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Research Article

Human health risk assessment of heavy metal(oid)s in four fish species harvested from the Bizerte lagoon (Tunisia)

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Abstract: This study investigated the levels of cadmium (Cd), lead (Pb), arsenic (As), iron (Fe), copper (Cu), and zinc (Zn) exposure in the child and adult populations in Tunisia. The heavy metal(loid) (HM) content in muscle and the organ mixture of four commercial species of fish widely available and consumed were determined (*Sparus aurata*, *Dicentrarchus labrax*, *Liza aurata, Sarpa salpa*).

The fish samples were prepared using a wet digestion method, and the HM analysis was carried out using an inductively coupled plasma mass spectrometry (ICP-MS). The results indicated that all fish organs had high amounts of Pb and As and high levels of Cd and Zn in some organs. Based on the exploratory data analysis, fish species can be differentiated according to their diet.

The estimated daily intake values for the children's population have exceeded the permissible levels for Cd and As, whereas they were only exceeded for As for the adult population. Only Fe's Target Hazard Quotient exceeded the permissible limit. The evaluation of the carcinogenic risk associated with the consumption of the four fish species from the Bizerte lagoon indicated a potential carcinogenic effect related to lead (Pb) exposure. In this lagoon, HM contamination of fish is requiring both a short-term and long-term approach to ensure the safety of marine products.

Keywords: Human health; Heavy metals; Fish; Bioaccumulation; Analysis; Contamination; Commercial species; Consumer protection; Carcinogens.

1. Introduction

The Mediterranean diet consists largely of fish. This food item is recognized as a good source of high-quality proteins, lipids, vitamins, and minerals (Gil and Gil, 2015). In addition, fish lipids are particularly rich in omega-3 polyunsaturated fatty acids, known for their important role in the prevention and treatment of many cardiovascular diseases (Gammone et al., 2019). For these reasons, it is recommended to consume fish at least twice a week (Rimm et al., 2018). However, the frequent intake of these products may pose a significant health risk to the consumer due to the high ability of several marine fish to accumulate contaminants, especially heavy metals (HMs) (Saha et al., 2016). In this regard, fish are considered a main source of HM contamination for humans (Annabi et al., 2013).

While some essential heavy metals, such as Fe, Cu, and Zn, are required for certain biochemical and physiological functions in the body, their excessive levels can have disastrous health consequences (Tuzen, 2009). For instance, excess iron can gradually accumulate in internal organs, causing potentially fatal damage to the brain and liver. The early symptoms of iron poisoning may include stomach pain, nausea, and vomiting (Yuen and Becker, 2021). Acute copper exposure can affect the gastrointestinal system (Gamakaranage et al., 2011). In addition, chronic exposure to high Cu doses can influence other organs, such as the liver, in sensitive subjects (Jaishankar et al., 2014). At high concentrations, zinc causes lung diseases, gastroenteritis, fever, vomiting, problems with muscular coordination, and dehydration (Gotteland et al., 2001). Most other "non-essential" heavy metals, such as lead, cadmium, and arsenic, are harmful to the body even at relatively low concentrations (Jaishankar et al., 2014). For example, arsenic has been associated with a variety of complications affecting the integumentary, nervous, respiratory, cardiovascular, hematopoietic, immune, endocrine, hepatic, renal, and reproductive systems (Kim and Kim, 2015). Enhanced lead concentration affects behavior, cognitive performance, postnatal growth, delays puberty, and reduces hearing capacity in infants and children (Wani et al., 2015). The clinical consequences of lead poisoning in children are essentially anemia and neurological toxicity, with an impact on psychomotor development correlated with the severity of intoxication. It is a reportable disease that warrants medical and environmental management (Rouzi et al., 2022). In adults, Pb causes cardiovascular, central nervous system, kidney, and fertility problems (Assi et al., 2016). Cadmium can induce epigenetic changes in cells that can lead to the development of various cancers (Genchi et al., 2020). Chronic cadmium poisoning mainly causes renal tubulopathy and may cause osteomalacia and diffuse osteoporosis. It is classified as a certain human carcinogen by the International Agency for Research on Cancer (IARC) (Andujar et al., 2010).

As a result of human activities such as transport, agriculture, industry, and urbanization, HMs are known to be emitted into the marine environment (Robu et al., 2015). In the Mediterranean and elsewhere, coastal lagoons have been identified as the primary ultimate receptacles of polluting inputs. They are particularly prone to heavy metal accumulation in sediment and suspended particulate matter in the water column (Martins et al., 2015), which may pose serious threats to the biota and ultimately to human health. They are highly productive ecosystems and provide a wide range of environmental and socioeconomic benefits (Pérez-Ruzafa et al., 2020).

The Bizerte lagoon is located in the north of Tunisia between latitude 37° 8′ and 37° 16′ N and longitude 9° 46′ and 9° 56′ E. This lagoon covers an area of approximately 150 km^2 and has an

average depth of 8 meters. The lagoon's northeast end communicates with the Mediterranean Sea via a channel (5 km long; 300 m wide) that empties into the sea via an artificial canal (1500 m length; 140 m width; 12 m depth). Surface waters flow in a southeasterly direction; however, the flow at the lagoon's bottom is in the opposite direction Béjaoui et al. (2017).
The Bizerte Lagoon is highly influenced by human activity on its banks, such as urban expansion, and receives both urban effluents and industrial discharges from the cement factory, metallurgical industry, El Fouledh steelworks, tire factories, and other sources (Ben Said et al., 2008). In addition, the lagoon receives agricultural runoff comprising fertilizers and pesticides, as well as waste from bivalve aquaculture and fisheries (Kamel et al., 2014).

