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Original Research

Health Risk Assessment of Some Heavy Metal Residues in Some Fish Retailed in Sharkia Governorate, Egypt

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Abstract

The aim of this study was to explore the residual concentrations of four heavy metals; mercury (Hg), arsenic (As), lead (Pb), and cadmium (Cd) in Mullet, Brush tooth lizard, and Coral fish collected from fish markets in Zagazig city, Sharkia Governorate, Egypt. Besides, metal's dietary intake and public health risk were calculated. Atomic absorption spectrometry (AAS) was used to analyze these toxic heavy metals in 60 fish samples of Mullet, Brush tooth lizard, and Coral (20 of each). The concentration (mg/ kg) of Hg were the same value (0.02±0.01) in Mullet, Brush tooth lizard, and Coral fish. However, for As were 0.35±0.10, 0.32±0.10, and 0.30±0.08, respectively; meanwhile, Pb concentration was 0.09±0.04 for both Mullet and Brush tooth lizard and it was 0.10±0.04 for Coral fish Cd concentrations were 0.02±0.01 in all examined fish. The estimated daily intake (EDI) of Hg, As, Pb, and Cd were 0.011, 0.191, , 0.049 and 0.011µg/ kg BW/ day , respectively in Mullet; 0.011, 0.174, 0.049, and 0.011 µg/ kg BW/ day in, respectively Brush tooth lizard; 0.011, 0.163, 0.054, and 0.011 µg/ kg BW/ day , respectively in Coral fish, that was below the tolerable daily intake (TDI) recommended by Joint Expert Committee on Food Additives (JECFA). The Target hazard quotient (THQ) of Hg, As, Pb, and Cd from consumption of examined fish was less than one which means that there was no hazard from consumption of these fish.

KEYWORDS
Fish, Heavy metals, Estimated Daily Intake, Tolerable Daily Intake, Target Hazard Quotient

INTRODUCTION

Fish is an important source of nutrition for human beings because it has nutrients as proteins, minerals, essential fatty acids, particularly omega 3, vitamins, and is frequently low in fat, which has drawn interest from consumers due to its benefits for health (Morshdy *et al*., 2022). Because, fish is at the peak of the aquatic food chain, it is able to accumulate heavy metals from their diet, water, or sludge in the environment, making them a useful biological measure for the assessment of metal at different levels (Aljohani *et al*., 2023). Heavy metals are one of the most contaminants in water that can pollute it, and their presence can have a negative impact on aquatic life and humans worldwide (Atia *et al*., 2018). Cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) are among the toxic metals that pose serious health risks. Exposure to such metals in humans can result in a variety of acute and chronic negative consequences, including cancer and neurotoxic effects (Saei-Dehkordi and Fallah, 2011). Cadmium has been linked to numerous serious disorders of the kidney, liver, bone, and respiratory systems. Additionally, there was a positive correlation between Cd and teratogenic, mutagenic, and carcinogenic effects (Morshdy *et al*., 2019). The primary consequences of lead involve destruction to the gastrointestinal tract, nervous system impairment, renal failure, high blood pressure, and reproductive disorders (Rubio *et al*., 2005). Furthermore, Pb exhibited considerable toxicity in human liver (HepG2) cells at food-relevant levels (Darwish *et al*., 2016). Arsenic exposure can have a negative impact on a variety of body functions, in particular the brain and nervous system, which may result in changes in neurological functioning and cognitive deficits (De la Ossa *et al*., 2023). Besides, AS is considered a class 1 carcinogen (IARC, 2012). Human exposure to metals such as mercury has a negative effect on biological macromolecules as deoxyribonucleic acid (DNA), which results in cell deterioration and the development of oxidative stress (Valko *et al*., 2005).

