



Health risk assessment of heavy metal intake due to fish consumption in the Sistan region, Iran

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Received: 6 June 2017 / Accepted: 5 October 2017 / Published online: 25 October 2017

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Abstract The heavy metal (Pb, Cd, Cr, and Ni) content of a fish species consumed by the Sistan population and its associated health risk factors were investigated. The mean concentrations of Pb, Cd, and Cr were slightly higher than the standard levels. The Ni content of fish was below the maximum guideline proposed by the US Food and Drug Administration (USFDA). The average estimated weekly intake was significantly below the provisional tolerable intake based on the FAO and WHO

standards for all studied metals. The target hazard quotients (THQ) of all metals were below 1, showing an absence of health hazard for the population of Sistan. The combined target hazard quotient for the considered metals was 26.94×10^{-3} . The cancer risk factor for Pb (1.57×10^{-7}) was below the acceptable lifetime carcinogenic risk (10^{-5}). The results of this study reveal an almost safe level of Pb, Cd, Cr, and Ni contents in the fish consumed by the Sistan population.

Highlights

- There is only scarce data regarding heavy metal concentrations in fishes captured from lakes and their human health risks.
- The heavy metal (Pb, Cd, Cr, and Ni) content of *Hypophthalmichthys molitrix* consumed by the Sistan population and its associated health risk factors were investigated.
- The average of the estimated weekly intake was significantly below the provisional tolerable intake.
- There is no health hazard for the population of Sistan through the consumption of studied fish.
- The cancer risk factor for Pb was below the acceptable lifetime carcinogenic risk.

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Keywords Heavy metals · Fish consumption · Health risks · Cancer risk · Sistan and Baluchestan

Introduction

In the last few decades, rapid population increase and economic development have caused an increase in global concern about heavy metal pollution, due to the stable, non-biodegradable, and persistent properties of heavy metals (Ghaneian et al. 2014; Saha et al. 2016; Sanchooli Moghaddam et al. 2016). Heavy metals such as cadmium, lead, nickel, chromium, and mercury can be classified as potentially toxic elements (Makedonski et al. 2017; Taghavi et al. 2015).

One of the main sources of the total human exposure to toxic chemicals such as heavy metals is the dietary intake of contaminated food, especially seafood (Zhao et al. 2014; Shadborestan et al. 2013; Nemati et al. 2014). Heavy metals can be introduced to water bodies through domestic, industrial, and agricultural activities, as well as atmospheric deposition (Zazouli et al. 2013). Therefore, aquatic ecosystems, especially lakes, are known as the main receptors of heavy metals either directly or indirectly (Elkady et al. 2015). Fish is an important part of human diet due to its high nutritional quality, especially its high protein content and omega-3 fatty acids and vitamins (Hao et al. 2013; Taweel et al. 2013). The environmental conditions of living fish can result in the bioaccumulation of some heavy metals in the fish tissue (Elkady et al. 2015). However, many factors such as the environmental conditions, the contaminant levels, the length of the food chain, and the physiochemical properties of the contaminants are involved in the trophic transfer; the relatively high concentration of heavy metals results in the uptake of heavy metals by fish

(Elkady et al. 2015; Hao et al. 2013). Heavy metals can get bioaccumulated in fish through direct absorption from water through their gills and/or skin and also by ingestion of contaminated food or particles (Mziray and Kimirei 2016). The concentration of bioaccumulated heavy metals in the fish body is a function of some environmental parameters, including pH, temperature, alkalinity of the environment, pollutant type, sampling site, and the ecological and physiological characteristics of the species such as food habits, size, sex, life span, habitat, and position in the aquatic food chain (Mziray and Kimirei 2016). However, the bioaccumulation of heavy metals in fish can lead to potential health risk to humans through fish consumption (Hao et al. 2013; Taweel et al. 2013). Many studies have been conducted on the metal accumulation in fish captured from the sea (Saha et al. 2016; Mziray and Kimirei 2016; El-Moselhy et al. 2014), rivers (Baharom and Ishak 2015; Liang et al. 2016), and lakes (Elkady et al. 2015; Hao et al. 2013; Monroy et al. 2014) all over the world.

