



Evaluation of the heavy metal content in the muscle tissue of common carp (*Cyprinus carpio* L.) reared in groundwater in Basrah province, Iraq

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Abstract

The concentration of heavy metals (Cu, Zn, Cr, Ni, Hg, Pb, and Cd) in the muscles of common carp (*Cyprinus carpio*) reared in groundwater in Khor Al-Zubair, Basrah province (in the south of Iraq) were assessed using X-ray fluorescence (XRF) spectroscopy. XRF is a powerful technique for element analysis in different environmental samples with many advantages compared with conventional laboratory methods. The mean concentration of the studied metals in the edible parts of the fish (Cr= 11.42, Ni= 2.75, Hg=1.53, Pb= 1.93, and Cd=4.42 mg/ kg dry weight) exceeded the recommended maximum acceptable levels proposed by the Food and Agriculture Organization (FAO)/World Health Organization (WHO), The commission of the European Communities (EC), and Food and Drug Administration (FDA). The results suggest that the tested fish muscle tissue was not safe for human consumption and that the groundwater in the Khor al-Zubair area is possibly contaminated with heavy metals, mainly owing to industrial activity.

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Introduction

The Shatt al-Arab river is the only source of freshwater in the Basrah governorate, south of Iraq. The Shatt al-Arab river faces major challenges, including a decrease in the amount of freshwater received from the Tigris and Euphrates rivers (1), an increase in the quantity of pollutants that reach it owing to agricultural, industrial, and domestic activities (2), and, the most difficult challenge, the phenomenon of frequent saltwater intrusion (SWI) from the Arabian Gulf (3). The SWI problem has caused the elimination of the vast majority of fish farms along the Shatt al-Arab River (in earthen pond and cage systems) (2).

Because of the availability of groundwater, a limited number of fish farms have been established for raising fish in earthen ponds or plastic tanks to avoid the economic losses caused by the exposure of fish to the direct environmental changes in the Shatt al-Arab river and sudden fluctuations in water quality resulting from SWI (2). The western part of Basrah, including Khor al-Zubair, is

the industrial zone of the province, in which many of the industrial facilities are located such as oil refining sites, the south gas company, the Khor Al-Zubair gas power station, a petrochemical plant, a fertilizers plant, cement factories, iron and steel plants, which emit a large amount of pollutants (4).

Fish take heavy metals from the water, which accumulate in the tissue to a hazardous level without any visible signs (5). Fish are good indicators of metal contamination and are usually used to evaluate the risk of human ingestion of fish contaminated by metals and other potentially toxic elements (6). Therefore, the concentration of heavy metals in fish meat should be measured and compared with the maximum limits established by the international legislation such as FAO/WHO, European Commission and Food and Drug Administration to ensure its suitability for human consumption (7).

X-Ray fluorescence (XRF) is a fast, economic, and nondestructive analytical method for the analysis of a variety of hazardous materials and environmental samples.

It can be used to identify up to 30 elements at the same time (8). XRF is usually used in archaeological studies to investigate the content of metals in human and animal fossil bones, in which the samples are precious and their destruction should be avoided (9). Total reflection XRF (TXRF) spectroscopy is a powerful analytical technique for elemental analysis of small masses. In this context, Bilo *et al.* (10) used TXRF to study Pb and Zn accumulation in zebrafish embryos. Similarly, Limburg *et al.* (11) used the X-ray fluorescence technique to investigate fish otolith trace element maps.

The aim of this study was to evaluate the heavy metals concentration in the edible part of common carp (*Cyprinus carpio*) reared in groundwater in Khor al-Zubair, Basrah province to determine their suitability for human consumption using the XRF technique.

Materials and methods

The fresh fish were purchased in January 2019 from one of the fish farms located in Khor al-Zubair, Basrah province, southern Iraq (Figure 1).



Figure 1: The yellow pin on the map shows the location of the fish farm in Khor al-Zubair, Basrah province.

The farm completely depends on well water 24 m depth, which is pumped to an earthen pond before it is distributed to the fish culturing system. The initial weight of the fish was 100 g and fish were kept in the rearing system 12×3×2 m for 10 months (from March 2018 to January 2019). During the rearing period, fish were fed different types of fish diets including locally made fish diets and imported fish feed depending on availability in the local market and the purchasing power of the farmers.

