**Original Paper****Occurrence of some heavy metals in shellfish: Dietary intakes and health risk assessment**

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01/07/2021**ABSTRACT**

Shellfish is considered as important source for high quality protein, polyunsaturated fatty acids, vitamins, and minerals. The present study aimed to study the incidence of four heavy metals including lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) in four of the shellfish including shrimp, crab, oyster, and mussels. Moreover, the dietary intakes and the potential non-carcinogenic human health risks were calculated for Egyptian consumers. The obtained results revealed that shrimps had the lowest residual concentrations for both Pb, and Cd; whereas mussels had the highest Pb content, while oyster had the highest Cd content. The high content of heavy metals in the examined shellfish indicates the contamination of their living environment with heavy metals. The inter-species differences in their accumulation of heavy metals indicate their physiological differences their xenobiotic metabolizing enzymes. All examined shellfish had considerable concentrations of Cu, and Zn suggesting that these species can provide humans with part of their needs for these essential trace elements. Calculation of the potential non-carcinogenic risks for the tested metals associated with the consumption of shellfish indicated that the average consumption of these shellfish would not pose any risks for the Egyptian population.

**1. INTRODUCTION**

Shellfish such as shrimp, crab, oyster, and mussels are important sources for the high-quality protein, polyunsaturated fatty acids, vitamins, and minerals. However, such shellfish might contain high levels of certain environmental pollutants including heavy metals, polycyclic aromatic hydrocarbons, and pesticides (Morshdy et al., 2019; Thompson and Darwish, 2019).

Heavy metals such as lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) characterized by their bioaccumulation and biomagnification characters. Pb, and Cd have no function for the body; however, Zn, and Cu play important roles in the regulation of the function of many enzymes in the body (Morshdy et al., 2013).

Lead intoxication, particularly among children was reported in many countries such as China, Nigeria, and Zambia (Darwish et al., 2016). Furthermore, Pb has several adverse effects on the gastrointestinal tracts, nervous system and the learning abilities (Cunningham and Saigo, 1997).

Cadmium has several bad effects on the kidney a, liver, breast, testes, and bone. Cadmium was the causative agent for the itai-itai disease in Japan, which is characterized by osteomalacia, and renal failure (Nishijo et al., 2017). Furthermore, Cd is classified as a group B1 carcinogen (IARC, 2016).

Zinc is an essential trace element which plays essential roles for the normal function of many enzymes in the body and is needed for the regulation of the gene expression of many cell components (Pogorzelska-Nowicka et al., 2018; Roohani et al., 2013).

Copper is another essential element that is regarded as a key player in the biochemistry and physiology of the living organisms as it acts as a co-factor for several enzymes. Furthermore, Cu is an important element for the cellular respiration. However, excess exposure to Cu might induce oxidative stress to the cell organelles (Darwish et al., 2014).

Therefore, this study was undertaken to estimate the residual contents of four heavy metals including Pb, Cd, Zn, and Cu in four shellfish, namely, shrimp, crab, oyster, and mussels retailed in Kalyobia Governorate, Egypt. In addition, dietary intakes and the potential non-carcinogenic risks for the detected metals were calculated. The public health significance of the studied metals was further discussed.

**2 .MATERIAL AND METHODS****2.1 .Collection of samples**

A total of 80 random shellfish samples including shrimp, crab, oyster, and mussels (20 of each) were collected from fish markets in Kalyobia Governorate, Egypt. The collected samples were transferred cooled without delay to the laboratory for heavy metal measurements .

**2.2 .Sample preparation and extraction**

At first the inedible parts were removed from the examined shellfish. Then one gram from each sample was homogenized in an acid digestion solution (10 mL) consists of three parts of nitric acid and two parts perchloric acid

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(Darwish et al., 2015). The mixture was left standing overnight at room temperature for digestion, and then placed at heated water bath (70°C) for 3 h. Metals' concentrations were directly measured using an atomic absorption spectrophotometer (PerkinElmer 2380).

