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Evaluation of Insulin-like growth factor-1 (IGF-1), antioxidant enzymes, and heavy metals in *Oreochromis niloticus* collected from different stations along the River Nile

Soha Mohamed Hamdy[1], Reham Taha Mansy[1,*], Hala Elshahat Ghannam[2]

1- Chemistry Department, Faculty of Science, Fayoum University, Fayoum, Egypt

2- Pollution Laboratory, Freshwater and Lakes Division, National Institute of Oceanography and Fisheries, Cairo, Egypt

[*]Corresponding Author: rehammansy30@gmail.com

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ABSTRACT

This study was carried out to assess the insulin-like growth factor-1 (IGF-1), levels of some physicochemical parameters and heavy metals in water collected from the River Nile. The results showed that the physicochemical concentrations of the water samples were as follows: temperature 16.3-36.5°C, transparency 54.3-80.2 cm, electrical conductivity 268-320 µS/cm, pH 7.5-8.3, dissolved oxygen 7.3-11.2 mg/l, biochemical oxygen demand 1.3-3.3 mg/l, chemical oxygen demand 6.3-10.7 mg/l, and ionized ammonia 0.57-0.96 mg/l. Heavy metals (Fe, Mn, Cu, Zn, and Pb) in water showed maximum values as follows: (5.8, 0.51, 0.350, 0.230 and 0.90 mg/l) and (5.2, 0.42, 0.13, 0.47, and 0.27 mg/l) in summer and winter, respectively. The levels of metals detected in the tilapia muscle were higher than those found in water. An increased activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was detected in blood analysis of *O. niloticus*. Levels of urea, uric acid, glucose, cholesterol and insulin-like growth factor-1 (IGF-1) were high in fish collected from polluted sites (iron and steel factory at Helwan, sugar cane factory at El-Hawamdia, Road El-Farag, and Electricity station at Shobra El-khima). In addition, a significant increase ($P \leq 0.05$) was shown in the activities of catalase (CAT), superoxide dismutase (SOD), and level of malondialdehyde (MDA) when compared to reference station (V) EL-Qanater. The results indicate that Nile tilapia resisted oxidative stress induced by heavy metal exposure by antioxidant mechanisms.

INTRODUCTION

Water is one of the most important components of the ecosystem because it is necessary for all living organisms on the earth to survive and grow. Water is used for drinking, eating, farming and industry and other fields; transportation and recreation are the most two popular uses of water **(Basavaraja *et al.*, 2011)**. Water of good quality is necessary for life, but when physicochemical characteristics and heavy metals exceed the allowed limits, it becomes harmful to drink. Because of the usage of contaminated drinking water, the human population suffers from a variety of waterborne diseases; the quality of drinking water should be examined on a regular basis (**Kiros *et al.*, 2021**).

Insulin-like growth factor 1 (IGF-1) is crucially involved in the regulation of growth, differentiation, and reproduction by selectively promoting mitogenesis and differentiation **(Jones & Clemmons, 1995; Reinecke & Collet, 1998)**. Among the nonmammalian classes of vertebrates, bony fish are the mostly studied with respect to IGF-1 **(Reinecke *et al.*, 2005; Wood *et al.*, 2005)** mainly due to their unique development from the larval to the adult life, their high growth potential, and their importance in aquaculture. Similar to mammals, IGF-1 is mainly produced in fish liver. The principal source of the circulating (endocrine) IGF-1 is under the influence of growth hormone (GH). In addition, IGF-1 is expressed in parenchymal cells of numerous extrahepatic sites of adult **(Reinecke *et al.*, 1997)** and developing fish **(Perrot *et al.*, 1999; Radaelli *et al.*, 2003; Berishvili *et al.*, 2006a, b)**, and most likely stimulates organspecific functions by paracrine/autocrine mechanisms. In a recent study on the tilapia, *Oreochromis niloticus*, it was noticed that, the early life exposure to elevated concentrations of 17a-ethinylestradiol (EE2), a major constituent of contraceptive pills, has long-lasting consequences on growth, IGF-1 serum levels, and IGF-1 expression in liver as well as in gonads **(Shved *et al.*, 2007)**. Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissue, directly from water through respiration and also through their diet (**Velkova *et al.*, 2008**). Fish are constantly exposed to the prooxidant effects of many contaminants commonly found in the aquatic environment. Catalase (CAT) and superoxide dismutase (SOD) have been found in a wide range of mammalian cells. Those enzymes have a vital role in shielding cells against the potentially harmful effects of contaminants in the environment **(Kuthan *et al.*, 1986; Hamdy *et al.*, 2021)**. Thus, this work was focused on the investigation of physicochemical parameter of water, heavy metals in water and fish muscle, biochemical parameter, antioxidant activity and Insulin growth factor-1(IGF-1) in the Nile tilapia collected from different sites along the River Nile.

