

MONITORING OF ORGANOCHLORINE PESTICIDE RESIDUES IN *OREOCHROMIS NILOTICUS* COLLECTED FROM SOME LOCALITIES IN EGYPT

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Abstract: *Tilapia nilotica* (*Oreochromis niloticus*) is the major fish species consumed in Egypt, particularly due to its high nutritive value, palatability and relatively low price compared with other kinds of fishes or red meat. In Egypt, tilapia fish is caught directly from river Nile or cultured in a specified aquaculture. Organochlorine pesticides (OCPs) have been extensively used in Egypt and many African countries in the past century for the control of the agricultural pests. A major character of OCPs is their persistent bio-accumulation in the environment, especially in the food chain, where they can get reach to humans. There is few reports had investigated the current scenario of OCPs contamination in fish in Egypt, particularly in Upper Egypt cities such as Sohag. Additionally, fish is consumed cooked in Egypt not raw like many Asian countries. Therefore, this study aimed at monitoring the residue levels of different OCPs in tilapia fish caught from Upper Egypt (Sohag) and compared with either that from northern part of Egypt (Damietta) or with fish cultured in a control location (Abbasa). Additionally, the effect of different cooking methods (boiling, grilling and pan-frying) on the residue levels of OCPs was investigated. The tested OCPs included pp-DDT and its metabolites pp-DDD and pp-DDE; hexachlorohexanes (HCHs) including α HCH and γ HCH; heptachlor and heptachlor epoxide; aldrin and endrin; chlordane, methoxychlor and hexachloride benzene and were detected using electron capture gas chromatography equipped with Ni63 – electron capture detector. The recorded results revealed that tilapia collected from Damietta had the highest incidence of OCPs' contamination (75%), over than that collected from Sohag (60%) compared to control value (35%). All examined samples had OCPs residues within the maximum permissible limits (MPLs) set by world health organization. Pan-frying had the highest reduction effect on the OCPs' residues followed by grilling and boiling. The public health significance of the examined OCPs was also discussed.

Key words: Tilapia; organochlorine pesticides; cooking methods; Egypt

Introduction

Fish such as tilapia species (*Oreochromis niloticus*) plays important roles as a food supply for Egyptian population. Fish is rich in high quality protein, essential amino acids, fatty acids and minerals such as calcium and phosphorus (1). Tilapia is the most famous fish species on the Egyptian table due its high palatability and relatively lower price compared with other fish species, red meat and poultry meats.

Organochlorine pesticides (OCPs) are persistent organic pollutants that were used over the past century in Egypt as well as in many African countries including those of the Nile basin for the control of pests and for malaria vector control. OCPs are characterized by their high bioaccumulation in the environment including aquatic system, where they can get reach to aquatic organisms and subsequently to human bodies after ingestion of contaminated fish or water (2,3).

OCPs had detected in the human milk and sera in many countries of the Nile basin including Egypt, Congo republic and Sudan (4-7). Additionally, there were positive association between para, para, dichlorodiphenyl trichloroethane (pp-DDT) and breast adenocarcinoma, reproductive disorders and fetal anomalies (8,9). OCPs are banned in Egypt and most of the world since 1980s (10). However, still some OCP compounds are used illegally to increase the productivity of the agricultural crops and for pest control; and subsequently can find their way into the water body through wastewater drainage systems. Among the most important OCPs are DDTs, hexachloride benzene (HCB), chlordane, heptachlor, aldrin, dieldrin and endrin (11). There are few reports indicating the current scenario of OCPs contamination in fresh water fish as tilapia species in Egypt, particularly in Upper Egypt cities such as Sohag.

