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Original Research Article

RESIDUAL LEVELS OF ORGANOCHLORINE PESTICIDES AND HEAVY METALS IN SHELLFISH FROM EGYPT WITH ASSESSMENT OF HEALTH RISKS

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Abstract: This study investigated the levels of organochlorine pesticides (OCPs) and heavy metal residues in shellfish (shrimp, oyster and crab) collected from three Egyptian governorates (Ismailia, Damietta and Alexandria). Levels of 12 OCPs such as hexachlorocyclohexanes (HCHs), aldrin, endrin and dichlorodiphenyltrichloroethanes (DDTs) residues were determined. The dominant detected OCPs were ß-HCH, p,p-DDE and endrin. The contamination pattern of OCPs was in the order of other OCPs (HCB, heptachlor, heptachlor-epoxide, aldrin, endrin and γ chlordane) > HCHs > DDTs. Residual levels of some heavy metals and trace elements were also estimated. The highest residual levels of OCPs and heavy metals were found in oysters collected from Damietta. The health risk assessment was determined by calculating hazard ratio and hazard index. Concentrations of OCPs and heavy metals in examined shellfish were below the maximum residual level set by United States Food and Drug Administration and FAO. Therefore, shellfish collected from these studied sites could be considered safe for human consumption.

Key words: organochlorine pesticides; heavy metals; shellfish; health risks; Egypt

Introduction

Organochlorine pesticides (OCPs) are considered as persistent pollutants all over the world due to their persistence in the environment; bioaccumulation; their magnification ability in food chain and induction of toxicity for human and wildlife (1). These pollutants enter the aquatic environment and could be transported into food chains, then accumulated in the aquatic organisms. Lastly, OCPs might reach human via consumption of fish and fish products, drinking water and agriculture foods (2). In Egypt since 1980s, several OCPs and their metabolites were found with different levels in fish where the OCPs use has been banned (3, 4).

Heavy metals, as major environmental contaminants, has detrimental effects on the aquatic organisms and human (5).Consequently, these metals reach the aquatic ecosystem through the natural and anthropogenic sources and causing serious threats due to their toxicity, bioaccumulations in food chain and non-biodegradable nature in the aquatic environments (6). The levels of heavy metals were elevated in natural water because of increasing the industrial and agricultural activities (7). Metals such as copper, chromium, zinc and nickel and iron are

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essential trace elements playing a vital role in the biological systems, whereas non-essential metals including lead, cadmium, arsenic and mercury are bioaccumlated in tissues leading to intoxication, damage of cells and tissues, reduced fertility, cell death and organ dysfunctions (8, 9).

Fish and shellfish are delicious food that support humans with high quality protein, various minerals, polyunsaturated fatty acids and vitamins. In addition, fish and shellfish are considered as one of the valuable bioindicators for pollution in the aquatic habitats as a result of their lower detoxification enzymes (e.g. mono-oxygenases) than those in mammals and thereby allowing a higher bioaccumulation for toxicants (10, 11). Besides, these metals will provide more reliable information on the impact on public health arising from seafood consumption (12). Information available about OCPs and heavy metal residues in shellfish in Egypt is very limited. This study aimed to investigate the levels of OCPs and heavy metal residues in three shellfish species (shrimp, oyster and crab) collected from Ismailia, Damietta and Alexandria Governorates, Egypt, and to assess the potential risks on the public health arising from shellfish consumption.

Materials and methods

Study area and sample collection

Ismailia is a province situated near the Delta with low industrial and agricultural activities, while Damietta is close to Nile Delta, where agricultural activities and industries are predominant. Alexandria is located in the North West of Delta extending for about 75 km along the Egyptian Mediterranean coast with medium industrial and agricultural activities (Fig. 1). Sixty-three samples of three shellfish species (shrimp, crab and oyster) were collected from fish markets at Ismailia, Damietta and Alexandria Governorates at Egypt during March to June 2015. The collection scheme was twenty-one samples per each governorate (as divided seven shellfish samples per each species).

The collected shellfish species; including white shrimp (*Penaeus setiferus*), blue crab (*Callinectes sapidus*) and oyster (*Crassostrea gigas*); were stored at -20°C until analysis. The residues of OCPs and heavy metals were analyzed in the edible portions of shellfish samples.

