Assessment of organochlorine pesticides residues in tilapia fish (*Oreochromis Niloticus*)

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**ABSTRACT**

In the present study, Organochlorine pesticides (OCPs) residues were determined in muscle tissues of Tilapia (*Oreochromis niloticus*) with different body weights collected randomly from different regions at Cairo and Giza markets using Gas Chromatography. The total mean values of DDT, DDE, DDE, Endrin, Dieldrin, Endosulfan, γ-chlordane, δ-BHC, Heptachlor epoxide, Methoxychlor and Heptachlor residues in the examined samples of tilapia were 16.82 ± 4.7, 4.80 ± 4.65, 8.34 ± 1.42, 26.54 ± 3.13, 10.91 ± 2.72, 6.20 ± 1.17, 21.32 ± 3.44, 16.17 ± 3.07, 5.33 ± 10.67, 1.24 ± 0.31 and 1.28 ±0.31 ppb, respectively. Accumulation of organochlorine pesticides in fish tissues was related to rate of pollution, lipid content, feeding behavior, rate, size and age. There were significant differences between organochlorine pesticides residues and different weights of tilapia. Most of the examined samples were within the maximum permissible limits of set by US-FDA (2008) and Codex Alimentarius Commission (1996). Human health assessment risk was discussed, and consumption of these fish had no potential hazard to human health as hazard ratio was less than one.

1. INTRODUCTION

Fish consumption has increased simultaneously with the growing interest of their nutritional and therapeutic benefits. Tilapia (*Oreochromis niloticus*) is the major fish species consumed in Egypt, due to its high nutritive value, palatability and relatively low price compared with other kinds of fishes (Morshdy et al., 2018). One of the major sources of human exposure to environmental contaminants could be consumption of contaminated fish (Yahia and Elsharkawy, 2014).

Organochlorine pesticides are classified as a class of the Persistence Organic pesticides depend on their toxicity, persistence, high lipid solubility, bioaccumulation nature and long-range transport potential (Luzardo et al., 2009) which tend to bio-concentration and bio-magnification in food chain (Neves Dias et al., 2015). Aquatic environment is contaminated with organochlorine pesticides through surface run off, discharge from surface, pesticides application, carless disposal of empty containers and equipment washings (Lu et al., 2011). Storage and elimination of pesticides from the fish depend on habitat, physiological factors, lipid content, feeding behavior, rate and routes of biotransformation of pesticides, species, size, age and sex of fish (Ribeiro et al., 2005).

Pesticides concentration, time of exposure, toxicity of active ingredients and individual’s health status are several factors for pesticide effects (Debnath and Khan, 2017). Organochlorine contaminants persist in the environment as they resist physical, chemical, biological and photochemical breakdown processes (Mrema et al., 2013). Reproductive failures and birth defects, endocrine disruptions, immune system malfunction and cancers are a health hazards of organochlorine pesticides (Afful et al., 2010). Exposure to organochlorine pesticides is associated with type 2 diabetes (Jayaraj et al., 2016). Organochlorine pesticides affect the nervous, immune, reproductive, renal and hepatic systems (Polder et al., 2014). Therefore, this study was carried out to determine organochlorine pesticides residues in different weights of Tilapia (*Oreochromis niloticus*) and to compare the obtained results with the maximum permissible limits of US-FDA (2008) and Codex Alimentarius Commission (1996) and also evaluation of human health risk associated with fish consumption.

2. MATERIAL AND METHODS

2.1. Collection of samples:

One hundred random samples of tilapia fish (*Oreochromis niloticus*) were divided into 3 groups according to their weights (Group A: up to 200 gm, n = 30), (Group B: 200-400 gm, n=40) and Group C: 400-600gm, n=30). They were collected from different localities at Cairo and Giza markets for determination of organochlorine pesticides residues in muscle tissues using Gas Chromatography.

2.2. Sample preparation:-(AOAC, 1996).

The soft parts of fish samples were removed. A muscle tissue sample (50 gm) was taken from the dorsal muscle and prepared for extraction and clean up procedures at the same day of collection.

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2.3. Extraction of OCP (AOAC, 1996).
Fifty gm of the sample blending with 100 gm anhydrous sodium sulphate and 150 ml petroleum ether for 2 min, poured through filter funnel into a suction flask and put in rotary evaporator till complete evaporation of petroleum ether and obtaining only fat content.

2.4. Sample clean up and injection into GC apparatus (AOAC, 1996)
The obtained fat film from previous step was cleaned up by petroleum ether-acetonitrile partitioning and finally clean up by florisil column by eluting 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 2 ml n-hexane and transferred to autosampler vials.

