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The use of biomarkers in the Nile Tilapia *(Oreochromis niloticus*) as biological signals to track Nile contamination in Egypt.

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ABSTRACT

In aquatic ecosystem biomonitoring, the use of biota and their habitat provides a good indication of conditions and potential threats to any water body. The potential for heavy metal poisoning in humans because of consuming tainted fish has gotten a lot of press around the world. Heavy metal pollutants in water are mostly caused by human activities such as waste disposal, organic fuel combustion, phosphate fertilizers, plastics, and pesticides. The aim of this study was to see how much heavy metals contamination there was in Nile tilapia *Oreochromis niloticus* and how physicochemical characteristics affected the biomarkers. Heavy metals accumulation (cadmium, lead, zinc, copper, and iron) was studied on the serological indices of a freshwater fish *O. niloticus*. Fish, water, and sediments were collected at three locations along the Nile River. The findings of this study showed a significant difference in water quality indices in response to pollution levels among the sites studied. Consequently, the current results confirm that the distribution of heavy metals in tissues could potentially deteriorate biochemical parameters of *O. niloticus*.

INTRODUCTION

Most environmental pollutants pose a threat to human and environmental health, as well as ecological integrity and productivity. As a result of increased human activity, chemical emissions have increased, adversely impacting both terrestrial and marine environments. Heavy metals are among the most hazardous pollutants (**Huang *et al.,* 2020; Khattab *et al.,* 2021**). Heavy metals are one of the most common pollutants because they can remain in the environment, accumulate in food chains, and cause toxicity in a variety of tissues and organs (**Dural *et al.,* 2006 ; Qiu 2015; Briffa *et al.,* 2020**). Contaminants in runoff from agricultural and industrial industries deteriorate the physical and chemical properties of water bodies, including salts, nutrients like phosphorus and nitrogen, and pesticide residues (**Sharma and Bhattacharya, 2017**). Heavy metals contaminants in water are mainly caused by industrial and agricultural discharges such as coal and oil combustion, phosphate fertilizers, sewage disposal plastics, mining activities and pesticides (**Hamada *et al.,* 2018; Zhong *et al.,* 2018; Vergilio *et al.,* 2020**). In aquatic ecosystems, heavy metals have been found to accumulate in faunal organisms in general and in fish as they dominant the food web (**Osman *et al.,* 2012; Wariaghli *et al.,* 2013; Authman *et al.,* 2015**). Fish is one of Egypt's most popular foods, whether farmed in aquatic systems or caught in natural ecosystems, so biomonitoring and evaluating ecosystem components are national responsibility. Metals occur in fish at much higher rates than in water and soil because fish are also at the top of the aquatic food chain.

Heavy metals may be absorbed by fish through their skin's epithelial or mucosal surface, gills, and gastrointestinal tract (**Jovanovic *et al.*, 2011**). Since serological indices are critical in determining the structural and functional state of fish exposed to environmental stressors, they have emerged as a good biomarker for assessing the effects of water pollution on fish (**Begum *et al.*, 2013; Osman *et al.*, 2018**). The current study aimed to assess the Nile River's water quality by evaluating key parameters on the one hand, and to evaluate biomarker responses in fish *O. niloticus* obtained from different locations on the other.

MATERIALS AND METHODS

The study area

Three Nile River water bodies are included in the study area. The first site is in the governorate of Assiut, near the Nile's main channel. The second location is the Manqabad Phosphate Fertilizer Plant, which is situated between the Nile's western bank and the El-Ibraheimia Canal and is connected to the main express roads of Assiut-Cairo and Assiut-Aswan, as well as the railway station. Nubaria Canal is the third location (the main irrigation canal at the northwest of Egypt, located at Al Beheira Governorate). Agricultural drainages run through this location. Long line and nets were used to catch Nile tilapia *O. niloticus* from the selected sites.