### 2.3 .Dietary intakes of heavy metals

The estimated daily intake (EDI) (mg/kg/day) values for the tested heavy metals were calculated using the following equation

$$EDI = C * FIR / BW \text{ (USEPA, 2010);}$$

Where C is the concentration of the tested metal in the sample (ppm wet weight); FIR is the shellfish ingestion rate in Egypt, which was estimated at 48.57 g/day (FAO, 2003); BW is the body weight of Egyptian adults, which was set at 70 kg (Darwish et al., 2015).

### 2.4 .Health risk assessment

The non-cancer risks associated with the consumption of the metal-contaminated shellfish among the Egyptian populations were calculated using the equations described by USEPA (2010) as following:

$$HR = EDI / RfD * 10^{-3}$$

Where HR is the hazard ratio; EDI is the estimated daily intake for each metal; RfD is the recommended reference doses for each metal (0.001 mg/kg/day for Cd, 0.004 mg/kg/day for Pb, and 0.3 mg/kg/day for Zn); there is no reported RfD for Cu (USEPA, 2010).

The hazard ratios can be summed to calculate a hazard index (HI) for estimation of the health risks associated with mixed contaminants .

$$HI = \sum HR_i$$

where i represents each metal

If the value of HR and/or HI exceeded one, this indicates a potential risk to human health, whereas a result less than or equal one indicates no risk.

### 2.5. Statistical analysis

The Tukey-Kramer HSD difference test (JMP) (SAS Institute, Cary, NC, USA) was used for statistical comparisons ( $p < 0.05$ ). In all analyses,  $P < 0.05$  was taken to indicate statistical significance (Gomez and Gomez, 1984).

## 3.RESULTS

The results recorded in Fig. 1 showed that the mean  $\pm$  SE values (mg/kg ww) for the residual concentrations of Pb in the examined shellfish were  $1.11 \pm 0.09$ ,  $0.71 \pm 0.09$ ,  $0.64 \pm 0.08$ , and  $0.34 \pm 0.05$  in the examined mussels, oyster, crab, and shrimp, respectively .The obtained results in Fig. 2 showed that the mean values for the residual concentrations of Cd were  $0.16 \pm 0.03$ ,  $0.10 \pm 0.01$ ,  $0.10 \pm 0.02$ , and  $0.04 \pm 0.01$  mg/kg ww in the examined oyster, crab, mussels, and shrimp, respectively .

Figure 3 showed the accumulated concentrations of Cu in the examined samples. The recorded mean concentrations were  $2.08 \pm 0.18$ ,  $1.83 \pm 0.15$ ,  $1.49 \pm 0.07$ , and  $0.77 \pm 0.10$  mg/kg ww in the examined crab, shrimp, mussels, and oyster, respectively .Zn was measured in the examined shellfish in the present study. The recorded results showed

that the average Zn concentrations were  $10.74 \pm 0.19$ ,  $10.10 \pm 0.43$ ,  $7.60 \pm 0.24$ , and  $6.69 \pm 0.38$  mg/kg ww in the examined crab, shrimp, mussels, and oyster, respectively (Fig. 4).

The calculated dietary intakes and associated non-carcinogenic risks were reported in Table 1, where HR and HI values were below 1.

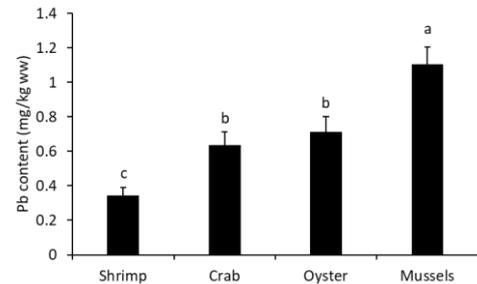


Figure 1: Lead residual contents in some shellfish  
Lead (Pb) residual concentrations mg/kg wet weight in the examined shellfish (n=20). Columns carrying different superscript letter is significantly different at  $p < 0.05$ .

