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Pesticide accumulation in tissues of cultured *Oreochromis niloticus* and *Mugil cephalus*: effects on biochemical biomarkers and associated human health risks

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Abstract

Background Egypt's food security depends heavily on aquaculture, however fish farmed on farms that receive agricultural drainage water may be subjected to pesticide pollution. Such contamination can impair fish health, alter physiological functions, and pose potential risks to consumers. This study investigated the occurrence and distribution of pesticide residues in water and in the gill, liver, and muscle tissues of two economically important species, *Oreochromis niloticus* and *Mugil cephalus*, collected from four fish farms that differ in their water sources in Fayoum Governorate, Egypt. The control farm was irrigated with fresh water, while the other farms were irrigated with agricultural drainage water. It also evaluated the biochemical and stress-related responses in fish and assessed potential risks associated with human consumption.

Results Farms supplied with drainage water exhibited higher salinity and nutrient pollution compared to the freshwater control farm. Multiple classes of pesticides, including organochlorine insecticides, pyrethroid insecticides, and herbicides, were detected in both water and fish tissues, with the highest concentrations found at the El-Bats and Dayer El-Berka farms. Pesticide accumulation varied by species and organ: *O. niloticus* accumulated the highest levels in the liver, whereas *M. cephalus* accumulated more in the gills. Fish from polluted farms showed reduced activities of the liver enzymes aspartate aminotransferase and alanine aminotransferase, alongside increased concentrations of triglycerides, cholesterol, and total lipids in muscle tissue. Oxidative and cellular stress markers, including malondialdehyde and caspase-12, were elevated in all examined tissues, while catalase activity was reduced.

Conclusions Fish raised in agriculture drainage-fed aquaculture systems showed clear evidence of pesticide exposure, organ-specific accumulation patterns, and associated biochemical and stress responses. Despite exceedances of regulatory residue limits for some compounds, the overall dietary risk to consumers was found to be low based on estimated intake calculations. Nevertheless, the high prevalence of pesticide mixtures in water and fish highlights the need for continuous monitoring and management of agricultural runoff to maintain aquaculture productivity and ensure safe consumption.

Keywords Aquaculture, Pesticide residues, Oxidative stress, Endoplasmic reticulum stress, Caspase-12, Risk assessment

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Introduction

According to recent studies, aquaculture has rapidly grown over the past 10 years to meet the rising demand for seafood as a vital source of nutrition for people globally [1, 2]. Approximately 17% of the world's protein intake comes from fish, making it a vital source of animal protein [3]. Aquaculture is very important in Egypt, which ranks seventh globally in terms of output volume and is the leading producer in Africa, accounting for 63.2% of the continent's total fish production [4]. Egypt produced 2.2 million metric tons (MMT) of fish in 2021, with 1.7 MMT coming from aquaculture. By 2025, plans are in place to boost production to 3 MMT [5].

One of the primary factors affecting fish quality in natural settings is water pollution [6]. Because of its toxicity, durability, and tendency to bioaccumulate in fish tissues through the food chain, pesticide pollution in aquatic habitats is especially concerning for human health [7, 8].

Pesticides are hazardous chemicals used in agriculture to improve crop yield, protect against pests, and control insect-borne diseases [9, 10]. They can be classified by target organisms (insecticides, herbicides, fungicides) [11] or by chemical nature. Inorganic pesticides contain substances such as arsenic and fluoride, while organic pesticides include organochlorines, organophosphates, carbamates, and pyrethroids [12].

Among these, organochlorine pesticides (OCPs) are of particular concern due to their carcinogenic, mutagenic, endocrine-disrupting, persistent, and bioaccumulative properties [13]. Although worldwide prohibited for almost forty years, including in Egypt, due to their persistence, lipid solubility, and toxicity, OCPs are nonetheless employed illicitly in numerous poor countries [14]. Subsequently, pyrethroids became widely used as substitutes for OCPs, organophosphates, and carbamates, because of their comparatively lower mammalian toxicity and persistence [15]. However, fish are highly sensitive to pyrethroid toxicity, which primarily targets the gills, liver, and muscular tissues [16]. They are known to disrupt antioxidant enzyme activity, increasing susceptibility to oxidative stress [17]. Since fish lack carboxylesterase, the enzyme responsible for metabolizing pyrethroids, the toxic effects can persist for extended periods [16].

Fish biomarkers are a proven method for assessing chemical exposure in aquatic settings [18, 19]. Under stress, fish undergo metabolic adaptations [20], and alterations in the activities of enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) can serve as indicators of pesticide-induced liver damage and overall health impairment [21, 22]. Similarly, alterations in lipid profiles, total lipids, triglycerides, and cholesterol reflect nutritional status, endocrine function, and organ integrity [23].

Pesticides, due to their lipophilic characteristics, can easily infiltrate the cell membranes of aquatic creatures, leading to oxidative stress through the generation of excessive reactive oxygen species (ROS). This leads to molecular damage, unless counteracted by antioxidant defense systems [10, 24, 25]. Particularly at risk is the endoplasmic reticulum (ER), an essential organelle involved in the synthesis of proteins, lipids, and sterols. By upsetting redox equilibrium, pesticides can damage ER function, resulting in ER stress and apoptosis [26, 27]. Caspase-12 has not yet been assessed as a biomarker of ER stress and apoptosis in fish from pesticide-contaminated habitats, even though numerous studies have documented pesticide-induced apoptosis in fish and shellfish [28–30].

In recent years, the potential of numerous pesticide exposures has become a recurring worry in environmental and human health investigations [31]. At environmentally relevant low doses, combinations of pesticides can have cumulative harmful effects even when the individual substances are not physically comparable [32]. Furthermore, exposure levels that surpass their corresponding no observed adverse effect levels (NOAELs) may result in toxicokinetic interactions [33]. *Oreochromis niloticus* (*O. niloticus*) and *Mugil cephalus* (*M. cephalus*), which rely on agricultural drainage water from El-Bats, El-Wadi, and other subsidiary drains, are two species for which Egypt's government has encouraged the expansion of aquaculture and the intensification of culture techniques in Lake Qarun areas due to water resource regulations [34, 35]. Concerns about the possible cumulative effects of pesticide residues in drainage water on fish health and, consequently, human consumers are raised by these situations.

Since *O. niloticus* and *M. cephalus* are two of the most commonly cultivated and consumed fish species in Egypt, they were chosen for the current study due to their ecological and economic significance [36]. Both species exhibit a high tolerance to a broad range of environmental conditions, making them suitable bioindicators in polluted aquaculture systems [35, 37]. Additionally, they feed on different food sources, which may influence their exposure to environmental contaminants. For instance, *O. niloticus* is primarily omnivorous, with a tendency to feed on phytoplankton and detritus [38], whereas *M. cephalus* is a detritivorous and benthic feeder that ingests sediment-associated organic matter [39, 40]. These differences enable a comparative evaluation of species-specific patterns

of pesticide bioaccumulation, tissue distribution, and biomarker responses. Therefore, evaluating both species offers a more thorough knowledge of the threats to human health and the environment posed by pesticide mixtures in Egyptian aquaculture systems.

Thus, the current study aimed to use a biomarker-based approach to examine the potential hazards of pesticide mixtures in cultured Nile tilapia (*O. niloticus*) and flathead grey mullet (*M. cephalus*) and to evaluate the risks to public health of consuming these species when reared in pesticide-contaminated aquaculture systems.

Materials and methods

Ethics approval

The fish sampling was conducted with approval from the National Institute of Oceanography and Fisheries (NIOF) committee for ethical care and use of animals/aquatic animals (NIOF-IACUC) according to the certificate number NIOF–FW3–F-25-R-024. In order to ensure that the research strategy is in line with accepted ethical standards and protects the welfare of the experimental animals, all study techniques strictly followed the ARRIVE guidelines v2.0 [41].

Sampling sites

Water and fish samples of *O. niloticus* and *M. cephalus*, were gathered from three fish farms around Lake Qarun in the El-Fayoum Governorate, Egypt, representing different water sources, in addition to a reference farm that depends only on irrigation water (freshwater) (Fig. 1) as follows:

Site 1 (control fish farm): depends on irrigation water (freshwater from the river Nile). GPS: 29.249961,30.860355;

Site 2 (El-Bats fish farm): depends on agricultural drainage water from El-Batts drain. GPS: 29.512957,30.831260;

Site 3 (El-Wadi fish farm): depends on agricultural drainage water from El-Wadi drain. GPS: 29.443235,30.659846;

Site 4 (Dayer El-Berka fish farm): depends on agricultural drainage water from Dayer El-Berka drain as a water source. GPS: 29.438820,30.694388.

Water and fish sampling

Surface water samples (n = 6 per farm) were taken at a depth of roughly 50 cm beneath the surface using pre-cleaned polyethylene bottles. Bottles were pre-washed with detergent, deionized water, and 2 M nitric acid to avoid contamination, and then they were rinsed once more with surface water and deionized water. Samples were filtered on-site, acidified with 10% HNO_3, and then transferred to the lab in ice-cooled containers for preservation.

From the same sites, a total of 288 adult *O. niloticus* and *M. cephalus* (36 fish per species per farm) were collected between May and July 2025. The fish were measured to the closest centimeter and weighed to the closest gram. The *O. niloticus* specimens ranged from 23.8 to 31.2 cm in total length and 230.5 to 510 g in weight, while *M. cephalus* ranged from 33.6 to 39.1 cm and 290 to 685.5 g. Fish were randomly selected, deeply anesthetized with MS-222 (0.1–0.2 g/L tricaine methane sulfonate), and euthanized by severing the head to obtain gill, liver, and muscle samples. The organs were carefully excised, and six pooled samples of 18 individuals were prepared per site per organ. A Glas-Col motor-driven homogenizer was used to homogenize the tissues in 5 mL of cold 50 mM potassium phosphate buffer (pH 7.4) for every gram of tissue. For biochemical biomarker studies, homogenates were centrifuged at 100,000 × g for 15 min at 4 °C. The resultant supernatants were then kept at − 80 °C.

For pesticide-residue determination, another six pooled samples (each sample 8 g) of 18 individuals per site were collected and stored at − 80 °C until subsequent pesticide extraction and instrumental analysis.

