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Linking Ecophysiological Alterations and Pesticide Bioaccumulation in *Tilapia zillii* Inhabiting Wadi El-Rayan Lakes, Egypt

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ABSTRACT

Wadi El-Rayan lake system, a Ramsar-designated wetland is one of the most ecologically and economically important freshwater bodies in Fayoum Governorate, Egypt. This study investigated the seasonal and spatial variations in pesticide bioaccumulation and the associated physiological impacts on *Tilapia zillii* from Wadi El-Rayan lakes, Egypt. Twenty pesticide compounds—including organophosphates, triazoles, carbamates, and pyrethroids—were quantified in fish tissues. Significantly higher concentrations were detected in Lake 1 during winter, with compounds such as terbufos, malathion, and methyl parathion showing notable accumulation. Morphometric traits and relative organ weights indicated severe impairments in growth and organ function, particularly in Lake 2 fish, which exhibited reduced muscle mass along with enlarged gills and livers. The condition factor (K) also varied significantly across lakes and seasons, correlating with levels of contaminant exposure. These findings highlight the ecotoxicological risks posed by agricultural pollutants and emphasize the need for regulatory actions and continuous ecological monitoring.

INTRODUCTION

Freshwater ecosystems in arid regions such as Egypt are increasingly threatened by anthropogenic pressures, particularly agricultural runoff containing a variety of chemical pollutants. Among the most ecologically and economically important freshwater bodies is the Wadi El-Rayan lake system, a Ramsar-designated wetland in the Fayoum Governorate, which has been experiencing significant ecological deterioration due to various forms of pollution, including heavy metals and pesticides **(Noaemy *et al*., 2020; Nassif & Amer, 2022)**.

Several studies have documented the deteriorating water quality and associated risks to aquatic organisms in Wadi El-Rayan. Comparative ecotoxicological research between Lake Qarun and Wadi El-Rayan highlighted the vulnerability of both wetlands to agrochemical contamination, especially through the accumulation of pesticides and heavy metals in key environmental compartments **(Mansour & Sidky, 2002)**. Bio assessment studies utilizing zooplankton and benthic macroinvertebrates have also revealed significant ecological stress within the Wadi El-Rayan system, emphasizing the need for continuous monitoring and ecotoxicological evaluation **(Nassif & Amer, 2022)**. In parallel, fish species inhabiting these ecosystems are widely used as bioindicators due to their sensitivity to environmental changes and their ability to bioaccumulate toxic substances. *Tilapia zillii*, a dominant species in Wadi El-Rayan, serves as a valuable model for assessing pollution impacts. Previous studies have established a strong association between metal contamination and physiological disturbances in *T. zillii*, including oxidative stress, histopathological alterations as well as genotoxic effects **(Abdel-Khalek *et al*., 2020)**.

However, despite the extensive research on heavy metal contamination, the ecophysiological and ecotoxicological effects of pesticide residues on fish in Wadi El-Rayan remain insufficiently explored. This gap is particularly concerning given recent findings that report pesticide bioaccumulation in fish tissues, with possible consequences for both fish health and human consumption **(IJAR, 2016)**. Toxicity represents one of the most immediate and visible environmental impacts of pesticides. Different classes of pesticides exhibit varying mechanisms of action and toxicity profiles, with organochlorines, organophosphates, carbamates, and pyrethroids being among the most widely studied groups **(Aktar *et al*., 2009)**.

Bioaccumulation represents another critical environmental concern, particularly for lipophilic pesticides that readily dissolve in fatty tissues. From an ecotoxicological standpoint, pesticides can disrupt endocrine, metabolic, and growth-related processes, leading to measurable changes in body morphometry, organ weights, and tissue integrity. Ecophysiological responses such as alterations in liver, muscle, and gill indices are important markers of sublethal stress, providing insight into how aquatic organisms cope with chemical exposure under field conditions **(Copping & Menn, 2000; Chandler *et al*, 2011)**.

In light of these considerations, the present study aimed to assess the bioaccumulation of various agricultural pesticides in *T. zillii* from Wadi El-Rayan lakes and to investigate associated ecophysiological alterations in terms of growth performance and organ integrity. The study integrates ecotoxicological and ecophysiological approaches to provide a comprehensive evaluation of the sublethal impacts of pesticide exposure, offering a critical update on the environmental status of one of Egypt's most threatened freshwater.

MATERIALS AND METHODS

1. Study area

The study was carried out in Wadi El-Rayan Lakes, a protected wetland located in the Fayoum Depression, Egypt. The system comprises two interconnected lakes. It is formed of two artificial lakes, the first (upper) and the second (lower) lakes. The upper Wadi El-Rayan Lake (UWRL) was completely filled in 1980 and water was allowed to inflow to the lower Wadi El-Rayan Lake (LWRL) **(Khedr *et al*., 2023)**:

- **Site 1 (Rayan 1):** Upper lake (~10 m below sea level); directly receives wastewater from the El-Wadi drain. *GPS: 29°14'10"N, 30°28'42"E*
- **Site 2 (Rayan 2):** Lower lake (~18 m below sea level); receives overflow from the upper lake and discharges from nearby fish farms. *GPS: 29°11'28"N, 30°24'52"E*

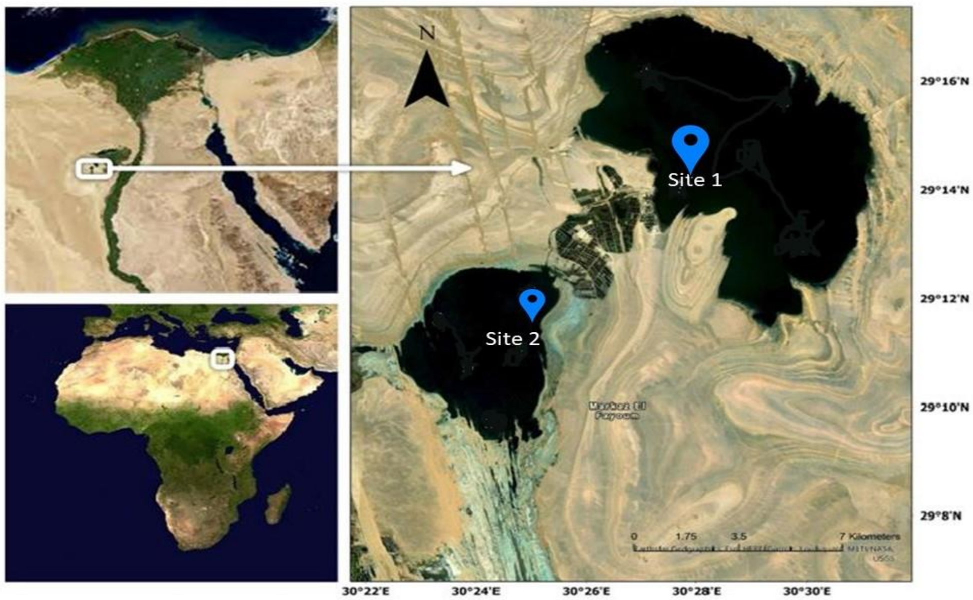

Fig. 1. Map of the studied aquatic protected areas in Fayoum Governorate **(Khedr *et al*., 2023)**

Water and fish Sampling from the studied wadi El-Rayan lakes was conducted during two distinct seasons: Season 1 (Summer) and Season 2 (Winter)**.**

2. Fish collection and sampling

A total of 20 adult specimens of *Tilapia zillii* were collected from two sites in Wadi El-Rayan lakes during two sampling seasons (winter and summer of 2023). Five fish were sampled per site per season, resulting in a total of 20 individuals.

