JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 25(12), 60–69
https://doi.org/10.12911/22998993/193755
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.09.21
Accepted: 2024.10.20
Published: 2024.11.01

Assessing Heavy Metal Contamination in *Anadara tuberculosa* within a Protected Ecosystem

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ABSTRACT

This study aimed to assess the levels of heavy metal contamination in *Anadara tuberculosa* from a protected area in Ecuador. Ten samples were collected from a 50 m² area, dissected, and analyzed for nine heavy metals (As, Cd, Hg, Pb, Ni, Cr, Cu, Zn, and V) using inductively coupled plasma (ICP). The results revealed significant bioaccumulation of several toxic elements, with maximum concentrations ranging from 2.37 mg/kg for Cr to 73.70 mg/kg for Zn. These elevated levels raise concerns about the potential health risks to both the bivalves themselves and human consumers. Continuous and comprehensive monitoring is crucial to identify and address the environmental impacts associated with heavy metal pollution.

Keywords: bioaccumulation, monitoring, protected area, *Anadara tuberculousa.*

INTRODUCTION

Heavy metal pollution is an escalating global issue, posing significant risks to both environmental and human health (Li et al., 2022). Toxic elements like arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), zinc (Zn), and vanadium (V) can accumulate in aquatic organisms and their habitats, adversely impacting water quality and the well-being of marine species (Zakaria et al., 2021). Coastal wetlands—such as salt marshes, mangrove forests, and seagrass beds—are particularly susceptible to heavy metal contamination due to their critical role in the land-ocean transition and their exposure to pollutants from terrestrial, oceanic, and atmospheric sources (Huang et al., 2020; Zerizghi et al., 2022). Human activities, including agriculture, aquaculture, and wastewater discharge, significantly exacerbate this issue by transporting heavy metals into these ecosystems via water and air (Jiménez-Oyola et al., 2021). Additionally, oceanic activities, such as mining and oil spills, further increase the heavy metal load in coastal wetlands, which often act as sorbents, removing these pollutants from the water (Yan et al., 2022).

In this context, *Anadara tuberculosa* emerges as a crucial bioindicator for assessing heavy metal contamination in marine ecosystems (Lucero-Rincón et al., 2023). The ability of this bivalve to accumulate metals in its tissues provides a clear indication of environmental contamination levels. Elevated concentrations of metals, such as Zn and Cu may signal substantial impacts on marine ecosystems and potential risks to human health through the consumption of contaminated seafood. Although Ecuador has legislation regulating permissible heavy metal limits in water and soil, past studies have reported contamination by Cd and Pb in soils from Guayas and Oro (Pozo et al., 2011; Chávez et al., 2015), as well as As in water, soil, and cultivated rice (Otero et al., 2016). Additionally, Pb, Cr, and Ni contamination has been observed in the Santiago River in Esmeraldas (Cruz et al., 2015), while Vinces et al. (2024) documented elevated metal levels in *A. tuberculosa* from the Gulf of Guayaquil, with Zn and Cu being most prevalent. Despite these findings, there has been no specific study on heavy metal contamination in the species from the Corazón and Fragatas Islands Wildlife Refuge (REVISICOF) in Manabí Province, Ecuador. The proximity of the refuge to the Chone River, known for its high contaminant load, heightens the ecological risks in the area (Vera, 2023; Pozo-Miranda, 2016). The Ecuadorian Ministry of Environment, Water, and Ecological Transition (MAATE, 2014) has identified solid waste dumps, residual discharges, shrimp farm effluents, and agrochemicals as significant contributors to heavy metal contamination. The study aimed to determine the degree of bioaccumulation of nine heavy metals, such as arsenic (AS), cadmium (CD), mercury (Hg), lead (PB), nickel (Ni), chrome (CR), copper (Cu), zinc (zinc (Zn) and vanadium (V) in the tissues of *A. tuberculosa*, establishing a baseline for evaluating contamination in this vulnerable ecosystem. The results will be crucial for developing effective environmental management strategies and implementing control measures to safeguard marine ecosystems and public health.