Water quality analysis

A combined meter (pH/EC/TDS/temperature, Mi 805) was used to measure temperature and pH; Winkler's iodometric method was used to measure dissolved oxygen (DO) [42]; a refractometer (VITAL Sine SR-6, China) was used to measure salinity; the phenate method was used to estimate ammonia [43]; colorimetric methods were used to determine nitrite and nitrate [44] and the automated hydrazine reduction method [45], respectively. Transparency was measured by measuring light penetration.

Pesticide extraction and analysis

Gas chromatography–mass spectrometry (GC–MS), extraction, and cleanup were used in the multiresidue detection of pesticide residues in fish tissues and water. Water samples were extracted following Mann [46] using liquid–liquid extraction with dichloromethane to transfer pesticides into the organic phase. Fish tissues were homogenized and subjected to acetonitrile–petroleum ether partitioning, where pesticides were solubilized in acetonitrile and transferred to the petroleum ether phase. Crude extracts were then purified using florisil solid-phase extraction columns with sequential elution using diethyl ether–petroleum ether mixtures at 6%, 15%, and

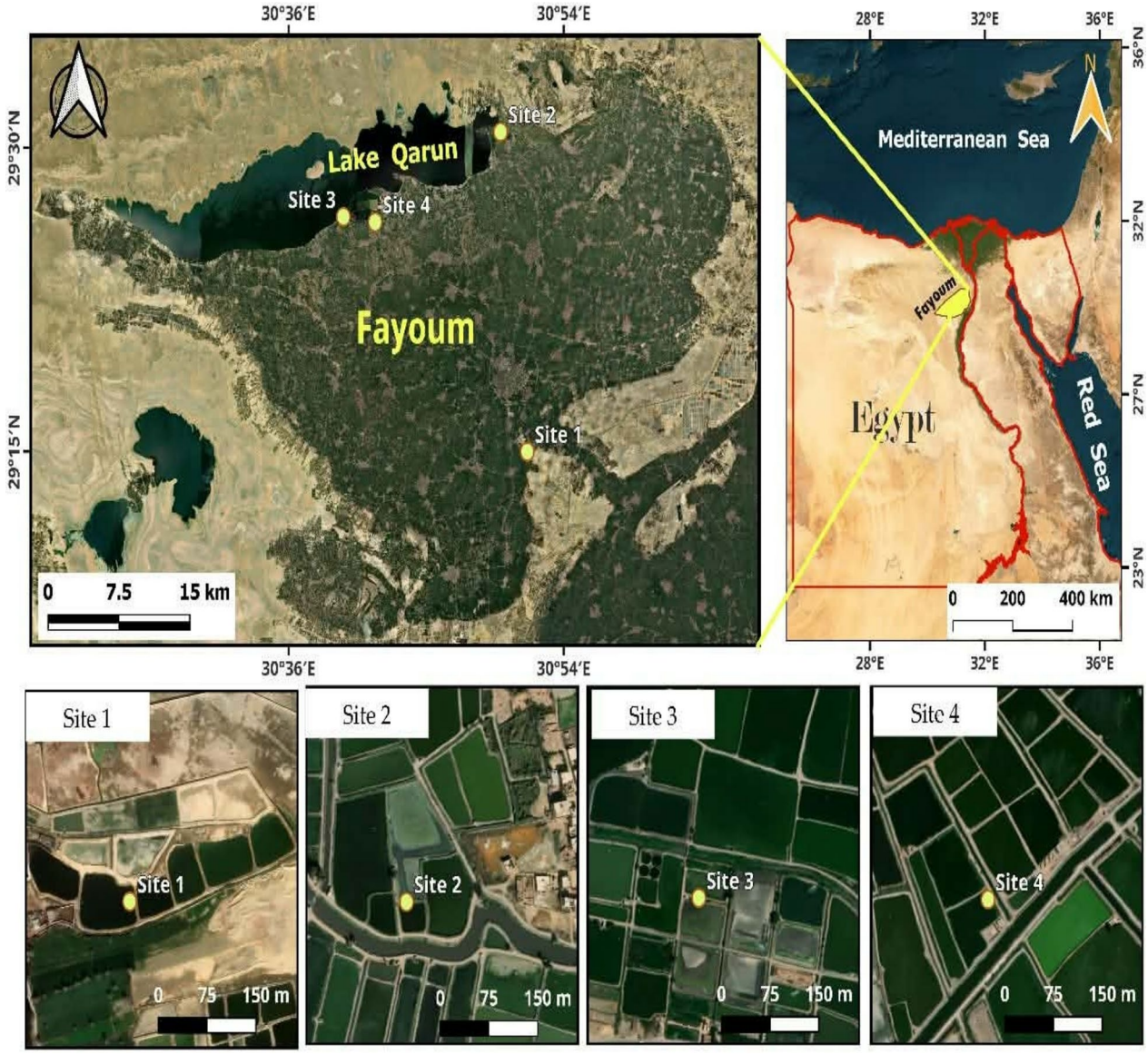

Fig. 1 A map showing the location of the studied fish farms

50%, according to AOAC [37] and EPA Method 3620C [48].

A Shimadzu system with a non-polar capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used to evaluate purified extracts by GC–MS. Helium was used as the carrier gas and injection was carried out in splitless mode at 250 °C at a rate of 1 mL/min. The oven temperature was set to rise from 100 °C with a 2-min hold to 280 °C at a rate of 10 °C per minute, with a final 10-min pause. Sensitive and selective detection was provided by the use of electron impact ionization (70 eV) and selected ion monitoring (SIM). Target pesticides included organochlorine insecticides (e.g., Endosulfan I & II, Dieldrin, Aldrin, HCH isomers, DDT and metabolites), fungicides (Pencycuron, Thiram, Diniconazole), herbicides (Butachlor, Atrazine), and pyrethroids (Deltamethrin, Fenvalerate). The findings were reported as mg/L for water and mg/kg wet weight for fish.

Metabolic markers

Liver AST and ALT activities were measured using the kinetic UV method of Gella et al. [49], where aminotransferase activity is monitored through NADH oxidation at 340 nm at 37 °C. Enzyme activities were expressed as U/L of tissue homogenate. Muscle

triglycerides were quantified following Wahlefeld [50] using an enzymatic colorimetric assay based on sequential triglyceride hydrolysis and peroxide-dependent dye formation, and results were expressed as mg/dL of homogenate. Cholesterol concentration was determined according to the CHOD–PAP method of Amundson and Zhou [51], and values were expressed as mg/dL of homogenate. Total lipids were measured using the vanillin–phosphoric acid colorimetric method of Knight et al. [52] after chloroform–methanol extraction, and total lipid content was expressed as g/dL of tissue homogenate.

Stress biomarkers

MDA levels were quantified in homogenized muscle, liver, and gill tissues following the thiobarbituric acid reactive substances (TBARS) method of Ohkawa et al. [53]. Briefly, tissue homogenates were mixed with sodium dodecyl sulfate, acetic acid, and thiobarbituric acid, then heated in a boiling water bath to allow formation of the MDA–TBA adduct. After cooling and centrifugation, the absorbance of the supernatant was measured at 532 nm using the Biodiagnostic (Egypt) commercial kit. MDA concentrations were calculated according to the kit instructions and expressed as nmol/g tissue. Catalase activity was determined according to the method of Aebi [54] using the Biodiagnostic (Egypt) CAT assay kit. Tissue homogenates were incubated with hydrogen peroxide, and the decomposition rate of H_2O_2 was monitored spectrophotometrically at 240 nm. Enzyme activity was calculated based on the decline in absorbance over time and expressed as U/g tissue. A sandwich ELISA kit (MyBioSource, USA) was used to quantify caspase-12 levels in homogenized tissues in accordance with the manufacturer's instructions. After adding standards and samples to antibody-coated wells, streptavidin-HRP and a biotin-conjugated detection antibody were incubated. Each well received an addition of a stop solution. When the stop solution was added, the liquid turned yellow. The standard curve was used to calculate concentrations after absorbance was measured at 450 nm. The results were given in ng/g tissue.

Public health risk assessment

The United States Environmental Protection Agency's [55] recommendations, which provide standardized methods for risk assessment, were followed in the human health risk assessment for consuming fish contaminated with environmental pollutants.

The assessment involved comparing the measured concentrations of pesticides in fish samples with established maximum residue limits (MRLs) [56–58], which represent safe exposure thresholds designed to protect both human health and the environment [59]. In cases where MRLs were unavailable, the default value of 0.01 mg/kg recommended by the Egyptian National Food Safety Authority [60] was applied.

In addition, human exposure was estimated by calculating the Estimated Daily Intake (EDI, mg/kg body weight) using the following equation:

$$EDI\left(mg/kg\ bw\right) = C \times D / BW$$

where C represents the mean concentration of the pesticide in fish samples (mg/kg), D is the daily fish consumption rate (kg/day/capita), and BW is the average adult body weight (70 kg) as recommended by WHO [61]. The daily fish consumption rate in Egypt was assumed to be 50.6 g/person/day, based on FAO [62].

Risk characterization was performed by calculating the Hazard Index (HI) for each pesticide as:

$HI = EDI/ADI$.

where ADI stands for Acceptable Daily Intake (mg/kg bw). Adverse health effects are uncommon if the $HI < 1.0$, but they may be possible if the $HI \geq 1.0$. The overall cumulative risk was determined by summing the HI values across all analyzed pesticides [55].

Statistical analysis

The Kolmogorov–Smirnov test and Levene's test were used to evaluate the data for normality and homogeneity of variances before analysis. To compare means among farms, the analysis of variance (One-way ANOVA) followed by the Duncan Waller test ($P \leq 0.05$) was used. A T-test was used to compare between the two species. Data was shown as means ± standard deviation and statistical package software (SPSS, 2015) Version 23. was used to perform the statistical analysis.

