Quantification of Heavy Metal Content in *Anadara tuberculosa* from the Gulf of Guayaquil Using ICP-OES: Assessing Marine Contamination

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Abstract: The present study was conducted to quantify the heavy metals cadmium, lead, copper, chromium, nickel, and zinc in the bivalve mollusk concha prieta (*Anadara tuberculosa*) using inductively coupled plasma optical emission spectroscopy (ICP-OES). This research aims to identify whether the bioaccumulate content of heavy metals exceeds the maximum limits established by various public health bodies such as the ONU, FAO, Codex Alimentarius, EEC, and NHI. Samples of the species were collected randomly from three locations in Puerto El Morro, Playas Municipal Market, and La Libertad Seafood Market in the Gulf of Guayaquil. Thirty-three soft tissue samples of (*Anadara tuberculosa*) were evaluated, and the values quantified in mg/kg were in the following order: Zn>Cu>Cd>Ni>Cr>Pb.

Keywords: pollution; Anadara tuberculosa; Ecuador; shellfish; gulf; ICP-OES

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1. Introduction

In marine and coastal ecosystems, one of the main pollutants is heavy metals (HMs), which are chemical elements whose density is greater than 5 g/cm³. Some of the most common HMs in aquatic environments include cadmium (Cd), lead (Pb), arsenic (As), zinc (Zn), copper (Cu), and mercury (Hg). HMs can enter the marine environment through various sources, including natural sources and anthropogenic activities [1,2]. Agricultural, industrial, and domestic activities are considered anthropogenic, and their increase is associated with one of the leading causes of progressive pollution of water resources [3].

HMs can also enter the sediment from water bodies with a progressive increase in their concentrations over time. The physical and chemical characteristics of sediments, such as texture, organic composition, and pH, can affect the bioavailability and toxicity of HMs in marine ecosystems [4]. Their diffusion phenomenon in the aquatic environment is relatively low; however, many HMs can be transferred or mobilized from sediments to surface waters, representing a continuous ecological threat to species inhabiting sediments and water bodies [5]. The persistent, toxic, and bioaccumulative properties of HMs induce a series of pathologies, such as liver, kidney, and muscle dysfunctions, which alter the growth, development, metabolism, behavior, and adaptation of marine species [6].

If HMs contaminate benthic fauna, it negatively impacts the survival and repopulation of its consumers in the trophic chain, which is significant. In addition, the supply of micronutrients and macronutrients from marine resources, such as mollusks and fish, is essential for human health; therefore, their entry will be linked to biomagnification of HMs [7]. As top predators, humans can come into contact with HMs by consuming species that live in contaminated sediments, such as bivalve mollusks, which are potentially bioaccumulating species of these elements in high concentrations [8].

Bioaccumulation of cadmium (Cd) in the body causes damage to the liver, testes, kidneys, and bones. Its chronic accumulation reduces the reabsorption of nutrients and low molecular weight proteins [9]. The reported health effects of Pb exposure include cardiovascular disease, idiopathic intellectual disability, and idiopathic intellectual development [10]. Hexavalent chromium (Cr VI) is reduced to trivalent chromium (Cr III) by a biochemical process that occurs after absorption into the body; chromium reduction leads to DNA damage causing histological damage [11]. A common pathology is skin hypersensitivity, caused by Nickel(Ni) allergy that develops dermatitis [12]. Aluminum (Al) causes numerous diseases, such as Alzheimer's disease and pulmonary fibrosis, and generates acute neurotoxicity. In humans, certain types of cancer and coronary heart disease are correlated with high levels of exposure [13].

One way of biomonitoring HMs contamination is by quantifying these metals in aquatic organisms [14]. Bivalve mollusks have been used as bioindicators of HMs for several decades because they are susceptible to specific species and have a high bioaccumulation capacity. The concentration of HMs in the tissues of bivalve mollusks reflects the presence and availability of these contaminants in their environment. Therefore, assessment of the abundance of HMs in the tissues of bivalve mollusks can be used to identify spatial and temporal trends in HMs contamination [7].

Anadara tuberculosa is a filter-feeding bivalve mollusk endemic to the American Pacific mangroves. It develops mainly in muddy substrates, especially clays and clayey silts [15]. Because of its ability to accumulate heavy metals, *A. tuberculosa* has been proposed as a potential biomarker for biomonitoring metal pollution in coastal mangrove ecosystems.

The Gulf of Guayaquil (GG), the largest estuarine ecosystem on the Pacific coast of South America, is home to many aquatic species, including *A. tuberculosa*. However, research in recent years indicates that the GG is experiencing increasing pollution from anthropogenic activities, such as industry, agriculture, fishing, aquaculture, and urbanization [16]. This pollution represents a risk to the health of ecosystems and the human communities that depend on them [17].

