Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(3): 711 – 723 (2022)
www.ejabf.journals.ekb.eg

Biochemical alterations of Nile tilapia fish in El-Manzala Lake as an indicator of pollution impacts

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ARTICLE INFO

Article History:
Received: June 4, 2022
Accepted: June 11, 2022
Online: June 26, 2022

Keywords:
Manzala Lake,
O. niloticus,
Creatinine,
Urea and Uric acid.

ABSTRACT

This work is concerned with studying the effect of various pollutants in Lake Manzala on fish quality by investigating the biochemical characteristics of Nile tilapia fish collected during the winter and summer seasons 2020. The biochemical parameters of Nile tilapia fish were significantly different between the two seasons at all investigated stations. However, blood creatinine, urea, and uric acid showed the highest levels in fish collected from Bahr El-Baqer and El-Matariya during the study. Fish from polluted stations recorded an increase in serum cholesterol levels in cold season, and triglycerides levels of *O. niloticus* showed remarkable variations. Alterations in the physiological blood parameters reflect changes in the organism's metabolic and biochemical factors as a result of the effects of various pollutants, and they allow researchers to evaluate the mechanisms of pollutants' impacts.

INTRODUCTION

Lake Manzala is a brackish lake in the Nile Delta in northeastern Egypt. It is the largest of Egypt's northern delta lakes, covering around 500 km^2 and contributing approximately 34.2 percent of the total fish production of Egypt's northern lakes (**GAFRD, 2016**). Pollution from domestic, industrial, and agricultural sources is abundant in Lake Manzala. The wastewater discharge carries climate gases, huge quantities of particulate matter, bacteria, nutrients, heavy metals, and hazardous organics to Manzala Lake (**Ismail** and **Hettiarachchi, 2017**).The El-Serw Drain, Bahr El-Baqer Drain, Matariya Drain, Ramsis Drain, Hadous Drain, and Faraskour Drain all collect about 7500 million cubic metres of untreated industrial, domestic, and agricultural drainage water annually into the lake. Following the construction of the El-Salam Canal, the volume of wastewater was reduced to around 4000 million cubic metres (**El-Ghazali *et al.*, 2015; Ismail** and **Hettiarachchi, 2017**). Fish have very close interaction with their habitat, with only a thin epithelial barrier separating the fish's blood from the environment. Fish are highlydsc susceptible to physico-chemical changes, which are reflected in the components of their blood (**Ribelles *et al.*, 1995**).

.Blood is expected to possess pathological changes prior to the emergence of exterior poisoning symptoms. Blood parameters are regarded as whole-body pathophysiological markers. Biochemical characteristics of blood and tissues are among the important indices of the status of internal environment of the fish organism, and therefore, they're crucial for determining the structural and functional state of fish exposed to environmental toxins (**Adhikari *et al.*, 2004**). Alterations in the biochemical blood profile show changes in the organism's metabolism and biochemical processes as a result of various pollutants' impacts, and they help researchers to investigate the causes of pollution's impacts (**Luskova *et al.*, 2002**). The degree of variance in the hematological and biochemical responses are a useful tool for diagnosing fish health, and it can vary depending on stressor stimulation, therapy, parasitic or viral disorders (**Silveira-Coffigny *et al.*, 2004**).

The biochemical profile of fishes has been shown to be a sensitive measure used to assess the metabolism of fish under pollution stress. Studies proved that, fish exposed to pollutants had decreased amounts of proteins, lipids, and ALT and AST activity in their muscles (**Atli** and **Canli, 2007; Mohamed** and **Gad, 2008**).A variety of factors, including biological functions, ambient conditions, and species, influence hematological and biochemical parameters (**Ighwela *et al.*, 2012**). So this study aims to evaluate the biochemical parameters in **Nile tilapia** fish collected from El-Manzala Lake as a sensitive indicator of environmental stress for any chemical pollutant.

