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Bio-Estimation of Selected Heavy Metals in Shellfish and Their Surrounding Environmental Media

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***Abstract*—**Due to the determination of the pollution status of fresh resources in the Egyptian territorial waters is very important for public health; this study was carried out to reveal the levels of heavy metals in the shellfish and their environment and its relation to the highly developed industrial activities in those areas. A total of 100 shellfish samples from the Rosetta, Edku, El-Maadiya, Abo-Kir and El-Max coasts [10 crustaceans (shrimp) and 10 mollusks (oysters)] were randomly collected from each coast. Additionally, 10 samples from both the water and the sediment were collected from each coast. Each collected sample was analyzed for cadmium, chromium, copper, lead and zinc residues using a Perkin Elmer atomic absorption spectrophotometer (AAS). The results showed that the levels of heavy metals were higher in the water and sediment from Abo-Kir. The heavy metal levels decreased successively for the Rosetta, Edku, El-Maadiya, and El-Max coasts, and the concentrations of heavy metals, except copper and zinc, in shellfish exhibited the same pattern. For the concentration of heavy metals in shellfish tissue, the highest was zinc and the concentrations decreased successively for copper, lead, chromium and cadmium for all coasts, except the Abo-Kir coast, where the chromium level was highest and the other metals decreased successively for zinc, copper, lead and cadmium. In Rosetta, chromium was higher only in the mollusks, while the level of this metal was lower in the crustaceans; this trend was observed at the Edku, El-Maadiya and El-Max coasts as well. Herein, we discuss the importance of such contamination for public health and the sources of shellfish contamination with heavy metals. We suggest measures to minimize and prevent these pollutants in the aquatic environment and, furthermore, how to protect humans from excessive intake.

***Keywords*—**Atomic absorption, heavy metals, sediment, shellfish, water.

I. Introduction

RECENTLY, marine environmental pollution with heavy metals (cadmium, chromium, copper, lead and zinc) has garnered public attention, especially in coastal areas. Metals generally enter the aquatic environment through atmospheric deposition, the erosion of a geological matrix or as by-products of anthropogenic activities, such as industrial effluents, domestic sewage and mining wastes [1].

Because of increased urbanization and industrialization, the anthropogenic inputs of metals currently exceed the natural inputs. Industrial waste, atmospheric deposition from crowded cities and other domestic waste are among the major sources of heavy metals in the surface water, ground water and soils [2]. The marine sediment in coastal areas or bays and seawater play an important role as an agrochemical discriminator [3]. Saxena and D'Suoza [4] reported that heavy metal pollution is of special concern because these metals are non-degradable and therefore persist in the ecosystem. The public health concerns related to shellfish consumption are prompted by the fact that they pump and filter large quantities of water as a part of their feeding process, which results in the rapid intake and concentration of heavy metals in their bodies [5]. Moreover, shellfish live near shores or at estuaries [6], which are exposed to many types of heavy metals, as well as on the surface of mud or sand, where high concentrations of heavy metals are present due to sedimentation [7]. Hence, shellfish may contain higher levels of heavy metals than are found in the water and sediment in which the shellfish grow and may thus constitute a public health hazard [8]. Moreover, heavy metals have a relatively high density and are toxic or poisonous even at low concentrations [9]. Additionally, mollusks (oysters) are frequently consumed raw or lightly cooked, and shellfish are consumed whole, including all organs, whereas only the muscle tissue of fish is consumed. Regarding the level of Egyptian natural pollution, very limited data concerning cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) in shellfish have been reported. Therefore, the aim of this study was to monitor the levels of selected heavy metals in certain shellfish at the Alexandria and El-Behera governorate beaches to ensure the safety of the shellfish.

II. Materials and Methods

A. Shellfish Samples

A total of 100 crustacean (shrimp) and mollusk (oyster) samples (10 of each) were randomly collected from the coasts of Rosetta, Edku, El-Maadiya, Abo-Kir and El-Max. Each sample was placed in a clean plastic bag. All samples were transported in a box of ice to the laboratory where they were examined for heavy metal residues in their soft tissues.

B. Sediment

According to Aboul Dahab and Halimm [10], the surface of the sea bottom sediment (5 cm in thickness) at the site of shellfish collection was collected and placed in a clean plastic bag. The samples were transported in a box of ice to the laboratory where they were analyzed for heavy metal residues.

C. Water

The water samples were taken from the same localities as the shellfish. Samples were collected manually from the

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middle of the water stream, 10 cm below the surface using polyethylene bottles. One liter of each sample was preserved with 10 ml of 6N nitric acid and stored at 5^{o}C. The water samples for heavy metal analysis were either filtered through Whatman filter paper No.1, and 1000 ml of the filtered samples were acidified to pH 2 with 20 ml of 6 N HNO_3, or after extraction in methyl-isobutyl-ketone (MIKB). Standard solutions for Zn, Cu, Pb, Cr and Cd were prepared according to the analytical methods for atomic absorption spectrophotometry.