The Chah Nime lakes are an important fishery resource in the Sistan and Baluchestan provinces (I.R. Iran). The environmental pollution of the Chah Nime lakes has previously been documented by measuring the concentrations of Cr, Pb, Cd, and Ni in water and sediment samples (Ghaleno et al. 2015; Rajaei et al. 2012). However, there is only scarce data regarding heavy metal concentrations in fish captured from the Chah Nime lakes and the related human health risk in the Sistan region. Since the Chah Nime lakes receive water from the Helmand River, the longest river in Afghanistan and the primary watershed for the endorheic Sistan basin, such fish pollution by heavy metals reflects somehow the environmental pollution by several local activities (industrial, agricultural, etc) and can be therefore considered as a general model for such pollution. Therefore, this study aims to determine the residual levels of selected heavy metals in the edible part of fish collected from the Chah Nime lakes. The human health risk assessment of the heavy metals by dietary intake through fish is also investigated.

Materials and methods

Sampling location

The Chah Nime lakes are three semi-natural wells along the river Helmand, at the place where this river separates

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into the Sistan and the common Parian rivers. These cavities are located in the south of the Sistan plain in southeastern Iran (between 61° 37' to 61° 46' longitude and 30° 43' to 30° 52' latitude). After the construction of the fourth basin, with 900 million m³, in recent years, the storage capacity reached a total of 1530 million m³, making the Chah Nime lakes the second most important water storage basin in the Helmand basin. Samples of fish for heavy metal analysis were taken from the fourth lake. Figure 1 shows the location of the Chah Nime Lakes.

Sampling design and procedure

A total of 40 *Hypophthalmichthys molitrix*—a widely consumed fish—were taken by the cooperation of a local fisherman from the fourth Chah Nime lake on four occasions. After sampling, the length and weight of the fish were measured. The fish were placed in plastic bags, kept in cool boxes at 0 °C, and

immediately transferred to the laboratory within 2 h to perform heavy metal analysis.

Heavy metal analysis

In the laboratory, the fish were washed with distilled water. A total of 20 g of fish muscle was removed and dried at 105 °C for 24 h and then transferred to a desiccator until it reached a constant weight. Then, 0.5 g of fish sample was powdered and transferred to a beaker containing 5 mL of nitric acid. The mixture was heated up to 140 °C to obtain a clear solution. The solution was then filtered and diluted to 50 mL and used for metal analysis. The concentrations of Cd, Cr, Ni, and Pb in the digested solutions were measured using an atomic absorption spectrophotometer (Varian AA-7000). Standard heavy metal solutions (1000 mg/L) were used to do the calibration curves after the appropriate dilutions.

