

Detection of Organochlorine Pesticides Residues in Nile Fish and Its Risks in Qena City**Mostafa Abu Elmagd Hassan¹, Ali Meawad Ahmed², Nabil Mohamed Marzouk³, Mohamed Abdelfatah Maky⁴**

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Abstract

Fifteen organochlorine pesticides (OCP) residues were determined in Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and Long fin catfish (*Chrysichthys auratus*) in Qena city, Egypt by gas mass chromatography. Nile tilapia fish significantly ($P < 0.05$) had the highest concentrations of Dichlorodiphenyldichloroethylene (DDE), endrin, methoxychlor, Dichlorodiphenyldichloroethane (DDD) and endosulfan sulfate with mean \pm SD concentration of 6.27 ± 0.68 , 25.02 ± 0.01 , 6.98 ± 1.88 , 33.35 ± 1.76 and 47.82 ± 12.51 ppb respectively. Long fin catfish significantly had the highest concentrations of D-chlordane, alpha hexachlorobenzene (a-BHC) and aldrin with mean \pm SD concentration of 82.27 ± 0.45 , 11.89 ± 0.56 , 11.73 ± 0.48 ppb respectively. Finally, African catfish significantly had the highest concentration of dieldrin with mean concentration 3.53 ± 0.03 ppb and had significant lowest concentration of endrin 1.99 ± 1.39 ppb. OCP residues in all examined raw fish samples were below the maximum permissible limits (MPL) set by Food and Drug Administration. The effect of pan frying on OCP residues showed that the highest reduction rate in OCP in Nile tilapia was 100% for dieldrin and endosulfan sulfate while reduction rate in African catfish was 100% for dieldrin, methoxychlor and endosulfan sulfate. On the other hand, the reduction rate in Long fin catfish was 100% for dieldrin, endrin, methoxychlor, heptachlor epoxide and endosulfan sulfate. The lowest reduction rate was detected in DDD, DDE, b-BHC, heptachlor and DDT respectively. Consumption of studied fish had no potential hazard to human health as hazard ratio was below one. Carcinogenic risk (CR) value considered acceptable risk for b-BHC and considered level of concern for heptachlor epoxide, total DDTs, lindane and total heptachlor.

Keywords: organochlorine, pesticides, fish.

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Introduction

Fish is a desirable and highly nutritive food and considered as a rich source of calcium, phosphorous and vitamins, long-chain omega-3 polyunsaturated fatty acids which have many health benefits (Mohamed et al., 2015). Pesticides are substances used to control organisms, including insects, water weeds and plant diseases. Pesticides used in agricultural fields to control pests is extremely toxic to non-target organisms like fish and affects fish health through impairment of metabolism and sometimes leading to death (Shankar et al., 2013). During those days, increased human population with rapid industrialization induced problem of disposal of waste waters. The domestic wastes and untreated or partially treated industrial effluents, supplemented with pollutants like heavy metals, pesticides and many organic compounds lead to massive fish death of aquatic ecosystems (Pazhanisamy and Indra, 2007).

Organochlorines have several forms of toxic effects including effects on liver, immunity 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 (Kallenborn, 2006).

Nile tilapia, African catfish and Long fin catfish are the most common types of fish in Qena city and most consumed fish due to their high palatability and economic price. This study was planned to evaluate OCP concentration in muscle of these fish species and human risk associated with fish consumption.

Materials and Methods

Collection of samples:

One hundred and fifty random samples of three different fish species were random collected from different fish

markets in Qena city. Each sample was kept frozen in a separate sterile plastic bag and transferred to the laboratory in an insulated ice box as fast as possible. Average length of Nile tilapia, African catfish and Long fin catfish was 15-20, 20-30 and 10-15 cm respectively while average weights was 150-200, 250-300 and 150-180 g respectively. Initially screened positive samples which contaminated with OCP were heat treated by pan-frying in corn oil at 150 C for 10 minutes (Darwish et al, 2015) to evaluate the reduction rate in OCP concentrations.

Preparation of Samples:

Fish samples prepared according to (AOAC, 1996). Fish samples were washed several times with deionized water to clean them from sediments and other adhesive materials. The fish samples were identified, scaled and the heads were removed using a stainless-steel knife, the flesh and other edible portions removed from the bone and entrails. Fifty grams from the dorsal muscle were ground in meat chopper and frozen until analysis and given unique identification codes.

Extraction of OCP:

One hundred g of anhydrous sodium sulfate were added to combine with water to disintegrate sample with a spatula and blender until well mixed, scrape down the sides of blender jar and break up the caked material with spatula then 150 ml petroleum ether were added and blended at high speed for 2 min. The extract then decanted through 12 cm Buchner funnel (containing two 12 cm Whatman filter paper No. 1) into a suction flask. The residue in the blender cup was re-extracted with 75 ml petroleum ether, blended at high speed for 2 minutes decanted through the Buchner funnel and collected with the first extract then the obtained extract was put in rotary evaporator till complete

evaporation of petroleum ether and obtaining only fat content. Extraction method was done according to (AOAC, 1996).

