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Insights into Histopathological and Ecotoxicological Implications of Heavy Metal Contamination in *Siganus rivulatus* from the Red Sea, Egypt

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ABSTRACT

The contamination of heavy metals (HMLs) causes serious harm to aquatic species and can affect human health when consumed. This study determined a comprehensive evaluation of metal contamination in *S. rivulatus*, focusing on bioaccumulation behaviour, inter-metal relationships, and associated human health risks across different age groups. The Pearson correlation matrix revealed significant positive correlations among HMLs in fish organs, notably between Cr–Cd (r = 0.87) and Cr–Ba (r = 0.76). Cluster analysis identified three distinct metal groupings based on levels of HMLs in fish: (1) Fe and Al, (2) essential elements (B, Cu, Zn), and (3) non-essential or toxic elements (e.g., Cr, Pb, Ba, As, Cd, Ni, Mn), with variable distributions indicative of cumulative environmental exposure. Tissue-specific clustering showed that liver and gills recorded the highest metal burdens, while muscle and intestine exhibited lower bioaccumulation levels. Bioaccumulation factor (Bio-AF) and biota-sediment factor (Bio-SF) analyses revealed that muscle tissues generally acted as de-concentrators, while gills and liver showed moderate to high bioaccumulation, particularly for Cu, Zn, B, and Ni. Target hazard quotient (THQ-HMLs) estimated daily intake (EDI-HMLs), and target cancer risk (TCR-HMLs) were conducted for children, young and adults consumers. All THQ-HMLs values across all age groups indicated no significant non-carcinogenic risk. However, TCR values for As, Cr, and Ni indicated potential carcinogenic risks in children. Histopathological examination revealed degradation of muscle fibres, intestinal villi shortening and fusion, liver cell vacuolation and congestion, and alterations in the gill epithelium In conclusion, the study underscores tissue-specific bioaccumulation patterns and identifies certain metals due to their bioaccumulative potential and associated health risks, particularly for vulnerable populations such as children. These findings emphasize the necessity of continuous environmental monitoring and implementating effective risk management strategies to ensure the safety of fish intake.

INTRODUCTION

Fish is a vital source of protein for human health, and due to its low saturated fat, high protein, and high omega-3 fatty acid content, fish consumption has rapidly expanded

worldwide **(Younis *et al.*, 2021; Kourany *et al.*, 2024)**. It is particularly vulnerable to HML pollution because they're an essential part of the aquatic food web. A key worry that has a detrimental effect on the associated aquatic organisms, particularly fish, is the presence of HMLs in aquatic environments **(Shahjahan *et al.*, 2022)**. Their ability of fish to bioaccumulate metals within their tissues makes them effective bioindicators of environmental pollution. The bioaccumulation patterns are influenced by factors such as species, age, feeding habits, and the physicochemical properties of the surrounding water **(Elhaddad *et al.*, 2022; Hamada *et al.*, 2024)**. Both the water and the sediments of aquatic habitats may include HMLs that may affect marine organisms, especially fish **(Emon *et al.*, 2023)**. Numerous sources of pollution, such as household sewage, vehicle exhaust, wastes discharge, agricultural and fisheries wastewater, metal mining, fertilizer residues, and smelting, industrial effluent, etc., impacting the level of HMLs in the water bodies, fuel leaks and antifouling coatings **(Li *et al.*, 2023; Khedr *et al*., 2024; Islam *et al.*, 2015)**. According to bodily requirements, metals are classified into two categories; essential (zinc (Zn), cupper (Cu), iron (Fe), and selenium (Se)) or non-essential (nickel (Ni), cadmium (Cd), lead (Pb), and mercury (Hg)) (**Abbas, 2024; El-Kady *et al.*, 2025**). These pollutants pose serious risks to aquatic organisms due to their persistence in the aquatic environment, and, consequently, the health of humans via the food chain **(Salaah *et al*., 2022)**. Fish growth and reproduction are adversely affected by HMLs pollution, which lowers the fish's gonadosomatic index, fertilization, fecundity, and hatching rate **(Emon *et al.*, 2023)**.

Histopathological alterations in fish tissues serve as early warning signs of environmental stress and toxicity. Organs such as the gills, liver, kidneys, and intestines are primary targets for HMLs accumulation and subsequent damage **(Muñoz *et al.*, 2015; Ghannam *et al*., 2025)**. In fish, gills are directly in contact with the external environment, previous studies reported several structural changes in gills due to exposure to toxins **(Mahboob *et al.*, 2020; Ahmed *et al*., 2022)**. The liver is the primary organ for detoxification, often exhibits histological changes in response to metal accumulation **(Pereira *et al.*, 2017)**.

Fish bioaccumulation of HMLs not only harms fish health but also endangers human consumers. Habitual intake of contaminated seafood lead to metals bioaccumulation in human tissues, trigging several health problems, such as renal failure, neurological conditions, and carcinogenic effects **(Mitra *et al.*, 2022)**. Metals are known to pose both non- carcinogenic and carcinogenic risks to human health **(Radwan *et al.*, 2022; Irshad *et al.*, 2024)**. These risks arise from their persistent and non-degradable nature within the body's internal organs **(Kim *et al.*, 2015)**. Therefore, assessing the levels of HMLs in edible fish’s tissues and evaluating the associated health risks are crucial for public health safety.

In the context of Red Sea's environment, *S. rivulatus* species pose significant ecological and economic importance. *S. rivulatus* is known for its benthopelagic and

herbivorous behavior, influencing both benthic and pelagic ecosystems through nutrient cycling **(Escalas *et al.*, 2022)**.

Previous studies have reported elevated levels of in Egyptian Red Sea coast **(El-Moselhy *et al.*, 2014; Younis *et al.*, 2021; Abbas *et al.*, 2024 a&b; El-Shorbagy *et al.*, 2024; Abbas & Alnasser, 2025; El-Kady *et al.*, 2025)**, highlighting the concerns about the safety of consuming fish and the overall health of marine environment. This study was conducted to investigate the bioaccumulation of HMLs in water and sediment from the Red Sea, along with four essential organs (liver, intestines, muscles, and gills) of *S. rivulatus*, assessing the associated health risks and the histopathological alterations in fish. The study also aims to perform two-way cluster analysis, exploring the potential organs-metals relationships.

MATERIALS AND METHODS

Collection of samples

Water and sediment samples (three replicates) were collected from three different areas in the Gulf of Suez during the summer of 2024. At the same time, fish samples were obtained from fishermen in Suez City. The sampled species, the sigan fish (*Siganus rivulatus*, 5 individuals), is a herbivorous member of the Siganidae family. The fish had an average total length of 20.34 ± 2.01 cm, a standard length of 18.11 ± 1.08 cm, and an average total weight of 175.77 ± 3.52 g.

The fish were immediately placed in an ice-filled container and transported to the laboratory. After dissection, tissue samples of at least 10 grams were collected. These samples were then frozen and stored in plastic bags for later analysis.

Heavy metal levels (HMLs) in water and sediment were analyzed solely for the calculation of Bioaccumulation Factor (Bio-AF) and Biota-Sediment Accumulation Factor (Bio-SF). Therefore, detailed results of HML concentrations in water and sediment are not included in the main text, as their purpose was limited to supporting the evaluation of element accumulation in fish tissues through these derived indices.

Trace element measurements:

The barium (Ba), arsenic (As), nickel (Ni), copper (Cu), (Zn), iron (Fe), lead (Pb), manganese (Mn), chromium (Cr), aluminium (Al), cadmium (Cd), and boron (B) levels were recorded in samples (sediment, water, and fish organs, n=5). 500mL of water samples were filtered through a filter (0.45µm), digested with HNO_3 (65%) and HCl (37%), according to APHA guidelines **(APHA, 2023)**. 100g of sediment samples were homogenized, dried at 105°C, and sieved via a mesh (63µm). After that, 1.0g of the sieved samples added to a Teflon vessel with 9mL of HNO_3 and 3mL of HCl **(USEPA, 2023)**. About 0.5g of fresh muscle was placed in digestion tubes with 1mL of H_2O_2 (30%) and 5mL of ultrapure HNO_3 (65%). On a hot plate, the mixture was heated until it was

completely digested. Following digestion, the samples were diluted with 1% HNO_3 to a final volume of 25mL **(AOAC, 2012)**.

The HMLs levels in samples (sediment, water, and fish tissues) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, USA). A quality control sample, an external reference, and standard reference materials were used to guarantee the precision and accuracy of the findings. The recovery rates for standard reference metals ranged between 90 and 110%. Water levels were reported in µg/L, whilst fish muscle levels were represented on a wet weight basis (ww-b) and sediment on a dry weight basis (dw-b), both quantified in mg/Kg. The limits of detection (LOD) values of HMLs were established at 0.0066 mg/kg for Ni, 0.0029 mg/kg for Zn, 0.025 mg/kg for Cu, 0.01 mg/kg for B, 0.0029 mg/kg for Mn, 0.0029 mg/kg for Fe, while the limits of quantification (LOQ) values were at 0.025 mg/kg for Ni, 0.0096 mg/kg for Zn, 0.084 mg/kg for Cu, 0.037 mg/kg for B, 0.0095 mg/kg for Mn, 0.0095 mg/kg for Fe.

Bioconcentration factor calculation

According **to Adolfsson-Erici *et al.* (2012)**, the bioaccumulation factor (Bio-AF) was computed using equation (1):

$$\textbf{Bio-AF = C-fish / C-water.} \quad \textbf{Eq. (1)}$$

Where, C-fish is the HMs in fish organs (µg/kg) and C-water is the HMs in water (µg/L). However, the bio-sedimentation factor (Bio-SF) was computed using equation (2) according to **Usero *et al.* (2005)**:

$$\textbf{Bio-SF = C-fish/C-sediment} \quad \textbf{Eq. (2)}$$

Where, C-fish, the metals in fish organs (µg/g), and C-sediment, the metals in sediments (µg/g).

Health risk assessment

The US-EPA's technique was used to assess the possible health risks associated with consuming fish muscle tissue that contains HMLs **(USEPA, 2018)**. This evaluation involved calculating the EDI-HMLs values as well as cancirogenic and non- cancirogenic risk indexes based on the trace elements' levels in the muscle tissues. To evaluate exposure levels, the EDI-HMLs, which is the average daily intake of a specific HMLs throughout a lifetime as a result of consuming fish muscle, was calculated. The EDI-HMLs was computed using equation (3) according to **Mwakalapa *et al.* (2019)**:

$$\textbf{EDI-HMLs values} = ((EP \times IR \times FM \times ER) \div (BW \times AT)) \times 10^{-3} \quad \textbf{Eq. (3)}$$

Where, EP represents the duration of the exposure period (70 years), and IR represents the fish ingestion rate 62.25 g/day **(CAMPAS, 2017; GAFRD, 2017)**. The metal level in fish muscle in µg/g ww-b is denoted by frequency of meals (FM), 365 days per year is the exposure rate by ER. BW refer to the body weights of consumers, where 70, 40, and 15 Kg were used for adult, young adults, and child, and the mean lifespan (365 days x 70 years = 25550) by AT **(USEPA, 2018)**. In this study, uniform values for EP, IR, and ER were applied across all age groups based on standardized assumptions recommended by **USEPA (2011)** for preliminary risk assessment.

To identify non-cancerogenic health concerns related to consuming metals bioacculumated in fish muscles, the target hazard quotient (THQ-HMLs) was employed. This measure evaluates the potential adverse health effects by comparing the EDI-HMLs of a HMLs to its RfD (oral reference dose). Equation (4) was used to determine the THQ-HMLs:

THQ-HMLs values = EDI-HMLs / RfD Eq. (4).

Where, EDI-HMLs represents the metal's estimated daily intake and RfD represents the oral reference dosage. The following are the RfD (mg/kg/day) values for the metals being studied: Lead 0.00357, iron 0.7, As 0.003, Ni 0.02, Mn 0.14, Cu 0.04, Zn 0.3, and Ba 0.2 **(USEPA, 2018)**.

The hazard index (HI-HMLs) value, according to **Cui *et al.* (2015)**, is an additional computational calculation (Eq., 5) that adds up the THQ-HMLs values for the metals under study to show the effect of non-cancer risk.

HI-HMLs values = ∑ THQ-HMLs Eq. (5).

Histo-pathological analysis

To investigate the histopathological structure, tissues such as gills, muscles, liver, and intestines of *S. rivulatus* were carefully excised and fixed in Bouin's solution for 48 hours. The organs were then dehydrated through a graded ethanol series with xylene and embedded in paraffin wax (melting point: 58°C). Using a rotary microtome, tissue sections were cut at a thickness of 4–5µm for histological examination. The sections were stained with hematoxylin and eosin (H&E) to reveal general tissue architecture following the method of **Humason (1979)**. Microscopic analysis was conducted using a light microscope (XSZ-N107T) at various magnifications, and representative images were captured using a digital camera (ToupCam, Ver. 3.7) for documentation and descriptive analysis.