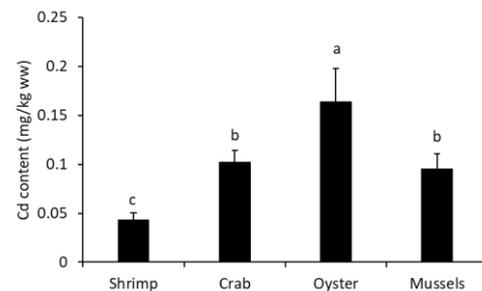


Figure 2: Cadmium residual contents in some shellfish  
Cadmium (Cd) residual concentrations mg/kg wet weight in the examined shellfish (n=20). Columns carrying different superscript letter is significantly different at  $p < 0.05$ .

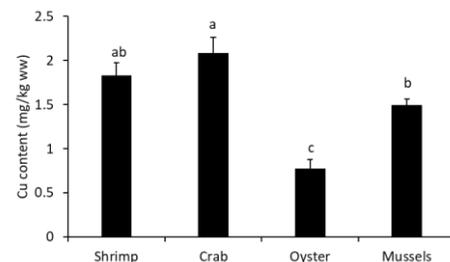


Figure 3: Copper residual contents in some shellfish  
Copper (Cu) residual concentrations mg/kg wet weight in the examined shellfish (n=20). Columns carrying different superscript letter is significantly different at  $p < 0.05$ .

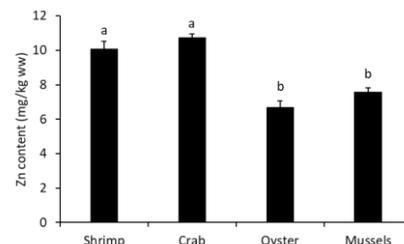


Figure 4: Zinc residual contents in some shellfish  
Zinc (Zn) residual concentrations mg/kg wet weight in the examined shellfish (n=20). Columns carrying different superscript letter is significantly different at  $p < 0.05$ .

Table 1: Dietary intakes and calculated hazard ratio and index of Pb, Cd, Cu, and Zn due to consumption of shellfish in Kalyobia governorate (n=20)

	Pb			Cd			Cu			Zn			
	%	EDI	HR	%	EDI	HR	%	EDI	HR	%	EDI	HR	HI
Shrimp	20	0.24	0.06	15	0.03	0.03	0	1.27	NA	0	7.01	0.02	0.11
Crab	70	0.44	0.11	90	0.07	0.07	0	1.45	NA	0	7.45	0.02	0.2
Oyster	60	0.49	0.12	90	0.11	0.11	0	0.54	NA	0	4.64	0.015	0.245
Mussels	90	0.77	0.19	70	0.07	0.07	0	1.04	NA	0	5.27	0.02	0.28

:%Percentage of samples exceeding the established maximum permissible limits for Pb (0.3 ppm), Cd (0.05 ppm), Cu (5.0 ppm), and Zn (50.0 ppm)

