Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(6): 185 – 211 (2022)
www.ejabf.journals.ekb.eg

Histopathology and Ultrastructure in Liver and Gonads of the Nile Tilapia (*Oreochromis niloticus*) as a Bio-Monitor for Water Quality Assessment in Lake Manzalah

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ARTICLE INFO

Article History:
Received: Oct. 15, 2022
Accepted: Nov. 7, 2022
Online: Nov. 25, 2022

Keywords:
Macroinvertebrates,
Gonads,
Liver,
Manzalah Lake,
Oreochromis niloticus

ABSTRACT

Despite being one of Egypt's most significant fish production resources, Lake Manzalah (Northeast Egypt) is subject to substantial inputs of pollution from domestic, agricultural and industrial sources. The liver and gonads of *Oreochromis niloticus* were evaluated for histological and ultrastructural alterations in the current study to monitor the possible effects of Lake Manzalah pollution at two different areas, (A) near Hadous drain and (B) near the Bahr El-Baqar drain during autumn and spring seasons. Heavy metal concentrations in water, sediment, and fish tissues were assessed. The results showed that Fe, Mn, and Ni levels were greater than the standard allowable values in area B compared to area A, and in autumn rather than spring. Both studied areas showed heavy metal deposition in *O. niloticus* tissues with greater values in autumn, compared to spring. The maximum concentration of the heavy metal in tissues was found in the fish liver of area (B) in autumn. In the autumn and area B, there were significant histopathological and ultrastructural damages to the liver and gonads. It was concluded that, the noted biomarker responses were possible indicators of health deterioration in Lake Manzalah fish populations. Long-term monitoring is therefore required to assess the eco-health of the Manzalah Lake ecosystem by using bio-indicator species such as *Oreochromis niloticus*, which offer accurate, dependable measures of environmental quality.

INTRODUCTION

Lake Manzalah is a brackish lake in northeastern Egypt at the Nile Delta. It is the biggest northern Delta lakes of Egypt with an area of approximately 500 km^2, representing 34.2% of the overall fish output of the northern natural lakes in Egypt **(Amer & Ahmed, 2019)**. The lake is exposed to significant pollution inputs from residential, agricultural and industrial sources. The wastewater discharge carries climate gases, significant volumes of particulate matter, nutrients, bacteria, heavy metals, and hazardous organics to the lake **(Ismail & Hettiarachchi, 2017)**. Around 7500 million cubic meters of untreated industrial, household and agricultural drainage water were dumped into the lake and annually discharged through six main drains. This volume of wastewater recently decreased to around 4000 million cubic meters with the establishment of El-Salam Canal **(Abdel-Fattah *et al.*, 2018)**. The drains transport a significant quantity of nutrients, notably from the Bahr-El-Baqar, Ramsis, and Hadous drains, which are heavily contaminated by sewage and industrial effluent **(Mustafa *et al.*, 2015)**. Bahr El Baqar drain, which travels about 170km and dumps approximately 1.5 million m^3 of domestic and industrial wastewater every day, of which 1.25 million m^3/day originates exclusively from Cairo Governorate, transports these wastewater to Lake Manzala. **(Amer & Ahmed, 2019)**.

Pollutants in lake water are pesticides and fertilizers besides industrial and sewage effluents supplying water bodies and sediment with large amounts of inorganic anions and heavy metals **(Saeed & Shaker, 2008)**. In sediment, the majority of heavy metals bind to certain particles leaving only a small amount to be dissolved in water and disperse widely in food chains, especially those at the pinnacle of the food chain **(King, 2013)**. Metal ions can be integrated into food chains and accumulated in aquatic organisms, and subsequently affect their physiological state **(Wang, 2002)**.

Bioassessment was principally used to estimate water bodies' health as it relies on living organisms as a bioindicator exposed to the incorporation of conditions in the watershed **(Moreno *et al*., 2007)**. Fish and benthic macro-invertebrates are the most commonly used aquatic organisms to estimate water quality or to monitor the effects of contaminants in the aquatic environment **(López-López & Sedeño-Díaz, 2015)**. Macroinvertebrates are sensitive to local stressors such as chemical and riparian degradation as they are long-living with limited mobility organisms **(Cheimonopoulou *et al*., 2011)**. One of these macroinvertebrates is the non-biting midges (Chironomidae, Diptera), which are widely used in ecotoxicology; besides, it is the most abundant group of insects found in a freshwater environment **(Raunio *et al.*, 2011)**.

Fish are deemed one of the main bio- monitors in the aquatic ecosystem used for estimating heavy metal pollution **(Mehana *et al.*, 2020)**. Moreover, fish can accumulate metals besides being at the top of the food chain, thus metals can be transferred to humans through fish consumption causing diseases **(Hembrom *et al.*, 2020)**. Accumulation of contaminants as metals directly affect the fish's health and tissues **(Sures, 2006)**. Microscopic examination of tissues is the most important stage in assessing the hazard of pollutants through histopathological parameters **(Van der Oost *et al.*, 2003)**. Adverse impacts of pollutants can be observed in fish tissues before appearing in the behavior and external appearance of fish **(Mahboob *et al.*, 2020)**. *Oreochromis niloticus* is commonly present in Manzalh Lake. It is one of the most used fish species in laboratory research, fundamental field, and environmental and toxicological studies **(Mekkawy *et al.*, 2008)**. It has several characteristics that can qualify it to be a suitable model and an indicator in bio-monitoring programs.

Recently, the Egyptian government concentrated its work on the development of the lakes, raised its efficiency, and decreased the discharge of the factories to the lake. The government's work resulted in a change in the hydrological, chemical, and biological characteristics of Manzalah Lake. The current work aimed to assess the lake water quality by the histopathology and ultrastructure of the liver and gonads of tilapia fish as a bioindicator for the lake quality during the governmental development project process at Manzalah Lake. The results of this study would participate in the literature on the heavy metals and physicochemical parameters of the water and sediment in the study area, providing a baseline for future investigations.

MATERIALS AND METHODS

Study area

Lake Manzalah is a brackish, shallow, and eutrophic semi-tropical lake that receives its saltwater from the Suez Canal and the Mediterranean Sea through Boughaz El-Gamil and Boughaz El-Sheikh Ali. It receives a yearly influx of fresh water from the following drains: Hadous, Bahr El-Baquar, Ramsis, El-Matariya pump station, and Faraskur pump stations **(Lotfi *et al*., 2016)**. This study was carried out in two areas. Area (A) is near to Hadous drain (32° 08′ 00″ E and 31° 09 ′ 00 ″ N), while area (B) is near Bahr El-Baqar drain (31° 12′ 00″ E and 31° 11′ 00″ N). There are 7 kilometers between the two areas (Fig. 1). Bahr El-Baqar and Hadous drains are the senior drains in the Egyptian Delta. Both have low levels of dissolved oxygen and high pollution that affect the aquatic life **(Mohammed & Mahran, 2022)**. Bahr El-Baqar drain serves an agricultural area of about 119.2km^2, covering almost 25% of the total discharge into the lake, providing the largest flow rates and pollution loads which contribute heavily to the deterioration of the water quality of the lake **(Zahran *et al*., 2015)**. Hadous is more than a typical agricultural serving some agricultural land of about 1756.96km^2. It participates to about 49% of the total inflow **(El-Amier *et al*., 2021)**. Although it is not contaminated with industrial or sewage discharge, it carries the biggest loads of pesticides (**Wahaab & Badawy, 2004**). Recently, the government has largely eradicated unlawful infringements from the lake’s fillers for housing or agriculture or the creation of unlicensed fish farms, which was abolished between 2017 and 2021 (Fig. 2).

