



Heavy metals bioaccumulation in fish of southern Iran and risk assessment of fish consumption

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Abstract

Background: This study purposed to assess the health risks of heavy metals including nickel (Ni), chromium (Cr), mercury (Hg), and lead (Pb) in three widely-consumed fish from the coast of Bandar Abbas (Persian Gulf) and to determine the maximum amounts allowed for consumption according to the degree of contamination.

Methods: This cross-sectional study was conducted in the summer and fall of 2013. *Thunnus tonggol* and *Liza klunzingeri* were selected from the Bandar Abbas market, and *Pleuronectiformes* were caught from the sea. Heavy metal concentrations were determined by atomic absorption. Risk assessment and allowable consumption levels were determined according to methods provided by the US Environmental Protection Agency (USEPA).

Results: The highest average levels of Ni and Cr in *Thunnus tonggol* were 0.059 and 0.234 ($\mu\text{g/g}$ dry weight), respectively. The highest average level of Hg in *Pleuronectiformes* was 0.095 ($\mu\text{g/g}$ dry weight), and the highest average level of Pb in *Liza klunzingeri* was 0.006 ($\mu\text{g/g}$ dry weight). The target hazard quotient (THQ) for all analyses of metals in the studied species and the hazard index (HI) were less than 1 for all three species.

Conclusion: The results showed that despite Ni, Cr, Hg, and Pb pollution, the consumption of up to 4 (kg/d) of *Thunnus tonggol* by adults and up to 2.2 (kg/d) by children does not cause health problems in terms of the tested heavy metals. For *Liza klunzingeri*, these levels are 7.32 and 4.02 (kg/d), respectively.

Keywords: Nickel; Chromium; Mercury; fish; Risk assessment.

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Introduction

The presence of heavy metals in the environment is a cause for major concern. Even at low concentration levels, they can cause problems as a result of toxicity and their tendency to permeate the food chain (1). The term 'heavy metals' refers to metals whose specific gravity is greater than 5 g/cm^3 in their standard state (2). These metals penetrate aquatic ecosystems through agricultural effluents and discharged industrial wastewater which have different chemical compounds, including heavy metals (3). Fish accumulate metals by ingesting particulate material suspended in water, through food, through the ion-exchange of dissolved metals across lipophilic membranes (e.g., the gills), and through adsorption onto tissue and membrane surfaces (4). Fish are considered bio-indicators of the quality of ecosystems because of their ability to bio-concentrate and integrate a contaminant load (5).

According to statistics compiled by Food and Agriculture Organization (FAO), the consumption of fish accounts for 16% of animal protein consumed and 6% of total protein intake by people of the world (6). Having high levels of unsaturated fats and low cholesterol and being high in protein makes fish one of the most important sources of protein. It is considered an important food ingredient in many countries, including Iran, and its production is increasing (7).

Heavy metals may enter the human body through the consumption of fish and thereby create a serious health hazard (8). The measurement of heavy metals in fish can be useful to assessing potential health risks to humans associated with the consumption of fish (9).

Health risk assessment is defined by the US Environmental Protection Agency (USEPA) as the description of the potential adverse health effects of humans as a result of



exposure to contaminants (10).

Because of the importance of measuring heavy metal concentrations in fish and assessing the health risk associated with exposure to heavy metals through the consumption of fish, the accumulation of heavy metals in fish has been widely studied throughout the world (3,11-13). Furthermore, numerous studies have evaluated potential health risks associated with exposure to heavy metals through fish consumption (7,9,14-17).

The present study was undertaken to determine the levels of heavy metals in the three most frequently used fish caught from Bandar Abbas waters. As a result of this study, consumers and authorities can become better aware of the possible risks of contamination through daily intake of fish.

Methods

This cross-sectional study was conducted in the summer and fall of 2013. Sampling was conducted on three of the most widely consumed fish caught from the coast of Bandar Abbas, i.e. *Thunnus tonggol*, *Pleuronectiformes* (*Pseudorhombus elevatus* and *Euryglossa orientalis*), and Klunzinger mullet that were being sold at the town market or were caught directly from the sea. Five samples from each fish species were tested three times at 2-month intervals (overall 15 samples for each fish species). A total of 180 tests were conducted on the samples. Concentrations of the four metals nickel (Ni), chromium (Cr), mercury (Hg), and lead (Pb) were measured.

