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Bioaccumulation of heavy metals muscle of common carp fish (Cyprinus carpio L, 1758) from Ala gul and Alma gul wetlands of Golestan and consumption risk assessment

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ABSTRACT

Heavy metals are importantly concerned due their biological accumulation and toxicity in terms of human health. Common carp is one of the most popular seafood in the North of Iran especially in Golestan province. In this descriptive-analytical research, about 30 samples of common carp were collected from Ala gul and (n: 24) and Alma gul (n: 6) wetlands. Once the samples were prepared by polarography device, the concentration of lead, cadmium, zinc and copper were measured in the muscle tissue of carp. The value of THQ in children and adults was estimated according to the equations of risk assessment. The mean concentration of zinc, lead, copper and cadmium was 120.9 ±106, 7.92 ±7.9, 5.84 ±5.22 and 0.027 ±0. 043 mg/kg in Ala gul wetland and 67.42±33.43, 3.24± 1.04, 4.36±2.77 and 0.005±0.008 mg/kg in Alma gul wetland , respectively. The order of heavy metals' concentration in both wetlands was Cd <Pb <Cu <Zn. Although the concentration of copper, lead and zinc was significantly higher than the standard limit, the results of risk assessment showed that THQ was less than 1 for all the heavy metals except for lead (in children group); therefore, it can be concluded that the consumption of common carp fish in Ala gul and Alma gul wetlands does not hazard adult's health.

Keywords: Heavy Metals, common carp; ala gul, alma gul, wetlands and risk assessment

INTRODUCTION

In recent decades, the aquatic environment has been contaminated with a wide range of heavy metals. Since toxic metals are refractory and non-biodegradable, concerns about the risks of heavy metals have been considerably increasing globally [1,2]. Industrial development, excessive increase of population in cities and villages and, subsequently, agricultural development and the use of fertilizers and pesticides cause a large amount of urban and industrial wastage as well as agricultural effluents which contain various chemical compounds particularly heavy elements enter aquatic ecosystems [3,4]. Amongst the contaminants entered aquatic ecosystems, heavy metals are noteworthy due to their toxicity and bioaccumulation potential in many aquatic species [5]. The contamination of aquatic ecosystems with a variety of pollutants can be verified by analyzing water, sediments and aquatic organisms [6]. The accumulation of heavy metals in each of these components can lead to serious ecological changes [7] . Amongst sources of food, fish are constantly exposed to water contaminated with the heavy metals; these metals can accumulate in the muscle tissues of fish in various amounts depending on the size and age of fish [8-10]. Heavy metals enter the body of live organisms by inhalation, absorption and eating. Thus, the consumption of marine organisms such as shellfish and fish may endanger human health [11]. Nowadays, the consumption of fish has been increasing due to its crucial role in health because fish is a rich source of unsaturated fatty acids in addition to easily digestible protein [12]. With respect to the fact that a wide range of pollutants have been consistently entering marine environments, fish have not been immune from the risk of contamination. On the other hand, due to the bioaccumulation potential of toxins in the muscles and other tissues of fish, eating such fish can seriously threaten human health [13,14]. Therefore, fish are appropriate bio-indicators to determine the concentration of heavy metals in aquatic ecosystems [15, 16]. Fish are considered as carriers of heavy metals in the food chain of an ecosystem. Since they feed on tiny sea creatures and marine plants, they are capable of accumulating heavy metals in their body [17]. Intoxication of human with heavy metals, caused by consumption of contaminated fish, was first observed in Minatama Bay, Japan in 1953 during which more than 43 local inhabitants died because of eating fish contaminated with the sewage of an industrial factory and more than 700 people were permanently disabled [18]. Amongst heavy metals, lead and cadmium are xenobiotic i.e. these elements are not required for body's metabolism and even tiny amount of these metals are harmful to the body [19]. The most important adverse effect of cadmium is Itai-Itai disease which was first reported in Japan due to the consumption of rice contaminated with this metal. The other toxic effects of cadmium include kidney tissues destruction. The destructive effects of lead are serious damage to the central and peripheral nervous system. However, the other group of heavy metals are elements which are needed for body in small quantities. In fact, the consumption of these metals higher than the standard amounts can lead to adverse effects. Such metals are copper and zinc [20, 21]. Cadmium is one of the few elements that does not have any critical productive role in the structure human body and even a tiny amount of it can be toxic and cause iron deficiency [22]. Zinc is an essential element in biochemical reactions and is balance in hemostatic manner [23, 24]. Seafood are the main source of zinc [25]. It is very vital for fish in small amounts and plays a crucial role as a catalyst in the most active enzymes involved in energy metabolism; hence, its tiny amounts are not considered a serious threat [26]. Regarding Atrak basin which encompasses a wide range of agricultural lands and its pass through areas with different soil textures, the entrance of heavy metals including cadmium, lead, zinc and copper were selected as prominent elements used in agricultural pesticides, chemical fertilizers and soil texture. Furthermore, the use of shotguns, whose main composition is lead and zinc, for hunting birds in these wetlands probably increases the concentration and accumulation of heavy metals in the ecosystems of wetlands. Considering the significance of the health of heavy metals, the present study intended to measure and evaluate the effect of four heavy metals, including lead, copper, cadmium and zinc, on the muscle tissues of common carp (Cyprinus Carpio L, 1758) in Ala gul and Alma gul wetlands in Golestan province located in the south of Caspian Sea in Iran. moreover, the potential risk of the consumption of common carp for human health was estimated in terms of mean daily intake and target hazard quotient (THQ) for both adults and children.