Fig. 1. The sampling locations at El-Manzalah Lake

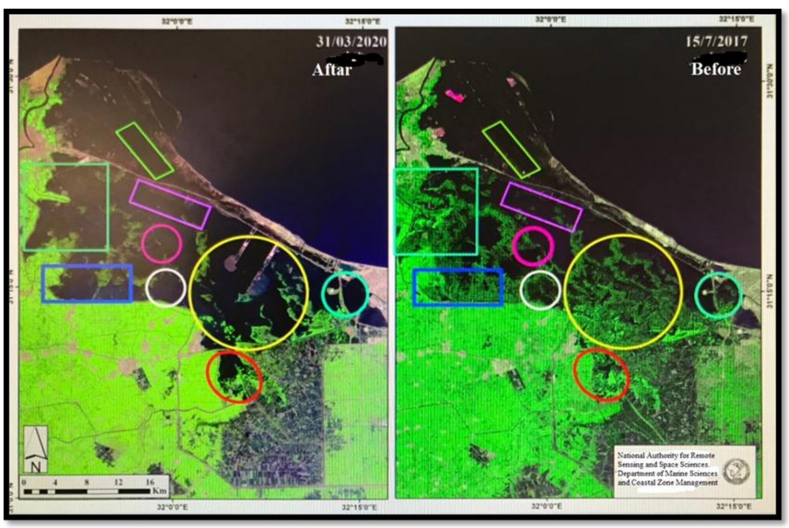

Fig. ٢. Changes in Lake Manzalah from July 2017 to March 2020 (before and after cleaning) (National Authority for Remote Sensing and Space Sciences, Department of Marine Sciences and Coastal Zone Management)

Sample collection

Water and sediment samples were randomly collected at 8.00 and 10.0 am in 2020 from six spots in triplicate at each site per season. Macro-invertebrates were collected using dip-netting and kick-netting methods. Samples were taken from the bottom and the surface of the water layer at the two sites in three replicates to evaluate the macro-invertebrate community through a sieve screen, with a mesh size of 500m. The samples were washed to get rid of any silt or dirt. In 80% glycerol alcohol, the obtained samples were stored. The samples were once again cleaned in a net with a mesh size of 500m in the lab before being categorized using a binuclear dissecting microscope. Keys were primarily used for taxa identification (**El-Azim *et al.*, 2018; Saad El-Din & Gad El-HaK, 2017)**, and they were able to identify them down to the family level, and the main description of a species was based on larvae specimens.

A total of 288 live Nile tilapia (*Oreochromis niloticus*), male and female, were gathered and brought to the lab in battery-aerated tanks containing the same water for analysis. After being delivered to the lab, fish were dissected, and the liver and gonads were removed and divided into three sections. One of which was quickly frozen to determine the content of heavy metals. The other two pieces were devoted to histological and ultrastructure examination.

Physical and chemical examination

The physical and chemical parameters of water and sediment were examined according to the methodology of **El-Hak *et al*. (2021)**. A digital pH meter was used to measure pH, and a hydro lab was utilized for measuring conductivity. Water alkalinity was instantly measured using phenolphthalein and methyl orange indicators. Chloride was measured using Mohr's technique, whereas sulfate was measured using a turbidimetric method. **Nan *et al.* (2016)** determined bicarbonate and carbonate levels. Magnesium and calcium were determined using direct titration with EDTA solution, whereas Na+, K+, Mg++, and Ca++ were determined using a flame photometer model "Jenway PFP, U.K."

Heavy metals assessment

Heavy metals in water, sediment and fish tissues (liver and gonads) were assessed following the methodology of **El-Hak *et al*. (2022)**. Water samples were obtained using polypropylene containers that were preserved at 4°C while being bathed in 10% nitric acid, deionized water, and 0.2% v/v nitric acid. According to the procedure of **ávan den Akker and Delft (1991)**, the sediment samples were dried at 105°C, ground, and sieved before being digested with a concentrated H_2O_2, HCl, and HNO_3 combination for around 1.0gm. Analysis was done on Cu, Fe, Mn, Ni, and Pb levels. According to **Gad El-Hak and Mobarak (2020)**, the accumulation of heavy metals in the liver and gonads was measured.

Following the steps of **El-Shenawy (2004)**, metal determinations were conditioned. A PERKIN ELMER Optima 2000 DV equipment was used to perform atomic emission spectrophotometry, with inductive coupling plasma (ICP-OES) to measure the levels of copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), lead (Pb), cadmium (Cd), nickel (Ni) and zinc (Zn).

Histopathological & electron microscopy examination methods

Histopathological examination

A portion of the liver and gonads was taken and preserved for 24 hours in 10% neutral formalin before being washed in tap water, dehydrated with an escalating ethanol series, injected with xylene, and finally embedded in paraffin wax. According to **Fischer *et al.* (2008)**, paraffin blocks were cut into sections and then stained with hematoxylin and eosin (H&E). Finally, slides were taken, analyzed, and blindly photographed.

Ultrastructure examination

Ultrastructure preparations were carried out based on the study of **Gad El-Hak and Mobarak (2020)**. Fresh tissues from the liver and gonads were cut into cubes which were not bigger than $1mm^3$. The samples were then fixed by quickly immersing them in 3% of glutaraldehyde solution overnight at 4°C, dehydrated in an escalating sequence of alcohols before being embedded in Araldite resin after being rinsed in sodium cacodylate buffer at 4°C and post-fixed with 1% osmium tetroxide for 1 hour. The ultra-thin sections were put on copper mesh grids, stained with uranyl acetate and lead citrate and then studied by TEM. The semi-thin sections were prepared for microscopic examination.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA), followed by the post hoc Duncan test.

RESULTS

1. Macroinvertebrates

Two species of Mollusca and two freshwater arthropod species were discovered during a survey of Lake Manzala's at two locations. These arthropods were a chironomid (Family: Chironomidae; Suborder: Nematocera) and a tabanid (Family: Tabanidae, Suborder: Brachycera) belonging to order Diptera of class Insecta (Fig. 3). When compared to the overall amount of aquatic biota, Chironomid larvae were numerous at both sites, with a higher number in Bahr Elbaqar station, compared to Hadous station.

Fig. 3. (a) Chironomidae and (b) Tabanidae

2. Physical and chemical analysis

Tables (1, 2) show the physical and chemical parameters of water and sediment in the two examined areas of Lake Manzalah in spring and autumn. The regional and seasonal physical and chemical parameters of water and sediment were significantly varied ($P \geq 0.05$).

Table 1. Water's physical and chemical parameters (mean ±SE) from studied areas of Manzalah Lake in the spring and autumn seasons

Parameter	Spring		Autumn	
	Area (A)	Area (B)	Area (A)	Area (B)
pH	7.16±0.1	7.23±0.04	7.38±0.2	7.39±0.1
Electrical conductivity (EC) (µs/cm)	0.32±0.04	0.3±0.04	0.30±0.04	0.32±0.03
Chloride (Cl−1) (mg/L)	1.3±0.2[a]	0.75±0.4[b]	0.93±0.4[b]	1.17±0.2[a]
Sulfate (SO4−2) (mg/L)	0.36±0.05	0.27±0.1	0.37±0.1	0.47±0.07
Bicarbonate (HCO3-) (mg/L)	1.67±0.1	1.43±0.2	1.73±0.1	1.77±0.1
Sodium (Na+1) (mg/L)	1.03±0.03[a]	0.75±0.3[b]	0.7±0.2[b]	1.13±0.2[a]
Potassium (K+1) (mg/L)	0.25±0.03	0.22±0.05	0.2±0.04	0.27±0.03
Magnesium (Mg+2) (mg/L)	1.2±0.1[a]	0.87±0.1[b]	1.07±0.2[a]	0.96±0.07[b]
Calcium (Ca+2) (mg/L)	1.06±0.03[a]	1.13±0.07[a]	1.06±0.05[a]	1.17±0.14[b]

Data with different letters in the same column are statistically different at $P < 0.05$ level.

