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Detection of Heavy Metals in Some Fish and Shellfish Marketed in Egypt: Bioaccumulation, Dietary Intake and Health Risk Assessment

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Abstract

IN MANY parts of the world particularly Egypt, fish and shellfish are widely consumed because of their palatability and nutritive value. Despite that, it can be contaminated by chemical residues particularly, heavy metals which accumulate in organs and muscles resulting in various adverse health effects on consumers. A total of 105 samples of tilapia, mullet, and shrimp muscles were purchased from various markets in Damietta Province, Egypt to detect their contamination by lead (Pb), cadmium (Cd), copper (Cu), and mercury (Hg) using atomic absorption spectrophotometer as well as determination of the estimated daily intake, hazard quotient and hazard index to the detected metals. The obtained results revealed that Pb, Cd, Cu, and Hg were detected in 41.9%, 24.8%, 100%, and 27.6% of the examined fish and shellfish muscles, respectively. The mean concentrations (mg/kg) of pb in tilapia, mullet and shrimp muscles were 0.40±0.11, 0.20±0.06, and 0.22±0.06, respectively; while for Cd the concentrations were 0.03±0.01, 0.01±0.01, and 0.03±0.01, respectively; furthermore 4.67±0.56, 3.29±0.16, and 4.99±0.32 were detected for Cu respectively, and they were 0.59±0.14, 0.08±0.05, and 0.18±0.08 for Hg, respectively. The examined fish and shrimp muscle samples were accepted in varying proportions for their concentration of Pb (65.7%), Cd (82.9%), Cu (100%), and Hg (73.3%). Health risk assessment of the detected heavy metals illustrated that the hazard index was 0.99, 0.21, and 0.39 for tilapia, mullet and shrimp, respectively. Therefore, consumption of these samples may have no health hazard effect on consumers because the HI is less than 1.

Keywords: Fish, Shellfish, Heavy metals, Bioaccumulation, Health hazards.

Introduction

Fish and shellfish are very important sources of high-quality protein, minerals, vitamins and lipids particularly omega-3 [1] which are incorporated into the cell membranes phospholipids and are important for the functions of heart, cardiovascular system and brain in addition to its beneficial role in controlling the inflammatory conditions [2]. Nile tilapia (*Oreochromis niloticus*) is one of the most popular fish species in Egypt originating from the Nile River. Because of its nutritive value and affordable price for everyone, it is highly consumed by Egyptians [3]. It has a calorie content of 1kcal/g, protein (18.46%), amino acids (21.56%), calcium content (74.58 mg/100 g) and 30.39% total fatty acids consisting of saturated fatty acids and unsaturated fatty acids [4]. Grey mullet (*Mugil cephalus*) is found mainly in the coastal water of many tropical and subtropical areas along the coast in estuaries and freshwater [5]. Globally, Egypt is the first country in the world to produce the mullet [6,7]. Mullet is rich in protein (18.5%), saturated fatty acids (36.9%), mono unsaturated fatty acids (22.6%), poly unsaturated fatty acids (33%) [7].

In Egypt, the Mediterranean coast is very rich with shrimp resources belonging to the family Penaeidae. *P. semisulcatus* is rich in protein (38.45%), carbohydrates (3.1%), fat (11.01%), in

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addition to 12 amino acids (9 essential and 3 non-essential) as well as vitamin B1 and B2 (0.282 and 0.351 mg/ 100g, respectively) [8]. Also, shrimp is a main source of various minerals such as calcium, phosphorus, potassium, zinc and iron [9]. Despite these nutritive values, toxic heavy metals reach to the aquatic environment from natural and anthropogenic sources; then absorbed by fish [10]. Fertilizers, pesticides, weathering, industries wastes and earth crusts are the principal sources of these metals [11].

