



Risk Assessment of Heavy Metal Bioaccumulation in Raw Crab and Prawn Flesh Market in Egypt

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ABSTRACT

Background: Heavy metal toxicity at low levels damages the function of the brain, lungs, kidney, liver, blood composition, and other important organs. Long-term exposure leads to gradual disease progression in multiple sclerosis, Parkinson's disease, Alzheimer's disease, muscular dystrophy, and cancer. The present work aims to determine the pollution caused by the levels and effects of heavy metals, *i.e.* nickel, zinc, chromium, and copper, in marine organisms (crabs and shrimps).

Methods: In total, 100 crustacean samples (50 crabs and 50 prawns) were analyzed in terms of nickel, zinc, chromium, and copper residues using an atomic absorption spectrophotometer. The health risk assessment method of the consumption of tested tissues was investigated through determining the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI).

Results: The concentrations of nickel, zinc, chromium, and copper in the crab samples were 0.292 ± 0.02 , 20.688 ± 3.06 , 1.158 ± 0.01 , and 22.304 ± 4.04 $\mu\text{g/g}$ of wet weight, respectively. Moreover, the values in the prawn samples were 0.373 ± 0.01 , 16.204 ± 2.01 , 0.844 ± 0.01 , and 18.524 ± 1.03 $\mu\text{g/g}$ of wet weight, respectively.

Conclusion: Our findings could lay the groundwork for monitoring the heavy metal contamination of marine organisms. The estimated daily detection intake of nickel, zinc, chromium, and copper was below the reported the Provisional Tolerable Daily Intake (PTDI) of each element. In addition, THQ and HI values of the heavy metals were below 1.00 in the crab and shrimp samples, suggesting no significant risks to the community health due to the consumption of the crab and shrimp samples.

1. Introduction

Industrial clearance, downtown stormwater throw-out, domestic wastefulness, husbandry wastes, and other human effluents cause heavy metals, pesticides, aliphatic/aromatic compounds, phthalate esters, nutrients, and organic

discharge pollution in the aquatic habitat. These contaminants also lead to undesirable differences in the physicochemical or biological aspects of the environment, thereby disturbing the ecological harmony of the surroundings directly or indirectly. Among various pollutants, heavy metal residues pollution in the marine



environment has become a global concern due to the associated hazards persistence through several decades in the marine habitat [1].

Heavy metals may decrease energy levels and impair the function of the brain, lungs, kidney, liver, and other vital organs. Moreover, chronic exposure may lead to the gradual progress of physical, muscular, and neurological disorders, such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy. Furthermore, prolonged exposure to some metals and their compounds may cause cancer [2]. The toxicity level of a few heavy metals may be above the background concentrations that are naturally present in the environment. Therefore, a thorough knowledge of heavy metals is essential to the provision of proper defense measures against excessive contact with these hazardous elements [3].

Aquatic organisms are susceptible to heavy metal residues when the level of these metals increases in water and debris. This is particularly important in crabs and shrimp, which are the invertebrates that collect higher levels of heavy metals more than other fish due to the variations in the developmental approach maintained by different phyla. Heavy metals that are concentrated in crustaceans might be magnified in the food chain and affect human life [4]. However, no studies have been focused on the metal contamination level of the crustaceans of the Pulicat Lake, which is the second largest brackish water lagoon in India.

Thus, the present study was focused to measure the level of heavy metal residues in crab (*Scylla serrata*) and the most common accessible shrimp species (*Penaeus semisulcatus*, *Penaeus indicus*, and *Penaeus monodon*), which appear to have great economic and ecological importance in the Pulicat Lake [5]. The difference in metal concentrations might be due to metabolic rate, exposure mode, metal motility, availability, and breed. Additionally, environmental factors such as pH, temperature, salinity, nutrients, organic materials, organic carbon, and the parameters associated with the ecosystem stimulate the availability and accumulation rate of metals [6].

The environmental sources of chromium (Cr) have been investigated through the flaming of petroleum oil and coal, rebellious ferrochrome materials, oxidants, chromium steel, fertilizers, oil well drilling, and metal tanneries. Moreover, chromium is released through excrement and manure [7]. Cr (III) is inactive in its reduced form and released into water, while oxidized chromium is mostly water-soluble and movable [8]. Environmental oxygen enables the oxidation of Cr (III) to Cr (VI), which is extremely toxic and highly water-soluble [9]. The reactions between Cr (VI) and other reductive biological agents (e.g., thiols and ascorbate) lead to the formation of highly reactive oxygen species (e.g., superoxide ions, hydrogen peroxide, and hydroxyl radicals), which may cause oxidative disorders in the cells, as well as DNA and protein damage.

