Heavy metals (Cd, Cu, Pb and Hg) concentration in some imported frozen fish sold in Sharkia province, Egypt: Risk assessment for the consumers

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Abstract:

The study was conducted to determine the residual concentration of Cd, Cu, Pb and Hg of three imported frozen fish collected from Zagazig City markets, Sharkia province, Egypt, and to estimate the dietary intake of such metals, as well as to assess the potential health risks associated with the consumption of such fish. Therefore, a total of 60 fish samples, 20 each of, Brush tooth lizard fish, Mackerel fish and Horse mackerel fish, were analyzed for the residual concentration of these metals using atomic absorption spectrophotometer (AAS). The obtained results revealed that the mean residual concentrations of Cadmium (Cd) in Brush tooth lizard fish, Mackerel fish and Horse mackerel fish were 0.039±0.009, 0.037±0.04, 0.043±0.006 mg/kg, respectively, while those of Lead (Pb) were 0.70±0.16, 0.40±0.11, 0.75±0.25 mg/kg, respectively. Regarding Mercury (Hg), they were 0.076±0.036, 0.48±0.14, 0.38±0.12 mg/kg, respectively, and Copper (Cu) mean residual concentration accounted for 0.46±0.15, 0.49±0.19, 0.34±0.13 mg/kg, respectively. Some of the investigated fish samples showed higher heavy metals' concentrations of Cd, Pb and Hg that exceed the recommended safety limits outlined by EOS (2010), while all the examined fish samples were 100% within the maximum permissible limits of copper. The total estimated daily intakes (EDI) of Cd, Pb, Hg and Cu were 0.074, 1.17, 0.59 and 0.82 μg/kg bw/day, respectively, that were below the tolerable daily intake (TDI) recommended by the Joint Expert Committee on Food Additives (JECFA). Referring to the potential health risks of investigated fish, it was estimated that the target hazard quotient (THQ) of Hg (1.03) and the total THQs (1.12) of mackerel fish exceeded 1, indicating possible health risks, while the total THQs (TTHQs) of other two fish were lower than 1 as compared to the reference doses. The obtained results give us an alarm that the consumer could be under health risks so in-depth future studies on imported frozen fish in our country is recommended.

Keywords: Frozen fish, Heavy metals, PTDIs, THQ, Health hazards.

1. Introduction

Fish consumption has simultaneously increased with their nutritional and therapeutic importance. Fish is a perishable, high protein food that typically contains all of the essential amino acids, high level of vitamins, minerals and Omega-3 fatty acids which are essential for human life (Fuentes et al., 2009). Hence, Fish could partially share in solving the problem of animal protein shortage that required to the majority of human population, so, chemical quality determination of such fish is essential.

Heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg) are considered the most toxic heavy ones to animals and humans, and the hazards associated with them are numerous and diverse including neurotoxic and carcinogenic effects (ATSDR, 2003; Tokar et al., 2011). It was reported that the presence of Cd, Pb and Hg at low concentrations leads to metabolic disorders that cause adverse health effects such as heart and kidney failure (Howard, 2002). However, some trace elements such as copper (Cu) is essential for enzymatic activity and many biological processes but it becomes toxic at high concentrations (Bryan, 1976).

Fish are used as a biological indicator for metal levels investigation owing to their position at top of the aquatic food chain, and consequently they can accumulate heavy metals from food, water, or sediment in the surrounding environment. (Yılmaz et al., 2007; Zhou et al., 2008). Heavy metals are increasingly being introduced into the aquatic environment through several natural and anthropogenic sources, which include natural weathering of the earth's crust, industrial wastes، urban runoff, sewage effluents, pesticides, fungicides, air pollution fallouts and/or any other disease control agents applied to plants (Ming-Ho, 2005).

The human is mainly exposed for such toxic metals through ingestion of contaminated food and water which accounted for more than 90 % compared to inhalation and other exposure routes (Loutfy et al., 2006). Although toxicity and public health risks of any contaminant are a function of concentration, it is well known that prolonged exposure to these heavy metals at relatively low concentrations can also lead to many health problems (Castro-González and Méndez-Armenta, 2008). Recently, the accumulation of heavy metals in the environment acquired an increasing concern due to the food safety issues and the associated potential public health risks (McLaughlin et al., 1999; Raknuzzaman et al., 2016).