Figure 1: Location of sampling sites; Ismailia, Damietta and Alexandria

Analysis of OCPs residues

The processing and analysis of shellfish samples were carried out according to the previously described method by Yohannes et al. (13). In brief, approximately 10 gm of each edible shellfish sample was homogenized with anhydrous sodium sulfate, and then extracted with 150 mL hexane: acetone (3:1, v/v) for 6 h in a Soxhlet S306AK Automatic Extractor System (Gerhardt, Germany). Firstly, the extract was concentrated with a rotary evaporator to about 2 mL, and then secondly was diluted with hexane to 10 mL. An aliquot from the extract was cleaned-up after the evaporation of solvent on the glass column that is packed with 6 gm of activated florisil, then eluted with 80 mL hexane containing a diethyl ether of 25%. The rotary evaporator concentrated the elute, then was dried using gentle nitrogen flow. The extract was redissolved in n-decane (100 µL), then transferred to gas chromatography (GC) vials for the analysis process.

The analysis of twelve OCPs including hexachlorocyclohexanes (HCHs; α-, β- and γ-HCH), hexachlorobenzene (HCB), heptachlor, heptachlor-epoxide, aldrin, endrin, γ chlordane, and dichlorodiphenyltrichloroethanes (DDTs; p,p-DDE, p, p-DDD and p, p-DDT) was achieved with a gas-chromatography equipped with a detector of Ni electron capture (GCECD: Shimadzu GC-2014,Kyoto, Japan). An ENV8MS capillary column with the splitless injection was utilized to separate OCPs. One µl of each sample was injected. The temperature of GC oven was initially set at 100 °C for 1 min, then raised up to 180 °C at 20 °C/min, and then to 260 °C at 4 °C/min, finally was held for 5 min. The temperature of injector was 250 °C, while that of detector was 310°C. Helium was used as a carrier gas at a flow rate (1.0 mL/min), while nitrogen (as a make –up gas) was at a flow rate of 45 ml/min.

Heavy metal analysis

Fish samples were digested according to the method of Finerty et al. (14). In brief, one gm of each sample was mixed with 10 mL 3:2 nitric acid (65% v/v): Perchloric acid (70% v/v). The mixture was allowed to digest overnight at room temperature, then it was heated for three h in a water bath at 70 °C with whirling at intervals of 30 min for the accuracy of complete digestion. After cooling, the digested shellfish samples were diluted with 20 mL de-ionized water then filtered through *a Whatman* filter paper (*No. 42).* Similar procedure was applied for the blank. For determination of Pb, Cd, As, Cu, Cr, Zn, Ni and Fe residue levels in shellfish samples, the analysis of filtrate was performed using Buck V210GP atomic absorption spectrophotometer (Buck Scientific Instrument Manufacturing Co., Norwalk, CT, USA) using lambs of hollow cathode, equipped with air-acetylene flame. While, Hg was measured a cold vapor atomic absorption spectrophotometer (Varian VGA-77; Agilent Technologies, Santa Clara, CA, USA).

Quality assurance and quality control

The OCPs were identified by comparing their retention time with reference to the corresponding standard. The quality control was conducted by analysis of procedural blanks and spiked blanks for each 7 samples. The detection limits based on 3:1 signal to noise ratio (S/N) were between 0.05 and 0.1 ng/g for all analyzed OCPs. The recovery rate of OCPs was ranged from 80-102 % and the results have not been corrected for recoveries.

For testing the accuracy and validity of analytical procedures of heavy metals, the reference material; DORM-3 (Fish protein, the National Research Council, Canada) was used. Replicate analysis of this reference material demonstrated good accuracy with recovery rates ranged from 80% to 115%. The detection limits for heavy metals were 0.1 µg/g for lead (Pb), 0.005 µg/g for cadmium (Cd), 0.02 µg/g for arsenic (As), 0.2 µg/g for mercury (Hg), 0.02µg/g for copper (Cu), 0.05 µg/g for chromium (Cr), 0,005 µg/g for zinc (Zn), 0.01µg/g for nickel (Ni) and 0.005 µg/g for iron (Fe).

Estimated daily intake (EDI)

The EDI was calculated on the basis of incorporation of data from heavy metals analysis, rates of fish consumption, and body weight of Egyptian adults. EDI (µg/kg/day) for heavy metals was calculated by using the following equation which is explained by the Human Health Evaluation Manual (US Environmental Protection Agency, EPA) (15):

$$EDI = \frac{C_m * F_{IR}}{BW}$$

Where C_m is the metal concentration in the sample (mg/kg wet weight); F_{IR} is the food (fish) ingestion rate in Egypt, which was determined at 48.57 g/day (16); BW is the body weight of Egyptian adults, which was determined at 70 kg.