MATERIALS AND METHODS

The area under investigation is approximately 60 km long, starting at the city of Helwan in the South till Delta barrage in the North. It receives all the year round fresh water, domestic waste water effluent of cities and villages located on the water way. Agricultural runoff originates from the drainage water mixed with the industrial wastes of the factories located in bath. These different types of waste water affect the biota/flora of the area under investigation. Five stations were chosen to represent the investigational regions (Fig.1); namely, Station (I) that receives the factory's waste in front of the Iron and Steel Factory in Helwan (Latitude 29°48'0''N and Longitude 31°17'45''E); Station (II) is located in front of the Sugar Cane Factory at El-Hawamdia (Latitude 29°52'31''N and Longitude 31°17'3''E) and receives all the fermented waste products. Station (III) of Road El-Farag (Latitude 30° 5'27'' N, Longitude 31°14'1''E) obtains the same sewage and agricultural runoff. Station (IV) is found in front of the electricity station at Shobra El-

Khima (Latitude 30°7'29"N and Longitude 31°14'4" E) and suffers from the thermal pollution. Station (V) is situated at El-Qanater El-Khairia city (30°11'1"N and Longitude 31° 8'20 "E) and is considered as the control. Different types of water samples from the 5 stations were taken in summer 2019 and winter 2020 at the mid-stream of each station.

Fig. 1. Sampling stations from Helwan City to El-Qanater El-Khairia City, Egypt (Source: Google earth).

Water analysis

Physicochemical parameters of water were done in the field, including temperature, pH, electrical conductivity (EC), and transparency. While, other parameters were estimated at the laboratory, covering heavy metals, iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and lead as well as the dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), and NH_4^+. Water samples were analyzed according to the standard method for the examinations of water and wastewater (**APHA, 2012**). The metals concentrations (Fe, Cu, Pb, Mn, and Zn) were determined using atomic absorption model (Perkin Elmer 3110 USA) with graphite atomizer HGA-600, according to the method described in **APHA (2012).**

Fish analysis

The tilapia fish specimens were collected from all study sites during summer 2019 and winter 2020. Each sampling site yielded twenty-four (24) fish of various sizes, lengths and weights, with the mean ± standard deviation (±SD) of body weight (160±10 g) and body length (20± 5 cm) at the time of collection. Samples were dried from external water, and blood samples were collected immediately from freshly alive fish by tail cutting method, after severing the caudal peduncle **(Mazhar *et al.*, 1987)** and were then collected in small sterilized vials. The blood was left to clot then centrifuged at 3000 r.p.m for 10 minutes. Supernatant serum was obtained using micropipette and stored at 4°C till determination of glucose, cholesterol, urea, uric acid, AST and ALT. ALT and AST activities were determined kinetically according to the method of **Thomas (1998)**. Serum cholesterol was estimated following the process of **Artiss and Zak (1997)**. Serum glucose level was determined in accordance with the method of **Trinder (1969).** Urea concentration was estimated according to **Kaplan (1984)**. Uric acid concentration (mg/dl) was determined using the method of **Thomas (1998)**. The activity of catalase (CAT) was assessed according to **Aebi (1984)**. The activity of superoxide dismutase (SOD) was determined by colorimetric method using readymade kits provided by Bio-diagnostic, Egypt, following the method of **Nishikimi *et al.* (1972)**. In addition, Malondialdehyde was decided using Bio diagnostic kit according to the method described in **Satoh (1978)** and **Ohkawa *et al.* (1979)**. While, insulin growth factor-1 was specified using ELISA Kit stepping the method of **Binoux *et al.* (1986)**. After fish dissection, the muscle tissue was collected to determine heavy metal accumulation. The samples were digested according to **Goldberg *et al.* (1963)**. On the other hand, the determination of the concentrations of iron, lead, copper, manganese and zinc was performed by using atomic absorption spectrophotometer, Hitachi model 170 -30 with graphite atomizer (G.A.Z). The results were expressed in µg/g dry weight.

