Trop J Nat Prod Res, April 2024; 8(4):6765-6770 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Bioaccumulation of Heavy Metals in Five Species of Fish Obtained from the Estuary of Rabat, Morocco

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ARTICLE INFO

Article history:
Received 17 September 2023
Revised 04 April 2024
Accepted 11 April 2024
Published online 01 May 2024

ABSTRACT

In recent years, there has been a dramatic fall in the fish population. This is due to habitat destruction, overfishing, diseases, climate change, and metal contamination. Despite the advantages of eating aquatic foods, hazardous metals can bioaccumulate in fish, posing a threat to human health and marine ecosystems. There is a need to evaluate the danger to human health posed by several heavy metals linked to eating popular edible fish species that are readily available to consumers in the Rabat-Sale region. The study aimed to evaluate the concentrations and accumulation of heavy metals in the various organs of fresh fish obtained from the Bouregreg estuary. During the winter and spring of 2023, fish belonging to five different genera *(Anguilla*, *Diplodus*, *Solea*, *Mullet*, and *Lophius*) were collected in the Bouregreg estuary at three different locations: My Youssef, Hassan II, and Mohammed VI Bridges. The concentrations of heavy metals (Cr, Zn, Cd, Pb, Fe, and Cu) in the muscles, livers, and gills were determined using an ULTIMA2 type inductively coupled emission spectroscopy plasma (ICP-AES). The results showed that all of the fish samples examined were contaminated with the six metallic trace elements. The fish samples meet the World Health Organization's recommended standards, especially for the *Anguilla* species, which, in contrast to *Lophius*, are recognized to be the healthiest fish and have the lowest metallic levels. The findings of the study revealed that the Bouregreg estuary is contaminated with heavy metals, necessitating regular fish awareness, monitoring, and assessment due to the significant reliance of the surrounding communities on fish.

***Keywords*:** Aquatic foods, contamination, human health, marine ecosystem.

Introduction

Numerous marine life forms are disappearing as a result of human activities that discharge waste into marine ecosystems, such as heavy metals.[1] These activities can also have an impact on aquatic life, especially fish.[2] This is a result of the industries, including the manufacturing of chemicals, oil and gas, transportation, and others, developing quickly.[3] Aquatic foods are known as a complete protein diet for humans because they contain all the required amino acids.[4] Fish can either bioaccumulate toxins they eat with their food products or bioconcentrate them directly from the water through diffusion in the gills and skin.[5] Depending on the fish's nutritional status, age, size, and water quality, the rate of accumulation varies.[6] They prefer to accumulate toxins in animal lipid deposits because they are also fat-soluble, which allows them to spread from prey to predators.[7]

Heavy metal contamination harms several aquatic species as well as the delicate balance of the environment.[8] Fish are one of the animals used to evaluate the health of the aquatic ecosystem because pollutants like heavy metals build up in the food chain and have negative effects, including death.[9] The state of the aquatic environment and human impacts on it are regarded as two aspects that have received increasing attention lately.[10]

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Citation: Aarabi S, Kettani K, Chauiyakh O, Bouganssa T, El Fahime E, Et-tahir A. Bioaccumulation of Heavy Metals in Five Species of Fish Obtained from the Estuary of Rabat, Morocco. Trop J Nat Prod Res. 2024; 8(4):6765-6770. https://doi.org/10.26538/tjnpr/v8i4.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Fish and other marine animals are commonly used in programs to monitor marine pollution because they take in toxins from the environment.[11] The risk to human health should be evaluated, and fish should be used as a proxy for the health of aquatic ecosystems.[12] Due to their toxicity, persistence in the environment, and capacity to enter food systems, heavy metals are major contaminants.[13] They accumulate in marine organisms by a variety of mechanisms, such as respiration, adsorption, and ingestion, as they are not biodegradable.[14] There has not been enough research conducted on the heavy metal concentration of these marine fish that are marketed commercially in Morocco. To assess the risks of consuming this product, it seemed crucial to investigate the concentrations of these metals in the edible tissues of these species. The Bouregreg estuary continues to experience human disturbance due to the construction of dams, shoreline developments, and domestic discharges.

The present study was conducted to measure the concentration of six heavy metals (Cu, Zn, Pb, Cr, Cd, and Fe) in the livers, gills, and muscle tissues to estimate the bioaccumulation of heavy metals in fish from the Bouregreg wadi.