Statistical measurements

SPSS program (Version 22) was used to perform statistical analysis. Levene's test was used to evaluate the data distribution's normality and confirm the homogeneity of variance. One-way ANOVA was used to identify statistically significant differences ($P < 0.05$) between the levels of HMLs **(Dytham, 2011)**. Additionally, the statistical tables display the results as means ± standard deviation.

RESULTS AND DISCUSSION

Trace elements levels in fish organs

The analysis of HMLs concentrations in the examined organs of fish revealed distinct patterns of accumulation (Fig. 1). Among the twelve investigated elements (As, Al, Cr, Pb, Ba, Cd, Ni, Fe, Mn, B, Zn, and Cu), the highest levels of these metals recorded for Al in the liver (232.80 ± 13.22 mg/kg), suggesting a strong bioavailability and affinity of Al. Conversely, Cd recorded the lowest concentrations in muscles, intestine, liver and gills

(0.12 ± 0.05, 0.26± 0.03, 0.45 ± 0.05, and 0.67 ± 0.05, mg/kg, respectively), indicating limited accumulation or efficient detoxification mechanisms of Cd in these tissues.

In intestinal tissues, a different trend was displayed, where Fe was the highest accumulated among all HMLs (64.68 mg/kg), highlighting the intestine's role in absorbing and regulating of essential metals. Cd demonstrated the lowest accumulation in the intestine (0.26 mg/kg), consistent with its minimal presence in other organs.

The analysis of metal bioaccumulation across different fish organs revealed two distinct patterns of distribution. For the HMLs Fe, Cr, Pb, Ba, As, Al, Mn, and B, the order of accumulation followed the trend: Liver > Gills > Intestine > Muscle. In contrast, for the elements (Zn, Cu, Ni, and Cd), the observed accumulation pattern was: gills > liver > intestine > muscle. The species, size, tissues, and habitat of the fish, as well as the degree of water pollution, all affected the metal accumulation in the tissues **(Zaghloul *et al.*, 2024)**. Furthermore, the lifetime and metabolic metabolism of the fish may have an impact **(Hernandez-Saavedra *et al.*, 2020)**.

The bioaccumulation of HMLs was especially noticeable in fish gills and liver. This distribution pattern underscores the liver's vital function in detoxification and storage of HMLs, attributable to its high metabolic activity and capacity to accumulate toxicants **(Guo *et al.*, 2019; Sabra *et al.*, 2025)**. The gills, serving as the primary interface between the fish and its aquatic environment, exhibited considerable metal concentrations, reflecting their role in direct uptake from water. In contrast, the muscle tissue showed the lowest levels, likely due to its limited involvement in metal metabolism and storage **(Tsurkan *et al.*, 2020; Salaah *et al.*, 2025)**. These results highlight the differential affinity of HMLs for specific organs, governed by both the physiological roles of the tissues and the pathways of environmental exposure.

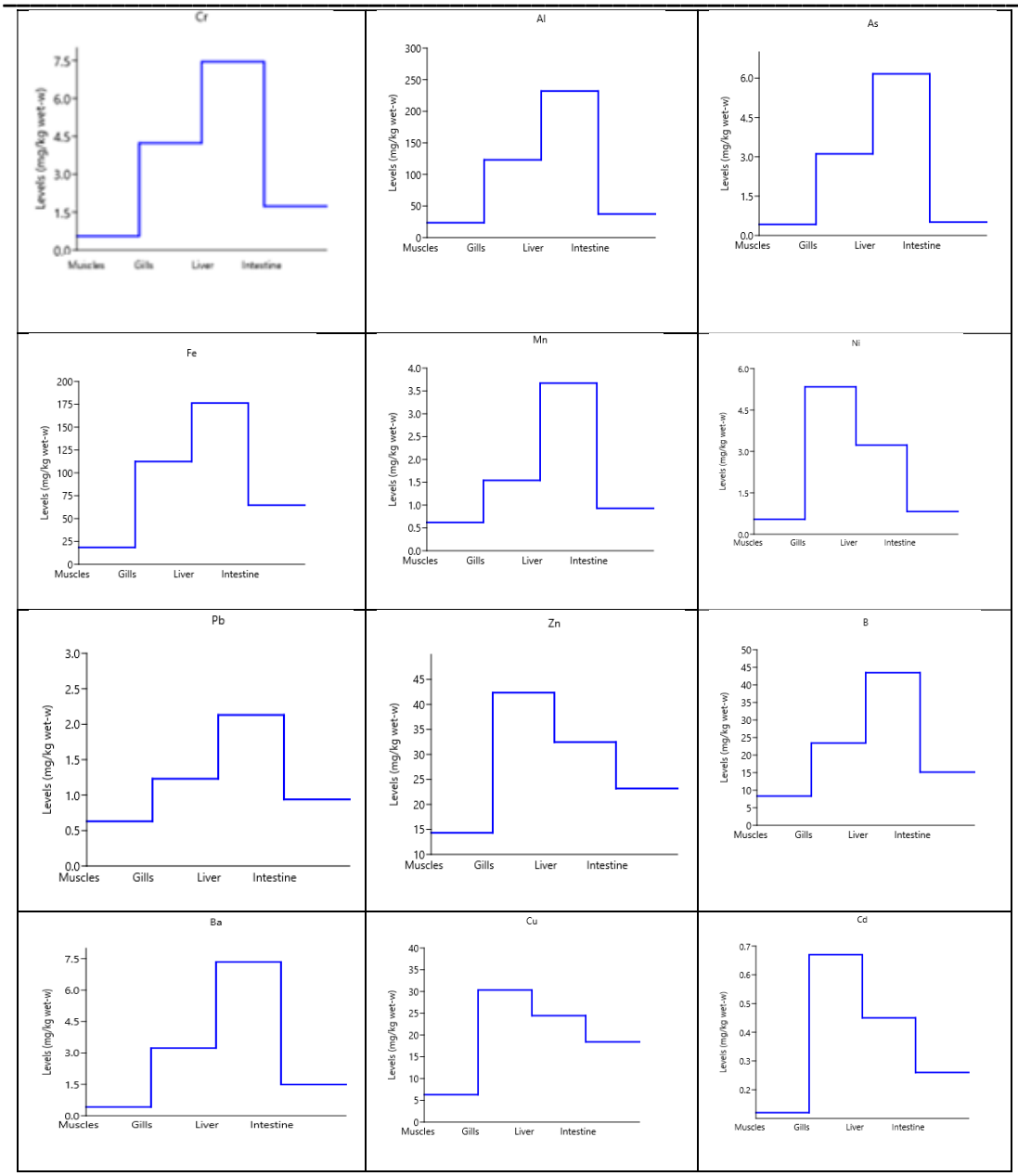


Fig. 1. The HMLs bioconcentration (mg/kg**)** in different organs of *S. rivulatus* from the Red Sea

Evaluating permissible limits and previous comparisons

The HMLs bioaccumulation in the studied *S. rivulatus*, muscles were mentioned in Table (1). The current study recorded that the concentration of Cr in fish muscles were within the permissible limit set by **FAO (1983)**, which is 2 mg/kg The Pb concentration in the studied muscle was 0.63 mg/kg, which is slightly higher than 0.5 mg/kg the limits set

by **FAO (1983)** and **FAO/WHO (1989)**, and it exceeds the **EU (2015)** limit of 0.3 mg/kg. When compared with previous studies, the current value is higher than those recorded by **Younis *et al.* (2021)** and **Hussein *et al.* (2023)**, but falls within the range documented by **Abdel-Wahab *et al.* (2017)**, **Zaghloul *et al.* (2022)** and **Abbas *et al.* (2024b)**, and is lower than the values reported by **Elaraby *et al.* (2024)**, and **El-Shorbagy *et al.* (2024)**. This suggests that while Pb levels in the present study are relatively moderate, they may still raise some concern regarding long-term exposure, especially when considering more stringent international safety standards.

The Ba concentration in fish muscles observed in the current study was 0.42 mg/kg. While comparative data from most previous studies and regulatory bodies are lacking, the measured level is significantly lower than the range reported by **Abbas *et al.* (2024b)**, which was between 1.00 and 2.54 mg/kg. This suggests that the Ba level in the current study is relatively low and may not pose a significant risk. However, due to the limited availability of guideline values and literature for Ba, further investigation is recommended to better assess potential health implications add references.

The outcomes of the current study revealed that the As concentration in fish muscles was 0.42 mg/kg, which is lower than the values reported in previous studies such as **Hussein *et al.* (2023)**, (1.03 to 1.13 mg/kg); **Abbas *et al.* (2024b),** (0.50 to 3.59 mg/kg) and **El-Shorbagy *et al.* (2024)**, (0.48 to 5.10 mg/kg). This concentration also falls within the permissible limit of 1 mg/kg **(FAO, 1983)**. These findings suggest that the fish samples analyzed in the current study are relatively safe for human consumption in terms of As content.

The current study showed that the concentration of Al in fish muscles reached 23.44 mg/kg, which is considerably higher than values reported in previous studies. For instance, **Hussein *et al.* (2023)** recorded Al levels ranging from 1.38 to 2.11 mg/kg, while **El-Shorbagy *et al.* (2024)** recorded levels between 1.93 and 4.36 mg/kg. Similarly, **Abbas *et al.* (2024b)** recorded levels between 0.80 to 4.21 mg/kg. In the current study, the Cd concentration in fish muscles was found to be 0.12 mg/kg. This value is slightly higher than the level reported by **Hussein *et al.* (2023)**, which ranged from 0.03 to 0.11 mg/kg, but it falls within the range observed by **El-Moselhy *et al.* (2014)** (0.04–0.38 mg/kg). Conversely, the results of **Abbas *et al.* (2024b)** and **El-Shorbagy *et al.* (2024)** indicated non-detectable (ND) levels of Cd. **Zaghloul *et al.* (2022)**, however, reported much higher concentrations (0.54 to 1.09 mg/kg). When compared with international standards, the Cd level in the current study exceeds the **FAO (1983)** guideline of 0.05 mg/kg and the **EU (2015)** limit of 0.25 mg/kg. However, it remains below the allowed limits set by the **FAO/WHO (1989)** at 0.5 mg/kg and **WHO (1989)** at 1 mg/kg. These findings suggest moderate Cd accumulation that warrants continued monitoring, particularly given the toxicological significance of Cd in seafood safety.

In the current study, the Ni concentration in fish muscles recorded 0.54 mg/kg. This value aligns closely with the lower range observed by **Zaghloul *et al.* (2022)** (0.332–

0.585 mg/kg). However, significantly higher Ni levels were reported by **Younis *et al.* (2021)** (3.60–19.19 mg/kg) and **Abbas *et al.* (2024b)** (1.46–4.86 mg/kg), indicating variability possibly due to differences in geographic location, pollution sources, or fish species. The values reported by **El-Shorbagy *et al.* (2024)** (1.20–1.76 mg/kg) were also higher than the present study. When compared to the international safety limits, the current Ni concentration is well below the **USEPA (2000)** guideline of 2 mg/kg and the **EU (2015)** maximum permissible limit of 80 mg/kg, suggesting that the Ni levels in the studied fish samples do not pose a toxicological risk based on these standards. In the current study, the concentration of Fe in fish muscles was 18.42 mg/kg. This value falls within the range reported by **Younis *et al.* (2021)** (17.0–39.29 mg/kg) and is comparable to that found by **Zaghloul *et al.* (2022)** (13.6–29.1 mg/kg). It also exceeds the range reported by **El-Moselhy *et al.* (2014)** (1.15–10.9 mg/kg), indicating a higher Fe accumulation in the present samples. However, it is lower than the concentrations reported by **Abbas *et al.* (2024b)** (36.86–135.96 mg/kg) and **El-Shorbagy *et al.* (2024)** (31.80–81.35 mg/kg), suggesting geographical and environmental influences on Fe levels. Importantly, the recorded concentration is below the permissible level (100 mg/kg) established by the **WHO (1989)**, indicating that the iron levels in the studied fish muscles are within safe consumption limits.

In the present study, the Mn concentration in the muscle tissue was found to be 0.62 mg/kg, which falls within the range reported by previous research. For instance, **El-Moselhy *et al.* (2014)** reported Mn levels (0.10 and 0.93 mg/kg), while **Younis *et al.* (2021)** observed slightly higher concentrations (0.65 to 1.13 mg/kg). Similarly, **Zaghloul *et al.* (2022)** recorded levels between 0.264 and 0.897 mg/kg, and **El-Shorbagy *et al.* (2024)** recorded levels between 0.50 to 1.31 mg/kg. In contrast, **Abbas *et al.* (2024b)** recorded higher concentrations, ranging from 1.27 to 2.50 mg/kg. It is important to note that the **WHO (1989)** has reported a reference value of 1 mg/kg for manganese in tissues, underscoring the need to monitor Mn levels to ensure food safety and minimize potential health risks associated with excessive exposure. Accordingly, the findings of this study fall within scientifically accepted limits, suggesting that the observed Mn concentrations are within safe levels for human consumption.