The results of quantification using ICP-OES in *Andara tuberculosa* from the present research allow for the establishment of findings of elevated concentrations of heavy metals in bivalve mollusks. This indicates the presence of contamination by HMs in the habitat where their life cycle unfolds. This indicator suggests considering high concentrations of heavy metals as potential hazards to microflora, macroflora, plants, animals, and humans [18]. It is representative of studies conducted on the content of HMs in aquatic organisms such as bivalve mollusks due to the direct correlation with contamination in marine sediment. Additionally, their position in the trophic network threatens species that feed on bivalve mollusks [19].

The present study focuses on the quantification by inductively coupled plasma optical emission spectroscopy (ICP-OES) of the heavy metals cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn) in bivalve mollusks of the *Anadara tuberculosa* species known as "concha negra" or "concha prieta" in three sectors of the Gulf of Guayaquil (GG) to identify the degree of bioaccumulation of heavy metals in the species.

2. Materials and Methods

2.1. Study Area

The Gulf of Guayaquil (GG) has an area of 13.711 Km^2 of the Ecuadorian continental shelf [20]. The GG is located in Ecuador between the provinces of Guayas and El Oro 81º00'00" W and 03º23'34" S. It has a tropical climate with two seasons, a rainy season from December to May and a dry season from June to November [17]. The GG is home to Guayaquil, a rapidly growing city and represents 80% of Ecuador's total mangrove area [21].

The study area comprises 3 study sectors selected for the characteristics they represent in relation to the GG. The Ministry of Environment of Ecuador (MAE) considers the mangrove El Morro to be a wildlife refuge, and according to the regulation (MAATE-2021-005 of November 5, 2021), the area

has an extension of 35,737.4 ha [22]. Another factor to consider was identifying a coastal city in which tourist, commercial, and port activities make it an ideal study area for this study. The MAE considers the beaches of the General Villamil canton as a National Recreation Area; according to reports from the entity, this area receives more than 1.25 million local and foreign tourists [23].

The second most important province for the GG is Santa Elena, a strategic place for monitoring marine species that fishermen of the GG market. It is the municipal market of La Libertad, which has 140 trading posts and is considered one of the most important markets in the province.

2.2. Sampling

In February 2023, 33 samples of *A. tuberculosa* were randomly collected from 3 sectors of the Gulf of Guayaquil: Puerto El Morro (n=11), General Villamil Playas (n=11) and La Libertad (n=11) Figure 1. Samples of concha prieta intended for direct consumption by the sector's inhabitants were collected at seafood markets. The samples were stored in a polyethylene bag with a zip closure at a temperature of 4 ºC. The collected samples were taken to the laboratory for processing.
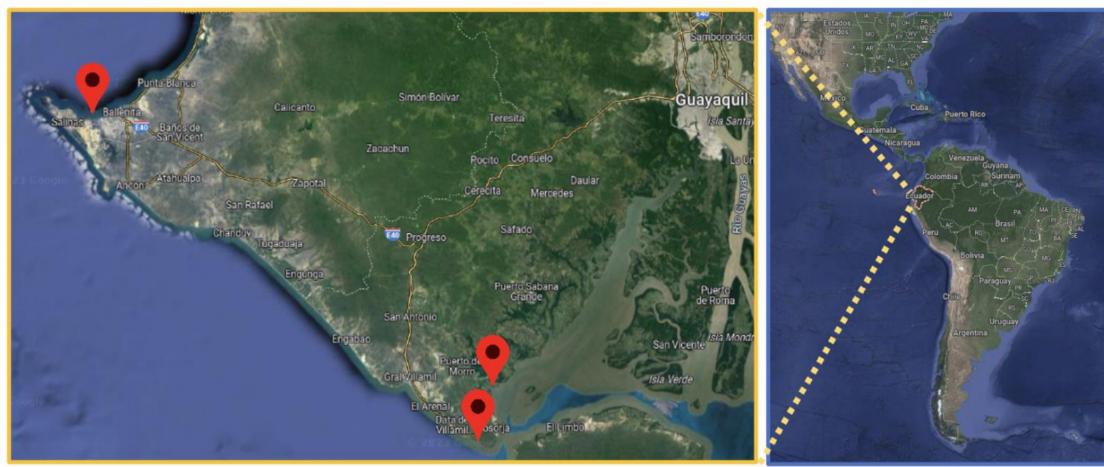
Figure 1. Section of Ecuador's map showing the location of the three *A. tuberculosa* sampling sectors using Google Earth.

