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Effect of Seasonal Changes in Heavy Metals on the Histomorphology of the Liver and Gills of Nile Tilapia (*Oreochromis niloticus* L.) in Burullus Lake, Egypt

Norhan Ezzat[1], Ibrahim I. Al-Hawary[1], Ahmed E. Elshafey[2], Norah Althobaiti[3] and Zizy I. Elbialy[1*]

[1] *Department of Fish Processing and Biotechnology, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafr El-sheikh 33516, Egypt. norhanezzat59@gmail.com hawaryib@fsh.kfs.edu.eg zeze_elsayed@fsh.kfs.edu.eg ORCID: 0000-0002-1654-0015*

[2] *Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafr El-sheikh 33516, Egypt. ahmed.emad.elshafey@gmail.com ORCID: 0000-0001-6359-6241*

[3] *Biology Department, College of Science and Humanities-Al Quwaiiyah, Shaqra University, Al Quwaiiyah 19257, Saudi Arabia, nalthobaiti@su.edu.sa*

Abstract

THIS study aimed to assess the impact of seasonal changes on heavy metal concentrations in water and their effects on Nile Tilapia (*Oreochromis niloticus*) liver and gill histomorphology in Burullus Lake, Egypt in 2021. Six designated points were selected for water and fish (length ±5cm) sampling in both summer and winter. The results indicated that Iron (f.e) had the highest overall levels across all seasons and sites, while Zinc (Zn), Lead (Pb), Copper (Cu), and Cadmium (Cd) showed variations. Notably, Pb, Cu, and Cd exhibited the widest ranges in concentration. Bioaccumulation of heavy metals in *O. niloticus* muscles revealed that Zinc concentrations were significantly highest in summer and winter at Site 3 ($p < 0.05$), while Iron levels were significantly highest in winter and lowest in summer ($p < 0.05$). Cadmium content varied, and lead concentrations remained low level. Histomorphological analysis of Nile tilapia liver and gills showed significant differences between summer and winter months. In summer, the liver exhibited severe hepatocyte degeneration, pancreatic blood vessel congestion, and pancreatic acinar cell degeneration, affecting liver development stages. Winter liver histomorphology was comparatively better than summer but showed some signs of central vein congestion, mononuclear cell infiltration, vacuolar degeneration, hypertrophy, and pancreatic acinar cell degeneration. Gill histomorphology in summer showed mild to severe congestion, hyperplasia, desquamation, edema, fusion, and degenerative changes, while winter gills displayed mild degeneration in secondary lamellae, hyperplasia of the epithelial lining, congested branchial blood vessels, and interstitial edema. Conclusively, these findings highlight the impact of seasonal changes on the health of Nile tilapia, with more severe consequences observed during the summer months.

Key words: *Oreochromis niloticus*; heavy metals; Histopathology; Burullus Lake; Egypt.

Introduction

The meal of fish serves as one of the most crucial traditional ingredients in Egyptian culinary recipes, offering a low-cost protein alternative compared to other animal sources. In developing countries, fish contributes over 30% of the total per capita animal protein intake [1]. Nile tilapia (*Oreochromis niloticus*), a member of the Cichlidae family native to African freshwater ecosystems, stands out as a low-cost fish compared to other farmed options due to its lower dependence on higher trophic level food sources [2]. This affordability has earned it the nickname "aquatic chicken" and sometimes positions it as a staple protein for vulnerable populations in developing and developed countries [2]. Tilapia plays a crucial role in rural development across these regions, contributing to poverty alleviation, hunger eradication, and improved human health [3]. This impact stems from several key benefits: domestic fish supply for food security, revenue generation

*Corresponding author: Zizy I. Elbialy, E-mail: zizyelbialy74@gmail.com, Tel.: 01004060315
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through export earnings, increased income for producers, and job creation opportunities [4, 5].

Northern lakes, encompassing El-Manzala, El-Burullus, Maryot, and Edko, with a combined area of 1430 km², yield the highest proportion of the harvested fish [6]. Next come the Mediterranean Sea and the Red Sea, respectively [7]. Burullus Lake, situated in the central Nile Delta, stands as a UNESCO-protected region and one of Egypt's most prominent wetland habitats, recognized under the RAMSAR convention since 1971 [8]. Unfortunately, over the past decades, it has faced various types of pollution, negatively impacting its water and sediment quality [9]. While natural streams typically contain trace levels of heavy metals, many pose dangers even at these minute concentrations. Arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, zinc, and selenium are all highly toxic metals, even in small quantities [10]. Their ability to accumulate in soft tissues without being metabolized by the body makes them especially hazardous, as this buildup can reach the point of being poisonous[11]. The rate at which metals are absorbed by fish can be influenced by a multitude of factors beyond simply their presence in the environment. These include dietary habits, specific food choices, feeding behaviors, the fish's size and age, its sex and genetic makeup, the geographical location of its habitat, its swimming patterns and activity levels, and even its reproductive cycle [12].

Upon interaction with humans in agricultural, manufacturing, pharmaceutical, industrial, or residential settings, heavy metals can enter the body through ingestion, inhalation, absorption through skin contact, or even through water sources. Similarly, fish naturally accumulate these metals through their diet, water, and the surrounding sediments. This ongoing accumulation within the aquatic environment concentrates heavy metals within fish tissues, potentially posing risks to their health and ultimately to consumers [13].

This study aimed to assess two key aspects of heavy metal pollution in Burullus Lake. The first is environmental quality and the second is the health of its resident organisms. To achieve this, the levels of six heavy metals (Mn, Zn, Fe, Ni, Cu, and Pb) were measured in both lake water and the muscles of Nile tilapia (Oreochromis niloticus) during the winter and summer of 2021. Additionally, histopathological changes in the liver and kidneys of impacted fish were evaluated. This combined approach provides valuable insights into the extent of metal contamination in the lake, its potential effects on aquatic life, and ultimately, assists in effective monitoring of the entire ecosystem.