Materials and Methods

Selection of sample locations

The Bouregreg estuary is located in Morocco at the confluence of the Bouregreg and Sebou rivers. It is located between the cities of Rabat and Salé on the Atlantic coast in the northwest of the country.[15] This estuary is very important ecologically because it supports a wide variety of animal and plant species, including migratory birds that stop here on their annual migration.[16] Additionally, the port activity in the estuary makes a substantial contribution to the expansion of the local economy.[17,18] The fishes were collected from three locations over the winter and spring of 2023 in Oued Bouregreg (Plate 1). Different

techniques have been employed to fish for benthic fish at different sites, depending on the significance of the methods. Station 1 is located about 4.3 km from the mouth of the Hassan II Bridge (34°01'40"N 6°49'10" W); Station 2 is 6.6 km from the mouth of the My Youssef Bridge (34°01'16"N 6°48'24"W), and Station 3 is 23 km from the mouth of the Mohammed VI Bridge (33°56'17"N 6°45'44"W).

Sampling and chemical analysis

At four separate locations along the estuary, pelagic trawling was utilized to sampling the five fish species (*Solea*, *Anguilla*, *Mullet*, *Lophius*, and *Diplodus*) that were in great abundance.[19] The fishes were immediately archived and kept frozen at -20°C until further investigation could be conducted.[20] They were then brought to the laboratory, and stored in a refrigerator (Model: MRFec 4001, Variant l47 made by Liebherr in Germany) with dry ice cubes.[20] After the samples were well cleaned with distilled water, the length (cm) and weight (g) of each sample were recorded.[21] The collected species were then divided into groups according to genus and then according to sex (Table 1). The frozen fish were defrosted and dissected at room temperature using a stainless-steel knife.[22] Cutting out the muscles, gills, and liver allowed for the use of composite samples weighing 5 g each for the analysis.[23] Each moist sample was lyophilized for 24 hours, and 1 g aliquots of each were obtained. The samples were ground in mortar.[24] The glassware was thoroughly cleaned by soaking it in 2 M HNO_3 for 48 hours before rinsing it five times in distilled water and five times in deionized water.[25] Analytical grade HNO_3:$HClO_4$:HCl (3:2:9) was used to digest 5 g of the identified tissues (dry) for 4-6 hours on a hot plate (series ZH manufactured by SGS in China).[26] Following cooling, the samples were run through Whatman No. 42 filter paper, and the samples were diluted in 50 mL of distilled water.[27]

Statistical analysis

The variability of the metal contents was analyzed using the analysis of variance (ANOVA) on the HP PROBOOK computer (450 G7 Windaus 10). Also, a one-way ANOVA was used to test whether the mean values of the univariate measurements differed. The statistical package for the social sciences (SPSS) was employed to calculate the components. Statistical significance was set at $p < 0.05$.

Plate 1: Locations of the three stations as determined by Google Earth (34°01'40"N 6°49'10"W, 34°0 1'16"N 6°48'24"W, and 33°56'17"N 6°45'44"W).

Table 1: The characteristics of fish caught in the Bouregreg estuary

Species name	Sex			Size (cm)	Weight (kg)
	Age (year)	Number	Female	Male	
Solea	2-5	10	6	4	15-40	0.150-0.430
Anguilla	3-7	5	4	1	40-100	0.250-3.2
Mullet	2-6	14	4	10	10-19	0.03-0.07
Diplodus	2-4	10	3	7	16-30	0.030-0.5
Lophius	3-5	9	4	5	25-37	0.52-1.5

Results and Discussion

The concentrations of trace metals in the five species of fish (*Solea*, *Anguilla*, *Mullet*, *Lophius*, and *Diplodus*) collected in the Bouregreg estuary are summarized in Table 2. The maximum average revealed the following hierarchy of heavy metals in fish: Fe > Zn > Cu > Cr > Cd > Pb (Figure 1). These values satisfied Morocco's food safety guidelines and did not exceed the international food safety guidelines. The five fish species in the Bouregreg estuary had a relatively high concentration of metals in their liver tissues, compared to muscle tissues. The maximum accumulation of metals was recorded in *Mullet* fish, with an average of 7.44 mg/kg, which meets the international limit. The livers of *Lophius* were found to have a rather high quantity of Cd, above the recommended limits. Fish livers are rarely consumed, but they can serve as effective environmental metal biomonitors. *Solea* has the lowest recorded rate of chromium concentration, at 0.0039 mg/kg. The metabolism of glucose depends critically on chromium.[28] *Mullet* had a higher total Cr concentration than *Anguilla*, although the amounts of Cr in both species are still within the acceptable limits set by the United Nation Food and Agriculture Organization (FOA).[29]