Table 2. Sediment's physical and chemical parameters (mean ±SE) from studied areas of Manzalah Lake in the spring and autumn seasons

Parameter	Spring		Autumn	
	Area (A)	Area (B)	Area (A)	Area (B)
pH	7.56±0.035	7.54+0.09	7.52+0.034	7.4+0.14
Electrical conductivity (EC) (μs/cm)	2.29±0.087[a]	2.5+0.32[a]	0.48+0.003[b]	0.53+0.06[b]
Chloride (Cl−1) (mg/L)	15.67±3.6[b]	19.67+4.9[a]	4.67+2.19[d]	6.7+2.05[c]
Sulfate (SO4−2) (mg/L)	30.67±6.6[a]	28.87+6.4[b]	26.37+7.67[c]	29.77+0.42[b]
Bicarbonate (HCO3-) (mg/L)	3.33±0.46	3.36+0.4	3.1+0.47	3.53+0.26
Sodium (Na+1) (mg/L)	6.33±2.59[a]	11.8±4.41[a]	2.53±2.27[b]	3.43±1.05[b]
Potassium (K+1) (mg/L)	0.67±0.11[ab]	0.93±0.25[a]	0.53±0.07[b]	0.4±0.047[b]
Magnesium (Mg+2) (mg/L)	3.37±0.82	2.93±0.89	2.63±0.62	3.03±1.02
Calcium (Ca+2) (mg/L)	4.37±1.05[a]	3.17±0.19[ab]	2.4±0.32[b]	2.77±0.54[b]

Data with different letters in the same column are statistically different at $P < 0.05$ level.

3. Heavy metals assessment in water, sediment and fish (liver and gonads)

The evaluation of heavy metals in Lake Manzala's water and sediment during the seasons of spring and autumn is presented in Tables (3, 4), respectively. In water and sediments, most of the heavy metals (Fe, Mn, Cu, Ni, Zn, Pb and Co) were significantly ($P \leq 0.05$) higher in autumn than in spring and in area (B) than in area (A). The largest concentrations were found in Fe, followed by Mn and Ni. It was only in autumn that the zinc was detected in water, whereas it was only found in sediment in area (B) during both seasons. It is worthy to mention that, Cd wasn't detected in water and sediment during the seasons of spring and autumn.

Heavy metals concentrations (Fe, Mn, Cu, Ni, Zn and Pb) in spring and autumn in fish liver and gonads are presented in Table (5). Generally, fish tissues possess significantly higher heavy metals concentration ($P \leq 0.05$) in autumn than in spring in both areas. However, metal accumulation was significantly higher in area (B) than in area (A) in spring, and Pb accumulation was recorded in tissues only in area (B) in autumn. The order of metal accumulation in tissues was Fe > Zn > Mn > Cu > Ni. Fe recorded the highest concentration of heavy metal in tissues in the fish liver of region (B) in autumn. Generally, the accumulation of heavy metals reached its highest in the liver, followed by the testis and ovary. Whereas, cobalt and cadmium weren't detected in both tissues collected from the two sites area.

Table 3. Heavy metals assessment in water (mean ± SE) from two studied areas of Lake Manzalah in the spring and autumn seasons

Heavy metal	Spring		Autumn	
	Area (A)	Area (B)	Area (A)	Area (B)
Iron, Fe (mg/l)	0.23±0.006[c]	5.19±0.7[b]	1.46±0.02[c]	49.1±2.7[a]
Manganese, Mn (mg/l)	0.015±0.002[c]	0.14±0.02[b]	0.093±0.02[b]	0.34±0.03[a]
Copper, Cu (mg/l)	0.0063±0.003[b]	0.023±0.007[b]	0.02±0.007[b]	0.095±0.05[a]
Nickel, Ni (mg/l)	0.15±0.02[b]	0.19±0.02[b]	0.37±0.007[a]	0.177±0.07[b]
Zinc, Zn (mg/l)	Nd	Nd	0.021±0.004[b]	0.059±0.006[a]
Lead, Pb (mg/l)	0.175±0.001[c]	0.18±0.01[a]	0.0174±0.0007[c]	0.089±0.03[b]
Cobalt, Co (mg/l)	0.2±0.003[ab]	0.174±0.01[b]	0.24±0.02[a]	0.139±0.03[b]

Data with different letters in the same column are statistically different at $P < 0.05$ level; Nd: not found.

Table 4. Heavy metals assessment in sediment (mean ± SE) from two studied areas of Lake Manzalah in the spring and autumn seasons

Heavy metal	Spring		Autumn	
	Area (A)	Area (B)	Area (A)	Area (B)
Iron, Fe (mg/l)	24764±2052[c]	34007±2373[b]	34426+6669[b]	35335±7043[a]
Manganese, Mn (mg/l)	122.2±11.9[d]	240.67±26.9[c]	304.3±39.2[b]	658.7±110.6[a]
Copper, Cu (mg/l)	11.3+1.5[d]	15.3+2.1[c]	44+4.1[a]	24.16+4.6[b]
Nickel, Ni (mg/l)	20.67+4.4[c]	18.7+2.8[cd]	369.3+80.1[b]	429.2+35.1[a]
Zinc, Zn (mg/l)	Nd	21.5+3.1[a]	Nd	21.74+3.2[a]
Lead, Pb (mg/l)	13.7+2.1[c]	68.7+8.8[b]	67.3+9.4[b]	130.1+8.9[a]
Cobalt, Co (mg/l)	126.3±9.1[d]	140.7±25.47[c]	264.6±12.9[a]	162.2±15.87[b]

Data with different letters in the same column are statistically different at $P < 0.05$ level; Nd: not found.

Table (5): Heavy metals assessment (mean ±SE) in fish tissues (liver, testis and ovary) of *O. niloticus* from two studied areas of Lake Manzalah in spring and autumn

Heavy metal	Spring						Autumn					
	Area (A)			Area (B)			Area (A)			Area (B)		
	Liver	Ovary	Testis	Liver	Ovary	Testis	Liver	Ovary	Testis	Liver	Ovary	Testis
Fe (mg/l)	998.2 ±4.4^{a}	198.5 ±4.2^{h}	133.6 ±6.8^{i}	942 ±17.2^{c}	211 ±6.5^{g}	231 ±9.1^{g}	975.7 ±20.1^{b}	247.1 ±10.1^{g}	219.3 ±8.9^{g}	912 ±6.4^{d}	397.3 ±5.2^{e}	310.4 ±5.1^{f}
Mn (mg/l)	4.02 ±0.4^{h}	3.19 ±0.1^{i}	4.29 ±0.12^{h}	11.8 ±0.1^{d}	5.6 ±0.2^{g}	7.8 ±0.1^{e}	6.5 ±0.3^{f}	5.5 ±0.1^{g}	6.74 ±0.3^{f}	17.8 ±1.1^{a}	15.6 ±0.1^{b}	12.6 ±0.2^{c}
Cu (mg/l)	1.27 ±0.1^{g}	0.6 ±0.03^{h}	1.47 ±0.2^{f}	3.39 ±0.1^{e}	5.1 ±0.1^{c}	5.42 ±2.8^{b}	4.23 ±0.9^{d}	3.32 ±0.3^{e}	1.19 ±0.1^{g}	11.7 ±0.28^{a}	5.3 ±0.08^{b}	11.3 ±0.14^{a}
Ni (mg/l)	0.83 ±0.1^{h}	0.37 ±0.1^{i}	0.55 ±0.5^{g}	1.53 ±0.2^{f}	1.2 ±0.1^{g}	1.9 ±0.1^{c}	1.82 ±0.1^{d}	1.32 ±0.1^{e}	1.3 ±0.1^{e}	2.37 ±0.2^{a}	1.43 ±0.09^{f}	2.22 ±0.2b
Zn (mg/l)	166.3 ±7.9^{k}	127.6 ±9.5^{l}	173.5 ±7.17^{j}	2926 ±1.1^{a}	2089 ±7.5^{c}	469.6 ±9^{h}	350 ±7.1^{i}	454.9 ±13.9^{g}	602.9 ±6.8^{f}	1135 ±6.3^{e}	2749 ±10.1^{b}	1514 ±6.7^{d}
Pb (mg/l)	nd	nd	nd	nd	Nd	nd	nd	nd	nd	0.041± 0.004^{a}	0.031± 0.003^{c}	0.034± 0.004^{b}

Data with different letters in the same column are statistically different at $P< 0.05$ level; Nd: not found.

4. Histopathological examination

4.1 *Examination of the liver*

The results showed that the liver of *O. niloticus* obtained during spring from the area (A) had a normal fish liver structure, consisting of polyhedral hepatocytes organized in cords around a central vein and divided by sinusoids (Fig. 4a), while liver collected from area (B) showed hepatocytes with fatty degeneration (Fig. 4b). On the other hand, liver collected in autumn from both sites area presented an array of histopathological alternations (Fig. 4c). Liver sections from area (A) showed that most of the hepatocytes were fatty degenerated. Focal necrosis with pyknotic nucleus and area of degenerated cells were recorded in liver sections area (B). Hepatic granuloma surrounding the encysted metacercaria was only detected in area (B) during autumn (Fig. 4d).