2.5. Quantitative determination of organochlorine pesticides
The extracts were injected into gas chromatography apparatus (Agilent, model 6890) equipped with a Ni63 electron capture detector, capillary column of 30 m length, 0.32 mm internal diameter, and 0.25 μm film thickness. The oven temperature was programmed from an initial temperature 160 °C (2 min hold) to 280 °C at a rate of 5 °C/min and maintained at 280 °C for 10 min. Injector and detector temperatures were maintained at 280 and 320 °C, respectively. Nitrogen was used as a carrier gas at flow rate of 4 ml/min and injection volume of 1 μl. The pesticide residues were identified based on comparison of relative retention times to those of known standards. The following equation was used to calculate the concentration of organochlorine pesticides was calculated:

\[ C = \left( \frac{\text{peak area(sample)} \times \text{dilution(2ml)} \times \text{standard conc. (ng)}}{\text{peak area standard} \times \text{injection volume(μl)} \times \text{sample weight(gm)}} \right) \times 1000 \]

*Limit of Detection (LOD) = 0.001 ppb

2.6. Human health risk assessment:
It can be determined through the following parameters:

2.6.1. Estimated daily intake:
EDI = C × DR/BW (WHO, 1987)
Where: C is the concentration of the OCP (mg/kg) in raw fish, DR is the daily consumption of fish (Kg/day) and BW is the average body weight set at 60 kg (WHO, 2010). The estimated daily consumption rate in Egypt was conservatively set at 55.9 g/day per person in Cairo governorate (FAO, 2005).

2.6.2. Carcinogenic Risk:
Both cancer risk (CR) and hazard (HR) ratios were calculated according to CR=EDI×CSF (USEPA, 2005)
Where CSF is cancer slope factor (mg/kg per day). Its value was 0.34 for DDT, 0.35 for ΣCHL, 1.6 for HCB, 16 for dieldrin, 9.1 for heptachlor epoxide (USEPA, 2000).
If the CR is smaller than 10-4 is considered “Unacceptable risk”, between 10-6 and 10-4 are considered “levels of concern” and CR more than 10-6 is considered “acceptable risk” (USEPA, 2005).

2.6.3. The Hazard Ratios (HR):
To evaluate the potential noncarcinogenic health risk, it was assessed by calculating the hazard index (HI) HI = EDI / ADI (US EPA, 1991)
Where, EDI: Estimated Daily Intake (mg/kg bw) and ADI: Acceptable Daily Intake (mg/kg bw).
For a preliminary quantitative risk assessment, HI ≤ 0.2 is considered to indicate negligible adverse health effects as a result of exposure, while HI values exceeding this threshold require a further detailed risk assessment or risk management measures to be undertaken (Health Canada, 2004).

Hazard ratio (HR) was evaluated for carcinogenic effects, it was calculated using the following equation

HR=EDI/BMC (USEPA, 2005; Jiang et al., 2005):
Where the BMC is the benchmark concentration derived from the USEPA CSF: BMC = (Risk × BW) / (Fish consumption × CSF) Where the risk is set at one in a million chances for lifetime exposure, and fish consumption is the amount of fish consumed per day (Kg/d) relative to body weight (kg). An HR greater than one indicates a potential risk to human health (Dougherty et al., 2000).

2.7. Statistical analysis
Data obtained from the current study was statistically analyzed by using the Statistical Package for the Social Sciences (SPSS) software (Corp, 2013). A value of P <0.05 was considered significant.

3. RESULTS
According to the results in table and figure (1) the organochlorine pesticides were:
(1) DDT: the total mean values of DDT in tilapia were 16.82±4.7 ppb. The mean values of DDT in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were ND, 6.64±1.28 and 47.21±14.26 ppb, respectively. DDT was accepted in 100% of samples. There was high significant difference (P<0.05) between different weights of tilapia.
(2) DDE: the total mean values of DDE in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were 8.29±1.60, 50.62±5.13and 73.54±10.97 ppb, respectively. DDT was accepted in 100% of samples. There was high significant difference (P<0.05) between different weights of tilapia.
(3) DDE: the total mean values of DDE in tilapia were 8.34±1.42 ppb. The mean values of DDE in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were 1,78±0.64, 10.92±2.07 and 11.46±3.57 ppb, respectively. DDE was accepted in 100% of samples. There was a high significant difference (P<0.05) between different weights of tilapia.
(4) Endrin: the total mean values of Endrin in tilapia were 26.54±3.13 ppb. The mean values of Endrin in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were 1.55±0.41, 24.05±3.04 and 53.52±6.94 ppb, respectively. Endrin was accepted in 98% of samples. There was a high significant difference (P<0.05) between different weights of tilapia.
(5) Dieldrin: the total mean values of dieldrin in tilapia were 10.91±2.72 ppb. The mean values of Dieldrin in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were 0.18±0.07, 0.40±0.12 and 35.66±7.33 ppb, respectively. Dieldrin was accepted in 100% of samples. There was a high
significant difference ($P<0.05$) between different weights of tilapia.