MATERIALS AND METHODS

Sampling

In this descriptive-analytical research (2013-2014) which investigated some heavy metals (Mn, Cu, Cd, Pb and Zn) to determine their concentration in the muscle tissue of fish, the SD limit (σ), degree of error (d) were equal to 0.25 and 1% respectively assuming 95% confidence level (α = 0.05) (z=1.96) and were estimated through Equation 1 below.

Equation 1

$$N = \frac{(Z_{(1-\frac{\alpha}{2})} \times 2 \times \sigma^2)}{d^2}$$

The number of required sample was estimated to be 30 fish (24 from Ala gul and 6 from Alma gul wetlands) [27]. In this study, 30 mature carp (weighing 1000±300 g) collected from Ala gul wetland.

2.2.Analysis procedures

The fish species caught from these wetlands were immediately placed into an icebox and sent to the laboratory. They were kept at the fridge until preparation. Next, the fish were thoroughly rinsed and washed with distilled water; then, about 10 – 20 grams of the fillet of each fish were weighed and kept in an oven at 105˚C for 4 hours. Afterwards, the samples were transferred to a Dessiccateur to be pulverized into powder in a porcelain mortar once they reached a constant weight. About 0.5 grams of the fully powdered fish was mixed with 5 ml of concentrated nitric acid and were heated on a bain-marie heater at 100 ˚C so that the intended elements were converted to a transparent solution. The prepared suspensions were filtered using Whatman filter paper (0.45 microns); the filtered solution was poured into a scaled flask and was brought to a volume of 50 ml. Once the solution was thoroughly stirred to obtain a uniform solution, the concentration of the intended heavy metals was determined using a polarography device (Metrohm 797, Switzerland). This concentration was converted to a ppm value per unit dry weight of actual concentration of sample through Equation 2 below [28].

Equation 2

$$C_{(ac)} = \frac{(C_{(pc)} \times V_1)}{m}$$

In this equation C_{ac} is actual concentration, C_{pc} is polarography concentration, V_1 is total volume of sample and m is dry weight of sample .

The inclusion criteria for samples was having a weight of 1000±300 grams. The exclusion criteria for fish samples was weighing under 700 grams.

Target Hazard Quotients Estimation

The potential health risk of carp was estimated by THQ equation for all the consumers of carp. If THQ was less than 1 for each metal, the inhabitants of that society would be at a safe condition. THQ was estimated through Equation 3 below [29].

Equation 3

$$THQ = \frac{E_F E_D F_{IR} C}{R_{DF} W_{AB} T_A} \times 10^{-3}$$

In this Equation : E_F is exposure factor (365 days/year), E_D is exposure duration (70 years) [30], F_{IR} is daily food intake (g/person/day), C is Concentration of heavy metals in fish (mg/g), R_{FD} is oral reference dose (μg/g/day), W_{AB} is mean body weight (Kg), T_A is mean time of exposure for non-carcinogenic metals (365 days/year number of exposure years) According to the assumptions of EPA for error analysis, the mean body weight for adults and children was 55.9 kg and 32.7 kg respectively [31].

Health Threat Caused by Consumption of Carp

The oral reference dose for zinc, cadmium, lead and copper was 0.3, 0.001, 0.004, and 0.04 mg/g-day, respectively [32].

Daily Intake of Fish and Statistical Analysis

The daily intake of carp for adults and children was 57.56 and 92.6 g/person/day , respectively[32]. Data, in the present study, was statistically analyzed using $SPSS_{19}$ and Excel 2013. The heavy metals of both wetlands were compared using independent sample t test. Moreover, in order to compare the concentration of heavy metals with standard limits, one sample t test was used. The significance level (P-value) for the t tests was calculated <0.05.

