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Trace elements in freshwater killifish, *Aphanius stoliczkanus*, from Oman: A food safety issue

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ABSTRACT

Freshwater killifish, *Aphanius stoliczkanus*, were collected from industrial and non-industrial areas in Oman to assess trace element levels in muscle and liver tissues and estimate potential human health risks using the Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ). Samples were obtained from Al Amarart, Al Khawd, and Surur and analyzed using Atomic Absorption Spectroscopy. Fish from Al Amarart exhibited significantly higher concentrations of toxic elements, particularly arsenic (As), cadmium (Cd), and lead (Pb), suggesting a strong influence of industrial activities. In contrast, trace element concentrations in fish from Al Khawd and Surur were lower, reflecting reduced anthropogenic inputs. In almost all sites, most metals accumulated at higher levels in the liver than in muscle, indicating increased bioavailability for essential elements or detoxifying function for toxic metals. Despite the variation in contamination levels, THQ values for all elements remained below the threshold of concern (THQ < 1), suggesting that individual exposure through fish consumption does not pose immediate health risks. Similarly, the calculated cancer risk (CR) for As was within acceptable limits at all sites. However, while the direct risk from *A. stoliczkanus* consumption appears low, potential cumulative effects from multiple dietary and environmental sources should not be overlooked. Bioaccumulation through trophic transfer and exposure from water and other food sources may lead to long-term health concerns. Overall, these findings highlight *A. stoliczkanus* as a valuable bioindicator of trace element contamination in industrial regions. Continued environmental monitoring and stricter pollution control measures are recommended to mitigate contamination sources and ensure long-term food safety. Further studies should explore temporal trends and additional toxicity biomarkers to provide a more comprehensive understanding of ecological and human health risks.

1. Introduction

Freshwater ecosystems play a fundamental role in sustaining both the environment and human societies. They serve as essential sources of water, food, and natural resources, supporting biodiversity and economic development across multiple sectors, particularly agriculture, fisheries, and industry. These ecosystems are critical for food security, benefiting both rural and urban populations [68]. Among their many contributions, fisheries and aquaculture stand out as vital sources of nutrition and livelihoods for communities worldwide. In Oman, fish consumption per capita is among the highest globally [8]. Fish is not only a dietary staple for local populations but also a key economic commodity, supplying urban markets and supporting exports. Consequently, the fishing industry plays a crucial role in the national economy [13,9]. However, Oman is undergoing rapid industrialization, urbanization, and agricultural expansion, all of which exert increasing pressure on aquatic ecosystems[12,7]. Industrial activities could generate significant waste, much of which eventually contaminates freshwater bodies. This challenge is not unique to Oman but represents a global concern, as pollutants from industrial and agricultural sources continue

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to degrade water quality worldwide [37,59,60]. Addressing these environmental pressures requires sustainable management strategies to protect freshwater ecosystems while balancing economic development and ecological conservation.

Freshwater ecosystems are particularly susceptible to contamination by trace elements (TEs) such as the most toxic lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) [40,53,62,73]. These elements are known to cause a range of adverse health effects, including neurological damage, cancer, and cardiovascular problems [11,36,39,77]. For instance, human exposure to Pb can result in developmental issues in children and is linked to cognitive impairments, while exposure to Hg can cause significant neurological damage. Similarly, Cd is associated with kidney damage and bone deterioration, and As is a known carcinogen [69]. Due to their toxicity, persistence, and ability to bioaccumulate, these pollutants represent a serious environmental threat. Once introduced into aquatic systems, trace elements are absorbed by plants and animals, progressively biomagnifying through the food chain [15,41]. This phenomenon has significant ecological and human health implications, especially in regions where fish consumption is high.

To assess and mitigate the impact of pollution in freshwater ecosystems, bioindicator species are widely used as effective tools for environmental monitoring, tracking contamination trends and assess ecosystem health, supporting strategies to reduce pollution and protect aquatic biodiversity These organisms accumulate contaminants in their tissues, offering valuable insights into pollution levels within their habitat [2,55,6]. Algae, fish and aquatic invertebrates are particularly useful bioindicators due to their sensitivity to variations in water quality and trace element concentrations [24,34,67]. Among the species used for environmental monitoring, the killifish *Aphanius stoliczkanus* has been recognized as a valuable bioindicator of aquatic ecosystem health. A member of the Cyprinodontidae family, this species is particularly suited for pollution assessment due to its resilience in contaminated habitats [61]. Endemic to Iran and Oman, *A. stoliczkanus* inhabits diverse freshwater environments, including watersheds, marshes, ponds, streams, and shallow watercourses known as wadis [26,35]. Its territorial behavior and limited mobility result in prolonged exposure to local pollutants, allowing researchers to assess site-specific contamination levels with high reliability [14].

Unfortunately, there is no specific data on the consumption patterns of killifish among the Oman population, nor are there published estimates of its annual consumption rate or catch volume. However, killifish remain a staple in traditional cuisine, particularly in rural communities, where they are commonly incorporated into local dishes. Despite their modest size (4.5–8.0 cm), they hold cultural and nutritional significance, as they are often consumed whole, providing a valuable source of protein and essential micronutrients. This raises concerns regarding potential health risks from consuming potential contaminated fish [16,56]. With the continued expansion of industrial activities in Oman, the risk of aquatic contamination by trace elements is increasing. These pollutants not only threaten ecosystem health but also pose serious risks to human populations, especially in communities where fish constitutes a dietary mainstay.

This study aimed to investigate the levels of trace elements—arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn)—in the muscle and liver tissues of *Aphanius stoliczkanus* (Day, 1872) collected from industrial and non-industrial areas in Al Amarart, Al Khawd, and Surur. We assessed the potential human health risks associated with consuming these fish through a comprehensive risk evaluation, including both non-carcinogenic and carcinogenic assessments. The findings provide valuable insights into the current state of freshwater ecosystems in Oman and the potential hazards of trace element contamination. Furthermore, they highlight the need for stricter regulations and pollution control measures to safeguard both aquatic life and public health. By monitoring trace element levels in bioindicator species such as *A. stoliczkanus*, we can better mitigate contamination risks and ensure the safety of fish for human consumption.

2. Material and methods

2.1. Sampling and study area

Sampling was conducted between November and December 2022 in Al Amarart (site 1: 23° 29' 59"N, 58° 29' 52"E), Al Khawd (site 2: 23° 37' 26" N, 58° 33' 45" E), and Surur (site 3: 23° 22' 41" N, 58° 6′ 17" E). Al Khawd and Al Amarat are in Muscat Governorate and are affected by industrial areas and sewage activities. Al Khawd is a village in Muscat, in northeastern Oman, near the Rusayl industrial area. It is at risk of various industrial and domestic pollutants from steel manufacturing plants, steam generators, plastic injection molding factories, pumping and pipeline manufacturers, aluminum window frame production, and steel furniture factories. However, no published data exists on the effect of industrial activities in Al Khawd's natural habitats. Al Amarat is the fifth-most populated city in Muscat, near the Al-Atkia industrial area. It has industrial activities such as metal hydraulic repair service, signs and banner manufacturing, metal scrap supply, and sewage and medical waste treatment. In this regard, according to Al Raisi et al. [7], Surur is far from industrial and heavy human activities and has a clean watercourse (Fig. 1).

Forty killifish were collected from each area. The specimens were placed in labeled plastic bags, refrigerated at 4 °C, and promptly transported to the laboratory. Upon arrival, the fish were dissected to obtain liver and muscle samples, which were then stored at –20 °C for further analysis, ensuring a storage period of no more than one month.

2.2. Analytical procedures

The procedure used to measure trace element concentration in fish samples was described previously [3]. Trace element concentrations were measured in the muscle and liver tissue samples using standard methods with a minor modification. According to the procedure outlined in our prior work, edible tissue aliquots (0.3 g, wet weight) were weighed and digested by high-pressure decomposition vessels [25]. The samples were digested with an acid mixture of 4 mL of 68 % nitric acid (Suprapur; Romil Ltd., Cambridge, UK), 4 mL of 30 % hydrogen peroxide (Suprapur; Merck, Darmstadt, Germany), and 1 mL of concentrated perchloric acid (Suprapur; Merck, Darmstadt, Germany). For Hg determination, 45 mg of vanadium pentoxide was added to the samples for the digestion step. Then, they will be diluted to 50 mL with 20 mL of distilled water and potassium dichromate (2 %). Digestion will take three hours on a hotplate at 90 ^{0}C or until the solution is transparent and all particles have turned white. Digested samples were filtered with a 0.45 mm nitrocellulose membrane filter and diluted with high-purity deionized water at a ratio of 1:5. Finally, the digestions were analyzed with flame atomic absorption spectrophotometry (Thermo M5 Series AA, Germany) equipped with a microcomputer-controlled acetylene flame. The determination of total mercury was performed using cold vapor hydride generation (Thermo VP100 Vapor system). Reference material (RM) TORT-3 was analyzed in triplicates to determine the recovery percentage and evaporation during digestion. The recovery percentage was between 88 % and 95 %.