In the current study, Cu concentration in muscle tissue was measured at 6.29 mg/kg, which is notably higher than the levels measured in previous studies. For example, **El-Moselhy *et al.* (2014)** recorded much lower ranges of 0.170–0.740 mg/kg. **Younis *et al.* (2021)** documented concentrations between 1.60 and 3.94 mg/kg, while **Zaghloul *et al.* (2022)** reported levels (0.381 to 0.970 mg/kg). **El-Shorbagy *et al.* (2024)** observed a wider range between 2.11 and 10.29 mg/kg, overlapping with the present study. **Abbas *et al.* (2024b)** reported even higher concentrations (10.33 to 25.85 mg/kg). Regulatory guidelines provide reference limits for Cu in food tissues, with the **FAO (1983)** and **WHO (1989)** both recommending a maximum level of 30 mg/kg, the **FAO/WHO (1989)** suggesting 3 mg/kg, and the **USEPA (2000)** setting a limit of 20 mg/kg. The concentration observed in

this study is within these safety thresholds, though it is important to continue monitoring Cu levels due to its potential toxicity at elevated concentrations.

In the current study, the Zn concentration in muscle tissue was measured at 14.31 mg/kg, which is higher than the ranges reported in several previous studies. For instance, **El-Moselhy *et al.* (2014)** documented Zn levels (2.70 and 8.23 mg/kg). **Zaghloul *et al.* (2022)** observed levels between 5.90 and 11.9 mg/kg, and **El-Shorbagy *et al.* (2024)** recorded levels between 7.02 to 19.75 mg/kg. **Abbas *et al.* (2024b)** found even higher concentrations (11.95 to 35.18 mg/kg). Regulatory standards for Zn in food tissues vary, with **FAO (1983)** recommending a maximum level of 30 mg/kg, **FAO/WHO (1989)** setting it at 40 mg/kg, **WHO (1989)** at 100 mg/kg, and **USEPA (2000)** at 50 mg/kg. The Zn concentration found in this study falls within these safety limits, indicating that although elevated compared to some reports, it remains within acceptable thresholds for human consumption. In the current study, the concentration of B in muscle tissue was found to be 8.32 mg/kg, which is slightly higher than the range metioned by **Abbas *et al.* (2024b)**, who documented levels between 2.50 and 8.07 mg/kg.

Overall, the HMLs concentrations in the analyzed fish exhibited considerable variation when compared to previous reports, reflecting differences in environmental conditions, pollution sources, fish species, and geographic locations add references. Most metal levels remain within internationally accepted safety limits, though elevated Pb and Cd concentrations warrant continuous monitoring to reduce any possible health concerns related to prolonged exposure add references.

Table 1. HMLs (mg/kg, Mean ± SD) bioaccumulation levels in the studied *S. rivulatus* (muscles) compared to previous studies

	Cr	Pb	Ba	As	Al	Cd	Ni	Fe	Mn	Cu	Zn	B
Current study (muscles)	0.55 ±0.07	0.63 ±0.07	0.42 ±0.05	0.42 ±0.05	23.44 ±2.33	0.12 ±0.01	0.54 ±0.07	18.42 ±1.78	0.62 ±0.07	6.29 ±0.34	14.31 ±1.04	8.32±0.53
El-Moselhy *et al.* (2014)	-	0.25-0.50	-	-	-	0.04-0.38	--	1.15-10.9	0.10-0.93	0.170-0.740	2.70-8.23	--
Younis *et al.* (2021)*	7.63-23.6	0.15-0.17	-	-	-	-	3.60-19.19	17.0-39.29	0.65-1.13	1.60-3.94	--	--
Zaghloul *et al.* (2022)	-	0.26-1.04	-	-	-	0.54-1.09	0.332-0.585	13.6-29.1	0.264-0.897	0.381-0.970	5.90-11.9	--
El-Shorbagy *et al.* (2024)	1.97-5.25	2.12-6.83	-	0.48-5.10	1.93-4.36	ND	1.20-1.76	31.80-81.35	0.50-1.31	2.11-10.29	7.02-19.75	--
Abbas *et al.* (2024b)	2.29-5.43	0.59-4.81	1.00-2.54	0.50-3.59	0.80-4.21	ND	1.46-4.86	36.86-135.96	1.27-2.50	10.33-25.85	11.95-35.18	2.50-8.07
FAO (1983)	2	0.5	-	1	-	0.05	-	-	-	30	30	-
FAO/WHO (1989)	-	0.5	-	-	-	0.5	-	-	-	3	40	-
WHO (1989)	-	2	-	-	-	1	-	100	1	30	100	-
USEPA (2000)	-		-	-	-		2	-	-	20	50	-
EU (2015)	-	0.3	-	-	-	0.25	80	-	-	-	-	-

*HMLs in fish samples measured as dry weight (dw-b) was converted to wet weight (wet w-b) using a conversion coefficient of 4.8 **(Rahman *et al.*, 2012)**.

Pearson correlation coefficient

Significant correlation along with a similar level of concentration denotes the release of the metals from similar sources with identical features and mutual dependence while transportation **(Ahmed *et al.*, 2019)**.

The Pearson correlation matrix of metal concentrations in the organs of *S. rivulatus* revealed several statistically significant relationships (Fig. 2), suggesting common sources of contamination or similar bioaccumulation behaviors among certain elements. A strong positive correlation was observed between Cr-Cd (r = 0.87), and Cr-Ba (r = 0.76), indicating potential co-occurrence in the aquatic environment and possible similarity in uptake mechanisms. Cd also showed strong positive correlations with As (r = 0.68), As (r = 0.68), Mn (r = 0.76), and B (r = 0.76), implying that these elements may share pathways of accumulation or originate from similar anthropogenic sources.

Fe demonstrated high correlations with As (r = 0.88), Zn (r = 0.88), and Cu (r = 0.75), which may reflect their tendency to accumulate in metabolically active tissues, especially the liver and gills. A particularly notable correlation was found between As and Cu (r = 0.92), which could be attributed to their involvement in oxidative stress responses or shared detoxification pathways. On the other hand, some negative correlations were observed, such as between Cr-As (r = -0.68), and Al-Mn (r = -0.90), indicating divergent environmental behaviors or tissue affinities. Moreover, Pb showed strong negative correlations with several elements, including Fe (r = -0.90) and Ni (r = -0.86), suggesting differences in source or bioavailability. Interestingly, B was positively correlated with Ni (r = 0.92) and Zn (r = 0.77), yet negatively associated with Ba (r = -0.78) and Al (r = -0.68), highlighting the complex interaction of essential and non-essential metals in the studied tissues.

Overall, these correlations provide insight into the possible common sources and accumulation behaviors of various elements in the aquatic environment. The patterns observed warrant further investigation into the environmental inputs and physiological mechanisms underlying these associations in fish.

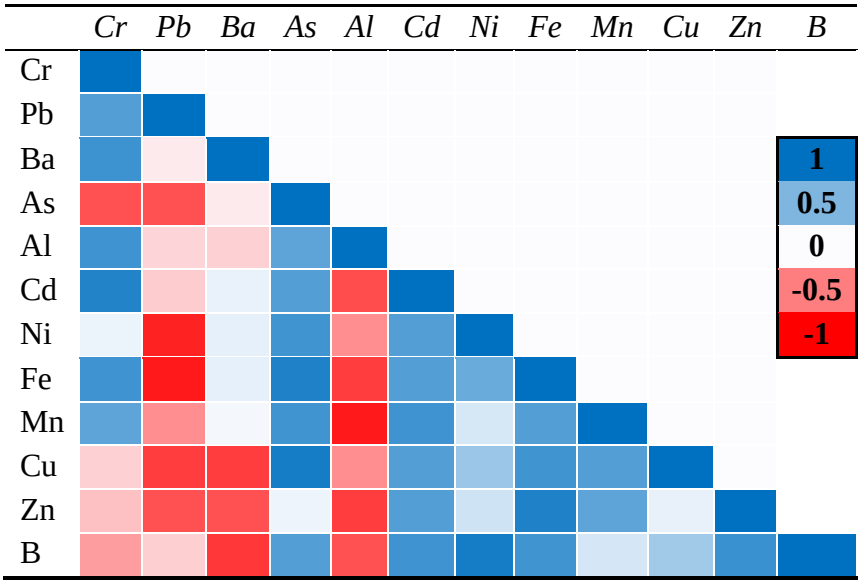


Fig. 2. Pearson correlation coefficient for the investigated metals levels in the studied fish organs

Cluster analysis (Two-way)

The results of the two-way cluster analysis, performed using the Ward linkage method and Euclidean distance, revealed a distinct pattern in the distribution of metal elements among the different fish organs of *S. rivulatus* (Fig. 3). Three clusters of metal elements were identified. The first cluster included Fe and Al, which exhibited the highest concentrations among all analyzed elements, reflecting their strong tendency to accumulate in metabolically active tissues such as the liver and gills. The second cluster comprised B, Cu, and Zn, which are essential trace elements involved in various physiological functions. Their moderate concentrations likely reflect homeostatic regulation within the fish. The third cluster contained the remaining elements (Cr, Pb, Ba, As, Cd, As, and Mn) most of which are non-essential or potentially toxic. Their variable concentrations suggest cumulative environmental exposure over time.

Regarding the tissue-specific distribution, two major clusters were observed. The first included the liver and gills, which showed the highest concentrations of metals due to their direct involvement in metal absorption, metabolism, and detoxification processes. The second cluster comprised the intestine and muscle tissues, where the lowest metal concentrations were recorded. This can be attributed to their limited role in metal accumulation, particularly in muscle tissue, which is generally not a primary site for HMLs metal storage.

These findings highlight a clear heterogeneity in the tissue-specific bioaccumulation of metals, driven by the physiological roles of different organs **(Soliman *et al.*, 2023)**. The use of cluster analysis proved valuable in elucidating the complex interactions between environmental contaminants and biological systems in aquatic organisms.

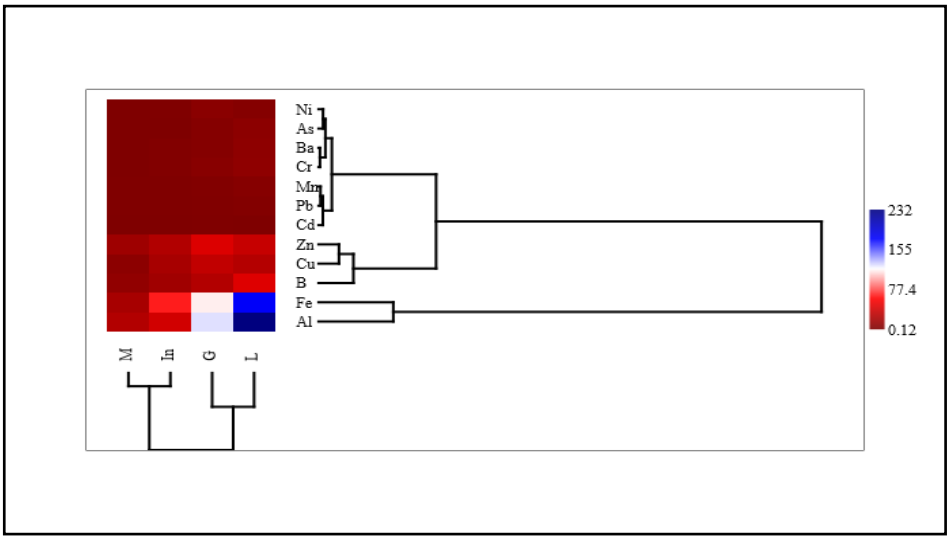


Fig. 3. Two-way cluster analysis among the metals' levels and organs in *S. rivulatus* from the red sea

Bioaccumulation factor calculation

HMLs bioaccumulation is influenced by species characteristics, tissue metabolism, exposure pathways, and habitat conditions **(Elhaddad *et al.*, 2022)**. Bio-AF values less than 1000 indicate negligible accumulation, values between 1000 and 5000 denote bio-accumulative organisms, and values exceeding 5000 suggest extreme accumulation **(Ahmed *et al.*, 2019; Ali *et al.*, 2020)**. The bioaccumulation assessment of metals (Fig. 4) across different tissues (muscle, gills, liver, and intestine) revealed distinct accumulation potentials characterized by varying Bio-AF values, reflecting differences in tissue function, metabolic activity, and exposure pathways. In muscle tissue, metals such as Cr (26), Ba (85), Mn (124), Pb (160), Ni (689), As (723), and Fe (777) exhibited low accumulation with BAFs below 1000, while Zn (1765), B (1821), Al (3113), and Cu (3699) were moderately bioaccumulative, with Cu and Al showing the highest affinity for muscle tissue. In contrast, gills demonstrated a broader accumulation range; Cr (201), Pb (313), Ba (659), and Mn (308) showed negligible accumulation, Fe (4740) exhibited moderate bioaccumulation, and several metals surpassed the extreme accumulation threshold including As (5409), Zn (5221), B (5131), Ni (6846), Al (16335), and Cu (17847). The notably high BAFs for Cu and Al in gills underscore their role as the primary interface with the aquatic environment, facilitating significant metal uptake. The liver presented a

distinctive profile with negligible accumulation for Cr (355), Pb (542), and Mn (734), moderate accumulation for Ba (1498), Ni (4141), and Zn (4001), and extreme accumulation for As (10721), Al (30810), Fe (7440), Cu (14382), and B (9513). The liver's high metabolic activity and detoxification function explain its prominent role as a HMLs reservoir, especially for highly toxic elements add reference.