Results of the *O. niloticus* hepatopancreas collected during spring from area (A) and area (B) showed normal structure among the hepatocytes. The hepatopancreas might be considered as islets with their lamellar lining in an acinar pattern surrounding a branch of the portal vein (Figs. 5a, c). While, the hepatopancreas of *O. niloticus* collected in autumn from area (A) showed irregular walls, elongated appearance and infiltration of inflammatory cells (Fig. 5b). Whereas, the hepatopancreas of *O. niloticus* collected in autumn from area (B) showed reduction and rounded appearance surrounded with adipocytes (Fig. 5d).

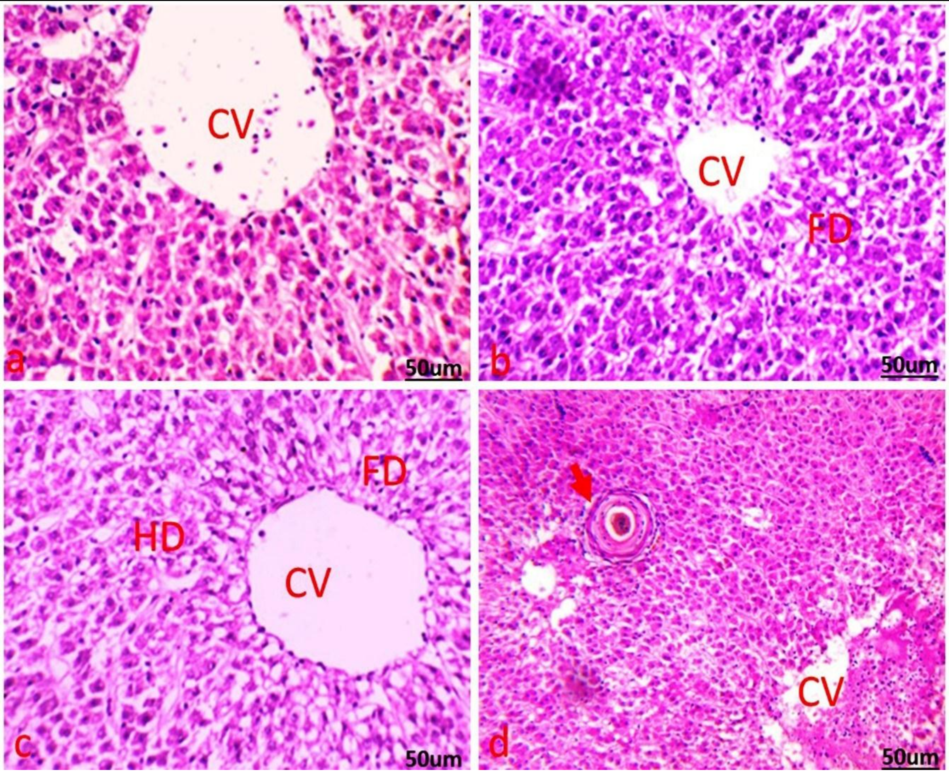

Fig. 4. Light micrographs of *O. niloticus* liver sections (H&E, 200X) showing: **(a)** Liver from area (A) during spring with normal liver architecture; **(b)** Liver from area (A) during autumn with fatty degenerated hepatocytes (FD); **(c)** Liver from area (A) during spring with degenerated hepatocytes (FD) & (HD), and **(d)** A substantial region of necrotic tissue around the central vein (CV) and granuloma encased encysted metacercaria (arrow) in the liver from area (B) during autumn.

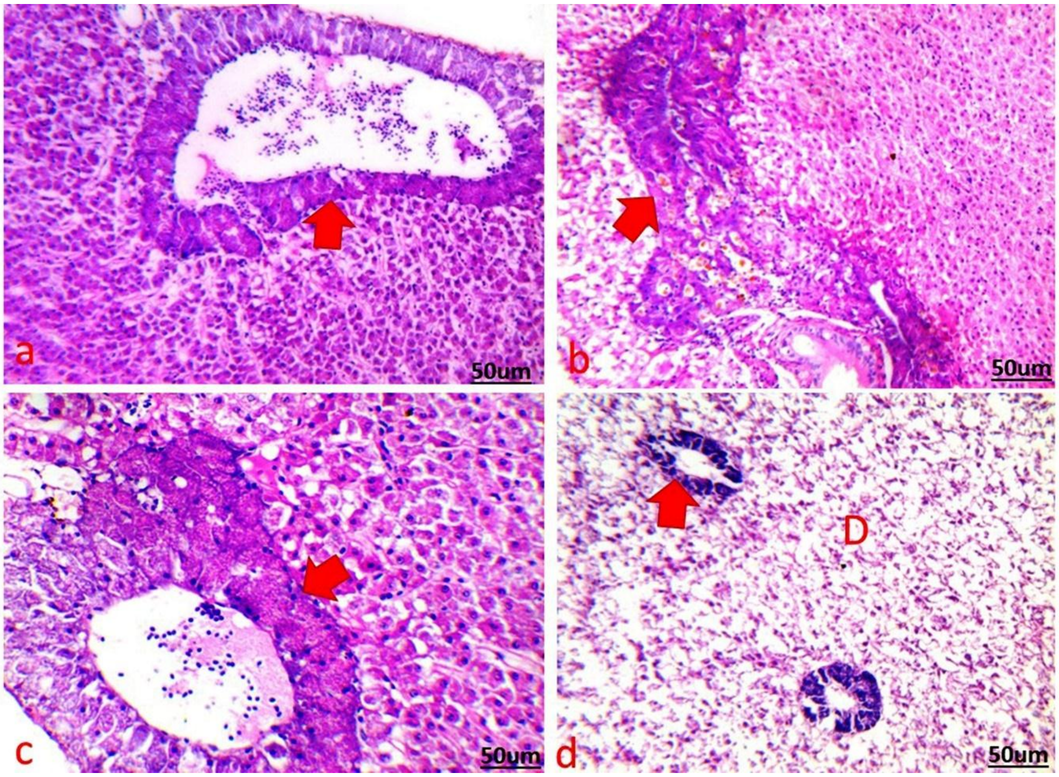

Fig. 5. Light micrographs of *O. niloticus* liver sections (H&E, 200X) showing: **(a)** Liver from area (A) during spring with normal hepatopancreas architecture (arrow); **(b)** Liver from area (A) during autumn exhibiting elongated hepatopancreas and infiltration of inflammatory cells; **(c)** Liver from area (A) during spring with normal hepatopancreas architecture (arrow), and **(d)** Liver from area (B) during autumn displaying rounded and small hepatopancreas surrounded with degenerated hepatocytes.

4.2 *Examination of the gonads*

O. niloticus testes were obtained from the two-region Manzalah Lake throughout the two seasons (spring and autumn), and their histological analysis revealed seminiferous tubules with some degenerative alterations. They showed up empty in several spermatogenic cells. All the seminiferous tubules also had collapsed interstitial cells. In the seminiferous tubules, spermatids are reduced (Fig. 6).

The histological study of *O. niloticus* ovaries obtained from the two-region area of Manzalah Lake during spring and autumn revealed deformation from their ideal forms, thickness, and detachment of the membrane in all stages of oocytes. Furthermore, necrotizing region and atresia in oocytes were observed (Fig. 7).