According to the literature, Cr (VI) is significantly more hazardous than Cr (III), It penetrates to the cells more easily

than Cr (III) and is conclusively reduced to Cr (III). Due to the teratogenic and carcinogenic properties of Cr (VI), it has been categorized as a class I human mutagenesis by the International Agency for the Research on Cancer [10].

The functional origins of chromium have been reported to be protective metal coats, metal combinations, magnetic lines, color pigments, rubber, cement, paper, wood conservatives, leather tanning, and metal foil [11]. Cigarettes contain 390 g/kg of chromium, while no findings have been reported regarding the quantity of chromium inhaled by smokers [12]. High concentrations of chromium in humans lead to the inhibition of erythrocyte glutathione reductase, which decreases the capability to reduce methemoglobin to hemoglobin [13]. Chromate composite could induce DNA impairment through numerous mechanisms, thereby causing chromosomal aberrations, mutated replication, and DNA transcription [14].

Inputs of Copper (Cu) into the marine environment is most probably through municipal wastewaters, manure, fertilizers, antifouling measures (e.g., paint and wood preservatives), and manufacturing industrial wastes. On the other hand, copper is the least expensive and most commonly used pesticide in the aquaculture industry and other aquatic systems [15].

Chromium plays a key role in glucose and lipid metabolism as an essential nutrient [16]. The presence of chromium in food is of utmost importance as it is associated with insulin function and lipid assimilation [17]. Therefore, the presence of a small amount of chromium is essential to the growth of prawns. However, anthropogenic activities such as smelting, metal m1mng, vehicle emission, fossil fuel burning, disposal of household, municipal, and industrial wastes, fertilizer use, and organic manures also contribute to the nickel pollution of the aquatic environment [18].

Copper is essential to health, while the excessive absorption of it may cause destructive health consequences in the form of liver and kidney disorders [19]. Zinc is fundamental in most metabolic pathways of humans, and its insufficiency leads to the loss of appetite, growth slowdown, skin mutation, and immunological dysfunction [20]. Nickel is often found in extremely small amounts in nature and contributes to various pulmonary disorders, such as lung inflammation, dysfunction, emphysema, and tumors [21].

The current research aimed to determine the level and effects of heavy metals pollution, including nickel, zinc, chromium, and copper, in marine organisms (crabs and shrimps).

2. Materials and Methods

2.1. Sample Collection

In total, 100 crustacean samples, including 50 crabs (*Callinectes sapidus*) and 50 prawns (*Parapenaeus longirostris*), were analyzed in terms of nickel, zinc, chromium, and copper residues. The samples were collected

from five cities on the Mediterranean Sea, i.e. Alexandria, Port Said, Ras El-Bar, Gamsa, and New Damietta, in which ten samples of each species were collected from each city. Raw samples were purchased chilled from the fish markets in Mansoura, Egypt. The sampled crustacean was individually packed into clean polyethylene bags, marked, and transferred to the laboratory of Food Hygiene and Control Department of the Faculty of Veterinary Medicine at Mansoura University, Egypt at the temperature of 4°C in an icebox with minimum delay (Code R/56). Following that, the samples were treated and prepared (digested) for the analysis of heavy metal contents in their flesh.

In the laboratory, each sample was properly cleaned by rinsing with distilled water to remove debris, planktons, and other external adherent dirt and washed with deionized water. Afterwards, each cleaned sample was packed separately in a clean plastic polyethylene bag, labeled with an identification number and collection date of collection, and kept frozen at the temperature of -18°C for two days until the final preparation and digestion.

2.2. Reagents

The reagents used in this study (E-Merck, Darmstadt, Germany) have an ultra-pure quality, including 70% perchloric acid (HClO₄), 37% hydrochloric acid (HCl), 65% nitric acid (HNO₃), and 30% hydrogen peroxide (H₂O₂). The laboratory wares used for the preparation and handling of the samples were immersed in soap and water for a minimum of two hours, rinsed several times with potable tap water, and rinsed with distilled water. A thread mixture (200 ml concentrated 37% HCl, 250 ml deionized water, 80 ml 30% H₂O₂), washing acid (100 ml concentrated 37% HCl, 900 ml deionized water), and deionized water were also prepared and dried on a clean bench.