Clean-up by Petroleum ether-Acetonitrile partitioning:

The extracted fat was transferred into 125 ml separatory funnel and 15 ml petroleum ether and 30 ml acetonitrile saturated with petroleum ether were added. Shake the funnel vigorously for 1 min and hold the separatory funnel in horizontal position and allow the layers to separate then drain the acetonitrile layer (the lower one) into a 1 L another separatory funnel containing 650 ml distilled water, 40 ml saturated sodium chloride solution and 100 ml petroleum ether. Set aside thoroughly mixed for 1 minute and leave to separate. Drain the ether layer (the upper one) over cotton wool and anhydrous sodium sulfate conditioned with petroleum ether into 250 ml round bottom flask and evaporate till complete evaporation of petroleum ether (AOAC, 1996).

Florisil Column Clean-up:

A glass column 22 mm internal diameter was prepared, blocked from bottom with glass wool and filled with activated florisil (60-100 mesh pesticides residues grade activated at 130 °C for 12 hours) to a height of 10 cm topped with 1 cm anhydrous sodium sulfate. Pre-wet the column with 40-50 ml petroleum ether then add 2g anhydrous sodium sulfate and 20 ml petroleum ether to the obtained petroleum ether solution of sample extract from the above step and then passed through the prepared column at the rate of 5ml/minute. The column was eluted at the same rate (5ml/minute) using 20 ml from every one of three eluting solvents (6% diethyl ether in petroleum ether, 15% diethyl ether in petroleum ether and 50% diethyl ether in petroleum ether). The eluate was concentrated to a dry film using the rotary evaporator then dissolved in 2ml

n-hexane and transferred into auto sampler vial (AOAC, 1996). The concentrated eluate is now ready for injection into Gas chromatography/Mass Spectroscopy (GC/MS) A TSQ triple quadruple GC-MS/MS instrument coupled with a Thermo Scientific™ TRACE™ 1300 GC (Thermo Scientific, Austin, TX, USA) was used. Sample introduction was performed an auto sampler (Thermo Scientific™ AS3000) and chromatographic separation using a Thermo Scientific™ Trace GOLD TG-5MS 30 ml 0.25 mm ID 0.25 µm film capillary column. GC/MS and Injector condition were TRACE 1310 GC, 1.0 (µl) injection volume, Liner SSL single taper (P/N: 453A2342), 240 (°C) inlet temperature, splitless inlet module and mode, carrier gas is He, 1.2(ml/min). Oven temperature program is 60 (°C) for temperature 1, 1 min hold time, 180 (°C) for temperature 2 with rate 50 (°C/min), 320 (°C) for temperature 3 with Rate 35 (°C/min) and hold time 4 min.

Mass Spectrometer Condition was TSQ 8000 Evo Mass Spectrometer, 280 (°C) Transfer Line, Ionization type is EI, 320 (°C) Ion source, 70 (eV) Electron Energy, Acquisition Mode: t-SRM, Q2 Gas Pressure (argon)(psi) is 60 , 0.7 (Da) Q1Peak Width and 0.7 (Da) Q3 Peak Width. The pesticide residues were identified based on comparison of relative retention times to those of known standards. Stock standard solutions of pesticide were prepared by dissolving the compound in hexane and stored in amber bottles. Mixed standard solutions were prepared from the individual stock solutions with known concentrations to create the calibration curves at R² = 0.9995. A series of calibration standards were prepared by diluting the mixed standard solution to produce final concentrations in hexane.

Calculation of organochlorine pesticides:

$$C = \frac{\text{peak area(sample)} \times \text{dilution(2ml)} \times \text{standard conc. (ng)}}{\text{peak area(standard)} \times \text{injection volume(1}\mu\text{l)} \times \text{sample weight(gm)}} \times 1000$$

Where C= Concentration of OCP (ppb).

Experimental trial:

- Experimental part aimed to control of OCP residues in fish by heat treatment. Fish samples contaminated with OCP were treated by pan-frying by cooking the positive fish samples in a hot pan containing a cooking oil (corn oil) at 150 C for 10 minutes then 50 g of heat treated fish muscle were prepared, extracted and pass through the steps of clean up by petroleum ether- acetonitrile partitioning and florisil column clean up to get the concentrated eluate for injection into GC/MS for detection of pesticides concentrations as mentioned before in preparation and extraction of pesticides in raw fish. Reduction rate % (R.R) of pesticides due to the effect of heat on OCP residues in fish muscles were calculated according to the following equation:

$$R.R = \frac{\text{Mean conc. of OCP in raw fish} - \text{Mean conc. of OCP in heat treated fish}}{\text{Mean conc. of OCP in raw fish}} \times 100$$

Human health risk assessment:

It can be determined through the following parameters:

Estimated Daily Intake (EDI)

The estimated daily dietary intake of OCPs from fish consumption was calculated according to (WHO, 1987) using the following equation:

$$\text{EDI} = C \times \text{DR} / \text{BW}$$

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 48 g/day per person in Upper Egypt area (FAO, 2003).

