

Article

Monitoring and Assessment of the Trace Element Accumulation in the Polychaete *Hediste diversicolor* from Tunisian Coastal Localities (Southwest of Mediterranean Sea)

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Abstract

The study of the impact of anthropogenic and natural pollution on living organisms has become a major social issue. In this context, the objective of this work is to assess the use of the polychaete annelid *Hediste diversicolor* as a bioindicator organism for the quality of the marine environment. The concentration of four heavy metals (lead, copper, zinc, and cadmium) was determined in natural populations of *H. diversicolor* captured from four locations along the Tunisian coast using atomic absorption spectroscopy. Concentration ranges ($\mu\text{g/g}$ dry weight) across all sites were as follows: Cd (0.12–0.43), Cu (3.80–6.45), Zn (18.35–42.78), and Pb (22.64–63.91). Statistical analysis confirmed significant spatial variation (Pb: $F = 12.15$, $p < 0.001$; Zn: $F = 3.32$, $p = 0.04$; Cd: $F = 48.66$, $p < 0.001$; Cu: $F = 9.08$, $p < 0.001$), with peak Pb in Bizerte and Cu in Sfax. These results highlight the influence of local environmental factors, such as industrial and urban pollution on metal accumulation in *Hediste diversicolor*. In this study, the accumulation of the analyzed elements in the tissues of *H. diversicolor* follows an increasing order as follows: Cd < Cu < Zn < Pb. Additionally, lead metal concentrations were higher than those of cadmium, zinc, and copper for all four studied locations. To our knowledge, this is the first study in Tunisia to assess heavy metal accumulation in *H. diversicolor*. The recorded levels were similar to, or lower than, those reported in other studies worldwide. These findings underscore the potential of *H. diversicolor* as a sensitive and effective bioindicator for monitoring coastal contamination and guiding environmental management strategies in Tunisia.

Keywords: bioaccumulation; heavy metals; aquatic ecosystem; *Hediste diversicolor*; Tunisia



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1. Introduction

The planet's ecosystems are currently being significantly impacted by human development and the resulting pollution [1]. This environmental threat also extends to aquatic ecosystems in Tunisia, from the Mediterranean Sea to lakes, rivers, and inland wetlands. These diverse environments are home to a rich biodiversity, providing essential ecosystem services such as biodiversity, fishing and aquaculture [2–7]. However, the sustainability

of these ecosystems is threatened by a variety of factors, including pollution, overfishing, global change, and habitat destruction [2,8].

In this context, we focus on metal pollution, which arises when trace-metal concentrations (e.g., Zn, Cu, Pb, Hg, Cd, Cr) deviate from their natural background levels. These micropollutants persist in water, sediments, and groundwater and exhibit high bioavailability, enabling uptake by aquatic organisms. Through bioaccumulation, metals are absorbed faster than they are eliminated, and via trophic transfer, they undergo biomagnification, leading to progressively higher tissue burdens at successive food-web levels. Consequently, metal concentrations in the tissues of marine organisms can exceed ambient environmental levels by several orders of magnitude, posing significant risks to biodiversity and ecosystem health [9]. In general, the two main sources of environmental contamination by trace elements are natural soil erosion processes linked to the nature of the parent rock, which directly influences the metallic composition of the soil [10], and human activity (air pollution, use of pesticides, fertilizers, urban and industrial waste), also contributing to the enrichment of ecosystems in heavy metals. In specific contexts and under certain conditions, the presence of metals in toxic concentrations can cause significant ecological damage [11,12]. Lead (Pb) and cadmium (Cd) are non-biodegradable and toxic, even at low concentration levels. It should be noted that even zinc (Zn), although essential to the proper functioning of the ecosystem, can become toxic at high doses. This form of pollution is of major concern due to its devastating effects on aquatic ecosystems, posing a threat to marine life and biodiversity. In certain environments, the introduction of chemicals into the marine environment can lead to the extinction of certain animal and/or plant species, causing the trophic chain to malfunction. The harmful effects of metal pollution are not limited exclusively to aquatic flora and fauna but also affect human beings. Contaminated water poses a direct threat to human health and can cause serious illness. A case in point was the situation in Japan in the 1950s–1960s, where cadmium contamination triggered multiple kidney and bone disorders, leading to significant mortality among populations in the affected areas [13].

In the northwestern coastal region of Tunisia, research on heavy metal contamination and bioaccumulation in marine ecosystems remains limited, despite the significant ecological importance and economic value of these coastal habitats. This study aims to assess the level of contamination of this ecosystem by measuring concentrations of metals (Cd, Pb, Cu, and Zn) in the tissues of the marine polychaete worm *H. diversicolor*. This species was chosen due to its widespread presence, ecological importance, and recognized role as a bioindicator of metal pollution. Although these aquatic organisms are recognized as excellent bioindicators due to their ability to accumulate metals, as well as their sessile and euryhaline lifestyle [14], marine worms are of great commercial importance and have been proposed as a very interesting novel feed ingredient for aquafeeds. The choice of inanimate (sediment and water) and living (polychaetes) substrates is justified by their ability to bind and accumulate various mineral and organic toxins [15]. This marine polychaete worm, belonging to the Nereididae family, is highly tolerant of extreme variations in temperature, salinity, and oxygen levels in its environment. It adapts to various types of sediment, from muddy to sandy, and survives in harsh and relatively polluted environments. As well as being an important food source for other animals, this *Hedistus*, often called *Nereis* in ancient literature, plays a crucial role in the mixing, aeration, and cycling of carbon and nitrogen in sandy and muddy sediment layers, through the phenomenon of bioturbation, similar to that of earthworms in soils.

In this context, this study focuses on a specific form of metal pollution, namely the bioaccumulation of heavy metals, by highlighting the marine worm species *H. diversicolor*. This benthic polychaete plays an essential role in aquatic ecosystems and is recognized as a

significant indicator of the ecological and environmental consequences of metal contamination. Over time, the gradual accumulation of metals is becoming an increasing threat both to this species and to the entire aquatic food chain.

Various scientific studies have highlighted the risks associated with the accumulation of heavy metals in aquatic ecosystems, underscoring the imperative of in-depth analysis to understand bioaccumulation mechanisms and assess potential risks to biodiversity and human health. The variety of chemical compounds present in polluted marine ecosystems intensifies the phenomenon of bioaccumulation in the tissues and organs of marine fauna. Thus, polychaete marine worms become agents of this contamination, while providing indications of the degree of pollution of their natural environment. In this study, we examined the bioaccumulation of four trace metals (cadmium, lead, copper, and zinc) in worms (*H. diversicolor*) at four sites in different Tunisian aquatic ecosystems (Bizerte Lagoon, Sfax beach, El Hicha Gabes, and Djerba Island).

