



Eco-Toxicological Risk Assessment of Possible Effects of Potentially Toxic Heavy Metals on Water Quality and Performance of Nile Tilapia (*Oreochromis niloticus*) in Burullus Lake, North Delta, Egypt



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**T**HIS study aimed to evaluate the effects of toxic metals (Cu, Fe, Ni, Cd and Pb) on Burullus Lake water and *Oreochromis niloticus*. To assess contamination grade and associated ecotoxicological risk use Bio-Concentration Factor (BCF), Contamination Factor (CF), Pollution Load Index (PLI), and Metal Pollution Index (MPI) and in addition to the effect of toxic metals on water quality and fish performance in sixteen sampling sites across the lake. Results revealed a significant ( $p<0.05$ ) lower heavy metals concentration in water, liver and muscles in eastern sector (ES) compared to other sectors. While, middle sector (MS) showed significant ( $p<0.05$ ) lower BCF of Fe in liver and muscle. The ES showed the lowest CF, PLI and MPI values in water, liver and muscles except for Fe in muscle CF. Furthermore, ES recorded the lowest salinity, conductivity, total dissolved salts, total ammonia and un-ionized ammonia and the highest dissolved oxygen. Moreover, ES showed the highest fish body weight ( $p>0.05$ ) and condition factor and the lowest hepatosomatic index ( $p<0.05$ ). In conclusion, there was a variation in heavy metals content in different sections of the Burullus lake water, ES showed lower heavy metals concentration in water, liver and muscles compared to other sectors. MS showed significant lower BCF of Fe in liver and muscle. The ES showed the lowest CF, PLI and MPI values in water, liver and muscles except for Fe in muscle CF. The ES may consider the lowest contaminated area of Burullus Lake and consequently showed better water quality and fish performance.

**Keywords:** Fish performance, *Oreochromis niloticus*, Heavy metals, Lake Burullus, Water quality.

## Introduction

Burullus Lake is a part of Kafr El-Sheikh governorate that located in the northern Delta, Egypt. The surface area of the Lake is about 410km<sup>2</sup>. It takes an oblong shape bounded from southern edge by agricultural lands and a sand bar separating the lake from the Mediterranean Sea in the north. The lake depth fluctuates from area to area and ranged from 20cm near to the coastline of the eastern sector

and 200cm in the middle sector and sea-lake connection. Burullus Lake is one of the largest lakes along the Mediterranean Sea and is considered an important source of fish production that could be related to its high environmental diversity. Nowadays, the lake is suffering from changes in environmental and fish components, especially water quality that may have originated from the large amount of industrial, agriculture and waste water discharged into the lake [1]. Roughly, the lake

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obtains around 4 billion m<sup>3</sup> of drainage water per year from various sources [2], including agricultural, domestic, and industrial wastewater that may represent 97% of the water emissions to the lake [3,4]. The drainage water discharged into Burullus Lake through seven drains; drain 7, drain 8, drain 9, drain 11, Nasser drain, Burullus East and West drain.

Many studies were performed to evaluate the various effects of heavy metals due to their toxic effect, bioaccumulation, and prolonged action not only on soil and water but also on different aquatic species [5-7]. Seasonal and physico-chemical properties of water may have an important role in heavy metals concentration in tissues of different aquatic organisms [8]. The source of heavy metals in the lake aquatic environment is related to industrial development in the delta region, massive use of fertilizers, increased livestock manure, air pollution and increases in pesticide usage in the different agriculture purposes [9,10]. Furthermore, [11] stated that heavy metal contamination may be caused by the huge amount of domestic sewage, industrial and agriculture flow into the aquatic environment.

Several studies were used fish as a biological indicator to screen the heavy metal pollution degree in aquatic environments [12], due to the ability of the fish to concentrate and bioaccumulate various toxic heavy metals from the water into their body organs [13]. The concentration of heavy metals in fish differs from organ to organ, therefore, the concentration of heavy metals in gills is very similar to concentration in water because gills are in continuous exposure to water [14]. The portal of heavy metal entry to the fish body is mainly through gills (the main site for direct metal uptake due to its highly absorptive capacity), digestive tract (the moderate site for metal uptake) and external surface of the fish body (the minor part of metals uptake) respectively [15]. Fish growth performance, physiological response, survival rate and reproduction efficiency can be affected by heavy metal toxicity [16].

Fish is a good source of easily digestible protein, high vitamins, essential minerals, and polyunsaturated fatty acids especially omega 3 and 6. At the same time, it is reported as a very dangerous store of toxic heavy metals [17]. By ingestion of fish flesh contaminated with toxic heavy metals, the metals are transferred into human digestive tract causing severe deteriorative effects and may alter human health status in form of digestive troubles, hepatotoxicity, and renal toxicity [18]. For that reason, heavy metals should not exceed the worldwide permissible limits and the recommended safety concentrations for humans and fish to avoid its toxic effects [19]. Therefore, many advanced methods are updated for toxic heavy metals

remediation as bioremediation [20]. Therefore, the aim of this study was to 1) evaluate the potentially available heavy metals (Cu, Fe, Ni, Cd and Pb) in Burullus Lake water and existing fish (muscle and liver tissues), 2) to evaluate the degree of contamination and the related eco-toxicological risk using different contamination indices as Bio-Concentration Factor (BCF), Contamination Factor (CF), Pollution Load Index (PLI), and Metal Pollution Index (MPI), 3) to evaluate the effect of potentially available toxic heavy metals (Cu, Fe, Ni, Cd and Pb) on water quality and fish performance.

## Experimental

### Study area, site description and data collection

This study was conducted in Burullus Lake which is located in Northern Nile Delta, Egypt (31° 22'–31° 26'N and 30° 33'–31° 07' E) and considered as one of the largest Egyptian northern lakes (Figure 1). It is connected to the Mediterranean Sea at its northeast side by a narrow outlet called Boughaz. Untreated agricultural, industrial, and domestic drainage from towns and villages near the lake directly flows into the southern part of the lake.

Sixteen sampling sites are selected from the whole lake during the summer season (from May to July 2018). Each site was sampled only one time and triplicate samples were collected during the sampling process. The studied area was divided into three sections. The western sector "WS": it is the narrowest part of the lake comprising four sampling sites (1, 2, 3, and 4) and it includes the western part of the studied area in the lake. The middle sector "MS": it includes the middle part of the studied area in the lake and comprises six sampling sites (5, 6, 7, 14, 15, and 16). The eastern sector "ES": it is the widest part of the lake comprising six sampling sites (8, 9, 10, 11, 12, and 13) and it includes the eastern part of the studied area in the lake. Total: it includes all the studied areas in the lake comprising all the sixteen sampling sites (Fig. 1). Water and fish (*Oreochromis niloticus*) samples were collected in triplicates from each site using a boat and guided with GPS (Figure 1). Each replicate was treated separately for each site.

For water samples collection from each site, one thousand ml (one liter) sterilized glass bottle was inverted 20 cm under the water surface. All samples (3 separate replicates / site) were transferred to the central laboratory for environmental studies, faculty of Agriculture, university of Kafrelsheikh in ice box to assess the concentration of some toxic heavy metals. Sampled fish are harvested using nylon net (0.5 cm mesh size) by fishermen in the same place and time of water sampling. Thirty fish were collected from each site (10 fish of average size per

each replicate). The harvested fish were weighed, measured, and dissected on the boat to obtain muscle and liver samples. Muscle and liver tissues were transferred to laboratory in ice box and then frozen until toxic metal analysis.

#### Assessment of heavy metals in water and fish samples

Five toxic heavy metals (Cu, Fe, Ni, Cd and Pb) were assessed in this study both in lake water and fish tissues (muscles and liver). The concentrations of heavy metals were measured by atomic absorption

spectrophotometer (GBC Avanta E, Victoria, Australia; Ser. No. A5616).

