



## Bioaccumulation of heavy metals in common carp fish (*Cyprinus carpio*) and its relationship with the protein content

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### Abstract

The objective of the present study was to investigate the concentration of some heavy metals in the dorsal muscle, gills, and liver of common carp (*Cyprinus carpio*) from the Dukan lake, Suleimani, Iraq, and its relationship with fish protein content. The sampling was carried out in both the summer and winter seasons of 2021. Fish samples were collected from the water of Dukan lake. Biometry analysis and determining of protein content were carried out. Moreover, the heavy metal concentrations were measured in different tissues and organs of fish (the dorsal muscle, gills, and liver). The metal concentration varied considerably among different tissues and organs. Overall, the mean metal concentration revealed an order of Zn > Fe > Cr > Cd > Cu > As > Pb. The mean concentrations of Zn and Pb were determined as the maximum and minimum concentrations of metals in the fish organs, respectively. Metal accumulation was higher in the gills and liver than in the muscle (except Cr). The content of protein showed a close relationship with some of the metal concentrations. The concentrations of metals like As, Cd, and Cu were higher during the summer than in the winter, except for Cr and Fe. Due to the concerns related to the adverse effects of heavy metals in fish meat on human health, investigation of the concentration of pollution and regular monitoring of the physicochemical and heavy metal contents and its relationship with those measured in fish is strongly recommended.

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### Introduction

Fish are the most common vertebrate on the planet and due to its great nutritional value, fish consumption is increasing nowadays. Fish are an essential part of the food chain as they are good sources of high-quality proteins, minerals like calcium (Ca), iron (Fe), phosphorous (P), zinc (Zn), magnesium (Mg), potassium (K), vitamins like D3, B2, B12, and E, also, they are considered as good sources of high unsaturated fatty acids such as omega-3, which play essential roles in human health (1,2). Therefore, the public interest in fish farming in artificial or natural environments such as inland lakes, flood plains, rivers, coastal lagoons, permanent or temporary lakes, and barrages has increased. However, the anthropogenic and natural sources of toxic metals are

contaminating water at the surface or underground. These sources can be released from mine disposals, industrial effluent discharge, domestic waste water, agricultural evacuations, precipitation of air-borne contaminants, and petrol or liquid oil spillage into the water bodies (3,4). Consequently, human health is threatened as a result of polluted water and also consumption of fish grown in such a contaminated environment. Many researchers have reported the contamination of heavy metals in different native and commercial fish (5-8). Many heavy metals, including zinc (Zn), iron (Fe) and copper (Cu), are essential minerals by contributing to biological reactions, but could be toxic in higher concentrations and cause several health problems. Otherwise, some heavy metals are vitally toxic even at trace or low concentrations, such as arsenic (As), cadmium (Cd),

chromium (Cr), and lead (Pb) in both humans and animals (9). Owing to the toxicity, persistence, and cumulative behavior of heavy metals, the bioaccumulation of metals could happen in different tissues or organs of fishes (8,10). Like other marine organisms, the bioaccumulation of metals in fish tissues and organs depends on environmental factors. These factors can be divided into controllable and uncontrollable factors and summarized as sex, age, species, feeding habits, physiological behavior, exposure to contaminants, environmental conditions, location, season, day light, temperature, trophic level, and environmental preference (11,12). Among different fish organs, the gills are responsible for the bioaccumulation of metals from water, but researches showed that different organs may have a different sensitivity to the accumulation of specific metals (7). In the fish body, muscle tissues have more active sites for metal biotransformation, and the liver has a critical role in the storage and redistribution of contaminants in the body and acts as a vital organ in the detoxification and transformation of pollutants and pathogens (8,10). Monitoring the level of heavy metals in fish as a source of protein in the human food chain exposes meaningful information on the pollution of aquatic environments (13).

The aim of the study was to investigate the concentration of selected heavy metals such as As, Cd, Cu, Cr, Fe, Pb and Zn in common carp fish samples collected from eight locations in the Dukan lake over two seasons of summer and winter in 2021.