MATERIALS AND METHODS

Fish sample collection

Nile tilapia fish, *Oreochromis niloticus,* were collected during winter and summer seasons of the year 2020 from seven stations (El-Gamel, New El-Gamel, El-Bogdady, middle of Manzala Lake, Bahr El-Baker drain, El-Matariya, and El-Serw drain). The studied station were showed in **Table (1)** and graphically represented in **Figure (1).** A total of 70 fish were collected with a total weight (280±2.50 g) and a standard length (29± 3.0 cm).

Blood samples

After samples collection, using a 2 ml plastic syringe, blood was collected from the caudal peduncle. A small portion was mixed with EDTA dipotassium salt as an anticoagulant and placed in Eppendorf tubes, while the other portion was allowed to coagulate at room temperature for serum in a plain centrifuge tube. Serum was extracted from a blood sample and centrifuged (3000 rpm for 10 minutes at 4°C) before being stored in Eppendorf tubes at –20°C until analysis.

Table 1. The latitude and longitude of sampling stations at El-Manzala Lake (GPS).

Station	Features of station	Latitude	Longitude
1	El-Gamil	31.246898 N	32.198973 E
2	New El-Gamil	31.280964 N	32.165232 E
3	El-Boughdady	31.246898 N	32.198973 E
4	Middle of El-Manzala Lake	31.280161 N	32.024334 E
5	Bahr El-Baqer drain	31.201003 N	32.20768 E
6	El-Matariya	31.190336 N	32.03935 E
7	El-Serw drain	31.23626 N	31.971521 E

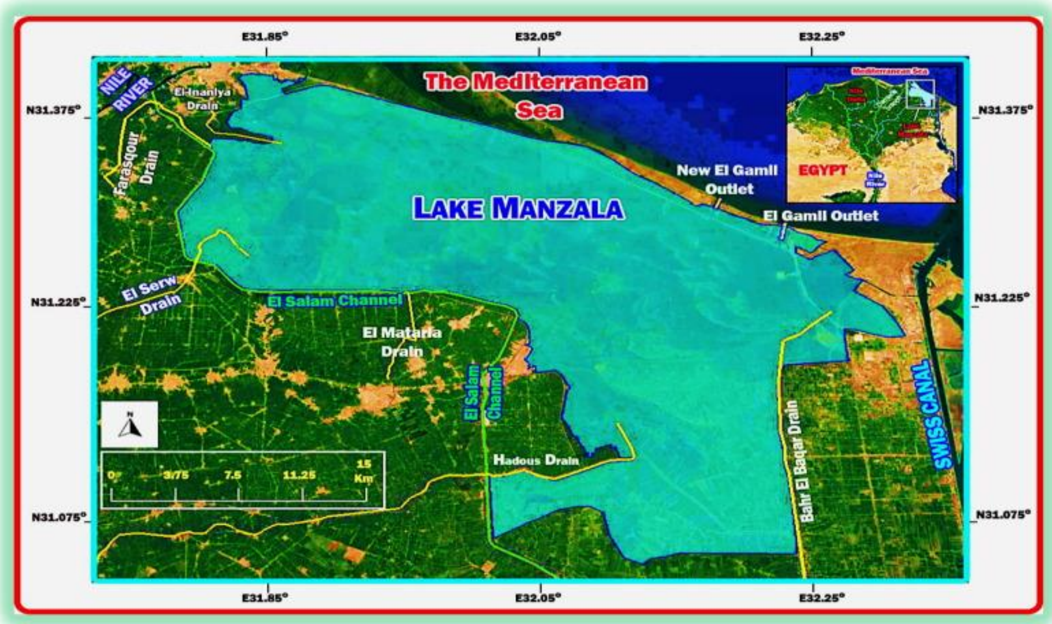

Figure 1. A map showing sampling sites at Lake Manzala

Biochemical investigations

The serum glucose concentration was estimated enzymatically according to **Trinder (1969).** The serum total proteins and albumin were measured colorimetrically according to the method described by **Gornall (1949)** and **Doumas (1986),** respectively. Serum creatinine was determined according to **Tietz (1986)**. While, the concentration of serum urea and uric acid were measured enzymatically according to **Tietz (1990).** The serum activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically using readily made kits according to the method described by (**Reitman** and **Frankal, 1957**). The serum total lipids, triglycerides, and cholesterol were estimated to **Frings** and **Dunn (1970).**

