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Research Article

Risk Assessment of Some Heavy Metals in Two Fish Species Oreochromis Niloticus and Clarias Gariepinus from Sharqia Province, Egypt

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Abstract

Accumulation of heavy metals in fish is considered a critical problem for human health. Therefore, the study aimed to quantify the concentrations of iron (Fe), zinc (Zn), manganese (Mn), and lead (Pb) in *Oreochromis niloticus* and *Clarias gariepinus* from two areas in Al Sharqia governorate, Egypt, from September 2017 to August 2018. A human health risk assessment was conducted to evaluate the potential hazards associated with fish consumption. Metals concentrations (mg/kg dry weight) in muscles of catfish ranged 1.88-221.26 for Fe; 1.78-19.77 for Zn; BDL-238.51 for Mn; BDL-22.75 for Pb. In muscles of tilapia fish metals concentrations ranged 7.96-149.10 for Fe; 1.20-19.77 for Zn; BDL-230.82 for Mn; BDL-25.93 for Pb. Pb had Hazard quotients (HQs) which indicated potential health risks to tilapia consumers at both study areas and catfish consumers at the Faqous area. Fishermen were at higher risk compared to the other consumers.

INTRODUCTION

Heavy metals are one of the major contaminants affecting aquatic environments [1-3]. Some heavy metals are essential for biochemical and physiological processes in living organisms such as copper (Cu), zinc (Zn), iron (Fe), and nickel (Ni) [4,5]. However, these essential metals become toxic at high concentrations [6, 7]. Other heavy metals such as lead (Pb), mercury (Hg), and cadmium (Cd) are non-essential and toxic].

Contamination of aquatic environments with heavy metals has attracted global attention due to their persistence, non-biodegradability, toxicity, and bioaccumulation in aquatic organisms [7-11]. Fish and other aquatic organisms can accumulate heavy metals up to 10^6 times the concentrations detected in the water column through food, gills, oral consumption of water, and absorption through the skin surface [12] . In most cases, the accumulation of heavy metals in fish tissues does not affect mortality rates; however, it is important to monitor heavy metals concentration in fish for consumers' safety [7, 12].

Fish is nutritious, rich in low-fat protein, omega-3 fatty acids, vitamin D, and trace elements such as selenium and iodine [14, 15]. In Egypt fish have been used as a cheap source of animal protein and as a substitute for red meat [16,17]. This has contributed to the increase in fish consumption by 27.5 % in the past 15 years [16,17]. *Oreochromis niloticus* (Tilapia) and *Clarias gariepinus* (African catfish) are two of the most widely consumed fish species in Egypt [18,19]. They are omnivorous species so they can bioaccumulate high levels of metals [20]. Common sources of *O. niloticus* and *C. gariepinus* include aquaculture and fish caught from the river Nile as well as the associated irrigation canals. Fish inhabiting the river Nile and its associated irrigation canals might be at risk of accumulating heavy metals as several studies have reported the contamination of these water bodies [8,18,21,22]. Therefore, assessing the levels of heavy metals in these fish species and evaluating the health risk associated with their consumption is essential.

This study aimed to: (1) determine the concentration of Fe, Cu, Zn, Pb, and manganese (Mn) in the two fish species *O. niloticus* and *C. gariepinus* caught from two rivers Nile associated irrigation canals namely; Muweis and Faqus Canals in Al Sharqia governorate, Egypt, (2) to assess the potential health risks associated with the consumption of these two fish species.

With the consumption of these two fish species among fishermen which are considered a high consuming group and other residents of both sites.

MATERIALS AND METHODS

Study area and fish sampling

Fish samples (*O. nilotica* and *C. gariepinus*) were captured by local fishermen from two freshwater canals branched from the Damietta Nile branch in Al Sharqia governorate located in the eastern part of the Nile Delta in Egypt. These two canals were: (1) Muweis canal in Zagazig city, which receives wastewater from Miser oil and soap factory, and (2) San El-Hagar canal in Faqus city which receives domestic and agricultural wastewater. Al Sharqia governorate is the third most populous city in Egypt with around 77% of the population living in rural areas. The *O. niloticus* and *C. gariepinus* are the most commonly consumed species in the study area [18]. Samples were collected monthly from September 2017 to August 2018.