Accumulation of toxic metals in fish varies depending on sex, age, species and size [12] as well as the activity of organs [13]. The toxic effect of heavy metals on kidneys includes hemorrhage, glomerular inflammation, connective tissue formation, necrosis, and tubular damage, whereas no damage to the fish muscles occur [14]; meanwhile its effect on liver includes inflammation, hemorrhage, degeneration, and necrosis [15]. Histo-pathological examination of gills revealed many alterations which include edema, cellular hypertrophy and lamellar fusions [16].

Public awareness considers exposure of fish to different contaminants which accumulate in fish muscles and organs is of great concern and has an effect on fish consumption [17]; resulting in bioaccumulation of these toxic metals in human body causing serious health problems such as malformations, neuromuscular disorders, mental retardation, kidney dysfunction, and cancers [18].

Lead (Pb) has hepatotoxic effect on liver tissue and increases the liver function test parameters [19]. Cadmium (Cd) is toxic and can cause damage to different organs including liver, kidney, spleen, testis and bone. Its toxic effect on liver includes elevation of liver enzymes, increase in urea and cholesterol levels [20], meanwhile on kidney; cadmium has nephrotoxic effect causing damage to the kidney tubules [21]. Furthermore, many compounds of cadmium such as chloride, oxide, and sulphate compounds have carcinogenic effect [22]. Copper (Cu) is an essential trace element; while a high dose is toxic to liver causing damage to liver tissue in different forms including cellular degeneration and hepatic necrosis as well as many adverse effects on the lining epithelium [23,24]. Mercury (Hg) exists in two forms; mercuric chloride is the inorganic form as well as methyl and ethyl mercury which are the organic forms of Hg that are toxic and have different implications on health. Fish and shellfish contaminated by toxic heavy metals are the principal sources of human exposure to methyl Hg [25].

Recently heavy metals in fish are of great concern due to its health and economic impacts because these metals contaminate the aquatic environment and subsequently accumulate in fish muscles and organs resulting in many health problems to consumers [26]. Therefore, our study aimed to detect Pb, Cd, Cu and Hg in *O. niloticus, M. cephalus*, and *P. semisulcatus* as well as determination of the daily intake from these metals and some hazardous indicators to these heavy metals.

Material and Methods

Samples collection

One hundred and five samples of tilapia (*O. niloticus*), mullet (*M. cephalus*) and shrimp (*P. semisulcatus*), 35 from each sample were collected randomly from many fish markets at Damietta Province, Egypt. Samples were kept in the refrigerator till measurements completed in the Animal Health Research Institute, Dokki, Giza, Egypt.

Samples preparation

From each fish and shellfish sample, one gram of muscle was taken to be digested by addition of 5 ml of the following mixture; 3 ml nitric acid in a concentration of 65% and 2 ml perchloric acid in a concentration of 70%. After that the test tubes were left at room temperature for twelve hours; then incubated at 70°C for three hours in hot water bath with regular stirring every thirty minutes. After digestion and at room temperature, the samples were diluted by addition of 20 ml double distilled water then filtered using filter papers. At room temperature, the filtrated tubes were kept till measurement of heavy metals as reported by Darwish et al. [27,28].

Estimation of toxic heavy metals.

Atomic absorption spectrophotometer (AAS), AA240 FS (VARIAN, Australia) was used to measure Pb, Cd and Cu using the graphite furnace atomic absorption spectroscopy, meanwhile, Hg was measured by the hydride vapour atomic absorption spectroscopy [22]. The conditions recommended by the instrument instruction was tabulated in Tabulated in (Table 1). Absorbency of Pb, Cd, Cu and Hg was recorded from the digital scale and its concentration was calculated according to the following equation:

$$C=R \times (D/W)$$

Where, C is the concentration of heavy metal (wet weight); R Reading of digital scale of AAS.; D Dilution of the sample.; W= Weight of the sample.