Thus, the purpose of this study was to determine the residual concentration of Cd, Cu, Pb and Hg of three imported frozen fish collected from Zagazig City markets, Sharkia province, Egypt, and to estimate the dietary intake of such metals, as well as to assess the potential health risks associated with the consumption of such fish.

2. Materials and Methods

2.1. Sampling:

A total of 60 imported frozen Brush tooth Lizard fish, Mackerel fish and Horse mackerel fish samples (20 of each) were randomly collected from Zagazig city, Sharkia province, Egypt. The collected fish samples were packed in a cold polyethylene page then transferred to the central laboratory, Faculty of Veterinary Medicine, Zagazig University for further analysis.

2.2. Heavy metals analysis:

The concentration of heavy metals (Cd, Cu, Pb and Hg) was determined with atomic absorption spectrophotometer (AAS) (Per Kin Elmer model (spectra-AA10, USA). Cadmium, copper and lead concentrations were recorded directly from the digital scale of AAS and they were expressed as µg/g wet weight (ppm) to compare them with (EOS, 2010). Briefly, sample digestates were typically prepared by diluted one gram of each sample with 5 ml of acid digestion mixture (3 ml nitric acid (HNO_3) : 2 ml perchloric acid ($HCLO_4$)) (Zantopoulos et al., 1996). Quantitative determination of heavy metals was conducted by "Buck scientific 210VGP Atomic Absorption Spectrophotometer" using air / Acetylene flow

(5.5/1.11/m) flame. Owing to the volatilization of mercury that occurred below 100°C, the determination of mercury (Hg) at minimal temperature was conceded by Diaz et al. (1994).

2.2.1. Estimated Daily Intake (EDI):

It is important to assess the daily intake of metals from fish and to compare it with the total acceptable daily intake (ADI) values set by international organizations for health safety. This can be obtained by using the following equation described by the Human Health Evaluation Manual of US Environmental Protection Agency (USEPA, 2010)**:**

$$EDI = (Cm \times FIR)/BW$$

where Cm is the concentration of the heavy metal in the sample (mg/kg wet weight); FIR=fish ingestion rate is 38.13 g/day (FAO, 2013)**;** BW is the body weight of Egyptian adults, which was estimated at 70 kg. Then compared to tolerable daily intakes (TDIs) (FAO/WHO, 2011).

2.2.2. Target Hazard Quotient (THQ):

Health risk to Egyptian people from frozen fish intake was determined by the Target Hazard Quotient (THQ). That is the ratio among the exposure and the oral reference doses (RfD). The reference dose is the daily exposure of a contaminant estimation to which the population continually exposed along a lifetime without a significant risk (Okoro et al., 2010). The reference oral dose value for Cd, Cu, Pb and Hg is 0.001, 0.04, 0.004 and 0.0003 (mg/kg bw/day), respectively (USEPA, 2000). Human will pose no hazard if the ratio is less than 1 and if the ratio is the same or higher than 1, the people will pose health risk. The Risk assessment was calculated by the following equation (Yi et al., 2011):

$$THQ = \frac{\text{EF x ED x FIR x C}}{\text{RFD x BW x AT}} x10^{-3}$$

Where, THQ is the target hazard quotient, EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years, average duration), FIR is the food ingestion rate (g/day), C is the heavy metal concentration in fish (µg/g); RfD is the oral reference dose (mg/kg/ day), BW is the average body weight of adult (70 kg) and AT is the exposure time average (365 days/ year × number of exposure years, assume 70 years).

2.2.3. Hazard index (HI):

To assess the human health potential risk among more than one metal, the hazard index (HI) has been established by USEPA (1989). The hazard index is the calculation of the hazard quotients as mentioned in the following equation:

$$HI = \Sigma\, TTHQs = THQ\ Cd + THQ\ Cu + THQ\ Pb + THQ\ Hg$$

Where Σ TTHQs is the summation of hazard quotients of all metals and THQ Cd; THQ Cu; THQ Pb and THQ Hg are the hazard quotients for cadmium, copper, lead and mercury, respectively. It is assumed that quantity of adverse hazard will be relatively the sum if multiple metal exposures. When the hazard index increase than 1.0, there is alarm for possible human health risk (Huang et al., 2008).