Sampling

Thunnus tonggol and *Liza klunzingeri* samples were selected from the town market, and *Pleuronectiforme* samples were caught from the sea and transferred to the Persian Gulf and Oman Sea Ecological Research Laboratory. The standard length of the fish after cleaning was measured by biometric boards, and their weight was measured by a laboratory scale with an accuracy of 0.1 g. Fish muscle is not an active site for heavy metal accumulation. In other words, fish muscle compared with other tissue has the lowest concentration of heavy metals (6). Therefore, because of its role in the transfer of heavy metals to the human body, muscle tissue was chosen as the target organ in risk assessment. Samples of 15-20 g of fish muscle tissue were selected and washed once with both municipal and distilled water. They were stored in plastic bags and, after coding, were frozen at -19°C.

Sample preparation and device analysis

Samples were prepared according to the Manual of oceanographic observations and pollutant analyses methods (MOOPAM), 1999 method. Heavy metal concentrations (Cr, Ni, and Pb) were assessed using graphite furnace atomic absorption spectrometry. Hg concentrations were measured using an atomic absorption spectrometer equipped with a hydride generator. Data quality was checked by analyzing standard reference materials (DORM-2 National Research Council, Canada). Recovery values of collaborative recovery model (CRM) samples were in the range of 95.2%-97%.

Statistical analysis

Data was tested for normality using the Shapiro-Wilk test. Since the data did not show a normal distribution, the Mann-Whitney and Kruskal-Wallis tests were used to compare average heavy metal concentrations in different fish species. The Pearson correlation coefficient was used for normal data to test the correlation between fish length and concentration of heavy metals; for abnormal data, the Spearman correlation coefficient was used. A level of 0.05 was considered significant. All tests were performed by SPSS version 18, and Excel software was used to draw diagrams.

Comparison with guidelines and standards

Since there is no standard for heavy metals involving fish in Iran, amounts of Ni, Cr, Hg, and Pb in the tested fish were compared with guidelines from the World Health Organization (WHO), FAO, The Joint FAO/WHO Expert Committee Report on Food Additives (JECFA), the European Commission, standards formulated by the American Food and Drug Administration (USFDA), the Ministry of Agriculture, Fisheries, and Food of the United Kingdom (MAFF), and China's national regulations regarding (Table 1).

Estimated daily intake and estimated weekly intake of heavy metals

Based on the method proposed by Fathi et al (23), daily and weekly absorption rates were calculated using equation 1:

$$EWI = C \times IR / BW \quad (1)$$

where estimated weekly intake (EWI) (µg/kg-week) is the EWI of pollutant; C (µg/g) is the average concentration of pollutant; IR (g/week) is the amount of food intake; and BW (kg) is the body weight of the consumer.

Table 1. Guidelines of WHO, FAO, JECFA, MAFF, the European Commission, standards formulated by the USFDA, and China's national regulations regarding heavy metals (mg/kg)

Codify by	Year	Ni	Cr	Hg	Pb	References
JECFA	1989	-	-	0.5	0.5	(18)
Europe Commission(EC)	2006	-	-	0.5-1	0.3	(18)
WHO	1996	-	-	-	2	(18)
FAO	1983	-	-	-	0.5	(19)
USFDA	1993	70-80	12-13	-	-	(20)
MAFF	2000	-	-	0.3	2	(21)
China national regulations	-	-	0.5	0.3	1	(22)

Risk assessment

Risk assessment is a process to calculate or estimate the risk for a given population exposed to certain pollutants (24). This risk assessment study investigated the target hazard quotient (THQ) for three groups: children, normal adults (non-fishermen), and fishermen. THQ is defined as the daily intake of pollutants higher than the reference dose. Estimates were based on the method proposed by Yi et al (25) and equation 2:

$$\text{THQ} = (\text{Efr} \times \text{EDtot} \times \text{FIR} \times \text{C}) / (\text{RFD} \times \text{BW} \times \text{ATn}) \times 10^{-3} \quad (2)$$

where THQ (unitless) is the THQ; EFr (day/year) is the exposure frequency; EDtot (year) is the exposure duration; FIR (g/d) is the food ingestion rate; C (µg/g) is the concentration of heavy metals in fish; BWa (kg) is the average body weight of consumers; RFD (µg g⁻¹ day⁻¹) or (mg kg⁻¹ day⁻¹) is the reference dose; and ATn (day) is the average time for exposure to non-carcinogens.

