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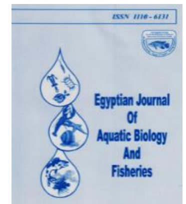

Organochlorine Pesticide Distribution in Muscle Tissues of *Oreochromis niloticus*, *Cyprinus carpio*, and *Sander lucioperca* from Moroccan Continental Waters: Implications for Health Risk Assessment

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ABSTRACT

Despite the prohibition of organochlorine pesticides (OCPs) in Morocco since 1984, our study revealed a widespread presence of these chemicals in the muscles of three fish species from the Al Massira Reservoir. Average concentrations of OCPs varied among the species, with higher levels found in *Sander lucioperca* compared to *Oreochromis niloticus* and *Cyprinus carpio*. Specifically, the average concentrations of the sum of hexachlorocyclohexane isomers (ΣHCHs) were 1.46 ± 1.14 for *Oreochromis niloticus*, 2.05 ± 1.55 for *Cyprinus carpio*, and 3.38 ± 2.22 for *Sander lucioperca*. For the sum of aldrin and dieldrin isomers (ΣDrins), concentrations were 2.40 ± 2.18 for *Oreochromis niloticus*, 2.61 ± 2.41 for *Cyprinus carpio*, and 6.01 ± 3.97 for *Sander lucioperca*. The average concentrations of the sum of dichlorodiphenyltrichloroethane isomers (ΣDDT) were 1.83 ± 1.72, 2.57 ± 2.36, and 4.70 ± 4.65, respectively, for *Oreochromis niloticus*, *Cyprinus carpio*, and *Sander lucioperca*. Despite these findings, none of the samples exceeded the maximum residue limits (MRLs) set by the European Commission. Risk assessments for both cancer and non-cancer risks associated with consuming these fish indicating that there is no significant threat to public health. However, ongoing monitoring of aquatic ecosystems is essential to ensure food safety and to protect the well-being of local populations. Addressing global concerns about human exposure to persistent organic pollutants (POPs) requires coordinated efforts and continued vigilance.

INTRODUCTION

Organochlorine pesticides (OCPs) represent a class of chemical compounds widely used in the past for their effectiveness as insecticides, fungicides, and herbicides **(Bharti *et al*., 2020)**. Their widespread use dates back to the 1940s and 1950s, when products such as dichlorodiphenyltrichloroethane (DDT) were extensively employed in disease vector control and agriculture **(Gardes *et al*., 2020)**. However, over time, increasing concern

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regarding their adverse effects on human health and the environment has led to restrictions and bans on their use in many countries **(Chareonviriyaphap *et al*., 1999; Van Den Berg *et al*., 2012)**.

The OCPs (Organochlorine Pesticides) are distinguished by their persistence in the environment, bioaccumulation in food chains, and long-distance transport through atmospheric and aquatic currents. Their ability to resist chemical and biological degradation makes them particularly concerning, as they can remain present in the environment for countless years, thus exposing wildlife, flora, and humans to health risks **(Taiwo, 2019; Mit *et al*., 2021; Chen *et al*., 2023)**.

Polycyclic organochlorines (OCPs) can contaminate aquatic environments from multiple sources, including agricultural runoff, industrial waste, urban discharges, landfills, and atmospheric emissions. These pesticides can be transported into streams, lakes, estuaries, and oceans through surface water, groundwater, and precipitation **(Al Mamoun, 2017; Olisah *et al*., 2021)**, and tend to accumulate in living organisms, particularly in fatty tissues.

Fish, crustaceans, and other aquatic organisms can absorb these compounds through skin contact, inhalation, or ingestion of contaminated food. Due to their low biodegradability and persistence, OCPs can build up in organisms over time, leading to high concentrations in higher trophic levels of the food chain. This biomagnification phenomenon can have adverse effects on predators and top-level organisms, including humans **(Miglioranza *et al*., 2022; Dione *et al*., 2023)**.

Due to their potential impact on human health and the ecosystem, OCPs have been subject to rigorous monitoring and regulation in many countries. Despite bans and restrictions, these compounds persist in the environment, underscoring the ongoing importance of monitoring their presence, assessing risks to public health, and implementing effective control measures **(Mahire *et al*., 2023)**.

It is worth noting that data regarding contamination by organochlorine pesticides (OCPs) in continental waters are largely scarce. Alarmingly, no study has yet been undertaken to assess the degree of contamination by OCPs in the waters, sediments, and fish populations of the Al Massira Dam Lake.

Our objective was to assess the degree of contamination by organochlorine pesticides (OCPs) in three fish species: *O. niloticus*, *C. carpio*, and *S. lucioperca*. These species were selected due to their different feeding habits, namely omnivorous, benthivorous, and carnivorous, respectively. It is important to note that these species are among the most consumed freshwater fish. The levels of OCPs in the studied fish species not only indicate bioaccumulation but also contribute to the database of fish species explored for POPs in Morocco. Thus, our research holds crucial importance in assessing the risks associated with consuming contaminated fish and would contribute to effective management of aquatic resources.

MATERIALS AND METHODS

1. Study area

The Al Massira Dam Lake is one of Morocco's main freshwater reservoirs, situated on the main course of the Oued Oum Erbia, approximately 70km south of Settat. The area surrounding the dam features a semi-arid to arid Mediterranean climate, with reservoir water known for its high hardness and a pH typically between 8 and 9, categorizing it as an oligotrophic to mesotrophic system.

Impounded in February 1979, the Al Massira Dam Lake is a vital resource supporting various sectors including potable water supply, agriculture, industrial activities, and energy production. It plays a central role in the watershed development plan of the Oum Rabia basin in Settat province, around 120km southeast of Casablanca. The continental climate around the reservoir is characterized by significant temperature fluctuations and high insolation, influenced by seasonal precipitation and fluctuations in water volume **(Ferraj *et al*., 2021; Bounif *et al*., 2023)**.

