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Estimation of organochlorine pesticides and heavy metal residues in two species of mostly consumed fish in Sharkia Governorate, Egypt

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Abstract

The current study aimed to afford baseline information on the level of twenty one organochlorine pesticides (OCPs) and four heavy metals in muscles of *Oreochromis niloticus and Mugil cephallus*. This will help elucidating the present status as chemical contaminants in the fish species. A total number of thirty fish from each species were collected to set up this study. *Nile tilapia (Oreochromis.niloticus),* was noticed to contain higher levels of most estimated types of ∑ HCH in (40% of *Tilapia*) and ∑ CHLRs. While Mullet (*Mugil cephallus*) was higher only in DDTs. Meanwhile, ∑ HCB, ∑ DRINs and ∑ HPTs were the lowest. The recorded results demonstrated that organochlorines were within the permissible limit according to FAO except for the CHLRs, it exceeds the permissible limit in 100% (N=30) of tested samples of *Nile tilapia* for all isomer. The analytical data obtained from such study declared that the level of Cd, Zn and Cu were significantly ($p \leq 0.05$) higher in Mullet than Tilapia but vice versa for Pb. It was evident, that all the detected metals were within the permissible limits according to FAO and WHO. Except for Zn, It exceeded the permissible limits in 50% of the analyzed samples in Mullet.

Key words: Organochlorine pesticide, Heavy metals, Residue, Fish, Egypt.

Introduction

Fish is a desirable and highly nutritive food. It is considered as a rich source of calcium, phosphorous and vitamins, long-chain omega-3 polyunsaturated fatty acids DHA (Docosahexaenoicacid) and Eicosapentaenoic acid which are associated with many health benefits [23]. The pollution of water with a broad range of pollutants has become a great matter of concern in the last few decades [29]. Water pollution referred to the contamination of water by any excess of foreign material which is harmful to human, animals and fish [28]. Organochlorine pesticides (OCPs) are of immense concern as environmental contaminants owing to their bioaccumulation , potential for magnification in food chain and adverse effects on human and wild life [20]. They are widely used in control of pests and diseases since 1940s causing wide spread contamination of the environment [24]. In developed countries the production and use of most compounds was banned since 1970s.However they are still used in developing countries and represent an environmental issue of concern, these compounds are bio-accumulated through food chain and reach a significant level in the top predators. Organochlorines have several forms of toxic effects; including effects on liver, immune, reproductive performance and nervous system viability in addition to skin irritation signs and cancer. They are accused of mimicking the function of hormones and of disrupting the endocrine system [18]

Heavy metals usually enter the aquatic ecosystem from natural and anthropogenic sources. Anthropogenic impacts including domestic sewage, industrial sources such as galvanizing, electrical conductors, batteries, cement, pigments and minning activities. This resulting in exposure of the aquatic animals and fish to elevated levels of heavy metals. These elements continuously enter the aquatic ecosystem and pose serious threat due to bioaccumulation in food chain and being permanent addition to the aquatic environment and their different organisms [2]. These metals cannot be destroyed by heat treatment so the potential to reach human was highly expected [28]. This study aimed to establish definite data borders about the level of twenty one OCPs and four heavy metals (Cd,Pb,Cu and Zn) in muscles of two species of mostly consumed fish, *Oreochromis niloticus and Mugil cephallus,* this will in turns help elucidating the status of chemical contaminants and bioaccumulation of OCPs and heavy metals.

Material and Methods

Sampling:

A total number of sixty fish (30 fish of each specie). Nile Tilapia (*O.niloticus*) ranged from (200-250 g) and about 10 cm length while Mullet (*Mugil cephallus*) ranged from (350-400 g) and (18-20 cm) in length. The sample collection was done during June and July 2014, from the local market in Zagazig city. The samples were immediately washed with distilled water then, the edible portion of the meat was taken, enclosed with clean and dry plastic film, then aluminum foil and stored frozen in -80 °C in liquid nitrogen tanks for analysis.