2.3. Statistical analysis

Statistical differences in concentrations of heavy metals among the different fish analyzed were evaluated by one-way analysis of variance (ANOVA) accompanied with Tukey-Kramer HSD test if necessary. The results were reported as mean values ± standard errors (SEs) and significance was considered at ($p < 0.05$). All the statistical analyses were done using SPSS software package, version 20 (SPSS Inc., Chicago, Ill).

3. Results and Discussion

The contamination of the environment and food with toxic metals has reached unprecedented levels over the past decade and that human exposure to toxic metals has become a major health risk (Yabe et al., 2010). The higher the contamination of heavy metals in the water body, the higher bio-accumulation of heavy metals contained in the network aquatic organisms and this represents a serious threat to humans (Tapia et al., 2012).

Table (1): Statistical analytical results of some heavy metal residues (ppm) in the examined fish samples (No= 60 of each) expressed in mg kg^{-1} WW (mean values ± standard error).

Fish sample	Cadmium	Copper	Lead	Mercury
Brush tooth lizard fish	0.039 ± 0.009^{a}	0.46 ± 0.15^{a}	0.70 ± 0.16^{a}	0.076 ± 0.036^{b}
Mackerel	0.037 ± 0.04^{a}	0.49 ± 0.19^{a}	0.40 ± 0.11^{b}	0.48 ± 0.14^{a}
Horse Mackerel	0.043 ± 0.006^{a}	0.34 ± 0.13^{a}	0.75 ± 0.25^{a}	$0.38 \pm 0.12^{a,b}$

Means on the same column carrying different subscripted letter are significant different ($p<0.05$).

The results obtained in Table (1) showed that the mean concentrations of Cd was 0.039±0.009, 0.037±0.04 and 0.043±0.006 ppm in Brush tooth Lizard fish, Mackerel and Horse mackerel fish, respectively. The cadmium concentration levels were: in Horse mackerel>Brush tooth Lizard fish> Mackerel fish with no significant effect ($p>0.05$). These results were lower than 0.18±0.03 and 0.15±0.01 ppm in Mackerel and Brush tooth Lizard fish, respectively (Morshdy et al., 2007), 0.17±0.08 ppm (Ahmed, 2015) in Mackerel fish and 0.23 ± 0.02 and 0.11 ± 0.01 in group (A), 0.19 ± 0.01 and 0.09 ± 0.01 in group (B) for the examined Mackerel and Brush tooth Lizard fish samples, respectively in Menoufiya governorate (Hassan et al., 2017). However, lower cadmium concentration of 0.027 ±0.003 ppm in canned Mackerel fish (Morshdy et al., 2013). Higher Cd concentration in Mackerel fish (0.057) and lower level in Horse mackerel fish (0.028) were recorded in Saudi Arabia (Gawish and Hosni, 2017).The results in Table (2) illustrated that70%, 90% and 80% of the examined Brush tooth Lizard fish, Mackerel and Horse mackerel fish, respectively were within the accepted permissible limits (not exceeding than 0.05 ppm) recommended by (EOS, 2010) and is considered safe for human consumption.

Table (2): Percentage of samples within or exceeding the maximum permissible limit of heavy metals in examined Fish samples.

Samples		Cadmium	Copper	Lead	Mercury
Brush tooth Lizard fish	Within PL	70%	100%	10%	80%
	Exceed PL	30%	0%	90%	20%
Mackerel	Within PL	90%	100%	40%	40%
	Exceed PL	10%	0%	60%	60%
Horse Mackerel	Within PL	80%	100%	40%	40%
	Exceed PL	20%	0%	60%	60%
PL (EOS.7136, 2010)		0.05	15	0.1	0.2