Because humans are at the top of the food chain, it is crucial to assess their dietary exposure to such pollutants (El Bayomi *et al*., 2018). Furthermore, evaluation of human health risk from the consumption of fish contaminated with heavy metals- is an important aspect in food hygiene. As a result, the purpose of this study was to estimate the concentrations of some heavy metals (Hg, As, Pb, and Cd) in fish samples (Mullet, Brush tooth lizard, and Coral) collected from fish markets in Zagazig city, Sharkia Governorate, Egypt, and to assess the risks related to their consumption. Additionally, metal-metal correlations were investigated to determine inter-species differences in metal accumulation pattern.

MATERIALS AND METHODS

Collection of samples

A total of 60 samples from Mullet, Bruch tooth Lizard, and Coral (20 samples each) were randomly collected from fish mar-

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kets in Zagazig city, Sharkia Governorate, Egypt. Ten grams from each sample were identified and wrapped separately in polyethylene bags, then transported to the Central Laboratory, Faculty of Veterinary Medicine, Zagazig university in an icebox, then prepared for metals measurements.

Digestion and analysis of samples

Five ml of an acid mixture, consisting of 3 ml of nitric acid (HNO_3) and 2 ml of perchloric acid (HCLO4), were added to 1 g of each fish muscle sample (Darwish *et al*., 2015). The solutions that were produced were then tested for the presence of cadmium, lead, and total arsenic. A blank solution was created to test for any metal trace in the deionized water or the acids used for dilution and sample digestion. This process was established in accordance with Diaz *et al*. (1994) for the determination of mercury at minimal temperature due to mercury volatilization that occurred at temperature below 100°C.

Samples Analysis

An atomic absorption spectrometer (Perkin Elmer model specta-AA10, USA) was used to determine the presence of heavy metals in the samples. While, Hg was determined using the cold vapor technique using a flame atomic absorption spectrophotometer set with a mercury hydride system. Cd, Pb, and As were all analyzed using an air/acetylene flow (5.5/1.11ml) flame atomic absorption spectrophotometer (AAS). The findings obtained were displayed in terms of µg/g wet weight (mg/ kg), and they were evaluated in relation to ES-7136 (Egyptian Standard, 2010).

Estimated Daily Intake (EDI)

The Egyptian population's dietary daily intake of the measured metals due to fish consumption was described using the equation provided by the United States Environmental Protection Agency (USEPA, 2010): EDI (µg/kg/day) = Cm × FIR/BW, where Cm is the metal's concentration in the sample (mg/kg ww); FIR is Egypt's fish consumption rate, which has been determined as 48.57 g/day (FAO, 2003); and BW is the average adult weight in Egypt, which was determined to be 70 kg

Health risk assessment

The non-cancer risk (Hazard Quotient) of heavy metals for Egyptian fish consumers was calculated by the United States Environmental Protection Agency (USEPA, 1989) using the following equation: HQ = EDI / RFD, where HQ represents the hazard quotient, EDI indicates the estimated daily intake of each metal, and RFD stands for the recommended reference dose (0.001, 0.003, 0.004, and 0.0016 mg/kg Bw/day for Cd, As, Pb, and Hg, respectively) (USEPA, 2000).

THQ value is estimated using the following equation: THQ=(EF x ED x FIR x C)/(RFD x BW x AT) x10-3 (Yi *et al*., 2011). THQ is the target hazard quotient; EF is exposure frequency (365 days/year); ED is the exposure duration (70 years, average lifetime); FIR is the food ingestion rate (g/day); C is the heavy metal concentration in meat (µg/g); RfD is the oral reference dose (mg/kg/ day); BW is the average adult body weight (70 kg); and AT is the averaging exposure time (365 days/ year × number of exposure years, assuming 70 years).

A hazard index (HI) was also calculated for assessing the risk of combined contaminants using the following formula: HI = ∑ HQi, where i stands for each metal, A HQ and/or HI of greater than one means there is a potential risk to human health, whereas a result of one or less reveals that there is no risk of adverse health consequences.

