



Monitoring heavy metal contamination on the Iranian coasts of the Persian Gulf using biological indicators: risk assessment for the consumers

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Abstract This research was conducted to determine the concentration of heavy metals (Cu, Pb, and Ni) in the sediments as well as the gill and muscle tissue of *Siganus javus* and two species of algae (*Padina australis* and *Sargassum vulgare*) collected from the Persian Gulf coasts of Bushehr province, which were studied using standard laboratory methods. The general form and trend of metal uptake at different stations in the gill and muscle tissue was $Cu > Ni > Pb$. The results of the study of metal uptake in both algae showed that the uptake of all three metals was higher in *Padina* species ($Pb < Cu < Ni$). The estimated daily intake (EDI), estimated weekly intake (EWI), allowable fish consumption rate limit (CR_{lim}), and the target

hazard quotients (THQ) for the consumption of this fish were also calculated. It was found that the concentration of heavy metals in the edible parts of the fish did not exceed the permissible limits proposed by the WHO, MAFF, JECFA, and NHMRC for human consumption, but the Ni concentration was higher than standard. The consumer risk indexes for non-cancerous diseases due to all metals were lower than standard. Also, the total risk index (HI) in this study was 0.065.

Keywords Sea pollution · Heavy metals · Biological Index · Bushehr Shores

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Introduction

The study of aquatic habitats provides us with the knowledge of their biological and non-biological characteristics and a more comprehensive understanding of their ecological structure, which can help us with better conservation, as well as sustainable exploitation and management of such areas (Kamaruzzaman et al., 2011). Rapid development of industry and agriculture increases the pollution of aquatic ecosystems, especially rivers and lakes, with heavy metals. Heavy metals enter the rivers through polluted waters and eventually reach the seas and lakes (Yi et al., 2017). Heavy metals are carried from natural and synthetic sources into the bodies and tissue of organisms and accumulate there, which increases their toxic danger

(Khatri & Tyagi, 2015). Unlike other organic pollutants, metals are not destroyed or eliminated in the ecosystem but accumulate in sediments or living organisms and eventually enter the human body as part of a food chain. Direct transmission of heavy metals from sediments to aquatic organisms is the main transmission route for many aquatic species. Copper and nickel are very low in the body, but these metals and a number of other heavy metals can have adverse effects on human health and wildlife behaviors because when they exceed the acceptable limits, they might lead to slower growth, renal failure, and reproductive disorders (Ahmed et al., 2019; Hosseini et al., 2013). Fish are the best organisms for assessing the pollution of aquatic environments by toxic elements. Fish play an important role in the transfer of metal pollutants from the marine ecosystem to the sea since they are in direct contact with the sediments of the seafloor which are the site of final accumulation of heavy metals; moreover, fish are important owing to their role in the human diet (Balcioğlu, 2016). Sediments actually serve as a reservoir for the accumulation of heavy metals in aquatic ecosystems and often store up to 99% of metals in them (Laxmi Priya et al., 2011). *Siganus javus* of the family of Siganidae lives in the waters of the Persian Gulf and the Oman Sea around rocky areas and coral reefs. This fish moves in small shoals, feeding on seaweed (El-Sayed et al., 1995). *Siganus javus* is one of the most widely consumed fish in the region and is mostly sold in Southeast Asian and Arab markets (Sadeghi, 2001). Padina algae belong to the group of brown algae and are widely found in tropical and temperate coastal areas in intertidal and subtidal zones. About 6 species of Padina have been identified so far on the northern coasts of the Persian Gulf (Amini et al., 2013). *Sargassum* algae are brown algae which grow mainly in the intertidal and subtidal zones as well as in shallow water areas and on rocky beds. The accumulation of pollutants is not the same in different types of tissue (muscle, gill, liver). Since muscles are the most edible part of fish, the potential risks of muscle contamination are of great concern to humans, and research on their pollutants is of great importance despite the muscles' low potential for concentration of metals compared to gills and liver (Neves et al., 2015). Therefore, it is important to measure the amount of heavy metal absorption in determining the general health status and in protecting the marine environment. Many researchers use health risk assessment