Heavy metal determination

Fish samples were stored in a deep freezer at -5 °C before processing. The fishes were defrosted for 2 hours, thereafter, gutted and descaled. Whole gutted samples were oven-dried overnight at 115 °C. The dry mass (g) was measured and the dried muscle tissues were used to determine the concentrations of iron, copper, lead, zinc, and manganese.

Muscle tissue (0.5 g) was placed into digestion flasks; 10 mL of ultrapure concentrated HNO_3 was added and left overnight. Then, the samples (n=12) were heated on a hot plate at 70 °C for 8 hours. After the solutions were cooled, 4 mL of H_2O_2 (30%) was added and heated again at 70 °C for 4 hours. The solution was diluted with deionized water to 25 mL (Ponnusamy et al. 2014). For quality assurance, a blank and two certified materials were included in every digestion. Heavy metals were determined by atomic absorption spectrophotometer (Varian AA-7000) using external calibration. A standard stock solution of 1000 mg/L multi-element (Merck, Germany) was used for instrument standardization. Each sample was analyzed three times and the variation coefficients were determined and accepted if they were below 5%. Analytical results of the quality control samples showed satisfactory metal determination performance within the range of 95-100% certified values. No drift was observed during the analysis as inferred by the results of the blank and drift standards. All reagents were of analytical grade; glassware was soaked in 10% nitric acid and subsequently washed with deionized water before use to avoid contamination of metal.

Exposure assessment

Estimated daily intake (EDI) of heavy metals: EDIs of the studied heavy metals were calculated using the average concentrations of heavy metals in the fishes' muscle, daily consumption, and average body weight of 70 kg using the following equation [23]:

EDI (µg /kg b.w/day) = [metals concentrations (µg/g wet weight) x fish consumption (g/day)] / body weight (kg). The metal concentrations (µ/g dry weight) were converted into µg/g fresh weight using factors of 0.36 and 0.47 for tilapia and catfish, respectively.

Fish consumption data were collected utilizing a quantitative food frequency questionnaire. A total of 150 residents' from both study areas were asked for their weekly consumption of the two types of fishes. The participants comprised 25 fishermen and 125 non-fisherman. The consumption rate for each fish type was estimated for fishermen and non-fishermen separately.

Risk characterization: Hazard Quotient (HQ) has been used to describe non-carcinogenic health effects arising from heavy metals toxicity by fish consumption according to the following formula: HQ= EDI/ RfD

Where, EDI is the estimated daily intake of metals through fish consumption, and RfD is the oral reference dose, which is an estimate of a daily exposure that is unlikely to cause appreciable risk or deleterious effects on human health [23].

To assess the risk associated with exposure to a mixture of heavy metal the hazard index (HI) was calculated as the sum of the individual HQs for each heavy metal according to the following equation:

HI= ΣHQs = HQ Fe + HQ Mn + HQ Pb + HQ Zn

The HQ or HI of less than or equal to 1 indicates low risk for human health and the risk increases when their values exceed 1 [23]. Exposure to more than two pollutants could cause an additive/interactive effect.

Data Analysis: A package of statistical programs for paleontological statistics (PAST) (2010) was used in data analysis. The independent samples t-test was used to compare the means of heavy metal concentrations of samples sites 1 and 2.

Result and discussion

The daily consumptions of Tilapia and Catfish among fishermen and other study participants from both study areas Zagazig and Faqous are presented in Table 1. The consumption of Tilapia was higher than that of the Catfish in the two areas (Table 1). Fishermen consumed more Tilapia and Catfish compared to the other study participants in the two study areas. Tilapia consumption was 2.4 and 1.3 times higher among fishermen from Faqous and Zagazig, respectively, compared to the other study participants from the same areas. For catfish, consumption was 4.2 and 4.1 times higher among fishermen in Faqous and Zagazig, respectively, compared to the other study participants from the same areas.

Table 2 presents the concentrations of Fe, Zn, Mn, and Pb (µg/g, dry weight) in the muscles of tilapia and catfish collected from the two sites at Zagazig and Faqous, Sharkia governorate, Egypt. The highest concentrations of Fe and Mn were found in catfish collected from Zagazig and Faqous, respectively. The mean concentration of Zn in the two fish species ranged from 2.57 to 10.20 µg/g dry weight, the highest concentration was found in tilapia collected from Faqous. The highest concentrations of Pb were detected in tilapia collected from Zagazig followed by catfish from Faqous with concentrations of 5.90 and 5.86 µg/g dry weights, respectively. These high concentrations of Pb are likely related to the discharge of industrial wastewater in the Muweis canal at Zagazig and the discharge of domestic wastewater in the San El-Hagar canal in Faqus.