Material and Methods

Ethical approval

The protocol and management of the current experiment were authorized by “the Institutional Aquatic Animal Care and Use in Research Committee, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt”.

Study area

Burullus Lake, encompassing approximately 460 square kilometers, lies within the Kafr El-Sheikh Governorate (30°22′–31°35′N; 30°33′–31°08′E). Situated on the eastern branch of the Nile River, the Rosetta branch, the lake receives an annual water volume of roughly 4.1 million cubic meters. This influx primarily arrives through an intricate eight-drainage system and the Brinbal freshwater canal, drawing from the surrounding catchment area's vast 998,000 acres, predominantly composed of agricultural drainage water.

Sampling protocol

Surface water and fish (*Oreochromis niloticus*) samples were collected seasonally from six sites representing different regions of Lake Burullus throughout the period from August 2021 to February 2022 where the temperature range was 21.54 to 39.87℃. Concentrations of five heavy metals (Cu, Zn, Cd, Fe, and Pb) were measured in both water and fish samples. Fish samples of approximately the same size length ±5cm) were collected, alongside water samples, at six designated points within Burullus Lake, points are, as indicated in Figure 1, Burullus East (St. 1), El-Boughaz (St. 2), Drain no.7 (St. 3), El-Shakloubah (St. 4), El-Hoksa (St. 5), Burullus North (St. 6).

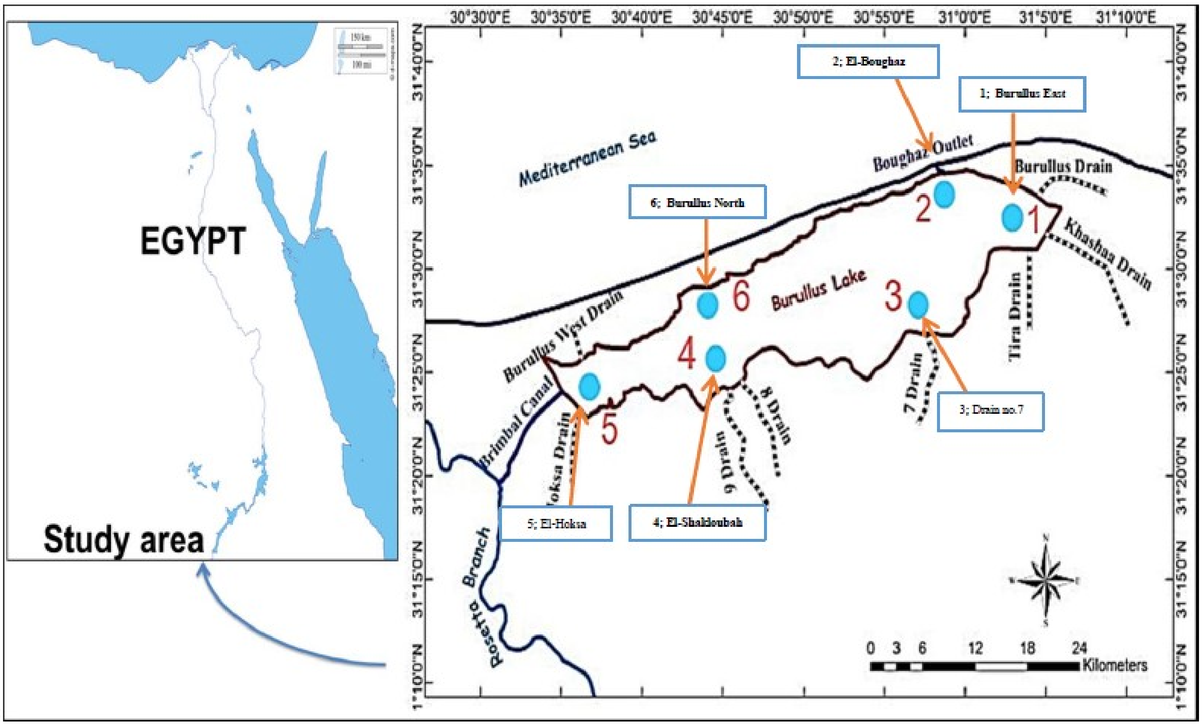

Fig. 1. Map of the sampling sites from Burullus Lake.

Collection Samples

Fish were collected using a combination of fishing nets and traps. For surface water samples, a pre-washed 1000 cm³ PVC vertical water sampler, as recommended by standard procedures [14], was used to collect water from a 50 cm depth at several locations within each chosen sampling site of the lake. The collected samples were then filtered, acidified with 65% HNO_3 to prevent metal precipitation, stored in an icebox, and transported to the laboratory for further analysis. Both water and fish samples were transported via a cold chain on the same day of collection to ensure analytical accuracy [15].

Determination of heavy metals in water and fish samples

Heavy metals in water

Water samples were collected in triplicate from the sampling points depicted in Figure 1, located within the drain site. Approximately 2 liters were collected at each point and subsequently filtered. One liter from each sample was acidified with 2% HNO_3 and then concentrated to a volume of 50 mL at a temperature of 64°C to prepare for elemental analysis. The concentrations of cadmium (C.d), copper (Cu), iron (F.e), lead (Pb), and zinc (Zn) within the digested samples were determined using graphite furnace atomic absorption spectrometry (GBC Avanta E, Victoria, Australia). This process involved selecting the appropriate wavelength for each specific element to ensure accurate quantification [15].

Heavy metals in fish

Triplicate fish samples were collected from the same locations as the water samples, ensuring consistency in site-specific analysis. The whole fish specimens were thoroughly washed with both tap water and distilled water to remove any external contaminants. They were then dried at a temperature of 65°C for of 48 hours to achieve complete dehydration. Following this, the dried samples were meticulously ground into a fine powder using a stainless-steel grinder and securely stored in airtight plastic bags until further analysis. For elemental analysis, one gram of the powdered fish material was subjected to ashing in a muffle furnace at 450°C for 5 hours. The resulting ash was then extracted with 20% hydrochloric acid to facilitate the release and measurement of heavy metals [16]. The precise concentrations of these heavy metals within the extracted samples were determined using the sensitive and reliable technique of atomic absorption spectrometry (AAS).