methods to determine the risk of consuming polluted materials for humans, and the calculation of risk indices can help human health decision-makers and authorities (Varol & Sünbül, 2018). Many previous studies have been carried out on algae as bioindicators for determining heavy metal contamination in water. Ali et al.'s study (2017) on the brown algae of Red Sea in Sudan and in Iran and Dadolahi et al.'s research (2010) on 13 dominant species of algae (including 3 species of green algae, 4 species of brown algae, and 6 species of red algae on the coasts of Bushehr) are two examples of this type of research. Therefore, the aim of this study was to assess the uptake of heavy metals (Cu, Pb, and Ni) in coastal sediments, gill and muscle tissue of fish and two species of *Padina australis* and *Sargassum vulgare* as bioindicators in order to help protect the marine environment and assess the risk for the fish consumers.

Materials and methods

The sampling of fish, algae, and sediments was carried out on the shores of Bushehr province (north of the Persian Gulf). For this purpose, 60 fish, 30 sediment, and 60 algae samples were collected from the Bushehr province coasts.

Sampling and preparation of chemical digestion of fish tissue

Samples were harvested with special fish cages by local fishermen to investigate the accumulation of heavy metals in the muscle tissue and gills of fish along the entire coasts of Bushehr. Samples were transferred to the University Research Laboratory (1:1) in baskets containing crushed ice. The fish biometric indices of weight in grams and total length in centimeters were measured by scales and a calibrated board, respectively. To perform chemical digestion, 10 g of muscle tissue and 10 g of gills were weighed and dried for 72 h in the oven at 50 to 55 °C. Then, 10 ml of concentrated nitric acid was added to 10 g of each sample and was placed on the hotplate at 95 °C for 3 h for complete digestion. After cooling completely, the specimens were filtered with Whatman filter paper 42, increased to 25 ml with distilled water, and placed in sterile capped tubes for injection into the atomic absorption unit (Moopam, 2010).

Sampling and preparation of chemical digestion of algae

After being collected, the specimens of algae from the coasts of Bushehr were transferred to the Research Laboratory using polyethylene containers. They were then rinsed twice with distilled water and completely dried at 80 °C. For preliminary digestion of algae samples, 10 ml of 65% nitric acid was added to 1 g of powdered algae and kept at room temperature for at least 12 h. Then, the samples were placed on a hotplate at 140 °C for 5 h for complete digestion. After digestion, 10 ml of 10% nitric acid was added to it. After filtration with Whatman filter paper 42, the solution was increased to 25 ml with double-distilled water and kept in the refrigerator at less than 4 °C before injection into the atomic absorption unit (Rajendran et al., 1993).

Calculating daily and weekly absorption

Fish are generally consumed in fresh weight. For this reason, metal accumulation should also be calculated in terms of wet weight by multiplying the amounts of metals in the fish muscles by the correction factor of 0.2 (UNEP, 1984). Con in WW: 1 – (the amount of moisture in the fish’s muscle/100) × Con in DW. In order to determine the amounts of contaminants absorbed through the nutrient per kilogram of body weight per day (EDI = estimated daily intake) and per week (EWI = estimated weekly intake), the US Environmental Protection Agency’s proposed method was used in Formulas 1 and 2 (Norouzi, 2020):

$$EDI = \frac{C \times MS_D}{BW} \tag{1}$$

$$EWI = \frac{C \times MS_w}{BW} \tag{2}$$

C = the average heavy metal concentration in fish (µg/g)

MS_D = rate of daily fish consumption (32.57 g day⁻¹ according to the EPA standard)

MS_w = rate of weekly fish consumption (228 g week⁻¹ according to the EPA standard)

BW = the average body weight (adults 70 kg, children 14.5 kg).