Statistical Analysis

All data were expressed as mean ± SD. The data were statistically analyzed by one way ANOVA. Results with p value $\leq$ 0.05 were considered significant. The statistical analysis was carried out by Bonferroni and confirmed by LSD with respect to the reference site (El-Kanater El-Khairia city (30°11'1"N and Longitude 31° 8'20 "E).

RESULTS AND DISCUSSION

Physical and chemical parameter in water

The physical parameters in summer and winter presented in Tables (1 & 2) show that water temperature reached its maximum value (36.5°C) during summer at site (II), while its minimum (16.3°C) was regarded in winter at site (III). Transparency showed the maximum value (80.2 cm) in summer at site (V) and the minimum (54.3 cm) in winter at site (I).The highest value (320 µS/cm) for electrical conductivity was in summer at site (I) while lowest was considered in winter at site (V). In winter, the high pH (8.3) was detected, whereas the low value (7.5) was recorded in summer.

The chemical parameters of the collected water samples during summer and winter are shown in Tables (2 & 3). The results illustrated that, the dissolved oxygen was maximum (11.2 mg/l) in winter at site (V) and minimum (7.3 mg/l) in summer at site (III). Biochemical oxygen demand recorded its highest value (3.3 mg/l) in summer and its lowest value (1.3 mg/l) in winter at site (II). The chemical oxygen demand of winter recorded (10.7mg/l) at site (I), while summer recorded (6.3 mg/l) at site (V). Ionized ammonia reached its highest (0.96 mg/l) during summer at site (I), while the lowest (0.57 mg/l) was presented during winter at site (V).

Table 1. Variations in physical parameters of water collected from selected stations in the River Nile during summer 2019 and winter 2020

Station	Temperature (°C)		Transparency (cm)		Electrical Conductivity (µS/cm)		pH	
	summer	winter	summer	winter	summer	winter	summer	winter
I	36	16.5	70.3	54.3	320	298	7.8	8.3
II	36.5	16.7	77.2	63.1	332	302	7.7	8.1
III	36.5	16.3	76.2	66.5	313	287	7.6	8.3
IV	36	16.6	73.5	70.3	303	285	7.7	8.2
V	35.7	16.5	80.2	77.8	307	268	7.5	8.3

The highest and lowest recorded values of water temperature with respect to seasons agree with those detected in the study of **Ghannam and Talab (2009**) who reported that, the increase in water temperature is related to the sampling time and number of hours exposed to of sunshine.

Table 2. Mean±SD of chemical parameters in the River Nile during summer 2019

Station		DO (mg/l)	BOD (mg/l)	COD (mg/l)	NH_4^+ (mg/l)
I	Mean	7.4	3.2	9.3	0.96
	±SD	±0.28	±0.14	±0.42	±0.028
	P value	0.10	0.03	0.05	0.01
II	Mean	7.5	3	8.7	0.87
	±SD	±0.14	±0.14	±0.14	±0.014
	p value	0.25	0.06	0.03	0.01
III	Mean	7.7	3.1	8.2	0.83
	±SD	±0.14	±0.07	±0.28	±0.028
	p value	0.32	0.04	0.06	0.06
IV	mean	7.3	3.3	7.6	0.77
	±SD	±0.28	±0.28	±0.21	±0.028
	p value	0.30	0.08	0.10	0.08
V	mean	7.5	2.8	6.3	0.72
	±SD	±0.21	±0.07	±0.28	±0.007

DO: Dissolved Oxygen; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand
NH_4^+: Ammonia; $P \leq 0.05$ significant differences; $P > 0.05$ non-significant differences

Table 3. Mean±SD of chemical parameters in the River Nile during winter 2020

Station		DO (mg/l)	BOD (mg/l)	COD (mg/l)	NH_4^+ (mg/l)
I	Mean ±SD P value	9.3 ±0.28 0.04	1.4 ±0.28 0.08	10.7 ±0.14 0.006	0.73 ±0.028 0.01
II	Mean ±SD P value	9.7 ±0.14 0.03	1.3 ±0.42 0.10	10.3 ±0.14 0.008	0.71 ±0.014 0.01
III	Mean ±SD P value	9.5 ±0.05 0.01	2.7 ±0.28 0.14	9.1 ±0.14 0.012	0.62 ±0.028 0.21
IV	mean ±SD P value	10.1 ±0.07 0.01	2.3 ±0.14 0.25	8.5 ±0.28 0.102	0.60 ±0.028 0.30
V	mean ±SD	11.2 ±0.14	2.5 ±0.14	8.3 ±0.21	0.57 ±0.021