Health risk assessment

The US EPA (15) quantitatively evaluates the health risks for humans in terms of non-cancer and cancer risks. This study was designed to quantify the non-cancer risks imposed on the three locations under the study at Egypt, by consumption of metal contained fish. The assessment of risks followed the guidelines adopted by the US EPA (2007)(15). For the non-cancer risks, EDI was compared with the recommended reference doses (RfD) (4E03, 1E03, 3E04, 5E04, 0.3, 3E03, 0.3, 2E02 mg/kg/d for Pb, Cd, As, Hg, Cu, Cr, Zn, Ni; respectively) (15), as was illustrated in the following equation:

$$\text{Hazard Ratio (HR)} = \frac{EDI}{RfD}$$

The hazard ratios (HRs) could be added together to estimate a hazard index (HI) to evaluate the risk of mixed contaminants. HI was measured using the following equation:

$$HI = \sum HRi$$

Where *i* represent each metal. HR and/or HI of >1 demonstrates that there is a potential risk for human health, while a result of ≤ 1 shows no risk for detrimental health effects.

Statistical analysis

The obtained data were expressed as the mean ± standard error (SE). The statistical analysis was performed using two-way analysis of variance (ANOVA) to evaluate the statistical differences in the concentrations of heavy metals between different shellfish species and localities followed by the post-hoc Duncan's test. This was carried out using IBM SPSS Statistics computer software (version 21). All the statistical analyses were done at the significance level of 0.05 ($P<0.05$).

Results and discussion

Concentration of OCPs

In this study, the residual levels of OCPs in shellfish on wet weight basis (ng/g ww) were dominated by other OCPs (HCB, heptachlor, heptachlor-epoxide, aldrin, endrin and γ chlordane) followed by HCHs (α-, β- and γ-HCH) then DDTs (p,p-DDE, p, p-DDD and p, p-DDT). OCPs concentrations were in the range of ND-63.53 ng/g ww (Table 1). The maximum concentration of OCPs was found in oysters collected from Damietta. This highest OCPs residual level in oysters could be due to their feeding habits, where these molluscan shellfish are filter feeders, and thus, they could concentrate these pollutants at higher levels than others found in the water environment (17). In addition, the highest residual levels of OCPs in shellfish from Damietta may be related to the increase in the agricultural and industrial activities.

HCHs

Shellfish revealed total HCHs within the range of ND-12.27 ng/g ww (Table 1). This concentration level was nearly similar to that reported in shellfish from Qiantang River, China (18). However, higher HCHs levels (16.20- 183.40 ng/g ww) were detected in mussels from the Red Sea (19).The highest concentration of total HCHs was recovered in oysters at Damietta. The β-HCH was the predominant isomer in HCHs for all shellfish followed by γ-HCH then α-HCH. Similar finding was cited in shellfish from Qiantang River, China (18). This result might be attributed to the stability, environmental existence, resistance for microbial degradation, long half-life with vapor pressure and low solubility of β-HCH (20). However, some studies reported that α-HCH was the predominant HCHs isomer in fish (10, 21).

DDTs

Total DDTs concentrations in examined shellfish had a range from ND to 8.64 ng/g ww (Table 1). Although, higher concentrations of total DDTs were recorded in shellfish from Qiantang River, China (18) and in mussels from the Red Sea (19). The highest level for total DDTs was also detected in oysters from Damietta. Moreover, these results may be accounted for the high chemical stability, hydrophobicity of p,p- DDE, its long half-life and persistence in the biotic and abiotic components of aquatic ecosystem (22).The high levels of DDE and low DDT concentrations in shellfish could indicate that the DDT had not been recently used in the agricultural activities after its ban (23).

Other OCPs

Total other OCPs concentrations in shellfish ranged from ND to 42.62 ng/g ww. Endrin was predominant among other OCPs with the range of ND–29.00 ng/g ww. Oysters collected from Damietta showed the highest residual levels of total other OCPs (Table 1). The residual levels of OCPs detected in fish samples were below the maximum residual limit (MRL) set by United States Food and Drug Administration (US FDA) (24) and Food and Agriculture Organization (FAO)(25) (Table 1).