## **Materials and methods**

### **Ethical approval**

This study was approved by the ethics committee of University of Sulaimani, approval code UoSCI-Bio 001, dated 25/08/2020.

### **Study areas and sampling**

Fish samples (both male and female) were collected from Dukan Lake. The Dukan lake is a water lake located in Suleimani city (36°08'N and 44°55'E). The lake is formed by the biggest concrete dam in Iraq, which is called the Dukan Dam. The surface area of the lake covers an area of about 270 square kilometers and the surface elevation is 515 meters above sea level (14).

### **Fish sampling and analyzing**

The samples of common carp fish (36 samples) were caught from the Dukan lake by a fishermen's net in two periods of time in August and December of 2021. For each season eighteen fish samples were washed thoroughly with fresh water and placed in ice boxes and transported to laboratory. The weight and length of fish were measured for each individual and recorded. The organs and tissues used in this study including dorsal muscle, liver, and gill were cut / prepared from each fresh sample's portions. After being cut

from the body, each sample was weighted and thoroughly mixed and placed in a drying oven at 420°C for 2 hours and 30 minutes (15). To obtain protein content, each portion was frozen and kept at -18°C. The dark meat, if any, was removed prior to the homogenization of the fish flesh. The homogenate samples were placed in a polyethylene bag. Store the sample in a refrigerator or on ice until required. Ensure that the prepared sample is still homogeneous prior to weighing (16).

### **Measurement of heavy metals**

The tissue samples were digested in perchloric acid (HClO<sub>4</sub>) and nitric acid (HNO<sub>3</sub>). One gram of muscle, gill, and liver tissues was separately weighted into 100 mL Erlenmeyer flasks, followed by adding 20 mL of nitric acid 65%. The mixtures were kept overnight and shaken on arbitrary shakers to be slowly digested. Afterward, 5 mL of perchloric acid 70% was added to each flask. The digestion process was continued by heating the flasks at 150°C for about 6 hours. After cooling, the solutions were filtered by Whatman No. 42 ashless filter paper and quantitatively transferred to polyethylene tubes and made up adequately with distilled water. The concentration of As, Fe, Cd, Cu, Cr, Pb, and Zn was determined using ICP (Optima 2100 DV, PerkinElmer, USA).

### **Measurement of fish protein content**

The total protein measurement was carried out by determining the nitrogen content in fish muscles by the Kjeldahl method (17). One gram of each sample was placed in the digestion tube, then 20 mL of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and a digestion mixture 1 gram as a catalyst which included 0.10 g Selenium, 3.496 g potassium, 4.52 g sulfate 0.5 copper sulfate were added. Blanks containing all these reagents were simultaneously processed. The tubes were placed in the preheated digestion block at 120°C for 150 min. The resulting solutions were cooled at room temperature and diluted by adding 30 ml of distilled water. The tubes were placed in the distillation-titration unit. The solutions were then distilled for 6 minutes after 20 ml of sodium hydroxide solution is automatically added. The ammonia collected in the receiving solution 30 ml was automatically titrated against the standard 0.1 N of hydrochloric acid (HCl) with colorimetric end point detection. An ammonium dihydrogen phosphate (NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>) standard was used to check the concentration of the titrating solution. The content of nitrogen was converted to protein by multiplying a factor of 6.25 (18).

### **Statistical analysis**

The data were analyzed using the SPSS Statistics software 23.0 (IBM SPSS Statistics for Windows, Version 23.0, IBM, Armonk, New York). The normal distribution of data was checked using Kolmogorov-Smirnov test. Analysis of Variance followed by Duncan's test was used to identify

statistical differences between means of dorsal muscle, gills and liver data. T test was used to identify statically differences between data obtained in two seasons of summer and winter. P value of equal or less than 0.05 was considered statistically significant.