A total of 10 fresh common carp were purchased from the farm gate and immediately placed in polyethylene bags, put into an icebox, and brought to the laboratory. The fish length was measured from tip to tail and weighed; data for the individual fish were recorded. The samples were

prepared according to AOAC (12). Briefly, fish samples were cleaned using a stainless steel knife; the edible part of the fish (tissue from the dorsal side of the body, which is the consumed part of fish) was taken without the skin, cut to small pieces, and washed with distilled water. The samples were dried in a drying oven at 65°C for approximately 24 hours and homogenized in a porcelain mortar. Approximately 0.5 g of the powdered fish of each sample (10 samples) was weighed and mixed with 3 drops of organic binder and the sample was pressed to form pellets ready for metal analysis

XRF Spectro, Shimadzu, Japan was used to qualitatively and quantitatively determine the metals present in the tissue samples according to Klockenkämper (13). The results were expressed in mg/ kg dry weight and the results were compared, where available, to WHO Guidelines, EC, Environmental Quality Standards, and FDA Consumer Advisory.

Statistical analysis

The statistical analysis (min, max, mean, standard deviation, and range) was conducted using Microsoft excel. All the results are presented as mean ± standard deviation (SD). SPSS program was used to test the normality of distribution and the Pearson correlation.

Results

In the current study, we determined the concentration of heavy metals in common carp reared in earthen ponds that were filled with water from a well (24 m depth) in Khor al-Zubair, Basrah, southern Iraq. The average weight and length of the fish used in the current study were 755.7 ±76.8 g and 29.74 ±1.65 cm respectively.

The results of the present study were compared with the international standard limits to determine if these fish were suitable for human consumption. The minimum, maximum, mean concentration, and SD of heavy metals in the edible tissues of the common carp from Khor Al-Zubair farm compared with the standard limits are given in Table 1. Table 2 and 3 represent the descriptive statistics of heavy metal concentrations (mg/kg) in the muscle of common carp.

Discussion

The maximum concentration of Cu in the muscle tissue was 5.30 mg/ kg, which was lower than the FDA (14) maximum guidelines (30 mg/ kg). Similarly, the mean concentration of Zn recorded in the current study was 42 mg/ kg, which was lower than the FAO/WHO (7) maximum guidelines (50 mg/ kg). It has been suggested that Cu and Zn, as essential metals, tend to accumulate in the liver of fish and not in the edible part owing to their role in metabolism (15).

Table 1: Content of heavy metals (mg/ kg dry weight) in the edible parts of the common carp (*Cyprinus carpio*) reared in ground water compared with standard limits

	Cu	Zn	Cr	Ni	Hg	Pb	Cd
Min	3.60	23.50	7.50	2.10	1.00	1.60	2.60
Max	5.30	61.00	15.60	3.50	1.90	2.40	10.50
Mean	4.33	42.00	11.42	2.75	1.53	1.93	4.42
SD	0.66	14.18	2.86	0.55	0.34	0.28	3.10
WHO, 1985	3.00	10.70	0.15	0.60		2.00	
FAO/WHO, 2004	10.00	50.00	1.00		1.00	1.00	
FDA, 2003	30.00				0.50	0.30	0.05
EC, 2006						0.50	0.10
FAO, 1983	30.00					0.50	0.05

Table 2: Descriptive statistics of heavy metal concentrations (mg/kg) in the muscle of common carp

	Cr	Ni	Cu	Zn	Hg	Pb	Cd
Mean	11.42	2.75	4.33	42.00	1.53	1.93	4.42
Standard Deviation	2.86	0.55	0.66	14.18	0.34	0.28	3.10
Sample Variance	8.18	0.31	0.44	201.06	0.11	0.08	9.62
Kurtosis	-0.24	-1.58	-1.31	-1.44	-0.51	0.67	4.34
Skewness	0.13	0.13	0.52	-0.02	-0.71	0.88	2.08
Range	8.10	1.40	1.70	37.50	0.90	0.80	7.90
Minimum	7.50	2.10	3.60	23.50	1.00	1.60	2.60
Maximum	15.60	3.50	5.30	61.00	1.90	2.40	10.50
Confidence Level (95.0%)	3.00	0.58	0.70	14.88	0.36	0.29	3.25