In winter, the average body weights were 175.12 ± 2.09 g at Site I and 113.68 ± 3.09 g at Site II, while the corresponding total lengths were 25.20 ± 3.57 cm and 13.71 ± 0.57 cm, respectively. In summer, the total lengths were 18.38 ± 0.64 cm at Site I and 16.50 ± 0.89 cm at Site II.

Fish were sampled from a localized area of approximately 0.5 km² surrounding each predetermined GPS point, using gill nets and baited traps with the assistance of local fishermen. All specimens were transported alive in aerated plastic containers equipped with portable oxygen pumps to the wet Lab, Faculty of Science, Fayoum University. Upon arrival, each fish was measured for total length (cm) and total weight (g). Fish were then euthanized, and liver, muscle, and gill tissues were carefully dissected, weighed, and stored at −20°C for subsequent biochemical and pesticide residue analyses.

3. Determination of pesticide residues

a. Sample preparation and extraction

Pesticide residue analysis was conducted on muscle, liver, and gill tissues collected from *Tilapia zillii* specimens (n= 20). Each tissue sample was homogenized, and a 10g aliquot was extracted following a modified QuEChERS protocol **(Anastassiades *et al*., 2003; Zhao & Mao, 2011)**. Briefly, 10mL of acetonitrile was added to each sample and was homogenized at 12,000 rpm for 3 minutes. The mixture was treated with 4g of anhydrous $MgSO_4$ and 1g of NaCl, shaken vigorously, and centrifuged at 4000 rpm for 5 minutes. 6mL aliquot of the supernatant was subjected to clean-up using 400mg primary secondary amine (PSA) and 1200mg $MgSO_4$. The purified extract was filtered through a 0.45µm Teflon syringe filter, acidified with 5% formic acid in acetonitrile (to reach pH 5–5.5), then evaporated to dryness. The residue was reconstituted in acetonitrile and stored at 4°C until instrumental analysis was conducted.

b. GC-MS instrumental analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using an Agilent 7890B GC system coupled with a 5977A mass selective detector (Agilent Technologies, USA), equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness).

Instrumental conditions followed the U.S. EPA Method 8081B (U.S. EPA, 2007) and were optimized based on the study of **Zhao (2013)** for trace-level pesticide detection. The oven temperature program was: initial 60°C (held for 1 min), ramped to 170°C at 2°C/min, then to 285°C at 5°C/min. The injector temperature was maintained at 250°C. Helium was used as the carrier gas at a constant flow rate of 1.0mL/ min.

Mass spectra were acquired in both full scan mode (m/z 40–550) and selected ion monitoring (SIM) mode for quantitative analysis. Analytical quality assurance was ensured through the use of procedural blanks, matrix spikes, and certified reference standards.

4. Morphometric and physiological measurements

Relative organ weights were determined as follows:

Relative organ weight (%) = Total body weight (g) X 100

This included:

- Relative Liver Weight (RLW)
- Relative Muscle Weight (RMW)
- Relative Gill Weight (RGW) **(Froese, 2006).**

5. Statistical analysis

Data were expressed as mean ± standard deviation. A two-way ANOVA was used to assess the effects of season**,** lake, and their interaction on all measured parameters using SPSS version 25.0**.** Statistical significance was considered at $P < 0.05$ **(Zar, 2010)**.

RESULTS AND DISCUSSION

There are several ways to be exposed to pesticides, such as through home use, polluted drinking water, food residues, and occupational contact. The greatest exposure hazards are faced by agricultural workers, and organophosphate poisoning is a major global health concern, especially in developing nations where safety training and protective gear may be insufficient **(Hayes *et al*., 2010)**. Children are more vulnerable because of their growing organ systems and higher relative intake rates, and chronic low-level exposure has been linked to neurological impairments, reproductive issues, and some types of cancer. According to epidemiological research, children who are exposed to pesticides are more likely to suffer developmental abnormalities, Parkinson's disease, and Alzheimer's disease **(Tejal *et al*., 2023)**. Endocrine disrupting pesticides can interfere with hormone function, potentially affecting reproductive health, thyroid function, and metabolic processes. The widespread presence of pesticide residues in food and water supplies means that even nonoccupational populations face continuous low-level exposure, raising concerns about cumulative health effects over lifetime exposure periods.

Pesticide residues in fish tissues

Residual analysis of 20 pesticide compounds in *Tilapia zillii* sampled from Wadi El-Rayan lakes revealed marked spatial **and** seasonal variations in both retention time and compound-specific detectability. The detected pesticides represented a wide spectrum of chemical groups, including organophosphates**,** carbamates**,** triazoles**, and** pyrethroids, reflecting varied physicochemical behaviors and bioaccumulation potentials.

Retention time variability

A two-way ANOVA (Table 1) revealed that season**,** compound type, and their interactions had significant effects on pesticide retention time:

- A highly significant main effect of compound type ($F = 166.811$, $P < 0.001$) indicated strong variability in chromatographic behavior across chemical classes.
- Season also exerted a significant influence ($F = 19.568$, $P < 0.001$), suggesting temporal shifts in bioaccumulation or degradation dynamics.
- The lake effect alone was not significant ($P = 0.822$), implying that geographic location had less influence than temporal and chemical factors.

- Several significant interaction terms (e.g., Season × Compound, Lake × Compound) highlighted complex interplays between biotic and abiotic conditions affecting pesticide dynamics.

Table 1. Results of two-way ANOVA evaluating the effects of season, lake, and compound type on pesticide retention time in fish tissues

Variation source	SS	df	MS	F- calculated	*P*-value
Season	26.06	1.00	26.06	19.568	0.000
Lake	0.07	1.00	0.07	0.051	0.822
Compound	4220.37	19.00	222.13	166.811	0.000
Season * Lake	0.29	1.00	0.29	0.217	0.642
Season * Compound	618.21	19.00	32.54	24.435	0.000
Lake * Compound	191.32	19.00	10.07	7.562	0.000
Season * Lake * Compound	189.07	19.00	9.95	7.473	0.000
Error	213.06	160.00	1.33		

df: Degree of freedom, MS: Mean square, SS: Sum of squares.
P<0.000: Represent significant effects.

These results emphasize the compound-dependent nature of pesticide behavior in fish tissue, modulated by seasonal influences likely related to temperature**,** fish physiology, and aquatic chemistry.