METHODOLOGY

Description of the study area

In REVISICOF, red mangroves dominate, creating an ecosystem of immense ecological importance. These mangroves play crucial roles, including coastal protection, water filtration, and providing habitat for diverse flora and fauna (MAATE, 2024). Additionally, the mangroves are vital to local communities, supporting activities such as fishing, logging, and ecotourism (Bayas et al., 2023). Figure 1 illustrates the geographical location of the study area.

REVISICOF covers 2,811.67 hectares and is situated in the Chone River estuary, straddling the cantons of Sucre, San Vicente, Chone, and Tosagua. Established in 2002, this protected area is renowned for its mangroves and estuarine water bodies, which hold significant ecological value. REVISICOF experiences a warm and humid climate, with average monthly temperatures ranging from 24.61 °C to 26.81 °C (MAATE, 2015).

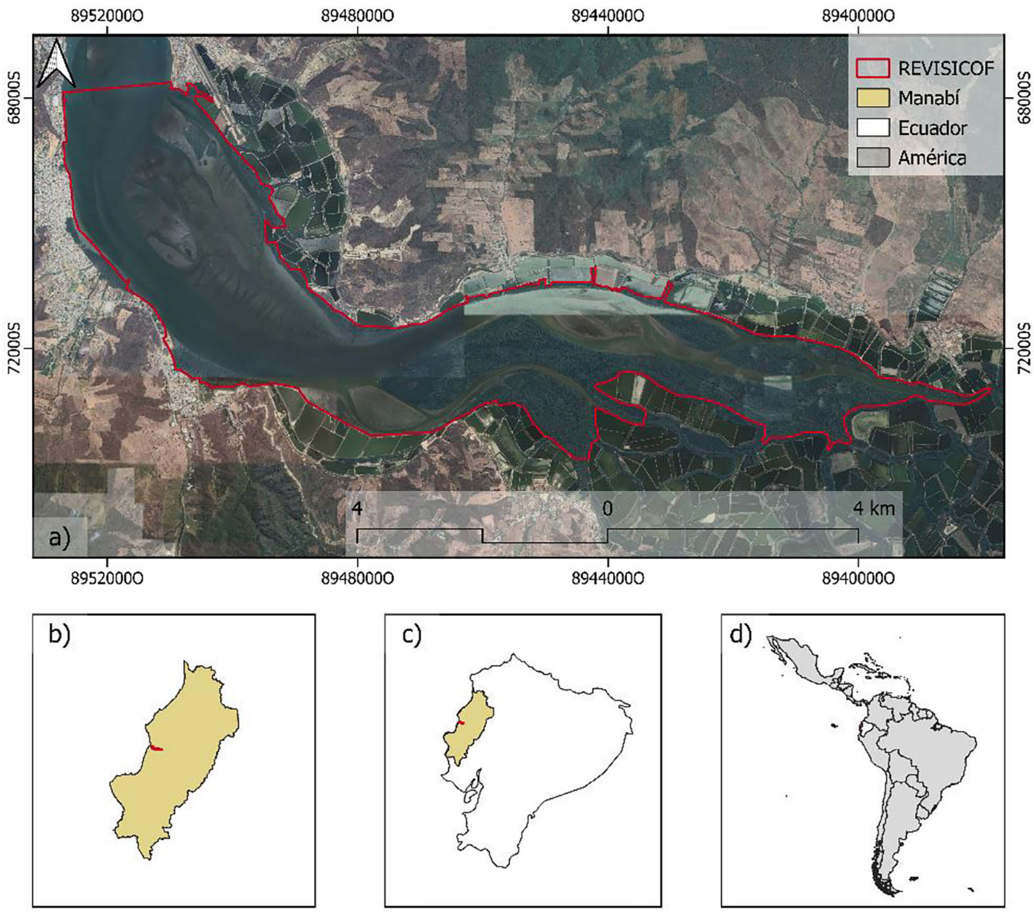

Figure 1. a) Geographical delimitation of REVISICOF according to MAATE; spatial location: b) provincial, c) national and d) continental.

