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Original Research Article

Organochlorine residues in fishes collected from different water sources in El-Fayoum Governorate

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ABSTRACT

A total of 120 fish samples were collected from different water sources in El Fayoum Governorate, (Bahr El Banat agricultural drainage, different fish farms and Al Rayaan Lake). and represented by *Clarias gariepinus* from Bahr El Banat agricultural drainage, different fish farms (15 each), *Mugil cephalus* from different fish farms and Al Rayaan Lake (15 each), *Solea solea* (30 samples) and *Oreochromis niloticus* from Bahr El Banat agricultural drainage, different fish farms and Al Rayaan Lake (10 each). Fourteen organochlorine compounds were analyzed by gas chromatography. Fish samples from Bahr El Banat revealed the highest mean levels of p,p'-DDD, endrin, endosulfan, γ-chlordane, heptachlor and γ-HCH, while fish samples from Al Rayaan Lake have shown the highest mean level of methoxychlor, p,p'-DDT, p,p'-DDE, dieldrin, heptachlor epoxide, δ-HCH and α-HCH. Most of the examined fish samples from different species, are within the maximum residue limits and should not pose a health risk to consumers. The public health hazards were discussed as well as recommendations were done.

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1. Introduction

Organochlorine pesticides have been widely used and become a worldwide concern due to their persistence, bio-accumulative potential, chronic toxicity, and potential negative impacts on humans and wildlife, it is known that most of the total intake of pesticide residues by human beings is through the food chain (Martinez et *al.*, 1997). Fish are known to biomagnify pesticides from the surrounding environment and transfer the pesticides to humans when consumed, (Mackay and Fraser, 2000).

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Organochlorine residues were detected in *Oreochromis niloticus* and in *Clarias gariepinus* by (Salem, 2003 and Said *et al.*, 2008) but Mohammed (2016) failed to detect chlordane, aldrin, dieldrin, heptachlor and heptachlor epoxide in both species.

Organochlorine pesticides were detected in Bouri and Mousa from Damietta Governorate were detected by (El Nemr and Abd-Allah, 2004) and from the Bizerte Lagoon, Tunisia, lindane (γ-HCH), heptachlor epoxide, dieldrin, p,p'-DDT, p,p'-DDE, p,p'-DDD were detected, while lindane, chlordane, dieldrin and heptachlor were undetected by (Ameur *et al.*, 2013).

The maximum residue limit of aldrin and dieldrin together is 300 ppb while that of chlordane is 300 ppb and the same for sum of heptachlor and heptachlor epoxide but much higher limit of 5000 ppb was specified for total DDTs, US-FDA (2008), While the maximum residue limit of endrin is 100 ppb, but specified higher limit of heptachlor and lindane (200 ppb) for each of them, aldrin and dieldrin (300 ppb) for each of them Codex Alimentarius Commission, (2009). On the other hand, high maximum residue limit of p,p'-DDE of 100 ppb was specified by EU (2011).

Several organochlorine pesticides could interfere with the estrogen-controlled pathway, cause weak estrogenic or antiestrogenic response and some of them even be harmful to human nervous systems (Dalvie *et al.*, 2004). Evidence is emerging on the thyroid disrupting effects of other pesticides, such as methoxychlor and endosulfan (Boas *et al*., 2006). Presence of DDT and its breakdown product, DDE, in maternal serum is associated with a decrement in mental development in 12- and 24-month old infants and an impaired psychomotor development at 6 and 12 months (Eskenazi *et al.*, 2006).

Therefore, this work was carried out to estimate organochlorine pesticides residues in fish species (*Oreochromis niloticus*, *Clarias gariepinus*, *Mugil cephalus* and *Solea solea*) from different water sources (Bahr El Banat, fish farms and El Rayaan Lake) in El-Fayoum Governorate, as well as public health significance of organochlorine pesticides residues in fish was discussed.