## Results

The results of fish samples' biometry including sex, total body weight, total length, gill weight and liver weight and also the protein content of fish samples are presented in tables 1. The total weight of fish ranged from 1021.2 to 1231.4 g. There were no significant differences in the total weight regarding the sex or time of sampling. The weight of the gill and liver varied between 20.0 to 22.50 g and 7.0 to 7.4 g, respectively. There were no significant differences in

gill and liver weight between sexes or seasons. Fish length varied between 368.6 and 404.0 mm. The main factors that may influence the growth of fish are related to environmental conditions, including feeding availability, temperature, pH, dissolved oxygen, total alkalinity, and etc. However, studies reported that the significant influence of these factors could be diagnosed after specific times in life stages. There were no significant differences in protein content between different sexes and seasons, as shown in tables 1.

Heavy metal concentrations (mg/kg dry wt.) in muscle, gill and liver of fish samples are presented in table 2. The results showed that there were no significant differences among metals in sex and season.

Table 3 show the most common permissible limits of heavy metals in aquatic environments for fish.

Table 1: Statistical results of fish samples' biometry

| Season                        | Mean ± SD     |               |              |              | Total        |
|-------------------------------|---------------|---------------|--------------|--------------|--------------|
|                               | Winter        |               | Summer       |              |              |
| Sex                           | Female (9)    | Male (9)      | Female (8)   | Male (10)    | 36           |
| Total weight (g)              | 1021.2±149.9  | 1162.2±184.7  | 1196.8±237.4 | 1231.4±258.8 | 1158.4±217.2 |
| Gill (g)                      | 20.0±2.24     | 21.80±3.04    | 21.20±3.42   | 22.50±4.71   | 21.53±3.54   |
| Gill weight (g)               | 45.11±2.38    | 42.05±3.62    | 41.3±2.17    | 42.6±4.03    | 42.76±3.11   |
| Liver(g)                      | 7.00±0.82     | 7.40±0.52     | 7.00±0.71    | 7.40±1.07    | 7.25±0.80    |
| Liver weight (g)              | 16.7±0.95     | 15.1±1.05     | 14.8±1.40    | 15.6±0.78    | 15.55±1.12   |
| Length (mm)                   | 368.6±37.6    | 404.0±25.9    | 398.0±37.7   | 401.0±43.1   | 394.4±37.2   |
| Protein content (%)/1g sample | 73.65±3.32 ** | 73.62±2.64 ** | 70.63±1.38** | 73.37±1.97 * | 73.08±2.59   |

\*\*Correlation is significant at the 0.01 level (2-tailed). \*Correlation is significant at the 0.05 level (2-tailed).

Table 2: Heavy metal concentrations (mg/kg dry wt.) in muscle, gill and liver of fish samples collected at two time periods

| Metals (mg/kg) | Muscle        |               | Gill            |               | Liver          |               |
|----------------|---------------|---------------|-----------------|---------------|----------------|---------------|
|                | Mean (SD)     | Range         | Mean (SD)       | Range         | Mean (SD)      | Range         |
| As             | 1.75 (0.54)   | 1.00-3.01     | 1.78 (0.54)     | 1.01-3.00     | 2.28 (1.03)    | 1.00-5.50     |
| Cd             | 39.19 (15.07) | 21.00-73.00   | 56.13 (12.46)   | 35.00-80.00   | 36.38 (12.46)  | 21.00-67.01   |
| Cu             | 6.22 (1.45)   | 4.50-12.85    | 7.03 (0.95)     | 5.01-8.80     | 6.80 (1.18)    | 2.00-8.40     |
| Cr             | 27.01 (3.51)  | 17.21-32.32   | 24.19 (5.01)    | 16.40-33.02   | 15.63 (3.85)   | 9.60-24.00    |
| Fe             | 74.31 (9.63)  | 56.20-95.01   | 86.66 (15.03)   | 61.10-125.02  | 229.99 (33.01) | 168.21-301.72 |
| Pb             | 0.14 (0.01)   | 0.12-0.16     | 0.14 (0.01)     | 0.12-0.15     | 0.15 (0.01)    | 0.13-0.16     |
| Zn             | 56.23 (15.27) | 27.00 - 85.60 | 456.39 (106.34) | 313.56-674.91 | 264.02 (41.87) | 206.2-375.70  |