Unlike many Asian countries such as China and Japan, fish is consumed cooked in Egypt. However, the effect of different cooking methods such as boiling, grilling and pan-frying on OCPs residue levels are less informed. Therefore, this study was undertaken to firstly

monitor the residue levels of twelve OCPs in the edible tissue of tilapia species collected from Sohag (Upper Egypt) and compared with either Damietta from Northern Egypt or a control location (Abbasa aquaculture). Then the recorded levels were compared with the maximum permissible limits of different OCPs set by world health organization (WHO). Secondly, the effects of different cooking methods of fish used in Egypt (boiling, grilling and pan-frying) on the accumulated OCPs were investigated. Lastly, the public health significance of the detected OCPs was discussed.

Material and methods

Research plan guidelines of Zagazig University, Egypt were followed during conducting this research study.

Sampling

Sixty *Oreochromis niloticus* fish samples were randomly purchased from local anglers at Sohag (Upper Egypt), Damietta (Northern Egypt) and from Abbasa aquaculture in Sharkia Govern ate, Egypt that there is little information available about OCPs scenario in the selected locations. Additionally, 15 *Oreochromis niloticus* samples (initially screened as positive for OCPs contamination) were heat-treated via boiling, grilling and pan-frying (n= 5 each treatment) (12). In brief, the boiled group was placed in boiled water at 100°C for 10 min, the grilled group was placed in an electric oven and grilled at 180°C for 10 min, the pan-fried group was deep-fried in corn oil at 150°C for 5 min on each side. Fish samples were homogenous in the weight and length for all sampling sites and had an average weight of 180.0±20.0 g and length of 20.0±3.0 cm. Samples were transferred into the laboratory in a cooled condition. Organochlorine pesticides were extracted and measured at Agricultural Research Center, Dokki, Giza, Egypt.

Detection of organochlorine compounds Chemicals

Standard OCPs including pp-DDT, pp-DDD, pp-DDE, α HCH, γ HCH, heptachlor, heptachlor epoxide, aldrin, endrin, chlordane, metho-

ychlor and HCB were obtained from Sigma–Aldrich Chemie GmbH (Kappelweg, Schenldor, Germany). Other chemicals were of the highest quality available and purchased from Merck (Darmstadt, Germany). Florisil was purchased from Silica (Silica Co., USA). All solvents were of pesticide grade. Florisil was activated at 130°C for 24 h and cooled to room temperature.

Extraction and preparation of samples

Each muscle sample (50g) was mixed with anhydrous sodium sulfate (100g) and petroleum ether for three successive extraction steps (2 min each) (13). Samples were filtered, and then subjected to solvent evaporation using a rotary evaporator at 40°C until dryness.

Partitioning of the extract

The method of the Association of Official Analytical Chemists (14) was followed during partitioning of the extracted samples. The extracted samples were separated with a 100 ml mixture of 80:20 n-hexanes: acetonitrile. The acetonitrile layer was collected after 3 repetitive times of partitioning followed by evaporation on a rotary evaporator to a 10-ml volume that used in florisil cleanup.

Cleanup of the extract

Cleanup of the extracted samples was performed in a chromatographic column containing 20g activated florisil. After cleanup, the resultant eluent was dried on a rotary evaporator and dissolved in hexane to a volume of 10 ml. An aliquot of each extract was transferred to 2-ml injection vials to be ready for the analysis with the electron capture gas chromatography (13).

Determination of organochlorine pesticide residual concentrations

Organochlorine pesticide residues were analyzed using electron capture gas chromatography (Hewlett Packard GC Model 6890) equipped with Ni63 – electron capture detector. GC conditions: HP- 5MS capillary column (30 m length X 0.32 mm internal diameter, X 0.25µm film thickness, carrier gas: N₂ at a flow rate of 4 ml/min; injector and

detector temperatures were 230°C and 300°C respectively). The extract was injected into a single inlet that was split into the dual columns. Instrumental settings were as follows: injector and detector temperatures were 230°C and 300°C, respectively; the gas chromatography oven temperature program was initiated at 150°C for 5 min, raised to 170°C (at a rate of 5°C/min) and held for 10 min, then raised to 220°C (at a rate of 10°C/min) and held for 20 min (with a total run time of 44 min); the injection volume was 1, µl, and the flow rates of nitrogen gas was 20 ml/min.