Water analysis

Some physicochemical parameters including water pH, dissolved oxygen (DO mg/l), conductivity (µScm-1) and turbidity (NTU) are measured by using water checker device. The other water criteria, total solids (TS mg/l), hardness mg/l, chemical oxygen demand (COD mg/l), nitrate (NO_3 mg/l), sulfate (SO_4 mg/l), total phosphate (PO_4 mg/l) and fluoride (F mg/l) are measured according to **APHA** (**2005**). Also, heavy metals in water including lead (Pb µg/l), copper (Cu µg/l), zinc (Zn µg/l), iron (Fe µg/l) and cadmium (Cd µg/l) were measured after digestion using graphite furnace. Also, sediment samples were collected for total heavy metals, sediment samples were air-dried in a circulating oven at 30°C and sieved mechanically using a 2 mm sieve. For the digestion of samples, 1 gram of sieved sediment was digested with repeated addition of nitric acid and hydrogen peroxide according to **Jackwerth and Würfels** (**1994**) method. Liver tissues were selected as the target organs for metals accumulation. After dissection tissues was HNO_3 digestion according to **McDaniel** (**1991**). Heavy metals concentrations in water, tissues and sediments were determined by a Perkin–Elmer spectrometer with a specific–hollow cathode lamp for each metal. The metal concentration was calculated in µg/g wet weight for tissue, µg/g for sediment and µg/l for water.

Biochemical determinations

Three blood samples were collected from cardiac puncture as described by **Osman and Harabawy** (**2010**). After centrifugation, the fresh sera subjected to biochemical analysis. Serum aspartate aminotransferase (ASAT), alanine aminotransferase *(*ALAT), were determined spectrophotometrically using reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostic mbH, Germany; while serum total cholesterol, triglycerides, creatinine, urea, glucose, albumin and total protein levels were determined spectrophotometrically using reagent kits purchased from DiaSys Diagnostic System GmbH, Germany.

RESULTS

Water physicochemical parameters

Site 3 had the highest pH (8.26±0.55), followed by sites 2 and 1 with (8.07±0.85) and (7.26±0.35), respectively (Table1). Site 3 had the lowest dissolved oxygen O_2 (5.92±0.13 mg/l), while sites 2 and 1 had higher levels (6.68±0.41mg/l) and (7.57±0.42 mg/l), respectively. Site 3 had the highest water conductivity (394.95±6.27 µScm-1), while sites 2 (289.15 µScm-1) and 1 (248.37 µScm-1) had the lowest. Along with conductivity and water temperature, site 3 had the highest chemical oxygen demand (25.50 mg/l), while sites 2 (15.82 mg/l) and 1 (11.86 mg/l) had the lowest. Total solids (TS) decreased significantly from 261.66 mg/l at site 3, then 212.66 mg/l at site 2 to 204 mg/l at site 1. The mean concentration of nitrates NO_3 was 2.06 mg/l in site 3, while it was 0.23 mg/l in site 2 and 0.11 mg/l in site 1. Site 3 had the highest fluoride concentration (0.46 mg/l), followed by site 2 (0.23 mg/l) and site 1 (0.18 mg/l). The mean concentration of sulphates SO_4 was reported at site 3 with a mean concentration of (59.66 mg/l), then declined at sites 2 (28.33 mg/l), and 1 (22 mg/l), like fluoride, phosphates have peaked at site 3 (13.21 mg/l) before decreasing at sites 2 (8.67 mg/l) and 1 (6.97 mg/l). When comparing the means of most processed parameters between the investigated sites, the LSD (Table 2) test showed that there were substantial differences ($0.05 > p < 0.01$).

Table 1. Means ±SD of water physicochemical parameters of the studied sites.

Parameters(unit)	Site 1	Site 2	Site 3
pH	7.26±0.35	8.07±0.85	8.26±0.55
DO (mg/l)	7.57±0.42	6.68±0.41	5.92±0.13
Conductivity (µScm-1)	248.37±3.60	289.15±28.24	394.95±6.27
Turbidity (mg/l)	10.43±0.20	12.42±1.86	19.81±1.64
TS (mg/l)	204±3.60	212.66±11.01	261.66±7.63
Hardness (mg/l)	82.33±10.78	98.66±11.50	114.66±1.52
COD (mg/l)	11.86±0.63	15.82±3.26	25.50±0.16
NO_3 (mg/l)	0.11±0.005	0.23±0.11	2.06±0.90
SO_4 (mg/l)	22 ±4	28.33±5.50	59.66±4.50
PO_4 (mg/l)	6.97±1.33	8.67±3.01	13.21±1.20
F (mg/l)	0.18±0.005	0.23±0.043	0.46±0.050

Table 2. LSD multiple comparisons between the ecological factors at the studied sites.