2. Material and methods

2.1. Samples collection:

A total of 120 fish samples were collected from different water sources in El Fayoum Governorate. The samples were collected from Bahr El Banat agricultural drainage, different fish farms and Al Rayaan lake. The collected samples were represented by *Clarias gariepinus* from Bahr El Banat agricultural drainage, different fish farms (15 each), *Mugil cephalus* from different fish farms and Al Rayaan lake (15 each), *Solea solea* (30 samples) and *Oreochromis niloticus* from Bahr El Banat agricultural drainage, different fish farms and Al Rayaan lake (10 each). The samples were kept in ice box and transported without delay to Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, either alive or freshly dead for extraction and clean-up procedures.

2.2. Sample preparation:

The fish samples were washed several times with deionized water to clean them from sediments and other entangled materials, the fish samples were identified and given unique identification codes. Each fish sample was gutted to remove intestines, the scales, head, tails, fins and bones were also removed using a stainless-steel knife.

The soft parts of fish samples were removed. A muscle tissue sample (50g) was taken from the dorsal muscle and prepared for extraction and clean up procedures at the same day of collection according to AOAC Official method 970.52 (1996) which is a multi-residue method for organochlorine and organophosphorus pesticide residues.

2.3. Sample extraction:

Extraction by blending 50 g of the sample with 100 g anhydrous sodium sulphate and 150 ml petroleum ether for 2 minutes then poured through filter funnel into a suction flask then evaporated till dryness in rotary evaporator.

2.4. Sample clean up and injection into GC apparatus:

The obtained fat film from previous step was cleaned by acetonitrile partitioning and finally clean up by florisil column by eluating the obtained extract through the column 3 times at the same rate with 20 ml of 6, 15 and 50% diethyl ether in petroleum ether, respectively and concentrate the eluate in rotary evaporator till obtaining a dry film which is then was dissolved in 2ml n-hexane and transferred to autosampler vials.

2.5. Adjustment of GC apparatus

The extracts were injected into gas chromatography apparatus (Agilent, model 6890) equipped with an Ni^{63} electron capture detector, capillary column of 30 m length, 0.32mm internal diameter, and 0.25 µm film thickness. The oven temperature was programmed from an initial temperature 160 ºC (2 min hold) to 280 ºC at a rate of 5º C /min and maintained at 280ºC for 10 min. Injector and detector temperatures were maintained at 280 and 320 ºC, respectively. Nitrogen was used as a carrier gas at flow rate of 4 ml /min and injection volume of 1µl. The pesticide residues were identified based on comparison of relative retention times to those of known standards.

3. Results and discussion

3.1. Organochlorine residues in fish species from Al Rayaan Lake:

Organochlorine was detected in fishes collected from Al Rayaan Lake by different percentages. Regarding *Solea solea*, δ-HCH was the most abundant compound with a mean value of 193.86±33.79 ppb, followed by methoxychlor with an average concentration of 11.07±7.61 ppb. Gamma-chlordane recorded the lowest mean value among all other organochlorine pesticides with a mean value of 0.07±0.04ppb (Table**,** 1).

Concerning *Oreochromis niloticus* samples from Al Rayaan Lake, it is evident that δ-HCH has the highest mean concentration among all other organochlorine pesticides compounds with a mean value of 9.77±2.39 ppb followed by p,p'-DDD with a mean value of 3.65±0.99 ppb. While p,p'-DDT recorded the lowest average concentration of 0.064±0.04 ppb. In Mugil cephalus samples, δ-HCH recoded the highest mean value of 2.16±0.29 ppb, followed by γ-HCH (lindane) with a mean value of 0.33±0.18 ppb while Dieldrin, endosulfan, gamma chlordane and α-HCH were all below the limit of detection (Table**,** 1).