Quality assurance of analytical procedures

Analysis was done based on the comparison with the created standard curves. To validate the analytical procedures, the standard reference material, SRM 1947 (Lake Michigan Fish Tissue) was similarly processed and analyzed like the samples. The recovery percentages of the tested OCPs ranged from 88% to 106%.

Statistical analysis

All recorded values were normally distributed and expressed as means ± SE. Statistical significance was judged using one-way analysis of variance (ANOVA) followed by the post-hoc Tukey–Kramer HSD test (JMP statistical package; SAS Institute Inc., Cary, NC).

Results and discussion

Organochlorine pesticides were extensively used in Egypt over the past century. These chemicals are characterized by their high accumulation potential, so the occurrence of these OCPs in the muscles of tilapia fish was examined. The results indicated in Figure 1A showed that the tested OCPs were detected in tilapia collected from Upper and Northern Egypt as well as the reference site with varying percentages. Only pp-DDT (25%), γ -HCH (15%) and HPT (15%) were detected in the reference site. This may be attributed to the persistence nature of these OCPs on the environment. Tilapia fish collected from Damietta had the highest incidence for the tested OCPs compared with that from Sohag. The total OCPs' residual concentration (ng/g

ww) was very minute (14.2 ± 1.8) in the reference site, when compared with samples collected from Sohag (69.7 ± 8.54) and Damietta (508.83 ± 56.55) (Figure 1B). Similarly, OCPs were detected in tilapia from the Nile basin countries as in Egypt, Ethiopia, Tanzania and Uganda (10,11,15-17).

Fish samples collected from Damietta had significantly ($p < 0.05$) the highest pp-DDT and its metabolites pp-DDD and pp-DDE with average residual concentrations (ng/g ww) of 98.11 ± 11.69 , 55.23 ± 7.11 and 44.15 ± 9.76 . However, only the parent compound pp-DDT was detected in the fish samples collected from Sohag (21.33 ± 5.12) and Abbasa (12.5 ± 4.55) (Figure 2A). Similarly, fish samples collected from Damietta had significantly the highest residues (ng/g ww) of α -HCH (52.16 ± 6.11) and its isomer γ -HCH (121.0 ± 10.25) compared with samples collected from Sohag and Abbasa (Figure 2B). The recorded concentrations in the current study were far below than the established maximum permissible limits (MPL) set by World Health Organization (WHO) (200 ng/g ww for each of DDTs and HCHs) (18). The recorded concentrations of either DDTs or HCHs were comparable to that recorded in Ethiopia and Tanzania (13,15).

Heptachlor and its epoxide metabolite were detected in the examined samples. Samples coming from Damietta had significantly the highest heptachlor and heptachlor-epoxide residues, the average concentrations (ng/g ww) of these chemicals were 18.55 ± 1.44 and 14.33 ± 2.11 , respectively. These concentrations were 5.23 ± 0.55 and 3.4 ± 0.56 , respectively in samples collected from Sohag. Samples collected from Abbasa had minute concentrations of HPT only (1.2 ± 0.2) as indicated in Figure 3 A. All samples had HPT levels below MPL set by WHO (150 ng/g ww) (16). Heptachlors and its metabolites were also detected in comparable levels in fish consumed in Ethiopia (19).

Drins were tested in the presented study and were not detected in samples collected from the reference site (Abbasa). Aldrin was not detected in any fish sample. Endrin was significantly high in fish samples collected from Damietta (15.65 ± 2.66) when compared

with samples collected from Sohag (4.5 ± 0.32) (Figure 3B). It notes worthy to confirm that the recorded drin levels were below MPL set by WHO (150 ng/g ww) (16). Nearly similar concentrations were recorded in tilapia in Ethiopia (13,19).

