

Original Article

Heavy metal concentrations in gill and liver tissues of *Rutilus kutum* and *Chelon aurata* in the coast of Babolsar, southern Caspian Sea

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Abstract: Heavy metal accumulation in the aquatic ecosystems is a main concern which threatens human health. In this study two commercial fish species, *Rutilus kutum* and *Chelon aurata* were selected for assessing heavy metal (Cd, Pb, Zn) concentrations in gill and liver tissues at Babolsar's coast, the southern Caspian Sea, Iran. Babolsar is one of the important fishery stations in the southern Caspian Sea. The results showed that liver tissue of *C. aurata* significantly accumulated higher concentration of Cd, Pb and Zn compared to that of *R. kutum*, but these results were not significant for gill tissue. Liver tissue accumulated higher concentration of Cd and Pb compared to gill tissue in *C. aurata*, but these results were not significant for *R. kutum*. It is concluded that the liver tissue of *C. aurata* has higher potential to accumulate heavy metal pollution compared to liver tissue of *R. kutum*.

Article history:

Received 22 December 2015
Accepted 15 April 2016
Available online 25 April 2016

Keywords:

Fish tissues
Zinc
Cadmium
Lead
Caspian Sea

Introduction

The Caspian Sea is a valuable ecosystem, continuously suffering from various sources of pollution, mainly due to the domestic, industrial and agricultural wastewater. Among the different pollutants entering the aquatic environment, heavy metals are known as the main important pollutants because of their toxic effects and potential of accumulation in many aquatic species, including fishes (Blevins and Pancorbo, 1986). Metals act differently in aquatic organisms; they can be absorbed, accumulated or excreted via the organs of aquatic organisms.

Among different heavy metals, lead and cadmium as non-essential elements are highly toxic for living organisms (Wood et al., 2012). Fishes are very sensitive to the toxic effects of these metals, especially during early life stages (Plumlee, 2001). Some heavy metals, like zinc is essential element for living organisms. Zinc is required for basic biological processes, including proteins, nucleic

acids, carbohydrates and lipids metabolisms and also contributes in the functions of immunity, neurotransmission and cell signaling (Murakami and Hirano, 2008). However, the high amount of this metal can be toxic for fishes and other aquatic organisms (Bodar et al., 2005).

The liver, kidney and gill as the most sensitive tissues to toxic elements that absorb the high amounts of metals and transmit them to the other tissues through blood circulation (Karaytug et al., 2007). Due to direct contact of the large surface of gill tissues with aquatic environment, they are susceptible to absorb high amount of metals and can be used as suitable tissue for surveying the impact of pollutants in fish (Heath, 1995; Simonato et al., 2008). Liver as high sensitive tissue to pollutants is used as a suitable bio-indicator of environmental pollutions in organisms (Thophon et al., 2003; Camargo and Martinez, 2007). Accumulation of metals in living organisms depends on different factors such as organism size, diet, metabolic

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Table 1. Mean concentrations (mg.Kg⁻¹ d.w.) and standard deviations of Cadmium (Cd), Lead (Pb), and Zinc (Zn) in liver and gill tissues of *Rutilus kutum* and *Chelon aurata* from the south Caspian Sea.

Fish	Tissue	Cd	Pb	Zn
<i>Rutilus kutum</i>	Liver	8.07±0.44 ^a	30.13±2.23 ^b	154.40±18.78 ^c
	Gill	7.52±3.88 ^a	25.08±3.87 ^b	182.96±19.19 ^c
<i>Chelon aurata</i>	Liver	11.02±2.47 ^a	36.44±4.64 ^b	199.3±39.48 ^c
	Gill	6.39±0.91 ^a	23.15±6.09 ^b	150.93±17.58 ^c

Different letters indicate significant difference ($P \leq 0.05$) between different metal concentrations separately for each tissue of fish ($P < 0.05$).

activities, different life stages, concentration of heavy metals in water and sediment, fishing season (Canli and Atli, 2003). They may also depend on different chemical and physical characteristics of water and sediment such as pH, alkalinity, hardness, organic and inorganic materials (Wood et al., 2012). According to various sources of pollutions, mainly due to untreated domestic wastewater and agricultural activity in the southern Caspian Sea, high amount of absorption and accumulation of heavy metals by aquatic organisms is possible. Therefore, this study aimed to evaluate the accumulation of Pb, Cd, and Zn in gill and liver tissues of two commercially important fishes, including *Rutilus kutum* and *Chelon aurata* at the Babolsar's coast, the southern Caspian Sea.