The lagoon of Bizerte is an important socio-economic pole in northern Tunisia. Its shores and maritime space host lots of human activities, such as coastal fishing, shellfish farming, maritime traffic, military activity, recreational fishing, and nautical sports. The evolution of fisheries production in the Bizerte lagoon is strongly correlated to the environmental condition of the water body and the configuration of the passes. Over the past decade, the cumulative output of the lagoon has averaged approximately 270 tons annually. Within this yield, fishing activities contribute to 25%, equating to around 70 tons, while the remaining 75% stems from shellfish farming endeavors (DGPA, 2020).
The lagoon of Bizerte is described as a contaminated ecosystem that has been impacted by both urbanization and industrialization (cement works, metallurgical industry, boatyards, tire manufacturers) (Béjaoui et al., 2008; Afli et al., 2009). The industry discharges roughly 32,000 m^3 of effluent into the Bizerte lagoon daily, whereas urban sources discharge 20,000 m^3 daily (MAWRF, 2015). Many investigations have been conducted in the Bizerte lagoon to assess the heavy metal concentrations in water, sediment, and benthic organisms (Yoshida et al., 2002; Ben Garali et al., 2010; Srarfi et al., 2010; Barhoumi et al., 2014; Zaaboub et al., 2015, 2016; Martins et al., 2016; Ben Mna et al., 2017; Saidi et al., 2019; Ghribi et al., 2020). According to Ben Garali et al. (2010) the superficial sediments in some locations of the Bizerte lagoon have been contaminated by nickel because of its proximity to the industrial zone and by lead, as a result of detrital material rich in lead from the surrounding geological lands.
The contamination of superficial sediments in the lagoon's southern and eastern sectors is mainly caused by copper, zinc, nickel, and iron. This pollution is related to the steelworks El Fouledh, the military arsenal, and Menzel Bourguiba waste disposal. Saidi et al. (2019) reported that the channel connecting the Bizerte lagoon to the Mediterranean Sea is characterized by moderately contaminated superficial sediments, primarily manganese, lead, zinc, chromium, barium, and arsenic, and to a lesser extent by nickel. The authors highlighted the impact of heavy metals on the spatial distribution of meiofaunal and microbial communities in relation with the pollution sources in the Bizerte lagoon. In addition, Ben Mna et al. (2017) reported that the Bizerte lagoon biota is affected by heavy metals based on enrichment and geo-accumulation variables. Although about 16 fish species are harvested in the Bizerte lagoon (Beji, 2000), there is scarce data regarding heavy metal concentrations in fish captured from the Bizerte lagoon and the related human health risk.

Because contaminated fish are the source of the greatest amounts of heavy metals in humans, determining metal concentrations in commercial fish species is a public health concern. Therefore, this study aims to determine the heavy metal(oid) contents (Cd, Pb, As, Fe, Cu, and Zn) in different organs of the four most consumed and most abundant fish species caught in the

Bizerte lagoon and assess the potential health risk for child and adult consumers. Our findings will provide valuable data for public health authorities and consumer protection organizations.

2. Materials and Methods

All chemical reagents used in the present study are of analytical reagent grade. The deionized water was produced by a Milli-Q purification system (Merck Millipore, Massachusetts, USA). The inductively coupled plasma mass spectrometry (ICP-MS) (Spectro Arcos, Germany) was used to measure the concentration of HMs.

2.1. Sample collection

A total of 64 fish specimens belonging to *Liza aurata* (Risso, 1810) (n = 18, length: 31.5 – 35.0 cm, age: 3.5 – 4.0 years), *Dicentrarchus labrax* (Linnaeus, 1758) (n =18, length: 31.5 – 3.5 cm, age: 3.0 – 3.5 years), *Sparus aurata* (Linnaeus, 1758) (n = 18, length: 23.2 – 25,6, age: 3.0 – 4.0 years), *Sarpa salpa* (Linnaeus, 1758) (n = 18, length: 23,0 - 24.5, age: 3.5 – 4.0 years), which are common in the Bizerte lagoon, were purchased from local fishermen operating in the Bizerte lagoon in May 2023. To avoid size-dependent differences in analysis, individuals of similar size were selected for each species. Fish samples were split into three pools, with six specimens per species. The samples were placed in a polyethylene bag, kept in cool boxes at 4°C, and transferred to the laboratory within 1 h. After biometry measurements and dissection, the fish organs (liver, gills, muscle, kidney, gonads, and brain) were stored at -20°C until analysis. It should be noted that the muscle samples (without the skin) were collected from the dorsal part of each fish.

2.2. Heavy metal(oid) analysis

In the laboratory, each fish specimen was washed with distilled water. An initial five-gram sample of fish was taken, and dried at 105 °C for 24 hours, and then stored in a desiccator until its weight was constant. Following this, 5 mL of nitric acid was added to a beaker containing 0.5 g of powdered fish sample. For a clear solution, the mixture was heated to 140 °C. The solution was then filtered and diluted to 50 mL and used for HM analysis. To determine the concentrations of Fe, Cu, and Zn, we used flame atomic absorption spectroscopy (Varian®, AA 240Z) equipped with a graphite furnace (Model, GTA 120) set with a transverse Zeeman corrector for background correction, whereas concentrations of As, Pb, and Cd were determined using an atomic absorption spectrophotometer (Varian®, AA 240Z) equipped with a graphite furnace (AAS-GF) (Model, GTA 120). Standard curves were created using reference material (Qhemis High Purity®), and recovery rates in all investigations varied from 80% to 120%. The concentrations of HMs were measured in mg kg^{-1} dry weight. The heavy metal(oid) analysis was performed for the following organs: liver, gonads, gills, muscle, kidney, and brain.

