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ORIGINAL ARTICLE

# Human Health Risks from Heavy Metals in Fish of a Fresh Water River in Iran

**Majid Majlesi[1], Safoora Pashangeh[2], Sayed Omid Salehi[3], Enayat Berizi[4*]**

1. Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
2. Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
3. Students Research Committee, Yasuj University of Medical Sciences, Yasuj, Iran
4. Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

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**Corresponding author:*
Enayat Berizi,
Department of Food Hygiene and Quality Control,
School of Nutrition and Food Sciences,
Shiraz University of Medical Sciences,
Shiraz, Iran
**Tel:** +98-71-37251001
**Email:** eberizi@sums.ac.ir

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ABSTRACT

**Background:** Aquatic organisms as one of the most important source of human nutrition are widely exposed to heavy metals, which even at low concentrations causes harmful effects on human health. To assess the human health risks, estimating of nutritional exposure to metals through fish consumption and comparing these factors with the recommended values is of great importance.

**Methods:** Measurement of mercury, lead and cadmium in edible tissues of three wild fresh water fish species (*Esox Lucius*, *Oncorhynchus mykiss* and *Cyprinus carpio*) was carried out by using the atomic absorption method with the help of Perkin Elmer 4100. By the handling of the mean concentration of heavy metals, factors such as estimated daily intake (EDI), target hazard quotients (THQ) and the maximum amount of consumption ($CR_{lim}$ and $CR_{mm}$) were evaluated.

**Results:** The level of EDI in the samples was far below the tolerable daily intake (TDI). Mercury showed the lowest levels of EDI and the lead had the highest level in all three fish species. The highest levels of THQ were found for lead (0.66) and the lowest for cadmium (0.014), which has not gone further of the hazard threshold of 1. Lead in all three species of fish showed the lowest and cadmium indicated the highest level of $CR_{Lim}$.

**Conclusion:** Mercury and cadmium concentration and human health risk through fresh water fish consumption was lower than the recommended and reference values, but the evaluation of these factors in relation to the lead has not show a quite favorable condition.

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## Introduction

Fish is one of the most important parts of the human diet, which contains a large amount of nutrients that are not normally found in other food sources. Fish is an excellent source of protein, omega-3 fatty acids, vitamins and minerals (1, 2). Contrary to the beneficial effects of fish consumption on human health, it can contain undesirable compounds such as heavy metals, pesticides and other toxic compounds that have harmful effects on human health (3). Water pollution and subsequent contamination of aquatic animals increase the concentration of heavy metals and cause harmful effects on human health. Water pollution can be caused by various sources such as (agricultural) drainage, industrial wastewater discharge, discharge drainage of sewage, accidental disposal of chemical wastes, metal smelting plants and mines (3-5).

Metals are imported into aquatic ecosystems by natural resources or through human manipulation, which are considered to be a serious health risk for consumers due to their high toxicity, high stability and bio-accumulate in the food chain (6). Heavy metals can accumulate in the body of the fish, and when they are received in excessive amounts, they cause undesirable effects, such as liver and kidney damage, nervous system disorders, cardiovascular diseases, hematological effects, growth anomalies, harmful effects on reproduction and cancer (1, 7, 8).

In recent years, attention has been paid to the potential risks to human health caused by fish contaminated with heavy metals (9). Nonetheless, fish are considered as reliable biomass indicators for the accumulation of metals in aquatic ecosystems (8, 10). Hence, many studies have been carried out to determine the amount of heavy metals in farmed fish of fresh water and wild fish for assessing human health risks (9, 11, 12). Toxic metals such as arsenic, cadmium, lead and mercury do not have known nutritional or beneficial effects on human health and their entrance into the human body over a long period of time, even at low concentrations, causes harmful effects on human health (13, 14).

Cadmium, mercury and lead are among the most important heavy metals that cause the greatest damage to human health (15). Mercury is one of the toughest heavy metals that can be found in aquatic tissues of contaminated water. This element can result in multiple toxic effects such as vision and hearing impairment, dizziness, vomiting, muscle weakness, allergies, immune system complications, depression, brain damage, and even death in humans (16). Cadmium poisoning, especially through oral exposure, can lead to renal failure as the main target tissue, musculoskeletal disorders, and disability in the peripheral and central nervous system (17, 18).