DO: Dissolved Oxygen; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand
NH_4^+: Ammonia; $P \leq 0.05$ significant differences; $P > 0.05$ non-significant differences

The decrease of transparency in winter may be due to the increase in the amount of phytoplankton, the suspended solid and organic matter found in water body, and the increase in electrical conductivity resulted from the presence of a large amount of organic and inorganic constituents diffused in water **(Ghannam, 2021)**. **Ebenezer (2014)** stated that, the level of the dissolved oxygen in natural water decreases dramatically when water temperature rises, and added that the organic concentrations rise due to increased decomposer activities.

Heavy metals in water samples

The concentration of different heavy metals in water samples collected during summer and winter are recorded in Tables (4 & 5). In both seasons, site (I) demonstrated the highest values in all metals analyzed as follows: 5.8, 0.51, 0.350, 0.230, and 0.90 mg/L in summer and 5.2, 0.42, 0.13, 0.47, and 0.27 mg/L in winter for Fe, Mn, Cu, Zn, and Pb, respectively. According to **WHO (2008)**, the permissible concentrations of Fe, Mn, Cu, Zn, and Pb are 0.3, 0.5, 0.5, 0.2, and 0.01 mg/L, respectively. From the analyzed results (Tables 4 & 5), all water samples recorded concentrations above the permissible limits of **WHO (2008)** for Fe, Zn, and Pb. Although iron does not pose any health risks, it does give water a bitter taste when it is present in high proportions. Those who drink iron-rich water complain about the flavour, colour, and corrosion of their plumbing systems, as well as liver damage. However, those exposed to low concentrations would be highly susceptible to anaemia **(Ocheri *et al.*, 2014)**. Elements of Cu and Mn were below the detection limit in all water samples of the study area. Hence, these elements may not pose an immediate hazard to the health of those who consume drinking water in the research area.

Table 4. Mean ±SD of heavy metal concentrations (mg/l) in water samples collected from different studied sites during summer 2019

Station		Fe	Mn	Cu	Zn	Pb
I	Mean ±SD *P* value	5.8 ±0.14 0.006	0.51 ±0.014 0.045	0.350 ±0.028 0.022	0.230 ±0.028 0.045	0.090 ±0.01 0.021
II	Mean ±SD *P* value	5.3 ±0.14 0.006	0.48 ±0.028 0.135	0.205 ±0.002 0.007	0.150 ±0.028 0.099	0.080 ±0.014 0.072
III	Mean ±SD *P* value	5.2 ±0.56 0.025	0.41 ±0.014 0.147	0.125 ±0.002 0.007	0.120 ±0.014 0.080	0.070 ±0.002 0.015
IV	mean ±SD *P* value	3.6 ±0.11 0.0004	0.39 ±0.014 0.25	0.090 ±0.002 0.028	0.120 ±0.003 0.027	0.025 ±0.002 0.194
V	mean ±SD	0.3 ±0.007	0.37 ±0.042	0.080 ±0.001	0.08 ±0.001	0.015 ±0.007

$P \leq 0.05$ significant differences; $P > 0.05$ non-significant differences

Table 5. Mean ±SD of heavy metal concentrations (mg/l) in water samples collected from different studied sites during winter 2020

Station		Fe	Mn	Cu	Zn	Pb
I	Mean ±SD *P* value	7.8 ±0.14 0.003	0.59 ±0.014 0.008	0.15 ±0.014 0.06	0.63 ±0.042 0.028	0.34 ±0.014 0.013
II	Mean ±SD *P* value	5.2 ±0.02 0.001	0.42 ±0.007 0.008	0.13 ±0.002 0.021	0.47 ±0.014 0.016	0.27 ±0.014 0.018
III	Mean ±SD *P* value	4.3 ±0.14 0.009	0.38 ±0.007 0.009	0.12 ±0.03 0.009	0.41 ±0.002 0.002	0.19 ±0.014 0.029
IV	Mean ±SD *P* value	2.2 ±0.14 0.017	0.32 ±0.007 0.015	0.11 ±0.001 0.17	0.35 ±0.004 0.008	0.08 ±0.01 0.028

$P \leq 0.05$ significant differences; $P > 0.05$ non-significant differences

Heavy metals in muscle of fish samples Nile tilapia

The anthropogenic activities pose a crucial environmental and human health problem, producing toxic materials as heavy metals. Some metals such as Cu, Ni, Cr, and Zn are trace metals essential for the living organisms, however at high concentrations they become a significant environmental pollutants with toxic effect **(Nagajyot *et al.*, 2010; Jaishankar, *et al.*, 2014)**. The concentrations of heavy metals (iron, manganese, zinc, copper and lead) in muscle tissues of the Nile tilapia during summer 2019 and winter 2020 are illustrated in Tables (6 & 7).