Thus, their human health risk assessment was not assessed in this study. Concerning the Commission Regulation of European community (EC) No 396(26) and amendments (27, 28, 29), we found that MRLs of pesticide residues in fish not applicable until the individual products are identified and listed. OCPs residues in studied shellfish may be attributed to unauthorized use of pesticides in the agriculture or as a result of marine water contamination

Heavy metal concentrations

The concentration of heavy metals was expressed as µg/g ww in shellfish samples (Tables 2 and 3). There was no a significant difference in the toxic metal levels between different species from the same locality. On the other hand, the mean concentrations of trace elements were significantly ($P<0.05$) different among different species. Moreover, edible portions of oysters harboured the highest residual levels of Cu and Ni. This was supported by Rainbow (17), who declared that the highest trace elements levels in oysters might be attributed to their feeding habits. Furthermore, there was asignificant difference in heavy metal concentrations among different localities. Our data depicted that the highest heavy metal concentrations were noticed in Damietta due to an increase in industrial and agricultural activities.

Lead (Pb) is a toxic heavy metal causing retardation in growth, anemia and neuronal defects in children. In addition, Pb chronic posioning could induce toxicity in different organs such as liver, kidney and brain (30).The present study showed a range for pb concentrations in shellfish (0.84 to 1.63µg/g ww) with a mean value of 1.19 µg/g ww (Table 2). The highest Pb level was observed in oysters from Damietta, while the lowest concentration was found in crabs from Ismailia. Pb levels in this study were within the range (0.67-0.99 µg/g ww) recorded in mussels from Alexandria, Egypt (31). Conversely, it was higher than that reported (ND-0.55 µg/g ww) in fish from Palestine (32). It is surprising, Pb levels in this study exceeded the maximum permissible limit (MPL) (0.5 µg/g ww) recommended by FAO (25) in fish and Commission Regulation (EC) No 1881(33) in crustacean fish. Although, these Pb levels were lower than MPL proposed by US FDA (24) in shellfish and Commission Regulation (EC) No 1881(33) in oysters .

Acute cadmium (Cd) intoxication in humans is manifested by nausea, vomiting, diarrhea, pain in abdomen and shock. While, the Cd chronic toxicity causes dysfunction of renal tubules and appearance of Itai–itai disease (34). The Cd residual concentrations in shellfish ranged from 0.21-0.55 µg/g ww with a mean value of 0.38 µg/g ww (Table 2). The highest concentration was detected in oysters from Damietta; whereas the lowest level was found in crabs from Ismailia. In the present study, Cd concentrations were nearly close to levels (0.16-0.65 µg/g ww) that reported in fish, Giza, Egypt (35). However, lower levels (ND-0.09 µg/g ww) were detected in fish from Palestine (32). Cd levels in the present study were below the MPL adopted by FAO (25) (0.5 µg/g ww) in fish, US FDA (24) and Commission Regulation (EC) No 1881(33) in shellfish.

Arsenic (As) is a toxic element that has carcinogenic effects and non-carcinogenic effects including genotoxicity and immunotoxicity (36, 37). The range of As levels in shellfish was 0.81 to 1.45µg/g ww with an average concentration of 1.13 µg/g ww (Table 2). It was clear that shellfish collected from Damietta showed the highest As residual levels. However, lower mean concentrations of As were recovered in shellfish from Ismailia. On the contrary, the residual As levels in this study were lower than those reported in fish from New Jersey (38). However, it was higher than those detected in seafood from Mumbai, India (39). In addition, As levels in this study were lower than the MPL for shellfish set by US FDA (24).

Mercury (Hg) is a highly toxic element causing different adverse health effects that include neurologigal, immune, renal and developmental disorders (40). The concentrations of Hg in shellfish were varied from 0.53 to 1.16 µg/g ww with a mean value of 0.83 µg/g ww (Table 2). The highest Hg levels were found in shellfish from Damietta, while the lowest concentrations were detected in the collected samples from Ismailia. The observed values of Hg in shellfish were higher than those found in fish from Mumbai Harbor, India (0.01-0.23 µg/g ww) (41).The levels of Hg in the present study were below MPL of 0.5-1 µg/g ww (33, 42) except Hg concentrations in oysters from Damietta and Alexandria.

