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ORIGINAL ARTICLE

The Concentration of Mercury, Cadmium and Lead in Muscular Tissue of Fishes in Khersan River

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ABSTRACT

Background: Aquatic ecosystems including fish in rivers may be widely contaminated by heavy metals released into waters from industrial, agricultural and other human activities.This study aimed to investigate the level of contamination of theses heavy metals (mercury, cadmium and lead) in fish caught in Khersan river, the largest tributary of Karun river.

Methods: The heavy metals level of mercury, cadmium and lead was investigated by atomic absorption spectrometry using Perkin Elmer 4100ZL in rainbow trout, pike and common carp species caught in Khersan river.

Results: The average concentration of mercury, cadmium and lead in muscular tissue of trout, pike and common carp in all three breeding areas were (0.023,0.110, 1.12), (0.026, 0.162, 1.34) and (0.027, 0.155, 1.45) mg/kg, respectively and only lead metal was more than the level determined by the World Health Organization and European Commission regulations. The concentration of cadmium and lead in muscular tissue of caught fishes indicated an inverse and significant relationship with increasing weight and reducing fish metabolism (r=-0.568 and r=-0.517). The concentration of heavy metals of fishes in the river reduced by an increase in fish age, length and weight.

Conclusion: The level of mercury and cadmium in fishes of Khersan river was shown not to be threatening for their consumers, but the increase for lead concentration reflects the fact that the entry of urban, rural and agricultural wastewater affects the concentration of heavy metals in fishes of the river that may be threatening for their consumers.

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Introduction

The presence of heavy metals in daily diet has certain effects on human and animal health even at low concentrations (1). Heavy metals are a group of natural compounds that enter the environment through various processes (2). The development

of recent industrial activities including mining, melting and casting and the production of synthetic compounds have led to an increase in the amounts of heavy metals released into the atmosphere, water and soil (3). Fresh waters pollution with a wide range of pollutants has become one of the most disturbing problems in the recent years (4). Aquatic ecosystems may be widely contaminated by heavy metals released into waters from industrial and other human activities (5). These metals have great effects on the balance of the environment and a wide range of aquatic organisms (4). Among animal inhabitants of the waters, fish has no option to get rid of the effects of these specific pollutants (4). Heavy metals enter the blood and tissues of fish from skin, digestive tract and gills. Fish is one of an important source of heavy metal accumulation that potentially results in an increase in toxic contaminants in the food chain (6). Heavy metals of human and geological resources are increasingly released into the natural resources of water (7). Heavy metal contamination in aquatic ecosystems has attracted a growing global attention, and many studies have been published regarding this issue (8). Fish are also considered as one of the most important indicators of freshwater systems for the estimation of metal pollution level and the determination of the risk for human consumption (9). Heavy metals pollution in the aquatic environment is known as one of the most serious environmental problems (10).

Heavy metals accumulate in tissues of aquatic animals that are toxic to fish and consumers. Mercury often accumulates as methyl mercury in fish tissues (11). Methyl mercury poisoning through fish diet has occurred in humans and was determined in Minamata incident in Japan for 50 decades causing death for 41 people (11). Cadmium is a heavy metal that may be present in aquatic ecosystems considered as a pollutant of two industry and agriculture sectors. Aquatic vertebrates have more susceptibility and vulnerability to exposure of cadmium poisoning than the dried vertebrates (12). Lead is one of the four metals that has the most adverse human health effects. Disorders of hemoglobin synthesis and anemia, high blood pressure, kidney disease, abortion and infant failure, nervous system disorder, brain damage, male infertility, children's learning and behavioral disorders are negative side effects of the increases lead concentrations in the human body (13). According to the World Health Organization (WHO, 1985), the maximum standard values of heavy metals of mercury, cadmium and lead based on ug/gr are respectively 0.1, 0.2 and 0.5 in fish tissue.

