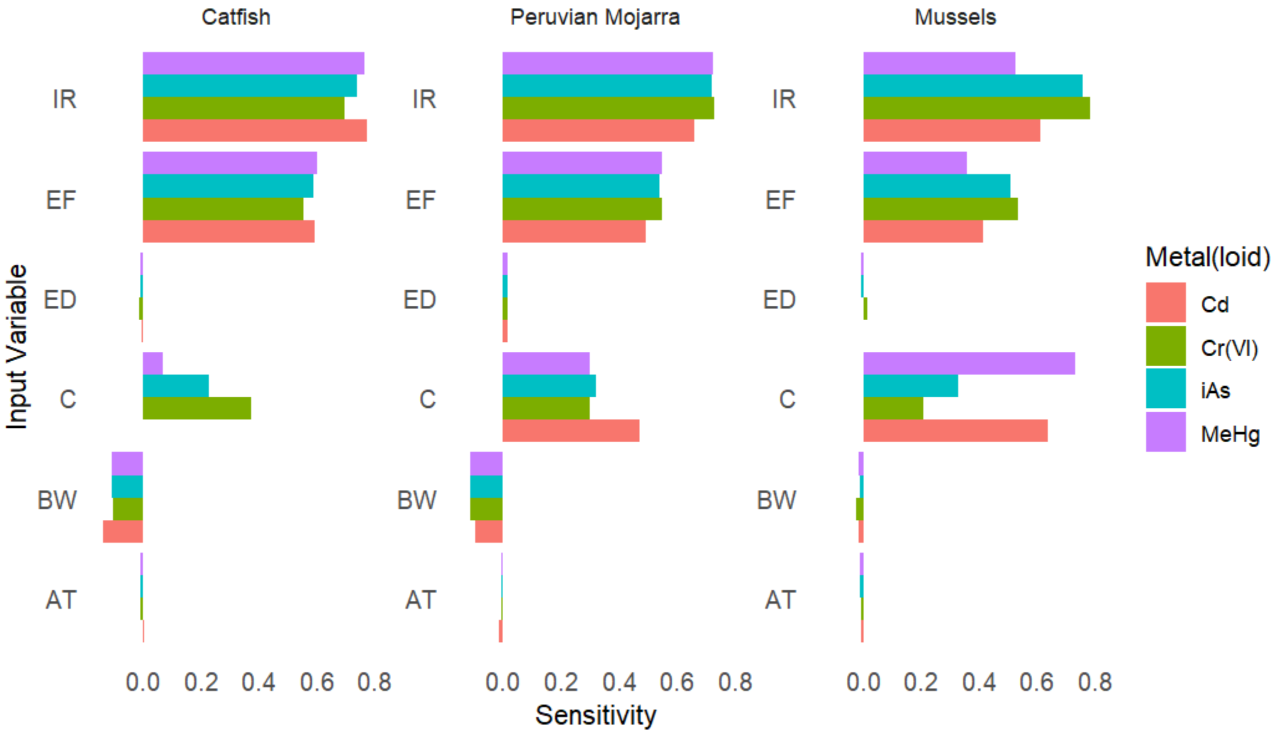


Figure 1.12 Sensitivity analysis of ingestion rate (IR), exposure frequency (EF), exposure duration (ED), concentration (C), body weight (BW) and average time (AT) in catfish, Peruvian mojarra and mussels.

For Pb, all MOE values for catfish, Peruvian mojarra, and mussels were above 100, indicating unlikely potential health risk for the population (Table 1.14) related to Pb exposure. Mussels exhibited the highest EDI values, followed by Peruvian mojarra and catfish.

Table 1.14 Margin of exposure (MOE) results for catfish, Peruvian mojarra and mussels for Pb.

	EDI($\frac{mg}{kg\ day}$) **mean**	**BMDL** ($\frac{mg}{kg\ day}$)	**MOE mean**
Catfish	4.47×10^{-6}	0.5	1.12×10^{5}
		0.63	1.41×10^{5}
		1.5	3.35×10^{5}
Peruvian Mojarra	8.38×10^{-6}	0.5	5.96×10^{4}
		0.63	7.51×10^{4}
		1.5	1.79×10^{5}
Mussels	8.52×10^{-6}	0.5	5.87×10^{4}
		0.63	7.39×10^{4}
		1.5	1.76×10^{5}

EDI: Estimated daily intake; BMDL: Benchmark-dose lower bound; MOE: Margin of exposure

Discussion

1.4.1 Sediment

The sediment sample analysis showed that Ag, Al, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sn, V and Zn were higher in "within city limits" than in "city border area" (Table 1.5). For both sites combined, the mean concentration in rank order was Fe> Al> Mn> Zn> Ba> Cu> V> Cr> Ni> Pb> Co> As> Mo> Se> Sn> Cd> Be> Ag> Sb> Hg.

Of the 20 metal(loid)s studied, Ag, As, Cd, Cr and Pb exhibited concentrations above the ERL guidelines, while Al, Ba, Be, Co, Fe, Mn, Mo, Sb, Se, Sn, and V were below this threshold (Fig. 1.4), suggesting a lower probability of adverse effects for these elements on sediment-dwelling biota[93]. In contrast, four metal(loid)s (Cu, Ni, Hg and Zn) exceeded the ERM values (Fig. 1.4), indicating that negative ecological effects are more likely[93]. These exceedances suggest that certain elemental concentrations in mangrove sediments pose an ecological risk. Hg sediment concentrations exceeded ERL values for both sites. However, the values were lower than in previous years[35]. This could be due to either a temporal trend or spatial variation in the sampling sites. Additional studies are needed to differentiate these possibilities.

Urban runoff entering the mangrove ecosystem at RPFMS has led to increased chemical contamination, potentially compromising the health of the environment[35]. Our results are consistent with those of Calle *et* al. (2018)[35] and Cadena *et* al. (2014)[94], which also showed that RPFMS is affected by metal(loid) pollution. Due to the lack of sediment quality guidelines in Ecuadorian legislation, our comparisons reference studies that employ soil baseline values. Medina *et* al. (2017)[28] found high level of Cd, Hg and Pb, surpassing the Ecuadorian soil limits according to the Texto Unificado de Legislación Secundaria de Medio Ambiente (TULSMA), in RPFMS in the "within city limits" area. In the current study, metal(loid) concentrations in sediment imply that both sampling zones within RPFMS ("within city limits" and "city border area") are impacted by human activities with elevated concentration of multiple toxic metal(loid)s in the sediment. However, the "within city limits" site appears to be more affected, likely due to its proximity to the city, which is associated with higher urban development[13], industrial activity[95], and increased wastewater effluents[96]. Those activities have been linked previously to mangrove pollution around the world[94].

1.4.2 Fish

Fish were captured in Puerto Hondo due to the low species richness and limited fish availability "within city limits" in the RPFMS. Catfish showed higher concentrations of most metal(loid)s compared to Peruvian mojarra (Figs. 1.5, 1.7 & 1.8), presumably due to their higher trophic level. Catfish are part of the Ariidae family[97] and are bottom dwellers with an omnivorous bentophagous diet[98]. They are tertiary consumers that occupy a higher position in the food web (trophic level: 4.5)[99], which leads to greater bioaccumulation and trophic transfer of toxic contaminants[100]. However, Peruvian mojarra showed higher concentrations of Cr and Se. Overall, the lower concentrations of Cr and Se could be due to their proximity to strong estuarine currents with higher water flow.

PC1 accounted for 41.38% of the variance of metal(loid) concentration in fish (Fig. 1.6). Sn, Zn, Fe and Al had high positive scores, suggesting they are driving the primary axis of variation in toxic metal(loid) concentration in fish (Table 1.7). PC2 accounted for 14.96% of the variance; most elements loaded negatively (Table 1.7). PC1 and PC2 were positively correlated with fish length (Fig. 1.7), indicating bioaccumulation of metal(loid)s as fish have indeterminant growth and hence larger fish are typically older[99,101]. The Peruvian mojarra samples may have been juveniles, which would limit their bioaccumulation potential compared to the typically older and larger catfish, which, along with the lower trophic level of Peruvian mojarra, could explain their generally lower concentrations of metal(loid)s. Multiple metal(loid)s (Ag, Co, Cr, Cu, Fe, Hg, Sb, Se, Sn, V, and Zn) were highly associated with fish length ($p < 0.05$) (Table 1.8), suggesting bioaccumulation of these contaminants. Sex appeared to have a negligible effect (Table 1.8).

1.4.3 Mussels

Bivalves have been widely used in pollution biomonitoring programs due to their sessile nature, ecological role as filter feeders[35], commercial value, and capacity to accumulate contaminants[102,103]. In general, intrinsic and extrinsic factors such as species, age, sex, genotype, reproductive activity, and environmental location and hydrodynamics of the sampling site influence the toxic metal(loid) content of these organisms[104-106]. In different provinces around Ecuador, bivalves, including mussels, have been found to contain elevated levels of Cd, Pb and Hg[107]. Particularly in the "within city limits" area of RPFMS, *M. guyanensis* presented high levels of Pb, but no detectable Cd levels[108]. *Mytella trautwineana* was the only species found at both sampling sites (Fig. 1.9) with higher metal(loid) concentrations in the "city border area", excluding Pb and Sn. *Mytella trautwineana* exhibited greater maximum metal(loid) concentrations compared to *M. guyanensis* and *M. strigata* in "city border area" for almost all metal(loid)s, excluding Cd, Co, Hg, Pb and Sb (Fig. 1.10). V, Fe, Cr, Mo, and Al had high negative scores, suggesting they are driving the primary axis of variation in toxic metal(loid) concentration in mussels, while Sb, Co, As, Ag and Cd had positive scores. *Mytella guyanensis* and *M. strigata* were only found in "city border area". Mussel biodiversity is low at stations located "within city limits" (Fig. 1.10), possibly due to high pollution levels in the city. The concentrations of metal(loid)s observed in mussels "within city limits" (Fig. 1.10) may be biased, because elevated pollution levels at these substations may result in only those mussels with low concentrations surviving. Indeed, we found fewer individuals "within city limits" compared to the "city border area". Additional sampling "within city limits" is necessary to resolve whether the lower concentrations are actual or due to survivorship bias.

1.4.4 Risk to Puerto Hondo

While previous investigations have focused on metal(loid) pollution in RPFMS, specifically Puerto Hondo[96,109], this study represents the first attempt to provide a preliminary health risk assessment for the Puerto Hondo community. Earlier studies in Ecuador (El Oro province) identified health risks from consuming a shellfish species, *Anadara tuberculosa*, particularly due to Cd and Pb contamination[62]. Our findings suggest similar concerns for the species examined in this study for Pb (Table 1.14).

The EDI values for metal(loid)s were higher in Peruvian mojarra than in catfish for iAs, Cr(VI), Pb and MeHg (Table 1.12). Even though there are greater metal(loid) concentrations in bottom-feeding species (Fig. 1.5), the IR and EF results for catfish are lower than they are for Peruvian mojarra, leading to greater EDI values in Peruvian mojarra (Table 1.12). THQ values did not exceeded 1 in both fish species and mussels, which indicates not expected health risks related to the analyzed metal(loid)s (Table 1.12). As a result, HI, which reflects the cumulative non-carcinogenic risk, was also below 1 in both species, supporting no health risk (Table 1.12). In mussels, no metal(loid) surpassed the THQ threshold or the HI guideline of 1 (Table 1.12). This could be related to lower use of mussels in the diet of Puerto Hondo residents compared to fish, as found in our survey. Despite employing a conservative value of MeHg present in mussel tissue (89%), the risk was below the baseline. The proportion of MeHg in the collected mussels must likely contain a lower percentage of MeHg in Hg. Nonetheless, the risk will fall in the threshold area lower than the one reported in the present study (Table 1.12).

For CR values, the lower value of 10^{-6} is the desired goal where the risk is considered minimal, and no further action is needed. However, in environments where it is not possible to achieve, EPA has allowed 10^{-4} acceptable risk in regulatory settings[110]. In this study, four of the six CR target values were between 10^{-6} and 10^{-4}. These values may represent risks that need to be attended to. iAs CR values for all fish species and mussels and Cr(VI) CR results for Peruvian Mojarra were above the critical threshold of 10^{-4}, where management decisions must be considered (Table 1.12). Overall, these results raise carcinogenic concerns regarding fish and mussel consumption in Puerto Hondo.

The sensitivity analysis from the Monte Carlo simulation indicated that IR and EF variables were the primary drivers of risk for both fish species, followed by C (Table 1.13, Fig.1.12). This is closely associated with the high concentrations of iAs and Cr found in catfish and Peruvian mojarra from RPFMS, combined with the frequent consumption of Peruvian mojarra by Puerto Hondo residents. Similarly, IR and EF were the most influential variables in the analysis for mussels, followed by C. Local residents reported consuming fish, with IR values ranging from 0.042 to 0.091 $\frac{kg}{day\ person}$, compared to 0.032 $\frac{kg}{day\ person}$ reported across Latin America and the Caribbean[111]. In contrast, mussel consumption was lower than fish consumption with a reported IR of 0.019 $\frac{kg}{day\ person}$.

Cd negative health endpoints involves effect on kidneys[65,112,113]. MeHg non-carcinogenic adverse outcomes include developmental neuropsychological impairment in children if consumed during pregnancy[87]and cardiovascular effects in adults[114]. Levels above the permissible limits for iAs and Cr(VI) are linked to both non-carcinogenic and carcinogenic health effects. According to EPA's Integrated Risk Information System (IRIS), iAs is associated with skin, bladder, and lung cancer, as well as skin lesions. iAs is also linked to diabetes and ischemic heart disease (IHD)[86]. Even at low concentrations, iAs may act as an endocrine disruptor[115,116]. Cr(VI) is primarily related to toxicity in the respiratory and gastrointestinal tracts, as well as liver damage and developmental effects in humans[67].

MOE values for Pb were all above the threshold of 100 (Table 1.14), indicating low to no potential public health concern from exposure in both fish species and mussels. The higher the MOE, the lower the risk over a 100 baseline. Pb exposure reduces IQ in children[117], and has numerous neurological effects[118]. Pb also causes kidney damage and adverse heart outcomes in adults[89].

1.5 Limitations

We measured total Hg rather than MeHg, although the health risk assessment for fish was based on MeHg. However, the EPA recommends using total Hg as a substitute for MeHg when comparing results to their consumption guidelines for unlimited intake and nearly all Hg in fish is MeHg[70].

The low number of mussels found "within city limits" could affect the overall comparison between zones in RPFMS limiting our statistical power to detect differences. Only six samples of *M. trautwineana* were found in the Velero substation. Elevated pollution levels "within city limits" may have led to poor sampling success in this area.

Given that ICP-MS was employed in the study, only the total concentrations of the metal(loid)s were reported. Speciation for iAs, Cr(VI), and MeHg in the fish and mussel samples was not conducted. Therefore, the proportions used in the health risk assessment were based on literature recommendations.

1.6 Conclusions

Our result revealed significant differences in concentrations of metal(loid)s in sediment, fish and mussels in the “within city limits” area compared to the “city border area” in RPFMS. For sediment, Ag, Al, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sn, V and Zn were higher in “within city limits” than in “city border area”. Sediments showed high concentrations of toxic metal(loid)s that surpassed the SQGs. Ag, As, Cd, Cr and Pb showed concentrations above the ERL values, while Cu, Ni, Hg and Zn exceeded both the ERL and ERM values. Fish samples exceeded EPA guidelines for unlimited consumption. iAs and MeHg surpassed the DCM and NDCM guidelines, while Cd and Se exceeded only the NDCM thresholds in both species, with catfish having higher values for most metal(loid)s compared to Peruvian mojarra. Species, sex and length were all significant predictors of PC1 and PC2. For *M. trautwineana*, except for Pb and Sn, all metal(loid)s had lower mean concentrations in “within city limits” compared to the “city border area” with Zn surpassing the FAO guidelines with one sample. This was the only species found in both sampling areas of RPFMS. *Mytella guyanensis* and *M. strigata* were only found in “city border area”. In general, mussels showed higher concentration of contaminants in “city border area” with most negative loadings for PC1 and positive loadings for PC2 possibly due to the higher pollution in ‘within city limits’ site. Peruvian mojarra from Puerto Hondo presented higher EDI values than catfish, with iAs and Cr(VI) posing a carcinogenic health risk due to fish consumption (iAs and Cr(VI) for Peruvian mojarra and only iAs for catfish), and iAs posing a carcinogenic health risk due to consumption of mussels. HI values were below the threshold limit of 1 in both fish species and mussels. MOE values related to Pb health effects were above the baseline for fish and mussels, suggesting no health concern.