Key findings on retention dynamics of specific pesticide compounds

- The retention time of Ethoprophos showed a significant seasonal reduction in Lake 1, decreasing from 8.18 ± 0.006 min in Season 1 to 7.87 ± 0.006 min in winter.
- Kresoxim-methyl and Chlorfenapyr were exclusively detected during Season 2 at Lake 2, suggesting potential season-specific application or degradation patterns.
- Compounds such as Pirimicarb**,** Profenofos, and others consistently showed undetectable levels (0.00min), possibly due to degradation, non-use, or low bioaccumulation.

Table 2. Retention times (min) of detected pesticides across lakes and seasons; Superscripts denote significant differences (P< 0.05).

	Winter		**Summer**	
	Lake 1	**Lake 2**	**Lake 1**	**Lake 2**
Ethoprophos	8.18 ± 0.006^{bB}	8.18 ± 0.017^{bB}	7.87 ± 0.006^{bA}	8.18 ± 0.012^{bB}
Atrazine	8.52 ± 0.012^{cA}	8.61 ± 0.023^{bB}	8.66 ± 0.017^{bB}	8.61 ± 0.020^{bB}
Terbufos	8.66 ± 0.017^{dA}	8.66 ± 0.017^{bA}	8.72 ± 0.040^{bA}	8.66 ± 0.035^{bA}
Diazinone	8.79 ± 0.020^{eA}	8.79 ± 0.012^{bA}	8.79 ± 0.029^{bA}	8.79 ± 0.040^{bA}

Pirimicarb	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}
Methyl Parathion	9.37 ± 0.020^{fA}	9.37 ± 0.023^{bA}	9.32 ± 0.058^{bA}	9.36 ± 0.462^{bA}
Pirimiphos methyl	9.54 ± 0.023^{gA}	9.58 ± 0.040^{bA}	9.52 ± 0.462^{bA}	9.57 ± 0.693^{bA}
Malathion	9.74 ± 0.017^{hA}	9.74 ± 0.035^{bA}	9.74 ± 0.635^{bA}	9.74 ± 1.155^{bA}
Chlorpyrifos	10.11 ± 0.012^{iA}	9.59 ±0.058^{bA}	9.59 ± 0.808^{bA}	9.59 ± 1.386^{bA}
Cyprodinil	10.25 ±0.006^{jA}	10.24 ±0.577^{bA}	10.42 ±1.212^{cA}	10.24 ±0.924^{bA}
Penconazole	10.27 ±0.017^{jB}	10.21 ±0.693^{bB}	10.26 ±1.328^{bcB}	0.00 ± 0.000^{aA}
cis-Chlorfenvinphos	10.57 ±0.023^{kA}	10.57 ±1.155^{bA}	10.56 ±1.097^{cA}	10.57 ±0.964^{bA}
Profenofos	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}
Kresoxim-methyl	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	12.31 ±1.155^{cdB}	12.31 ±1.097^{dB}
Chlorfenapyr	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	12.16 ±1.155^{cdB}
Diniconazole	12.53 ±0.029^{lA}	12.51 ±1.156^{cA}	12.51 ±0.837^{cdA}	12.51 ±1.674^{deA}
Ethion	12.67 ±0.035^{mA}	12.68 ±1.270^{cA}	12.67 ±0.808^{dA}	12.66 ±0.866^{eA}
EPOXICONAZOLE	14.48 ±0.046^{nA}	14.47 ±0.982^{cdA}	14.47 ± 0.751^{deA}	14.47 ±1.016^{efA}
Bifenthrin	15.10 ±0.058^{oA}	15.09 ±1.097^{dA}	15.10 ± 0.760^{eA}	15.46 ±1.132^{fA}
Fenpropathrin	15.70 ±0.087^{pA}	15.48 ±1.270^{dA}	15.77 ±1.156^{eA}	15.46 ±1.270^{fA}

- In each column, values marked with the same superscript small letter are insignificantly different(P>0.05), whereas those marked with different letters are significantly different(P<0.05).
- In each row, values marked with the same superscript capital letter are insignificantly different (P>0.05), whereas those marked with different letters are significantly different (P<0.05).

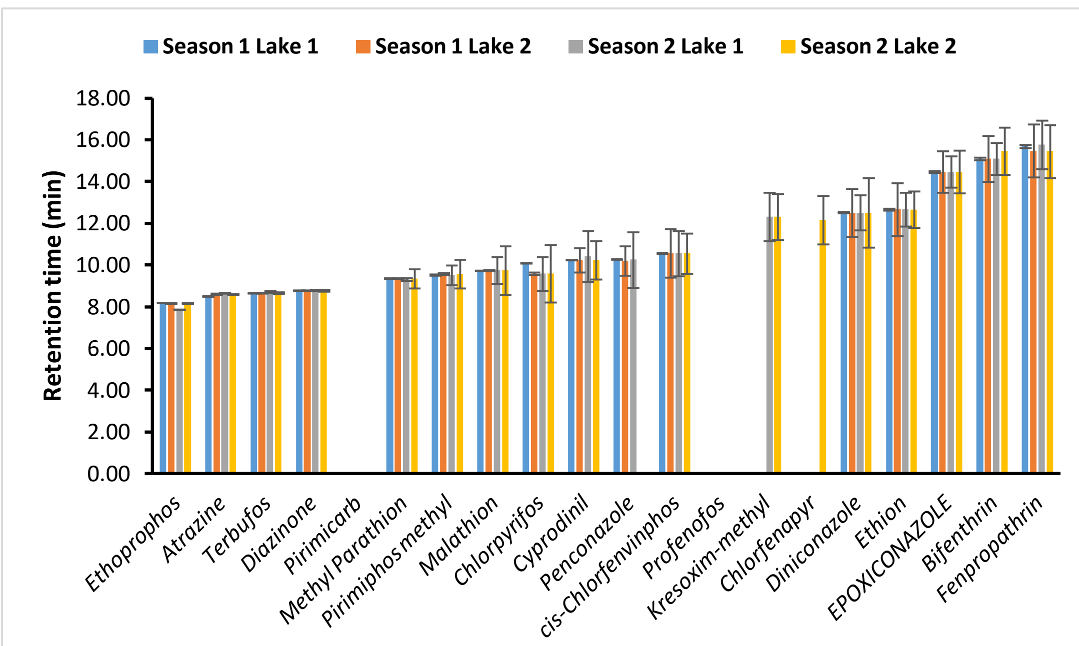

Fig. 2. Mean retention times (min) of pesticides across lake–season combinations
Bars sharing the same superscript letter are not significantly different (P> 0.05); different letters indicate significant differences (P< 0.05).

Pesticide concentration variations

The concentrations of pesticides in fish tissues showed similarly marked variability. The highest concentrations were detected in fish from Lake 1 during summer**,** particularly for:

- Terbufos (108.88 ± 3.12 ng/ml);
- Malathion (99.46 ± 2.31 ng/ml);
- Methyl Parathion (92.15 ± 1.03 ng/ml).