2.3. Sample Preparation and Digestion

Frozen clean samples were kept to thaw in the refrigerator (0-5 °C). The incision of the outer shell was carried out using a stainless steel scalpel and forceps on a clean polyethylene work surface. A portion of the muscle (~1.5 g) was also excised from each sample to determine the content of heavy metal residues. The samples of muscles were homogenized by Braun MULTIQUEICK 9 Hand Blender 1000 WATT BLACK MQ9087X, Germany.

In this study, we used the wet digestion method proposed by Finerty *et al.* (1990) [22]. Briefly, 1.5 g of each muscle sample was excised, macerated, and transferred into a clean and previously washed screw-capped tube (20 ml) containing a mixture of 65% concentrated nitric acid (6 ml) and 60% concentrated perchloric acid (3 ml). The screw-capped tubes represented the correspondent samples and were closed and incubated overnight in a water bath adjusted at the temperature of 53°C to complete the digestion. After cooling to room temperature, the digest of

each tube was filtrated into a clean glass beaker using a Whatman filter paper No. 1 [23]. Following that, the filtrate was diluted by adding 40 milliliters of deionized water, and the diluted filtrate was poured into a clean screw-capped bottled, labeled with a number, season, fish species, and stored at room temperature until the evaluation of the heavy metal contents.

At the next stage, blank solutions were prepared to assess the possible traces of the metals that may have been present in the acids and deionized water used in the preparation, digestion, and dilution of the samples. One blank solution consisted of nitric acid and perchloric acid at the ratio of 3:1, which was preserved in a water bath at the temperature of 60°C for six hours and diluted with 20 milliliters of deionized water. However, the standard solutions for the calibration curves were prepared by diluting a stock solution of each analyzed element (1,000 mg/l) with acidified ultrapure water (5% v/v HNO₃). In addition, strength standard solutions of 0.005, 0.01, 0.1, and 0.2 µg/g were used [22].

2.4. Heavy Metal Analysis

At this stage, the filtered samples were analyzed in terms of heavy metal residues (nickel, zinc, chromium, and copper) in accordance with the AOAC standards [24] using an atomic absorption spectrophotometer (AAS; Buck Scientific 210 VGP Inc.) (58 Fort Point St. East Norwalk, CT 06855, USA). The characteristic of wavelengths were element-specific and accurate to 0.01-0.1 nm. The apparatus had a digital absorbance capable of operating at wavelengths of 232.0, 213.9, 357.9, and 324.8 nm for nickel, zinc, chromium, and copper, respectively with the detection limits of 0.05, 0.005, 0.04, and 0.005 mg/L for nickel, zinc, chromium, and copper, respectively (Table 1).

The beam source used in the atomic absorption spectrophotometer (AAS) was a single hollow cathode lamp. The analysis of nickel, zinc, chromium, and copper was conducted by air-acetylene flow flame atomic absorption spectrometry (FAAS). The AAS had a digital absorbance and concentration readout capable of operating at the recommended parameters of the instrumental instructions, which was used for the quantitative determination of the investigated elements based on the air-acetylene flow rate of 1.0, 0.9, 1.1, and 1.0 l/min flame AAS for nickel, zinc, chromium, and copper, respectively (Table 1).

2.5. Calculation of Heavy Metals in Samples

The process involved a direct drawing of the tested samples, standard solution, and blanks into the flame for analysis. The metal contents were expressed as µg/g of the wet weight of the fresh fish (ppm). In addition, the heavy metal levels were recorded directly from the digital AAS scale and calculated using the following equation:

$$\text{Element (ppm)} = R \times D / W$$

Table 1: Standard conditions in determination of different elements and their detection limits by AAS

Metals	Lamp wavelength (nm)	Slit width (nm)	Detection limit (mg/L)	Lamp current (mA)	Fuel flow rate (L/min)	Burner height (cm)	Detection limit (µg/kg)
Nickel	232.0	0.2	0.05	4.0	1.0	7.0	0.05
Zinc	213.9	0.7	0.005	7.0	0.9	7.0	0.005
Chromium	357.9	0.7	0.04	5.0	1.1	7.0	0.04
Copper	324.8	0.7	0.005	5.0	1.0	7.0	0.005

Where R is the reading of the element concentration, ppm from the digital AAS scale, D shows the dilution of the prepared sample (ml), and W represents the wet weight of the samples (g).

The concentration of the absorbance value of the heavy metals in the blank samples was also calculated and subtracted from each analyzed sample [25].

