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Research Article

Accumulation of heavy metals in different organs of the Caspian kutum, Pikeperch, and their intestinal parasites from the southern Caspian Sea

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Abstract

This study aimed to determine some heavy metal concentrations in the tissues of the Caspian kutum (*Rutilus kutum*) and Pikeperch (*Sander lucioperca*) as well as their parasites. The fish samples were collected from the coasts of Guilan province, Anzali port for 6 months in 2020. The fish muscle, gonad, liver, and intestine organs and their intestinal parasite samples of both fish species were collected and analyzed for Cu, Pb, Cd, and Zn concentration using the tissue dissolution technique and an absorption spectrophotometer. The results confirmed that the concentrations of Zn and Cu in the intestine and liver tissues were significantly higher than those in the muscle and gonad. Cd was not detected in the *S. lucioperca* tissues and its parasites, but it was detected in the liver and intestine of *R. kutum*. The mean concentration of Zn in Anisakis simplex parasite of *R. kutum* was 29.23 times higher than in the host intestine and 15.82 times higher than in the host liver. Also, Zn concentration in *Rafidascaris acus* (male) and *Eustrongylides excisus* (female) nematodes of *S. lucioperca* was 22.4 and 6.01 times higher than in the host intestine, respectively. The absorption rate of the four heavy metals in the muscle of *R. kutum* and *S. lucioperca* was lower than all international standards and risk index target hazard quotient (THQ) for adults and children. Pb, Cd, Cu, and Zn were not detected in the fish samples. In the contaminated fish with heavy metals, measuring the level of heavy metals in their parasites (nematodes) can also be used as an indicator for heavy metal contamination. Also, the accumulation of metals in the muscle of both fish species was lower than the world standards.

Article info

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Introduction

Recently, the widespread use of heavy metals and the production of thousands of toxic chemicals have polluted the environment and aquatic ecosystems, which lead to accumulation in the sediments of lakes and seas (Khandker *et al.*, 2009; Sadeghi Bajgiran *et al.*, 2016). Fish are an important part of aquatic food chains that usually accumulate heavy metals from water and cause serious physiological and biochemical changes in their body. Therefore, the heavy metals concentration in the body of fish can persist in the food chain and transfer to the human body (Zhao *et al.,* 2012; Micheline *et al.*, 2019). The main challenge in consuming as an essential food resource considered by most researchers is the poisonous heavy metals. It is a long time since we know about the harmful impacts of heavy metals on public health (Castro-González and Méndez-Armenta, 2008). These impacts comprise biochemical disorders including liver damage, kidney failure, and heart disease, and eventually, they can lead to death (Al-Busaidi *et al*., 2011). Heavy metals such as zinc, copper, cadmium, and lead are present in the natural ecosystem and accumulate in fish muscle, liver, gonad, and intestine tissues (Najm *et al.,* 2014). Cadmium (Cd) as a trace element causes some problems in the behavior, growth, and physiology of aquatic organisms. The average concentration of this metal in the surface and ground waters is usually <1 µg/L which inhibits the electron transfer chain in mitochondria and stimulates reactive oxygen species (ROS) production in fish (Wang *et al.*, 2004).

In fish and other vertebrates, Cu as an essential trace metal, is one of the elements required for the body's metabolic enzymes and glycoprotein (Nordberg *et al.,* 2007). When Cu concentration is higher than 20 ppb, it accumulates in tissues and causes damage to the nervous system, respiratory system, liver function, and immune system (Sfakianakis *et al.,* 2015). Lead (Pb) is one of the most hazardous heavy metals present in most aquatic ecosystems and its concentration of more than 0.2 mg/Liter causes toxicity for aquatic animals (Ebrahimpour and Mushrifah, 2008; Zarbock *et al.,* 2018; Xu *et al.,* 2020). Zinc (Zn) is a ubiquitous trace element of the earth's crust. Although it has been proven that Zn has little toxicity to humans, long-term consumption of high concentrations can lead to physical effects such as fatigue and dizziness (Prasad, 2012). According to the WHO (2004), it stated that the permissible limit of heavy metals such as Cd, Cu, Pb, and Zn in fresh fish meat for a human weighing 70 kg is 0.2, 10, 0.3, and 100 mg/g, respectively (Saei-Dehkordi and Fallah, 2011; Hamida *et al.,* 2018; Skalnaya and Skalny, 2018; Wu *et al.,* 2018).