2.3. Sample Preparation and Analysis

Each sample was washed externally with deionized water (DDW) during sample processing. Then, the contents of the mollusk were extracted, and the moisture was removed until it was weighed in an oven. The dried samples were crushed in a laboratory manual mortar and pestle and individually packed in polyethylene bags. The microwave acid digestion technique was used in the digester (Mars 6, CEM) for sample digestion. Approximately 0.5 g of the sample was weighed, and 10 mL of ultrapure-grade nitric acid was added to a Teflon digestion vessel and heated for 15 min at 200 °C. After cooling, filtration was performed and brought to a volume of 50 mL. Finally, the concentration of heavy metals in the studied elements in the solution was quantified using an inductively coupled plasma optical emission spectrometer (ICP-OES; iCAP 7400 Duo, Thermo Scientific, Waltham, MA, USA) and following the guidelines of the United States Environmental Protection Agency (EPA) Method 6010D [24].

2.4. Quality Assurance and Quality Control (QA/QC)

Duplicate samples and reagent blanks were used in the study. The R^2 value for the calibration curves of each element was greater than 0.999.

Figure 2 shows the limit of detection (LoD) for each chemical element. The values are as follows: Cd 0.0004 ppm, Pb 0.0023 ppm, Cr 0.0008 ppm, Ni 0.0009 ppm, Cu 0.001 ppm, and Zn 0.0004 ppm.
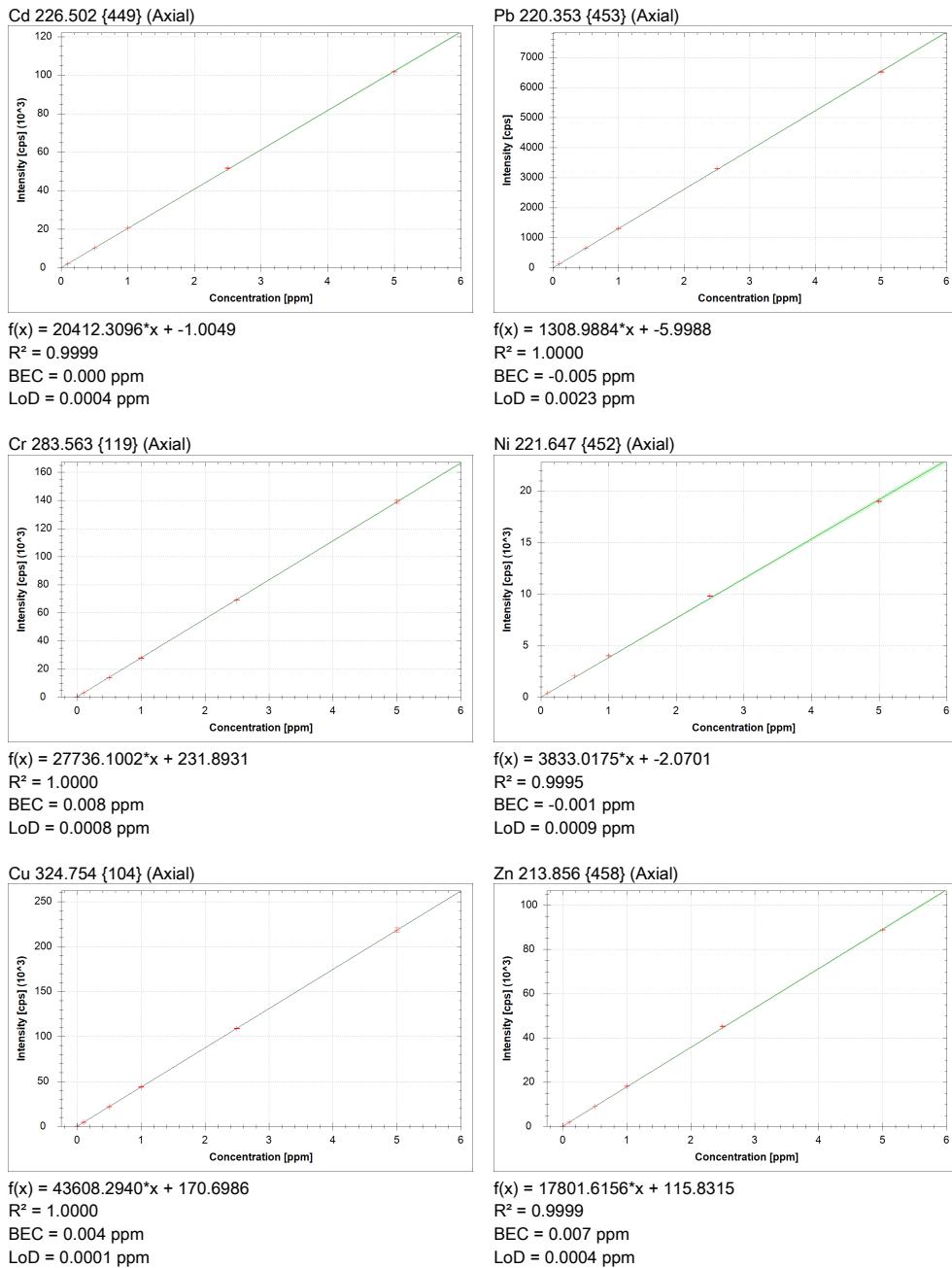
Figure 2. Calibration graphs for the six metals Cd, Pb, Cr, Ni, Cu, and Zn.

