

Organochlorine pesticide residues in some marketed fish species in Egypt

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ABSTRACT

Fish can be considered as an ideal food as it contains high protein content, minerals, vitamins and omega 3 fatty acids. Organochlorine pesticides (OCPs) are characterized by their low cost, severe toxicity against a wide array of pests, long duration of action and stability in the environment. OCPs have been used for many years in African countries, particularly in Egypt. Despite being outlawed everywhere, OCPs are still being used illegally. One of the main responsibilities of the food safety and public health sectors is to guarantee the safety and wholesomeness of such food products before they are made available to the general public. In order to determine the residual OCP contents in retailed tilapia, catfish, mullet, saurus, and pagrus, this study was conducted. The obtained results in the current investigation revealed the detection of OCPs at 80%, 50%, 20%, 15%, and 15% in the examined catfish, tilapia, mullet, saurus, and pagrus, respectively. Different OCPs were detected at variable concentrations; however, such concentrations were within the established permissible limits in Egypt. In conclusion, OCPs are still detected in different aquatic species and residual concentrations can be detected in different fish species retailed in the Egyptian fish markets. Therefore, it is highly advised to continuously check for OCP residues in fish.

Introduction

Fish production is one of the food industries experiencing rapid expansion, supplies approximately 50% of the world's fish consumption and exhibited a growth rate of 6.2% in 2011 (FAO, 2013). In low and middle-income nations, small-scale producers raise over 70% of farmed fish in freshwater (Hastein *et al.*, 2006; Morshdy *et al.*, 2022). Fish is a notable provider of protein-rich, low-fat foods that are also abundant in omega-3 and omega-6 fatty acids, which have been found to offer protection against detrimental health consequences such as stroke and coronary heart disease (Morshdy *et al.*, 2013; 2019). Egypt ranks among the top ten producers worldwide and holds the distinction of being the leading producer of aquaculture in Africa. In 2015, the country produced 1.5 million tons of aquaculture (Eltholth *et al.*, 2015). Additionally, concerns are mounting regarding the potential transfer of chemical and microbial gastrointestinal hazards to humans via fish consumption. Due to these concerns, the demand for domesticated fish might decrease (Smallwood and Blaylock, 1990). This phenomenon could potentially result in adverse consequences for fish producers, as well as a reduction in the consumption and utilization of animal-derived foods. In Egypt and several African nations, the most well-known aquatic species are *Tilapia nilotica*, catfish, saurus, pagrus and mullet species. These fish species significantly contribute to Egypt's animal protein shortage, primarily due to their brief life cycle, low cost, and large-scale production. The hazards to public health posed by such species in Egyptian fish value chains are the subject of few published studies. A comprehensive understanding of any possible contamination of this substantial delicacy is crucial, particularly considering the remarkable output and consumption of freshwater fish in Egypt in 2011 that surpassed 500,000 tons (Macfadyen *et al.*, 2012). An analysis of the production, marketing, and consumption patterns of fish, particularly Tilapia in the Nile Delta revealed a multitude of possible sites of contam-

ination (Eltholth *et al.*, 2015). Tilapia, catfish, saurus, and pagrus and mullet have been implicated in the transportation of chemical contaminants, including heavy metals (Morshdy *et al.*, 2019), antibiotics (Morshdy *et al.*, 2022), and pesticides (Morshdy *et al.*, 2018).

Organochlorine pesticides (OCPs) have been extensively utilized for decades due to their extended duration of activity, economical price, and broad-spectrum toxicity against pests (Pirsahab *et al.*, 2015; Darwish and Thompson, 2023). Dichlorodiphenyltrichloroethane (DDT), Aldrin, and Dieldrin were three OCPs that were extensively utilized in agriculture during the 1950s. Rapidly, the toxicity and extensive biomagnification of DDT in other species were identified. At first, it was believed that DDT exhibited deleterious effects solely on insects. Lipophilic chlorine residues are accumulated by animals from OCPs, and species at the apex of the food chain demonstrate biomagnification. 179 countries have ratified the Stockholm Convention on Persistent Organic Pollutants (POPs) since its inception in May 2001 (UNEP, 2002). The first twelve persistent organic pollutant (POP) substances to be prohibited by this convention were aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, and toxaphene. Although the use of certain OCPs in agriculture has been restricted, others continue to be employed as pesticides in numerous countries. Several African countries continue to employ DDT despite the existence of a valid exemption for the purpose of disease vector control. In this scenario, bed nets are treated with insecticides or indoor residual sprinkling (IRS) is utilized to apply chemicals inside residences. Certain locations may contain chemical stockpiles or obsolete materials that are being maintained in potentially hazardous environments. The identification of these substances is contingent upon the examination of environmental samples for signs of contamination (Thompson *et al.*, 2017, 2018).