The Pikeperch (*Sander lucioperca* Linnaeus, 1785), is one of the most abundant species belonging to the Pikeperch family (Percidae). In Iran, they are distributed only in the Caspian Sea and its basins. This fish is a carnivore and has delicious meat. This species lives in inland freshwaters, estuaries, and brackish water with a salinity of less than 23 ppt (Abdolmalaki and Psuty, 2007). The Caspian Kutum, *Rutilus kutum,* is also one of the most important commercial species in the southern Caspian Sea. It includes more than 70% of the yearly Iranian bony fish that are commonly caught in the Caspian Sea and the rivers because they are anadromous fish species (Najm *et al.*, 2014; Forouhar Vajargah *et al.*, 2022).

Several studies have shown that intestinal helminthic parasites of fish are as good biological indicators over the last few years because helminth parasites are organisms that can accumulate in their tissues to higher concentrations levels of heavy metals than their host tissues (Hassan *et al.*, 2016). Also, several studies have indicated that parasitic nematodes accumulate essential metals rather than toxic ones (Khaleghzadeh-Ahangar *et al.*, 2011; Amini *et al.*, 2013). Some parasites live symbiotically with these two fish in the Caspian Sea. *Abramis brama, Chalcalburnus chalcoides, Barbus capito, Capoeta capoeta,* and *Paradiplozoon chazaricum* can be mentioned among the most critical parasites reported in the Caspian kutum (Mohamamad and Mahsa, 2019). Regarding pikeperch, some of the identified parasites that coexist with it are *Diplastomum spathaceum, Dactylogyrus* sp., *Trichodina* sp., *Achtheres percarum, and Eustrongylides excisus* (Movahed *et al.*, 2015). The purpose of this study was to evaluate and compare the signs of zinc, copper, cadmium, and lead concentrations in different tissues of the *Rutilus kutum* and *Sander lucioperca* fish and their parasites from the Southwest Caspian Sea, Guilan province, northern Iran.

Materials and methods

Samples collection and preparation

In this study, 60 *Rutilus kutum* and *Sander lucioperca* were caught using gillnets from the southwest coast of the Caspian Sea, Anzali city, Guilan province (49º28′E, 37º28′N), northern Iran between September 2020 and March 2021. Three stations of catchments were sampled (Fig. 1). The fish were transported to the laboratory at a temperature of 4°C. Then, the genus and species of fish were identified using the key reference and biometric features (the weight and the total length) measured for each fish (Table 1). To remove any contamination, the samples were cleaned using distilled water. Also, the gonads, muscle tissue, intestine, and liver of the fish were isolated, washed using tap water, and stored at –20ºC until the metal analysis assay (Lavilla *et al.*, 2008).

Heavy metals analysis

In order to investigate the accumulation of the heavy metals in the examined organs, 20 to 30 g of homogenous tissues were taken and completely dried at 105ºC for 24 hours. Next, they were thoroughly pounded in a porcelain mortar until they were completely powdered and became uniform (Adel *et al.*, 2016). The amount of 0.5-1 g of dried tissue was chemically digested in a hot vigorous acidic mixture (HNO_3/$HClO_4$) and using a hot plate. At the same time, the control sample was also used for each series of samples (4 control samples) as prepared separately. The remaining solution and the samples containing filtered suspended particles were transferred to 25 ml volumetric flasks and then soaked in 10% nitric acid and finally placed in a polyethylene container to inject into the atomic absorption device (Cheggour *et al.*, 2001). The concentration of standard solutions and heavy metals was specified using flame atomic absorption spectrometry (SHIMADZU AA/680, Tokyo, Japan) (APHA, 1992). Depending on the type of metal being analyzed, all the standard solutions used were prepared from the main standard (Merck), salt or chemical reagents tetrazole standard (stock) with a concentration of 1000 mg/L. Then, by diluting and making the standards with suitable concentrations (6 standards) injecting them into the atomic absorption device, and drawing the curve using the calibration equation, the device automatically determined the concentration of heavy metals (Adel *et al.*, 2016; Aweez *et al.*, 2021).

Figure 1: Location of sampling stations in the southwest of Caspian Sea (Anzali port in Guilan province). S1: Karimbakhsh; S2: Motahari; S3: Mian Poshteh.

Table 1: Biometry parameters results of two fish species.