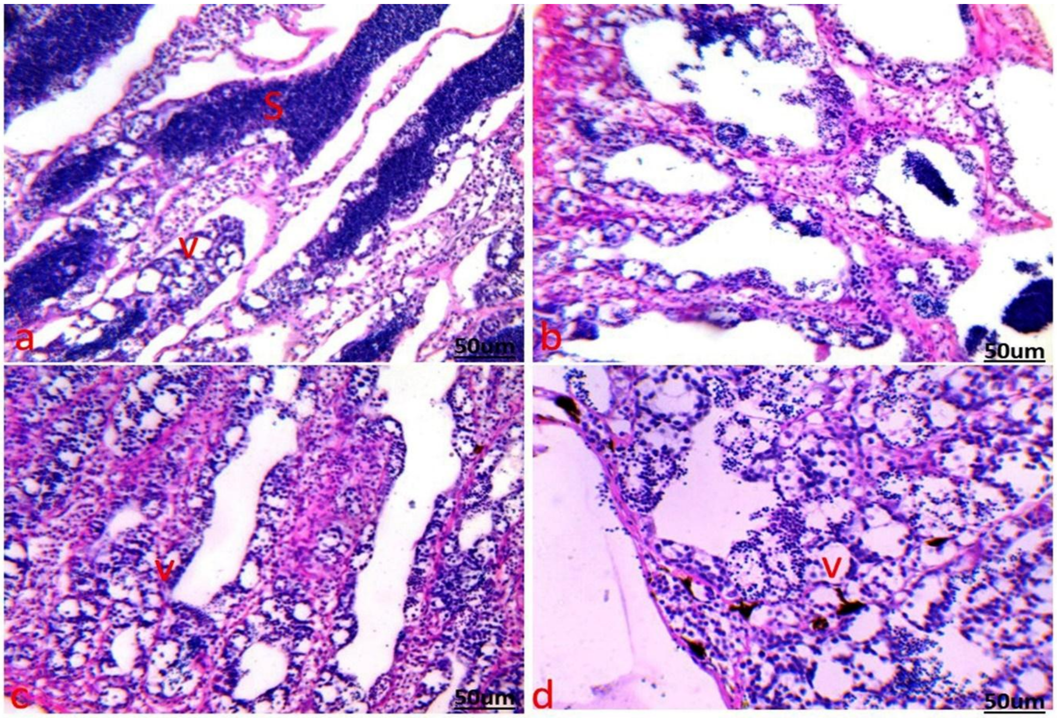

Fig. 6. Light micrographs of *O. niloticus* testicular sections (H&E, 200X) showing: **(a & c)** Testicular section from area (A) during the spring with degeneration in the spermatogenic stage (V); **(b)** Testicular section from area (A) during autumn displaying degeneration in the spermatogenic stage, and **(d)** Testicular section from area (B) during autumn exhibiting lost spermatogenic identity, with irregular arrangement and severe degeneration in spermatogenic stage (V).

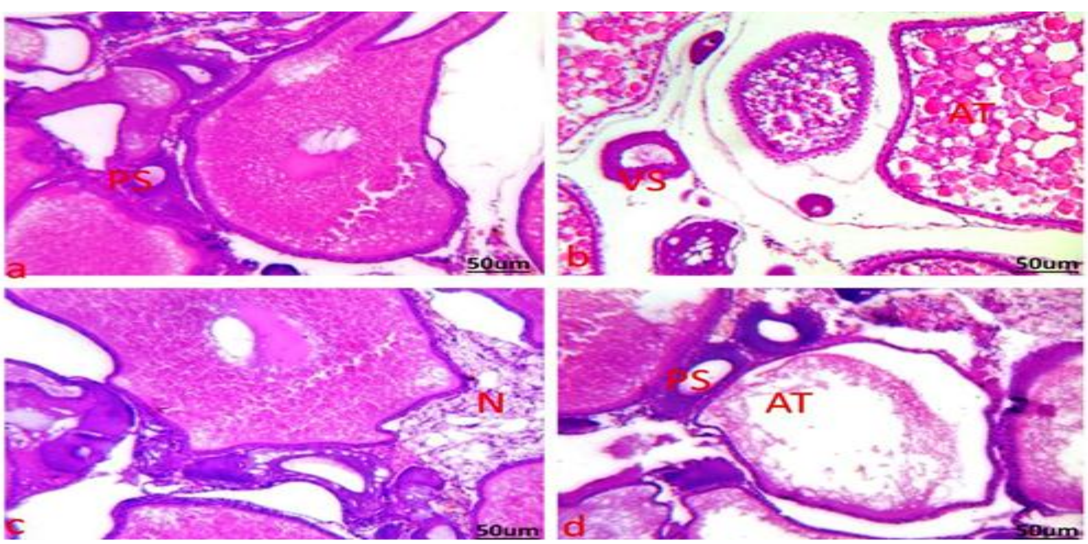

Fig. 7. Light micrographs of ovary sections of *O. niloticus* (H&E, 200X) showing: **(a)** Ovarian section from area (A) during spring with thickened wall in previtellogenic stage (PS) and absence of its nucleus; **(b)** Ovarian section from area (A) during autumn displaying thickened walls of vitellogenic stage (VS) and atretic cell (AT); **(c)** Ovarian section from area (A) during spring with hyperplasia and necrotic stroma accompanied by infiltration of inflammatory cells (N), and **(d)** Ovarian section from area (B) during autumn exhibiting degenerated atretic cells and thickened wall of previtellogenic stage (PS) and absence of their nucleus.

5. Ultrastructure alterations

5.1 TEM examination of the liver

The liver of *O. niloticus* obtained in spring and autumn from areas A and B (Figs. 8a, b) show typical ultrastructure composition with a massive nucleus with a dense nucleolus and several rough endoplasmic reticula (RER) arranged around the nucleus. Few mitochondria and small lysosomes were also recorded. Glycogen granules were abundant through the cytoplasm and the peripheral margins of the hepatocytes. In contrast, the ultrastructure of tilapia hepatocytes in autumn was markedly altered. Hepatocytes of the liver from area (A) showed the appearance of different-sized lipid droplets, secondary lysosomes with heterogeneous inclusions beside some degenerated mitochondria (Fig. 8c). However, hepatocytes of liver from area (B) showed that most hepatocytes lost intracellular organization. Besides, lipid droplets and secondary lysosomes, and autophagic vesicles were also recorded. RER and mitochondria were fractionated and degenerated, and the nucleus was aberrated. Many cells were necrotic and degenerated (Fig. 8d).