(6) Endosulfan: the total mean values of endosulfan in tilapia were 6.20±1.7 ppb. The mean values of Endosulfan in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were 0.82±0.26, 3.95±0.97 and 14.57±3.20 ppb, respectively. Endosulfan was accepted in 100% of samples. There was a high significant difference ($P<0.05$) between different weights of tilapia.

(7) γ-chlordane: the total mean values of γ-chlordane in tilapia were 21.32±3.44 ppb. The mean values of γ-chlordane in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were 0.25±0.08, 17.56±3.82 and 47.42±8.33 ppb, respectively. γ-chlordane was accepted in 100% of samples. There was a high significant difference ($P<0.05$) between different weights of tilapia.

(8) δ-BHC: the total mean values of δ-BHC in tilapia were 16.17±3.07 ppb. The mean values of δ-BHC in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were 2.82±0.59, 7.09±1.44 and 41.64±8.42 ppb, respectively. δ-BHC was accepted in 100% of samples. There was a high significant difference ($P<0.05$) between different weights of tilapia.

(9) Heptachlor-epoxide: the total mean values of Heptachlor-epoxide in tilapia were 5.33±10.67 ppb. The mean values of Heptachlor-epoxide in tilapia at weights up to 200 gm, 200-400 gm and 400-600 gm were ND, 4.18±6.65 and 12.19±5.79 ppb, respectively. Heptachlor-epoxide was accepted in 100% of samples. There was a high significant difference ($P<0.05$) between different weights of tilapia.

(10) Methoxychlor: the total mean values of Methoxychlor in tilapia were 1.24±0.31 ppb. Methoxychlor was not detected at weights up to 200 gm, and 400-600 gm. While, the mean values of Methoxychlor in tilapia at weights 200-400 gm were 3.09±0.68 ppb. Methoxychlor was accepted in 100% of samples. There was a high significant difference ($P<0.05$) between different weights of tilapia.

(11) Heptachlor: the total mean values of Heptachlor in tilapia were 1.28±0.31 ppb. Heptachlor was not detected at weights up to 400 gm. While, the mean values of Heptachlor in tilapia at weights 400-600 gm were 4.27±0.82 ppb. Heptachlor was accepted in 100% of samples. There was a high significant difference ($P<0.05$) between different weights of tilapia.

The acceptability of each OCPs residue are evaluated according to permissible limit in table (2).

**4. DISCUSSION**

Improper use of pesticide in agricultural production lead to pesticides or their metabolite residues in products which adversely affect aquatic life and human health (Acara et al., 2006). Organochlorine pesticides have long residual action and persist in the environment for long periods without losing their toxicity (Agbeve et al., 2014). Fish has a moderate ability to metabolize organochlorines so, contaminant loading in fish is well reflective of the state of pollution in surrounding environments (Guo et al., 2008), kidney problem, birth defects, impaired reproductive system, tumor development, cancer and death are adverse effects of Long-term exposure to pesticides (Debnath and Khan, 2017).

According to table (1) there were high significant differences between different weights of tilapia fish as large weight fish have more residues of organochlorines pesticides than small weight fish. Larger and/or fatty fish often contain higher organic contaminant concentrations than smaller and leaner fish (USEPA, 2000). Also, increase of organochlorine pesticides concentrations in large fish is explained by physiological changes resulting in their slow clearance (Olsson et al., 2000). Concentrations of OCPs vary among contaminants in ecosystems, different fish species, size classes within a fish species and fish tissues furthermore, chemical contaminants are not bio-accumulated to the same degree in all fish species (Hassan et al., 2020).