2.5. Statiscal Analysis

Descriptive statistics of the data were calculated with SPSS 26 (IBM, Armonk, NY, USA), and Minitab 19 was used for the graphs. To determine if there is a significant difference with the permissible limit values for heavy metals, a t-test was used, with a confidence level of 95 %. Rstudio, version 4.3.2 (2023-10-31 ucrt) was used to elaborate the mean diagram of the interaction of variables.

3. Results and Discussion

3.1. Concentrations of Cd, Pb, Cr, Ni, Cu, and Zn.

The concentrations of Cd, Pb, Cr, Ni, Cu, and Zn in samples of *A. tuberculosa* concha prieta are presented in Table 1 and Figure 3. The maximum allowable concentration of the heavy metals Cd and

Pb in bivalve molluscs established by the European Union [25] was used for comparison with this study. For the heavy metals Cr, Ni, Cu and Zn, the daily intake limit issued by the National Institutes of Health (NHI) and the Spanish Agency for Food Safety and Nutrition (AESAN) was recognized.
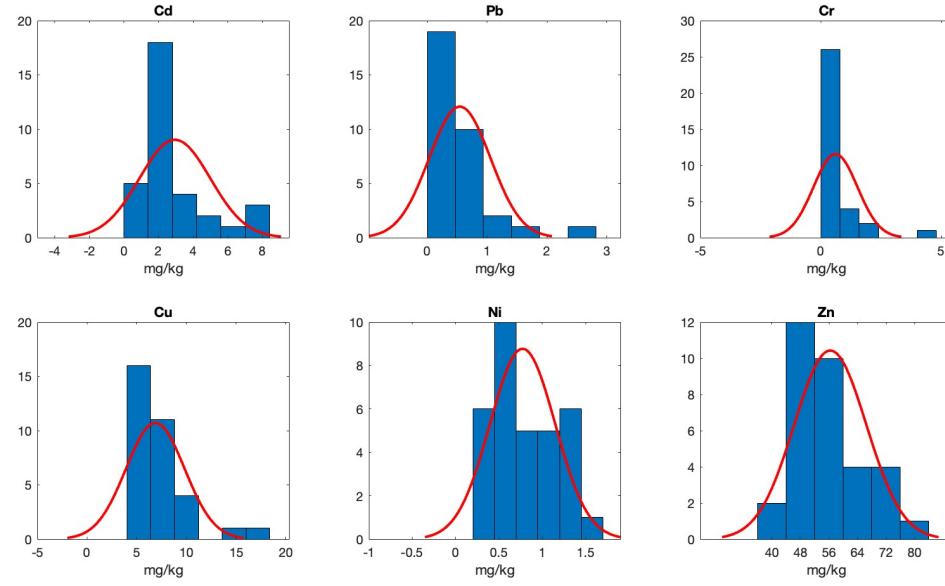
Figure 3. Histogram of Cd, Pb, Ni, Cr, Cu, and Zn concentrations (mg/kg) in *A.Tuberculosa*.
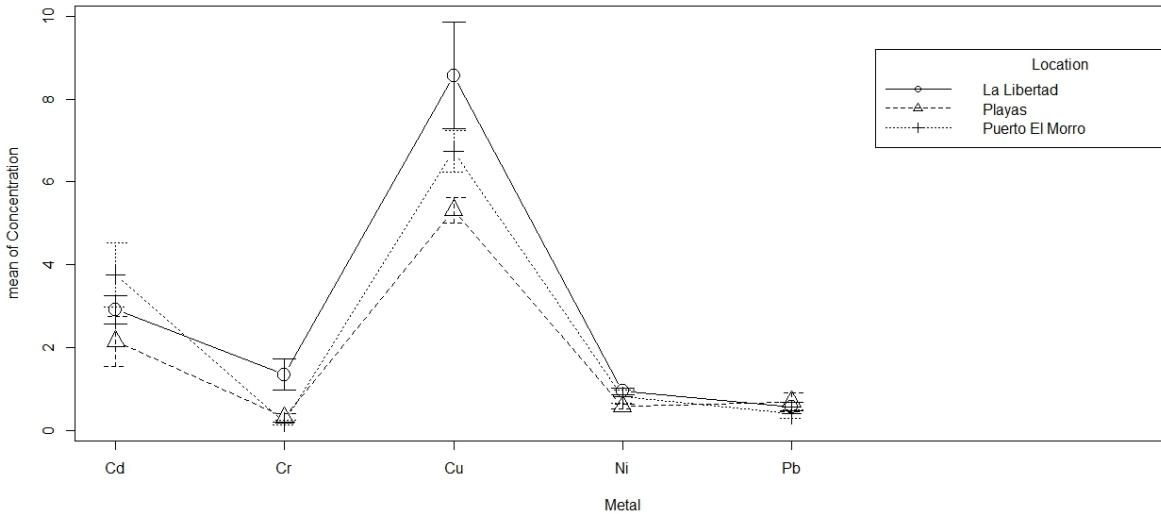
Figure 4. Interaction plot of the means (mg/kg) of five metals from *A. tuberculosa* sampled in three sectors of the Gulf of Guayaquil.
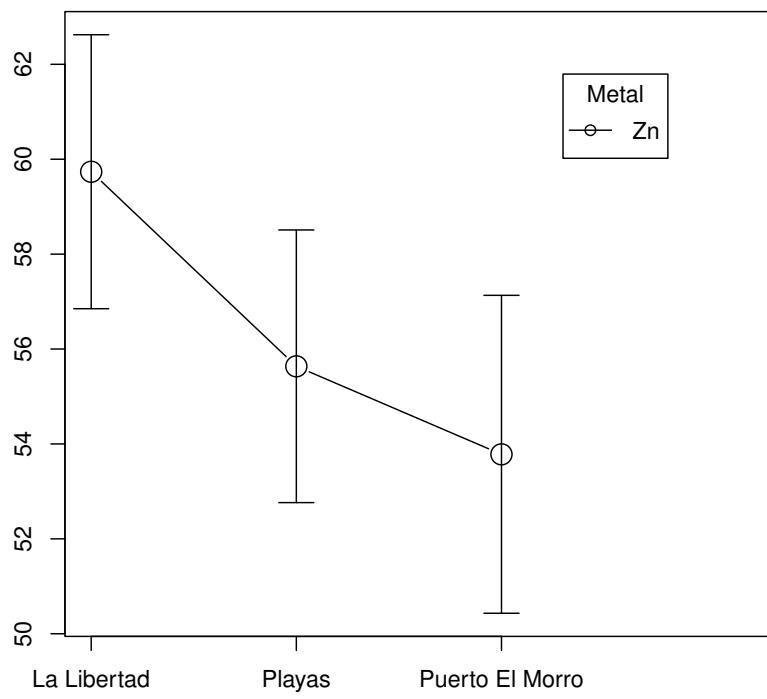
Figure 5. Interaction plot of the means (mg/kg) of Zn from *A. tuberculosa* sampled in three sectors of the Gulf of Guayaquil.