Determination of the estimated daily intake (EDI)

The estimated daily intake for the detected toxic metals was estimated based on this following equation [29]:

$$EDI= \frac{Cm \ x \ FIR}{BW}$$

Where; (FIR) is the fish intake rate (48.57 g/day) by the Egyptians according to FAO [6]; (Cm) is the concentration of heavy metal; and (BW) is the adult Egyptian body weight (70 kg).

Evaluation of health risks

Health risks were assessed by determination of the non-carcinogenic effect of the measured heavy metal which was expressed by hazard quotient (HQ) based on the following equation [29]:

$$HQ = \frac{EDI}{RFD}\ x\ 10-3$$

Where; (RFD mg/kg/day) is the reference dose; its value for lead, cadmium, copper, and mercury are 0.004, 0.001, 0.04, and 0.0005, respectively [29].

Furthermore, hazard index (HI) was calculated to assess non-carcinogenic effects of mixed metals [30]. $HI = \sum HQi$

Where; (i) is the HQ for each metal. When HQ and HI values are higher than 1, there are possible risks on human health, but there are no health risks when the value is less than 1 [30].

Statistical analysis

It was conducted by SPSS-21; Duncan's multiple range test was used to compare the samples by one-way ANOVA (SAS Institute, Cary, NC). Statistical significance was considered at $p < 0.05$. The data were presented as means ± standard error (S.E.).

Results

Results tabulated in (Table 2) revealed that Pb, Cd, Cu and Hg were detected in the examined fish and shellfish muscle samples in a prevalence of 41.9%, 24.8%, 100% and 27.6%, respectively. It was found that Pb was detected in 51.4%, 34.3% and 40% of *O. niloticus, M. cephalus* and *P. semisulcatus*, respectively, while Cd was detected in 28.6%, 20% and 25.7% of *O. niloticus, M. cephalus* and *P. semisulcatus*, respectively (Table 2). In a prevalence of 45.7%, 14.3%, and 22.9%, Hg was found in *O. niloticus, M. cephalus* and *P. semisulcatus, respectively*, meanwhile, Cu was detected in all examined muscle samples (Table 2).

Concentration of Pb in *O. niloticus, M. cephalus* and *P. semisulcatus*, varied from 0.06-1.3, 0.03-0.96 and 0.14-1.33, respectively; with a mean value of 0.40±0.11, 0.20±0.06 and 0.22±0.06 (mg/kg), respectively; while Cd level (mg/kg) ranged from 0.03-0.13, 0.01-0.06 and 0.03-0.29 with mean concentrations of 0.03±0.01, 0.01±0.01 and 0.03±0.01, respectively as found in (Figure 1). There were no statistical significance differences (p>0.05) between the examined muscle samples in their concentrations of Pb and Cd by one-way ANOVA. Furthermore, Cu concentrations ranged from 1.50-14, 2.20-5.30 and 1.10-8.9 mg/kg, with mean concentrations of 4.67±0.56, 3.29±0.16 and 4.99±0.32 mg/kg, while Hg concentrations ranged from 0.38-2.45, 0.22-1.54 and 0.38-1.89 mg/kg with mean concentrations of 0.59±0.14, 0.08±0.05 and 0.18±0.08 mg/kg in *O. niloticus, M. cephalus* and *P. semisulcatus,* respectively as illustrated in (Figure 1). Concerning to Cu concentration, there were significance differences between the examined samples (p<0.05), with individual variation between *O. niloticus and M. cephalus* as well as between *M. cephalus and P. semisulcatus* by one-way ANOVA; meanwhile, a significance difference at p<0.05 was detected between *O. niloticus* and *M. cephalus* as well as *P. semisulcatus* by one-way ANOVA.