Fish from estuaries tend to accumulate heavy metals since they are benthic, and Pb is one of these dangerous metals that have carcinogenic consequences for both the aquatic environment and human health.[30] In this study, *Mullet* accumulated more Pb than *Diplodus* in all organs (Figure 1). The highest accumulation was observed in liver (0.1218 mg/kg), followed by gills (0.0268 mg/kg), and then muscles (Figure 2). The results of the present study revealed that *Diplodus* is capable of exhibiting exceptionally elevated levels of Fe (7.102 mg/kg) and Zn (3.586 mg/kg) in various tissues examined, particularly the liver. This poses a risk to human health, even though the species is not well-known for its propensity to accumulate heavy metals and its extreme toxicity. This evidence indicates that long-lived fish that are not predatory should be considered at an equally high risk to predatory fish. In the study, only *Lophius* exceeded the FAO for cadmium limits. The highest Cd value was 0.697 mg/kg, with an average Cd value of 0.234 mg/kg. As shown in Table 3, metals were discovered to be strongly correlated linearly in the tissues of several fish species ($p < 0.05$).

The metal content of muscle tissues was lower than that of the liver and gills, as illustrated in Figure 2. On the other hand, it also helps the body eliminate metal ions. Therefore, the high metal content of the tissue may be linked to the organ's ability to accumulate during its activity in the depuration and absorption of ingested heavy metals.[31] The concentrations of heavy metals in the muscle tissues of five fishes obtained from Bouregreg estuary are represented in Figures 3a-e. *Lophius* is the fish with the highest average concentration of Cd (0.234 mg/kg), exceeding the WHO's recommended limit of 0.05 mg/kg.[32] With 0.0039 mg/kg of concentrate, *Solae* has the least amount. Chromium plays a critical role in the metabolism of glucose.[33] *Mullet* had a higher total Cr concentration than *Anguilla* (Figure 3a). The organ with the highest concentration of Cr in mules is the liver (0.6122 mg/kg), followed by the muscle. The lowest metallic Cr concentration was obtained by *Solea* (0.0011 mg/kg) as depicted in Figure 3c. All values observed were approved by WHO, including the maximum level of Cu (3.917 mg/kg) in *Lophius* and the lowest level of Cu (0.0007) in *Solea* muscle.

Table 2: The mean heavy metal concentration (mg/kg) in five fish species collected from Bouregreg River, Rabat, Morocco.

Fish species	Tissues	Heavy metal					
		Cr	Zn	Cd	Pb	Fe	Cu
	Liver	0.0851	2.607	0.0095	0.0381	25.987	1.987
	Gill	0.0185	1.101	0.0017	0.015	4.765	0.0524
Solea	Muscle	0.0011	0.0776	0.0005	-0.0238	0.2779	0.0007
	Liver	0.0532	1.771	0.0078	0.0245	3.609	0.1214
	Gill	0.0244	1.291	0.0019	0.0232	2.819	0.0472
Anguilla	Muscle	0.0118	1.039	0.0019	0.0041	0.8001	0.0535
	Liver	0.6122	3.275	0.0097	0.1218	28.209	1.47
	Gill	0.0821	1.406	0.0073	0.0268	6.205	0.0837
Mullet	Muscle	0.3306	0.1648	0.0046	0.0088	1.496	0.0103
	Liver	0.0503	3.586	0.0439	0.0272	7.102	0.1806
	Gill	0.0406	2.506	0.0064	0.0193	6.58	0.0712
Diplodus	Muscle	0.00077	0.1389	0.0045	0.0003	0.3319	0.0045
	Liver	0.064	4.617	0.697	0.02222	9.052	3.917
	Gill	0.0417	1.274	0.0062	0.0017	7.753	0.1807
Lophius	Muscle	0.0292	0.0976	-0.0001	0.0009	6.58	0.0045

Table 3: Descriptive statistics of the six heavy metals (mg/kg) in fish of Bouregreg

Heavy metal	N	Minimum	Maximum	Sum	Average		Standard deviation	Variance	Kurtosis	
	Statistics	Statistics	Statistics	Statistics	Statistics	Erreur standard	Statistics	Statistics	Statistics	Standard error
Cr	15	.0011	.6122	1.4228	.094855	.0423420	.1639898	.027	7.636	1.121
Pb	15	.0003	.1218	.3385	.022567	.0077277	.0299291	.001	9.651	1.121
Fe	15	.2779	28.2090	111.5549	7.436993	2.1883327	8.4753759	71.832	2.911	1.121
Cu	15	.0007	3.9170	8.6091	.573940	.2831072	1.0964696	1.202	6.138	1.121
Zn	15	.0776	4.6170	25.0939	1.672927	.3594767	1.3922471	1.938	-.260	1.121
Cd	15	.0001	.6970	.8027	.053513	.0460439	.1783272	.032	14.870	1.121