OCPs have the potential to infiltrate the body via various routes, including dermal contact, ingestion of contaminated food or water, and

airborne inhalation (Mahmoud *et al.*, 2013, 2016; Thompson *et al.*, 2018). Animal products contaminated with OCP are probably the most significant source of pesticide exposure for humans (Hassal, 1990). Neonatal maternal transfer to neonates and placental transfer to the foetus are both possible via breast milk. A number of adverse health effects may result from the use of these OCPs (Sallam and Morshdy, 2008; Morshdy *et al.*, 2018). Varying quantities of these substances are found in living organisms, contingent upon their environment and position in the food chain (Zhou *et al.*, 2007).

Ensuring the integrity and safety of food products prior to their distribution to the public constitutes a fundamental obligation of the public health and food safety industries. Consequently, the residual concentrations of OCPs in retailed tilapia, catfish, mullet, saurus, and pagrus were investigated in this study.

Materials and methods

A hundred samples of tilapia, catfish, mullet, saurus, and pagrus (20 each) were selected at random from fish markets in Zagazig City, Sharkia province, Egypt. Each fish sample is individually packaged in polyethylene sachets. The specimens were transported in a refrigerated container to the laboratory of the Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt. Agricultural Research Centre personnel extracted and quantified organochlorine pesticides in Dokki, Giza, Egypt.

Chemicals

Standard OCPs, including pp-DDT, pp-DDD, pp-DDE, HCH, HCH, heptachlor, heptachlor epoxide, aldrin, endrin, chlordane, methoxychlor, and HCB, were obtained from Sigma-Aldrich (Germany). Methylene chloride, petroleum ether, diethyl ether, n-hexane, acetonitrile, and anhydrous sodium sulphate were all purchased from Merck (Darmstadt, Germany). Silica (Silica Co., USA) was the supplier of PR Grade florisil (60-100 micron). Every solvent was of the utmost purity available, or HPLC grade. Following a 24-hour period of activation at 130°C, florisil was lowered to ambient temperature.

Extraction and preparation of samples

All three extraction phases, each lasting two minutes, involved mixing and homogenizing 50 g of each sample with 150 g of anhydrous sodium sulphate. Following that, the homogenates were thoroughly combined with 100 g of anhydrous sodium sulphate and 150 ml, of petroleum ether, as mentioned previously (Mahmoud *et al.*, 2013). Sodium sulphate anhydrous aids in the dissolution of the substance by eliminating water. After each extraction process, the samples underwent filtration using a vacuum pump. The solvent underwent rotary evaporation at a temperature of 40°C until it became completely dry.

Partitioning of the extracts

The procedure outlined by the Association of Official Analytical Chemists (AOAC, 1999) was adhered to in order to partition the extracted samples. Before segregating and utilizing each solvent individually, 500 ml of n-hexane and an equivalent volume of acetonitrile were combined in a separating funnel to partition the sample. After placing the extracted substance into a 100-ml separating funnel containing a solution of 20 ml acetonitrile and 80 ml n-hexane, the funnel was vigorously shaken for two minutes. Following its separation into two solvent layers and removal of moisture with anhydrous sodium sulphate, acetonitrile was accumulated in a flask. In order to repeat the partitioning procedure as described previously, 20 ml of additional acetonitrile was added to the n-hexane three times. Ultimately, less than 10 ml of acetonitrile was evaporated using a

rotary evaporator for use in the florisil cleaning; n-hexane was discarded.