Our findings suggest biomonitoring additional contaminants in the RPFMS reserve in aquatic organisms (*A. tuberculosa*) and water. For Puerto Hondo, recommendations may include reducing the weekly consumption frequency of contaminated species and diversifying diets with fish from lower trophic levels. Epidemiological studies are also suggested for local residents collecting biomarkers. Given the economic constraints faced by Puerto Hondo, whose livelihood depends on aquatic resources, external and governmental aid will be fundamental. Overall, implementing a remediation plan that includes depuration strategies for toxic metal(loid)s in the RPFMS is needed to safeguard the reserve’s environmental quality.

Chapter 2: Ecotoxicology of RPFMS and REVISMEM

2.1 Introduction

Mangroves are ecologically central components of tropical estuarine ecosystems that provide economic and ecological services to communities living on their surroundings[4,7], including coastal zone protection[119-121], nursery habitat for many marine species[122], improved water quality[123], and habitat for diverse species[124]. However, these ecosystems are under threat. Anthropogenic activities including agriculture, aquaculture and urban development fueled by the increasing demand for coastal resources are contributing to the degradation of mangrove forests[13] on a global scale[125].

Over the past decades, environmental monitoring efforts have focused on sediments and aquatic organisms (e.g., fish, bivalves, gastropods) within mangrove areas[126-129]. Monitoring the toxic metals and metalloids in sediment and benthic organisms facilitates an understanding of contamination profiles of mangrove ecosystems and their link to human activities. These metal(loid)s are frequently used as environmental indicators of overall pollution[126,130-132]. Bivalves, in particular, are widely recognized as effective environmental indicators due to their ability to bioaccumulate contaminants, their sessile life history that indicates localized exposure, and their ecological, economic, and nutritional importance[133].

Two mangrove reserves in the Gulf of Guayaquil, Ecuador provide a robust case for comparison. Reserva de Producción Faunística Manglares El Salado (RPFMS) is part of the estuarine network in the Gulf of Guayaquil and it is one of the most productive bioregions in South America[30,134] spanning an area of approximately 156 km^2 [37,135]. Over the years, wastewater discharges, dense urban expansion, and irregular population settlements have introduced pollutants into the reserve[29,30,136,137]. In 2010, the Ecuadorian government signed an agreement to implement a management plan with the goal of protecting RPFMS[40]. Despite this, the close proximity of the reserve to the largest city of Ecuador, Guayaquil, continues to disrupt the reserve's ecological balance[35], affecting the quality of the mangrove across multiple environmental parameters. For example, elevated Hg concentrations were detected surpassing guideline thresholds for both sediments and aquatic organisms[35]. Sediment concentrations of Cd and Pb were also above the permissible soil local guidelines, Texto Unificado de Legislación Secundaria de Medio Ambiente (TULSMA), within Guayaquil's city limits[96].

The Refugio de Vida Silvestre Manglares El Morro (REVISMEM), also located in the Guayas province (2°S, 80°W)[138], was declared a protected area in 2007 by Ministerial Agreement No. 266 according to the MAE. It covers 101.36 km^2 [139]. REVISMEM harbors a high biodiversity of birds and mammals[140]. It also serves as habitat for multiple mangrove tree species including *Rhizophora harrizonii, Laguncularia racemosa, Conocarpus erecta* and *Avicennia germinans*[140]. Multiple species of mollusks, including *Mytella guyanensis*, *Mytella strigata*, *Crassostrea columbiensis* and *Anadara tuberculosa*, represent a significant dietary resource for nearby coastal communities[141].

Despite the ecological and socioeconomic value of these mangrove forests, metal(loid) contamination in both reserves remains understudied. Existing data are scarce, especially regarding the bioaccumulation of these pollutants in local aquatic fauna and their distribution in sediments. A comprehensive assessment of metal(loid) concentrations in sediment and aquatic animals is necessary to evaluate ecological risk, address fate and transport of toxic elements, and understand the influence of human activities on the ecological health of mangrove forests. This chapter provides an initial assessment of toxic metal(loid)s for RFPMS and REVISMEM that can inform local management and conservation strategies.

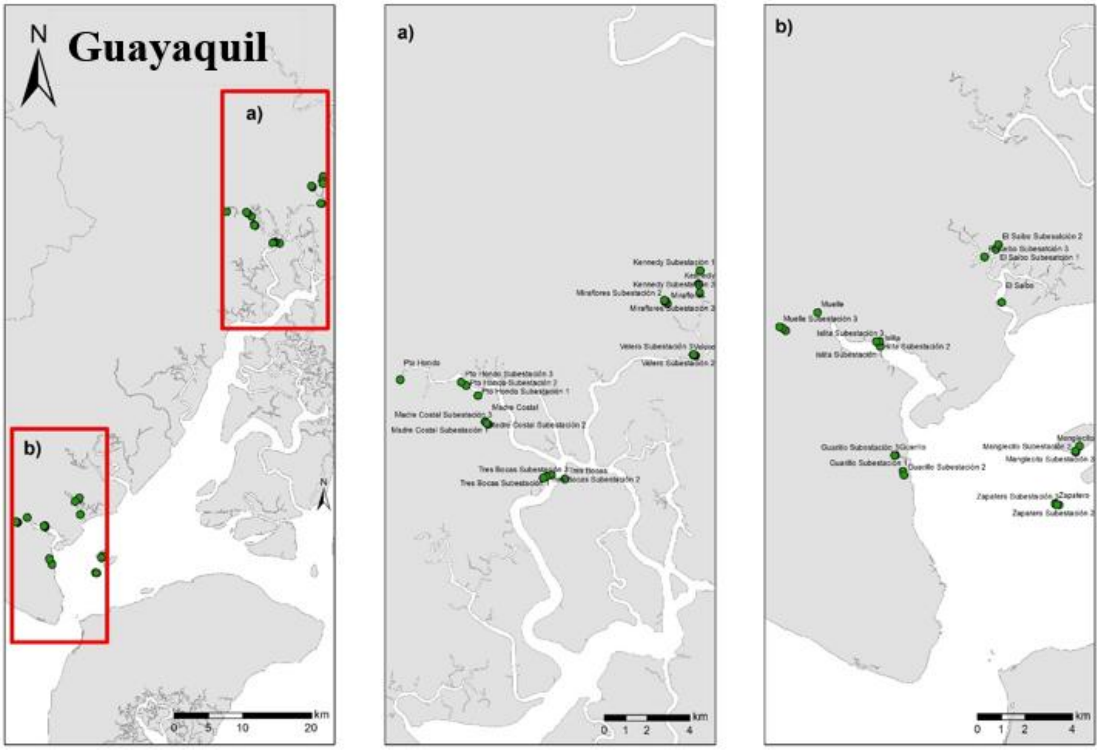


Figure 2.1 Ecuadorian reserves: a) Reserva de Producción Faunística Manglares El Salado (RPFMS) and b) Refugio de Vida Silvestre Manglares El Morro (REVISMEM), with sampling substations. For RPFMS, a total of six substations were sampled: three "within city limits" (Miraflores, Kennedy, and Velero) and three within "city border area" (Puerto Hondo, Madre Costal, and Tres Bocas). For REVISMEM, a total of six substations were also sampled: three "near the city" (Guarillo, Islita, and Muelle) and three "far from the city" (El Saibo, Manglecito, and Zapatero). Maps created by Daniel Garcés.

2.2 Methodology

2.2.1 Sample collection, storage and preparation

Sediment and mussel samples were collected in RPFMS and REVISMEM during dry season of 2023 with the research permit No. MAATE-ARSFC-2023-3173. For RPFMS, a total of six substations were sampled: three "within city limits" (Miraflores, Kennedy, and Velero) and three within the "city border area" (Puerto Hondo, Madre Costal and Tres Bocas). For REVISMEM, a total of six substations were also sampled: three "near the city" (Guarillo, Islita, and Muelle) and three "far from the city" (El Saibo, Manglecito, and Zapatero). Samples were stored in labeled Zip-lock bags on ice inside a cooler before being transported to the ESPOL Ecotoxicology, Quality and Environmental Health laboratory and kept in -80°C freezers. At the laboratory, the samples were freeze-dried and homogenized, and then shipped to the University of Arizona.

2.2.2 Metal(loid) extraction and analysis

A total of 72 sediment samples (36 for RPFMS and 36 for REVISMEM) and 90 composite mussel samples of 40 individuals for each sample (75 for RPFMS and 15 for REVISMEM) were analyzed at the Arizona Laboratory for Emerging Contaminants (ALEC) at the University of Arizona. Sample preparation was performed using microwave-assisted acid digestion with a CEM Model MARS6 system (Matthews, North Carolina) and 20 mL Teflon Xpress vessels. Digestion temperatures were set at 180 °C for sediment samples following EPA Method 3051, and 200 °C for mussel samples according to the CEM Method for animal tissue. Each digestion involved 0.1 g of sample treated with ultrapure concentrated HNO_3, using 1 mL for soils and 2 mL for tissues. For each digestion, 1 mL of ultrapure water was added before sealing the vessels. Upon completion of the digestion, the samples were transferred to acid-washed polypropylene tubes and diluted 1:10 with a solution of 1% HNO_3 and 1% HCl to stabilize Hg in solution prior to analysis.

Metal(loid) concentrations were quantified using coupled plasma-mass spectrometry (ICP-MS) with an Agilent 8900 triple quadrupole instrument (Santa Clara, CA). Laboratory-grade reagents served as blanks, and both samples and calibration standards were prepared in accordance with EPA Method 200.8. Analytical quality control was ensured using certified reference materials: National Institute of Standards and Technology (NIST) 2711 Montana soil for sediment and NIST 2976 mussel tissue for mussels. Results were reported in dw as $\frac{\mu g}{g}$.

2.2.3 Data standardization and regulatory thresholds

Contaminant levels of sediments were compared to the US National Oceanic and Atmospheric Administration (NOAA) “effects range median” (ERM) and “effects range low” (ERL) guidelines based on dw with a calculation of ~49% water content (Table 1.1). Contaminant levels of mussels were compared with the consumption guidelines of the Food and Agriculture Administration of the United Nations (FAO) (Table 1.3) with a calculation of ~85% water content for shellfish.

2.2.4 Statistical analysis

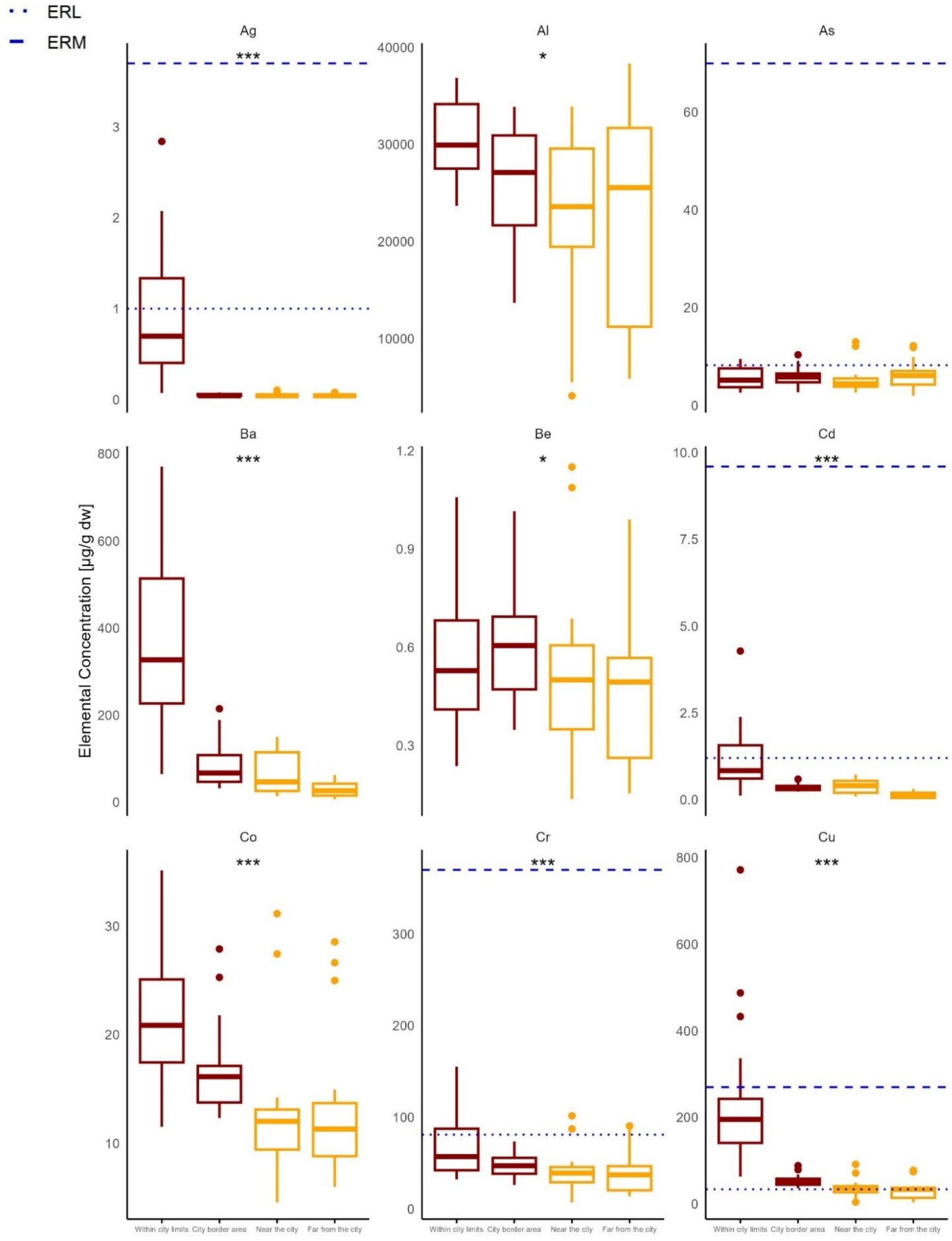
Statistical analyses were completed in R version 4.3.1. Metal(loid) concentration values below the limit of detection (LOD) were replaced with $\frac{LOD}{\sqrt{2}}$. For sediment and mussel samples, we employed both non-parametric (Mann–Whitney U (MWU) test) and parametric (Student’s t-test or Welch's t-test) analyses to compare reserves for each metal(loid). Normal distribution was assessed with the Shapiro-Wilk test. Equal variance was examined with the Levene’s test. The parametric tests were performed on log-transformed data for the metal(loid)s. Additionally, to assess differences between four sampling sites (“within city limits”, “city border area”, “near the city”, and “far from the city”) for sediment samples that followed a normal distribution, a one-way analysis of variance (ANOVA) was performed, followed by Tukey Honestly Significant Difference (HSD) test. For non-normally distributed data, the Kruskal-Wallis test was used instead, with Dunn’s test with Benjamini-Hochberg correction. We conducted a principal component analysis (PCA) using log-transformed data for mussels to show the differences of species per reserve.