Parameters	*Rutilus kutum*	*Sanders lucioperca*
Total weight (g)	910.731 ± 276.01^{a}	700.076 ± 173.29^{b}
Total length (cm)	45.51 ± 5.45^{a}	43.74 ± 5.00^{a}
Age (years)	5.38 ± 0.8^{a}	4.28 ± 1.08^{a}

Parasites survey and identification

To measure the concentration of heavy metals in possible multicellular gastrointestinal parasites in the target fish, first, their different organs were studied in terms of parasitic contamination. After isolating the parasites based on standard methods and valid diagnostic kits, the parasites were identified by species (Moravec, 2013). Then, based on the method above, the amounts of heavy metals in each parasite were gauged. In the end, the numbers obtained from heavy metals in different organs and parasites were compared with the global standard values and tables (Table 1).

Calculation of daily and weekly absorption

The determination of contaminants that were absorbed via food per kilogram of body weight every day (EDI) and every week (EWI) based on the procedure that the

US EPA *i.e.*, Environmental Protection Agency (2000), was calculated using the following equations (Yaghobzadeh *et al.*, 2014):

$$EDI = \frac{C \times MS_D}{BW}$$

$$EWI = \frac{C \times MS_W}{BW}$$

EDI = daily absorption of metals through eating fish (µg/g / body weight/day); EWI = Weekly absorption of metals via eating fish (µg/g / body weight/week); C=Concentration of metal in the muscle of the consumed fish (µg/g); MSD=daily rate of eating fish; 13–14 kg per year (38 g per day based on per capita fish consumption on the southern coasts of the Caspian Sea); MSW= rate of fish-eating per week; (266 g per week based on each capita fish consumption on the southern coasts of the Caspian Sea).

Calculation of the limit of fish consumption

One of the most important methods of determining the permissible limit of fish consumption is the method proposed by the EPA *i.e.*, US Environmental Protection Agency (2000). The method is based on the amount of metals in the edible tissues of fish by applying RfD which the reference dose is following formula 4 presented. It can be used to gain an acceptable amount of fish and fishery products consumption in a certain period. The following formulas were also used to calculate the number of allowed meals each month.

$$CR_{lim} = \frac{RfD \times BW}{C} \times 7$$

CR_{lim} =permissible fish consumption (kg/day); BW=body weight (adults 70 kg, children 14.5 kg); RfD=reference dose (µg/g of body weight per day) (Table 2); CR=Carcinogenic risk

$$CR_{mm} = \frac{CRlim \times Tap}{MS}$$

CRmm=Permissible rate of eating fish (each month); MS=amount per serving (adults 227 g, children 114 g); Tap=mean period (4.3 weeks per month).

Table 2: Metal reference doses (RfD) (Qu *et al.*, 2012).

Metal	RFD (mg/ kg -d)	Source	RFC (mg/m^3)	Source
Ag	5.0E-3	IRIS[a]	-	ATSDR[b]
Cd	l.0E-3	IRIS	1.0E-5	
Cr	1.5E+0	IRIS	-	
Cu	4.0E-2	HEAST[c]	-	
Ni	2.0E-2	IRIS	9.0E-5	ATSDR
Pb	104E-4	Oak Ridge	-	
Se	5.0E-3	IRIS	2.0-2	Cal EPA[e]
Ti	3.0E-6	IRIS	-	
Zn	3.0E-1	IRIS	-	
Hg	1.6E-4	Cal EPA	3.0-4	IRIS

[a] Integrated Risk Information System, U.S. EPA;
[b] The Agency for Toxic Substances and Disease Registry, U.S.;
[c] Health Effects Assessment Summary Tables, U.S. EPA;
[d] Oak Ridge National Laboratory, U.S.;
[e] California Environmental Protection Agency, U.S;
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Target hazard quotient (THQ)

Hazard potential is the proportion of the element's condensation to the greatest condensation of the metal without causing issues in the body. Formula 6, suggested by the US EPA, was applied to estimate the risk to individuals with non-cancerous diseases. It should be noted that to estimate the mentioned index, several items must be the default title, including the sum of metal imported, equal to the sum absorbed in the body (National Pollutant Discharge Elimination System, 2014); The mechanism of this index is such that the resulting number which is higher than one signifies a strong probability of non-cancerous diseases. Also, when the resulting number is less than one, it suggests that aquatic consumption does not have a detrimental consequence on the users. HI (the aggregate hazard index), was gained from the sum of the hazards of 4 metals that accord following as below (Chien *et al.*, 2002):

$$THQ = \frac{EF \times ED \times IR \times C}{BW \times RfD \times AT}$$

THQ=risk index; EF=exposure frequency (frequency of exposure) 365 days per year; ED=entire exposure duration (exposure rate) 70 years; IR=daily consumption rate of fish; (38 g per day based on per capita fish consumption on the southern shores of the Caspian Sea); AT=average days of exposure (2550 days=70 years×365 days).