Accordingly, this study engages in an in-depth exploration of heavy metal bioaccumulation in *H. diversicolor* within various aquatic ecosystems in Tunisia. It aims to quantify metal concentrations, assess spatial variation, and compare the results with values reported in the literature, in order to provide essential baseline data for sustainable ecosystem management and to confirm the usefulness of *H. diversicolor* as a reliable bioindicator species for monitoring metal contamination in coastal environments.

2. Materials and Methods

2.1. Studied Sites

A studied sample was carried out in 4 different areas: Bizerte, Sfax, Gabes, and Djerba as shown in Figure 1.

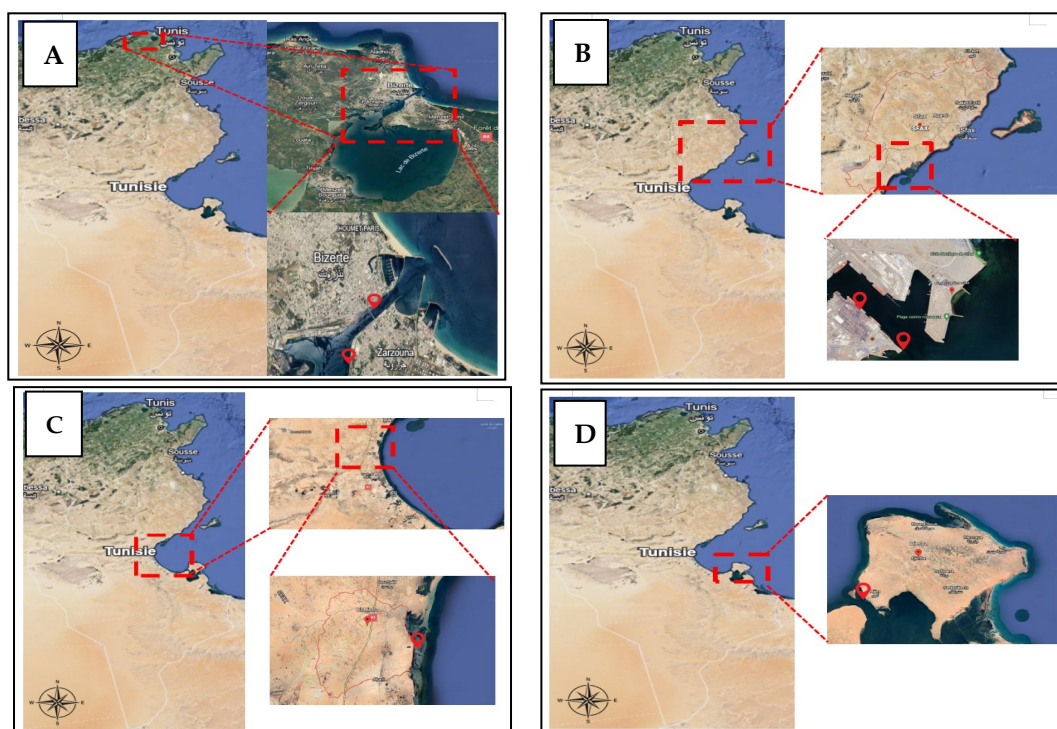


Figure 1. Studied sites (A) Bizerte; (B) Sfax; (C) Gulf of Gabes; (D) Djerba.

2.1.1. Bizerte Lagoon

The Bizerte Lagoon, located in northern Tunisia (Figure 1), covers an area of approximately 128 km² and holds significant ecological and geostrategic importance. It connects

to the Mediterranean Sea via a navigable channel (1500 m long, 300 m wide, and 12 m deep) and to Lake Ichkeul through OuedTinja, a narrow channel approximately 5 km in length [6–18]. Historically, the lagoon benefited from a natural balance between freshwater inputs and seawater exchange, supporting diverse biological communities. However, in recent decades, rapid urbanization and the establishment of major industrial facilities along the shoreline have altered its ecological equilibrium. The construction of dams upstream, particularly in the catchment areas of Lake Ichkeulhas, significantly modified freshwater inflows and increased salinity levels within the lagoon [19]. The region's Mediterranean climate is characterized by hot, dry summers and mild, rainy winters, with dominant northwesterly winds that further influence local hydrodynamics [20].

2.1.2. Sfax Coast

Sfax, located on the southeastern coast of Tunisia, is one of the country's most industrialized urban centers. Since the 1950s, rapid industrial growth, urban expansion, and population increase have contributed to significant discharges of both industrial and domestic wastewater. These inputs have resulted in heavy metal contamination, particularly affecting marine sediments along the coastline [21–23]. The sampling sites in this study are situated between latitudes 34°43' and 34°46' N and longitudes 10°46' to 10°49' E.

2.1.3. Gulf of Gabes

The study area is situated in southern Tunisia, approximately 35 km north of Gabes and just a few kilometers inland from the Mediterranean Sea. Spanning roughly 300 km², it is bordered by the Gulf of Gabes to the east, El Hamma to the west, and Skhira to the north [24–26]. Although it is recognized for its high marine productivity and plays a central role as Tunisia's main fishing ground also ranking among the most important in the Mediterranean, the Gulf of Gabes has suffered continuous ecological degradation over the past decades. This deterioration is largely due to rapid, unregulated industrial development and the discharge of both industrial and domestic waste into the marine environment.

2.1.4. Djerba Island

Located in south-eastern Tunisia, the island of Djerba is geographically close to the mainland, with two outposts on either side: Jorf and Ajim to the west, Zarzis and El Kantara to the east (Figure 1). Ajim is separated from Jorf by a 2 km strait, crossed by shuttles, while on the Zarzisside, a 7.5 km bridge links the island to the mainland. Although Jerba is increasingly perceived as a peninsula thanks to these land links, the majority of tourists reach it by air.

2.2. Sampling

The samples (N = 15 for each site) were taken at low tide, in the intertidal zone, more precisely in the lower mediolittoral of the Gulf. Individuals of *H. diversicolor* were carefully extracted from their sandy tubes and placed in clean 0.5 L plastic bottles of mineral water, thus creating an environment conducive to their preservation, protecting them from external factors. After a period of 15 to 30 min, the samples were stored in the refrigerator at a temperature of 6 °C to inhibit crystallization within the annelid tissues and promote elimination of their digestive tract contents. Before being used for analysis, samples were left to thaw at room temperature, preparing them for subsequent analysis.