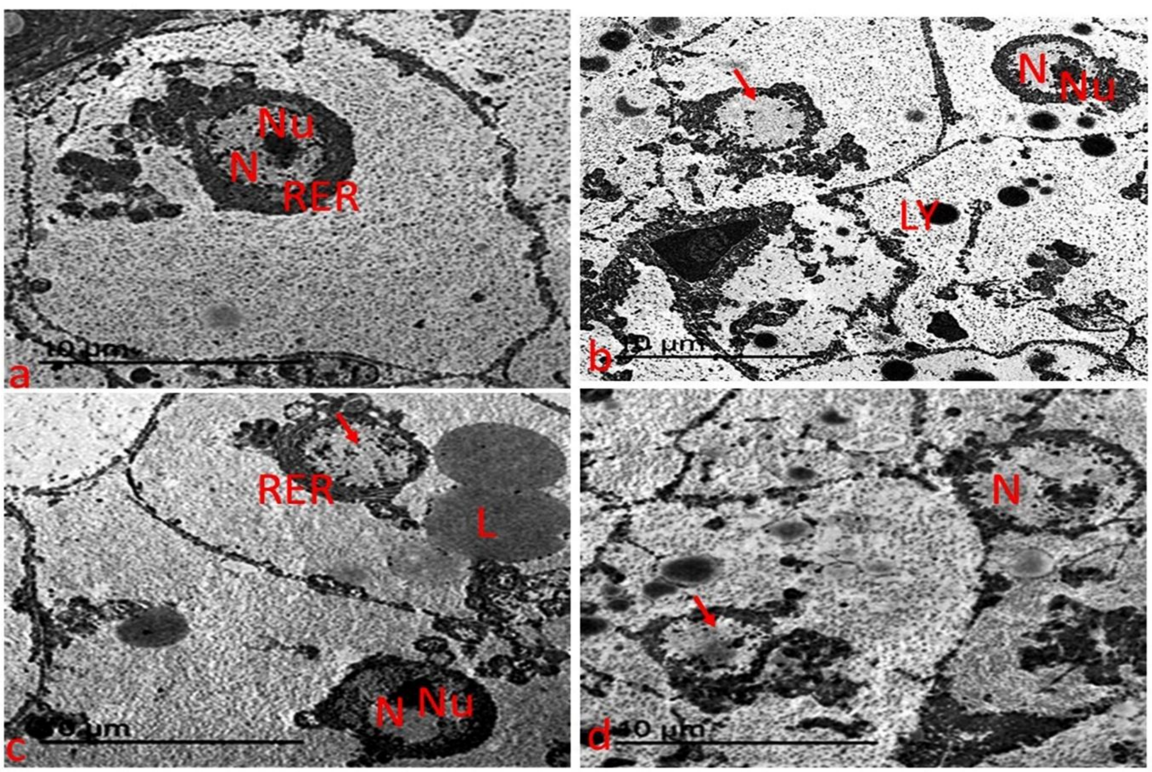

Fig. 8. Transmission electron micrographs of liver of *O. niloticus* showing: **(a)** From area (A) in spring showed normal ultrastructure organization, nucleus (N), nucleolus (Nu) surrounded with rough endoplasmic reticulum (RER); **(b)** Samples from area (A) in autumn with normal ultrastructure organization of nucleus (N), nucleolus (Nu) surrounded with rough endoplasmic reticulum (RER) and lysosomes (Ly). In addition, the nucleus without nucleolus is surrounded by degenerated rough endoplasmic reticulum

(arrow); **(c)** Samples from area (B) in spring displaying condensed nucleus (N) and nucleolus (Nu) surrounded with the compact rough endoplasmic reticulum (RER). In addition, nucleus without nucleolus is surrounded by degenerated rough endoplasmic reticulum (RER) and many lipid droplets (L), and **(d)** Samples from area (B) in autumn with a condensed nucleus (N) and necrotic nucleus (left side down) and a nucleus without nucleolus with degenerated rough endoplasmic reticulum (arrow).

5.2 TEM examination of the gonads

The results illustrated that, *O. niloticus* testis collected in spring and autumn from area A and area B showed spermatid cells with a strongly condensed nucleus. The plasmalemma is tightly bound to the nucleus. The cytoplasm is now constricting around the flagella, and the surrounding collar is forming. The ultrastructure examination of testes of *O. niloticus* from area (A) in spring and autumn showed normal ultrastructure organization of spermatid with few cells with vacuolation in the plasmalemma (Figs. 9a, b). Testes of *O. niloticus* from area (B) in spring showed vacuolation in the basal lamina and myeloid cells, while testes of *O. niloticus* from area (B) in autumn showed an increase in mast cells and spermatids with vacuolation in the plasmalemma (Figs. 9c, d).

The results revealed that, *O. niloticus* ovary collected in spring and autumn from area A and B showed normal ultrastructure organization of presynaptic oocytes, indicating that the nucleus is characterized by dense chromatin material; there is a large number of mitochondria, endoplasmic reticulum and Golgi bodies in the cytoplasm of the presynaptic oocyte. The oocyte showed the migration of the nucleus toward the animal pole. The ultrastructure examination of ovary of *O. niloticus* from area A in spring and autumn showed condensed nucleated necrotic oocyte and degenerated basement membrane (Bm), degenerated oocyte and edema (Figs. 10a, b). Ovary of *O. niloticus* from area B in spring and autumn showed condensed presynaptic oocyte, increased in the degenerated oocyte with vacuolated degenerated basement membrane and vitelline layer and edema (Figs. 10c, d).

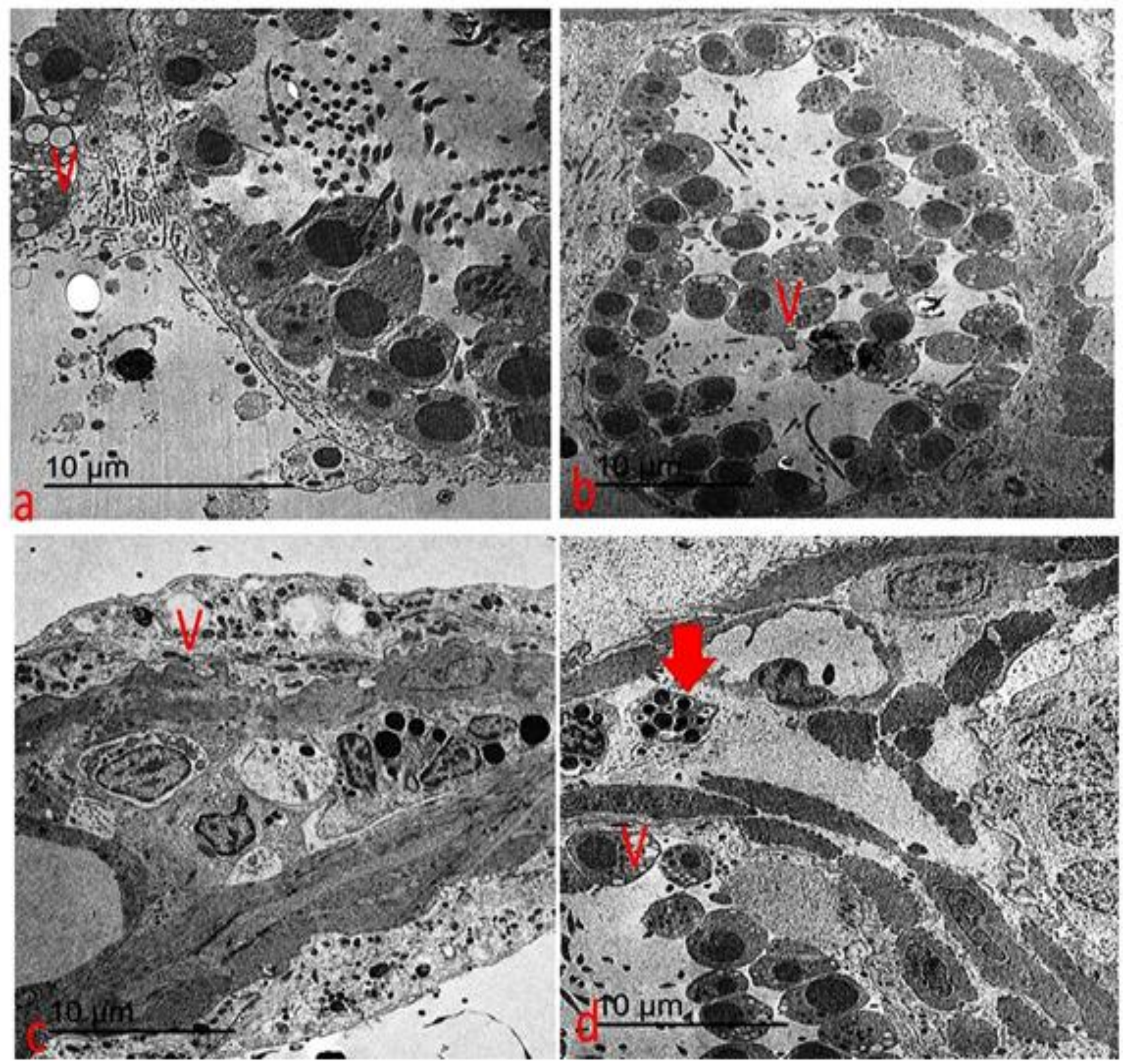

Fig. 9. Micrographs of transmission electron microscopy of testes of *O. niloticus* showing: **(a)** Samples from area (A) in spring with a normal ultrastructure organization of spermatid with few cells vacuolated in the plasmalemma (V); **(b)** Samples from area (A) in autumn exhibiting a normal ultrastructure organization of spermatid with few cells showing vacuolation (V) in the plasmalemma; **(c)** Those from area (B) in spring displaying vacuolation (V) in the basal lamina and myeloid cells, and **(d)** From area (B) in autumn showing an increase in mast cells (arrow) and spermatid with vacuolation (V) in the plasmalemma.