Histological Examination

For histopathological examination, six fish each from the summer and winter treatment groups were chosen. Specimens from their gills and livers were excised and immediately fixed in 10% formalin for 48 hours. Following fixation, the tissue samples were systematically dehydrated through an ascending series of ethyl alcohol solutions (from 70% to absolute alcohol). After clearing in xylene, they were impregnated with paraffin wax and prepared for histological examination. Tissue sections of 4-5 µm thickness were stained with hematoxylin and eosin (H&E) stain according to the method described by [17]. Subsequently, the stained sections were examined and imaged using a light microscope (Olympus CX40).

Statistical Analysis

GraphPad Prism software, version 8.01, was used to do statistical analysis after evaluating the data for both normality and homogeneity of variance. Two-way analysis of variance (ANOVA) was used to analyze and compare the data for significant differences between the experimental groups. All sample numbers are equal, where there are 6 samples per group. Tukey's post-hoc multiple comparison test was used also. When the p-value was less than 0.05, differences were considered significant and were assigned separate superscription letters. The means and SEM are used to express all results.

Results

Estimation of Heavy metals in water

Table 1 demonstrates the seasonal variations in the concentrations of the studied metals in the water, with Fe consistently showing the highest levels, followed by Zn, Pb, Cu, and Cd. Fe values fluctuated from a significantly high level ($p < 0.05$) at St.2 in summer to a significantly low level at St.3 in winter ($p < 0.05$). Similarly, Zn concentrations varied, reaching a significantly high level at St.6 in winter ($p < 0.05$) and a minimum level at St.3 in summer. Lead (Pb) records significantly high levels at St.5 in summer ($p < 0.05$) and significantly low levels at St.1 in summer ($p < 0.05$). Copper (Cu) concentrations in water displayed the highest value of St.4 in summer and the lowest at St.6 in summer. The content of cadmium (Cd) exhibited the widest range, with a maximum Level at St.6 in summer and a minimum level at St.5 in summer.

Table 1. Average values of heavy metals (mg/L) in surface water of Burullus Lake during winter and summer 2021 (Mean±SEM)

Heavy metal (mg/l)	Season	Site For Water Sampling						p- value		
		St.1	St.2	St.3	St.4	St.5	St.6	Interaction	(Row Factor) Season	(Column Factor) sites
Fe	Summer	52.267±1.357^{aA}	60.233±2.887^{bA}	12.723±1.123^{cA}	33.413±0.116^{dA}	32.650±0.074^{dA}	32.073±0.163^{dA}	0.0001	0.0001	0.0001
	Winter	46.277±1.026^{aB}	33.373±0.465^{bdB}	11.363±0.214^{cA}	38.850±0.242^{dA}	32.057±0.815^{bA}	14.023±1.530^{bB}			
Cu	Summer	1.030±0.006^{aA}	0.460±0.058^{aA}	1.673±0.214^{bA}	2.550±0.006^{cA}	0.583±0.289^{adA}	0.072±0.014^{bdA}	0.0001	0.0001	0.0001
	Winter	1.070±0.006^{aA}	1.023±0.009^{aB}	2.410±0.061^{bB}	1.480±0.115^{acB}	1.253±0.156^{acB}	1.830±0.055^{bcB}			
Zn	Summer	5.413±1.166^{aA}	4.230±1.709^{aA}	1.443±0.046^{aA}	4.030±0.006^{aA}	3.527±0.990^{aA}	3.360±0.205^{aA}	0.1723	0.0312	0.0168
	Winter	4.303±0.234^{abA}	5.270±1.448^{abA}	1.853±0.015^{aA}	5.507±2.302^{abA}	5.347±1.013^{abA}	8.220±0.059^{bA}			
Cd	Summer	0.940±0.006^{abA}	0.630±0.058^{aA}	1.080±0.133^{abA}	1.127±0.093^{bA}	0.630±0.058^{aA}	1.500±0.231^{bA}	0.0005	0.2799	0.0002
	Winter	0.953±0.026^{aA}	0.890±0.058^{aA}	0.787±0.066^{aA}	0.850±0.058^{aA}	1.020±0.040^{aA}	1.057±0.022^{aA}			
Pb	Summer	2.317±0.069^{aA}	2.700±0.247^{abA}	3.670±0.348^{abA}	2.650±0.173^{abA}	4.040±0.017^{bA}	3.280±0.040^{aA}	0.3080	0.7768	0.0008
	Winter	2.367±0.061^{aA}	3.143±0.003^{aA}	3.503±0.192^{aA}	2.877±0.112^{aA}	3.247±0.717^{aA}	3.823±0.578^{aA}			

Estimation of Heavy metals in Nile tilapia tissue

Table 2 demonstrates the bioaccumulation of heavy metals in the musculature of *O. niloticus*, revealing seasonal variations in the concentrations of the studied metals. The metals consistently followed the sequence of zinc > iron > cadmium > lead > copper. Zinc concentrations exhibited the significantly highest levels ($p < 0.05$), reaching their highest value in both summer and winter at St.3, while the significantly lowest values were observed in winter at St.1 and St.6, respectively ($p < 0.05$). Iron levels had similarly significantly high values in both seasons at St.3 ($p < 0.05$), with the significantly lowest values recorded at St.6 in winter ($p < 0.05$). Cadmium content displayed a range, with the highest values detected at St.1 in winter and St.6 in summer, while the significantly lowest values were found at St.2 in summer ($p < 0.05$). Lead concentrations in the muscles remained consistently below a specific threshold across all sites in both summer and winter.