Rainbow trout (*Oncorhnchus mykiss*) is the most well-known salmon in the world (14), and is also considered as an economical fish and the most popular fish in Iran that is often caught in Khersan river with Pike (*Esox lucius*) and Common carp. Heavy metals are persistent pollutants; unlike organic compounds, they are not decomposed by chemical and biological processes in the nature. The most important results of the persistent heavy metals are their high breadth in the food chain, so that the result of this process in the trout shown that this amount in the food chain is increased several times more than the amount found in water or air. It is necessary to study the heavy metals in aquatic animals as they are rapidly absorbed into their bodies (15).

Khersan river passes through several towns and villages, the urban, rural wastewater and animal fertilizers may enter the river, so it is necessary to study the concentration of heavy metals of mercury, cadmium and lead in muscle tissue and edible fish caught often by local fishermen and in distant cities and villages that this study determines.

Methods and Materials

In this cross-sectional study, trout, pike and common carp were caught by local fishermen in May and June 2016 by traditional tours (Earshot tour) from Khersan river, the largest tributary of Karun river that originates from Tange Sorkh and "Kandahar Soeda" mountains located in 43 km southeast of Yasuj and 15 km northwest of Ardakan city of Fars and passes through Yasuj city with the name of "Boshar" river and enters Lordegan county in Chaharmahal-o-Bakhtiari province with the name of Khersan river (Figure 1).

Six fish species were collected and placed on ice and delivered to lab immediately. The hunting length and weight of the fish were assessed by a biometric board with a precision of one millimeter and a digital scale with a precision of 0.01 g, and then the muscles were dissected. The fish muscular tissue was stored frozen until testing at -70 °C for the measurement of heavy metals.

The samples were placed in an oven at 65 °C for 120 to 150 min until a constant weight obtained, then removed from the oven. Wet digestion method was used for samples that 0.5 gram poured into a 250 ml balloon and then 25 ml of concentrated sulfuric acid, 20 ml of 7 M nitric acid and1 ml of 2% sodium molybdate solution were added and boiling stones for uniform and regular heating were used. Twenty milliliter of a mixture (1:1 ratio of concentrated nitric acid and concentrated perchloric acid) were added slightly above the refrigerant to the cooled sample. The mixture was heated until the white fumes of acid were removed completely. The mixture was cooled

Fig. 1: Fish Sampling and fishing location in Khersan River.

and while the balloon containing the sample shaken, 10 ml of distilled water were added slightly above the refrigerant. The resulting sample was heated for 100 min to obtain a completely clear solution, then it was cooled and transferred to volumetric flask and brought to volume as described before (16).

The level of heavy metals of mercury, cadmium and lead was measured by atomic absorption spectrometry using Perkin Elmer 4100ZL as described before (17). The determination of mercury, cadmium and lead levels were respectively based on a hybrid system and graphite furnace atomic absorption spectrometry. First, 5 ml of ammonium pyrolidine solution of 5% carbamate was added to 10 ml of the sample digestible solution for the measurement of these metals and the samples were mixed for 20 minutes to dissolve the elements in the form of an organic metal, Then, 2 ml of methyl isobutyl ketone were added to these samples and mixed for 30 minutes. Then, the samples were centrifuged at 2500 g for 10 min and the elements were transferred to the organic phase. The calibration curve of these metals was drawn using the standards of these elements and the palladium matrix was modifier by Winlab 32 software after adjusting the furnace and the source of the cathode ray tube production (EDL) system and optimizing atomic absorption device, and the amount of these heavy metals was measured in the prepared solution.

SPSS statistical software (version 19, Chicago, IL, USA) was used for statistical analysis and the mean of heavy metals in various samples was compared by one way ANOVA test. Pearson correlation coefficient was used to determine the relationship between length and weight of fish and the concentration of metals. $p<0.05$ was considered for significant level.

Results

Mean±standard deviation of heavy metals for mercury (Hg), cadmium (Cd) and lead (Pb) in muscular tissue of trout, pike and common carp was shown in Table 1. The mean±SD of mercury metal in the mentioned three fish species was 0.027±0.005, 0.026±0.006and 0.023±0.004mg/kg (ppm), respectively. The data showed that the average of heavy metal concentration of mercury in each of the three fish species was not more than the acceptable values set by World Health Organization (WHO), Food and Agriculture Organization (FAO), Food and Drug Administration (FDA), European Commission (EC) (Table 2). Also, there was no significant difference between the three studied species in terms of this heavy metal ($p<0.05$).