Extract purification and OCP concentration measurement

To purify the extracted samples and eliminate any residual fat, the extract was loaded onto a glass chromatographic column coated with anhydrous sodium sulphate and containing 20 g of activated florisil (60–100 micron). Once the extracted sample had been washed with 50 mL of petroleum ether, it was deposited onto the constructed column. The column was eluted with 200 ml of an eluent consisting of 10% petroleum ether and 10% anhydrous diethyl ether. For the second elution, 100 ml of an additional eluent containing 1% acetonitrile, 29% n-hexane, and 70% methylene chloride was utilized. The recovered eluent was subjected to concentration via rotary evaporation prior to its dissolution in 10 mL of hexane. To facilitate electron capture gas chromatography analysis of each extract, a corresponding aliquot of each extract was transferred into injection containers with a capacity of 2 ml.

Organochlorine residues were identified through the utilization of an electron capture gas chromatograph (Hewlett Packard GC Model 6890) equipped with a Ni63-electron capture detector to analyze the samples. In the GC, the HP-5MS capillary column was utilized under the subsequent conditions: At a flow rate of 4 ml/min, N₂ was used as the carrier gas; the injector and detector temperatures were 230°C and 300°C, respectively; the film measured 30m in length, 0.32mm in internal diameter, and 0.25m in thickness. The gas chromatography oven temperature program commenced at 150°C for a duration of 5 minutes, was increased to 170°C at a rate of 5°C/min and maintained for an additional 10 minutes, and finally reached 220°C at a rate of 10°C/min and maintained for an additional 20 minutes (for a total run time of 44 minutes); the nitrogen make-up gas flow rate was 20 ml/min and the injection volume was 1.0. On the basis of the calibrated standard curves, residual concentrations of the OCPs that were evaluated were calculated and expressed as ng/g.

Statistical analysis

Each value is expressed as the mean ± standard error, and every measurement was conducted in triplicate. JMP statistical software (SAS Institute Inc., Cary, NC) was employed to ascertain statistical significance via the Tukey-Kramer HSD test, which utilizes the comparison of means method. A significance level of $p < 0.05$ was established for this purpose.

Results and Discussion

Organochlorine pesticides have been subject to prohibition in Egypt since the 1980s. Nevertheless, their enduring presence has enabled their detection in agricultural crops, water, animal tissues, and soil (Mahmoud *et al.*, 2013, 2016; Darwish and Thompson, 2023). OCPs are largely used in the Nile basin's country like Ethiopia (Yohannes *et al.*, 2013; Teklit, 2016), Kenya (Mitema and Gitau, 1990), and Sudan (Elbashir *et al.*, 2015). The drainage of irrigation water usually carries large quantities of OCPs residues to the river stream that reach Egypt at the end of the Nile route. Fish living in the Nile and other aquaculture species can be exposed to such pollutants. Subsequently the human body may become exposed to OCP residues through the consumption of contaminated fish.

The findings of the present study indicated that OCPs were detected at concentrations of 80%, 50%, 20%, 15%, and 15%, in the catfish, tilapia, mullet, saurus, and pagrus respectively, that were analyzed (Fig. 1). OCPs were also detected in the sediment, water, and fish tissues collected from Manzala Lake, and river Nile in Egypt as indicated by Yamashita *et al.* (2000). OCPs were also detected in Nile fish including tilapia and catfish retailed in Qena city, Egypt (Hassan *et al.*, 2020).

The findings presented in Fig. 2 indicated that the concentrations of Σ DDTs in the catfish (155 ± 11.2 ng/g ww) and tilapia (50 ± 2.1 ng/g ww) were significantly higher than those in the mullet (6.6 ± 0.3 ng/g ww), sau-

rus (4.6 ± 0.2 ng/g ww), and pagrus (2.1 ± 0.3 ng/g ww), in that order. In the positive samples, various DDT isomers were identified, including pp-DDT, pp-DDD, and pp-DDE.

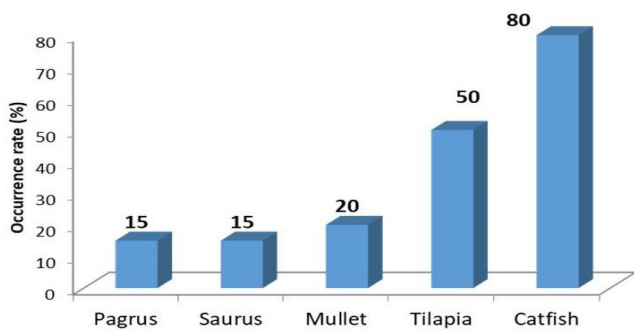


Fig. 1. Detection rates (%) of OCPs in the examined fish species (n=20/each).