The coefficient of variation on replicates, spiked samples ranged up to 10 %. The detection limits for As. Cd. Cr, Co, Cu, Hg, Mn, Ni, Pb, Zn were 0.004, 0.006, 0.05, 0.01, 0.02, 0.005, 0.002, 0.05, 0.001, and 0.05 μg g^{-1}, respectively. The samples were analyzed in triplicate, and the results were collected on a wet-weight basis. Blanks are processed in the same way as the samples.

2.3. Risk assessment

For risk assessment, both the simplified Estimated Daily Intake (EDI), the Target Hazard Quotient (THQ) and the Cancer Risk (CR) were

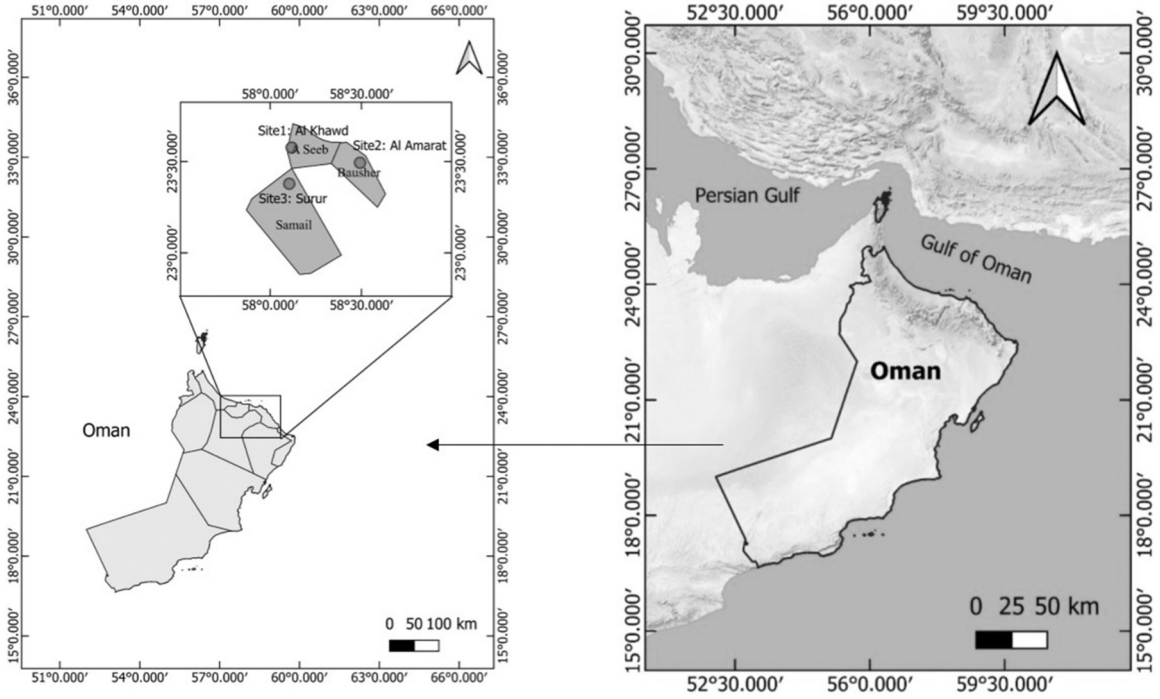

Fig. 1. Sampling and study area in Oman. Site 1: Al Amarart; Site 2: Al Khawd; and site 3: Surur.

calculated. These calculations were performed to estimate the potential health risks associated with the consumption of metals through fish, allowing us to evaluate whether oral exposure levels might pose a threat to human health.

The Estimated Daily Intake (EDI) of metals through the consumption of fish was calculated using a simplified approach. The EDI was estimated based on the concentration of metals in the edible tissues of *A. stoliczkanus* and an assumed ingestion rate equivalent to a typical adult meal portion, as defined by the Environmental Protection Agency (EPA). The following formula was applied:

$$EDI = (C \times FIR)/BW$$

Whereas: C is the concentration of the metal in fish (mg/kg); FIR is the Food Ingestion Rate of fish (kg/day) (0227 kg), BW is the average adult Body Weight (70 kg)

The Target Hazard Quotient (THQ) is a risk assessment tool used to estimate the potential non-carcinogenic health risks associated with exposure to hazardous substances during the lifetime. This approach allows us to evaluate the potential health risks posed by contaminants present in food and it is determined by comparing the estimated lifetime intake of a substance to a reference dose considered safe. A THQ value greater than 1 suggests a potential risk of adverse health effects due to prolonged exposure [46]. The following formula was applied:

$$THQ = (EF \times ED \times FIR \times C)/(BW \times AT \times RfD \times 10^{-3})$$

Whereas: EF is the Exposure frequency (350 days/year for people who eat fish); ED is the Exposure duration (70 years, equivalent to the average lifetime); FIR is the Food Ingestion Rate (0.009 kg/person/day for the Iranian population); C is the trace elements concentration in the sample (mg/kg); BW is the average body weight of an adult (70 kg); AT is the average exposure time for non-carcinogenic substances (350 days × ED); RfD is the oral Reference dose (mg/kg body weight/day). In particular, the Reference Doses (RfD) established for each metal by the Environmental Protection Agency are as follows: 0.0003 for As (set for Inorganic As), 0.0001 for Cd, 0,0003 for Co, 0.003 for Cr (set for Cr IV), 0.04 for Cu, 0.00014 for Hg (set for Methyl Hg) 0.14 for Mn, 0.011 for Ni and 0.3 for Zn [74]. Regarding Pb, since the US-EPA has not established a specific oral Reference Dose (RfD), and Pb is recognized as a toxic substance with no safe threshold, researchers and regulatory agencies use alternative approaches to calculate the Target Hazard Quotient (THQ) for Pb exposure. In the absence of a RfD, many studies use the Lowest Observed Adverse Effect Level (LOAEL) of Pb which was set as 0.0012 mg/Kg [75].

The Cancer Risk (CR) associated with the consumption of fish contaminated with metals was calculated to estimate the probability of developing cancer over a lifetime due to oral exposure. Cancer Risk thus expresses the likelihood of an individual developing cancer over a lifetime due to chronic exposure to the contaminant through the consumption of fish. A CR greater than 1×10^{-6} (one in a million) is considered to indicate a potential health risk, while values below this threshold are deemed acceptable by regulatory standards. The Cancer Risk was assessed using the following formula:

$$\text{Cancer Risk: } (EF \times ED \times FIR \times C)/(BW \times AT) \times CSF$$

The cancer slope factor (mg/kg/day) has been determined only for As (1.5, set for Inorganic As). The Cancer Risk was performed assuming that inorganic As represents 3 %, 10 %, and 25 % of the total arsenic concentration. These percentage values were chosen based on scientific literature, which suggests that the proportion of inorganic As in freshwater fish can vary widely and, in some cases, reach elevated levels, although such high values remain rare [33,43,48,76].

2.4. Statistical analysis

For the statistical analyses, all values below the limit of quantification (LOQ) were substituted with half the limit of detection (LOD/2), a widely accepted approach to manage non-detects and maintain consistency in data interpretation. All subsequent statistical analyses were conducted using this adjusted dataset. The Kolmogorov–Smirnov test was used to assess the normality of data distribution, while Bartlett's test evaluated the homogeneity of variances. Differences among sampling sites and between tissues were analyzed using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons. Results are presented as means ± standard deviation (SD). All analyses were

performed using STATISTICA v. 12.0 for Windows (StatSoft, Inc.).

3. Results

3.1. Trace elements analysis

40 specimens per site of *A. stoliczkanus* were collected between November and December 2022, with a mean total length of 3.44 ± 0.59 cm and a mean body weight of 0.73 ± 0.24 g (Table S1) No significant differences among sizes ($p = 0.472$) or weights ($p = 0.610$) were observed per site.

The distribution of trace elements followed the trend: Zn > Cu > Ni > Mn > Pb > Co > Cd > As, with Cr concentrations below the limit of quantification (LOQ) at all sites. Traces of mercury (Hg) were detected exclusively at the Al Amarart site, although its values were very close to the limit of detection (LOD). Table 1 presents the concentrations of various metals in the liver and muscle tissues of *A. stoliczkanus* collected from three distinct study areas: Al Amarart, Al Khawd, and Surur. The results indicate significant spatial variations in metal concentrations, with Al Amarart generally exhibiting the highest levels for most of the analyzed elements.

A statistical comparison of metal concentrations between muscle and liver for each sampling site revealed that, as expected, metal levels were generally higher in the liver, with only a few exceptions. The differences in statistical significance levels, as reported in Table 1, were site specific. Notably, distinct trends were observed for Cd, Co, and Cu across the sampling sites. The concentration of Cd was significantly higher in the liver of fish sampled at Al Amarart ($p < 0.05$). However, in fish from Al Khawd, no significant differences were found between muscle and liver. In contrast, at Surur, Cd levels were significantly higher in muscle ($p < 0.01$). Co levels were significantly higher in the liver of fish sampled at Al Amarart ($p < 0.001$) and Surur ($p < 0.01$), while no significant differences were observed between tissues in fish from Al Khawd. Cu concentrations were significantly higher in the muscle of fish sampled at both Al Amarart and Al Khawd ($p < 0.05$ for both), whereas no significant differences were found between tissues in fish from Surur.

To evaluate spatial variations in metal accumulation, a statistical comparison of metal concentrations among the three sampling sites was performed separately for liver and muscle.