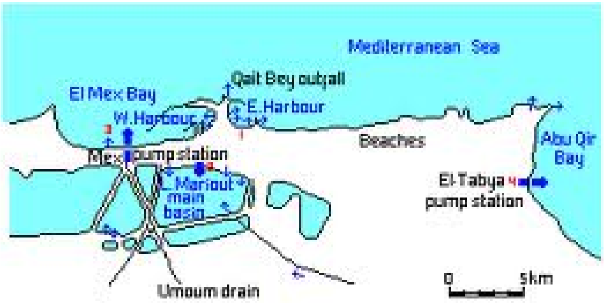

Fig. 1 Map Showing the Locations of Study Area

D. Preparation of Samples

These samples were kept in a deep freeze (-21°C) until analysis and prepared according to international standard methods [11]. The samples were weighed and digested with conc. HNO_3: $HClO_4$ (5:1) (extra pure Merck) under reflux and filtered. Sediments were collected with a shovel. Each sediment sample oven dried at 60°C for 24 h and digested with conc. HCl: HNO_3 (3:1) (extra pure Merck) under reflux and then filtered through Watman 40 filter paper. All samples were diluted with bidistilled water and analyzed.

E. Digestion, Analysis and Calculation

The muscle samples were taken (approximately 3-5 g) from the dorso-lateral surface of each fish and weighed. The analysis and calculations were carried out according to the methods used in EOSQC 1806 [12]. Each collected sample was analyzed for cadmium, chromium, copper, lead and zinc residues using a Perkin Elmer atomic absorption spectrophotometer 603 (AAS).

III. Results and Discussion

The extent of occurrence or accumulation of heavy metals by organisms in different tissues is dependent on the route of entry, that is, either from surrounding medium or in the form of food or chemical form of material available in the media [13]. Heavy metals may be accumulated by shrimps either through food or water [14].

As presented in Tables I and II, the highest cadmium levels were found in the shrimp and oysters from Abo-Kir and decreased successively for the Rosetta, Edku, El-Maadiya, and El-Max coasts. These cadmium levels directly reflect the degree of environmental contamination because the highest cadmium levels in the water and sediment were found at the Abo-Kir coast and then decreased successively for the Rosetta, Edku, El-Maadiya, and El-Max coasts. The cadmium levels in all examined fish samples were lower than the Egyptian permissible limit (0.1 ppm), as recommended by the Egyptian Organization for Standardization and Quality Control [15] and the World Standard [16], which states that the cadmium level should be below 0.5 mg/kg. Higher cadmium concentrations were obtained by Jursa and Blanosa [8], who reported 0.42±0.017 mg/kg fresh weight. The hazards of cadmium as a food contaminant are renal dysfunction, lung impairment, anemia and prostate cancer and arise from long-term exposure to cadmium [17]. Furthermore, bone changes may occur from bone diseases such as Ita Ita disease, which is a syndrome that affected a man in Japan in 1964 after the ingestion of rice that was heavily contaminated with cadmium [18]. In 1993, the joint FAO/WHO Expert Committee on Food Additives (JECFA) established a PTWI for cadmium of 7 µg/kg bwt. Additionally, the JECFA estimated that the dietary intake of cadmium is typically 1-4 µg/kg bwt/week and recognized that there is a relatively small safety margin between exposure in the normal diet and exposure that produces deleterious effects. Consequently, the JECFA recommended that the cadmium levels in foods and total diets should continue to be monitored and should not undergo further increases [19]. The results of a study carried out by the European Committee in 1995 and 1996 indicated that dietary exposure to cadmium in the 15 states of the EU ranged from 0.007-0.057 mg/kg/day [20]. Dietary exposure estimates have also been reported for the USA at 0.015 mg/day and Canada at 0.024 mg/day [21], France at 0.02 mg/day [22] and Egypt at 0.24 mg/day [23].