Parameters(unit)	Site 1& Site 2	Site 1& Site 3	Site 2& Site 3
pH	-(.162) ^{NS}	-(.096) ^{NS}	-(.721) ^{NS}
DO (mg/l)	(.021) *	(.001) **	(.037) *
Conductivity (µScm-1)	-(.028) *	-(000) **	-(000) **
Turbidity (mg/l)	-(.142) ^{NS}	-(000) **	-(.001) **
TS (mg/l)	-(.234) ^{NS}	-(000) **	-(000) **
Hardness (mg/l)	-(.071) ^{NS}	-(005) **	-(.076) ^{NS}
COD (mg/l)	-(.045) *	-(000) **	-(.001) **
NO3 (mg/l)	-(.794) ^{NS}	-(.004) **	-(.005) **
SO4 (mg/l)	-(.151) ^{NS}	-(.000) **	-(.000) **
PO4 (mg/l)	-(.344) ^{NS}	-(.009) **	-(.034) *
F (mg/l)	-(.218) ^{NS}	-(.000) **	-(.000) **

*: The mean difference is significant at the 0.05 levels.
**: The mean difference is significant at the 0.01 levels.
NS: The mean difference is not significant

Heavy metals in water

Based on the current data, metals concentrations in water were found in the following order: Fe > Zn > Cu > Cd >Pb in site 1 (Table 3), whereas they follow the order of Fe > Cu > Zn > Pb > Cd in sites 2 and 3. The mean concentration of cadmium fluctuated as (0.002 µg/l) at site 1, (1.106 µg/l) at site 2 and (3.933 µg/l) at site 3. Lead concentration increased from (.0008 µg/l) at site 1 to value (1.923 µg/l) at site 2, then increased remarkably to (8.116 µg/l) at site 3. The mean concentrations of zinc were (87.33 µg/l), (112.333 µg/l) and (236.000 µg/l) from site 1 to site 3 respectively. Copper achieved its maximum concentration at site 3 (420.66 µg/l) and decreased to (253.66 µg/l) and (83.66 µg/l) at site 2 and 1, respectively; as well as copper, iron exhibited remarkable increase (124.33 µg/l), (399.66 µg/l) and (818.33 µg/l) at site 1, 2 and 3 respectively. The LSD statistics (Table 4) concluded that the mean concentration of all reported metals varied substantially between site 1 and site 3 and site 2 and site 3 ($p < 0.01$), except for the mean difference of copper between site 2 and 3 (insignificant).

Table 3. Means ±SD of heavy metals concentrations (µg/l) in water at the investigated sites.

Heavy metals (µg/l)	Site 1	Site 2	Site 3
Cd	.002±.002	1.10±1.05	3.938±0.208
Pb	.0008±.0006	1.92±1.72	8.11±0.63
Zn	87.33±2.51	112.33±23.86	236±12.16
Cu	83.66±2.51	253.66±173.09	420.66±33.50
Fe	124.33±4.04	399.66±255.86	818.33±112.53

Table 4. LSD multiple comparisons between the concentration of heavy metals in water at the studied sites.

Heavy metals (µg/l)	Site 1& Site 2	Site 1& Site 3	Site 2& Site 3
Cd	-(.072) ^{NS}	-(000) **	-(.001) **
Pb	-(.068) ^{NS}	-(.000) **	-(000) **
Zn	-(.096) ^{NS}	-(000) **	-(000) **
Cu	-(.087) ^{NS}	-(.007) **	-(.091) ^{NS}
Fe	-(.082) ^{NS}	-(.002) **	-(.019) **

Heavy metals in sediment

The mean concentration of cadmium in sediment (Table 5) analyzed from site 3 was (0.67 µg/g) followed by (0.38 µg/g) in site 2, then (0.51 µg/g) in site 1. The measured lead from site 3 was (6.01 µg/g) followed by (1.33 µg/g) in site 2 then (0.44 µg/g) in site 1. Zinc peaked in site 3 (74.90 µg/g), then decreased in site 2 to (49.81 µg/g) and gave the value (42.68 µg/g) at site 1. Copper was higher in site 3 (45.52 µg/g) and showed closed values in site 2 (32.32 µg/g) and site 1 (20.45 µg/g). Like copper, the lowest concentration of iron was observed in site 1 with mean (275.36 µg/g) and gave negligible differences between site 2 (283.43 µg/g) and site 3 (288.31 µg/g). To clarify the interaction between sites in relevant to their concentration of heavy metals, LSD (Table 6) test has revealed that the means of Zn, Pb and Cu differed significantly between site 1& site 3 and site 2& site 3 ($0.05 > p < 0.01$).