In the liver tissue, As was most concentrated in Al Amarart (0.055 ± 0.022 μg g^{-1}), with statistically significant differences across the three locations ($p < 0.01$). Similarly, Cd concentrations were significantly higher in Al Amarart (0.052 ± 0.030 μg g^{-1}) than in the other sites ($p < 0.001$), indicating potential localized contamination. Interestingly, Cr concentrations in the liver were below the detection limit in all areas, making it impossible to detect any meaningful differences (n.d.). Co also followed the trend of higher concentrations in Al Amarart (0.082 ± 0.019 μg g^{-1}), again showing statistically significant differences between the study areas ($p < 0.01$). Cu concentrations, although slightly elevated in both Al Amarart and Al Khawd, were significantly different across the sites ($p < 0.05$). One notable finding is that Hg was only detected in the liver tissue of *A. stoliczkanus* from Al Amarart (0.009 ± 0.002), with levels below the limit of quantification (LOQ) in the other two areas. Mn, Ni, Pb, and Zn all exhibited their highest concentrations in Al Amarart's liver samples, with significant differences between study areas ($p < 0.01$).

In the muscle tissue, the trend is quite similar, with Al Amarart displaying higher concentrations for most metals. As (0.021 ± 0.017 μg g^{-1}) and Cd (0.041 ± 0.024 μg g^{-1}) again showed significantly elevated levels in Al Amarart compared to the other sites ($p < 0.01$). Cr concentrations remained below the detection limit in all areas, as was the case in the liver, preventing meaningful comparison. Cu concentrations in muscle tissue were not significantly different across the three areas ($p = 0.441$), which contrasts with its variability in liver samples. Interestingly, Co concentrations suggest a difference compared to other metals, as it was found to be higher at the Al Khawd site

Table 1
Descriptive statistics (mean, standard deviation, minimum, and maximum – mg/Kg wet weight) of trace metals analyzed in the muscle and liver tissues of Killifish (*Aphanius stoliczkanus*) samples from Oman. Lowercase superscript letters indicate significant differences in metal concentrations between study areas within the same tissue, as determined by the Tukey post hoc test: a= $p < 0.001$, b=$p < 0.01$; c=$p < 0.05$; Uppercase superscript letters indicate significant differences in metal concentrations between tissues within the same study area, as determined by the Tukey post hoc test A= $p < 0.001$, B=$p < 0.01$; C=$p < 0.05$. n.a: Not Available; n.d.: Not detected.

Element	EC No. 915/2023	FAO 66/ 2003	CODEX STAN 193–1995	Liver			Muscle		
				Al Amarart	Al Khawd	Surur	Al Amarart	Al Khawd	Surur
As	n.a.	n.a.	0.1*	0.055 ± 0.022^{bB} (0.010–0.100)	0.030 ± 0.013 ^{A} (0.010–0.050)	0.030 ± 0.017 ^{A} (0.010–0.060)	0.021 ± 0.017^{b} (0.007–0.06)	0.005 ± 0.003 (<0.004–0.01)	0.008 ± 0.001^{b} (0.006–0.010)
Cd	0.05	0.05	n.a.	0.052 ± 0.030^{aC} (0.010–0.100)	0.021 ± 0.012 (0.008–0.040)	0.011 ± 0.005 (<0.006–0.02)	0.041 ± 0.024^{b} (0.010–0.080)	0.021 ± 0.013 (0.008–0.05)	0.020 ± 0.013^{B} (<0.006–0.05)
Cr	n.a	n.a	n.a	< 0.05 (<0.05–0.08)	< 0.05 (<0.05-<0.05)	< 0.05 (<0.05-<0.05)	< 0.05 (<0.05–0.06)	< 0.05 (<0.05-<0.05)	< 0.05 (<0.05-<0.05)
Co	n.a	n.a	n.a	0.082 ± 0.019 ^{aA} (0.050–0.110)	0.050 ± 0.017 (0.030–0.080)	0.040 ± 0.021^{B} (0.010–0.080)	0.042 ± 0.032^{c} (0.010–0.100)	0.060 ± 0.019^{b} (0.020–0.100)	0.021 ± 0.011^{b} (0.008–0.040)
Cu	n.a	n.a	n.a	0.142 ± 0.030^{cC} (0.100–0.190)	0.140 ± 0.038^{cC} (0.070–0.190)	0.108 ± 0.024^{b} (0.070–0.150)	0.122 ± 0.051 (0.040–0.200)	0.127 ± 0.055 (0.050–0.230)	0.104 ± 0.019 (0.080–0.140)
Hg	0.5	0.5	n.a	0.009 ± 0.002 (<0.005–0.013)	< 0.005 (<0.005–0.007)	< 0.005 (<0.005–0.003)	0.006 ± 0.002 (<0.005–0.010)	< 0.005 (<0.005–0.007)	< 0.005 (<0.005–0.006)
Mn	n.a	n.a	n.a	0.108 ± 0.031^{bC} (0.060–0.170)	0.080 ± 0.015^{cC} (0.050–0.100)	0.059 ± 0.020^{B} (0.020–0.090)	0.084 ± 0.026^{a} (0.050–0.130)	0.049 ± 0.024^{b} (0.010–0.090)	0.022 ± 0.013 (0.010–0.050)
Ni	n.a	n.a	n.a	0.143 ± 0.030^{bB} (0.090–0.200)	0.100 ± 0.021^{cB} (0.060–0.140)	0.087–0.032^{B} (<0.05–0.150)	0.070 ± 0.028^{c} (<0.05–0.100)	0.069 ± 0.025^{c} (<0.05–0.100)	0.044 ± 0.022 (<0.05–0.08)
Pb	0.3	0.2	0.3	0.122 ± 0.024^{bC} (0.080–0.170)	0.105 ± 0.040^{cA} (0.050–0.200)	0.078 ± 0.027^{bA} (0.050–0.130)	0.080 ± 0.028^{a} (0.030–0.130)	0.042 ± 0.020^{b} (0.020–0.090)	0.031 ± 0.014^{b} (0.010–0.050)
Zn	n.a	n.a	n.a	0.324 ± 0.061^{bB}	0.333 ± 0.071^{bB}	0.233 ± 0.048^{B}	0.235 ± 0.047^{a}	0.201 ± 0.048^{a}	0.148 ± 0.039^{b}

* Edible fat and oil

($p < 0.01$). Hg, once again, was found only in muscle tissue of fish sampled in Al Amarart, indicating a slight contamination or a natural higher background Al Amarart. Mn, Pb, and Zn all followed a pattern similar to that seen in liver tissue, with significantly higher concentrations in Al Amarart muscle samples ($p < 0.01$). Ni, on the other hand, was found in higher concentrations in Al Amarart and Al Khawd compared to the levels detected in Surur ($p < 0.05$).

3.2. Risk assessment

Results obtained from the risk assessment are reported in Table 2. The results of the Estimated Daily Intake (EDI), calculated based on a single meal ingestion rate of 227 g, reveal important insights into metal exposure across the three study areas. Notably, for none of the trace elements analyzed, EDI was found to exceed the Reference Dose (RfD), indicating that the exposure levels for these elements remain below thresholds considered potentially harmful to human health. This suggests that the immediate risk from consuming fish in these regions, based on a realistic meal size, is relatively low.

The results regarding the Target Hazard Quotient (THQ) calculations indicate that oral exposure to the trace metals analyzed in the muscle tissue of the fish species does not pose a risk of developing non-carcinogenic effects over the lifetime, as each value of THQ is below 1. This suggests that long-term intake of these metals through fish consumption is unlikely to result in adverse health effects. Furthermore, when calculating the total THQ for each study site, the cumulative values also remain well below 1, with totals of 0.09 for Al Amarart, 0.007 for Al Khawd, and 0.04 for Surur. These findings confirm that combined exposure to all analyzed trace metals at each site does not pose a significant non-carcinogenic health risk.

The Cancer Risk associated with oral exposure to *A. stoliczkanus* was calculated under the assumption that inorganic arsenic constituted 3 %, 10 %, and 25 % of the total arsenic measured in muscle tissue. In all three scenarios and across the three studied sites, the estimated values remained below the Acceptable Risk Level of 1×10^{-6}, indicating that the consumption of this species does not pose a significant carcinogenic risk based on the parameters considered (Table 2). Notably, in the site with the highest total As concentration, the Cancer Risk (CR) calculated under the assumption that inorganic arsenic constituted 25 % of the total arsenic reached a value of 9.70E-07. This corresponds to a probability of 9.7 cases per 10,000,000 inhabitants, which remains below the Acceptable Risk Level of 0.000001. Despite this, the variation in arsenic concentrations across sites highlights the importance of site-specific risk assessments to account for potential localized differences in exposure

4. Discussion

The accumulation of trace elements in freshwater fish is influenced by a complex interplay of biological and environmental factors, including species-specific physiology, trophic interactions, habitat conditions, and the physicochemical properties of the water. Understanding these dynamics is crucial for assessing the potential health risks associated with fish consumption and for monitoring environmental contamination. Aphaniidae is a family of killifishes recognized for its high adaptability to various aquatic habitats [21]; however, like other freshwater fishes, it remains vulnerable to trace elements contamination, particularly in ecosystems exposed to anthropogenic pollution. Among the studied sites, Al Khawd and Al Amarat are located in industrial areas with substantial sewage discharge, whereas Surur is farther from industrial zones and heavy human activity. The latter site is characterized by a relatively unpolluted watercourse, which likely accounts for the lower trace element concentrations found in fish from this location [16,42,46,9].