2.3. Risk assessment

The values of heavy metal concentration in the muscle and the mixture of organs (liver, gonags, gills, kidney, brain) of the targeted fish species were used to determine the estimated daily intake (EDI), the target hazard quotient (THQ), the hazard index (HI), and the target cancer risk (TCR) according to the U.S. Environmental Protection Agency (USEPA, 2000). These indices were separately calculated for the adult and children's populations. Table 1 shows all the parameters used for health risk estimation. The Estimated daily intake (EDI) of metals is measured using the following equation:

$$\mathrm{EDI} = \frac{\mathrm{MC\ x\ IR}}{\mathrm{BW}}$$

where parameters are already explained in Table 1.

The target hazard quotient (THQ) represents the ratio of exposure level to HMs over a specified period to the reference dose of that HMs. Thus, THQ ≥1 indicates potential health hazards associated with the consumption of fish. THQ values were calculated using the formula given by USEPA (2015):

$$\mathrm{THQ} = \frac{\mathrm{MC\ x\ FIR\ x\ 10^{-3}\ x\ EF\ x\ ED}}{\mathrm{RfD\ x\ BW\ x\ AT}}$$

where all parameters are already explained in Table 1.

The hazard index (HI) is used to evaluate the potential risk of adverse health effects from a mixture of toxic metals. It was calculated as the sum of THQ for each metal:

$$\mathrm{HI} = \mathrm{THQ_{Pb}} + \mathrm{THQ_{As}} + \mathrm{THQ_{Cd}} + \mathrm{THQ_{Fe}} + \mathrm{THQ_{Cu}} + \mathrm{THQ_{Zn}}$$

When HI < 1.0, it is unlikely that there will be obvious adverse effects, while HI > 10 indicates high risk and chronic or even acute effects (EFSA, 2012). The target cancer risk (TCR) is used to indicate the carcinogenic risk. The model for estimating TCR is provided in USEPA Region III Risk-Based Concentration Table 1 (USEPA, 2011). TCR was calculated by the following equation:

$$\mathrm{TCR} = \frac{\mathrm{EF\ x\ ED\ x\ FIR\ x\ MC\ x\ \ CSF}}{\mathrm{BW\ x\ AT}}\ \mathrm{x\ 10^{-3}}$$

where all parameters are already explained in table 1.

3. Results and discussion

3.1. Heavy metal(loid) concentrations in fish

The average concentrations of HMs in different fish organs (including liver, gonads, gills, muscle, kidney, and brain) as well as in their mixture are presented in Table 2. The obtained results showed that there is no common pattern for the HM distribution in organs among the different fish species. Moreover, significant variations ($p<0.05$) of HM concentration across organs and species (except for Cd among species) were revealed by the ANOVA two-way analysis given in Table 3.

As given in Table 2, essential metals, including Fe, Zn, and Cu, appeared to be the most abundant.

While focusing solely on the edible parts of fish may seem more directly relevant to human consumption, analyzing non-edible parts provides valuable data for broader ecological and environmental assessments. Non-edible portions can also be used as animal powder for a variety of applications. Moreover, in some cultures, certain non-edible organs are consumed by humans, further emphasizing their importance in metal analysis.

2.4. Statistical analysis

Data analyses were performed using R software version 4.0.5. (R Core Team, 2021). The HM concentrations of fish species organs are presented as mean ± standard deviation. The graphs were generated using Excel version 2016. Furthermore, the Shapiro-Wilk test was accomplished to analyze the normality of the data distribution, while the variance equality was tested using Leven's test. To assess differences in HM concentrations among organs and species, a one-way ANOVA was employed, followed by a Tukey's test ($p<0.05$).

The principal component analysis (PCA) and clustered heatmap were performed using R packages "devtools", "ggbiplot", and "pheatmap" (Wickham et al., 2021; Kolde, 2019; Vu, 2011 respectively).

Table 1. Parameters and values used in the human health risk models.

Parameter	Unit	Children	Adults	References
MC: Metal concentration	mg kg^{-1}			
FIR: Fish ingestion rate	g $person^{-1}$ day^{-1}	16	32	MassDEP (2008)
BW: Average body weight	Kg	33	70	Alidadi et al. (2019) Zrelli et al. (2020)
EF: Exposure frequency	Days/year		365	Alidadi et al. (2019)
ED: Exposure duration	Years		70	
AT: Average exposure time	Days		BW x EF	U.S. EPA (2012)
RfD: Oral reference dose	mg kg^{-1} day^{-1}		Fe: 7E-01 Cu: 4E-02 Zn: 3E-01 As: 3E-04 Cd: 1E-03 Pb: 3.5E-03	
CSF: Cancer slop factor	mg kg^{-1} day^{-1}		As: 1.5E-00 Pb: 8.5E-03	U.S. EPA (2012)

Results showed that Zn concentrations in all samples surpassed the permitted limits set by WHO (1989) and FAO (1983), except for the muscles of *D. labrax* and *S. salpa* which displayed the lowest Zn concentrations (24.75 and 18.62 mg kg^{-1} ww, respectively).

Fish absorb zinc primarily through the ingestion of contaminated water or food. Once absorbed, zinc spreads throughout their bodies, accumulating in tissues at varying rates.

While muscles generally accumulate lower levels of zinc compared to organs like the liver or kidneys due to selective uptake and retention mechanisms, factors like selective accumulation, detoxification mechanisms, and metabolic differences influence zinc concentrations in fish muscles. *D. labrax* and *S. salpa* may exhibit lower muscle zinc levels due to these factors, reflecting the intricate interplay of exposure, uptake, distribution, and elimination processes in fish. For Cu, it was found that the recorded concentrations in all fish species organs did not exceed WHO (1989) and FAO (1983) permissible levels except the liver of *L. aurata* which exhibited the highest recorded level (47.88 mg kg^{-1}ww). The lowest Fe concentrations, which were below the WHO (1989) permissible level, were detected in the muscle tissues of the different analyzed fish species. In contrast, the tissue mixtures displayed higher Fe concentrations, surpassing their corresponding limitation value of 100 mg kg^{-1} (WHO, 1989).