#### Assessment of heavy metals in water samples

One liter of each water sample was gently evaporated until dryness in a rotary evaporator (Binder, Germany) and then the sample was dissolved in 5 ml concentrated HNO<sub>3</sub>. For sample digestion, 5-10 drops of hydrogen peroxide were added to each sample. The dried residue was eluted in 1 ml HNO<sub>3</sub>, and the target metals were measured as described by some authors [21].

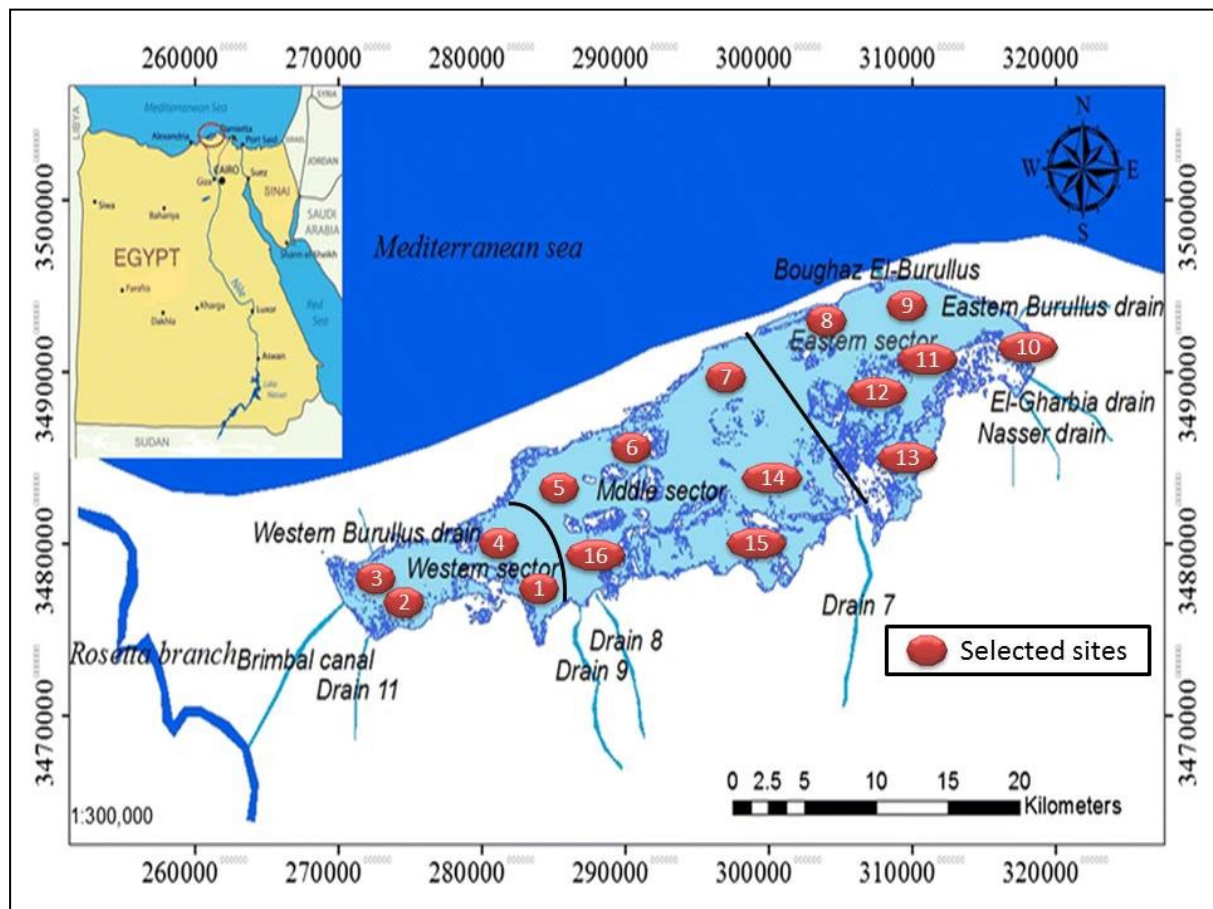


Fig. 1. Study area

#### Assessment of heavy metals in fish samples (muscles and liver)

In a muffle furnace (Binder, Germany), each sample was prepared at 450 °C for 5 h. Extraction was done using 20 % hydrochloric acid [22]. Half gram of dry-ached fish muscles mass and liver sample was used.

#### Quality control

All glass and plastic wares were washed and kept overnight in 10 % (v/v) nitric acid solution. After that, it was rinsed thoroughly with ultra-pure water and dried. The quality assurance and accuracy of the

analytical method was achieved by duplication of the sample. The analytical validation was accompanied by the analysis of certified reference samples (APHA, AAM.0500). The calibration standard solutions and blanks were analyzed by the same procedures as the sample solutions. All chemicals used for sample preparation and extraction of samples are purchased from Sigma Aldrich, Egypt. The obtained results of Cu, Fe, Ni, Cd and Pb heavy metals coincided with the certified reference samples. Standard deviation of replicate analysis was below 5%, but in a very few cases the standard deviation of the measurements was above 5% so

these were not included in the statistical analyses. The detection limits obtained for Cu, Fe, Ni, Cd and Pb were 0.1, 0.2, 0.1, 0.03 and 0.1 mg/L, respectively. The heavy metals concentrations in the digested samples were measured using atomic absorption spectrometry (GBC Avanta E, Victoria, Australia; Ser. No. A5616).

### **Eco-toxicological risk assessment of potential toxic heavy metals**

#### **Bio-Concentration Factor (BCF)**

It is the ratio of the metal ion concentrations in the fish tissue (muscle or liver) versus the concentration in water [6]. The BCF was calculated using the following equation [23]:  $BCF = \text{concentration of a metal in fish tissue} / \text{concentration of the same metal in water}$ . The higher the ratio of BCF, the more severe the bio-concentration of pollutants in fish tissue in this study.

#### **Contamination factor (CF)**

The CF was calculated using the following equation [24]:  $CF = C_{\text{metal}} / C_{\text{background}}$ , Where  $C_{\text{metal}}$  is the metal concentration and  $C_{\text{background}}$  is the background concentration of each metal. In this study, the lowest concentration was used as baseline/or background values [25]. The authors have used these background reference concentrations due to the lack of local element background information and to have a uniform scale for all elements studied. The indication of CF value;  $CF < 1$  it indicates low contamination,  $1 \geq CF < 3$  it indicates moderate contamination,  $3 \geq CF \geq 6$  means considerable contamination and  $CF > 6$  means very high contamination [26].

#### **Pollution load index (PLI)**

The PLI was calculated using the following equation [27]:  $PLI = (CF_1 \times CF_2 \dots \times CF_n)^{1/n}$

Where  $n$  is the number of metals and  $CF$  is the contamination factor. The indication of PLI value:  $PLI < 1$  it indicates a low proposed pollution,  $PLI$  is equal one indicates the presence of pollutants baseline level, however  $PLI$  is above one indicates progressive degree of pollution.

#### **Metal pollution index (MPI)**

The MPI was calculated using the following equation [28-30]:  $MPI = (M_1 \times M_2 \dots \times M_n)^{1/n}$

Where  $n$  is the metal number and  $M$  is metal concentration, respectively. Value indications as reported for PLI ( $MPI < 1$  it indicates a low proposed pollution,  $MPI$  is equal one indicates the presence of pollutants baseline level, however  $MPI$  is above one indicates progressive degree of pollution).