Table 3: The most common permissible limits of heavy metals in aquatic environments for fish

| Heavy Metals | Freshwater (mg/L) | Seawater (mg/L) | References |
|--------------|-------------------|-----------------|------------|
| Pb           | 0.18-1.00         | 0.02-0.05       | WHO (19)   |
| Fe           | 434.78            | 531             | FAO (20)   |
| Cd           | 0.05              | 1.0             | WHO (21)   |
| Cr           | 5                 | 5               | FAO (22)   |
| Cu           | 0.1               | 2               | B.C (23)   |
| Ni           | 0.1               | 2               | NSW (24)   |

The gill had the highest concentration of metals among the organ tissues, corresponding to Zn, followed by the mean concentration of Fe in liver tissues. Also, for Zn, there were significant differences among different organs and tissues. High mean concentrations of Cd and Zn were observed in the gills. However, the concentration of Fe was highest in the liver organ 229.99 g g<sup>-1</sup>. There were no significant differences among organs and tissues in As, Cu, and Pb concentrations. The mean concentration of Cr was the lowest in the liver tissues. The results of statistical analysis showed that changes in the concentration of some metals in one of

the organs were related to changes in the concentration of the same metal in other organs. The outcomes showed a substantial correlation between changes in Pb, Cd, and Zn metal levels in the liver and these metal concentrations in the gills and muscles. In contrast, the changes in As and Cu were not significant in the studied parts. This can be due to the presence of bioaccumulation phenomena in a particular organ.

## **Discussion**

Our findings demonstrated that the liver has a greater average content of these two metals than the gills and muscles. The alteration in the concentration of metals in the gill was significant, with changes in the metals in the muscle at a lower level at Cr and Cd. Also, changes in iron concentration in the liver or muscle were not significant with changes in the gill. This case may indicate the physiological relationship of iron consumption in the muscle structure or the extent of the blood supply network in the muscle tissue (17). The amount of Cr in the gill tissue was not statistically significant compared to the concentration in the muscle. The ranking of significance based on the amount was related to Zn, Cd, and Pb respectively. Overall, the mean metal concentration revealed an order of  $Zn > Fe > Cr > Cd > Cu > As > Pb$ . This case shows that raising fish in polluted water can lead to the accumulation of harmful substances in their bodies, and water chemistry is important in aquaculture. The weight of gill and liver tissues was found to be significantly related to the total weight of fish in both sexes. The weight of the gill was closely related to the weight of the liver. Although the total weight of fish was not affected by the concentration of any metal, the content of protein was significantly affected by the total concentration of Pb, Fe, Cr, and Cd in tissues. Metal accumulation was higher in the gills and liver than in the muscle (19). Because the gills are directly exposed to the water and consequently toxic compounds, there are more pollutants in them than in muscle (25).

Studies showed that Cd and Cu may affect the enzyme activity and response in the cytochrome of the liver of fish in low concentrations (4). The reports revealed that the inhibitory potency of the metals on the activity of enzymes could have the following order:  $Cd > Ni > Cu > Zn > Pb$  (3).

Knowledge of heavy metal concentration in tissues of a fish's body could increase awareness of human health and environmental management. The assessment of changes in metal concentrations in water is critical to understanding the possibility of toxic substance bioaccumulation in aquatic life and can provide a valuable vision in aquaculture industry management. The toxicity of metals could directly enter the human food chain upon consumption of fish and threaten human health. In the present investigation, the seven metals (As, Cd, Cu, Cr, Fe, Pb, and Zn) were studied as the common metals that could be found in artificial lakes with incoming

sources from urban regions. Apparently, the assimilation and toxicity level of metals in fish tissues and organs are consequences of different factors and their interactions. Many studies have found that different organs and tissues have a different tendency to accumulate high-value metals than other parts of the body (26,27).