This result is attributed to the highest Fe concentrations mainly recorded in the liver, gills, and kidney. For the non-essential metals, the highest average levels of As, Pb, and Cd were found in the gills of *L. aurata* (1.15 ± 0.17 mg kg^{-1} ww), the liver of *L. aurata* (2.04 ± 0.27 mg kg^{-1} ww), and the liver of *S. aurata* (1.52 ± 0.25 mg kg^{-1}ww), respectively.

Table 2. Heavy metal(oid) concentrations expressed as mean (mg kg^{-1}ww) ± standard deviation (sd) in the organs of fish species.

Species	Organs	As	Pb	Cd	Fe	Cu	Zn
Sparus aurata	Liver	0.86[a] ± 0.07	1.43[a] ± 0.22	1.52[a] ± 0.25	275.46[a] ± 23.95	26.70[a] ± 2.56	113.87[a] ± 16.33
	Gonads	0.65 ± 0.06	0.86[b] ± 0.09	0.81[b] ± 0.08	49.72[b] ± 6.44	9.89[b] ± 0.86	107.85[a] ± 19.48
	Gills	0.82[ab] ± 0.12	1.42[a] ± 0.18	0.64[b] ± 0.07	203.88[c] ± 19.24	10.21[b] ± 1.25	147.76[b] ± 22.59
	Muscle	0.44[c] ± 0.07	0.43[c] ± 0.08	0.45[c] ± 0.06	9.29[d] ± 1.02	7.89[c] ± 0.64	67.41[c] ± 9.77
	Kidney	0.81 ± 0.23	0.74[b] ± 0.08	0.39[c] ± 0.05	378.63[e] ± 39.78	9.89[bc] ± 0.98	100.03[a] ± 11.35
	Brain	0.73[ab] ± 0.13	0.98[b] ± 0.09	0.58[bc] ± 0.06	273.95[a] ± 25.66	6.14[d] ± 0.62	116.08[b] ± 14.36
Dicentrarchus labrax	Liver	0.78[a] ± 0.12	1.25[a] ± 0.21	0.25[a] ± 0.21	243.18[a] ± 27.34	12.56[a] ± 2.04	114.06[a] ± 13.55
	Gonads	0.72 ± 0.08	0.92[b] ± 0.09	0.82[bc] ± 0.15	43.49[b] ± 4.58	8.42[b] ± 0.87	114.39[a] ± 11.32
	Gills	1.08[a] ± 0.16	1.02[ab] ± 0.15	1.01[b] ± 0.21	165.01[c] ± 17.68	3.55[c] ± 0.46	85.05[b] ± 6.78
	Muscle	0.42[b] ± 0.09	0.32[c] ± 0.05	0.25[a] ± 0.09	20.25[d] ± 2.14	2.67[cd] ± 0.32	24.75[c] ± 2.15
	Kidney	0.75 ± 0.15	0.41[c] ± 0.06	0.63[c] ± 0.08	133.11[c] ± 13.74	2.27[d] ± 0.28	93.76[b] ± 7.87
	Brain	0.83[a] ± 0.14	0.66[d] ± 0.07	0.08[d] ± 0.03	80.76[e] ± 6.85	6.63[b] ± 0.62	88.72[b] ± 6.85
Liza aurata	Liver	0.87[a] ± 0.16	2.04[a] ± 0.27	1.44[a] ± 0.27	352.01[a] ± 28.91	47.88[a] ± 4.66	127.29[a] ± 13.88
	Gonads	0.94 ± 0.14	0.32[b] ± 0.05	0.71[b] ± 0.06	216.05[b] ± 17.14	7.18[b] ± 0.88	149.04[a] ± 17.54
	Gills	1.15[a] ± 0.17	1.94[a] ± 0.31	0.88[b] ± 0.07	304.05[a] ± 26.43	8.92[b] ± 0.76	117.44[a] ± 19.95
	Muscle	0.57 ± 0.06	1.15[c] ± 0.13	0.59[bc] ± 0.09	96.11[c] ± 7.53	9.44[b] ± 0.85	70.98[b] ± 7.86
	Kidney	0.80 ± 0.13	1.76[a] ± 0.22	0.82[b] ± 0.08	228.22[b] ± 23.11	15.25[c] ± 2.02	87.73 [b] ± 7.13
	Brain	0.95[a] ± 0.14	1.68[a] ± 0.19	0.43[c] ± 0.07	144.41[d] ± 12.88	22.85[d] ± 3.24	95.46 [ab] ± 6.51
Sarpa salpa	Liver	0.94[a] ± 0.08	1.33[a] ± 0.25	1.07[a] ± 0.17	266.34[a] ± 24.23	21.34[a] ± 2.85	114.10[a] ± 9.55
	Gonads	0.82 ± 0.07	0.54[b] ± 0.08	1.05[a] ± 0.12	88.47[b] ± 7.62	14.33[b] ± 1.55	85.75[b] ± 7.05
	Gills	0.86[a] ± 0.10	1.02[a] ± 0.13	1.23[a] ± 0.17	302.46[ac] ± 31.62	18.84[ab] ± 2.01	93.88[ab] ± 8.75
	Muscle	0.55 ± 0.06	0.35[c] ± 0.05	0.47b[c] ± 0.06	87.22[b] ± 9.31	7.28[c] ± 0.79	18.62[c] ± 1.56
	Kidney	0.64 ± 0.09	0.74[b] ± 0.08	0.68[b] ± 0.08	367.91[c] ± 36.84	29.78[d] ± 2.87	115.26[a] ± 11.54
	Brain	0.75[ac] ± 0.18	1.11[a] ± 0.14	0.13[c] ± 0.04	225.86[a] ± 21.68	19.49[a] ± 1.88	98.64[ab] ± 8.78
FAO (1983)			0.5	0.05		30	30
EFSA (2009)		0.1	—	—	—	—	—
WHO (1989)		0.02	2	1	100	30	100
FAO (2003)		—	0.2 - 0.4	0.05 – 0.1	—	—	—
MAWRF (2015)		—	0.3	0.5	—	—	—

Values with different letters in the same column are significantly different (P < 0.05).