Statistical analysis

Two-way ANOVA was employed to evaluate the variability of the concentration of each metal with respect to different seasons and stations, using the software CoStat ver. 6.4 (CoStat, CoHort software, USA) and when significant differences were noted, multivariate, post hoc Tukey assessments were used to calculate the statistical difference between seasons. The analyzed data were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Blood biochemical parameters

Blood glucose levels

Serum glucose levels in *O.niloticus* were showed in **Table (2).** In winter, the glucose levels in *O. niloticus* recoded alterations, the lowest values were found at the middle of Manzala Lake (204.33±2.6 mg/dL) followed by El-Boughdady and El-Serw drain (216.3±2.3 and 264.16±9.6 mg/dL). The higher glucose levels were recorded in Bahr El-Baqer drain pursued with El-Matariya and New El-Gamil (363.15±25.4, 355.26±30.7 and 307.7±2.2mg/dL), respectively, in *O. niloticus* fish, serum glucose in *O.niloticus* showed a reduction, as compared to winter. Bahr El-Baqer drain recorded the highest glucose level (318.21±27.5 mg/dL) followed by El-Matariya (246.3±9.4 mg/dL) and El-Serw (195.1±4.6 mg/dL), while middle of Manzala Lake recorded the lower values (154.9±30.7 mg/dL). In winter, the hyperglycemia is a general secondary response of fish to acute toxic effects and is regarded a reliable sign of environmental stress. This indicates that the fish is stressed and is intensively creating higher glucose to create energy,which is utilized to treat stress caused by pollution in the environment (**Vosyliene, 1999**). Increase glucose in some stations at Lake Manzala might be attributed to the effect of pollution sources in that sites, as sewage and domestic wastes at Baher El-Baqer drain and sewage of El-Matariya and Port Said drain. On the other hand, glucose levels in summer were significantly decreased as compared to winter data. In fact, **Chavin and Young (1970)** and **Ottolenghi *et al.* (1995)** found that as the temperature increases, the evaluation of blood glucose concentration is reduced. In toadfish, **Nace *et al.* (I964)** discovered greater blood glucose concentrations in the winter than those in the summer, implying that surrounding temperature influences blood glucose levels in some way. The seasonal variation in glucose levels could be attributed to the varied sugar metabolism in different seasons. The silver catfish conserves glucose in low temperatures, resulting in gluconeogenesis, whereas it presumably eats glucose in warm water (**Lermen *et al.*, 2004**). According to **Tandon** and **Joshi (1974),** serum glucose levels in *Clarias batrachus* were elevated as the temperature dropped. During this time, they noticed the highest blood glucose level.

Serum total proteins and albumin

Total protein and albumin levels in *O.niloticus* showed in **Table (2).** In winter, total protein and albumin levels in *O. niloticus* were higher in middle of Manzala Lake (3.8±0.03 g/d) followed by El-Boughdady (3.43±0.5g/dL), New El-Gamil (3.16±0.33 g/dL), while Bahr El-Baqer drain recorded the lowest levels (2.19±0.20 g/dL) followed by El-Matariya (2.45±0.31 g/dL) and El-Serw drain (3.05±0.04 g/dL), respectively.