Overall, our results indicate that trace element concentrations in fish from these regions remain within the safety levels established by regulatory organizations [27,30,29,31]. While regulatory limits primarily apply to Cd, Pb, and Hg, the Codex Alimentarius also sets specific thresholds for As. *Aphanius stoliczkanus* appears to be safe for human consumption based on these standards. However, if whole fish were consumed, Cd intake could be slightly higher at the Al Amarart site, where liver concentrations reach the legal threshold.

Moreover, the detected levels are generally lower than those reported in previous studies examining both freshwater and saltwater fish species from the same geographical area [1,17,42,5,50,53,65,66,72] (Table 3). These differences likely reflect variations in environmental exposure and species-specific bioaccumulation capacities. One possible explanation for the relatively low trace element concentrations in killifish is their position at the lower end of the food chain. Unlike larger predatory species, which tend to accumulate higher levels of contaminants through biomagnification, killifish primarily feed on plankton, small invertebrates, and detritus, resulting in a lower overall bioaccumulation of trace elements. Additionally, factors such as metabolic detoxification mechanisms, growth rates, and habitat-specific water chemistry may also influence accumulation in this species [22].

Once trace elements are absorbed into the organism, the accumulation of metals is influenced by their chemical properties, their endogenous metabolism role if present, and their specific affinity for different tissues, leading to varying concentrations in organs such as the liver, kidneys, and gills [29,46,9]. Commonly, muscle tissue shows a lower accumulation of toxic elements than other tissues [41,42,47,53], while

Table 2
Risk assessment of trace elements in the muscle of Killifish (*Aphanius stoliczkanus*) from Oman. The table presents the average concentrations of metals (mg/kg), the Estimated Daily Intake (EDI) (mg/kg body weight), the Target Hazard Quotient (THQ) (unitless) and the Cancer Risk calculated for As.

Study Areas	Metal	As	Cd	Cr	Co	Cu	Hg	Mn	Ni	Pb	Zn
Al Amarart	Mean	0.021	0.041	<LOD	0.042	0.122	0.006	0.084	0.070	0.080	0.235
	EDI	6.67E−05	1.32E−04	-	1.35E−04	3.95E−04	1.95E−05	2.73E−04	2.27E−04	2.59E−04	7.62E−04
	THQ	8.63E−03	5.05E−02	-	1.73E−02	3.76E−04	8.63E−03	7.40E−05	7.85E−04	3.29E−03	9.66E−05
	$CR_{3\,\%}$	1.16E−07	-	-	-	-	-	-	-	-	-
	$CR_{10\,\%}$	3.88E−07	-	-	-	-	-	-	-	-	-
	$CR_{25\,\%}$	9.70E−07	-	-	-	-	-	-	-	-	-
Al Khawd	Mean	0.005	0.021	<LOD	0.060	0.127	<LOD	0.049	0.069	0.042	0.201
	EDI	1.70E−05	6.70E−05	-	1.95E−04	4.11E−04	-	1.59E−04	2.24E−04	1.35E−04	6.51E−04
	THQ	2.05E−03	2.59E−02	-	2.47E−02	3.91E−04	-	4.32E−05	7.73E−04	1.73E−03	8.26E−05
	$CR_{3\,\%}$	2.77E−08	-	-	-	-	-	-	-	-	-
	$CR_{10\,\%}$	9.25E−08	-	-	-	-	-	-	-	-	-
	$CR_{25\,\%}$	2.31E−07	-	-	-	-	-	-	-	-	-
Surur	Mean	0.008	0.020	<LOD	0.022	0.104	<LOD	0.022	0.046	0.031	0.148
	EDI	2.59E−05	6.57E−05	-	6.97E−05	3.38E−04	-	7.03E−05	1.49E−04	1.00E−04	4.81E−04
	THQ	3.29E−03	2.47E−02	-	9.04E−03	3.21E−04	-	1.94E−05	5.27E−04	1.27E−03	6.08E−05
	$CR_{3\,\%}$	4.44E−08	-	-	-	-	-	-	-	-	-
	$CR_{10\,\%}$	1.48E−07	-	-	-	-	-	-	-	-	-
	$CR_{25\,\%}$	3.70E−07	-	-	-	-	-	-	-	-	-

Table 3
Concentrations of trace elements (mean ± standard deviation or mean range μg g^{-1} wet weight) in muscle of different fish species from Oman.

Species		Study	As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Zn
Freshwater	*Aphanius stoliczkanus*	This study	0.011 ± 0.012	0.027 ± 0.020	0.041 ± 0.028	<LOD	0.118 ± 0.044	<LOD	0.052 ± 0.034	0.060 ± 0.028	0.051 ± 0.030	0.195 ± 0.057
	Garra shamal	[6]	0.012	0.012	0.040	0.0324	0.087	<LOD	0.039	0.058	0.032	0.203
	Aphanius dispar dispar, Oreochromis niloricus, Clarias gariepinus, Poecilia latipinna	[52]*	0.67–1.85	0.35–0.68	NA	0.22–0.53	4.07–0.08	NA	4.09–6.64	0.72–1.72	0.50–1.09	41.1–92.8
Seawater	*Otolithes ruber*	[42]*	NA	0.033	NA	NA	0.121	NA	0.0528	NA	0.4906	0.7612
	Sillago indica, Sillaginopodys chondropus	[17]*	1.21–1.84	0.017–0.019	NA	0.07–0.12	0.55–0.62	0.17–0.19	NA	0.04–0.05	0.27–0.29	9.01–9.33
	Parastromateus niger	[72]	NA	0.004–0.007	NA	NA	NA	NA	NA	NA	0.002–0.003	0.011–0.017
	Tigertooth croaker	*[5]	0.1034	0.00176	0.006	0.0616	0.528	0.0374	0.044	0.0242	0.02574	1.034
	Euthynnus affinis, Katsuwonus pelamis, Thunnus albacares	[65]*	NA	NA	NA	NA	0.51–1.57	NA	NA	NA	0.15–0.28	3.39–19.6
	Otolithes ruber	[64]*	0.55–1.57	14.2–18.6	NA	1.92–2.24	NA	NA	NA	1.15–3.94	0.20–0.41	NA
	Otolithes rube, Sphyraena genie, Rastrelliger kanagurta, Acanthopagrus latus, Trichiurus lepturus	[66]*	0.09–0.27	0.05–0.11	NA	0.09–0.19	0.14–0.99	NA	NA	0.29–0.63	0.12–0.37	2.43–5.87
	Epinephelus coioides	[1]*	NA	0.011	NA	NA	0.0748	NA	NA	NA	0.044	NA
	Scomberoides commersonnianus, Cynoglossus arel	[50]	NA	0.39–0.49	NA	1.20–1.27	0.71–0.82	NA	NA	NA	0.44–0.97	8.84–10.37

* The results were converted from dry weight to wet weight based on a moisture content of 78 % [50].

the liver is a metabolic, active, and excretory tissue and considered a target tissue for elements such as Cd and As [3,4]. Our findings indicate that trace elements, when essential, may exceed the physiological requirements of fish, while toxic elements are actively sequestered by detoxifying organs, leading to their accumulation in the liver in both cases [32], particularly at Al Amarat, the site most impacted by industrial pollution. However, site-specific variations suggest differences in environmental exposure and metal bioavailability. Notably, Cr and Hg were either absent or evenly distributed between liver and muscle across all sites, indicating low environmental contamination. Additionally, Cd showed a uniform distribution between liver and muscle at Al Khawd, while at Surur, it was higher in muscle tissue. This pattern suggests both lower environmental contamination compared to Al Amarat and a more effective homeostatic regulation [51]. These findings emphasize the complex interactions between environmental contamination, metal bioavailability, and specific physiological responses. The variability in metal distribution across sites underscores the importance of site-specific risk assessments and highlights the need for further studies on metal speciation, bioaccumulation dynamics, and metabolic processing in fish.