Results

Water quality parameters

Table 1 displays the water physicochemical characteristics of the samples from the various fish farms. Water temperature ranged from 29.5 to 31.5 °C with no significant differences between farms. The pH values across all sites were alkaline, with the highest mean pH at El-Bats fish farm and the lowest value at the control fish farm. Dissolved oxygen (DO) concentrations were highest at the control fish farm, and lowest at El-Bats fish farm. Regarding salinity, ammonia, nitrite, and nitrate levels, the highest mean values were recorded at Dayer El-Berka fish farm. In contrast, the lowest concentrations for these parameters were found at the control fish farm. Water transparency was highest at the control fish farm and lowest at El-Bats fish farm.

Table 1 Water quality parameters

Parameters	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Temperature, °C	31.50 ± 0.71	29.50 ± 0.71	30.00 ± 1.41	31.00 ± 1.41
pH	7.67 ± 0.08^{b}	8.31 ± 0.03^{a}	8.28 ± 0.02^{a}	8.30 ± 0.01^{a}
Dissolved oxygen, mg/L	6.65 ± 0.18^{a}	5.74 ± 0.35^{b}	6.01 ± 0.16^{ab}	6.27 ± 0.35^{ab}
Salinity, g/L	0.52 ± 0.14^{c}	2.19 ± 0.51^{b}	1.48 ± 0.18^{bc}	14.94 ± 0.83^{a}
Total ammonia, mg/L	0.277 ± 0.022^{b}	0.476 ± 0.028^{a}	0.354 ± 0.039^{b}	0.591 ± 0.066^{a}
Nitrite, mg/L	0.119 ± 0.004^{c}	0.221 ± 0.030^{ab}	0.192 ± 0.022^{b}	0.254 ± 0.011^{a}
Nitrate, mg/L	3.81 ± 0.26^{b}	10.07 ± 0.64^{a}	9.84 ± 0.55^{a}	10.42 ± 0.29^{a}
Secchi disc, cm	17.59 ± 1.51^{a}	10.19 ± 0.47^{c}	13.51 ± 1.17^{b}	10.47 ± 0.49^{c}

The values are displayed as mean ± standard deviation. Significant differences across farms are indicated by different superscript letters (a,b,c) (ANOVA-test; $p \leq 0.05$). Values sharing the same letter are not significantly different

Table 2 Pesticide residues in water (mg/L)

Pesticides	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Organochlorine i Pesticides residues nsecticides				
Aldrin	ND	ND	ND	ND
Chlordane	ND	0.086 ± 0.008	ND	0.058 ± 0.007
Dieldrin	ND	ND	ND	ND
Endosulfan-I	0.007 ± 0.001	0.049 ± 0.008	0.029 ± 0.008	0.039 ± 0.005
Endosulfan-II	ND	ND	ND	ND
Endrin	ND	0.019 ± 0.004	ND	ND
α-HCH	ND	ND	ND	ND
β-HCH	ND	ND	ND	ND
δ-HCH	ND	ND	ND	ND
γ-HCH	ND	0.019 ± 0.003	ND	ND
Heptachlor	ND	ND	ND	ND
o,p'-DDT	0.009 ± 0.001	0.025 ± 0.006	0.047 ± 0.006	0.096 ± 0.005
p,p'-DDD	ND	ND	ND	ND
p,p'-DDE	ND	ND	ND	ND
p,p'-DDT	0.009 ± 0.001	0.025 ± 0.006	0.047 ± 0.006	0.096 ± 0.005
Fungicides				
Diniconazole	ND	ND	ND	ND
Pencycuron	ND	ND	ND	ND
Thiram	ND	ND	ND	ND
Herbicides				
Atrazine	ND	ND	0.017 ± 0.003	ND
Butachlor	0.013 ± 0.001	0.079 ± 0.004	0.036 ± 0.004	0.026 ± 0.004
Pyrethroid insecticides				
Deltamethrin	ND	0.985 ± 0.011	ND	0.588 ± 0.063
Fenvalerate	ND	ND	ND	0.286 ± 0.074
Σ Total pesticides	0.029 ± 0.004^{b}	1.261 ± 0.044^{a}	0.129 ± 0.022^{b}	1.088 ± 0.163^{a}

The values are displayed as mean ± standard deviation. Significant differences across farms are indicated by different superscript letters (a,b,c) (ANOVA-test; $p \leq 0.05$). Values sharing the same letter are not significantly different. ND = not detected (below the limit of detection, LOD = 0.001 mg/L)

Pesticide residues in water

As shown in Table 2, organochlorine, pyrethroid insecticides, and herbicides were among the pesticide residues found in water samples from the four fish farms under study, while fungicides were not found. Regarding organochlorine insecticides, Endosulfan-I and o,p'-DDT were found in all farms, Endrin was detected only in El-Bats, while Chlordane was identified in both El-Bats and

Dayer El-Berka farms. Pyrethroid insecticides were also present, with Deltamethrin detected in El-Bats and Dayer El-Berka, and Fenvalerate found exclusively in Dayer El-Berka. For herbicides, Butachlor was the only compound identified, with the highest concentration in El-Bats farm. Regarding the total concentration of all detected pesticides (Σ pesticides), the greatest value was recorded in El-Bats and Dayer El-Berka, while the lowest value was observed in the control farm.

Pesticides residues in fish tissues

Pesticide residues in fish gills

The gills of *O. niloticus* from the control farm contained residues of Endosulfan-I and Chlordane. At El-Bats farm, the gills accumulated several organochlorine insecticides, including Endosulfan-I, Endrin, Chlordane, and various DDT isomers (p,p′-DDT and o,p′-DDT), along with δ-HCH and the herbicide Butachlor. At El-Wadi farm, the gills showed the presence of multiple pesticide residues, including Endosulfan-I, Endrin, Heptachlor, Chlordane, DDT isomers, γ-HCH, and the fungicide Diniconazole. At Dayer El-Berka farm, Endosulfan-I, Dieldrin, Chlordane, γ-HCH, o,p′-DDT, and Butachlor were detected in gill tissues (Table 3).

M. cephalus gills contained residues of Endosulfan-I, Dieldrin, Aldrin, and Chlordane in the control farm. At El-Bats farm, several organochlorine compounds were detected, including Endosulfan-I, Dieldrin, Chlordane, DDT isomers (p,p′-DDT and o,p′-DDT), and γ-HCH, along with the herbicides Butachlor and Atrazine. At El-Wadi farm, the gills showed residues of Endosulfan-I, Endrin, Aldrin, Chlordane, DDT derivatives, and γ-HCH, as well as the pyrethroid Fenvalerate. At Dayer El-Berka farm, *M. cephalus* gills exhibited a wide variety of pesticide residues, including Endosulfan-I, Dieldrin, Aldrin, Chlordane, o,p′-DDT, Butachlor, Atrazine, and Diniconazole (Table 4).

Pesticide residues in fish liver

Regarding *O. niloticus* from the control farm, the liver contained detectable residues of Endosulfan-I, Endosulfan-II, and p,p′-DDD, while all other compounds were below detection limits. At El-Bats farm, a wider range of pesticides was detected in the liver, including Endosulfan-I and II, several DDT derivatives, HCH isomers (α-, γ-, and δ-HCH), and the fungicide Pencycuron. The herbicides Butachlor and Atrazine were also present, with Butachlor being particularly dominant. At El-Wadi farm, the liver exhibited the presence of Endosulfan-II, Dieldrin, Heptachlor, p,p′-DDD, o,p′-DDT, α-HCH, and Pencycuron. At Dayer El-Berka farm, the liver showed residues of Endosulfan-I and II, Dieldrin, and the pyrethroid insecticide Fenvalerate, while other compounds were not detected (Table 5).

For *M. cephalus* from the control farm, the liver contained several organochlorine insecticides, including Endosulfan-I and II, Dieldrin, Aldrin, and p,p′-DDD. At El-Bats farm, a more diverse array of pesticide residues was observed, including Endosulfan-I and II, Endrin, Dieldrin, Aldrin, DDT derivatives, α- and γ-HCH isomers, and the herbicide Butachlor. The liver samples from El-Wadi farm contained residues of Endosulfan-I and II, Endrin, Dieldrin, Aldrin, α-HCH, γ-HCH, Pencycuron, and Butachlor. At Dayer El-Berka farm, the liver exhibited residues of Endosulfan-I and II, Butachlor, Atrazine, and Fenvalerate (Table 6).

Pesticide residues in fish muscles

In the control farm, *O. niloticus* muscle tissues contained traces of organochlorine insecticides, including Endosulfan-I, Endosulfan-II, Dieldrin, and DDT metabolites (p,p′-DDE and p,p′-DDD). At El-Bats farm, muscle samples exhibited a broader range of contaminants, such as Endosulfan-I and II, Dieldrin, Heptachlor, DDT derivatives (p,p′-DDT, p,p′-DDE, and p,p′-DDD), and several HCH isomers (α-, γ-, and δ-HCH). The herbicide Atrazine was also present. At El-Wadi farm, the muscles contained a variety of organochlorine compounds, including Endosulfan-I and II, DDT derivatives, Chlordane, and several HCH isomers, with Heptachlor showing relatively higher presence among the detected residues. The fungicide Thiram and the herbicide Atrazine were also observed. In Dayer El-Berka farm, muscle tissues of *O. niloticus* contained multiple pesticide residues, including Endosulfan-I and II, DDT derivatives, Heptachlor, Atrazine, and minor traces of Endrin (Table 7).

M. cephalus muscles from the control farm contained low residues of DDT metabolites (p,p′-DDE and p,p′-DDD) and Endosulfan isomers. At El-Bats farm, muscle tissues revealed contamination by several organochlorine insecticides, including Endosulfan-I and II, DDT derivatives, Chlordane, and α- and δ-HCH isomers, as well as the herbicide Atrazine. At El-Wadi farm, *M. cephalus* muscles contained Endosulfan-I and II, Endrin, Dieldrin, Aldrin, and DDT metabolites. At Dayer El-Berka farm, residues of Endosulfan-I and II, DDT derivatives, Aldrin, and fungicides such as Pencycuron and Diniconazole were detected, along with the pyrethroid Fenvalerate (Table 8).