Table 3: Pearson correlation between heavy metals concentrations (mg/kg) in the muscle of common carp

	Cr	Ni	Cu	Zn	Hg	Pb	Cd
Cr	1						
Ni	0.621659**	1					
Cu	-0.58007*	-0.07085	1				
Zn	-0.47907	-0.04888	0.448491	1			
Hg	0.313247	0.56496**	0.484519	-0.22951	1		
Pb	0.103898	0.694959**	0.520372**	0.243899	0.533468**	1	
Cd	-0.55887*	-0.30669	-0.20188	0.489874	-0.81194*	-0.24679	1

* Correlated at 5% significance level, ** Correlated at 1% significance level.

The minimum level of Cr in the muscles of carp was approximately 7 times higher than the maximum acceptable limit recommended by FAO/WHO (Table 1). The western part of Basrah province is the center of the main industrial activities in the city. There are more than 9 industrial plants that release solid, liquid, and gaseous wastes directly to the surrounding environment, causing significant environmental damage (4). Cr is used as an anticorrosion agent in cooling water in different factories such as iron and steel factories and petrochemical plants and the wastewater is directly discharged to the environment without treatment. Therefore, it is possible that groundwater in Khor al-Zubair is contaminated with Cr.

High levels of Ni were found in the edible parts of tested fish, as shown in Table 1. The mean concentration

(2.75 ± 0.55 mg/ kg) was higher than the acceptable limit reported by WHO (16), which is 0.6 mg/ kg. Al-Gburi *et al.* (17) suggested that the presence of Ni in environmental samples results from agricultural activities and waste from oil extraction. Khor al-Zubair is the center of vegetable farms in the city, which consume a huge amount of chemical fertilizers that end up in the soil and groundwater.

The mean concentration of Hg in the fish tested in the present study was 1.53 ± 0.34 mg/ kg. These results were higher than the maximum level recommended by WHO (16) and FDA (14) (1.0 and 0.5 mg/ kg), respectively. It has been suggested that Hg from atmospheric deposition contributes to elevated Hg concentrations in groundwater (18). Furthermore, in addition to pesticides, Hg could be introduced to soils from fertilizers (19). The exposure of

humans to Hg through food and fish is a major source of methyl mercury exposure (20). Inorganic mercury is converted to organic compounds, such as methyl mercury, which is very stable and accumulates in the food chain (21). A high dietary intake of mercury from consumption of fish has been hypothesized to increase the risk of coronary heart disease (22)

Lead (Pb) is the most toxic heavy metal for fish owing to its negative effects, which include delayed embryonic development, suppressed reproduction, inhibition of growth, increased mucous formation, enzyme inhibition, and kidney dysfunction (23). The mean concentration of Pb in the muscle tissue of tested fish was 1.93 ± 0.28 mg/ kg. The mean concentration of Pb was higher than the maximum guidelines of FDA (14), WHO (16), FAO/WHO (7), and FAO, (24), as shown in Table 1. It has been reported that Pb pollution is mainly owing to Pb emissions from petrol (21). It is possible that the groundwater in the western part of Basrah is contaminated with Pb because Khor al-Zubair is located within the industrial zone of the city, which is rich in oil fields. Lead causes renal failure and liver damage in humans (25) and is known to reduce the intellectual performance of children and increase blood pressure and cardiovascular disease in adults (26).

Cadmium (Cd) levels in fish tissue tested in the current study ranged between 2.60 and 10.50 mg/ kg, whereas the maximum level of Cd permitted by the FAO, (24) and FDA (14) is 0.05 $\mu\text{g/ g}$ and 0.1 $\mu\text{g/ g}$ by the commission regulation (27) (Table 1). Cadmium compounds are used in rechargeable nickel-cadmium batteries and are often dumped together with household waste (21). In addition, cadmium compounds are used as stabilizers in PVC products, color pigment, and several metallic cadmium alloys have been used as anticorrosion agents (21). Cadmium may accumulate in the human body and may induce kidney dysfunction, skeletal damage, and reproductive deficiencies (28).