Carcinogenic Risk

Both cancer risk (CR) and hazard ratios (HR) were calculated according to the (USEPA, 2005).

$$\text{CR} = \text{EDI} \times \text{CSF}$$

Where CSF is cancer slope factor (mg/kg per day). Its value was 1.6 for HCB, 0.35 for Σ CHL 16 for dieldrin, 9.1 for heptachlor epoxide, 1.3 for lindane and 0.34 for Σ DDT (USEPA, 2000).

If the CR is more than 10^{-6} it is considered 'acceptable risk', between 10^{-6} and 10^{-4} are considered 'levels of concern' and a CR smaller than 10^{-4} is considered 'Unacceptable risk' (USEPA, 2005).

The Hazard Ratios (HR)

To evaluate the potential non-carcinogenic health risk, it was assessed by calculating the hazard index (HI) (US EPA, 1991).

$$\text{HI} = \text{EDI} / \text{ADI}$$

Where, EDI; Estimated Daily Intake (mg/ kg bw) and ADI; Acceptable Daily Intake (mg/ kg bw). For a preliminary quantitative risk assessment, a $\text{HI} \leq 0.2$ 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).

For carcinogenic effects evaluation, hazard ratio (HR) was calculated using the following equation according to (USEPA, 2005) and (Jiang et al. 2005):

$$\text{HR} = \text{EDI} / \text{BMC}$$

Where the BMC is the benchmark concentration, derived from the USEPA CSF:

$$\text{BMC} = (\text{Risk} \times \text{BW}) / (\text{Fish consumption} \times \text{CSF})$$

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).

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 (Significant at $P < 0.05$).

Results

From the result recorded in table (1) and figure (1,2,3), it was obvious that Alpha-hexachlorocyclohexane (a-BHC) concentrations level were ranged from 0.93–8.42, 0.29–3.75 and 11.14–12.64 ppb with mean value 4.77 ± 3.59 , 2.21 ± 1.43 and 11.89 ± 0.56 ppb in Nile tilapia, African catfish and long fin catfish respectively. Beta- hexachlorocyclohexane (b-BHC) concentrations were 4.54–4.65, 4–6.54 and .21–5.36 with mean value 4.61 ± 0.04 , 5.27 ± 0.92 and 5.26 ± 0.06 ppb in Nile tilapia, African catfish and long fin catfish respectively.

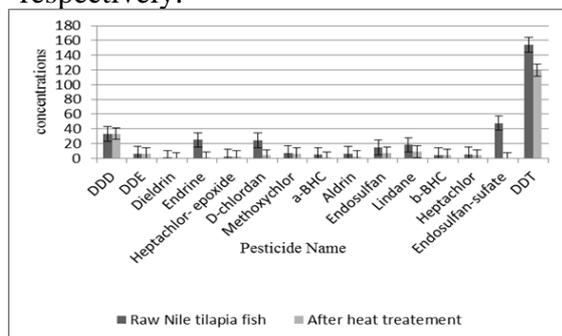


Figure 1. Mean concentration of organochlorine pesticides residues in Tilapia Nilotica fish before and after heat treatment.

Lindane concentrations level were ranged from 8.5–27.86, 28.87–29.32 and 17.4–28.68 ppb with mean value 18.18 ± 9.11 , 29.11 ± 0.20 and 23.03 ± 4.55

ppb in Nile tilapia, African catfish and long fin catfish respectively. The highest lindane level was detected in African catfish. Heptachlor mean concentrations were 5.03, 4.95 ± 0.04 and 4.14 ± 0.26 ppb in Nile tilapia, African catfish and long fin catfish respectively while heptachlor-epoxide mean values were 2.43 ± 0.01 , 2.37 ± 0.10 and 2.53 ± 0.20 ppb in Nile tilapia, African catfish and long fin catfish respectively. Aldrin mean concentrations were 5.94 ± 3.67 , 5.59 ± 3.18 and 11.73 ± 0.48 ppb in Nile tilapia, African catfish and long fin catfish respectively. The mean concentrations of dieldrin and endrin were ranged from 0.5–3.53 and 1.99–25.02 ppb respectively.

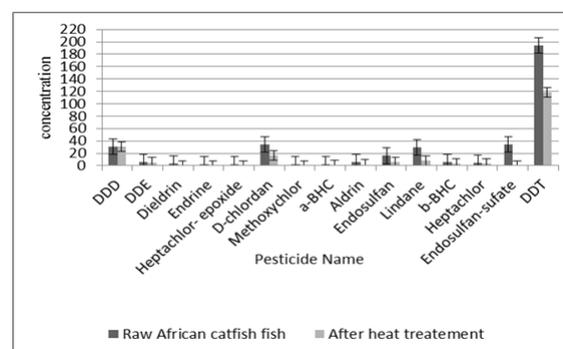


Figure 2. Mean concentration of organochlorine pesticides residues in African catfish before and after heat treatment.