Hazard Index (HI) = ΣTHQ = THGPb + THGCd + THGCu + THGZn

Data analysis

Statistical examination of the obtained data was done by SPSS software version 22. All data were stated as a mean ± standard deviation. The Shapiro-Wilk test was used to assess the normality of the data. After the normalization of the data, the t-student test and one-way variance test were applied to compare the mean of the data. Tukey's test was also used to determine significant differences between groups. The difference was considered at the 95% confidence level ($p<0.05$).

Results

Comparison of the heavy metal concentrations in different organs of Rutilus kutum and parasites

The concentration of the heavy metals including Zn in the liver of infected fish was significantly higher than in other organs of fish ($p<0.05$), whereas, Cd had the least concentration of heavy metals in muscle and gonads (zero). Cu in the liver and intestine indicate a higher concentration in infected fish than in other organs and parasite tissue, and the difference was significant ($p<0.05$). Also, significant bioaccumulation was shown for Cu and Zn metals in gonad organs in comparison to males and females ($p<0.05$). Also, the concentration of Cu metal in the muscle organs of 7-year-old fish was significantly higher than that of 6-year-old fish ($p<0.05$), whereas in the gonad organs of 4-year-old fish, this difference was significantly higher than that of 7-year-old fish ($p<0.05$). The concentration of Zn in *Anisakis simplex* parasite (male and female) tissue was significantly higher than all organs infected fish ($p<0.05$) (Table 3). Moreover, the concentration of Cd, Pb, and Cu metals in parasite tissue was lower than in all the tissues of infected fish ($p>0.05$). Also, the concentration of Zn metal in the parasitic tissue of 4-year-old fish was significantly higher than that of 6-year-old fish (*p<0.05*).

Comparison of the heavy metal concentrations in different organs of Sander lucioperca and parasites

According to Table 4, Cd metal had the least concentration of heavy metals in all organs of infected fish and parasite tissue, whereas the highest amount of these four metals was related to Zn in the intestine tissue of infected fish. Also, the concentration of Cu metal in the intestine organs of 6-year-old fish was significantly higher than that of 4-year-old fish (*p<0.05*). The concentration of Zn metal in *Rafidascaris acus* (Male) parasite tissue was significantly more than *Rafidascaris acus* (female) parasite, whereas Zn metal concentration in *Eustrongylides excisus* (female) parasite tissue was significant (*p<0.05*) (Table 4). Also, Cu showed a higher concentration in *Eustrongylides excisus* (female) than in the muscle, gonad, liver, and intestine of infected fish, but Zn metal in *Rafidascaris acus* (male) was higher than in all organs of fish (*p<0.05*). Also, Zn metal concentration in the *Rafidascaris acus* and *Eustrongylides excisus* parasite tissues of fish at 4 years old were significantly higher than fish at 6 and 3 years old, respectively (*p<0.05*).

Table 3: The concentration of heavy metals (Cd, Pb, Zn, and Cu) in different organs of *Rutilus kutum* in comparison to the *Anisakis simplex* parasites tissue from the southwest Caspian Sea, northern Iran.

Wet weight	Cd (μg/g)	Pb (μg/g)	Zn (μg/g)	Cu (μg/g)
Muscle	ND	0.0008±0.0007	0.0005±0.0001	0.042±0.024
Female Gonad	ND	0.0006±0.0005	0.0005±0.029	0.033±0.017
Male Gonad	ND	0.0006±0.0005	0.075±0.034	0.0005±0.014
Liver	0.388±0.238	ND	72.185±30.100	40.835±26.754
Intestine	0.31±0.018	0.019±0.006	39.063±26.357	10.612±4.254
Anisakis simplex (Male)	ND	ND	1142.042±325.084	ND
Anisakis simplex (Female)	ND	ND	696.552±114.025	ND

(ND: Not detected)

Table 4: The concentration of heavy metals (Cd, Pb, Zn, and Cu) in different organs of *Sander lucioperca* in comparison to parasite tissue from the southwest Caspian Sea, northern Iran