Cumulative pesticide burden (Σ pesticides)

The cumulative pesticide burden (Σ pesticides) in gill, liver, and muscle tissues of both *O. niloticus* and *M. cephalus* differed markedly among the four farms ($P \leq 0.05$). The highest Σ pesticide levels were observed at El-Bats

Table 3 Pesticides in *O. niloticus* gills (mg/kg wet weight)

Pesticides	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Organochlorine Insecticides				
Aldrin	ND	ND	ND	ND
Chlordane	0.004 ± 0.001	0.043 ± 0.002	0.017 ± 0.001	0.010 ± 0.003
Dieldrin	ND	ND	ND	0.023 ± 0.003
Endosulfan-I	0.005 ± 0.001	0.034 ± 0.002	0.029 ± 0.001	0.026 ± 0.001
Endosulfan-II	ND	ND	ND	ND
Endrin	ND	0.023 ± 0.003	0.125 ± 0.019	ND
α-HCH	ND	ND	ND	ND
β-HCH	ND	ND	ND	ND
δ-HCH	ND	0.021 ± 0.002	ND	ND
γ-HCH	ND	ND	0.035 ± 0.004	0.064 ± 0.002
Heptachlor	ND	ND	0.080 ± 0.003	ND
o,p′-DDT	ND	0.290 ± 0.001	0.009 ± 0.002	0.017 ± 0.001
p,p′-DDD	ND	ND	ND	ND
p,p′-DDE	ND	ND	ND	ND
p,p′-DDT	ND	0.059 ± 0.001	0.004 ± 0.002	ND
Fungicides				
Diniconazole	ND	ND	0.048 ± 0.002	ND
Pencycuron	ND	ND	ND	ND
Thiram	ND	ND	ND	ND
Herbicides				
Atrazine	ND	ND	ND	ND
Butachlor	ND	0.349 ± 0.019	ND	0.630 ± 0.003
Pyrethroid Insecticides				
Deltamethrin	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	ND

Values are presented as mean ± standard deviation. ND = not detected (below the limit of detection, LOD = 0.001 mg/kg)

Table 4 Pesticides in *M. cephalus* gills (mg/kg wet weight)

Pesticides	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Organochlorine Insecticides				
Aldrin	0.004 ± 0.001	ND	0.018 ± 0.001	0.015 ± 0.001
Chlordane	0.006 ± 0.001	0.052 ± 0.002	0.020 ± 0.003	0.014 ± 0.002
Dieldrin	0.005 ± 0.001	0.018 ± 0.001	ND	0.019 ± 0.003
Endosulfan-I	0.007 ± 0.002	0.032 ± 0.001	0.020 ± 0.003	0.024 ± 0.003
Endosulfan-II	ND	ND	ND	ND
Endrin	ND	ND	0.035 ± 0.001	ND
α-HCH	ND	ND	ND	ND
β-HCH	ND	ND	ND	ND
δ-HCH	ND	ND	ND	ND
γ-HCH	ND	0.059 ± 0.002	0.029 ± 0.005	ND
Heptachlor	ND	ND	ND	ND
o,p′-DDT	ND	0.725 ± 0.015	0.019 ± 0.001	0.017 ± 0.001
p,p′-DDD	ND	ND	ND	ND
p,p′-DDE	ND	ND	ND	ND
p,p′-DDT	ND	0.085 ± 0.002	0.027 ± 0.003	ND
Fungicides				
Diniconazole	ND	ND	ND	0.553 ± 0.042
Pencycuron	ND	ND	ND	ND
Thiram	ND	ND	ND	ND
Herbicides				
Atrazine	ND	0.045 ± 0.002	ND	0.209 ± 0.009
Butachlor	ND	0.954 ± 0.006	ND	0.524 ± 0.018
Pyrethroid insecticides				
Deltamethrin	ND	ND	ND	ND
Fenvalerate	ND	ND	0.078 ± 0.002	ND

Values are presented as mean ± standard deviation. ND = not detected (below the limit of detection, LOD = 0.001 mg/kg)

and Dayer El-Berka, while the control farm exhibited the lowest overall contamination. In gill tissue, *M. cephalus* accumulated significantly more pesticides than *O. niloticus* at both El-Bats and Dayer El-Berka ($P \leq 0.05$), but significantly fewer at El-Wadi ($P \leq 0.05$); no interspecies difference was detected in the control farm ($P > 0.05$). In the liver, *M. cephalus* showed a significantly higher Σ pesticide than *O. niloticus* at the control and El-Wadi farms ($P \leq 0.05$) but a significantly lower Σ pesticide burden at El-Bats and Dayer El-Berka ($P \leq 0.05$). Muscle tissues, by contrast, revealed no significant difference in Σ pesticide between the two species at any site ($P > 0.05$) (Table 9).

Organ-specific and species-specific distribution of pesticide residues

The distribution of pesticide groups in the organs of the two fish species followed distinct orders. In *O. niloticus*, the order of pesticide group contributions was as follows: gills, herbicides > organochlorine insecticides > fungicides; liver, herbicides > pyrethroid insecticides > organochlorine insecticides > fungicides; muscles, organochlorine insecticides > herbicides > fungicides. In *M. cephalus*, the order was: gills, herbicides > organochlorine insecticides > fungicides > pyrethroid insecticides; liver, organochlorine insecticides > herbicides > pyrethroid insecticides > fungicides; muscles, organochlorine insecticides > pyrethroid insecticides > fungicides > herbicides (Fig. 2). Furthermore, the total pesticide accumulation (Σ pesticides) in the organs exhibited species-specific patterns. In *O. niloticus*, pesticide accumulation followed the order: liver > gills > muscles, while in *M. cephalus*, the order was: gills > muscles > liver (Fig. 3).

Metabolic changes

Table 10 displayed the biochemical findings for O. niloticus and M. cephalus. When compared to fish from the control farm, fish from the contaminated farms showed a substantial rise ($P < 0.05$) in muscle triglycerides, cholesterol, and total lipids, along with

Table 5 Pesticides in *O. niloticus* liver (mg/kg wet weight)

Pesticides	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Organochlorine Insecticides				
Aldrin	ND	ND	ND	ND
Chlordane	ND	ND	ND	ND
Dieldrin	ND	ND	0.029 ± 0.004	0.048 ± 0.002
Endosulfan-I	0.014 ± 0.002	0.058 ± 0.006	ND	0.034 ± 0.001
Endosulfan-II	0.010 ± 0.003	0.006 ± 0.004	0.024 ± 0.003	0.040 ± 0.001
Endrin	ND	ND	ND	ND
α-HCH	ND	0.019 ± 0.002	0.053 ± 0.003	ND
β-HCH	ND	ND	ND	ND
δ-HCH	ND	0.020 ± 0.003	ND	ND
γ-HCH	ND	0.059 ± 0.009	ND	ND
Heptachlor	ND	ND	0.010 ± 0.003	ND
o,p′-DDT	ND	0.006 ± 0.003	0.006 ± 0.001	ND
p,p′-DDD	0.009 ± 0.001	0.058 ± 0.001	0.037 ± 0.002	ND
p,p′-DDE	ND	ND	ND	ND
p,p′-DDT	ND	0.004 ± 0.001	ND	ND
Fungicides				
Diniconazole	ND	ND	ND	ND
Pencycuron	ND	0.153 ± 0.018	0.025 ± 0.004	ND
Thiram	ND	ND	ND	ND
Herbicides				
Atrazine	ND	0.029 ± 0.001	ND	ND
Butachlor	ND	1.392 ± 0.047	ND	ND
Pyrethroid insecticides				
Deltamethrin	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	0.585 ± 0.003

Values are presented as mean ± standard deviation. ND = not detected (below the limit of detection, LOD = 0.001 mg/kg)

Table 6 Pesticides in *M. cephalus* liver (mg/kg wet weight)

Pesticides	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Organochlorine Insecticides				
Aldrin	0.020 ± 0.001	0.071 ± 0.004	0.043 ± 0.001	ND
Chlordane	ND	ND	ND	ND
Dieldrin	0.016 ± 0.001	0.024 ± 0.002	0.023 ± 0.001	ND
Endosulfan-I	0.015 ± 0.002	0.076 ± 0.001	0.028 ± 0.001	0.035 ± 0.001
Endosulfan-II	0.008 ± 0.001	0.014 ± 0.003	0.022 ± 0.001	0.029 ± 0.001
Endrin	ND	0.012 ± 0.002	0.017 ± 0.001	ND
α-HCH	ND	0.059 ± 0.001	0.081 ± 0.003	ND
β-HCH	ND	ND	ND	ND
δ-HCH	ND	ND	ND	ND
γ-HCH	ND	0.034 ± 0.003	ND	ND
Heptachlor	ND	ND	ND	ND
o,p′-DDT	ND	0.005 ± 0.003	ND	ND
p,p′-DDD	0.014 ± 0.004	0.059 ± 0.004	ND	ND
p,p′-DDE	ND	ND	ND	ND
p,p′-DDT	ND	0.014 ± 0.002	0.015 ± 0.002	ND
Fungicides				
Diniconazole	ND	ND	ND	ND
Pencycuron	ND	ND	0.027 ± 0.001	ND
Thiram	ND	ND	ND	ND
Herbicides				
Atrazine	ND	ND	ND	0.205 ± 0.002
Butachlor	ND	0.089 ± 0.002	0.054 ± 0.002	0.086 ± 0.003
Pyrethroid Insecticides				
Deltamethrin	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	0.046 ± 0.004

Values are presented as mean ± standard deviation. ND = not detected (below the limit of detection, LOD = 0.001 mg/kg)

a significant decrease ($P \leq 0.05$) in liver AST and ALT activity. A t-test comparison between the two species revealed significant differences ($P \leq 0.05$) in liver AST activity at El-Bats, El-Wadi, and Dayer El-Berka farms; liver ALT activity at El-Bats and El-Wadi farms; muscle triglycerides across all four farms; and muscle cholesterol only at Dayer El-Berka farm. In contrast, muscle total lipid levels showed no significant changes ($P > 0.05$) between *O. niloticus* and *M. cephalus* across the farms under investigation.