Although the concentrations of trace elements were found to be low, it is important to emphasize that even small amount of heavy metals or excessively high essential metals, such as copper (Cu) and zinc (Zn), can exert adverse effects on fish health. Some elements such as As, Cd, Pb and Hg, due to their potential high toxicity, persistence, and potential for bioaccumulation, can disrupt physiological processes, impair growth and reproduction, and compromise immune function, even at sublethal concentrations [3]. Arsenic is commonly used in fungicides and insecticides in agriculture, while the concentrations of Pb, Cd, and Hg in aquatic systems primarily originate from mining, metallurgy, oil activities, and urban waste [10,38,57,63]. It was found with significantly higher concentrations at the Al Amarart site and it could be of public concern, as inorganic arsenic is a known potential carcinogen and its toxic effects are largely attributed to its ability to induce oxidative stress, disrupt cellular function, and cause DNA damage, ultimately promoting carcinogenesis [23,45]. However, for a complete risk assessment, it would be essential to further investigate the specific chemical species of arsenic accumulated in the tissues, as different forms of arsenic vary in their toxicity [33]. Pb was the most abundant toxic trace element in fish tissues at all three sites. Pb is the toxic trace element more abundant in biota, freshwater, and marine habitats. It is associated with electronic and painting wastes, the dumping of steel scrap in landfills, and oil activity such as drilling and pumping in the Persian Gulf [17,42,7]. It has previously been observed that Pb levels have decreased since the shift to unleaded gasoline [58]. However, in regions with high oil and metallurgical activity, there are still elevated concentrations of this pollutant in the environment [78]. This is caused by fuel and waste combustion, which releases Pb into the environment, is transported through biogeochemical cycles, and ultimately ends up in aquatic habitats [70]. Certain elements, such as Cd, tend to exhibit greater bioaccumulation in freshwater fish, while As is more prevalent in seawater species [15]. Notably, Cd is among the most toxic trace elements and the primary source of exposure to this contaminant is through trophic chains; it represents a significant health hazard to humans and wildlife [19,49,78]. At concentrations of 1.4 μg g^{-1} —three times higher than those observed in this study—it could induce physiological stress and disrupt the reproductive status of killifish [14]. Regarding Hg levels, it was detected only in the tissues of fish sampled at the industrial site of Al Amarart, though not at concentrations considered harmful to human health. However, Hg levels, like those of other trace elements, can fluctuate based on trophic level, reproductive stage, and seasonal variations, all of which are influenced by natural and anthropogenic factors or element speciation [20]. For instance, rising water temperatures can enhance the methylation of Hg, leading to the formation of methylmercury ($MeHg^{+}$), the most toxic form of mercury, which typically accumulates in fish muscle (accounting for 70–100 % of total Hg) [28].

Regarding the risk assessment conducted, the results are favorable, indicating that exposure levels to the analyzed trace elements remain within safe limits. This study found that the accumulation of trace elements in the muscle of *Aphanius stoliczkanus* from Oman was relatively low, further supporting its safety for human consumption. Our findings suggest no significant risk associated with regular dietary intake. The Estimated Daily Intake (EDI) provides an immediate exposure estimate, reflecting the intake based on a typical fish portion consumed in a single meal. This approach is particularly relevant for assessing short-term exposure, especially for individuals who do not regularly consume fish but may experience higher contaminant intake from an occasional meal. Meanwhile, the Target Hazard Quotient (THQ) for each trace metals analyzed consistently remained below 1, indicating that the non-carcinogenic health risks associated with consuming *A. stoliczkanus* from the study areas in Oman are minimal. A THQ below 1 suggests that the daily intake of these metals through fish consumption is unlikely to cause adverse health effects over a lifetime. Importantly, the sum of the THQs for all metals also remained below the safety threshold of 1, further supporting the conclusion that cumulative exposure does not pose a significant health risk. This finding is particularly relevant, as it highlights that even in environments where trace metal contamination is a concern, exposure levels through the consumption of this species remain within internationally recognized safety limits. These results align with guidelines from global health and environmental agencies, reinforcing the conclusion that consuming *A. stoliczkanus* does not pose a significant health risk from trace metal contamination.

The results of the Cancer Risk (CR) assessment for arsenic (As) offer valuable insights into the potential long-term health risks associated with the consumption of *Aphanius stoliczkanus*. Arsenic is a well-recognized human carcinogen, as extensively documented in the scientific literature, and its presence in food sources warrants careful evaluation. Numerous studies have demonstrated that prolonged exposure to arsenic, particularly through contaminated water and food sources, can lead to an increased risk of developing various cancers, including skin, lung, bladder, and liver cancer [18,44,54,71]. The International Agency for Research on Cancer (IARC) classifies arsenic and arsenic compounds as Group 1 carcinogens, indicating sufficient evidence of their carcinogenicity to humans. The findings suggest that the long-term cancer risk associated with the ingestion of *A. stoliczkanus* is low, given that the estimated exposure to inorganic arsenic does not exceed the established threshold for Cancer Risk. However, vigilance remains essential, as carcinogenic risk from dietary exposure should not be assessed in isolation but rather within the broader context of cumulative exposure.

5. Conclusions

Aphanius stoliczkanus has proven to be an excellent bioindicator, effectively reflecting trace element levels in both industrial and non-industrial areas of Oman. This study confirms that fish from industrialized regions accumulate higher trace element concentrations compared to those from less impacted areas. However, the levels detected in *A. stoliczkanus* from Al Amarart, Al Khawd, and Surur were lower than those reported in other fish species from the region, which in turn exhibit lower concentrations than fish from heavily contaminated areas such as the Persian Gulf. Notably, while trace element concentrations in *A. stoliczkanus* remained within safe limits for the Target Hazard Quotient (THQ) and Cancer Risk (CR) assessments, metals such as arsenic warrant attention from regulatory bodies. Moreover, the potential synergistic effects of multiple contaminants—ingested through other dietary sources or absorbed via alternative exposure pathways—could amplify long-term health risks. Individual susceptibility factors, such as genetic predisposition and variations in arsenic metabolism, may further modulate these effects.

To mitigate contamination risks, targeted policy measures and environmental strategies should be implemented. Regular monitoring programs should track trace element levels in aquatic ecosystems, considering both small-sized bioindicators like *A. stoliczkanus* and higher trophic levels to assess biomagnification effects. Strengthening industrial regulations to limit heavy metal discharge into freshwater bodies, alongside sustainable waste management practices, is essential. Additionally, habitat restoration initiatives—such as wetland buffer zones and phytoremediation using aquatic plants—could aid in pollutant filtration. Public awareness campaigns and community engagement should be encouraged to promote responsible waste disposal and industrial compliance. Future research should integrate these mitigation strategies while continuing to assess the ecological and human health risks posed by trace element contamination in freshwater systems in Oman.

CRediT authorship contribution statement

Adel Milad: Writing – original draft, Investigation, Conceptualization. **Al Jufaili Saud M.:** Funding acquisition, Data curation, Conceptualization. **Ley-Quiñonez Cesar P:** Writing – review & editing, Formal analysis, Data curation. **Ferrante Margherita:** Writing – review & editing, Supervision. **Grasso Alfina:** Writing – original draft, Methodology. **Copat Chiara:** Writing – review & editing, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Milad Adel reports financial support was provided by Sultan Qaboos University. Milad Adel reports a relationship with Sultan Qaboos University that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2025.102023.

Data availability

Data will be made available on request.

References

[1] S.A. Abdul-Wahab, I.S. Al-Husaini, A. Rahmalan, Using grouper fish as bio-indicator of Cd, Cu, Pb and V in the vicinity of a single buoy mooring (SBM3) at Mina Al Fahal in the Sultanate of Oman, Bull. Environ. Contam. Toxicol. 91 (2013) 684–688, https://doi.org/10.1007/s00128-013-1126-0.

[2] M. Adel, C. Copat, G. Oliveri Conti, F. Sakhaie, Z. Hashemi, G. Mancini, A. Cristaldi, M. Ferrante, Trace elements in the muscle tissue of Hemiculter leucisculus and Abramis brama orientalis from the Anzali International wetland, south-west of Caspian Sea: an exposure risk assessment, Mar. Pollut. Bull. 180 (2022) 113756, https://doi.org/10.1016/j.marpolbul.2022.113756.

[3] M. Adel, K. Mohammadmoradi, C.P. Ley-Quiñonez, Trace element concentrations in muscle tissue of milk shark, (Rhizoprionodon acutus) from the Persian Gulf, Environ. Sci. Pollut. Res. Int. 24 (2017) 5933–5937, https://doi.org/10.1007/s11356-016-8358-6.

[4] M. Adel, H.N. Saravi, M. Dadar, L. Niyazi, C.P. Ley-Quinonez, Mercury, lead, and cadmium in tissues of the Caspian Pond Turtle (Mauremys caspica) from the southern basin of Caspian Sea, Environ. Sci. Pollut. Res. Int. 24 (2017) 3244–3250, https://doi.org/10.1007/s11356-015-5905-5.

[5] H. Agah, Ecological risk assessment of heavy metals in sediment, fish, and human hair from Chabahar Bay, Makoran, Iran, Mar. Pollut. Bull. 169 (2021) 112345, https://doi.org/10.1016/j.marpolbul.2021.112345.

[6] S.M. Al Jufaili, M. Adel, S.P.H. Shekarabi, C. Copat, J. Velisek, Trace elements in the muscle and liver tissues of Garra shamal from the freshwater ecosystem of Oman: an exposure risk assessment, Environ. Sci. Pollut. Res. 31 (2024) 15199–15208, https://doi.org/10.1007/s11356-024-32229-w.

[7] S.A.H. Al Raisi, H. Sulaiman, F.E. Suliman, O. Abdallah, Assessment of heavy metals in leachate of an unlined landfill in the Sultanate of Oman, Int. J. Environ. Sci. Dev. 5 (2014) 60.

[8] M.A. Al-Busaidi, D.J. Jukes, S. Bose, Seafood safety and quality: an analysis of the supply chain in the Sultanate of Oman, Food Control 59 (2016) 651–662, https://doi.org/10.1016/j.foodcont.2015.06.023.