Statistical analysis

Heavy metal concentrations were recorded as means±standard errors. The results were analyzed using one-way analysis of variance (ANOVA) (SPSS version 22). Duncan's test was applied for statistical analysis. T-test was used to compare metals in the samples. The value of P < 0.05 was used to represent statistically significant differences. Correlation analyses among the analyzed metals were performed using the JMP program (SAS Institute, Cary, NC, USA).

RESULTS

The results in Table 1 declared the residual concentrations of heavy metals (Hg, As, Pb, and Cd) in Mullet, Brush tooth lizard, and Coral fish obtained from different fish markets in Zagazig city, Sharkia Governorate, Egypt. In Mullet, the average of investigated heavy metals residual concentrations (mg/kg) were 0.02±0.01, 0.35±0.10, 0.09±0.04, and 0.02±0.01, respectively. While, in Brush tooth lizard, the values (mg/kg) were 0.02±0.01, 0.32±0.10, 0.09±0.04, and 0.02±0.01, respectively. The recorded results (mg/kg) in Coral fish were 0.02±0.01, 0.30±0.08, 0.10±0.04, and 0.02±0.01.

The EDI of Hg, As, Pb, and Cd was 0.011, 0.191, 0.049, and 0.011, respectively in Mullet. While, it was 0.011, 0.174, 0.049, and 0.011, respectively in Brush tooth lizard and 0.011, 0.163, 0.054, and 0.011, respectively in coral fish (Table 2).

The THQ for Hg, As, Pb, and Cd due to the consumption of investigated fish samples was listed in Table 3. The THQ values were 0.0068, 0.635, 0.0122, and 0.01089, in Mullet, respectively, 0.0068, 0.581, 0.0122, and 0.01089 in Brush tooth lizard, respectively, and 0.0068, 0.544, 0.0136, 0.01089 in Coral, respectively. The hazard index (HI) of the metals screened in fish samples was < 1

Spearman's coefficient factors were determined to assess the inter-metal`s propensity to accumulate as cleared in Table 4. Significant negative correlations were observed between mercury and lead (r=0.961*-, P = 0.039) in Bruch tooth lizard; mercury-cadmium (r=-1.000**, P = 0.029) in mullet; arsenic- lead (r =

Table 1. Statistical analytical results of Hg, As, Pb, and Cd residues (mg/ kg) in the examined fish samples (No=10 of each).

Samples	Mullet			Brush tooth lizard			Coral		
	Mini.	Max.	Mean±S.E	Mini.	Max.	Mean±S.E	Mini.	Max.	Mean±S.E
Hg	0.01	0.03	$0.02±0.01^b$	0.01	0.04	$0.02±0.01^b$	0.01	0.04	$0.02±0.01^b$
As	0.17	0.54	$0.35±0.10^a$	0.14	0.5	$0.32±0.10^a$	0.16	0.43	$0.30±0.08^a$
Pb	0.02	0.15	$0.09±0.04^b$	0.02	0.17	$0.09±0.04^b$	0.02	0.17	$0.10±0.04^b$
Cd	0.01	0.03	$0.02±0.01^b$	0.01	0.04	$0.02±0.01^b$	0.01	0.05	$0.02±0.01^b$

Means within the same column of each sample carrying different subscripted letters are significantly different (p < 0.05) based on Duncan's multiple comparisons. mg/ kg: Part per million, NO: Number, Mini.: Minimum, Max.: Maximum, S.E: Standard error of mean. Hg: Mercury, As: Arsenic, Pb: lead, Cd: Cadmium.

-0.986*, p = 0.014) in mullet, in Bruch tooth Lizard (r = --0.999**, p = 0.001), and in Coral (r = 0.991, p = 0.009). A Significant strong positive correlation was observed in Coral fish between mercury and arsenic (r = -0.975 *, p = 0.025).