RESULTS

Table 1. Concentration of heavy metals of zinc, cadmium, lead and copper in the muscle tissues of carp in ala gul wetland (mg/kg)

Number sample	Zn	Cd	Pb	Cu
1	1.150	0.002	1.570	2.100
2	129.000	0.012	5.500	9.300
3	14.580	0.000	1.240	1.229
4	87.000	0.001	2.760	1.740
5	160.000	0.001	3.280	3.760
6	477.000	0.110	6.700	4.200
7	134.000	ND*	6.300	16.500
8	102.530	0.140	ND	5.240
9	138.500	ND	4.750	16.070
10	266.890	0.031	5.760	13.690
11	223.710	ND	8.210	7.780
12	304.120	0.100	18.260	4.800
13	102.960	0.002	3.700	9.700
14	52.830	0.043	2.900	8.750
15	126.000	0.003	21.860	11.610
16	75.780	0.020	7.960	39.430
17	78.670	0.110	4.200	4.700
18	36.200	ND	2.900	6.620
19	96.080	0.060	11.570	3.400
20	79.700	0.001	3.600	4.140
21	72.770	0.006	2.300	2.600
22	62.560	ND	9.980	5.830
23	17.050	0.005	2.680	3.060
24	62.700	0.001	2.200	3.890
Mean	**120.908**	**0.027**	**5.841**	**7.922**
SD	106.250	0.043	5.226	7.994
Max	477.000	0.140	21.860	39.430
Min	1.150	ND	ND	1.229

**Not Detected*

Table 1 presents the concentration of heavy metals including zinc, cadmium, copper and lead in the muscle tissues of common carp in Ala gul wetland. The order of heavy metals was Cd (0.027 ±0.043 mg/kg) <Pb (5.84 ±5.22 mg/kg) <Cu (7.92 ±7.9mg/kg) <Zn (120.9 ±106 mg/kg) in terms of concentration. The range of concentration was 4.77-1.15 mg/kg for Zn, 0.14-ND mg/kg for Cd, 21.8-ND mg/kg for Pb, 39.4-1.22 mg/kg for Cu.

Table 2 presents the concentration of heavy metals including zinc, cadmium, copper and lead in the muscle tissues of common carp in Alma gul wetland. The order of heavy metals was Cd (0.005±0.008mg/kg) <Pb (4.36±2.77 mg/kg) <Cu (3.24±1.04mg/kg) <Zn (67.42±33.43 mg/kg). The range of concentration was 19-177 mg/kg for Zn, ND-0.02 mg/kg for Cd, 2.1-8.7 mg/kg for Pb and 1.6-4.4 mg/kg for Cu.

Table 2. Concentration of heavy metals of zinc, cadmium, lead and copper in the muscle tissues of carp in alma gul wetland (mg/kg)

Number sample	Zn	Cd	Pb	Cu
1	86.080	0.020	8.700	2.400
2	71.700	0.001	3.200	4.400
3	117.400	0.004	2.100	1.600
4	61.600	ND	6.980	3.830
5	19.050	0.003	2.800	3.460
6	48.700	0.001	2.400	3.800
Mean	67.422	0.005	4.363	3.248
SD	33.432	0.008	2.772	1.044
Max	117.400	0.020	8.700	4.400
Min	19.050	0.000	2.100	1.600

As shown in Table 3, there was a significant difference between the concentration of zinc, cadmium and copper in Ala gul and Alma gul wetlands (P-value <0.05); however, there was not any significant difference in the concentration of lead (P-value <0.05).

Table 3. Difference between the concentration of heavy metals in the muscle tissues of carp in ala gul and alma gul wetlands

HM	Ala Gol	Alma Gol	P value
Zn	120.9±106	67.42±33.4	**0.047**
Cd	0.027±0.043	0.005±0.008	**0.026**
Pb	5.84±5.22	4.36±2.77	0.357
Cu	7.92±7.99	3.24±1.04	**0.01**

DISCUSSION

The consumption of common carp does not endanger the health of adults. Fish is one of the most important sources of human exposure to metals [33]. Since carp intake in high in the northern area of Iran (Golestan), the identification of environmental conditions where carp grows in Ala gul and Alma gul wetlands is essential [34]. Thus, the continuous monitoring of the concentration of heavy metals in these wetlands has got health significance. The current study indicated that the order of heavy metals in both wetlands was Cd <Pb <Cu <Zn. That is, zinc had the maximum and cadmium had the minimum concentration in the muscle tissues of carp in both sites.

Like the study of Adel et al., the order of heavy metals' concentration in pike (Esox Lucius Linnaeus, 1758) in four Anzali Wetlands was determined [4]. It is worth noting that some certain factors such as fish species, sampling site, season and diet all influence fish metabolism and may change the order of concentration [35, 36]. The maximum and the minimum concentration of zinc in the muscle tissues of carp was observed in Ala gul wetland (Table 1). The mean zinc concentration in both wetlands of Ala gul and Alma gul was significantly more than the maximum limit (50 mg/kg) proposed by the organization of Food and Agriculture. Therefore, zinc can endanger the health of carp consumers [37]. The health limit of copper in the muscle tissues of carp is 30.0 mg/kg for human health [38] . In both Ala gul and Alma gul wetlands, the concentration of copper in common carp wad significantly less than the standard limit; thus, the intake of carp with this amount of copper cannot endanger human health. Cadmium with a concentration of 1 mg/kg causes chronic and severe toxicity. It is also potentially more lethal than other metals [39,40] . The maximum concentration of cadmium belonged to Ala gul wetland (0.140 mg/g) and its minimum amount was related to both Ala gul and Alma gul wetlands (ND).