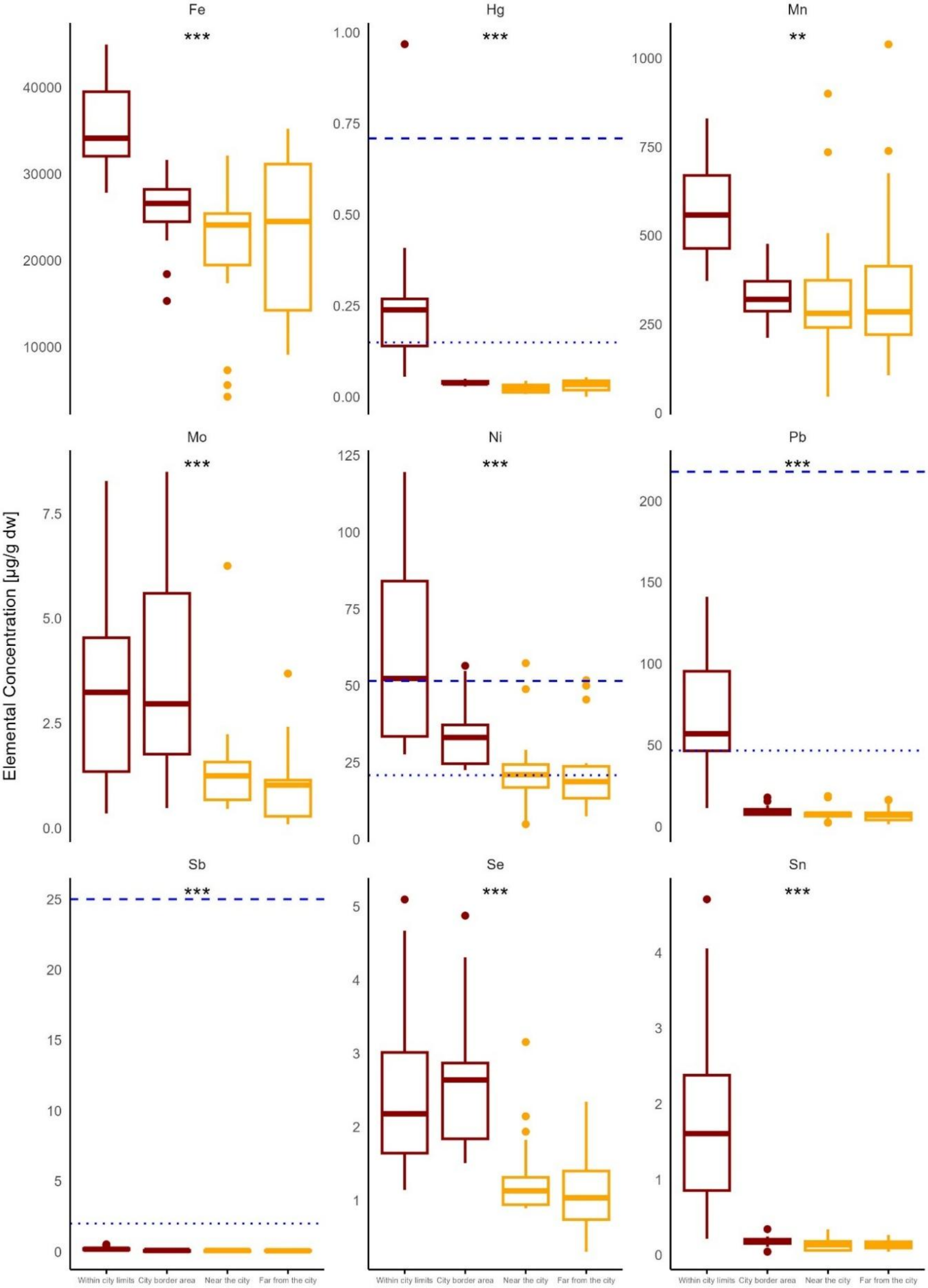
2.3 Results

2.3.1 Sediment

Concentrations of some metal(loid)s in sediment samples in RPFMS and REVISMEM were elevated compared to sediment quality guidelines (SQGs): As, Cr and Cu exceeded the ERL and Ni exceeded the ERM in both reserves. Ag, Cd, Hg, Pb, and Zn concentrations exceeded the ERL only for RPFMS. All analyzed metal(loid) concentrations in sediment from RPFMS were higher than were sediment concentrations from REVISMEM (Table 2.1, Fig. 2.2,). This could be given proximity to industrial sectors near RPFMS. Nine-five percent of analyzed metal(loid)s showed statistically significant differences between the two reserves (Fig. 2.2), with As being an exception.

The significance of analyses remained consistent when the four areas in both reserves were analyzed by one-way ANOVAs and Kruskal Wallis tests for all metal(loid)s except Be (Fig. 2.3). After the post-hoc tests (Tukey HSD and Dunn with correction) (Table 2.2), significant differences between sites in RPFMS ("within city limits" and "city border area") (Fig. 2.3) were largely consistent with the sediment results presented in Chapter 1 (Fig. 1.4), except for Al, Co, Cr, and V. None of the metal(loid)s significantly differed in REVISMEM stations ("near the city" and "far from the city") (Fig.2.3). Most metal(loid) presented significant differences between "within city limits" (RPFMS) and "city border area" (RPFMS), "near the city" (REVISMEM) and "far from the city" (REVISMEM), except for Al, As, Be, Co, Cr, Mo, Se and V (Fig. 2.3). As and Be concentrations did not differ significantly among the four sites in the two reserves (Fig. 2.3).


ERL
ERM
Elemental Concentration [µg/g dw]
Ag
***
0
1
2
3
Al
*
10000
20000
30000
40000
As
0
20
40
60
Ba
***
0
200
400
600
800
Be
*
0.3
0.6
0.9
1.2
Cd
***
0.0
2.5
5.0
7.5
10.0
Co
***
10
20
30
Cr
***
0
100
200
300
Cu
***
0
200
400
600
800
Within city limits
City border area
Near the city
Far from the city


Fe
***
40000
30000
20000
10000
Hg
***
1.00
0.75
0.50
0.25
0.00
Mn
**
1000
750
500
250
0
Elemental Concentration [µg/g dw]
Mo
***
7.5
5.0
2.5
0.0
Ni
***
125
100
75
50
25
0
Pb
***
200
150
100
50
0
Sb
***
25
20
15
10
5
0
Se
***
5
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3
2
1
Sn
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4
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0
Within city limits
City border area
Near the city
Far from the city

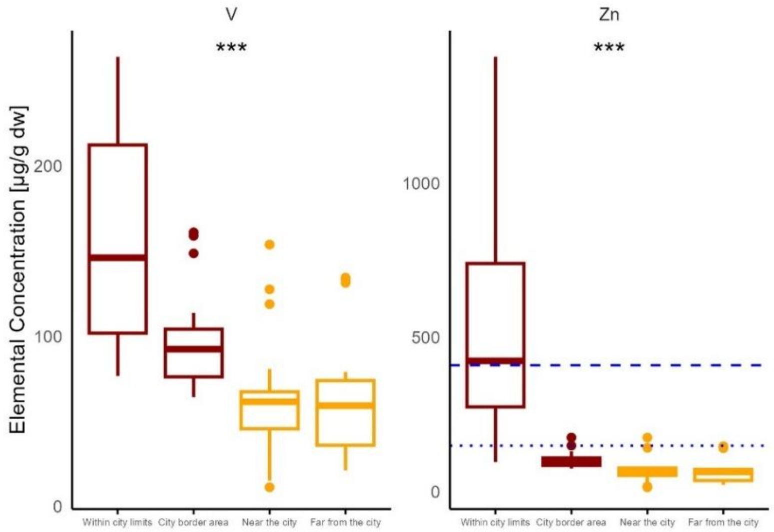


*Figure 2.2 Metal(loid) concentrations in sediment in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM). The dashed horizontal line indicates the "Effect Range Median" (ERM) guidelines. The dotted horizontal line indicates the "Effect Range Low" (ERL) guidelines. *<.05 **<.01 ***<.001 for statistical significance difference per reserve.*

Table 2.1 Sediment sample results ($\frac{\mu g}{g}$ dw) in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM).

Reserve	**RPFMS** **N= 36**			**REVISMEM** **N= 36**				
	mean ± sd **range**			**mean ± sd** **range**				
	within city limits	**city border area**	**combined**	**near the city**	**far from the city**	**combined**	**test**	***p*-value**
Metal(loid)								
Ag	0.96 ± 0.77 (0.07, 2.84)	0.05 ± 0.01 (0.04, 0.08)	0.51 ± 0.71 (0.04, 2.84)	0.05 ± 0.02 (0.04,0.10)	0.05 ± 0.01 (0.04,0.08)	0.51 ± 0.01 (0.04, 0.10)	MWU	**< 0.001**
Al	30566.87 ± 4573.05 (23682.47, 36860.96)	25957.93 ± 5751.53 (13698.72, 33859.40)	28262.40 ± 5629.16 (13698.72, 36860.96)	22611.46 ± 9087.80 (4118.98, 33894.38)	22831.17 ± 11976.74 (5890.29, 38321.78)	22721.31 ± 10478.49 (4118.98, 38321.78)	MWU	**< 0.05**
As	5.70 ±2.28 (2.59, 9.49)	5.89 ± 1.95 (2.67, 10.34)	5.79 ± 2.09 (2.59, 10.34)	5.20 ± 2.87 (2.60, 13.01)	6.06 ± 3.00 (1.95, 12.21)	5.63 ± 2.93 (1.95, 13.01)	MWU	NS
Ba	370.49 ± 215.01 (64.56, 770.69)	86.25 ± 55.33 (31.64, 214.54)	228.37 ± 211.46 (31.64, 770.69)	65.71 ± 46.90 (13.69, 149.81)	29.95 ± 18.00 (7.10, 62.29)	47.83 ± 39.43 (7.10, 149.81)	MWU	**< 0.001**

Be	0.57 ± 0.22 (0.24, 1.06)	0.63 ± 0.20 (0.35, 1.02)	0.60 ± 0.21 (0.24, 1.06)	0.51 ± 0.27 (0.14, 1.15)	0.46 ± 0.27 (0.15, 0.99)	0.49 ± 0.27 (0.14, 1.15)	MWU	**< 0.05**
Cd	1.19 ± 0.98 (0.11, 4.28)	0.34 ± 0.10 (0.23, 0.59)	0.77 ± 0.81 (0.11, 4.28)	0.39 ± 0.19 (0.09, 0.72)	0.12 ± 0.09 (0.02, 0.31)	0.25 ± 0.20 (0.02, 0.72)	MWU	**< 0.001**
Co	21.62 ± 6.18 (11.52, 35.15)	16.81 ± 4.29 (12.34, 27.91)	19.21 ± 5.78 (11.52, 35.15)	12.58 ± 6.82 (4.56, 31.16)	13.09 ± 6.84 (5.99, 28.56)	12.83 ± 6.74 (4.56, 31.16)	MWU	**< 0.001**
Cr	69.72 ± 36.03 (32.16, 155.29)	48.51 ± 13.47 (26.17, 73.58)	59.12 ± 28.88 (26.17, 155.29)	39.89 ± 23.87 (7.09, 101.56)	40.85 ± 24.90 (13.77, 90.50)	40.37 ± 24.04 (7.09, 101.56)	MWU	**< 0.001**
Cu	241.86 ± 172.73 (63.59, 771.82)	54.48 ± 13.87 (36.31, 88.71)	148.17 ± 153.67 (36.31, 771.82)	36.29 ± 21.02 (4.54, 91.80)	32.43 ± 24.04 (3.91, 78.12)	34.36 ± 22.34 (3.91,91.80)	MWU	**< 0.001**
Fe	35673.13 ± 5060.75 (27819.59, 44940.81)	25659.17 ± 3990.33 (15332.62, 31629.05)	30666.15 ± 6779.37 (15332.62, 44940.81)	21277.47 ± 8078.93 (4279.70, 32126.76)	22946.89 ± 9521.87 (9141.33, 35215.90)	22112.18 ± 8743.94 (4279.70, 35215.90)	MWU	**< 0.001**
Hg	0.24 ± 0.20 (0.06, 0.97)	0.04 ± 0.01 (0.03, 0.05)	0.14 ± 0.18 (0.03, 0.97)	0.02 ± 0.01 (0.01, 0.04)	0.03 ± 0.02 (0.00, 0.05)	0.03 ± 0.02 (0.00,0.05)	MWU	**< 0.001**
Mn	578.65 ± 140.39 (372.12, 830.69)	326.31 ± 79.41 (212.12, 476.56)	452.48 ± 170.32 (212.12, 830.69)	323.52 ± 216.06 (45.71, 900.31)	364.78 ± 241.47 (106.08,1039.57)	344.15 ± 226.79 (45.71,1029.57)	MWU	**< 0.01**
Mo	3.43 ± 2.42 (0.35, 8.28)	3.54 ± 2.34 (0.48, 8.50)	3.48 ± 2.35 (0.35, 8.50)	1.47 ± 1.33 (0.46, 6.25)	1.08 ± 0.95 (0.09, 3.69)	1.27 ± 1.15 (0.09, 6.25)	MWU	**< 0.001**
Ni	59.12 ± 28.32 (27.69, 119.65)	33.72 ± 10.30 (22.59, 56.53)	46.42 ± 24.64 (22.59, 119.65)	22.47 ± 13.24 (4.95,57.39)	22.04 ± 13.64 (7.55, 51.85)	22.25 ± 13.25 (4.95, 57.39)	MWU	**< 0.001**
Pb	67.88 ± 34.59 (11.49, 141.12)	9.99 ± 3.08 (7.01,17.91)	38.94 ± 38.04 (7.01, 141.12)	7.89 ± 4.38 (2.38, 18.79)	7.31 ± 4.61 (1.45, 16.49)	7.60 ± 4.44 (1.45, 18.79)	MWU	**< 0.001**
Sb	0.21 ± 0.14 (0.03, 0.53)	0.08 ± 0.05 (0.00, 0.14)	0.15 ± 0.12 (0.00, 0.53)	0.07 ± 0.04 (0.01, 0.11)	0.07 ± 0.03 (0.01, 0.12)	0.07 ± 0.03 (0.01, 0.12)	MWU	**< 0.001**
Se	2.52 ± 1.23 (1.14, 5.10)	2.67 ± 0.95 (1.51, 4.88)	2.59 ± 1.09 (1.14, 5.10)	1.32 ± 0.59 (0.89, 3.16)	1.15 ± 0.61 (0.30,2.34)	1.24 ± 0.60 (0.30, 3.16)	MWU	**< 0.001**
Sn	1.86 ± 1.25 (0.21, 4.71)	0.18 ± 0.07 (0.04, 0.34)	1.02 ± 1.22 (0.04, 4.71)	0.14 ± 0.09 (0.05, 0.34)	0.14 ± 0.07 (0.05, 0.27)	0.14 ± 0.08 (0.05, 0.34)	MWU	**< 0.001**