2. Sampling

Twenty-four specimens of *O. niloticus* (n=24), *C. carpio* (n=24), and *S. lucioperca* (n=24) were obtained from the Al Massira Dam Lake between July 2022 and June 2023. Upon purchase from local fishermen, the fish were immediately placed in an ice chest, transported to the laboratory, and stored in a freezer at -20°C. Prior to dissection, the fish were rinsed with distilled water, weighed using an electronic balance accurate to 0.1 grams, and their total length was measured with an ichthyometer with a precision of 0.1 centimeters. Subsequently, each fish was dissected along the vertebral column to the dorsal fin, and the skin was removed to expose the muscle tissues. The weight of samples before and after lyophilization was recorded for each fish, and the condition index (KI = total weight x 100 / total length3) was subsequently calculated.

3. Extraction and clean-up

The first step involved preparing the samples. After lyophilization and pulverization of the biota to ensure maximum homogeneity, 5g of each sample was accurately weighed. The samples were then placed in Teflon tubes, and 30mL of a hexane and acetone mixture (27mL of hexane and 3mL of acetone) was added. Each tube was supplemented with 100µL of an internal standard.

Following this meticulous preparation, the tubes were subjected to digestion in a microwave oven at 120°C for 30 minutes, with a gradual heating phase. Once digestion was complete, the resulting liquid was recovered in a glass ampoule, and the tubes were rinsed with hexane, adding the rinse liquid to the ampoule. The liquid was then condensed under nitrogen until a volume of 1mL was obtained and transferred to graduated tubes.

Next, 5mL of sulfuric acid (H2SO4) was added and agitated, followed by centrifugation at 4500rpm for 5 minutes to recover the hexane phase in a flask. For fractionation, a florisil SPE cartridge was used. The cartridge was pre-rinsed with 10mL of hexane, and the samples were added. An additional 10mL of hexane was added to recover the first fraction of pesticides in a flask. The process was repeated with a mixture of hexane and dichloromethane (9mL of hexane and 1mL of dichloromethane) to obtain the second fraction of pesticides in the same flask. It was essential to control the drip rate under a pressure not exceeding 0.2 bar. Finally, the liquid was condensed under nitrogen until a volume of 1mL was obtained, then transferred to a 1mL vial for further analysis **(Kabriti, 2023)**.

4. Instrumental analysis

For the analysis of organic micropollutants, an Agilent 7890B gas chromatograph, coupled with an Agilent 5977B single quadrupole mass spectrometer and equipped with an automatic sample changer, was used. The qualitative and quantitative data were processed separately by two different software programs provided with the instrument, named "MassHunter Qualitative Analysis" and "MassHunter Quantitative Analysis." One of the software programs allows visualization of peaks and detection of the most abundant molecules, while the other enables quantification of elements targeted by the methods.

Fractionated samples were analyzed by gas chromatography (GC) equipped with an electron capture detector (ECD). Injection was performed in splitless mode with an injector temperature of 240°C and a purge flow rate of 3mL/ min. The samples were separated on two HP-5 MS capillary columns (15m x 0.25µm x 0.25µm each).

The column oven temperature program was configured as follows: the initial temperature was held at 60°C for 2 minutes, then increased at a rate of 20°C/ min until reaching 150°C. Subsequently, the temperature was increased at a rate of 10°C/ min until 200°C, where it was held for 20 minutes. Finally, the temperature was increased to the final temperature of 260°C, where it was held steady for 10 minutes. The total method time was 28.5 minutes **(Kabriti, 2023)**.

5. Statistical analysis

Statistical analyses were carried out using R software version 4.4.0 and Excel to conduct descriptive statistics. Analysis of variance (ANOVA) and Tukey tests were employed in R to compare the concentrations of organochlorine pesticides (OCPs) across various fish muscle samples. Statistical significance was defined as $P < 0.05$. For comparisons involving multiple OCP levels in fish tissues using ANOVA, data points below the detection limit (< BDL) were treated as zero based on the approach described by Cohen **(US EPA, 2006)**.

6. Human health risk assessment

The **US EPA (2000)** has set standards and guidelines to assess health risks associated with consuming fish contaminated with environmental pollutants. Health risk evaluations usually involve comparing average contaminant levels to international standards. However, these assessments often fail to consider key factors such as fish consumption rates and dietary patterns specific to the exposed populations. Consequently, this study incorporated not only mean values but also the 50 and 95th percentiles of detected contaminant concentrations to offer a comprehensive assessment of the health risks linked to consuming fish contaminated with organochlorine pesticide residues.

6.1.Estimation of daily intake (EDI)

The estimated daily intake (EDI) is a measure used in risk assessment to estimate the amount of a given substance that an individual is likely to ingest daily through diet or environmental exposure. The EDI is calculated taking into account several factors, including the concentration of the substance in the food, the dietary habits of the target population, including the frequency and quantity of consumption of foods containing the substance, as well as individual characteristics such as body weight **(Mahmood *et al*., 2014; Verhaert *et al*., 2017)**.

The estimated dietary intake (EDI) of all identified organochlorine pesticide residues in fish muscle was computed using the formula:

$$EDI = C \times IR/Bw$$

Where, C represents the average concentration of the individual organochlorine pesticide residue in ng/g wet weight (ww); - IR is the ingestion rate (g/day); and Bw is the body weight (kg).

For this study, the exposure parameters used were an average body weight of 73.3kg and an average fish ingestion rate of 38g/ day for the Moroccan population **(Kasmi *et al*., 2023)**.