Overall, the recorded concentrations of As in all fish organs were higher than the permissible level established by EFSA (2009). Similarly, the concentrations of Pb were higher than the Tunisian recommended guidelines (MAWRF, 2015) in all analyzed samples. Moreover, the Cd levels exceeded the threshold set by FAO (2003) in all organs taken separately or mixed.

Table 3. Two-way ANOVA showing variations in trace metal concentrations between organs and fish species.

	Source	df	F	p
Zn	Species	1	4.500	0.016
	Organ	1	8.631	0.000
Cu	Species	1	5.603	0.007
	Organ	1	3.689	0.014
Fe	Species	1	4.725	0.013
	Organ	1	7.905	0.000
As	Species	1	7.331	0.002
	Organ	1	17.650	0.000
Pb	Species	1	9.480	0.001
	Organ	1	6.193	0.001
Cd	Species	1	1.878	0.170
	Organ	1	4.270	0.008

Overall, the high levels of HMs in the studied fish are consistent with the relatively high contamination levels reported in water, sediments, and some other marine organisms from the Bizerte lagoon (Barhoumi et al., 2016; Hammami et al., 2016; Ben Mna et al., 2017). The herein recorded levels of Cd and Pb in all fish species were higher than the levels reported by Zrelli et al. (2021) in other fish species harvested in the north and south of Tunisia. However, higher levels of some HMs such as As, Pb, Cd, and Zn were reported by Ben Salem and Ayadi (2016) in some fish species from Sfax, located in the central eastern part of Tunisia.

By comparing the edible part (muscle tissue) and the total mixed fish tissues (all organs included), marked differences between the concentration and the distribution of toxic metals were observed (Fig. 1). According to the obtained results, the tissue mixture of *D. labrax* and *L. aurata* displayed similar bioaccumulation patterns following this order: Fe>Zn>Cu>Pb>AS>Cd, while *S. aurata* and *S. salpa* shared the following common pattern: Fe>Zn>Cu>Pb>Cd>AS. However, species-specific pattern was detected for the muscle tissue. Figure 1 showed that mixed fish tissues exhibited significantly higher HM concentrations than muscle. Moreover, *L. aurata* appeared to have the highest toxic metal concentrations, followed by *S. salpa*, and *S. aurata*; while *D. labrax,* displayed the lowest HM levels for both muscle and tissue mixtures. These differences in HM concentrations between fish species could be principally attributed to their diet. Indeed, omnivorous and bottom feeder fish such as *L. aurata* are more capable of accumulating HMs than carnivore and herbivore fish (Tesser et al., 2021) Other factors, including biological and physiological parameters (such as age, gender, size, growth rate, genetic tendency, and analyzed tissues), could also contribute to the metal accumulation in fish (Ali et al., 2019).

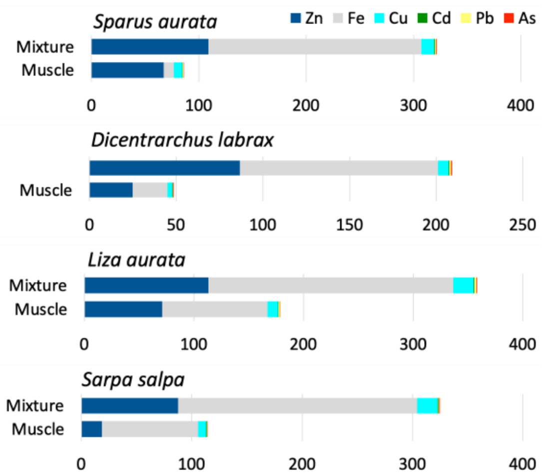

Figure 1. Concentration of heavy metal(oids) (Cd, Pb, As, Fe, Cu, and Zn) in the muscle tissue and the total mixed fish tissues of *Liza aurata*, *Dicentrarchus labrax, Sparus aurata,* and *Sarpa salpa.*

The toxicokinetic of MHs in fish has also a significant influence on the residual level of different contaminants (Kumar et al. (2024).

3.2. Explorative data analysis

The first two principal components (PC) explained 90.8% of the total variance of the HM level data set of muscle and organ mixtures of the four fish species (Figure 2, Table 4). PC1 explained 82.4% of the total variance with the positive contribution of all HMs, whereas PC2 accounted for 8.4% of the total variation and showed As, Zn, and Pb contributing positively to this axis while Fe, Cd, and Cu contributing negatively. Although some overlap ellipses occur, a clear tendency toward segregation of fish species HM bioaccumulation by PCA is observed. Along the PC2 axis, the *D. labrax* scores were noticeably skewed toward positive values, while the *S. salpa* scores were deviated toward negative ones. They performed a perfect separation between each other and the other two fish scores. However, we observed an overlap between *S. aurata* and *L. aurata* ellipses that showed near scores between *L. aurata* muscle and the *S. aurata* mixture (Figure 3). Although the results obtained for *D. labrax* and S. salpa seem to point towards a separation based on the feeding preferences of carnivorous and herbivorous species, the overlap between *S. aurata* and *L. aurata* may indicate a lower probability of separation between carnivorous and omnivorous species.