Lead also may cause complications, such as cardiovascular, hematologic, renal, fertility, neurological, and growth disorders in humans (ATSDR, 2007). The Estimation of nutrient exposure to metals in seafood and the assessment of human health risks and its comparison with provisional tolerable weekly intake (PTWI) are required as recommended by the Joint Committee of Food Professionals (JECFA), the international organizations of the Food and Drug Organization (FAO) and the World Health Organization (WHO). The PTWI values for Cd, Hg and Pb vary from 0.005 to 0.025 mg kg-1 body weight per week (19). The tolerable weekly dose of JECFA for the listed metals is 0.35, 1.75 and 0.49 mg, respectively, for each person of 70 kg (20).

The maximum standard values for heavy metals of mercury, cadmium, and lead in the fish muscles, according to the WHO, are 0.1, 0.2 and 0.5 (ug/gr), respectively (21). The consumption of fish in the world is 20 kilograms per capita and in Iran, which is one of the least-consumed countries in this area have been reported to be 6.25 kilograms in 2004, and which increased up to 9.2 kilograms in 2014, while seafood consumption is rising rapidly around the world (22). To assess the dangers of heavy metals in human health based on the concentration of mercury, lead, and cadmium in edible tissues of wild fresh water fish, values of risk factors such as the daily intake level (DIL), Target Hazard Quotients; (non-cancerous fish consumption risks) (THQ), that indicating potential non-cancer risks, maximum amount of fish consumption ($CR_{lim}$ and $CR_{mm}$) and cancer risk (CR) in relation to types of metals through fish consumption and allowance fish consumption were evaluated.

## Materials and Methods

In this study, species of of pike, rainbow trout and common carp were caught by by local fishermen using traditional tours from the Khersan River, the largest tributary of the Karoon River (23). Six fish were caught from each species and immediately laid beside the ice and transferred to the laboratory. In the laboratory, the edible muscular tissues were removed through dissection and then the samples were kept in a freezer at -70°C until the test was performed to measure the heavy metals.

The edible specimens of the caught fish were placed in an oven at 65°C for 120 to 150 minutes and by reaching the certain weight the thermal process was stopped. For digestion of the samples, the wet digestion method was used, and half a gram of sample was poured into a 250 ml balloon, then 25 ml of concentrated sulfuric acid, 20 ml of nitric acid 7 molar and 1 ml of 2% sodium molybdate solution were added and in order to uniform heating a few pounds boiling stones was used.

To the cooled sample, a mixture of 20 ml of concentrated nitric acid and concentrated perchloric acid with 1:1 ratio was added slowly from the top of the refrigerant. The mixture was heated until the white acid vapor was completely removed. The mixture was cooled and, while the balloons were shaken, 10 ml of distilled water was added slowly from the top of the refrigerant. The obtained sample was heated for 100 minutes to obtain a completely clear solution, then it is cooled and transferred to the volumetric flask and charged to the desired volume (24).

Measurement of mercury, lead and cadmium was carried out by the atomic absorption method with the Perkin Elmer 4100 device (25, 26). Mercury has been measured by applying the hybrid system, and lead and cadmium were measured by using the graphite furnace atomic absorption spectrometry. In order to measure these metals, 10 ml of the edible specimen solution were added to 5 ml of an ammonium borilidine carbamate solution 5%, and for 20 minutes the samples were mixed to make the elements in the form of organic metal in solution. Then the samples were added 2 ml of methyl isobutyl ketone and mixed for 30 minutes. After 10 minutes, the samples were centrifuged at 2500 g for 10 min and the elements were transferred to the organic phase. After adjusting the furnace and the system for generating the cathode ray tube and optimizing the atomic absorption device, the calibration curve of these metals was prepared using the standards of these elements and the high-speed modifiers matrix by Winlab 32 software and the amount of these heavy metals in the prepared solutions has been measured (25, 27).