2.3. Chemical Analysis

As a first step, tissue samples were dried in a G-Therm115 oven at 65 °C for 24 h to obtain a constant dry weight. After dehydration, the dry weight of each sample was

recorded, and the samples were then prepared for mineralization. The concentrations of Cd, Zn, Pb, and Cu were determined following the procedure described by [27]. During the mineralization step, organic matter was removed using an acid digestion method. Dried samples (10 mg) were taken and digested with concentrated nitric acid (3 mL) at 120 °C. After dilution to 30 mL with ultrapure water, the digested solution was stored at 4 °C until analysis by flame atomic absorption spectrometry (Avanta GBC spectrometer, A6600 model, Melbourne, Australia). A hollow cathode lamp was utilized as a light source for Zn, Cd, Pb, and Cu at wavelengths of 213.9, 228.8, 283.3, and 248.3 nm, respectively, for the determination of each respective metal.

The equipment underwent calibration using NIST-traceable atomic absorption standards for metals to establish a calibration curve. Linear calibration curves were established with linear regression values exceeding $R^2 > 0.989 \pm 0.10$. The limit of detection (LOD) for each metal was determined as follows: Cu 0.0045 mg/L, Zn 0.0033 mg/L, Cd 0.0020 mg/L, and Pb 0.013 mg/L. Heavy metal concentrations are expressed in µg/g dry weight. The precision of the analytical procedure was assessed through triplicate analysis, and the relative standard deviation (%RSD) for each metal was calculated. The %RSD values obtained were found to be less than 10%.

To assess repeatability and verify the analytical procedure, samples were spiked with known concentrations of heavy metals. Each test was performed in triplicate. The spiked samples were digested and analyzed using the same protocol as for the original specimens. Recoveries for the analyzed metals ranged from 77% to 95%, and recovery correction was applied to each sample accordingly.

All reagent and standard solutions were prepared using ultrapure water. Only analytical-grade chemicals were used throughout the analysis. To minimize contamination, all glassware was soaked in 3% nitric acid for 24 h, rinsed thoroughly with ultrapure water, and dried in an oven prior to use.

The BSAF (bio-sediment accumulation factor) was also calculated. It is the ratio of the contaminant concentration in tissue to the contaminant concentration in sediment.

2.4. Statistical Analysis

Metal concentrations are expressed as means \pm standard deviations (SD). Prior to statistical analysis, data normality was assessed. One-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test was performed using StatView software (version 5.0, by SAS Institute Inc., Cary, NC, USA). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Trace Metal Accumulation in *H. diversicolor* Tissues

Figure 2 shows the results of assays for the metals studied bioaccumulated in the organs and tissues of *H. diversicolor*. The analyses revealed the presence of these xenobiotics in the tissues of the target worm, with heterogeneous levels. The highest levels of Pb were observed at all study sites. From the results obtained, we noted that in the four study sites, heavy metal levels follow a concentration scale in the following ascending order: Cd < Cu < Zn < Pb.

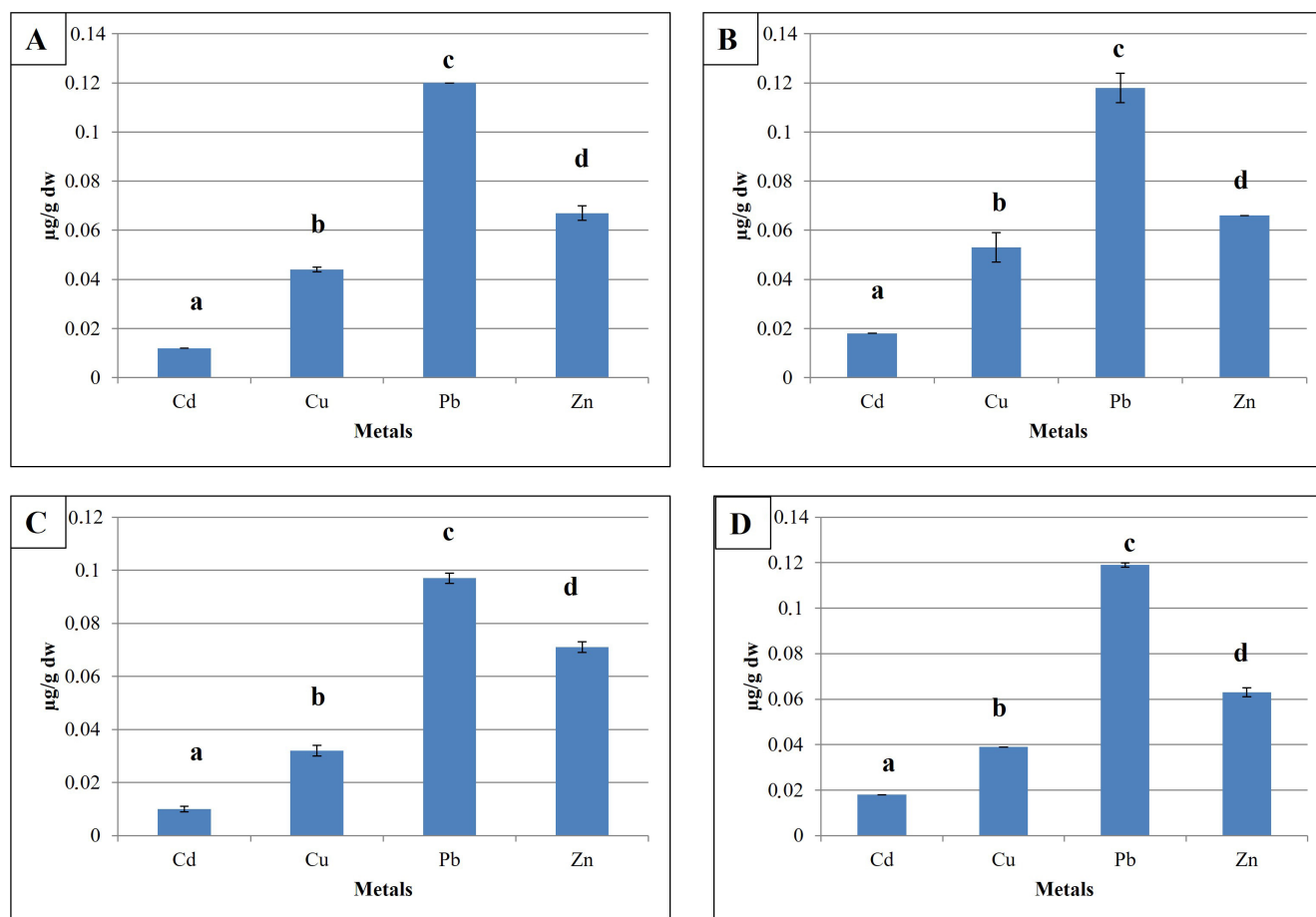


Figure 2. Trace elements levels (mean \pm SE; $\mu\text{g/g}$ dry weight) in the studied station (A) Bizerte; (B) Sfax; (C) Gabes; (D) Djerba. Different letters indicate statistically significant differences.

