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Assessment of seasonal and spatial variations of heavy metals in the muscles of *Oreochromis niloticus* and *Clarias gariepinus* at Sharkia province, Egypt: Biochemistry and Histology Evaluation

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Abstract

The most significant pollutants are the heavy metals in the aquatic network due to their toxicity, accumulation, and bio-magnification. In an attempt to characterize the physical-chemical and demonstrate the potential water impact of the heavy metal content of wastes in two lakes in Sharkia province, Egypt, this study was developed to determine their toxicity and the potential waste impacts in biochemistry and histology of fish muscle. Samples of water and fish muscles were collected and analyzed for heavy metals using atomic absorption of two different fish species (*Oreochromis niloticus* and *Clarias gariepinus*) along two canals in Sharkia province in Egypt during the four seasons in 2018. The water samples were also analyzed for the physicochemical parameters. The impact of heavy metal on the enzymatic antioxidant (superoxide dismutase and catalase) as well as the reduced glutathione content, besides the oxidative stress marker presented by lipid peroxidation levels in fish muscles, were evaluated. Results showed the largest amount of chloride (Cl^-) and iron (Fe) in the Sharkia water sample, while Faquas water sample had the highest level of HCO_3 and iron (Fe). The manganese concentration in the fish muscle was the highest in Sharkia water and the highest level of Fe was detected in the fish muscles collected from Faquas area. These high levels of some of the physicochemical parameters and heavy metals in the two lakes with two different wastes as seen in this study may be a source of water pollution. The highest accumulation of metals in the muscle of the two fish species, suggesting risk for human consumption. There were seasonal variations in the level of LPO in muscle tissue of two types of fishes with a reverse relationship with antioxidant parameters. This accumulation varied seasonal and spatial as well as according to the species of fish. Increasing the level of heavy metals effect on the biochemistry and histology of fish.

Keywords: Heavy metals; Muscle; Oxidative/Antioxidant, Histology, *Clarias gariepinus; Oreochromis niloticus*

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Introduction

The heavy metal introduction to the region of water has pulled in worldwide concern due to natural change, environmental impacts and bioaccumulative and tireless nature [1]. Expansive amounts of metals discharge into water bodies due to quick populace development and seriously residential exercises, as well as growing mechanical and rural generation [2,3]. Water biological systems around the world had been influenced by both common and anthropogenic activities [4].

Moreover, numerous metals that show in the aquatic environment, are known to construct up in animal tissues to exceptionally high levels, posturing a potential risk to the higher living organisms within the food chain counting humans [5,6]. Evidence of heavy metal toxic effects have been reported in fish and consumers of the contaminated food [7]. They tend to accumulate depending on the entire amount, the bioavailability of each metal within the natural medium and the course of take-up, capacity, and mechanisms [8,9,10]. The foremost vital trace and poisonous elements from an aquatic contamination point of view are Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn. Living organisms actually exposed to high metal concentrations follow various mechanisms to counter potential toxicity [11,12]. Different organisms respond in different ways; one of the most extreme responses is death or migration to another habitat and inhibition of certain enzyme systems necessary for normal metabolism. Once the responses of specific aquatic organisms to any given changes have been identified, they can be used to determine water quality in terms of its suitability for aquatic life [13,14].

In spite of the fact that heavy metals are terrestrially created by industrial and agricultural activities, they stream into the water asset through effluents and sewage to the waterfront [15]. In numerous surface water regularly have a lower release or recharging rates, subsequently, toxin defilement from industries have a high negative effect on the physicochemical and natural quality of the water [16,17]. Fish are widely used as they are at the top of the food supply chain hence, they may bioaccumulate high concentrations from their nourishment sources [18]. As fish are frequently utilized as a human food source the comes about are straight forwardly important to human wellbeing. Their potential utilizes as biomonitors are subsequently noteworthy within the assessment of bioaccumulation and biomagnification of contaminants in the environment. The region of accumulation of heavy metals within fish varies according to the uptake route, type of heavy metals, and species of fish [19].

Based on these fundamentals, this study focuses on the two localities that are Muweis Canal (Zagazig canal at Adab Bridge near oil and soap Factory) and Faqus Canal, at Sharkia Governorate-Egypt, are considered as a highly valued fish resource. However, they receive several sources of pollution, as agricultural, industrial, and domestic wastes. Thus, a water quality assessment study was conducted on the two locations and samples were taken in four different seasons with distinct hydrological

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characteristics to quantify and evaluate the quality status of water columns. In addition, two types of in situ fish have been used to demonstrate the integrated effects of all impacts on the water body and can be used to compare relative changes in water quality from two distinct locations over a period of time. The combination of biota samples with samples of water taken from the lakes could be used to provide information on the intensity of adverse effects resulting from specific anthropogenic influences, assessment of the potential environmental impact of substances or effluents discharged into surface water systems. Moreover, the objective of this study to transform water quality data for these two locations in different seasons into understandable information by the public. Different variable such as water's physical and chemical properties as well as seasonal shifts in heavy metals are the justification for a substantial increase in metals of muscle fish [15]. Therefore, the anthropogenic influences on water bodies (physicochemical characters and concentrations of heavy metals) in combination with the effect of these factors on muscle biochemistry and histology of the fishes were evaluated.