TABLE 2. Mean values of heavy metals (mg/kg) in muscles of Nile tilapia at Burullus Lake during winter and summer 2021.

Heavy metal (mg/Kg)	Season	Site For Tilapia Sampling						p- value		
		St.1	St.2	St.3	St.4	St.5	St.6	Interaction	(Row Factor) Season	(Column Factor) sites
Fe	Summer	39.300±5.774^{aA}	38.767±5.687^{aA}	124.500±1.155^{bA}	40.500±0.231^{aA}	30.000±5.774^{aA}	27.333±0.882^{aA}	0.0010	0.0001	0.0001
	Winter	40.833±7.224^{aA}	120.267±5.780^{bB}	181.760±35.854^{cB}	44.500±1.155^{adA}	98.430±0.464^{bdB}	24.527±0.520^{aA}			
Cu	Summer	ND	ND	ND	ND	ND	ND	-	-	-
	Winter	ND	ND	ND	ND	ND	ND			
Zn	Summer	45.287±1.453^{aA}	102.500±1.155^{bA}	147.450±0.621^{cA}	42.313±1.693^{aA}	26.320±1.706^{dA}	55.290±0.592^{eA}	0.0001	0.0001	0.0001
	Winter	19.367±0.367^{aB}	24.313±0.656^{aB}	116.113±0.644^{bB}	71.370±0.529^{cB}	84.377±2.357^{dB}	8.550±0.429^{eB}			
Cd	Summer	0.532±0.004^{aA}	0.075±0.006^{aA}	0.242±0.030^{aA}	0.105±0.046^{aA}	0.650±0.058^{aA}	1.847±0.137^{bA}	0.0001	0.0001	0.0001
	Winter	2.307±0.121^{aB}	1.300±0.300^{bB}	1.770±0.064^{abB}	1.357±0.035^{bB}	1.227±0.152^{bA}	0.359±0.036^{cB}			
Pb	Summer	0.380±0.058^{aA}	0.380±0.058^{aA}	0.387±0.012^{aA}	0.327±0.027^{aA}	0.380±0.006^{aA}	0.437±0.038^{aA}	0.8007	0.3590	0.3736
	Winter	0.380±0.006^{aA}	0.357±0.034^{aA}	0.380±0.006^{aA}	0.330±0.025^{aA}	0.380±0.006^{aA}	0.360±0.036^{aA}			

Histomorphological examination of Nile Tilapia samples

The study identified varying degrees of pathological changes in the organs of *O. niloticus* across different seasons. Winter exhibited the most pronounced alterations, particularly in the skin and muscles, characterized by edema and parasite presence. Conversely, summer presented milder histological changes, primarily restricted to degeneration and edema in the muscle layer.

I. Liver histomorphology in summer

Figure.2. explains the **liver** histomorphology in summer where Figure 2.A. displays St.1, Degeneration and nuclear pyknosis of hepatocytes (black arrowheads), dilation and congestion of hepatic sinusoids (black arrow) and pancreatic blood vessels (white arrow), and degeneration of pancreatic acinar cells (white arrowheads) are observed. Figure 2; B. displays St.2, where Necrosis of hepatocytes (black arrows), dilation of hepatic sinusoids (black arrowhead), degeneration of pancreatic acinar cells (white arrowheads), and congestion of pancreatic blood vessels (white arrow) are evident. Figure 2.C. displays St.3, where Nuclear pyknosis (black arrows), vacuolar degeneration of hepatocytes (black arrowhead), degeneration of pancreatic acinar cells (white arrowheads), and congestion of pancreatic blood vessels (white arrow) are present. Figure 2.D. displays St.4, where Necrosis of hepatocytes (black arrows) and congested blood sinusoids (black arrowhead) are apparent. Figure 2.E. displays St.5, where Nuclear pyknosis of hepatocytes (black arrowheads), vacuolar degeneration of pancreatic acinar cells (white arrowheads), and congestion of both pancreatic blood vessels (white arrow) and hepatic sinusoids (black arrows) are noted. Figure 2.F. Displays St.6, where Necrosis of hepatocytes (black arrowheads), dilated blood sinusoids (black arrows), vacuolar degeneration of pancreatic acinar cells (white arrows), and congestion of pancreatic blood vessels (white arrows) are observed.