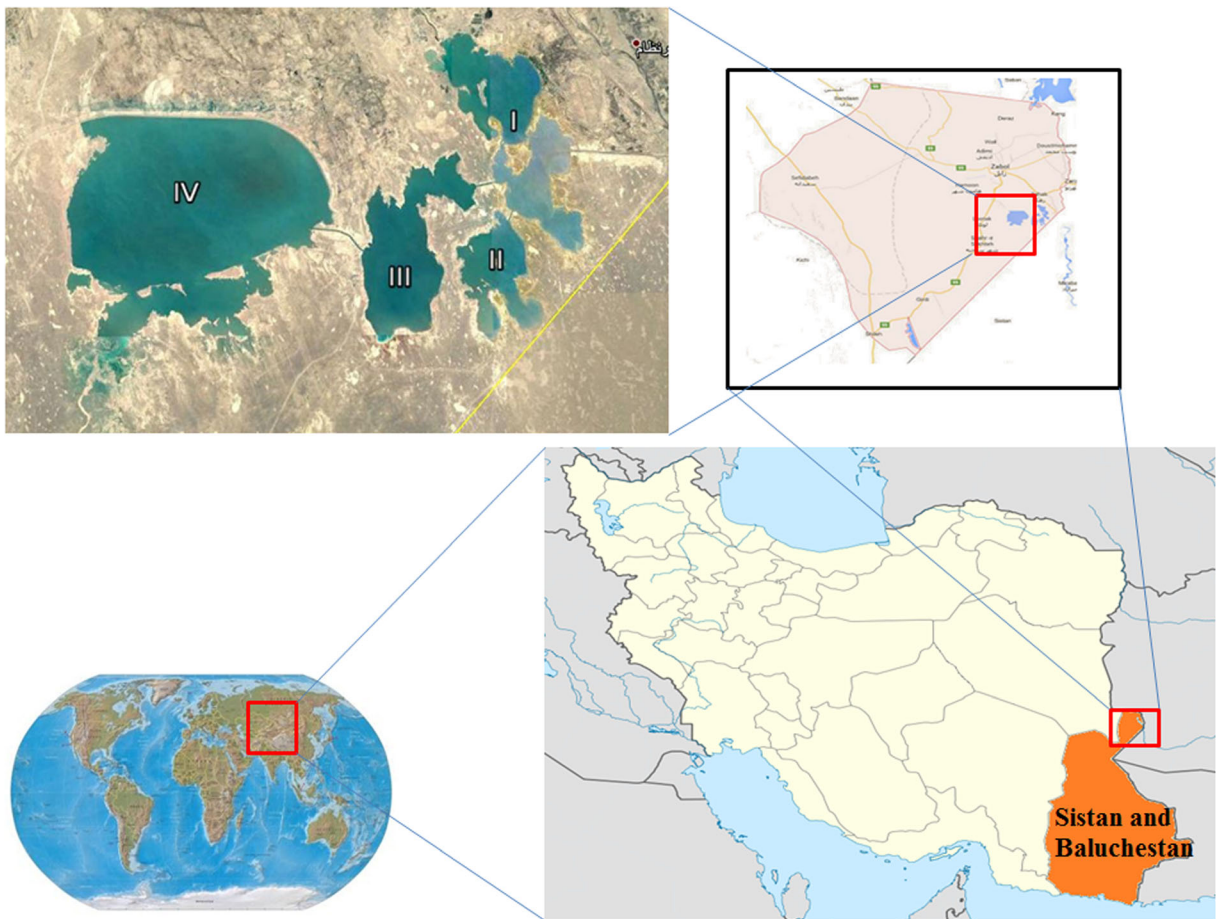


Fig. 1 Location of the four lakes—Chah Nime, Zabol, Sistan, and Baluchistan, Iran

Consumption rate limits

Estimated daily/weekly intake

The estimated daily intake was calculated by the following equation:

$$EDI = \frac{E_F \times E_D \times F_{IR} \times C_F \times C_m}{W_{AB} \times T_A} \times 10^{-3} \quad (1)$$

where E_F and E_D are the exposure frequency (365 days/year) and the exposure duration (60 years) (Saha et al. 2016), respectively; F_{IR} is the ingestion rate of fish (25.2 g/day for Iranian); C_F is the conversion factor to convert fresh weight to dry weight (moisture content of fish fillet equal to 79%), which is equal to 0.208 (Saha et al. 2016); C_m is the metal concentration in the fish tissue ($\mu\text{g/g}$ dry weight basis); W_{AB} is the average body weight for adults (65 kg, for Iranian); and T_A is the average exposure time for non-carcinogens (equal to $E_F \times E_D$) (Saha et al. 2016).

The percentage of provisional tolerable weekly intake

The percentage of provisional tolerable weekly intake was calculated for each heavy metal by the well-recognized safety criterion provisional tolerable weekly intake (PTWI) (Ostos et al. 2015; Yeh et al. 2016). The PTWI is a reference dose set by the joint FAO/WHO Expert Committee on Food Additive (JECFA). It represents a safe weekly intake of pollutants.

$$\%PTWI = \frac{EWI}{PTWI} \times 100 \quad (2)$$

where EWI is the estimated weekly intake of metals (mg/kg bw/week) and PTWI is the provisional tolerable weekly intake (mg/kg bw/week).

Daily consumption limit

The daily consumption rate limit (CR_{lim}) of fish, based on the carcinogenic effect of the contaminants, was calculated by the following equation:

$$CR_{lim} = \frac{(ARL \times W_{AB})}{CSF \times C_m} \quad (3)$$

Based on the non-carcinogenic effects of the contaminants, the maximum allowable daily consumption of fish was determined using the following equation:

$$CR_{lim} = \frac{(RfD \times W_{AB})}{C_m} \quad (4)$$

where CR_{lim} is the maximum allowable daily consumption of contaminated fish (kg/day), ARL indicates the maximum acceptable individual lifetime risk level (in the present study, 10^{-5} was used (Yu et al. 2014)), W_{AB} is the mean body weight of consumer population (kg), CSF shows the cancer slope factor; RfD stands for the oral reference dose (mg/kg-day), and C_m is the metal concentration in the edible part of fish (mg/kg) (Alipour et al. 2015).