Chlordane mean concentration was 24.17 ± 1.15 , 33.93 ± 19.57 and 82.27 ± 0.45 ppb in Nile tilapia, African catfish and long fin catfish respectively. The obtained mean concentrations of endosulfan in Nile tilapia, African catfish and long fin catfish were 14.45 ± 3.12 , 15.89 ± 6.45 and 12.74 ± 1.03 ppb and endosulfan sulfate mean concentrations were 47.82 ± 12.51 , 34.28 ± 0.27 and 35 ± 0.20 ppb respectively. Methoxychlor mean concentrations in Nile tilapia, African catfish and long fin catfish were 6.98 ± 1.88 , 1.84 ± 0.09 and 0.79 ± 0.43 ppb. Total DDTs and its metabolites (DDD, DDE and DDT) concentrations

were 193.72 (33.35, 6.27 and 154.1 ppb) in Nile tilapia, 230.44 (30.75, 5.35 and 194.52 ppb) in African catfish and 234.93 (30.8, 5.56 and 198.57 ppb) in long fin catfish respectively.

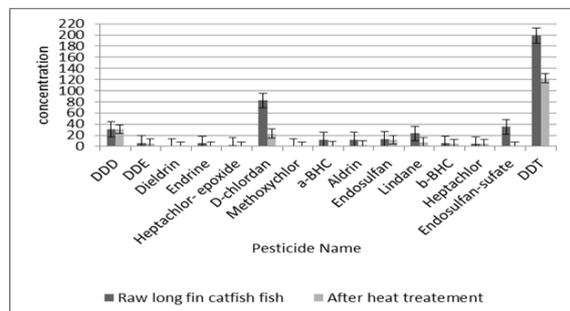


Figure 3. Mean concentration of Organochlorine pesticides residues in long fin catfish before and after heat treatment.

Nile tilapia fish had significantly the highest mean concentration of DDD, DDE, endrin, methoxychlor and endosulfan sulfate and the lowest mean concentration of DDT at $P < 0.05$ among different fish species. African catfish had significantly the highest mean concentration of dieldrin and the lowest mean concentration of endrin among different examined fish species while aldrin, a-BHC and chlordane recorded the most significant highest mean concentration in Long fin catfish comparing with other examined fish species at $P < 0.05$.

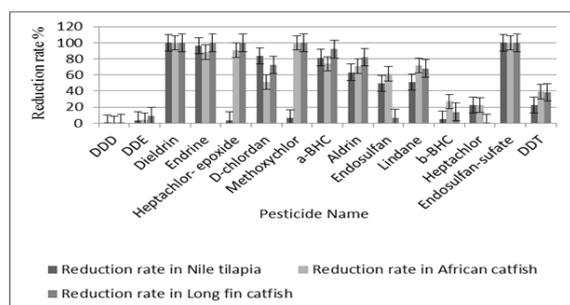


Figure 4: Reduction rate of organochlorine pesticides residues in fish species after pan frying.

The results obtained in table (2) and figure (4) revealed that dieldrin and

endosulfan sulfate cannot be detected after heat treatment that record 100% reduction rate (R.R) and the lowest R.R was recorded in DDD, DDE, heptachlor epoxide, b-BHC and methoxychlor with rate varied from 0.12 to 6.59% in pan-fried Nile tilapia.

Table (2) showed that dieldrin, methoxychlor and endosulfan sulfate were undetected after pan-frying with 100% R.R while R.R of heptachlor epoxide, endrin, a-BHC, lindane, aldrin, endosulfan, D chlorodan, DDT, b-BHC and heptachlor varied from 22.42% up to 90.72%. The lowest R.R was 0.07% and 3.93% for DDD and DDE respectively in pan-fried African catfish.

Pan-frying of long fin catfish reduced five organochlorine pesticides with 100% R.R including dieldrin, endrin, heptachlor epoxide, methoxychlor and endosulfan sulfate while R.R for a-BHC, aldrin, D chlorodan, lindane, DDT and b-BHC were 92.01, 81.93, 72.32, 68.04, 38.35, 14.07% respectively and the lowest R.R was 0.09, 0.48 and 8.81% for DDD, heptachlor and DDE respectively.

Discussion:

In the current study mean value of Alpha-hexachlorocyclohexane (a-BHC) was varied from 2.21 to 11.89 ppb in examined fish species which was below permissible limits (300 ppb) set by FDA (2001). The obtained results were higher than those detected by Abdallah and Morsy (2013) who found a-BHC in catfish and tilapia fish collected from El-Manzala Lake with range of 0.27 ng/g and 0.17-37.15 ng/g respectively while it was lower than the result obtained by Salah El-Dien and Mahmoud (2011) who revealed that concentration of a-BHC was 24.26 ppb in catfish collected from Sharkia market. The obtained results were similar to the results recorded by Abdallah and EL-Greisy (2006) who study the a-BHC concentration in male and female *Tilapia zillii* in El-Manzala Lake (1.8 and 2.2 ppb) and El-

Mekkawi et al.(2009) where mean value of α -BHC varied from 8.5-15.3 ng/g in three fish farms in Sharkia Governorate, Egypt.