DDT, total mean value was 16.82±4.7 ppb. Lower findings were obtained by Morshdy et al. (2018) (12.5±4.55 ppb , Ali et al. (2016) (0.06±4.04 ppb) and Marzouk et al. (2016) (420.8 ppb). Whereas, higher findings were obtained by Hassan et al. (2020) (154.1 ppb in Nile tilapia), Morshdy et al. (2018) (DDT in tilapia from Damietta and Sohag were
Endosulfan, total mean value was 6.2±1.17 ppb. Lower findings were recorded by Marzouk et al. (2016) (1.24±0.4 ppb) and Botaro et al. (2011) (in muscle of adult and juveniles farmed Nile tilapia 0.103±0.089 and 0.033±0.026 ppb). Also, Nasr et al. (2009) (3.836 ppb in El-Embaby drain at El Menofiya Governorate). Whereas, higher values were obtained by Hassan et al. (2020) (14.45±3.12). Endosulfan remains in the environment for longer periods and bio-accumulates in plants and animals which leads to contamination of food consumed by humans (Brizet et al., 2011). γ-chordane, total mean value was 21.32±3.44 ppb. Lower findings were obtained by Morshdy et al. (2018) (in tilapia from Damietta and Sohag were 16.44±1.22 and 10.4±2.78 ppb) and Nasr et al. (2009) (1.650 ppb in fish from El-Bagoria canal at El Menofiya Governorate). Whereas, Higher values were recorded by Hassanen et al. (2016) (in Otochromis niloticus from Manzala Lake and El-Ryiah El-Tawfiky were 90±11 and 20±1.1 ppb).

Heptachlor epoxide, total mean value was 5.33 ppb. Nearly similar results were obtained by Nasr et al. (2009) (4.541 ppb in fish from Miet Rabiha drain at El Menofiya Governorate) which could be attributed to the drainage canal receives industrial and productive waste-waters. Lower findings were obtained by Kamel et al. (2015) (0.113±0.052 ppb). Higher findings were obtained by Morshdy et al. (2018) (in tilapia from Damietta 14.33±2.11 ppb) and Talab and Ghanam (2015) (14 ppb in raw Nile tilapia fillets from local market at EL-Kanater El-Khairia City).

Levels of OCPs contaminants can vary significantly within the same fish species depending on the area where the fish was caught, the age and the fat content (Pandelova et al., 2008).

Methoxychlor, total mean value was 1.24±0.31 ppb. Higher findings were obtained by Hassan et al. (2020) (6.98±1.88) and Morshdy et al. (2018) (in tilapia from Damietta and Sohag were 31.88±5.21 and 8.55±2.12 ppb). the high ratio of OCPs in fish from Damietta may be because of the extensive past use of these OCPs in agriculture and nonagricultural activities (Azab et al., 2013).

Hepatochlor, total mean value was 1.28±0.31 ppb. similar values were obtained by Ogunfowokan et al. (2012) (the monthly mean values in fish in the rainy and dry season monthly were 1.36±1.00 ppb and 1.89±1.75 ppb). Lower findings were obtained by Yahia and Elsharkawy (2014) (in Nile tilapia from Mankbud area 0.18±0.01 ppb). whereas, Higher findings were recorded by Hassan et al. (2020) (5.03 ppb) and Morshdy et al. (2018) (in tilapia fish from Damietta 18.55±1.44 ppb). Whereas, Talab and Ghanam (2015) (in Nile tilapia from local market at El-Kanater El-Khairia City 15 ppb). While, too much high values were recorded by Abd El-Gawad and Abou El Elifa (2014) measured Hepatychlor residues at Kafr El-Zayat were 206.6 ppb as industrial pesticide of Kafr El-Zayat pesticides Production Company. Pesticides end up in the tissue of aquatic organisms and bio-accumulate with time (Yahia and Elsharkawy, 2014). Many organochlorine pesticides and their metabolites are highly toxic and have been implicated in a wide range of adverse health effects such as cancer, neurological damage, reproductive system deformities, birth defect, and damage to the immune system (Leena et al., 2012). Children have higher values of cancer risks than adults from consumption of fish contaminated with organochlorine pesticides

98.11±11.69 and 21.33±5.12 ppb). Reproductive disorders, fetal anomalies and breast adenocarcinoma are positively related to DDT (Thompson et al., 2017).

DDD, total mean value was 44.80±4.65 ppb. Lower findings were recorded by Hassan et al. (2020) (33.35±1.76 ppb in Nile Tilapia), Hassanen et al. (2016) (30±2.3 ppb of DDD from agricultural drainage), Kamel et al. (2015) (DDD in tilapia from Manzala Lake 0.087±0.008 ppb). Whereas, higher findings were obtained by Morshdy et al. (2018) (55.23±7.11 ppb), Hassanen et al. (2016) (170±21 ppb DDD in O. niloticus from El-Ryiah El-Tawfiky). Moreover, Marzouk et al. (2016) (80±8 ppb in Tilapia).