Materials and Methods

Sampling and sample preparation: Twenty male fishes of *R. kutum* and *C. aurata* were collected from the southern Caspian Sea (Babolsar's coast) in March 2015. The selected fish species are the most important commercial fishes in the southern Caspian Sea. The collected fishes were immediately transferred to a laboratory where their total length, fork length, standard length (in cm) and the weight (in gram) were measured using a biometric ruler, and an electronic digital balance with an accuracy of 0.01, respectively. The liver and gill tissues were carefully dissected and washed with distilled water. The washed tissues were put into a Petri dish and dried in the oven at 70°C for 5-6 days to obtain the constant weight.

Chemical analysis: The dried tissues were homogeneously powdered by a mortar and stored in a

polyethylene container. For the digestion of tissue samples, 8 ml of HNO₃ (Merck, Darmstadt Germany) was added to 1 g of each powdered sample tissue, and this solution was placed in an oil bath at 100°C for one hour. The solution was removed from the oil bath and after temperature reduction, 2 ml of H₂SO₄ was added to the solution and placed in an oil bath at 100°C for one hour to complete dissolving the samples. The solution was passed through the Watman filter paper with a pore size of 42 µm, then its volume increased to 25 ml with distilled water. It was injected into the Atomic Absorption spectrophotometer (Analytik Jena, model novAA 400, Germany) for the determination of metals concentrations. The metals concentrations were presented as mg.kg⁻¹ dry weight.

Statistical analysis: The normality of the data was tested by Kolmogorov-Smirnov tests, and the data that did not meet normality, were normalised by logarithmic transformations. The differences between the two studied fish species i.e. *R. kutum* and *C. aurata* and their tissues (liver and gill) were analyzed using a two-way analysis of variance (ANOVA) procedure and a Duncan's test. The correlation between all considered parameters was performed through Pearson's correlation matrix. All analyses were performed in R 2.15.3 software (R Development Core Team, 2013).

Results

The statistical analysis of Cadmium (Cd), Lead (Pb), and Zinc (Zn) concentrations in the liver and gill tissues of the *R. kutum* and *C. aurata* are presented in Table 1. In both species, the concentrations of metals were in order: Zn > Pb > Cd in the liver and