Chlordane, HCB and methoxychlor were not detected in tilapia caught from the reference site (Abbasa). However, all of these OCPs were detected in fish samples collected from Sohag and Damietta. Fish collected from Damietta had significantly higher levels of these OCPs compared with that from Sohag. The average residual concentrations (ng/g ww) of chlordane, HCB and methoxychlor were 10.1 ± 2.78 , 9.44 ± 1.51 and 8.55 ± 2.12 , respectively, in samples collected from Sohag and 16.44 ± 1.22 , 41.33 ± 6.33 and 31.88 ± 5.21 , respectively in fish samples collected from Damietta (Figure 3C). These levels were below MPLs set by WHO (18). The recorded concentrations of these OCPs were corresponding to the levels recorded in Nile tilapia in Ethiopia and Uganda (13,16). The reasons for the high load of OCPs in fish collected from Damietta compared with other sampling sites may be due to the extensive past use of these OCPs in agricultural and non-agricultural activities (10).

As fish is consumed heat-treated in Egypt and many countries worldwide, so the effects of different heat treatments on the accumulated OCPs were investigated and the results were recorded in Figure 4. Pan-frying had the highest reduction effects on such OCPs. The reduction percentages were 80%, 75%, 85% and 90% on the total DDTs, HCHs, HPTs and drins, respectively. Grilling came second after pan-frying with reduction percentages of 50%, 55%, 60%, and 65% on the total DDTs, HCHs, HPTs and drins, respectively. Boiling had the lowest reduction percentages compared with other common cooking methods in Egypt as indicated in Figure 4. These results go in agreement with Mahmoud et al. (20) who observed a clear reduction in the OCPs levels in the edible offal of cattle under different cooking methods. Therefore, it is highly recommended for fish consumers to have their fish efficiently cooked before consumption, especially in areas with high OCPs' contamination.

Organochlorine pesticides were detected in low concentrations in the present study. However, such chemicals are lipophilic and can accumulate in the breast milk to find their way into infants leading to endocrine disrupting effects (21). Additionally, such toxicants can cross the placental barriers leading to spon-

taneous abortion, fetal anomalies and other reproductive tract related cancers in both sexes (8,9). Therefore, continuous monitoring plans for OCPs residues in foods should be followed. Additionally, strict legislations should be adopted to avoid the illegal use of OCPs in the agricultural activities.

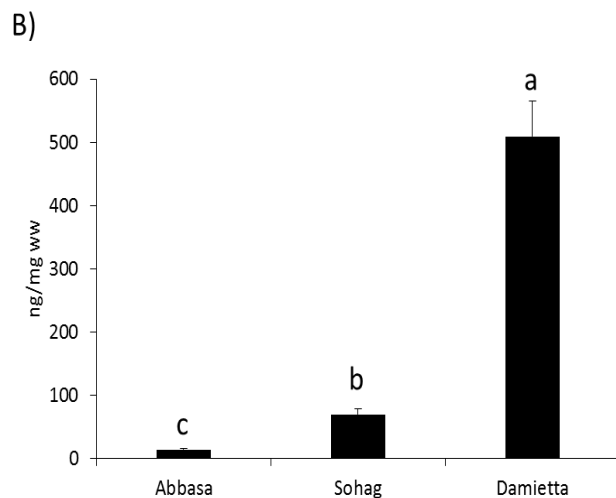
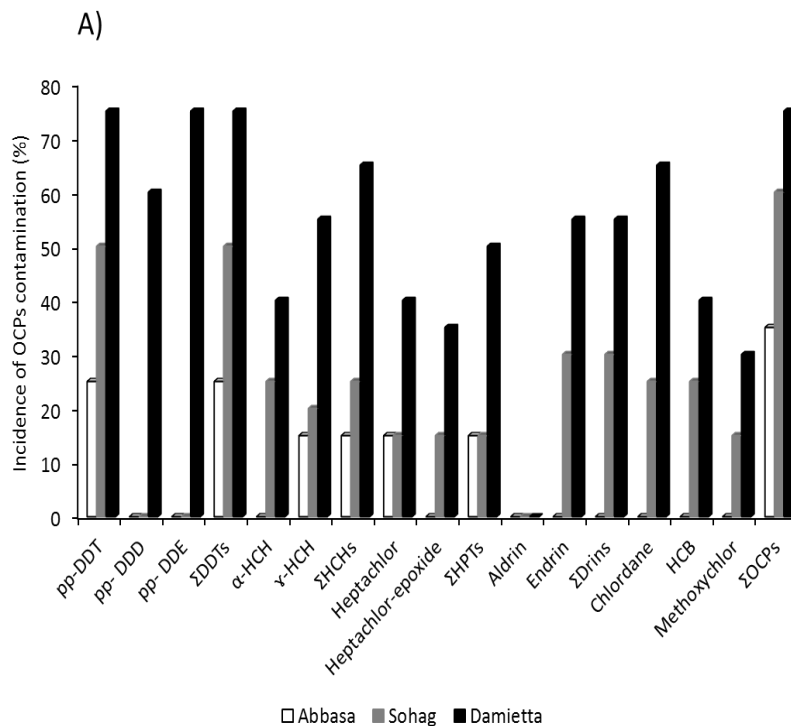