The mean concentration of cadmium in Ala gul wetland was higher than the standard limit proposed by European committee (0.02 mg/g) while it was lower in Alma gul wetland [41]. The standard limit of cadmium was 5.5 and 1 mg/g according to western Australian and Spanish authorities respectively [42, 43]. The high concentration of cadmium can increase bioaccumulation and human health risk in the long run [44]. The range of lead's concentration in both Ala gul and Alma gul wetlands was 21.8-ND mg/kg (Table 1 & 2). The maximum tolerable limit of lead in the muscle tissues of carp is 0.3 mg/g according to WHO and FAO [45]. The mean concentration of lead in both wetlands was significantly higher than the guidelines (P-values<0.05). Lead and cadmium are heavy metals that known to be toxic. These metals take a long time to be absorbed and can endanger human health even in low concentrations [46, 47]. The reason contributing to the existence of lead and cadmium in common carp in both intended wetlands is the increase in industrial and agricultural activities (pesticides and chemical fertilizers) following the contamination of wetlands. Karadede et al. studied the heavy metals in water, sediments and fish in Ataturk dam in Turkey based on Atomic Absorption Spectroscopy (AAS). They found that the concentration of Cu, Ni, Ci, Pb and Zn was lower than the standard limit. These findings were not in line with the results of the present study [28]. The difference in the results lies in the difference in the site, fish species and concentration of heavy metals under study [48]. As shown in Table 3, the concentration of zinc, cadmium and copper was significantly higher in Ala gul than Alma gul wetland indicating that the source of emission is more in Ala gul wetland. Table 4 shows the estimation of THQ for heavy metals in common carp in both Ala gul and Alma gul wetlands. Except for the Lead's THQ for children, the THQ of none of the heavy metals was less than 1 for adults and children. The results indicated that the consumer population of common carp were in an immune zone except for the THQ od lead for children. Like the present study, several research has been done to determine the THQ of heavy metals in other areas which recommends valuable suggestions for controlling the human health risk caused by seafood intake [4, 49, 50] .

Table 4. Estimation of THQ of heavy metals as a result of carp intake.

Adult	Ala gol	Alma gol	THQ
Cd	0.011	0.002	0.007
Pb	0.013	0.409	0.211
Cu	0.074	0.074	0.074
Zn	0.151	0.004	0.078
Children	Ala gol	Alma gol	THQ
Cd	0.028	0.005	0.017
Pb	1.509	1.127	1.318
Cu	0.205	0.205	0.205
Zn	0.417	0.011	0.214

The value of THQ for cadmium, lead, copper and zinc was 2.4, 6.2, 2.85 and 3 times higher for children than adults, respectively. Consequently, the order of THQ for adults and children was Cd<Cu<Zn<Pb. As shown in Figure 1, the maximum and minimum proportion of THQ belonged to lead and cadmium.

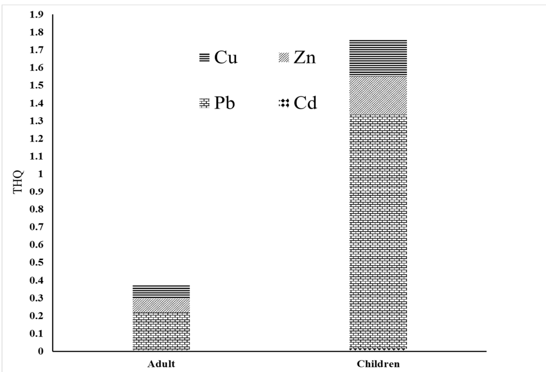

Figure 1. Values of THQ of heavy metals in common carp for children and adults

CONCLUSION

Environmental pollution has become a global problem and heavy metals are considered as one of the most contaminants due to their bioaccumulation nature. When the concentration of heavy metals is higher than the threshold limit, they can be toxic. The results of the present study showed that unlike cadmium, the concentration of other heavy metals including lead, copper and zinc was higher than the standard limit in the muscle tissues of common carp in Ala gul and Alma gul wetlands. The value of THQ was larger than 1 for children; therefore, they are in the unsafe zone. Regarding the value of THQ for four heavy metals of lead, cadmium, copper and zinc, the consumption of common carp caught from Ala gul and Alma gul wetlands does not endanger the health of adults.

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DECLARATION OF INTEREST

We declare that we have no conflicts of interest.

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