### **Water quality parameters**

Water quality parameters were measured in triplicates for each site. Three water samples were collected from each site by inverting 250-ml sterilized glass bottle 20 cm under the lake water surface to determine the total ammonia (TAN) using a portable colorimeter (Martini MI 405), pH, temperature, salinity, electrical conductivity (EC) and total dissolved solids (TDS) using Multiparameters probe meter (HI9829, Hanna Instruments Inc., Woonsocket, RI 02895 USA). Un-ionized ammonia (UIA) was calculated depending on the pH and temperature values of the same sample. Dissolved oxygen (DO) was determined in the different selected sites at 20 cm below the lake water surface using a dissolved oxygen meter (AQ 600 Milwaukee, Romania) [31].

### **Fish performance, condition factor, hepatosomatic index, and length-weight relationship**

To remove the excess water before weighing, fish samples were dried using a clean and sterile filter paper directly after fishing. Fish and dissected liver were weighed using digital balance (PW Balance, ADAM equipment Co., USA). Hepatosomatic index (HSI) was calculated as:  $HSI = (\text{liver weight} / \text{total fish weight}) \times 100$ . The length and width of fish were measured using a measuring board, while the fish thickness was measured by digital caliber (mm). The total length was measured as the distance from the mouth to the beginning of the caudal fin. The length and weight of fish were recorded to the nearest mm and 0.1 g, respectively. The length-weight relationship (LWR) was calculated using the logarithmic regression formula:  $W = a \times L^b$  while condition factor ( $K$ ) was calculated as  $K = 100 \times W/L^3$ , where  $W$  is the total weight (g) and  $L$  is the total length (cm), whereas  $a$  and  $b$  are the regression slope and intercept (regression coefficient), respectively [32-34].

### **Ethical approval**

Experiments were conducted in accordance with the Committee of Aquatic Animal Care and Use in Research, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt.

### **Statistical analysis**

Data were tested for the distribution normality and homogeneity of the variance. Data were analysed by a one-way ANOVA followed by Tukey's multiple range test using Graph Pad Prism 6. Results are presented as means and SD. The LWR was calculated by linear regression analysis of Graph Pad Prism 6 using logarithmic weight and length data. Significance level was set at  $p < 0.05$ .

## Results

### Concentration of the heavy metals (Cu, Fe, Ni, Cd and Pb) in Burullus Lake water and fish (muscle and liver):

#### Evaluation of heavy metals (Cd, Fe, Ni, Cu, and Pb) in Burullus Lake water:

The average values of heavy metals (Cd, Fe, Ni, Cu, and Pb) in surface water of Burullus Lake are presented in table 1. There was a significant difference between means of Fe in the different

sectors of the lake (p- value: 0.013). Also, there was a significant difference between means of Pb in the different lake sectors (p- value: 0.009). The means of Fe and Pb in total were 1.322 and 0.317 mg/L, respectively. The concentration of Ni, Cu and Cd in lake water was not detected. The highest value of Fe in Burullus Lake water was 1.632 mg/L in the MS whereas the lowest value of Fe was 1.1mg/L in ES. The highest value of Pb in Burullus Lake water was 0.396mg/L in the MS whereas the lowest concentration of Pb was 0.264 mg/L in ES.

**TABLE 1. The concentration (mg/L) of some toxic heavy metals (Cu, Fe, Ni, Cd, Pb) in Burullus Lake water, North delta, Egypt**

	Concentrations from the present study (mg/L)				PL	Mean concentrations from previous studies (mg/L)	
	WS	MS	ES	Total		Value	Reference
<b>Cu</b>	Min.	ND	ND	ND	0.05	0.023	[19]
	Max.	ND	ND	ND		0.012	[63]
<b>Fe</b>	Min.	0.870	0.880	0.590	0.3	0.029	[25]
	Max.	1.740	2.680	1.630		0.015	[19]
	Mean	1.191 <sup>ab</sup>	1.632 <sup>a</sup>	1.100 <sup>b</sup>		1.920	[63]
	SD	0.333	0.588	0.410		0.522	
<b>Ni</b>	Min.	ND	ND	ND	0.01	0.007	[25]
	Max.	ND	ND	ND		0.027	[19]
<b>Cd</b>	Min.	ND	ND	ND	----	0.03	
	Max.	ND	ND	ND		0.03	[64]
<b>Pb</b>	Min.	0.210	0.210	0.190	0.05	0.006	[25]
	Max.	0.320	0.670	0.375		0.012	[19]
	Mean	0.267 <sup>a</sup>	0.396 <sup>b</sup>	0.264 <sup>a</sup>		0.080	[56]
	SD	0.053	0.150	0.051		0.120	0.007

PL (permissible limits, mg L<sup>-1</sup>) according to guidelines in [41], WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area), ND (not detected). Means within the same row and have different superscript letters are significantly different at  $p < 0.05$ .

### Assessment of heavy metals (Cd, Fe, Ni, Cu, and Pb) in fish tissue samples (Muscles and liver):

#### Assessment of heavy metals concentration in muscle tissue:

Table 2 shows the average concentrations of heavy metals (Cd, Fe, Ni, Cu, and Pb) in muscles of *O. niloticus* fish. The means of heavy metals Cu, Fe and Ni in the muscles of *O. niloticus* fish were 12.2, 90.55 and 1.464 mg/kg dry weight, respectively. The concentrations of heavy metals in muscle of fish follow the following order Fe > Cu > Ni. There was a significant difference between means of Fe and Ni in the different sectors (p- value: 0.005 and 0.030 respectively). The highest concentration of Fe in fish muscles was 102.15 mg/kg at the WS whereas the lowest concentration of Fe was 84 mg/kg at the ES. The highest value of Ni in fish muscles was 1.5 mg/kg dry weight at the MS whereas the lowest concentration of Ni was 1.363 mg/kg dry weight at the ES. There was not a significant difference

between means of Cu in the different sectors (p-value: 0.999) however, the concentration of Cu in eastern and middle sector was not detected. The concentration of Cd and Pb in muscles was not detected.

#### Assessment of heavy metals concentration in liver:

The obtained concentrations of heavy metals (Cd, Fe, Ni, Cu, and Pb) in liver of *O. niloticus* fish are presented in table 3. The means of heavy metals Cu, Fe and Ni in the liver of *O. niloticus* fish were 43.9, 179.7 and 63.65 mg/kg dry weight, respectively. The concentrations of heavy metals in liver of fish follow the following order Fe > Ni > Cu. There was a significant difference between the means of Fe and Ni in the different lake sectors (p- value: 0.022 and 0.001 respectively). The highest concentration of Fe in fish liver was 204.65 mg/kg dry weight at the WS whereas the lowest concentration of Fe was 150 mg/kg at the ES.

The highest concentration of Ni in fish liver was 74.25 mg/kg dry weight at the WS whereas the lowest value of Ni was 49.9 mg/kg dry weight at the ES. There was not a significant difference between means of Cu in the different lake sectors (p- value: 0.947) however, the highest concentration of Cu in

fish liver was 48.35 mg/kg dry weight at the WS whereas the lowest value of Cu was 43 mg/kg dry weight at the ES.

The concentration of Cd and Pb in liver was not detected.