Beta-hexachlorocyclohexane (β -BHC) mean value were varied from 4.14-5.27 ppb in fish species which was higher than those detected in catfish, bayad and mackerel measured by Marzouk et al.(2016). It was lower than result of Choudhury et al.(2013) where it ranged from 0.001 to 0.0016 $\mu\text{g/g}$ in five species of fish in Jorhat district of Assam, India. Also, Witczak and Patrzykat (2011) detected β -BHC with range 2.89 to 18.28 (ng/g dry mass) in five fish species in Poland. The obtained results of β -BHC in current study were closely resembled to the values obtained by Salah El- Dien and Mahmoud (2011). On the other hand, Barakat et al.(2017) revealed abnormal high concentration of β -BHC was found in muscle of Mugil species (543 mg/kg w/wt) and in Tilapia (0.11 mg/kg w/wt) collected from Qarun Lake, Egypt which exceeded the maximum residue level recommended by various organizations. Qarun Lake is suffering from serious water pollution problems due to uncontrolled solid and liquid domestic and industrial waste disposal practices, in addition to agrochemical contamination and lack of sustainable wastewater management (Hussein et al. 2008).

Lindane (Gamma-hexachlorocyclohexane) used primarily for controlling wood-inhabiting beetles and as a seed treatment. Lindane is also used as a prescription pharmaceutical to control head lice and mites (scabies) in humans. Lindane mean values were varied from 18.18- 29.11 ppb. Its concentration was below permissible limits (0.3 ppm) set by FDA (2001). In current study, the highest

concentration of lindane was detected in African catfish. It was higher than results of Daoud et al. (2011) who recorded the mean value (0.002 $\mu\text{g/kg}$) in Tilapia collected from Qena market, Fianko et al.(2011) who revealed the mean concentration of γ -HCH in examined fish samples collected from Densu river basin in Ghana was 4.94 $\mu\text{g/kg}$ and (Witczak and Patrzykat, 2011) who measured mean concentration of γ -HCH (2.84 to 6.54 ng/g dry mass).

Heptachlor and Heptachlor-epoxide were classified as a probable human carcinogen (B2) by EPA. Heptachlor and Heptachlor-epoxide mean values were varied from 4.14-5.03 and 2.37-2.53 ppb respectively and the detected levels were within the permissible limits (300 ppb) set by FDA (2011) And US-FDA (2000) while those detected by Botaro et al.(2011) were varied from 0.01-0.04 $\mu\text{g/kg}$ in farmed tilapia from Brazilian fish farms. Heptachlor was the highest average concentration (18.5ng/g) in catfish samples detected by (Rahmawati et al., 2013). Obtained results were below the average concentrations of heptachlor and heptachlor-epoxide residues in fish samples collected from Damietta while it was close to these concentrations in samples collected from Sohag measured by Morshdy et al. (2018). Elbially et al. (2015) measured the range of heptachlor (3.2-10.89) and heptachlor epoxide (15.2-83.69) $\mu\text{g/kg}$ in farmed Nile tilapia in Kafr Elsheikh Governorate, Egypt which is higher than current study results.

Aldrin mean concentration was varied from 5.59-11.73 ppb in examined fish species. It was within permissible limits (0.3 ppm) set by FDA (2001). Aldrin concentration was 2.56 $\mu\text{g/kg}$ measured by Daoud et al.(2011), 0.61 ppb in tilapia

Table (1) Concentration levels of Organochlorine Pesticides residues (ppb) in raw fish species (No=50).

Pesticide	Raw Nile tilapia fish					Raw African catfish					Raw Long fin catfish				
	Contaminated fish sample					Contaminated fish sample					Contaminated fish sample				
	No	%	Min	Max	Mean±SD	No	%	Min	Max	Mean±SD	No	%	Min	Max	Mean±SD
DDD	9	18	31.13	35.56	33.35±1.76	10	20	30.52	30.63	30.57±0.03	7	14	30.69	30.95	30.80±0.08
DDE	9	18	5.45	7.09	6.27±0.68	10	20	5.32	5.39	5.35±0.03	7	14	5.33	5.62	5.56±0.01
Dieldrin	7	14	0.07	0.95	0.75±0.13	5	10	3.49	3.56	3.53±0.03	4	8	0.49	0.51	0.50±0.01
Endrin	5	10	25	25.03	25.02±0.01	10	20	0.34	3.46	1.99±1.39	5	10	5.3	5.32	5.31±0.01
Heptachlor-epoxide	5	10	2.41	2.44	2.43±0.01	10	20	2.22	2.51	2.37±0.10	7	14	2.29	2.77	2.53±0.20
D-chlordane	9	18	22.97	25.35	24.17±1.15	10	20	8.91	58.94	33.93±19.57	2	4	81.81	82.73	82.27±0.45
Methoxychlor	2	4	4.94	9.02	6.98±1.88	10	20	1.71	1.94	1.84±0.09	7	14	0.3	1.27	0.79±0.43
a-BHC	6	12	0.93	8.42	4.77±3.59	9	18	0.29	3.75	2.21±1.43	7	14	11.14	12.64	11.89±0.56
Aldrin	9	18	2.1	9.77	5.94±3.67	10	20	1.57	9.6	5.59±3.18	7	14	11.15	12.3	11.73±0.48
Endosulfan	9	18	11.2	17.7	14.45±3.12	10	20	6.8	24.99	15.89±6.45	7	14	11.63	13.84	12.74±1.03
Lindane	8	16	8.5	27.86	18.18±9.11	10	10	28.87	29.32	29.11±0.20	7	14	17.4	28.68	23.03±4.55
b-BHC	7	14	4.54	4.65	4.61±0.04	10	20	4	6.54	5.27±0.92	7	14	5.21	5.36	5.26±0.06
Heptachlor	1	2	5.03	5.03	5.03	5	10	4.89	4.99	4.95±0.04	7	14	3.9	4.44	4.14±0.26
Endosulfan-sulfate	9	18	34.68	60.96	47.82±12.51	4	8	33.99	34.57	34.28±0.27	7	14	34.67	35.21	35.00±0.20
DDT	9	18	118.54	189.66	154.1±31.62	10	20	186.08	202.96	194.52±5.68	7	14	192.78	204.35	198.57±5.47