Stress-related responses

In *O. niloticus* and *M. cephalus* gathered from polluted farms, levels of the oxidative damage marker malondialdehyde (MDA) and the ER stress-related marker caspase-12 were significantly elevated ($P \leq 0.05$) in gill, liver, and muscle tissues compared with fish from the control farm. Conversely, the antioxidant enzyme catalase showed a significant decline ($P \leq 0.05$) under the same conditions. A t-test comparison between the two species indicated no significant differences ($P > 0.05$) in their responses across the studied farms (Table 11).

Health risk assessment

Comparison of pesticide residues in muscles with international maximum permissible residue limits (MRLs) revealed that Σ Endosulfan concentrations exceeded the MRLs in the muscle tissues of *O. niloticus and M. cephalus* from the El-Bats, El-Wadi, and Dayer El-Berka farms. The concentration of α-HCH exceeded the MRL in *M. cephalus* from the El-Bats farm. δ-HCH in *O. niloticus* exceeded the MRL only at the El-Bats and Dayer El-Berka farms, while in *M. cephalus* exceeded the MRL at the El-Bats and El-Wadi farms. Atrazine concentrations surpassed the MRLs in *O. niloticus* from El-Bats, El-Wadi, and Dayer El-Berka. Thiram residues were above the MRLs in *O. niloticus* muscles collected from El-Wadi. Furthermore, Pencycuron, Diniconazole, and Fenvalerate

Table 7 Pesticides in *O. niloticus* muscles (mg/kg wet weight)

Pesticides	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Organochlorine insecticides				
Aldrin	ND	ND	ND	ND
Chlordane	ND	ND	0.015 ± 0.002	ND
Dieldrin	0.003 ± 0.001	0.042 ± 0.003	0.017 ± 0.002	ND
Endosulfan-I	0.004 ± 0.002	0.059 ± 0.002	0.037 ± 0.002	0.049 ± 0.002
Endosulfan-II	0.005 ± 0.002	0.082 ± 0.002	0.033 ± 0.002	0.082 ± 0.001
Endrin	ND	ND	ND	0.018 ± 0.001
α-HCH	ND	0.010 ± 0.001	ND	ND
β-HCH	ND	ND	ND	ND
δ-HCH	ND	0.027 ± 0.003	0.013 ± 0.003	0.029 ± 0.002
γ-HCH	ND	0.058 ± 0.004	0.083 ± 0.001	0.028 ± 0.001
Heptachlor	ND	0.026 ± 0.001	0.102 ± 0.002	0.081 ± 0.001
o,p′-DDT	ND	ND	ND	ND
p,p′-DDD	0.006 ± 0.001	0.087 ± 0.001	0.034 ± 0.004	0.057 ± 0.001
p,p′-DDE	0.006 ± 0.001	0.018 ± 0.001	0.013 ± 0.004	0.021 ± 0.002
p,p′-DDT	ND	0.017 ± 0.002	ND	0.009 ± 0.002
Fungicides				
Diniconazole	ND	ND	ND	ND
Pencycuron	ND	ND	ND	ND
Thiram	ND	ND	0.043 ± 0.002	ND
Herbicides				
Atrazine	ND	0.015 ± 0.002	0.033 ± 0.001	0.095 ± 0.004
Butachlor	ND	ND	ND	ND
Pyrethroid insecticides				
Deltamethrin	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	ND

Values are presented as mean ± standard deviation. ND = not detected (below the limit of detection, LOD = 0.001 mg/kg)

Table 8 Pesticides in *M. cephalus* muscles (mg/kg wet weight)

Pesticides	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Organochlorine insecticides				
Aldrin	ND	ND	0.041 ± 0.003	0.038 ± 0.001
Chlordane	ND	0.041 ± 0.002	ND	ND
Dieldrin	ND	ND	0.054 ± 0.001	ND
Endosulfan-I	0.004 ± 0.001	0.084 ± 0.003	0.030 ± 0.001	0.050 ± 0.003
Endosulfan-II	0.005 ± 0.001	0.087 ± 0.003	0.035 ± 0.001	0.074 ± 0.002
Endrin	ND	ND	0.07 ± 0.001	ND
α-HCH	ND	0.047 ± 0.002	ND	ND
β-HCH	ND	ND	ND	ND
δ-HCH	ND	0.029 ± 0.001	0.015 ± 0.002	ND
γ-HCH	ND	ND	ND	ND
Heptachlor	ND	ND	ND	ND
o,p′-DDT	ND	ND	ND	ND
p,p′-DDD	0.005 ± 0.002	0.072 ± 0.001	0.036 ± 0.001	0.066 ± 0.001
p,p′-DDE	0.006 ± 0.001	0.021 ± 0.002	0.033 ± 0.002	0.024 ± 0.004
p,p′-DDT	ND	0.017 ± 0.001	ND	0.008 ± 0.002
Fungicides				
Diniconazole	ND	ND	ND	0.047 ± 0.006
Pencycuron	ND	ND	ND	0.095 ± 0.004
Thiram	ND	ND	ND	ND
Herbicides				
Atrazine	ND	0.012 ± 0.001	ND	ND
Butachlor	ND	ND	ND	ND
Pyrethroid insecticides				
Deltamethrin	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	0.158 ± 0.005

Values are presented as mean ± standard deviation. ND = not detected (below the limit of detection, LOD = 0.001 mg/kg)

concentrations exceeded the MRLs in *M. cephalus* from the Dayer El-Berka farm (Fig. 4).

Table (12) summarizes the assessment of the health risks associated with feeding muscle tissue from *O. niloticus* and *M. cephalus* that contains pesticides.For all studied pesticides in both *O. niloticus* and *M. cephalus* from the investigated fish farms, the EDIs were lower than the ADIs, and thus the calculated HI values for muscle consumption were < 1, indicating no significant health risk from individual pesticide exposure. Also, the cumulative risk assessment in all the studied farms indicated no potential unacceptable health risks (ΣHI < 1) for *O. niloticus and M. cephalus* consumers from these farms.

Discussion

The physicochemical properties of water have a major impact on fish biology and physiology [65]. The present investigation found that the water quality of the various fish farms under investigation varied significantly depending on the water resources used for irrigation. Due to the shallowness of most tropical fishponds, location variances were the primary cause of the observed changes in water temperature. These results aligned with those of Ali et al. [66]. The observed rise in pH at El-Bats Fish Farm could be the result of increased phytoplankton photosynthetic activity. This could be explained by the large fluctuation in phytoplankton between fish ponds and feeder/drains, where a variety of biotic and abiotic processes interact to cause surface water pH changes [34, 66]. The highest value of dissolved oxygen in the control fish farm may be attributed to the actual source of irrigation, virtual mixing and water current of the river Nile branch that fed this fish farm [67]. However, according to Hamdy et al. [68] & Zaghloul et al. [69], the reduction in dissolved oxygen in water samples taken from El-Bats fish farm may be caused by oxygen depletion from the oxidation of significant amounts of organic compounds from agricultural wastes. As previously reported by Zaghloul

Table 9 Σ pesticides (mg/kg wet weight)

Organs	Species	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Gills	*O. niloticus*	0.009 ± 0.003^{c}	0.817 ± 0.030^{a}	0.291 ± 0.083^{b}	0.769 ± 0.013^{a}
	M. cephalus	0.022 ± 0.006^{d}	$1.968 \pm 0.033^{a*}$	$0.244 \pm 0.017^{c*}$	$1.368 \pm 0.032^{b*}$
Liver	*O. niloticus*	0.032 ± 0.004^{d}	1.802 ± 0.095^{a}	0.183 ± 0.005^{c}	0.706 ± 0.006^{b}
	M. cephalus	$0.072 \pm 0.009^{d*}$	$0.456 \pm 0.021^{a*}$	$0.309 \pm 0.012^{c*}$	$0.399 \pm 0.011^{b*}$
Muscles	*O. niloticus*	0.023 ± 0.006^{b}	0.439 ± 0.023^{a}	0.419 ± 0.026^{a}	0.469 ± 0.018^{a}
	M. cephalus	0.019 ± 0.006^{d}	0.406 ± 0.018^{b}	0.323 ± 0.013^{c}	0.558 ± 0.028^{a}

Significant differences (ANOVA-test; $p \leq 0.05$) within the same species across different farms are indicated in superscript letters (a,b,c). For a given parameter, values that share the same letter do not differ significantly. Significant differences (T-test; $p \leq 0.05$) across species at the same farm are indicated by an asterisk (*)

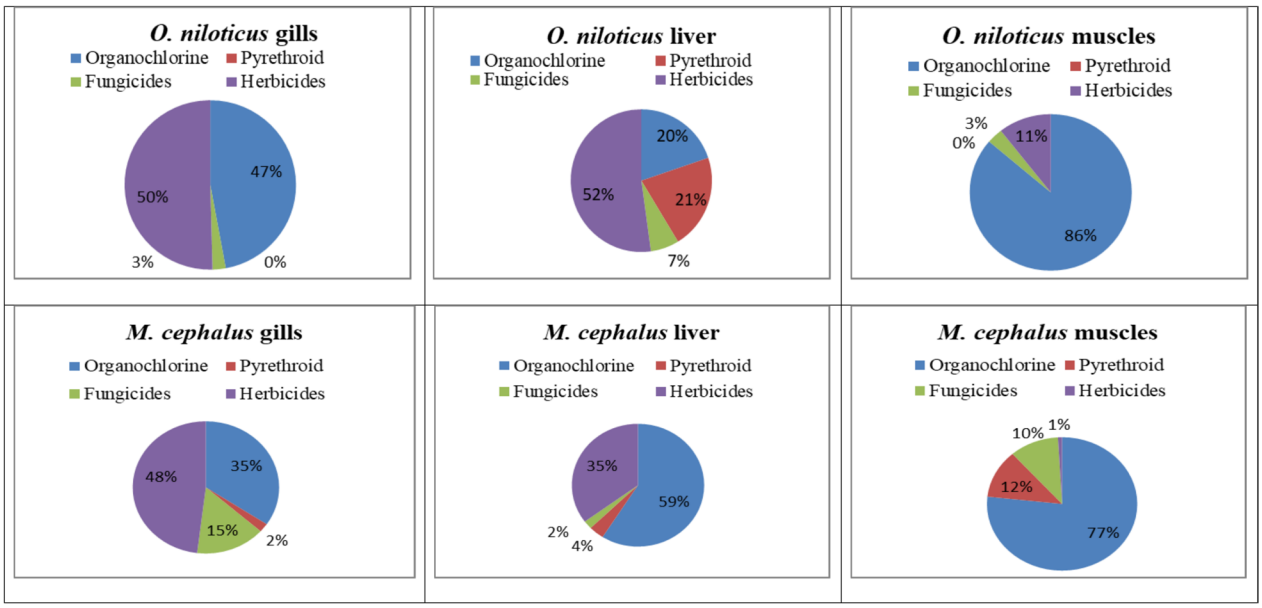

Fig. 2 The distribution percentages of pesticide groups in the organs of the two examined fish species