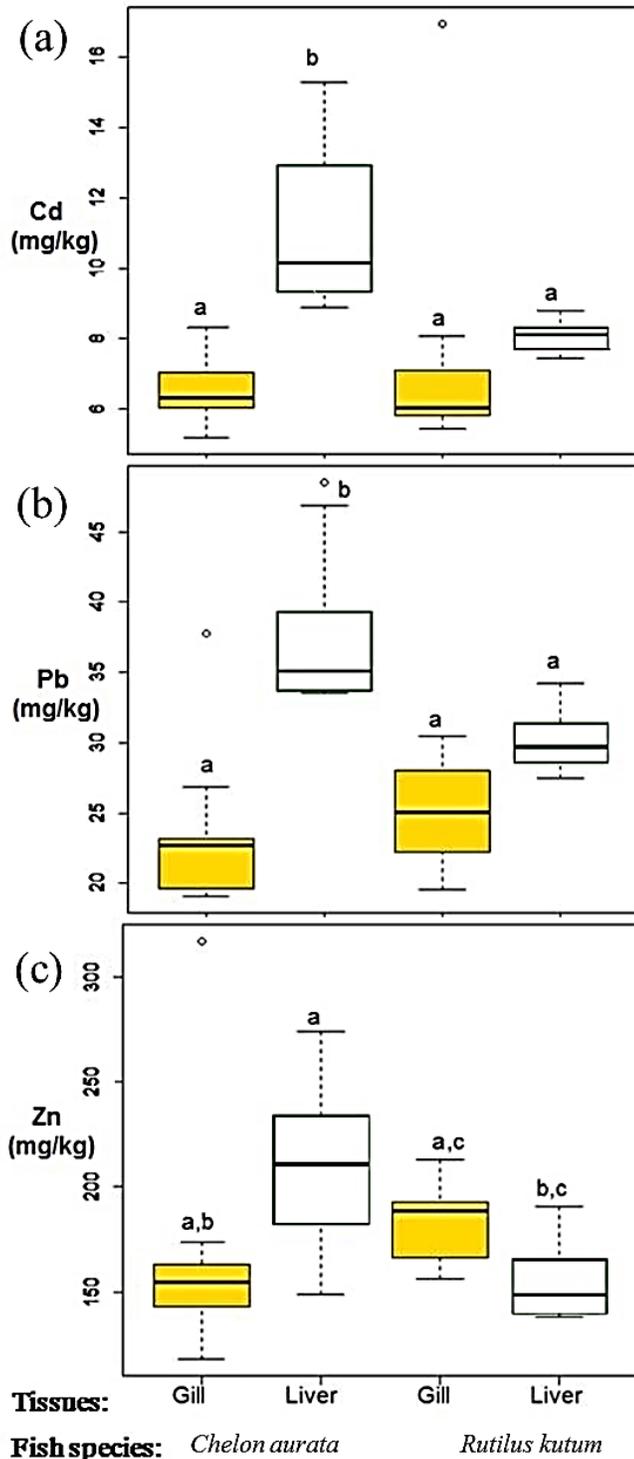


Figure 1. Mean concentrations ($\text{mg}\cdot\text{Kg}^{-1}$ d.w.) and standard deviations of (a) Cd, (b) Pb, and (c) Zn in liver and gill tissues of *Rutilus kutum* and *Chelon aurata* from the south Caspian Sea. Different letters indicate significant difference ($P \leq 0.05$) between the fishes and their tissues.

gill tissues.

Cadmium (Cd) concentration in the tissues of fish species: The results showed that the liver of *C. aurata*

has a significantly higher concentration of Cd (37.5%) compared to that of *R. kutum*, but the difference was not significant for the gill tissue (Fig. 1a). Although the liver Cd concentration in *C. aurata* was significantly higher compared to its gill tissue (Fig. 1a). Also, in *R. kutum*, the liver tissue accumulated a higher concentration of this metal compared to gill tissue, but this result was not significant (Fig. 1a).

Lead (Pb) concentration in the tissues of fish species: The liver concentration of Pb in *C. aurata* was significantly higher (24.7%) than that of *R. kutum* (Fig. 1b). According to the results, the liver tissue of *C. aurata* accumulated a significantly higher concentration of Pb compared to gill tissue, but this result was not significant for *R. kutum* (Fig. 1b).

Zinc (Zn) concentration in the tissues of fish species: The results revealed that only the liver tissue of *C. aurata* had a significantly higher concentration of Zn (35.1 %) than that of *R. kutum* (Fig. 1c). For both studied species, there was no significant differences between liver and gill tissue in regard to Zn accumulation (Fig. 1c).

Correlation between considered parameters: The results showed a positive and significant correlation between weight, standard length, fork length and total length of both considered fish species (Table 2). A significant positive correlation was observed between Cd and Pb concentration in both the gill and liver tissues in *C. aurata* (Table 2). There was no significant relationship between the fish biometric parameters (length and weight) and the concentration of the measured metals in the gill and liver of both species.

Discussion

Comparison of zinc, lead and cadmium concentration: Zn had the highest concentration in the gill and liver tissues of both fish species (*C. aurata* and *R. kutum*) followed by Pb and Cd. The finding of the present study confirms the results of other works from different aquatic ecosystems (Canli and Atli, 2003; Anan et al., 2005; Ali et al., 2011). For instance, a higher concentration of Zn

Table 2. Pearson's correlation matrix for different considered parameters (W= weight, SL= standard length, FL= fork length, TL= total length, Cdgil= Cd in gill, Cdliv= Cd in liver, Pbgil= Pb in gill, Pbliv= Pb in liver, Zngil= Zn in gill, Znliv= Zn in liver) in a) *Rutilus kutum* and b) *Chelona aurata*.