These elevated concentrations may reflect increased agricultural runoff**,** enhanced bioavailability, and the lipophilicity of these compounds, promoting accumulation in fish tissues.

Two-way ANOVA (Table 3) revealed significant effects of:

- Season (F= 2898.21, P< 0.001);
- Lake (F= 183.115, P< 0.001);
- Compound type (F= 5496.79, P < 0.001);
- All interaction terms, including Season × Compound and Lake × Compound (P< 0.001).

Table 3. Two-way ANOVA showing the effect of season, lake, compound type, and their interactions on pesticide concentrations in *Tilapia zillii* tissues. (*SS = Sum of Squares, MS = Mean Square, df = degrees of freedom*)

Variation source	**SS**	**df**	**MS**	**F-calculated**	***P*-value**
Season	4466.90	1	4466.90	2898.21	0.000
Lake	282.23	1	282.23	183.115	0.000
Compound	160967.75	19	8471.99	5496.791	0.000
Season * Lake	1.02	1	1.02	0.665	0.416
Season * Compound	18251.21	19	960.59	623.25	0.000
Lake * Compound	33293.20	19	1752.27	1136.909	0.000
Season * Lake * Compound	23406.83	19	1231.94	799.306	0.000
Error	246.60	160	1.54		

These statistical results underscore the complex interplay between ecological drivers and chemical characteristics that shape bioaccumulation patterns.

Specific compound trends (Table 4) included:

- Fenpropathrin reaching 162.43 ± 2.33 ng/ml in Lake 2 during Season 2 – the highest level detected;
- Persistent high levels of Methyl Parathion and Malathion in Lake 1 across both seasons.

Table 4. Changes in the mean concentrations (ng/ml) of 20 pesticide compounds detected in *Tilapia zillii* across lakes and seasons

	Winter		Summer	
	Lake 1	**Lake 2**	**Lake 1**	**Lake 2**
Ethoprophos	1.41 ± 0.002^{aC}	0.79 ± 0.035^{aB}	14.92 ± 0.012^{deD}	0.68 ± 0.035^{aA}
Atrazine	0.75 ± 0.003^{aB}	0.20 ± 0.023^{aA}	1.37 ± 0.035^{abC}	0.13 ± 0.038^{aA}
Terbufos	24.88 ± 0.004^{dB}	25.64 ± 1.155^{dB}	108.88 ± 3.12^{hC}	19.20 ± 0.577^{eA}
Diazinone	1.34 ± 0.052^{aA}	2.02 ± 0.404^{abA}	9.73 ± 0.924^{cB}	1.42 ± 0.289^{abA}
Pirimicarb	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}
Methyl Parathion	89.72 ± 1.155^{eC}	63.38 ± 1.617^{eA}	92.15 ± 1.039^{gC}	67.67 ± 0.520^{fB}
Pirimiphos methyl	0.03 ± 0.001^{aA}	0.04 ± 0.001^{aB}	0.08 ± 0.003^{aC}	0.05 ± 0.006^{aB}
Malathion	99.46 ± 2.309^{fD}	73.48 ± 1.732^{fA}	93.72 ± 1.155^{gC}	$79.54 \pm .693^{gB}$
Chlorpyrifos	4.31 ± 0.577^{bA}	2.80 ± 0.289^{bA}	13.21 ± 1.386^{dB}	2.50 ± 0.462^{bcA}
Cyprodinil	0.37 ± 0.017^{aA}	0.96 ± 0.289^{aA}	4.11 ± 1.039^{bB}	1.04 ± 0.029^{abA}
Penconazole	0.97 ± 0.023^{aA}	0.17 ± 0.040^{aA}	4.36 ± 1.068^{bB}	0.00 ± 0.000^{aA}
cis-Chlorfenvinphos	6.34 ± 0.289^{cB}	2.73 ± 0.052^{bA}	29.49 ± 0.895^{fC}	2.99 ± 0.000^{cA}
Profenofos	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}
Kresoxim-methyl	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	16.38 ± 0.751^{eB}	17.73 ± 0.577^{eB}
Chlorfenapyr	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	5.92 ± 0.693^{dB}
Diniconazole	3.77 ± 0.323^{bB}	1.09 ± 0.046^{abA}	12.53 ± 0.693^{dC}	0.36 ± 0.017^{aA}
Ethion	0.01 ± 0.001^{aA}	0.11 ± 0.023^{aB}	0.09 ± 0.006^{aB}	0.02 ± 0.003^{aA}
Epoxiconazole	0.69 ± 0.058^{aB}	0.27 ± 0.023^{aA}	2.17 ± 0.040^{abC}	0.39 ± 0.003^{aA}
Bifenthrin	0.12 ± 0.007^{aA}	0.33 ± 0.029^{aB}	0.56 ± 0.040^{aC}	1.34 ± 0.012^{abD}
Fenpropathrin	0.06 ± 0.007^{aA}	14.22 ± 0.046^{cB}	0.43 ± 0.035^{aA}	162.43 ± 2.33^{hC}

Different superscript letters indicate statistically significant differences at $P<0.05$.

In each column, values marked with the same superscript small letter are insignificantly different ($P>0.05$), whereas those marked with different letters are significantly different ($P<0.05$).

In each row, values marked with the same superscript capital letter are insignificantly different ($P>0.05$), whereas those marked with different letters are significantly different ($P<0.05$).

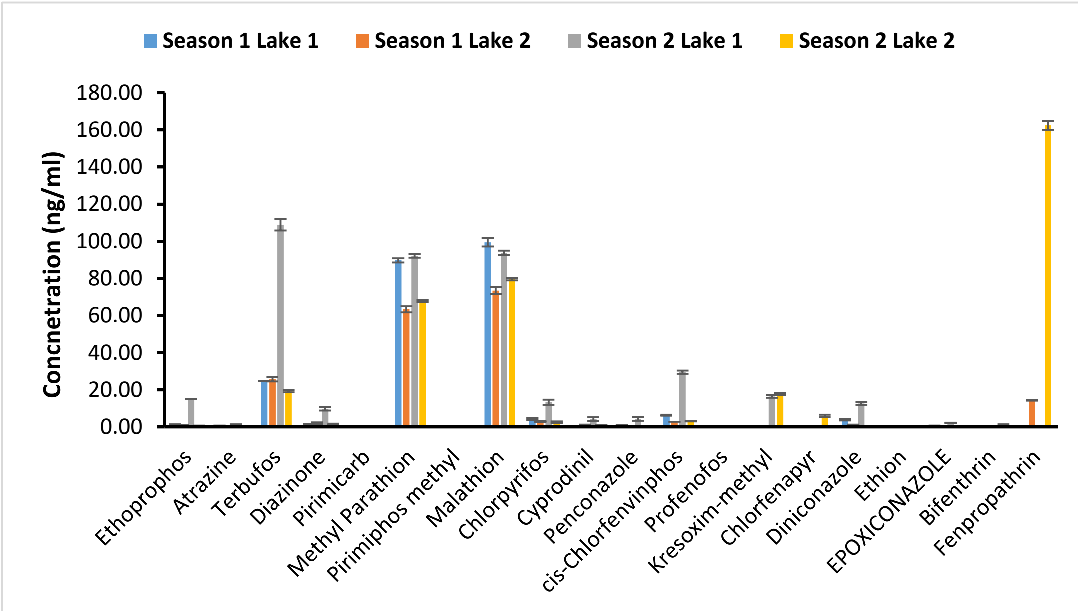

Fig. 3. Graphical representation of spatial and seasonal variation in pesticide concentrations(ng/ml)

Bars sharing the same letter are not significantly different (*P*> 0.05), while different letters indicate significant differences (*P*< 0.05).