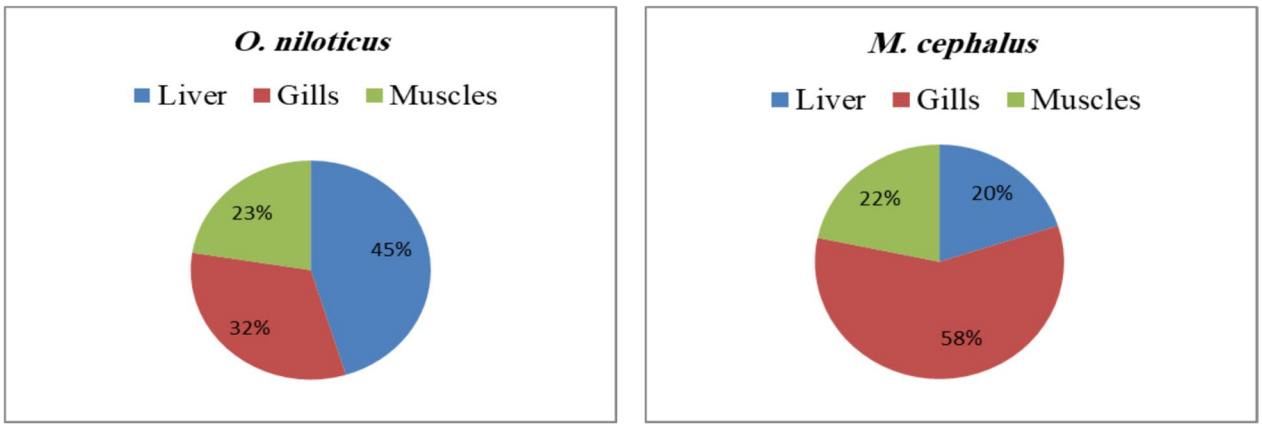

Fig. 3 The distribution percentages of Σ pesticides between organs for each fish species

Table 10 Biochemical markers in *O. niloticus* and *M. cephalus* from the studied farms

Biomarker	Species	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
Liver					
AST, U/L	*O. niloticus*	243.00 ± 4.24^{a}	181.00 ± 2.12^{b}	188.00 ± 4.24^{b}	144.00 ± 6.36^{c}
	M. cephalus	254.00 ± 2.83^{a}	$152.00 \pm 4.98^{c*}$	$149.00 \pm 10.61^{c*}$	$176.00 \pm 6.35^{b*}$
ALT, U/L	*O. niloticus*	120.00 ± 7.75^{a}	69.00 ± 4.25^{bc}	79.00 ± 2.13^{b}	62.00 ± 4.93^{c}
	M. cephalus	130.00 ± 3.53^{a}	$91.00 \pm 4.92^{bc*}$	$104.00 \pm 6.38^{b*}$	83.00 ± 6.34^{c}
Muscle					
Triglyceride, mg/dL	*O. niloticus*	138.00 ± 4.25^{c}	326.00 ± 9.19^{a}	255.00 ± 7.79^{b}	335.00 ± 7.99^{a}
	M. cephalus	$116.00 \pm 5.66^{d*}$	$258.00 \pm 11.31^{b*}$	$317.00 \pm 10.61^{a*}$	$170.00 \pm 14.85^{c*}$
Cholesterol, mg/dL	*O. niloticus*	2.81 ± 0.77^{c}	7.95 ± 0.62^{ab}	9.12 ± 0.84^{a}	6.39 ± 0.62^{b}
	M. cephalus	4.22 ± 0.09^{c}	6.21 ± 0.87^{b}	10.94 ± 0.77^{a}	$9.99 \pm 0.66^{a*}$
Total lipid, g/dL	*O. niloticus*	1.45 ± 0.33^{c}	4.67 ± 0.35^{a}	3.71 ± 0.26^{b}	5.49 ± 0.25^{a}
	M. cephalus	1.60 ± 0.38^{c}	4.89 ± 0.48^{ab}	3.84 ± 0.39^{b}	5.94 ± 0.30^{a}

Significant differences (ANOVA-test; $p \leq 0.05$) within the same species across different farms are indicated in superscript letters (a,b,c). For a given parameter, values that share the same letter do not differ significantly. Significant differences (T-test; $p \leq 0.05$) across species at the same farm are indicated by an asterisk (*)

Table 11 Stress biomarkers in *O. niloticus* and *M. cephalus* from the studied farms

Bio-marker	Organs	Species	Control farm	El-Bats farm	El-Wadi farm	Dayer El-Berka farm
MDA (nmol/g)	Gills	*O. niloticus*	314.00 ± 4.93^{d}	805.00 ± 21.21^{b}	741.00 ± 12.02^{c}	856.00 ± 5.66^{a}
		M. cephalus	320.00 ± 6.36^{d}	807.00 ± 17.68^{b}	755.00 ± 14.85^{c}	866.00 ± 7.77^{a}
	Liver	*O. niloticus*	372.00 ± 8.49^{d}	639.00 ± 4.95^{b}	557.00 ± 10.61^{c}	667.00 ± 6.36^{a}
		M. cephalus	376.00 ± 7.78^{d}	647.00 ± 7.78^{b}	563.00 ± 11.31^{c}	674.00 ± 7.07^{a}
	Muscle	*O. niloticus*	191.00 ± 4.97^{d}	359.00 ± 13.44^{b}	269.00 ± 3.54^{c}	382.00 ± 5.65^{a}
		M. cephalus	200.00 ± 9.89^{c}	368.00 ± 4.99^{a}	279.00 ± 8.49^{b}	387.00 ± 8.47^{a}
CAT (U/g)	Gills	*O. niloticus*	58.00 ± 3.54^{a}	41.00 ± 2.11^{bc}	46.00 ± 1.41^{b}	38.00 ± 2.12^{c}
		M. cephalus	61.00 ± 4.95^{a}	42.00 ± 2.12^{b}	47.00 ± 2.11^{b}	40.00 ± 2.10^{b}
	Liver	*O. niloticus*	59.00 ± 5.66^{a}	33.00 ± 3.54^{bc}	44.00 ± 4.95^{b}	26.00 ± 2.12^{c}
		M. cephalus	61.00 ± 4.94^{a}	36.00 ± 4.24^{bc}	46.00 ± 4.24^{b}	29.00 ± 2.11^{c}
	Muscle	*O. niloticus*	31.00 ± 1.42^{a}	14.00 ± 2.83^{b}	24.00 ± 4.92^{a}	11.00 ± 2.15^{b}
		M. cephalus	34.00 ± 2.10^{a}	18.00 ± 4.24^{bc}	27.00 ± 6.36^{ab}	13.00 ± 1.40^{c}
Caspase-12 (ng/g)	Gills	*O. niloticus*	8.53 ± 1.44^{c}	31.50 ± 2.12^{a}	23.50 ± 3.54^{b}	30.00 ± 1.41^{a}
		M. cephalus	9.50 ± 1.58^{b}	33.00 ± 2.83^{a}	26.00 ± 4.24^{a}	34.00 ± 2.83^{a}
	Liver	*O. niloticus*	16.50 ± 2.12^{c}	48.00 ± 4.24^{a}	38.00 ± 1.41^{b}	46.00 ± 2.83^{a}
		M. cephalus	18.00 ± 2.83^{c}	50.50 ± 3.54^{a}	40.50 ± 2.12^{b}	49.50 ± 2.12^{a}
	Muscle	*O. niloticus*	1.58 ± 0.06^{c}	9.89 ± 0.47^{a}	8.07 ± 0.78^{b}	9.10 ± 0.82^{ab}
		M. cephalus	1.79 ± 0.15^{c}	10.81 ± 0.81^{a}	8.34 ± 0.64^{b}	9.46 ± 1.10^{ab}

Significant differences (ANOVA-test; $p \leq 0.05$) within the same species across different farms are indicated insuperscript letters (a,b,c). For a given parameter, values that share the same letter do not differ significantly

et al. [70], the highest concentrations of salinity, ammonia, nitrite, and nitrate found in the Dayer El-Berka fish farm could be attributed to the quality of the agricultural drainage water that supplies the farm, which is brackish water rich in chemicals and fertilizers. Large amounts of algae can bloom when nitrate and nitrite concentrations are high. According to this study, dead algae can lead to oxygen depletion issues, which can kill fish and other aquatic life [71]. Water transparency shows an increased link with plankton abundance because Secchi disc visibility measures water turbidity, which is typically caused by suspended soil particles and/or plankton abundance [72]. According to the current findings, the El-Bats fish farm had poorer visibility, which might be attributed to a higher phytoplankton accumulation. In contrast, the control farm had higher

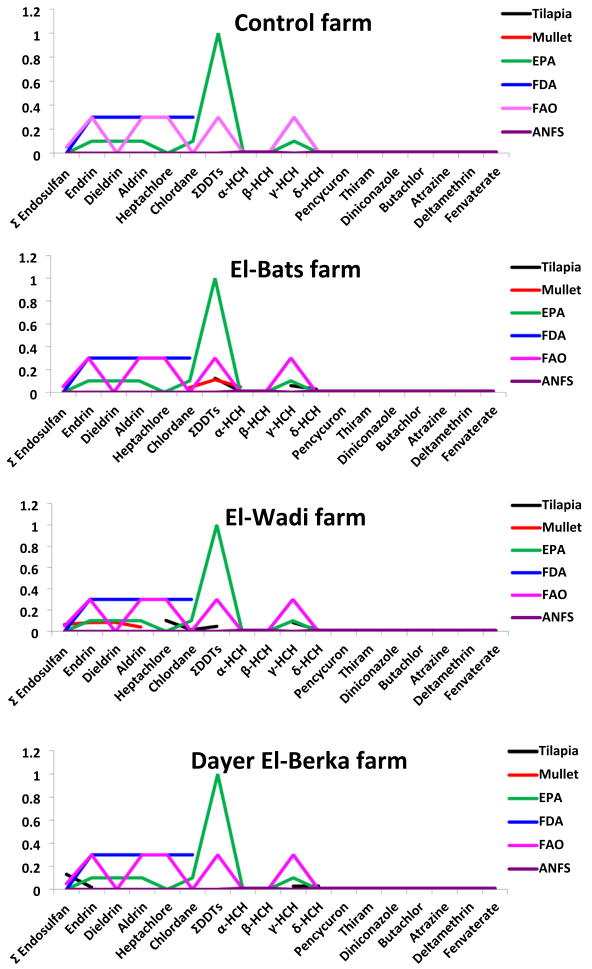

Fig. 4 Comparison of pesticide concentrations in muscles of *O. niloticus* and *M. cephalus* from the studied farms with maximum residue level (MRL) of different standards

transparency because of a lower phytoplankton accumulation. This is consistent with what Soltan et al. [73] found.