Copper is acting as an essential element which mostly accumulates in muscle and liver but it may cause chronic toxicity for animals and man when its concentrations increase than the safe permissible limits (Bradl, 2005). The mean copper values in Brush tooth Lizard fish, Mackerel and Horse mackerel fish samples were 0.46±0.15, 0.49±0.19, 0.34±0.13 ppm, respectively (Table 1). The highest concentration levels for copper were recorded in Mackerel fish samples and the lowest concentration levels for copper were recorded in Horse mackerel fish samples. These copper results were lower than 18.8±0.37 and 18.6±0.50 ppm in Mackerel and Brush tooth Lizard fish, respectively (Morshdy et al., 2007)**,** 0.79±0.057ppm in Mackerel fish (Ahmed, 2015)**,** 0.807 and 0.726 in Horse mackerel and Mackerel fish samples (Gawish and Hosni, 2017). However, copper residue in this study was higher than (Morshdy et al., 2013) who found that the mean copper residue level in canned Mackerel fish was 0.08 ± 0.02 ppm. According to copper permissible limit of EOS (2010) **(**15.0 µg/g**)**, all the examined samples (100%) were within the permissible limits and considered safe for human consumption (Table 2).

Higher concentrations of Pb can occur in aquatic organisms close to anthropogenic sources. The mean concentrations level for lead residues in Brush tooth Lizard fish, Mackerel and Horse mackerel fish samples were 0.7±0.16, 0.4±0.11 and 0.75±0.25 ppm (Table 1). The lead concentration level was, Horse mackerel>Brush tooth Lizard fish>Mackerel fish, it was cleared that the fish samples had a significant effect ($P>0.05$) on the lead levels in all the examined samples. Nearly similar results (0.4±0.03) in Mackerel fish collected from in group (A) in Menoufiya governorate (Hassan et al., 2017). However, Lower results (0.128±0.009 and 0.16±0.005 ppm) were recorded by Morshdy et al. (2007) in Mackerel and Brush tooth Lizard fish, respectively, 0.023± 0.01 ppm in the examined canned Mackerel (Morshdy et al., 2013), 0.27±0.02 and 0.14±0.01 in the Mackerel and Brush tooth Lizard fish samples, respectively in Menoufiya governorate (Hassan et al., 2017), 0.197 in Horse mackerel fish and 0.33 in Mackerel samples in Saudi Arabia (Gawish and Hosni, 2017). Meanwhile, higher lead concentration (0.9940±0.05219) in Mackerel fish were obtained by Ahmed (2015). The permissible limits proposed by EOS (2010) for lead residues in meat must be not exceeds 0.1 mg/kg and the majority of examined samples (90%, 60% and 60%) for Brush tooth Lizard fish, Mackerel and Horse mackerel fish samples, respectively, were exceed the permissible limits and considered unsafe for human consumption (Table 2).

The mean values of Hg in Brush tooth Lizard fish, Mackerel and Horse mackerel fish were 0.076±0.036, 0.48±0.14 and 0.38±0.1 ppm (Table 1), the mercury concentration level was, Horse mackerel>Brush tooth Lizard fish>Mackerel fish. Higher Hg levels of 0.54±0.03 and 0.56±0.01 ppm in Brush tooth Lizard fish and Mackerel fish were reported by Morshdy et al. (2007). Lower Hg levels (0.3±0.05 ppm) in Mackerel fish were obtained by Ahmed (2015). It was cleared that the fish samples had a significant effect ($p>0.05$) on the mercury levels in the examined samples (Table 1). The EOS (2010) is put an acceptable limit for mercury residues in meat which must be not exceed than 0.2 µg/g. According to this limit, 20%, 60% and 60% of Brush tooth Lizard fish, Mackerel and Horse mackerel fish, respectively, were exceeding the permissible limit (Table 2).