The levels of Cu in shellfish was ranged between 1.13 and 5.72 µg/g ww with an average value of 3.33µg/g ww (Table 3).The maximum

Table 1: Concentrations (range) of OCPs residues (ng/g ww) in the examined fish species from different localities

Loactions	Shellfish	α-HCH	β-HCH	γ-HCH	HCB	Hepta-chlor	Heptachlor-epoxide	Aldrin	Endrin	γ Chlordane	p,p-DDE	p, p-DDD	p, p-DDT	ΣHCHs	ΣDDTs	Σother OCPs	Σ OCPs
Ismailia	Shrimp	ND - 0.30	ND - 2.32	ND - 2.15	ND - 0.25	ND - 0.33	ND - 4.80	ND - 0.70	ND - 9.44	ND - 0.56	ND - 1.50	ND - 0.22	ND - 0.48	ND - 4.77	ND - 2.20	ND - 16.08	ND - 23.05
	Oyster	ND - 0.39	ND - 4.29	ND - 2.21	ND - 0.35	ND - 0.46	ND - 5	ND - 1.05	ND - 10.18	ND - 0.62	ND - 2.44	ND - 0.34	ND - 0.54	ND - 6.89	ND - 3.32	ND - 17.66	ND - 27.87
	Crab	ND - 0.31	ND - 2.45	ND - 2.10	ND - 0.29	ND - 0.36	ND - 4.20	ND - 0.65	ND - 9.00	ND - 0.20	ND - 1.90	ND - 0.20	ND - 0.40	ND - 4.86	ND - 2.50	ND - 14.70	ND - 22.06
Damietta	Shrimp	ND - 1.12	ND - 4.15	ND - 2.75	ND - 0.36	ND - 4.00	ND - 6.00	ND - 0.85	ND -28.00	ND - 0.78	ND - 2.70	ND - 0.53	ND - 0.60	ND - 8.02	ND - 3.83	ND - 12.27	ND - 24.12
	Oyster	ND - 1.25	ND - 8.22	ND - 2.80	ND - 0.54	ND - 4.20	ND - 6.60	ND - 1.40	ND - 29.00	ND - 0.88	ND - 5.40	ND - 2.44	ND - 0.80	ND - 12.27	ND - 8.64	ND - 42.62	ND - 63.53
	Crab	ND - 1.15	ND - 5.21	ND -2.70	ND - 0.34	ND - 3.96	ND - 6.30	ND - 0.81	ND - 27.32	ND - 0.72	ND -2.30	ND - 0.49	ND - 0.65	ND - 9.06	ND - 3.44	ND - 39.45	ND - 51.95
Alexandria	Shrimp	ND - 0.89	ND -3.76	ND - 2.43	ND - 0.28	ND - 0.71	ND - 5.34	ND - 0.77	ND - 14.87	ND - 0.72	ND - 2.26	ND - 0.46	ND - 0.54	ND - 7.08	ND - 3.17	ND - 22.69	ND - 32.94
	Oyster	ND - 1.07	ND - 6.01	ND - 2.53	ND - 0.46	ND - 0.97	ND - 5.60	ND - 1.23	ND - 15.02	ND - 1.23	ND - 2.70	ND -0.56	ND - 0.75	ND - 9.61	ND - 4.01	ND - 24.51	ND -38.13
	Crab	ND - 0.96	ND - 4.02	ND - 2.38	ND - 0.31	ND - 0.76	ND - 5.30	ND - 0.79	ND - 14.80	ND - 0.78	ND - 2.00	ND - 0.40	ND - 0.61	ND - 7.36	ND - 3.01	ND - 22.74	ND - 33.11
MRL			300[1,2]	300[1,2]		300[1,2]	300[1]	300[1,2]	300[1,2]	300[1]					5000[1] 300[2]		

ND; Non detetected (1): US FDA (24) (2):FAO (25)

Table 2: Toxic metal concentrations (μg/g ww) in the examined fish species from different localities

	Sampling sites	Shrimp	Crab	MPL		
		Mean ± SE	Mean ± SE	Regulation(EC) No 1881(33)	US FDA(24)	FAO (25)
Pb	Ismailia	0.85 ± 0.007^{aB}	0.84 ± 0.003^{aB}	Crustacean 0.5	Crustacean 1.5	
	Damietta	1.47 ± 0.002^{aA}	1.49 ± 0.007^{aA}	Oysters 1.5	Oysters 1.7	0.5
	Alexandria	1.23 ± 0.004^{aAB}	1.24 ± 0.002^{aAB}			
Cd	Ismailia	0.25 ± 0.011^{abB}	0.21 ± 0.019^{bB}	Crustacean 0.5	Crustacean 3	
	Damietta	0.44 ± 0.011^{aA}	0.37 ± 0.011^{aA}	Oysters 1	Oysters 4	0.5
	Alexandria	0.42 ± 0.008^{aA}	0.36 ± 0.016^{aA}			
As	Ismailia	0.81 ± 0.016^{aB}	0.82 ± 0.013^{aB}			
	Damietta	1.32 ± 0.009^{aA}	1.30 ± 0.013^{aA}	-	Crustacean 76 Oysters 86	-
	Alexandria	1. 14 ± 0.011^{aAB}	1.14 ± 0.014^{aAB}			
Hg	Ismailia	0.53 ± 0.007^{aB}	0.55 ± 0.011^{aB}			
	Damietta	0.96 ± 0.010^{aA}	0.96 ± 0.012^{aA}	0.5-1	-	-
	Alexandria	0.82 ± 0.009^{aA}	0.82 ± 0.009^{aA}			