Calculating allowable fish consumption rate

One of the most important methods of determining fish consumption limits is the method proposed by the US Environmental Protection Agency (EPA) (USEPA, 2000). In this method, based on the amount of metals in edible fish tissue and using a reference dose (RfD) according to Formula 3, the maximum acceptable amount of fish and fishery products to be consumed within a specific period can be calculated (Pinzón-Bedoya et al., 2020):

$$CR_{lim} = \frac{RfD \times BW}{C} \tag{3}$$

CR_{lim} = maximum allowable fish consumption rate (g/day)

RfD = reference dose (mg/kg/day)

Risk Calculation – Risk Index (THQ = Target Hazard Quotients)

The potential hazard is the ratio of the concentration of an element to the maximum concentration of the element that does not cause problems in the body. Formula 4, presented by the EPA, was used to calculate the probability of people being at risk of non-cancerous diseases. According to this index, if the number is higher than one, it indicates a high risk of non-cancerous diseases (USEPA, 2009), while an index number of less than one indicates no adverse effect on consumers. The total hazard indicator (HI) was obtained using Formula 5 (Chien et al., 2002; Dee et al., 2019):

$$THQ = \frac{EFr \times ED \times FIR \times C}{BW \times RFD_o \times ATn} \times 10^{-3} \tag{4}$$

$$HI = \sum THQ = THQPb + THQCu + THQNi \tag{5}$$

THQ = target hazard quotient

HI = hazard index

EFr = exposure frequency (365 days per year)

ED = total exposure time (exposure duration) (70 years)

FIR = daily fish ingestion rate (32.5 g/day according to the EPA standard)

ATn = average exposure time (70 years × 365 days)

One-way ANOVA was used for data analysis, and the Pearson correlation test was used to determine the

relationship between factors with a 95% confidence level.

Results and discussion

Fish

The *Siganus javus* fish caught in various stations of Bushehr's coasts were 100.48 ± 15.67 g in average weight and 18.59 ± 0.75 cm in length. The difference in weight was significant ($P < 0.05$), but the difference in length was not significant ($P > 0.05$). The general form and trend of metal uptake in the gill and muscle tissue was $Cu > Ni > Pb$. It is important to note that the non-significance of some metals in fish tissue depends on different metabolic activities and physiological changes over time, which may lead to a negative correlation between the fish size and the amount

of metals in its muscle (Fard et al., 2015). The results of analyzing metal accumulation in the gill and muscle tissue of *Siganus javus* were compared with those of other species in the Persian Gulf based on international standards (Table 1).

The results of this comparison showed that the concentrations of lead and copper were lower than normal (but on the borderline), and the concentration of nickel was higher than normal by international standards. Various factors are responsible for the high amount of metal accumulation in the gill and muscle tissue of *Siganus javus*. Fishing boats as well as urban, agricultural, and domestic wastewater are some major contributors to the contamination of water resources (Luoma & Rainbow, 2008). The other major source of heavy metals is the anti-corrosion and anti-clogging compounds and paint used to protect the body of ships and boats against algae and barnacles, with about 15 to

Table 1 Comparison of the metals of gill and muscle tissue of *Siganus javus* ($\mu\text{g/g}$) with those of other species based on international standards

Regions of study	Fish species	Tissue	Ni	Pb	Cu	References
	<i>Liza klunzingeri</i>	Muscle	–	3.25–14.16	10–16.66	Bastami et al. (2015)
	<i>Alepes djedaba</i>	Muscle	0.059	0.015	0.404	Akhbarizadeh et al. (2018)
	<i>Epinephelus coioides</i>	Muscle	0.098	0.007	0.235	
	<i>Sphyraena jello</i>	Muscle	0.085	0.008	0.359	
	<i>Platycephalus indicus</i>	Muscle	0.052	0.012	0.226	
	<i>Anodontostoma chacunda</i>	Muscle	–	1.10	0.83	Keshavarzi et al. (2018)
	<i>Liza abu</i>	Muscle	2.03	0.86	1.33	Abdolahpur Monikh et al. (2013)
	<i>Euryglossa orientalis</i>	Muscle	2.17	0.63	3.12	
	<i>Johnius belangerii</i>	Muscle	1.96	0.94	1.56	
	<i>Liza abu</i>	Gills	10.42	1.74	4.71	
	<i>Euryglossa orientalis</i>	Gills	14.61	1.22	8.32	
	<i>Johnius belangerii</i>	Gills	8.47	2.30	2.16	
Hendijan Port, Iran	<i>Euryglossa orientalis</i>	Muscle	–	5.20	5.08	Sadough Niri et al. (2012)
Deilam Port, Iran		Muscle	–	4.69	3.08	
Southeast China	<i>Aristichthys nobilis</i>	Gills	–	0.102	0.562	Jiang et al. (2018)
	WHO		0.4	0.5–1.5	30	WHO (1996)
	USEPA		–	0.005	0.1	Tuzen (2009)
	MAFF		–	1.5–2	20	MAFF (1995)
	FAO		–	5	30	FAO (1983)
	NHMRC		–	1.5	10	Maher (1986)
	WHO (1989)		0.5	0.4	–	Gholamhosseini et al. (2021)
Present study	<i>Siganus javus</i>	Muscle	3.08	0.56	13.98	
		Gills	2.07	0.55	13.12	