Wet weight	Cd (μg/g)	Pb (μg/g)	Zn (μg/g)	Cu (μg/g)
Muscle	ND	ND	0.040±0.017	0.005±0.002
Female Gonad	ND	0.003±0.002	0.203±0.130	0.007±0.002
Male Gonad	ND	0.0005±0.0001	0.100±0.076	0.029±0.018
Liver	ND	0.0007±0.0004	0.107±0.036	0.018±0.012
Intestine	ND	ND	39.382±20.717	7.345±0.008
Rafidascaris acus (Male)	ND	ND	883.333±25.425	ND
Rafidascaris acus (female)	ND	ND	7.284±1.520	ND
Eustrongylides excisus (Male)	ND	4.774±1.235	7.118±3.250	ND
Eustrongylides excisus (female)	ND	ND	236.4±141.9	21.242±7.142

(ND: Not detected)

Comparison of the heavy metal levels in different organs of Sander lucioperca and Rutilus kutum tissues and their parasites

The biometry data of fish indicated that *Rutilus kutum* fish weight was significantly higher than that of *Sander lucioperca* (*p<0.05*) (Table 1). The lowest concentration of heavy metals was observed in the muscles of both fish (Table 3). So the highest amount of heavy metals was related to Zn (0.060±0.022 µg/g) in *Rutilus kutum*, whereas for other metals there were no significant differences in both fish. The most significant amount of these metals was related to Zn metal in the female gonad organ of *Sander lucioperca* (0.203±0.130 µg/g) which was higher than in the male gonad. Moreover, in *Sander lucioperca*, the highest concentration of heavy metals was related to Zn and Cu (39.282±20.717 µg/g and 7.345±0.008) in the intestine, respectively. However, statistical analysis of variance (one-way ANOVA) displayed a highly significant difference in concentrations of Cu, Zn, and Cd metals in the two fish ($p<0.05$), whereas, the Pb metal was the same in the two fish ($p>0.05$). The most remarkable observation obtained from the comparison of data was linked to the collection of investigated metals in the liver of two *Rutilus kutum* and *Sander lucioperca*. Zn, with the highest amount (72.185±30.100 µg/g) in the liver of Caspian kutum, was the leader in the accumulation of metals, and Cu, with a relatively high amount (40.835±26.754 µg/g), was in second place in this fish. Fortunately, the two metals, including cadmium and Pb, did not have significant amounts in both types of fish ($p>0.05$), to the extent that the amount of lead in the liver of Caspian kutum was negligible. Accordingly, there is a significant difference between the two kinds of fish in the concentration of Zn and Cu metals in the intestine ($p<0.05$). But the amount of Zn (>39) was at a higher level than Cu metal (<11) in both fish species. The results of the one-way variance test in these two kinds of fish were not significantly different in terms of Cu content ($p>0.05$). But in Zn, this difference was significant ($p<0.05$). As shown in Tables 3 and 4, the mean Zn concentration in the infected fish with *Anisakis simplex* was slightly higher than this in the infected fish with *Rafidascaris acus* and *Eustrongylides excisus* parasites ($p<0.05$), but no significant positive correlations were indicated between Cd or Pb concentrations in fish tissues and their parasites ($p>0.05$). Moreover, the results showed that parasites have a high ability to accumulate heavy metals in their tissues.

Comparison of the heavy metals absorption in muscle of Rutilus kutum

In comparison the amount of absorption of the four metals with the fresh weight of Caspian kutum muscle among adults and children, the amount was lower than all international standards. This amount of absorption was reminiscent of the amount of accumulation of these metals in different organs. Thus, Zn and Cu had the highest, and other metals had the lowest amount of absorption (Table 5). The comparison with WHO (2011) and USEPA (2012) guidelines indicated pollution of the liver and intestine as Cd and Cu were well above the safe limit. The data showed that the risk index of metal accumulation (0.00±0.00 µg/g) in Caspian kutum muscle was lower than their permissible limit in international standards (Table 6).

A comparison of the absorption amount of the heavy metals in muscle organs of Sander lucioperca fish

Similar to *Rutilus kutum*, in *Sander lucioperca* muscle, the amount of absorption was lower than all world standards, and this ratio of Zn to Cu and other metals was facing a decreasing trend (Table 7). An exciting and pleasant finding was the lower risk index of the investigated metal accumulation compared to the world standards in the muscle of *Sander lucioperca* (Table 8).

Table 5: Caspian kutum muscle tissue uptake (wet weight), the permissible limit of heavy metals consumption, daily and weekly uptake of toxic and non-toxic metals in the body of adults and children (mean±SD).