**TABLE 2. The concentration of some toxic heavy metals (Cu, Fe, Ni, Cd, and Pb) in muscle tissue of Nile tilapia (*Oreochromis niloticus*) reared in Burullus Lake, North delta, Egypt**

	Concentrations from the present study (mg/Kg)				PL	Mean concentrations from previous studies (mg/kg)		
	WS	MS	ES	Total		Value	Reference	
<b>Cu</b>	Min.	10.8	ND	ND	30	0.39	[25]	
	Max.	12.75	ND	ND		0.36	[19]	
	Mean	12.2	----	----		12.2	4.90	[66]
	SD	2.44	----	----		2.44		
<b>Fe</b>	Min.	86	70	51.5	100	10.62	[25]	
	Max.	124	104.5	106		124	27.14	[19]
	Mean	102.15 <sup>a</sup>	85.15 <sup>b</sup>	84 <sup>b</sup>		90.55 <sup>ab</sup>	21.44	[7]
	SD	7.6	5.8	7.5		4.3		
<b>Ni</b>	Min.	1.230	1.180	0.940	0.5-1	0.52	[25]	
	Max.	1.690	1.630	1.550		1.690	1.4	[19]
	Mean	1.405 <sup>ab</sup>	1.500 <sup>a</sup>	1.363 <sup>b</sup>		1.464 <sup>ab</sup>	6.31	[66]
	SD	0.125	0.147	0.212		0.127		
<b>Cd</b>	Min.	ND	ND	ND		0.01-	[67]	
	Max.	ND	ND	ND		0.03		
<b>Pb</b>	Min.	ND	ND	ND	2	0.46	[25]	
	Max.	ND	ND	ND		1.29	[19]	

PL (permissible limits) (average daily intake in wet weight) according to WHO guidelines, 1989 [42], WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area), ND (not detected). Means within the same row and have different superscript letters are significantly different at  $p < 0.05$ .

**TABLE 3. The concentration of some toxic heavy metals (Cu, Fe, Ni, Cd, and Pb) in liver tissue of Nile tilapia (*Oreochromis niloticus*) reared in Burullus Lake, North delta, Egypt**

	Concentrations from the present study (mg/ kg)				PL	Mean concentrations from previous studies (mg kg <sup>-1</sup> )		
	WS	MS	ES	Total		Value	Reference	
<b>Cu</b>	Min.	25.1	19.9	7.7	20-30	12.7	[68]	
	Max.	74.95	111.3	73.95		111.3	165.4	[43]
	Mean	48.35	43.5	43		43.90		
	SD	11.0	15.0	11.3		17.40		
<b>Fe</b>	Min.	138	126	70.5	----			
	Max.	274.5	260	227.5		274.5		
	Mean	204.65 <sup>a</sup>	192.85 <sup>ab</sup>	150 <sup>b</sup>		179.7 <sup>ab</sup>		
<b>Ni</b>	Min.	65	64	37.5	----			
	Max.	86	77.5	67		86		
	Mean	74.25 <sup>a</sup>	70.4 <sup>ac</sup>	49.9 <sup>b</sup>		63.65 <sup>c</sup>		
	SD	7.309	3.448	9.806		13.03		
<b>Cd</b>	Min.	ND	ND	ND	0.5	8.8	[43]	
	Max.	ND	ND	ND		ND		
<b>Pb</b>	Min.	ND	ND	ND	0.5	2.193	[68]	
	Max.	ND	ND	ND		ND	108	[43]

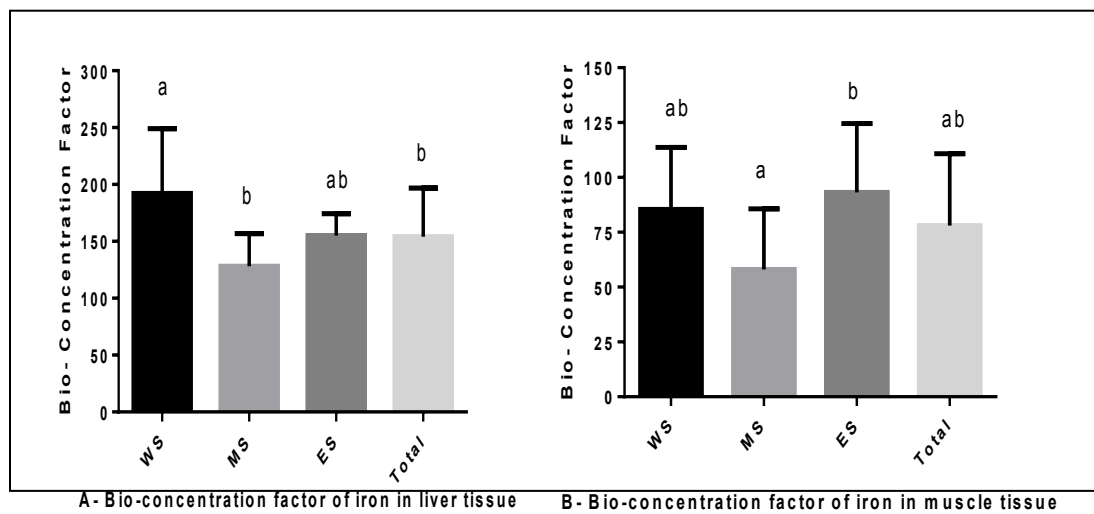
PL (permissible limits) according to Egyptian authority for environmental affairs, law 48 [43], WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area), ND (not detected). Means within the same row and have different superscript letters are significantly different at  $P < 0.05$ .

**Assessment the degree of contamination and the related eco-toxicological risk using Bio-Concentration Factor (BCF), Contamination Factor (CF), Pollution Load Index (PLI), and Metal Pollution Index (MPI) for heavy metals (Cu, Fe, Ni, Cd and Pb) in Burullus Lake water and fish (muscles and liver):**

**Estimation of Fe BCF in Burullus Lake *O. niloticus* fish:**

The BCF of Fe in the muscles of fish in different Burullus Lake sectors is represented in Figure 2.

There was a significant difference in BCF of Fe in the muscles of *O. niloticus* fish between the different sectors ( $p$ -value: 0.0087). The ES recorded the highest BCFs of Fe (93.21) however the MS had the lowest BCFs of Fe (58.12). Also, there was a significant difference in BCF of Fe in the liver tissues of *O. niloticus* fish between the different lake sectors ( $p$ -value 0.0006). The maximum BCF of Fe in liver (192.0) was observed at the WS, whereas the minimum BCF of Fe in liver (128.0) was recorded at the MS.



**Fig. 2. Bio-Concentration Factor (mean ±SD) of iron (Fe) in Nile tilapia (*Oreochromis niloticus*) liver tissues ( $p$ -value = 0.0006) “Left side” and muscle tissue ( $p$ -value = 0.0087) “right side” in Burullus Lake, North delta, Egypt.**

Means lack common superscripts differ significantly (Tukey’s multiple comparison test,  $p < 0.05$ ). WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area)

**Estimation of the CF, PLI, and MPI for fish and water in Burullus Lake:**

Table 4 shows the calculated values of the CF for *O. niloticus* fish collected from different sectors of Burullus Lake. The means of CF of Cu, Fe and Ni in the liver of fish were 5.701, 2.549 and 1.698, respectively. The CF of heavy metals in liver of fish follow the following order Cu > Fe > Ni. It was observed that the WS had the highest CF of Cu, Fe and Ni in liver tissues, while the ES had the lowest CF of these metals in the liver. The means of CF of Cu, Fe and Ni in the muscles of *O. niloticus* fish were 1.121, 1.351 and 1.558, respectively. The CF of

heavy metals in muscle of fish follow the following order Ni > Fe > Cu. The mean of CF of Cu in fish muscle in the WS was 1.121, while the values of CF of Cu in fish muscle in the eastern and middle sectors were not detected. The highest record of CF of Fe in muscle of fish was 1.524 at the WS whereas the lowest value of Fe was 1.221 at the MS. The highest value of CF of Ni in fish muscle was 1.657 at the MS while, the lowest concentration of Ni was 1.388 at the ES. The highest values of CF of Fe and Pb in water were 2.824 and 2.084, respectively at the MS whereas the lowest values of Fe and Pb in water were 1.746 and 1.495, respectively at the ES.