Figure 1: Incidence of *Oreochromis niloticus* OCPs contamination from some localities in Egypt. A) Frequency (%) B) Total OCPs concentrations (ng/g ww) in the examined *Oreochromis niloticus* from different locations in Egypt. Data represent means \pm SE (ng/g ww) (n=20 from each location). Columns with different superscript letter are significantly different at $p < 0.05$

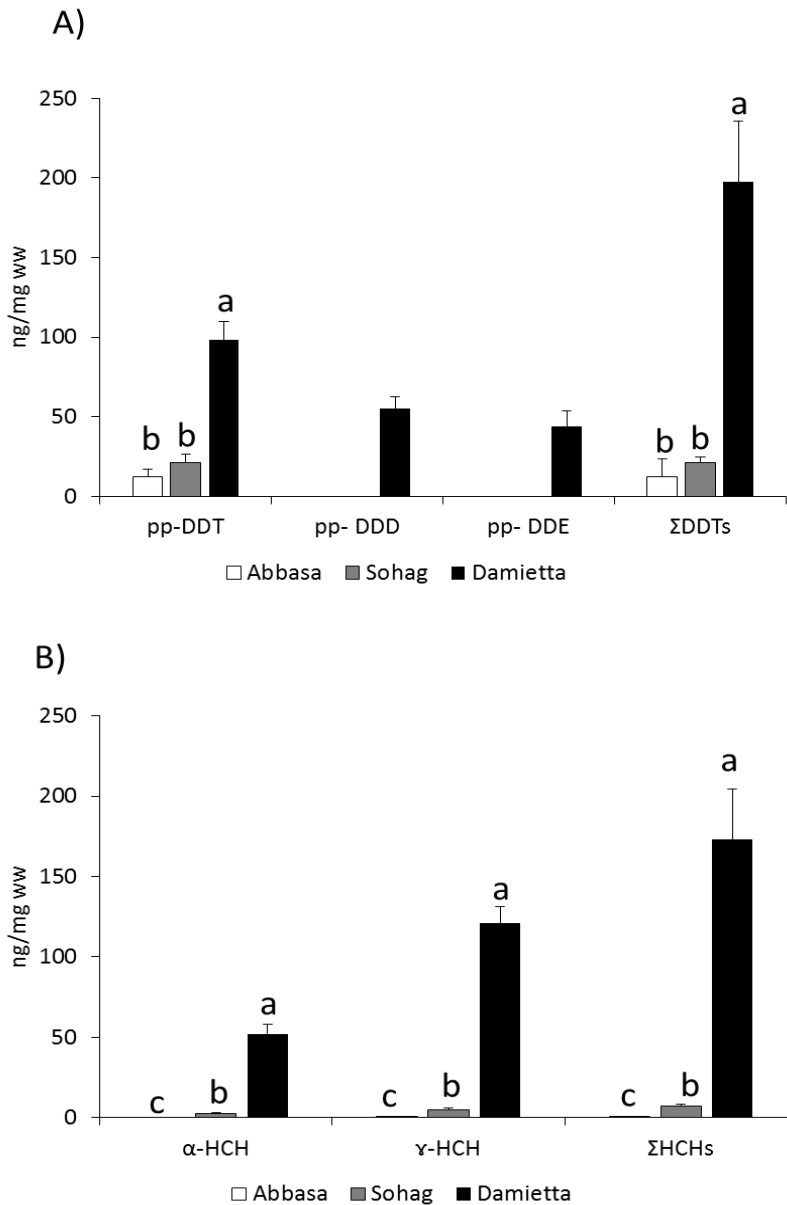


Figure 2: Levels of DDT and HCHs in *Oreochromis niloticus* marketed in Egypt. A) Total DDT and its metabolites B) Total HCH and its isomers residues in the examined *Oreochromis niloticus* collected from different locations in Egypt. Data represent mean \pm SE (ng/g ww) (n=20 each from each location). Columns that carry different superscript letter among the same chemical are significantly different at $p < 0.05$