The conversion of pesticide concentrations in fish muscles, expressed in ng/g dry weight, to ng/g wet weight (ww) **(Cresson *et al*., 2017)**, was performed using the following relationship:

$$Cww=Cdw/FC$$

Where, *Cww* represents the concentration in ng/g wet weight (ww); - *Cdw* is the concentration in ng/g dry weight (Dw); - FC is the conversion factor.

6.2.Estimation of hazard ratio

To evaluate the potential health risks associated with consuming *O. niloticus, C. carpio,* and *S. lucioperca,* we applied a method based on the ratio of estimated daily intake (EDI) to cancer benchmark concentration (CBC), as outlined by **Jiang *et al*. (2005)**. The CBC was determined based on a lifetime cancer risk of 1 in 1,000,000. For assessing non-carcinogenic effects, the CBC is derived from reference dose values provided by the US EPA for each analyte. In contrast, for carcinogenic effects, the slope factor for cancer from the US EPA's Integrated Risk Information System (IRIS) 2012 is utilized. Three risk ratios were calculated, along with their means and the 50th and 95th percentiles, to assess the cancer and non-cancer risks associated with consuming fish contaminated with organochlorine pesticide residues. The CBC was calculated using the following formula:

$$CBC = (RL/OSF) \times Bw / IR$$

Where, RL represents the maximum acceptable risk level of 10^(-6); OSF refers to the oral slope factor (mg/kg/day) obtained from the US EPA's IRIS system; Bw is the average body weight (kg), and IR is the ingestion rate (kg/day). The Cancer Benchmark Concentration (CBC) for assessing cancer risk ensures a lifetime risk of 1 in 1,000,000. For non-carcinogenic effects, the risk assessment involves comparing the estimated daily intake (EDI) with the US EPA reference dose values established for each analyte.

RESULTS AND DISCUSSION

1. *Estimation of biological parameters*

The total length of *O. niloticus*, *C. carpio*, and *S. lucioperca* ranged from 14.2 to 49.5cm, 14.2 to 45.8cm, and 19 to 54 cm, respectively. Correspondingly, their total weights spanned from 50 to 1005g, 45.8 to 1505g, and 70 to 1025g. The condition factor (KI) values varied between 0.78 to 3.28 (mean ± SD: 1.64 ± 0.52), 0.64 to 4 (mean ± SD: 1.60 ± 0.48), and 0.78 to 1.18 (mean ± SD: 0.87 ± 0.18) for *O. niloticus*, *C. carpio*, and *S. lucioperca*, respectively. Some overlap in KI values was observed among the three species. A KI value greater than 1 indicates good health status of the fish prior to sampling **(Tricklebank *et al*., 2002; Jisr *et al*., 2018)**.

2. Distribution of OCPs among fish muscles

The average concentration of ΣOCPs in *O. niloticus* muscle ranged from BDL to 8.445ng/ g wet weight (1.8421 ± 1.6684), while in *C. carpio*, it ranged from BDL to 11.8073ng/ g wet weight (2.5765 ± 2.3653), and in *S. lucioperca*, it ranged from BDL to

28.676ng/ g wet weight (4.5375 ± 3.8484). Analysis of variance revealed significant differences in ΣOCP levels among the muscles of the three species ($P = 1.76 \times 10^{-3}$). Tukey's post hoc test further indicated significant differences between *S. lucioperca* and *C. carpio* (Padj = 3.9×10^{-3}), as well as between *S. lucioperca* and *O. niloticus* (Padj = 1.6×10^{-4}).

Organochlorine pesticides (OCPs) are organic pollutants known for their hydrophobic-lipophilic nature, making them particularly prone to binding to fatty substances and accumulating in aquatic ecosystems. In addition to their physicochemical properties such as the octanol/water partition coefficient (Kow) and lipid content in organisms, the bioaccumulation of OCPs is influenced by species-specific biological characteristics. These concentration differences among species, as evidenced by significantly different results in the analysis of variance and Tukey's test, can be explained by factors such as length, body mass, age, sex, metabolic activity, diet, and body composition of the fish **(Eqani *et al*., 2013; Van Ael *et al*., 2013)**. These biological characteristics can affect a fish's ability to metabolize and eliminate OCPs, as well as its capacity to store them in its adipose tissues. Therefore, although the chemical properties of OCPs remain constant, their bioaccumulation can vary significantly from one fish species to another due to their biological and ecological differences.

According to our study, a higher concentration of OCP was found in the muscles of *S.lucioperca*, followed by *C.carpio*, and then *O.niloticus* (Fig. 1). In the aquatic ecosystem, position in the food chain and longevity are key factors that can influence contaminant accumulation. The pike-perch, as a top predator, occupies a high position in the food chain. Consequently, it is more likely to feed on prey that have already bioaccumulated OCPs by consuming contaminated organisms lower in the food chain. Additionally, the pike-perch is generally a large fish and can live for several years. This increased longevity allows it more time to accumulate contaminants throughout its life, which can lead to higher concentrations of OCPs in its muscle tissues **(Borgå *et al*., 2022)**.

In comparison, the Nile tilapia, although it may also be exposed to OCPs in its environment, often occupies a lower position in the food chain by primarily feeding on phytoplankton, algae, and plant debris. Consequently, it may bioaccumulate fewer contaminants than the pike-perch due to its diet and position in the food chain. Additionally, although the Nile tilapia can reach a considerable size and have moderate longevity, these factors may play a less significant role in OCP accumulation compared to the pike-perch due to its less carnivorous diet **(Rao *et al*., 2015)** .

For the common carp, it typically occupies a similar position to the Nile tilapia in the food chain, primarily feeding on aquatic plants, invertebrates, and detritus. Although the common carp can also reach a large size and live for several years, its diet and position in the food chain may result in less significant bioaccumulation of OCPs compared to the pike-perch **(Dadebo *et al*., 2015)**.