Finally, the intestine showed negligible accumulation for Cr (83), Pb (240), Ba (303), As (881), and Mn (187), moderate accumulation for Ni (1052), Fe (2729), Zn (2858), and B (3316), while Al (4968) approached the extreme accumulation range and Cu (10840) clearly exceeded it. These findings highlight the intestine's importance in initial metal absorption and storage due to its exposure to dietary and waterborne contaminants and its involvement in nutrient and metal transport. This overall pattern suggests that the liver and gills exhibiting the highest accumulation capacities, and muscle showing comparatively lower affinity for metal accumulation, emphasizing tissue-specific bioaccumulation influenced by both physiological and environmental factors.

The Bio-SF values for metals across different tissues reflect varying accumulation patterns relative to sediment exposure, following **Dallinger's (1993)** classification where values > 2 indicate macro-concentrators, values between 1 and 2 represent micro-concentrators, and values < 1 denote de-concentrators. In muscle tissue, all metals displayed BSF values below 1, classifying them as de-concentrators, with Cu (0.41) and Zn (0.59) showing the highest but still limited sediment accumulation. The gills exhibited micro-concentration for Ni (2.77), Cu (1.97), Zn (1.76), and B (1.06), while Cr (0.85), As (0.99), and Ba (0.28) remained de-concentrators. Liver tissue showed micro-concentration for Cr (1.50), As (1.95), Ni (1.67), Cu (1.59), Zn (1.35), and B (1.97), with other metals as de-concentrators. In the intestine, Cu (1.20) was the sole micro-concentrator, while Zn (0.96) and B (0.69) were close to this range, and the rest of the metals remained de-concentrators with values well below 1. These results highlight tissue-specific differences in sediment metal accumulation, with gills and liver showing higher sediment-related accumulation likely due to their active roles in filtration and detoxification, whereas muscle and intestine exhibit lower sediment affinity.

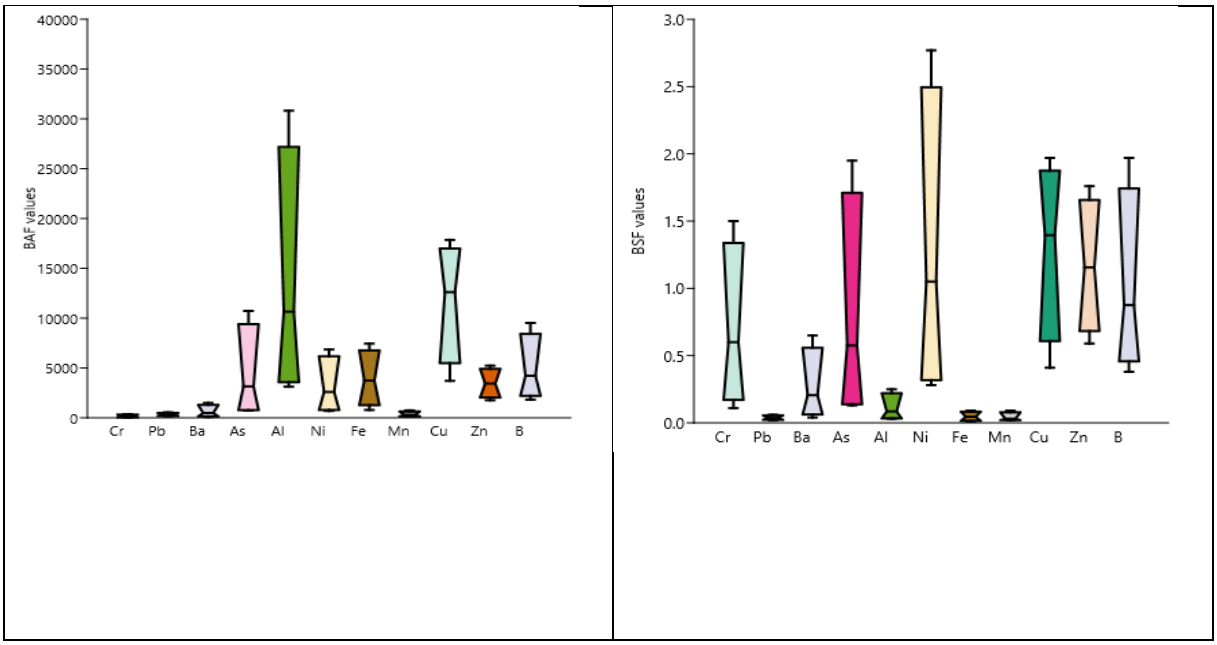


Fig. 4. Bio-AF and Bio-SF values of the investigated metals levels in the studied fish organs

Health risk assessment

The EDI-HMLs was calculated for three age groups: children, young adults, and adults (Table 2). The EDI-HMLs values ranged for children from 5.0×10^{-4} (Cd) to 1.0×10^{-1} mg kg^{-1} day^{-1} (Al). For young adults, the range was from 1.8×10^{-4} (Cd) to 3.6×10^{-2} mg kg^{-1} day^{-1} (Al), while adults showed lower values ranging between 1.1×10^{-4} (Cd) and 2.1×10^{-2} mg kg^{-1} day^{-1} (Al). Generally, children had the highest EDI values, indicating a greater exposure to these metals compared to young adults and adults. Metals such as Al, Fe, and Zn exhibited relatively high intake values across all groups, with a gradual decrease from children to adults. The values of Allowed Tolerable Daily Intake (ATDI) were (0.20, 0.03, 0.20, 2.0, 0.14, 0.08, 0.04, 70, 1.4, 50, 50, and 0.20 mg kg^{-1} day^{-1} for Cr, Pb, Ba, As, Al, Cd, Ni, Fe, Mn, Cu, Zn, and B according to **USEPA (2018)**. In general, children's consumers often had higher EDI values than other consumers. Similar findings were conducted by previous studies **(Saha *et al.*, 2016; Radwan *et al.*, 2022; Tytła & Widziewicz-Rzońca 2023; Afifi *et al.*, 2024)**. In all cases, the EDI-HMLs values for children, young adults, and adults were found to be well below their respective ATDI thresholds. These results indicate that the consumption of the studied fish species poses no significant risk of elemental toxicity based on current intake levels and established ATDI guidelines, ensuring consumer safety across all age groups.

The THQ-HMLs is used to detect the potential non-cancer risks associated with exposure to metals through fish consumption. A THQ-HMLs value > 1 indicates a possible health risk due to prolonged exposure to the studied metals. In this study, the THQ-HMLs

values for all metals in children, young adults, and adults were below the critical threshold of 1, suggesting no significant non-cancer risk from consumption (Table 2). However, the higher values of THQ for children compared to young and adults were recorded. These results are similar to those found in other studies **(Abbas *et al.*, 2022 a&b; Abdel-Aziz *et al.*, 2022; Afifi *et al.*, 2024)**. Overall, the THQ-HMLs assessment confirms that the studied muscles do not pose significant non-cancer risks through metal exposure for all age groups under the current consumption levels.

The values of hazard index (HI-HMLs) indicate the overall non-cancer risk from exposure to multiple metals through fish consumption. In this study, the HI-HMLs values (Table 2) were highest in children (4.06), followed by young individuals (1.48), and lowest in adults (0.85). Since an HI value > 1 suggests potential health risks, the results imply that children and young consumers may be at risk of adverse health effects from metal exposure through fish consumption. In contrast, the HI value for adults was consistently below 1, indicating that fish consumption poses no significant health risk for this group.

The target cancer risk (TCR-HMLs) values were evaluated following the USEPA guidelines, where TCR-HMLs values below 10^{-6} (negligible); values of 10^{-6}-10^{-4} (deemed acceptable); and values > 10^{-4} (an unacceptable carcinogenic risk) **(Ullah *et al.*, 2018)**. In this study, TCR-HMLs values for the analyzed metals in children, young adults, and adults showed a range of carcinogenic risk levels (Table 3). Cr and As exhibited the highest TCR-HMLs values across all age groups, with Cr reaching up to 1.2×10^{-3} in children and As up to 2.7×10^{-3}, both of which exceed the acceptable range, indicating a potentially unacceptable carcinogenic risk from prolonged exposure. As also showed elevated TCR-HMLs values, with a maximum of 3.9×10^{-3} in children, which is above the acceptable threshold, suggesting a carcinogenic concern. In contrast, Cd and Pb displayed TCR-HMLs values within or near the acceptable range. Lead had the lowest TCR-HMLs values, with a maximum of 2.3×10^{-5} in children, indicating negligible to acceptable carcinogenic risk levels. Cd showed intermediate values, with the highest being 1.9×10^{-4} in children, falling within the acceptable risk range. Overall, the TCR-HMLs assessment highlights a potential carcinogenic risk primarily from Cr, As, and Ni exposure, particularly in children, highlighting the necessity for continuous monitoring and risk management strategies for these metals in fish consumption.

Table 2. EDI-HMLs, THQ-HMLs, and HI-HMLs values of the investigated metals levels in the studied fish muscles

	EDI			THQ		
	Children	**Young**	**Adults**	**Children**	**Young**	**Adults**
Cr	2.3×10^{-3}	8.5×10^{-4}	4.9×10^{-4}	7.8×10^{-1}	2.8×10^{-1}	1.6×10^{-1}
Pb	2.7×10^{-3}	9.8×10^{-4}	5.6×10^{-4}	7.5×10^{-1}	2.7×10^{-1}	1.6×10^{-1}
Ba	1.8×10^{-3}	6.5×10^{-4}	3.7×10^{-4}	8.9×10^{-3}	3.2×10^{-3}	1.8×10^{-3}
As	1.8×10^{-3}	6.5×10^{-4}	3.7×10^{-4}	5.9×10^{-1}	2.2×10^{-1}	1.2×10^{-1}
Al	1.0×10^{-1}	3.6×10^{-2}	2.1×10^{-2}	1.0×10^{-1}	3.6×10^{-2}	2.1×10^{-2}
Cd	5.0×10^{-4}	1.8×10^{-4}	1.1×10^{-4}	5.0×10^{-1}	1.8×10^{-1}	1.1×10^{-1}
Ni	2.3×10^{-3}	8.4×10^{-4}	4.8×10^{-4}	1.1×10^{-1}	4.2×10^{-2}	2.4×10^{-2}
Fe	7.9×10^{-2}	2.9×10^{-2}	1.6×10^{-2}	1.1×10^{-1}	4.1×10^{-2}	2.3×10^{-2}
Mn	2.7×10^{-3}	9.7×10^{-4}	5.5×10^{-4}	1.9×10^{-2}	6.9×10^{-3}	4.0×10^{-3}
Cu	2.7×10^{-2}	9.8×10^{-3}	5.6×10^{-3}	6.7×10^{-1}	2.4×10^{-1}	1.4×10^{-1}
Zn	6.1×10^{-2}	2.2×10^{-2}	1.3×10^{-2}	2.0×10^{-1}	7.4×10^{-2}	4.2×10^{-2}
B	3.6×10^{-2}	1.3×10^{-2}	7.4×10^{-3}	2.1×10^{-1}	7.6×10^{-2}	4.4×10^{-2}
HI				4.1	1.5	0.85

Table 3. CR values of the investigated metals levels in the studied fish muscles

	CR		
	Children	**Young**	**Adults**
Cr	1.2×10^{-3}	4.3×10^{-4}	2.4×10^{-4}
Pb	2.3×10^{-5}	8.3×10^{-6}	4.8×10^{-6}
As	2.7×10^{-3}	9.7×10^{-4}	5.5×10^{-4}
Cd	1.9×10^{-4}	7.0×10^{-5}	4.0×10^{-5}
Ni	3.9×10^{-3}	1.4×10^{-3}	8.1×10^{-4}