EDI: Estimated daily intake

HR: Hazard ratio

HI: Hazard index

#### 4. DISCUSSION

Shellfish is regarded as a valuable source for many nutrients such as proteins, vitamins, minerals, and omega-3-fatty acids. However, their living environment near the bottom of the sea, and their feeding behavior give high chances for accumulation of environmental pollutants, particularly heavy metals (Atia et al., 2018). In the present study, all examined shellfish accumulated Pb to certain limits; where, 90%, 60%, 70%, and 20% of the examined mussels, oyster, crab, and shrimp exceeded the established maximum permissible limits (MPL) for Pb (0.3 mg/kg ww) according to the Egyptian Organization for Standardization (EOS, 2010). Mussels had significantly ( $p < 0.05$ ) the highest Pb residues, followed by oyster, crab and shrimp, respectively. The obtained results in the present study agree with those recorded by Atia et al. (2018) who reported comparable levels in the shrimp, and crab collected from Ismailia, Egypt. Helmy et al. (2018) reported relatively higher Pb levels in oyster, shrimp, and crab collected from Kalyobia, Egypt. Globally, Olemedo et al. (2013) recorded similar Pb concentrations in shellfish samples collected from Spain. Unlikely, Vázquez-Boucard et al. (2014) detected higher Pb concentrations in oysters collected from Mexico. However, lower Pb levels were recorded in shrimp, mussels, and crab collected from Brazil (Silva da Araújo et al., 2016). Like Pb, all examined shellfish had residual concentrations of Cd. Oyster, and crab had the highest Cd residues, followed by mussels, and shrimp. Where 90% of the sampled oyster and crab, and 70% of mussels, and 15% of the collected shrimps had Cd residues higher than the established MPL (0.05 mg/kg ww) (EOS, 2010). The recorded Cd concentrations in the present study go in agreement with those recorded by Atia et al. (2018) who recorded Cd residues in the range of 0.3-0.9 mg/kg ww in oyster, crab and mussels collected from Ismailia city, Egypt. In addition, Helmy et al. (2018) detected similar levels in oyster, shrimp, and crab collected from Kalyobia, Egypt. Besides, Darwish et al. (2019) reported nearly similar levels (0.4-0.61 mg/kg ww) in crab, oyster, and shrimp collected from Zagazig city, Egypt. Higher Cd levels were reported in oyster sampled from France (Baudrimont et al., 2005). Lower Cd levels were demonstrated in shellfish collected from Brazil (Silva da Araújo et al., 2016). Copper was detected in all examined shellfish samples with levels that did not exceed the established MPL (5 mg/kg ww) (EC, 2006). Crab had the highest residual concentrations of Cu, followed by shrimp, mussels, and oyster, respectively. In agreement with the recorded Cu residues in the current work, Gong et al. (2020) detected Cu in oyster, mussels, and clams sampled from China. Higher Cu levels that recorded in the present study were also

estimated in shellfish collected from Spain (Velasco-Reynold et al., 2008).

In addition, high Cu levels (9.3-13.12 mg/kg ww) were recorded in shrimp and crabs from India (Kumar et al., 2021).

Zinc was like Cu as it was detected in all examined shellfish samples with levels that did not exceed the established MPL (50 mg/kg ww) (EC, 2006). Shrimp and crab had significantly ( $p < 0.05$ ) the highest Zn residues followed by mussels and oyster, respectively. The recorded Zn levels in the examined shellfish were comparable to that recorded by Kumar et al. (2021) in shrimp and crab from India. Higher Zn levels (16.3-511 mg/kg ww) were recorded in mussels and oyster sampled in Croatia (Bilandžić et al., 2015).

The collected shellfish in the present study mainly originates either from the Mediterranean or the Red sea. Detection of high levels of heavy metals in the examined shellfish indicates potential contamination of their living environment with heavy metals. The sources of the contamination of the water bodies with heavy metals include the direct release of industrial and agricultural wastes (Atia et al., 2018). One possible explanation for the inter-species difference in their bioaccumulation of heavy metals is their physiological differences in their xenobiotic metabolizing enzymes (Darwish et al., 2019).

Dietary intakes and the potential non-carcinogenic risks associated with the consumption of shellfish in Kalyobia governorate were also calculated. The obtained results were presented in Table 1, the results clearly showed that shellfish contribute significantly to cover part of the human needs from the essential trace elements (Cu, and Zn). Calculation of the hazard ratio and hazard index for all examined metals were less than 1, which indicates that consumption of such levels would not pose any non-carcinogenic risks among the Egyptian population. Unlikely, Gong et al. (2020) indicated that children will be at high risks-associated with the consumption of shellfish contaminated with heavy metals in China. Furthermore, Kumar et al. (2021) reported that excessive consumption of shellfish in India will represent a great risk among the Indian population due to the high residual concentrations of the toxic metals in these shellfish.

#### 5. CONCLUSION

In conclusion, the current study demonstrated the occurrence of heavy metals and trace elements residues in the shellfish mostly consumed in Egypt including shrimp, crab, oyster, and mussels. Generally, mussels and oyster had the highest residues for Pb, and Cd. While shrimp, and crab had the highest contribution for the dietary supplementation for both Cu, and Zn. Therefore, it is highly advisable to consume shrimp, and crab than oyster and mussels.

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