The main contributors to ΣDDTs in the muscles of *O. niloticus*, *C. carpio*, and *S. lucioperca* were respectively: DDT (49.42%), DDE (39.06%), and DDT (37.98%). β-HCH constituted the majority of ΣHCHs in *O. niloticus* (50.4%), *C. carpio* (46.08%), and *S. lucioperca* (40.99%). Dieldrin was the primary contributor to Σdrins in *O. niloticus* (53.5%), while in *C. carpio* and *S. lucioperca*, aldrin predominated (54.22 and 55.02%, respectively) (Fig. 2).

The beneficial physicochemical characteristics of DDT, along with its elevated partition coefficient between octanol and water (Kow), contribute to its strong tendency for bioaccumulation in organisms **(Fisk *et al*., 2001)**. This study found increased levels of DDT and its metabolites in the muscle tissues of *S. lucioperca* (Table 2). Ratios like DDE/DDT or DDD/DDT are frequently employed to distinguish between recent and historical exposures to DDT. However, it is important to recognize that DDT undergoes degradation within living tissues, yielding persistent metabolites such as DDE and DDD. Hence, the presence of these metabolites in tissues cannot always serve as a reliable indicator of recent exposure to DDT.

Technical mixtures of HCH typically consist of α-HCH (55–80%), β-HCH (5–14%), γ-HCH (8–15%), and δ-HCH (2–16%), whereas lindane predominantly contains over 90% γ-HCH **(Yang *et al*., 2005; Eqani *et al*., 2013)**. The present study identified all HCH isomers in the three species. The limit of detection (LOD) indicated that ∑HCHs were not detected in 13.88% of *O. niloticus* samples, 18.05% of *C. carpio* samples, and 19.44% of *S. lucioperca* samples, likely due to agricultural insecticide usage **(Shroyer *et al*., 1990)**.

The α/γ-HCH and β/γ-HCH ratios provide valuable insights into the exposure profiles of three fish species, *O. niloticus*, *C. carpio*, and *S. lucioperca*, from the Al Massira Reservoir to HCH compounds. The α/γ-HCH ratios for *O. niloticus*, *C. carpio*, and *S. lucioperca* are 0.6502 ± 0.6739, 0.8581 ± 0.6441, and 1.0039 ± 0.9084, respectively. These ratios suggest a relative predominance of γ-HCH for *O. niloticus* and *C. carpio* in their exposure, likely indicating widespread use of lindane. Meanwhile, there is a predominance of α-HCH for *S. lucioperca.*

As for the β/γ-HCH ratios, variations were observed. For *O. niloticus*, the ratio was 1.5615 ± 1.4141; for *C. carpio*, it was 1.8678 ± 1.1552, and for *S. lucioperca*, it was 1.6007 ± 1.0414. These ratios indicate a predominance of β-HCH in the three fish species.

The predominance of γ-HCH (lindane) in exposure suggests current or recent use of this compound. However, as lindane can persist in the environment for some time after its use, some of the exposure may also be attributed to historical use.

Variations in the β/γ-HCH ratio can also provide indications of the timing of exposure. A higher β/γ-HCH ratio may suggest more recent exposure to technical mixtures of HCH, as β-HCH is typically associated with these mixtures, while a lower ratio may indicate older exposure **(Kumari *et al*., 2014; Chakraborty *et al*., 2017)**.

3. Comparison of OCP in fish muscles of *O. niloticus, C. carpio* and *S. lucioperca* with those reported in other studies

Carnivorous fish, positioned at the top of the food chain in ecosystems, are noted for their elevated lipid content compared to other fish with varying feeding habits. Previous studies have consistently demonstrated that carnivorous fish tend to accumulate higher levels of organochlorine pesticides (OCPs) such as DDTs in their muscle tissues. These findings underscore that the bioaccumulation of OCPs in fish is influenced by specific ecological traits, including feeding behaviors and habitat preferences. Our research corroborates these observations, with *S. lucioperca* showing higher OCP concentrations compared to *O niloticus* and *C. carpio* (Tables 1, 2). This aligns with a previous research by **Rongbing *et al*. (2016)**, who similarly reported elevated OCP levels in carnivorous fish compared to species with different feeding strategies.

The findings of this study have emphasized a notable concentration of the DDE isomer relative to DDT and DDD isomers in the muscles of *Sander lucioperca*. This observation is consistent with previous research where DDE was identified as the predominant contaminant in these tissues, averaging 3.9ng/ g wet weight, constituting over 95% (98% on average) of the total DDT content. DDE, a metabolite of DDT, typically predominates as the residual form of DDT in fish samples **(Colombo *et al*., 2011)**. Environmental studies on bivalves suggest a half-life of DDT ranging from 10 to 20 years, during which DDT transforms into DDE and DDD **(Sericano *et al*., 1990)**. Subsequent investigations in the 1990s reaffirmed that, despite DDT's ban in the 1970s; DDE accounted for 50 to 70% of the total DDT residues **(Newsome & Andrews, 1993)**. The composition and concentration of DDT residues detected in the fish samples in this study strongly imply historical agricultural use of DDT as the primary source of these pollutants. A similar study conducted in Pakistan also identified DDT as the predominant contaminant in all fish samples, underscoring agricultural runoff as the main contamination source **(Riaz *et al*., 2021)**.