Heavy metals (µg/g)	Metal absorption	Wet metal absorption	Standard WHO (2011)	Reference dose (RfD) USEPA (2012)	Daily dose adults	Weekly dose Adults	Daily dose children	Weekly dose children	PTWI
Pb	0.0007 ± 0.0008	0.0001	0.3	0.0035	0.0000 ± 0.0000	0.0005 ± 0.0006	0.0004 ± 0.0004	0.0028 ± 0.0030	25
Cd	0.0003 ± 0.0001	0.0000	0.2	0.001	0.0000 ± 0.0000	0.0002 ± 0.0001	0.0001 ± 0.0000	0.0013 ± 0.0006	7
Cu	0.0518 ± 0.0282	0.0056	10	0.037	0.0056 ± 0.0030	0.0393 ± 0.0214	0.0271 ± 0.0148	0.1900 ± 0.1036	3500
Zn	0.0214 ± 0.0487	0.0095	100	0.3	0.0023 ± 0.0052	0.0163 ± 0.0363	0.0112 ± 0.0250	0.0788± 0.1757	7000

Units: metal absorption and metal absorption: µg/g; Reference Dose (RfD): µg/g of body weight per day; daily intake (EDI): µg/g per day; Weekly absorption rate (EWI): µg/g per week; Standard Maximum Permissible Weekly Intake (PTWI): µg/g/body weight 70 kg/week.

Table 6: Mean and standard deviation of risk index, consumption limit, and number of promises per month in *Rutilus kutum*.

Heavy metals (µg/g)	Risk index	Limit Adult consumption	Limit Consumption of children	Permitted rates Adult consumption	Permitted rates children consumption
Pb	0.00 ± 0.00	3957.58± 4651.79	819.78± 693.59	74.97± 88.12	30.92±36.35
Cd	0.00 ± 0.00	1499.37± 333.70	310.58± 69.12	28.40±6.32	11.72± 2.61
Cu	0.00 ± 0.00	1170.34± 831.46	242.43±172.23	22.17± 15.75	9.14±6.50
Zn	0.00 ± 0.00	1034.34±754.35	214.26± 156.26	19.59±14.29	8.08±5.89

Units: allowable fish consumption rate (CRmm): serving per month; Permissible limit of fish consumption in terms of (Crlim): kg per day.

Table 7: *Sander lucioperca* muscle tissue uptake, the permissible limit of heavy metal consumption, daily and weekly uptake of toxic and non-toxic metals in the body of adults and children (mean ± SD).

Heavy metals (µg/g)	Metal adsorption	Wet metal adsorption	Standard WHO (2011)	Reference dose (RfD) USEPA (2012)	Daily dose adults	Weekly dose adults	Daily dose children	Weekly dose children	PTWI
Pb	0.0012 ± 0.0013	0.0002	0.3	0.0035	0.0001 ± 0.0001	0.0009 ± 0.0010	0.0006 ± 0.0007	0.0044 ± 0.0051	25
Cd	ND	ND	0.2	0.001	ND	ND	ND	ND	7
Cu	0.0059 ± 0.0022	0.0011	10	0.037	0.0006 ± 0.0002	0.0045 ± 0.0016	0.0031 ± 0.0011	0.0218 ± 0.0081	3500
Zn	0.0405 ± 0.0156	0.0081	100	0.3	0.0044 ± 0.0016	0.0308 ± 0.0118	0.0212 ±0.0081	0.1487 ± 0.0572	7000

Units: metal absorption and metal absorption: µg/g; Reference Dose (RfD): µg/g of body weight per day; daily intake (EDI): µg/g per day; Weekly absorption rate (EWI): µg/g per week; Standard Maximum Permissible Weekly Intake (PTWI): µg/g/body weight 70 kg/week. ND: Not detected

Table 8: Standard and Mean deviation of risk index, consumption limit, and number of promises per month in *Sander lucioperca*.