a)	SL	FL	TL	Cdgil	Cdliv	Pbgil	Pbliv	Zngil	Znliv
W	0.90**	0.79*	0.89**	0.41	-0.15	0.31	-0.42	0.04	-0.57
SL		0.98***	0.98***	0.04	-0.20	0.01	-0.22	0.02	-0.61
FL			0.97**	-0.14	-0.31	-0.05	-0.13	0.00	-0.63
TL				0.02	-0.38	0.14	-0.26	-0.03	-0.69
Cdgil					0.15	0.65	-0.48	-0.13	-0.27
Cdliv						-0.58	0.08	0.11	0.55
Pbgil							-0.54	-0.23	-0.44
Pbliv								0.74*	0.40
Zngil									0.49

b)	SL	FL	TL	Cdgil	Cdliv	Pbgil	Pbliv	Zngil	Znliv
W	0.84**	0.88**	0.86**	-0.39	0.35	-0.19	0.05	-0.44	-0.39
SL		0.99***	0.99***	-0.32	0.43	-0.27	-0.04	-0.35	0.02
FL			0.99***	-0.30	0.42	-0.22	0.00	-0.40	-0.05
TL				-0.32	0.42	-0.24	-0.02	-0.35	-0.01
Cdgil					0.06	0.79**	0.05	0.15	0.43
Cdliv						-0.20	0.75**	-0.13	0.54
Pbgil							-0.11	-0.10	0.04
Pbliv								-0.04	0.34
Zngil									0.42

*: significant at $P=0.05$, ** significant at $P=0.01$, ***: significant at $P=0.001$

(114.83 mg.kg⁻¹ dry weight) compared to Pb (18.52 mg.kg⁻¹ dry weight) and Cd (4.06 mg.kg⁻¹ dry weight) was observed for sturgeon species in the South Caspian Sea (Mashroofeh et al., 2013).

It is not surprising that the concentration of Zn is higher than Pb and Cd in this stud, since this metal is an essential element for aquatic organisms and its content is more than other non-essential elements on the earth's crust, but its level can be increased as a result of industrial, agricultural and mining activities (Saghali et al., 2012; Wood et al., 2012). Therefore, it is released increasingly into the aquatic environments, and increase its absorption and bioaccumulation in the tissues and organs of aquatics (Canli and Atli, 2003; Wood et al., 2012). It is characterized by a higher solubility and a slower absorption relative to the amount of bioaccumulation (Wood et al., 2012).

Pb and Cd are considered as toxic and non-essential elements that have no known role in biological systems (Wood et al., 2012) and even low concentrations of these metals threat aquatic

organisms' health (Olmedo et al., 2013). These heavy metals transfer through water and sediments in different parts of aquatic organisms, including fish (Rashed, 2001). High concentrations of heavy metals in water, sediments and organisms have reported in different areas of the Caspian Sea (De Mora et al., 2004; Parizanganeh et al., 2006; Karbassi et al., 2008; Bastami et al., 2015).

The concentrations of Cd, Pb and Zn in liver and gill of the both fish species i.e. *C. aurata* and *R. kutum* were higher than the limits of international standards viz. WHO (World Health Organization), FDA (Food and Drug Administration) and MAFF (Ministry of Agriculture, Fisheries and Food, United Kingdom) (Table 3). High concentration of heavy metals in the aquatic environments also is reported by other studies e.g. Canli and Atli (2003), Ali et al. (2011) and Mashroofeh et al. (2013).

Comparison of heavy metal accumulation in the fish species: Based on the results, the accumulation of metals in the fish tissues were species specific. This can be attributed to the different diets and feeding

Table 3. International Standards for heavy metals (mg. kg⁻¹ dry weight).

Standards	Zn	Pb	Cd
WHO	100	0.5	0.2
MAFF	50	2	0.2
FDA	40	5	1

behaviors of the two studied fishes. Sediments are considered as an important source of heavy metals and play a key role in metal transition through the food chain (Bastami et al., 2015). Heavy metals are accumulated in the sea bed due to their low mobility in sediments, and can be easily available in large quantities for aquatic organisms, particularly benthic dwellers or eater (Gnandi et al., 2006). Studies have shown that fish species accumulate high levels of heavy metals such as Zn, Pb and Cd in their tissues, depending on their feeding behaviors (Canli and Atli, 2003; Voigt, 2004; Evans et al., 2005; Ben Salem et al., 2014). Thus, the higher values observed for *C. aurata* can be due to its feeding behavior which causes it to spending more time close to sediments relative to *R. kutum*. However, many factors may effect on the absorption or accumulation of metals by an organism such as biological and physiological factors, environmental parameters, and fish habitats (Evans et al., 2005). Canli and Atli (2003) observed the highest Zn (110.03 mg.kg⁻¹ dry weight), Pb (41.24 mg.kg⁻¹ dry weight) and Cd (4.5 mg.kg⁻¹ dry weight) in the livers of *Mugil cephalus*, *Atherina hepsetus*, and *Trigla cuculus*, respectively.