The ΣDDTs concentrations observed in this study were comparable to those reported for *O. mossambicus* in Lake Bosomtwi, Ghana, which ranged from 7.5 to 11.9ng/ g wet weight (ww) **(Darko *et al*., 2008)**; *O. niloticus* in Lake Edward, Uganda, with values from not detected (ND) to 51ng/ g ww **(Ssebugere *et al*., 2009)**; and *O. niloticus* in Lake Ziway, Ethiopia, ranging from 0.9 to 5.1ng/ g ww **(Yohannes *et al*., 2014)**. However, the ΣDDTs levels in the fish species examined are lower than those reported for *Ethamalosa fimbriata* in Lagos Lagoon, Nigeria, which ranged from 1.6 to 369ng/ g ww **(Williams *et al*., 2013)**, and for eight fish species in the Ga-Selati River in South Africa, where concentrations varied from 1.9 to 5643ng/ g ww **(Govaerts *et al*., 2018)**.

The ΣHCHs concentrations were found to be below detection limits (BDL) to 8.445ng/ g in *O. niloticus*; to11.8073ng/ g in *C. carpio*, and to 28.676ng/ g in *S. lucioperca*. These levels are lower compared to those reported by **Buah Kwofie *et al*. (2018)** for *C. gariepinus* (32–179ng/ g ww) and *O. mossambicus* (9.8–132.6ng/ g ww). The HCH compounds demonstrated a significant correlation with lipid content **(Rosca, 2022)**, highlighting a notable contribution of ΣHCHs in the muscle tissues of *C. carpio* and *S. lucioperca*.

The results of another study revealed that among the hexachlorocyclohexane (HCH) congeners, only the β-HCH and γ-HCH isomers were identified. Regarding the DDT congeners, only DDE was detected. Furthermore, this study showed that *C. carpio* exhibited the highest concentrations of contaminants compared to *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, and *Hypophthalmichthys nobilis*. It was also demonstrated that contaminant concentrations were higher in the ventral muscle than in the dorsal muscle, thus confirming the findings of **Yin *et al*. (2020)**.

In this study, ΣDrins in *O. niloticus* ranged from below the detection limit (BDL) to 8.445, in *C. carpio* from BDL to 11.807, and in *S. lucioperca* from BDL to 17.102. These concentrations were found to be similar to those reported in Mexico and Vietnam (Table 1). Differences and similarities observed in contamination levels result from the specific characteristics of each fish species, such as their metabolic rate, body size, and detoxification capacity. These individual factors can influence the accumulation and metabolism capacity of contaminants in fish muscle tissues, as well as pollution levels in each study area **(Redondo-López *et al*., 2022)**.

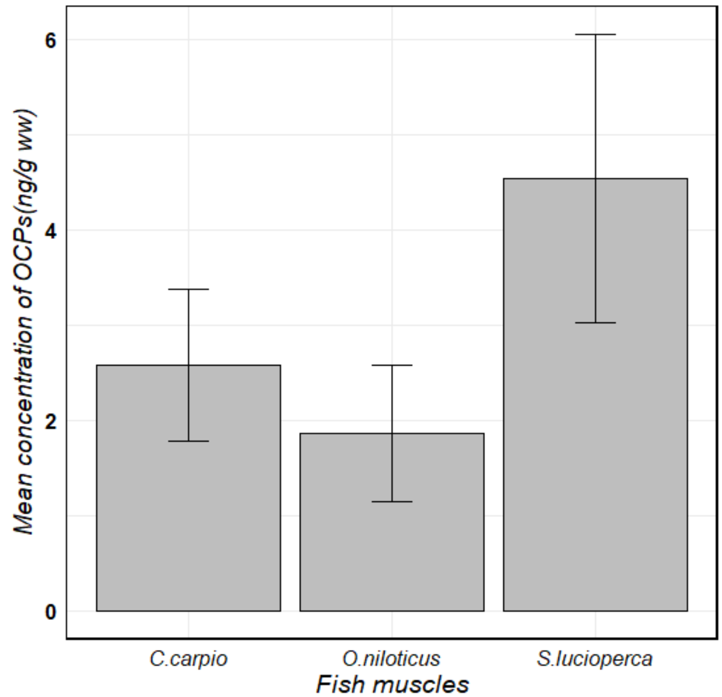

Fig. 1. Mean concentration (± SE) of OCPs in fish muscles of *C. carpio*, *O. niloticus*, and *S. lucioperca*

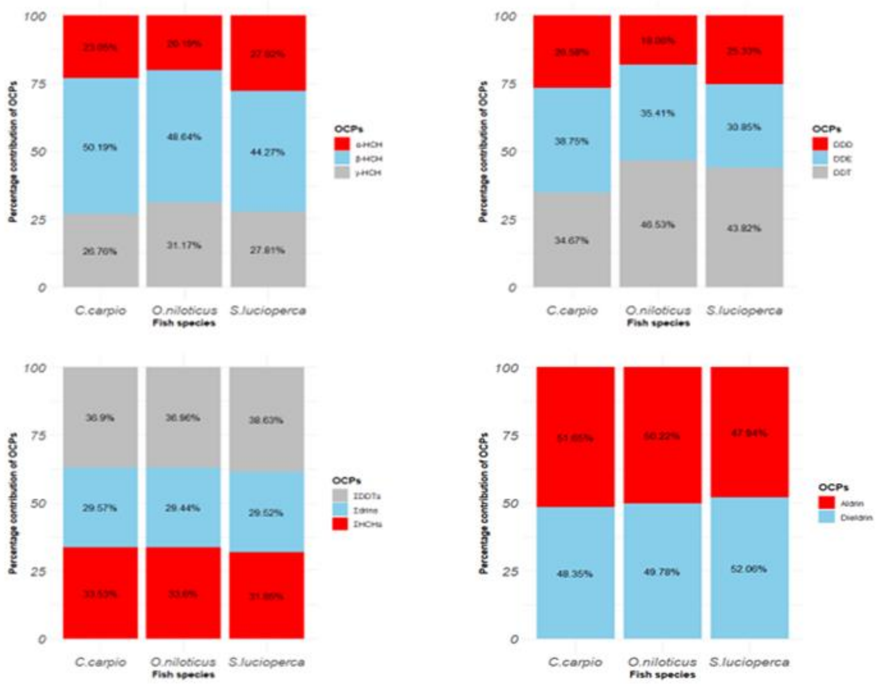

Fig. 2. Percentage contributions of ΣDDTs, ΣHCHs and Σdrins, in muscles of *C. carpio*, *O. niloticus* and *S. lucioperca*