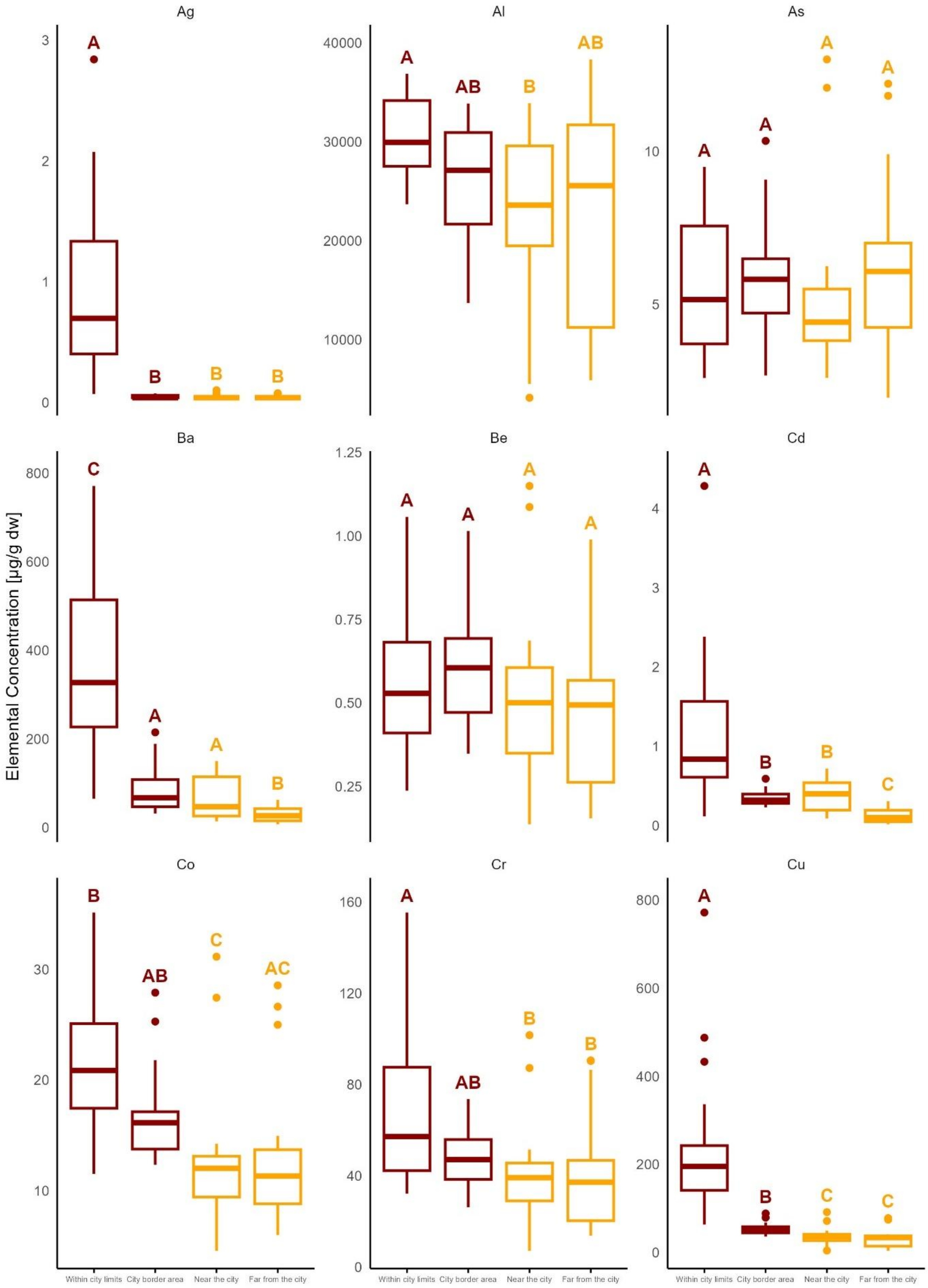
V	158.30 ± 62.69 (76.69, 264.19)	97.86 ± 30.49 (64.27, 161.08)	128.08 ± 57.44 (64.27, 264.19)	64.08 ± 37.47 (11.31, 153.91)	64.78 ± 36.14 (21.27, 134.48)	64.43 ± 36.28 (11.31, 153.91)	MWU	**< 0.001**
Zn	538.43 ± 327.47 (97.01, 1409.25)	104.58 ± 26.99 (75.50, 176.19)	321.51 ± 317.55 (75.50, 1409.25)	69.69 ± 39.21 (14.98, 175.86)	68.00 ± 40.05 (22.66, 147.86)	68.84 ± 39.07 (14.98, 175.86)	MWU	**< 0.001**

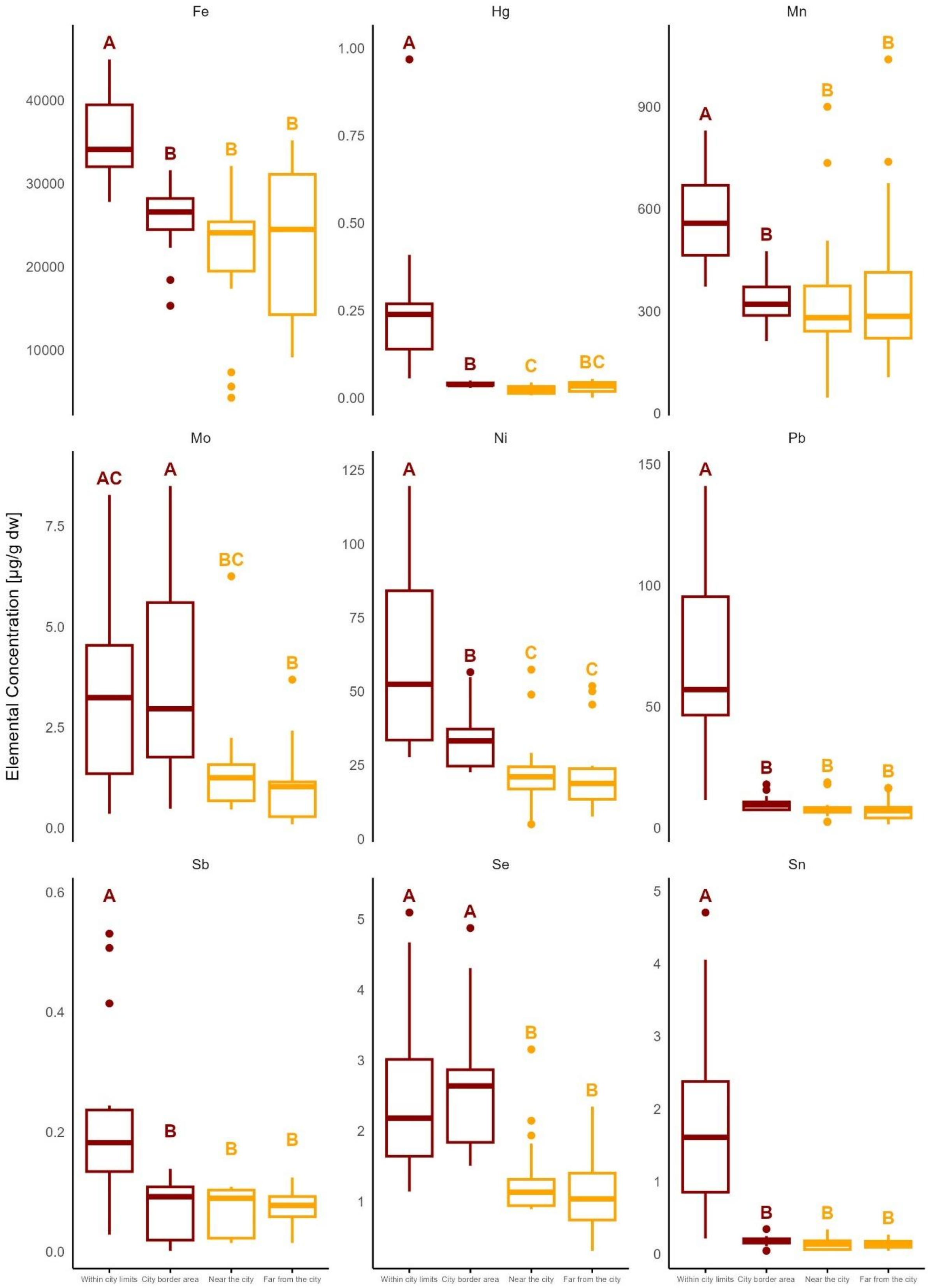
Statistical analysis: MWU test for non-normal distribution
Significant coefficients ($p < 0.05$, $p < 0.01$, $p < 0.001$, bold numbers)

Table 2.2 Post-hoc tests in sediment samples.

Metal(loid)	**test**
Ag	Dunn BH
Al	Dunn BH
As	Dunn BH
Ba	Tukey HSD
Be	Tukey HSD
Cd	Dunn BH
Co	Tukey HSD
Cr	Dunn BH
Cu	Dunn BH
Fe	Dunn BH
Hg	Dunn BH
Mn	Dunn BH
Mo	Tukey HSD
Ni	Dunn BH
Pb	Dunn BH
Sb	Dunn BH
Se	Dunn BH
Sn	Dunn BH
V	Tukey HSD
Zn	Dunn BH

Dunn BH: Dunn’s test with Benjamini-Hochberg correction
Tukey HSD: Tukey Honestly Significant Difference test


Elemental Concentration [µg/g dw]
Ag
0
1
2
3
A
B
B
B
Al
10000
20000
30000
40000
A
AB
B
AB
As
5
10
A
A
A
A
Ba
0
200
400
600
800
C
A
A
B
Be
0.25
0.50
0.75
1.00
1.25
A
A
A
A
Cd
0
1
2
3
4
A
B
B
C
Co
10
20
30
B
AB
C
AC
Cr
0
40
80
120
160
A
AB
B
B
Cu
0
200
400
600
800
A
B
C
C
Within city limits
City border area
Near the city
Far from the city


Fe
A
B
B
B
40000
30000
20000
10000
Hg
A
B
C
BC
1.00
0.75
0.50
0.25
0.00
Mn
A
B
B
B
900
600
300
0
Mo
AC
A
BC
B
7.5
5.0
2.5
0.0
Ni
A
B
C
C
125
100
75
50
25
0
Pb
A
B
B
B
150
100
50
0
Sb
A
B
B
B
0.6
0.4
0.2
0.0
Se
A
A
B
B
5
4
3
2
1
Sn
A
B
B
B
5
4
3
2
1
0
Elemental Concentration [µg/g dw]
Within city limits
City border area
Near the city
Far from the city

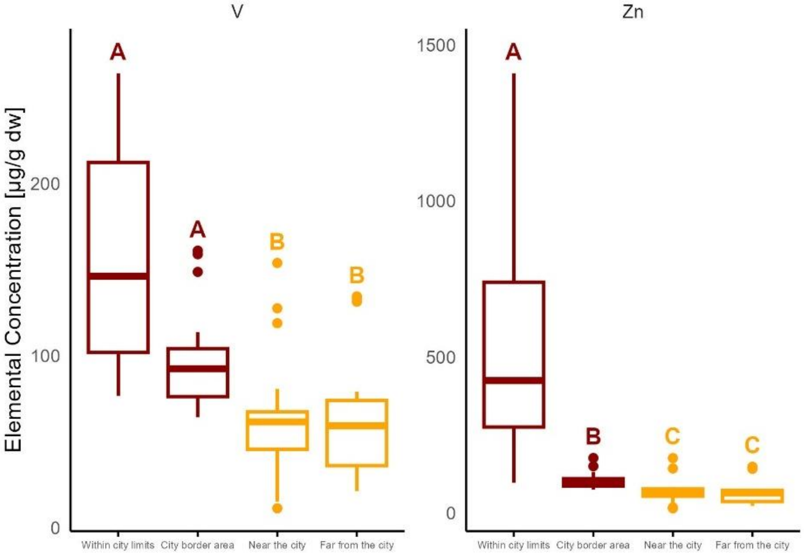
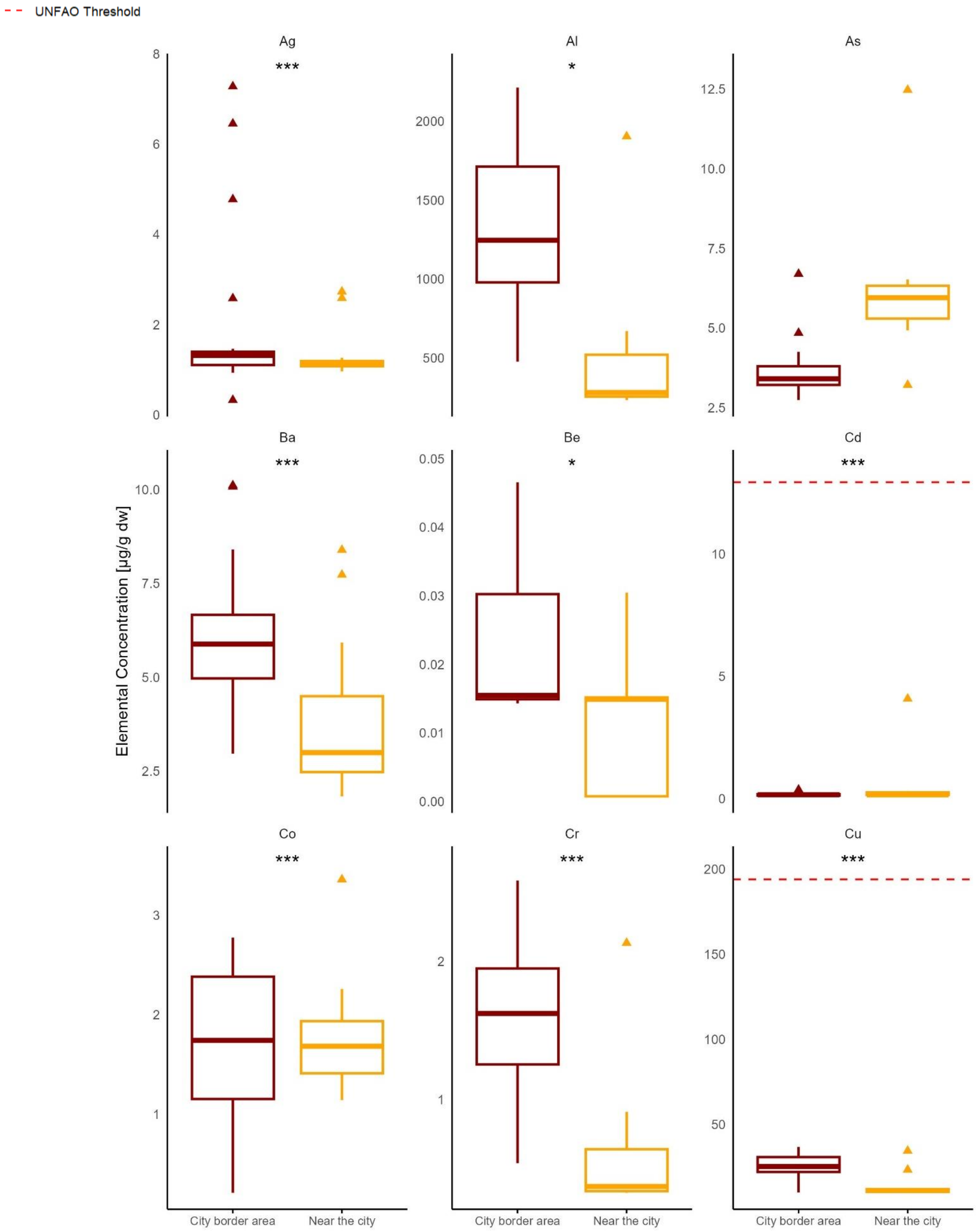
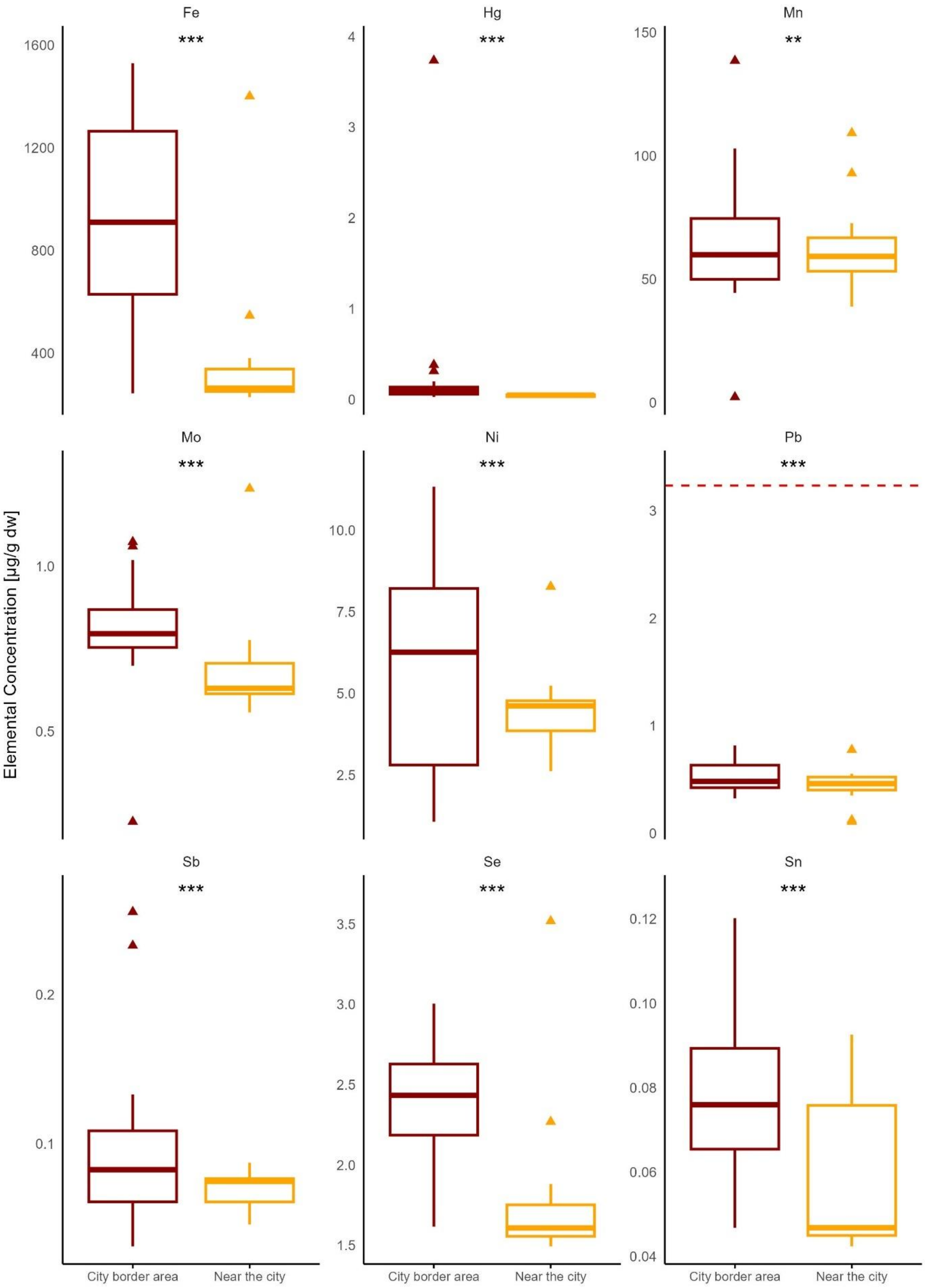


Figure 2.3 Metal(loid) concentrations in sediment in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM). Different letters indicate significant difference between four sites ("within city limits", "city border area", "near the city", "far from the city") (ANOVA, Tukey HSD test; Kruskal-Wallis, Dunn test; p < 0.05).