3.1.1. Bizerte

Figure 2A shows the levels of trace metals (Cd, Cu, Pb, and Zn) bioaccumulated in *H. diversicolor* tissues (in $\mu\text{g g}^{-1}$ dry weight) in the Bizerte region. According to the graph, Pb concentrations were the highest ($0.12 \pm 2.54 \times 10^{-4} \mu\text{g g}^{-1}$ dry weight), compared with Zn ($0.067 \pm 0.003 \mu\text{g g}^{-1}$ dry weight), Cu ($0.044 \pm 0.001 \mu\text{g g}^{-1}$ dry weight), and Cd ($0.012 \pm 2.01 \times 10^{-5} \mu\text{g g}^{-1}$ dry weight). Statistical analysis revealed a significant difference in the levels of these elements ($F = 882.21$; $p < 0.001$).

3.1.2. Sfax

Figure 2B shows the concentrations of trace metals (Cd, Cu, Pb, and Zn) bioaccumulated in the tissues of *H. diversicolor* caught in the Sfax region. Pb levels at this site were significantly higher ($F = 103.761$; $p < 0.001$) at $0.118 \pm 0.006 \mu\text{g g}^{-1}$ dry weight, compared with Zn ($0.066 \pm 1.54 \times 10^{-4} \mu\text{g g}^{-1}$ dry weight), Cu ($0.053 \pm 0.006 \mu\text{g g}^{-1}$ dry weight), and Cd ($0.018 \pm 2.75 \times 10^{-4} \mu\text{g g}^{-1}$ dry weight).

3.1.3. Gabes

Results for trace metal content in samples of *H. diversicolor* caught in the Gabes region, presented in Figure 2C, show that Pb was the most important element, with levels in the order of $0.097 \pm 0.002 \mu\text{g g}^{-1}$ dry weight, followed by Zn ($0.071 \pm 0.002 \mu\text{g g}^{-1}$ dry weight), Cu ($0.032 \pm 0.005 \mu\text{g g}^{-1}$ dry weight), and Cd ($0.01 \pm 0.001 \mu\text{g g}^{-1}$ dry weight). Statistical analysis revealed significant differences between these levels ($F = 512$; $p < 0.001$).

3.1.4. Djerba

Analyses of samples collected in the Djerba region (Figure 2D) show that cadmium is the element with the lowest concentration ($0.018 \pm 3.28 \times 10^{-4} \mu\text{gg}^{-1}$ dry weight), compared with lead, which has the highest concentration ($0.119 \pm 0.001 \mu\text{gg}^{-1}$ dry weight). Zn and Cu contents were of the order of $0.063 \pm 0.002 \mu\text{gg}^{-1}$ dry weight and $0.039 \pm 2.21 \times 10^{-4}$, respectively. Statistical analyses showed significant differences between these different grades ($F = 1387.194$; $p < 0.001$).

3.2. Site-Specific Variations in Average Heavy Metal Concentrations

3.2.1. Lead

Pb concentrations at the four study stations are shown in Figure 3A. The results show that Pb levels were lowest in samples collected in the Gabes region ($0.097 \pm 0.002 \mu\text{gg}^{-1}$ dry weight), compared with levels in the other three study stations (Table 1). Statistical analysis revealed a statistically significant difference between samples from the Gabes site and the other three stations ($F = 12.15$; $p < 0.001$).