The heavy metal concentration of cadmium in three species of trout, pike and common carp was 0.110±0.028, 0.162±0.023 and 0.155±0.018 mg/kg (ppm), which was less than the standard value set by the related organizations, respectively (Table 1 and 2). However, it is observed that the concentration of this metal in trout fish was significantly lower than other two species ($p<0.05$). The heavy metal concentration of lead in three species of trout, pike and common carp was 1.12±0.13, 1.34±0.079 and 1.45±0.086 mg/kg (ppm), respectively which is more than the recommended value set by the above organizations in all three studied fish species. The amount of this metal in muscular tissue and edible portions of fish was significantly lower than the other two species ($p<0.05$).

Khersan river were shown in Table 3. Also, the

Table 1: Mean±standard deviation of heavy metals concentration of mercury, cadmium and lead in trout, pike and common carp.

Fish species	Heavy metals		
	Mercury	Cadmium	Lead
Esox Lucius (pike)	0.026±0.006 [a]	0.162±0.023 [a]	1.340±0.079 [a*]
Oncorhynchus mykiss (rainbow trout)	0.023±0.004 [a]	0.110±0.028 [b]	1.120±0.130 [b*]
cyprinus carpio (common carp)	0.027±0.005 [a]	0.155±0.018 [a]	1.450±0.086 [a*]

* Indicates a higher level of heavy metal than the WHO standard and EC Regulation No. 1881/2006 and different letters indicates a significant difference in each column ($p<0.05$).

Table 2: An accepted concentrations of heavy metals of mercury, cadmium and lead in fish

Standard	Heavy metal (mg/kg)		
	Mercury	Cadmium	Lead
FAO (2000)	0.5	0.5	2
WHO (2000)	0.5	0.5	0.5
ROPME (1999)	0.5	0.07 – 0.75	0.01 – 1.28
FDA (2001)	0.5 – 1	4	1.7
EC Regulation No. 1881/2006	0.5 – 1	0.5	1
NOAA (2009)	0.5	4	1.28

FAO: Food and Agriculture Organization; WHO: World Health Organization; ROPME: Regional conventions On the Protection of the Marine Environment; FDA: Food and Drug Administration; EC Regulation No.: European Commission Regulation No. 1881 and NOAA: National Oceanic and Atmospheric Administration of America

Table 3: Mean±standard deviation of the length and weight of rainbow trout, pike and common carp caught in Khersan River.

Fish species	Physical characteristics	
	Weight (gr)	Length (cm)
Esox lucius Pike	666±86	38±7
Oncorhynchus mykiss rainbow trout	534±112	34±16
cyprinus carpio common carp	423±123	26±25

Table 4: Correlation between concentrations of heavy metals of mercury, cadmium and lead with Physical Characteristics (Length and Weight). The sign * represents the correlation at the 0.05 level.

Physical properties	Heavy metal		
	Mercury	Cadmium	Lead
Length	-0.246	-0.312	-0.283
Pearson Correlation	0.393	0.195	0.165
Sig. (2 - tailed)			
weight	-0.256	-0.568*	-0.517*
Pearson Correlation	0.275	0.032	0.018
Sig. (2 - tailed)			

correlation and association of heavy metals with the length and weight of fish were shown in Table 4. The results showed that with increasing length and weight, the concentration of all three heavy metals in fish muscular tissue decreased, so the accumulation of cadmium and lead had an inverse and significant relationship with increasing fish weight according to the data obtained from the Pearson correlation coefficient (r=-0.568 and r=-0.517 for the relationship between cadmium and lead with fish weight).