However, in summer total protein exhibited higher levels of total protein in blood serum of *O. niloticus.* North of the lake recorded (4.78±0.73 g/dL) the highest value compared to other sites, followed by middle of Manzala Lake, El-Serw drain and El-Matariya while Bahr El-Baker drain recorded the lower levels (4.36± 0.67, 3.2±0.49, 2.8±0.14 and 2.63±1.3 g/dL), respectively. Although, albumin showed variation upon seasons, that the highest levels of albumin were recorded in El-Serw drain, El-Gamil, El-Matariya and Bahr El-Baker drain (1.69±0.03, 1.55±0.06, 0.7±0.11 and 0.60±0.22 g/dL, respectively). The present hypoproteinemia and hypoalbuminemia may be related to acceleration of protein synthesis in the liver or/and activating the metabolic systems as a result of pollutants' exposure in these areas (**Al-Attar, 2005**).

Serum AST and ALT

AST and ALT activities in *O.niloticus* showed in **Table (2).** In winter, *O. niloticus* fish from middle of Manzala Lake exhibited the lowest activity of ALT and AST (33.4±3.4 and 105.2±4.05 U/ml) followed by El-Boughdady (42.93±1.3 and 117.03±1.86 U/ml) and El-Serw drain (49.0±3.6 and 136.6±23.7 U/ml). However Bahr El-Baqer drain recorded the highest activities (74.03±3.2 and 265.57±35.8 U/ml) followed by El-Matariya (65.58±2.4 and 198.88±14.6 U/ml) and El-Gamil (64.85±0.85 and 194.75±1.48 U/ml). Highest activities may be attributed to the effect of pollution sources in that sites, as sewage and domestic wastes at Bahr El-Baqer drain and sewage of El-Matariya and Port Said drain. In summer, both ALT and AST activities in *O. niloticus* recorded their highest activities in Bahr El-Baker drain (63±2.5 and 195.17±14.8 U/mL), while El-Matariya recorded (56.7±2.62 and 170.2±7.9 U/mL). However, the activity of ALT and AST in Bahr El-Baker drain and middle of Manzala Lake recorded 46.75±2.48, 117.5±3.5 and 35±1.41, 95.5±0.71 U/mL. El-Serw drain showed lower ALT and AST activities (35.6±0.57, 89.5±0.71). The present serum AST and ALT activities in *O. niloticus* exhibited variations due to the atmosphere temperature. In cold conditions, both ALT and AST activities were elevated, that could be caused by a variety of disorders, such as muscle damage, gastrointestinal and hepato-pancreatic injuries, and hazardous hepatitis caused by pollution in these environments. (**Chen *et al.*, 2004**).

Renal function tests

Renal function impairment, renal insufficiency, renal tubular necrosis, muscular injury, and altered nitrogen metabolism were all diagnosed using urea, creatinine, and uric acid (**Murray *et al.*, 1990**). Renal function tests in *O.niloticus* showed in **Table (3).** In winter, creatinine along with urea and uric acid showed the same pattern, as fish from the middle of Manzala Lake recorded the lowest levels of these parameters (3.6±0.31, 15.3±2.1 and 3.3±0.5 mg/dL) pursued with El-Boughdady (4.4±0.21, 15.7±1.9 and 3.03±25.3) and El-Serw drain (4.6±0.47, 13.99±1.23 and 4.38±0.42 mg/dL), respectively. On the other hand, Bahr El-Baqer drain reported the highest levels of creatinine (9.9±0.36, 33.19±4.9 and 10.9±0.2 mg/dL), followed by El-Matariya (8.2±0.3, 30.5±0.8

and 10.98±0.57 mg/dL), respectively, in *O. niloticus* fish. In summer season, levels of creatinine, urea, and uric acid were decreased as compared to winter. Bahr El-Baqer station recorded the highest levels of creatinine, urea, and uric acid (7.30±0.58, 30.67±1.33 and 10.19±1.4 mg/dL), respectively. El-Matariya and El-Serw drain recorded (6.27±0.42, 22.43±0.55, 8.21±1.34 and 3.68±0.18, 8.95±1.03, 3.76±0.49 mg/dL) in creatinine, urea and uric acid, respectively. Regarding the seasonal variations, Creatinine, urea, and uric acid showed the highest levels in Bahr El-Baqer and El-Matariya during the study that may be correlated to the high amount of sewage and domestic wastes at Baher El-Baqer drain and sewage of El-Matariya

Table 2. Glucose, total serum protein, and albumin, as well as ALT and AST activities of Nile tilapia fish from Lake Manzala during winter and summer (2020).