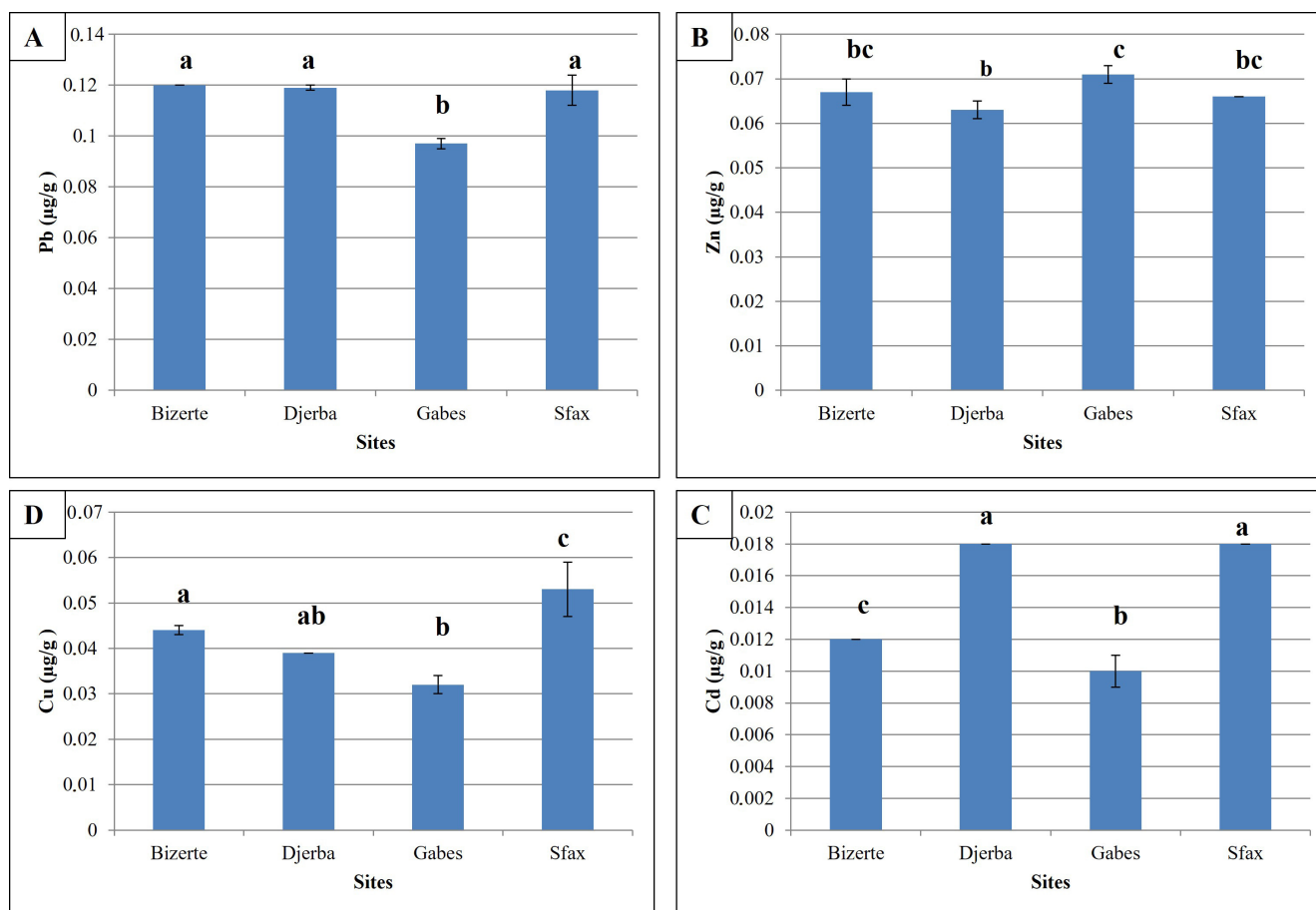


Figure 3. Trace elements levels (mean \pm SD; μgg^{-1} dry weight) between study stations. (A) Pb; (B) Zn; (C) Cd; (D) Cu. Different letters indicate statistically significant differences.

3.2.2. Zinc

The results for Zn levels at the study stations showed that samples from the Djerba region had the lowest values ($0.063 \pm 0.002 \mu\text{gg}^{-1}$ dry weight), while the highest levels were observed in samples from Gabes (Figure 3B; $0.071 \pm 0.002 \mu\text{gg}^{-1}$ dry weight) (Table 1). Statistical analyses showed a significant difference between these two study stations, while

there were no statistically significant differences between concentrations at the Bizerte and Sfax stations.

Table 1. Results of statistical analyzes between study sites for Pb, Zn, Cd, and Cu contents (μgg^{-1}).

	Sites	Mean \pm SE	F	p
Pb	Bizerte	$0.12 \pm 2.54 \times 10^{-4}$	12.15	<0.001
	Djerba	0.119 ± 0.001		
	Gabes	0.097 ± 0.002		
	Sfax	0.118 ± 0.006		
Zn	Bizerte	0.067 ± 0.003	3.32	0.04
	Djerba	0.063 ± 0.002		
	Gabes	0.071 ± 0.002		
	Sfax	$0.066 \pm 1.54 \times 10^{-4}$		
Cd	Bizerte	$0.012 \pm 2.01 \times 10^{-5}$	48.66	<0.001
	Djerba	$0.018 \pm 3.28 \times 10^{-4}$		
	Gabes	0.01 ± 0.001		
	Sfax	$0.018 \pm 2.75 \times 10^{-4}$		
Cu	Bizerte	0.044 ± 0.001	9.082	<0.001
	Djerba	$0.039 \pm 2.21 \times 10^{-4}$		
	Gabes	0.032 ± 0.002		
	Sfax	0.053 ± 0.006		

3.2.3. Cadmium

Cadmium levels in the samples studied showed that the Djerba and Sfax regions had the highest values, $0.018 \pm 3.28 \times 10^{-4}$ and $0.018 \pm 2.75 \times 10^{-4} \mu\text{gg}^{-1}$ dry weight, respectively (Figure 3C; Table 1), while the lowest concentrations were reported in the Bizerte and Gabes regions (in the order of $0.012 \pm 2.01 \times 10^{-5}$ and $0.1 \pm 0.001 \mu\text{gg}^{-1}$ dry weight). Analysis of Cd concentrations at the various study stations revealed statistically significant differences between samples from Bizerte and those from Djerba, Gabes and Sfax ($p < 0.001$). Also, levels in the Gabes region were significantly lower than levels in the Djerba and Sfax regions ($p < 0.001$).

3.2.4. Copper

Figure 3D revealed the levels of lead, zinc, cadmium and copper in tissues from the studied sites. The lowest levels of these four heavy metals were found in samples collected from the two stations, Gabes and Djerba, while the other two stations, Bizerte and Sfax, contained the highest heavy metal values (Table 1). Significant differences were obtained between levels in the Sfax region with Djerba, Bizerte and Gabes, while there was no significant difference with levels in Bizerte. The grades of samples from Bizerte show statistically significant differences with grades from the Gabes region. There were no differences between Djerba and Gabes/Bizerte.

4. Discussion

This study confirmed the effective presence of these xenobiotics in the tissues of the target annelid, with highly variable levels. It is important to note that cadmium was identified as the least bioaccumulated metal among samples from all four sites studied, while lead was detected as the most bioaccumulated metal in samples from all four sites studied. The presence of copper and zinc varied from site to site.

The results revealed that lead levels were the highest among the four stations sampled, with maximum concentrations recorded in organisms sampled in the Bizerte Lagoon. The average for this pollution was $0.12 \pm 2.54 \times 10^{-4} \mu\text{gg}^{-1}$ dry weight. On the other hand, the

lowest lead concentration was observed in Gabes, with an average of $0.097 \pm 0.002 \mu\text{g g}^{-1}$ dry weight. Lead can have a natural origin, but its main sources of emission are the lead industries and especially road traffic. It is used in construction, mechanical engineering, batteries, cables, and pigments. This substance tends to accumulate in fish and mammals, presenting potential risks for fertility, neurotoxicity, and immune responses [28].

Similarly to lead, mean zinc concentrations recorded in *H. diversicolor* samples showed a diversity of variations. Significant values were observed in worms collected in the El Hicha region of Gabes. Indeed, the average for this pollutant was 0.071 ± 0.002 and $0.063 \pm 0.002 \mu\text{g g}^{-1}$ dry weight, respectively, in annelids from Gabes and Djerba. These higher zinc values, like those of lead, could originate from common anthropogenic sources such as industrial effluents from phosphate and chemical processing plants. Moreover, the lack of sufficient water dilution and circulation in the Gabes coastal zone may limit the dispersion of contaminants, enhancing their retention in sediments and increasing their bioavailability to benthic organisms like *H. diversicolor*.