Estimated Daily Intake (EDI) is related to heavy metals on the food, which depends on the concentration of these elements on the fish muscle tissue and their daily intake. The daily intake of metals in adults is calculated as EDI=(MC×IRD)/BW. MC is the metal concentration in the muscle tissue of fish (mg/kg), IRD is the fish consumption per day. Based on the Iranian fish consumption per capita, which is 9200 grams per year, the daily consumption will be about 25 grams. The body weight (BW) is considered to be 70 kg for each adult person (28). EDI levels have been reported in µg/kg/day.

The risk of non-carcinogenicity associated with fish consumption is calculated using the Target Hazard Quotients (THQ) formula, which is the ratio between the estimated dose of the contaminant and the reference dose (RFD). The risk estimation method was based on THQ by USEPA (1989) which was described using the formula (29): $THQ=[(EF×ED×IR×MC)/(RFD×BW×AT)]×10^{-3}$.

THQ is the target hazard quotients, EF is the exposure frequency (365 days per year), ED related to exposure duration (70 years), IR is the intake rate of fish (25 g/day), MC is metal concentration in muscle tissue (mg/kg), RFD is the oral reference dose (mg/kg/day), BW defines the mean of adult body weight (70 kg), and AT is the average time for non-carcinogens, which is calculated to be 70 years in this study. If the THQ is less than one (THQ<1), it is unlikely that the exposed population will experience obvious adverse effects and if THQ>1, there is a potential health hazard (30). In this study, the total THQ was obtained for three heavy metals of mercury, cadmium and lead by using the fish consumption with the formula: total THQ=THQ(Hg)+THQ(Cd)+THQ(Pb).

For the effects of cancerous or non-cancerous metals, the maximum amount of fish consumption for adults is estimated to determine the amount of fish that can be used safely during a specific period. The following equation has been used to calculate the maximum amount of fish consumed (31). $CR_{lim}=(RFD×BW)/MC$). $CR_{lim}$ is the maximum amount of fish consumption in terms of Limiting consumption rate (kg/day). The maximum acceptable amount of fish consumption $CR_{mm}$ of the number of acceptable fish meals intake per month is calculated according to the formula (31): $CR_{mm}=(CR_{Lim}×Tap)/MS$. Tap is related to the time average period (365.25 days/12 months = 30.44 days per month) and MS is meal size (0.227 kg fish/meal for adults) (31). In this study, the average concentration of three metals of mercury, cadmium and lead in the muscle tissue of the three species of fish was calculated in order to estimate THQ, $CR_{Lim}$, $CR_{mm}$ and EDI.

The Carcinogenic risk (CR) is indicative of a possible increase in the incidence of cancer, due to a potential carcinogenic during the lifespan. Since the cancer scope factor (CSF) for lead is defined by the USEPA (32), the carcinogenic risk of this metal is estimated by using the equation: CR=CSF×EDI. All the statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) software, version 19. The one way ANOVA test was used for comparing the mean of heavy metals in different samples. P values of <0.05 were considered statistically significant.

## Results

The average concentration of lead, mercury and cadmium, the EDI value and the TDI reference dose (JECFA, 2011) are presented in Table 1. The value of EDI in tested fish were significantly lower than TDI (Table 1), which exhibited the tact that consumption of these wild river fishes can not lead to health hazards in the consumers. The ratio of TDI/EDI in all three species of fish for mercury, cadmium and lead was 28.75, 16 and 3.22, respectively (Table 1).