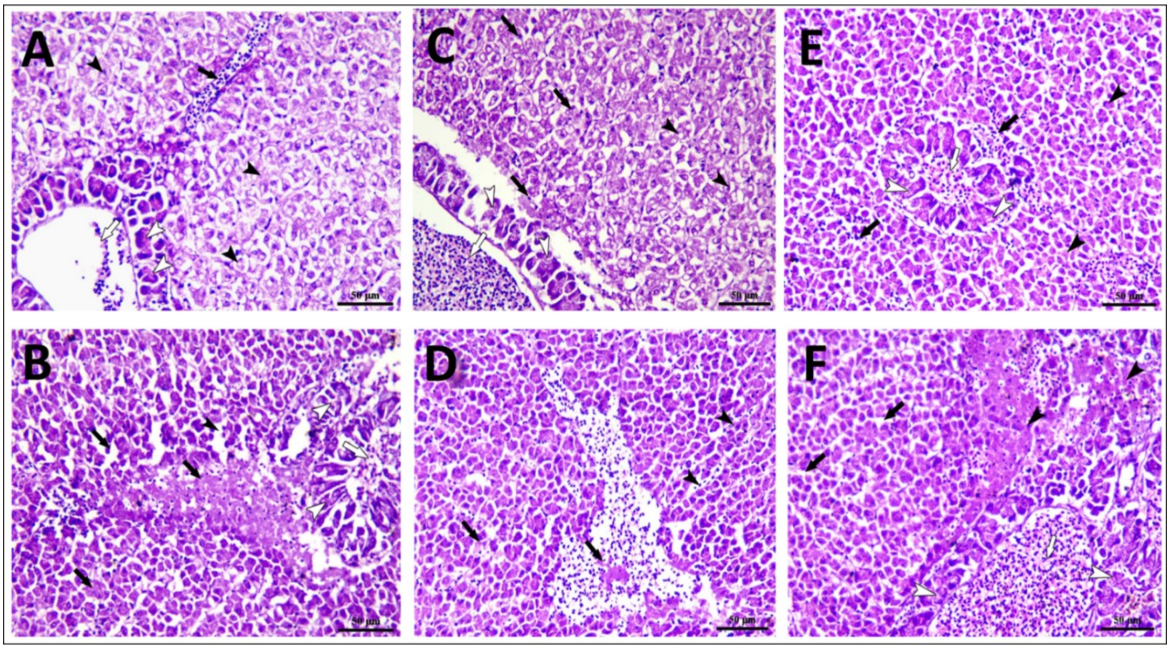

Fig. 2. Liver of O. niloticus collected from Lake Burullus in summer in different site. (At 400× magnifi cation and using haematoxylin and eosin stain, 50 µm scale bar for all images)

II. Liver histomorphology in winter

Figure (3) explains the **liver** histomorphology in winter where Figure 3.A displays St.1, where the central vein (CV) is evident, along with mild peri-vascular infiltration of mononuclear cells (black arrows) and mild degeneration of some hepatocytes (black arrowheads). Additionally, slight congestion of hepatic sinusoids is observed (white arrows). Figure 3.B displays St.2, where vacuolar degeneration of hepatocytes (black arrowheads) and pancreatic acinar cells (black arrows) is evident, alongside dilated hepatic sinusoids (white arrows). Figure 3.C displays St.3, where vacuolar degeneration of hepatocytes (black arrowheads) and dilated hepatic sinusoids (white arrows) are present, accompanied by mild infiltration of mononuclear cells (white arrowheads). Figure 3.D displays St.4, where Hypertrophy and vacuolar degeneration of hepatocytes (white arrowheads) are observed, along with congested hepatic sinusoids (white arrows) and pancreatic blood vessels (black arrow). Additionally, degeneration of some pancreatic acinar cells is evident (black arrowheads). Figure 2.E displays St.5, where Congestion of both hepatic sinusoids (white arrows) and pancreatic blood vessels (black arrow) is notable, along with mild infiltration of mononuclear cells (white arrowheads) and some degeneration of pancreatic acinar cells (black arrowheads). Figure 2.F displays St.6, where hypertrophy, nuclear pyknosis, and vacuolar degeneration of hepatocytes (black arrows) are evident, alongside dilated and congested hepatic sinusoids (white arrows) and infiltration of mononuclear cells (white arrowheads).

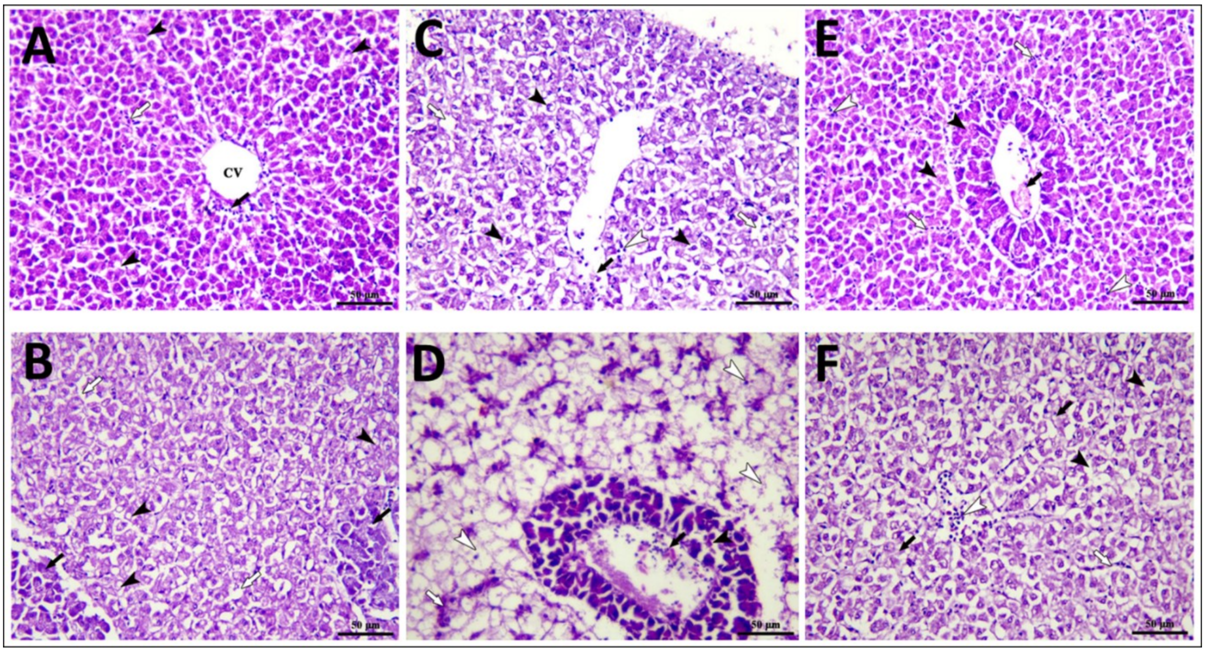

Fig. 3. Liver of O. niloticus collected from Lake Burullus in winter in different site. (At 400× magnifi cation and using haematoxylin and eosin stain, 50 µm scale bar for all images)