Detection of organochlorine compounds

Extraction and preparation of samples:

The organic solvents used in detection of organochlorine compounds (hexane, acetone, dichloromethane, and n-decane) were of HPLC grade. Fish muscle samples were processed and analyzed using a method described by Yohannes et al. [32]. The aliquot of 20% of the extract was for clean-up process after solvent. Clean-up was carried out on a glass column packed with about 6 g of the activated florisil material. Then it was concentrated to 2 ml elute on rotatory evaporator, dried under gentle nitrogen flow. The extract was re-dissolved in 100 μL n-decane and transferred to gas-chromatography (GC) vials for analysis.

Determination of OCPs residual concentrations:

It was carried out with a gas-chromatography equipped with Ni electron capture detector (GC-ECD: Shimadzu GC-2014, Kyoto, Japan). An ENV-8MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with splitless insertion or injection was used to separate the OCPs. One μl of each sample was instilled. The oven temperature of column was initially set at 100 °C for one minute, increased to 180 °C at 20 °C/min and then to 260 °C at 4 °C/min, and was held for only five minutes. The injector was 250 °C and of detector 310 °C. Helium used as carrier gas at a flow rate of 1.0 ml/min and nitrogen at flow rate of 45 ml/min was used as make-up gas.

Heavy metal analysis:

Muscle tissues of fish were subjected to digestion according to the method illustrated by [27]. The blank solution was prepared according to the method described by [25]. The quantitative determination of lead, cadmium, zinc and copper residues was conducted by using Thermo Jarrell

Ash Atomic Absorption Spectrophotometer, in Central Laboratory in Zagazig University.

Concentration of metal in samples= AXB÷W, where A=metal concentration (ppm) in the prepared samples from the digital scale reading of Atomic Absorption Spectrophotometer, B= the final volume of the prepared samples, W= weight of samples in gram [26].

Statistical analysis:

All values are expressed as mean ±SE, statistical significance was evaluated by the Tukey-Kamer (HSD test) (JMP statistical package; SAS Inistitute Inc.,Cary,NC)

Results and Discussion

Organochlorine compounds

Organochlorine pesticides have become large scale concern owing to their chronic toxicity, tendency to accumulate and persist in biota and potential ruinous impacts on humans and wildlife 34). They are broad-spectrum, persistent toxicants which accumulate in the food chain with high risks to the ecosystem and human health [29].

Polychlorinated biphenyl residues which investigated in two fish species, Hexachlorocyclohexanes (HCHs) which detected 40% of the tested samples of tilapia (Table 1) were exceeding the permissible limit of 13and significantly ($p \leq 0.05$) higher than Mullet. This result is much higher than that recorded in a study on tilapia (37.215 ng/g wet weights) in a shallow lagoon, Egypt by [22], in mullet, ∑ HCH (Table 1) was within the safe value. The result of this study were higher than that found by Ferreira et al., in Douro Estuary of Portugal who found the concentration in Mullet 0.321- 0.562 $\mu g\ g^{-1}$ wet weight by [16]. This may be attributed to the illegal use of HCH as an insecticide although its use was banned in Canada and USA since 1970s, it still applied in some developing countries. It may reach to fish through contamination of surface water that may occur as a result of surface run-off from agricultural or land especially because it is highly soluble in water.

Concerning heptachlor, table (1) showed that the maximum value of ∑HPTs was detected in the Nile Tilapia and it is significantly ($p \leq 0.05$) higher than mean concentration in Mullet. The obtained results were higher than that obtained by Farshid et al., [16] who found the concentration ranged from 0.24-0.44 in fish muscles in Iran. The result regarding DRINs (aldrin, dieldrin and endrin), the results of two species recorded in table (2) indicating the absence of endrin fraction in both types of fish. The mean of ∑DRINs in Tilapia is higher than that of Mullet. Dieldrin is the epoxide metabolite of aldrin is found to be higher by more than two folds indicating significant increase ($p \leq 0.05$) in *O. niloticus* than *Mugil cephallus*.

Although the total chlordans were not previously studied in fish tissues from Sharkia Governoratedue to the absence of any recommendation concerning their levels. Cis- and trans-chlordane were detected in 80% of Tilapia flesh with exceeding the maximum Austrian level (10 μg/kg). In Mullet the Cis- and trans-nonachlor and oxychlordane were noticeably prevalent with different levels (Table 2). This may be attributed to the previous historical use of commercial products in which chlordanes are the active compounds in agricultural applications that may be leaked to fish sources through the drainage of excessive water, especially because of the long time bioaccumulation and slow degradation of chlordanes. This type was used for crops like corn or citreous fruits. Moreover, they may be used as fumigating agents in areas near to the collection sites of studied fish, so the droplets was fallen in water and stored in fish muscles. These residues reach human leading to testicular, liver breast, brain, and prostate cancer[7].