Table 5. Means ±SD of heavy metals concentrations (µg/g) in sediment of investigated sites.

Heavy metals (µg/g)	Site 1	Site 2	Site 3
Cd	0.51±0.27	0.38±0.21	0.67±0.11
Pb	0.04±0.24	1.33±1.22	6.01±1.37
Zn	42.68±1.25	49.81±8.16	74.90±4.79
Cu	20.45±2.04	32.32±8.16	45.52±4.44
Fe	275.36±0.91	283.43±9.04	288.31±2.66

Table 6. LSD multiple comparisons between the concentrations of heavy metals in sediment at the studied sites

Heavy metals (µg/g)	Site 1& Site 2	Site 1& Site 3	Site 2& Site 3
Cd	-(.474) ^{NS}	-(.393) ^{NS}	-(.143) ^{NS}
Pb	-(.186) ^{NS}	-(000) **	-(.002) **
Zn	-(.165) ^{NS}	-(000) **	-(.001) **
Cu	-(.048) *	-(.002) **	-(.034) *
Fe	-(.121) ^{NS}	-(.027) *	-(.317) ^{NS}

Heavy metals in liver

Regarding the three sites, the mean concentration (Table 7) of cadmium in liver was (1.23 µg/g) in site 3, (0.64 µg/g) in site 2 and was (0.59 µg/g) in site 1. Lead concentration was maximum in site 3(1.13 µg/g) but exhibited the mean values as (0.48 µg/g) and (0.13 µg/g). at site 2 and site 1, respectively. Also, Zinc peaked in site 3 (119.11 µg/g), then declined in site 2 (77.15 µg/g) and site 1 (46.47 µg/g). The mean value of copper was (51.49 µg/g), (61.75 µg/g) and (149.10 µg/g) from site 3 to site 1, respectively. The mean records (113.14 µg/g), (62.70 µg/g) and (40.94 µg/g) were the values of iron at site 3, 2 and 1, respectively. On the other hand, LSD test has verified that the difference of means of all metals (except Cd) were significantly (Table 8) differed between site 1 &site 3 and site 2 & site 3($0.05 \geq p < 0.01$). To better understand the bioaccumulation of heavy metals in *O. niloticus* tissue (liver) and the surrounding environment, correlation analysis (Table 9) showed that the mean values of all heavy metal concentrations in liver (except Cd in sediment) correlated positively (0.05>p0.01) with heavy metals distribution in the environment (water and sediments).

Table 7. Means ±SD of heavy metals concentrations (µg/g) in the liver of *O. niloticus* of investigated sites.

Heavy metals (µg/g)	Site 1	Site 2	Site 3
Cd	0.59±0.48	0.64±0.70	1.23±0.17
Pb	0.13±0.005	0.48±0.49	1.13±0.15
Zn	46.75±4.76	77.15±23.88	119.11±1.05
Cu	61.75±30.11	51.49±12.92	149.10±4.43
Fe	40.94±6.90	62.70±25.72	113.14±1.67

Table 8. LSD multiple comparisons between the concentrations of heavy metals in tissues of *O. niloticus* collected from the investigated sites.

Heavy metals	Site 1& Site 2	Site 1& Site 3	Site 2& Site 3
Cd	-(.912) ^{NS}	-(.173) ^{NS}	-(.202) ^{NS}
Pb	-(.103) ^{NS}	-(.004) **	-(.037) *
Zn	-(.037) *	-(.001) **	-(.011) *
Cu	(.535) ^{NS}	-(001) **	-(001) **
Fe	-(.134) ^{NS}	-(001) **	-(007) **

Table 9. Means ±SD of Correlations between metal in tissues and environment.

liver	Cd		Pb		Zn		Cu		Fe	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
Cd	**.69***	**.29**^{NS}								
Pb			**.93****	**.94****						
Zn					**.94****	**.94****				
Cu							**.89****	**.75***		
Fe									**.96****	**.84****

Biochemical analysis (serological parameters)