The maximum allowable consumption level of fish contaminated with heavy metals in terms of meals per month can be obtained by Eq. 5, in which the maximum allowable daily consumption in terms of kilograms is converted to meal consumption per month considering meal size (Shakeri et al. 2015).

$$CR_{mm} = \frac{CR_{lim} \times T_{ap}}{MS} \quad (5)$$

where CR_{mm} is the maximum allowable consumption rate (meals/month), T_{ap} is the average time period (30.44 day/month), and MS is the meal size (0.227 kg fish/meals) (Shakeri et al. 2015).

Health risk assessment

Target hazard quotient

Non-carcinogenic risk was investigated using the target hazard quotient (THQ), which is the ratio between the estimated exposure (estimated daily intake (EDI)) and the oral reference dose (RfD). RfD (mg/kg bw/day) represents an estimate of the daily oral exposure of the human population that is likely to be without an appreciable risk of deleterious effects. The RfDs were 0.004, 0.001, 1.5, and 0.02 (mg/kg bw/day) for Pb, Cd, Cr, and Ni, respectively (Saha et al. 2016). The following equation was used to calculate the THQ:

$$THQ = \frac{EDI}{RfD} \quad (6)$$

A THQ less than 1 reveals that there is no adverse hazard of the exposed population. A THQ equal to 1 shows that the concerned receptors may experience non-carcinogenic health risk, while the probability should increase as the THQ value increases. In calculating the THQ, the effect of cooking on the concentration of

contaminants was not considered and the ingestion dose was assumed to be equal to the absorbed dose of the contaminant (Saha et al. 2016).

Based on the literature, exposure to two or more pollutants may cause additive and/or interactive effects, and hence, the combined target hazard quotient (CTHQ) may be calculated. The CTHQ gives an overview of health risks of the four studied metals (Pb, Cd, Cr, and Ni) together through fish consumption. The CTHQ was calculated according to the following equation:

$$CTHQ = \sum_{j=1}^4 THQ \tag{7}$$

where *j* is the index of the studied heavy metals (Sim et al. 2016).

Cancer risk

The cancer risk (CR) over a lifetime of exposure to Pb was estimated using the cancer slope factor according to Eq. 8 (Peng et al. 2016; Shaheen et al. 2016):

$$CR = \frac{E_F \times E_D \times F_{IR} \times C_F \times C_m \times CSF}{W_{AB} \times T_A} \times 10^{-3} \tag{8}$$

where CSF is the cancer slope factor (mg/kg/day), while the other parameters have been defined previously. The US Environmental Protection Agency set an acceptable lifetime carcinogenic risk of 10⁻⁵ (Saha et al. 2016).

Relative risk

Yu et al. (2014) define the relative risk (RR) of contaminants for both carcinogenic and non-carcinogenic effects, which can be helpful for managers to recognize the most harmful contaminants. The RR equation is as follows:

$$RR = \frac{C_m}{RfD} \tag{9}$$

where all parameters have been previously defined. Human health risk through fish consumption should increase with an increase in the RR value (Yu et al. 2014).

Statistical analysis

Statistical analysis of data was performed using the SPSS software (SPSS 18.0 for Windows). Spearman’s rank correlation coefficient was used in order to

examine the relation between heavy metal concentration and the relation of heavy metal content with the length and weight of the fish. The statistical significance was considered for *p* < 0.05 (two-tailed tests).

Results and discussion

Heavy metal concentration

The mean concentrations of Pb, Cd, Cr, and Ni in fish muscles are illustrated in Fig. 2. The concentrations of Pb, Cd, Cr, and Ni were 0.23, 0.15, 0.17, and 0.17 µg/g, respectively. Maximum and minimum heavy metal concentrations are given by the bars. As observed, all values remain in a limited range, except for Ni, which shows a large gap between the maximum and mean values.

The maximum Pb concentrations in fish samples allowed by the WHO, the FAO, and the European Community (EC) are 2, 0.5, and 0.2 µg/g, respectively (Gu et al. 2015; El-Moselhy et al. 2014). The Pb concentration in this study did not exceed the WHO and FAO limits, but was a little higher than the EC-recommended value.