Histopathological measurements

Histopathological alterations were found in the liver, gills, muscles, and intestinal tissue of *S. rivulatus*. Chemicals, herbicides, and dangerous HMLs are commonly found in fish muscles **(Dutta *et al.,* 2017)**. The mechanical tissue produced by the active, high-protein muscles of fish helps the animal navigate through the water **(Tasneem & Yasmeen, 2020)**. They don't engage in any metabolic activity. Muscular activity provides the body with the majority of its energy. All muscle cells need ATP, which is created during the metabolism of lipids and carbs, as energy. Fish muscles have normal myotomes, with muscle bundles that are uniformly spaced. The main location of exposure is the skin muscle, and pollutants had an immediate impact on the epidermis **(Rakhi *et al.,* 2013)**. The skin and muscle tissue come into direct contact with toxins dissolved in water as gills, which can cause severe thickening and separation of muscle bundles, haemolysis, necrosis, lesions with decreased compactness, pronounced intramuscular oedema, and degeneration in muscle bundles with inflammatory cell aggregations, among other histopathological changes **(Srivastava, 2019)**. Pollutant levels in fish muscle are influenced by many factors

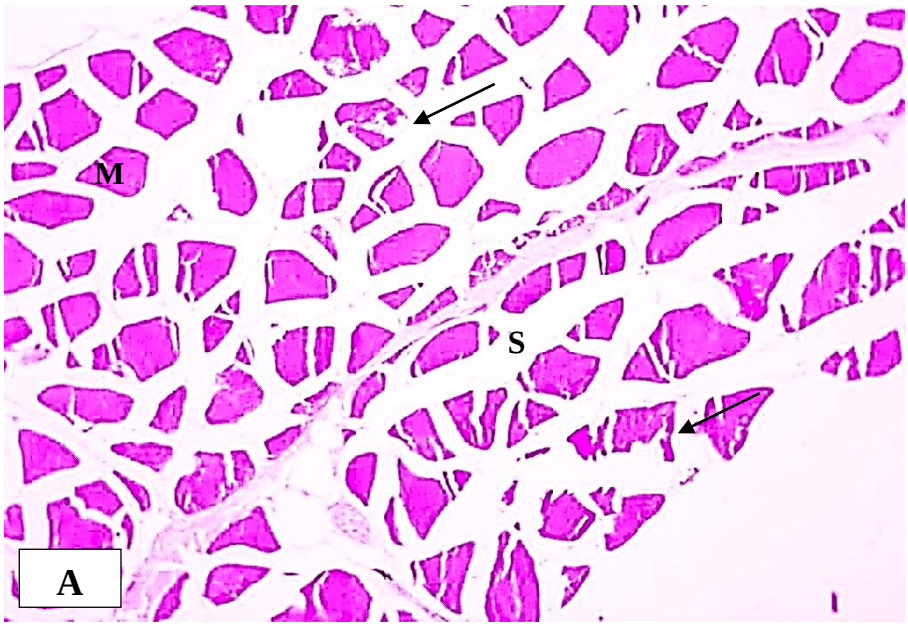
such as fish length, species, trophic level, habitat type, feeding habitat, and fish movement patterns and time spent in contaminated areas **(Bonito *et al.,* 2016)**. The histological abnormalities in the muscle of *S. rivulatus* in this study included muscle fiber degeneration and increasing inter muscular spaces (Fig. 5 A&B).

The liver of fish is a vital organ for detoxification and a reliable indicator of health due to its numerous functions, which include controlling blood flow, detoxification, biotransformation, storing glycogen, releasing glucose into the bloodstream, breaking down red blood cells, and synthesizing different components of blood plasma **(Maurya *et al.,* 2019; Sharmin *et al.,* 2021; Suchana *et al.,* 2021; salaah *et al*., 2022)**. According to **Montaser *et al.* (2010)**, **Liu *et al*. (2017)** and **Kobayashi *et al.* (2019)**, the liver is the primary organ targeted by xenobiotics and insecticides, serving as the main site of parenchymal injury. **Karami-Mohajeri and Abdollahi (2010)** also emphasized that the liver's extensive blood supply and central role in metabolism make it particularly susceptible to toxicants. Consequently, alterations in its histochemical structure can disrupt the metabolism of proteins, lipids, and carbohydrates. In the present study, significant histopathological changes were observed in the liver of *S. rivulatus*, including hepatic vacuolation, aggregation of melanomacrophages, increased infiltration of inflammatory cells, and the presence of necrotic cells (Fig. 6A & B).

The second most important portion of a fish alimentary canal is the intestine, which is responsible for absorption of food add references. It is exposed to a variety of hazardous substances either directly through the ingestion of contaminated food or indirectly through the blood and/or lymph **(Maurya *et al.,* 2019)**. In *S. rivulatus,* intestinal tissue displayed severe histopathological alternations, including abnormal appearance, intestinal villi shortening, fusion and atrophy of muscular layer (Fig. 7 A&B). Histopathology of the intestine indicate raising of columnar epithelium of villi and hyperplasia as responses representing defense mechanisms, with villi rupture, loss of structural integrity of mucosal folds, and degeneration and necrosis of the submucosa in the intestine **(Samuel *et al.,* 2019)**. The same was observed in the present study with degenerated epithelium, autolysis of mucosa, degenerated and necrotic villi.

The gills of fish have many roles in vital processes **(Dolenec & Kuzir, 2009)**, such as respiration, acid-base balance, osmoregulation, and are the principal location for oxygen intake in fish **(Maurya *et al.,* 2019; Shah & Parveen, 2020)**. The cells that comprise gill structure are similar, even though the gills of different fish species differ anatomically **(Wilson & Laurent, 2002)**. Gills are the primary target for toxicans exposure, trigging significant histological alterations **(Singh & Pandey, 2021)**. In the present study, the gills of *S. rivulatus* displayed significant histological alterations like damage of gill structure as epithelial desquamation and loss of lamellar architecture organization, abnormal arrangement of cartilage cells, hyperplasia and bending or curling of secondary gill lamellae, rupture of pillar cells and capillaries that led to ballooned like lamellae packed with erythrocytes aneurism or lamellar telangiectasis were observed epithelial alterations as loss of lamellar architecture organization, hyperplasia of secondary gill lamellae with partial oedema, and rupture of primary filaments (Fig. 8 A&B). These changes indicate toxic stress caused by TMLs accumulation. According to **Winkaler *et al.* (2001)**, fish's response to the harmful substances in water and sediment caused histological alterations. Additionally, according to **Subashkumar and Selvanayagam (2014)**, exposure to toxins

causes an increase in certain histological alterations in fish gills, such as hyperplasia, epithelial lifting, and fusion of lamellae, which leads to respiratory disruption and fish death. Various researchers, including **Handy** and **Maunder (2009)**, **Wu *et al.* (2010)**, **Gomes *et al.* (2011)** and **Rajkumar *et al.* (2016)**, have interpreted the gills tissue damages.



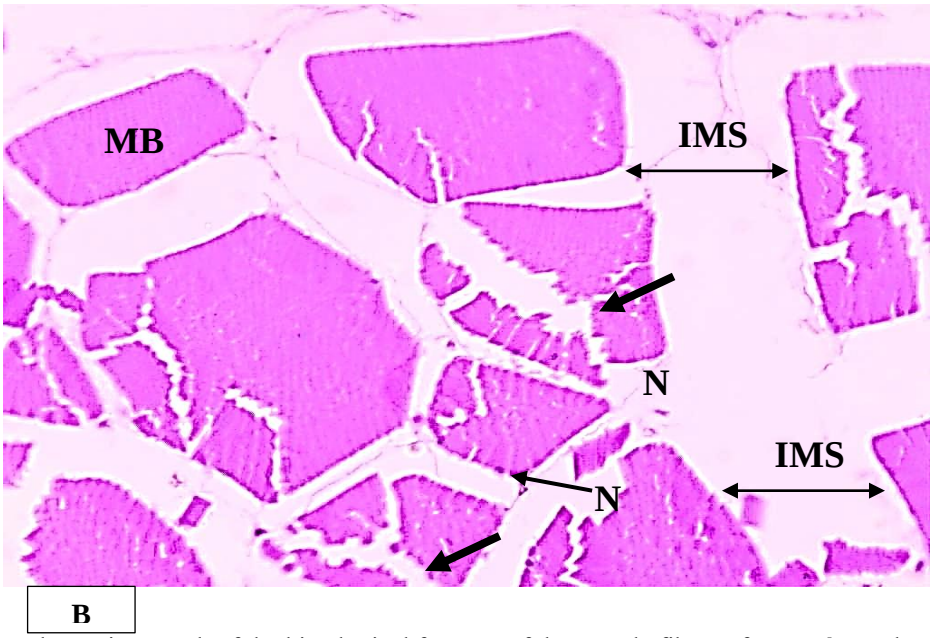


Fig. 5. A: Photomicrograph of the histological features of the muscle fibers of *S. rivulatus* showing muscles bundles (**MB**); spaces (**S**), between muscle bundles; N, nucleus of the muscle bundle; disintegrated fibers (**arrow**) (**Hx &E; 100 X**). **B:** Higher magnification Photomicrograph showing the histological features of the muscle fibers of *S. rivulatus* muscles bundles (**MB**); increasing inter muscular spaces (**IMS**) between muscle bundles; nucleus (**N**) of the muscle bundle; and disintegrated fibers (**arrow**) (**Hx &E; 400 X**)

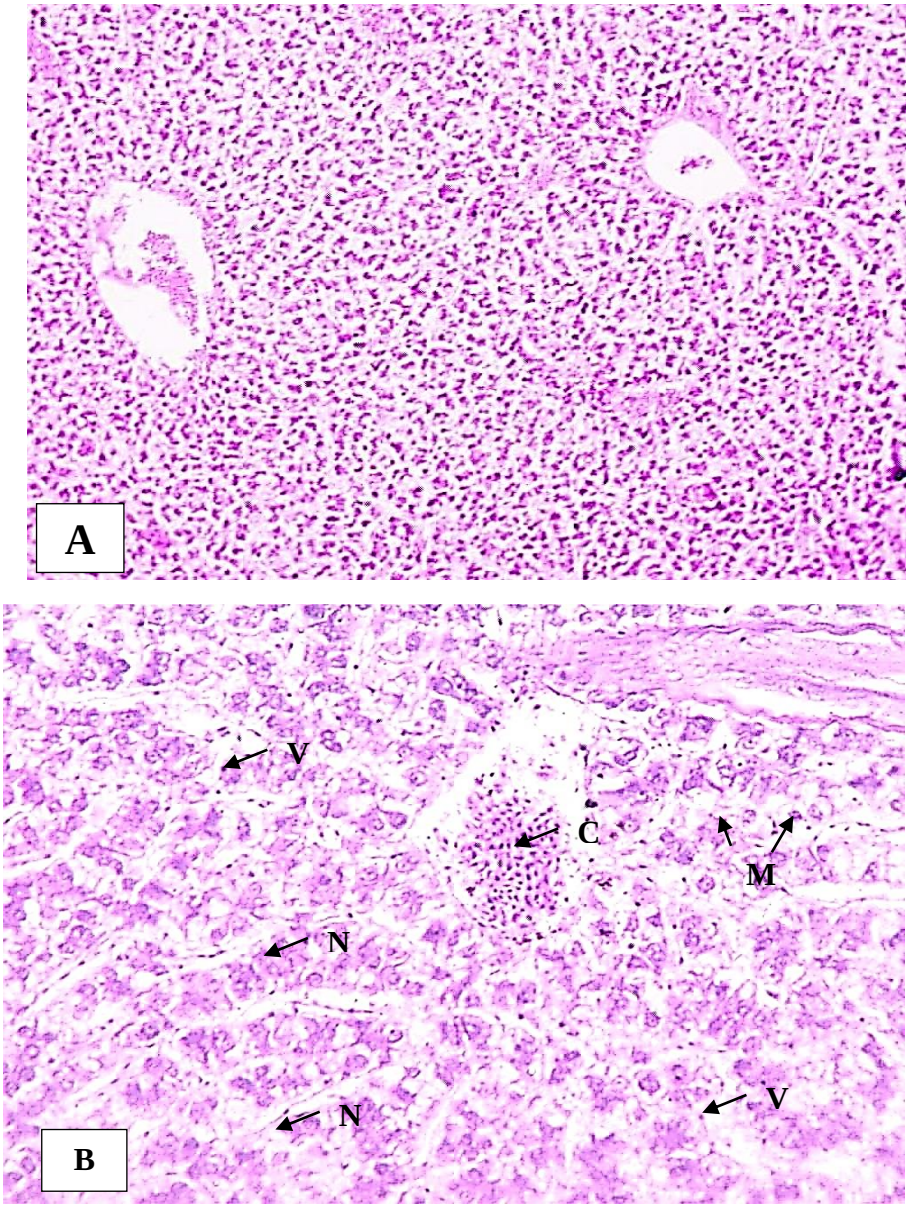


Fig. 6. A: Photomicrograph of the histological features of the liver of *S. rivulatus* showing severe degeneration of hepatocytes giving highly accumulation of large vacuoles were recorded (**Hx &E; 100 X**), **B:** Higher magnification Photomicrograph of the histological features of the liver of *S. rivulatus* showing congested blood vessels (**C**), aggregation of melanomacrophages (**M**), and severe degeneration of hepatocytes giving highly accumulation of large vacuoles (**V**) with increase of inflammatory cells, necrotic cells (**N**) were noticed (**Hx &E; 400 X**)