Table 3: Trace element concentrations (μg/g ww) in the examined fish species from different localities.

	Sampling sites	Shrimp	Oyster	Crab	MPL	
		Mean ± SE	Mean ± SE	Mean ± SE	US FDA (24)	FAO (25)
Cu	Ismailia	1.13 ± 0.02[cC]	2.44 ± 0.06[aC]	1.86 ± 0.02[bB]	-	30
	Damietta	4.75 ± 0.10[aA]	5.72 ± 0.08[aA]	4.56 ± 0.06[aA]		
	Alexandria	3.14 ± 0.02[bB]	4.41 ± 0.06[aB]	1.97 ± 0.03[cB]		
Cr	Ismailia	0.84±0.01[aA]	1.02±0.01[aA]	0.86 ± 0.01[aA]	crustacean 12 oysters 13	-
	Damietta	1.18±0.01[aA]	1.37±0.02[aA]	1.17 ± 0.01[aA]		
	Alexandria	0.96±0.10[aA]	1.18± 0.01[aA]	1.06 ± 0.01[aA]		
Zn	Ismailia	11.25 ± 0.01[aB]	13.33 ± 0.01[aC]	11.71±0.16[aB]	-	40
	Damietta	19.19 ± 0.04[abA]	22.19± 0.08[aA]	15.94±2.27[bAB]		
	Alexandria	17.06 ± 0.03[aAB]	18.17± 0.05[aB]	17.18 ± 0.03[aA]		
Ni	Ismailia	0.15±0.01[bC]	0.25±0.02[aC]	0. 17±0.01[bC]	crustacean 70 oysters 80	-
	Damietta	0.52±0.01[cA]	1.30 ±0.01[aA]	0.72 ± 0.01[bA]		
	Alexandria	0.34±0.01[bB]	0.55±0.03[aB]	0.44 ± 0.01[abB]		
Fe	Ismailia	34.49± 1.01[bB]	46.17 ± 0.94[aB]	34.89 ± 0.86[bB]	-	-
	Damietta	173.95 ± 0.90[aA]	189.00 ± 0.85[aA]	173.64 ± 0.76[aA]		
	Alexandria	154.51 ± 0.79[aA]	165.75 ± 0.82[aA]	153.38 ± 0.80[aA]		

Table 4: Estimated daily intakes (EDI) and hazard ratio (HR) of toxic metals through consumption of shellfish from different studied areas

	Sampling sites	Shrimp		Oyster		Crab	
		EDI	HR	EDI	HR	EDI	HR
Pb	Ismailia	0.58	0.15	0.68	0.17	0.58	0.14
	Damietta	1.03	0.26	1.13	0.28	1.03	0.26
	Alexandria	0.85	0.21	0.93	0.23	0.86	0.22
Cd	Ismailia	0.17	0.17	0.23	0.23	0.15	0.15
	Damietta	0.30	0.30	0.38	0.38	0.25	0.25
	Alexandria	0.30	0.30	0.37	0.37	0.25	0.25
As	Ismailia	0.56	1.87	0.68	2.27	0.56	1.86
	Damietta	0.92	3.05	1.01	3.36	0.90	3.00
	Alexandria	0.79	2.64	0.86	2.87	0.79	2.64
Hg	Ismailia	0.37	0.73	0.45	0.89	0.36	0.73
	Damietta	0.66	1.33	0.80	1.60	0.66	1.33
	Alexandria	0.57	1.14	0.71	1.41	0.57	1.14

Table 5: Estimated daily intakes (EDI) and hazard ratio (HR) of trace elements through consumption of shellfish from different studied areas