Table 1. Comparison of OCPs (ng/g ww) reported in muscles of fish species from regions in the world

Species	ΣHCHs	ΣDDTs	ΣDrins	ΣOCPs	References
O. niloticus	BDL-5.305	BDL- 7.571	BDL- 8.445	BDL-5.6264	This study
C. carpio	BDL- 6.4242	BDL- 11.8073	BDL- 11.8073	BDL-4.77	This study
S. lucioperca	BDL- 9.74	BDL- 28.676	BDL- 17.102	BDL-17.3667	This study
C. gariepinus	6-179	11-264		2-267	Buah-Kwofie *et al*. (2018)
C. gariepinus	BDL-515	BDL-601	BDL-315	BDL-601	Buah-Kwofie *et al*. (2018)
O.mossambicus	0.8-132.6	3.7-111.7	2.4-23.3	0.8-132.6	Buah-Kwofie *et al*. (2018)
O.niloticus		20-30			Buah-Kwofie *et al*. (2018)
O.mossambicus	0.7-1.4	3.4-11.9	0.3-0.6		Buah-Kwofie *et al*. (2018)
O.niloticus		BDL-51			Buah-Kwofie *et al*. (2018)
P. lineatus	01-18.0	220-380			Colombo *et al*. (2011)
O. bonariensis	1.14–13.81	1.01–3	BDL- 0.11		Menone *et al*. (2000)
Mix of species	1.82-8.38	0.05–10.3			Fair *et al*. (2018)
Mix of 3 species			BDL–18.00		Granados-Galv´*et al*. (2015)
O. niloticus	5.81–8.17d	6.81–9.31			Abbassy (2018)
P. commersonnil	BDL-191.1	BDL-27.1	BDL-232.2		Olisah *et al*. (2019)
M. cephalus	0.6–57.8	BDL-6.8	3.9–186		Olisah *et al*. (2019)
B. sinensis	0.80–6.56	2.62–19.91	0.13–9.00		Tong *et al*. (2019)
O. ophuyseni, C. lucidus		4.80–210.0			Sun *et al*. (2015)

4. Risk assessment of OCP residues in *O. niloticus*, *C. carpio* and *S. lucioperca*

Fish consumption represents a major pathway for human exposure to both organic and inorganic contaminants. To assess the impact of these concentration levels on human health, contaminant concentrations in the muscles of *O. niloticus*, *C. carpio*, and *S. lucioperca* were compared to international standards, as indicated in Table (2). None of the concentrations of organochlorine pesticides (OCPs) in the muscles of the three fish species exceeded the maximum international residue limits (MRL).

4.1.Non-cancer and cancer risk assessments

The presence of organochlorine compounds (OCPs) in the muscles of the fish species listed in Tables (3, 4) highlight both the persistence of these pollutants in the study area and the potential risk of human exposure through fish consumption. The estimated daily intakes (EDIs) for *O. niloticus* ranged from 0.45 to 1.41 for mean concentrations, 0.44 to 1.58 at the 50th percentile, and 1.04 to 4.22 at the 95th percentile. For *C. carpio*, EDIs varied from 0.72 to 1.79 for mean concentrations, 0.68 to 1.77 at the 50th percentile, and 1.36 to 4.67 at the 95th percentile. In *S. lucioperca*, EDIs ranged from 1.46 to 3.39 for mean values, 1.39 to 3.03 at the 50th percentile, and 3.02 to 8.69 at the 95th percentile. According to Table (3), all reported EDI values for OCPs in muscle tissues remained below the acceptable daily intake (ADI) levels set by the U.S. EPA.

Our findings contrast with those of previous studies where the estimated daily intake (EDI) levels of Aldrin and Dieldrin exceeded the acceptable daily intake (ADI) for *Clarias gariepinus* and Aldrin for *Tilapia zilli* **(Ozekeke Ogbeide *et al*., 2016)**. Similar findings have been observed in other fish species in Ethiopia. The estimated daily intakes (EDIs) of HCH and DDT at the 50th and 95th percentiles were significantly below the acceptable daily intake (ADI), suggesting that current consumption of these species, *O. niloticus*, *T. zillii*, *Carassius* spp., and *C. gariepinus*, do not present a health risk **(Yohannes *et al*., 2014)**. Comparable results have also been reported in South Africa, including for *C. gariepinus* and *O. mossambicus* from iSimangaliso Wetland National Park **(Buah-Kwofie *et al*., 2018)**, as well as for *H. vittatus*, *S. zambezensis*, and *C. rendalli* from the Phongolo River floodplain **(Volschenk *et al*., 2019)**.

The disparities observed in the values of the estimated dietary intake (EDI) index among various studies may stem from multiple factors influencing pesticide concentrations in fish and the dietary consumption patterns of the populations involved. Pesticide concentrations in fish are subject to variations depending on several parameters such as geographical location, time of year, local agricultural and industrial practices, as well as the level of pollution present in the surrounding waters.