In this study the obtained value of DDT significantly ($P \leq 0.05$) higher in Mullet Table 3. These values were lower than that found in fish samples caught from the black sea (0.047-0.138 ppm) and from Miditerranean sea coast Tunisia

(0.014-0.016 ppm wet weight)[6]. The EPA, in 1987, classified DDT as a main cause for human cancer. In 2007 a Canadian study found a positive association between DDE and the disease of non-Hodgkins Lymphoma.

From the previously mentioned results, it was observed that ∑HCH, CHLRs in *Oreochromis. niloticus* and ∑DDTs in *Mugil cephallus* were the superior concentrations. While, the values of ∑HCB, ∑ HPTs and ∑ DRINs recorded the lowest concentrations among the measured organochlorine pesticide residues. These residues explained by their environmental long persistence and / or the illegal using of these pesticide groups. Organochlorine toxicity leads to serious public health troubles as impairment of immune function, disruption of the endocrine system and promotion of cancer[11].

Heavy metal

The natural aquatic system has been extensively polluted with heavy metals released from industrial, domestic, agricultural and the different man-made activities[8]. Among animal species, fish are the inhabitants which cannot escape from the damaging effects of these pollutants[27].

Cadmium (Cd)

There was considerable variation of cadmium levels among the examined species. The depicted results in table (4) are lower than that of Essa and Rateb[11] and Ekop[10] with mean value 0.976 and 0.001 ppm respectively in Tilapia and Mullet. Higher than that obtained from Awassa Lake, Ethiopia[32]. Lower cadmium residues in mullet fish detected in Gaza Strip Markets by Muzyed[24]. There was significant difference between the examined fish species ($p \leq 0.05$).

Zinc (Zn):

The results of this study indicated that the concentration of zinc in examined species arranged in descending manner as following tilapia then mullet. Higher residues detected in a study by Amal, M. and Nahed [4] and the concentration was 56±11 ppm for tilapia muscle tissues while studying accumulation of some heavy metals and the biochemical change in muscles of O.niloticus from the River Nile in Upper Egypt. Lower results for mullet than that recorded in the present study, detected by Karadede et al., [19] who recorded 7.74mg.kg-1 in Turkey. Furthermore, lower results found in mullet caught in Iskenderun bay in Turkey with means values of zn were 30.44 ppm. [5]

Lead (Pb):

Pb concentration in the present study was lower than those found by Essa and Rateb[12] during assessment of heavy metals in fish tissues, from Assuit city market, Egypt and found the average of lead residue in tilapia nilotica samples 0.125 ppm. Higher residues in Tilapia (0.49 ± 0.33 ppm) recorded in Taiwan[21], while comparable residues obtained in Tilapia from Awassa Lake, Ethiopia when they detect 0.004 ppm[32]. The examined samples were significantly different ($p \leq 0.05$) between the two tested fish species depending on the habitat of fish in water, the pelagic fish species which present in shallow water in which Pb dissolved more than deep water could accumulate Pb in deep water inhabitant fish[8].

Copper (Cu):

The detected Copper concentration in examined samples (Table 4) were lower than that detected in fish fillets of tilapia (8.926 mg. g-1 fish) from El –Sharkia Governorate by [4,17] with mean value of 3.5 ppm. But nearly similar to the results of Ling et al.,[21]. Lower results detected in muscle tissues of Mullet samples by Karadede et al.[19] with mean values 1.36 ppm and another with mean 0.244, 1.39 and 0.99 ppm caught in Iskenderun bay in different periods[5]. Collectively from the observed data of heavy metal analysis in table 4 Zn exceeded the permissible limit in *Mugil cephallus* but still in its safe limit in *Oreochromis.niloticus*. This variation may be resulted from the different sources of fish according to condition of water, sediment, industrial or agricultural contaminants.