Discussion

This study aimed to evaluate the concentration of heavy metals of Hg, Cd and Pb in fish muscular tissue and food of rainbow trout, pike and common carp in Khersan river. The results of this study indicated

that heavy metals concentrations of mercury and cadmium were below permitted levels determined by (WHO, 1998) (18), and (EC regulations, 2006) (19), so the fish caught in this river were not harmful for human consumption. However, the lead metal in the muscular tissue of all three fish species was higher than WHO standards (18). The lead metal in the studied fish was more than the standard value set by EC regulations (20). In a study conducted on the heavy metals concentration of breeding trout fish in the Zayandehrud river and fish farms in Chahar-Mahal Bakhtiari province, the lead metal in fish muscular tissue of fish farms (breeding species) and river (wild species) was 33.3% and 41.6%, respectively which was more than the standard value set by EC (20). Mercury metal was less than the standard level in all samples and cadmium level in both breeding and wild species was higher than the level set by this standard in only 16.6% of the samples (21). Moreover, Demirak*et al.* conducted a study in Turkey in 2006 that heavy metal level in muscle tissue of different fishes was at lower risk of human consumption (22). The heavy metals levels of mercury, cadmium and lead in Shard fish of Bandar Abbas was less than the standards set by the global organizations.

The heavy metals concentration in fish tissues of Chiln Lake in Malaysia was lower than the standards level and also indicated that fish of this lake were not dangerous for human consumption (23). In another study conducted on the evaluation of heavy metals level in consumed fish in Khorramabad, it was shown that the concentration of most of these metals was less than the standard value set by WHO. In this study, the concentrations of lead, mercury and cadmium in different types of fish was less than the standard value set by FDA, WHO and EC in breeding trout. In a study done by Saee Dehkordi and Fallah on valuable commercial species of Persian Gulf determined that the daily consumption of lead, copper and zinc in fish meats was below the standard value set by global organizations (24). The amount of zinc and copper in four species of Pamvotis lake in Greece was in acceptable limit and safe for human consumption (9). The level of lead was higher than the standard value set by WHO in breeding fish of upper part of Ghadirabad in Pakistan, while cadmium and chromium were less than the standard value (25), which is consistent with the results of this study. In a study conducted in North Mexico, the amount of some heavy metals was investigated in freshwater fish and found that heavy metal of lead (4.298 mg/kg) was higher than the standard value set by EC and the Almintarius Codex, because of the adjacent industrial activity (26), which is consistent with the results of this study due to the heavy metal concentration of lead.According to the mentioned contents, heavy metals level of mercury and cadmium in fresh water fish was lower than the standard value of human consumption in most studies, and it has also been shown that lead increased more under the influence of seasonal factors, and agricultural and industrial activities and exceeded the limits set by global organizations in fish (26) that is consistent with the results of this study.

It has been reported that industrial pollution of fresh waters caused an increase in heavy metals accumulation in fish tissues. The release of industrial wastewater and pollutants of human activities into rivers increased the lead level in Liza abu fish in Karun River (27). In another study conducted on heavy metals of lead, cadmium and mercury in commercial fish in Cochin coast of India, it was reported that these metals concentrations were more than the standard value in fish because of the industrial activities in that area (28). Wild carps in the downstream regions of Ravi and Indusrivers in Pakistan were reported a higher rate of heavy metals contamination than upper area (17).

In a study done by Mostafa *et al.* (2015), it has been shown that heavy metal concentrations of cadmium and lead in muscular tissue of breeding fish consumed from two different sources of water were significantly different (29). Although the lead and cadmium metals in freshwater fish of Chiln were lower than the standard value, compared to other freshwater fish due to the contamination of adjacent lakes with various sources, including agricultural activities related to palm oil processing and the plastic materials industry were higher than the standard value (22).

The heavy metals concentration, including lead in *Mugil cephalus* and *Trachurus mesiteraneus* fish was high in Gulf of Alexandria, in contaminated areas and close to industrial areas (30). High amounts of cadmium and nickel in fish of Kuetsjarvi area was due to the increase in heavy metals of the surrounding area and nearby metal smelting factories, so cadmium was more affected. Heavy metals accumulation in fish will be lower than the standard value at long distance between factories and pollutant sources (4).