A thorough evaluation of the health risks, linked to the consumption of muscle tissue from *O. niloticus*, *C. carpio*, and *S. lucioperca* containing organochlorine compounds (OCPs), was conducted and is summarized in Table (4). Hazard Quotient (HQ) values calculated for non-carcinogenic risks associated with the muscle tissues of these fish species were consistently below one across mean concentrations, the 50th percentile, and the 95th percentile. This indicates that the consumption of these fish species poses no significant non-carcinogenic health risks. However, contrasting results were observed in a previous study, where hazard ratios (HRs) for cancer risk, particularly at the 95th percentile, showed varying trends. For *O. niloticus*, the carcinogenic risk associated with HCH exceeded one, whereas it was below one for DDT. Conversely, for *T. zillii*, *Carassius* spp., and *C. gariepinus*, HRs for DDT were above one. Cumulatively, daily exposure to OCPs through fish consumption could lead to a lifetime cancer risk exceeding one in a million. These findings emphasize ongoing concerns regarding these compounds due to their persistent presence and potential health impacts **(Yohannes *et al*., 2014)**.

The cancer risk assessment based on hazard ratios (HRs) for all OCPs detected in the muscle tissues of *O. niloticus*, *C. carpio*, and *S. lucioperca* at mean, 50th, and 95th percentile concentrations is presented in Table (4). For *O. niloticus*, HRs at mean concentrations ranged from 8.6×10^{-5} to 1×10^{-2}, at the 50th percentile from 8.8×10^{-5} to 1.3×10^{-2}, and at the 95th percentile from 8.4×10^{-4} to 2.1×10^{-2}. In *C. carpio*, HR values ranged from 8.9×10^{-4} to 1.4×10^{-2} at mean concentrations, from 9.6×10^{-4} to 1.4×10^{-2} at the 50th percentile, and from 7.7×10^{-4} to 3.9×10^{-2} at the 95th percentile. For *S. lucioperca*, HRs ranged from 8.3×10^{-4} to 2.4×10^{-2} at the mean, from 7.9×10^{-4} to 2.5×10^{-2} at the 50th percentile, and from 7.6×10^{-4} to 6.2×10^{-2} at the 95th percentile. All HR values were below 1, indicating that daily exposure to these OCPs through consumption of these fish species does not result in a lifetime cancer risk of 1 in 1,000,000.

While these results indicate that consuming these fish species is currently safe, it is crucial to remain aware of the potential health risks related to aquatic resource consumption. The ongoing presence and extensive distribution of certain organochlorine pesticides (OCPs) highlight the need for ongoing monitoring to ensure the long-term safety of consuming these fish species.

Furthermore, this study may not account for other important factors such as variations in fishing practices, the location of fish within the reservoir, or the dietary habits of local populations. Vulnerable populations such as children, the elderly, and heavy fish consumers may be more exposed to risks associated with OCP contamination. Similarly, the average daily fish intake rate of 38 grams per day and the average population weight of 73.3kg may not accurately reflect local and individual consumption habits. However, despite these limitations, the study provides valuable information on the health risk assessment associated with exposure to OCPs through the consumption of fish from the Al Massira Reservoir for this specific population.

Table 2. Mean concentration ± SE and range (in bold) of organochlorine pesticide residues (ng/kg ww) in muscles of *O. niloticus*, *C. carpio* and *S. lucioperca*

Analyses	MRL (ng/g) A	MRL (ng/g) b	MRL (ng/g) C	*O.niloticus* n=24	*C.carpio* n=24	*S.lucioperca* n=24
α-HCH	200			0.887 ±0.65 **BDL- 2.171**	1.40 ±0.95 **BDL- 3.782**	2.82±1.94 **BDL- 7.545**
γ-HCH (lindane	200			1.364 ±0.97 **BDL-3.535**	1.63 ±1.47 **BDL- 4.496**	2.81 ±2.14 **BDL- 7.376**
β-HCH				2.130±1.37 **BDL- 5.305**	3.05±1.7 **BDL- 6.4242**	4.5±2.23 **BDL- 9.640**
ΣHCHs				1.46± 1.14 **BDL-5.305**	2.05±1.55 **BDL- 6.4242**	3.38±2.22 **BDL- 9.64**
Aldrin	200	300		2.416±2.65 **BDL- 8.445**	3.47±3.19 **BDL- 11.807**	5.63±2.91 **BDL- 11.323**
Dieldrin	200	300		2.5±1.75 **BDL- 5.611**	3.4±3.14 **BDL- 10.675**	6.39 ±4.85 **BDL- 17.102**
ΣDrins				2.409±2.18 **BDL- 8.445**	2.61±2.41 **BDL- 11.807**	6.01±3.97 **BDL- 17.102**
DDD	1000	5000	5000	0.957 ±0.69 **BDL- 2.226**	1.95 ±1.52 **BDL- 4.337**	3.42±2.39 **BDL- 7.454**
DDE	1000	5000	5000	1.87±1.33 **BDL- 4.634**	2.84 ±2.66 **BDL- 8.377**	4.15±3.07 **BDL- 8.875**
DDT	1000	5000	5000	**2.73 ±2.26** **BDL- 7.571**	2.83 ±2.47 **BDL- 8.174**	6.55 ±6.76 **BDL- 28.676**
ΣDDTs				1.830±1.72 **BDL- 7.571**	2.57±2.36 **BDL- 11.807**	4.7±4.64 **BDL- 28.676**

a – Maximum residue limit set by European Commission Directives 2006/53, 2006/59, 2006/60, 2006/61 and 2006/62.

b – Maximum residue limit set by US food and drug administration.

c - Maximum residue limit set by health Canada (Health Canada, 2015), BDL – Below detection limit.