Gills histomorphology in summer

Figure (4) explains the **Gills** histomorphology in summer where Figure 4.A displays St.1 where the primary filament (PF) and secondary lamellae (SL) are visible, along with mild congestion in some branchial blood vessels (arrowheads). Hyperplasia of the gill epithelium (red arrows) is observed, accompanied by mild epithelial desquamation (blue arrows) and interstitial edema (black arrows). Figure 4.B displays St.2, where there are revealed severe congestion of branchial blood vessels (arrowheads), moderate interstitial edema (black arrows), and loss of some secondary lamellae (blue arrows). Furthermore, the fusion of some lamellae is evident (red arrows). Figure 4.C displays St.3, where mild interstitial edema (black arrows) in addition to degenerative changes and epithelial lifting in the secondary lamellae (blue arrows). Figure 4.D displays St.4, which shows mild interstitial edema (black arrows) and epithelial lifting of secondary lamellae (blue arrow). Degeneration of these structures is also evident (red arrow). Figure 4.E displays St.5, which shows interstitial edema (black arrows) and degenerative changes with epithelial lifting of secondary lamellae (red arrow). Additionally, a fusion of some lamellae is observed (blue arrow). Figure 4.F displays St.6, which there is revealed interstitial edema (black arrows) and a specific abnormality known as telangiectasis at the tips of secondary lamellae (red arrow), alongside degenerative changes and epithelial lifting (blue arrow).

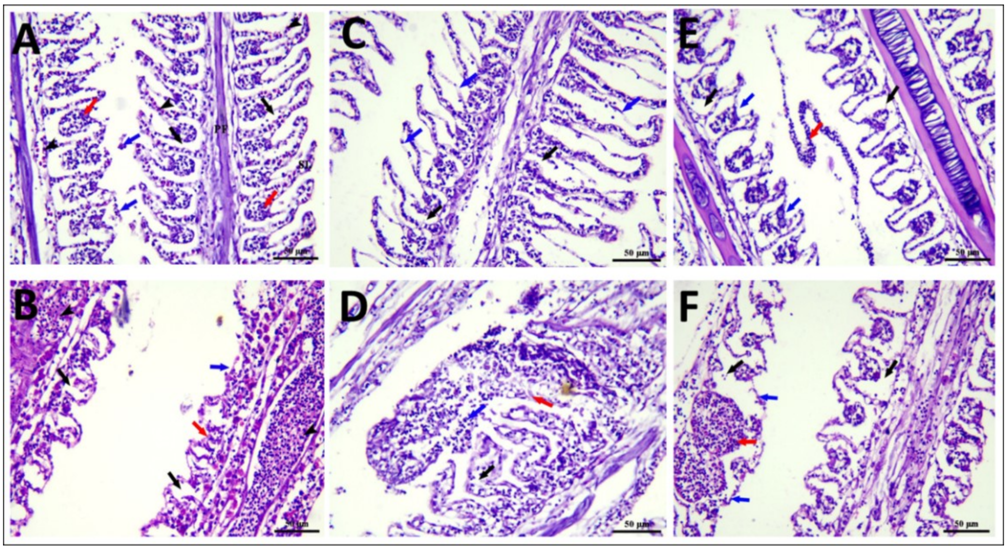

Fig. 4. Gills of *O. niloticus* collected from Lake Burullus in summer in different site. (At 400× magnification and using haematoxylin and eosin stain, 50 µm scale bar for all images)

Gills histomorphology in winter

Figure (5) explains the **Gills** histomorphology in winter where Figure 5.A shows St.1, which shows mild degeneration in some secondary lamellae (black arrows). Notably, hyperplasia of the epithelial lining is evident (red arrow). Figure 5.B shows St.2, where there are revealed congested branchial blood vessels (arrowheads), mild epithelial desquamation (red arrows), and mild interstitial edema (black arrows). Figure 5.C show St.3, where congested branchial blood vessels (arrowheads) are observed, along with mild epithelial lifting (black arrows). However, this group also shows hyperplasia of the lamellar epithelium (red arrow). Figure 5.D shows St.4, where there are revealed mild interstitial edema (black arrows) and hyperplasia of the lamellar epithelium (red arrow), similar to the pre-discharge group. Additionally, partial fusion of some secondary lamellae is evident (blue arrows). Figure 5.E shows St.5, which displays congested branchial blood vessels (arrowheads) and hyperplasia of the lamellar epithelium (red arrow). Furthermore, mild degeneration is observed in some secondary lamellae (black arrows). Figure 5.F shows St.6, which shows partial fusion of some lamellae (blue arrows) and hyperplasia of the lamellar epithelium (red arrow), consistent with the pre-discharge group. Additionally, interstitial edema is evident (black arrows).

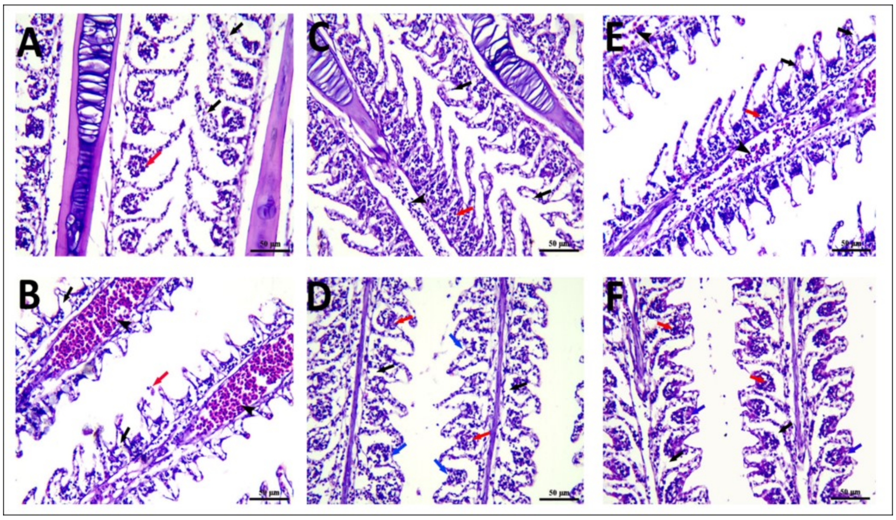

Fig. 5. Gills of *O. niloticus* collected from Lake Burullus in winter in different sites, (At 400× magnification and using haematoxylin and eosin stain, 50 µm scale bar for all images).