The highest chromium levels were present in the water and sediment from the Abo Kir coast, and the levels decreased successively at the Rosetta, Edku, El-Maadiya, and El-Max coasts. These levels resulted in higher chromium concentrations in the shrimp and oysters from Abo Kir, which then decreased according to the aforementioned order. This finding suggests a more extensive contamination of the shellfish growing at the Abo Kir coast. These high chromium levels in the water and sediment from the Abo Kir shellfish habitat may arise from industrial sources, such as the textile, ceramic, dye and fertilizer companies that dominate the Abo Kir bay, as well as from sewage sludge, which contains chromium levels as high as 900 mg/kg. Moreover, at the Rosetta coast, high chromium contamination may be attributable to its location as the furthest eastern part of the Abo Kir bay; as a result, it receives different types of pollutants from the vast quantity of agricultural fresh water that is drained from Edku Lake via Bughas El-Maadiya and the industrial pollutants that are discharged through the El Tabia pumping station from the dye and textile companies at Kafr El Dawar. In all examined shellfish samples from all coasts, the level of chromium was lower than the standard permissible limit of 5.5 µg/g wet weight [24]. Chromium is currently banned in seafood in developed countries; however, the results presented herein do not suggest that chromium is currently a threat to public health because the values are below the FAO/WHO limit (13.0 mg/kg tissues) [16]. Because of the carcinogenicity of hexavalent Cr to humans and other mammals and the prevalence of its use in the leather and textile industries, there is significant justification to continue the monitoring of shellfish for this metal. The clinical features of cadmium toxicity are vomiting, diarrhea, hemorrhagic diathesis and blood loss into the gastrointestinal tract causing cardiovascular shock. If the patient survives approximately 8 days, the lasting effects are necrosis and enlargement of the liver and edema in the brain [25]. Additionally, a number of effects can result from occupational exposure to foodborne chromium, including irritative lesions of the skin and upper respiratory tract as well as allergic lesions and cancer of the respiratory tract. The data concerning other effects in the gastrointestinal, cardiovascular, and urogenital systems were insufficient for evaluation.

The highest copper concentrations were found in the water and sediment from Rosetta, and the copper level decreased successively at the Edku, El-Maadiya, Abo-Kir and El-Max coasts. Reflecting the degree of environmental contamination, the contamination levels in the shellfish exhibited the same order. The copper level of all examined shellfish samples was lower than the permissible limit (20 ppm) recommended by the EOSQC [15]. The maximum acceptable daily load is 0.05-0.5 mg/kg bwt [26]. Copper is an essential element for humans because it is necessary for iron utilization to promote hematopoiesis and is essential for the biological activity of many enzymes [27]. Both deficiency and excess of copper in mammalian systems result in undesirable effects [28]. The symptoms of copper poisoning include nausea, vomiting, diarrhea, hematemesis and jaundice. Chronic diseases from excessive copper storage are epitomized by Wilson's disease, which is characterized by excessive copper deposition in most organs, especially the liver, kidneys, brain and eyes. This disease also inhibits the liver alkaline phosphatase and catalase enzymes and initially induces hemolytic anemia as well as Monday morning fever [29].

The water and sediment of the Rosetta coast presented the highest lead levels. The levels of this metal decreased successively at the Edku, El-Maadiya, Abo-Kir and El-Max coasts, and the contamination levels in the shellfish from each coast exhibited the same pattern, reflecting the degree of environmental contamination. All examined shellfish samples were lower than the permissible limit (0.1 ppm) recommended by the EOSQC [15]. Higher lead levels were previously obtained by Jursa and Blanosa [8] in the *Elliptio complanata* bivalve, while significantly higher levels were detected by Fakhry and Eissa [30] from the tissues of Egyptian shellfish.

Over time, considerable quantities of lead have been mobilized into the environment. The primary sources of lead are industrial smelters, mining, refining, discarded batteries, the burning of garbage and wood with old paint, the petrochemical industry, cement companies, and oil pipe terminals. Additionally, excessive fuel dust from traffic near the coasts can lead to air pollutants and lead in the water and sediment from the coasts. The lead derived from all previously mentioned sources contributes not only to intake through inhalation but also to intake through ingestion as a result of fallout from vehicle exhaust onto nearby food crops [31]. Lead is recognized as a known neurotoxic substance and is a major public health concern that causes both acute and chronic intoxication [32]. It also causes encephalopathy in children [33]. The provisional weekly intake of lead in food must not exceed 0.05 ppm, as recommended by WHO [34]. However, Carl [33] postulated that the acceptable limit ranges from 0.05-0.2 ppm.

The highest zinc levels were found in the water and sediment from the Rosetta coast, and the zinc levels decreased successively for the Edku, El-Maadiya, Abo-Kir and El-Max coasts. The same pattern was detected with regard to the concentrations in the shellfish from these coasts. The higher levels at the Rosetta coast may be attributable to the fact that dissolved zinc levels in small rivers are ten-fold higher than those in marine estuaries [35]. The Nile River sediment contains 108.8 µg/g of zinc [36], which is discharged through water and sediment influx onto the Rosetta coast [37]. Additionally, agricultural drainage and sewage are important sources of zinc [38]. However, the level of zinc in all examined shellfish from all coasts is lower than the permissible limit recommended by the FDA [39], which is 50 ppm.