Materials and Methods

Study area

Fish samples (Oreochromis niloticu and Clarias gariepinus) were collected from two localities at Sharkia Government in the east of the Nile Delta and is circumscribed by coordinates 30° 34′ 0″ N, 31° 30′ 0″ E that are considered as natural sources for the fishery. These localities lie east to Damietta Branch (Nile water). The two localities are Muweis Canal (Zagazig canal at ADab Bridge near oil and soap Factory) that presented by area (A) where it receives industrial wastes from oil and soap factory. San El-Hagar canal (Faqus canal at south San El-Hagar Bridge) were selected and presented as area (B) where it receives domestic and agricultural wastes. Samples were collected monthly during the period from September 2017 to August 2018 at Muweis and San El-Hagar Canal. Sites were invariably human-influenced. The climate of the region representing the study location is tropical with distinct seasons; a hot and dry summer, and a cool and rainfall winter. The region received rainfall during the winter season.

Sample collection

Water sampling bottles were rinsed twice with the distilled water before sampling was done at each site. Monthly collected water samples were stored in an ice-containing isolated cooler and delivered the same day to the laboratory and maintained at 4 °C before processing and analysis. For each water sample, one bottle was used for heavy metal partition analysis and physicochemical analysis.

Fish samples were collected monthly, from each site by fisherman's net. The collected fish was with average body weight for tilapia (165.8 ± 7.35 g, n= 120), and an average body length (21.2 ± 0.44 cm) while, for African catfish (n=100) the weight was 279.52 ± 25.56g and the length was 36.51 ±1.7cm during the four seasons. After dissection of fish, muscle tissues were separated for estimation of heavy metal residues, an oxidative stress marker (lipid peroxidation) and antioxidant (superoxide dismutase; SOD, catalase; CAT and glutathione; GSH) in the two fish species and the two location areas. During the collection and treatment of samples, all the precautions suggested by [20] to minimize risks of sample contamination were pursued.

Physicochemical determinations of water samples

The aim of this research was to investigate several water quality parameters, including pH with a portable Hanna HI98129/30 pH meter, total dissolved solids (TDS) major anions, (e.g.,

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chloride and phosphate; $Cl^-$and SO_4^{2-}) and major cations (calcium, magnesium, sodium and potassium; Ca^{2+}, Mg^{2+}, Na^+, and K^+, respectively). The data obtained in the present studies were compared with the guideline values suggested by the World Health Organization [21]. Cl^- was measured using Mohr´s method and sulfate by turbidimetric methods. Ca^{2+} and Mg^{2+} were determined by direct titration using EDTA solution. Na^+ and K^+ were measured directly using the flame photometer model.

Determination of heavy metals in water and fish samples

Heavy metal concentrations iron (Fe), copper (Cu), zinc (Zn), lead (Pb), manganese (Mn) and nickel (Ni) in water were determined by atomic absorption spectrophotometer (Perkin Elmer, 2280). All standard solution for the target element was supplied by Merck Germany with the highest purity level (99.98%). Ultra-pure HNO_3 was used for sample digestion. All other acids and chemicals were pure and received from Merck Germany or Scharlau, Spain. The samples El-shenawy prepared and analyzed according to EL-SHENAWY et al. [22] sequentially for Fe, Zn, Cu, Pb, Cd, Mn, and Ni. To 50 mL of unfiltered water sample (in 500 mL Taylor flask) 0.50 mL of concentrated sulphuric acid was added. This was boiled down to obtain white fumes, cooled and 1.0 mL of 60% $HCLO_3$ and 5.0 mL of concentrated HNO_3 was added. The resulting mixture was digested until a clear digest was obtained. This was cooled, filtered (Whatman paper No. 44) into 500 mL volumetric flask, diluted to volume and mixed. Fish from each variety dissected to separate muscles. Deionized water was used to rinse fish samples to remove surface adherents that could have adsorbed metals. The separated muscles were put in Petri-dishes to dry at 120 ^oC until reaching a constant weight after removing the fish scales and skin. The separated muscles were put into digestion flasks and ultrapure Conc. HNO_3 and H_2O_2 (1:1 v/v) was added. The digestion flasks were heated to 130 ^oC until the tissue dissolved, diluted with water and evaluated for heavy metal concentration using atomic absorption Spectrometer.

Oxidative stress/antioxidant of fish muscles evaluation

Tissue homogenates were prepared from trunk muscle samples in 10 volumes of 0.1 M Tris-EDTA buffer (pH 7.4), centrifuged at 1,000 × g at 4°C for 30 min then the kit's instruction was followed for determining the different oxidative stress/antioxidant parameters. Lipid peroxidation (LPO) was determined by a colorimetric method of (Kei, 1978) according to the details given in the kit's instructions. Superoxide dismutase activity (SOD) was determined spectrophotometrically at 560 nm, followed the kit's instruction according to the method of Nishikimi et al. (1972). The method based on the ability of the SOD enzyme to inhibit the phenazine methosulphate mediated reduction of nitroblue tetrazolium dye. Catalase (CAT) activity was measured following the kit's instruction according to the method described by Aebi (1984). The CAT reacts with a known quantity of H_2O_2, and the reaction is stopped after 1 min with a CAT inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3,5- dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore, with a color intensity inversely proportional to the amount of CAT in the sample. The absorbance was measured at 510 nm. The reduced glutathione (GSH) level was assayed following the kit's instruction using a method of (Beutler et al., 1963) based on the reductive cleavage of 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) by a sulfhydryl (-SH) group to yield a yellow color. The reduced chromogen (absorbance measured at 412 nm) is directly proportional to the GSH concentration.