DISCUSSION

Contaminations of fish with metals like arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd) can pass through the food chain to humans, and may have adverse effects on humans and endangering their health (Ali *et al*., 2022; Mielcarek *et al*., 2022). Fish pick up metals from food, water, and sediment, the amount of metals that accumulate in fish tissues varies depending on the amount of metal in the environment, the fish's natural habitat, their feeding habits, their age, trophic status, the length of time they were exposed, as well as the specific metal and the rate at which it was absorbed, deposited, and excreted (Xie *et al*., 2020). In the current study the mean concentration of Hg was 0.02±0.01 mg/ kg in all investigated fish samples (mullet, Brush tooth lizard, and coral). Nearly similar level was identified by Elnabris *et al*. (2013) who recorded Hg concentration of 0.031±0.02 from mullet in Palestine., in addition, Ozuni *et al*. (2023) revealed concentration (mg/kg ww) of 0.052±0.089 from small fish size red mullet and 0.060±0.076 from large fish size red mullet in Tirana, Albania. Furthermore, Girolametti *et al*. (2022) recorded a concentration of 0.20±0.15 mg/ kg ww from muscle tissue of red mullet in Adriatic Sea (Central Mediterranean Sea). Higher concentration of Hg (0.497±0.233 mg/kg ww) was detected by Bošković *et al*. (2023) from red mullet in Montenegro. In addition, a higher level of 0.48±0.09 mg/kg ww was observed in red mullet in Italy by Perugini *et al*. (2014). In brush tooth lizard, higher Hg concentration (0.076±0.036 mg/ kg ww) was articulated by El Bayomi and Mahmoud (2018). In addition, higher Hg level of 0.54±0.03 mg/kg ww was reported by Morshdy *et al*. (2007).The variance in results may be explained by the fact that fish's capacity to accumulate heavy metals depends on environmental requirements, metabolic processes, and the level of pollution in sediment, water, and food (Burger and Gochfeld, 2005). All the examined fish samples (100%) were within mercury permissible limit of EOS NO 7136 (Egyptian Standard, 2010).

Arsenic is thought to be a highly toxic metal even in extremely low concentrations, as a result of industrial discharges, coastal areas frequently have high concentrations of toxic elements, which can contaminate marine organisms, particularly fish, with arsenic (Suresh Kumar *et al*., 2013). The mean concentrations level (mg/ kg) of arsenic residues in mullet, brush tooth lizard, and coral were 0.35±0.10, 0.32±0.10, and 0.30±0.08, respectively. Higher results of 0.57±0.05 mg/ kg ww from grey mullet in Manzala Lake, Egypt were screened by Sallam *et al*. (2019). Additionally, Higher level of As (50.34±39.59 mg/kg ww) from red mullet in Turkey was cited by Varol *et al*. (2019). Moreover, Martínez-Gómez *et al*. (2012) noted a higher concentration of As (19.8±1.6 mg/kg ww) from red mullets in Spain. On contrary, lower results of 0.06±0.03 mg/kg ww from red mullet in the south-eastern Mediterranean Sea were demonstrated by Ramon *et al*. (2021). All the examined fish samples (100%) were within arsenic permissible limits of EOS NO 7136 (Egyptian Standard, 2010).

Lead comes into the aquatic system in Egypt primarily through human activity such as mining industries, burning of rice straw in agricultural lands and afterward discharge into lakes and rivers, and domestic and industrial wastes (Morshdy *et al*., 2023). The current study showed that the concentrations level (mg/ kg) for lead residues in mullet, brush tooth lizard, and coral were 0.09±0.04, 0.09±0.04, and 0.10±0.04, respectively. The levels of Pb found in mullet in this study were comparable to those found in mullet collected from Bafa Lake, Turkey (Aydin-Onen *et*

Table 2. Estimated daily intake (EDI) µg/ kg body weight of different metals in comparison to the permissible tolerable daily intake (PTDIs) µg/ kg body weight.

Heavy metals	Mullet EDI	Brush tooth lizard EDI	Coral EDI	Overall EDIs	PTDIs
Hg	0.01	0.01	0.01	0.01	0.57
As	0.19	0.17	0.16	0.18	2.14
Pb	0.05	0.05	0.05	0.05	3.57
Cd	0.01	0.01	0.01	0.01	1

Overall EDIs: ∑ EDI of the three investigated fish; PTDIs: Permissible tolerable daily intake; PTDIs are according to FAO/WHO (2011).