2.6. Health Risk Assessment of the Consumption of Tested Tissues

2.6.1. Estimated Daily Intake (EDI)

The EDI of the tested heavy metals was calculated using the following formula:

$$EDI = Mc \times \text{Consumption Rate}$$

Where Mc is the metal concentration (µg/g) of the flesh-tested crustacean samples on a wet weight basis.

The mean daily consumption rate of the crab and prawn samples by a person weighing 70 kilograms in Egypt was estimated at 1.2 g/day (0.0012 kg/day) based on the FAO standards [26].

2.6.2. Target Hazard Quotient (THQ)

THQ was calculated using the formula proposed by the USEPA [26], as follows:

$$THQ = FIR \times C/RfD \times W_{AB}$$

where FIR is the food ingestion rate of the crustacean flesh (1.2 g/day/person) [26], C shows the metal concentration (µg/g) in the samples on a wet weight basis, RfD is the oral reference dose as the daily intake of a contaminant over a lifetime that is expected to have adverse health effects [27] (estimated at 20, 300, 1.5, and 40 for nickel, zinc, chromium, and copper, respectively as established by the USEPA [27] and EFSA [28]), and WAB represents the average adult body weight of a consumer in Egypt (70 kg).

2.6.3. Hazard Index (HI)

HI could be expressed as the sum of THQs [26], as follows:

$$HI = THQ_{Ni} + THQ_{Zn} + THQ_{Cr} + THQ_{Cu}$$

3. Results and Discussion

3.1. Heavy Metal Residues in the Flesh of Crab and Prawn Samples

Table 2 shows the heavy metal concentrations in the flesh of the crustaceans that were collected from Egypt. Accordingly, copper and zinc were the predominant heavy metals in the crab and prawn flesh, with the mean concentration of copper estimated at 22.304 and 18.524 µg/g, respectively. The mean zinc concentration was 20.688 and 16.204 µg/g in the crabs and prawns, respectively. Additionally, nickel levels increased from 0.019 to 0.564 µg/g and from 0.058 to 0.687 µg/g in the crabs and prawns, respectively. The chromium values also increased from 0.044 to 2.271 µg/g and from 0.061 to 1.627 µg/g in the crabs and prawns, respectively.

According to the findings, the heavy metals residues in the flesh of the raw prawns were higher compared to the raw crab samples [29]. In addition, the nickel residues in the flesh of the raw crabs and prawn in the present study were higher compared to the reported values by Turkmen *et al.* (2008) in the fish samples collected from Marmara, Aegean, and the Mediterranean Sea (0.02–3.97 µg/g of wet weight) [30]. In addition, Lavilla *et al.* (2008) reported the concentration range of 2.94–46 µg/g of wet weight in fish and shellfish samples [31]. Similarly, a higher concentration of nickel residues was reported by Raknuzzaman *et al.* (2016) in the fish samples collected from the coastal areas of Bangladesh (0.1–0.56 µg/g/wet weight) [32]. The differences in the reported values of nickel residues in the raw flesh of crabs and prawns could be due to the nature of this element, as well as environmental factors, physical reaction, physiological tolerance, tissue threshold, and regulatory mechanisms [33].

According to the results of the present study, the mean level of the zinc residues in the flesh of *Parapenaeus longirostris* was 14.57 µg/g of the wet weight, which is approximately equal to the lower mean level of the residues of this element in other shrimp species (e.g., *Palaemon serratus*) as reported by Gokoglu *et al.* (2008) (6.25 µg/g of wet weight) [34]. The lower minimum and maximum zinc levels in the collected fish samples from Iskenderun Bay (Turkey) has also been reported to be 0.60–11.57 µg/g of wet weight [35]. On the other hand, higher zinc levels have been estimated Rahman *et al.* (2012) in some fish species in Bangladesh (42.8–418 µg/g of wet weight) [36], as well as the cached fish harvested from the Black Sea in Turkey in the

Table 2: Heavy metal residues in flesh of raw crabs and Prawns ($\mu\text{g/g}$ of wet weight)

Metals	Raw crabs			Raw prawns		
	Min	Max	Mean \pm SE	Min	Max	Mean \pm SE
Nickel	0.019	0.564	0.292 \pm 0.02	0.058	0.687	0.373 \pm 0.01
Zinc	0.682	40.653	20.688 \pm 3.06	0.479	31.928	16.204 \pm 2.01
Chromium	0.044	2.271	1.158 \pm 0.01	0.061	1.627	0.844 \pm 0.01
Copper	0.467	44.140	22.304 \pm 4.04	0.345	36.702	18.524 \pm 1.03
Nickel	0.019	0.564	0.292 \pm 0.02	0.058	0.687	0.373 \pm 0.01

Min: minimum, Max: maximum, SE: standard error of mean values.

study by Tuzen (2009) (38.8–93.4 $\mu\text{g/g}$ of wet weight) [20]. In another study, Raknuzzaman *et al.* (2016) reported the higher maximum range of zinc residues in analogous samples to be 31–138 $\mu\text{g/g}$ of wet weight [32].