Table 6. Mean±SD of heavy metal concentrations (mg/kg dry weight) in muscle tissues of Nile tilapia collected from different studied sites during summer 2019

Station		Fe	Mn	Cu	Zn	Pb
I	Mean ±SD *P* value	14.6 ±0.14 0.003	11.2 ±0.014 0.001	3.7 ±0.014 0.001	45.7 ±0.141 0.004	2.7 ±0.141 0.011
II	Mean ±SD *P* value	14.3 ±0.28 0.007	10.2 ±0.141 0.045	3.5 ±0.028 0.001	41.3 ±0.014 0.024	2.6 ±0.014 0.001
IV	Mean ±SD *P* value	12.2 ±0.28 0.015	10 ±0.141 0.071	2.2 ±0.014 0.081	39.7 ±0.0282 <0.001	1.3 ±0.028 0.005
V	Mean ±SD	7.5 ±0.042	9.6 ±0.028	2.1 ±0.021	38.5 ±0.282	0.04 ±0.035

P<0.001 highly significant; *P*≤0.05 significant differences; *P*> 0.05 non-significant differences.

Table 7. Mean±SD of heavy metal concentrations (mg/kg dry weight) in muscle tissues of Nile tilapia collected from different studied sites during winter 2020

Station		Fe	Mn	Cu	Zn	Pb
I	Mean ±SD P value	7.8 ±0.28 0.006	14.2 ±0.14 0.003	7.3 ±0.02 0.006	43.7 ±0.28 0.003	2.7 ±0.014 0.012
II	Mean ±SD P value	6.7 ±0.01 0.008	12.2 ±0.28 0.012	6.8 ±0.01 0.003	35.9 ±0.14 0.002	2.3 ±0.014 0.012
IV	Mean ±SD P value	4.5 ±0.02 0.018	8.5 ±0.02 0.004	6.2 ±0.14 0.036	33.7 ±0.04 0.001	2 ±0.028 0.027
V	Mean ±SD	3.1 ±0.14	7.8 ±0.04	5.2 ±0.02	23.7 ±0.01	1.3 ±0.070

P<0.001 highly significant; *P*≤0.05 significant differences; *P*> 0.05 non-significant differences

In summer 2019, the concentration of Zn in station (IV) showed a highly significant increase (*P*<0.001) compared to reference station (V) EL-Qanater. While, the concentrations of Fe and Pb in stations (I to IV), and the concentrations of Mn, Cu, and Zn in stations (I & II) showed significant increase ($P \leq 0.05$) compared to the reference site. The concentrations of Mn and Cu in station (IV) recorded non-significant differences ($P > 0.05$) compared to reference station (V) EL-Qanater. In winter, 2020 the concentrations of Fe, Mn, Cu, Zn, and Pb in stations (I to IV) showed significant differences ($P \leq 0.05$) when compared to the reference site.

Metals accumulate in fish tissues through a direct contact via gills and dermal exposure or by absorption in the digestive tract of fish **(Tunçsoy *et al.*, 2017)** and give a negative impact on the ecological balance of the environment and the diversity of aquatic organisms **(Ashraj, 2005; Vosyliene & Jankaite, 2006)**. Fish are generally used as bio-indicators to evaluate aquatic pollution **(Ezemonye, *et al.*, 2019; Belal *et al.*, 2021**) and the bioavailability of heavy metals influenced by the physiological activities of fish during different seasons **(Tekin-Özan & Kir, 2008)**.