In this study, the muscle protein content had a close relationship with the total concentration of Cr, Fe, Pb, and Cd in tissues. Overall, the protein content of muscle tissues was affected by the mean concentrations of Pb in liver and muscle, Fe in gill, Cr in muscle and liver, Cd in muscle and liver, and Zn in muscle tissues (26,27). There is evidence that Cd builds up in a variety of fish tissues, causing pathological changes in the head kidney and other tissues and organs (28). Free radicals, particularly ROS, are produced by redox reactions brought on by Cd bioaccumulation, and these radicals damage and kill fish cells (29). While gills showed high concentrations of Cr, Mn, and Zn, scales showed high concentrations of Cr, Ni, and Sr. The elements' increasing rate was caused by their extraction, concentration, and accumulation over time from the water body and during the scale-forming process. (30). A study reported that the lethal concentrations of  $CuSO_4$  at 10 mg/l led to killing the fish, and mortality was 100% during 24 hours, while the concentration at five mg/l was the median lethal concentration which led to killing 50% of treated fish (31,32).

The accumulation of heavy metals causes histological changes in fish muscles, providing an opportunity to assess fish health and information on possible health hazards from their environment (33). Using natural products such as algae can barely enhance fish performance, leading to good immunity and good protein content (34,35). A study showed the possibility of serious fish diseases such as septicemia when fish are under stressed or unsuitable environmental conditions. this is considered the main cause of economic losses and effects on public health due to eating, handling, and transporting (36-39).

## **Conclusion**

The metal concentration varied considerably among different tissues and organs. Overall, the mean metal concentration revealed an order of  $Zn > Fe > Cr > Cd > Cu > As > Pb$ . The mean concentrations of Zn and Pb were determined as the maximum and minimum concentrations of metals in the fish organs, respectively. Metal accumulation was higher in the gills and liver than in the muscle (except Cr). The content of protein was linked to the chemical quality of the water. The content of protein showed a close relationship with some of the metal concentrations and water characteristics of the lake. In the investigated properties, there was no significant difference between the sexes. Therefore, regular monitoring of the water physicochemical characteristics and the content of metals in fish is strongly recommended.

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## Conflict of interest

The authors declare that they have not any conflict of interest (The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report).

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## التراكم الحيوي للمعادن الثقيلة في أسماك الكارب الشائع وعلاقته بمحتوى البروتين

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### الخلاصة

الهدف من هذه الدراسة هو فحص تركيز بعض المعادن الثقيلة في العضلات الظهرية والخياشيم والكبد لأسماك الكارب الشائع من بحيرة دوكان، السليمانية، العراق وعلاقتها بمحتوى البروتين السمكي. تم أخذ العينات في كل من موسمي الصيف والشتاء لعام ٢٠٢١. تم جمع عينات الأسماك من مياه بحيرة دوكان. تم إجراء تحليل القياسات الحيوية وتحديد محتوى البروتين. علاوة على ذلك، تم قياس تراكيز المعادن الثقيلة في أنسجة وأعضاء مختلفة للأسماك (العضلات الظهرية والخياشيم والكبد). يختلف تركيز المعدن بشكل كبير بين الأنسجة والأعضاء المختلفة بشكل عام، أظهر متوسط تركيز المعدن ترتيباً من الرصاص > الزرنيخ > النحاس > الكاديوم > الكروم > الحديد > الزنك. تم تحديد متوسط تراكيز الزنك والرصاص على أنهما الحد الأقصى والأدنى لتركيزات المعادن، على التوالي، في أعضاء الأسماك. كان تراكم المعادن أعلى في الخياشيم والكبد منه في العضلات (باستثناء الكروم). أظهر محتوى البروتين علاقة وثيقة مع بعض تركيزات المعادن. وجد أن تركيز المعادن مثل الزرنيخ والكاديوم والنحاس أعلى خلال فصل الصيف مقارنة بموسم الشتاء، باستثناء الكروم والحديد. بسبب المخاوف المتعلقة بالآثار الضارة للمعادن الثقيلة في لحوم الأسماك على صحة الإنسان. يوصى بشدة بفحص تركيز الملوثات والرصد المنتظم لمحتويات المياه الفيزيائية والكيميائية والمعادن الثقيلة وعلاقتها بتلك المقاسة في الأسماك.