No relation was observed between the types of heavy metals and their accumulation levels in the two studied fish species. This

Table 1: Daily consumption of tilapia and catfish among fishermen and other study participants from both study areas Zagazig and Faqous, Egypt.

	Zagazig			Faqous		
	Percent %	Tilapia consumption (g/day)	Catfish consumption (g/day)	Percent %	Tilapia consumption (g/day)	Catfish consumption (g/day)
Fishermen	6.0	76.7	38.4	10.7	118.8	42.3
Other	94.0	57.1	9.3	89.3	49.50	10.0

Table 2: Concentrations of Fe, Zn, Mn, and Pb (μg/g, dry weight) in the muscles of Tilapia and Catfish collected from two sites (Zagazig and Faqous) during the time 2017-2018.

	Zagazig			Faqous	
Heavy metals	Tilapia	Catfish		Tilapia[c]	Catfish
Fe	53.6 ± 12.3	70.7 ± 16.7		46.5± 9.57	41.89 ± 6.29
Zn	2.90 ± 0.30	2.57 ± 0.17		10.2 ± 1.36	5.08 ± 0.79
Mn	42.8 ± 18.3	6.02 ± 0.95		43.6 ± 22.3	62.63 ± 28.45
Pb	5.90 ± 2.50	0.60 ± 0.32		4.80 ± 2.48	5.86 ± 2.89

Note: Data presented as mean ± standard error where [a] n = 15, [b] n = 18, [c] n = 15, [d] n = 11.

Table 3: Estimated daily intake of Fe, Zn, Mn, and Pb (μg/kg BW/day) based on the consumption of two types of fish in two different areas.

	Zagazig				Faqous				RfD (μg/kg BW/day)	Reference
	Tilapia		Catfish		Tilapia		Catfish			
Heavy metals	Fishermen High consumption	others	Fishermen High consumption	others	Fishermen High consumption	others	Fishermen High consumption	others		USEPA (regional screening level
Fe	21.13	15.73	18.24	4.42	28.39	11.83	11.47	4.45	0.7×10^3	
Zn	1.12	0.84	0.66	0.16	6.23	2.59	1.39	0.58	0.3×10^3	(EPA, 1991)
Mn	16.89	12.57	1.55	0.38	26.63	11.10	17.57	16.01	0.014×10^3	(EPA, 1995)
Pb	2.33	1.74	0.15	0.04	2.92	1.22	1.66	1.53	0.5	(WHO, 2014)

*According to the USEPA 2019, it is difficult to establish a RfD for Pb that would be considered health-protective and the WHO (2011) assigned an exposure margin of 0.5 (μg/kg BW/day).

could be related to the variability in water metals concentrations in the two study areas in our previous study [18]. Furthermore, the age difference, body weight, feeding habits, and water intake may have affected metal bioaccumulation in the two studied fish species [10,24,25].

Fe, Zn, and Mn are important for the biochemical mechanisms in fish and their levels were comparable to other studies. In Egypt, [26] reported average concentrations of Fe and Zn of 50.3 ± 3.0 and 7.2 ± 1.9 mg/kg dry wt., respectively, in tilapia collected from El-Max Fish Farm. Likewise, tilapia collected from lake Edku had concentrations of 18.8 ± 3.3, 17.7 ± 12.4, and 2.6 ± 2.4 for Fe, Zn, and Mn, respectively [27]. In comparison to studies from other parts of the world, Leung et al. [28] reported that concentrations of Zn in catfish and tilapia from China ranged from 0.01 to 38.2 mg/kg wet weight (ww) as well as Mn concentrations ranged from 1.2 to 9.3 mg/kg ww. Mn concentrations were 0.76-11.23 mg/g ww in fish collected from River Chenab in Pakistan resembled those in the current study. However, Zn concentrations (15.5-88.3mg/g, ww) were higher than the ones reported in the study [29].