Table 3. The estimated daily intake (EDI) and OCPs concentrations in muscles of *O. niloticus*, *C. carpio* and *S. lucioperca* from Al Massira Dam Lake, Morocco. EDIs were compared against US EPA acceptable daily intake (ADI) values

OCPs	ADI(ng/kg/d)	Concentration (ng/g ww)									EDI (ng/kg bw/day)								
		Mean			50th percentile			95th percentile			Mean			50th percentile			95th percentil		
		O.N	C.C	S.L	O.N	C.C	S.L	O.N	C.C	S.L	O.N	C.C	S.L	O.N	C.C	S.L	O.N	C.C	S.L
α-HCH	**5000**	0.88	1.40	2.82	0.86	1.32	2.70	2.04	2.64	5.84	0.45	0.72	1.46	0.44	0.68	1.39	1.04	1.36	3.02
β-HCH	**5000**	2.13	3.05	4.50	1.94	3.28	4.85	4.20	5.58	7.68	1.10	1.57	2.33	1	1.69	2.51	2.17	2.89	3.97
γ-HCH	**5000**	1.36	1.63	2.81	1.13	1.61	3.33	2.84	4.02	5.85	0.70	0.84	1.45	0.58	0.83	1.72	1.47	2.08	3.03
Aldrin	**100**	2.41	3.47	5.63	1.47	3.42	5.86	8.16	8.08	9.97	1.24	1.79	2.91	0.76	1.77	3.03	4.22	0.04	5.16
Dieldrin	**100**	2.50	3.40	6.39	3.06	3.20	5.85	5.02	9.03	15.26	1.29	1.76	3.31	1.58	1.65	3.03	2.60	4.67	7.90
DDD	**10000**	0.95	1.95	3.42	0.97	2.07	3.77	2.18	3.99	6.78	0.49	1.01	2.23	0.5	1.07	1.95	1.12	2.06	3.51
DDE	**10000**	1.87	2.84	4.15	1.99	2.32	4.60	4.05	7.37	8.31	0.96	1.47	2.14	1.03	1.20	1.38	2.09	3.81	4.30
DDT	**10000**	2.73	2.83	6.55	2.70	3.02	4.95	6.65	7.65	16.78	1.41	1.46	3.39	1.39	1.56	2.56	3.44	3.96	8.69

Table 4. Cancer benchmark concentrations and hazard ratios (HRs) for OCP residues detected in muscles of *O. niloticus* (n = 24), *C. carpio* (n=24) and *S. lucioperca* (n = 24) from Al Massira Dam Lake, Morocco

OCPs	OSF (mg/kg/day)	CBC (ng/kg/day)	Mean HRs			50th Percentile HRs			95th Percentile HR		
			O.N	C.C	S.L	O.N	C.C	S.L	O.N	C.C	S.L
α-HCH	1.1	1753.6	2.6×10^{-4}	4.1×10^{-4}	$8 .3\times 10^{-4}$	2.5×10^{-4}	3.9×10^{-4}	7.9×10^{-4}	5.9×10^{-4}	7.7×10^{-4}	1.7×10^{-3}
β-HCH	1.1	1753.6	6.3×10^{-4}	8.9×10^{-4}	1.3×10^{-3}	5.7×10^{-4}	9.6×10^{-4}	1.4×10^{-3}	1.6×10^{-3}	1.6×10^{-3}	2.3×10^{-3}
γ-HCH	1.1	1753.6	4×10^{-4}	4.8×10^{-4}	8.3×10^{-4}	3.3×10^{-4}	4.7×10^{-4}	9.8×10^{-4}	8.4×10^{-4}	1.2×10^{-3}	1.7×10^{-3}
Aldrin	16	120.559	1×10^{-2}	1.4×10^{-2}	2.4×10^{-2}	6.3×10^{-3}	1.5×10^{-2}	2.2×10^{-2}	3.2×10^{-2}	3.3×10^{-4}	4.3×10^{-2}
Dieldrin	16	120.559	1×10^{-2}	1.4×10^{-2}	2.7×10^{-2}	1.3×10^{-2}	1.4×10^{-2}	2.5×10^{-2}	2.1×10^{-2}	3.9×10^{-2}	6.5×10^{-2}
DDD	0.34	5673.4	8.6×10^{-5}	1.8×10^{-4}	3.9×10^{-4}	8.8×10^{-5}	1.9×10^{-4}	3.4×10^{-4}	2×10^{-4}	3.6×10^{-4}	6.2×10^{-2}
DDE	0.34	5673.4	1.7×10^{-4}	2.6×10^{-4}	3.8×10^{-4}	1.8×10^{-4}	2.1×10^{-4}	4.2×10^{-4}	3.7×10^{-4}	6.7×10^{-4}	7.6×10^{-4}
DDT	0.34	5673.4	2.5×10^{-4}	2.6×10^{-4}	6×10^{-4}	2.4×10^{-4}	2.7×10^{-4}	4.5×10^{-4}	6×10^{-4}	7×10^{-4}	1.5×10^{-3}

CONCLUSION

This study represents the first assessment of organochlorine pesticide (OCP) accumulation in the freshwater ecosystem of Morocco's Al Massira Reservoir. The results indicated that the pike perch (*S. lucioperca*) accumulated higher levels of OCPs in their muscles compared to the Nile tilapia (*O. niloticus*) and common carp (*C. carpio*). Despite widespread contamination in all three species' muscle tissues, none exceeded the maximum residue limit (MRL) set by the European Commission. Risk assessments based on average hazard ratios (HR) at the 50th and 95th percentiles indicated no potential cancer risk from consuming muscles of *O. niloticus*, *C. carpio*, and *S. lucioperca*. Cancer risk assessments for all OCPs in these species, at various percentiles, were below 1, suggesting daily consumption poses no lifetime cancer risk of 1 in 1,000,000. Nonetheless, given the elevated OCP concentrations found, residents consuming these fish species are likely experiencing higher OCP exposure levels.

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