Our findings regarding chromium residues are consistent with the values reported for the crustacean collected from the coastal areas of Bangladesh in the study by Raknuzzaman *et al.* (2016) (0.15–2.2 $\mu\text{g/g}$ of wet weight) [32]. Furthermore, the similar range of 0.07–6.46 $\mu\text{g/g}$ of wet weight has been reported in the fish species collected from Iskenderun Bay in Turkey [34]. On the other hand, higher ranges of chromium levels have been estimated to be 0.80–1.40 and 0.11–0.23 $\mu\text{g/g}$ of wet weight in some fish species [37, 38].

Despite the similarity of our findings with the mean values of copper residues in the flesh of raw prawns reported in some studies, lower ranges of 1.3–1.4 $\mu\text{g/g}$ of wet weight have also been estimated in the crustaceans collected from the coastal areas of Bangladesh in the study by Raknuzzaman *et al.* (2016) [32], as well as the samples obtained from Iskenderun Bay (Turkey) in a research by Turkmen *et al.* (2005) (0.04–5.43 $\mu\text{g/g}$ of wet weight) [35]. Similarly, higher ranges of copper residues have been detected by Ahmed *et al.* (2009) (5.17–94.5 $\mu\text{g/g}$ of wet weight) in the collected fish from Dhaleshwari River in Bangladesh [39].

In another study, the findings regarding the seasonal changes in trace element connotations (e.g., Cr, Ni, Cu, Zn) in different tissue of male and female green tiger shrimp (*Penaeus semisulcatus*) collected from Iskenderun Bay (northeastern Mediterranean Sea, Turkey) indicated that the trace element contents varied in terms of the type of metals, season, and sex. Furthermore, their accumulations differed significantly in some cases, and the concentrations of metals were reported to be higher in male gonads and lower in the flesh of all the collected shrimp samples [40].

According to the literature, the concentration of several heavy metals (e.g., Cr, Cu, Ni, and Zn) in the muscle tissues of tiger prawn (*Penaeus monodon*) resists the build-up of specific metals, while allowing the entry of others to the extent of exceeding the proportion that occurs in the environment. Some of the controlling factors in this regard include the nature of the metals, environmental factors, physical reaction, physiological tolerance, tissue threshold, and regulatory mechanisms [33]. In another study, the concentrations of a wide range of metallic elements (e.g., Cr, Ni, Cu, and Zn) in commercial fish and the crustaceans harvested from the coastal areas of Bangladesh have been estimated at 0.15–2.2, 0.1–0.56, 1.3–1.4, and 31–138 mg/kg of

wet weight [32]. On the other hand, the nickel residues in the in fish samples cached from Marmara, Aegean, and the Mediterranean Sea were estimated at 0.02–3.97 mg/kg of wet weight [30]. Another study indicated the range of 0.11–12.88 mg/kg of wet weight in the fish species collected from Iskenderun Bay [35], while the range of 2.94–46 mg/kg of wet weight has also been quantified in the fish and shellfish samples in the study conducted by Lavilla *et al.* (2008) [31]. Notably, the World Health Organization (WHO) has recommended the range of 100–300 mg/kg bw/day for daily nickel consumption [41].

The chromium residues in the fish species obtained from Iskenderun Bay (Turkey) have been calculated to be 0.07–6.46 mg/kg of wet weight [35], while the concentration has been reported to be 0.04–1.75 mg/kg of wet weight in the fish collected from Marmara, Aegean, and the Mediterranean Sea in Turkey [30]. On the other hand, higher levels of chromium (0.47–2.07 mg/kg of wet weight) have been quantified in the fish consumed in Bangladesh. Other findings have indicated the chromium residues in the edible tissue of *Silurus glanis* to be within the range of 0.80–1.40 [37] and 0.11–0.23 mg/kg of wet weight [38].