As recorded in (Table 3), the acceptability *O. niloticus, M. cephalus* and *P. semisulcatus* for their contents of Pb were 57.1%, 71.4% and 68.6%, respectively; Cd were 74.3%, 94.3% and 80%, respectively; Hg were 54.3%, 88.6% and 77.1%, respectively; and Cu were 100% for all samples with a total acceptability of 65.7%, 82.9%, 100% and 73.3% for Pb, Cd, Cu and Hg, respectively

The EDI and HQ of fish and shellfish muscle samples according to their heavy metals were illustrated in (Table 4). The EDI (µg/ kg/ day) from Pb, Cd, Cu and Hg was 0.278, 0.021, 3.24 and 0.409, respectively for *O. niloticus*; 0.13, 0.007, 2.28 and 0.056, respectively for *M. cephalus*; and 0.153, 0.021, 3.46 and 0.123, respectively for *P. semisulcatus*. Furthermore, the HQ due to Pb, Cd, Cu and Hg contamination to *O. niloticus* was 0.070, 0.021, 081 and 0.818, while it was 0.033, 0.007, 0.057 and 0.112 to *M. cephalus* and 0.038, 0.021, 0.087 and 0.246 to *P. semisulcatus*. As shown in (Figure 2), the calculated HI due to heavy metal in *O. niloticus, M. cephalus and P. semisulcatus* was 0.99, 0.21 and 0.39, respectively.

Discussion

The present study threw a light on the most commonly consumed species of fish (tilapia and mullet) and shellfish (shrimp) in Egypt to evaluate their contamination by heavy metals and its impact on consumer health. The examined samples were highly contaminated by Pb compared to other heavy metals. Generally, Pb was detected at highest levels in *O. niloticus* followed by *P. semisulcatus*, while *M. cephalus* had the lowest level of Pb contamination. The mean concentration of Pb varied between the examined samples; *O. niloticus* was the highest concentrations followed by *P. semisulcatus*, while, *M. cephalus* was the lowest in Pb concentrations. This result disagreed with another study conducted by Ighariemu et al. [31] reported higher concentration of Pb (0.53± 0.03 mg/kg) in shrimp as well as Ali et al. [25] who reported 0.3226 mg/kg Pb in *M. cephalus*. While lower concentration (0.03, 0.12, 0.02, 0.26) was reported in Indonesia, Palestine, India, and Sri Lanka by Takarina et al. [32], Elnabris et al. [33], Damodharan and Reddy [34] and Jinadasa and Edirisinghe [35] in tilapia. According to the Egyptian Organization for Standardization (EOS) [36] which stated not more than 0.1 mg/kg as the maximum limit of Pb in fish and shellfish muscles, most of the examined samples (65.7%) was accepted especially *M. cephalus.*

Exposure of fish and shellfish to high doses of Pb results in damages in the blood and nerve cell through inhibition of three important enzymes [37]. Furthermore, Pb can cause nephrotoxicity, hepatotoxicity, neurotoxicity and various other adverse health effects in humans [38].

M. cephalus had the lowest level of Cd contamination followed by *P. semisulcatus*. The mean concentration of Cd in *O. niloticus* was the same as in *P. semisulcatus*; but *M. cephalus* had the lowest concentration of Cd. Nearly similar concentrations were reported by Hassan et al. [22], Jinadasa and Edirisinghe [35] in Sri Lanka and Lin et al. [39] in Taiwan and Kong et al. [40] in China. Higher concentration of Cd (0.0518 and 0.04628) was reported in tilapia and mullet by Ali et al. [25]. Compared to other studies world-wide, higher concentrations of Cd in tilapia (0.14, 1.7 and 0.09) were reported in Iran, Nigeria and China by Hemmatinezhad et al. [41], Regina and Kingsley [42], Lin et al. [39]. According to EOS [36] which stated that the permissible limit of Cd in muscles must not exceed 0.05 mg/kg, most of the examined samples were accepted. Naturally, Cd is found in the environment due to activities of the industrial and agricultural sectors; but prolonged exposure to it can cause osteoporosis and disturbances in kidney function, as well as many types of cancer [43,44].