According to these findings, DDTs continue to be detected in the fish retailed in Egypt. Likely, Khalaf *et al.* (2018) detected different DDTs isomers in tilapia collected from Bahr Shebeen Canal (BSC), a River Nile Canal. Besides, Morshdy *et al.* (2018) detected pp-DDT, pp-DDD, and pp-DDE at relatively similar concentrations in tilapia muscles that were collected from Damietta and Sohag. Consistent with the results of this investigation, Hassan *et al.* (2020) detected DDD, and DDE in Nile tilapia, African catfish, and long fin catfish in Qena city, Egypt.

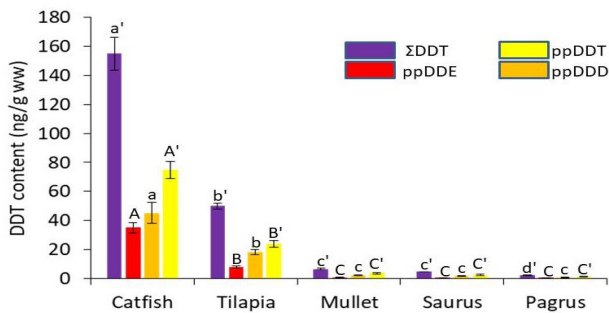


Fig. 2. DDTs concentrations (ng/g ww) in the examined fish species. Values represent means \pm SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

The residual concentrations (ng/g ww) of Hexachlorocyclohexanes (HCHs) in the samples under investigation were depicted in Fig. 3. The analyzed samples contained, in order, the following average total HCH concentrations: 115.9 ± 4.2 , 47.6 ± 2.5 , 23.5 ± 0.8 , 9.0 ± 0.5 , and 4.8 ± 0.2 for catfish, tilapia, mullet, saurus, and pagrus, respectively. Additionally, variable rates of detection were observed for α -HCH and lindane (γ -HCH), the most stable and active isomer of HCH. Fresh water fish (catfish, and tilapia) contained the highest residual concentrations of HCHs, whereas marine water fish as saurus and pagrus contained the lowest concentrations. The HCH concentrations documented in this study were relatively similar to those found in tilapia collected from Damietta, Sohag, and Qena (Morshdy *et al.*, 2018; Hassan *et al.*, 2020). Besides, HCHs were also detected at comparable levels in the retailed mullet and saurus in Mansoura city (Hussein *et al.*, 2022).

Epoxides of heptachlors were identified in every positive sample that tested positive for OCP contamination. The catfish muscles exhibited the greatest quantity of residues, with tilapia, mullet, saurus, and pagrus following suit, as shown in Fig. 4. Heptachlors were presumably identified in relatively comparable concentrations in the tilapia retailed in Damietta and Sohag (Morshdy *et al.*, 2018), as well as in catfish (Hassan *et al.*, 2020), mullet and saurus (Hussein *et al.*, 2022). Higher concentrations were detected in fish sampled in Ethiopia (Yohannes *et al.*, 2013), and Mozam-

bique (Thompson *et al.*, 2018).

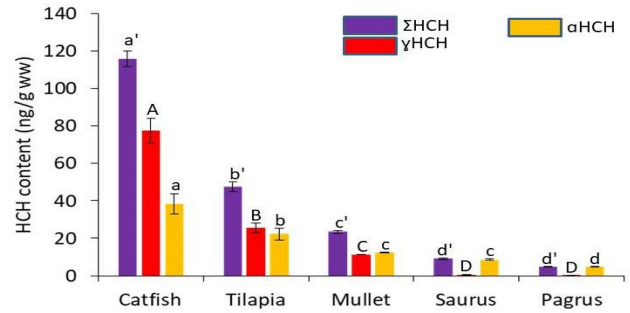


Fig. 3. HCHs concentrations (ng/g ww) in the examined fish species. Values represent means \pm SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