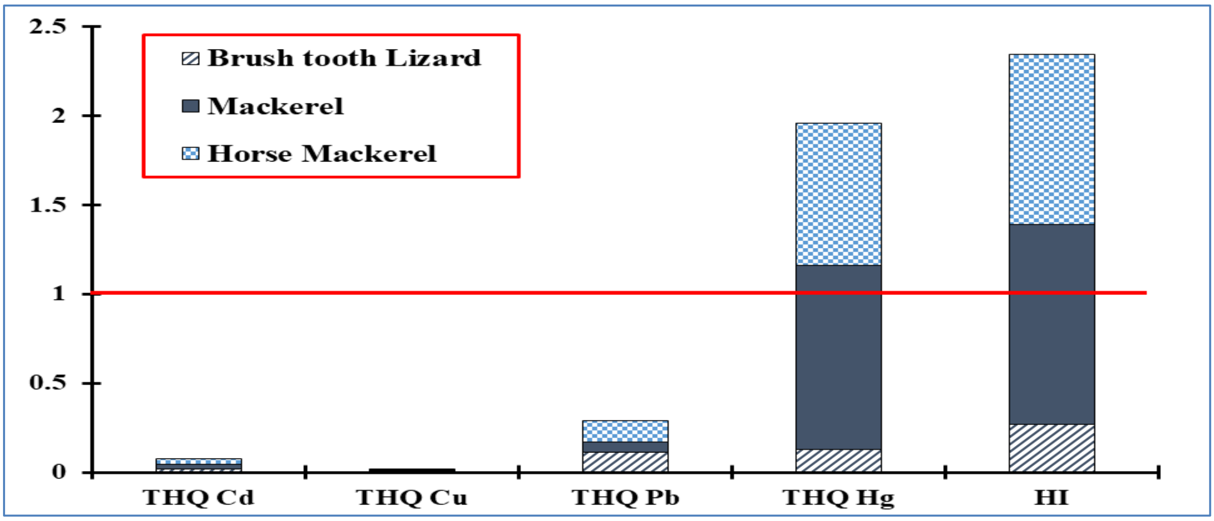
Table (3): Estimated daily intake (µg/kg bw/day) of different metals in the investigated fish samples compared to the permissible tolerable daily intake (µg/kg bw/day).

Fish / Metal	Brush tooth Lizard	Mackerel	Horse Mackerel	Total EDIs	PTDIs
Cadmium	0.02	0.024	0.03	0.074	1
Copper	0.29	0.31	0.22	0.82	500
Lead	0.44	0.25	0.48	1.17	3.57
Mercury	0.04	0.31	0.24	0.59	0.71

Total daily dietary intakes (TDIs) = Σ EDI of the three investigated fish.
PTDIs = Permissible tolerable daily intakes.

Foods having toxic metals represent a toxic hazard for the consumer which is dependent on metal concentration in food and the amount of consumed food (Hajeb and Jinap, 2009). The 'tolerable intake' is commonly used to explain 'safe' intake levels and can express on either a daily basis (TDI) or a weekly basis (TWI). The tolerable intake of heavy metals that set by the FAO/WHO (2011) is the maximum amount of a contaminant to which a person can be exposed per day over a lifetime without an undesirable danger of health. The estimated tolerance daily intake (ETDI) was calculated and presented in Table (3). The cadmium PTWI has been set by JECFA, as 7 µg/kg bw/week (corresponding to 1 µg/kg bw/day), 0.5 mg/kg body weight/day which was equivalent to 500 µg/kg body weight/day for copper, 25 µg/kg bw/week (corresponding to 3.57 µg/kg bw/day) for lead and 5 µg/kg bw/week, which is equivalent to 0 .71 µg/kg bw/day for total mercury. The ETDI in this study were 0.024, 0.27, 0.39 and 0.20 µg/kg bw for cadmium, copper, lead and total mercury daily consumption, respectively (Table 3). The estimated dietary daily intakes of studied heavy metals through consumption of the imported frozen fish in Egypt lie within the recognized PTDI guidelines and considered to be safe to consumers.

For estimation of the possible human health risks of heavy metals in fishes, risk evaluation is the best methods to estimate the hazard risk on human health and to determine the health levels that liable to solve the environmental problem in daily life (Zhang et al., 2012). Figure 1 summarizes the results of THQ for Cd, Cu, Pb and Hg caused by the three fish consumption. The estimated THQ for cadmium was (0.02, 0.024 and 0.03); for copper was (0.007, 0.008 and 0.006), for lead was (0.11, 0.06 and 0.12) and (0.27, 1.03 and 0.80) for mercury in Brush tooth Lizard fish, Mackerel and Horse mackerel fish, respectively. Although THQs of studied heavy metals did not exceed 1 through the consumption of fish, which theoretically representing that people not experience major health risks from the



ingestion of individual metals through fish consumption. It was estimated that THQ of Hg due to Mackerel fish consumption was higher than 1, indicating possible health risks for the consumers. Additionally, the estimated THQs in the current study was similar to Zohra and Habib (2016), who demonstrated that the target quotients for individual metal due to fish consumption from the Mediterranean Sea (Southern coast of Sfax, Tunisia) was decreased in following sequence: Hg > Cd > Pb > Cu. Concerning, TTHQs of the investigated fish species it was obvious that the TTHQ of frozen Mackerel fish was over 1 (1.12) which indicates possible health hazards, meanwhile the TTHQs of other fish species were below 1, recommended by US Environmental Protection Agency (USEPA, 2010).