Copper is an essential trace element that naturally present in living organisms but in high levels it can cause toxicity to different organs and tissues [45]. In our study, muscle samples of *P. semisulcatus* had the highest concentration of Cu followed by *O. niloticus*; meanwhile, *M. cephalus* had the lowest concentration. Nearly similar result of Cu in shrimp and *O. niloticus* was reported by Mortuza and Al-Misned [46], and Hassan et al. [22], respectively. Lower concentrations (2.497 and 0.229 mg/kg) were reported in *O. niloticus* and *M. cephalus* by Ali et al [25]. Also, Al-Weher [47] reported 2.9 ± 0.3 mg/kg in *O. niloticus* and Kaoud and El-Dahshan [48] reported 2.5 ± 0.05 mg/kg in tilapia. While elevated concentration (5.4, 6.19, 16.2 mg/kg) were reported in Turkey, Indonisia, Bangladesh in shrimp by Turkmen et al. [49], Takarina et al. [32] and Abdel-Baki et al. [50]. Muscle under examination didn't exceed the EOS [36] limit, which stated that the maximum permissible limit of Cu in fish and shellfish muscles shouldn't more than 15 mg/kg, so all samples were 100% accepted for Cu. The principal sources of contamination by copper are uses of copper compounds in fungicides, pesticides, and algicides in soil treatment [32].

The highest level of contamination by Hg was found in *O. niloticus* followed by *P. semisulcatus*; while *M. cephalus* had the lowest level of Hg contamination. The mean concentration of Hg varied in the examined samples; *O. niloticus* was highly contaminated followed by *P. semisulcatus* and *M. cephalus*. Nearly similar result to Hg concentration in *M. cephalus* was reported by Ali et al. [25]. Higher results (0.89±0.01, 1.18±0.12 and 0.94±0.10) were obtained by Shokr et al. [51], Hamada et al. [52] and Shaltout et al. [53]. Lower results (0.105±0.005, 0.003±0.002, and 0.013± 0.001) were recorded by Marzouk et al. [54], Abdel-Baki et al. [50] and Hashim et al. [55]. According to EOS [36] permissible Hg limit (0.02 mg/kg), most of the examined samples (73.3%) were accepted. One of the most widespread contaminants in aqua culture is mercury. Its organic form is the most hazardous form; it can cause neurotoxicity [56]; in addition to bioaccumulation in different tissues and organs [57].

Sewage and untreated discharges from both industrial and agricultural sector as well as misuse of chemicals, herbicides and insecticides are the primary sources of aquatic environment contamination. The variation of toxic metal in fish and shellfish muscle samples could be attributed to the difference in tissue accumulation. Also, many factors including size, feeding, and ecological zone can affect the heavy metal accumulation [58].

In his study some of the examined samples exceeded the MPL according to EOS [36]; therefore, the EDI, HQ, and HI were calculated to evaluate the expected health hazardous effect of consumption of these fish species on human. The calculated HQ value for each of the detected heavy metal revealed values lower than one, also, the HI for the examined samples was below one; therefore, no possible adverse health effects were related to these metals from the examined fish and shellfish. This result conceded with what had been reported by Morshdy et al. [30]

Conclusion

This study aimed to detect some heavy metals in the highly consumed fish and shellfish species in Egypt as well as determination of some hazardous indicators to these heavy metals. Concerning to the previous results lead, cadmium, copper and mercury were found in fish and shellfish at variable levels (65.7%, 82.9%, 100% and 73.3%). Some of the samples were contained by heavy metals above the maximum permissible levels, particularly Pb (34.3%) and Hg (26.7%). There were no expected public health hazards calculated for the Egyptians after computing EDI, HQ, and HI because HI was lower than 1 in the examined samples. Due to the bioaccumulations and biomagnifications nature of heavy metals, it is necessary to take care about these presumptions. Strict hygienic measures should be considered by the responsible authorities to eliminate the environmental pollution and increase citizens' awareness.

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Conflict of Interest

There is no conflict of interest.