[9] M. Al-Busaidi, P. Yesudhason, S. Al-Mughairi, W. a K. Al-Rahbi, K.S. Al-Harthy, N. A. Al-Mazrooei, S.H. Al-Habsi, Toxic metals in commercial marine fish in Oman with reference to national and international standards, Chemosphere 85 (2011) 67–73, https://doi.org/10.1016/j.chemosphere.2011.05.057.

[10] H. Ali, E. Khan, Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—concepts and implications for wildlife and human health, Hum. Ecol. Risk Assess. Int. J. 25 (2019) 1353–1376, https://doi.org/10.1080/10807039.2018.1469398.

[11] B. Ali-El-Dein, M. Abdelgawad, M. Tarek, M. Abdel-Rahim, M.E. Elkady, H. H. Saleh, M.M. Zakaria, H.H. Tarabay, M. Laymon, A. Mosbah, A. Stenzl, Bladder cancer associated with elevated heavy metals: investigation of probable carcinogenic pathways through mitochondrial dysfunction, oxidative stress and mitogen-activated protein kinase, Urol. Oncol. 43 (2025) 190.e11–190.e20, https://doi.org/10.1016/j.urolonc.2024.09.009.

[12] Aljuboury, D.A., Palaniandy, P., Maqbali, K.S.A.A., 2019. Evaluating of performance of landfills of waste in Al-Amerat and Barka, in Oman. Mater. Today In: Proc., 6th International Conference on Recent Advances in Materials, Minerals & Environment (RAMM) 2018, RAMM 2018, 27 - 29 November 2018, Penang, Malaysia 17, 1152–1160. https://doi.org/10.1016/j.matpr.2019.06.546.

[13] S. Al-Mughairi, P. Yesudhason, M. Al-Busaidi, A. Al-Waili, W.A.K. Al-Rahbi, N. Al-Mazrooei, S.H. Al-Habsi, Concentration and exposure assessment of mercury in commercial fish and other seafood marketed in Oman, J. Food Sci. 78 (2013), https://doi.org/10.1111/1750-3841.12150.

[14] A. Annabi, K. Said, I. Messaoudi, Heavy metal levels in gonad and liver tissues-effects on the reproductive parameters of natural populations of Aphanius facsiatus, Environ. Sci. Pollut. Res. Int. 20 (2013) 7309–7319, https://doi.org/10.1007/s11356-013-1745-3.

[15] M. Ariyaee, A.H. Hamidian, S. Eagderi, S. Ashrafi, M. Khazaee, Comparative STudy of Cadmiumand Arsenic Accumulation in Toothed Carp (Aphanius sophiae)in fresh and salt water, Iran. J. Toxicol. 9 (2015) 1342–1346.

[16] S.M. Azam, M. Naeem, N. Ahmad, R. Yaqoob, M. Khalid, V. Lal, Elemental composition of Talang Queenfish (Scomberoides commersonnianus Lacépède, 1801) in relation to body size and condition factor from Pakistan, Braz. J. Biol. Rev. Brasleira Biol. 84 (2022) e262533, https://doi.org/10.1590/1519-6984.262533.

[17] H.K. Barani, M.S. Alavi-Yeganeh, A.R. Bakhtiari, Metals bioaccumulation, possible sources and consumption risk assessment in five Sillaginid species, a case study: Bandar Abbas (Persian Gulf) and Chabahar Bay (Oman Sea), Iran, Mar. Pollut. Bull. 187 (2023) 114551, https://doi.org/10.1016/j.marpolbul.2022.114551.

[18] J. Boyle, M.H. Ward, S. Koutros, M.R. Karagas, M. Schwenn, A.T. Johnson, D. T. Silverman, D.C. Wheeler, Modeling historic arsenic exposures and spatial risk for bladder cancer, Stat. Biosci. 16 (2024) 377–394, https://doi.org/10.1007/s12561-023-09404-7.

[19] M. Camacho, J. Orós, L.D. Boada, A. Zaccaroni, M. Silvi, C. Formigaro, P. López, M. Zumbado, O.P. Luzardo, Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (*Caretta caretta*): Results from a nesting colony from Cape Verde, West Africa, Mar. Environ. Res. 92 (2013) 15–22, https://doi.org/10.1016/j.marenvres.2013.08.002.

[20] M.I. Castro-González, M. Méndez-Armenta, Heavy metals: implications associated to fish consumption, Environ. Toxicol. Pharmacol. 26 (2008) 263–271, https://doi.org/10.1016/j.etap.2008.06.001.

[21] E.A. Charmpila, A. Teimori, J. Freyhof, A. Weissenbacher, B. Reichenbacher, New osteological and morphological data of four species of Aphaniops (Teleostei; Aphaniidae), J. Appl. Ichthyol. 36 (2020) 724–736, https://doi.org/10.1111/jai.14074.

[22] H.Y. Çoğun, T.A. Yüzereroğlu, Ö. Firat, G. Gök, F. Kargin, Metal concentrations in fish species from the Northeast Mediterranean Sea, Environ. Monit. Assess. 121 (2006) 431–438, https://doi.org/10.1007/s10661-005-9142-0.

[23] K.L. Cooper, R. Liu, X. Zhou, Particulate arsenic trioxide induces higher DNA damage and reactive oxygen species than soluble arsenite in lung epithelial cells, Toxicol. Appl. Pharmacol. 457 (2022) 116320, https://doi.org/10.1016/j.taap.2022.116320.

[24] C. Copat, M. Rizzo, A. Zuccaro, A. Grasso, P. Zuccarello, M. Fiore, G. Mancini, M. Ferrante, Metals/metalloids and oxidative status markers in saltwater fish from the ionic coast of Sicily, Mediterranean Sea, Int. J. Environ. Res. 14 (2020) 15–27, https://doi.org/10.1007/s41742-019-00237-1.

[25] M. Dadar, R. Peyghan, H.R. Memari, Evaluation of the bioaccumulation of heavy metals in white shrimp (Litopenaeus vannamei) along the Persian Gulf Coast, Bull. Environ. Contam. Toxicol. 93 (2014) 339–343, https://doi.org/10.1007/s00128-014-1334-2.

[26] H.R. Esmaeili, S.A. Jufaili, A.H. Masoumi, F. Zarei, Ichthyodiversity in southeastern Arabian Peninsula: annotated checklist, taxonomy, short description and distribution of Inland fishes of Oman, Zootaxa 5134 (2022) 451–503, https://doi.org/10.11646/zootaxa.5134.4.1.

[27] EU, 2023. Regulation - 2023/915 - EN - EUR-Lex.

[28] D.C. Evers, J.T. Ackerman, S. Åkerblom, D. Bally, N. Basu, K. Bishop, N. Bodin, H.F. V. Braaten, M.E.H. Burton, P. Bustamante, C. Chen, J. Chételat, L. Christian, R. Dietz, P. Drevnick, C. Eagles-Smith, L.E. Fernandez, N. Hammerschlag, M. Harmelin-Vivien, A. Harte, E.M. Krümmel, J.L. Brito, G. Medina, C.A. Barrios Rodriguez, I. Stenhouse, E. Sunderland, A. Takeuchi, T. Tear, C. Vega, S. Wilson, P. Wu, Global mercury concentrations in biota: their use as a basis for a global biomonitoring framework, Ecotoxicology 33 (2024) 325–396, https://doi.org/10.1007/s10646-024-02747-x.

[29] FAO, 1983. Compilation of legal limits for hazardous substances in fish and fishery products. Fisheries Circular. No. 764. | منظمة الأغذية والزراعة | مصايد الأسماك الداخلية للأمم المتحدة [WWW Document]. URL https://www.fao.org/inland-fisheries/topic/detail/ar/c/1150083/ (accessed 3.15.25).

[30] FAO, 2003. Heavy Metals Regulations (L.N. No. 66 of 2003). | FAOLEX.

[31] FDA, 2025. Alerts, Advisories & Safety Information [WWW Document]. FDA. URL https://www.fda.gov/food/recalls-outbreaks-emergencies/alerts-advisories-safety-information (accessed 3.17.25).

[32] D. Fernandes, M.J. Bebianno, C. Porte, Hepatic levels of metal and metallothioneins in two commercial fish species of the Northern Iberian shelf, Sci. Total Environ. 391 (2008) 159–167, https://doi.org/10.1016/j.scitotenv.2007.10.057.

[33] M. Ferrante, S. Napoli, A. Grasso, P. Zuccarello, A. Cristaldi, C. Copat, Systematic review of arsenic in fresh seafood from the Mediterranean Sea and European Atlantic coasts: a health risk assessment, Food Chem. Toxicol. 126 (2019) 322–331, https://doi.org/10.1016/j.fct.2019.01.010.

[34] M. Ferrante, M. Vassallo, A. Mazzola, M.V. Brundo, R. Pecoraro, A. Grasso, C. Copat, In vivo exposure of the marine sponge Chondrilla nucula Schmidt, 1862 to cadmium (Cd), copper (Cu) and lead (Pb) and its potential use for bioremediation purposes, Chemosphere 193 (2018) 1049–1057, https://doi.org/10.1016/j.chemosphere.2017.11.144.