A THQ of THQ < 1 indicates that, based on current consumption rates and current concentrations of pollutants in fish, the consumption of fish has no adverse effects on the health of consumers over a lifetime (26).

EFr: In this study, an exposure frequency of 128 days was considered (7).

EDtot: Exposure time was considered to be 70 years based on life expectancy in Hormozgan province (27).

FIR: Based on the General Department of Fisheries of Hormozghan province, this study used a dose of 50 mg per day (28). This amount was considered to be 20 g per day for children and 2.4 times more than the normal range for fishermen (29).

The parameter of heavy metal concentrations in fish was extracted from the mean heavy metal concentrations in fish muscle in the present study.

BW: In this study, the average adult weight was considered to be 60 kg, based on a 2009 research in Iran by Hajizadeh et al (30), and child weight was considered to be 33 kg (29).

RFD: The USEPA in 2008 determined the reference doses for Cr and Ni to be 0.003 and 0.02 (mg kg⁻¹ day⁻¹), respectively (31), in 2009 determined the reference dose of 0.004 (mg kg⁻¹ day⁻¹) for Pb (32), and in 2011 set 0.0003 (mg kg⁻¹ day⁻¹) as the reference dose of Hg. These values were used in calculations in this study (16).

ATn: This time comes from multiplying the number of days during a year and number of years of exposure.

Total THQ of heavy metals in fish or a hazard index (HI) was calculated for each species (16).

Calculating permissible limits of consumption

Since man uses several fish species in his diet, he risks possible contamination from multiple pollutants. Equation 3, given by the USEPA (33), was used to calculate the allowable amount of exposure to multiple contaminants in many fish species:

$$\text{CR}_{\text{lim}} = \sum (\text{RfD} \cdot \text{Pm} / (\text{Cm}_j \times \text{P}_j)) \times \text{BW} \quad (3)$$

where, CR_{lim} is the allowable amount of fish consumption (kg/d); RFD is the reference dose (mg/kg-d); P_j is the por-

tion of fish species in the food diet; Pm is the portion of contaminants in the food diet; BW is the body weight of consumers (kg); and Cm is the concentrations of chemical matter (mg/kg).

Results

The amount of heavy metals in fish

The concentrations of Ni, Cr, Hg, and Pb were tested in the muscles of three species of fish (*Thunnus tonggol*, *Liza klunzingeri*, and *Pleuronectiformes*) collected from the Bandar Abbas coast during the summer and autumn of 2013. Results are listed in Table 2 as mean ± standard deviation (SD), maximum and minimum amounts according to µg/g dry weight.

In order of concentration amounts of Cr and Ni, the fish species are ranked as *Thunnus tonggol* > *Pleuronectiformes* > *Liza klunzingeri*; in terms of the amount of Hg they are ranked as *Pleuronectiformes* > *Thunnus tonggol* > *Liza klunzingeri*. In terms of contamination levels of Pb they are ranked as *Liza klunzingeri* > *Pleuronectiformes* > *Thunnus tonggol*.

Statistical analyses indicated a significant difference between the concentrations of Ni, Cr, and Pb in the tested fish species (*P* < 0.05).

Correlation between heavy metal concentrations and fish length

The correlation between Ni, Cr, Hg, and Pb concentrations fish length in tested species is shown in Table 3.

Table 3 shows that there is no significant correlation between Ni, Cr, or Hg and the length of the tested fish species, but there is a strong, significant correlation between Pb and fish length.

Estimated daily intake and estimated weekly intake of heavy metals

To describe the safe levels of absorption of various pollutants, tolerable daily intake (TDI) and tolerable weekly intake (TWI) values have been determined by the JECFA. They are shown as Provisional tolerable daily intake (PTDI) and Provisional tolerable weekly intake (PTWI) because of their temporary nature. PTWI is the maximum

Table 2. Mean concentrations and standard deviation of heavy metals (µg/g dry weight) in three tested fish species