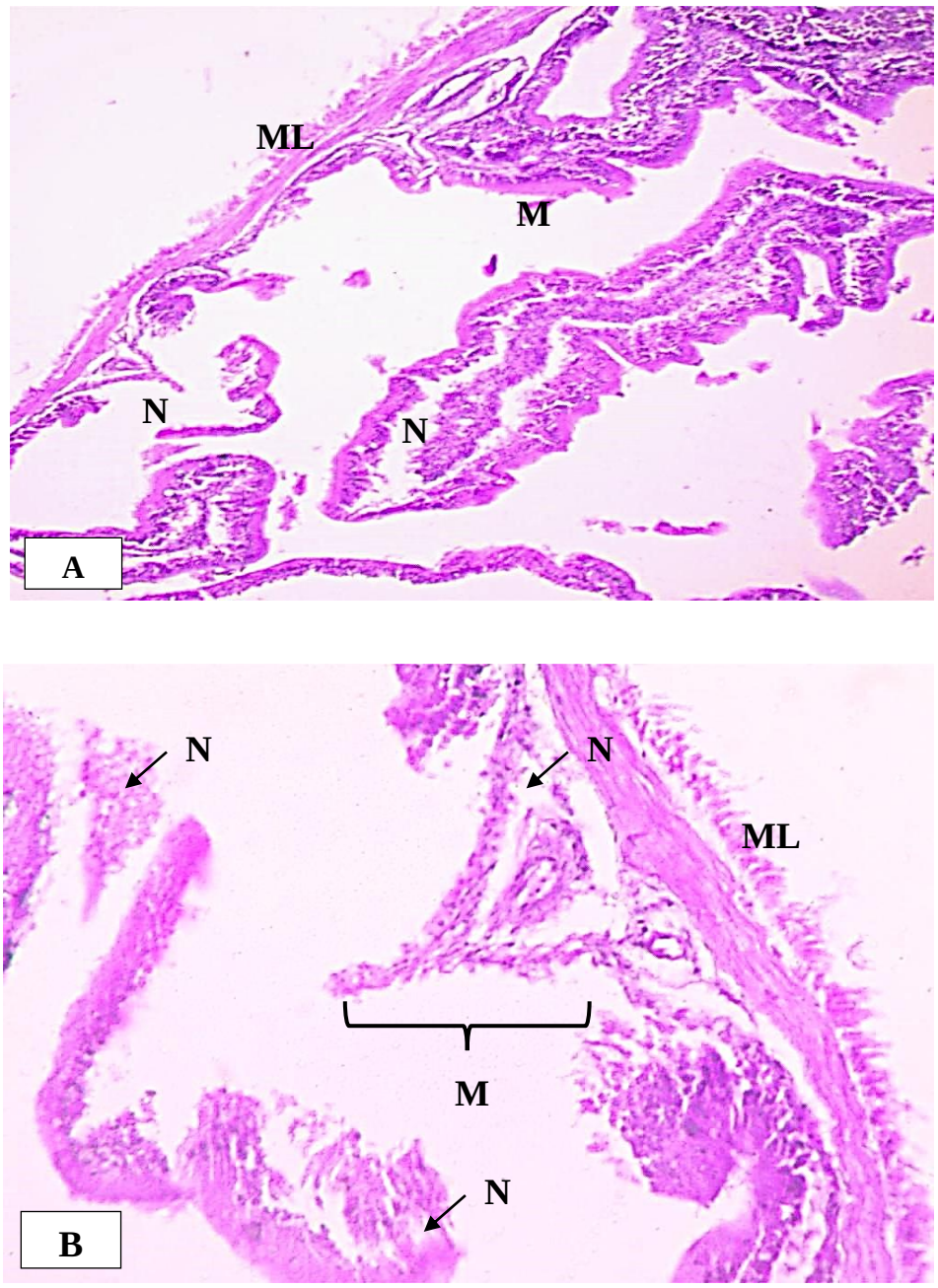


Fig. 7. A: Photomicrograph of the histological features of the intestine of *S. rivulatus* showing severe damage of Mucosa layer (**M**) complete atrophy of muscular layer (**ML**) and necrotic areas (**N**) were observed (**Hx &E; 100 X**) **B:** Higher magnification Photomicrograph of the histological features of the intestine of *S. rivulatus* showing severe damage of Mucosa layer (**M**) complete atrophy of muscular layer (**ML**) and necrotic areas (**N**) were observed (**Hx &E; 400 X**)

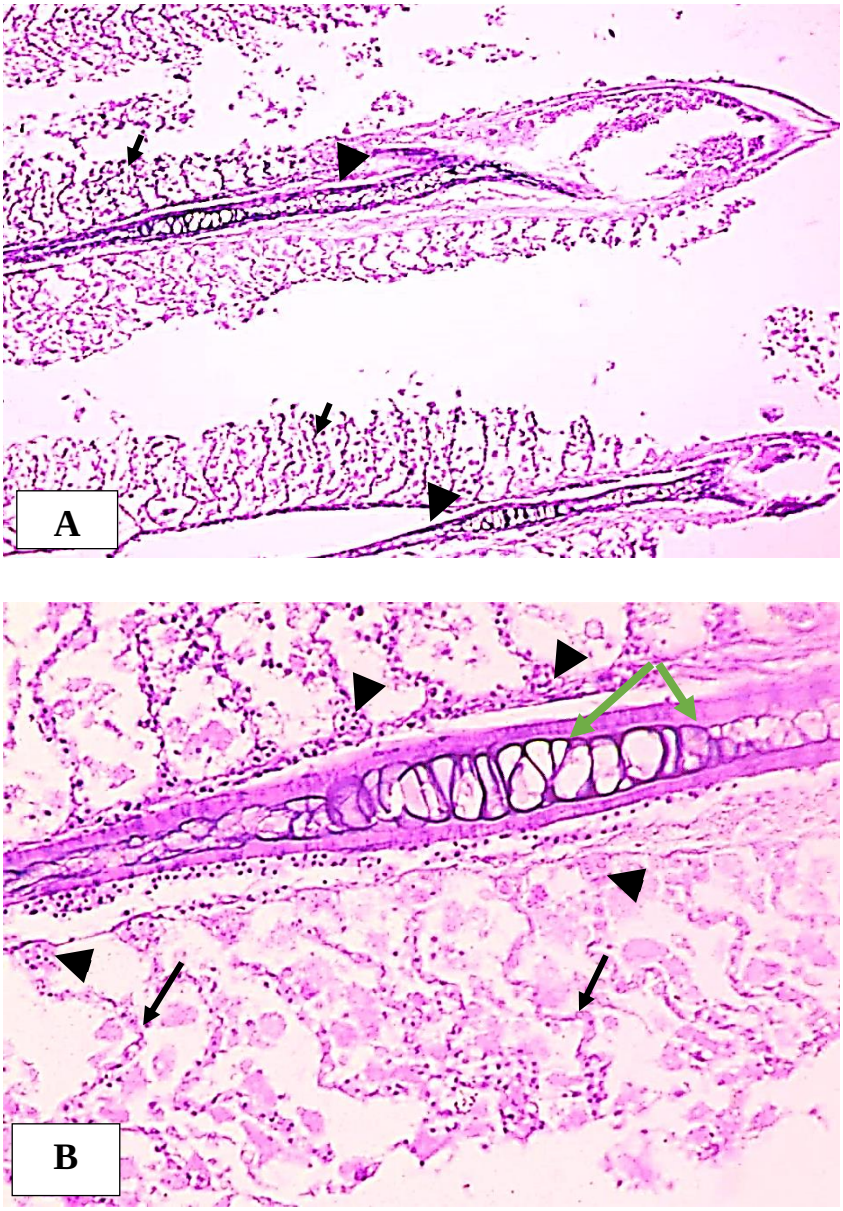


Fig 8: A: A Photomicrograph from gills S. *rivulatus* group showing: abnormal gill structure, loss of lamellar architecture organization, hyperplasia of secondary gill lamellae with partial oedema (arrow), rupture of primary filaments (black arrowhead). **(Hx &E; 100 X), B:** higher magnification Photomicrograph from gills *S. rivulatus* group showing: severe damage of gill structure, epithelial desquamation and loss of lamellar architecture organization, abnormal arrangement of cartilage cells (green arrow), hyperplasia and bending or curling of secondary gill lamellae (arrow), rupture of pillar cells and capillaries that led to ballooned like lamellae packed with erythrocytes aneurism or lamellar telangiectasis (black arrow head) were observed. **(Hx &E; 400 X)**

CONCLUSION

This study presents a detailed evaluation of HMLs bioaccumulation in various tissues of *S. rivulatus*, highlighting tissue-specific accumulation trends, inter-metal relationships, and potential health risks. The results revealed the muscles had the lowest levels of HMLs, while the liver and gills had the highest levels, as well as the highest levels of iron and aluminium. Bioaccumulation and biota-sediment factor analyses confirmed the role of muscle as a de-concentrator and emphasized the accumulation of specific elements like Cu, Zn, B, and Ni in metabolically active organs. Histopathological examination confirmed toxic stress in exposed fish, with clear alterations in muscles, liver, intestines, and gills. Human health risk assessment models indicated no significant non-carcinogenic risks across age groups. However, children showed elevated carcinogenic risks from Cr, As, and Ni, exceeding the recommended thresholds. Finally, this research emphasizes the need for ongoing environmental monitoring, enhanced pollution regulation, and raising public health awareness to minimize exposure from seafood intake.

REFERENCES

Abbas, M.M.M.; Abd El-Aziz, M.A.E.; Kaddah, M.M.Y.; Hassan, A.K.; El-Naggar, H.A.; Radwan, M.; Tabakh, M.A.M.; Afifi, M.A. and **Bashar, M.A.E. (2022a).** Bioaccumulation, biosedimentation, and health hazards of elements in crayfish (*Procambarus clarkii*) from El-Rahawi Drain and El-Qanatir in the River Nile, Egypt. *Biological Trace Element Research* 1(6), 3050–3059. https://doi.org/10.1007/s12011-022-03380-7.

Abbas, M.M.M. Shehata, S.A.; Talab, A.A. and **Ghanem, M.H.M. (2022b).** Effect of Traditional Processing Methods on the Cultivated Fish Species, Egypt. Part I. Mineral and Heavy Metal Concentrations, Biological Trace Element Research. https://doi.org/10.1007/s12011-021-02840-w.

Abbas, M.M.M.; EL-Sharkawy, S.M.; Mohamed, H.R.; Elaraby, B.E.; Shaban, W.M.; Metwally, M.G. and **Farrag, D.M.G. (2024a).** Heavy Metals Assessment and Health Risk to Consumers of Two Commercial Fish Species from Polyculture Fishponds in El-Sharkia and Kafr El-Sheikh, Egypt: Physiological and Biochemical Study. *Biological Trace Element Research*, 202, 4735-4750. https://doi.org/10.1007/s 12011-023-04007-1

Abbas, M.M.M.; El-Kady, A.A. Ghanem, M.H.; Embaby, M.A. El- Shorbagy, S.M. Ahmed, D.F.I. and **Abdel-Monie, S.M. (2024b).** Heavy Metals Accumulation in Marine Fish Muscles from the Suez Gulf, Egypt: Ecological and Health Consequences. *Egyptian Journal of Aquatic Biology and Fisheries*, 28(6), 1331-1356. doi: 10.21608/ejabf.2024.396343

Abbas, M.M.M. (2024). Heavy metal levels and carcinogenic risk assessments of the commercial Denis, *Sparus aurata*, collected from Bardawil Lake and private fish farm waters as a cultured source, Egypt. *Biological Trace Element Research*. https://doi.org/10.1007/s12011-023-03880-0.

Abbas, M.M.M. and **Alnasser A.S. (2025).** Trace element concentrations in two coral reef fish species, El-Shaour fish (*Lethrinus nebulosus*) and El-Hareed fish (*Chlorurus sordidus*): A study of contamination degree, environmental and health risks. Mar Pollut Bull. 2025 Apr;213:117658. doi: 10.1016/j.marpolbul. 2025.117658.

Abdel-Aziz, M.E.; Hassan AM, El-Naggar HA.; Abbas, M.M.M. and Bashar, M.A. (**2022**). Potential cancer and non-cancer health risks of heavy metals ingestion from consumption of the crayfish, *Procambarus clarkii* in El-Rahawy Drain and ElKanater in the River Nile, Egypt. Egypt. J. of Aqu. Biolo. and Fishe. 26:667–686. https://dx.doi.org/10.21608/ejabf.2022.244364.