Station	Seasons	Glucose (mg/dL)	T. protein (g/dL)	Albumin (g/dL)	ALT (U/mL)	AST (U/mL)
El-Gamil	Winter	301.4±1.77 A	3.05±0.04 B	0.97±0.02 B	64.85±1.06 A	194.75±1.48 A
	Summer	175.1±7.07 B	3.70±0.14 A	1.30±0.14 A	35.01±1.41 B	95.50±0.707 B
	Average	238.2±89.4	3.38±0.46	1.14±0.23	49.93±21.11	145.10±70.2
New El-Gamil	Winter	307.7±2.21	3.16±0.33	1.20±0.05	55.37±4.17	161.50±5.21
	Summer	NA	NA	NA	NA	NA
El-Boughdady	Winter	216.3±2.25	3.43±0.49	1.65±0.05	42.93±1.30	117.03±1.86
	Summer	NA	NA	NA	NA	NA
Middle of Manzala Lake	Winter	204.3±2.59 A	3.80±0.07 B	1.55±0.06 B	35.60±0.60 A	105.20±4.05 A
	Summer	154.9±30.74 B	4.35±0.67 A	1.80±0.05 A	33.43±3.39 B	89.50±0.71 B
	Average	179.6±35.0	4.08±0.39	1.67±0.18	34.52±1.53	97.40±11.10
Bahr El-Baqer drain	Winter	363.2±25.44 A	2.20±0.20 B	0.47±0.08 B	74.03±3.21 A	265.57±35.80 A
	Summer	318.2±27.48 B	2.63±1.30 A	0.60±0.23 A	63.01±2.48 B	195.17±14.75 B
	Average	340.7±31.8	2.41±0.31	0.53±0.09	68.52±7.80	230.40±49.80
El-Matariya	Winter	355.3±30.62 A	2.45±0.31 B	0.66±0.06 B	65.58±2.36 A	198.88±14.60 A
	Summer	246.3±9.35 B	2.82±0.14 A	0.70±0.11 A	56.74±2.62 B	170.21±7.87 B
	Average	300.8±77.0	2.64±0.26	0.68±0.03	61.16±6.25	184.50±20.30
El-Serw drain	Winter	264.2±9.55 A	3.09±0.41 B	1.37±0.05 B	49.01±3.61 A	136.62±23.7 A
	Summer	195.1±4.63 B	3.24±0.49 A	1.69±0.03 A	46.75±2.48 B	117.50±3.54 B
	Average	229.6±48.9	3.16±0.10	1.53±0.22	47.88±1.59	127.10±13.5

* At P<0.05, results from the same column with different alphabetic letters are statistically different.
* NA= not available.

Furthermore, the current increase in ALT and AST activities in polluted areas could be due to renal function impairment and acute kidney injury induced by renal tubule necrosis, which is directly linked to a decline in urea, creatinine, and uric acid excretion, as well as an increase in plasma (**Gowda *et al.*, 2010**). Also, numerous studies have shown that hepatic enzymes levels act as good prognostic markers in chronic kidney disease (**Ray *et al.*, 2015** and **Oyelade *et al.*, 2020).** Furthermore, pollution-induced kidney injury can lower renal blood flow and glomerular filtration rates, resulting in azotemia, a condition marked by a rise in blood urea nitrogen, uric acid, and creatinine (**Chang *et al.*, 1996**).

Lipid profile

Cholesterol and triglycerides are the two main lipid categories found in plasma or serum. It is a member of a category of steroid chemicals known as the basic phenanthrene derivatives. Cholesterol is a constituent of cellular membrane, the outer layer of plasma lipoproteins, and the source to all steroid hormones (**Yang** and **Chen, 2003**).