Morphometric measurements of *Tilapia zillii*

Morphometric assessment revealed significant spatial and seasonal differences in the total body dimensions of *Tilapia zillii*. The highest mean body length **(**25.20 ± 3.57 cm**)** and width (10.10 ± 1.49 cm**)** were recorded in Lake 1 during Season 1, while the lowest measurements were observed in Lake 2, particularly in the same season (Tables 6–7).

Two-way ANOVA results (Table 5) confirmed the significant effects of lake and the season × lake interaction on both total length and width (*P*< 0.05), while the main effect of season alone was not significant (*P*> 0.05). These findings suggest that the deterioration of water quality in Lake 2 may have adversely impacted somatic growth in fish exposed to environmental stressors.

Table 5. Two-way ANOVA for the effects of season and lake on body morphometrics and organ weights of *Tilapia zillii*

Parameter	Variation Source	SS	df	MS	F-Calculated	*P*-value
Total length	Season	23.788	1	23.788	1.412	0.251
	Lake	218.52	1	218.52	12.97	0.002
	Season * Lake	110.809	1	110.809	6.577	0.02
	Error	286.408	17	16.848		
Width	Season	3.835	1	3.835	1.336	0.264
	Lake	31.491	1	31.491	10.972	0.004

	Season * Lake	18.434	1	18.434	6.423	0.021
	Error	48.792	17	2.87		
Relative liver weight	Season	3.518	1	3.518	13.794	0.002
	Lake	0.797	1	0.797	3.124	0.095
	Season * Lake	0.48	1	0.48	1.883	0.188
	Error	4.336	17	0.255		
Relative muscle weight	Season	19.982	1	19.982	6.662	0.019
	Lake	3011.505	1	3011.505	1004.092	0.000
	Season * Lake	2.658	1	2.658	0.886	0.36
	Error	50.987	17	2.999		
Relative Gill weight	Season	6.243	1	6.243	1.568	0.227
	Lake	3119.811	1	3119.811	783.748	0.000
	Season * Lake	2.426	1	2.426	0.61	0.446
	Error	67.671	17	3.981		

df: Degree of freedom, MS: mean square, SS: sum of squares.
P<0.05, P<0.000: represent significant effects.

Table 6. Changes in total body length of *Tilapia* sp.

		Total Length (Cm)
Season 1	Lake 1	25.20 ± 3.57^{b}
	Lake 2	13.71 ± 0.57^{a}
Season 2	Lake 1	18.38 ± 0.64^{a}
	Lake 2	16.50 ± 0.89^{a}

In the same column, values marked with the same letter are insignificantly different (P>0.05), whereas those marked with different letters are significantly different (P<0.05).

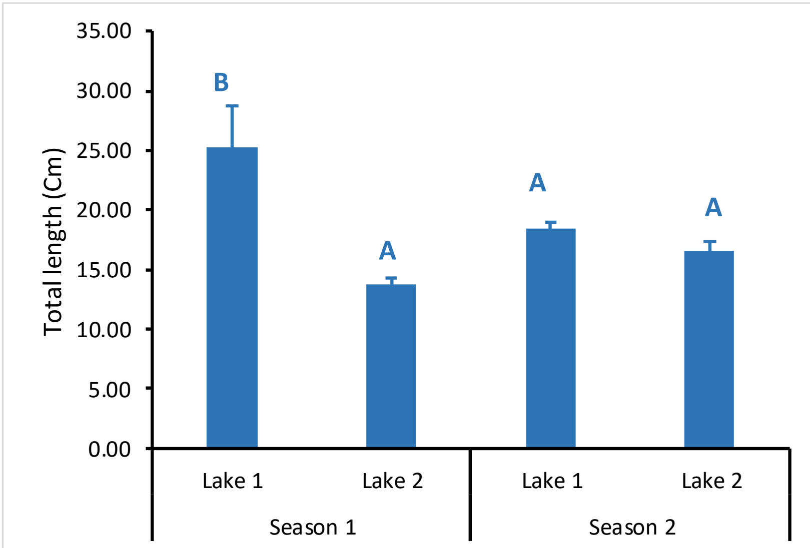

Fig. 4. Changes in total body length of Tilapia sp.

Bars marked with the same letter are insignificantly different (*P*>0.05), whereas those marked with different letters are significantly different (*P*<0.05).

Table 7. Changes in total body width of *Tilapia* sp.

		Total Width (Cm)
Season 1	Lake 1	10.10 ± 1.49^{b}
	Lake 2	5.61 ± 0.26^{a}
Season 2	Lake 1	7.33 ± 0.21^{a}
	Lake 2	6.75 ± 0.25^{a}

In the same column, values marked with the same letter are insignificantly different (*P*>0.05), whereas those marked with different letters are significantly different (*P*<0.05).

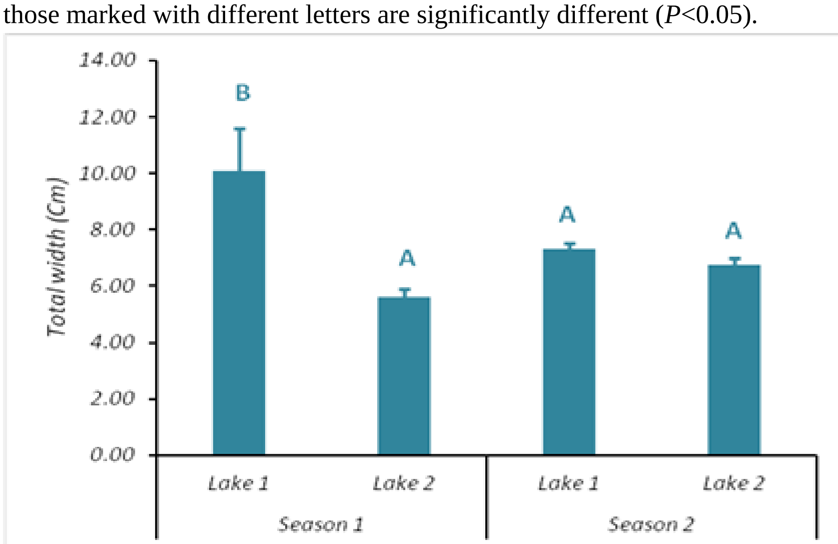

Fig. 5. Changes in total width of Tilapia sp.