2.3.2 Mussels

Mytella strigata species was only found in one station from each reserve: “city border area” (RPFMS) and “near the city” (REVISMEM) (Fig. 2.5). For *M. strigata*, all of the 20 analyzed metal(loid)s except As showed statistically significant differences between RPFMS and REVISMEM (Fig. 2.4). Only As, Cd, and Co showed lower concentrations in “city border area” from RPFMS compared to “near the city” from REVISMEM (Table 2.3, Fig. 2.4). This could be due to the pollution coming from vessels entering the REVISMEM reserve. Across all mussel species*, M. guyanensis*, *M. strigata*, and *M. trautwineana*, just one sample of *M. trautwineana* in Zn exceeded the FAO guideline thresholds (Fig. 2.5). *Mytella trautwineana* was the only species observed “within city limits” (RPFMS),while *M. strigata* was the only species found “near the city” (REVISMEM). *Mytella guyanensis*, *M. strigata,* and *M. trautwineana* were all found in “city border area” (Fig. 2.5).


UNFAO Threshold
Elemental Concentration [µg/g dw]
Ag
***
0
2
4
6
8
Al
*
500
1000
1500
2000
As
2.5
5.0
7.5
10.0
12.5
Ba
***
2.5
5.0
7.5
10.0
Be
*
0.00
0.01
0.02
0.03
0.04
0.05
Cd
***
0
5
10
Co
***
1
2
3
Cr
***
1
2
Cu
***
50
100
150
200
City border area
Near the city
City border area
Near the city
City border area
Near the city


Elemental Concentration [µg/g dw]
Fe
***
1600
1200
800
400
Hg
***
4
3
2
1
0
Mn
**
150
100
50
0
Mo
***
1.0
0.5
Ni
***
10.0
7.5
5.0
2.5
Pb
***
3
2
1
0
Sb
***
0.2
0.1
Se
***
3.5
3.0
2.5
2.0
1.5
Sn
***
0.12
0.10
0.08
0.06
0.04
City border area
Near the city
City border area
Near the city
City border area
Near the city

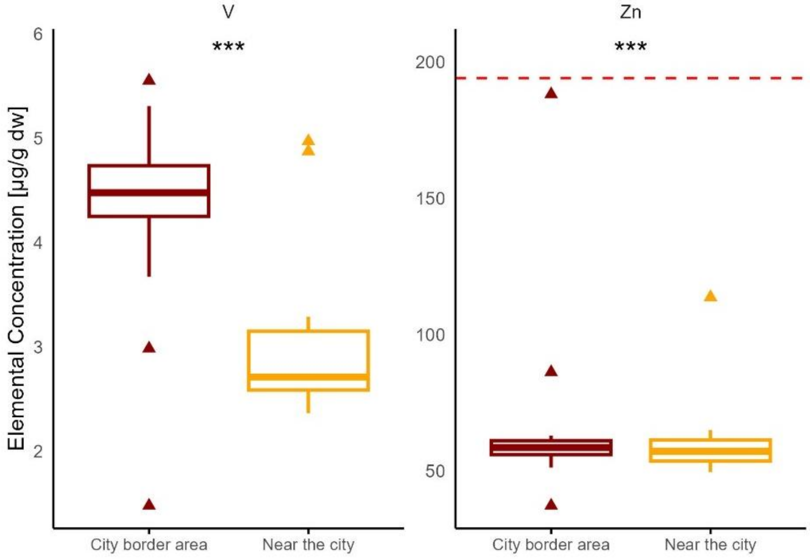


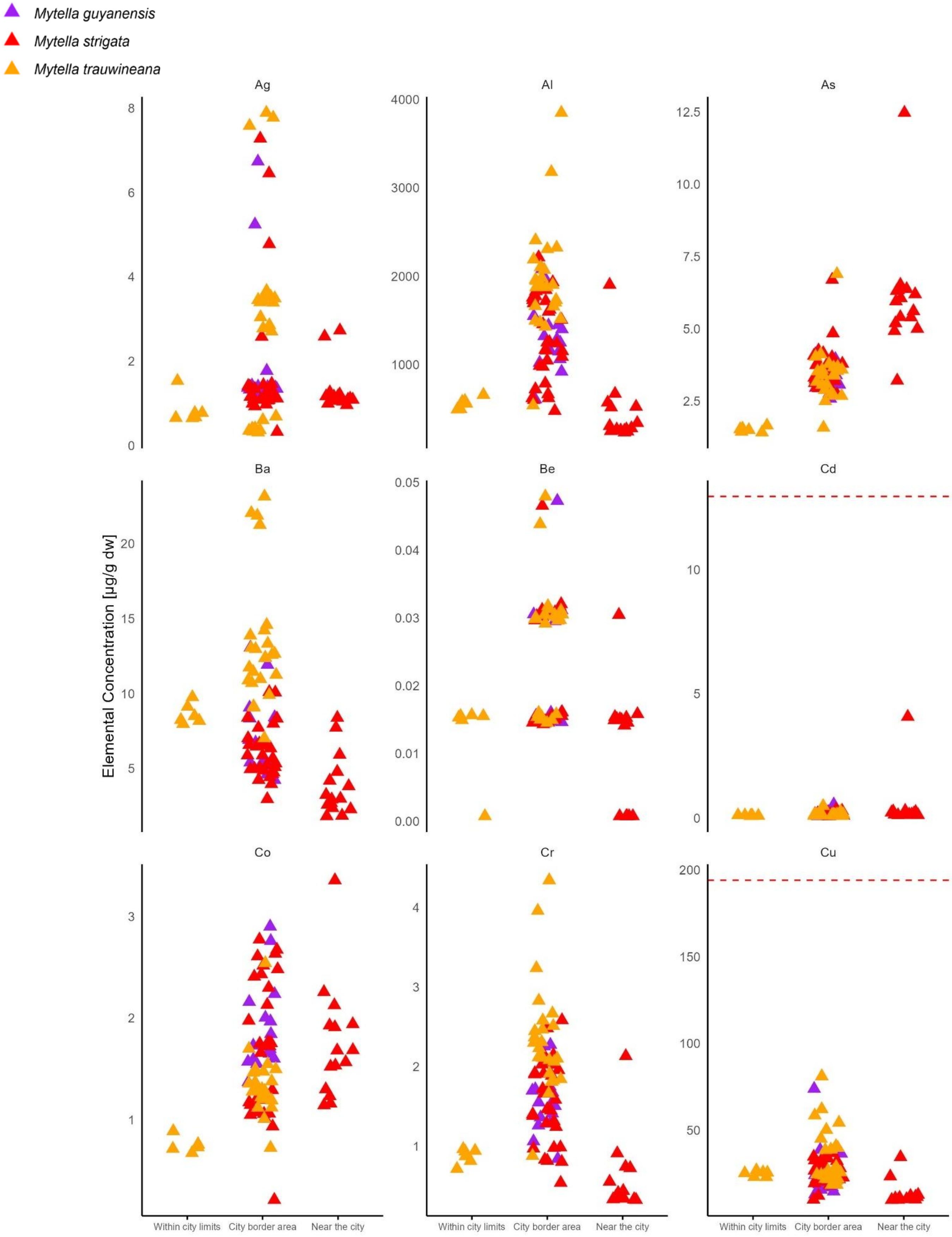
*Figure 2.4 Metal(loid) concentrations in mussels (Mytella strigata) in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM). *<.05 **<.01 ***<.001. No mussels were found in "within city limits" in RPFMS and "far from the city" in REVISMEM.*

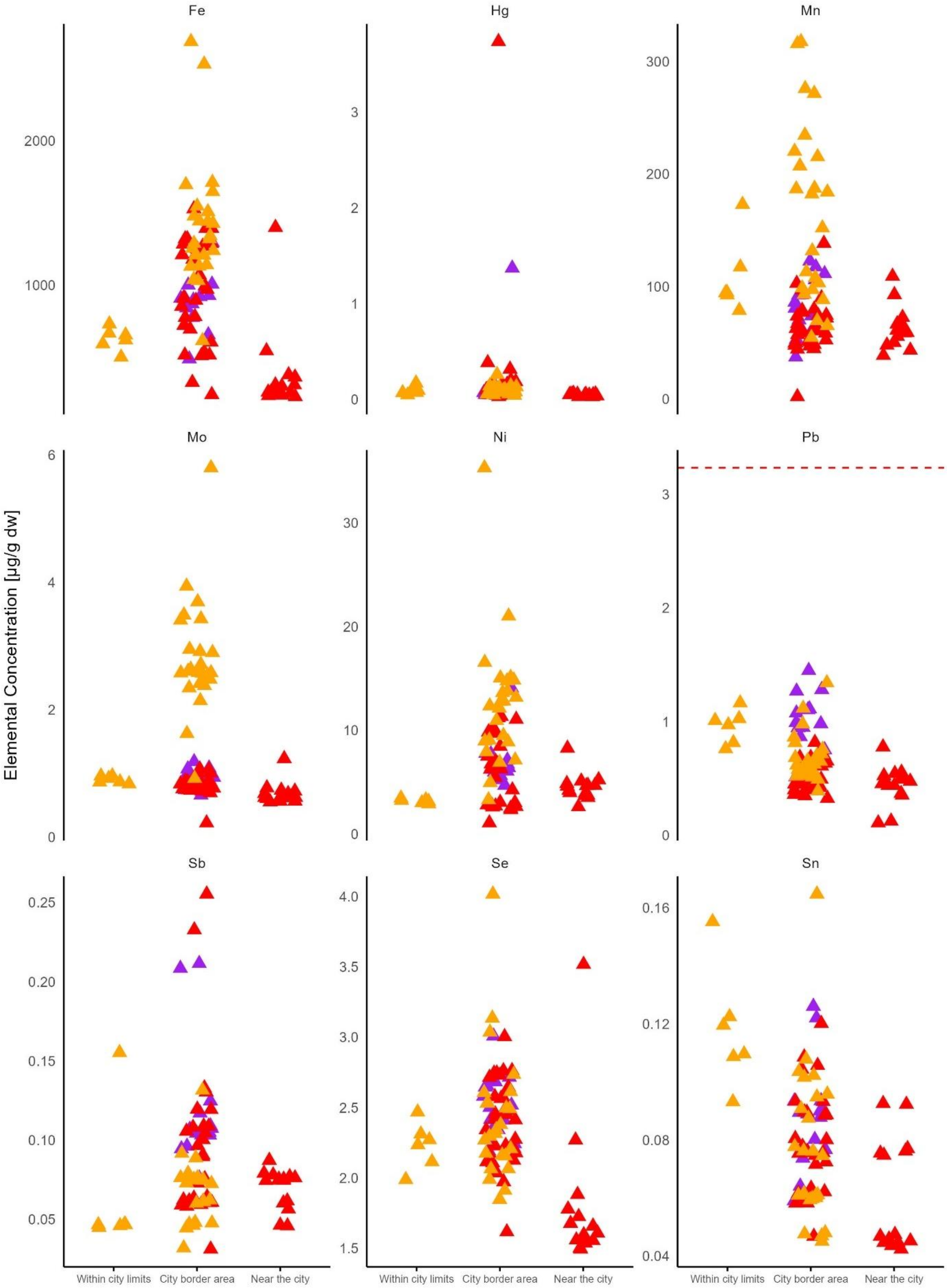
Table 2.3 Metal(loid)s concentrations of mussels (Mytella strigata) ($\frac{\mu g}{g}$ dw) in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM). No mussels were found "within city limits" in RPFMS or "far from the city" in REVISMEM.

Reserve	**RPFMS** **N= 30**	**REVISMEM** **N= 15**		
	mean ± sd **range**	**mean ± sd** **range**		
	city border area	**near the city**	**test**	***p*-value**
Metal(loid)				
Ag	1.73 ± 1.58 (0.33, 7.28)	1.32 ± 0.55 (0.96, 2.73)	MWU	**< 0.001**
Al	1283.64 ± 473.09 (476.94, 2213.94)	458.93 ± 424.23 (233.22, 1903.71)	MWU	**< 0.05**
As	3.63 ± 0.74 (2.74, 6.7)	6.07 ± 1.96 (3.21, 12.47)	MWU	NS
Ba	6.12 ± 1.69 (2.97, 10.11)	3.85 ± 2.04 (1.83, 8.39)	MWU	**< 0.001**
Be	0.02 ± 0.01 (0.01, 0.05)	0.01 ± 0.01 (0, 0.03)	MWU	**< 0.05**
Cd	0.17 ± 0.07	0.44 ± 1.01	MWU	**< 0.001**

	(0.09, 0.36)	(0.13, 4.08)		
Co	1.72 ± 0.67	1.76 ± 0.56	MWU	**< 0.001**
	(0.21, 2.77)	(1.14, 3.36)		
Cr	1.57 ± 0.52	0.58 ± 0.47	MWU	**< 0.001**
	(0.54, 2.58)	(0.32, 2.13)		
Cu	25.78 ± 6.41	13.55 ± 6.68	MWU	**< 0.001**
	(10.14, 36.9)	(10, 34.59)		
Fe	927.11 ± 355.65	373.23 ± 295.76	MWU	**< 0.001**
	(243.12, 1529.25)	(228.42, 1400.54)		
Hg	0.23 ± 0.67	0.05 ± 0.01	MWU	**< 0.001**
	(0.03, 3.74)	(0.03, 0.06)		
Mn	64.21 ± 23.78	63.38 ± 18.13	MWU	**< 0.01**
	(2.23, 138.52)	(38.94, 109.23)		
Mo	0.81 ± 0.15	0.69 ± 0.17	MWU	**< 0.001**
	(0.23, 1.07)	(0.56, 1.24)		
Ni	5.88 ± 3.21	4.53 ± 1.24	MWU	**< 0.001**
	(1.07, 11.33)	(2.62, 8.27)		
Pb	0.52 ± 0.13	0.44 ± 0.16	MWU	**< 0.001**
	(0.32, 0.82)	(0.11, 0.78)		
Sb	0.09 ± 0.05	0.07 ± 0.01	MWU	**< 0.001**
	(0.03, 0.26)	(0.05, 0.09)		
Se	2.4 ± 0.3	1.8 ± 0.52	MWU	**< 0.001**
	(1.62, 3)	(1.49, 3.52)		
Sn	0.08 ± 0.02	0.06 ± 0.02	MWU	**< 0.001**
	(0.05, 0.12)	(0.04, 0.09)		
V	4.38 ± 0.75	3.03 ± 0.82	MWU	**< 0.001**
	(1.48, 5.55)	(2.37, 4.97)		
Zn	62.6 ± 24.79	60.89 ± 15.27	MWU	**< 0.001**
	(37.32, 188.11)	(49.6, 113.75)		

Statistical analysis: MWU for non-normal distribution
Significant coefficients ($p < 0.05$, $p < 0.01$, $p < 0.001$, bold numbers)


Mytella guyanensis
Mytella strigata
Mytella trauwineana
Ag
0
2
4
6
8
Al
1000
2000
3000
4000
As
2.5
5.0
7.5
10.0
12.5
Ba
5
10
15
20
Be
0.00
0.01
0.02
0.03
0.04
0.05
Cd
0
5
10
Co
1
2
3
Cr
1
2
3
4
Cu
50
100
150
200
Elemental Concentration [µg/g dw]
Within city limits
City border area
Near the city
Within city limits
City border area
Near the city
Within city limits
City border area
Near the city


Fe
Hg
Mn
Mo
Ni
Pb
Sb
Se
Sn
Elemental Concentration [µg/g dw]
2000
1000
3
2
1
0
300
200
100
0
6
4
2
0
30
20
10
0
3
2
1
0
0.25
0.20
0.15
0.10
0.05
4.0
3.5
3.0
2.5
2.0
1.5
0.16
0.12
0.08
0.04
Within city limits
City border area
Near the city
Within city limits
City border area
Near the city
Within city limits
City border area
Near the city

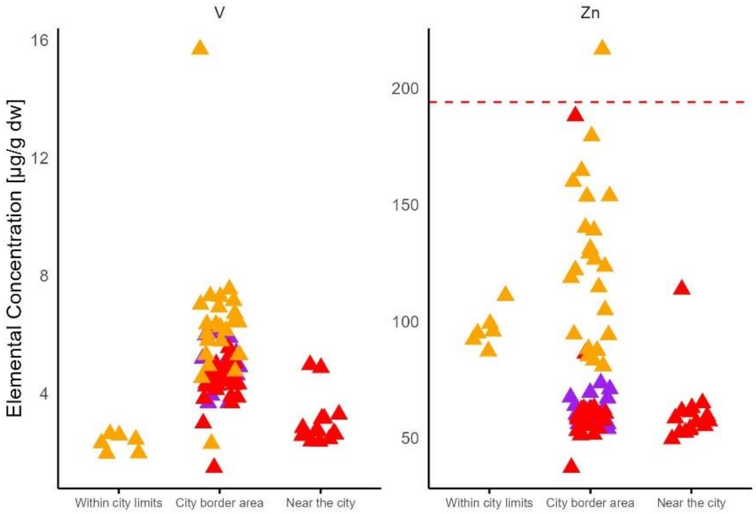


Figure 2.5 Metal(loid) concentrations in mussels (Mytella guyanensis, Mytella strigata and Mytella trautwineana) in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM). No mussels were found in "far from the city" station in REVISMEM.