Comparison with standards and previous studies

The results indicated that fish in the present investigation had significantly lower levels of Cd than fish in the Meghna Estuary in Bangladesh.[33] Chemical fertilizers may be associated with this observation, as they are the cause of the rise in Cd in aquatic environments. The metal buildup found in the gills of *Lophius* fish is different from that found in freshwater fish gathered in Pakistan by Kakar (2020),[34] especially when it comes to the three metals (Cu, Fe, and Cd). The *Lophius* of Bouregreg contains considerably higher concentrations of these metals than the ones in Pakistan.[35] Changes in the diet and way of life of the species may be the cause of variations in the bioaccumulation of heavy metals in *Lophius*. However, the findings of the current study were not in agreement with those previously reported for the southern Atlantic coast of Spain,[36], and the Ionian Sea Dam *Mullet* in Italy.[37] On the other hand, Moiseenko and Gashkina demonstrated that the concentrations of heavy metals in the muscles of fish from the North Dvina River were higher than what was observed in the present study.[38] This can be explained by the low salinity of this ravine as well as the pollution of this water body by acidic leachates.[39] The results in the present study are still encouraging and acceptable compared to those of Wing in South China,[40] where they reported a variety of metals, including Cd, which was bioaccumulated in the muscles and viscera of tilapia and the muscles of the snakehead fish.[41] The *Solae* fish, which lives in the Bouregreg estuary, was determined to be healthy, and the levels of the six metallic elements (Zn, Fe, Cr, Cu, Pb, and Cr) investigated (Figure 3) meet the international standards recommended by the European Community. This is consistent with the findings of El Agri (2021), who found that *Solae aegyptiaca* accumulated six heavy metals (Fe, Cu, Zn, Cd, Pb, and Ni) in Lake Qarun, meeting international standards.[42]

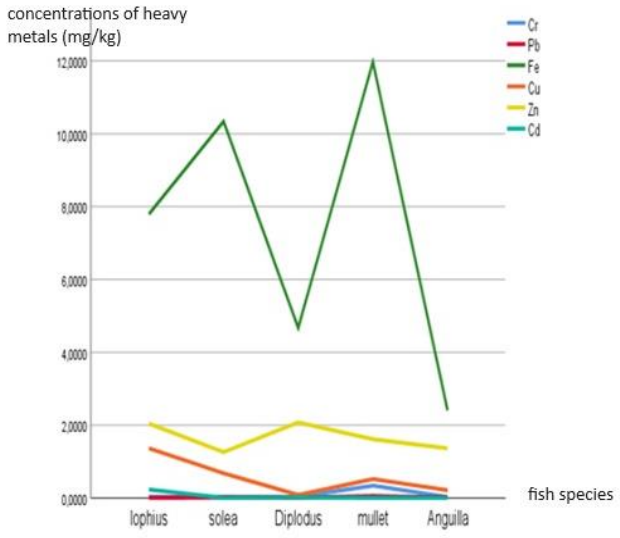

Figure 1: The average concentrations of heavy metals(mg/kg) in the different species of Bouregreg estuary fish.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

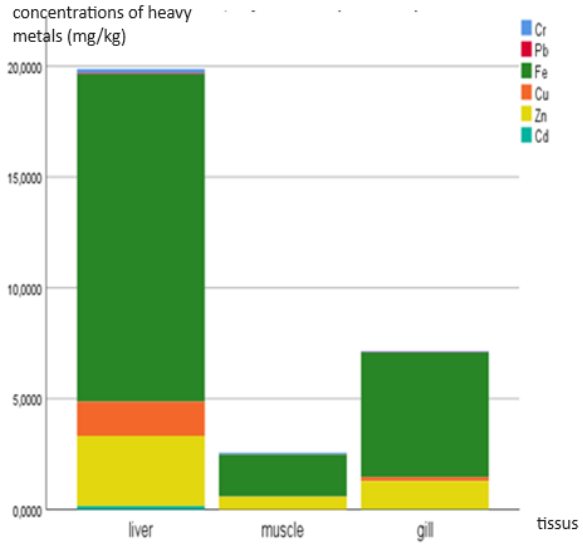

Figure 2: Averages of heavy metals(mg/kg) in the three tissues of different fish species from Bouregreg.

