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Original Article

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Measurement of Heavy Metals (Nickel, Chromium, and Cobalt) in Wild and Farmed Carps (*Cyprinus carpio*) of Hamadan Province

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Abstract
Fish consumption has been considerably increased in Iran recently. On the other hand, the increase in aquatic ecosystem pollution can cause the accumulation of heavy metals in aquatic animals – the fish, in particular. Therefore, measuring the amount of heavy metals in fish is of great importance for consumers' health. This study aimed to measure the amount of heavy metals (nickel, chromium, and cobalt) in wild and farmed carps (*Cyprinus carpio)* by using an ICP device. In this cross-sectional study which was performed in Hamadan province in 2018, 40 carp samples (20 wild carp samples and 20 farmed ones) were randomly collected from Anouch and Shirinsoo dams and from breeding centers of the province and, after completing the digestion phase, the amount of heavy metals in the samples were measured using the ICP device. The study results showed that the amounts of nickel metal in wild and farmed carps were equal to 0.108±0.086 and 0.102±0.108 mg/kg dry weight, respectively; and the amounts of chromium in wild and farmed fish samples were 0.348±0.396 and 0.136±0.074 mg/kg dry weight in muscle tissue, respectively. Cobalt metal was not observed in wild and cultured samples. According to the results from the statistical analysis, no significant difference was detected between wild and farmed fishes regarding contamination with nickel metal. However, a difference was found between wild and farmed fishes ($P<0.05$) regarding chromium. Taking into account the higher amount of nickel and chromium metals in the wild carp samples compared to the farmed ones, it was recommended that necessary measures be taken to control the sources responsible for polluting the aquatic environment and to prevent the penetration of industrial effluents and other polluting factors into the dams of Hamedan province in order for avoiding the increase in the amount of heavy metals in the province's water sources.
Keywords: Cobalt, Chrome, Nickel, *Cyprinus carpio*, Heavy metals

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1. Introduction

Heavy metal toxicity poses a significant threat to the environment and public health since it increases the pollution level which, in turn, causes serious problems for living organisms. The second sentences should be replaced with the following phrase:Therefore, the information about their level in various products consumed living organism is important (1, 2). In health sciences, fish is argued to serve as a reliable indicator for assessing heavy metal pollution level in seas, dams, and rivers (3). These metals are one of the most important pollutants that harm aquatic environment and seriously endanger the health of aquatic living beings by, for example, poisoning the fish (4). Aquatic environment pollution is usually caused by the industrial effluents, sewage, petroleum products, and gasoline from ships and boats (5). Nowadays, aquatic products – fish, in particular, are considered as rich protein sources for human body. In addition, fish provides human body with rich sources of vitamin and omega-3 fatty acids (6). Common carp is one of the most commercially important fish in the world which is farmed commercially and wildly. Carps are omnivorous, but usually feed on sediments in the water and may accumulate more heavy metals than other types of fish (7). Farmed fishes have been noted for their freshness and low price. Eating fish twice a week has been highly recommended by the American Heart Association (8). Heavy metals in effluents from factories and industrial sites may find their way into seawater and pollute the aquatic ecosystem. These metals are resistant to decomposition