Rainfall is heaviest from November to May, while September and October are the driest months. The average monthly relative humidity fluctuates between 84.45% and 89.6%. The region is also affected by the Humboldt and El Niño marine currents, which further influence its environmental conditions (MAATE, 2023).

Sample collection

In a defined sampling area of 50 m², ten individuals of the study species were collected on three separate occasions. A sample size of 10 individuals was chosen due to a combination of factors, including resource limitations, ethical concern (minimizing harm to animals) and adherence to previous research practices. The specimens were then transported to the REVISICOF laboratory, where their soft tissues were dissected. Representative samples, each weighing between 3 and 5.5 grams, were carefully placed in hermetically sealed bags, labeled, and refrigerated (Vinces et al., 2024). These samples were subsequently transported to the University of the Americas laboratory in Quito for heavy metal analysis. The Inductively Coupled Plasma (ICP) technique was employed to determine the concentrations of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), zinc (Zn), and vanadium (V), adhering to standardized protocols for the preparation and analysis of biological tissue samples (Environmental Protection Agency, 2014).

RESULTS AND DISCUSSION

To gain a comprehensive understanding of the environmental conditions in REVISICOF, it is crucial to consider the findings of Álava and Velásquez (2022). Their study revealed high levels of heavy metals in the sediment, with Pb concentrations reaching up to 63.52 mg/kg and Cd levels as high as 1.51 mg/kg. These elevated levels were attributed primarily to anthropogenic sources, posing a significant threat to both biota and human health. Furthermore, the soft tissues of *Anadara tuberculosa,* a common species in the region, exhibited considerable variability in arsenic (As) concentrations, ranging from 0.92 mg/kg to 3.76 mg/kg, with an overall average of 2.53 mg/kg. As depicted in Figure 2, Sample 1 demonstrated the highest As concentration, while Samples 2 and 3 exhibited notably lower levels.

The variability in As concentrations in the soft tissue of *Anadara tuberculosa* ranging from 0.92 mg/kg to 3.76 mg/kg – can be attributed to several factors. Environmental variations, such as fluctuations in As levels in water and sediments due to geographic location, anthropogenic activities, and geological features, play a significant role. Additionally, physiological and behavioral differences among organisms influence As absorption and accumulation, while seasonal factors can cause variations throughout the year due to changes in environmental conditions and feeding patterns (Roldán-Wong et al., 2023). Vieira et al. (2021) reported high As concentrations in

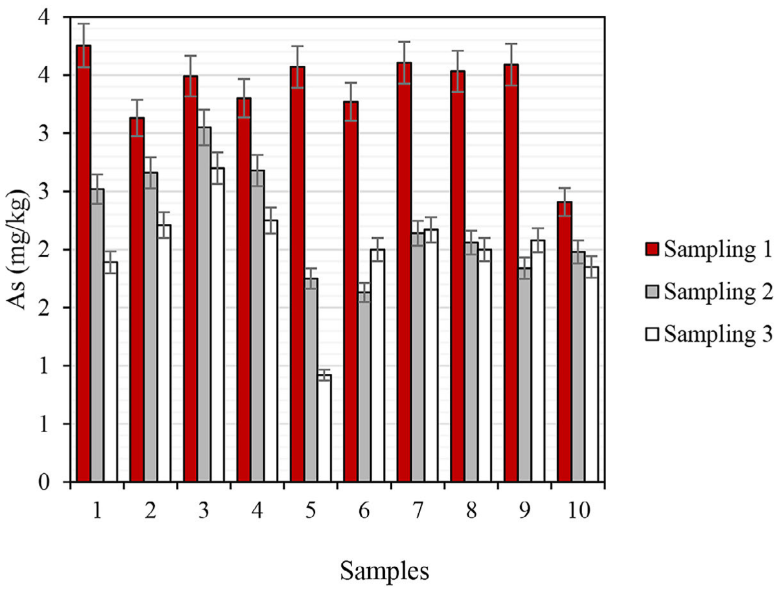

Figure 2. As levels during the 3 samplings, with an average of 2.53 mg/kg

the oysters from Vitória Bay, Brazil, demonstrating the bioaccumulation of this metal in marine organisms. Comparative studies suggest that female bivalves might accumulate more As (Kato et al., 2020). Modestin et al. (2022) highlighted the health risks associated with consuming contaminated bivalves, noting that the intake estimates of up to 6 mg/day of As (from a 240 g bivalve meal) can exceed recommended safety limits.