Abdel-Wahab, H.; Abdel-Hamid, M. and Alnady, A. (2017). Ecological study on water and sediments in a section of the River Nile, Aswan, Egypt as indication for water quality with the existence of the tourists ships floating.," *Al-Azhar Bulletin of Science*: Vol. 28: Iss. 1, Article 15. https://doi.org/10.21608/absb.2017.8165

Adolfsson-Erici, M.; Åkerman, G. and **McLachlan, M.S. (2012).** Measuring bioconcentration factors in fish using exposure to multiple chemicals and internal benchmarking to correct for growth dilution. *Environmental Toxicology and Chemistry* 31, 1853–1860. https://doi.org/10.1002/etc.1897.

Afifi, M.A.M.; Radwan, M.; Abbas, M.M.M.; Hwihy, H.M.; Alabssawy, A.N. and **Khalaf-Allah, H.M. (2024).** Threat of heavy metal pollutants and parasites to freshwater fish with special reference to their risk of carcinogenicity to humans in Egypt. *Aquaculture* 587, 740833. https://doi.org/10.1016/j.aquaculture.2024.740833.

Ahmed, N. M.; Salaah, S. and Tayel, S. (2022). Accumulation and risk assessment of heavy metals-induced biochemical and histopathological alterations in O. niloticus from Lake Nasser. *Egyptian Journal of Aquatic Biology and Fisheries*, *26*(2), 609-625.

Ahmed, A.; Sultana, S.; Habib, A.; Ullah, H.; Musa, N.; Rahman, M.M. and **Sarker, M.S. (2019).** Bioaccumulation of heavy metals in commercially important fish species from the tropical river estuary suggests higher potential child health risk than adults. *PLoS One* 14(10), e0219336. https://doi.org/10.1371/journal. pone.0219336

Ali, M.M.; Ali, M.L.; Proshad, R.; Islam, S.; Rahman, Z. and **Kormoker, T. (2020).** Assessment of trace elements in the demersal fishes of a coastal river in Bangladesh: a public health concern. *Thalassas: An International Journal of Marine Sciences* 36(2), 641–655. https://doi.org/10.1007/s41208-020-00227-7.

AOAC, Association of Official Analytical Chemists, (2012). Official methods of analysis. 15th ed. Association of Official Analytical Chemists, Inc.; Washington, DC, USA, 478p.

APHA, American Public Health Association, (2023). American Water Works Association, Water Environment Federation. Lipps, W.C.; Braun-Howland, E.B.; Baxter, T.E. (Eds.), *Standard Methods for the Examination of Water and Wastewater*. 24th ed. APHA Press, Washington, DC.

Bonito, L. T.; Hamdoun, A. and Sandin, S. A. (2016). Evaluation of the global impacts of mitigation on persistent, bioaccumulative and toxic pollutants in marine fish. Peer J, 4, e1573.

Bosch, A. C.; O'Neill, B.; Sigge, G. O.; Kerwath, S. E. and Hoffman, L. C. (2016). Heavy metals in marine fish meat and consumer health: A review. *Journal of the Science of Food and Agriculture*, 96(1), 32–48. https://doi.org/10.1002/jsfa.7360

CAMPAS. *Annual Bulletin of Statistics Fish Production in the Arab Republic of Egypt for 2015*. Central Agency for Public Mobilization and Statistics, Ref. No. 71-22112-2015 (2017).

Cui, L.; Ge, J.; Zhu, Y.; Yang, Y. and **Wang, J. (2015).** Concentrations, bioaccumulation, and human health risk assessment of organochlorine pesticides and heavy metals in edible fish from Wuhan, China. *Environmental Science and Pollution Research International* 22, 15866–15879. https://doi.org/10.1007/s11356-015-4752-8.

Dallinger, R. (1993). Ecotoxicology of metals in invertebrates. In *Strategies of metal detoxification in terrestrial invertebrates* (pp. 246–332). Lewis Publisher.

Dolenec, M. and Kuzir, S. (2009). Anatomy and histology of bony fish gills as a basis of their multiple roles. Veterinarska Stanica, 40(4), 209–217.

Dutta, J.; Choudhary, G. R. and Mitra, A. (2017). Bioaccumulation of toxic heavy metals in the edible fishes of Eastern Kolkata Wetlands (EKW), the designated Ramsar site of West Bengal, India. International Journal of Aquaculture and Fishery Sciences, 3(1), 18–21.

Dytham, C. (2011). *Choosing and Using Statistics: A Biologist's Guide.* John Wiley & Sons.

Elaraby, B.E.; EL-Sharkawy, S.M.; Elfeky, K.S.; Afifi, M.A.; El-Tabakh, M.A.; Ali, R.M.; Abbas, M.M.M. and Alabssawy, A. N. (2024). Physiological Responses and Histopathological Indices against Acrylamide Toxicity Treated with Metformin and Propolis *in Clarias gariepinus*. Egyptian Journal of Aquatic Biology and Fisheries, 28(1): 105-130. https://dx.doi.org/10.21608/ejabf.2024.336220

Elhaddad, E., Salaah, S. M., Salama, H. M., El-Sherif, D. M. and Gaber, H. S. (2022). Risk assessment and hazardous effects of metal contamination in *O. niloticus* and *S. galilaeus* from four islands of the River Nile. *Bulletin of Environmental Contamination and Toxicology*, *109*(5), 839-851.

El-Kady, A.A.; Abbas, M.M.M. and **Wade, T.L. (2025).** Health risks of trace element concentrations in commercial fish gathered from Greater Cairo fish markets, Egypt. *J. Food Compos. Anal.* https://doi.org/10.1016/j.jfca.2025.107320.

El-Moselhy, Kh.M.; Othman, A.I.; Abd El-Azem, H. and **El-Metwally, M. E. A. (2014).** Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. Egyptian *Journal of Basic and Applied Sciences*, 1, 97-105. https://doi.org/10.1016/j.ejbas.2014.06.001

El-Shorbagy, M.A.; Abdel-Moniem, S.M.; Ghanem, M.H.; Abbas, M.M.M. (2024). Elucidating the Environmental and Health Risks of Trace Element Pollution in Red Sea Fish from Nuweiba City, Aqaba Gulf, Egypt. *Biological Trace Element Research.* https://doi.org/10.1007/s12011-024-04246-w.

Emon, F. J., Rohani, M. F., Sumaiya, N., Jannat, M. F. T., Akter, Y., Shahjahan, M., Kari, Z. A., Tahiluddin, A. B. and Goh, K. W. (2023). Bioaccumulation and bioremediation of heavy metals in fishes—A review. *Toxics*, *11*(6), 510. https://doi.org/10.3390/toxics11060510

Escalas, A., Avouac, A., Belmaker, J., Bouvier, T., Clédassou, V., Ferraton, F., Rieuvilleneuve, F., Rilov, G., Rovirosa Mulet, A., Shapiro Goldberg, D. and Villéger, S., (2022). An invasive herbivorous fish (*Siganus rivulatus*) influences both benthic and planktonic microbes through defecation and nutrient excretion. *Science of The Total Environment*, 838(Part 3), p.156207. https://doi.org/10.1016/j.scitotenv.2022.156207

EU, (2015). Commission regulation (EU) No. 1005/2015 of 25 June 2015 amending regulation (EC) No. 1881/2006 as regards maximum levels of lead in certain foodstuffs. L 161/9. Off. J. Eur. Union. Available online: http://eur-lex.europa.

FAO, Food and Agriculture Organization, (1983). Compilation of legal limits for hazardous substances in fish and fishery products. *FAO Fishery Circular* No. 464. Rome, Italy. Available online: http://www.fao.org/docrep/014/q5114e/q5114e.pdf.

FAO/WHO, (1989). Evaluation of Certain Food Additives and the Contaminants Mercury, Lead and Cadmium. WHO Technical Report Series No. 505.

GAFRD. *Fish Statistics Year Book 2017.* General Authority for Fish Resources Development, Ministry of Agriculture and Land Reclamation, Cairo, Egypt (2017).

Ghannam, H. E., Khedr, A. I., El-Sayed, R., Ahmed, N. M. and Salaah, S. M. (2025). Oxidative stress responses and histological changes in the liver of Nile tilapia exposed to silver bulk and nanoparticles. *Scientific Reports*, *15*(1), 15390.

Gomes, T., Pinheiro, J.P., Cancio, I., Pereira, C.G., Cardoso, C. and Bebianno, M.J. (2011). Effects of copper nanoparticles exposure in the mussel Mytilus galloprovincialis, Environ. Sci. Technol. 45 (21) 9356–9362 . DOI: 10.1021/es200955s.

Guo J, Zhou Y, Zhao H, Chen WY, Chen YJ, and Lin SM. (**2019**). Effect of dietary lipid level on growth, lipid metabolism and oxidative status of largemouth bass.

Micropterus salmoides Aquaculture. 2019;506:394400. doi: 10.1016/j.aquaculture.2019.04.007.

Hamada, N. H. S., Ali, R. A. S., El-Tabakh, M. A. M., & Bream, A. S. (2024). Environmental monitoring: Tobara fish as bioindicators of heavy metal pollution in a coastal ecosystem. *Regional Studies in Marine Science*, *69*, 103335. https://doi.org/10.1016/j.rsma.2023.103335

Handy, R.D. & Maunder, R.J. (2009). The biological roles of mucus: importance for osmoregulation and osmoregulatory disorders of fish health, in: R.D. HandyN.R. Bury, G. Flik (Eds.), Osmoregulation and Ion Transport: Integrating Physiological, Molecular and Environmental Aspects, Society for ExperimentalBiology, Cambridge, pp. 203–235 .

Hernandez-Saavedra D, Sanders L, Freeman S, Reisz JA, Lee MH, Mickael C, Kumar R, Kassa B, Gu S, D'Alessandro A. (**2020**). Stable isotope metabolomics of pulmonary artery smooth muscle and endothelial cells in pulmonary hypertension and with TGF-beta treatment. Sci Rep. 2020;10:1–13. doi: 10.1038/s41598-019-57200-5.

Humason, G. L. (1979). Animal tissue techniques (4th ed.). W. H. Freeman. Freeman Company, San Francisco, USA, 661 pp.

Hussein, M.A.; Morsy, N.S.; Mahmoud, A.F.; Darwish, W.S.; Elabbasy, M.T.; Zigo, F.; Farkašová, Z. and Rehan, I.F. (2023). Risk assessment of toxic residues among some freshwater and marine water fish species. Frontiers in Veterinary Science, 25 (10). doi.org/10.3389/fvets.2023.1185395.

Irshad, M.A.; Nawaz, R.; Nasim, I.; Irfan, A.; Hussain, A.; Al-Mutairi, A.A. and **Zaki, M.E. (2024).** Bioaccumulation of carcinogenic metals in river fish: A quantitative investigation of public health risk. *Ecol. Indic.* 162, 112057. https://doi.org/10.1016/j.ecolind.2023.112057

Islam M. S., Ahmed M. K., Raknuzzaman M., Habibullah -Al- Mamun M., Islam M. K. (2015). Heavy metal pollution in surface water and sediment: A preliminary assessment of an urban river in a developing country. *Ecol. Indic.* 48, 282–291. doi: 10.1016/j.ecolind.2014.08.016.

Khedr, A. I., Ahmed, N. M., Salaah, S. M., Tayel, S. I., Soliman, Y. A. and Goher, M. E. (2024). Assessment of PAH pollution in mediterranean lakes and health implications for fish and consumers, case study: Manzala Lake, Egypt. *Water Cycle*, *5*, 199-214.

Kim, N.H.; Hyun, Y.Y.; Lee, K.B.; Chang, Y.; Ryu, S.; Oh, K.H. and **Ahn, C. (2015).** Environmental heavy metal exposure and chronic kidney disease in the general population. *J. Korean Med. Sci.* 30(3), 272–277. https://doi.org/10.3346/jkms.2015.30.3.272

Kobayashi, I., Kondo, M., Yamamori, S., Kobayashi-Sun, J., Taniguchi, M., Kanemaru, K., Katakura, F. and Traver, D. (2019). Enrichment of hematopoietic stem/progenitor cells in the zebrafish kidney. Scientific Reports, 9, 14205.

Kourany, M. S.; Mohamed, H. R.; El-Sherif, S.A.; Abbas, M.M.M. and **El-Lahamy, A. A. (2024).** Assessing the quality of tilapia fillets (*Oreochromis niloticus*) and the impact of frozen storage periods on their chemical composition. Mediterranean Aquaculture Journal, 11(2), 36-42. doi: 10.21608/maj.2024.411264

Li, Z., Liu P., Chen S., Liu X., Yu Y., Li T., et al. (2023). Bioinspired marine antifouling coatings: Antifouling mechanisms, design strategies and application feasibility studies. *Eur. Polymer J.* 190, 111997. doi: 10.1016/j.eurpolymj. 2023.111997

Liu, X., Wang, H., Liang, X. and Roberts, M. S. (2017). Hepatic metabolism in liver health and disease. In Liver pathophysiology, Academic Press. (pp. 391–400).