**TABLE 4. Values of the Contamination Factor (CF) of potential toxic heavy metals (Cu, Fe, Ni, and Pb) in fish (liver, muscle) and water in Burullus Lake, North delta, Egypt**

			WS	MS	ES	Total
Liver	Cu	Minimum	3.260	2.584	1.000	1.000
		Maximum	9.734	14.45	9.604	14.45
		<b>Mean</b>	<b>6.280</b>	<b>5.650</b>	<b>5.582</b>	<b>5.701</b>
		SD	2.489	4.158	3.053	3.367
	Fe	Minimum	1.957	1.787	1.000	1.000
		Maximum	3.894	3.688	3.227	3.894
		<b>Mean</b>	<b>2.902</b>	<b>2.735</b>	<b>2.128</b>	<b>2.549</b>
		SD	0.713	0.615	0.745	0.755
	Ni	Minimum	1.733	1.707	1.000	1.000
		Maximum	2.293	2.067	1.787	2.293
		<b>Mean</b>	<b>1.980</b>	<b>1.877</b>	<b>1.331</b>	<b>1.698</b>
		SD	0.195	0.092	0.262	0.348
Muscle	Cu	Minimum	1.000	ND	ND	1.000
		Maximum	1.181	ND	ND	1.181
		<b>Mean</b>	<b>1.121</b>	<b>ND</b>	<b>ND</b>	<b>1.121</b>
		SD	0.130	ND	ND	0.130
	Fe	Minimum	1.284	1.000	1.060	1.000
		Maximum	1.851	1.560	1.582	1.851
		<b>Mean</b>	<b>1.524</b>	<b>1.221</b>	<b>1.336</b>	<b>1.351</b>
		SD	0.198	0.206	0.165	0.220
	Ni	Minimum	1.000	1.564	1.106	1.000
		Maximum	1.995	1.734	1.649	1.995
		<b>Mean</b>	<b>1.496</b>	<b>1.657</b>	<b>1.388</b>	<b>1.558</b>
		SD	0.270	0.070	0.233	0.176
Water	Fe	Minimum	1.322	1.492	1.000	1.000
		Maximum	3.475	4.542	2.763	6.237
		<b>Mean</b>	<b>2.301</b>	<b>2.824</b>	<b>1.746</b>	<b>2.258</b>
		SD	0.948	1.038	0.705	1.365
	Pb	Minimum	0.579	1.105	1.000	0.579
		Maximum	1.6842	3.526	2.211	3.526
		<b>Mean</b>	<b>1.6184</b>	<b>2.084</b>	<b>1.495</b>	<b>1.840</b>
		SD	0.040	0.791	0.362	0.899

WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area), ND (not detected).

The PLI and MPI values are demonstrated in table 5. For the values of PLI recorded in liver tissue, the highest value was recorded in the WS (3.304) and the lowest in ES (2.51). The PLI values of muscle and water show the highest records in the MS (1.422 and 2.426) and the lowest in ES (1.362 and 1.616), respectively. The results of MPI values for muscle, it

recorded the highest value in the WS (12.05) and the lowest in ES (10.70), whereas the outcomes of MPI values for water, it recorded the highest value in the MS (0.804) and lowest in ES (0.531). The highest MPI values for liver samples were reported in WS and the lowest in ES.

**TABLE 5. Values of Pollution Load Index (PLI) and Metal Pollution Index (MPI) of potential toxic heavy metals (Cu, Fe, Ni, Cd, and Pb) in fish (liver, muscle) and water in Burullus Lake, North delta, Egypt**

		WS	MS	ES	Total
<b>PLI</b>	Liver (Cu, Fe and Ni)	3.304	3.072	2.510	2.911
	Muscle (Cu, Fe and Ni)	1.367	1.422	1.362	1.331
	Water (Fe and Pb)	1.929	2.426	1.616	2.038
<b>MPI</b>	Liver (Cu, Fe and Ni)	90.23	83.90	68.53	79.48
	Muscle (Cu, Fe and Ni)	12.05	11.30	10.70	11.70
	Water (Fe and Pb)	0.563	0.804	0.531	0.647

WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area)

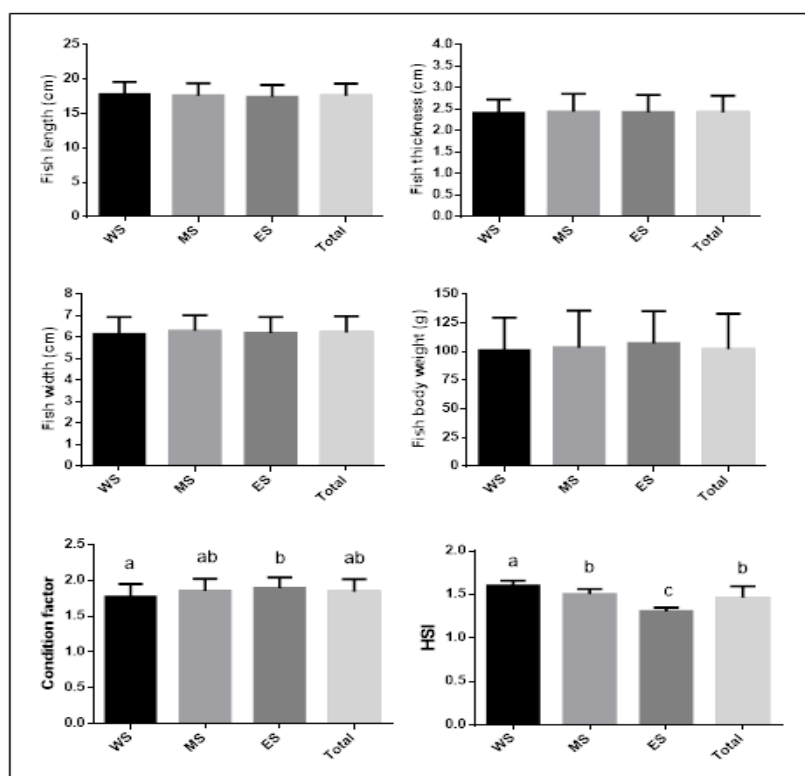


### Effect of potentially toxic heavy metals (Cu, Fe, Ni, Cd and Pb) on water quality:

Water quality parameters in Burullus Lake are presented in (Figure 3). There was a significant difference in salinity between the different lake sectors (p- value: 0.0017). The salinity of WS was higher than the MS and ES. The mean of salinity in WS, MS and ES were 7.775, 6.75 and 6.25 ppm, respectively. There wasn't a significant difference in electric conductivity (EC) between the different lake sectors (p- value: 0.334), conversely, the highest value of EC was recorded in WS (14.00  $\mu\text{S/L}$ ) whereas; the lowest value of EC was also recorded in the ES (11.66  $\mu\text{S/L}$ ).

There was a significant difference in TDS between the different lake sectors (p- value: 0.0179). The TDS of WS was higher than the MS and ES (p < 0.05). The mean of TDS in WS, MS and ES was 7.003, 6.158 and 5.502 mg/L, respectively. There was a significant difference in temperature between the different lake sectors (p- value: 0.0065). The temperature in WS was higher than the MS and ES (p < 0.05). The mean temperature in WS, MS and ES

was 28.6, 28.55 and 28.48°C, respectively. There wasn't a significant difference in pH between the different lake sectors (p- value: 0.1514), however, the highest value of pH was recorded in MS (8.307) whereas; the lowest value of pH was recorded in the WS (8.138). There was a significant difference in dissolved oxygen (DO) between the different lake sectors (p- value: < 0.0001). The DO in the MS recorded the lowest value compared with the WS and ES (p < 0.05). The mean DO value in WS, MS and ES was 2.817, 2.3 and 4.55 mg/L, respectively. There was a significant difference in total ammonia nitrogen (TAN) between the different lake sectors (p- value: 0.0019). The highest value of TAN (p < 0.05) was recorded in MS (1.300) whereas; the lowest value was recorded in the ES (0.973). The mean of TAN in WS, MS and ES was 1.208, 1.300 and 0.973 mg/L, respectively. Furthermore, there was a significant difference in un-ionized ammonia (UIA) between the different lake sectors (p- value: 0.0229). The UIA of MS was higher than the WS and ES (p < 0.05). The mean value of UIA in WS, MS and ES was 0.1235, 0.1809 and 0.1256 mg/L, respectively.