Conclusion

Based on our analyses of both Nile Tilapia and Mullet marketed in Sharkia governorate, Egypt, it can be concluded that all the estimated organochlorines and heavy metals were mostly detected in the safe limits admitted by FAO and WHO except for chlordan and Zn. Hence, further investigations about their levels in water, sediment and other types of fish sources in Sharkia governorate should be addressed.

Table 1: Levels of HCH and HPTs pesticides in Nile Tilapia (*O.niloticus*) and mullet (*Mugil cephallus*) muscles by ng/g wet weight (ppb). The frequency distribution of HCH and HPTs (n=30). The permissible limits of α-HCH and γ-HCH and Heptachlor epixoid are 300 ppb[13)]

HCH and HPTs	Min	Max	Mean ± SE	N.D. No	N.D. %	Within P.L. No	Within P.L. %	Exceed P.L. No	Exceed P.L. %
	Nile Tilapia (*O.niloticus*)								
α-HCH	37.16	139.15	69.22 ±15.64	0.0	0.0	30	100	0.0	0.0
β-HCH	27.28	82.83	56.32±12.41	5	16.6	25	83.4	0.0	0.0
γ-HCH	52.37	75.68	66.31±5.05	0.0	0.0	30	100	0.0	0.0
d-HCH	30.28	81.56	61.67±11.27	3	10	20	90	0.0	0.0
∑HCH	147.09	379.22	253.52±44.37*	6	20	12	40	12	40
Heptachlor	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
cis-Hep-epox	28.92	61.64	42.64±5.79	12	40	18	60	0.0	0.0
trans-Hep-epox	27.46	64.63	51.92±8.68	15	50	15	50	0.0	0.0
∑HPTs	56.37	126.28	94.57±14.48*	10	33.3	20	66.7	0.0	0.0
	Mullet (*Mugil cephallus*)								
α-HCH	6.07	143.0585	34.83±10.65	15	50	15	50	0.0	0.0
β-HCH	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
γ-HCH	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
d-HCH	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
∑HCH	6.07	143.06	34.83±10.65	15	50	15	50	0.0	0.0
Heptachlor	5.56	44.50	15.55±3.38	9	30	21	70	0.0	0.0
cis-Hep-epox	5.97	29.53	15.35±2.70	10	33.3	20	66.7	0.0	0.0
trans-Hep-epox	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
∑HPTs	12.14	200.07	58.23±17.75	13	43.3	17	56.7	0.0	0.0

n=30 replicates, ND=Non detected and * Value with superscript significant from the other fish species ($P \leq 0.05$)

Table 2: Levels of DRINs and CHLRs pesticides in Nile tilapia (*O.niloticus*) and Mullet (*Mugil cephallus*) Muscles by ng/g wet weight The Frequency distribution of DRINs and CHLRs. The permissible limits of DRINs 300 ppb, (FAO 1996). CHLRs is 10 ppb[13)]

DRINs CHLRs	Nile tilapia (*O.niloticus*)								
	Min	Max	Mean± SE	N.D.		Within P.L.*		Exceed P.L. *	
				No	%	No	%	No	%
Aldrine	39.12	93.39	63.86±11.25	3	10	27	90	0.0	0.0
Dieldrin	40.51	42.52	41.51±0.58	6	20	24	80	0.0	0.0
Endrine	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
∑DRINs	79.62	135.91	105.37±11.84*	3	10	27	90	0.0	0.0
Oxy-Chlordane	23.09	56.57	39.83±9.70	0.0	0.0	0.0	0.0	30	100
trans -chlordane	40.54	171.31	76.93±21.90	0.0	0.0	0.0	0.0	30	100
trans-Nonane	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
cis-Chlordane	26.78	63.81	48.58±6.20	0.0	0.0	0.0	0.0	30	100
cis-Nonach	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
∑ CHLRs	90.40	291.69	165.34±37.80*	0.0	0.0	0.0	0.0	30	100
			Mullet (*Mugil cephallus*)						
Aldrine	13.37	134.15	41.90±10.09	6	20	24	80	0.0	0.0
Dieldrin	10.24	22.65	18.17±1.99	13	43.3	17	56.7	0.0	0.0
Endrine	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
∑DRINs	23.61	156.80	60.07±12.08	11	36.7	19	63.3	0.0	0.0
Oxy-Chlordane	3.16	83.08	25.05±7.32	0.0	0.0	0.0	0.0	30	100
trans-chlordane	5.54	53.93	29.73±9.88	0.0	0.0	0.0	0.0	30	100
trans-Nonane	3.77	22.63	13.10 ±2.26	0.0	0.0	0.0	0.0	30	100
cis-Chlordane	4.25	18.80	12.04±1.74	0.0	0.0	0.0	0.0	30	100
cis-Nonach	5.04	42.49	15.53±3.73	0.0	0.0	0.0	0.0	30	100