30% of this paint being composed of some metals such as zinc, copper, and lead (Keshavarzi et al., 2015). In addition, the physiological differences and locations of different types of tissue in fish can affect the bioaccumulation of each metal. Levels of metal accumulation are controlled by physical factors such as severity of contamination, exposure time, water pH, alkalinity, and temperature, while biological factors such as size, age, feeding habits, and reproductive cycle are the main factors for controlling the degree of contamination in fish (Milošković et al., 2016; Mziray & Kimirei, 2016). The Persian Gulf is one of the most important sites in Iran that hosts a variety of benthic and pelagic species. The benthic species live and feed in the sediments, while the pelagic ones reside in water columns and surface layers (Naccari et al., 2015). In general, the Persian Gulf is one of the areas surrounded by large deserts of the world and receives a high volume of sediments each year, especially in cold seasons when wind speeds are higher. On the other hand, as the wind speed increases, waves are created, thereby increasing the potential for the water bed sediments to mix with the water columns and the living creatures in these areas. These conditions provide grounds for increasing concentrations of pollutants such as heavy metals found in sediments and fish and can lead to higher levels of contamination (Gelsleichter et al., 2020). Due to the fact that *Siganus javus* is a pelagic kind of fish and lives in surface areas, the sources of water contamination have caused an increase in the accumulation of metals in its tissue. *Siganus javus* has a vegetarian diet and omnivorous behavior and is capable of utilizing low levels of food in the aquatic environment (Boonyaratpalin, 1997). It seems that the high concentration of metals in the gill tissue is due to the mixing of elements with the gill mucosa, which makes it impossible to completely move the elements from the gill hinge when preparing the tissue for testing (Heath, 2018). In dealing with metals, gills apply four mechanisms: reducing the water uptake, detoxifying the metals with metallothionein proteins, protecting cellular structures by binding to proteins, and excreting metals (Yilmaz, 2009). Since different species accumulate metals and eliminate toxic elements in different ways, it is important to sample different species (Koide et al., 2016).

Algae

The Persian Gulf, with a depth of 40 m, is a shallow and semi-enclosed sea connected to the Indian Ocean through the Strait of Hormuz (Fard et al., 2015; Hosseini et al., 2016). Over the past few decades, rapid industrial development and population growth in the Persian Gulf states have led to a significant increase in heavy metals in aquatic ecosystems (Pourkerman et al., 2017). In the Persian Gulf area, the Moussa river and Mahshahr Port, there are two coastal ecosystems that host the fauna and some unique plants. These ecosystems receive large amounts of pollution from municipal and agricultural effluents, petrochemicals, and large transportation ports. The results of the study of metal uptake in both genera of algae showed that the uptake of all three metals was higher in *Padina* species. The metal uptake behavior was $Pb < Cu < Ni$ in *Padina* species and $Pb < Ni < Cu$ in *Sargassum* species. There are two major pathways for the biosorption of heavy metals within plants. The first is the entry of pollutants through the extracellular space and the cell wall, which is reversible and occurs at a high speed. The second is through the cell membrane, which is slow, energy consuming, and irreversible. Because copper takes the first path and lead the second path to penetrate into the plant tissue and accumulate there, the highest and lowest adsorption rates were related to copper and lead in *Sargassum* species. The high levels of nickel and copper in the algae are due to the high consumption of fossil fuels for industrial and transportation purposes in the Persian Gulf. Urban wastewater can also be a contributing factor (Dadolahi et al., 2010). High amounts of heavy metals travel toward the coasts through surface running waters, dry land, and the land surrounding industries. Temperature changes and solar energy also have a considerable effect on the bioaccumulation of metals because a decrease in the light and heat of the sun or high temperature stresses without efficient transpiration makes for a reduction of in plants' metabolic activity (Mahan & Burke, 2015), which leads to further accumulation of metals in them (Strezov & Nonova, 2005).