The Hierarchical Clustering on Principal Components (HCPC) and the heatmap of the HM accumulation were also plotted (Figures 3 and 4, respectively). Both figures clearly showed three distinct clusters. Cluster 1 refers to the fish organ mixture, except those of *D. labrax*. Cluster 2 groups the *L. aurata* muscle and *D. labrax* organ mixture, while cluster 3 includes fish muscles except those of *L. aurata*. These findings confirmed the ability of the fish organs to differentially accumulate HMs. According to the literature, the fish muscle tends to accumulate less metal concentration than other tissues such as the liver, gills, and kidney, which are metabolically active organs (Ben Salem and Ayadi, 2016; Mensoor et Said, 2018).

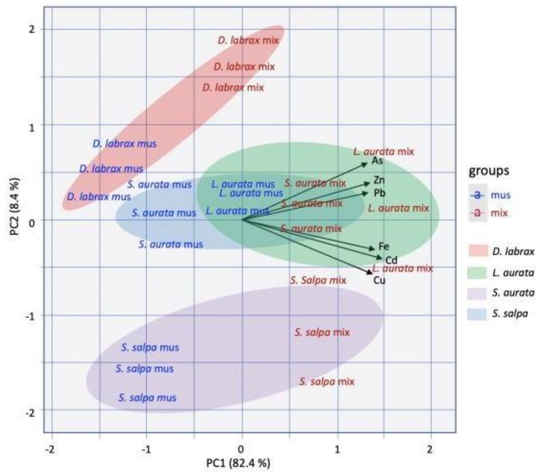

Figure 2. The Principal Component Analysis (PCA) biplot of fish metal bioaccumulation data, displaying the loading of each heavy metal(oid) and the scores of fish muscle (Mus) or mixture (Mix). For each fish species, 90 % bivariate ellipses of the scores are provided. The length of the arrows approximates heavy metal(oid) variance, while the angles between them approximate their correlations. Fish organs close together correspond to observations that have similar scores.

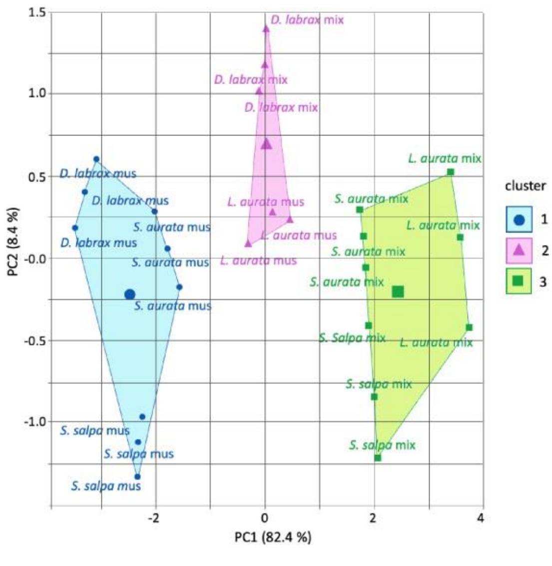

Figure 3. Hierarchical Clustering on Principal Components (HCPC) of heavy metals and metalloid in the muscle and mixed organ of four fish species: *Liza aurata*, *Dicentrarchus labrax, Sparus aurata,* and *Sarpa salpa.*

3.3. Health risk assessment

Estimated Daily Intake (EDI) values computed in the muscle and organ mixture of the targeted fish species are grouped in

Table 4. For the adult population, EDI values are significantly below their respective metal Acceptable Daily Intake (ADI) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1980,2003,2005), except for As which exhibited EDI values higher than the limit level set by JEFCA for all fish mixtures.

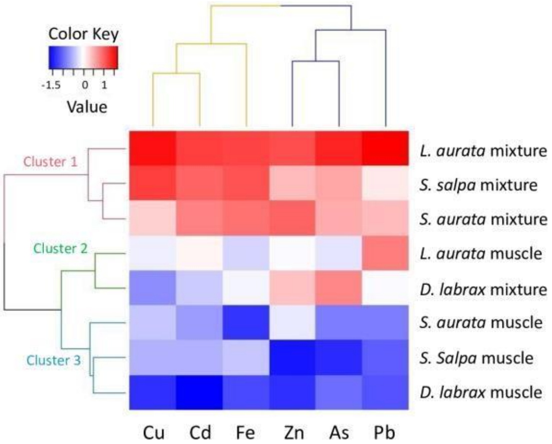

Figure 4. Hierarchal clusters of heavy metals and metalloid accumulation in the muscle and organ mixture of four fish species: *Dicentrarchus labrax*, *Liza aurata*, *Sparus aurata* and *Sparpa salpa*. The heatmap was performed with "pheatmap" R package.

As for the child population, the recorded EDI values are higher than the ADI level in all samples for As. High EDI values were also reported for Cd in all samples except the *S. aurata* and *D. labrax* muscles. Target Hazard Quotient (THQ) values recorded for the metals were below the USEPA permissible limit of 1 for both adult and children's populations.

However, exceptions were noticed for Fe in the organ mixture of *S. aurata* and *S. salpa* which displayed values higher than 1 for children (Table 5).

The cumulative intake of HMs in the form of Hazard indices (HI) was also below the USEPA permissible limit for the adult population; however, for children, the HI is higher than 1 for all fish species mixtures (Table 6). The Pb TCR values ranged from 6.25E-05 to 2.90E-04 for adults and from 3.66E-07 to 1.35E-03 for children and exceeded the USEPA level of 1.0E-04.

The Pb TCR values of mixed organs pose a considerable expected carcinogenic health risk to both child and adult populations. Hence, biological monitoring should be implemented for children using a urine indicator (Nilkarnjanakul et al., 2022). It will therefore be required to implement an alarm system to warn about possible As and Pb toxicity for people who may be vulnerable to the direct negative influence of Pb on their general health.