### Effect of potentially toxic heavy metals (Cu, Fe, Ni, Cd and Pb) on fish performance:

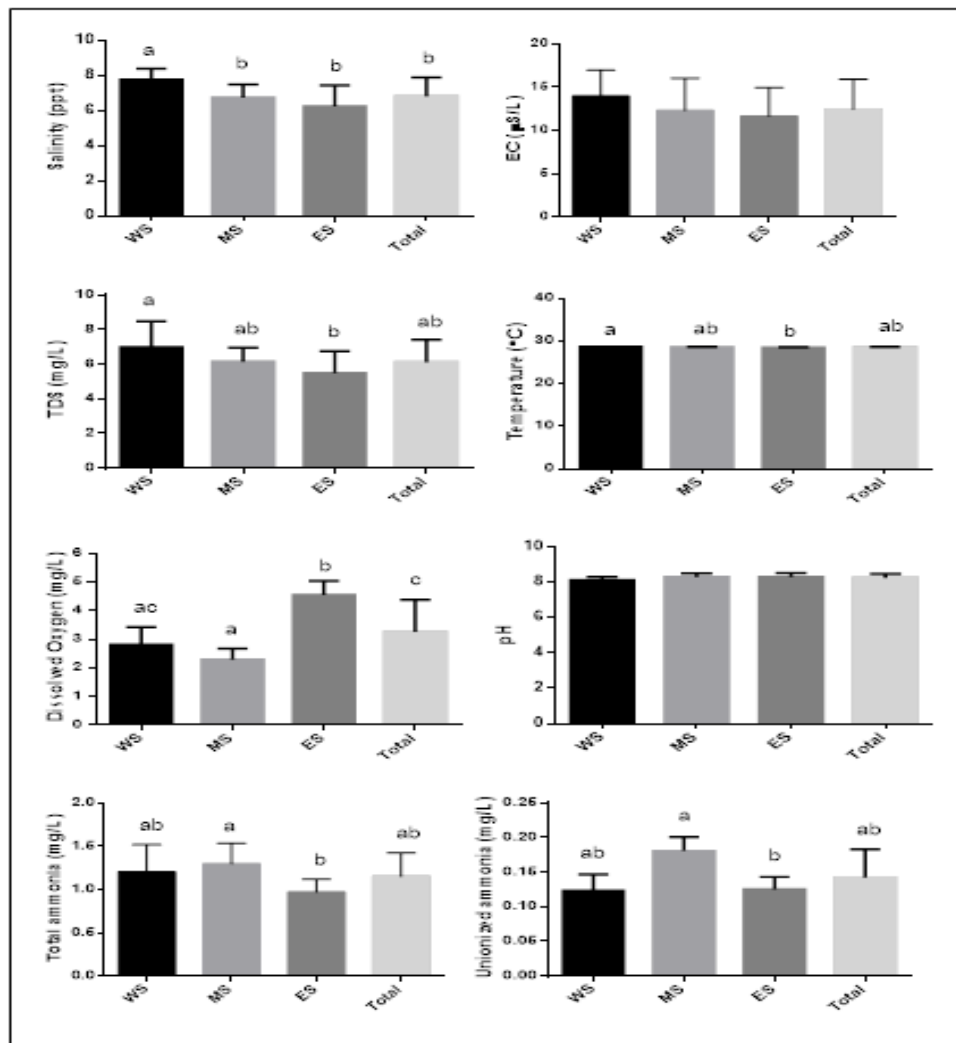
**Fig. 3.** Water quality parameters (mean  $\pm$ SD) in Burullus Lake, North delta, Egypt (summer, 2018).

Means lack common superscripts differ significantly (Tukey's multiple comparison test,  $P < 0.05$ ). WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area).

Effects of some potentially available toxic heavy metals on Nile tilapia (*Oreochromis niloticus*) performance in Burullus Lake are presented in Figure 4. There wasn't a significant difference in fish total length between the different lake sectors (p-value: 0.9994), however, the highest value of fish total length was recorded in ES (17.65 cm). Moreover, there wasn't a significant difference in fish thickness between the different lake sectors (p-value: 0.8978), however, the highest value of fish thickness was recorded in ES (2.481 cm) whereas; the lowest value of fish thickness was recorded in the WS (2.417 cm). Furthermore, there wasn't a significant difference in fish width between the different lake sectors (p-value: 0.8042), however, the highest value of fish width was recorded in WS (6.311cm) and the lowest

value was recorded in the ES (6.159 cm). Moreover, there wasn't a significant difference in fish body weight (FBW) between the different lake sectors (p-value: 0.8208), however, the highest record of FBW was recorded in ES (107.2 g) and the lowest value was recorded in the WS (101 g).

There was a significant difference in the condition factor (K) between the different lake sectors (p-value: 0.0406). The condition factor of ES was higher than the MS and WA. The mean condition factor in WS, MS and ES was 1.772, 1.852 and 1.894, respectively. Moreover, there was a significant difference in hepatosomatic index (HSI) of fish between the different lake sectors (p-value: 0.0001). It was observed that the ES had the highest HSI (1.6) while, the WS had the lowest HSI (1.306).

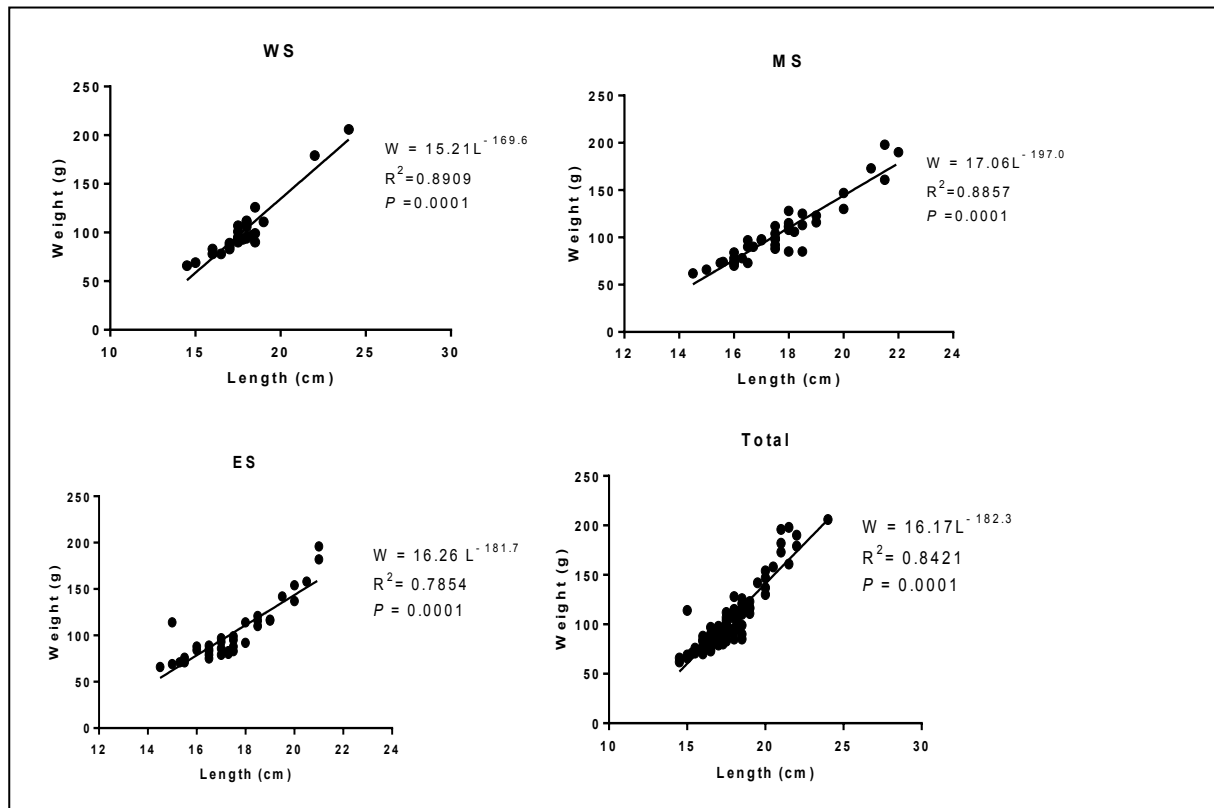


**Fig. 4. Effect of some toxic heavy metals on Nile tilapia (*Oreochromis niloticus*) performance (mean  $\pm$ SD) in Burullus Lake, North delta, Egypt.**

Means lack common superscripts differ significantly (Tukey's multiple comparison test,  $P < 0.05$ ). WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area).