Regarding Cd, the highest concentration was found in Sample 9 during the third monitoring period, at 4.09 mg/kg. Samples 1 and 3 showed similar Cd levels, while the second sample from the second monitoring had the lowest Cd concentration at 1.58 mg/kg (Figure 3). The Cd levels observed in this study are consistent with previous research. Vinces et al. (2024) reported a mean Cd concentration of 2.93 ± 2.04 mg/kg in *Anadara tuberculosa* from the Gulf of Guayaquil, exceeding the EU maximum limit of 1.0 mg/kg as per Regulation (EC) No. 1881/2006. Similarly, Romero-Estévez et al. (2020) found up to 0.948 mg/kg of Cd in the *A. tuberculosa* from Santa Rosa, Ecuador, suggesting widespread Cd contamination along the Ecuadorian coast. Cu, being an essential element, was present in higher concentrations than As and Cd. Monitoring 3 showed the highest Cu levels across all samples, with a maximum concentration of 11.58 mg/kg. The trends for Cu in monitoring periods 1 and 2 were similar, with Sample 5 from monitoring period 2 having the lowest Cu concentration at 4.14 mg/kg, as shown in Figure 4.

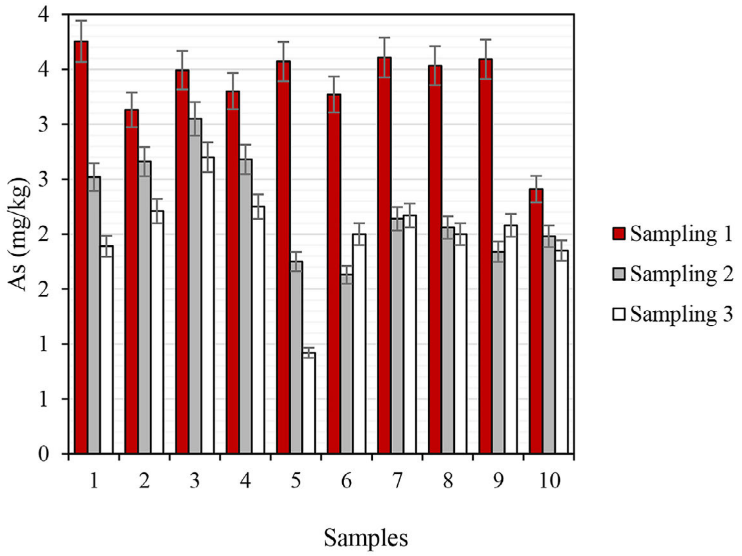

Figure 3. Cd levels during the 3 samplings, with an average of 2.67 mg/kg

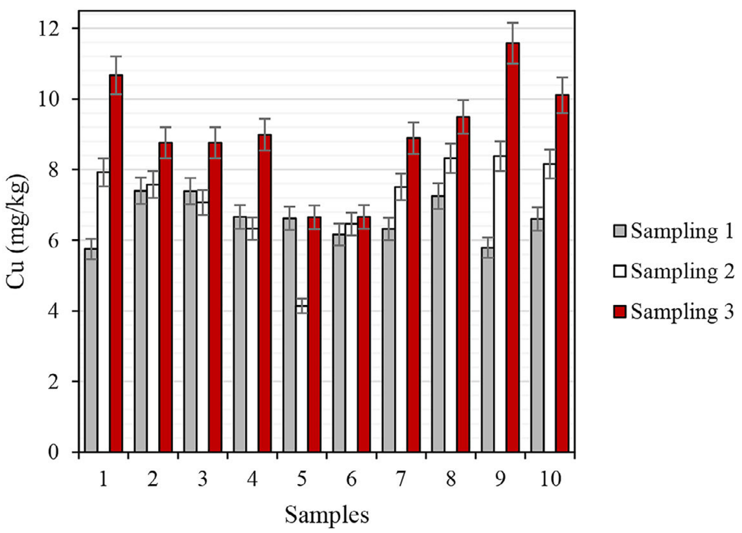

Figure 4. Cu levels during the 3 samplings, with an average of 7.61 mg/kg