Table 1. Mean residue levels of organochlorine pesticides (ppb, wet weight) in fish species from Al Rayaan Lake

Compound name	Tilapia *(Oreochromis niloticus)*		Sole fish *(Solea solea)*		Mullet *(Mugil cephalus)*	
	Mean	±S.E	Mean	±S.E	Mean	±S.E
Methoxychlor	0.768[a]	0.3941	11.076[a]	7.616	0.264[a]	0.0612
P,p'-DDT	0.064[a]	0.0443	0.392[a]	0.392	0.2133[a]	0.04462
P,p'-DDD	3.652[a]	0.9986	2.984[a]	0.5805	0.0266[b]	0.02162
P,p'-DDE	2.296[a]	0.6255	4.650[b]	0.2180	0.01066[c]	0.007268
Endrin	2.808[a]	0.7643	7.53[b]	0.2582	0.0266[c]	0.01008
Dieldrin	0.528[a]	0.1473	0.542[a]	0.1205	ND[b]	ND
Endosulfan	0.08[a]	0.03955	0.1966[a]	0.09519	ND[a]	ND
Gamma chlordane	0.32[a]	0.08844	0.0793[b]	0.04437	ND[b]	ND
Aldrin	0.28[a]	0.07752	0.6386[b]	0.06753	0.0053[a]	0.00533
Heptachlor epoxide	0.392[a]	0.107	0.723[b]	0.03853	0.0266[c]	0.01008
Heptachlor	1.208[a]	0.4835	1.944[b]	0.4715	0.0533[a]	0.01008
Delta HCH	9.776[a]	2.395	193.861[b]	33.791	2.168[a]	0.2938
Gamma HCH	0.888[a]	0.4523	1.242[a]	0.4137	0.3373[a]	0.1804
Alpha HCH	0.176[a]	0.07234	1.632[b]	0.2627	ND[a]	ND

Means with different superscripts are significantly different at p≤ 0.05

There were significant differences in levels of the most analyzed organochlorine pesticides between fish species from Al Rayaan Lake at p≤0.05. This may be due to their different feeding habits as well as the differences in their abilities to metabolize and excrete these contaminants. This agrees with that mentioned by Gbeddy *et al.*, (2012).

3.2. Organochlorine residues in fish species from different fish farms:

Regarding *Oreochromis niloticus* samples collected from fish farms, p,p'-DDD recorded the highest mean concentration of 8.39±2.77 ppb followed by methoxychlor with a mean concentration of 1.72±0.59 ppb whereas p,p'-DDT, p,p'-DDE, aldrin and HCH isomers were all (Table 2).

Table 2. Mean residue levels of organochlorine pesticides (ppb) in fish farms

Compound name	Tilapia *(Oreochromis niloticus)*		Cat fish *(Clarias gariepinus)*		Mullet *(Mugil cephalus)*	
	Mean	**±S.E**	**Mean**	**±S.E**	**Mean**	**±S.E**
Methoxychlor	1.728^{a}	0.5944	0.2546^{b}	0.05966	1.188^{ab}	0.3730
P,p'-DDT	ND^{a}	ND	0.2986^{b}	0.05162	ND^{a}	ND
P,p'-DDD	8.394^{a}	2.775	0.0546^{b}	0.01492	5.764^{a}	0.4462
P,p'-DDE	ND^{a}	ND	ND^{a}	ND	2.253^{b}	0.4922
Endrin	1.442^{a}	0.7362	0.0053^{a}	0.0053	6.809^{b}	0.5391
Dieldrin	0.184^{a}	0.1228	ND^{ab}	ND	0.448^{a}	0.1280
Endosulfan	1.304^{a}	0.8728	ND^{a}	ND	0.6533^{a}	0.1298
Gamma chlordane	0.542^{a}	0.2384	ND^{a}	ND	1.364^{b}	0.1841
Aldrin	ND^{a}	ND	ND^{a}	ND	0.4026^{b}	0.0593
Heptachlor epoxide	0.26^{a}	0.1069	ND^{b}	ND	0.5973^{c}	0.03728
Heptachlor	1.008^{a}	0.6746	0.0266^{a}	0.01008	4.273^{b}	0.9722
Delta HCH	ND^{a}	ND	ND^{a}	ND	35.27^{b}	7.732
Gamma HCH	ND^{a}	ND	ND^{a}	ND	1.384^{b}	0.3090
Alpha HCH	ND^{a}	ND	ND^{a}	ND	0.054^{a}	0.0372

Means with different superscripts are significantly different at p≤ 0.05

Concerning *Clarias. gariepinus*, results in Table (2) revealed that p,p'-DDT has the highest mean concentration of 0.29±0.05 ppb followed by methoxychlor with a mean concentration of 0.25±0.05 ppb while, p,p'-DDE, dieldrin, endosulfan, aldrin, γ-chlordane, heptachlor epoxide and HCH isomers were all below the limit of detection.