Biochemical Parameter

The activities of ALT and AST, levels of cholesterol, urea, uric acid, and glucose, and the level of IGF-1 in serum of fish under study are illustrated in Tables (8-10). In summer 2019, in comparison with the reference station (V) of EL-Qanater, the following observations were recognized. The level of cholesterol in stations (I & IV), and the level of urea in station (I) showed highly significant increase ($P < 0.001$). The activities of ALT and AST in stations (I to IV), and the level of cholesterol in station (II) noted a significant increase ($P \leq 0.05$). On the other hand, the level of urea in stations (II & IV), and the level of Uric Acid in stations (I & II) recorded a significant increase ($P \leq 0.05$). The level of uric acid in station (IV) showed non-significant differences ($P > 0.05$). Furthermore, the level of IGF-1 in stations (I to IV) showed significant differences ($P \leq 0.05$).

In winter, when compared to reference station (V) of EL-Qanater, the level of cholesterol in station (I), and the level of glucose in station (II) showed highly significant increase ($P < 0.001$). In addition, the activities of ALT and AST, and levels of urea and uric acid in stations (I to IV) marked a significant increase ($P \leq 0.05$). The level of cholesterol in stations (II to IV) and the level of glucose in stations (I and IV) showed significant increase ($P \leq 0.05$). While, the level of IGF-1 in stations (I to IV) showed significant differences ($P \leq 0.05$).

The present results presented increased activities of AST and ALT and levels of urea and uric acid in the blood of *O. niloticus* fish sample at site (I), which may be due to the existance of heavy metals in water. Similarly, **Zaki *et al.* (2009)** recorded a significant higher levels in those parameters regarding the Nile tilapia and related it to some heavy metals exposure. High level of plasma uric acid can be used as rough indicators of glomerular filtration rate and kidney disease **(Abu *et al.*, 2009)**. Additionally, the increase of urea and uric acid may be attributed to sewage **(Hassaan, 2011)**.

Table 8. Mean±SD of biochemical parameter in serum of Nile tilapia collected from different studied sites during summer 2019

Station		GPT (ALT) (U/l)	GOT (AST) (U/l)	Cholesterol (mg/dl)	Glucose (mg/dl)	Urea (mg/dl)	Uric Acid (mg/dl)
I	Mean	25.3	37.8	187.5	76.3	35.2	5.2
	±SD	±0.14	±0.028	±0.07	±0.14	±0.07	±0.14
	P value	0.01	0.0003	<0.001	0.004	<0.001	0.03
II	Mean	24.7	34.4	160.2	71.2	33.2	4.7
	±SD	±0.28	±0.014	±0.28	±0.28	±0.14	±0.14
	P value	0.01	0.0002	0.0008	0.003	0.003	0.04
IV	Mean	20.3	32.5	132.4	53.3	30.2	4.1
	±SD	±0.04	±0.042	±0.04	±0.14	±0.56	±0.21
	P value	0.04	0.0008	<0.001	0.030	0.047	0.06
V	Mean	19.5	20.8	90.5	49.2	28.4	3.3
	±SD	±0.14	±0.03	±0.02	±0.63	±0.07	±0.42

P<0.001 highly significant; *P*≤0.05 significant differences; *P*> 0.05 non-significant differences

Blood IGF-1 level has been used as an indicator of environmental stress to reflect changes in carbohydrate metabolism under stress conditions **(Kamal & Omar, 2011)**. The level of IGF-1 was high in the blood of *O. niloticus* fish collected from polluted sites; due to the existence of chemical pollutants such as heavy metals, causing hyperglycaemia by activating the glycogenolysis in fish **(Levesque *et al.*, 2002)** and other pollutants **(Adedeji *et al*., 2009; El-Gaar *et al*., 2021)**. Moreover, this can be attributed to the alteration in the activity of glucose-6-phosphate dehydrogenase and lactate dehydrogenase as previously detected in the study of **Osman *et al.* (2010)**.

Table 9. Mean±SD of biochemical parameter in serum of Nile tilapia collected from different studied sites during winter 2020

Station		GPT (ALT) (U/l)	GOT (AST) (U/l)	Cholesterol (mg/dl)	Glucose (mg/dl)	Urea (mg/dl)	Uric Acid (mg/dl)
I	Mean	23.5	32.4	153.8	80.3	30.6	4.5
	±SD	±0.028	±0.01	±0.14	±0.42	±0.07	±0.014
	P value	0.0002	0.009	<0.001	0.0008	0.004	0.02
II	Mean	17.6	30	150	66.7	29.6	4.3
	±SD	±0.028	±1.4	±0.42	±0.28	±0.42	±0.282
	P value	0.0006	0.01	0.0017	<0.001	0.003	0.02
IV	Mean	16.3	28.3	122	60.4	22.4	4.2
	±SD	±0.070	±0.14	±0.05	±0.56	±0.35	±0.282
	P value	0.0016	0.01	0.0007	0.0121	0.009	0.02
V	Mean	12.5	15.1	78.3	44.6	20.6	3.1
	±SD	±0.042	±0.7	±0.14	±0.28	±0.28	±0.141

P<0.001 highly significant; *P*≤0.05 significant differences; *P*> 0.05 non-significant differences.