The results for Cu in *Anadara tuberculosa* indicate significant spatial and temporal variability, aligning with Vinces et al. (2024), who reported an average Cu concentration of 6.86 ± 0.51 mg/kg, with a range of 4.10–18.20 mg/kg. The maximum Cu concentration detected (11.58 mg/kg) far exceeds natural levels in marine organisms (Cao et al., 2022), suggesting potential anthropogenic impacts and climatic variations. This raises concerns about the health of coastal ecosystems and the food safety for the populations consuming these bivalves.

Figure 5 illustrates Zn levels, another essential element, which peaked during sampling 3, with Sample 9 reaching 73.70 mg/kg. Monitoring periods 1 and 2 showed similar trends, with the lowest Zn value recorded in Sample 5 of monitoring period 2 (41.82 mg/kg). These results are consistent with Vinces et al. (2024), who found an average Zn concentration of 56.38 ± 1.75 mg/kg (range: 40.00–81.60 mg/kg). The high Zn concentrations may be due to its increased absorption and bioaccumulation in sediments or water (Roldán-Wong et al., 2023). To confirm this, further studies are needed to assess the bioavailable Zn in sediments and water as well as correlate it with the Zn concentrations in bivalve tissues. Similar to Zn, Cu, and Cd, V also showed its highest concentration during sampling 3, with a peak value of 5.95 mg/kg. While the three sampling periods displayed similar trends in V distribution, sampling 1 had significantly lower levels, with the lowest recorded value of 0.44 mg/kg in Sample 6 (Figure 6). These concentrations are notably higher than those found in the *Circenita callipyga*