The results of biochemical parameters of *O. niloticus* are shown in (Table 10). The tabulated data indicated that the total protein of fish collected from sites 1 and 2 are similar in contrast to that of site 3 was lower (4.35 g/dl). As well as protein, albumin decreased from 3.93 g/dl (site 1) to 3.04 g/dl (site 2) and 2.07 g/dl at site 3. The mean value of glucose from site 1 was (87±5.45 mg/dl), while it decreased to 82.56±8.03 mg/dl at site 2 and dramatically increased again at site 3 (99 ±3 mg/dl). Cholesterol and triglyceride exhibited the same trend; they increased significantly from site 1 (127 ±2 mg/dl, 117.33±10.50 mg/dl) to site 3 (183 ±26.90 mg/dl, 239.66±18.61 mg/dl) at site; as well as glucose, creatinine dropped from 0.53±0.27 mg/dl (site1) to 0.45±0.12 mg/dl (site 2) and increased again to the value 1.03±0.11mg/dl (site 3). Urea showed fair increase around the value (6) from site1 to site 3. The mean records of AST and AL elevated remarkably from site 1 (83.33±5.50 U/I and 79 ±8.18 U/I) to site 3 (359.66±54.63 U/I and 391.66±77.68 U/I) respectively. On the other hand, the multiple comparisons LSD (Table 11) test has concluded that all biochemical parameters (except protein) differed significantly between the first and third site and the second and third site ($0.05 \geq p \leq 0.01$). The means of protein correlated (Table 12) negatively and strongly with the heavy metal accumulations in liver ($0.05 > p < 0.01$). As well as protein, albumin correlated negatively and strongly ($0.05 > p < 0.01$) with the heavy metal accumulations in liver (except in the case of Cd was insignificant). In contrast to protein and albumin, the rest of serological parameters of *O. niloticus* exhibited positive increase ($0.05 \geq p \leq 0.01$) with heavy metals increase (Table 12) in liver (glucose, triglycerides and urea were insignificant with Cd).

Table 10. Means ±SD of biochemical parameters of investigated sites

Parameters (unit)	Site 1	Site 2	Site 3
Protein (g/dl)	**6.76±1.71**	**6.58±1.86**	**4.35±0.31**
Albumin (g/dl)	**3.93±0.55**	**3.04±0.61**	**2.07±0.28**
Glucose (mg/dl)	**87 ±5.45**	**82.56±8.03**	**99 ±3**
Cholesterol (mg/dl)	**127 ±2**	**147.66±20.25**	**183 ±26.90**
Triglyceride (mg/dl)	**117.33±10.50**	**137 ±17.43**	**239.66±18.61**
Creatinine (mg/dl)	**0.53±0.27**	**0.45±0.12**	**1.03±0.11**
Urea (mg/dl)	**19.56±1.80**	**24.20±4.73**	**30.46±2.13**
ASAT (U/L)	**83.33±5.50**	**153 ±65.93**	**359.66±54.63**
ALAT (U/L)	**79 ±8.18**	**145 ±51.73**	**391.66±77.68**

Table 11. LSD multiple comparisons between the concentrations of Biochemistry in at the studied sites.

Parameters (unit)	Site 1& Site 2	Site 1& Site 3	Site 2& Site 3
Protein (g/dl)	**(.88)** ^{NS}	**(.092)** ^{NS}	**(.114)** ^{NS}
Albumin (g/dl)	**-(.074)** *	**-(.004)** **	**-(.057)** *
Glucose (mg/dl)	**-(.391)** ^{NS}	**-(.046)** *	**-(.014)** **
Cholesterol (mg/dl)	**-(.241)** ^{NS}	**-(.013)** **	**-(.068)** ^{NS}
Triglyceride (mg/dl)	**-(.0481)** ^{NS}	**-(000)** **	**-(000)** **
Creatinine (mg/dl)	**-(.63)** ^{NS}	**-(.017)** *	**-(.009)** **
Urea (mg/dl)	**-(.124)** ^{NS}	**-(.006)** **	**-(.052)** *
ASAT (U/L)	**-(.136)** ^{NS}	**-(000)** **	**-(.002)** **
ALAT (U/L)	**-(.186)** ^{NS}	**-(000)** **	**-(.001)** **