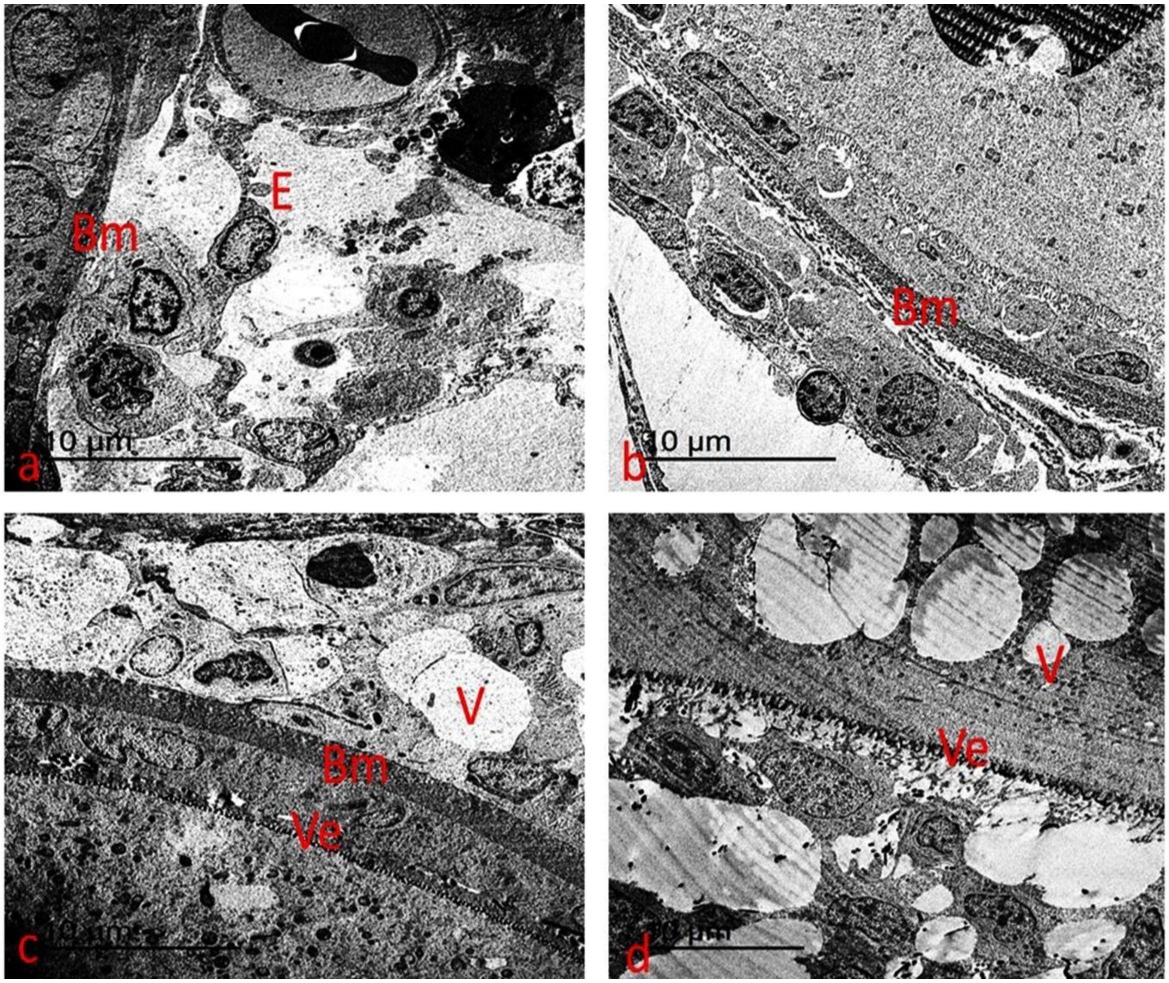

Fig. 10. Micrographs of transmission electron microscopy of the ovary of *O. niloticus*
(a) From area (A) in spring showed normal ultrastructure organization of presynaptic oocyte and basement membrane. Some cells showed degenerated oocytes and edema (E); **(b)** Area (A) in autumn showed condensed nucleated necrotic oocytes and degenerated basement membrane (Bm); **(c)** From area (B) in spring showed condensed presynaptic oocyte, vacuolated degenerated cells with normal basement membrane (Bm) and vitelline layer (Ve), and **(d)** Area (B) in autumn showed an increase in vacuolated degenerated oocytes (v) and degenerated vitelline layer (Ve).

DISCUSSION

Lake Manzalah is one of Egypt's largest and most productive lakes **(Mageed, 2007**). However, it is exposed to robust anthropogenic pressure as it is considered a cesspool for agricultural, industrial, and domestic sewage disposal and as a result, the quality of water and sediment is directly affected (**Abou-Elela, 2017**). Alteration in water quality directly influences the equilibrium of the aquatic environment, affecting water-living organisms (**Carpenter *et al.*, 2011)**, and thus the periodic analysis of water parameters and heavy metals contents is highly recommended.

Recently, the Egyptian government's efforts to clean the Manzala lake caused a change in the hydrological, chemical and biological characteristics of the lake. The present study showed that the pH of the water and sediment were both alkaline (7.1–7.5) which is optimal for fish to live (**Boyd, 2017**). Chloride, potassium and sodium are essential elements for all living organisms. However, chloride, potassium and sodium values in both water and sediment were less than the minimum acceptable levels **(Awadh & Ahmed, 2013**). **Valdebenito *et al.* (2015)** found that low chloride, sodium and potassium levels in water negatively affect fish productivity by directly affecting fish ovulation and egg quality. The levels of sulfate were lower than the maximum in both seasons and at both locations. The maximum sulfate concentration in water should not exceed 90 mg/L. (**Mutlu *et al.*, 2016**). Ca and Mg levels in sediment and water, as well as seasonal variations, are below-permitted limits. (**Jiries *et al.*, 2001**). According to **Westman & Savolainen (2002)** low Ca concentration in water may slow down fish growth.

Heavy metals are one of the major aquatic contaminants environments, because of their extreme toxicity, persistence, non-biodegradability, and bioaccumulative nature (**Isangedighi & David, 2019**). The current investigation found heavy metals in both water and sediment samples, with concentrations greater in sediments than in water. This might be due to heavy metals being quickly transported from water to sediments, leading to concentrated heavy metals in sediment (**Zhang *et al.*, 2019**). Additionally, heavy metal levels were greater at area (B) than at area (A) during the autumn than during the spring. This result agreed with **Helmreich *et al.* (2010)** who showed that the concentration of heavy metals was three times higher in the cold seasons than in the warmer ones. Moreover, the levels of heavy metals obtained from Bahr El Baqar drains were higher in samples than that obtained from Hadous drains depending on the types and amounts of waste discharged (**Zahran *et al.*, 2015**). The current results showed that values of all measured metals were higher than standard permissible levels according to WHO **(Hespanhol & Prost, 1994**) and at area (B) than area (A). This result is following **Omran (2016)** who found that Mn, Zn, Cu, and Pb levels were increased at the site which received domestic wastes discharged from Bahr-El Baqar drain. According to **Elhaj Baddar *et al.* (2021)** increased Cu values during autumn may be attributed to Cu deposition on sediment as CuS. While **Masoud *et al.* (2005)** explained that increased Mn values in autumn than spring might be related to Mn mobility from sediment to water as a result of microbial activity that decomposes organic debris. **Abdel-Satar (2005)** showed that increased Fe concentration is due to the large volume of agricultural, manufacturing, and domestic pollutants discharged during autumn. The maximum Pb concentration was detected in autumn in area (B) and this may be due to this site receiving domestic sewage which contains both dissolved and particulate forms of Pb in high concentration ***(*Casiot *et al*., 2009**). Pb is a very toxic non-essential heavy metal that has no biological role in living organisms. Thus, its presence in even low concentrations could affect fish health

(**Ali & Khan, 2018)**. Heavy metals can accumulate in fish tissues in very high concentrations directly from water and diet which could reach hundreds/thousands times over their levels in water, sediment and diet (**Laws, 2000**). Bioaccumulations of heavy metal in Tilapia tissues were greater in autumn than in spring, and within area (B) than in area (A). Fe was the most abundant metal in tissues, followed by Zn, Mn, and Ni. These findings are consistent with the current study's findings on heavy metal levels in water and sediment. This result is consistent with the findings of **Saeed & Shaker (2008), El-Hak *et al.* (2021); El-Hak *et al.* (2022),** who discovered that Fe, Mn, and Zn were the most abundant metals in tissues recovered from Lake Manzalah fish. Metal concentrations in the liver are often higher than in the gonads, suggesting that the liver is the initial target tissue for heavy metals in fish (**Cavas *et al*., 2005**). The failure of the liver's detoxifying processes as a result of increased metal intake, according to **Annabi *et al.* (2013),** is the cause of metal poisoning. The Nile tilapia (*Oreochromis niloticus*) liver at Bahr El Baqar station showed the greatest value for Fe concentration in the spring and autumn seasons due to the highest concentrations of Fe in the water and sediment during those seasons.