Discussion

Heavy metals are a group of metallic elements that have high atomic weights and densities. They occur naturally in the Earth's crust but can also be introduced into the environment through human activities such as industrial processes, mining, and agriculture. Due to their toxicity and persistence, heavy metals have become a significant concern in environmental science and public health. The measurement of heavy metals is crucial for assessing their presence, concentration, and potential effects on organisms, particularly fish. Heavy metals can have various detrimental effects on fish. The specific impacts depend on factors such as the type of metal, its concentration, exposure duration, and the species' sensitivity. Overall, understanding the effects of heavy metals on fish is crucial for assessing the health of aquatic ecosystems and ensuring the safety of fish as a food source for humans.

Our study reveals a clear seasonal pattern in heavy metal concentrations, with a consistent trend of peak levels observed in summer and lower concentrations during winter in Burullus Lake, Egypt these results line with [18, 19]. This observation can be attributed to summer heat intensifies evaporation, concentrating pollutants in the remaining water [20]. Additionally, increased agricultural activity and industrial discharges during summer months contribute to higher metal influx [21]. On another hand during winter, cooler temperatures and reduced agricultural/industrial activity result in lowered evaporation and decreased pollutant input, leading to lower metal concentrations [22]. Elevated metal concentrations in summer pose a significant threat to aquatic ecosystems and potentially even human health through bioaccumulation. So there is a need for seasonal monitoring and targeted remediation efforts focused on the summer months when metal pollution is most severe in Burulls Lake as mentioned in [23, 24]. Furthermore, understanding the seasonality of metal concentrations can inform better management practices for agricultural effluents and industrial discharges, potentially mitigating their impact on water quality [25]. This study sheds light on the dynamic interplay between seasonal changes and metal pollution, providing valuable insights for environmental management and conservation strategies.

Our analysis showcases a distinct pattern in the bioaccumulation of heavy metals within the muscles of O. niloticus. Notably, zinc shows the highest concentrations in the Nile tilapia body during the winter and summer seasons and all sites of Burullus Lake [26]. Can be attributed to variations in diet [27], feeding habits [28], environmental sources of zinc [29], species-specific differences [30], and individual physiological factors [28]. Zinc's high levels likely stem from its widespread presence in aquatic environments due to agricultural runoff and industrial effluent [31]. Additionally, its efficient uptake and retention by fish gills and internal organs contribute to its elevated bioaccumulation [32]. On the other hand, Copper is not detected in the body of Nile tilapia during summer and winter and in all sites as lined with [33]. The seasonal variations, with peaks at St.3, potentially point to increased heavy metal bioavailability in the body of Nile Tilapia during certain periods, possibly linked to fluctuations in water temperature or algal blooms as mentioned in [34]. Furthermore, the consistently low levels of lead identified in the body of Nile tilapia are an encouraging sign, suggesting this metal may not pose an immediate threat to *O. niloticus* populations at the studied sites. This knowledge forms a valuable foundation for holistic water quality management strategies aimed at protecting aquatic ecosystems and preserving the health of valuable fish species like *O. niloticus*.

Histopathological analysis revealed seasonal fluctuations in *O. niloticus* liver health, with summer being a major risk. In summer there is cellular degradation, vascular congestion, and altered pancreatic function. That can be attributed to the exposure of Nile tilapia to higher metal concentrations or algal blooms in the water of Burullus Lake during the summer season [35]. This heavy metal can further stress the liver, exacerbating cellular damage [36, 37]. A compromised liver poses a serious threat to the overall health and survival of *O. niloticus*, impacting individual fitness and potentially affecting entire populations [38]. ON the other hand, during warmer months, fish require more energy, pushing their metabolic machinery to its limits [39]. This can lead to oxidative stress and cellular damage [40], evident in the observed necrosis [41], pyknosis [42], and vacuolar degeneration of hepatocytes [43]. This study highlights the vital link between environmental factors, seasonal changes, and fish health. Our findings pave the way for further research into the specific mechanisms behind summer-induced liver damage and inform the development of holistic management strategies for protecting aquatic ecosystems and ensuring the well-being of valuable fish species like *O. niloticus*.

While the Nile Tilapia endures significant cellular distress during summer, winter reveals a remarkable shift towards resilience with minimal challenges. Its liver, though not entirely stress-free, navigates winter with significantly less damage compared to the summer months [44]. This resilience likely stems from potentially better-suited winter food sources alleviating summer deficiencies [45]. Also, reduced environmental temperatures lowered heavy metal burdens and even a low-level immune response to residual toxins [46].However, subtle winter stress persists, evident in mononuclear cell infiltration around blood vessels, vacuoles in hepatocytes, and increased blood flow in liver sinusoids. This comparison illuminates the multifaceted nature of fish health and the importance of seasonal variations. Winter, while offering respite from summer's onslaught, doesn't guarantee complete recovery [47]. Continuous research and proactive management remain crucial for safeguarding the health of Nile Tilapia and the environment it inhabits.

According to our results, the fragile gill structures of Nile tilapia have sustained considerable damage, which could seriously jeopardize the fish's ability to breathe and their general health in the warmer months. This summer onslaught manifests in a cascade of pathological features, highlighting the vulnerability of this crucial respiratory organ that lines with [48]. This could be attributed to higher heavy metal concentrations in warmer water [49]. These can directly damage sensitive gill tissues, disrupting gas exchange and triggering inflammatory responses [50]. Higher temperatures can favor the growth and activity of aquatic pathogens, leading to increased gill infections and further compromising respiratory function [51]. This study's findings highlight the need for holistic approaches to safeguarding fish health, by considering the interplay between environmental factors, seasonality, and respiratory function; we can develop effective strategies for protecting *O. niloticus* populations and maintaining the integrity of aquatic ecosystems.