Comparison of heavy metal accumulation in liver and gill tissues: The results showed that the accumulation of heavy metals in liver tissue was significantly higher than gill tissue for both fish species i.e. *C. aurata* and *R. kutum*, except for Zn in the gill of *R. kutum*. Most studies confirm that the liver is a tissue which accumulates the highest concentration of heavy metals compared to gill tissue (Canli and Atli, 2003; Ozturk et al., 2009; Paulino et al., 2014; Beg et al., 2015). The higher concentrations of metals in the liver compared to the gill can be due to the high capacity of liver tissue to induce the metal-binding protein metallothionein (Canli and Atli, 2003). These metallothionein can

bind to certain metals and leads to higher concentrations of those metals in the liver (Beg et al., 2015). Most elements tend to react with carboxylate oxygen, amino groups, and nitrogen or mercapto-group sulfur in metallothionein protein (Cheung et al., 2004; Scudiero et al., 2005). Thus, the highest concentration of metals can be seen in the liver and this causes the liver to be the major tissue for metal accumulation (Okocha and Adedeji, 2011).

Relationship between all studied parameters: The results revealed that there is a significant correlation between Cd and Pb in the liver and gill of *C. aurata* which indicates a common contamination source of these metals. Moreover, these results indicated that *C. aurata* is potentially a better bioindicator of heavy metal pollution in aquatic environments than *R. kutum*, because of its feeding behavior.

The results also showed that the relationship of Zn with Cd and Pb was positive and not significant. These results might be due to two reasons: (i) Zn has a higher solubility and slower absorption and plays an important role in fish metabolism relative to Cd and Pb, and (ii) there are more various sources for Zn compared to Cd and Pb in an aquatic environment.

As conclusion, the present study revealed the liver tissue of *C. aurata* accumulated significantly higher concentration of Cd, Pb and Zn compared to that of *R. kutum*, but these results were not significant for gill tissue. Moreover, liver tissue accumulated significantly higher concentration of Cd and Pb compared to gill tissue in *C. aurata*, but these results were not significant for *R. kutum*. From the results of this study we can conclude that the liver tissue of *C. aurata* has higher potential to accumulate heavy metal pollution compared to liver tissue of *R. kutum*.

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چکیده فارسی

غلظت فلزات سنگین در بافت‌های آبشش و کبد ماهی سفید (*Rutilus kutum*) و ماهی کفال طلایی (*Chelon aurata*) در ساحل بابلسر، جنوب دریای خزر

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چکیده:

تجمع فلزات سنگین در اکوسیستم‌های آبی مهمترین نگرانی است که سلامت انسان‌ها را تهدید می‌کند. در این مطالعه دو گونه تجاری، ماهی سفید (*Rutilus kutum*) و ماهی کفال طلایی (*Chelon aurata*) جهت بررسی فلزات سنگین (Cd, Pb, Zn) در ساحل بابلسر، جنوب دریای خزر انتخاب گردید. بابلسر یکی از مهمترین ایستگاه‌های ماهیگیری در جنوب دریای خزر است. نتایج نشان داد که غلظت فلزات Cd, Pb و Zn در بافت کبد ماهی کفال طلایی در مقایسه با بافت کبد ماهی سفید به طور معنی‌داری بیشتر می‌باشد، اما این نتایج برای بافت آبشش معنی‌دار نبودند. غلظت تجمع فلز سرب و کادمیوم در بافت کبد در مقایسه با بافت آبشش در ماهی کفال طلایی بیشتر بود، اما این نتایج برای ماهی سفید معنی‌دار نبود. می‌توان نتیجه گرفت که بافت کبد ماهی کفال طلایی توانایی بیشتری نسبت به کبد ماهی سفید در تجمع فلزات دارد.

کلمات کلیدی: بافت‌های ماهی، روی، کادمیوم، سرب، دریای خزر.