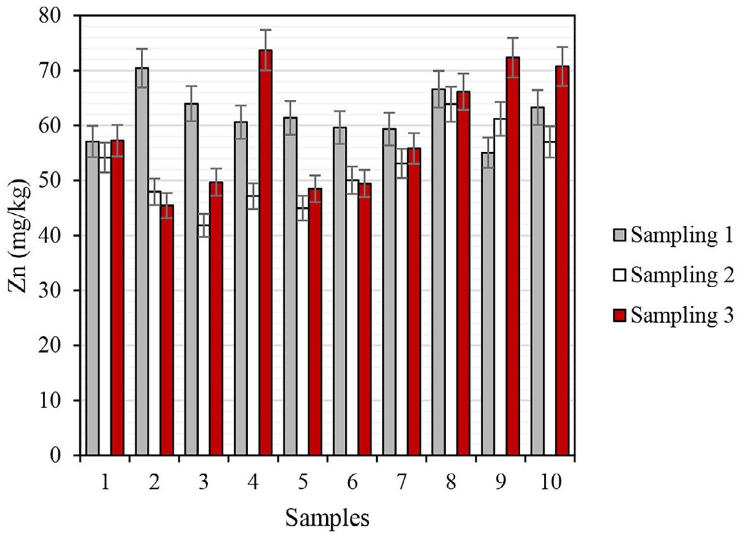

Figure 5. Zn levels during the 3 samplings, with an average of 57.59 mg/kg

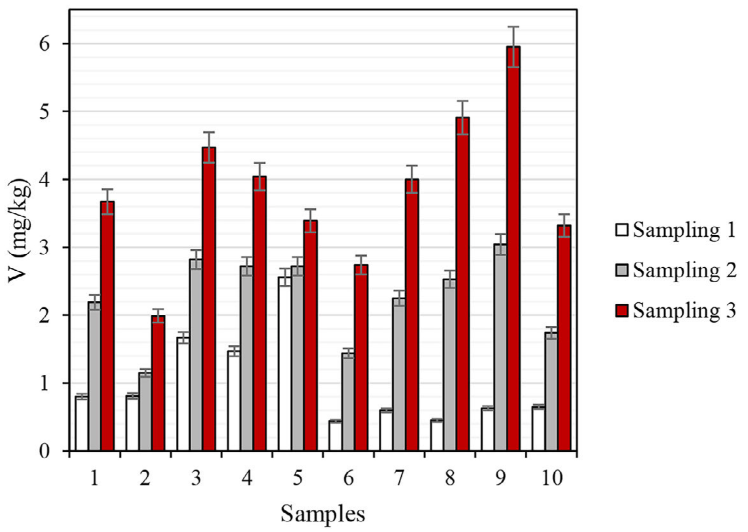

Figure 6. V levels during the 3 samplings, with an average of 2.37 mg/kg

bivalve, where the V levels peaked at 0.64 mg/kg in the northern Persian Gulf, attributed to the low accumulation potential for this metal exhibited by this species (Emami et al., 2024). The higher bioaccumulation capacity of V in *Anadara tuberculosa* suggests that this species could serve as an effective bioindicator for monitoring V contamination. As noted by Ścibior et al. (2021), bioindicators are crucial for assessing environmental risks and safeguarding human health.

The concentration of Hg exhibited significant variability across the three monitoring sessions. The highest Hg level, 0.2 mg/kg, was observed in Sample 9 from the third sampling. In contrast, Hg was detected in only three samples each during Samplings 1 and 2. Notably, Hg was present in 8 of the 10 samples from the first sampling, but concentrations remained consistently around 0.01 mg/kg (Figure 7). Nasevilla et al. (2022) found Hg contamination in *Anadara tuberculosa* from a market in Quito, Ecuador, with levels up to 0.055 mg/kg, attributing this to illegal mining activities in the region of species origin and suggesting a link between environmental contamination and Hg levels in bivalves.

Lucero-Rincón et al. (2023) reported that Hg concentrations of 0.25 mg/kg can cause tissue lesions in *Anadara tuberculosa*, particularly affecting sexual cells. This underscores the potential health risks of elevated Hg levels for bivalves and their possible reproductive impact. Emami et al. (2024) found a maximum Hg concentration of 0.088 mg/kg in *Circenita callipyga*, indicating some Hg accumulation in this bivalve as well. Additionally, Pan and Han (2023) reported up to 0.111 mg/kg of Hg in the wet tissue of bivalves on the southeastern coast of China, highlighting the presence of this heavy metal in the region. Hansen et al. (2024) concluded that total Hg concentrations in bivalves (*Mercenaria mercenaria, Crassostrea virginica* and *Geukensia demissa*) were largely independent of the animal's size.

Pb levels exhibited significant variability between monitoring sessions. Unlike Hg, Pb was not detected in any samples from the second sampling. However, a maximum Pb concentration of 2.97 mg/kg was observed in Sample 3 from the first monitoring session, while in the third sampling session, four samples with detectable Pb concentrations exceeded 0.5 mg/kg (Figure 8). Tanaviyutpakdee and Karnpanit (2023) reported Pb levels up to 1.445 ± 0.062 mg/kg in clams from Thailand, although these levels were not considered harmful to human health. In contrast, Vinces et al. (2024) found an average Pb concentration of 0.54 ± 0.51 mg/kg in *A. tuberculosa* from the Gulf of Guayaquil, which is below the EU permissible limit of 1.5 mg/kg set by Regulation (EC) No. 1881/2006. Roldán-Wong et al. (2023) emphasize the need for ongoing monitoring of elements like Pb in foods due to their potential health risks.