The zinc concentration in forage plants, feed, and water as well as pasture silage ranged between 17-60 ppm, but industrial fallout can increase these values. Zinc was also present in some pesticides and fungicides [40].

Zinc is an essential trace element for plant, animal and human life. It is found in all natural water and soils as well as the atmosphere. It is present at 76 ppm in the Earth's crust and, in normal soils, averages approximately 50 ppm [41].

The American daily intake (ADI) of zinc is approximately 12.6 mg, which is mostly derived from food [42]. The maximum acceptable daily load is 0.3-1.0 mg/kg bwt, and zinc toxicity from excessive ingestion is uncommon, although gastrointestinal disturbances and diarrhea have been recorded [43]. The highest level of heavy metal residue in shellfish was detected for zinc, and the levels decreased successively for copper, lead, cadmium and chromium.

TABLE I
MEAN VALUES ± S.E. OF CADMIUM, CHROMIUM, COPPER, LEAD &ZINC (PPM) IN EDIBLE PORTION OF SHELLFISH AS WELL AS WATER AND SEDIMENT OF THE ROSETTA, EDKU AND EL-MAADIYA COAST

Samples	Cadmium	Chromium	Copper	Lead	Zinc
Rosetta coast					
Water	0.026±0.001	0.02±0.006	0.21±0.002	0.004±0.003	0.65±0.004
Sediment	0.095±0.003	0.09±0.002	0.42±0.003	0.03±0.035	5.34±0.62
Crustaceans (Shrimp)	0.017±0.002	0.72±0.051	3.85±1.0	0.017±0.01	7.21±2.11
Mollusca (Omel-Kholoul)	0.018±0.002	0.75±0.062	4.72±1.12	0.018±0.021	8.11±2.41
Edku coast					
Water	0.021±0.001	0.07±0.004	0.18±0.01	0.003±0.002	0.37±0.003
Sediment	0.073±0.002	0.21±0.002	0.51±0.02	0.07±0.03	4.97±0.8
Crustaceans (Shrimp)	0.015±0.002	0.65±0.05	2.15±0.14	0.016±0.002	6.34±1.51
Mollusca (Omel-Kholoul)	0.017±0.003	0.68±0.05	3.24±0.15	0.017±0.003	7.21±1.13
El-Maadiya coast					
Water	0.012±0.001	0.05±0.003	0.15±0.012	0.002±0.001	0.29±0.002
Sediment	0.019±0.002	0.10±0.005	0.72±0.021	0.06±0.003	4.15±0.9
Crustaceans (Shrimp)	0.014±0.002	0.61±0.015	1.65±0.14	0.13±0.002	5.34±1.5
Mollusca (Omel-Kholoul)	0.015±0.003	0.064±0.01	2.94±0.17	0.014±0.004	6.72±1.8
Permissible Limit (EOSQC,1993)	0.1	5.5	20	0.1	50

TABLE II
MEAN VALUES ± S.E. OF CADMIUM, CHROMIUM, COPPER, LEAD &ZINC (PPM) IN EDIBLE PORTION OF SHELLFISH AS WELL AS WATER AND SEDIMENT OF THE ABO-KIR AND EL-MAX -COAST

Samples	Cadmium	Chromium	Copper	Lead	Zinc
Abo-Kir coast					
Water	0.012±0.001	0.16±0.02	0.13±0.012	0.002±0.001	0.29±0.002
Sediment	0.014±0.002	0.91±0.07	0.25±0.022	0.05±0.004	4.14±0.03
Crustaceans (Shrimp)	0.012±0.002	0.91±0.091	1.36±0.23	0.012±0.001	6.22±1.6
Mollusca (Omel-Kholoul)	0.013±0.002	0.87±0.04	2.23±0.22	0.014±0.002	6.93±1.7
El-Max -coast					
Water	0.011±0.001	0.031±0.001	0.12±0.002	0.002±0.001	0.21±0.002
Sediment	0.012±0.002	0.14±0.002	0.96±0.004	0.04±0.003	3.92±0.3
Crustaceans (Shrimp)	0.011±0.002	0.55±0.12	1.21±0.02	0.012±0.002	4.22±0.82
Mollusca (Omel-Kholoul)	0.012±0.002	0.57±0.17	1.76±0.04	0.013±0.003	5.65±1.22
Permissible Limit (EOSQC,1993)	0.1	5.5	20	0.1	50

In conclusion, follow-up studies of these toxic heavy metal residues in both shellfish and their environments must be continued and intensified to reduce and/or avoid hazards for the population and to minimize metal release into the environment.

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