Table 3. Target hazard quotient (THQ) and Hazard index (HI) of different metals from consumption of the examined fish samples.

Samples	Mullet	Brush tooth lizard	Coral	RFD
THQ (Hg)	0.01	0.01	0.01	0.00
THQ (As)	0.64	0.58	0.54	0.00
THQ (Pb)	0.01	0.01	0.01	0.00
THQ (Cd)	0.01	0.01	0.01	0.00
HI	0.67	0.61	0.57	

Table 4. Correlation analysis among the examined metals in the samples.

Metals	Mullet		Bruch tooth Lizard		Coral	
	r	p	r	p	r	p
Hg As	0.943-	0.06	0.95	0.05	0.975*	0.03
Hg Pb	0.88	0.12	0.961*-	0.04	0.93	0.07
Hg Cd	-1.000**	0.03	0.26	0.74	-0.78	0.23
As Pb	-0.986*	0.01	-0.999**	0.00	-0.991**	0.01
As Cd	0.94	0.06	0.45	0.55	-0.60	0.40
Pb Cd	-0.88	0.12	-0.88	0.12	-0.66	0.34

r: spearman's correlation factor; p: probability and values in bold show significant correlation between each two metals.

al. 2015) and in canned fish marketed in Egypt (Morshdy *et al*. 2013). Higher concentrations level of 3.59±1.79 and 8.00±2.91 mg/kg ww were detected by Yilmaz (2005) and Mansour and Sedky (2002) in mullet samples from Turkey and Egypt, respectively. In brush tooth lizard, nearly similar results of 0.16±0.005 and 0.14±0.01 mg/ kg were obtained by Morshdy *et al*. (2007) and Hassan *et al*. (2017) in Egypt, respectively. The exposure to Pb in fish has been linked to impaired gill function as well as the induction of oxidative stress and erythrocyte destruction (Ercal *et al*., 2001). The permissible limits for lead residues in fish as determined by ES No 7136/2010 (ES, 2010) must not exceed 0.1 mg/kg and 50% of all examined samples exceeded the accepted permissible limits of Pb.

Cadmium levels in freshwater are naturally less than 0.1 mg/L. This concentration could be increased by human activities such as the leakage of agricultural and industrial wastewater into aquatic systems (Banerjee and Gupta, 2012). Such high Cd concentrations may get into freshwater fish like tilapia and mullet, where they bioaccumulate and cause multiple pathological conditions and damage to organs (Dang and Wang 2009) . In the present study the level of Cd was 0.02±0.01 mg/ kg for all examined fish (mullet, brush tooth lizard, and coral). The obtained results were in parallel with those obtained by Khemis *et al*. (2017) and Keskin *et al*. (2007) who detected Cd level in mullet with a mean of 0.016±0.013 and 0.012±0.0054 mg /kg ww from Tunisia and Turkey, respectively. A higher level of 0.07±0.00 and 0.35±0.14 mg/ kg were demonstrated by George *et al*. (2023) and Ogan and Bob-Manuel (2022) from mullet fish in Nigeria, respectively. Higher Cd levels may be attributed to being widely used in battery manufacturing, galvanized pipes, solders, and some metal fittings (Taha *et al*., 2019). Regarding brush tooth lizard, Morshdy *et al*. (2007) detected higher level of Cd (0.15±0.01 mg/ kg) in Egypt. According to EOS NO 7136 (Egyptian Standard, 2010), all samples were within the cadmium permissible limit.