Copper quantities in the fish harvested from Dhaleshwari River in Bangladesh have been reported to be within the range of 5.17–9.45 mg/kg of wet weight [39]. In another study, copper concentrations have been estimated at 0.04–5.43 mg/kg of wet weight in Iskenderun Bay [35]. In the present study and in line with the international limitations, the level of copper residues was considered to be within permissible limits.

Zinc concentrations in the fish consumed in Bangladesh have been reported to be within the range of 42.8–418 [36] and 38.8–93.4 mg/kg of wet weight in the samples cached from the Black Sea in Turkey [20]. Furthermore, other studies have detected the range of 0.60–11.57 mg/kg of wet weight in the fish harvested from Iskenderun Bay [35]. In the current research, the copper content was within the range of 1.82–6.22 and 4.24–7.40 mg/kg of wet weight in the crustacean samples. Therefore, no serious hazard was detected in the samples in terms of the heavy metal contents. The contents of the trace elements in some species of shrimp such as *Penaeus semisulcatus*, *Parapenaeus longirostris*, and *Palaemon serratus* have been estimated at 6.19, 1.33, and 5.59 $\mu\text{g/g}$ for copper and 30.84, 14.57, and 6.25 $\mu\text{g/g}$ for zinc, respectively [35].

In another study, higher levels of copper, zinc, and chromium were observed in crabs and the associated sediments collected from the mangrove wetlands in Qi'ao Island in South China. In addition, the mean ranges of sediments were reported to be 65-91, 212-247, and 8.8-14 mg/kg of dry weight for the three elements, respectively. Meanwhile, the residues of the elements in the crab tissues were within the range of 36-528 and 44-280 mg/kg of dry weight for copper and zinc, respectively, while chromium was only quantified in the hepatopancreas (7.8 ± 1.7 mg/kg of dry weight) and carapace (13.2 ± 3.7 mg/kg of dry weight). In the mentioned study, metal pollution was detected in the area of collection, and the bottom-dwelling habits of crab were reported to precipitate more heavy metals. Furthermore, the results of the total target hazard quotient investigation indicated that adults might be exposed to significant potential health hazards by the consumption of these crab species.

In another research, the level of zinc in the eyes of the crabs (*M. miu*) harvested from the middle coast of Zhejiang Province (China) was reported to be 5.59 ± 2.61 μ g/g of wet weight, while it was 16.35 ± 4.38 μ g/g of wet weight in the flesh, which was higher than the gill content (8.59 ± 3.84 μ g/g of wet weight). In the mentioned study, the higher copper concentrations in the gills (35.09 ± 19.73 μ g/g of wet weight) was considered significant since it is a component of hemocyanin, which is a respiratory pigment with its site of action in the gills. Meanwhile, the heavy metal residues in the flesh stemmed from accumulation through the blood supply.

3.2. Comparison of Detected Heavy Metals in Crab and Prawn Samples with the Maximum Permissible Limit (MPL)

Table 3 shows the comparison of the detected heavy metal residues in the crab and prawn samples to their maximum permissible limit (MPL). Accordingly, the MPL of nickel, zinc, chromium, and copper was 0.4, 24, 0.8, and 24 μ g/g of wet weight based on the FAO guidelines [26], respectively. Moreover, the exceeding percentages from the MPL were recorded in the crab samples, which increased by 34% and 30% for copper and zinc, respectively. Furthermore, copper and zinc increased by 24% and 22% in the prawn samples as

the exceeding percentages from the MPL, respectively. On the other hand, the exceeding percentages from the MPL were estimated at 12% and 16% for chromium in the crab and prawn samples, respectively. Finally, the minimum exceeding percentages from the MPL were detected for nickel and calculated to be 8% in the crab and prawn samples, respectively.

3.3. Provisional Tolerable Daily Intake (PTDI) of Heavy Metals in Crab and Prawn Muscles

Tables 4 and 5 show the provisional tolerable daily intake (PTDI) of nickel, zinc, chromium, and copper. Accordingly, the values were estimated to be 5, 1,000, 3, and 500 μ g/70 kg of person/day according to the FAO guidelines [26], respectively. In addition, the data in these tables indicated that the mean daily consumption rate of the crab and prawn muscles by a person weighing 70 kilograms in Egypt was 1.2 grams per day, which equals 0.0012 kilograms per day.