According to **Keçi *et al.* (2012),** macro-benthic fish and fauna are regarded as reliable indications of the aquatic ecosystem's biological and environmental health. Due to the vital work done by various species in the community, biodiversity plays a crucial role in the aquatic ecosystem. Since contaminated ecosystems have fewer species, this represents a loss of biodiversity and contributes to habitat damage (**Saad El-Din, 2006).** Only Two macro-benthic species were identified in the present study. They were represented in one phylum namely Arthropoda. The present studies showed Midges belonging to the family Chironomidae (Insecta: Diptera) are the most prevalent appeared in the studied region area (B) habitat. They are present in high densities. They were present due to the high amount of food and nutrients and the lack of competitors and predators in this area (**Langdon *et al.,* 2010**). Chironomid midges were highly accumulated heavy metals and were considered a significant component of the diet of several fish species (**Gerhardt, 1993**).

An essential element of aquatic habitats is fish (**Ormerod, 2003**). They are one of the most representative species in freshwater systems and a source of protein for humans in addition to being extremely sensitive to changes in their environment **(Altshuler *et al.*, 2011).** They are typically regarded as the best species for determining how environmental changes affect organisms, and they are employed as bio-indicators to track water pollution in aquatic ecosystems **(Kuklina *et al.,* 2013).** The exposure of fish in Lake Manzalah to diverse pollutants causes several pathological changes in the fish organs **(Yacoub *et al.*, 2021).**

The histopathological and ultrastructure changes observed in liver and gonads of *O. niloticus* are consistent with the tissues' bioaccumulation of heavy metals. The present study's results indicated that the liver and gonads of Tilapia gathered from area (B) showed several alterations than that collected from area (A). Hepatocellular necrosis, pyknosis, hydrobic and fatty degeneration and parasitic infection were the main observed histopathological alterations. Other fish species that live in polluted habitats have been described with these changes **(El-Hak *et al.*, 2021).** According to **Padmini & Usha Rani (2008)**, the direct impacts of pollutants on hepatocytes may be the cause of alterations in fish liver cells. The imbalance in lipid metabolism is thought to be the cause of lipid buildup and fatty degeneration in fish hepatocytes (**Lu *et al.*, 2012**). **Geeraerts & Belpaire (2010)** suggested that lipid accumulation due to metal exposure may be a prenecrotic stage for the fish. Furthermore, **Mehana *et al.* (2020)** suggest that parasite infection may be brought on by a weakened immune system brought on by the buildup of heavy metals in fish organs. This study discovered a change in *O. niloticus*'s hepatopancreas concurrent with an increase in the concentration of heavy metals that accumulated in the autumn.

Fish hepatopancreas cells had the most pathogenic changes, according to **Molina *et al.* (2005).** This is likely because of the organ's function and its high concentration of hydrolytic enzymes. This shows that the heavy metal that collected in the water and sediment may have contained an irritant that caused changes in the hepatopancreas of *O. niloticus*. The tilapias taken from the two areas in the autumn season showed the following characteristics in their histological sections: uneven walls, seemingly shrinking, with changed forms, others rounded vacuolized and expanded elongated. The loss of glycogen and/or lipids in the hepatocytes, a typical morphological reaction in the liver of fish exposed to toxic substances, may be the cause of these changes, according to **Wolf & Wolfe (2005).**

Fish population dynamics are significantly influenced by the gonad anatomy **(Pope *et al*., 2010)**. One of the numerous environmental conditions that might lead to a seriously harmed fish reproductive system is the presence of contaminants (**Mansour *et al.*, 2018**). According to the current study, the environmental effects of Lake Manzalah led to a noticeable decline in the gonad activity of the fish that were the subject of the study. This decline was evident in the histological and ultrastructural changes that occurred to the gonads of both sexes in the two study areas during the two seasons of spring and autumn. These effects could interfere with the growth of germ cells and inhibit fish reproduction (**Carnevali *et al.*, 2018)**. Additionally, the spermatogenesis and lobular structures of the fish whose testes were removed from the contaminated water were shown to be impaired, and **Elgaml *et al.* (2019)** research noted a suppression of sperm production. **Corriero *et al.* (2021)** reported follicular atresia, which is brought on by exposure to contaminants. According to **Monson *et al.* (2019),** there is a connection between pollution and the development of follicular atresia, which is brought on by decreased gonadotropin (GTH) and other estrogen hormone activities. Fish yolk degeneration and oocyte disruption were caused by problems in the reproductive hormone caused by pollution **(Jobling & Tyler, 2003** and **Zulfahmi *et al.*, 2018**).

Although the Lake's pollution discharges have decreased, it was discovered that *O. niloticus* gonads have deformed from their optimal forms. In the autumn and region (B), the ovary and testis deformations were visible.

At the ultrastructure level, the liver of Tilapia showed a normal ultrastructure appearance in spring in the area (A). In contrast, several varied ultrastructure changes were observed in spring in area (A) and in autumn from both sites including loss of intracellular organization, the formation of different-sized lipid droplets and the degeneration of RER. The histopathology analysis and the heavy metal bioaccumulation results in tissues are consistent with the ultrastructural alterations seen in the liver of *O. niloticus*. According to **Abdel-Moneim (2014),** cytoskeletal abnormalities brought on by chemicals and disturbances in hepatocellular homeostasis may be the cause of intracellular organization loss. Fish treated with Pb and Cd showed the production of lysosomes in the current investigation **(Atta *et al*., 2012)**. According to **Köhler *et al*., (2002),** an increase in lysosomes is the result of the cell's requirement to eliminate more cellular components that have been destroyed as a result of toxins.

When *O. niloticus* testes were examined ultrastructurally, the spermatids, basal lamina, and myeloid membrane underwent significant alterations that may have been brought on by the inflammatory effects of heavy metal buildup. Similar findings were made by **Loir *et al.* (1995),** who discovered that the seminiferous tubules' basement membrane, which is crucial for spermatogenesis, appeared vacuolated and wrinkled, which may have slowed fish sperm production. According to **Rocha *et al.* (2018),** heavy metal pollution may cause fish spermatozoa to produce more reactive oxygen species (ROS), which can damage the lipid matrix in spermatozoa's membranes and cause axonemal damage, decreased sperm viability, an increase in mid-piece abnormalities, decreased mitochondrial membrane potential, and even complete inhibition of spermatogenesis. The current study shows that the spermatid contains a significant number of mast cells. Heavy metal toxins and parasites may cause the recruitment of mast cells to the infection site **(Lauriano *et al*., 2012).** Acute mast cell activation is another characteristic of several forms of tissue damage (**Galli & Tsai, 2010).** Oogenesis in *O. niloticus* fish was shown to be slowed down by the current histological and ultrastructural analyses of the ovaries. The current work demonstrated that oogenesis was inhibited by inducing cell death, which was visible in the ultrastructure as a condensed nucleus.

CONCLUSION

The studied tissues' histopathological and ultrastructural changes were consistent with the levels of heavy metals present in the water, sediments, and tissues, showing that *O. niloticus* was not in good condition throughout the fall, which may have something to do with heavy metal pollution. As a result, the research showed that *O. niloticus* is regarded as a good model for a biomonitor of environmental health. Last but not least, despite the government's attempts to clean Manzalah Lake, the study revealed that more efforts are required to raise the lake's water quality and reduce environmental pollution, particularly in the fall to enhance the health of the fish that are produced. It is important to note that this research was conducted while the lake was being developed, hence it is strongly advised to conduct another evaluation of the water quality in Manzalah Lake once the development project has been completed for at least one year to provide more accurate results.

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