Concerning *Mugil cephalus* samples from different farms, organochlorine pesticides were predominated in which δ-HCH showed the highest level with a mean value of 35.27±7.73 ppb followed by Endrin with a mean value of 6.8±0.53 ppb. On the other hand, P,p'-DDT was not detected in any of *Mugi cephalus* samples collected from farms in Fayoum Governorate.

There were significant differences between fish species from different farms in the mean values of most organochlorine pesticides at p≤0.05, which may be due to the environmental conditions, level of exposure, nature of the pesticide, its solubility, the fish species, and its ability to excrete these compounds. (Edward,s 1973). The presence of organochlorine residues in fishes from different farms may be due to the distinct land uses which lead to the contamination of aquatic environments by persistent organic pollutants mainly through runoff, direct discharges and wet or dry deposition. This is in accordance with that mentioned by Roche *et al.*, (2000). These differences may be also due to fish accumulates some organic chemicals particularly organochlorine pesticides in its fatty tissues due to its inability to metabolize them in the food; they eat and from the intake of particulates in water and sediment. (Baird, 2005).

3.3. Organochlorine residues in fish species from Bahr El Banat

Results in table (3) showed that the highest mean for organochlorine residue in tilapia *(Oreochromis niloticus)* was for delta HCH with a mean concentration of 13.03±6.52 ppb followed by p,p'-DDD with a mean value of 7.49 ppb, while p,p'-DDT and α-HCH were not detected in tilapia species from Bahr El Banat.

On the other hand, the analysis of *Clarias gariepinus* samples from Bahr El Banat revealed that δ-HCH has the highest mean value of 119.76±17.89 ppb followed by p,p'-DDD with a mean value of 7.36±1.03 ppb, while p,p'-DDT was below the limit of detection.

Table 3. Mean residue levels of organochlorine pesticides residues (ppb, wet weight) in different fish species from Bahr El Banat

Compound name	Tilapia *(Oreochromis niloticus)*		Cat fish *(Clarias gariepinus)*	
	Mean	±S.E	Mean	±S.E
Methoxychlor	1.888[a]	0.917	1.29[a]	0.916
P,p'-DDT	ND[a]	ND	ND[a]	ND
P,p'-DDD	7.496[a]	0.3714	7.362[a]	1.031
P,p'-DDE	1.696[a]	0.5655	2.602[a]	0.5424
Endrin	5.376[a]	0.1885	5.32[a]	0.2257
Dieldrin	0.112[a]	0.1120	0.517[a]	0.1136
Endosulfan	0.528[a]	0.1821	1.769[a]	0.8052
Gamma chlordane	1.032[a]	0.3506	1.164[a]	0.2325
Aldrin	0.064[a]	0.3331	0.261[b]	0.0248
Heptachlor epoxide	0.152[a]	0.0777	0.516[b]	0.039
Heptachlor	4[a]	1.330	3.333[a]	0.213
Delta HCH	13.032[a]	6.525	119.76[b]	17.891
Gamma HCH	1.672[a]	0.4659	1.357[a]	0.3396
Alpha HCH	ND[a]	N.D	0.24[a]	0.09336

Means with different superscripts are significantly different at p≤ 0.05

There were significant differences in the mean residual levels of organochlorine pesticides residues between *Oreochromis niloticus* and *Clarias gariepinus* samples from Bahr El Banat at p≤0.05, Which may be attributed to different feeding habits because *Clarias. gariepinus* is a carnivorous which has tendency for bioaccumulation while *Oreochromis niloticus* is almost herbivorous fish species. This agrees with that reported by Holtan, (1998) and Yohannes *et al.,* (2014).