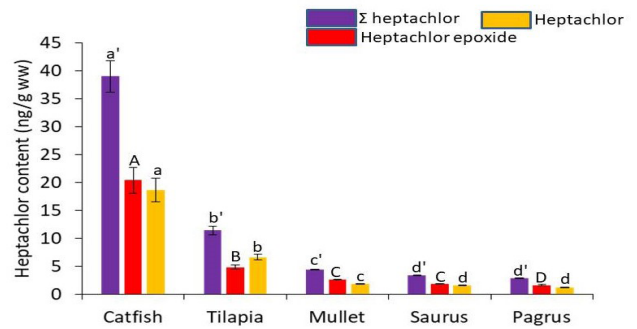


Fig. 4. Heptachlors concentrations (ng/g ww) in the examined fish species. Values represent means \pm SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

The measured concentrations of aldrins in the samples under investigation were illustrated in the results depicted in Fig. 5. Catfish still contained the highest levels of residual aldrins. Tilapia, mullet, saurus, and pagrus followed suit, in that order. The aldrin concentrations that were documented were consistent with those reported in other studies that examined fish retailed in Egypt Morshdy *et al.* (2018) (tilapia), Hassan *et al.* (2020) (catfish), and Hussein *et al.* (2022) (saurus and mullet).

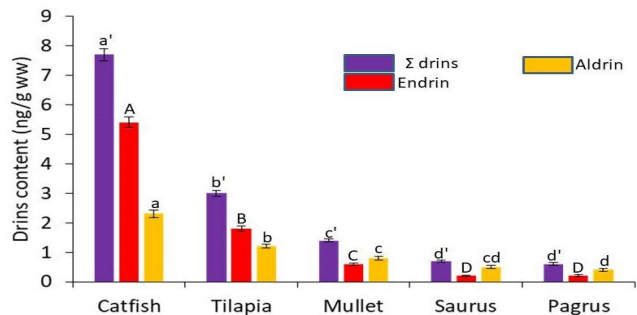


Fig. 5. Drins concentrations (ng/g ww) in the examined fish species. Values represent means \pm SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

The results presented in Fig. 6 illustrated the variable rates of detection of chlordane, HCB, and methoxychlor in the samples under investigation. These results were consistent with the results reported by Morshdy *et al.* (2018) for tilapia, Hassan *et al.* (2020) for catfish, and Hussein *et al.* (2022) for saurus and mullet. It is noteworthy that the concentrations of all identified OCPs were found to be lower than the maximum allowable limits (MPL) for OCPs in Egypt, which are as follows: HCHs (1000 ng/g), DDTs (5000 ng/g), drins (600 ng/g), HCB (200 ng/g), and chlordane (200

ng/g) (EOS, 1992). On the contrary, OCPs have been linked to a number of detrimental health effects, such as teratogenesis, stunted development, infertility, and an increased risk of developing cancer (Thompson *et al.*, 2017; Thompson and Darwish, 2019).

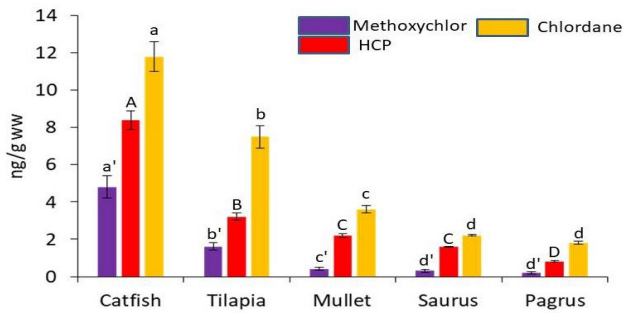


Fig. 6. Chlordane, HCB, and methoxychlor concentrations (ng/g ww) in the examined fish species. Values represent means \pm SE, and columns for each tested parameter carrying different characters are statistically different at $p < 0.05$.

Conclusion

The findings of the present investigation indicated that OCPs were still detected in the retailed fish in Egypt. Ongoing surveillance for OCP residues in consumables derived from fish and other animal species is strongly advised, notwithstanding the fact that the levels fall below the MPL of OCPs in Egypt. Additionally, raising producers' awareness regarding the detrimental consequences of OCPs is strongly recommended.

Conflict of interest

The authors affirm that they do not possess any conflicts of interest.

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