Bars marked with the same letter are insignificantly different ($P>0.05$), whereas those marked with different letters are significantly different ($P<0.05$).

Relative organ weights

Relative liver weight (RLW)

The relative liver weight (RLW) showed a notable increase in fish from Lake 2 during Season 2 (1.87 ± 0.27%), possibly indicating hepatomegaly as an adaptive response to chronic exposure to environmental contaminants.
Two-way ANOVA (Table 5) revealed a significant effect of season ($P= 0.002$), whereas lake and season × lake interaction were not statistically significant.

Table 8. Changes in relative liver weight of *Tilapia* sp.

		Relative liver weight (%)
Season 1	Lake 1	0.64 ± 0.25[a]
	Lake 2	0.72 ± 0.21[a]
Season 2	Lake 1	1.16 ± 0.10[a]
	Lake 2	1.87 ± 0.27[b]

In the same column, values marked with the same letter are insignificantly different ($P>0.05$), whereas those marked with different letters are significantly different ($P<0.05$).

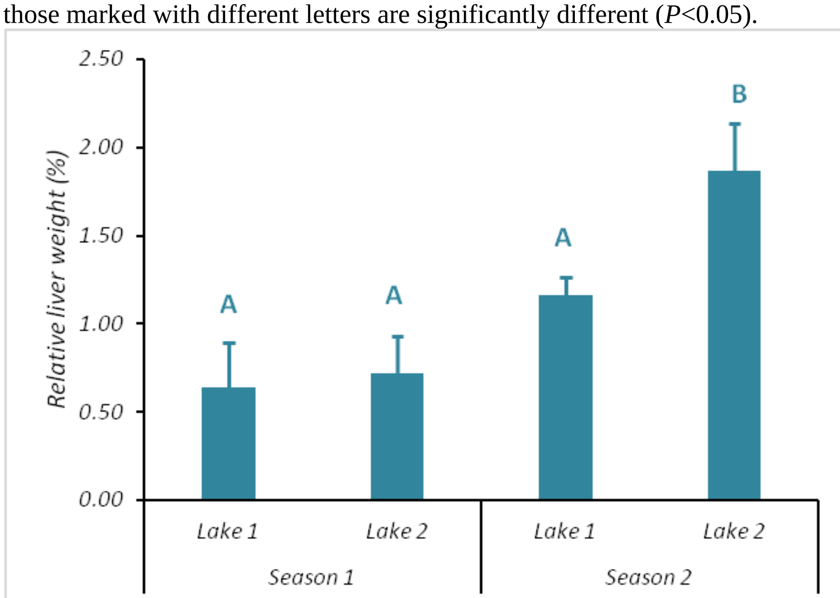

Fig. 6. Changes in relative liver weight of Tilapia sp.
Bars marked with the same letter are insignificantly different ($P>0.05$), whereas those marked with different letters are significantly different ($P<0.05$).

Relative muscle weight (RMW)

Relative muscle mass exhibited a drastic decline in Lake 2, dropping to 3.07 ± 0.19% in Season 2, compared to 26.63 ± 0.96% in Lake 1. This dramatic atrophy likely reflects growth retardation**,** protein catabolism, or energy reallocation due to toxicant-induced stress.

Two-way ANOVA showed that both lake and season had statistically significant effects (P< 0.001 and P< 0.05, respectively).

Table 9. Changes in relative muscle weight of Tilapia sp.

		Relative muscle weight (%)
Season 1	Lake 1	29.33 ± 0.95^{c}
	Lake 2	4.32 ± 0.40^{a}
Season 2	Lake 1	26.63 ± 0.96^{b}
	Lake 2	3.07 ± 0.19^{a}

In the same column, values marked with the same letter are insignificantly different (P>0.05), whereas those marked with different letters are significantly different (P<0.05).

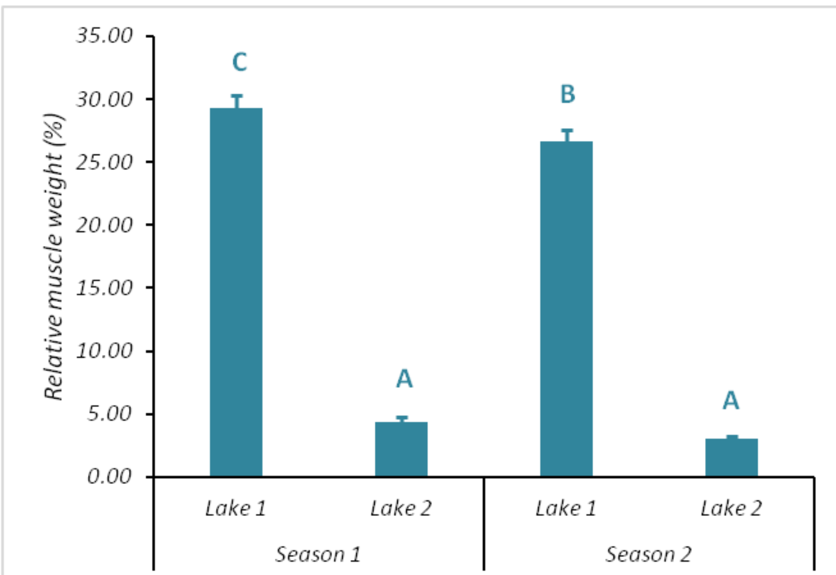

Fig. 7. Changes in relative muscle weight of Tilapia sp.

Bars marked with the same letter are insignificantly different (P>0.05), whereas those marked with different letters are significantly different (P<0.05).

Relative gill weight (RGW)

Fish from Lake 2 exhibited remarkably elevated RGW values in both seasons (28.90 ± 0.80% and 27.10 ± 1.98%), in contrast to the relatively low values recorded in Lake 1 (~3%). This may reflect gill hypertrophy, inflammation, or compensatory swelling in response to poor water quality or pollutant-induced hypoxia. Two-way ANOVA showed that the effect of lake was highly significant (p < 0.001), while season and interaction effects were not.

Table 10. Changes in relative gill weight of Tilapia sp.

		Relative gill weight (%)
Season 1	Lake 1	3.49 ± 0.20[a]
	Lake 2	28.90 ± 0.80[b]
Season 2	Lake 1	3.07 ± 0.15[a]
	Lake 2	27.10 ± 1.98[b]

In the same column, values marked with the same letter are insignificantly different (*P*>0.05), whereas those marked with different letters are significantly different (*P*<0.05).