The value of THQ for each metal in the three wild river fish species and the carcinogenic risk (CR) related to lead are listed in Table 2. The highest levels of THQ was related to the lead (0.126) and the lowest was evaluated for cadmium (0.014) which is found to have not exceeded the threshold of THQ, which is 1, for all three metals and in all three species of

**Table 1:** Mean±standard deviation of mercury, cadmium and lead concentration; the amount of EDI and TDI; the reference value (JEFCA, 2011) in pike, rainbow trout and common carp.

| Fish species | Hg | | | Cd | | | Pb | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean (µg/kg) | EDI | TDI | Mean (µg/kg ww) | EDI | TDI | Mean (µg/kg ww) | EDI | TDI |
| Esox Lucius (pike) | 0.026±0.006 | 0.009 | 0.23 | 0.162±0.023 | 0.057 | 0.8 | 1.34±0.079 | 0.478 | 1.50 |
| Oncorhynchusmykiss (rainbow trout) | 0.023±0.004 | 0.008 | | 0.110±0.028 | 0.039 | | 1.12±0.13 | 0.4 | |
| cyprinuscarpio (common carp) | 0.027±0.005 | 0.009 | | 0.155±0.018 | 0.055 | | 1.45±0.086 | 0.517 | |

Hg: Mercury; Cd: Cadmium; Pb: Lead; EDI: Estimated Daily Intake; TDI: Tolerable Daily Intake

**Table 2:** The amount of RFD, THQ and total THQ value for each metal in three fish species of pike, rainbow trout and common carp. CR value was evaluated only for lead.

| Fish species | Hg | | Cd | | Pb | | | tTHQ |
|---|---|---|---|---|---|---|---|---|
| | RFD (µg/kg/ bw/day) | THQ | RFD (µg/kg/ bw/day) | THQ | RFD (µg/kg/ bw/day) | THQ | CR | |
| Esox Lucius (pike) | 0.08 | 0.042 | 1.0 | 0.021 | 1.50 | 0.116 | 0.004 | 0.179 |
| Oncorhynchusmykiss (rainbow trout) | | 0.037 | | 0.014 | | 0.097 | 0.003 | 0.146 |
| Cyprinus carpio (common carp) | | 0.043 | | 0.020 | | 0.126 | 0.004 | 0.254 |

Hg: mercury; Cd: cadmium; Pb: lead; RFD: reference dose; THQ: Target Hazard Quotients; tTHQ: Total Target Hazard Quotients; CR: Carcinogenic risk

**Table 3:** $CR_{lim}$ and $CR_{mm}$ factor for mercury, cadmium and lead in three fish species of pike, rainbow trout and common carp.

| Fish species | Hg | | Cd | | Pb | |
|---|---|---|---|---|---|---|
| | $CR_{lim}$ (kg/day) | $CR_{mm}$ (meals/ month) | $CR_{lim}$ (kg/day) | $CR_{mm}$ (meals/ month) | $CR_{lim}$ (kg/day) | $CR_{mm}$ (meals/ month) |
| Esox Lucius (pike) | 0.215 | 28.80 | 0.432 | 87.87 | 0.078 | 10.44 |
| Oncorhynchusmykiss (rainbow trout) | 0.243 | 32.55 | 0.636 | 85.20 | 0.093 | 12.45 |
| Cyprinuscarpio (common carp) | 0.207 | 27.73 | 0.451 | 60.41 | 0.072 | 9.65 |

Hg: mercury; Cd: cadmium; Pb: lead; $CR_{lim}$: Maximum authorized daily consumption range; $CR_{mm}$: Maximum authorized monthly consumption range

fish, pike, rainbow trout, and common carp. The carcinogenic risk (CR) for lead in all three fish species is more than $10^{-4}$ (Table 2). To assess the non-cancerous effects, the maximum allowable daily consumption limits/permissible limit ($CR_{Lim}$) has been calculated for all three metals and in all three fish species (Table 3).

Value of $CR_{Lim}$ for lead, cadmium and mercury in all three fish species was more than $10^{-3}$ (kg/day). The lead $CR_{Lim}$ value in all three species of fish showed the lowest (0.072) and cadmium had the highest (0.63) value. The maximum allowable monthly consumption ($CR_{mm}$) is also calculated to determine how many meals of these three species of wild fish in the freshwater river can be used safely without undesirable non-cancerous effects per month (Table 3). In this study, the obtained $CR_{mm}$ for cadmium and mercury in all three fish species was more than 16 meals per month but for lead was less than 16 meals.