In the current study, kurtosis (a measure used to describe the normality of distribution) was the highest in the order $\text{Cd} > \text{Pb} > \text{Cr} > \text{Hg} > \text{Cu} > \text{Zn} > \text{Ni}$; the skewness for Cd, Pb, Cu, Cr, and Ni was relatively high (Table 2) and low for Hg and Zn, which indicated non-normality of the distribution for these heavy metals. The significant differences between the variance and the confidence level of all heavy metals also proved that they were not normally distributed. Furthermore, the variation of Zn, Cd, Cr were 201.06, 9.62 and 8.18, respectively, and higher than those of Cu, Ni, Pb, and Hg, suggesting that the former had greater variation among the fish in the current study.

Heavy metals have a significant contribution to the potential environmental threat according to the coefficients for $\text{Zn} > \text{Cd} > \text{Cr} > \text{Cu} > \text{Hg} > \text{Pb}$, which suggest that both natural and anthropogenic factors contribute to the heavy metal concentrations and its resulting effects on potential ecological risks (29).

We used Pearson correlation to determine whether the various metals in the fish had a common source. The metal couples Pb/Ni, C/Ni, Hg/Pb, Cu/Pb, and Ni/Hg (Table 3) had a strong positive relationship ($P < .001$). Cd had no interactions with Hg, Ni, and Cu. The clear negative comparison indicated that Hg/Cd, Cr/Cu, and Cr/Cd were likely to come from similar sources. Wastewater, municipal waste, industrial effluents, and agrochemical waste from nearby villages could provide a possible anthropogenic source of these metals (30).

The limitation of the current study is that the EDI was not calculated owing to the absence of data related to the daily consumption rate of fish in the city (g/day). For future studies, it is imperative to calculate the estimated daily intake (EDI) of heavy metals through fish consumption. The EDI of metals depends on the concentrations of metals in fish tissue and the amount of fish consumed (31).

Conclusions

The concentrations of Cr, Ni, Hg, Pb, and Cd heavy metals in the edible parts of common carp (*Cyprinus carpio*) reared in groundwater in Khor al-Zubair, Basrah province (Southern Iraq) exceeded the standard maximum guidelines, which indicates a health risk for human consumption. Fish species can be good indicators of water quality; however, more studies are needed to assess the quality of the groundwater in the Khor al-Zubair industrial zone and its suitability for agriculture and aquaculture enterprises. Further studies are required to determine the daily consumption rate of fish in the city to calculate the estimated daily intake (EDI) of heavy metals through fish consumption.

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Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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تقدير العناصر الثقيلة في عضلات اسماك الكارب الشائع المربأة في المياه الجوفية في منطقة خور الزبير في محافظة البصرة/ جنوب العراق

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الخلاصة

تم تقدير تراكيز بعض المعادن الثقيلة (النحاس والزنك والكروم والنيكل والزرنيق والرصاص والكاديوم) في عضلات اسماك الكارب الشائع المربأة في المياه الجوفية في منطقة خور الزبير الصناعية الواقعة في محافظة البصرة / جنوب العراق باستخدام تقنية فلورة الأشعة السينية. تقنية فلورة الأشعة السينية من التقنيات الفعالة في تحليل مدى واسع من العناصر في العينات البيئية المختلفة مع العديد من الفوائد مقارنة بالطرق المخبرية التقليدية. بينت نتائج الدراسة أن تركيز العناصر المذكورة في الأجزاء المأكولة (الكروم = 11,42 و النيكل = 2,75 و الزرنيق = 1,35 و الرصاص = 1,93 و الكاديوم = 4,42) ملغم/ كغم من الوزن الجاف قد تجاوز الحدود المقبولة والمسموح بها من قبل المنظمات العالمية مثل منظمة الغذاء والزراعة العالمية ولائحة المفوضية الأوروبية وهيئة الدواء والغذاء الأمريكية وعليه فان عضلات الأسماك المدروسة غير صالحة للاستهلاك البشري ومن المحتمل أن تكون منطقة خور الزبير ملوثة بالعناصر الثقيلة كنتيجة للنشاط الصناعي.