Table 12. Means ±SD of Correlations between biochemical factors and metals in tissues

Parameters	Heavy metals in liver				
	Cd	Pb	Zn	Cu	Fe
Protein	**-.806****	**-.734***	**-.757***	**-.772***	**-.848****
Albumin	**.394**^{NS}	**-.877****	**-.932****	**-.676***	**. -876****
Glucose	**.301**^{NS}	**.712***	**.618***	**.796****	**.683***
Cholesterol	**.631***	**.790****	**.880****	**.746***	**.873****
Triglyceride	**.589**^{NS}	**.879****	**.930****	**.888****	**.938****
Creatinine	**.801****	**.627***	**.658***	**.923****	**.758****
Urea	**.471**^{NS}	**.979****	**.919****	**.758****	**.898****
ASAT	**.880****	**.880****	**.950****	**.885****	**.963****
ALAT	**.60***	**.833****	**.919****	**.879****	**.926****

DISCUSSION

Environmental pollution has risen significantly in recent decades due to a range of sources, including industrial, commercial, agricultural, and domestic waste, effluents and pollutants, and hazardous substances. The Nile has recently become more polluted because of rising population and related anthropogenic activities. Egypt has also been named as one of the ten countries that will run out of water by 2025 due to its increasingly growing population (**Badr *et al.,* 2013**). Excessive use of pesticides and fertilizers in agriculture, as well as increased dumping of heavily contaminated domestic and industrial effluents into its rivers, are the key sources of Nile pollution in Upper Egypt. Nitrogen and phosphorus (N, P) are important macronutrients for aquatic fauna and flora growth; however, excessive nutrient inputs can cause eutrophication problems (**Varol and Şen, 2012**). Aluminum, agro-industrial, small private factories, and sugar cane, as well as many human activities along the Nile's coastal shore, are the main sources of Nile pollution. Increased point and non-point sources combined with a decline in environmental quality pose a significant threat to human life. The pH values found in this analysis were usual at site 1, but alkaline at sites 2 and 3, indicating that sites 2 and 3 are receiving effluents that increase the pH of the water, and this means that the buffering capacity has been impacted (**Carr and Neary, 2008**). COD is a water quality parameter that measures the oxygen equivalent of the organic matter content (**Emamgholizadeh *et al.,* 2014**). From site 1 to site 2 to site 3, the chemical oxygen demand COD increased significantly. This rise may be since site 2 receives wastewater containing various fertilizers, while site 3 receives a mix of wastes from various sources. This comparison allows us to understand why site 3 has higher phosphate, nitrate sulphate, turbidity, and total hardness than sites 2 and 1, as verified by the LSD test. Site 3 had the lowest dissolved oxygen and the highest chemical oxygen in this analysis. These rational conclusions are because urban waste discharges require more oxygen for the biodegradation of organic matter. As a result, the amount of dissolved oxygen available will decrease. On the other hand, the bioavailability of toxic metals is influenced by physicochemical properties of water, such as pH, alkalinity, and hardness, as well as the natural organic matter content in water (**Svobodová 1993; Sepe *et al.,* 2003**). In the present study, all metals are ranked as follow: Site 1 < site 2 < site 3. Bioaccumulation measurements are studies or methods that monitor the absorption and retention of contaminants such as metals or biocides in the organs and/or tissues of organisms like fish. When ingested, the pollutant is transferred by the blood

to a storage site (such as the bone) or to the liver for transformation and/or storage. Lead Pb, is one of the most toxic heavy metals. Lead is naturally present in the atmosphere, but anthropogenic sources have significantly increased its concentration (**Monteiro *et al.*, 2011; Sfakianakis *et al.*, 2015**). Cadmium toxicity is minimized as calcium and magnesium levels in the water increase. The maximum allowable cadmium concentration in water for salmonids is 0.0002 mg per liter, and 0.001 mg per liter for cyprinids (**Schreckenbach ,1982**). In natural surface waters, the concentration of zinc is usually below 10µg/l (**WHO, 2008**). Unfortunately, the mean zinc concentration fluctuated from (87.33±2.51µg/l), (112.33±23.86µg/l) and (1395.33±523.66µg/l), from south to north suggesting a non-healthy condition of the Nile in combination with the discharge of wastes from various sources. Iron compounds can damage fish in low-oxygenated waters with a low pH as mentioned above, where the iron is more in the form of soluble compounds. Since the fish's gill surface is alkaline, soluble ferrous iron may be oxidized to insoluble ferric compounds, which cover the gill lamellae and prevent breathing (**Svobodová, 1993**). Copper, like lead, can be a developmental poison in large doses, for example various skeletal and soft tissue malformations were found in rats exposed to various copper levels (**Lecyk, 1980**). The liver of the rainbow trout *Oncorhynkus mykiss* demonstrated dilation of sinusoids, congestion of blood vessels, and hepatocyte degeneration (**Atamanalp *et al.*, 2008**) after exposure to copper sulphate.