In contrast to summer, which depicted substantial damage, winter offers a less dire situation for *O. niloticus* gills. The absence of pronounced distress suggests improved respiratory function compared to the warmer months, although subtle signs of stress persist. This could be attributed to lower environmental temperatures often leading to reduced heavy metal concentrations, minimizing exposure to gill-damaging toxins [52], and offering some respite from external stressors [53]. Even while things have improved overall, there are still hints of winter stress. Even though they are not as bad as they are in the summer, congested branchial blood arteries indicate some persistent circulation problems or lasting inflammatory reactions [54, 55]. This comparative analysis underscores the multifaceted nature of fish health and highlights the importance of considering seasonal variations, while winter offers respite from the severe damage observed in summer, it doesn't guarantee complete recovery.

Conclusion

In conclusion, heavy metal concentrations in Burullus Lake, Egypt display a clear seasonal pattern, with peak levels in summer and lower concentrations in winter. This trend has significant implications for aquatic ecosystems and human health due to the potential for bioaccumulation. The Nile tilapia (*O. niloticus*) exhibits distinct bioaccumulation patterns for different metals, with zinc consistently showing high concentrations throughout the year. Histopathological analysis reveals that summer months pose a major risk to O. niloticus liver health, characterized by cellular degradation, vascular congestion, and altered pancreatic function. In contrast, winter months show a remarkable shift towards resilience in liver health. The fragile gill structures of Nile tilapia sustain considerable damage in summer, impacting their respiratory function. However, winter months offer a less dire situation for gill health. Understanding these seasonal patterns is crucial for developing holistic management strategies to protect aquatic ecosystems and ensure the well-being of valuable fish species like *O. niloticus*.

Data Availability Statement

All data related to the current work are provided in the manuscript.

Author contributions

Norhan Ezzat: methodology, sampling, sample analysis, writing draft paper; **Ibrahim I. Al-Hawary:** supervision, reviewing the manuscript; **Ahmed E. Elshafey:** data analysis, writing and reviewing the manuscript; **Norah Althobaiti**: Reviewing, editing the final manuscript; **Zizy I. Elbialy:** conceptualization, methodology, reviewing the final manuscript**.**

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Conflict of interest

The authors declared that there is no conflict of interest.

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تأثير التغيرات الموسمية في المعادن الثقيلة على التركيب النسيجي للكبد والخياشيم لسمك البلطي النيلي في بحيرة البرلس، مصر

نورهان عزت[1] ، إبراهيم إبراهيم الهوارى [1] ، أحمد عماد الشافعى[2] ، نوره الثبيتى[3] و زيزى إبراهيم البيلى[1]

[1] قسم تصنيع الأسماك والبيوتكنولوجى- كلية علوم الثروة السمكية والمصايد- جامعة كفرالشيخ- مصر.

[2] قسم الإستزراع المائى- كلية علوم الثروة السمكية والمصايد- جامعة كفرالشيخ- مصر.

[3] قسم البيولوجى- كلية العلوم والإنسانيات- جامعة الشقرا - المملكة العربية السعودية.

هدفت هذه الدراسة إلى تقييم تأثير التغيرات الموسمية على تركيزات المعادن الثقيلة في المياه وتأثيرها على أنسجة الكبد والخياشيم في البلطي النيلي في بحيرة البرلس بمصر خلال عام 2021. تم اختيار ست نقاط محددة للمياه والأسماك (الطول ± 5 سم) أخذ العينات في كل من الصيف والشتاء. أشارت النتائج إلى أن الحديد [56] كان له أعلى المستويات الإجمالية في جميع المواسم والمواقع، في حين أظهر الزنك (Zn)، الرصاص (Pb)، النحاس (Cu)، والكادميوم (Cd) اختلافات. والجدير بالذكر أن الرصاص والنحاس والكادميوم أظهروا النطاقات الأوسع في التركيز. أظهر التراكم الحيوي للمعادن الثقيلة في عضلات البلطي النيلي أن تركيزات الزنك كانت أعلى بشكل ملحوظ في الصيف والشتاء في الموقع 3 ($P < 0.05$)، بينما كانت مستويات الحديد أعلى بشكل ملحوظ في الشتاء وأدنى في الصيف ($P < 0.05$). وتباين محتوى الكادميوم، وظلت تركيزات الرصاص منخفضة. أظهر التحليل النسجي لكبد وخياشيم البلطي النيلي اختلافات معنوية بين أشهر الصيف والشتاء. في الصيف، أظهر الكبد تنكسًا حادًا في خلايا الكبد، واحتقان الأوعية الدموية البنكرياسية، وتنكس خلايا عنيبية البنكرياس، مما يؤثر على مراحل نمو الكبد. كان التشريح النسيجي للكبد في الشتاء أفضل نسبيًا من الصيف ولكنه أظهر بعض علامات احتقان الوريد المركزي، وارتشاح الخلايا وحيدة النواة، والتنكس الفراغي، والتضخم، وانحطاط الخلايا العنيبية البنكرياسية. أظهر التشريح النسيجي للخياشيم في الصيف احتقانًا خفيفًا إلى شديدًا، وتضخمًا، وتقشرًا، وذمة، واندماجًا، وتغيرات تنكسية، بينما أظهرت الخياشيم الشتوية انحطاطًا خفيفًا في الصفائح الثانوية، وتضخم البطانة الظهارية، واحتقان الأوعية الدموية الخيشومية، والوذمة الخلالية. بشكل قاطع، تسلط هذه النتائج الضوء على تأثير التغيرات الموسمية على صحة البلطي النيلي، مع ملاحظة عواقب أكثر خطورة خلال أشهر الصيف.

الكلمات المفتاحية: البلطى النيلى، المعادن الثقيلة ، علم أمراض الأنسجة ، وبحيرة بورولس ، مصر