## Discussion

The concentration of mercury and cadmium in none of the fish samples were not higher than the permissible values defined by World Health Organization, however, the level of lead in all three species of fish has been higher than the permissible recommended amount by the WHO (Table 1) (23). Investigation of the ratio of TDI/EDI in freshwater fishes is consistent with the results of Varol et al. (2017) (33), which evaluated the risk factors for farmed rainbow trout. The human health risks of consumption of *Oreochromis mosabicus*, *pangasius pangasius* and *Labeo rohita* in Bangladesh with regard to EDI based on heavy metals level such as cadmium and lead has not been observed (34). In this study, mercury indicated the lowest daily intake (EDI) in all three fish species, and lead showed the highest EDI amount (Table 1), which is consistent with the results of the study by AtiqueUllah et al. (2017) (35).

The amount of heavy metals in fish of tropical wetlands in India by estimating the provisional tolerable daily intake (PTDI) and provisional tolerable weekly intake (PTWI) does not pose a health hazard to consumers (36), that reinforces the results of this study. The EDI level for 8 heavy metals, including lead, cadmium and mercury in the upstream of the Yangtze River in China was lower than the reference dose, which is consistent with the results of this study with regard to low level of the daily intake of heavy metals in the surveyed fish (37). Intaking mercury, cadmium, and lead through the use of wild and farmed Salmon in Canada is a very small part of human exposure to these metals and is much lower than the provisional tolerable weekly intake (38).

Value of THQ reflects the fact that exposure to heavy metals of mercury, cadmium and lead in the three species of wild fish in Iranian adult consumers is lower than the recommended threshold dose. Therefore, it can be concluded that human exposure to the studied heavy metals in the examined fish does not have a harmful effect on the human health during the lifespan. By consumption of fish of fresh water rivers in Iranian adult people, the consumer health is not threatened. In the study of Varol et al. (2017) (33), the THQ level for 10 heavy metals, including cadmium and lead with the consumption of fresh water trout fish evaluated less than the specified unit of 1, which was consistent with the results of this study. The amount of THQ of cadmium and lead in adult Bangladeshi was less than one, which indicates that there is no adverse effects on the health of the consumers of cultured fish (34). Kumar and Mukherjee (2011) estimated the amount of THQ below the standard safe level of 1 for heavy metals such as arsenic, mercury, copper, nickel and zinc in tropical wetland fish (36).

If the CR level is less than $10^{-6}$, it is negligible, the values between $10^{-4}$ and $10^{-6}$ are acceptable and the CR level higher than $10^{-4}$ is unacceptable. Therefore, the potential risk to health for those who are exposed to lead by using fish should not be overlooked. In addition to fish, exposure to heavy metals through other foods and inhalation of dust should be considered (34). $CR_{Lim}$ for each metal is the maximum daily intake of fish throughout the life span that is not expected to have adverse non-cancerous effects (31). The maximum permissible daily intake for all three metals was less than the average daily consumption of fish (25g) which is consistent with the results of the study by Varol et al. (2017) (33).

According to USEPA (31), when $CR_{mm}$ of a meal is more than 16 meals per month (represented by > 16 meals / month), it is safe to consume. Therefore, adults can consume more than 16 meals of this species based on the cadmium and mercury metals. As shown in result, $CR_{mm}$ for lead in all three fish species is less than 16 units, which indicates non-cancerous harmful effects on the health of the consumer (10).

## Conclusion

Regarding human health assessment, the levels of mercury and cadmium in all three species of fish inhabit in the fresh water river of Khersan are safe. The concentration of these two metals in the muscle tissue of the fish was less than the recommended limit, and also the estimated EDI value was lower than the permissible amount of TDI for these two metals. Considering the THQ fishes inhabit in the fresh water river does not cause non-cancerous complications in Iranian adult consumers. However, the concentration of lead has exceeded the global standard values. The estimated THQ for lead is less than 1, but carcinogenicity factor (CR) is higher than the permissible limit and the evaluated maximum amount consumption was lower than the recommended measure. Therefore, based on lead hazard connsumer require more caution for consumption of these fish.

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## Conflict of Interest

None declared.

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