Electricity power stations can reduce the acidity of water and, consequently increase the solubility of lead and cadmium in water that increase these metals accumulation in aquatic species (21). Heavy metals concentration reduced in fish muscular tissue with increasing length and weight that showed a negative relationship. Therefore, there was significant and inverse relationship between fish weight and lead

and cadmium concentrations ($p<0.05$). There was an inverse and significant relationship between fish length and chromium concentration in liver of *Ather inahepsetus* fish and in gill of *Trigla cuculus*. This relationship was also observed about chromium and lead in liver and copper in all tissues of *Scomberes oxsaurus* fish. Generally, there was a negative correlation between heavy metals level in fish tissues and size of fish (length and weight) in the texts (31).

Level of lead metal in fish has significantly decreased with increasing its size (32), which is consistent with the results of this study. It has been suggested that fish metabolism plays a major role in heavy metals accumulation. For example, the amount of cadmium in the two-year-old *Noemacheilus barbatulus* fish reaches a constant level that does not change with increasing age (33).

It is also clear that metabolic activity in younger people is higher than elderly ones. Therefore, heavy metals accumulation in younger fish are more than older ones (31). In many studies, there is a positive correlation between the spectroscopic parameters of fish and heavy metals concentration, however, this association has been negatively reported in catfish and yellow catfish (34). The heavy metal concentration of lead decreased with increasing the length and weight of Poecilia reticulate fish (31), although the concentration of copper and zinc was not related to fish weight.

Other studies have reported negative relationship between fish size and heavy metals concentration (27, 30). There is no fixed and defined relationship between the fish size and the heavy metals concentrations. The heavy metals accumulation in fish reaches a constant status after a certain age (32). It was determined that heavy metals accumulation is increased with the increase in fish age, length and weight shown in a study done on Caspian Sea Sturgeon species, which is not consistent with the results of this study (35).

The heavy metal concentration of copper, lead, and zinc in *Abramis bramal* muscular tissue showed negative correlation with increasing age and length (34), which is consistent with the results of this study. Generally, there is an agreement on this issue that metals in organisms are accumulated, disinfected and eliminated by a special mechanism that significantly depends on metabolism in a certain weight (36). Therefore, the negative relationship between metal concentrations and the fish size did not necessarily mean that a certain amount of metals accumulated in fish body at the beginning of growth and did not subsequently absorb further metals (34).

There is also good agreement on this hypothesis that the absorption of metals in low contaminated water is affected more by nutrition and the amount of fish feed in reduced during growth and development (37). In other words, the amount of heavy metals in organs are significantly reduced in the maturation stage as the daily fish food is reduced with increasing age (38). Overfeeding is expected to result in the highest levels of heavy metal accumulation in fish (39). The heavy metal concentration of cadmium in the muscular tissue of pike and common carp was 0.052 and 0.045 mg/kg, respectively that was higher than trout. Also, the lead concentration in edible tissues of rainbow trout was respectively 0.22 and 0.33 mg/kg that waslower than two species of pike and common carp.

The accumulation of heavy metals depends on fish feeding habits, reproductive status, size, and gender (29). The habitat of organism is also one of the most important factors of the heavy metals accumulation in organisms (40). Deep deposits contain a large amount of heavy metals, and the parasite organisms occupies the deepest layers of the water and has the higher levels of heavy metals than the superficial creatures (39). A study conducted in Australia has reported that there is a high correlation between the heavy metals concentration in fish tissues and feeding habits of fish (40).

Conclusion

The level of heavy metals of mercury and cadmium in fish muscle tissue caught in Khersan river has been determined below permitted levels like many other studies conducted on fresh waters, so this valuable food source does not endanger human consumers. The lead metal was shown to be increased more under the influence of seasonal factors, agricultural and industrial activities and exceeded the limits set by global organizations in fish. It seems that the industrial pollution of fresh waters leads to an increase of the accumulation of heavy metals in fish tissues. The accumulation of heavy metals in fish was dependent on species, nutrition, size and habitat, or the degree of contamination of water that are important factors for health policy makers.

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Conflict of Interest

None declared.

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