Fish biochemical blood parameters react quickly to changes in the environment (**Atamanalp *et al.*, 2002; Coşkun *et al.*, 2016**). In the present study, the examined *O. niloticus* collected from different stations of the River Nile exhibited higher values of glucose at higher loads of contamination. This mean that, environmental stressors could potentially pose glycaemia in blooded animals (**Jahanbakhshi and Hedayati, 2013**). Level of glucose were previously recorded in blood of fishes exposed to heavy metals (**Lévesque *et al.*, 2002; Mekkawy *et al.*, 2010**). This can be attributed to the alteration in the activity of glucose-6-phosphate dehydrogenase and lactate dehydrogenase previously detected by **Osman and Harabawy** (**2010**). The concentration of proteins, lipids, and moisture) as well as energy value were found to be significantly lower in the tissues of the river fish due to oxidative stress induced by various contaminants (**Vaseem and Banerjee, 2016**). The chemical pollutants modulate the metabolism of carbohydrates, causing hyperglycemia by stimulating the glycogenolysis in fish (**Lévesque *et al.*, 2002**). Contrarily, protein value in serum in fish from site 3 and 2 was lower than that of site 1. protein depletion could be attributed to change in the water quality of River Nile as a result of the discharged effluents from different sources, including pesticides, hydrocarbons found in sewage wastes and heavy metals in the industrial and agricultural drainage (**Zaghloul, 2000**). *Oreochromis niloticus* exposed to malathion (**Hamed , 2015**) and *Clarias gariepinus* exposed to carbofuran (**Harabawy and Ibrahim 2014**) both showed similar results. This may be explained as the exposure to metals (as Pb and Zn). Moreover, decreased tissues protein in fish living in polluted environment may be a result of decreased insulin level caused by metal toxicity (**Zaghloul, 2001**). Since lipid homeostasis is one of the primary functions of the liver, changes in blood cholesterol and triglyceride concentrations are a sensitive indicator of liver dysfunction (**Kim *et al.*, 2020**). Heavy metals and other xenobiotic not only affect liver, but also can accumulate in

other tissues such as kidney, muscles, causing damaging effects. Renal dysfunction was widely documented during several studies dealt with ecotoxicology. The current results indicated alteration in renal – regulated products, namely creatinine and urea. For example, (**Zaki *et al.*, 2009**) tabulated high values of creatinine and urea from the serum of Mullet fish exposed to cadmium. Furthermore, the remarkable rise in ASAT and ALAT levels observed from site 1 to site 3 could be attribute to physiological and biofunction disruptions. A study conducted by (**Oyagbemi *et al.*, 2016; Adikwu and Bokolo, 2018)** has revealed decreased serum albumin accompanied with elevated serum ASAT and ALAT due to chemical stressors. Fish treated with fungicides (**Osman, 2019**) gave higher values of ALAT and ASAT compared to control ones, as a result of the release of these enzymes into the blood stream. Totally, liver damage caused by harmful chemicals and some medications is a common toxicological issue since the liver is the site of detoxification (**Chiang 2014; Abdel-Wahhab *et al.*, 2021**).

CONCLUSION

Over the last few decades, there has been a growing interest in determining heavy metal levels in aquatic environments, with particular emphasis on public food sources, especially fish. Because heavy metals cannot be degraded biologically, they persist in ecosystems, causing biotransformation and biomagnification via food webs. As a result, because fish is such a large part of the human diet, dietary intake of such metals poses a health risk. Protein, albumin, glucose, cholesterol, creatinine, urea, ASAT, and ASAT in the serum of Nile tilapia were analyzed to determine the extent of harm sustained by fish due to the contaminated condition of the investigated sites.

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