Heavy metals	Fish species	Mean± SD	Min.	Max.
Ni	<i>Thunnus tonggol</i>	0.234 ± 0.06	0.179	0.36
Cr	<i>Thunnus tonggol</i>	0.059 ± 0.03	0.018	0.097
Hg	<i>Thunnus tonggol</i>	0.083 ± 0.03	0.065	0.151
Pb	<i>Thunnus tonggol</i>	0.003 ± 0.001	0.0018	0.005
Ni	<i>Pleuronectiformes</i>	0.184 ± 0.07	0.118	0.36
Cr	<i>Pleuronectiformes</i>	0.033 ± 0.01	0.02	0.05
Hg	<i>Pleuronectiformes</i>	0.095 ± 0.003	0.036	0.153
Pb	<i>Pleuronectiformes</i>	0.004 ± 0.002	0.002	0.01
Ni	<i>Liza klunzingeri</i>	0.116 ± 0.04	0.081	0.232
Cr	<i>Liza klunzingeri</i>	0.011 ± 0.01	0.004	0.052
Hg	<i>Liza klunzingeri</i>	0.078 ± 0.003	0.054	0.175
Pb	<i>Liza klunzingeri</i>	0.006 ± 0.003	0.018	0.013

Table 3. Correlation between Ni, Cr, Hg, and Pb concentrations and fish length in tested species

Fish type	Ni		Cr		Hg		Pb	
	R	P	R	P	R	P	R	P
<i>Thunnus tonggol</i>	0.29	0.29	0.07	0.48	0.34	0.22	0.06	0.83
<i>Pleuronectiformes</i>	0.3	0.27	0.45	0.21	0.11	0.7	0.6	0.02
<i>Liza klunzingeri</i>	0.2	0.46	0.49	0.19	0.1	0.7	0.14	0.62

Table 4. EDI ($\mu\text{g/kg-day}$) and EWI ($\mu\text{g/kg-week}$) for heavy metals

Fish type	Ni		Cr		Hg		Pb	
	EDI	EWI	EDI	EWI	EDI	EWI	EDI	EWI
<i>Thunnus tonggol</i>	0.195	1.36	0.049	0.344	0.07	0.487	0.003	0.021
<i>Pleuronectiformes</i>	0.15	1.07	0.027	0.19	0.079	0.55	0.004	0.028
<i>Liza klunzingeri</i>	0.096	0.676	0.009	0.065	0.065	0.457	0.005	0.036

Abbreviations: EDI, Estimated daily intake; EWI, estimated weekly intake.

amount of a pollutant that a man can ingest during a life-time without any acceptable risk to his health (16). Weekly and daily intake rates of heavy metals including Ni, Cr, Hg, and Pb through the consumption of *Thunnus tonggol*, *Liza klunzingeri*, and *Pleuronectiformes* were calculated in micrograms per day and micrograms per week, respectively. The results are given in Table 4.

Result of THQ values

In the present study, THQ was calculated for each of the heavy metals (Ni, Cr, Hg, and Pb) in three fish species (*Thunnus tonggol*, *Liza klunzingeri*, and *Pleuronectiformes*) and for three different groups of people (children, normal adults, and fishermen). The results are given in Table 5. It is to be noted that results are unitless.

Because of the possibility of interference and intensification of the effects of heavy metals, the HI was calculated for Ni, Cr, Hg, and Pb in three species (*Thunnus tonggol*, *Liza klunzingeri*, and *Pleuronectiformes*), and the results are shown in Figure 1.

The lowest and highest HIs were associated with the *Liza klunzingeri* and *Pleuronectiformes* species, respectively. The maximum HI was seen in the fishermen group, and the lowest was in the category of children.

Calculating the permissible limit of consumption

The consumers of three different types of fish were divided into seven groups (*Thunnus tonggol* consumers, *Liza klunzingeri* consumers, *Pleuronectiformes* consumers, *Thunnus tonggol* and *Liza klunzingeri* consumers, *Thunnus tonggol* and *Pleuronectiformes* consumers, *Liza klunzingeri* and *Pleuronectiformes* consumers, and consumers of all three species). Permissible intake limits were calculated for the various consumers groups considering the degree of heavy metal contamination from fish consumption for children and adult groups. The results are given in Table 5. It was necessary to determine the proportion of each species in the diet of each consumer group in which members consume two or three species. The ratio of each fish species in the diet was determined based on information regarding consumption. These results are given in Table 6.

Maximum allowable levels of fish consumption belonged

to the *Liza klunzingeri* fish and for adults and children were 7.32 and 4.02 kg/d, respectively. Minimum allowable levels of fish consumption belonged to the *Thunnus tonggol* fish and were 4 and 2.2 kg/d, respectively.