DDD, total mean value was 8.34±4.22 ppb. Lower findings were obtained by Hassan et al. (2020) (6.27±0.68 ppb in Nile Tilapia). Kamel et al. (2015) (0.024±0.008 ppb in tilapia from Manzala Lake). While, Yahia and Elsharkawy (2014) (1.25±0.34 ppb in Nile tilapia from Elwastra area). Also, Afful et al. (2010) (0.6±0.01ppb in Tilapia from Weija along the Densu river in Ghana). Whereas, higher findings were reported by Morshdy et al. (2018) (44.15±9.76 ppb from Damietta). Mohamed et al. (2016) (95.55±19.55 ppb), Talab and Ghanam (2015) (20 ppb in Nile tilapia from El-Kanater El-Khairia).

Total DDTs include DDT metabolites (sum of DDT, DDD and DDE). In the present study the mean values of total DDTs and its metabolites (DDT, DDD and DDE) were 69.96 (16.82, 44.80 and 8.34 ppb) in tilapia fish. The obtained results were below permissible limits (5000 ppb) set by (US-FDA, 2008). Degradation resistance and High lipid solubility of DDT residues and metabolites (DDD and DDE) make them soluble in fats and lipids of animals therefore, when water is contaminated, fish and other aquatic organisms have the capacity to absorb them from water and concentrate them in their fatty tissues (WHO, 2010). Also, DDT oxidize to DDE in aerobic or oxidation environment and deoxidize to DDD under anaerobic or reducing environment (Guo et al., 2008).

Endrin, total mean value was 26.54±3.13 ppb. Nearly similar results were recorded by Hassan et al. (2020) (25.02±0.01ppb), while, Lower findings were obtained by Morshdy et al. (2018) (15.65±2.66 and 4.5±0.32 ppb in tilapia from Damietta and Sohag) and Omwengna et al. (2016) determined (0.04±0.05 ppb). Whereas, higher findings were obtained by Marzouk et al. (2016) (100±0.3 ppb). Abd El-Gawad and Abou El Elifa (2014) (in Damietta branch was 124 ppb, while at Helwan location was 76.66 ppb). Also, Gad (2010) (The detected endrin from Helwan was 40 ppb, El-Giza 10 ppb and El- Qalubia 1600 ppb).

Dieldrin, total mean value was 10.91±2.72 ppb. Nearly similar results were obtained by El-Mekkawi et al. (2009) who monitored the mean concentrations of dieldrin in three pools from Private Fish Farms residues in fish tissue from three pools in Private Fish Farms were (13.05, 9.7 and 4.2 ppb, respectively). While, Lower findings were obtained by Hassan et al. (2020) (0.75±0.13 ppb). Kuranchie-Mensah et al. (2011) (in Nile tilapia 4.09±0.65 ppb) and Daoud et al. (2011) (0.16±0.006 ppb in tilapia nilotica collected from Qena markets). Whereas, higher findings were recorded by Mohamed et al. (2016) (41.5±0.50 ppb) and Talab and Ghanam (2015) (30 ppb in Nile tilapia from El-Kanater El-Khairia City). In the environment or the body of an organism, aldrin is converted into dieldrin by the action of sunlight and bacteria which is resistant to bacterial and chemical breakdown (Afful et al., 2013). Dieldrin is more environmentally persistent as it presents lower biotransformation and evaporation ratios than aldrin (Cetesh, 2008).

Environmental breakdown of organochlorines in the environment is by the action of sunlight and bacteria which is resistant to bacterial and chemical breakdown (Afful et al., 2013). Dieldrin is more environmentally persistent as it presents lower biotransformation and evaporation ratios than aldrin (Cetesh, 2008).
(Wenaty et al., 2019). Human health risk from consumption of fish tissue from different body weight groups (A, B and C): the EDI, CR and HR were estimated for fish groups with different weights. The obtained data in Table (3) showed that hazard ratio (RH) of studied OCPs in fish of different weights was less than 1 that ensure consumption of these fish has no potential hazard risk to human health. Carcinogenic risk (CR) is considered acceptable risk (more than 10-6) for total DDT, dieldrin, γ-chlordane and δ-BHC in group A and dieldrin, γ-chlordane in group B while CR is considered level of concern (between 10-6 and 10-4) for total DDT and δ-BHC in group B,C and heptachlor epoxide in group B also, total heptachlor in group C. whereas, Carcinogenic risk (CR) is considered non acceptable risk (less than 10-4) for dieldrin and heptachlor epoxide in group C.

5. CONCLUSION
From the present study, it could be concluded that there is a variation in the levels of organochlorine pesticides residues in the examined samples of tilapia fish at different body weights from different regions at Cairo and Giza markets. The large weight fish has more levels of organochlorine pesticides residues in its muscle tissue than small weight fish. So, the smaller fish is preferred than larger fish within a species as they may have lower contaminant levels, while the larger fish may be more contaminated because they had more time to accumulate residues in their bodies.

6. REFERENCES


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