	Sampling sites	Shrimp		Oyster		Crab	
		EDI	HR	EDI	HR	EDI	HR
	Ismailia	0.78	0.003	1.69	0.01	1.29	0.004
Cu	Damietta	3.29	0.01	3.97	0.01	3.16	0.01
	Alexandria	2.18	0.01	3.06	0.01	1.37	0.005
	Ismailia	0.58	0.19	0.70	0.23	0.59	0.20
Cr	Damietta	0.82	0.27	0.95	0.32	0.81	0.27
	Alexandria	0.67	0.22	0.82	0.27	0.73	0.24
	Ismailia	7.80	0.03	9.25	0.03	8.13	0.03
Zn	Damietta	13.32	0.04	15.40	0.05	11.06	0.04
	Alexandria	11.84	0.04	12.61	0.04	11.92	0.04
	Ismailia	0.10	0.01	0.17	0.01	0.10	0.01
Ni	Damietta	0.36	0.02	0.50	0.02	0.90	0.04
	Alexandria	0.30	0.01	0.38	0.02	0.31	0.02
	Ismailia	23.93	ND	32.03	ND	24.21	ND
Fe	Damietta	120.70	ND	131.14	ND	120.48	ND
	Alexandria	107.21	ND	115.00	ND	106.43	ND

Table 6: Hazard index (HI) due to consumption of shellfish in different localities in Egypt

	Shrimp	Oyster	Crab
Ismailia	3.15	3.84	3.12
Damietta	5.28	6.02	5.2
Alexandria	4.57	5.22	4.55

concentration of Cu was found in oysters from Damietta; while the minimum value was found in shrimp from Ismailia. Cu concentrations in this study were comparable with those reported in fish from Alexandria, Egypt (31). On the other hand, the obtained results were higher than those detected in fish collected from Galas River and Beranang mining pool, Selangor (0.01-0.05 µg/g ww) (43) and Palestine (0.25-0.91 µg/g ww) (42). The Cu values observed in this study were lower than MPL (30 µg/g ww) in fish as permitted by FAO (25).

The concentrations of Cr in shellfish were in the range of 0.84-1.37 µg/g ww with an an average concentration of 1.07 µg/g ww (Table 3).The highest value of Cr was detected in oysters from Damietta while the lowest value was observed in shrimp from Ismailia. The Cr levels in this study were nearly similar with those detected (0.1-1.10 µg/g ww) in fish and shellfish from Calicut region, India (11). Although, lower concentrations were reported in (0.03-0.34 µg/g ww) in fish from New Jersey (38).The observed values of Cr were below MPL for shellfish recommended by US FDA (24).

The residual levels of Zn in shellfish were ranged from 11.25-22.19 µg/g ww with a mean concentration of 16.22 µg/g ww (Table 3). The highest level of Zn was found in oysters from Damietta; meanwhile, the lowest level was detected in shrimp from Ismailia. The observed values of Zn in shellfish were nearly similar with those detected (16.53-22.12 µg/g ww) in mussels from Alexandria, Egypt (31) and in investigated fish from Palestine (3.71-20.54 µg/g ww) (32). However, higher levels (3.35- 41.87 µg/g ww) were reported in fish collected from El Menofiya Governorate, Egypt (44). Zn concentrations in this study were below MPL (40 µg/g ww) in fish set by FAO (25).

The concentrations of Ni in shellfish was ranged between 0.15 and 1.30 µg/g ww with a mean concentration of 0.49 µg/g ww (Table 3). The highest levels were observed in shellfish from Damietta; whereas the lowest levels were detected in collected samples from Ismailia. Ni values observed in this study were comparable with those reported in blue crab from Mediterranean Lagoons (0.24-1.96 µg/g ww) (45). However, Baharom and Ishak (43) cited that concentrations of Ni in fish from Galas River and Beranang mining pool, Selangor were lower (0.06-0.07 µg/g ww) than those detected in this study. The levels of Ni detected in the present study were below MPL for shellfish adopted by US FDA (24).

Iron (Fe) was the most abundant trace element in shellfish in this study. The maximum concentration of Fe was found in oysters from Damietta (189.00 µg/g ww) and the minimum Fe concentration was detected in shrimp from Ismailia (34.49 µg/g ww). The average value of Fe reported in this study was 125.14 µg/g ww (Table 3). The observed Fe values in shellfish were nearly similar with those detected in fish from El Menofiya Governorate, Egypt (34.97-165.30 µg/g ww) (44) and in blue crab from Mediterranean Lagoons (25.50-170.00 µg/g ww) (45). On the other hand, higher levels were reported in mussels from Alexandria, Egypt (261.16-332.15 µg/g ww) (31). Fe levels in this study were higher than MPL in fish set by WHO/ FAO (46) (43 µg/g ww) except Fe values in shrimp and crab from Ismailia.