Zinc, a relatively common metal, is found in metalliferous seams, coal, bitumen, and oil, with frequent occurrence in mining areas. It can also be of anthropogenic origin. Industrial applications for zinc and its compounds are numerous, ranging from metal coatings to the manufacture of paint pigments, plastics, rubber, pharmaceuticals, and insecticides. Water-soluble zinc salts tend to accumulate in organisms, and zinc is an essential metal for all living organisms [28]. Zinc is mainly used in corrosion protection coatings, alloy manufacture (brass, bronze, light alloys), building construction, automotive equipment, railroads, and the production of rolled or formed products. It plays an intermediary role in the manufacture of other compounds, acting as a reducing agent in organic chemistry and as a reagent in analytical chemistry [29]. Although zinc is not generally considered a toxic metal, high concentrations can cause physiological disturbances in the body [30]. Although its toxicity to aquatic organisms does not classify it as a priority contaminant, effects on oyster reproduction and larval growth can be observed at high concentrations [29]. Zinc has the capacity to accumulate in aquatic organisms, with bioconcentration factors reported at 1000 for freshwater fish and 2000 for marine fish [31].

Lead is frequently found in association with zinc in ores, along with various other elements such as iron (Fe), copper (Cu), cadmium (Cd), bismuth (Bi), antimony (Sb), germanium (Ge), arsenic (As), silver (Ag), and gold (Au). These elements, with the exception of iron, are generally recovered during metallurgical operations. Mixed lead and zinc ores account for around 70% of lead mine production, while pure lead ores account for around 20%. Around 10% of lead production comes from co-production during the processing of copper, zinc, or other metal ores. Galena (PbS) is the main lead ore, often associated with sphalerite (zinc) and pyrite [32].

The different cadmium concentrations obtained show that the cadmium concentration varies according to the sampling site. Cadmium was detected in 100% of samples from the Djerba and Sfax sites. Maximum mean cadmium levels were recorded in *H. diversicolor* tissues, reaching $0.018 \pm 3.28 \times 10^{-4}$ and $0.018 \pm 2.75 \times 10^{-4} \mu\text{g g}^{-1}$ dry weight, respectively, at the Djerba and Sfax sites. Cadmium (Cd) is a silvery-white or blue metal, often associated with lead, copper, or zinc deposits. It is generally found as cadmium oxide, chloride, or sulfate/sulfide. In zinc deposits, cadmium sulfide is the most common form. Cadmium production depends on zinc demand, as it is often a by-product of zinc concentrate processing [33]. Cadmium metal is used in the anti-corrosion coating of metals (cadmium plating by electrolysis or dipping and spraying), in the manufacture of negative electrodes for rechargeable nickel-cadmium silver-cadmium batteries, and in the production of stabilizers for plastics (chloride, nitrate). Once across the biological barrier, the Cd^{2+} form is captured by numerous intracellular ligands, including metallothioneins (MTs), proteins

that regulate cellular concentrations of essential free metals such as zinc (Zn^{2+}) and copper (Cu^{2+}). Cadmium's high affinity for MTs leads to displacement of zinc (initially bound to the MT), thus disrupting zinc uptake and transport. Target tissues include biological barriers such as gills and the digestive tract, as well as detoxification organs such as the kidneys and liver. It should be noted that muscle does not appear to be a preferred storage site of this element [34].

Results concerning Cu content showed that the latter was particularly detected in high quantities in samples from the Sfax beach, with maximum mean values recorded in *H. diversicolor* tissues reaching $0.053 \pm 0.006 \mu\text{g g}^{-1}$ dry weight at Sfax. Copper is a metal that occurs quite frequently in nature. It is of major importance as an essential microelement for the respiration of many organisms and other enzymatic functions. In humans, copper is stored in the digestive gland (hepatopancreas) and gills of molluscan invertebrates. Excess copper in humans can damage liver tissue and affect blood pressure [28,35]. Similarly, in aquatic organisms, high concentrations of this metal can cause oxidative damage to lipids and proteins, as well as DNA alterations.

The accumulation of metals in organs is a very important parameter to study, since unlike other pollutants, metals can accumulate in organs to concentrations well above those present in the environment and can reach toxic thresholds. Our results on metal accumulation show inter-population variation in the levels of the elements measured. Generally speaking, polluted sediments indicate environmental degradation and pose significant risks to organisms that inhabit or interact with them, particularly because such areas often serve as critical nurseries and spawning grounds for benthic species [36]. An increase in metal accumulation in the tissues of the polychaete annelid *H. diversicolor* is, therefore, probably highly favored, given the ecobiological nature of this species. This partly explains the higher metal levels in the tissues of the Gabes population, since this site also has high sediment values. These observations corroborate earlier studies by Machreki-Ajmi and Hamza-Chaffai [37], who confirmed that the northern part of the Gulf of Gabes is the most polluted zone in this geographical area. This part of the gulf is subject to large-scale industrial and urban effluent discharges, in addition to a stock of phosphogypsum, leading to contamination of the environment mainly by metals. The contamination of the gulf course by these elements is essentially attributed to urban and industrial liquid discharges, as well as to pollution generated by public landfills. Characterization of these discharges has shown them to be highly polluting, particularly in terms of Zn.

The lead levels obtained in *H. diversicolor* tissues collected from some Tunisian aquatic ecosystems are closer to those reported in samples from: Venice, Italy, for the species *H. diversicolor* [38], and Oualidia lagoon, Morocco, for the species *H. diversicolor* [39] (Table 2). However, the concentration of this element at the tissue level is much lower than that reported in samples from Bouregreg Estuary, Morocco, for the species *H. diversicolor* [40], Buleji Karachi, Pakistan, for the species *Eurytho ecomplanata* [41] (Table 2).

Mean zinc values obtained in *H. diversicolor* tissues collected from a few Tunisian regions are very low compared to those reported in samples from (Table 2): JorfLasfar, Morocco, for the species *Sabellaria alveolata* [42]; Bouregreg Estuary, Morocco, for the species *H. diversicolor* [43]; and Mersey Estuary, UK, for the species *H. diversicolor* [44].

The average cadmium levels obtained in *H. diversicolor* tissues collected from several Tunisian aquatic ecosystems are closer to those reported in samples from: Homa Lagoon Izmir, Turkey, for the species *H. diversicolor* [45], and Oualidia lagoon, Morocco, for *H. diversicolor* [39]. In contrast, our results seem only slightly different from those of JorfLasfar, Morocco, for the species *Sabellaria alveolata* [42], and the Ross Sea, Antarctica, for the species *Perkinsiana littoralis* [46] (Table 2).