Discussion

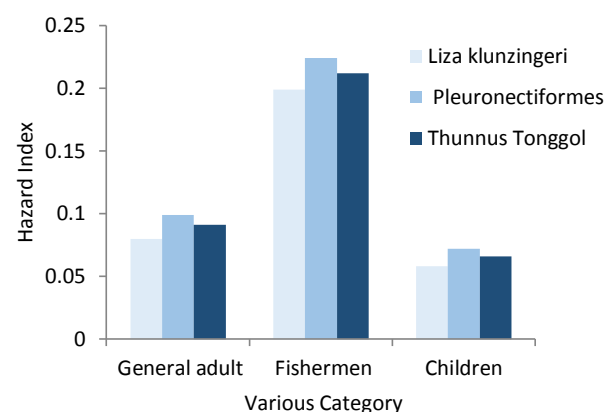
Heavy metal concentrations

Various concentrations of heavy metals ranging widely

Table 5. THQ values for heavy metals in three fish species for children, normal adults, and fishermen

Heavy metals	Fish species	THQ		
		Children	Adults	Fishermen
Ni	<i>Thunnus tonggol</i>	0.0025	0.0034	0.0082
Cr	<i>Thunnus tonggol</i>	0.0042	0.0057	0.014
Hg	<i>Thunnus tonggol</i>	0.0592	0.0814	0.19
Pb	<i>Thunnus tonggol</i>	0.0002	0.0002	0.0005
Ni	<i>Pleuronectiformes</i>	0.002	0.0027	0.006
Cr	<i>Pleuronectiformes</i>	0.0023	0.0032	0.008
Hg	<i>Pleuronectiformes</i>	0.067	0.092	0.21
Pb	<i>Pleuronectiformes</i>	0.0002	0.0003	0.0008
Ni	<i>Liza klunzingeri</i>	0.0012	0.0017	0.0041
Cr	<i>Liza klunzingeri</i>	0.0008	0.0011	0.0026
Hg	<i>Liza klunzingeri</i>	0.055	0.073	0.19
Pb	<i>Liza klunzingeri</i>	0.0003	0.0005	0.0011

Abbreviations: THQ, target hazard quotient.

**Figure 1.** HI of heavy metals in three species (*Thunnus tonggol*, *Liza klunzingeri*, and *Pleuronectiformes*).

from 0.0018 to 0.36 were observed in fish muscle tissue. In order of Cr and Ni concentrations, the fish species are classified as: *Thunnus tonggol* > *Pleuronectiformes* > *Liza klunzingeri*; in order of Hg concentrations, they are ranked as *Pleuronectiformes* > *Thunnus tonggol* > *Liza klunzingeri*. In order of contamination levels of Pb, the fish species are ranked as *Liza klunzingeri* > *Pleuronectiformes* > *Thunnus tonggol*.

Thunnus tonggol was larger than the two other tested species of fish and average concentrations of Ni and Cr were higher in it than in the other two species (*Liza klunzingeri* and *Pleuronectiformes*). One reason for the higher contamination of *Thunnus tonggol* by Cr and Ni may be the result of their feeding patterns. Indeed, this species feeds on shrimp, crabs, and small fish and has a wider food chain than the other two species.

Pleuronectiformes was smaller than *Thunnus tonggol*, but its average concentration of Hg was higher. Maximum accumulation and storage of heavy metals occurred in benthic fish eaters, plankton eaters, and carnivores. The high concentration of Hg in *Pleuronectiformes* could be attributed to this reason.

The concentration of all studied heavy metals except Pb was lower in *Liza klunzingeri* than in the two other species. Heavy metals accumulation was lower in phytophagous species than in carnivorous species (34); the *Liza klunzingeri* species is small and phytophagous, and that could be a reason for its low heavy metals concentration. Statistical analysis showed a significant difference between the concentrations of Ni, Cr, and Pb in the tested fish species ($P < 0.05$).

Comparison of results with guidelines and standards

The heavy metal concentrations of Ni, Cr, Hg, and Pb in three species of fish (*Thunnus tonggol*, *Liza klunzingeri*, and *Pleuronectiformes*) tested in this study were lower than the limits recommended by the guidelines of WHO,

FAO, JECFA, the European Commission, and the standards formulated by the USFDA, the MAFF, and China's national regulations, which are shown in Table 1.