collected from Assuit Governorate detected by Yahia and Elsharkawy (2014) and 1.9 ppb estimated by Salah El- Dien and Mahmoud (2011). It was lower than results measured by Eltholth et al. (2018). Aldrin was not detected in fish examined by Morshedy et al.(2018) and Marzouk et al.(2016).

Dieldrin and Endrin EPA has classified endrin as a Group D carcinogen (not classifiable as to human carcinogenicity). Children may be more sensitive to acute endrin exposure than adults, based on effects observed in children during a poisoning incident. Children appeared more susceptible to neurotoxic effects and have exhibited convulsions. The mean concentrations of dieldrin and endrin were ranged from 0.5-3.53 and 1.99-25.02 ppb respectively. It was within permissible limits detected (300 ppb) set by FDA (2011). Marzouk et al.(2016) did not detect dieldrin in examined fish but endrin mean value was 0.04 ppb. Both dieldrin and endrin lower than those exceeded the permissible limits

studied by Barakat et al. (2017) where the values were 43.2 and 3260 (mg/kg ww) in muscle of Nile tilapia and 449 and 409 (mg/kg wet ww) in Mugil species from Qarun Lake, Egypt. The obtained value was below the results of El Nahas et al.(2017). Obtained results were match with Eltholth et al.(2018) who measured concentration of dieldrin (16.78) and endrin (2.37 ppb) in tilapia from fish farms in Kafrelsheikh governorate which was within maximum permissible limits. Aldrin significant difference among fish samples and the sediments may be due to their specific habitats and feeding habits as well as a higher rate of biotransformation of aldrin to dieldrin and endrin to endrin aldehyde in fish.

Chlordane mean value was ranged from 24.17 to 82.27 ppb in studied fish which was within permissible limits set (300 ppb) set by FDA (2011). It was higher than those measured by Marzouk et al. (2016). Its concentration was below that detected by studied by El Nahas et al.(2017) in Tilapia fish collected from

Table (2): Organochlorine Pesticides concentration (ppb) in positive samples of fish species after heat treatment (No=50) *Significant at $P < 0.05$ among different fish species. R.R= Reduction Rate of OCP in fish after pan frying.

Pesticide name	Heat treated Nile tilapia					Heat treated African catfish					Heat treated Long fin Catfish				
	%	Min	Max	Mean \pm SD	R.R%	%	Min	Max	Mean \pm SD	R.R%	%	Min	Max	Mean \pm SD	R.R%
DDD	18	31.1	35.53	33.31 \pm 1.76*	0.12	20	30.49	30.61	30.55 \pm 0.04	0.07	14	30.67	30.89	30.77 \pm 0.07*	0.09
DDE	18	5.12	6.98	6.04 \pm 0.73 *	3.7	20	5.08	5.18	5.14 \pm 0.04*	3.93	14	5.01	5.11	5.07 \pm 0.04*	8.81
Dieldrin	14	0	0	0*	100	10	0	0	0*	100	8	0	0	0*	100
Endrin	10	0.94	0.95	0.95 \pm 0.004*	96.2	20	0	0.47	0.23 \pm 0.24*	88.44	10	0	0	0*	100
Heptachlor- epoxide	10	2.32	2.35	2.34 \pm 0.01*	3.7	20	0	2.21	0.22 \pm 0.69*	90.72	14	0	0	0*	100
D-chlordane	18	0	18.05	4.01 \pm 7.95*	83.41	20	1.82	31.52	16.67 \pm 12.66*	50.87	4	22.11	23.42	22.77 \pm 0.39*	72.32
Methoxychlor	4	4.02	9.01	6.52 \pm 2.35	6.59	20	0	0	0*	100	14	0	0	0*	100
a-BHC	12	0.85	0.91	0.88 \pm 0.03	81.55	18	0	0.92	0.58 \pm 0.34*	73.76	14	0.83	1.1	0.95 \pm 0.09*	92.01
Aldrin	18	2.01	2.3	2.18 \pm 0.12*	63.30	20	1.1	2.13	1.62 \pm 0.36*	71.02	14	2.1	2.16	2.12 \pm 0.02*	81.93
Endosulfan	18	5.15	9.42	7.29 \pm 2.08*	49.55	20	5.87	6.43	6.11 \pm 0.23*	61.55	14	10.65	13.23	11.88 \pm 1.05*	6.75
Lindane	16	8.41	9.15	8.86 \pm 0.13	51.27	10	7.95	8.48	8.22 \pm 0.25*	71.76	14	6.4	8.56	7.36 \pm 0.78*	68.04
b-BHC	14	4.31	4.45	4.37 \pm 0.05*	5.21	20	3.12	4.64	3.85 \pm 0.53*	26.94	14	4.43	4.72	4.52 \pm 0.13*	14.07
Heptachlor	2	3.9	3.9	3.9	22.47	10	3.8	3.89	3.84 \pm 0.04*	22.42	14	3.9	4.41	4.12 \pm 0.26	0.48
Endosulfan-sulfate	18	0	0	0*	100	8	0	0	0*	100	14	0	0	0*	100
DDT	18	117.89	121.48	119.69 \pm 1.59*	22.33	20	116.44	120.86	118.64 \pm 1.49*	39.01	14	121.21	123.63	122.42 \pm 1.01*	38.35