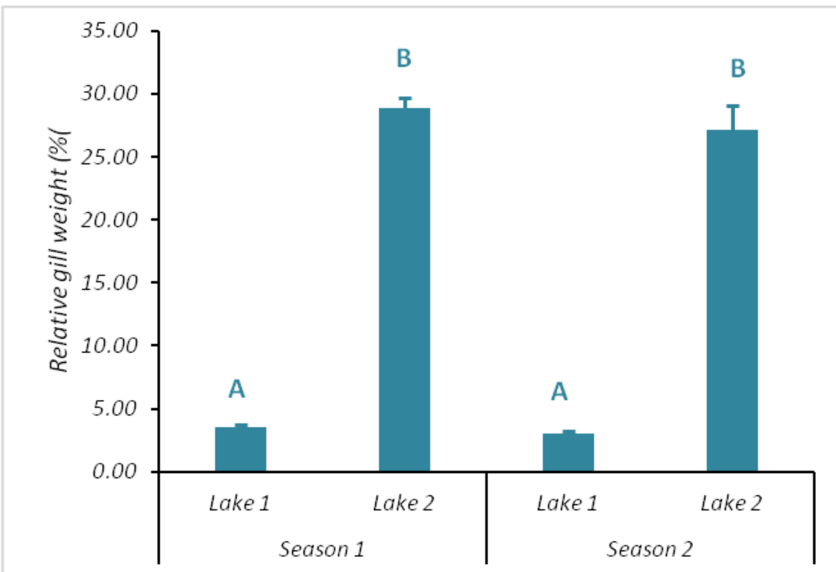

Fig. 8. Changes in relative gill weight of Tilapia sp.
Bars marked with the same letter are insignificantly different (*P*>0.05), whereas those marked with different letters are significantly different (*P*<0.05).

Evaluation of somatic condition using Fulton's condition factor (K)

The condition factor (K) is a widely accepted morphometric index that reflects the overall health, nutritional state, and physiological well-being of fish, providing an indirect measure of energy reserves relative to body length and weight. It is sensitive to environmental variables such as water quality, food availability, and chemical exposure **(Froese, 2006; Lloret *et al*., 2012)**. Higher K values typically indicate favorable somatic condition, while lower values may suggest physiological stress, malnutrition, or toxicant impact **(Le Cren, 1951)**.

The K values of *Tilapia zillii* exhibited statistically significant differences across lakes and seasons, highlighting spatial and temporal influences on fish health.

Two-way ANOVA results (Table 11) show significant effects of: Lake (*F*= 10.56, *P*= 0.004), Season (*F*= 7.63, *P*= 0.013) and Lake × Season interaction (*F* = 12.54, *P*= 0.002)

These findings indicate that both location and time, as well as their interaction, significantly influenced fish condition.

Table 11. Two-way ANOVA for the effects of lake, season, and their interaction on condition factor (K).

Variation Source	Sum of Squares	Df	Mean Square	F	*P*-value
Lake	1.74	1	1.737	10.56	0.004
Season	1.26	1	1.256	7.63	0.013
Lake × Season	2.06	1	2.064	12.54	0.002
Residuals	2.96	18	0.165		

Assumption checks

Prior to analysis, the following assumptions were verified:

- Levene's test for homogeneity of variance: $F = 1.34$, $P= 0.292$
- Shapiro–Wilk test for normality: $W = 0.963$, $P= 0.554$
- Q–Q plot analysis confirmed visual normality (Fig. 9)

These tests confirmed the validity of parametric assumptions, supporting the use of Two-way ANOVA.

Tukey's post hoc test

Multiple comparisons (Table 12) revealed the following:

- Lake 2 had significantly higher K values than Lake 1 ($P = 0.004$)
- K values were significantly higher in Season 1 compared to Season 2 ($P = 0.013$)
- A significant interaction was detected:
    - Lake 2 / Season 1 had the highest K values overall
    - A sharp seasonal decline was observed in Lake 2 ($P = 0.002$)
    - No seasonal effect was observed in Lake 1 ($P = 0.942$)

Table 12. Tukey's post hoc comparisons for condition factor (K) across lakes and seasons

Comparison	Mean Difference	SE	df	t	*P*-value
Lake1 vs Lake2	−0.574	0.177	18.0	−3.25	0.004
Season1 vs Season2	0.488	0.177	18.0	2.76	0.013
Lake1–S1 vs Lake1–S2	−0.1377	0.246	18.0	−0.560	0.942
Lake1–S1 vs Lake2–S1	−1.2003	0.238	18.0	−5.05	< 0.001
Lake1–S1 vs Lake2–S2	−0.0860	0.272	18.0	−0.316	0.989
Lake2–S1 vs Lake2–S2	1.1143	0.254	18.0	4.383	0.002

Q-Q Plot

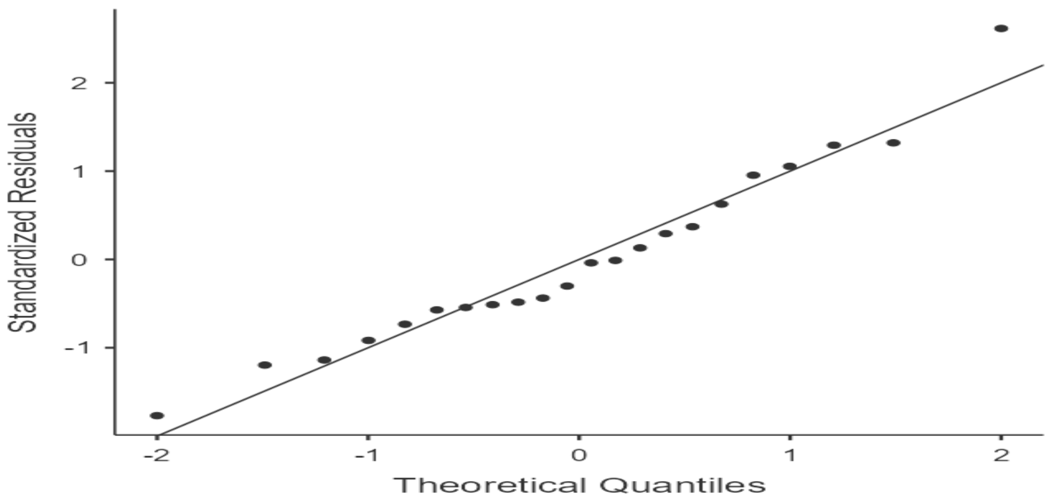

Fig. 9. Q–Q plot of residuals for condition factor (K), confirming normality assumption