The limits for Cd concentration in fish given by the FAO/WHO, the FAO, and the EC are 0.5, 0.05, and 0.05 µg/g, respectively (Baharom and Ishak 2015; El-Moselhy et al. 2014). The Cd concentrations observed in this study (Fig. 2) were significantly below the concentration threshold set by the FAO/WHO but above the level recommended by the FAO and the EC. The Cd contents were reported to be 0.235 and < 0.0025 µg/g in channel fish and < 0.0025 and 0.121 µg/g in green sunfish from the El Rejón and Chihuahua reservoirs in

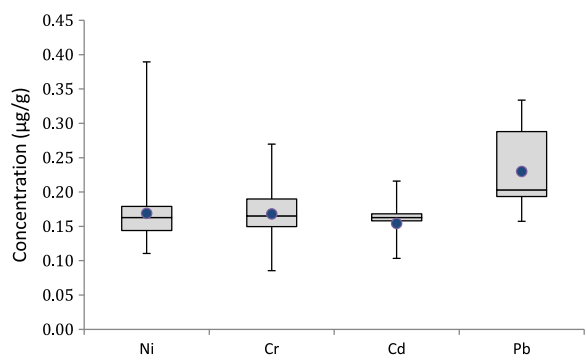


Fig. 2 Box plot of heavy metal concentration in the muscles of fish. Boxes represent the interquartile range of heavy metals. The maximum and minimum values, shown by whiskers and points, represent the mean concentrations of heavy metals

Mexico, respectively, during the summer season (Nevárez et al. 2015); 1.08 $\mu\text{g/g}$ in four edible fish species from the Rawal lake reservoir in Pakistan (Malik et al. 2014); and 0.275 $\mu\text{g/g}$ in fish from the Fosu lagoon in the Republic of Ghana (Akoto et al. 2014).

WHO set a maximum allowable concentration of 0.15 $\mu\text{g/g}$ for Cr (Shakeri et al. 2015). In the present study, the mean Cr concentration (Fig. 2) is slightly higher than the standard level recommended by the WHO. However, it is lower than the Cr concentration reported by Malik et al. (2014), Squadrone et al. (2016), and Hao et al. (2013).

The US Food and Drug Administration (USFDA) proposed a maximum guideline of 70–80 mg/kg for nickel concentration, and hence, the Ni concentrations found in the fish samples from the Chah Nime lakes were below the guideline level (Akoto et al. 2014). The Ni concentrations in this study (Fig. 2) are comparable to those reported in the available literature, by Monferran et al. (2016) for the silver side from the San Roque lake (0.56 and 1.04 $\mu\text{g/g}$ for dry and wet seasons, respectively), by Wei et al. (2014) for the edible part of 11 fish species from the Poyang lake (ranging from 0.017 to 0.114 $\mu\text{g/g}$), and by Zeng et al. (2012) for 5 fish species from the Meiliang bay (ranging from 0.43 to 1.69 $\mu\text{g/g}$) and the Xukou bay (ranging from 0.10 to 0.64 $\mu\text{g/g}$).

Consumption rate limits

The EDI of the four heavy metals studied is determined based on the assumption of 65-kg body weight per person and exposure duration of 60 years. From Table 1, it can be seen that the maximum daily intake is in the order $\text{Pb} > \text{Ni} > \text{Cr} > \text{Cd}$, while related to the percentage of PTWI (%PTWI), the order is $\text{Cd} > \text{Pb} > \text{Cr} > \text{Ni}$. In this study, the average EWI values are significantly

below the PTWI values set by the FAO/WHO for all the studied metals.

Health risk assessment

Based on the data presented in Table 2, daily consumption rate limits for contaminated fish based on non-carcinogenic effects range from 0.42 to 7.68 kg/day. Based on the carcinogenic effect of Pb, the daily consumption rate of contaminated fish is 0.33 kg/day.

The meal size of fish consumption per month based on carcinogenic and non-carcinogenic effects was determined, and the results are listed in Table 2. Considering the non-carcinogenic effect, the meal size based on contaminated fish by Pb, Cd, Cr, and Ni ranges from 56 to 1030 meals per month. In regard to the carcinogenic effect of Pb, the meal size is 44 meals per month.