Heavy metals (µg/g)	Risk index	Limit Adult consumption	Limit Consumption of children	Permitted rates Adult consumption	Permitted rates children consumption
Pb	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND
Cu	ND	1756.94 ± 148.63	363.93 ± 30.78	33.28 ± 2.81	132.72 ± 1.16
Zn	ND	1377.82 ± 372.55	285.40 ±77.17	26.09 ± 7.075	10.76 ± 2.91

Units: allowable fish consumption rate (CRmm): serving per month; Permissible limit of fish consumption in terms of (Crlim): kg per day. ND: Not detected

Discussion

Fish consumption is one of the main ways in which heavy metals are accumulated in the human body. As a result, ongoing research has been carried out on metal pollution in fish as an essential field of research because there is an essential correlation between the consumption of this delicious food and human health (Dadar *et al.,* 2016). Numerous reports have shown that the bioaccumulation of heavy metals depends on the relevant environment and the fish species (Orangi *et al*., 2021). The feeding habits of fish and the habitat type are effective in the accumulation of heavy metals (Bosch *et al.,* 2016). Also, various studies have proven the correlation between the level of pollution with the examined metals in fish and the intake of some rivers; however, a significant correlation between river input and pollution in the genus has not been observed (Dadar *et al.,* 2016). Conversely, due to agricultural activities on the shores of the Caspian Sea, as well as oil extraction in some areas of this sea, pollution such as polychlorinated phenyls, heavy metals, oil pollutants, and pesticides have endangered its aquatic life in the ecosystem. Toxic substances both exist in water and also accumulate in the muscle tissues of every fish and other marine creatures (Saha *et al.,* 2016; Amirgaliev *et al.,* 2022). Scientific authorities have divided the metals accumulated in seafood sources into two types: non-biologically essential or toxic metals (Pb, Cd, and Hg) that usually accumulate in excretory organs such as the intestine, and biologically essential metals including Zn, Cu, and Fe that collect in other body parts such as muscles and gonad, which plays an important role in the metabolic activities of organisms (Nachev *et al.,* 2013).

Several authors in the scientific field of environmental parasitology have discussed that some helminthic parasites such as (intestinal nematodes, cestodes, trematodes, and acanthocephalans) are able to accumulate heavy metals in concentrations higher than their hosts (Eira *et al.,* 2009; Brázová *et al.,* 2012).

The accumulation ability of Zn by *Anisakis simplex* parasite in the present study compares well with the results presented Hassan *et al.* (2016) stated an insignificant increase in zinc and copper metals concentration in fish infected with intestinal and stomach nematodes. Therefore, it was concluded that Zn metal was not recorded from the muscle of Caspian kutum fish but was found in the *Anisakis simplex* parasite. It confirmed how the whale worm may accumulate Pb and Cu to levels far in excess of their host tissues (Pascual and Abollo, 2003). The promising finding in the research was the low accumulation of the studied metals in the muscle of both Caspian kutum and Pikeperch. The outcomes of this research, matched the findings of Sattari *et al.* (2020), which indicates the low concentration of the metals in the edible tissues of these fish species. This value was lower than the international standards. Therefore, the consumption of the muscles of these fish will be safe (Mirzajani *et al.*, 2016).

The average amount of cadmium in the liver of Caspian kutum from Astara to Tenkabon was reported 0.23 µg/g, which is much lower than the average amount of cadmium in the present study (0.388±0.238 mg/g) (Forouhar Vajargah and Bibak, 2022). It seems that this difference is due to the larger area under research or the research time of the study mentioned above when a smaller amount of pollution enters the sea. A comparison between the standard level of cadmium in the WHO (2011) (0.2 µg/g of fresh fish meat for a 70 kg human) and the findings of this research (0.388 µg per weight) in the liver of Caspian kutum, indicates a higher contamination level in the Caspian Sea than the international standard level. The highest amount of Cu and Pb in the liver of the Caspian kutum was reported in Babolsar and Tonkabon regions, which is consistent with the results of this research (Soltani *et al.*, 2014), but the resultant amount of lead in this research was insignificant. The amount of copper and lead in the World Health Report (2011) is 10 and 0.3 µg, respectively, in the weight of fresh fish meat for a 70 kg human. Comparing the data of this research with the above report, we can conclude that the pollution of the liver and intestinal organs in Caspian kutum is more than the international standard. For Pikeperch, this situation is true only about the intestines. Regarding zinc, the standard of 100 µg/g of wet-weight fish meat has been announced for a 70-kilogram human, so comparing this number with these research findings, due to less accumulation in all organs, creates a good situation for the consumers of these two kinds of fish. The outcomes of this research displayed that in both Caspian kutum and Pikeperch, in the gonads, the amount of contamination with the investigated four metals was very insufficient. Also, no significant correlation was observed between the level of contamination with these metals and these organs. Sattari *et al.* (2020) obtained similar results.