In the current study, Pb concentrations were higher than 0.3 μg/g which is the maximum permissible concentration of Pb in fish established by the Codex Alimentarius Commission [30]. Also, Pb levels found in this study are higher than those recorded in other Egyptian studies [17,19], Turkey [31], Sweden [32], and Pakistan [29]. However, two studies from Egypt showed higher concentrations of Pb in tilapia collected from El-Max Fish Farm (10.45±6 mg/kg dry wt.) [26] and Damietta Branch of River Nile (14.0 ± 1mg/kg dry wt.) [33].

Table 3 shows the EDIs of Fe, Zn, Mn, and Pb (μg/kg BW/day) for the low and high fish consumption groups as well as the oral reference doses (RfD) values for the elements studied. Fe, Zn, and Mn are essential elements that play vital roles in metabolic and physiological processes in the human body, but the excess intake of these elements may pose a health risk [6,7]. In the present study, the EDIs of Fe, Zn, and Mn were below the recommended RfD for both the high consumption group and the general population in both study areas (Table 3). This indicated that the dietary intakes of Fe, Zn, and Mn through fish consumption in the two study areas are safe and do not pose toxic or harmful effects.

Pb is a toxic metal that causes neurodevelopmental effects, an increase in blood pressure, cardiovascular diseases, chronic kidney diseases and is classified as a probable human carcinogen by the EFSA and the U.S. EPA [34,35]. According to the WHO and U.S. EPA, a provisional tolerable weekly intake of Pb that would be considered health security cannot be identified [35,36]. In this context, the EFSA identified children's developmental neurotoxicity and cardiovascular effects and adult nephrotoxicity as critical risk assessment effects [34]. The assigned margins of exposure for Pb dietary intake according to its neurodevelopmental toxicity, cardiovascular and kidney effects were; 0.5, 1.50, and 0.63 μg/kg b.w. per day, respectively [34]. In the current study, the EDIs for Pb ranged from 0.04 to 2.33 μg/kg/day in the two studied fish species, which in most cases exceeded all margins of exposure established by EFSA (Table 3). This indicated that fish consumers in this study are at risk from Pb poisoning and might be at higher risk if they are exposed to Pb from other sources such as other food commodities, water, air, dust, and soil.

HQ is a parameter used to quantify the potential health threat associated with long-term chemical exposure [23]. The values of the HQs and HIs for the studied heavy metals based on the consumption of tilapia and catfish in the two study areas are shown in (Table 4). HQs for Fe, Zn, and Mn were all below 1 for the low and high consumers indicating that their intakes through tilapia and catfish consumption do not pose a health risk.

To calculate the HQ for Pb, the margin of exposure for neurodevelopmental toxicity was used as RfD as this value is considered protective for the other adverse effects of Pb [34]. HQs for Pb were greater than one for tilapia at both study areas and for catfish at Faqous area which indicated that consumers are subjected to health risk. The HQ was less than one for Pb in catfish collected from the Zagazig area for both consumer groups; this could be attributed to the lower consumption rate of catfish in Zagazig compared to Faqous. However, having Pb levels below one does not mean that consumers are exposed to no risk of experiencing toxicity effects as Pb has no established maximum safety limit [35,36].

The HI was calculated to estimate the potential health risk associated with exposure to all the studied heavy metals through tilapia and catfish consumption. Fishermen represented the high consumer group; they were at higher risk from tilapia consumption as the HIs were 4.82 and 6.10 for fishermen from Zagazig and Faqous, respectively. However, considering the high HQ values of Pb, it could be concluded that Pb was the major heavy metal giving rise to the high HI values. The HI for Zagazig study participants (low and high consumers) from catfish consumption was less than one. This indicated that the consumption rates of catfish for this study area did not pose a health risk.

CONCLUSION

Levels of Fe, Zn, and Mn in the muscles of both fish species were within the safe limits set by the Codex Alimentarius Commission [30]. In contrast, the levels of Pb in the muscles were up to 19.6 times higher than the levels set by the Codex Alimentarius Commission. Hazard quotients (HQs) for Fe, Zn, and Mn were less than 1 for both fish species based on their consumption patterns among study participants, which inferred that these metals do not pose harmful health effects. HQs for Pb were greater than one for tilapia at both study areas and for catfish at Faqous area which indicated that consumers are subjected to health risk. Fishermen are considered at more health risk because they consume more fish than other study participants. This study recommends regular monitoring of heavy metals in fish caught from both study areas to potential hazards to human health.

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