n=30 replicates and ND=Non detected * Value with superscript significant from other fish species (P ≤ 0.05)

Table 3: Levels of DDTs pesticides in Nile tilapia (*O.niloticus*) and mullet (*Mugil cephallus*) muscles by ng/g wet weight. The Frequency distribution of DDTs. The permissible limits of DDTs 5000 ppb[13)].

DDTs	Nile tilapia (*O.niloticus*)								
	Min	**Max**	**Mean± SE**	**N.D.**		**Within P.L.***		**Exceed P.L. ***	
				No	%	No	%	No	%
OP-DDE	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
PP-DDE	39.33	169.66	95.55±19.55	0.0	0.0	30	100	0.0	0.0
OP-DDD	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
PP-DDD	39.27	92.70	59.92±9.14	5	16.7	25	83.4	0.0	0.0
OP-DDT	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
PP-DDT	ND	ND	ND	30	100	0.0	0.0	0.0	0.0
∑DDTs	78.60	262.36	155.47±28.69	0.0	0.0	30	100	0.0	0.0
	Mullet (*Mugil cephallus*)								
DDTs	Min	Max	Mean± SE	N.D.		Within P.L.*		Exceed P.L. *	
				No	%	No	%	No	%
OP-DDE	11.02	29.44	21.23±2.71	7	23.3	23	76.6	0.0	0.0
PP-DDE	11.66	48.84	26.02±4.07	3	10	27	90	0.0	0.0
OP-DDD	8.09	38.75	25.60±4.20	6	20	24	80	0.0	0.0
PP-DDD	16.45	53.70	33.27±5.45	9	30	21	70	0.0	0.0
OP-DDT	13.75	59.77	30.00±5.26	15	50	15	50	0.0	0.0
PP-DDT	9.88	38.75	24.48±4.17	2	6.7	28	93.3	0.0	0.0
∑DDTs	70.86	269.24	160.60±25.86*	5	16.7	25	83.3	0.0	0.0

n=30 replicates and ND=Non detected* Value with superscript significant from the other fish species (P ≤ 0.05)

Table 4: Concentration of heavy metals in tilapia (*O.niloticus*) and mullet (*Mugil cephallus*) muscles and maximum permissible limit (MPL) of heavy metals in fish muscles [9,13,14]

Heavy metal (ppm)	Min	Max	Mean ± SE	N.D. No	N.D. %	Permissible limit (ppm)	Reference
Tilapia(*O.niloticus*)							
Cadmium (cd)	0.0005	0.002	0.001±0.00	15	50	0.05	13, 9
						0.5	14
Lead(Pb)	0.0056	0.077	0.022±0.01	6	20	0.5	14
						0.2	9
Zinc(Zn)	13.255	23.844	20.222±4.05	0.0	0.0	30	13
						40	14
Copper(Cu)	0.354	0.720	0.539±0.12	3	10	30	13
						30	14
Mullet (*Mugil cephallus*)							
Cadmium (cd)	0.0008	0.007	0.003±0.00	0.0	0.0	0.05	13,9
						0.5	14
Lead (Pb)	0.0032	0.0214	0.011±0.01	0.0	0.0	0.5	14
						0.2	9
Zinc (Zn)	24.505	67.410	49.853±15.74*	0.0	0.0	30	13
						40	14
Copper (Cu)	0.744	4.170	1.424±0.90*	6	20	30	13
						30	14

n=30 replicates and ND=Non detected.* Value with superscript significant from the other fish species ($P \leq 0.05$)

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