The liver in Caspian kutum had the highest pollution rank for zinc and copper. Since the data collection was done from the Anzali region, and this region is the source of many agricultural pollutants, the agrarians on the banks of the rivers leading to the Caspian Sea use macro fertilizers containing zinc and copper to strengthen the agricultural soil and achieve a better crop; these elements enter the body of these fish through food and are stored in the liver, which is responsible for purifying toxins. Regarding Pikeperch, there was no study on the contamination of these metals in the liver. The intestine is an excretory organ in all organisms, and it was expected that the amount of metal accumulation in this organ would be high in both types of fish (Mehana *et al.,* 2020). A significant correlation was observed between the accumulation of zinc and this organ indicating the high level of contamination in the beaches of Anzali port concerning this metal. The presence of high amounts of heavy metals in the liver was because of the role of this organ in the body's metabolism, and the existence of great amounts of heavy metals in the intestine was due to its role in food digestion, and the presence of intestinal villi and mucus was because the absorption of nutrients and elements is done through them. The chemical absorption of elements is affected by the compounds in the tissues, the liver, and intestines, somehow reducing the absorption in other organs such as muscles by absorbing harmful substances, and as a result, the amount of heavy metals in the muscles decreases.

Similar results of zinc accumulation difference, as seen in the *Rutilus kutum* Anisakis parasite, were also observed in Pikeperch. There was a significant difference in the amount of zinc accumulation in both male and female species of *Rafidascaris acus* and *Eustrongylides excisus* parasites and *Rafidascaris acus* had a much higher accumulation of zinc in males than in females. This situation was completely reversed in the case of *Eustrongylides excisus* parasite, so the accumulation of this metal was much higher in the female fish than in the male ones. One of the main differences is caused by the difference in the accumulation of metals in male and female parasites due to the correlation between these metals and the nature of gonadotropin hormones in both sexes, which is especially effective in reproduction and spawning in females. It has not reported a correlation between the collection of heavy metals and the fish species (Dadar *et al.,* 2016). Therefore, according to the findings of this study, it can be suggested that there is a logical correlation between the absorption of heavy metals and hormonal changes. The results of the current study are consistent with results from some previous investigations that nematodes are suitable bioindicators of heavy metals in the marine environment and the fish species (Tellez and Merchant, 2015).

The higher concentration of metals was determined in the intestine organ of fish host when infected by nematode *Raphidascaris* sp. and was low efficiency in accumulating metals (Hassan *et al.*, 2016). *Raphidascaris acus* parasite in *Esox lucius* host is a reliable species in the accumulation of heavy metal pollution and only Zn element was found in *R. acus* and fish liver, whereas, in the current study highest concentration of Zn metal was found in *R. acus* (male) parasite and fish intestine (Tekin-Ozan and Kir, 2007).

An intimate correlation indicated between *Eustrongylides* sp. nematode and essential elements such as Zn metal that these parasites could be a good surrogate for zinc pollution (Keke *et al.*, 2020). However, our study demonstrated that these parasites could absorb essential metals such as Zn and Cu of the muscle organ of infected fish and store them compared to cestoda and acanthocephalan larvae (Esmaili Sari, 2002). WHO (2011) has announced the allowable levels of heavy metals copper, zinc, lead, and cadmium to be 10, 100, 0.3, and 0.2 µg/g of fresh fish meat for a 70 kg human (WHO, 2011). However, the permissible levels of copper, zinc, lead, and cadmium in Pikeperch were in most cases lower than the permitted standards. Since the amount of heavy metals in the muscle and even the ducks' gills (in terms of food) was much lower than the allowable limit, it can be said that there is no problem in terms of human consumption. However, health care must be increased and taken to reduce these elements or how to consume fish because it is possible that with growing sources of pollutants and concentrations of contaminants, the condensation of these elements in fish muscle will increase. Therefore, it is better to avoid consuming other organs that can absorb and metabolize the high amount of heavy metals, and implement a good management program that leads to consume healthier organs.

In general, these findings prove the ability of nematodes to bioaccumulate heavy metals in different organs of Caspian kutum and Pikeperch. The results showed that present nematodes were capable of reducing heavy metal concentrations in the tissues of fish and tended to accumulate essential metals including Zn and Cu more strongly than non-essential ones. Considering the lower accumulation of Zn and Cu in the muscle tissue of Caspian kutum and Pikeperch compared to the world standard, using these two fish is not risky to consumers.

Conflict of interest

The authors have no conflict of interest to declare.

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