According to the information in Table 4, the mean concentrations of the investigated heavy metals were 292, 20,686, 1,158, and 22,304 μ g/kg for nickel, zinc, chromium, and copper, respectively. Furthermore, the EDI of the detected heavy metals in the crab samples was 0.350, 24.8, 1.38, and 26.7 μ g/day/person, which constituted 7%, 2%, 46%, and 5% of the PTDI of nickel, zinc, chromium, and copper, respectively.

According to the information in Table 5, the mean concentrations of the investigated heavy metals were 373, 16,204, 844, and 18,524 μ g/kg for nickel, zinc, chromium, and copper, respectively. In addition, the EDI of the detected heavy metals in the prawn samples was 0.447, 19.44, 1.013, and 22.22 μ g/day/person, which constituted 9%, 2%, 34%, and 4% of the PTDI of nickel, zinc, chromium, and copper, respectively.

3.4. THQ of Heavy Metals with Consumption of Crab Muscles

As indicated in Tables 6 and 7, the food ingestion rate (FIR) of the crab and prawn muscles in Egypt was 1.2 g/day/person, and the average adult body weight (WAB) of the consumers in Egypt was 70 kg. Furthermore, the oral reference dose (RfD) of nickel, zinc, chromium, and copper was 20, 300, 1.5, and 40 μ g/kg/day, respectively.

Table 3: Comparison of detected heavy metals in crab and prawn samples with their maximum permissible limit (MPL; μ g/g of wet weight)

Metals	Maximum permissible limits (MPL) ^[a]	Exceeded crab samples (n= 50)	Exceeded crab %	Exceeded prawn samples (n= 50)	Exceeded prawn %
Nickel	0.4	5	10	4	8
Zinc	24	15	30	11	22
Chromium	0.8	6	12	8	16
Copper	24	17	34	12	24

[a] MPL for Nickel, Zinc, Chromium, and Copper as recommended by FAO [26].

Table 4: Comparison of EDI of detected heavy metals in crab samples with Their PTDI

Metals	PTDI $\mu\text{g}/70\text{ kg person/day}$	Mean Conc. of heavy metals ($\mu\text{g}/\text{kg}$) in the present study	The average daily consumption rate of crab muscles by 70 kg person in Egypt	Estimated Daily Intake	
				EDI $\mu\text{g}/\text{day}/\text{person}$	% of PTDI
Nickel	5 [b]	292	1.2 g/day = 0.0012 kg/day [d]	0.350	7%
Zinc	1000 [a]	20686	1.2 g/day = 0.0012 kg/day [d]	24.8	2%
Chromium	3 [c]	1158	1.2 g/day = 0.0012 kg/day [d]	1.38	46%
Copper	500 [c]	22304	1.2 g/day = 0.0012 kg/day [d]	26.7	5%

[a] JECFA, [b] JECFA, [c] JECFA, [d] FAO [26].

Table 5: Comparison of EDI of detected heavy metals in prawn samples with Their PTDI

Metals	PTDI $\mu\text{g}/70\text{ kg person/day}$	Mean Conc. of heavy metals ($\mu\text{g}/\text{kg}$) in the present study	The average daily consumption rate of prawn muscles by 70 kg person in Egypt	Estimated daily intake	
				EDI $\mu\text{g}/\text{day}/\text{person}$	% of PTDI
Nickel	5 [b]	373	1.2 g/day = 0.0012 kg/day [d]	0.447	9%
Zinc	1000 [a]	16204	1.2 g/day = 0.0012 kg/day [d]	19.44	2%
Chromium	3 [c]	844	1.2 g/day = 0.0012 kg/day [d]	1.013	34%
Copper	500 [c]	18524	1.2 g/day = 0.0012 kg/day [d]	22.22	4%

[a] JECFA, [b] JECFA, [c] JECFA, [d] FAO [26].

Table 6: THQ [a] of heavy metals with consumption of crab muscles

Metals	FIR [b]	W_{AB} [c]	RfD [d, e] ($\mu\text{g}/\text{kg}/\text{day}$)	Crab muscles	
				C [f]	THQ
Nickel	1.2 g	70 kg	20	0.292	0.0002
Zinc	1.2 g	70 kg	300	20.688	0.001
Chromium	1.2 g	70 kg	1.5	1.158	0.013
Copper	1.2 g	70 kg	40	22.304	0.009
HI [g]					0.023

[a] $\text{THQ} = \text{FIR} \times \text{C} / \text{RfD} \times \text{WAB}$.

[b] FIR: food ingestion rate of crab muscles in Egypt (1.2 g/day/person).

[c] W_{AB} : average adult body weight of consumers in Egypt (70 kg).