TABLE 1. Instrumental parameters and instructions for Flame Atomic Absorption Spectrophotometer

Condition	Pb	Cd	Cu	Hg
Lamp wave length (nm)	283.3	228.8	324.8	253.7
Lamp current (mA)	3	2	10	18
Slit width (nm)	0.7	0.7	0.5	0.7
Detection Limit (DL)	0.05	0.01	0.6	0.05

C=R x (D/W)

Where, C is the concentration of heavy metal (wet weight); R Reading of digital scale of AAS.; D Dilution of the sample.; W= Weight of the sample.

TABLE 2. Incidence of samples contaminated by heavy metals (N=35 of each).

Samples	Pb	Cd	Cu	Hg
O. niloticus	18(51.4%)	10(28.6%)	35(100%)	16(45.7%)
M. cephalus	12(34.3%)	7(20%)	35(100%)	5(14.3%)
P. semisulcatus	14(40%)	9(25.7%)	35(100%)	8(22.9%)
Total	44(41.9%)	26(24.8%)	105(100%)	29(27.6%)

N: Number of examined samples.

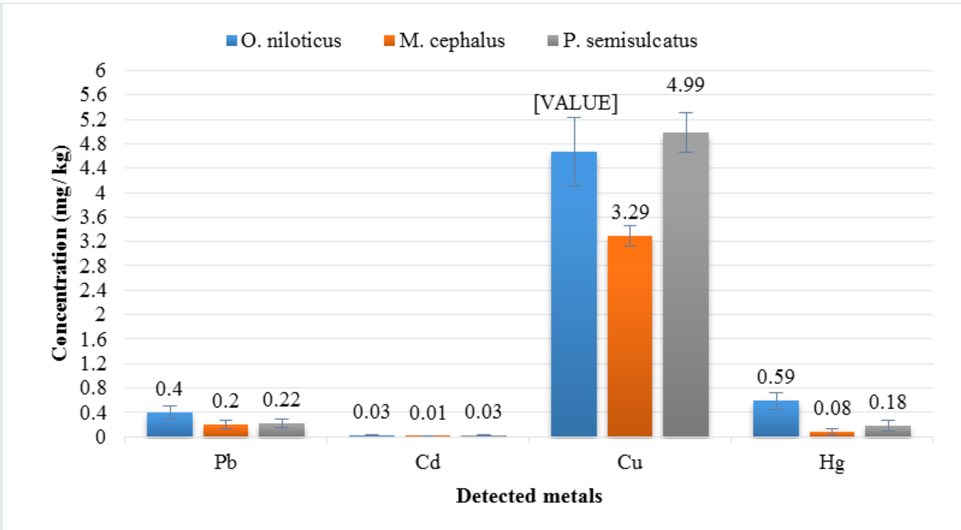

Fig. 1. Concentration of Pb, Cd, Cu and Hg (mean±S.E.) in the examined fish and shellfish samples (N=35).

TABLE 3. Accepted samples (N=35) according to their contents of heavy metals.

Fish species	MPL (mg/kg)	*O. niloticus*	*M. cephalus*	*P. semisulcatus*	Total
Pb	0.1	20(57.1%)	25(71.4%)	24(68.6%)	69(65.7%)
Cd	0.05	26(74.3%)	33(94.3%)	28(80%)	87(82.9%)
Cu	15	35(100%)	35(100%)	35(100%)	105(100%)
Hg	0.2	19(45.3%)	31(88.6%)	27(77.1%)	77(73.3%)

N: Number of examined samples.

MPL: Maximum Permissible Limit according to EOS [26].

TABLE 4. Estimated daily intake (EDI) and hazard quotient (HQ) of the detected heavy metals.