3.2. Tukey's test

Tukey's test was performed to determine which means are significantly different. Considering the p-values, no significant differences were found between the means of the variable concentration of metals associated with the locations.

We performed Tukey's test to contrast which pairs of means are significantly different. Taking into account the p-values obtained, for the usual α, significant differences between the means of the variable means of metals associated with the concentration of the Cu-Cd groups, as well as Zn-Cd, Cu-Cr, Zn-Cr, Zn-Cr, Ni-Cu, Pb-Cu, Zn-Cu, Zn-Ni and also Zn-Pb are appreciated.

3.3. Principal Component Analysis (PCA)

The first component has significant positive associations with Cr, Cu, and Zn; thus, this component mainly measures the long-term bioaccumulation of these elements. The second component identifies a sizeable negative association with the element Cd; this component mainly indicates persistent bioaccumulation of this metal. The Figure 6 graphically shows the two components, the first on the X-axis and the second on the y-axis.
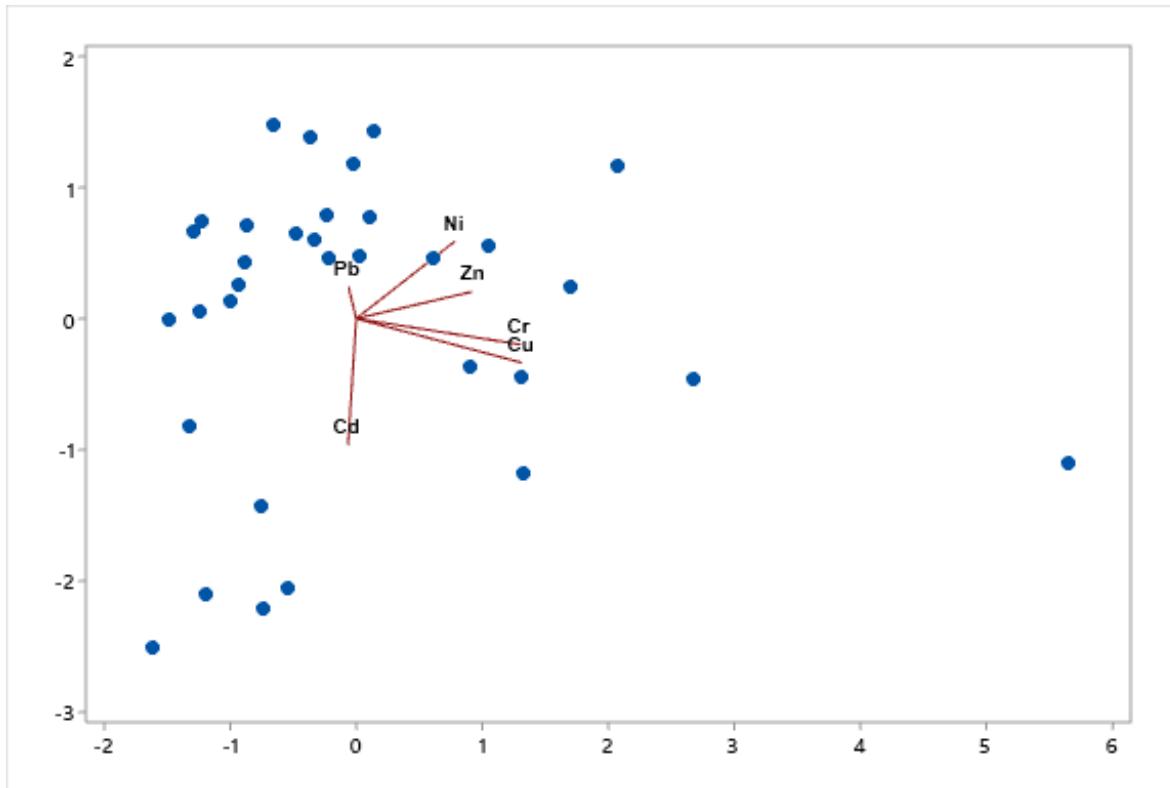
Figure 6. Principal Component Analysis (PCA) plot of *Anadara tuberculosa* samples about heavy metals.