Although pesticides have been employed to control a range of pests and disease vectors, their careless use poses serious problems for the aquatic ecosystem [74]. According to the overall trend of the results, nearly all of the water and fish samples from El-Bats and Dayer El-Berka farms were much more contaminated with pesticide residues than those from other farms. This may be attributed to the source of water for these farms (agricultural drainage water). Similarly, previous studies detected pesticides in water and fish of fish farms at El-Fayoum Governorate [35, 47, 75]. Because aquatic organisms like fish can bioaccumulate pesticide residue concentrations several times higher than the surrounding water, pesticide residues were detected in the tissues of the fish under study but not in the water [76]. This observation is consistent with the findings of Elbialy et al. [77] & Mohamed and Gad [47]. Moreover, the differences in the accumulation of pesticides in gills and liver between *O. niloticus* and *M. cephalus* may be due to the differences in metabolism and their ability to excrete the compounds [35].

The observed distribution patterns of pesticide groups in the organs of *O. niloticus* and *M. cephalus* reflect both species-specific and tissue-specific differences in pesticide accumulation. In *O. niloticus*, herbicides were predominant in the gills and liver, suggesting a higher affinity or exposure of this species to herbicide residues in the aquatic habitat. Organochlorine insecticides' dominance in muscle tissue may be related to the muscle's elevated lipid content, as organochlorine pesticides are lipophilic in nature and prefer to concentrate in tissues with a high fat level and low turnover rates [78, 79]. In contrast, *M. cephalus* exhibited a different accumulation profile. Organochlorine insecticides were the most prevalent group in both liver and muscle tissues, while herbicides dominated in the gills. Notably, pyrethroid insecticides contributed significantly to the liver and muscle burden in *M. cephalus*, unlike in *O. niloticus*, where they were more prominent only in the liver. These variations could be linked to habitat preference, metabolic capacity, and feeding habits unique to each species [80]. *M. cephalus*, being a benthic feeder [40], may have more frequent contact with sediment-associated pesticides, especially persistent insecticides such as organochlorines and pyrethroids.

The total organ pesticide accumulation (Σ pesticides) also varied between species. In *O. niloticus*, the liver showed the highest pesticide burden, followed by gills and muscles, consistent with the liver's central role in xenobiotic metabolism [81]. In *M. cephalus*, however, the gills accumulated the highest levels of pesticides, possibly due to greater filtration rates or longer exposure to waterborne contaminants [82]. These results emphasize how crucial it is to take species and tissue type into account when assessing bioaccumulation patterns and possible health hazards related to pesticide exposure in aquatic organisms.

When evaluating target organ toxicity and the general physiological state of organisms, biochemical markers are useful indications. They may also serve as early warning markers of stress in aquatic species [83]. Fish exposed to sub-lethal amounts of synthetic pesticides frequently exhibit changes in enzyme activity [84]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), two major enzymes involved in amino acid metabolism that are largely found in the liver [22], are frequently utilized as indicators of hepatic function. In the current investigation, the reduced AST and ALT activities in

Table 12 Acceptable daily intake (ADI), Estimated daily intake (EDI), Hazard index (HI), and the cumulative risk effect (ΣHI) for *O. niloticus* and *M. cephalus* consumers

Pesticides	ADI (mg/kg B.W)	Control Farm		El-Bats Farm		El-Wadi Farm		Dayer El-Berka Farm	
		EDI (mg/kg bw/day)	HI (EDI/ADI)	EDI (mg/kg bw/day)	HI (EDI/ADI)	EDI (mg/kg bw/day)	HI (EDI/ADI)	EDI (mg/kg bw/day)	HI (EDI/ADI)
O. niloticus									
Σ Endosulfan	0.006	0.00007	0.0012	0.0001	0.0166	0.00005	0.0083	0.00009	0.015
Endrin	0.0002	ND	–	ND	–	ND	–	0.00001	0.05
Dieldrin + Aldrin	0.0001	0.000002	0.02	0.00003	0.30	0.00001	0.10	ND	–
Heptachlor	0.0005	ND	–	0.00002	0.04	0.00007	0.14	0.00006	0.12
Chlordane	0.001	ND	–	ND	–	0.00001	0.01	ND	–
Σ DDTs	0.02	0.000009	0.0005	0.00009	0.0045	0.00003	0.0015	0.00006	0.003
Σ HCH	0.005	ND	–	0.00007	0.014	0.00007	0.014	0.00004	0.008
Deltamethrin	0.01	ND	–	ND	–	ND	–	ND	–
Fenvalerate	0.02	ND	–	ND	–	ND	–	ND	–
Pencycuron	0.2	ND	–	ND	–	ND	–	ND	–
Thiram	0.01	ND	–	ND	–	0.00003	0.003	ND	–
Diniconazole	0.02	ND	–	ND	–	ND	–	ND	–
Butachlor	0.01	ND	–	ND	–	ND	–	ND	–
Atrazine	0.02	ND	–	0.00001	0.0005	0.00002	0.001	0.00007	0.0035
ΣHI			0.022		0.114		0.278		0.200
M. cephalus									
Σ Endosulfan	0.006	0.000006	0.0017	0.0001	0.0166	0.00005	0.0083	0.00009	0.015
Endrin	0.0002	ND	–	ND	–	0.00006	0.253	ND	–
Dieldrin + Aldrin	0.0001	ND	–	ND	–	0.00009	0.687	0.00003	0.30
Heptachlor	0.0005	ND	–	ND	–	ND	–	ND	–
Chlordane	0.001	ND	–	0.00003	0.03	ND	–	ND	–
Σ DDTs	0.02	0.000008	0.0004	0.00008	0.004	0.00005	0.0025	0.00007	0.0035
Σ HCH	0.005	ND	–	0.00005	0.01	0.00001	0.002	ND	–
Deltamethrin	0.01	ND	–	ND	–	ND	–	ND	–
Fenvalerate	0.02	ND	–	ND	–	ND	–	0.0001	0.005
Pencycuron	0.2	ND	–	ND	–	ND	–	0.00007	0.0004
Thiram	0.01	ND	–	ND	–	ND	–	ND	–
Diniconazole	0.02	ND	–	ND	–	ND	–	0.00003	0.0015
Butachlor	0.01	ND	–	ND	–	ND	–	ND	–
Atrazine	0.02	ND	–	0.000008	0.0005	ND	–	ND	–
ΣHI			0.002		0.061		0.942		0.325

ADI: acceptable daily intake (mg/kg bw/day), established by WHO/FAO [63]; values for pencycuron and diniconazole were obtained from EFSA [64]. *EDI* estimated daily intake, *HI* hazard index (HI > 1 indicates potential health risk). ND: not detected. Σ Endosulfan = endosulfan I + II; Σ DDTs = p,p′-DDE + p,p′-DDD + p,p′-DDT + o,p′-DDT; Σ HCHs = α-, β-, γ-, and δ-HCH

the liver of *O. niloticus* and *M. cephalus* collected from pesticide-polluted farms suggest impaired transamination and oxidative deamination processes [85], as well as inhibition of intermediary metabolism [86]. Such reductions may also result from subcellular alterations that disrupt enzyme activity [87]. Previous studies have reported similar findings, where organophosphorus pesticide exposure led to a significant decrease in hepatic aminotransferase activity, attributed to hepatocellular damage and the inability of compromised cells to synthesize these enzymes [21, 88]. Likewise, when Cyprinus carpio were exposed to carbamazepine, Malarvizhi et al. [89] found lower levels of AST and ALT in the gill, liver, and muscle, suggesting that detoxification processes were not strong enough to offset the drug's harmful effects. Overall, the decline in metabolic enzyme activities under pesticide exposure suggests a direct inhibitory action of these compounds on enzyme systems [90]. In addition,

the significant differences in liver AST and ALT activities between *O. niloticus* and *M. cephalus* across several farms indicate differential hepatocellular stress.

Lipids play a vital role in animal metabolism, serving as a major energy source and as essential components of cell structure. They are stored as metabolites and mobilized to provide energy when organisms face adverse environmental conditions [91]. Total lipid content is composed of cholesterol, phospholipids, and triglycerides; thus, any alteration in the concentration of these components leads to changes in total lipid levels [92]. In the present study, triglycerides, total cholesterol, and total lipids in the muscle tissues of *O. niloticus* and *M. cephalus* were markedly sensitive to pesticide contamination. Their levels increased significantly ($p \leq 0.05$) in fish from El-Bats, El-Wadi, and Dayer El-Berka farms compared with those from the control farm. This elevation could be related to improved lipid production and/or decreased lipid catabolism. Furthermore, the observed rise in lipid content might indicate that organisms require more energy reserves, such as cholesterol, glucose, and triglycerides, to deal with stress [93]. The elevated triglyceride concentrations observed here could be linked to reduced activity of lipoprotein lipase, the enzyme responsible for triglyceride breakdown [94]. In addition, pesticide-induced stimulation of cholesterol production in the liver and other tissues, release of cholesterol from damaged cell membranes, decreased hepatic excretion, thyroid dysfunction, or inhibition of cholesterol conversion to sex steroids due to gonadal impairment and decreased activity of cytochrome P450 enzymes can all contribute to an increase in cholesterol levels [94]. Similar increases in triglycerides, total cholesterol, and total lipids following pesticide exposure have been documented in many fish species [77, 95–98]. In the present study, the significant interspecific differences observed in triglyceride content across all farms and in cholesterol levels at Dayer El-Berka, suggest that lipid metabolism in muscle is differentially affected in the two species, likely reflecting differences in energy mobilization and membrane lipid composition under pesticide stress. The absence of differences in total lipid levels between the species suggests that while the qualitative composition of lipid fractions is altered, the overall lipid content remains relatively stable.