Daily intake and human risk assessment

EDI of different heavy metals owing to consumption of shellfish in the three examined localities was evaluated in the present study as was found in Tables (4 and 5). It was clear that the highest EDI of the investigated heavy metals was found at Damietta especially due to oyster consumption. EDI values of Pb, Cd, As and Hg were 1.13, 0.38, 3.36 and 1.60 µg/Kg/day, respectively; due to consumption of oyster at Damietta (Table 4). The highest recorded EDI values of different trace elements were similarly reported at Damietta due to oyster consumption (Table 5). The recorded EDI values in this study were strongly higher than that recorded at Catalonia, Spain (47). For instance, they reported that EDI values of Pb, Cd, As and Hg due to consumption of shellfish are 0.06, 0.04, 2.52 and 0.11 µg/Kg/day; respectively. Therefore, further investigation for the hazard ratio (HR) and hazard index (HI) is necessary due to shellfish consumption (Tables 4, 5 and 6). Values of HR and/or HI increasing than 1 indicate that there is a potential risk to human health.

HR exceeded 1 for both As and Hg in all examined shellfish species (Table 4). However, HI of different heavy metals exceeded 1 in all localities and in different shellfish (Table 6). In spite of having heavy metal load within the permissible limits, consumption of shellfish in these geographic areas may constitute a public health hazard. Thereby, some adverse effects such as hepatic and renal dysfunctions may be expected. Future approaches necessitate finding solutions to reduce the concentrations of heavy metals in the edible shellfish. Continuous monitoring studies are highly warranted to screen heavy metal load in fish and shellfish.

In Conclusion, this study confirmed that the residual levels of OCPs (HCB, heptachlor, heptachlor-epoxide, aldrin, endrin and γ chlordane) were the predominant then followed by HCHs then DDTs. The highest concentrations of β-HCH HCH and p,p-DDE in shellfish were attributed to their resistance nature for the microbial degradation, and thus the long half life. Of interest, oysters from Damietta showed the highest residual levels of OCPs and heavy metals among the analyzed shellfish species. The levels of OCPs and heavy metal residues were within the recommended level adopted by US FDA, FAO and EU except in few instances. From the analysis of EDI, HR and HI values for the metals in examined shellfish, it was declared that heavy metal load in shellfish must be reduced to decrease the possible toxicological complications arises from shellfish consumption.

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RAVNI PREOSTANKOV ORGANOKLORINSKIH PESTICIDOV IN TEŽKIH KOVIN V LUPINARJIH IZ EGIPTA IN OCENA ZDRAVSTVENIH TVEGANJ

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Povzetek: V tej raziskavi smo proučevali ravni organokloriranih pesticidov (OCP) in ostankov težkih kovin v lupinarjih (kozice, ostrige in rakovice), zbranih iz treh egiptovskih provinc (Ismajlija, Damietta in Aleksandrija). Določene so bile vrednosti 12 OCP-jev, kot so heksaklorocikloheksani (HCH), aldrin, endrin in diklorodifeniltrikloroetani (DDT). Prevladujoči odkriti OCP-ji so bili ß-HCH, p,p-DDE in endrin. Vzorec kontaminacije OCP je bil v vrstnem redu drugih OCP-jev (HCB, heptaklor, heptaklor-epoksid, aldrin, endrin in γ klordan) > HCHs > DDT. Ocenjeni so bili tudi ostanki nekaterih težkih kovin in elementov v sledovih. Največje preostale količine OCP-jev in težkih kovin so bile najdene v ostrigah, zbranih v provinci Damietta. Ocena tveganja za zdravje je bila določena z izračunom razmerja nevarnosti in indeksa nevarnosti. Koncentracije OCP-jev in težkih kovin v pregledanih školjkah so bile pod najvišjo stopnjo preostankov, ki so jo določile Združene države Amerike za prehrano in zdravila ter FAO. Zato bi bili lupinarji, proučeni v tej raziskavi, varni za prehrano ljudi.

Ključne besede: organoklorirani pesticidi; težke kovine; lupinarji; tveganje za zdravje; Egipt