[35] J. Freyhof, A. Weissenbacher, M. Geiger, Aphanius kruppi, a new killifish from Oman with comments on the A. dispar species group (Cyprinodontiformes: Aphaniidae), Zootaxa 4338 (2017) 557–573, https://doi.org/10.11646/zootaxa.4338.3.10.

[36] X. Gao, X. Zheng, X. Wang, Z. Li, L. Yang, Environmental pollutant exposure and adverse neurodevelopmental outcomes: an umbrella review and evidence grading of meta-analyses, J. Hazard. Mater. 491 (2025) 137832, https://doi.org/10.1016/j.jhazmat.2025.137832.

[37] I. Giovos, M.V. Brundo, N. Doumpas, Z. Kazlari, D. Loukovitis, D.K. Moutopoulos, R.N.A. Spyridopoulou, A. Papadopoulou, M. Papapetrou, F. Tiralongo, M. Ferrante, C. Copat, Trace elements in edible tissues of elasmobranchs from the North Aegean Sea (Eastern Mediterranean) and potential risks from consumption, Mar. Pollut. Bull. 184 (2022) 114129, https://doi.org/10.1016/j.marpolbul.2022.114129.

[38] A.M. Góngora-Gómez, M. García-Ulloa, N.P. Muñoz-Sevilla, A.L. Domínguez-Orozco, B.P. Villanueva-Fonseca, J.A. Hernández-Sepúlveda, R. Ortega Izaguirre, A.M. Góngora-Gómez, M. García-Ulloa, N.P. Muñoz-Sevilla, A.L. Domínguez-Orozco, B.P. Villanueva-Fonseca, J.A. Hernández-Sepúlveda, R. Ortega Izaguirre, Heavy-metal contents in oysters (Crassostrea gigas) cultivated on the southeastern coast of the Gulf of California, Mexico, Hidrobiológica 27 (2017) 219–227.

[39] T. Guo, M.L. Najafi, J. Zhang, A systematic review of exposure to toxic elements and neurocognitive development in children, Ecotoxicol. Environ. Saf. 291 (2025) 117792, https://doi.org/10.1016/j.ecoenv.2025.117792.

[40] S.S. Habib, S. Maqaddas, F. Fazio, R. El Amouri, G.S. Shaikh, A. Rahim, K. Khan, J. Ullah, M. Mohany, V. Parrino, A. Al-Emam, Evaluation of lead exposure effects on tissue accumulation, behavior, morphological and hemato-biochemical changes in common carp, Cyprinus carpio, J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. GMS 86 (2024) 127523, https://doi.org/10.1016/j.jtemb.2024.127523.

[41] G.M.M.A. Hasan, A.K. Das, M.A. Satter, M. Asif, Distribution of Cr, Cd, Cu, Pb and Zn in organs of three selected local fish species of Turag river, Bangladesh and impact assessment on human health, Emerg. Contam. 9 (2023) 100197, https://doi.org/10.1016/j.emcon.2022.11.002.

[42] H. Hosseini, M. NaderI, S. Gholami, M. Hadipour, Toxic metals in the muscle and liver of five main commercially- important fishes from the Persian Gulf, Southern Iran, Casp. J. Environ. Sci. 16 (2018) 191–198.

[43] JFCFA, 1988. Evaluation of certain food additives and contaminants: thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives [WWW Document]. URL https://www.who.int/publications/i/item/9241207760 (accessed 3.15.25).

[44] J.-R. Jhuang, C.-H. Lee, C.-J. Chiang, C.-J. Chen, W.-C. Lee, Reduced burden of arsenic-related cancers after water mitigation in Taiwan, Environ. Int. 185 (2024) 108542, https://doi.org/10.1016/j.envint.2024.108542.

[45] A.H. Khoshakhlagh, M. Mohammadzadeh, A. Gruszecka-Kosowska, The preventive and carcinogenic effect of metals on cancer: a systematic review, BMC Public Health 24 (2024) 2079, https://doi.org/10.1186/s12889-024-19585-5.

[46] N. Kumar, N.K. Chandan, S. Bhushan, D.K. Singh, S. Kumar, Health risk assessment and metal contamination in fish, water and soil sediments in the East Kolkata Wetlands, India, Ramsar site, Sci. Rep. 13 (2023) 1546, https://doi.org/10.1038/s41598-023-28801-y.

[47] M. Latif, M. Zahoor, A. Muhammad, S. Naz, A.W. Kamran, R. Ullah, A.B. Shah, R. Almeer, A. Sayed, Bioaccumulation of lead in different organs of Ctenopharyngodon idella (grass fish) and Tor putitora (Mahseer) fish, Braz. J. Biol. Rev. Brasleira Biol. 84 (2022) e260355, https://doi.org/10.1590/1519-6984.260355.

[48] C. Lau, X. Lu, X. Chen, K.S. Hoy, T. Davydiuk, J.A. Graydon, M. Reichert, A. LeBlanc, C. Donadt, G. Jhangri, X.C. Le, Arsenic speciation in more than 1600