The toxicity and environmental behavior of metals in aquatic systems—such as their mobility and bioavailability—are largely influenced by their speciation, which refers to the distribution of an element among its various chemical forms or phases (both soluble and insoluble) [47]. These effects also depend on the characteristics of the exposed organism, including species, sex, age, developmental stage, and the metal concentration in specific organs [48,49]. Even if metal analyses in samples from different localities do not reveal situations of real concern, questions remain due, on the one hand, to the large volume of discharges and, on the other, to the bioaccumulation of heavy metals in aquatic species.

Numerous studies have shown that biological variability plays a key role in shaping organism responses to a broad spectrum of contaminants, often influenced by spatial and seasonal environmental fluctuations [42,50–56]. Moreover, metal bioaccumulation is governed by a complex interplay of factors. These include physical parameters such as temperature, salinity, pH, organic carbon content, food availability, dissolved oxygen levels, sediment granulometry, and the system’s hydrological characteristics; chemical aspects like metal concentration, speciation, and bioavailability; and physiological traits of the organism itself, including growth rate, weight fluctuation, sexual maturity, reproductive stage, metal uptake efficiency, and internal accumulation capacity [53,55–61].

Table 2. A compilation of Cd, Cu, Zn, and Pb ($\mu\text{g g}^{-1}$ dwt) contents in different polychaete species from the literature.

Locality	Species	Metals				References
		Cd	Cu	Zn	Pb	
Homa Lagoon–Izmir–Turkey	<i>Hediste diversicolor</i>	1.5–4.5	14.0–26.5	-	-	[62]
Homa Lagoon–Izmir–Turkey	<i>Hediste diversicolor</i>	1.6–2.7	24.2–28.5	-	-	[63]
Homa Lagoon–Izmir–Turkey	<i>Hediste diversicolor</i>	1.6–2.7	14.2–18.5	-	-	[64]
Homa Lagoon–Izmir–Turkey	<i>Hediste diversicolor</i>	0.03–0.43	17.2–41.0	-	6.5–19.1	[44]
Essaouira. Morocco	<i>Sabellariaaalveolata</i>	4.14 \pm 1.08	6.73 \pm 4.48	168.39 \pm 34.88	2.55–2.00	[42]
JorfLasfar. Morocco	<i>Sabellariaaalveolata</i>	38.44 \pm 8.83	16.06–35.10	522.39 \pm 140.26	-	
JorfLasfar. Morocco	<i>Arenicola grubii</i>	10.29 \pm 7.18	6.62–50.43	86.97 \pm 55.27	-	
Oualidialagoo. Morocco	<i>Hediste diversicolor</i>	0.09 \pm 0.1	6.8 \pm 2.5	115 \pm 29.5	1.0 \pm 0.5	[39]
Khnißs lagoon. Morocco	<i>Hediste diversicolor</i>	1.4 \pm 0.2	8.8 \pm 3.8	94 \pm 44.3	3.0 \pm 1.1	[43]
Bouregreg Estuary. Morocco	<i>Hediste diversicolor</i>	-	53.00	555–654	-	
Bouregreg Estuary. Morocco	<i>Hediste diversicolor</i>	-	22.11	102.27	33.17	
Mersey Estuary. United Kingdom	<i>Hediste diversicolor</i>	0.7 \pm 1.0	46 \pm 22	196 \pm 45	9.5 \pm 4.2	[44]
Buleji Karachi. Pakistan	<i>Eurythoe complanata</i>	2.11 \pm 0.38	3.67 \pm 1.26	14.86 \pm 1.64	11.12 \pm 1.18	[41]
Venice. Italy	<i>Hediste diversicolor</i>	0.15 \pm 0.09	24.91 \pm 9.89	-	1.12 \pm 0.4	[38]
Todosos Santos Bay. Brazil	<i>Chaetopterus variopedatus</i>	-	1.8–39	40.6–125	-	[65]
Zolotoi Rog Bay. Russia	<i>Ophryotrocha sp.</i>	0.30 \pm 0.02	3.1 \pm 0.10	24.4 \pm 0.73	-	[66]
	<i>Nereis vexillosa</i>	0.33 \pm 0.03	1.3 \pm 0.25	44.5 \pm 1.30	-	
	<i>Alitta brandti</i>	0.26 \pm 0.02	1.8 \pm 0.04	28.2 \pm 0.80	-	
	<i>Capitella capitata</i>	0.30 \pm 0.02	1.4 \pm 0.03	30.3 \pm 0.90	-	
	<i>Schistomeringos japonica</i>	0.49 \pm 0.04	3.5 \pm 0.07	32.7 \pm 0.96	-	

Table 2. Cont.

Locality	Species	Metals				References
		Cd	Cu	Zn	Pb	
Bengal Bay, India	<i>Perenereis cultifera</i>	-	36.5–52	36.5–52	-	[67]
	<i>Mastobranchius indicus</i>	-	25–44.5	150–320	-	
	<i>Namalycastis fauveli</i>	-	31.00	85.00	-	
	<i>Dendronerides arborifera</i>	-	33.00	140.00	-	
UK Estuaries	<i>Arenicola marina</i>	-	2.9–125	43.3–141	-	[68,69]
Estuaries SW England	<i>Hediste diversicolor</i>	0.2–0.6	44–3900	150–170	-	[70]
Atlantic Coast, France	<i>Hediste diversicolor</i>	0.1–0.3	11–26	110–170	-	[70]
Ross Sea, Antarctica	<i>Perkinsiana littoralis</i>	23–33	4–9	140–200	-	[46]

The bio-sediment accumulation factor (BSAF) is a key index used to relate metal concentrations in organisms to those in sediments [71]. Accordingly, we used BSAF to evaluate the ability of *H. diversicolor* to accumulate metals from sediments. The BSAF for Cd, Cu, Zn, and Pb was calculated following the methods described by [71,72]. The resulting values are presented in Table 3.

Table 3. Bio-sediment accumulation factors (BSAF) of Cd, Cu, Zn, and Pb in *H. diversicolor* tissues from Tunisian coastal sites.