The Target Cancer Risk (TCR) values for As in both muscle and mixture of the analyzed fish ranged from 3.66E-07 to 9.88E-07 for adults and from 7.97E-07 to 4.61E-06 for children, respectively (Figure 5). Since these TCR values are lower than the USEPA set value of 1.0E−05, we can report that the cancer risk of As appears to be negligible for children and adults. As for Pb data, the highest TCR was recorded for the mixture of *L. aurata* (1.35E-03) and the lowest one was recorded for the muscle of *S. salpa* (3.66E-07).

4. Conclusion

The Bizerte Lagoon is currently facing environmental degradation, mostly as a result of the sewage from the surrounding towns and the nearby industrial factories. In this study, the health risk assessments of HMs in four commonly consumed fish species gathered in the lagoon were determined. The findings indicated that all fish organs had significant amounts of Pb and As, as well as high levels of Cd and Zn in some organs. Based on the risk assessment of non-carcinogenic adverse effects in the analyzed organ mixture for children, HMs had hazard index values that were greater than 1.

Table 4. Estimated daily intake of heavy metals (Zn, Fe, Cu, Cd, Pb and As expressed in mg/ kg /day) in four fish species from Bizerte lagoon, consumed by children and adults. ADI: Acceptable Daily Intake. EDI values higher than the respective ADE are reported in bold. Mus: muscle, Mix: Mixture.

	Fish species	Organ	EDI Zn	EDI Fe	EDI Cu	EDI Cd	EDI Pb	EDI As
Children	*S. aurata*	Mus	1,4E-01	2,0E-02	1,7E-02	9,6E-04	9,2E-04	**9,4E-04**
		Mix	2,3E-01	4,2E-01	2,5E-02	**1,6E-03**	2,1E-03	**1,5E-03**
	D. labrax	Mus	5,3E-02	4,3E-02	5,7E-03	5,3E-04	6,8E-04	**9,0E-04**
		Mix	1,9E-01	2,4E-01	1,3E-02	**1,1E-03**	1,6E-03	**1,6E-03**
	L. aurata	Mus	1,5E-01	2,1E-01	2,0E-02	**1,3E-03**	2,5E-03	**1,2E-03**
		Mix	2,4E-01	4,8E-01	4,0E-02	**1,7E-03**	3,2E-03	**1,9E-03**
	S. salpa	Mus	4,0E-02	1,9E-01	1,6E-02	**1,0E-03**	7,5E-04	**7,0E-04**
		Mix	1,9E-01	4,6E-01	3,6E-02	**1,7E-03**	1,8E-03	**1,5E-03**
	Total	Mus	**3,9E-01**	4,5E-01	5,8E-02	**3,8E-03**	**4,8E-03**	**3,8E-03**
		Mix	**8,5E-01**	**1,6E+00**	1,1E-01	**6,0E-03**	**8,7E-03**	**6,6E-03**
Adults	*S. aurata*	Mus	3,1E-02	4,3E-03	3,6E-03	2,1E-04	2,0E-04	2,0E-04
		Mix	5,0E-02	9,1E-02	5,4E-03	3,3E-04	4,5E-04	**3,3E-04**
	D. labrax	Mus	1,1E-02	9,3E-03	1,2E-03	1,1E-04	1,5E-04	1,9E-04
		Mix	4,0E-02	5,2E-02	2,8E-03	2,3E-04	3,5E-04	**3,5E-04**
	L. aurata	Mus	3,3E-02	4,4E-02	4,3E-03	2,7E-04	5,3E-04	2,6E-04
		Mix	5,2E-02	1,0E-01	8,5E-03	3,7E-04	6,8E-04	**4,1E-04**
	S. salpa	Mus	8,5E-03	4,0E-02	3,3E-03	2,2E-04	1,6E-04	1,5E-04
		Mix	4,0E-02	9,9E-02	7,7E-03	3,5E-04	3,9E-04	**3,3E-04**
	Total	Mus	8,3E-02	9,7E-02	1,3E-02	8,1E-04	1,0E-03	**8,1E-04**
		Mix	1,8E-01	3,4E-01	2,4E-02	**1,3E-03**	1,9E-03	**1,4E-03**
	ADI		3,0E-01	5,0E-01	5,0E-01	1,0E-03	3,6E-03	3,1E-04
	References		JECFA (2003)	JECFA (1980)	JECFA (2003)	JECFA (2005)	JECFA (2005)	JECFA (2005)

Table 5. Estimated Target Hazard Quotient (THQ) for heavy metal(loid)s from fish consumption harvested in the lagoon of Bizerte. Values of THQ > 1 are reported in bold.

	Fish species	Organ	THQ					
			Zn	Fe	Cu	Cd	Pb	As
Children	*S. aurata*	Mus	4,8E-01	6,6E-02	5,6E-02	3,2E-03	3,1E-03	3,1E-03
		Mix	7,7E-01	1,4E+00	8,4E-02	5,2E-03	7,0E-03	5,1E-03
	D. labrax	Mus	1,8E-01	1,4E-01	1,9E-02	1,8E-03	2,3E-03	3,0E-03
		Mix	6,2E-01	8,1E-01	4,3E-02	3,6E-03	5,4E-03	5,4E-03
	L. aurata	Mus	5,1E-01	6,8E-01	6,7E-02	4,2E-03	8,2E-03	4,1E-03
		Mix	8,0E-01	**1,6E+00**	1,3E-01	5,8E-03	1,1E-02	6,3E-03
	S. salpa	Mus	1,3E-01	6,2E-01	5,2E-02	3,3E-03	2,5E-03	2,4E-03
		Mix	6,2E-01	**1,5E+00**	1,2E-01	5,5E-03	6,0E-03	5,1E-03
Adults	*S. aurata*	Mus	1,0E-01	1,4E-02	1,2E-02	6,9E-04	6,6E-04	6,7E-04
		Mix	1,7E-01	3,0E-01	1,8E-02	1,1E-03	1,5E-03	1,1E-03
	D. labrax	Mus	3,8E-02	3,1E-02	4,1E-03	3,8E-04	4,9E-04	6,4E-04
		Mix	1,3E-01	1,7E-01	9,2E-03	7,7E-04	1,2E-03	1,2E-03
	L. aurata	Mus	1,1E-01	1,5E-01	1,4E-02	9,0E-04	1,8E-03	8,7E-04
		Mix	1,7E-01	3,4E-01	2,8E-02	1,2E-03	2,3E-03	1,4E-03
	S. salpa	Mus	2,8E-02	1,3E-01	1,1E-02	7,2E-04	5,3E-04	5,0E-04
		Mix	1,3E-01	3,3E-01	2,6E-02	1,2E-03	1,3E-03	1,1E-03