Conclusion

The findings of the present study revealed that the *Anguilla* species meets WHO standards. Unlike *Lophius* and *Mullet*, with the highest concentrations in their livers, they exceeded the recommended limits. However, the five fish species in the Bouregreg estuary had a relatively high concentration of metals in their liver tissues compared to their muscle tissues, and gills. The Bouregreg estuary is contaminated with heavy metals (Cr, Zn, Cd, Pb, Fe, and Cu) and requires periodic fish awareness, monitoring, and assessment, as most communities along this location depend on fish.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

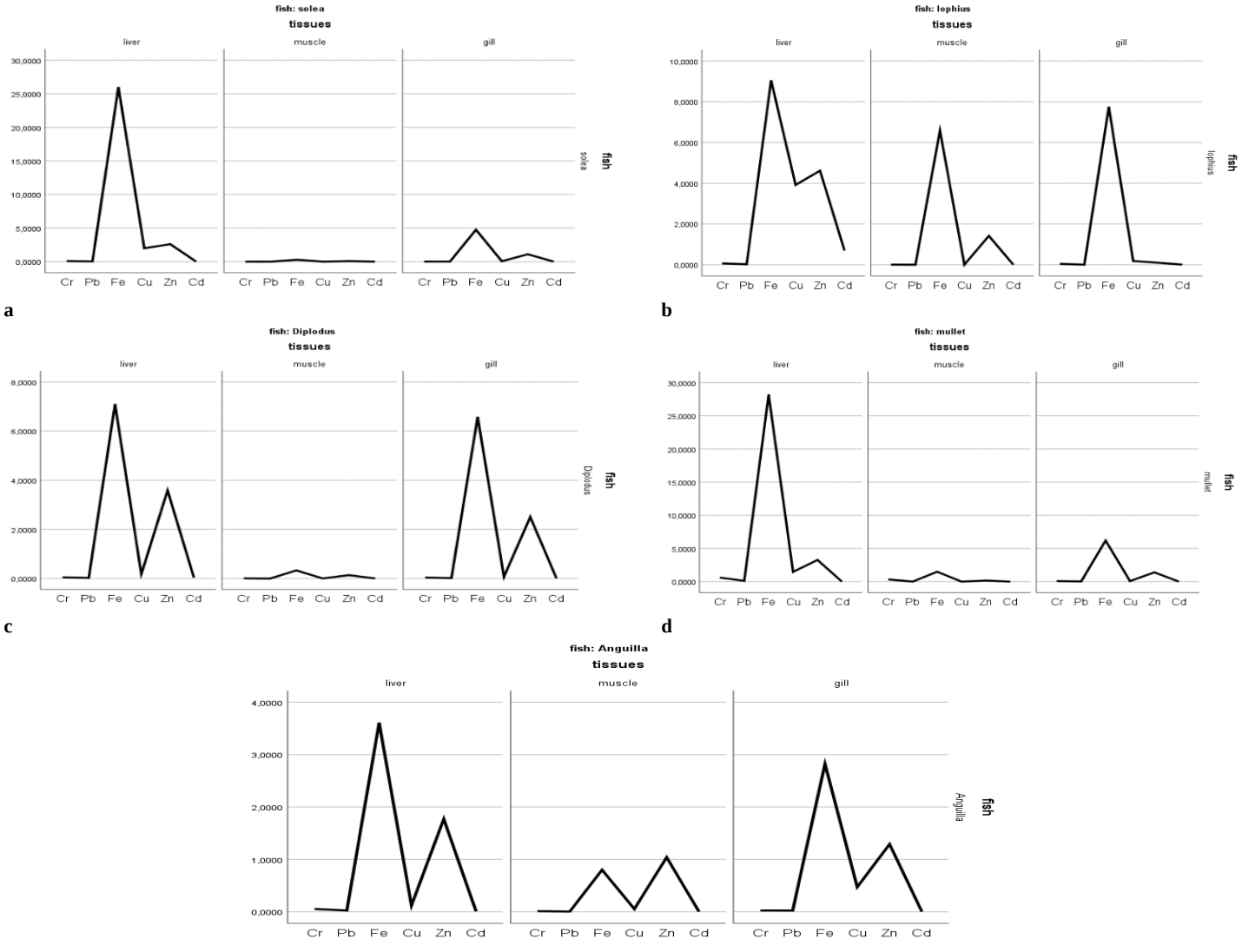

Figure 3: Heavy metal concentration (µg/g dry weight) in the muscle tissues of five fishes in Bouregreg. a: *Anguilla*; b: *Diplodus sparus*; c: *Solea*; d: *Mullet;* e: *Lophius*

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