and can accumulate in the aquatic living organisms (9). Chromium, cobalt, and nickel are among potentially toxic elements (PTEs) found in the aquatic environment, and the World Organization has warned against exposure to them (10). Chromium is a toxic and unnecessary metal found in soil and water sources, which can pose a serious threat to human health through the food chain. One of the side effects of multiple exposure to chromium (VI) is liver toxicity in human (11, 12). Chromium exists in two major stable oxidation states, hexavalent chromium (Cr (VI)) and trivalent chromium (Cr (III)) (13, 14). Cobalt is one of the most dangerous metals for living organisms, which is stable in the forms of Co + 3 and Co + 2 (15). Cobalt constituents are believed to cause cancer and respiratory tumors in human (16). Nickel metal which is a toxic element found in aquatic environment, air, and soil can cause respiratory cancer, dermatitis, and reproductive problems for human (2). Younesipour et al found that the levels of nickel and cobalt in the Caspian Sea carps were equal to 121 ± 14.3 and 323.5 ± 28.34 (µg/g dry weight), respectively (17). In the study by Solgi et al and Sarvai et al measuring the levels of heavy metals in common carp, it was revealed that the amounts of cobalt and nickel were lower than the limits defined by WHO (18-20). Heshmati et al also attempted to examine the levels of heavy metals in the Caspian Sea and discovered that the levels of nickel and cobalt in wild and farmed carps were not significantly different (21). In a study by Varol et al in Turkey, it was determined that the concentrations of cobalt, nickel, and chromium in carps were equal to 0.081 ± 0.035, 0.234 ± 0.407, and 0.074 ± 0.074 (mg/kg), respectively (22). According to that, little information was available about the amount of heavy metals in wild and farmed carp species in Hamadan province. Moreover, the evidence showed that some pesticides and environmental pollutants had penetrated into the Shirinsoo and Anouch dams due to the increasing agricultural activities and their water had been exposed to rural and urban sewage, posing a serious threat to carp consumers in the province due to the increasing toxicity of farmed fish. This study, therefore, aimed to measure the amount of heavy metals nickel, chromium, and cobalt in wild and farmed craps.

2. Materials and Methods

2.1. Study Area and Time Description

The areas investigated in the present study were Shirinsoo and Anouch dams and carp breeding centers of Hamadan province in the autumn of 2018. The geographical locations of the fishing sites are shown in Fig. 1.

2.2. Sample Collection

A total of 40 carp samples (20 wild carp samples from Shirinsoo and Anouch dams, and 20 farmed carp samples from fish breeding centers) were randomly collected and

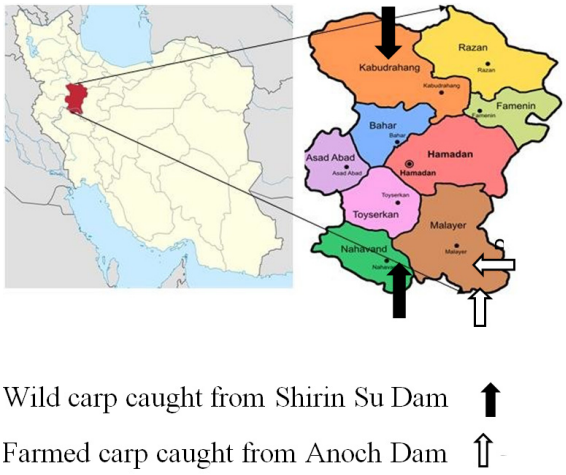

Fig. 1. Geographical Location of Carp Samples Caught in Shirinsoo and Anouch Dams of Hamadan Province. White arrow = Wild Carp Caught From Shirin-Su Dam; Black arrow =Farmed Carp Caught From Anoch Dam.

transferred to the Food and Nutrition Control Laboratory of Hamadan University of Medical Sciences and, then, they were stored in a freezer at minus 18°C.

2.3. Sample Preparation

Materials used in this study included hydrochloric acid 37%, nitric acid 65%, and hydrogen peroxide, all of which were purchased from the Merck Co., Germany. Prior to the experiment, the frozen fish were thawed and their weight and length were determined by a digital scale and a simple ruler in the next step. The means and standard deviations of length and weight of wild and farmed carps are shown in Table 1. Then some of the fish's edible muscles were removed, weighed, and placed on a clean plate. The plate containing the sample was transferred to an oven at 115°C and kept in it for 120 minutes to let the sample dry completely. the dried fish weight was measured and its moisture content was determined (21). The means and standard deviations of the dry and wet matter of wild and farmed carps are shown in Table 2. To complete the digestion phase, 1 g of the dried fish muscle was placed in Erlenmeyer and 5 cc of a mixture of 37% hydrochloric acid and 65% nitric acid in a ratio of 3 to 1 was added to Erlenmeyer; then it was placed on the heater at 95°C for 60 minutes. After 60 minutes, a mixture of 5 cc of hydrochloric acid and nitric acid was added to Erlenmeyer again and the heating by the heater was continued for another hour until a clear color appeared. Then 2 cc of hydrogen peroxide was added to the Erlenmeyer to make the color of the Erlenmeyer contents completely clear. After cooling Erlenmeyer, the samples were filtered using filter paper and bring up to the 25 mL in a Falcon tube. Heavy metals were measured by the ICP-OES (inductively coupled plasma - optical emission spectrometry) method using the ARCOS FHE12 model