Table 1. Concentrations of Cd, Pb, Cr, Ni, Cu, and Zn in samples of bivalve mollusks *Anadara tuberculosa* (n=33) for each sector of the Gulf of Guayaquil (mg/kg) on a dry basis.

Samples Area	Mean ± SD (mg/kg) (Minumum-Maximun)					
	Cd	Pb	Cr	Ni	Cu	Zn
Puerto El Morro	3.75 ± 2.56 (1.10 - 8.20)	0.39 ± 0.34 (0.0 - 1.20)	0.19 ± 0.17 (0.0 - 0.60)	0.80 ± 0.51 (0.20 - 1.70)	6.70 ± 1.64 (4.30 - 10.00)	53.78 ± 11.10 (81.60 - 40.00)
General Villamil Playas	2.14 ± 2.00 (0.50 - 8.00)	0.68 ± 0.75 (0.10 - 2.8)	0.30 ± 0.34 (0.0 - 1.1)	0.57 ± 0.21 (0.30 - 1.1)	5.3 ± 1.02 (4.1 - 7.50)	55.63 ± 9.52 (73.80 - 43.90)
La Libertad	2.90 ± 1.16 (1.60 - 5.20)	0.56 ± 0.33 (0.20 - 1.50)	1.34 ± 1.27 (0.10 - 4.50)	0.93 ± 0.24 (0.60 - 1.20)	8.56 ± 4.27 (4.20 - 18.20)	59.73 - 9.57 (75.30 - 45.70)

Table 2 identifies the differences in the concentrations of heavy metals detected in the *Anadara tuberculosa* species. The information presented in the table serves as a valuable frame of reference for interpreting the results of this research and provides an overview of the problem. There is sufficient evidence to determine the species as a bioindicator of heavy metal contamination in the Gulf of Guayaquil.

Table 2. Heavy metals content in Anadara tuberculosa: Review of previous studies.

Accumulation	Location	Sampling Year	Reference
Pb 0.87 ± 0.68 mg/kg Hg 0.57 ± 0.74 mg/kg	Colombia, Valle del Cauca	2023	Lucero Rincón et al., 2023 [26].
Cd 0.55 mg/kg Pb 0.03 mg/kg	Ecuador, Esmeraldas		
Cd 0.76 mg/kg Pb 0.06 mg/kg	Ecuador, Guayas	2023	Aldás et al., 2024 [27].
Cd 0.64 mg/kg Pb 0.02 mg/kg	Ecuador, El Oro		
Hg 0.055 mg/kg	Ecuador, Quito	2022	Nasevilla et al., 2022 [28].
Cd 0.096 ± 0.06 µg/g Cu 1.59 ± 1.10 µg/g	Golfo de Montijo, Panamá	2020	Tuñon et al.,2020 [29].
Pb 7.5 ± 0.45 mg/kg As 12.43 ± 0.08 mg/kg Hg 364.67 ± 91.37 mg/kg Cd 1.67 ± 0.27 mg/kg Cr 4.40 ± 2.15 mg/kg Co 2.71 ± 0.34 mg/kg	Ecuador, Puerto Bolívar.	2017	Collaguazo et al., 2017 [30].