Burullus Lake. On the other hand, its average value was 10.1(ng/g ww) in fish sample from Sohag and 16.44(ng/g ww) in fish from Damietta studied by Morshdy et al. (2018). Chlordane used extensively on agricultural crops, livestock, lawns, and for termite control. Because of concern over cancer risk, human exposure, and effects on wildlife, most uses were banned in 1978, and all uses were banned by 1988 (USEPA 2000). Due to its long half-life and ability to concentrate in biological materials, it is still widely distributed in fish in the United States.

Total DDTs included DDT metabolites (sum of DDD, DDE and DDT). In the current study the mean values of total DDTs and its metabolites (DDD, DDE and DDT) were 193.72 (33.35, 6.27 and 154.1 ppb) in Nile tilapia, 230.44 (30.75, 5.35 and 194.52 ppb) in African catfish and 234.93 (30.8, 5.56 and 198.57 ppb) in long fin catfish respectively. The obtained results were below permissible limits (5000 ppb) set by (Codex Alimentarius, 1999) while those studied by (Said and Hamed, 2005) were ranged from 161-3100 ng/g with average 1087 ng/g in

three fish species collected from El Tamsah and Bitter Lakes of the Suez Canal so, it exceeded the permissible limits set by various organization. Obtained results were higher than those evaluated by Yahia and Elsharkawy (2014) who estimated pp.DDE in fish in Assuit. It was closely similar to that obtained by Al-Shwafi et al.(2009) who investigated DDTs in imported fish and shellfish from the Gulf of Adan and Red Sea of Yemen Coast. Land based activities result from agricultural and municipal wastes are the main source of pollution by chlorinated pesticides in the Suez Canal area (Said and Hamed 2005). DDT is generally used against a wide variety of agricultural and forest pests and against insect pests including vectors such as mosquito and tse-tse fly. DDTs had been in continuous use over the past decade for agricultural and public health programs Muralidharan et al.(2009)

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

Table (3) Human risk (HR) and Carcinogenic risk (CR) of Organochlorine Pesticides

pesticide	CSF	ADI	EDI			CR			HR		
			Nile Tilapia	African catfish	Long fin catfish	Nile tilapia	African catfish	long fin catfish	Nile tilapia	African catfish	long fin catfish
Dieldrin	16	10000	7.5E-07	3.53E-06	5E-07	0.000012	5.65E-05	0.000008	0.0096	0.045184	0.0064
Heptachlor epoxide	9.1	5000	2.43E-06	2.37E-06	2.53E-06	2.21E-05	2.16E-05	2.3E-05	0.01769	0.017254	0.018418
D-chlordane	0.35	500	2.42E-05	3.39E-05	8.23E-05	8.46E-06	1.19E-05	2.88E-05	0.006768	0.0095	0.023036
a-BHC	1.6	100	4.77E-06	2.21E-06	1.19E-05	7.63E-06	3.54E-06	1.9E-05	0.006106	0.002829	0.015219
Lindane	1.3	5000	1.82E-05	2.91E-05	2.3E-05	2.36E-05	3.78E-05	2.99E-05	0.018907	0.030274	0.023951
b-BHC	1.6	ND	4.61E-06	5.27E-06	5.26E-06	7.38E-06	8.43E-06	8.42E-06	0.005901	0.006746	0.006733
Total DDT	0.34	10000	0.000194	0.00023	0.000235	6.59E-05	7.83E-05	7.99E-05	0.052692	0.06268	0.063901
Total heptachlor	4.5	100	7.46E-06	7.32E-06	6.67E-06	3.36E-05	3.29E-05	3E-05	0.026856	0.026352	0.024012

CSF= cancer slope factor according to US PA (2000).