Samples	Hazard assessment	Pb	Cd	Cu	Hg
O. niloticus	EDI (µg/kg/day)	0.278	0.021	3.24	0.409
	HQ	0.070	0.021	0.081	0.818
M. cephalus	EDI	0.13	0.007	2.28	0.056
	HQ	0.033	0.007	0.057	0.112
P. semisulcatus	EDI	0.153	0.021	3.46	0.123
	HQ	0.038	0.021	0.087	0.246

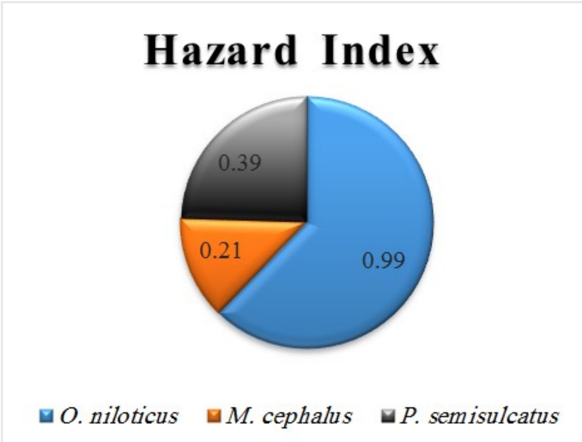

Fig. 2. Hazard index of the detected heavy metals.

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بقايا المعادن الثقيلة في بعض الأسماك وقشريات الأسماك المسوقة في أسواق السمك المصرية

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الملخص

هدفت هذه الدراسة إلي تقييم مدي تواجد المعادن الثقيلة وخصوصا الرصاص والكادميوم والنحاس والزئبق في بعض الأسماك (البلطي والبوري) وقشرياتها (الروبيان)، بالإضافة إلي دراسة معدل الإستهلاك اليومي والمخاطر الصحية الناتجة عن إستهلاكها. لذلك تم تجميع عدد 105 عينة من كل من أسماك البلطي والبوري والجمبري السويسي بواقع 35 عينة من كل نوع من أسواق السمك المختلفة بمحافظة دمياط ونقلها إلي معهد بحوث صحة الحيوان بالدقي- الجيزة- مصر لدراسة تركيز المعادن الثقيلة بها. أوضحت النتائج أن 41.9% و 24.8% و 100% و 27.6% من العينات تحتوي علي متبقيات من الرصاص والكادميوم والنحاس والزئبق علي الترتيب. كان متوسط تركيز الرصاص والكادميوم والنحاس والزئبق 0.11±0.40 و 0.01±0.03 و 0.56±4.67 و 0.59 ±0.14 مجم لكل كجم علي الترتيب في عينات البلطي، بينما كانت 0.06±0.20 و 0.01±0.01 و 0.16±3.29 و 0.05±0.08 مجم لكل كجم علي الترتيب في عينات البوري، كما كانت أيضا 0.06±0.22 و 0.01±0.03 و 0.32±4.99 و 0.08±0.18 مجم لكل كجم علي الترتيب في عينات الجمبري السويسي. طبقا للمواصفة القياسية المصرية لعام 2010، كانت العينات مقبولة بنسبة 65.7% و 82.9% و 100% و 73.3% في محتواها من الرصاص والكادميوم والنحاس والزئبق علي الترتيب. كما أظهرت النتائج أن حاصل الخطر المستهدف لكل من الرصاص والكادميوم والنحاس والزئبق في العينات أقل من الواحد، كما أن مؤشر الخطر الناتج عن إستهلاك تلك العيات أقل من الواحد مما لا يمثل خطر علي صحة المستهلكين. علي الرغم من ذلك نصت القواعد الصحية علي أن وجود المواد الكيميائية حتي ولو كانت غير سامة تمثل خطرا علي الصحة والسلامة العامة؛ لذلك توصي هذه الدراسة بضرورة الحفاظ علي البيئة وإتباع كافة الإجراءات الصحية للحد من الملوثات الكيميائية.

الكلمات الدالة: الأسماك، قشريات الأسماك ، المعادن الثقيلة ، التراكم الحيوي ، المخاطر الصحية.