Lipid profile in *O.niloticus* showed in **Table (3).** In winter, the present study revealed that the middle of Manzala Lake recorded the highest levels of total lipids (833.3±55.6 mg/dL), while Bahr El-Baqer drain (506.23±33.7 mg/dL) recorded the lowest level of total lipids pursued with El-Serw drain (532.04±28.4 mg/dL) and El-Matariya (633.9±13.3 mg/dL) in *O. niloticus* fish. Moreover, cholesterol levels in *O. niloticus* from middle of Manzala Lake showed the lowest level (169.3±13.8 mg/dL) followed by El-Gamil (174.8±16.2 mg/dL) and El-Boughdady (184.5±16.9 mg/dL). Whereas, the highest cholesterol levels were recorded at Bahr El-Baqer drain (253.9±5.9 mg/dL) followed by New El-Gamil (241.23±9.5.4mg/dL), El-Matariya (206.7±29.7 mg/dL), and El-Serw drain (202.4±11.72 mg/dL) in *O. niloticus* fish. The present increase may be because of sewage and domestic wastes at Baher El-Baqer drain and sewage of El-Matariya.

Fish from polluted sites recorded an increase in serum cholesterol levels in the present study; this may be due to the release of cholesterol and other lipids constituents from damaged hepatocytes and other organs into the circulation, or due to the effect of environmental pollution increases the levels of total lipids in the plasma (**Shalaby *et al.*, 2007**). The liver dysfunctioning shows hypothyroidism that results in increase in glycerides levels (**Mohiseni *et al.*, 2016**). Because triglycerides are thought to be components of the innate, non-adaptive immune response, their decrease in fish from contaminated environments in this study shows immunity inhibition. (**Javed** and **Usmani, 2015**).

Moreover, in cold season triglycerides levels of *O. niloticus* showed remarkable variations. The highest levels were recorded in middle of Manzala Lake (46.6±5.3 mg/dL), pursued with El-Boughdady (38.9±2.0 mg/dL) and New El-Gamil (36.03±4.04 mg/dL) in *O. niloticus* fish. While the lowest levels were reported at Bahr El-Baqer drain

(19.28±3.9 mg/dL) followed by El-Matariya (24.4±1.8 mg/dL) and El-Gamil (24.6±0.5 mg/dL) may be increase of sewage and domestic wastes at Baher El-Baqer drain and sewage of El-Matariya and Port Said drain. In summer, middle of Manzala Lake recorded the highest total lipid levels in *O. niloticus* (671.04±25.8 mg/dL) followed by El-Matariya, El-Gamil, and El-Serw (603.7±31.99, 594.0±2.83 and 578.4±8.9 mg/dL). While Bahr El-Baker drain showed the lowest levels of total lipid (269.25±26.8 mg/dL). Cholesterol recorded its highest levels in Bahr El-Baqer (208.8±12.4mg/dL) followed by El-Matariya, El-Serw (187.99±16.7 and 153.24±2.8 mg/dL), while El-Gamil and middle of Manzala Lake showed no difference (118.0±2.8 and 115.9±1.55). Triglycerides level in *O. niloticus* recorded the highest levels in middle of the Lake in summer as in the winter (33.5±0.71 mg/dL), pursued with El-Gamil (30.73±7.3.5 mg/dL), El-Serw (27.33±0.98 mg/dL), while Bahr El-Baqer drain recorded the lowest levels (21.76±2.32 mg/dL).

Table 3. Creatinine, urea, uric acid, total lipid, cholesterol, and triglycerides levels of Nile tilapia fish from Lake Manzala during winter and summer (2020).