freshwater fish samples from fifty-three waterbodies in Alberta, Canada, J. Environ. Sci. 153 (2025) 289–301, https://doi.org/10.1016/j.jes.2024.12.009.
[49] C.P. Ley-Quiñónez, N.A. Rossi-Lafferriere, T.L. Espinoza-Carreon, C.E. Hart, S. H. Peckham, A.A. Aguirre, A.A. Zavala-Norzagaray, Associations between trace elements and clinical health parameters in the North Pacific loggerhead sea turtle (Caretta caretta) from Baja California Sur, Mexico, Environ. Sci. Pollut. Res. Int. 24 (2017) 9530–9537, https://doi.org/10.1007/s11356-017-8556-x.
[50] M. Loghmani, M.M. Tootooni, S. Sharifian, Risk assessment of trace element accumulation in two species of edible commercial fish *Scomberoides commersonnianus* and *Cynoglossus arel* from the northern waters of the Oman Sea, Mar. Pollut. Bull. 174 (2022) 113201, https://doi.org/10.1016/j.marpolbul.2021.113201.
[51] M. López-Alonso, M. Miranda, I. Orjales, V. Pereira, B. Larrán, R. Rodríguez-Bermúdez, C. Veiras, Á. Robles, D. Bravo, R. Juncosa, C. Herrero-Latorre, Biomonitoring toxic and essential element accumulation in trout in the as pontes mine pit lake (NW Spain), Chemosphere 374 (2025) 144212, https://doi.org/10.1016/j.chemosphere.2025.144212.
[52] S. Mahboob, H.F. Alkkahem Al-Balwai, F. Al-Misned, K.A. Al-Ghanim, A study on the accumulation of nine heavy metals in some important fish species from a natural reservoir in Riyadh, Saudi Arabia, Toxicol. Environ. Chem. 96 (2014) 783–798, https://doi.org/10.1080/02772248.2014.957485.
[53] S. Mahboob, H.F. Alkkahem Al-Balwai, F. Al-Misned, K.A. Al-Ghanim, Z. Ahmad, A study on the accumulation of nine heavy metals in some important fish species from a natural reservoir in Riyadh, Saudi Arabia, Toxicol. Environ. Chem. 96 (2014) 783–798, https://doi.org/10.1080/02772248.2014.957485.
[54] D.R.S. Middleton, M.J. Watts, E.M. Hamilton, T. Fletcher, G.S. Leonardi, R. M. Close, K.S. Exley, H. Crabbe, D.A. Polya, Prolonged exposure to arsenic in UK private water supplies: toenail, hair and drinking water concentrations, Environ. Sci. Process. Impacts 18 (2016) 562–574, https://doi.org/10.1039/C6EM00072J.
[55] H. Monji, Amin Nematollahi, Chiara Copat, Margherita Ferrante, A.A. Fallah, A comparison of the metals and metalloid levels in wild and cultured Capoeta damascina fish and assessment of its potential health risks to humans in Iran, Toxin Rev. 41 (2022) 1179–1190, https://doi.org/10.1080/15569543.2021.1987265.
[56] N.A. Nasir, G.A. Al-Najar, Seasonal variations of heavy metal concentrations in tissues of Talang Queenfish (Scomberoides commersonianus) from Iraqi marine and coastal waters, Northwestern Arabian Gulf, Mesop. J. Mar. Sci. 30 (2015) 112–123, https://doi.org/10.58629/mjms.v30i2.114.
[57] F. Páez-Osuna, S. Álvarez-Borrego, A.C. Ruiz-Fernández, J. García-Hernández, M. E. Jara-Marini, M.E. Bergés-Tiznado, A. Piñón-Gimate, R. Alonso-Rodríguez, M. F. Soto-Jiménez, M.G. Frías-Espericueta, J.R. Ruelas-Inzunza, C.R. Green-Ruiz, C. C. Osuna-Martínez, J.-A. Sanchez-Cabeza, Environmental status of the Gulf of California: a pollution review, Earth-Sci. Rev. 166 (2017) 181–205, https://doi.org/10.1016/j.earscirev.2017.01.014.
[58] F. Páez-Osuna, M.F. Calderón-Campuzano, M.F. Soto-Jiménez, J.R. Ruelas-Inzunza, Lead in blood and eggs of the sea turtle, *Lepidochelys olivacea*, from the Eastern Pacific: concentration, isotopic composition and maternal transfer, Mar. Pollut. Bull. 60 (2010) 433–439, https://doi.org/10.1016/j.marpolbul.2009.10.004.
[59] B.P. Panda, Y.K. Mohanta, S.P. Parida, A. Pradhan, T.K. Mohanta, K. Patowary, W. A. Wan Mahari, S.S. Lam, A.A. Ghfar, G. Guerriero, M. Verma, H. Sarma, Metal pollution in freshwater fish: A key indicator of contamination and carcinogenic risk to public health, Environ. Pollut. 330 (2023) 121796, https://doi.org/10.1016/j.envpol.2023.121796.
[60] P. Rapisarda, G.O. Conti, E. Pulvirenti, A. Cristaldi, C. Favara, M. Fiore, C. Copat, A. Grasso, M. Castrogiovanni, G. Mancini, M. Ferrante, Ecotoxicological evaluation of urban wastewater treatment plants: a Sicilian study, Ecotoxicology (2024), https://doi.org/10.1007/s10646-024-02840-1.
[61] N.M. Reid, D.A. Proestou, B.W. Clark, W.C. Warren, J.K. Colbourne, J.R. Shaw, S. I. Karchner, M.E. Hahn, D. Nacci, M.F. Oleksiak, D.L. Crawford, A. Whitehead, The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish, Science 354 (2016) 1305–1308, https://doi.org/10.1126/science.aah4993.
[62] R. Roila, A. Piersanti, A. Valiani, D. Ranucci, T. Tavoloni, A. Stramenga, F. Griffoni, L. Pittura, S. Gorbi, R. Franceschini, F. Agnetti, G. Palma, R. Branciari, Carassius auratus as a bioindicator of the health status of Lake Trasimeno and risk assessment for consumers, Ital. J. Food Saf. 12 (2023) 11137, https://doi.org/10.4081/ijfs.2023.11137.
[63] J. Ruelas-Inzunza, G. Meza-López, F. Páez-Osuna, Mercury in fish that are of dietary importance from the coasts of Sinaloa (SE Gulf of California), J. Food Compos. Anal. 21 (2008) 211–218, https://doi.org/10.1016/j.jfca.2007.11.004.
[64] P. Sadeghi, M. Loghmani, E. Afsa, Trace element concentrations, ecological and health risk assessment in sediment and marine fish Otolithes ruber in Oman Sea, Iran, Mar. Pollut. Bull. 140 (2019) 248–254, https://doi.org/10.1016/j.marpolbul.2019.01.048.
[65] P. Sadeghi, M. Loghmani, S. Frokhzad, Human health risk assessment of heavy metals via consumption of commercial marine fish (Thunnus albacares, Euthynnus affinis, and Katsuwonus pelamis) in Oman Sea, Environ. Sci. Pollut. Res. 27 (2020) 14944–14952, https://doi.org/10.1007/s11356-020-07907-0.
[66] P. Sadeghi, M. Loghmani, D.J. Yousuf, Z. Taghizadeh Rahmat Abadi, Ecological and human health risk assessment of trace element pollution in sediments and five important commercial fishes of the Oman Sea, Mar. Pollut. Bull. 173 (2021) 112962, https://doi.org/10.1016/j.marpolbul.2021.112962.
[67] A. Salvaggio, R. Pecoraro, C. Copat, M. Ferrante, A. Grasso, E.M. Scalisi, S. Ignoto, V.S. Bonaccorsi, G. Messina, B.M. Lombardo, F. Tiralongo, M.V. Brundo, Bioaccumulation of Metals/Metalloids and Histological and Immunohistochemical Changes in the Tissue of the European Hake, Merluccius merluccius (Linnaeus, 1758) (Pisces: Gadiformes: Merlucciidae), for Environmental Pollution Assessment, J. Mar. Sci. Eng. 8 (2020) 712, https://doi.org/10.3390/jmse8090712.
[68] C.A. Sayer, E. Fernando, R.R. Jimenez, N.B.W. Macfarlane, G. Rapacciuolo, M. Böhm, T.M. Brooks, T. Contreras-MacBeath, N.A. Cox, I. Harrison, M. Hoffmann, R. Jenkins, K.G. Smith, J.-C. Vié, J.C. Abbott, D.J. Allen, G.R. Allen, V. Barrios, J.-P. Boudot, S.F. Carrizo, P. Charvet, V. Clausnitzer, L. Congiu, K. A. Crandall, N. Cumberlidge, A. Cuttelod, J. Dalton, A.G. Daniels, S. De Grave, G. De Knijf, K.-D.B. Dijkstra, R.A. Dow, J. Freyhof, N. García, J. Gessner, A. Getahun, C. Gibson, M.J. Gollock, M.I. Grant, A.E.R. Groom, M.P. Hammer, G. A. Hammerson, C. Hilton-Taylor, L. Hodgkinson, R.A. Holland, R.W. Jabado, D. Juffe Bignoli, V.J. Kalkman, B.K. Karimov, J. Kipping, M. Kottelat, P.A. Lalèyè, H.K. Larson, M. Lintermans, F. Lozano, A. Ludwig, T.J. Lyons, L. Máiz-Tomé, S. Molur, H.H. Ng, C. Numa, A.F. Palmer-Newton, C. Pike, H.E. Pippard, C.N. M. Polaz, C.M. Pollock, R. Raghavan, P.S. Rand, T. Ravelomanana, R.E. Reis, C. L. Rigby, J.A. Scott, P.H. Skelton, M.R. Sloat, J. Snoeks, M.L.J. Stiassny, H.H. Tan, Y. Taniguchi, E.B. Thorstad, M.F. Tognelli, A.G. Torres, Y. Torres, D. Tweddle, K. Watanabe, J.R.S. Westrip, E.G.E. Wright, E. Zhang, W.R.T. Darwall, One-quarter of freshwater fauna threatened with extinction, Nature 638 (2025) 138–145, https://doi.org/10.1038/s41586-024-08375-z.
[69] M.A.M Siddique, Md.S. Hossain, Md.M. Islam, M. Rahman, G. Kibria, Heavy metals and metalloids in edible seaweeds of Saint Martin's Island, Bay of Bengal, and their potential health risks, Mar. Pollut. Bull. 181 (2022) 113866, https://doi.org/10.1016/j.marpolbul.2022.113866.
[70] M.F. Soto-Jiménez, C. Arellano-Fiore, R. Rocha-Velarde, M.E. Jara-Marini, J. Ruelas-Inzunza, F. Páez-Osuna, Trophic transfer of lead through a model marine four-level food chain: tetraselmis suecica, Artemia franciscana, Litopenaeus vannamei, and Haemulon scudderi, Arch. Environ. Contam. Toxicol. 61 (2011) 280–291, https://doi.org/10.1007/s00244-010-9620-4.
[71] R.M. Speer, S.P. Nandi, K.L. Cooper, X. Zhou, H. Yu, Y. Guo, L.G. Hudson, L. B. Alexandrov, K.J. Liu, Arsenic is a potent co-mutagen of ultraviolet light, Commun. Biol. 6 (2023) 1–11, https://doi.org/10.1038/s42003-023-05659-4.
[72] N. Tabezar, P. Sadeghi, G. Attaran Fariman, Monsoon effect on heavy metal and chemical composition in parastromateus niger of the Oman Sea: health risk assessment of fish consumption, Biol. Trace Elem. Res. 201 (2023) 4093–4102, https://doi.org/10.1007/s12011-022-03475-1.
[73] R. Ullah, S. Mohiuddin, K. Yasmeen, E. Zahir, M.A. Ahmed, Risk assessment, interdependencies, and source appraisal of bioaccumulated heavy and essential metals in seafood as pollutants, Biol. Trace Elem. Res. 202 (2024) 3800–3809, https://doi.org/10.1007/s12011-023-03949-w.
[74] US EPA, 2015. Regional Screening Levels (RSLs) [WWW Document]. URL https://www.epa.gov/risk/regional-screening-levels-rsls (accessed 3.15.25).
[75] US-EPA, 1987. Tetraethyl lead CASRN 78-00-2 | IRIS | US EPA, ORD [WWW Document]. URL https://iris.epa.gov/ChemicalLanding/&substance_nmbr= 109 (accessed 3.15.25).
[76] WHO, 1981. WHO. Arsenic, Environmental Health Criteria. Vol. 18. Geneva: 1981.
[77] Y. Yan, L. Jin, J. Li, G. Chen, Association of cadmium and lead exposure with mortality in cancer survivors: a prospective cohort study, Ecotoxicol. Environ. Saf. 292 (2025) 117960, https://doi.org/10.1016/j.ecoenv.2025.117960.
[78] K.A. Zavala-Félix, M.A. Reyes-López, F.Y. Camacho-Sánchez, H.H. Acosta-Sánchez, C.E. Hart, A.A. Zavala-Norzagaray, V. Leal-Sepúlveda, R. Leal-Moreno, B. A. Espinoza-Romo, A.A. Aguirre, C.P. Ley-Quiñónez, Trace elements concentration in blood of nesting Kemp's Ridley turtles (Lepidochelys kempii) at Rancho Nuevo sanctuary, Tamaulipas, Mexico, PLOS One 17 (2022) e0269346, https://doi.org/10.1371/journal.pone.0269346.