BSAF (biosediment accumulation factor) was used to compare the capability of heavy metal accumulation in the studied algae. This indicator shows the ratio of heavy metal accumulation in algae and

Table 2 ANOVA test results and standard deviation \pm average amount of heavy metals in gill and muscle tissue of *Siganus javus*, two species of algae and sediments of Bushehr coasts

	Muscle ($\mu\text{g/g}$ Dry weight)			Gill ($\mu\text{g/g}$ Dry weight)		
	Pb	Ni	Cu	Pb	Ni	Cu
Total	0.56 \pm 0.31	2.7 \pm 1.02	13.12 \pm 7.49	0.55 \pm 0.31	3.08 \pm 1.85	13.98 \pm 5.31
Min–Max	0.05–1.5	0.8–4.5	1.02–26.8	0.0–1.60	1.18–7.30	5.20–23.21
Sig	**	**	**	**	**	**
	<i>Padina australis</i> ($\mu\text{g/g}$)			<i>Sargassum vulgare</i> ($\mu\text{g/g}$)		
Total	0.58 \pm 0.58	8.54 \pm 4.23	6.71 \pm 1.54	0.08 \pm 0.07	2.57 \pm 1.81	5.70 \pm 1.39
Min–Max	0.08–2	1.33–17.44	4.98–10.61	0.0–0.23	0–6	3.24–8.15
Sig	**	**	NS	NS	**	**
Index BSAF	0.07	0.78	0.9	0.008	0.27	0.78
	Sediments ($\mu\text{g/g}$)					
Total	8.43 \pm 6.34	9.63 \pm 7.49	7.42 \pm 1.5			
Min–Max	3.42–25.48	0.18–23	5.58–11.08			
Sig	*	**	NS			

NS not significant

*Significance level is 0.05; **Significance level is 0.01

related sediments. The results of this index are shown in Table 2.

In Table 3, the amount of heavy metal uptake in wet weight fish muscles and the reference dose, as well as the estimated daily intake (EDI) and weekly intake (EWI) rates of metal in adults and children and the maximum allowable weekly intake ($\mu\text{g/g}/70$ kg/week), are shown. Table 3 also shows the risk level and allowable fish consumption rate limit (CR_{lim}). According to these results, the amounts of heavy

metals in the fish muscles were lower than the heavy metal consumption limits allowed by international standards. The calculated values of the risk of contracting non-cancerous diseases due to the consumption of all three metals were less than 1, indicating that eating *Siganus javus* had no adverse effect on consumers. Finally, the total hazard index (HI) in this study was below 1 (0.064).

According to the results of the Pearson correlation test, there was a direct relationship between the

Table 3 Amounts of intake for the 3 metals ($\mu\text{g/g}$ wet weight) and average \pm standard deviation and comparison of the amounts with international standards

Heavy metal	Average con	EDI adults	EDI children	EWI adults	EWI children	CR_{lim} adults	CR_{lim} children
Pb	0.11 \pm 0.06	0.05 \pm 0.02	0.25 \pm 0.13	0.36 \pm 0.20	1.77 \pm 0.97	2.23 \pm 4.08	0.46 \pm 0.84
Cu	2.79 \pm 1.06	1.30 \pm 0.49	6.28 \pm 2.38	9.11 \pm 3.46	43.98 \pm 16.71	0.93 \pm 2.44	0.20 \pm 0.51
Ni	0.61 \pm 0.37	0.28 \pm 0.17	1.38 \pm 0.83	2.01 \pm 1.20	9.71 \pm 5.81	2.30 \pm 3.71	0.48 \pm 0.78
	Reference dose	MTDI	PTWI	THQ			
Pb	0.0035 ^a	0.21 ^a	25	0.015			
Cu	0.037 ^a	30.00 ^c	3500	0.035			
Ni	0.02 ^a	0.30 ^b	35	0.014			