PC1 loadings were mostly negative, except for As, Cd, and Co (Table 2.4). PC2 loadings were largely influenced by positive values, except Pb, Be, Fe, Sn, Al, Hg, Cr, and Cu, which loaded negatively on PC2 (Table 2.4). Mussels showed a separation along PC1 between the two reserves, with RPFMS mussel samples spread from slightly positive to negative along PC1 (Fig. 2.6). Additionally, *M. trautwineana* tended to be more negative on PC1 than the other two species. REVISMEM mussel samples were all positive on PC2. *Mytella strigata* was the only species found in REVISMEM in the "near the city" area (red circle); these samples are all located in the positive PC2 region, which indicates lower metal(loid) pollution.

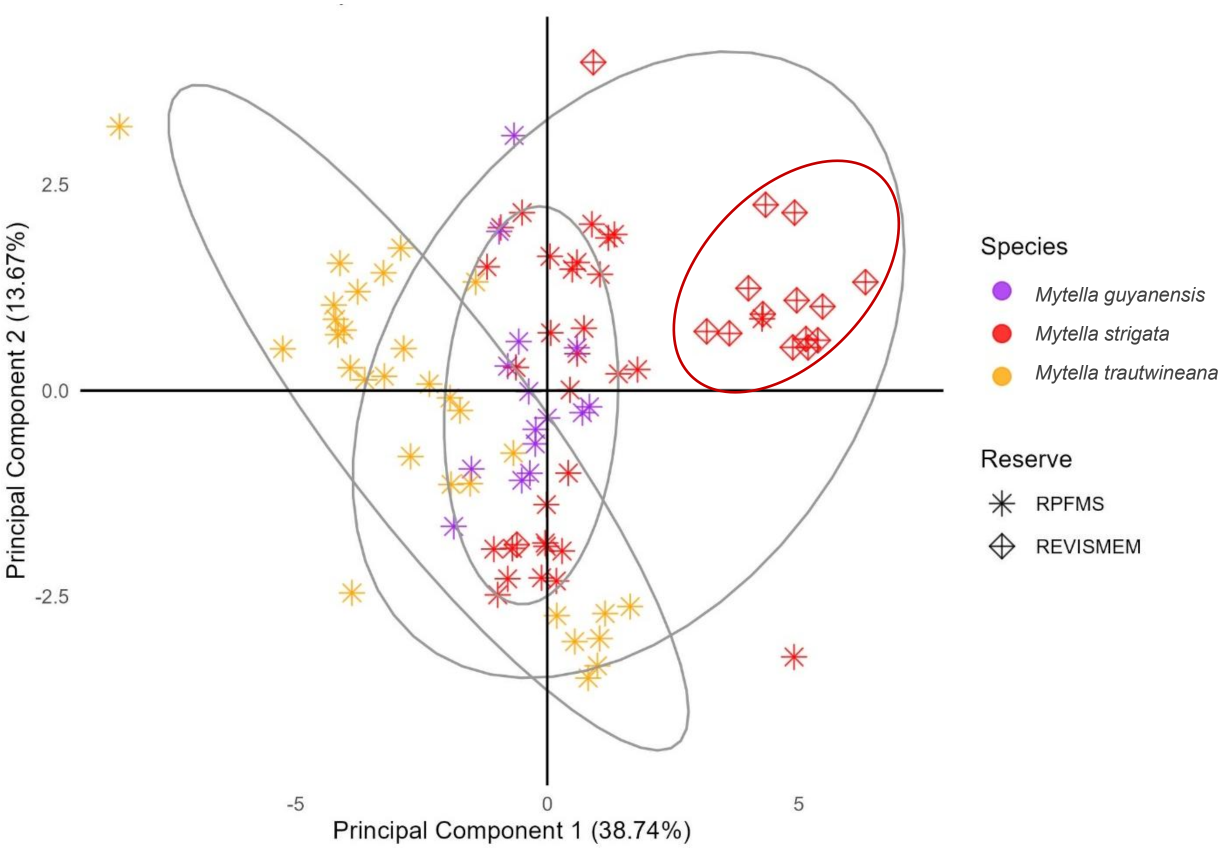


Figure 2.6 Principal component analysis (PCA) biplot for mussels: species and reserves in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM).

Table 2.4 Principal component loadings of log-transformed metal(loid) concentrations (n=90) in mussels, with elements organized for each PC by rank order of the absolute value of the loading.

Metal(loid)	**PC1**	**Metal(loid)**	**PC2**
Fe	-0.332	Co	0.477
Cr	-0.325	As	0.432
Al	-0.315	Ni	0.383
V	-0.310	Ag	0.322
Ba	-0.305	Pb	-0.303
Mo	-0.285	Sb	0.265
Cu	-0.275	V	0.178
Zn	-0.255	Be	-0.143
Be	-0.244	Mo	0.143
Se	-0.235	Fe	-0.124
Ni	-0.212	Sn	-0.122
Mn	-0.200	Al	-0.118

Pb	-0.155	Cd	0.113
Ag	-0.139	Hg	-0.112
As	0.121	Cr	-0.101
Sn	-0.092	Mn	0.099
Cd	0.075	Se	0.074
Hg	-0.041	Cu	-0.055
Sb	-0.038	Zn	0.052
Co	0.003	Ba	0.012

2.4 Discussion

2.4.1 Sediment

Contamination of sediment in RPFMS and REVISMEM is evident, with higher values in RPFMS (Table 2.1, Fig. 2.2). Only As and Be concentrations did not statistically differ in four sites across the two reserves (Fig. 2.3). Significant differences between stations in RPFMS ("within city limits" and "city border area") (Fig. 2.3) were mostly consistent with the sediment concentration results in Chapter 1 (Fig. 1.4), excluding Al, Co, Cr, and V, which may be due to the correction applied in the post-hoc tests. Previous research demonstrated the presence of toxic metals (Cd, Cu, Pb and Hg) in sediment in RPFMS (Table 2.5), with concentrations exceeding the ERL values, the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life, and the Ecuadorian sediment legislation quality guidelines[35,94,142]. In this study, higher concentrations of Cr (Table 2.1, Table 2.5) were found within city limits in RPFMS than the concentrations reported in Alcívar *et* al. (2012)[142]. Furthermore, our study revealed concentrations of Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Pb, V and Zn (Table 2.5) that exceeded the values reported by Fernández-Cadena (2014)[94]. We compared our results "within city limits" to Fernández-Cadena (2014) since both investigations were conducted inside the city of Guayaquil. Additionally, our study showed the highest concentrations of metal(loids) in RPFMS (Table 2.5), excluding Hg, surpassing many mangrove sediment levels reported globally[94,128,143-149].

Mero *et* al. (2012)[150] found that sediments in REVISMEM exhibited Cd and Pb concentrations surpassing the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life[151]. We found a higher maximum concentration of Pb in sediment compared to the maximum value reported by Mero *et* al., while our maximum Cd concentration was lower (Table 2.1, Table 2.5). However, the overall mean concentrations of both Cd and Pb in sediment were lower in our study (Table 2.1) than the mean values reported by Mero et *al.*

In RPFMS, Ag, Cd, Hg, Pb, and Zn exceeded ERL values, while Ni surpassed the ERM threshold (Fig. 2.2). In REVISMEM, As, Cr, and Cu exceeded ERL values, and Ni also exceeded ERM guidelines (Fig. 2.2). These data support the contention that urban activities are contributing to the degradation of mangrove forests. Forest degradation due to pollution is occurring simultaneously with deforestation. According to the Center for Integrated Natural

Resources Remote Sensing of Ecuador (CLIRSEN), mangrove losses since the early 2000s have reduced the extent of these ecosystems by up to 70%[152]. Human activities related to farming and cultivation of aquatic species are likely the main drivers of this habitat loss along the coast of Ecuador.

Table 2.5 Toxic metal(loid) concentrations ($\frac{\mu g}{g}$ *dw) in sediment in Reserva de Producción Faunística Manglares El Salado (RPFMS) in 'within city limits' and Refugio de Vida Silvestre Manglares El Morro (REVISMEM) in both sites.*

Reserve	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Mn**	**Ni**	**Pb**	**V**	**Zn**	**Reference**
RPFMS	0.96	5.70	1.19	21.62	69.72	241.86	0.24	578.65	59.12	67.88	158.30	538.43	This study
	0.003	0.012	0.002	0.021	0.095	0.254	-	0.469	0.082	0.081	0.196	0.678	Fernández-Cadena (2014)[94]
	-	-	[ND-9.50]	-	[11.99-43.47]	-	-	-	-	[13.47-69.47]	-	-	Alcívar *et* al. (2012)[142]
	-	-	-	-	-	-	2.58	-	-	-	-	-	Calle *et* al. (2018)[35]
REVISMEM	0.51	5.63	0.25	12.83	40.37	34.36	0.03	344.15	22.25	7.60	64.43	68.84	This study
	-	-	[ND-12.94]	-	-	-	-	-	-	[ND-13.93]	-	-	Mero *et* al. (2012)[150]

ND: Not detected
range:[min-max]

2.1.1 Mussels

Concentrations of all analyzed toxic metal(loid)s in mussels in our study were below the FAO threshold guidelines for shellfish (Fig. 2.5). Our results are compared with the range of Cd values reported for *M. strigata* collected from RPFMS by Kuffó (2013)[153] and Banguera (2021)[154] (Table 2.6). However, their investigations were conducted in 'within city limits', while our results are for 'city border area' for *M. strigata*. Cr values were higher and Pb and Hg values were lower in the current study (Table 2.6) possibly due to the method of metal(loid) analysis and difference on sites.

In REVISMEM, Banguera (2021)[154] found lower mean concentrations of Cd in 2016-2020 and Pb in 2016 in *Mytilus edulis*, but higher concentrations of Hg in 2016[154] than our results for Cd, Hg and Pb in *M. strigata* (Table 2.6). Given that mussels are natural biofilters, it is common for them to accumulate pollutants in their tissues from their surrounding environment. Increasing contamination is evident in both reserves, though levels are lower in REVISMEM. *Mytella trautwineana* is a species with more metal(loid) concentrations in comparison to *M. guyanensis* and *M. strigata* (Fig. 2.5). However, due to lower biodiversity in RPFMS, we compared values between reserves only for *M. strigata* since it is the most abundant mussel species in RPFMS[141]. Concentrations of Ag, Al, Ba, Be, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sn, V and Zn were all higher in *M. strigata* collected from RPFMS than in those collected from REVISMEM (Table 2.3).

Pb levels are likely introduced through anti-corrosive boat paint used by vessels entering the reserves, as well as from chemical discharges, including waste from electronics and lead-based materials used in fishing activities[155]. Elevated Cd levels may be due to industrial discharges into the mangrove waters of RPFMS and REVISMEM, including materials made from Cd-Ni alloys found in household items as well as plastics, glass, and batteries[155]. The decrease in Hg levels compared to previous investigations (Table 2.5, Table 2.6) may suggest that its metal(loid) concentration is being influenced by variation across sampling sites[156].

Table 2.6 Toxic metal(loid) concentrations ($\frac{\mu g}{g}$ dw) in bivalves collected in Reserva de Producción Faunística Manglares El Salado (RPFMS) and Refugio de Vida Silvestre Manglares El Morro (REVISMEM).

Reserve	**Site**	**Species**	**Cd**	**Cr**	**Hg**	**Pb**	**Reference**
RPFMS	city border area	*Mytella strigata*	0.17	1.57	0.23	0.52	This study
	within city limits		0.17	-	1.44	1.41	Banguera (2021)[154]
	within city limits		0.21	0	-	1.45	Kuffó (2013)[153]
REVISMEM	near the city	*Mytella strigata*	0.44	0.58	0.05	0.44	This study
	near the city	*Mytilus edulis*	0.11	-	0.09	0.13	Banguera (2021)[154]

2.2 Limitations

The small number of *M. strigata* found in RPFMS may influence the comparison between reserves since REVISMEM had a lower sample size of mussels. This limits the power to detect differences between reserves. Additionally, we did not find the other two mussel species in REVISMEM, and therefore we were unable to compare these species across reserves.

Conclusions

Concentrations of toxic metal(loid)s in sediment and mussels support the hypothesis that urban mangrove forests are more contaminated than rural forests. RPFMS is more polluted than REVISMEM, likely due to its proximity to Guayaquil, Ecuador's largest city and main port. Additionally, nearby industrial activity may contribute pollutants to RPFMS. However, we found that concentrations of As, Cd and Co were higher in mussels from REVISMEM, suggesting the potential for local sources or environmental dynamics that need further investigation.

Our study revealed significant differences in concentrations of metal(loid)s in sediment and mussels between both mangrove reserves. In sediment, As, Cr and Cu exceeded the ERL values and Ni exceeded the ERM guidelines in both reserves. There is not a statistical difference among the stations in REVISMEM for most metal(loid)s, excluding Ba and Cd. Most metal(loid) presented significant differences between "within city limits" (RPFMS) and "city border area" (RPFMS), "near the city" (REVISMEM) and "far from the city" (REVISMEM), except for Al, As, Be, Co, Cr, Mo, Se and V. As and Be did not show a significant difference between the four sites in the two reserves. For mussels (*M. strigata*), no metal(loid) exceeded FAO thresholds. As was the only toxic metalloid that presented statistical significance between reserves for *M. strigata*. As, Cd, and Co showed lower concentrations in "city border area" from RPFMS compared to "near the city" from REVISMEM. The PCA for mussels located higher contamination from toxic metal(loid)s in RPFMS compared to REVISMEM for all mussel species included in this study.

Additionally, our results revealed higher mean concentrations of Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Pb, V and Zn in sediment in RPFMS and lower mean concentrations of Cd and Pb in REVISMEM than the ones found in previous investigations. For mussels, RPFMS showed lower Cd and Pb concentrations than previous research possibly due to the sampling areas. On the contrary, in REVISMEM, Cd and Pb concentrations were higher than other studies. Despite being below the FAO limits, mussels also appeared to bioaccumulate higher concentrations of metal(loid)s than in previous studies for Cd and Pb[157,158].