Comparison with other studies

The heavy metal concentrations for the species of *Thunnus tonggol*, *Liza klunzingeri*, and *Pleuronectiformes* in this study were compared with other studies (Table 7). Comparing the data in Tables 2 and 7 indicates that concentrations of Ni, Cr, and Pb were higher in fish of Lake Taihu, China and Tagus Estuary in Portugal than the fish in this study. The Cr concentration in fish of rivers in Italy placed in the Cr range in this study, but concentrations of Hg and Pb were higher than the range of this study. Several factors are involved in the accumulation of metals in different organs of fish, such as age, length, weight, gender, food habitat, ecological demands, metal concentrations in water and sediment, fish shelf life in contaminated environments, the fishing season, and the physical and chemical properties of water (17). Therefore, one cannot explain why the results of other studies are higher than those of this study. One major reason could be the noted differences, such as the feeding habits of the studied species and contamination in their living environments.

Estimated daily and weekly intake

Guidelines of the JECFA in 2010 designated 1.75 and 0.35 ($\mu\text{g/kg-week}$) as PTWI for Pb and Hg levels, respectively (7). In 2004, the PTWI for Ni was determined to be 35 ($\mu\text{g/kg-week}$) (6). Recommended PTWI amounts of Ni and Pb are higher than the calculated weekly absorption rate in this study. In the case of Hg, however, it is lower.

Risk assessment

The THQ of tested metals in the studied species and for different groups (children, normal adults, and fishermen) was less than 1. Thus, the danger caused by each of the

Table 6. Contribution of each species in food diet and permitted consumption levels for each of the seven groups and three new cases.

Consumer category	The ratio of species in food diet			Consumption permitted levels (kg per day)	
	<i>Thunnus tonggol</i>	<i>Pleuronectiformes</i>	<i>Liza klunzingeri</i>	Children	Adults
Just <i>Thunnus tonggol</i>	1	0	0	2.2	4
Just <i>Liza klunzingeri</i>	0	1	0	2.8	5.09
Just <i>Pleuronectiformes</i>	0	0	1	4.02	7.32
<i>Thunnus tonggol</i> and <i>Liza klunzingeri</i>	0.7	0.3	0	2.66	4.84
<i>Thunnus tonggol</i> and <i>Pleuronectiformes</i>	0.6	0	0.4	2.9	5.27
<i>Liza klunzingeri</i> and <i>Pleuronectiformes</i>	0	0.4	0.6	3.55	6.46
All tree species	0.5	0.2	0.3	3.05	5.55

Table 7. Comparison of measured heavy metal concentrations in tested fish with concentrations taken from the literature ($\mu\text{g/g}$)

Sampling site	Ni	Cr	Pb	Hg	Reference
Lake Taihu, China	0.64-2.59	0.7-0.98	1.73-12.87	-	(11)
Tagus estuary in Portugal	3.12-14.35	0-0.56	8.55-46.47	-	(35)
Italy rivers	-	0.04	0.04	0.34	(12)
Shadegan wetland, Iran	2.59-98.64	0.0-0.7	1.37-12.87	-	(36)
Moses estuary in Iran	2.35	48.14	1.32	-	(37)

studied metals alone does not threaten the health of consumers. The results of this study are consistent with those of Tao et al (29) in 2012 in Lake Taihu, China and the results of Taweel et al (38) in 2013 in Langat River and Engineering Lake, Malaysia. In contrast to the results of this study, Krishna et al reported in 2014 a THQ higher than 1 for Pb, Cr, and Hg in fish from the Machilipatnam coast in India (14). A HI of less than 1 was achieved in each of the studied species. Therefore, the current consumption rate of each species poses no danger to consumer health (considering the interference and resonance effects of the studied metals).

Conclusion

The order of mean heavy metal concentrations in fish is $Ni > Hg > Cr > Pb$. The highest mean concentrations of Ni and Cr were obtained in *Thunnus tonggol*. The highest mean concentration of Hg was obtained in *Pleuronectiformes*, and the highest mean concentration of Pb was obtained in *Liza klunzingeri*. There was a significant difference between the average concentrations of similar metals (except Hg) in different species. Only levels of Pb were significantly associated with the length of the *Liza klunzingeri* species. Obtained estimations of daily and weekly intakes (except for Hg) were much lower than the PTDI and PTWI. The THQ of all tested metals in the studied species and the HI of all three species were less than 1. Therefore, consumption of the studied fish has no health risk for consumers. The consumption of up to 4 kg/d of *Thunnus tonggol* and 7.32 kg/d of *Liza klunzingeri* causes no health problems for general adult consumers.

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Ethical issues

The authors hereby certify that all data collected during the study is as stated in this manuscript, and no data from the study has been or will be published elsewhere separately.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed equally and were involved in the design of the study, data collection, and article approval.

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