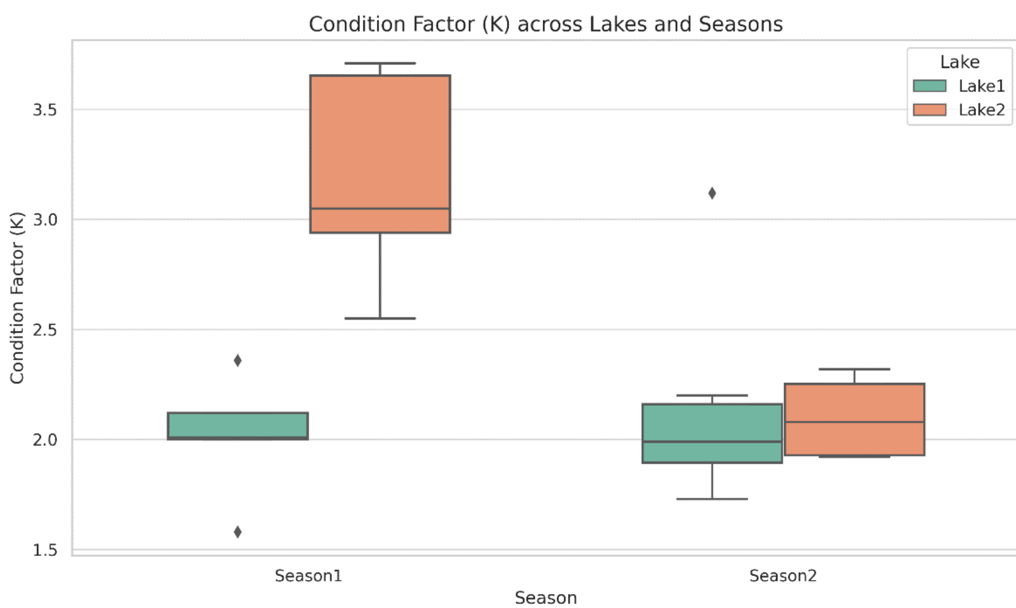

Fig. 10. Spatial and seasonal variations in condition factor (K) of *Tilapia zillii*.
Bars not sharing the same superscript letter differ significantly ($P < 0.05$); bars sharing the same letter indicate no significant difference.

Across species, physiological parameters have a major impact on pesticide uptake, dispersion, and elimination rates. The ability to store lipophilic pesticides is influenced by body composition, specifically lipid content, with adipose tissue acting as a key reservoir compartment. Both absorption kinetics and biotransformation capacity are influenced by metabolic rate; higher metabolic rates are typically linked to quicker removal of metabolizable substances **(Isman *et al*., 2006)**.

The findings of the present study revealed marked spatial and seasonal variation in the bioaccumulation of pesticides in *Tilapia zillii* collected from Wadi El-Rayan lakes. The highest concentrations were observed for organophosphate compounds such as malathion, methyl parathion, and terbufos, in addition to some pyrethroid pesticides like fenpropathrin. These were particularly elevated in fish sampled from Lake 1 during Season 2 (winter), likely due to intensified agricultural runoff and reduced water exchange during colder months **(Velisek *et al*., 2009; Ismail *et al*., 2014)**. The observed accumulation may be attributed to the lipophilic nature and chemical stability of these compounds, which facilitate their persistence and accumulation in fish tissues **(Ahouangninou *et al*., 2016)**.

These pesticide residues appear to have adversely affected fish morphometrics. Fish from Lake 2 exhibited significantly reduced total length and width, particularly during Season 1, suggesting possible growth retardation due to metabolic stress or reduced nutrient assimilation **(van der Oost *et al*., 2003; Al-Ghanim *et al*., 2020)**.

Through its effects on metabolic rates, membrane permeability, and chemical partitioning behavior, temperature has a significant impact on bioaccumulation processes. In addition to increasing absorption rates, higher temperatures may also improve metabolism and removal, resulting in intricate patterns of temperature-dependent accumulation **(Jacobsen & Hielmso, 2014)**.

Furthermore, physiological alterations in internal organ weights were evident. The relative liver weight (RLW) peaked in Lake 2 during Season 2 (1.87 ± 0.27%), likely indicating hepatomegaly as a detoxification response to chronic contaminant exposure. This aligns with previous studies linking hepatic enlargement to elevated biotransformation activities in polluted environments **(De Smet *et al*., 1998)**.

Muscle weight percentage showed a substantial decline in Lake 2, especially in Season 2 (3.07 ± 0.19%) compared to Lake 1 (26.63 ± 0.96%). This may reflect protein catabolism or impaired energy storage caused by toxic stress **(Begum, 2004; Cazenave *et al*., 2005)**. Similarly, relative gill weight (RGW) values in Lake 2 were abnormally elevated in both seasons (~27–29%), indicating possible gill hyperplasia, edema, or inflammatory responses due to exposure to waterborne toxicants or hypoxic stress **(Bhattacharya *et al*., 2008; Hedayati & Tarkhani, 2014)**.

The condition factor (K), which integrates length and weight to assess overall fish health, showed significant spatial and temporal variation. Fish from Lake 2 during Season 1 exhibited the highest K values, potentially due to organ hypertrophy rather than actual fitness. This suggests that condition factor alone may not always reflect true health, especially in chronically exposed populations. Conversely, a marked decline in K was noted in Lake 2 during Season 2, reflecting deteriorating health status and energy depletion under sustained environmental pressure **(Le Cren, 1951; Froese, 2006; Lloret *et al*., 2012)**.

Collectively, the morphometric and physiological findings underscore the toxicological impact of pesticide exposure on *Tilapia zillii*, affecting growth, organ function, and overall body condition. These biomarkers can serve as early warning indicators of ecological disturbance and chronic contamination in freshwater ecosystems. Such physiological disruptions may not only threaten individual fish survival but also impact population structure and ecosystem stability in the long term.

CONCLUSION

This study provides compelling evidence that pesticide contamination in Wadi El-Rayan lakes significantly affects the physiological and morphological health of *Tilapia zillii*. The bioaccumulation patterns of 20 pesticide compounds showed marked spatial and seasonal variability, with higher concentrations recorded in Lake 1 during Season 2. These elevated residues corresponded with noticeable reductions in fish body size, altered organ weights—particularly liver and gill hypertrophy—and significant declines in muscle mass. Additionally, condition factor (K) was significantly affected by both lake and seasonal conditions, reflecting the integrated physiological stress experienced by the fish.

The collective findings underscore the ecological risk posed by pesticide runoff and highlight the urgent need for environmental monitoring and regulation of agricultural discharge into freshwater ecosystems.

Recommendations

Based on the findings, the following recommendations are proposed:

1. **Regulatory Control**: Implement stricter controls on pesticide usage in agricultural zones surrounding Wadi El-Rayan to minimize chemical runoff into aquatic habitats.
2. **Routine Monitoring**: Establish a routine biomonitoring program using *Tilapia zillii* as a bioindicator species for assessing pesticide pollution and ecosystem health.
3. **Public Awareness Campaigns**: Educate local farmers on the environmental consequences of pesticide overuse and promote the adoption of integrated pest management (IPM) strategies.
4. **Further Research**: Conduct longitudinal studies to evaluate the long-term impact of chronic pesticide exposure on fish reproduction, immune response, and biodiversity in the lake system.
5. **Policy Integration**: Encourage collaboration between environmental agencies and agricultural stakeholders to integrate pesticide management into national water conservation policies.

Compliance with Ethical Standards

Ethical approval:

All experimental procedures involving *Tilapia zillii* fish were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals. According to Animal Care and Use Committee (IACUC) at the Faculty of Science, Fayoum University.

Conflict of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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