Table 10. Mean SD of insulin growth factor-1 related biomarker in blood of Nile tilapia collected from different studied sites during summer 2019 and winter 2020

Station		Insulin growth factor-1(IGF-1) (µg /L)	
		Summer	Winter
I	Mean	221	230
	±SD	±1.02	±5.05
	P value	0.0002	0.002
II	Mean	199	213
	±SD	±2.08	±1.5
	P value	0.0006	0.001
IV	Mean	171	203
	±SD	±6.1	±2.5
	P value	0.0009	0.001
V	Mean	121	148
	±SD	±3.2	±5.5

P≤0.05 significant differences; *P*> 0.05 non-significant differences

Antioxidant activity in muscles of Nile Tilapia

The level of MDA and activities of antioxidant activity of CAT and SOD in muscle tissues of the Nile tilapia are elaborated in Table (11).

Table 11. Mean±SD of oxidative stress parameter MDA level and antioxidant enzyme activities in liver tissues of Nile tilapia collected from different studied sites during summer 2019 and winter 2020

Station		Catalase (U/g)		SOD (U/g)		MDA (nmole/g)	
		Summer	Winter	Summer	Winter	Summer	Winter
I	Mean	85.3	171.3	91.7	103.3	64.06	41.05
	±SD	±2.8	±1.6	±1.1	±2.8	±0.7	±0.03
	P value	0.01	0.003	0.001	0.1	0.002	0.02
II	Mean	91.7	204.7	107.9	128.7	57.31	36.24
	±SD	±2.2	±1.3	±0.5	±2.0	±0.6	±0.09
	P value	0.02	0.004	0.028	0.01	0.001	0.04
IV	Mean	101.2	224.4	112.8	167.5	36.77	34.68
	±SD	±1.4	±1.8	±1.2	±37.2	±0.1	±1.4
	P value	0.02	0.041	0.007	0.46	0.002	0.01
V	Mean	120.3	237.6	120.1	170.1	10.79	23.12
	±SD	±0.5	±0.6	±0.9	±0.4	±0.1	±2.2

P≤0.05 significant differences; *P*> 0.05 non-significant differences

Compared to the reference station (V) of EL-Qanater, the activities of CAT and SOD, and the level of MDA in stations (I to IV) showed significant increase ($P \leq 0.05$) in summer 2019. In winter 2020, the activity of CAT in stations (I to IV) and the activity of SOD and level of MDA, in stations (I & II) witnessed a significant increase ($P \leq 0.05$).

The activity of SOD and level of MDA in stations (I to III) showed non-significant differences (*P*>0.05). The response to environmental pollution and toxic impact of the pollutant in the aquatic environment represents one of the possible reasons. According to **Velkova *et al.* (2008)**, the level of antioxidant enzymes in fish samples is affected by their age, nutrition and spawning. Moreover, **Zikic (2001)** determined that, cadmium induces the appearance of anaemia and alters the metabolism of carbohydrates and proteins in goldfish. Their conclusions also detected a decrease in SOD activity in goldfish erythrocytes after acute cadmium exposure, indicating the presence of ROS-induced peroxidation, which leads to RBC membrane damage.

CONCLUSION

This work addressed the estimation of pollution effect on the aquatic system using physicochemical parameter of water, heavy metals in water and fish muscle, biochemical parameter, IGF-1 and antioxidant activity. Although water samples analyses revealed concentrations above the permissible limits of **WHO (2008)** for Fe, Zn, and Pb, yet Cu and Mn showed no surpassing limits. Fish were used as bio-indicators to assess aquatic pollution and site I recorded the maximum concentrations of all metals in different seasons. The results of the present study exhibited increased activities of AST and ALT and levels of urea and uric acid in the blood of *O. niloticus* fish sample at site (I), which may be due to the presence of heavy metals in water. The level of MDA and activities of antioxidant activity of CAT and SOD in muscle tissues of the Nile tilapia were also used to indicate the effect of pollution.

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