The logarithmic regression of LWR and R<sup>2</sup> determination coefficient values are recorded in (Figure 5). The results revealed a significant correlation ( $p < 0.05$ ) between the total length and

the weight of the sampled fish among all lake sectors with a R<sup>2</sup> values equal to 0.8888, 0.8915, 0.7844 and 0.8435 and regression slopes of 0.9212, 1.383, 1.116 and 1.152 in WS, MS, ES and total, respectively.



**Fig. 5. Effect of some toxic heavy metals on Nile tilapia (*Oreochromis niloticus*) length weight relationship in Burullus Lake, North delta, Egypt .WS (western sector), MS (middle sector), ES (eastern sector), total (all the lake area).**

## Discussion

The present study revealed a variation in heavy metal content in different lake sectors. The highest values of Fe and Pb were recorded in MS. [35] stated that the high heavy metal concentration in the lake may be due to the massive amount of sewage overflow and the massive use of fertilizers and pesticides in the agriculture land around the lake that reach the lake through different drainage systems. Spreading of toxic elements in water bodies is controlled by many risk factors like hydro-soil texture, quality, amount, and type of effluent water. The high concentration of Fe in the lake water may be attributed to clay particles in the nature of lake soil that facilitate the distribution of Fe [21] and its release from sediments in form of sulphides [36]. While the source of contamination of lake with Pb may be due to the massive use of compounds containing Pb as gasoline that used as a source of

energy in fishery boats and antifouling paints used for small fishing boats [37]. Furthermore, the huge amount of pesticides, fertilizers and many other agriculture chemicals containing Pb that reach the lake through different drains [38]. In contrast, the Pb was undetected in the fish muscle and liver. This finding is similar to the result obtained by [39]. [40] reported that in four species of fish collected from northeastern Mediterranean Sea, the Pb concentration was not detected. Furthermore, the Cu was recorded in the fish muscle in the WS only and in all lake sectors in case of liver tissue in spite of its absences in lake water. The same trend was observed for Ni concentration; it was not detected in water and was detected in liver and muscle of fish with relatively higher concentrations in WS followed by MS. It indicates the low level of Cu and Ni concentration in the lake [39]. Overall, the results of heavy metal concentration were dissimilar to that recorded by [25]. They recorded Cu and Ni in the surface water

of the lake and Pb in fish muscle. This may be attributed to different sampling times and the condition of the effluents of the lake. Comparing the results of the present study with the permissible limit (PL) that approved by many national and international organizations; the detected toxic elements in lake water (Fe and Pb) were above the PL approved by guidelines of [26]. While the detected concentration of toxic metals in fish muscle fluctuated with the PL approved by [41] that adapted from [42]. The Cu concentration was below the PL and the Fe concentration was around the PL, but Ni concentration was above the PL. The detected concentration of Cu in fish liver was below the PL approved by Egyptian authority for environmental affairs, law 48 that adapted from [43]. Collectively, the concentrations above the PL may indicate an environmental and human health hazard.

The extent of heavy metals contamination could be estimated by assessing the BCFs, the CF, PLI and MPI. Previous studies showed that the value of BCFs is controlled by many factors such as the chemical types of the pollutant, the affected tissues, and the degree of environment pollution [44-46]. [47] mentioned that accumulation of heavy metal in fish body may be resulted from the affinity of the metal to certain fish tissues, the route of metal uptake into fish body and elimination rates. The higher BCFs in the muscles and liver of fish indicate higher release of heavy metals to the environment. The environmental pollution by heavy metals may be originated from the contaminated waste and agriculture water flow to the lake from different drains, the release of these elements from the soil of the lake and/or presence of high load of organic matter in the aquatic environment. These high loads of organic matter increase the rates of metals bioavailability and subsequently its accumulation in the fish food chain that facilitate its entrance to fish body through the digestive tract [48]. According to the results of our study, Fe was found to be the most prominent element in the fish tissues and recorded the highest concentration. This result is similar to that obtained by [49,25]. The higher concentration of Fe in fish muscle and liver may be attributed to multiple reasons such as it has many portals of entry to fish body (through mouth by ingestion, skin, and gills by absorption). In addition, Fe is accumulated in liver with relatively larger amount due to the presence of Fe containing enzymes and hemoglobin. On the other hand, the absence of route for Fe elimination from the fish body resulting in little loss and subsequently high concentration [49]. The increased BCFs may lead to pathological changes in the affected organs, hemato-biochemical deviations in the blood profile and in severe cases may lead to massive fish mortalities [46].

The heavy metals (Cu, Fe, Ni, Cd and Pb) involved in the present study are used to estimate the different pollution indices. The PLI makes an evaluation of the total toxicity status of the sample. Moreover, MPI is used to evaluate the quality of coastal areas and compare the total metal content in the different lake sites [24]. According to the estimated data resulting from CF, PLI and MPI, the pollution grade in the Burullus lake using fish as a biological indicator may be categorized as:  $WS > MS > ES$ . These data are in agreement with [25]. The CF of detected metals in fish liver (Fe and Ni), muscle (Cu, Fe and Ni) and lake water (Fe and Pb) ranged from above one to below three indicating moderate contamination. The only exception was for the value of Cu in liver which recorded CF ranged from above three to above six in few cases indicating considerable to very high contamination. The concentrations above the PL may symbolize an environmental and human health risks. These results were concluded according to the scale of contamination described by [27]. The values of PLI and MPI for the studied elements in fish liver (Cu, Fe and Ni), muscle (Cu, Fe and Ni) and lake water (Fe and Pb) were above one that indicate sever and progressive degree of pollution. The only exception was for the value of MPI for detected metals in lake water that recorded a value below one indicating low degree of pollution. These findings were demonstrated according to [25]. These findings indicate that Burullus lake is suffering from moderate to severe pollution indices that may cause severe damage to the aquatic environment. It may subsequently extend to cause sever health hazards not only to fish and other aquatic organisms but also human beings through the consumption of contaminated feed.