3.4. Cadmium

The mean Cd concentration in the *A. tuberculosa* bivalve samples from the GG was 2.93 ± 2.04 mg/kg (range: 0.50–8.20 mg/kg) Table 1. This value exceeds the European Union (EU) maximum permitted limit of 1.0 mg/kg, established in Regulation (EC) No. 1881/2006 [25].

Of the 33 samples of *A. tuberculosa* analyzed, 93.9% exceeded the permitted Cd limit. The mean Cd concentration (3.75 mg/kg) in the Puerto El Morro sector was almost four times the permitted limits. The average Cd concentrations in General Villamil Playas (2.14 mg/kg) and La Libertad (2.90 mg/kg) were also more than 200% higher than the permitted limit. These results coincide with the range of Cd concentration reported [16] in *A. tuberculosa* sampled in the GG (1.40-3.97 ppm).

Figure 4 shows that the mean cadmium (Cd) concentrations in the three sampled sites are similar. This result is consistent with reports [31], which indicate that Cd contamination is mostly of geogenic origin. However, the indiscriminate use of agricultural inputs and their residues from crop runoff also contribute to these high levels of Cd. Therefore, it is necessary to continue monitoring this metal's content and look for mitigation alternatives.

3.5. Lead

The mean concentration of Pb in the bivalve *A. tuberculosa* was 0.54 ± 0.51 mg/kg Table 1, which is below the permissible limit of 1.5 mg/kg established by the European Union in Regulation (EC) No. 1881/2006 [25]. The average concentrations of the three sampled sectors (General Villamil Playas, La Libertad, and Puerto El Morro) comply with this limit.

The General Villamil Playas sector had the highest Pb concentration (0.68 ± 0.75 mg/kg), followed by La Libertad (0.56 mg/kg) and Puerto El Morro (0.39 mg/kg). Only 3% of the samples exceeded the permissible limit, with values almost double this limit. These results coincide with the Pb concentration range reported in *A. tuberculosa* sampled in the Gulf of Guayaquil (0.11-7.52 ppm).

Figure 4 shows that the average concentrations of lead (Pb) in the three sampled sites are similar. According to the research [30], the combustion of gasoline from boats used in fishing activities is one of the primary anthropogenic sources of Pb in the landing areas of artisanal docks in Ecuador and a possible source of Pb in the sectors sampled. Although Pb concentrations are below the limits proposed by the European Union, a higher Pb content can be observed in Playas and La Libertad because they are fishing ports that harbor vessels a few kilometers from their shores daily. Unlike Puerto El Morro, a protected area and wildlife refuge since 2011, there is greater control and surveillance of fishing and ecotourism.

3.6. Chromium

The mean Cr concentration in the *A. tuberculosa* samples is 0.6121 ± 0.15 mg/kg (range: 0.0–4.50 mg/kg) Table 1. This value is similar to that reported. The highest mean concentration among the

sectors evaluated corresponds to La Libertad (1.34 mg/kg). Although maximum tolerable intakes or maximum acceptable limits for this metal have not yet been established in Ecuador, other institutions such as the NHI recommend a lower intake of 35 μg/day for adult men and 25 μg/day for adult women, considering an age of 19 to 50 years [32]. In addition, the US EPA has established maximum oral reference doses of 1.5 mg/kg per day for Cr (III) [33]. Only 9% of the data is above 1.5 mg/kg and corresponds to data from La Libertad, of which 3% are three times the maximum limit suggested by the US EPA. One of the primary sources of chromium is the manufacturing processes of tanneries and industrial electroplating.

These data will help improve future monitoring and analysis of industrial impact. It is proposed to identify the industries surrounding the sampling sector. This will allow us to characterize their effluents and evaluate their impact on chromium concentrations in La Libertad's water bodies, which could be affected by chromium residues.

3.7. Nickel

The mean concentration of Ni in the *A. tuberculosa* samples is 0.77 mg/kg ±0.37 with a range of 0.20–1.70 mg/kg Table 1. The lowest mean quantified in the study corresponds to the General Villamil Playas sector mean 0.57 ± 0.21 mg/kg (range: 0.30-1.10 mg/kg), while the sector with the highest mean 0.93 ± 0.24 mg/kg (range: 0.60–1.20 mg/kg).

Although there are no defined limits for nickel in bivalves in Ecuador, the Spanish Agency for Food Safety and Nutrition (AESAN) recommends a maximum average nickel intake of 150–900 μg/day [34]. In addition, Brazilian legislation on heavy metals establishes a tolerance of 5 mg/kg of nickel in food [35].

In contrast to other mangrove areas in Ecuador, such as Estero Salado, where nickel concentrations of up to 60.14 mg/kg have been recorded, the results of nickel analyses in the three sectors studied suggest that nickel concentrations are within the permissible intake limits established by the health authorities cited above. This is positive because the communities in these sectors depend on fishing as a source of food.