Table 1. Means and Standard Deviations of Length (cm) and Weight (g) of Wild and Farmed Carps Studied in Hamadan Province in 2018

Crap (*Cyprinus carpio*)	Weight (g) Mean ± SD	The Length Mean ± SD
Wild crap	76.480 ± 52.305	18.05 ± 3.48
Farmed crap	154.87 ± 127.894	22.15 ± 6.607
P value	0.006	0.028

Table 2. Means and Standard Deviations of Moisture Content (%) and Dry Matter (%) in Wild and Farmed Fish Studied in Hamadan Province in 2018

Crap (*Cyprinus carpio*)	Dry matter (%) Mean ± SD	Moisture (%) Mean ± SD
Wild crap	76.542 ± 4.694	23.457 ± 4.577
Farmed crap	75.537 ± 5.557	24.463 ± 5.557
P value	0.07	0.213

(AMETEK company, Germany) (23).

2.4. Statistical Analysis

SPSS software version 16 was employed for statistical analysis and Kolmogorov-Smirnov test was performed for determining the normal distribution of data. Man-Whitney test was also used to compare the data without normal distribution. If ($P < 0.05$), a significant difference was assumed.

3. Results and Discussion

The linearity range and calibration curve equation for the three metals are shown in Table 3. The *limit of detection* (*LODs*) of Ni, Cr, and Co were 0.000454, 0.000471, and 0.00208 (mg/kg^{-1}) respectively. The average concentrations of nickel in wild and farmed carps were equal to 0.086 ± 0.108 and 0.108 ± 0.102 mg/kg dry weight, respectively; and the average concentrations of chromium in muscle tissues of wild and farmed samples were equal to 0.396 ± 0.348 and 0.074 ± 0.136 mg/kg dry weight, respectively. However, cobalt metal was not detected in free and cultured carp samples, and its level was below the detection limit of the device. In this study, the amounts of metals measured in salmon and farmed fish in the forms of chromium > nickel > cobalt were also obtained. The amounts of chromium and nickel metals in wild carp samples were higher than the amount found in farmed carp samples; and the amounts of the given metals in wild carp samples were higher than those detected in farmed carp samples, which were lower than the global standards determined by of FAO, WHO, and FDA (24-26). Also, the amount of chromium metal in both farmed and wild carps was lower than the limit defined by global FDA (26). According to our study results from statistical analysis, no significant difference was observed between the edible tissues of wild and farmed fishes in terms of the concentration of nickel metal, while a noticeable difference was observed ($P < 0.05$) between wild and farmed fishes regarding chromium metal concentration. This significant difference may have been due to the presence of surface sediments and/or the entry of rural/agricultural effluents and toxic substances such as pesticides from adjacent agricultural lands into dams water (27,28). The mean and standard deviations of the wild and farmed carps are shown in Table 4. Due to the special importance attached to the determination of heavy metals in various food products in Iran and other parts of the world – wild and farmed fishes, in particular, this study aimed to investigate the concentrations of nickel, chromium, and cobalt in samples from wild and farmed carps. Chromium is one of the toxic metals which can be accumulated and disrupted in the host body. Nickel and cobalt are essential metals for the body and their excessive intake is harmful to human organs (29). In the studies by Nasrollahzadeh Saravi et al and Mansouri et al, the amounts of nickel metal and cobalt in the muscle tissues of carps had been discovered to be very insignificant and lower than the limits determined by FAO international standards, which were consistent with our study findings (19,30). Our study findings showed that the amount of nickel metals in wild carp was higher than that detected in farmed carp, but no significant difference was observed. This finding was in line with the finding from the study by Heshmati et al (21). Nickel is one of the most abundant elements in the ecosystem, whose high levels in wild fish can be due to the presence of silicate minerals and water pollution caused by industrial wastewater (31). In a

Table 4. Means and Standard Deviations (mg/kg dry **weight**) of the Studied Metals in Muscle Tissue of Wild and Farmed Fish in Hamadan Province in 2018

Metal	Farmed Crap (mg/kg)	Wild Crap (mg/kg)	*P* value
Nickel	0.108 ± 0.102	0.108 ± 0.086	0.54
Chromium	0.074* ± 0.136	0.348 ± 0.396*	0.002
Cobalt	ND	ND	ND

* The results have significant differences.
ND: not detected.