Figure (1) Target hazard quotient (THQ) and Hazard index (HI) of different metals from consumption of the imported fish, RfDo (mg $kg^{-1}day^{-1}$) = 0.001, 0.04, 0.004 and 0.0003 for Cd, Cu, Pb and Hg, respectively.

4. Conclusion

The study shows that heavy metals of interest though found in measurable quantities are still within safe limit for consumption. The health risks of humans with respect to heavy metals concentration (Cu, Cd, Pb, and Hg) in the imported frozen fish commonly consumed in Zagazig city were evaluated. The estimated dietary daily intakes of analyzed heavy metals due to consumption of such fishes lie within the recommended PTDIs. These intakes might be underestimated as they represent a part of exposure because there are different sources which contribute in elevating the dietary intakes of such metals by the consumers. Regarding the potential health risks of investigated fish, it was estimated that THQ Hg and TTHQs through Mackerel fish consumption exceeds 1, indicating possible health risks, while TTHQs of the other two fish were lower than 1 as compared to the reference doses. Finally, this work may offer valuable record for ongoing research on the frozen fish importation in our country. It is recommended to improve aquaculture-fishing practice as well as permanent monitoring of fish is suggested to minimize the health risks for the consumers.

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الملخص العربى

تركيز المعادن الثقيلة (الكادميوم، الرصاص، الزئبق والنحاس) فى بعض الأسماك المجمدة المسوقة فى محافظة الشرقية - مصر : تقييم المخاطر المحتملة على صحة المستهلكين

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قسم مراقبة الأغذية – كلية الطب البيطرى – جامعة الزقازيق– الزقازيق– مصر

هدفت هذه الدراسة إلي التقييم الكيميائي للأسماك المجمدة المستوردة ولذلك تم تجميع 60 عينة من أسماك المكرونة والماكريل والباغة المجمدة (20 من كل منها) من مختلف الأسواق في محافظة الشرقية ونقلها إلى المعمل المركزي بكلية الطب البيطري- جامعة الزقازيق وذلك للكشف عن تركيزات بعض المعادن الثقيلة .

أوضحت النتائج أن متوسط تركيزات الكادميوم 0.039 ± 0.009 و 0.037 ± 0.04 و 0.043 ± 0.006 وكان متوسط تركيزات النحاس 0.46 ± 0.15، 0.49 ± 0.19، 0.34 ± 0.13 وكان متوسط تركيزات الرصاص هو 0.7 ± 0.16، 0.4 ± 0.11، 0.75 ± 0.25 وكان متوسط قيم الزئبق 0.076 ± 0.036، 0.48 ± 0.14، 0.38 ± 0.12 جزء في المليون في أسماك المكرونة والماكريل والباغة على التوالي .كان معدل الاستهلاك اليومي المقدر للكادميوم والنحاس والرصاص و الزئبق من وزن الجسم في اليوم لأسماك المكرونة والماكريل والباغة أقل من الكمية اليومية المقبولة .كان حاصل الخطر المستهدف من الكادميوم والنحاس والرصاص والزئبق من استهلاك الأسماك المجمدة أقل من الواحد في اسماك المكرونة والماكريل والباغة على التوالي مما لا يمثل خطورة على المستهلك علما بأن القواعد الصحية تنص على أن وجود أي مادة كيميائية حتى ولو غير سامة فإنها تمثل خطرا على صحة وسلامة الإنسان.لذلك نوصي بالحفاظ على نظافة المياه ومعالجة النفايات والصرف الصحي وتجنب صيد الأسماك من الأماكن الملوثة بمخلفات المصانع واستخدام المرشحات البيولوجية . كما نوصي بتوعية الناس بمخاطر تلوث المياه من خلال برامج تدريب وذلك للحد من خطر المعادن الثقيلة.