In order to characterize the risk of fish consumption by the Sistan population, some indices were investigated; the results of health risk assessment are shown in Table 2. As observed, the maximum target hazard quotient is for Cd, followed by Pb, Cr, and Ni. The combined target hazard quotient for the four studied metals is 26.94×10^{-3} , i.e., below 1, showing the absence of potential significant health risk through the ingestion of fish.

The CR factor for Pb over a lifetime of exposure through contaminated fish consumption is 1.57×10^{-7} . As USEPA set the value of 10^{-5} (1 for 100,000) as an acceptable lifetime carcinogenic risk (Saha et al. 2016), the CR of Pb appears to be negligible.

The non-carcinogenic RR for the consumption of contaminated fish in terms of the four studied metals is shown in Table 2. The maximum and minimum RRs were found to be 46.25 and 2.53% of total risk for Cd and Ni, respectively. Therefore, the highest concern of

Table 1 The estimated EDI and EWI by the Sistan population through the consumption of captured fish from the fourth Chah Nime lake

| | PTWI (mg/kg bw/week) | EDI (mg/kg bw/day) | EWI(mg/kg bw/week) | %PTWI |
|----|----------------------|-----------------------|-----------------------|-------|
| Pb | 0.025 ^a | 0.18×10^{-4} | 1.30×10^{-4} | 0.52 |
| Cd | 0.007 ^b | 0.12×10^{-4} | 0.87×10^{-4} | 1.25 |
| Cr | 0.0233 ^c | 0.13×10^{-4} | 0.95×10^{-4} | 0.41 |
| Ni | 0.035 ^b | 0.14×10^{-4} | 0.95×10^{-4} | 0.27 |

^a Source: Iwegbue (2015)

^b Source: Yap et al. (2015)

^c Source: Alipour et al. (2015)

Table 2 Estimated CR_{lim} (carcinogenic and non-carcinogenic), CR_{mm}, THQ, CSF, CR, and RR

| Metal | RfD (mg/kg bw/day) ^a | Non-carcinogenic | | Carcinogenic | | THQ | CSF (mg/kg/day) | CR | RR (%) |
|-------|------------------------------------|-------------------------------|-----------------------------------|-------------------------------|-----------------------------------|------------------------|-----------------------|-----------------------|-----------|
| | | CR _{lim} (kg/day) | CR _{mm} (meals/month) | CR _{lim} (kg/day) | CR _{mm} (meals/month) | | | | |
| Pb | 0.002 | 0.56 | 75 | 0.33 | 44 | 9.28×10^{-3} | 8.5×10^{-3b} | 1.57×10^{-7} | 34.43 |
| Cd | 0.001 | 0.42 | 56 | – | – | 12.46×10^{-3} | – | – | 46.25 |
| Cr | 0.003 | 1.06 | 143 | – | – | 4.52×10^{-3} | – | – | 16.78 |
| Ni | 0.02 | 7.68 | 1030 | – | – | 0.68×10^{-3} | – | – | 2.53 |

^a Source: Alipour et al. (2015)

^b Source: Shaheen et al. (2016)

fish consumption for the Sistan population is related to Cd. Yu et al. (2014) assessed the RR posed by non-carcinogenic effects of some organic compounds, hexachlorocyclohexane (HCH), polybrominated diphenyl ether (PBDE), polychlorinated biphenyl (PCB), dichlorodiphenyltrichloroethane (DDT), and some heavy metals like Cd, Hg, As, and Cr, through fish consumption in China and found that 2.6, 37.2, 12.5, and 14.7% of RRs were derived from Cd, Hg, As, and Cr, respectively.

Statistical analysis

Table 3 shows the correlation between heavy metal concentrations and their correlation with the length and weight of fish. A correlation between Pb and Cd concentrations with a correlation coefficient of 0.505 at a significant level of 0.01 was shown (Table 3). The Ni concentration also showed a significant correlation with Pb and Cd concentrations (Table 3).

Chatta et al. (2016) found that there is a significant and positive correlation between the concentrations of

Cd and Pb in four farmed carp fish species, but Cr did not show a correlation with Cd and Pb. These results are in agreement with the findings of this study.

There is a significant positive relationship between heavy metal concentration and the weight and length of fish for all studied heavy metals. Yi and Zhang (2012) also report a positive relationship between fish sizes and Zn, Pb, Cu, Cd, Hg, and Cr in most species and negative relationships only between Cr and Hg contents and the size of catfish and yellow-head catfish.