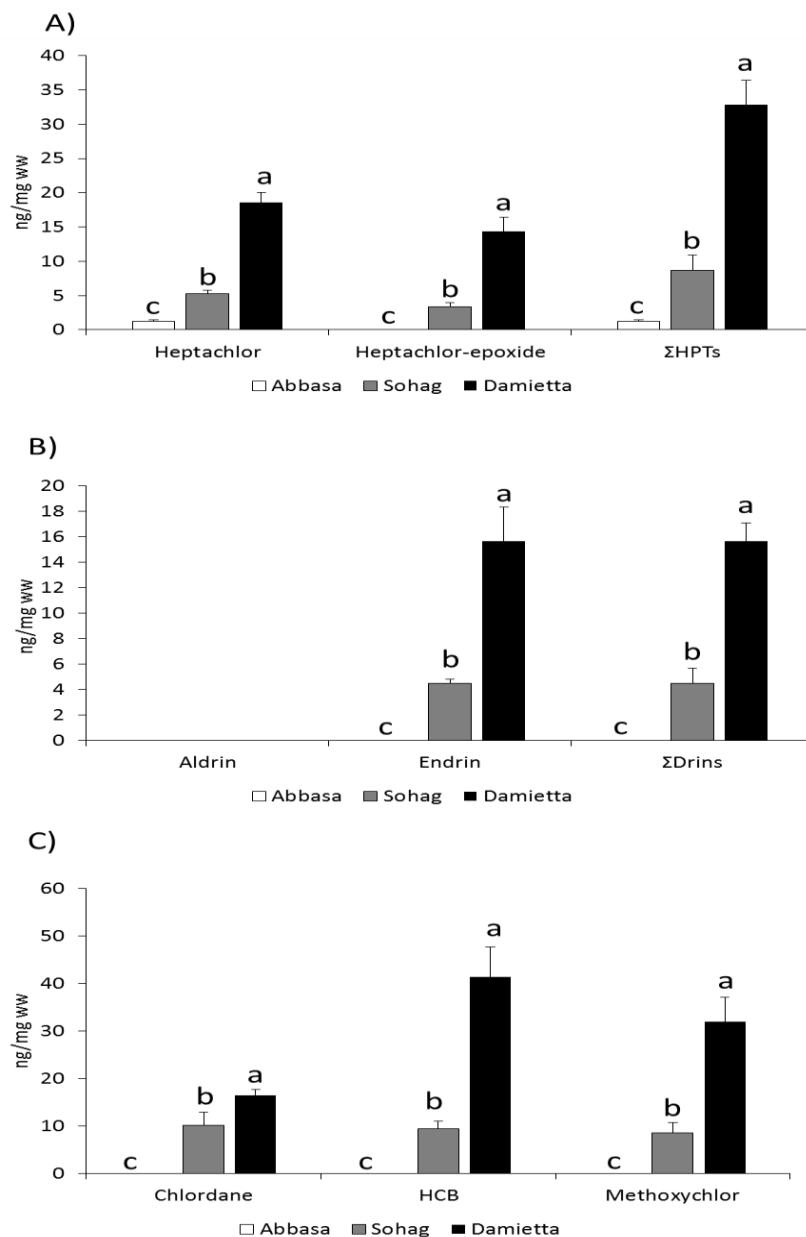


Figure 3: Levels of heptachlors, drins, and other OCPs in *Oreochromis niloticus* marketed in Egypt. A) Total heptachlor and its epoxide metabolite B) Total drins C) Chlordane, HCB and methoxychlor residues in the examined *Oreochromis niloticus* collected from different locations in Egypt Data represent mean \pm SE (ng/g ww) (n=20 each from each location). Columns that carry different superscript letter among the same chemical are significantly different at $p < 0.05$

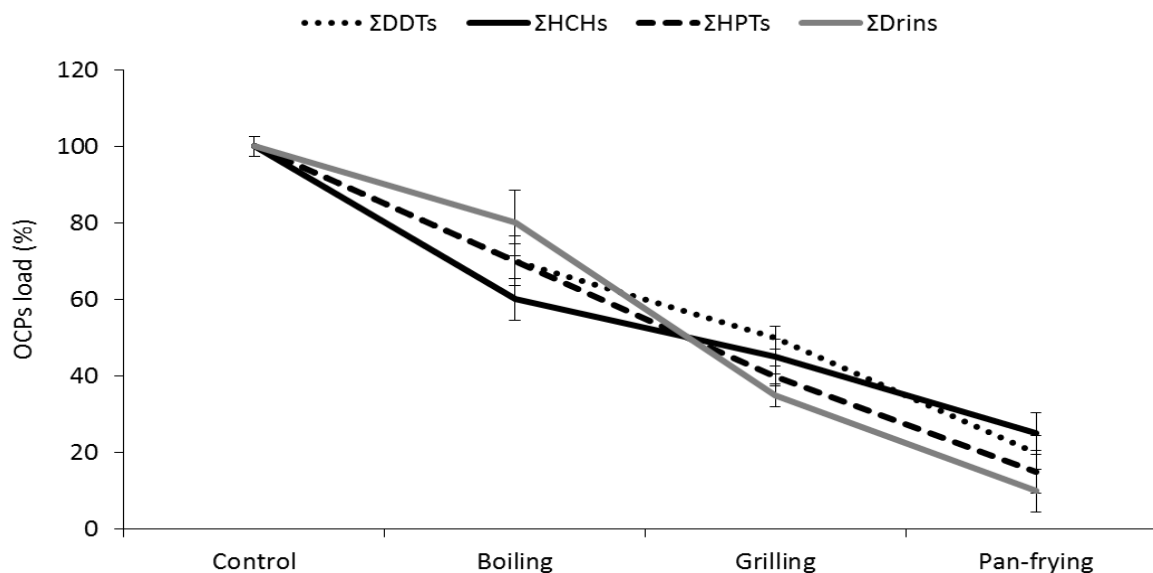


Figure 4: Effects of different cooking methods on total OCPs levels in the examined *Oreochromis niloticus*. Data show the effects of different cooking methods (boiling, grilling and pan-frying) compared with the non-heat treated (control). Data represent mean \pm SE (%) for the load of the total OCPs in the examined tilapia samples collected from Damietta (n=5 each treatment)

Conclusion

The achieved results revealed contamination of tilapia fish with OCPs at low concentrations, but did not exceed the maximum permissible limits. Therefore, continuous monitoring studies for the levels of OCPs in foods of animal origin should be continued in Egypt. Efficient cooking of fish, particularly, grilling and pan-frying strongly reduced the residue levels of OCPs in fish. Thus, it is highly advisable to expose fish to efficient heat treatment, especially in areas with high OCPs contamination.

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Conflict of interest

None of the authors have any conflict of interest to declare.

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