Stations	Parameters / Seasons	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL	T. lipids (mg/dL)	Cholesterol (mg/dL)	Tri-glycerides (mg/dL)
El-Gamel	Winter	7.45±2.19 A	23.86±0.18 A	7.45±0.21 A	670.15±38.5 A	174.8±16.19 A	33.5±0.707 A
	Summer	3.85±0.07 B	8.45±0.07 B	4.3±0.14 B	594±2.83 B	118±2.83 B	24.7±0.7 B
	Average	5.7±2.5	16.16±10.90	5.85±2.19	632.08±53.85	146.4±40.2	29.1±6.2
New El-Gamel	Winter	5.7±0.26	23.98±0.32	5.4±0.264	648.47±34.09	241.23±9.45	36.03±4.04
	Summer	NA	NA	NA	NA	NA	NA
El-Bogdady	Winter	4.43±0.21	15.68±1.86	3.033±0.9	651.93±25.27	188.5±16.99	38.93±2.00
	Summer	NA	NA	NA	NA	NA	NA
Middle of Manzala Lake	Winter	3.57±0.306	15.26±2.07	3.65±0.72	833.3±55.6	169.27±13.8	46.6±5.28
	Summer	3.55±0.502	10.11±2.07 B	3.27±0.93 B	671.04±25.8 B	115.9±1.55 B	28.17±2.53 B
	Average	3.6±0.0	12.69±3.64	3.46±0.27	752.17±114.74	142.6±37.7	37.4±13.0
Bahr El-Baqer drain	Winter	9.9±0.36 A	33.187±4.89 A	11.87±0.31 A	506.23±33.66 A	253.95±5.95 A	21.76±2.32 A
	Summer	7.303±0.58 B	30.67±1.33 B	10.19±1.42 B	269.25±26.8 B	208.84±12.4 B	19.28±3.94 B
	Average	8.6±1.8	31.93±1.78	11.03±1.19	387.74±167.57	231.4±31.9	20.5±1.8
El-Matariya	Winter	8.22±0.25 A	30.478±0.77 A	10.98±0.57 A	633.98±13.39 A	202.4±11.72 A	24.4±2.76 A
	Summer	6.27±0.42 B	22.43±0.55 B	8.21±1.34 B	603.7±31.99 B	187.99±16.74 B	23.26±9.48 B
	Average	7.2±1.4	26.45±5.69	9.60±1.96	618.84±21.41	195.2±10.2	23.8±0.8
El-Serw drain	Winter	4.56±0.47 A	13.99±1.23 A	4.38±0.42 A	578.39±8.89 A	206.7±29.63 A	34.18±3.58 A
	Summer	3.68±0.18 B	8.95±1.032 B	3.76±0.49 B	532.04±28.4 B	153.24±2.78 B	27.33±0.98 B
	Average	4.1±0.6	11.47±3.57	4.07±0.44	555.22±32.77	180.0±37.8	30.8±4.8

* At P<0.05, results from the same column with different alphabetic letters are statistically different.
* NA= not available.

In the present study, the biochemical parameter showed seasonal variations due to the change in the climate temperature. Temperature is one of the most significant abiotic parameters, influencing all biochemical responses and hence having a significant impact on fish physiology. Warm - blooded animals, such as fish, experience an exponential increase in metabolic rate as water temperatures rise, which, along with decreasing oxygen solubility, has a negative impact on growth efficiency (**Blaxter, 1991; Rombough, 1997; Katersky *et al.*, 2006;** and **Barnes *et al.*, 2011**).

CONCLUSION

According to the present study findings, *O. niloticus* from middle of Manzala Lake and El-Bogdady showed better biochemical parameters. However fish from Bahr El-Baker showed the worst health status upon the present physiological studies followed by drain El-Matariya and El-Gamel, due to the various sources of pollution at these sites. *O. niloticus* from mentioned sites showed higher levels of serum glucose, hepatic enzymes activities, creatinine, urea, uric acid and triglycerides, especially in winter. This indicates that fish is in stressful conditions, which increased in winter season associated with impaired of both liver and kidney functions. These conditions make fish weak and vulnerable to diseases.

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