Conclusions

Chapter 1 provides an overview of the growing pollution problem in the RPFMS, focusing on sediment and aquatic organisms (catfish, Peruvian mojarra and mussels), and includes a human health risk assessment for iAs, Cd, Cr(VI), MeHg, and Pb. The results showed that ERM and ERL values were exceeded for Cu, Hg, Ni, and Zn in sediment. Catfish exhibited higher concentrations of metal(loids) than Peruvian mojarra in most cases. *Mytella trautwineana* showed higher Zn concentrations, surpassing the FAO threshold with one sample. In Puerto Hondo, the health risk assessment revealed that EDI values were higher in Peruvian mojarra than in catfish for all metal(loid)s except Cd. No THQ values exceeded the baseline in both fish species, and HI values were below 1 in both cases. In mussels, due to the lower IR and EF reported in the survey, all THQ values for the metal(loid)s were below the threshold. CR exceeded the baseline for iAs and Cr(VI) in Peruvian mojarra and iAs in catfish, while for mussels, only iAs exceeded the baseline. All MOE values for Pb were above the margin of exposure, indicating no potential health risks from Pb exposure. Based on these findings, risk communication and management strategies are recommended for consideration.

Chapter 2 compares metal(loid) concentrations between two Ecuadorian reserves: RPFMS and REVISMEM. REVISMEM appears more pristine, with generally lower contamination levels, although toxic metal(loid)s were still found in both sediments and mussels. Our findings highlighted that As, Cr and Cu exceeded ERL guidelines and Ni exceeded ERM values in sediment in both reserves, and no metal(loid) surpassed the FAO guidelines in *M. strigata* for either reserves. *Mytella trautwineana* showed greater concentration of metal(loids) in RPFMS and REVISMEM. Given the pollution likely coming from anthropogenic activities, continued monitoring and improved urban management are recommended to protect environmental health in these increasingly impacted coastal areas in Ecuador. Special focus should be placed on investigating the distribution and bioaccumulation of toxic metal(loid)s and other contaminants in the biological parameters (size, weight) of estuarine fish and bivalve species.

References

1 *UNEP. Mangrove forests, < https://www.unep.org/topics/ocean-seas-and-coasts/blue-ecosystems/mangrove-forests> (2025).*

2 *Alongi, D. M. Carbon sequestration in mangrove forests. Carbon Management **3**, 313-322 (2012).*

3 *Hutchison, J., Spalding, M. & Ermgassen, P. Z. The role of mangroves in fisheries enhancement. (2014).*

4 *UNEP. The importance of mangroves to people: A call to action. 128 (Cambridge, 2014).*

5 *Quarto, A. Mangrove restoration—Natural protection from natural disasters. Biodiversity **6**, 3-12 (2005).*

6 *Sundaramanickam, A., Nithin, A. & Balasubramanian, T. in Mangroves: Ecology, biodiversity and management 257-278 (Springer, 2021).*

7 *López Rodríguez, F. V. in Threats to Mangrove Forests: Hazards, vulnerability, and management 557-578 (Springer, 2018).*

8 *Caiza Morales, L. Monitoring of the mangrove ecosystem in the province of Guayas-Ecuador during the period 2018-2022, Universidad Estatal Península de Santa Elena, (2023).*

9 *Carpio, P. L. Plant biodiversity of Ecuador: A neotropical megadiverse. Global Biodiversity **4**, 24 (2018).*

10 *MAE. Ministerio del Ambiente y Cámara Nacional de Acuacultura impulsan la gestión sostenible de los manglares. (2023).*

11 *Ágreda, A. E. & Del Pezo, D. Guía de los servicios ecosistémicos del manglar y conservación de aves playeras migratorias. Aves y conservación en Ecuador y humedales costeros. (2022).*

12 *Morocho, R. G., I.; Ferreira, T. O.; Otero, X. L. Mangrove forests in Ecuador: A two-decade analysis. **13**, 656 (2022).*

13 *López Rodríguez, F. Mangrove in Ecuador: Conservation and management. Coastal Environments **91** (2021).*

14 *ITC. List of exporters for the selected product in 2020 (Crustaceans, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine, ...). (2022).*

15 *Spalding, M., Blasco, F. & Field, C. World mangrove atlas. The International Society for Mangrove Ecosystems: Okinawa, Japan, 178 (1997).*

16 *Spurrier, G. Estudio del impacto de la acuicultura camaronera en el Ecuador, (2012).*

17 *Marcillo, F. Shrimp farming and the environment in Ecuador: Past and present. World Aquaculture **48**, 39-42 (2017).*

18 *Hinojosa-Ramos, M. V., León, M., León, P., Arevalo, R. & Redroban, C. Assessing management effectiveness: Manglares El Salado Fauna Production Reserve study case. International Conference on Computational Science and Its Applications, 51-63. Cham: Springer Nature Switzerland (2023).*

19 *Rebolledo Monsalve, E. & Verduga Vergara, L. Water and sediment quality changes in mangrove systems with shrimp farms in the Northern Ecuadorean Coast. Applied Sciences **13**, 7749 (2023).*

20 *Twilley, R. R., Montaño, M., Valdivieso, J. M. & Bodero, A. The environmental quality of coastal ecosystems in Ecuador: implications for the development of integrated mangrove*

and shrimp pond management. Mangrove Ecosystems in Tropical America. IUCN/NOAA/Instituto de Ecologia, Xalapa, Mexico, 199-230 (1999).

21 *Ashton, E. C. The impact of shrimp farming on mangrove ecosystems. CABI Reviews, 12 (2008).*

22 *Jones, A., O'donohue, M., Udy, J. & Dennison, W. Assessing ecological impacts of shrimp and sewage effluent: biological indicators with standard water quality analyses. Estuarine, Coastal and shelf Science **52**, 91-109 (2001).*

23 *Kautsky, N., Rönnbäck, P., Tedengren, M. & Troell, M. Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture **191**, 145-161 (2000).*

24 *Hempel, E., Winther, U. & Hambrey, J. Shrimp farming and the environment: Can shrimp farming be undertaken sustainably? (2002).*

25 *Cambien, N. et al. Using the soil and water assessment tool to simulate the pesticide dynamics in the data scarce Guayas river basin, Ecuador. Water **12**, 696 (2020).*

26 *Eurie Forio, M. A. et al. From field to plate: Agricultural pesticide presence in the guayas estuary (Ecuador) and commercial mangrove crabs. Environmental Pollution **289**, 117955 (2021).*

27 *Álvarez Arroyo, A. V., Pérez Cayeiro, M. L. & Macías Bedoya, A. Criterios de zonificación ambiental: Estudio de caso estero salado de Guayaquil - Ecuador. Revista Científica Ecociencia **2** (2015).*

28 *Medina, J. A. C., Chávez, G. F. F. & Sang, K. C. F. Estudio de las concentraciones por metales pesados e hidrocarburos en el ramal B del Estero Salado. Polo del Conocimiento: Revista científico-profesional **2**, 711-730 (2017).*

29 *MAE. Listado de industrias con descargas al Estero Salado Ministerio del Ambiente. (2012).*

30 *Twilley, R. et al. in Coastal Marine Ecosystems of Latin America 245-263 (Springer, 2001).*

31 *Alongi, D. M. Paradigm shifts in mangrove biology. Coastal Wetlands an Integrated Ecosystem Approach. Elsevier, 615-640 (2009).*

32 *Lee, S. Y. et al. Ecological role and services of tropical mangrove ecosystems: A reassessment. Global Ecology and Biogeography **23**, 726-743 (2014).*

33 *Barbier, E. B. Progress and challenges in valuing coastal and marine ecosystem services. Review of Environmental Economics and Policy (2012).*

34 *Spalding, M. D. et al. The role of ecosystems in coastal protection: Adapting to climate change and coastal hazards. Ocean & Coastal Management **90**, 50-57 (2014).*

35 *Calle, P. et al. Mercury assessment, macrobenthos diversity and environmental quality conditions in the Salado Estuary (Gulf of Guayaquil, Ecuador) impacted by anthropogenic influences. Marine Pollution Bulletin **136**, 365-373 (2018).*

36 *Cucalón, E. Temperature, salinity and water mass distribution off Ecuador during an El Niño event in 1976. Revista de Ciencias del Mar y Limnología **2**, 1-25 (1983).*

37 *Quinteros, A., Castro, J. & Leon, F. La Reserva de Producción Faunística Manglares El Salado (RPFMS) de Guayaquil, como recurso turístico sostenible. Revista Caribeña de Ciencias Sociales (2017).*

38 *MAE. Plan de manejo de la Reserva de Producciòn Faunística Manglares El Salado. (Guayaquil: Municipio de Guayaquil, 2013).*

39 *Miño, M. F. & Bermeo, C. A. C. Evaluación turística del recinto de puerto hondo, Ecuador. Mikarimin. Revista Científica Multidisciplinaria **6**, 147-156 (2020).*

40 *Rizzo, H. Diagnóstico del uso turístico y recreativo de la reserva de producción faunística manglares El Salado orientado a la implementación de una operadora turística de deportes acuáticos, Universidad de Guayaquil, (2015).*

41 *Olmedo, P. et al. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environment International* ***59****, 63-72 (2013).*

42 *De Wolf, H. & Rashid, R. Heavy metal accumulation in Littoraria scabra along polluted and pristine mangrove areas of Tanzania. Environmental Pollution* ***152****, 636-643 (2008).*

43 *Förstner, U. & Wittmann, G. T. Metal pollution in the aquatic environment. (Springer Science & Business Media, 2012).*

44 *Diop, C., Dewaelé, D., Cazier, F., Diouf, A. & Ouddane, B. Assessment of trace metals contamination level, bioavailability and toxicity in sediments from Dakar coast and Saint Louis estuary in Senegal, West Africa. Chemosphere* ***138****, 980-987 (2015).*

45 *Kulkarni, R., Deobagkar, D. & Zinjarde, S. Metals in mangrove ecosystems and associated biota: A global perspective. Ecotoxicology and Environmental Safety* ***153****, 215-228 (2018).*

46 *Le, D. Q., Satyanarayana, B., Fui, S. Y. & Shirai, K. Mercury bioaccumulation in tropical mangrove wetland fishes: evaluating potential risk to coastal wildlife. Biological Trace Element Research* ***186****, 538-545 (2018).*

47 *Domouhtsidou, G. & Dimitriadis, V. Ultrastructural localization of heavy metals (Hg, Ag, Pb, and Cu) in gills and digestive gland of mussels, Mytilus galloprovincialis (L.). Archives of Environmental Contamination and Toxicology* ***38****, 472-478 (2000).*

48 *Khan, M. I., Zahoor, M., Khan, A., Gulfam, N. & Khisroon, M. Bioaccumulation of heavy metals and their genotoxic effect on freshwater mussel. Bulletin of Environmental Contamination and Toxicology* ***102****, 52-58 (2019).*

49 *Wang, Q., Kim, D., Dionysiou, D. D., Sorial, G. A. & Timberlake, D. Sources and remediation for mercury contamination in aquatic systems—A literature review. Environmental Pollution* ***131****, 323-336 (2004).*

50 *Sanger, D., Holland, A. & Scott, G. Tidal creek and salt marsh sediments in South Carolina coastal estuaries: I. Distribution of trace metals. Archives of Environmental Contamination and Toxicology* ***37****, 445-457 (1999).*

51 *Sanger, D., Holland, A. & Scott, G. Tidal creek and salt marsh sediments in South Carolina coastal estuaries: II. Distribution of organic contaminants. Archives of Environmental Contamination and Toxicology* ***37****, 458-471 (1999).*

52 *Peng, X., Liu, F. & Wang, W. X. Organ-specific accumulation, transportation, and elimination of methylmercury and inorganic mercury in a low Hg accumulating fish. Environmental Toxicology and Chemistry* ***35****, 2074-2083 (2016).*

53 *Kidd, K., Clayden, M. & Jardine, T. Bioaccumulation and biomagnification of mercury through food webs. Environmental chemistry and toxicology of mercury, 455-500 (2012).*

54 *Burgess, N. M. & Meyer, M. W. Methylmercury exposure associated with reduced productivity in common loons. Ecotoxicology* ***17****, 83-91 (2008).*

55 *Lei, M., Zhang, Y., Khan, S., Qin, P.-f. & Liao, B.-h. Pollution, fractionation, and mobility of Pb, Cd, Cu, and Zn in garden and paddy soils from a Pb/Zn mining area. Environmental Monitoring and Assessment* ***168****, 215-222 (2010).*

56 *Kaur, R., Sharma, S. & Kaur, H. Heavy metals toxicity and the environment. Journal of Pharmacognosy and Phytochemistry* ***1****, 247-249 (2019).*

57 *Stöhrer, G. Arsenic: Opportunity for risk assessment. Archives of Toxicology* ***65****, 525-531 (1991).*

58 *Nordberg, G. F. et al. Risk assessment of effects of cadmium on human health (IUPAC Technical Report). Pure and Applied Chemistry* ***90****, 755-808 (2018).*

59 *Nag, R. & Cummins, E. Human health risk assessment of lead (Pb) through the environmental-food pathway. Science of the Total Environment* ***810****, 151168 (2022).*

60 *Ma, H., Hung, M. & Chen, P. A systemic health risk assessment for the chromium cycle in Taiwan. Environment International* ***33****, 206-218 (2007).*

61 *WHO. Lead poisoning and health, < https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health> (2023).*

62 *Romero-Estévez, D. et al. An overview of cadmium, chromium, and lead content in bivalves consumed by the community of Santa Rosa Island (Ecuador) and its health risk assessment. Frontiers in Environmental Science* ***8****, 134 (2020).*

63 *ATSDR. Toxicological profile for chromium. (Public Health Service, Atlanta, GA, 2000).*

64 *Satarug, S., Vesey, D. A. & Gobe, G. C. Current health risk assessment practice for dietary cadmium: Data from different countries. Food and Chemical Toxicology* ***106****, 430-445 (2017).*

65 *USEPA. Cadmium (CASRN 7440-43-9), <https://iris.epa.gov/ChemicalLanding/&substance_nmbr=141> (2001).*

66 *Amzal, B. et al. Population toxicokinetic modeling of cadmium for health risk assessment. Environmental Health Perspectives* ***117****, 1293-1301 (2009).*

67 *USEPA. Chromium(VI) CASRN 18540-29-9, <https://iris.epa.gov/ChemicalLanding/&substance_nmbr=144> (2001).*

68 *Kimbrough, D. E., Cohen, Y., Winer, A. M., Creelman, L. & Mabuni, C. A critical assessment of chromium in the environment. Critical Reviews in Environmental Science and Technology* ***29****, 1-46 (1999).*

69 *Wolfe, D. A. Biological effects of toxic contaminants in sediments from Long Island Sound and environs. Vol. 80 (US Department of Commerce, National Oceanic and Atmospheric Administration, 1994).*

70 *USEPA. Guidance for assessing chemical contaminant data for use in fish advisories. United States Environmental Protection Agency office of water.* ***1*** *(2000).*

71 *Nauen, C. E. Compilation of legal limits for hazardous substances in fish and fishery products. (1983).*

72 *USEPA. Issue paper on the human health effects of metals. (2004).*

73 *USEPA. National survey of mercury concentrations in fish. (2018).*

74 *USEPA. Revised technical support document: National-scale assessment of mercury risk to populations with high Consumption of self-caught freshwater fish in support of the appropriate and necessary finding for coal- and oil-fired electric generating units. (2015).*

75 *Montojo, U. M. et al. Levels and health risk assessment of mercury, cadmium, and lead in green mussel (Perna viridis) and oyster (Crassostrea iredalei) harvested around Manila Bay, Philippines. Food Control* ***124****, 107890 (2021).*

76 *USEPA. Risk assessment guidance for superfund. Volume I: Human health evaluation manual (part a). (EPA/540/1-89/002, 1989).*

77 *USEPA. Guidance for assessing chemical contaminant data for use in fish advisories. United States Environmental Protection Agency office of water.* ***2*** *(2000).*

78 *Cresson, P. et al. Underestimation of chemical contamination in marine fish muscle tissue can be reduced by considering variable wet: Dry weight ratios. Marine Pollution Bulletin* ***123****, 279-285 (2017).*