Pesticides have been shown to cause oxidative stress by producing reactive oxygen species (ROS), which damage biological components such as proteins, lipids, and DNA [99]. When fish are exposed to pesticides, disruption of mitochondrial electron transport, which results in electron leakage and superoxide radical production, is one of the main sources of ROS. Furthermore, phase I detoxification enzymes, especially cytochrome P450, create reactive intermediates during pesticide metabolism that exacerbate oxidative stress [100, 101]. Overproduction of ROS leads to lipid peroxidation, which damages polyunsaturated fatty acids (PUFAs) in cellular membranes. This produces lipid radicals and malondialdehyde (MDA), a well-known indicator of oxidative damage [102]. Ion gradients are disturbed, permeability is increased, and membrane integrity is compromised by lipid peroxidation [103]. Particularly in metabolically active regions like the liver and gills, elevated MDA levels in fish exposed to pesticides are a symptom of serious cellular damage, disrupting signaling pathways and possibly causing cell death [104, 105]. In the present study, significantly higher MDA concentrations in the gills, liver, and muscles of *O. niloticus* and *M. cephalus* exposed to pesticide pollution confirm pronounced oxidative damage, consistent with previous reports in pesticide-exposed fish [104, 106, 107].

To guard against oxidative damage, cells have defensive mechanisms that comprise both enzymatic and non-enzymatic antioxidants [19]. ROS and antioxidants are normally in balance in cells, but during pollution stress, the balance between ROS production and scavenging is often shifted in favor of free radical synthesis, which causes cell damage to accumulate [108]. The catalase enzyme, which is found in peroxisomes, shields fish against oxidative stress by triggering the decomposition of hydrogen peroxide into water and oxygen and avoiding its buildup in cells [109]. The significant inhibition of CAT activity observed in the gills, liver, and muscles of pesticide-stressed *O. niloticus* and *M. cephalus* from the polluted El-Wadi, El-Bats, and Dayer El-Berka fish farms, compared to reference fish, could result from an increased flux of superoxide radicals, which have been shown to inhibit CAT function [110], or direct pesticide damage to the enzyme's protein structure [111]. These results are in line with other research that found reduced CAT activity in the tissues of several fish species upon exposure to pesticides [112–114].

The endoplasmic reticulum (ER) is a crucial organ responsible for maintaining intracellular Ca^{2+} homeostasis, as well as for protein synthesis and folding [115]. Cellular homeostasis gets disrupted when unfolded or misfolded proteins build up in the ER lumen during stressful situations. Apoptotic signaling pathways can be triggered by prolonged or severe ER stress, which eventually results in programmed cell death [116]. A central component of apoptosis is the caspase family of cysteine proteases, among which caspase-12, an ER-resident enzyme, is specifically activated by disruptions in calcium balance or excessive protein misfolding [117].

In the current study, the most important finding was the significant induction of caspase-12 activity in the gills, liver, and muscle tissues of pesticide-exposed *O. niloticus* and *M. cephalus* collected from the polluted El-Wadi, El-Bats, and Dayer El-Berka fish farms, compared with reference fish. This observation strongly suggests that exposure to pesticides induces ER stress and activates ER-mediated apoptotic pathways in both species. Similar findings were reported by [118], who showed that exposure to pesticide mixtures (metolachlor, linuron, isoproturon, tebuconazole, aclonifen, atrazine, pendimethalin, and azinphos-methyl) at both 22 °C and 32 °C markedly increased apoptosis and CAS mRNA expression in the kidney tissues of goldfish (*Carassius auratus*). Similarly, Atamanalp et al. [119] stated that rainbow trout's kidney, gill, and liver tissues showed a marked increase in caspase activity following prolonged exposure to cypermethrin. Further supporting evidence comes from previous studies, which shown that certain pesticides, including cyfluthrin, 2,4-dichlorophenol, and chlorpyrifos, induce ER stress and apoptosis and alter the expression of genes associated with apoptosis, such as p53, caspase-12, and caspase-3 [120–122]. Finally, pesticide exposure is known to stimulate excessive ROS production in both intra- and extracellular spaces, leading to biomolecular damage, impaired cellular function, and apoptosis.

Interestingly, despite site- and tissue-specific differences in pesticide burdens and certain biochemical markers, there were no consistent species-specific differences in oxidative stress (MDA and catalase) or ER stress (caspase-12). This suggests that *O. niloticus* and *M. cephalus* share a broadly similar physiological vulnerability to pesticide-induced oxidative and ER stress, likely reflecting conserved stress response pathways among teleosts. The lack of species-specific variation in these key biomarkers strengthens their value as universal indicators of pollutant-induced cellular injury in ecotoxicological assessments.

Consuming fish with high pesticide residues may be harmful to the public's health because humans are one of the main receptors of aquatic pollutants [50]. To mitigate such risks, several international organizations, including the EPA, FDA, and FAO, have set maximum residue limits (MRLs) for pesticides in food items [6]. In Egypt, the National Food Safety Authority [60] applies a default MRL of 0.01 mg/kg for pesticides where specific limits are not defined, representing a more precautionary regulatory approach. In the present study, several pesticide residues in fish muscles exceeded established regulatory limits [56–58, 60]. Σ Endosulfan concentrations in both *O. niloticus* and *M. cephalus* from El-Bats, El-Wadi, and Dayer El-Berka farms exceeded the FAO MRL (0.05 mg/kg) as well as the Egyptian default limit (0.01 mg/kg). Similarly, α-HCH and δ-HCH, which are not assigned international MRLs but are regulated under Egyptian standards (0.01 mg/kg), exceeded permissible levels in multiple sites, particularly in El-Bats and Dayer El-Berka. Moreover, atrazine, thiram, pencycuron, diniconazole, and fenvalerate residues also surpassed the Egyptian default MRL (0.01 mg/kg), indicating concentrations exceeding nationally established guideline values. Since no FAO or FDA limits are specified for these compounds in fish, the exceedance relative to the Egyptian guideline suggests a potential concern that warrants further monitoring and risk assessment.

These results are partially in line with earlier investigations on *O. niloticus*, *M. cephalus*, and *C. gariepinus* from aquaculture systems in El-Fayoum [38, 57], which reported pesticide residues within acceptable limits. However, the higher concentrations observed in this study suggest a potential increase in contamination levels or differences in local environmental conditions and agricultural practices.

To evaluate the possible health concerns, we determined the health index (HI) for individual pesticides. The HI values for all single pesticides were < 1 in both fish species, indicating that consumption of any individual pesticide residue through muscle tissue is unlikely to pose significant health risks [55]. Similar observations have been reported in studies on fish from other aquatic systems, where estimated daily intakes of individual pesticides were lower than the acceptable daily intake (ADI) [7, 123, 124]. Furthermore, cumulative risk assessment revealed that the total HI (ΣHI) for all studied farms remained < 1, suggesting no unacceptable combined health risks for consumers of *O. niloticus* and *M. cephalus* from these aquaculture systems. This highlights that, under current pesticide contamination levels, both species are considered safe for consumption by humans with respect to the evaluated pesticide residues.

Conclusion

This study indicates that fish reared in agricultural drainage-water–fed aquaculture systems in Egypt are exposed to multiple pesticide residues. Both *O. niloticus* and *M. cephalus* showed metabolic alterations and oxidative stress responses, along with elevated levels of caspase-12, suggesting physiological disturbance under contaminated conditions. Pesticide accumulation exhibited clear species- and organ-specific patterns, likely influenced by biological and ecological differences between the two species. Furthermore, the estimated dietary exposure and hazard index values

remained below risk thresholds, indicating that there are no intolerable overall health hazards for consumers of *M. cephalus* and *O. niloticus* from these aquaculture systems. This indicates that both species are considered safe for human dietary intake under existing pesticide contamination levels based on the evaluated pesticide residues.

We recommend future studies integrating biochemical, histopathological, and molecular approaches (e.g., gene expression analysis of ER stress-related markers) to provide direct evidence and a more comprehensive understanding of pesticide-induced toxicity in fish, and enhancing the management of agricultural wastewater used in aquaculture, which is crucial to reducing contaminant inputs and promoting environmental sustainability and food safety.

Abbreviations

O. niloticus	*Oreochromis niloticus*
M. cephalus	*Mugil cephalus*
MMT	Million metric tons
OCPs	Organochlorine pesticides
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ER	Endoplasmic reticulum
NOAELs	No observed adverse effect levels
DO	Dissolved oxygen
GC–MS	Gas chromatography–mass spectrometry
AOAC	Association official analytical chemists
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FAO	Food and Agriculture Organization
MDA	Malondialdehyde
TBARS	Thiobarbituric acid reactive substances
CAT	Catalase
MRLs	Maximum residue limits
EDI	Estimated daily intake
HI	Hazard index
ADI	Acceptable daily intake
ND	Not detected
ROS	Reactive oxygen species
PUFAs	Polyunsaturated fatty acids

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
The fish sampling was conducted with approval from the National Institute of Oceanography and Fisheries (NIOF) committee for ethical care and use of animals/aquatic animals (NIOF-IACUC) according to the certificate number NIOF–FW3–F-25-R-024.

Consent for publication
All authors have reviewed the content of this manuscript and informed consent for its publication.

Competing interests
The authors declare no competing interests.

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