Ni levels displayed considerable variability, being detected in all samples across the three monitoring sessions. Sampling 3 showed the widest range, with Ni concentrations varying from 0.43 mg/kg to 1.54 mg/kg, and an overall arithmetic mean of 0.89 mg/kg. These findings align with Vinces et al. (2024), who reported a maximum Ni concentration of 1.70 mg/kg and an average of 0.77 mg/kg in the soft tissue of *Anadara tuberculosa*. This suggests a moderate level of Ni in this

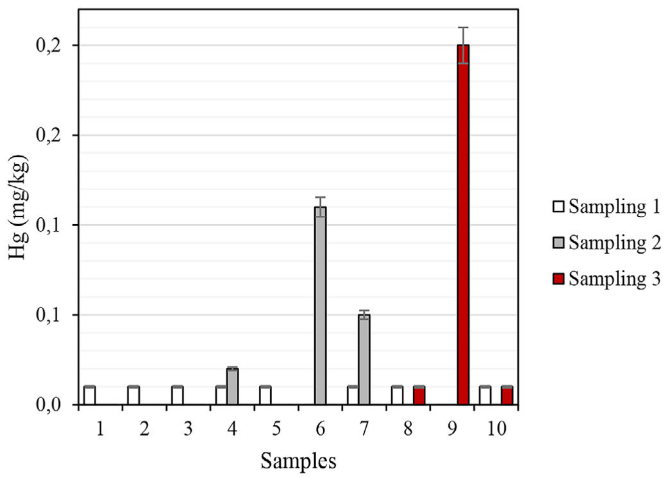

Figure 7. Hg levels during the 3 samplings, with an average of 0.02 mg/kg

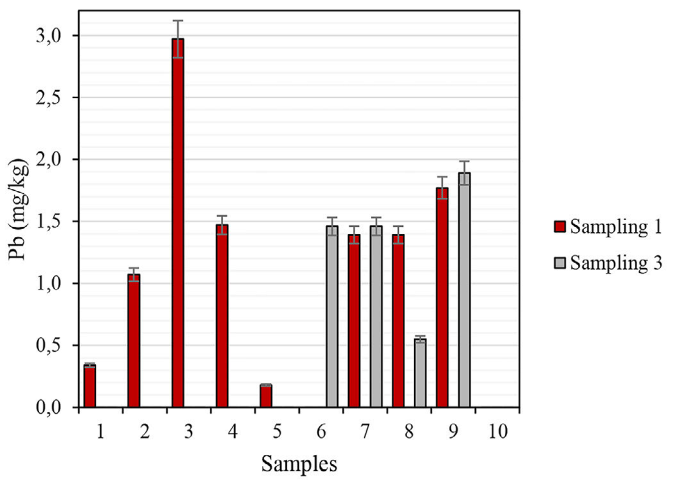

Figure 8. Pb levels during the 3 samplings, with an average of 0.53 mg/kg

bivalve species. In contrast, Singh et al. (2024) observed significantly higher Ni levels in *Paphia malabarica*, with concentrations reaching up to 6.88 mg/kg in the samples from Mumbai, India (Figure 9). This discrepancy highlights considerable variability in Ni concentrations among different bivalve species and geographic regions. Factors such as local environmental pollution, habitat characteristics, and species-specific differences in metal accumulation pathways could contribute to these variations (Morankar and Kurhe, 2023).

Finally, Cr was detectable in all samples across the three monitoring sessions, with Sampling 3 showing the highest concentration at 2.37 mg/kg and Sampling 1 recording the lowest at 0.46 mg/kg. The average Cr concentration across all samples was 1.11 mg/kg (Figure 10). These values are notably higher than those reported by Romero-Estévez et al. (2020), who found Cr levels of only 0.059 mg/kg in the *A. tuberculosa* from Santa Rosa, Ecuador. Conversely, Vinces et al. (2024) documented even higher Cr levels, up to 4.50 mg/kg, in the same species. This variability highlights the significant impact of local pollution, industrial activity, and environmental management practices on the accumulation of heavy metals in bivalves.

In this study of metal concentrations in *Anadara tuberculosa* from REVISICOF, significant levels of heavy metals were detected, indicating considerable environmental contamination. Pb reached a maximum of 2.97 mg/kg, while Cd peaked at 4.09 mg/kg, both exceeding safety limits and suggesting anthropogenic influence. As varied between 0.92 to 3.76 mg/kg, highlighting geographic and environmental factors in bioaccumulation. Cu concentrations soared to 11.58 mg/kg, indicating potential pollution, whereas Zn levels peaked at 73.70 mg/kg, consistent with local environmental conditions. Hg showed a maximum