HI=0.064

RfD reference dose (USEPA, 2000); PTWI standard for the maximum permissible limit of weekly absorption: $\mu\text{g/g}$ 70/kg bw/week (Norouzi, 2020); MTDI standard for the maximum tolerable daily dietary intake: mg/kg/day (Gholamhosseini et al., 2021)

^aProvisional tolerable daily intake (JECFA, 2000),(USEPA, 2011)

^bEstimated safe and adequate daily dietary intake (NRC, 1989), (USEPA, 2002)

^cProvisional maximum tolerable daily intake (JECFA, 1982)

Table 4 Determining the Pearson correlation of sediment metals and their accumulation in fish tissue and two species of algae

Sediment		Fish: Gill tissue			Algae: <i>Padina australis</i>		
		Ni	Pb	Cu	Ni	Pb	Cu
Ni	Pearson	0.680**	0.420	0.016	0.873**	0.709**	-0.222
	Sig	0.002	0.082	0.951	0.000	0.003	0.427
Pb	Pearson	-0.336	-0.331	-0.120	-0.092	-0.129	-0.414
	Sig	0.173	0.180	0.635	0.745	0.647	0.125
Cu	Pearson	-0.086	0.050	-0.399	0.118	0.418	-0.155
	Sig	0.734	0.843	0.101	0.676	0.121	0.581
Sediment		Fish: Muscle tissue			Algae: <i>Sargassum vulgare</i>		
		Ni	Pb	Cu	Ni	Pb	Cu
Ni	Pearson	0.884**	0.731**	0.801**	0.657**	0.401	0.070
	Sig	0.000	0.001	0.000	0.008	0.139	0.805
Pb	Pearson	-0.245	-0.276	-0.434	-0.056	0.236	-0.369
	Sig	0.328	0.268	0.072	0.843	0.397	0.176
Cu	Pearson	0.111	0.158	0.098	0.159	0.532*	-0.123
	Sig	0.662	0.530	0.699	0.573	0.041	0.663

NS not significant
 *Significance level is 0.05;
 **Significance level is 0.01

amount of nickel in the sediments with its content in both fish tissue and two species of algae. Furthermore, a significant positive relationship was found between the amount of copper adsorbed in the sediments and the amount of lead metal adsorbed in *Sargassum* algae (Table 4). Most well-known analyses indicate that most sources of metal contamination are related to crude oil. Due to the oil-related activities in the studied area over the past 50 years, it appears that these metals in fish originate in oil contaminants such as oil leakage or discharge in the sea (Osuji & Onojake, 2004).

Conclusions

The results of this study showed that considering the relevant standards, the accumulation of metals in the sediments was harmless to the environment. Also, it was found that the concentrations of copper and lead (but not nickel) in the gill and muscle tissue of *Siganus javus* caught in Bushehr waters were below the permissible limit based on international standards. Moreover, considering the risk assessment of the human consumption of fish, it seems that the consumption of this fish caught off the coast of Bushehr currently does not pose a serious threat to consumers. However, there are some considerations for the consumption of this fish by pregnant women and children. Since fish consumption patterns are not

the same all over Iran, the fishing community and even people living in coastal areas may frequently consume seafood. Although the consumption of fish does not pose a health risk to the consumers, caution should be exercised by pregnant women and children in eating fish because fetuses, infants, and children under 10 are sensitive. Moreover, the risk indexes for adults’ and children’s bodies weight are 70 kg and 14.5 kg, respectively. Clearly, the optimal intake rate also varies for overweight and underweight people. The increasing number of pollutant sources in the studied coasts seems to be related to industrialization and people’s activities for expanding their businesses, which have caused the decay of the aquatic ecosystems (Rajeshkumar et al., 2018; Hu et al., 2010; Padmini & Geetha, 2007; Huang, 2000). Because of the variability in diet, marine fish have a high level of metal accumulation in their tissue. Also, the data show that metal pollution is increasing in the studied region. For this reason, special attention should be paid to the safety of seafood in an effort to increase the per capita fish consumption and to change people’s consumption behavior.

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Data availability Data supporting Tables 1–4, are publicly available in Marine Data Archive repository, as part of this record: http://mda.vliz.be/directlink.php?fid=VLIZ_00001041_60f2bbdbe4a7e893968028

Declarations

Competing interests The authors declare no competing interests.

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