Mahboob, S., Al-Ghanim, K. A., Al-Balawi, H. F., Al-Misned, F. and Ahmed, Z. (2020). Toxicological effects of heavy metals on histological alterations in various organs in Nile tilapia (Oreochromis niloticus) from freshwater reservoir. Journal of King Saud University - Science, 32(1), 970–973. https://doi.org/10.1016/j.jksus.2019.07.004

Maurya, P. K., Malik, D. S., Yadav, K. K., Gupta, N. and Kumar, S. (2019). Haematological and histological changes in fish Heteropneustes fossilis exposed to pesticides from industrial waste water. Human and Ecological Risk Assessment, 25(9), 1251–1278.

McEneff, G.; Quinn, B.; Bennion, M.; Dolan, S.; O'Rourke, K.; and **Morrison, L. (2017).** Bioaccumulation of metals in juvenile rainbow trout (Oncorhynchus mykiss) via dietary exposure to blue mussels. Chemosphere 188, 548–556. https://doi.org/10.1016/j.chemosphere.2017.09.027

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., Idris, A. M., Khandaker, M. U., Osman, H., Alhumaydhi, F. A. and Simal-Gandara, J. (2022). Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science, 34(3), 101865. https://doi.org/10.1016/j.jksus.2022.101865.

Muñoz, L., Weber, P., Dressler, V., Baldisserotto, B. and Vigliano, F. A. (2015). Histopathological biomarkers in juvenile silver catfish (Rhamdia quelen) exposed to a sublethal lead concentration. Ecotoxicology and Environmental Safety, 113, 241–247.

Mwakalapa, E.B.; Simukoko, C.K.; Mmochi, A.J.; Mdegela, R.H.; Berg, V.; Bjorge Muller, M.H. and **Polder, A. (2019).** Heavy metals in farmed and wild milkfish (*Chanos chanos*) and wild mullet (*Mugil cephalus*) along the coasts of Tanzania and associated health risk for humans and fish. Chemosphere 224, 176–186. https://doi.org/10.1016/j.chemosphere.2019.02.063

Pereira, B. F.; da Silva Alves, R. M.; Pitrol, D. L.; Senhorini, J. A.; Rocha, R. G. and Caetano, F. H. (2012). Morphological gill analysis of fish species Prochilodus lineatus after exposure to pollutants. Journal of Environmental & Analytical Toxicology, 2, 3.

Pereira, E., Silvares, R. R., Flores, E. E. I., Rodrigues, K. L., Ramos, I. P., et al. (2017). Hepatic microvascular dysfunction and increased advanced glycation end products are components of non-alcoholic fatty liver disease. PLoS ONE, 12(6), e0179654. https://doi.org/10.1371/journal.pone.0179654

Radwan, M.; Abbas, M. M. M.; Afifi, M. A. M.; Mohammadein, A. and **Al Malki, J. S.** (2022). Fish parasites and heavy metals relationship in wild and cultivated fish as potential health risk assessment in Egypt. *Fro.in Enviro. Sci*, *10*, 890039. https://dx.doi.org/10.3389/fenvs.2022.890039.

Rahman, M. S.; Molla, A. H.; Saha, N. and **Rahman, A. (2012).** Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. *Food Chemistry*, 134(4), 1847-1854. DOI: 10.1016/j.foodchem.2012.03.099

Rajkumar, K.S., Kanipandian, N. and Thirumurugan R. (2016). Toxicity assessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish Labeo rohita , Appl. Nanosci. 6 (1), 19–29 .

Rakhi, S. F., Reza, A. H. M. M., Hossen, M. S., and Hossai, Z. (2013). Alterations in histopathological features and brain acetylcholinesterase activity in stinging catfish Heteropneustes fossilis exposed to polluted river water. International Aquatic Research, 5, 7.

Renu, and Agrwal, A. M. K. (2013). Lead induced histopathological changes in the gills of Heteropneustes fossilis. Journal of Experimental Zoology, India, 16(2), 603–606.

Sabra, M. S., Salaah, S. M., Kotob, M. H., Gazzar, W. B. E., Farag, A. A., Idriss, S. K. and Khalil, N. S. A. (2025). Hepato-Renal Damage of Hyaluronic Acid on the African Catfish (Clarias gariepinus). *Aquaculture Research*, *2025*(1), 6694475.

Saha, N.; Mollah, M.Z.I.; Alam, M.F. and **Safiur, R.M. (2016).** Seasonal investigation of trace elements in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. Food Control, 70: 110-118. https://doi.org/10.1016/j.foodcont.2016.05.040.

Salaah, S. M., Ali, E. H., Mostafa, A. B., Khalil, M. T. and El-Naggar, M. M. (2025). Assessing the Ecotoxicological Impact of Hospital Wastewater on Nile Tilapia and the Mitigating Effects of $NiFe_2O_4$ Nanocomposite. *Scientific African*, e02536.

Salaah, S. M., Zanaty, N. and El-Naggar, M. M. (2022). Evaluation of heavy metals contents and the possible risk in the surface water and fish of Lake Qarun, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, *26*(4), 1067-1091.

Samuel, T., Thresia, M., Loganathan, K., Arivoli, S., Raveen, R. and William, J. (2019). Biopesticide, azadirachtin induced histopathological alterations in the

intestine of the African catfish Clarias gariepinus (Burchell, 1822) (Teleostei: Clariidae). Proceedings of the Zoological Society of India, 18(1), 45–53.

Shah, Z. U. and Parveen, S. (2020). A review on pesticides pollution in aquatic ecosystem and probable adverse effects on fish. Pollution Research, 39(2), 309–321.

Shahjahan, M.; Taslima, K.; Rahman, M.S.; Al-Emran, M.; Alam, S.I. and **Faggio, C. (2022).** Effects of heavy metals on fish physiology – A review. Chemosphere 300, 134519. https://doi.org/10.1016/j.chemosphere.2022.134519

Sharmin, S., Islam, M. T., Sadat, M. A., Jannat, R., Alam, M. R. and Shahjahan, M. (2021). Sumithion-induced structural erythrocytes alteration and damage to the liver and kidney of Nile tilapia. Environmental Science and Pollution Research, 28(27), 36695–36706.

Singh, U. and Pandey, R. S. (2021). Fertilizer industry effluent induced hematological, histopathological and biochemical alterations in a stinging catfish, Heteropneustes fossilis (Bloch, 1794). Environmental and Sustainability Indicators, 10, 100110.

Stalin, A., Suganthi, P., Mathivani, S., Paray, B. A., Al-Sadoon, M. K., Gokula, V., and Musthafa, M. S. (2019). Impact of chlorpyrifos on behavior and histopathological indices in different tissues of freshwater fish Channa punctatus (Bloch). Environmental Science and Pollution Research, 26(17), 17623–17631.

Subashkumar, S. and Selvanayagam, M. (2014). First report on: acute toxicity and gill histopathology of fresh water fish Cyprinus carpio exposed to Zinc oxide (ZnO) nanoparticles, Int. J. Sci. Res. Publ. (3), 1-4.

Suchana, S. A., Ahmed, M. S., Islam, S. M. M., Rahman, M. L., Rohani, M. F., Ferdusi, T., Ahammad, A. K. S., Fatema, M. K., Badruzzaman, M. and Shahjahan, M. (2021). Chromium exposure causes structural aberrations of erythrocytes, gills, liver, kidney and genetic damage in striped catfish Pangasianodon hypophthalmus. Biological Trace Element Research, 199(10), 3869–3885.

Soliman, H. A.; Salaah, S. M.; Hamed, M. and Sayed, A. E. D. H. (2023). Toxicity of co-exposure of microplastics and lead in African catfish (Clarias gariepinus). *Frontiers in Veterinary Science*, *10*, 1279382.

Tasneem, S. and Yasmeen, R. (2020). Biochemical changes in carbohydrate metabolism of the fish Cyprinus carpio during sub-lethal exposure to biopesticide Derisom. Iranian Journal of Fisheries Sciences, 19(2), 961–973.

Tsurkan LV, Volichenko YM and Sherman IM. (**2020**). Ecological and hematological components of wintering of carp carpets in the conditions of the South of Ukraine. Water Bioresour Aquac. 2020;2:59–69. doi: 10.32851/wba.2020.2.6.

Tytła, M. and **Widziewicz-Rzońca, K. (2023).** Ecological and human health risk assessment of heavy metals in sewage sludge produced in Silesian Voivodeship, Poland: A case study. Environmental Monitoring and Assessment, 195(11): 1373. https://doi.org/10. 1007/s10661-023-11987-z.

Ullah, A. K. M. A., Akter, M., Musarrat, M. and Quraishi, S. B. (2018). Evaluation of Possible Human Health Risk of Heavy Metals from the Consumption of Two Marine Fish Species Tenualosa ilisha and Dorosoma cepedianum. Biological Trace Element Research, 191(2), 485–494. https://doi.org/10.1007/s12011-018-1616-3

USEPA, United States Environmental Protection Agency, (2000). Guidance for assessing chemical contaminant data for use in fish advisories, volume II. Risk Assessment and fish consumption limits, (EPA 823-B-00-008).

USEPA, United States Environmental Protection Agency (2011). Risk Assessment Guidance for Superfund. Volume I: (Part A: Human Health Evaluation Manual; Part E, Supplemental Guidance for Dermal Risk Assessment; Part F, Supplemental Guidance for Inhalation Risk Assessment). EPA US Environmental Protection Agency, Washington, DC. Available online at: https://www.epa.gov/sites/default/files/2015-09/documents/efh-chapter10.pdf.

USEPA, United States Environmental Protection Agency, (2018). US EPA Regional Screening Levels (RSLs), Dataset- California open data. https://data.ca.gov/dataset/us-epa-regional-screening-levels-rsls-november-2018/resource/b66c9172-a61a-4131-997f-01f45a473a08.

USEPA, United States Environmental Protection Agency, (2023). Microwave Assisted Acid Digestion of Sediments (3051A method), Sludges, Soils, and Oils, part of *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods.*

Usero, J.; Marilla, J. and **Graccia, I. (2005).** Heavy metal concentrations in molluscs from the Atlantic Coast of Southern Spain. Chemosphere 59, 1175–1181. https://doi.org/10.1016/j.chemosphere.2004.11.089.

Wani, A. A., Sikdar-Bar, M., Borana, K., Khan, H. A., Andrabi, S. S. M. and Pervaiz, P. A. (2011). Histopathological alterations induced in gill epithelium of African catfish, Clarias gariepinus, exposed to copper sulphate. Asian Journal of Experimental Biological Sciences, 2(2), 278–282.

WHO, World Health Organization, (1989). Toxicological evaluation of certain food additives and contaminants. In: Proceedings of the Thirty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, Number 24. Cambridge University Press, Cambridge.

Wilson, J. M. and Laurent, P. (2002). Fish gill morphology: Inside out. Journal of Experimental Zoology, 293(3), 192–213.

Winkaler, E.U., Silva, A.G., Galindo, H.C. and Martinez, C.B.R. (2001). Histological and physiological biomarkers to assess fish health in Londrina streams, state of Paraná, Acta Sci. Biolo. Sci. 23, 507–514 .

Wu, Y.L., Zhou, Q., Li, H., Liu, W., Wang, T. and Jiang, G. (2010). Effects of silver nanoparticles on the development and histopathology biomarkers of japanese medaka (Oryzias latipes) using the partial-life test, Aquat. Toxicol. 100, 160–167 .

Younis, E.M.; Abdel-Warith, A.A.; Al-Asgah, N.A.; Elthebite, S.A. and **Mostafizur R.M. (2021).** Nutritional value and bioaccumulation of heavy metals in muscle tissues of five commercially important marine fish species from the Red Sea. Saudi Journal of Biological Sciences, 28(3), 1860-1866. https://doi.org/10.1016/j.sjbs.2020.12.038

Zaghloul, G. Y.; Eissa, H. A.; Zaghloul, A. Y.; Kelany, M. S.; Hamed, M. A. and El Moselhy, K. M. (2024). Impact of some heavy metal accumulation in different organs on fish quality from Bardawil Lake and human health risks assessment. *Geochemical Transactions, 25*, 1. https://doi.org/10.1186/s12932-023-00084-2 F. Flores-Lopes , A.T. Thomaz , Histopathological alterations observed in fish gills as a tool in environmental monitoring, Braz. J. Biol. 71 (2011) 179–188

Zaghloul, G.Y.; Ezz El-Din, H.M.; Mohamedein, L.I. and **El-Moselhy, K.M. (2022).** Bio-accumulation and health risk assessment of heavy metals in different edible fish species from Hurghada City, Red Sea, Egypt. *Environmental Toxicology and Pharmacology*, 95, 103969. https://doi.org/10.1016/j.etap. 2022. 103969