Toxic metals found in foods pose a health hazard to consumers and vary depending on the level of the metal in the food and the amount consumed (Bhupander and Mukherjee, 2011). The term 'tolerable intake' is frequently used to describe 'safe' intake levels and can be expressed on a daily (TDI) or weekly (TWI) basis. The maximum level of a contaminant to which an individual can be exposed daily over the path of his lifetime without putting their health at risk is known as the tolerable intake of heavy metals, and it was established by the FAO/WHO (2011). The estimated daily intake (EDI) was calculated in the current study. The mercury PTWI has been set by JECFA as 5 µg/kg Bw/week (equal to 0.71 µg/kg Bw/day), 14.89 µg/kg Bw/week, which is equal to 2.14 µg/kg Bw/day for arsenic, 25 µg/kg Bw/week , which is corresponding to 3.57 µg/kg Bw/day for lead, and 7 µg/kg Bw/week, which is corresponding to 1 µg/kg Bw/day for cadmium. The estimated daily intakes of heavy metals from eating the examined fish samples in the current study lie within PTDIs guidelines and considered safe for consumers. Similar results were obtained by Varol *et al*. (2019) who showed that the estimated daily intakes of As, Cd, and Pb from consumption of mullet fish in Turkey were within the permissible limit. In addition, El Bayomi and Mahmoud (2018) proved that the estimated intakes of Cd, Pb, and Hg from consumption of brush tooth lizard fish in Egypt didn't exceed the permissible level proposed by FAO/WHO (2011).

The possible adverse health effects of heavy metals in fish have to be predicted in order to evaluate the hazard risk to people's health and to determine health levels that are liable to solve environmental issues (El Bayomi *et al*., 2019). The non-carcinogenic dangers associated with consumption of fish were evaluated using the THQs and HI. The target hazard quotient (THQ) of Hg, As, Pb, and Cd from the consumption of investigated fish in the current study was less than one, indicating that there was no risk from eating these fish. In contrast to the current study, Sallam *et al*. (2019) found THQ of Pb and As from the consumption of mullet fish in Manzala lake, Egypt was above 1.0, suggesting that consuming if such fish from Manzala Lake could pose a health risk for Egyptians, particularly those with a high fish consumption. Moreover, Storelli and Barone (2013) found THQ of Hg from the consumption of mullet fish in Italy was above 1 and they concluded that there was a significant health risk.

Hazard index (HI) is the total of the HQs for various substances or exposure pathways. In this study, the HI is assessed by adding the THQ values of all heavy metals investigated (USEPA, 2011). An HI with value < 1 is judged acceptable. The HI values for the four heavy metals identified in the three fish species examined in the present study were less than 1, supposing that these fishes are safe for consumption. The obtained results were in agreement with Mwakalapa *et al*. (2019) who observed that the HI of all metals from mullet fish samples in Tanzania was < 1, suggesting that there was no hazard from consumption of fish under study. On the other hand, Yi and Zhang (2011) detected an HI value > 1 from fish samples in Yangtze River, China, concluding that these samples represent a public health hazard.

In order to study the inter-metal`s tendency of accumulation, Spearman`s coefficient factors were calculated. In agreement with the obtained results, Bakhshalizadeh *et al*. (2022) screened a significant positive relationship between lead and mercury in mullet species from the Southern Caspian Sea. In contrast with the obtained results in the current study, Bachouche *et al*. (2017) demonstrated a significant strong correlation at $P < 0.05$ between mercury and cadmium Hg-Cd (0.6) in mullet fish from Algeria. According to Kükrer *et al*. (2014), a high correlation between two metals means the presence of a single source of pollution. If no correlation between elements is observed, it suggests the metals are not controlled by a single factor (Suresh *et al*., 2011).

CONCLUSION

The current study revealed the presence of heavy metal residues of Hg, As, Pb, and Cd in the investigated fish samples (mullet, brush tooth lizard, and coral) where the level of Hg, As, and Cd was within the permissible limit, while the level of Pb exceeded the permissible limit by a percentage of 50% in the examined samples. The estimated daily intake of metals was below the tolerable daily intake. The THQs of the all metals from fish samples consumption were lower than 1, suggesting that these fish consumption has no public health hazard.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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