79 *Satapathy, S., Panda, C. R. & Jena, B. S. Risk-based prediction of metal toxicity in sediment and impact on human health due to consumption of seafood (Saccostrea cucullata) found in two highly industrialised coastal estuarine regions of Eastern India: A food safety issue. Environmental Geochemistry and Health* ***41****, 1967-1985 (2019).*

80 *ATSDR. Evaluation of exposures to chemicals in shellfish and sediments. (U.S. Department of Health and Human Services, Public Health Service, Port Gamble Bay, Kitsap County, Washington, 2014).*

81 *Liang, L., Shi, J., He, B., Jiang, G. & Yuan, C. Investigation of methylmercury and total mercury contamination in mollusk samples collected from coastal sites along the Chinese Bohai Sea. Journal of Agricultural and Food Chemistry* ***51****, 7373-7378 (2003).*

82 *WHO. Complementary feeding, <https://www.who.int/health-topics/complementary-feeding#tab=tab_1> (2025).*

83 *WHO. Life expectancy, Ecuador, <https://data.who.int/countries/218> (2021).*

84 *Sharif, R., Chong, E. & Meng, C. K. Human health risk assessment of heavy metals in shellfish from Kudat, Sabah. Malaysian Journal of Nutrition* ***22****, 301-305 (2016).*

85 *Mohiuddin, M. et al. Human health risk assessment for exposure to heavy metals in finfish and shellfish from a tropical estuary. Journal of King Saud University-Science* ***34****, 102035 (2022).*

86 *USEPA. Inorganic arsenic (CASRN 7440-38-2), <https://iris.epa.gov/ChemicalLanding/&substance_nmbr=0278> (2001).*

87 *USEPA. Methylmercury (MeHg) (CASRN 22967-92-6), <https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=73> (2001).*

88 *ATSDR. Hazard quotients and cancer risk. (U.S. Department of Health and Human Services, 2022).*

89 *EFSA. in EFSA Journal Vol. 8 1570 (2010).*

90 *EFSA. Use of the 'margin of exposure' (MOE) approach for deriving risk-based action limits for carcinogens unintentionally present in food. (Parma, Italy 2012).*

91 *USEPA. RCRA delisting technical support document: Chapter 4 – Risk and hazard assessment. (Washington, DC, 2020).*

92 *ATSDR. Evaluate the evidence to examine cancer effects. (2025).*

93 *Long, E. R. & MacDonald, D. D. Recommended uses of empirically derived, sediment quality guidelines for marine and estuarine ecosystems. Human and Ecological Risk Assessment* ***4****, 1019-1039 (1998).*

94 *Fernández-Cadena, J. C., Andrade, S., Silva-Coello, C. & De la Iglesia, R. Heavy metal concentration in mangrove surface sediments from the North-west coast of South America. Marine Pollution Bulletin* ***82****, 221-226 (2014).*

95 *Ormaza-Gonzalez, F. I., Campi-Alvarez, P. A., Cárdenas-Condoy, J. W., Caiza-Quinga, R. & Statham, P. J. Further evidence for increasing global estuarine eutrophication from the Estero Salado, Guayaquil, Ecuador. Continental Shelf Research* ***278*** *(2024).*

96 *Monserrate, L., Medina, J. F. & Calle, P. Estudio de condiciones físicas, químicas y biológicas en la zona intermareal de dos sectores del Estero Salado con diferente desarrollo urbano, ESPOL, (2012).*

97 *Rodriguez, R. & Acero, A. Filogenia de los bagres marinos (Siluriformes: Ariidae) del nuevo mundo. Acta Biológica Colombiana* ***9****, 72 (2004).*

98 *Bastidas, J. J. M., Andres & Duque, G. Impact of contamination due to ingestion of microplastics on commercial fish in relation to their trophic habits. (2024).*

99 *Bagre pinnimaculatus, <https://www.fishbase.se/summary/Bagre-pinnimaculatus> (2025).*

100 *Saidon, N. B., Szabó, R., Budai, P. & Lehel, J. Trophic transfer and biomagnification potential of environmental contaminants (heavy metals) in aquatic ecosystems. Environmental Pollution* ***340****, 122815 (2024).*

101 *Diapterus peruvianus, <https://www.fishbase.se/summary/SpeciesSummary.php?ID=10430&AT=Peruvian+Mojarra> (2025).*

102 *Beyer, J. et al. Blue mussels (Mytilus edulis spp.) as sentinel organisms in coastal pollution monitoring: a review. Marine Environmental Research* ***130****, 338-365 (2017).*

103 *Chahouri, A., Yacoubi, B., Moukrim, A. & Banaoui, A. Bivalve molluscs as bioindicators of multiple stressors in the marine environment: Recent advances. Continental Shelf Research* ***264****, 105056 (2023).*

104 *Abdullah, M., Sidi, J. & Aris, A. Z. Heavy metals (Cd, Cu, Cr, Pb and Zn) in meretrix meretrix roding, water and sediments from estuaries in Sabah, North Borneo. International Journal of Environmental & Science Education* ***2****, 69-74 (2007).*

105 *Richir, J. & Gobert, S. The effect of size, weight, body compartment, sex and reproductive status on the bioaccumulation of 19 trace elements in rope-grown Mytilus galloprovincialis. Ecological Indicators* ***36****, 33-47 (2014).*

106 *Otchere, F. A. A 50-year review on heavy metal pollution in the environment: Bivalves as bio-monitors. African Journal of Environmental Science and Technology* ***13****, 220-227 (2019).*

107 *Tamayo León, J. D. Análisis de concentración de metales pesados en moluscos estuarinos en las provincias de Esmeraldas, Guayas, El Oro, Ecuador, 2009-2018, Universidad Estatal Península de Santa Elena, (2021).*

108 *Rodríguez, F. Cuantificación de cadmio, plomo y níquel en agua superficial, sedimento y Organismos (Mytella Guyanensis) en los puentes Portete Y 5 de Junio Del Estero Salado (Guayaquil). (2013).*

109 *Drouet Racines, D. A. & Lovato Mendoza, A. P. Distribución y abundancia de macrobentos en la reserva de producción faunística manglares del salado del golfo de Guayaquil para la estación seca del año 2014, (2019).*

110 *USEPA. Useful terms and definitions for explaining risk. (USEPA Superfund Program Washington, D.C., n.d).*

111 *Vergara, E., Pancetti, F., Zúñiga, L., Fabres, K. & Bahamonde, P. Risk map of human intake of mercury through fish consumption in Latin America and the Caribbean. Frontiers in Sustainable Food Systems* ***8****, 1470683 (2024).*

112 *Cheng, W. W. & Gobas, F. A. Assessment of human health risks of consumption of cadmium contaminated cultured oysters. Human and Ecological Risk Assessment* ***13****, 370-382 (2007).*

113 *Vahter, M., Berglund, M., Nermell, B. & Åkesson, A. Bioavailability of cadmium from shellfish and mixed diet in women. Toxicology and applied pharmacology* ***136****, 332-341 (1996).*

114 *EFSA. Scientific opinion on health benefits of seafood (fish and shellfish) consumption in relation to health risks associated with exposure to methylmercury. EFSA journal* ***12****, 3761 (2014).*

115 *Davey, J. C. et al. Arsenic as an endocrine disruptor: Arsenic disrupts retinoic acid receptor–and thyroid hormone receptor–mediated gene regulation and thyroid hormone–mediated amphibian tail metamorphosis. Environmental Health Perspectives* ***116****, 165-172 (2008).*

116 *Stoica, A., Pentecost, E. & Martin, M. B. Effects of arsenite on estrogen receptor-α expression and activity in MCF-7 breast cancer cells. Endocrinology* ***141****, 3595-3602 (2000).*

117 *Pan, S. et al. Effects of lead, cadmium, arsenic, and mercury co-exposure on children's intelligence quotient in an industrialized area of southern China. Environmental Pollution* ***235****, 47-54 (2018).*

118 *Smith, M. R. et al. Integrative bioinformatics identifies postnatal lead (Pb) exposure disrupts developmental cortical plasticity. Scientific Reports* ***8****, 16388 (2018).*

119 *Othman, M. A. Value of mangroves in coastal protection. Hydrobiologia* ***285****, 277-282 (1994).*

120 *Deb, M. & Ferreira, C. M. Potential impacts of the Sunderban mangrove degradation on future coastal flooding in Bangladesh. Journal of Hydro-Environment Research* ***17****, 30-46 (2017).*

121 *Horstman, E. M. et al. Wave attenuation in mangroves: A quantitative approach to field observations. Coastal Engineering* ***94****, 47-62 (2014).*

122 *Miththapala, S. Mangroves. Vol. 2 (IUCN, 2008).*

123 *Saenger, P. Mangrove ecology, silviculture and conservation. (Springer Science & Business Media, 2002).*

124 *Acharya, G. Life at the margins: The social, economic and ecological importance of mangroves. Madera y Bosques* ***8****, 53-60 (2002).*

125 *Makowski, C. & Finkl, C. W. Threats to mangrove forests: Hazards, vulnerability, and management. Vol. 25 (Springer, 2018).*

126 *Cuong, D. T. et al. Heavy metal contamination in mangrove habitats of Singapore. Marine Pollution Bulletin* ***50****, 1732-1738 (2005).*

127 *Yam, R. S., Fan, Y.-T., Tan, Z., Wang, T.-D. & Chiu, C.-Y. Assessing impacts of metallic contamination along the tidal gradient of a riverine mangrove: multi-metal bioaccumulation and biomagnification of filter-feeding bivalves. Forests* ***11****, 504 (2020).*

128 *Bodin, N. et al. Assessment of trace metal contamination in mangrove ecosystems from Senegal, West Africa. Chemosphere* ***90****, 150-157 (2013).*

129 *Marchand, C. et al. Heavy metals distribution in mangrove sediments along the mobile coastline of French Guiana. Marine Chemistry* ***98****, 1-17 (2006).*

130 *Liu, J., Cao, L. & Dou, S. Bioaccumulation of heavy metals and health risk assessment in three benthic bivalves along the coast of Laizhou Bay, China. Marine Pollution Bulletin* ***117****, 98-110 (2017).*

131 *Tobar, J., Ramírez-Muñoz, M., Fermín, I. & Senior, W. Concentración de Metales Pesados en Bivalvos Anadara tuberculosa y a. Similis del estero huaylá, provincia de El Oro, Ecuador. Boletín del Centro de Investigaciones Biológicas* ***51****, 19-30 (2017).*

132 *Venegas, M. C. Acumulación de metales pesados en bivalvos y sus efectos tóxicos en la salud humana: Perspectivas para el estudio en Costa Rica. Pensamiento Actual* ***15****, 173-181 (2015).*

133 *van der Schatte Olivier, A. et al. A global review of the ecosystem services provided by bivalve aquaculture. Reviews in Aquaculture* ***12****, 3-25 (2020).*

134 *Carvajal, R. & Alava, J. J. Mangrove wetlands conservation project and the shrimp farming industry in Ecuador: Lessons learned. World Aquaculture-Baton Rouge* ***38****, 14 (2007).*

135 *Negrete, J., Varela, J., Heras, E., Alcívar, M. & Rosero, P. in Primer Congreso Manglares de América.*

136 *Solorzano, L. & Viteri, G. Investigación química del Estero Salado. Rev. Ciencias del Mar y Limnología* ***3****, 227 (1993).*

137 *Ayarza, W. et al. Estudios geobioquímicos de la sección urbana del Estero Salado. Revista de Ciencias del Mar y Limnología* ***3****, 1-19 (1993).*

138 *Alava, J. J. et al. Persistent organic pollutants and mercury in genetically identified inner estuary bottlenose dolphin (Tursiops truncatus) residents of the Guayaquil Gulf, Ecuador: Ecotoxicological science in support of pollutant management and cetacean conservation. Frontiers in Marine Science 7, 122 (2020).*

139 *MAE. Plan de Manejo del Refugio de Vida Silvestre Manglares El Morro. 164 (General Villamil. Ecuador, 2010).*

140 *Mora, E., Moreno, J., Jurado, V. & Flores, L. La pesquería de la concha prieta (Anadara tuberculosa y Anadara similis) en el 2009: Indicadores pesqueros y condición reproductiva en la zona sur y norte de Ecuador. (2010).*

141 *Cruz, M. Estado actual del recurso malacológico (bivalvos y gasterópodos) de la zona infralitoral del Golfo de Guayaquil. (1992).*

142 *Alcivar, M. d. J. & Mosquera, J. L. Concentración de metales pesados (Cr total, Pb, Cd) en agua superficial y sedimentos en el estero salado (Guayaquil). Revista Universidad de Guayaquil* ***113****, 29-34 (2012).*

143 *Espinosa, L. F., Parra, J. P. & Villamil, C. Determinación del contenido de metales pesados en las fracciones geoquímicas del sedimento superficial asociado a los manglares de la ciénaga grande de Santa Marta, Colombia. Boletín de Investigaciones Marinas y Costeras-INVEMAR* ***40****, 7-23 (2011).*

144 *Mejías, C. L., Musa, J. C. & Otero, J. Exploratory evaluation of retranslocation and bioconcentration of heavy metals in three species of mangrove at Las Cucharillas marsh, Puerto Rico. Journal of Tropical Life Science* ***3****, 14-22 (2013).*

145 *El-Said, G. F. & Youssef, D. H. Ecotoxicological impact assessment of some heavy metals and their distribution in some fractions of mangrove sediments from Red Sea, Egypt. Environmental Monitoring and Assessment* ***185****, 393-404 (2013).*

146 *Tam, N. F. & Wong, Y. S. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. Environmental Pollution* ***110****, 195-205 (2000).*

147 *Tavakoly Sany, S. B. et al. Distribution and contamination of heavy metal in the coastal sediments of Port Klang, Selangor, Malaysia. Water, Air, & Soil Pollution* ***224****, 1-18 (2013).*

148 *Behera, B. et al. Impact of heavy metals on bacterial communities from mangrove soils of the Mahanadi Delta (India). Chemistry and Ecology* ***29****, 604-619 (2013).*

149 *Caeiro, S. et al. Assessing heavy metal contamination in Sado estuary sediment: An index analysis approach. Ecological Indicators* ***5****, 151-169 (2005).*

150 *Mero, M., Arcos, V., Egas, F., Siavichay, R. & Lindao, G. Determinación de metales pesados (Cd y Pb) en moluscos bivalvos de interés comercial (Anadara tuberculosa y A. grandis) de Puerto El Morro, Ecuador. Investigación, Tecnología e Innovación* ***4****, 19–32-19–32 (2012).*

151 *CCME. Canadian sediment quality guidelines for the protection of aquatic life. Canadian Council of Ministers of the Environment (2001).*

152 *Benavides, M. T. Conflictos en el ecosistema manglar de la costa del Ecuador: El desarrollo de la acuacultura industrial del camarón frente a los derechos de los pueblos de recolectores y pescadores de los estuarios. Periodo: 2008-2019. (Proyecto Equiterra, 2021).*

153 *Kuffó, A. Niveles de cadmio, cromo, plomo, y su bioacumulación por Mytella strigata delimitando la zona urbano-marginal en el Estero Salado de Guayaquil, Universidad de Guayaquil, (2013).*

154 *Banguera Garcés, T. M. Análisis de metales pesados cadmio, plomo y mercurio Cd, Pb, Hg en bivalvos de la familia Mytilidae y Ostreidae, en la provincia del Guayas y El Oro, 2010–2020, Universidad Estatal Península de Santa Elena, (2021).*

155 *Rodríguez Aguilar, J. A. & Alvarado Siguencia, D. F. Propuesta de un plan de manejo ambiental, en base a la presencia de metales pesados en el estero Huaylá, Pto. Bolívar, (2015).*

156 *Sonke, J. E. et al. Global change effects on biogeochemical mercury cycling. Ambio* ***52****, 853-876 (2023).*

157 *Oves, M., Saghir Khan, M., Huda Qari, A., Nadeen Felemban, M. & Almeelbi, T. Heavy metals: biological importance and detoxification strategies. Journal of Bioremediation and Biodegradation 7, 1-15 (2016).*

158 *Canli, M. & Atli, G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution* ***121****, 129-136 (2003).*