[d] RfD: oral reference dose.

[e] RfD: oral reference dose for Ni, Zn, Cr, and Cu as established by USEPA [27].

[f] C: metal concentration ($\mu\text{g}/\text{kg}$) in crab muscles on wet weight basis.[g] $\text{HI} = \text{THQ}_{\text{Ni}} + \text{THQ}_{\text{Zn}} + \text{THQ}_{\text{Cr}} + \text{THQ}_{\text{Cu}}$ [27].**Table 7:** THQ [a] of heavy metals with consumption of prawn muscles

Metals	FIR [b]	W_{AB} [c]	RfD [d, e] ($\mu\text{g}/\text{kg}/\text{day}$)	Prawn muscles	
				C [f]	THQ
Nickel	1.2 g	70 kg	20	0.373	0.0003
Zinc	1.2 g	70 kg	300	16.204	0.0009
Chromium	1.2 g	70 kg	1.5	0.844	0.009
Copper	1.2 g	70 kg	40	18.524	0.007
HI [g]					0.017

[a] $\text{THQ} = \text{FIR} \times \text{C} / \text{RfD} \times \text{WAB}$.

[b] FIR: food ingestion rate of prawn muscles in Egypt (1.2 g/day/person).

[c] W_{AB} : average adult body weight of consumers in Egypt (70 kg).

[d] RfD: oral reference dose.

[e] RfD: oral reference dose for Ni, Zn, Cr, and Cu as established by USEPA [27].

[f] C: metal concentration ($\mu\text{g}/\text{kg}$) in crab muscles on wet weight basis.[g] $\text{HI} = \text{THQ}_{\text{Ni}} + \text{THQ}_{\text{Zn}} + \text{THQ}_{\text{Cr}} + \text{THQ}_{\text{Cu}}$ [27].

According to the information in Table 6, the THQ of the investigated heavy metals was 0.0002, 0.001, 0.013, and 0.009 for nickel, zinc, chromium, and copper in the crab muscles, respectively. In addition, the calculated HI was 0.023. According to the information in Table 7, the THQ of the investigated heavy metals was 0.0003, 0.0009, 0.009, and 0.007 for nickel, zinc, chromium, and copper in the prawn muscles, respectively. Additionally, the calculated HI was 0.017.

RfD was used to evaluate the EDI of the heavy metals in the fish, which was estimated at 3, 20, 40, and 300 $\mu\text{g}/\text{kg bw}/\text{day}$ for chromium, nickel, copper, and zinc, respectively [27]. RfD represents an estimate of the daily exposure in the human population, which may be continual over a lifetime without any significant risk of deleterious effects [32].

The above-mentioned results could be used as an indicator for any further prospective changes that could occur regarding pollutions. The above-mentioned results

could be used as an indicator for any further prospective changes that could occur regarding pollutions. Lower doses than the RfD are not often associated with adverse health effects and are less likely to be of regulatory concern as well. As the frequency and/or magnitude of the exposures exceeding the RfD increases, the probability of adverse effects on the human population becomes higher. However, it should not be categorically concluded that all the doses below the RfD are acceptable or risk-free and all the doses above the RfD are unacceptable or exert adverse effects [27]. Several approaches to human exposure could be used to trace metallic elements, such as breathing and dermal exposure. However, food consumption is often regarded as one of the most important approaches in this regard.

4. Conclusion

According to the results, the low concentrations of the heavy metals in the crab and prawn muscles are important since muscles constitute the largest mass of consumed crabs and shrimp. Therefore, failure to control the exposure leads to severe complications in the future due to the adverse effects of heavy metals. Occupational exposure to heavy metals could be minimized by engineering solutions. Therefore, monitoring the exposure and interventions to reduce additional exposure to heavy metals in the environment and humans would be a momentous step toward the prevention of these hazards. Furthermore, National and international cooperation is essential to adopt appropriate tactics for the prevention of heavy metal toxicity.

Authors' Contributions

H.A.Z., A.H.M., S.E.H., and A.Y.E., conceived the presented idea, developed the theory, performed the computations, verified the analytical methods, and investigated and supervised the findings of this research. All the authors discussed the results and contributed to the final manuscript.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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The Faculty of Agriculture - Mansoura University certifies that the research under the title " Risk Assessment of Heavy Metal Bioaccumulation in Raw Crab and Prawn Flesh Market in Egypt " was conducted according to the ethics of scientific research and approval of the publication of the study. (Project No. 7239).

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