ADI= Accepted Daily Intake (ng/kg bw/day) according to WHO (2010).

EDI= Estimated Daily Intake (mg/kg bw/day).

CR= Carcinogenic Risk.

HR= Human Risk.

Bhuiyan et al (2009) stated that DDT and heptachlor are nearly insoluble in water and has a good solubility in most organic solvents, fats and oils. Moreover, these are highly stable molecule. OCP concentrations vary among different fish species, size classes within a fish species, fish tissues, and contaminants present in ecosystems. Chemical contaminants are not bioaccumulated to the same degree in all fish species. In addition, chemical contaminants are not distributed uniformly in fish tissues; some toxicants bind primarily to lipids and others to proteins. Fatty and/or larger fish often contain higher organic contaminant concentrations than leaner and smaller fish US EPA (2000). The nature of catfish habitat which lives in cloudy water and preys on another fish, worm and insects, in addition the high fat contents of catfish meat as previously said are satisfied reasons to explain these relative high frequency and concentrations of OCP residues Salah El- Dien and Mahmoud (2011).

Control of OCP residues in fish species by pan-frying:

Frying is a cooking method in which food is submerged in hot fat, most commonly in cooking oil. Typically, it is a rapid preparation technique that promotes physical and chemical changes in the products and leads to unique sensory properties of color, flavor, texture, palatability and has the greatest effect on nutrient composition in fish because of water loss, fat uptake, and changes in the profile of fatty acids Pérez- Palacios et al.(2014). Pan-frying is cooking the food in a hot pan containing a scant amount of cooking oil. Frying can affect the lipid composition and nutritional value of cooked products compared with those in the raw samples Domínguez et al.(2014). The reduction of pesticides is due to loss of lipids during cooking Bayen et al.(2005). Dressing fish that is the removal of head, viscera and tail also influences pesticide values in fish flesh however this depends on the species of fish and fatty fish has higher pesticide values.

The result obtained in table (2) showed the effect of pan-frying on OC pesticides residues in fish. The study revealed that there is significant reduction

of OCP residues (Significant at $P < 0.05$) by pan-frying. The reduction rate of dieldrin, endosulfan sulfate was 100% in all examined fish species. Methoxychlor reduction rate was 100% in both African catfish and Long fin catfish. Heptachlor epoxide reduced with rate 100% in long fin catfish and 96.2% in Nile tilapia. Endrin reduction rate reach up to 100% in Long fin catfish only. The lowest reduction rate was detected in DDD, DDE, DDT, b-BHC in all pan-fried fish species.

The obtained results were higher than the study of Witczak (2009) who demonstrated that frying reduced levels of persistent OCP in fish meat by 10-32% on average. Talab and Ghannam (2015) studied the effect of thermal processing methods microwave and halogen caused significant ($P < 0.05$) losses in the concentrations of most examined pesticides. Halogen cooking method was more effective than microwave in reducing the content of persistent organochlorine pesticides which is somewhat close to current study. The results detected by Morshdy et al.(2018) demonstrated that Pan-frying had the highest reduction effects on such OCPs. The reduction percentages were 80%, 75%, 85.5 and 90% on the total DDTs, HCHs, HPTs and drins (aldrin and endrin) respectively. Reduction rate in OC varied according to fish species and its locality according to results of Hassanen et al.(2016) who revealed that depletion rate in Tilapia samples from Manzala lake were, 100, 90, 11, 8, 67, 67, 67 % for α BHC, β BHC, γ -chlordane, HCB, heptachlor, aldrin, DDE respectively. Goyal and Aulakh (2017) studied the effect of frying on levels of pesticide residues in 3 fish species and depletion rate was 45.4, 40 to 44.5% for β -HCH, γ -HCH respectively. Finally pan-frying has significant reducing effect (Significant at $P < 0.05$) of different type of examined OCP and it considers of

effective cooking methods to reduce OC pesticides residues in fish.

Human health risk from consumption of fish tissue:

The ADI, EDI, HR and CR were estimated for each fish species .Data obtained in Table (3) showed that hazard ratio (HR) of all studied OCP in all examined raw fish species was less than 1 that ensure consumption of these fish has no potential hazard risk to human health in Qena city. Carcinogenic risk (CR) measured in examined raw fish species and its value considered acceptable risk (more than 10^{-6}) for b-BHC in all examined raw fish species, dieldrin in Long fin catfish, chlordane in Nile tilapia and a-BHC in both Nile tilapia and African catfish while studied CR considered level of concern (between 10^{-6} and 10^{-4}) for heptachlor epoxide, total DDTs, lindane and total heptachlor in all examined raw fish species. In addition to a-BHC in Long fin catfish, dieldrin in both Nile tilapia and African catfish, chlordane in both African catfish and Long fin catfish also considered level of concern. There is no level of OCP in examined raw fish considered non-acceptable risk (less than 10^{-4}).

Conflict of interest statement

The authors declare that they have no conflict of interest.

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