However, excessive consumption of nickel can have adverse health effects, such as allergies, dermatitis, respiratory problems, and even cancer. Therefore, it is recommended that people living in these areas limit their consumption of bivalves to once a week and choose those that have undergone quality controls.

3.8. Copper

The mean Cu concentration in the *A. tuberculosa* samples was 6.86 ± 0.51 mg/kg with a range of 4.10–18.20 mg/kg Table 1. According to FAO (1982), a daily intake of 2-3 mg of copper through the diet is sufficient to meet the nutritional needs of adults, establishing a maximum tolerable intake of 0.5 mg Cu/kg body weight per day. Other sources suggest an upper daily intake limit of 10 mg [32].The copper levels reported in the three sectors can be considered acceptable for the consumption habits of the region. Furthermore, these results are much lower than those reported in Estero Salado, Guayas province, which found copper concentrations of up to 204.1 mg/kg.

However, in Figure 4, it is observed that the letters of the Tukey statistical method indicate significant differences between the means of copper concentrations and the other metals. This means that it is likely that these differences are not due to chance but are due to real factors, such as environmental pollution, industrial activity, or soil geology.

In the same figure, it is observed that copper concentrations are higher in La Libertad (8.5636 ± 4.27 mg/kg; range 4.20–18.20 mg/kg) than in Puerto El Morro and General Villamil Playas (5.30 ± 1.02 mg/kg; range: 4.10–7.50 mg/kg). This suggests that La Libertad may be more contaminated with copper than the other two locations.

To determine the cause of the differences in copper concentrations, a more detailed analysis is required. This may include studying the sources of contamination in the area, soil and water conditions, and industrial activity.

3.9. Zinc

The mean Zn concentration in the *A. tuberculosa* samples was 56.38 ± 1.75 mg/kg (range: 81.60–40.00 mg/kg). According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the average daily intake of Zn should not exceed 20 mg/day for adults or a maximum tolerable daily intake of 0.3–1 mg/kg body weight [36].

Compared with other metals, Zn is in higher amounts in the tissues of A. tuberculosa. These results are consistent with those obtained by a previous study that also observed elevated Zn concentrations in bivalves of the genera Anadara, Perna, Crassostrea, Meretrix and Amusium [37].

These elevated Zn concentrations could be due to several causes. First, Zn is a natural constituent of bivalve tissues and is in considerable concentrations [36]. Zn is a trace element necessary for the proper functioning of living organisms but in quantities lower than those reported in Table 1. Therefore, its high concentrations in the tissues of this bivalve may be due to a greater facility for the uptake and bioaccumulation of Zn from the sediment or water. To confirm this hypothesis, the bioavailable zinc content in sediment and water should be evaluated and correlated with the zinc content in bivalve tissues in longitudinal studies.

Figure 4 also shows significant differences between the means of Zn concentrations and those of the other metals. Zn concentrations were higher in La Libertad (59.73 ±9.57 mg/kg; range: 45.70–75.30 mg/kg) and lower in Puerto El Morro (53.78 ± 11.10 mg/kg; range: 40.00 - 81.60 mg/kg). This suggests that the higher Zn levels in La Libertad are the result of some type of contamination and not only due to its natural content. To determine the causes, further analysis is needed considering the sources of contamination in the area, soil and water conditions, and industrial activity.

4. Conclusions

From a local public health perspective, the mean concentrations of the metals Pb, Cr, Ni, Cu, and Zn are below the maximum levels allowed by the EU, US EPA, AESAN, IHN, FAO, and JECFA, respectively. However, the average concentration of Cd in bivalves is twice the permitted limits suggested by the EU. In order not to exceed the admissible daily intake of Cd proposed by AESAN, it is recommended to limit the consumption of this food to 0.36 μ Cd/kg body weight which corresponds to a weekly dietary intake of 2.52 μ Cd/kg body weight. Based on the Cd averages in the three sectors, consumption of *A. tuberculosa* from General Villamil Playas is recommended, followed by La Libertad and Puerto El Morro.

These recommendations apply to the bivalves analyzed in this study. It is possible that Cd concentrations in other bivalves from the same area may be different. Therefore, it is recommended that individuals consuming bivalves consult local health authorities for specific information on safe consumption limits.

The differences in mean Cu and Zn concentrations for the other metals among the three sectors suggest that the causes of contamination may be different. To determine the cause of these differences, a more detailed analysis including sources of contamination in the area, effluents, soil and water conditions, and industrial activity is recommended.

According to several studies reviewed in this research, bivalves employ regulatory and detoxification processes that can modify their accumulation of metals. Therefore, it is recommended to evaluate the concentrations of these metals in bivalve tissues from the early to adult stages and correlate them with their content in sediment and water. On the other hand, it is recommended to evaluate processing techniques such as cooking and washing to determine if they reduce their concentrations.

Finally, this study highlights the importance of periodic food quality controls, especially in areas with a high risk of heavy metal contamination.

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