However, Liang et al. (2016) reported that there was no positive correlation between the length of fish and heavy metal (Cu, Zn, Pb, and Cr) concentrations in three farmed fish species—orange-spotted grouper, red snapper, and snubnose pompano.

The heavy metal content in the muscles of *H. molitrix*, as reported in the recent related literature published after 2016, is reviewed and summarized in Table 4. As seen in the table, the concentrations of Pb, Cd, and Ni are well within the reported ranges, while the Cr value reported in this study is higher than the values reported in the literature. In this latter

Table 3 The correlation between heavy metal concentrations and their correlation with length and weight of fish

| | Pb | Cd | Cr | Ni | Weight | Length |
|--------|---------|---------|---------|---------|---------|--------|
| Pb | 1 | | | | | |
| Cd | 0.505** | 1 | | | | |
| Cr | 0.274 | 0.274 | 1 | | | |
| Ni | 0.334* | 0.355* | 0.263 | 1 | | |
| Weight | 0.711** | 0.664** | 0.386* | 0.521** | 1 | |
| Length | 0.756** | 0.602** | 0.450** | 0.514** | 0.940** | 1 |

***Correlation is significant at the 0.05 level; Correlation is significant at the 0.01 level

Table 4 Comparison of heavy metal content in the muscles of *Hypophthalmichthys molitrix* sampled worldwide in the literature published since 2016

| Sampling site | No. of samples | Metal concentration ($\mu\text{g/g}$) | | | | Reference |
|---|----------------|---|--------------------|--------------------|--------------------|-----------------------|
| | | Pb | Cd | Cr | Ni | |
| Agri-product markets, Huainan, China | 9 | 0.027 ^a | 0.001 ^a | 0.650 ^a | NA | Cheng et al. (2017) |
| Heilongjiang River, China | 7 | mdl | mdl | 0.029 ^a | NA | Jiang et al. (2016) |
| Danube River, Belgrade region, Serbia | 5 | 0.048 ^b | 0.014 ^b | NA | NA | Milanov et al. (2016) |
| Market, district lower Dir, Khyber Pakhtunkhwa, Pakistan | 9 | 0.237 ^b | 0.050 ^b | NA | NA | Ullah et al. (2016) |
| Changhoz dam, district Karak, KPK, Pakistan | 3 | 3.651 | mdl | NA | 12.470 | Munir et al. (2016) |
| Arbela lake (KTS), district Haripur, Khyber Pakhtunkhwa, Pakistan | 9 | 0.260 | NA | 0.200 | 0.210 | Ahmed et al. (2016) |
| Fish farms, head Qadirabad, Pakistan | 5 | 0.184 ^a | 0.008 ^a | 0.031 ^a | NA | Chatta et al. (2016) |
| Fish market, fish farms, head Qadirabad, Pakistan | 5 | 0.325 ^a | 0.008 ^a | 0.049 ^a | NA | Chatta et al. (2016) |
| Chah Nime lake, Zabol, Iran | 40 | 0.230 ^b | 0.155 ^b | 0.168 ^b | 0.169 ^b | Present study |

NA not available, mdl method detection limit

^a Based on wet weight

^b Based on dry weight

case, the difference can be attributed to the sampling site, the different activities near the sampling location, and the water discharged into the lakes. As previously mentioned, the Chah Nime lakes receive water from the Helmand River, the longest river in Afghanistan. Therefore, the higher concentration of Cr may be due to agricultural and industrial activities in the vicinity of the Helmand River.

Conclusion

In the present study, the concentrations of four heavy metals (Pb, Cd, Cr, and Ni) in fish consumed by the Sistan population and their health risk were investigated. The results showed that there is only little concern with Pb, Cd, and Cr contents in fish, since their mean concentrations are slightly higher than the guidelines of some international organizations, namely, the WHO, the FAO, and the EC. The estimated weekly intake of the studied metals through fish consumption is below the provisional tolerable weekly intake. The health risk assessments show that exposure to the studied metals does not pose a risk to human health in the Sistan population. Based on the obtained results, among the considered metals, the main risk for human health can be related to the amount of Cd followed by Pb. Due to the possible accumulation of this metal to

toxic levels, it is recommended to continue the monitoring of heavy metal concentrations in the considered fish, *H. molitrix*, which is widely consumed in Sistan.

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