Table 3. Linear Equations and Regression Coefficient of the Calibration Curves and LOD and LOQ of Studied and Semi-essential Metals

LOQ (mg/kg)	LOD (mg/kg)	Regression Coefficient	Equation	Range (mg/L)	Metals
0.00154	0.000454	0.99999	Y=0.00002.996X-0.2065	0.000454-6	Ni
0.00160	0.000471	1	Y=0.000005.4454X-0.0744	0.000471-6	Cr
0.00707	0.00208	1	Y=0.000006.551X-0.0944	0.00208-6	Co

LOD, Limit of detection; LOQ, limit of quantification.

study by Kalyoncu et al, the amount of chromium in carp samples was found to have been higher than that of the nickel in the given fish, which was consistent with result from the present study (32). Chromium metal is known as a toxic element, and its increase in the environment and aquatic ecosystem may be due to the widespread use of pesticides near the seas, rivers, and dams (33, 34). In the study by Leung et al, however, the amount of nickel metal in the examined fish was detected to have been higher than that of chromium metal, which was not consistent with our study findings in this regard (35). The high amount of nickel metal found by this study may have been due to pollution caused by oil as well as the traffic of oil ships and motorboats Pearl river delta/china (36). In Elsagh's study, the concentration of chromium metal in carp from the Caspian Sea was reported to be higher than the level determined by international standards, which was contrary to the result obtained in the present study; this inconsistency may have been due to the higher pollution of the Caspian Sea and enormous increase in using pesticides in this area compared to water basins of Hamadan province (37). In a study by Tkachenko et al, the amount of chromium metal was determined to be higher than that of cobalt metal in farmed carp in Poland, which was in agreement with our study results (38). Salemi and Hosseini Alhashemi reported that the concentration of chromium in common carp from the shores of Dez was equal to 0.02 mg/kg dry weight and was lower than the limit defined by international standards, which was consistent with our study findings; however, they demonstrated that the concentration of nickel in this fish was equal to 1.41 mg/kg dry weight and was higher than the limit defined by international standards, which was inconsistent with the findings from this study (39). In another study by Saleem et al, it was found that the concentration of cobalt metal in Pakistani carp samples was higher than the amount of nickel and chromium, which was contrary to the results from our study since cobalt metal was not observed in wild and farmed carp samples examined in our study and its amount was below the detection limit of the device (40). The high amount of cobalt recorded by Saleem et al may have been attributed to the existence of agricultural lands and the extensive use of fertilizers and chemical materials in the investigated area (41). In a study by Ahmadi, no concentration of cobalt metal was observed in the Esox lucius from west of Anzali wetland and it was not within the detection of the device, which was consistent with our study findings (42).

4. Conclusion

Although the amount of cobalt metal was below the detection limit and the chromium level in the tested samples was lower than the one defined by the world standards, there was a possibility that the excessive use of pesticides as well as the agricultural discharge of effluents to nearby lands, dams, and water sources in fish farms would result in an increase in the amount of heavy metals in the given areas in the following years. Therefore, it was suggested that the local people and farmers be educated about the dangers of heavy metals, and the entry of environmental pollutants into the dams of Hamadan province be effectively controlled as much as possible. As the amounts of nickel and chromium metals in wild carp samples were higher than those in farmed carp samples, moreover, it was recommended that the necessary measures be taken in order to control the factors responsible for polluting the aquatic environment, and to prevent the entry of industrial effluents and other pollutants into the dams of Hamadan province for avoiding the increase in the amount of heavy metals in water sources.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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