Water pollution is a complex dynamic process affecting the aquatic ecosystem and has a sever public health importance. The functioning and stability of an aquatic ecosystem to support life forms depends on the physicochemical properties of water [50]. The salinity in the lake fluctuates greatly from lower values in ES to higher values in MS followed by WS. Despite this variation, the brackish water conditions remain the most dominant condition in the lake. Although the brackish condition is suitable for growth and reproduction of *O. niloticus* but some previous studies concluded the higher salinity (above 5 to 8 ppt) may affect its growth and performance [51]. This finding is in agreement with the present study as the lower body weight of fish coincided with higher salinity in the WS of the lake. The mean value of EC is higher in WS and lower in

ES. The increased level of EC is mainly due to an increase in TDS. In addition, every year the lake receives a huge amount of inorganic matter and toxic metals with untreated wastes in form of agricultural, industrial, and domestic wastes [3]. Furthermore, any variation of salinity or metal content is accompanied by changes in EC values [52]. This finding is in agreement with the present study as the higher salinity and metal values were recorded in the WS which have the highest EC level. The same trend of EC was observed in TDS. There is a narrow fluctuation in the temperature between the different lake sectors. The temperature range is around 28 °C which is considered as a favorable temperature for the growth of *O. niloticus* fish during summer season. Similar results were recorded by [4]. They recorded that the maximum water temperature recorded during June was 29.4 °C. Measuring of DO is a crucial test in assessment of water pollution and wastewater treatment process effectiveness [53]. The concentration of DO was varied between different lake sectors. The highest DO was recorded in the ES while the lowest was recorded in the MS. This may be due to the large amount of organic matter load reaching the lake through the drains accompanied by oxidation reduction processes [54]. Estimation of pH helps in the evaluation process of water quality in large water bodies and lakes. It has a strong relationship with other water parameters as DO and TAN as it affects many biological and chemical processes in the water streams. The pH values are directed toward the alkaline side with narrow non-significant variations. Similar findings were stated by [4]. This finding may be related to the increased photosynthesis by planktonic algae which mainly developed under the effect of high-water temperature in summer season and the high load of effluent water with organic matters [55]. It may result in changes in DO and TAN concentration and pH value. The highly significant low values in dissolved oxygen and the high values in TAN and UIA level in water samples collected from WS and MS in lake water in the present study could be attributed to the increase in the oxygen consumption due to breakdown of organic matter and oxidation of water chemicals [55].

The body weight of fish in ES was higher than the other lake sectors. These findings are in complete accordance with our findings in the present study. As, the ES is characterized by lower heavy metal concentration, salinity, EC, TDS, TAN, UIA and higher DO which has a direct promoting effect on growth performance of fish. The condition factor (K) is assessed to evaluate the effect of environmental changes in the ecosystem on growth performance of fish [56]. Therefore, the fish health status can be evaluated from the variation in condition factor. The obtained data are in agreement with [57] who found fluctuation in K values of fish and attributed these

changes to the fish feeding rate. The lower values of the K for *Oreochromis niloticus* collected from the WS and MS of the lake may be due to the relatively higher concentration of the different toxic elements accumulated at high levels in the fish tissues [58] and the inferior quality of water recorded in the present study. On the other hand, the high K values of collected fish from ES agrees with the finding obtained by (Kheir et al., 1998) who attributed these results to the increase of food consumption and improved water quality.

Hepatosomatic index (HSI) which is a biological indicator that helps in evaluation of growth rate and degree of stress response in fish [59]. There is a significant decrease in HSI of collected fish from WS indicates some liver function disturbances induced by exposure to toxic heavy metals which agrees with the finding obtained by [60]. It may be attributed to a depletion of glycogen of liver followed by hyperglycemia that reflects the need of fish for energy necessary to overcome stress. In addition, the deposition of high concentration of studied heavy metal in the liver may induce liver degeneration [61]. These findings agree with our findings in the present study that reflected on higher concentration of heavy metals, low-grade water quality and lower growth rate of fish in WS of lake.

Consequently, all the lake sectors show growth patterns lower than the ideal growth as the slope values were lower than the range of ideal growth (2.5-3.5) suggested by [33]. It was obvious in the slender growth of fish in different lake sectors. As WS shows the highest concentration of studied toxic heavy metals both in lake water and fish tissues (liver and muscles), it shows the lowest fish growth represented by very low slope. These findings are like that recorded by [15]. Moreover, [62] found negative correlation between concentration of toxic heavy metal and fish size that may be due to the difference in metabolic activity between small and large size fish. In addition, these findings indicate the bad effects of toxic heavy metals on growth form in lake fish.

## Conclusions

The present study gives a brief account on the toxic effects of heavy metals on the water quality and performance of *O. niloticus* fish in Burullus Lake, North Nile Delta, Egypt. In the present study, there was a variation in heavy metals content in different sections of the Burullus lake water, ES showed lower heavy metals concentration in water, liver and muscles compared to other sectors. MS showed significantly lower BCF of Fe in liver and muscle. The ES showed the lowest CF, PLI and MPI values in water, liver, and muscles except for Fe in muscle

CF. The ES may be considered the lowest contaminated area of Burullus Lake and consequently showed better water quality and fish performance. These outcomes may provide a clear mirror about the present situation of Burullus lake ecosystem and aquatic environment to protect the lake water, aquatic organisms and subsequently human against further pollution.

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#### Conflicts of interest

The authors declare that they have no conflict of interest.

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## تقييم المخاطر السمية البيئية للآثار المحتملة للمعادن الثقيلة السامة المحتملة على جودة

المياه وأداء البلطي النيلي في بحيرة البرلس، شمال الدلتا، مصر

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حمل التلوث لمجري المياه الكبيرة والبحيرات في حالة ديناميكية. تعتمد ديناميكيات التلوث بشكل أساسي على كمية ملوثات النفايات السائلة لمصادر المياه هذه. وبناء على ذلك، ينبغي أن يكون الرصد والتقييم المستمران للتلوث ممارسة أساسية لإنقاذ البيئة المائية. لذلك، هدفت هذه الدراسة إلى تقييم التأثير السام للمعادن الثقيلة (النحاس، الحديد، النيكل، الكاديوم والرصاص) في مياه بحيرة البرلس و البلطي النيلي (العضلات والكبد). لتقييم درجة التلوث والمخاطر السمية البيئية المرتبطة به، قدر المؤلفون عامل التركيز الحيوي، وعامل التلوث، ومؤشر حمل التلوث، ومؤشر التلوث المعدني بالإضافة إلى تأثير المعادن الثقيلة السامة على جودة المياه وأداء الأسماك في ستة عشر موقعا لأخذ العينات عبر البحيرة. أظهرت النتائج انخفاضا ملحوظا في تركيز المعادن الثقيلة في الماء والكبد والعضلات في القطاع الشرقي مقارنة بالقطاعات الأخرى. بينما أظهر القطاع الأوسط انخفاضا كبيرا في عامل التركيز الحيوي من الحديد في الكبد والعضلات. أظهر القطاع الشرقي أدنى قيم عامل التلوث و مؤشر حمل التلوث و مؤشر التلوث المعدني في الماء والكبد والعضلات باستثناء الحديد في عامل التلوث المعدني. علاوة على ذلك، سجل القطاع الشرقي أدنى ملوحة، و التوصيل الكهربائي، وإجمالي الأملاح الذائبة، والأمونيا الكلية والأمونيا غير المتأينة وأعلى أكسجين مذاب. علاوة على ذلك، أظهر القطاع الشرقي أعلى وزن لجسم السمكة وعامل الحالة وأدنى مؤشر كبدي. في الختام، كان هناك تباين في محتوى المعادن الثقيلة في أقسام مختلفة من مياه بحيرة البرلس، وأظهر القطاع الشرقي تركيز أقل للمعادن الثقيلة في الماء والكبد والعضلات مقارنة بالقطاعات الأخرى. أظهر مرض التصلب العصبي المتعدد انخفاضا كبيرا في معامل التركيز الحيوي من الحديد في الكبد والعضلات. أظهر القطاع الشرقي أدنى قيم وعامل التلوث و مؤشر حمل التلوث و مؤشر التلوث المعدني في الماء والكبد والعضلات باستثناء الحديد في عامل التلوث المعدني. قد يعتبر القطاع الشرقي أقل منطقة ملوثة في بحيرة البرلس وبالتالي أظهر جودة مياه وأداء أفضل للأسماك.

الكلمات الدالة: أداء الأسماك، البلطي النيلي، المعادن الثقيلة، بحيرة البرلس، جودة المياه.