Regarding organochlorine pesticides residues in *Oreochromis niloticus*, high levels of organochlorine pesticides were obtained by Hashem (1997) and Salem (2003) in *Oreochromis niloticus* samples from fish markets in Cairo, Kalyobia, Giza and Assiut.

On the contrary, Mohammed (2016) was not able to detect any of the organochlorine residues in *Oreochromis niloticus* samples collected from Gesr Bahr El baar agricultural drainage, Kaliobya Governorate.

Also, high values were obtained by. El Nemr and Abd-Allah (2004) in Bouri (*Mugil cephalus*) from Damietta Governorate, Nearly, similar figures were obtained by Abd-Allah et al., (1998) in *Mugil cephalus* samples from Abu-Qir, Gamasa, and Damietta markets.

Concerning *Clarias gariepinus*, high levels of organochlorine pesticides residues were obtained by Hashem (1997). On the other hand, Mohammed (2016) could not detect any of the organochlorine residues in *Clarias gariepinus* from Gesr Bahr El baar agricultural drainage, Kaliobya Governorate, with exception of p,p'-DDD (9±1.1) ppb and found no residues of organochlorine pesticides in catfish samples from El-Reiah El-Tawfeki.

Regarding *Solea solea*, high figures were obtained by Ameur *et al.,* (2013). On contrary, low figures were obtained by El Nemr and Abd-Allah (2004) in Mousa (*Solea solea*) samples from South Sinai Governorate.

The presence of organochlorine contamination in water bodies may be attributed to nonpoint sources via runoff, and leaching due to agricultural applications, this leads to fish contamination through ingestion, dermal absorption and respiration. (Afful *et al.,* 2010).

Fish samples from Bahr El Banat revealed the highest mean levels of p,p'-DDD (7.41±0.62 ppb), endrin (5.34±0.15), endosulfan (1.27±0.49), γ-chlordane (1.11±0.19), heptachlor (3.6±0.53) and γ-HCH (1.48±0.27) ppb, while fish samples from Al Rayaan Lake showed the highest mean level of methoxychlor (6.25±4.18), p,p'-DDT (0.28±0.21), p,p'-DDE (2,.95±0.31), dieldrin (0.39±0.07), heptachlor epoxide (0.47±0.04), δ-HCH (108.11±22.32) and α-HCH (0.922±0.177) ppb.

It is worth to mention that among all examined fish species, only 20% of *Solea solea* samples exceeded the MRL specified by Codex Alimentarius Commission (1996 and 1999) and US-FDA (2008) for δ-HCH, because it has the longest half-life of the HCH isomers, this agrees with that reported by (Willett et al., 1998).

The relatively high organochlorine levels in Al Rayaan Lake may be attributed to different sources of agricultural, municipal, and industrial contamination in El Fayoum region. Chemicals mainly reach water via evaporation, atmospheric fallout, surface run-off, and wastewater discharges. This is in accordance with that reported by Trabelsi and Driss, (2005).

Exposure to organochlorine insecticides such as aldrin, chlordane, DDT, dieldrin, heptachlor, lindane, and toxaphene is associated with the incidence of depression in wives and husbands living in Iowa and North Carolina (Beard *et al.,* 2013). Endosulfan raises the concern for the incidence of neurodevelopmental defects, such as Parkinson disease in the fetus during gestation and post-partum period (Wilson *et al.,* 2014). Concentrations of β-HCH, heptachlor, and p,p′-DDE were significantly higher in cases of breast cancer than in control cases (Arrebola *et al.,* 2015).

4. Conclusion

From the present study, it could be concluded that p,p'-DDT was not detected neither in *Clarias gariepinus* nor *Oreochromis niloticus* from fish collected from Bahr El- Banat. *Mugil cephalus* collected from fish farms showed the highest level of contamination with organochlorine pesticides in comparison with other fish species. Among all examined fish species, only 20% of *Solea solea* samples exceeded the MRL specified by Codex Alimentarius Commission and for this reason consuming fishes in Fayoum Governorate constitutes a little public health hazards to consumers.

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