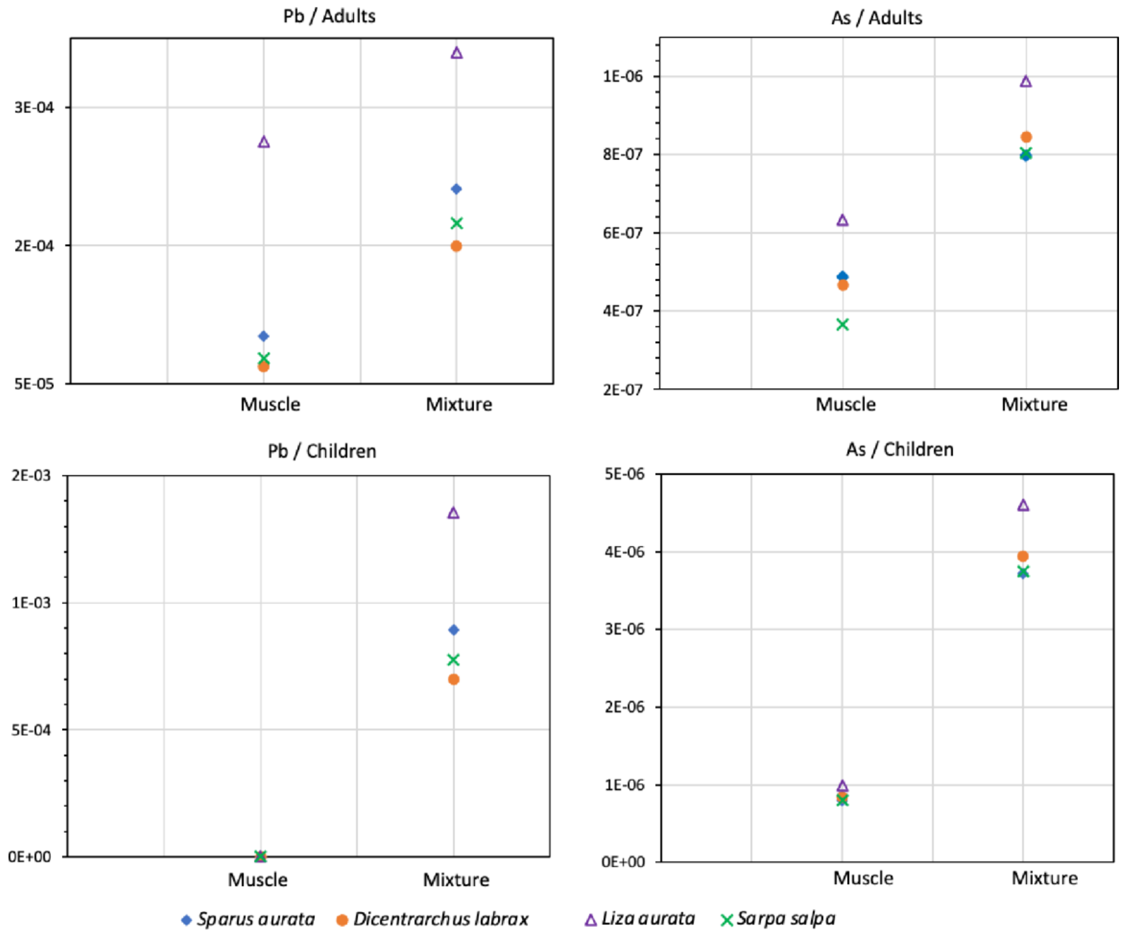

Figure 6. Target cancer risk (TCR) of fish heavy metal(oid) contamination assessed for adult and children's populations.

Table 6. Estimated Hazard Index (HI) for heavy metal(loid)s from fish consumption harvested in the lagoon of Bizerte. Values of HI>1 are reported in bold.

	Fish species	Organ	HI
Children	*S. aurata*	Mus	0.61
		Mix	**2.30**
	D. labrax	Mus	0.35
		Mix	**1.50**
	L. aurata	Mus	**1.30**
		Mix	**2.60**
	S. salpa	Mus	0.81
		Mix	**2.30**
Adults	*S. aurata*	Mus	0.13
		Mix	0.49
	D. labrax	Mus	0.07
		Mix	0.32
	L. aurata	Mus	0.27
		Mix	0.55
	S. salpa	Mus	0.17
		Mix	0.49

Our findings revealed that the carcinogenicity of Pb was found to be higher than the allowed level of 10^{-4}. HM contamination in the investigated fish species is a severe problem of concern in the Bizerte lagoon, especially for children. As a result, immediate action is required to decrease human exposure to hazardous HMs through periodic monitoring and evaluation of metal contamination sources in the lagoon. The development and execution of measures to reduce sources of metal contamination impact in the lagoon is a long-term approach. As a short-term solution, public awareness programs emphasizing that both children and adults should only consume the flesh of fish, not the other organs, and no more than once a week to prevent serious health hazards are urgently needed.

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