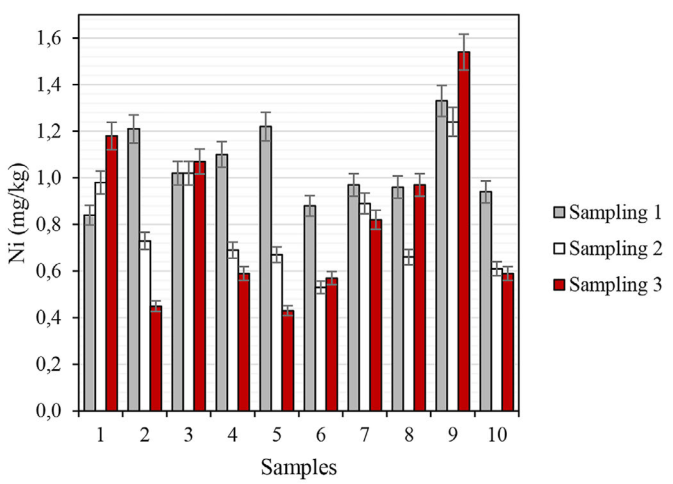

Figure 9. Ni levels during the 3 samplings, with an average of 0.89 mg/kg

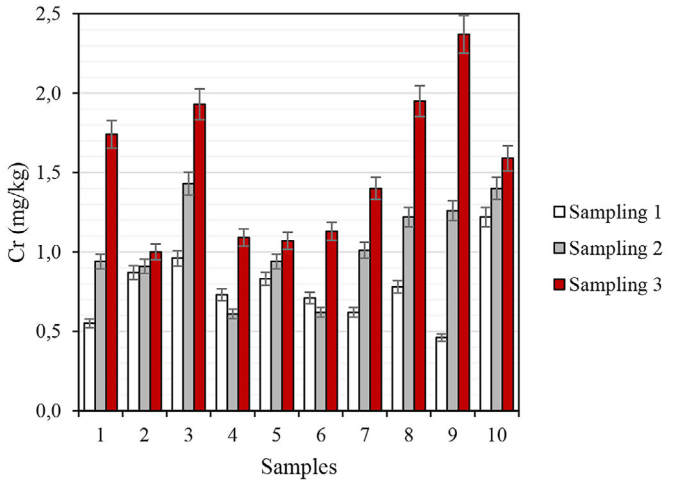

Figure 10. Cr levels during the 3 samplings, with an average of 1.11 mg/kg

concentration of 0.2 mg/kg, raising health concerns, Ni and Cr levels were also notable, indicating significant local pollution. Overall, the findings, as discussed above, reflect both regional contamination trends in Ecuador and align with global patterns of heavy metal pollution in coastal areas.

CONCLUSIONS

The analysis of heavy metals (As, Cd, Hg, Pb, Ni, Cr, Cu, Zn, and V) in the soft tissue of *Anadara tuberculosa* from REVISICOF establishes a crucial baseline for assessing pollution in this vulnerable ecosystem. The results reveal substantial accumulation of several toxic elements, with maximum concentrations recorded as follows: 2.37 mg/kg Cr, 1.54 mg/kg Ni, 0.2 mg/kg Hg, 2.97 mg/kg Pb, 5.95 mg/kg V, 4.09 mg/kg Cd, 11.58 mg/kg Cu, 73.70 mg/kg Zn, and 3.76 mg/kg As. Notably, the high levels of Zn and Cu indicate a significant heavy metal load in this bivalve species.

These elevated concentrations suggest potential risks to both bivalves and human health, underscoring the need for ongoing, detailed monitoring to identify and address environmental impacts. The observed variability in heavy metal concentrations across different studies highlights the importance of targeting local contamination sources and implementing effective control measures. Continued monitoring is essential to safeguard delicate marine ecosystems, ensure food safety, and mitigate the impact of heavy metals on aquatic environments.

Acknowledgements

The authors are grateful to Fabricio Enrique Alcívar Intriago, Patricio Javier Noles Aguilar†, Sergio Santiago Alcívar Pinargote and María Isabel Delgado Moreira.

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