	Cd Concentration in Sediment	Cd Concentration in Soft Tissue	BSAF Cd	Cu Concentration in Sediment	Cu Concentration in Soft Tissue	BSAF Cu	Zn Concentration in Sediment	Zn Concentration in Soft Tissue	BSAF Zn	Pb Concentration in Sediment	Pb Concentration in Soft Tissue	BSAF Pb
Bizerte Lagoon	1.40 [73]	0.01	0.01	8.60 [73]	0.04	0.01	76.30 [73]	0.07	8.78×10^{-4}	23.70 [73]	0.12	0.01
Sfax Coast	0.54 [74]	0.02	0.03	3.00 [74]	0.05	0.02	13.80 [74]	0.07	4.78×10^{-3}	13.90 [74]	0.12	0.01
Gulf of Gabes	6.53 [75]	0.01	0.00	13.94 [75]	0.03	0.00	225.20 [75]	0.07	3.15×10^{-4}	98.15 [75]	0.10	0.00
Djerba Island	1.28 [76]	0.02	0.01	16.16 [76]	0.04	0.00	56.57 [76]	0.06	1.11×10^{-3}	22.04 [76]	0.12	0.01

At Bizerte Lagoon, Cd and Cu showed the highest BSAF values (0.01), indicating similar bioaccumulation potential. Zn showed very limited bioaccumulation, while Pb was moderately accumulated. This suggests that Cd and Cu are more bioavailable, whereas Zn is least accumulated despite relatively high sediment concentrations. At Sfax Coast, Cd exhibited the highest bioaccumulation (0.03), followed by Cu and Pb, while Zn was the least accumulated but still higher than at other sites. This indicates a generally high bioavailability of all metals, with Cd being the most accumulated. In the Gulf of Gabes, all metals showed very low BSAFs, with Pb slightly higher than the others. This suggests that, despite high sediment contamination, metals are likely present in non-bioavailable forms or are not readily taken up by organisms.

At Djerba Island, Pb showed the highest BSAF, followed by Cd. Cu showed no measurable accumulation. This indicates selective bioaccumulation, where Pb and Cd are more bioavailable, and Cu is largely unavailable to organisms.

The inter-site comparison of BSAF values revealed distinct patterns of metal bioaccumulation across the four coastal sites. Sfax Coast stands out with the highest BSAFs for all metals, especially for Cd (0.03) and Cu (0.02), indicating higher bioavailability and uptake efficiency of these metals by biota, likely due to favorable environmental conditions or more labile metal forms. In contrast, the Gulf of Gabes, despite showing the highest sediment concentrations, exhibited extremely low BSAFs for all metals, suggesting that metals are mostly in non-bioavailable or strongly bound forms, limiting their transfer to organisms. Bizerte Lagoon showed moderate BSAFs for Cd and Cu (0.01), but much lower values

for Zn and Pb, indicating a selective accumulation pattern possibly influenced by metal speciation or sediment characteristics. Djerba Island displayed a similar trend, with Pb (0.0054) and Cd (0.01) showing the highest accumulation, while Cu is not accumulated at all, suggesting localized factors affecting metal mobility and organism exposure. Overall, while Sfax Coast poses the greatest ecotoxicological risk due to high bioaccumulation, the Gulf of Gabes may represent a contamination hotspot with limited biological impact, emphasizing the importance of assessing both sediment contamination levels and bioaccumulation potential for accurate ecological risk evaluation.

The differences in BSAF values between sites and metals reflect the combined influence of metal speciation, sediment characteristics, and local environmental conditions affecting metal bioavailability and organism uptake. Sfax Coast consistently showed the highest BSAF values for all metals, especially Cd and Cu, suggesting that metals there are present in more bioavailable forms, possibly due to factors such as finer sediment grain size, higher organic matter content, or more active benthic communities facilitating uptake. In contrast, the Gulf of Gabes, despite high total metal concentrations in sediments, showed extremely low BSAF values, indicating that metals are likely bound to stable mineral phases or complexed with substances that reduce their mobility and uptake by organisms. Bizerte Lagoon and Djerba Island show intermediate patterns, with Cd and Pb being more bioaccumulated than Cu and Zn, suggesting that metal-specific properties, such as ionic radius, affinity for organic matter, and redox sensitivity, influence their accumulation. Additionally, biological factors such as species metabolism, feeding strategy, and detoxification mechanisms may further explain the variability in BSAF values across both sites and elements.

According to [71], snail tissues can be categorized as macroconcentrators ($BSAF > 2$), microconcentrators ($1 < BSAF < 2$), or deconcentrators ($BSAF < 1$). In this study, the biota-sediment accumulation factors (BSAF) indicate that the soft tissues of *H. diversicolor* functioned as deconcentrators at all sampled sites. This suggests a limited capacity for metal uptake from sediments. Similarly, all calculated bioaccumulation factor (BAF) values were below 1000, indicating a low potential for significant bioaccumulation of heavy metals in this species. Based on the classification proposed by [77], BAF values are interpreted as follows: $BAF < 1000$ reflects negligible accumulation potential; values between 1000 and 5000 indicate moderate bioaccumulation; and $BAF > 5000$ denotes a high bioaccumulation risk. Generally, BSAF values greater than 1 imply that a metal or metalloid may accumulate in an organism's soft tissues, whereas values below 1 suggest minimal accumulation and weak association with sediment concentrations [78]. Thus, the presence of metals in the tissues of *H. diversicolor* likely reflects limited environmental uptake rather than direct accumulation from sediment.

Based on the observed heavy metal contamination, particularly the elevated levels of lead and the presence of cadmium, it is imperative to implement effective environmental management and protection strategies to safeguard Tunisia's aquatic ecosystems. Establishing regular biomonitoring programs using reliable bioindicator species such as *Hediste diversicolor* will be essential for tracking pollutant trends and identifying emerging risks. Strengthening regulations on industrial discharges and urban wastewater treatment is crucial to reducing the input of toxic metals into coastal waters. Furthermore, raising public awareness about the ecological and health risks associated with heavy metal contamination can enhance community engagement and support pollution prevention efforts. Sustainable coastal development practices, including habitat restoration and pollution source control, will be vital to preserving biodiversity and ensuring the long-term health of marine environments and the human populations that rely on them.

5. Conclusions

This study is the first to focus on the use of polychaete *H. diversicolor* as a sentinel species in Tunisia for the biomonitoring of aquatic ecosystems. There are few studies available in the literature regarding the use of *H. diversicolor* as a bioindicator species for monitoring heavy metals. Indeed, most of the studies carried out mainly concern the monitoring of organic pollutants. The different heavy metal levels recorded at the sites investigated during this work confirm the usefulness of using this polychaete species as a sentinel species in biomonitoring programs. As perspectives for this preliminary study, we intend to use this species as a sentinel for the monitoring of organic pollutants along the Gulf of Gabes and for the monitoring of metallic and organic pollutants in other Tunisian aquatic ecosystems.

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