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Histological evaluation

Fish muscles were fixed in 4 % paraformaldehyde, prior to being embedded in paraffin. Paraffin sections (5-µm thick) were prepared and stained with hematoxylin and eosin (H & E) according to standard procedures (Chen et al., 2013). All sections were examined under a light microscope.

Statistical analysis

The results obtained were subjected to mean, and standard error of means. ANOVA was determined and $p<0.05$ was considered to indicate statistical significance. SPSS (Statistical Package for the Social Sciences) version 16 was used for the analysis.

Results

Physicochemical evaluation

The pH values presented in Table 1 for all sampling locations ranged between 7.07 – 7.25. The minimum value of TDS, as shown in Table 1 was found in autumn, spring and winter in the two-location sample, and the maximum value was found in summer in the two-sample location. The given data in Table 1 showed that SO_4^{2-} and Cl^- concentrations ranged between 0.15 – 0.9 mg/L and 0.10–1.60) mg/L, respectively. Major cations available data illustrated by Table 1 showed that the concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} were naturally variable from season to another, their values ranged between 0.4 – 0.90 mg/L, 0.1 – 0.3 mg/L, 1.20 – 1.75 mg/L and 0.75 – 1.55 mg/L, respectively.

Table 1: Seasonal Physicochemical Characters Concentration of Water

Parameters		Site A				Site B			
		Summer	Autumn	Spring	Winter	Summer	Autumn	Spring	Winter
pH		7.25± 0.02^{e}	7.24± 0.01^{e}	7.11±0.04^{e}	7.24± 0.01	7.24± 0.02	7.14± 0.06	7.07± 0.04	7.19± 0.01^{d}
Total dissolved solids (mg L^{-1})		155.65± 0.2^{b}	0.35± 0.01^{a}	0.26± 0.05^{a}	0.35± 0.01^{a}	166.80± 0.1^{c}	0.40± 0.01^{a}	0.31± 0.01^{a}	0.38± 0.01^{a}
Major anions	Chloride (mg L^{-1})	0.10± 0.01^{a}	1.60± 0.01^{f}	0.95± 0.06^{b}	1.30± 0.04^{e}	1.15± 0.022^{c,d}	1.10 ±0.00^{d}	1.15 ±0.02^{d}	1.20± 0.01^{d}
	Sulfate (mg L^{-1})	BDL	0.40± 0.01^{b}	0.15± 0.02^{a}	0.90± 0.08^{d}	BDL	0.45± 0.02b	0.20± 0.01a	0.60± 0.01^{c}
Major cations	Calcium (mg L^{-1})	1.35± 0.02^{bc}	1.20± 0.01^{ab}	1.15± 0.05^{a}	1.750± 0.05^{e}	1.500± 0.04^{cd}	1.40± 0.13^{c}	1.20± 0.01^{ab}	1.60± 0.01^{d}
	Magnesium (mg L^{-1})	0.75± 0.02^{a}	1.20± 0.00^{b}	0.85± 0.02^{a}	1.40± 0.08^{c}	0.85± 0.02^{a}	1.55± 0.02^{d}	1.10± 0.01^{b}	1.20± 0.01^{b}
	Sodium (mg L^{-1})	0.41± 0.04^{a}	0.90± 0.01^{de}	0.55± 0.02^{b}	0.70± 0.04^{c}	0.45± 0.02^{ab}	0.95± 0.1^{e}	0.80± 0.01^{cd}	0.85± 0.01^{de}
	Potassium (mg L^{-1})	0.10± 0.01^{a}	0.30± 0.01^{d}	0.10± 0.01^{a}	0.18± 0.08^{b}	0.10± 0.01^{a}	0.19± 0.04b^{c}	0.10± 0.01^{a}	0.20± 0.01^{c}

The average of monthly samples (3 samples each month; n=9 for each season). Data presented as mean ± S.E. Different letter superscript is significant $p< 0.05$. BDL – below the detection limit.

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Heavy metals in water

Comparing the average levels of heavy metals in the two study sites, the data recorded in Table 2 showed annual variations in heavy metal concentrations in water samples. The levels had the order: Fe > Mn> Pb > Zn > Cu while Ni and Cd did not detect during summer for the two areas. In the spring, the levels of heavy metals had the order of Fe > Ni >Zn > Mn > Pb > Cu > Cd in area A while all the heavy metals were undetectable in area B.

Table 2: Seasonal Concentration of Heavy Metal in the Water Sample in the Two locations

	Season	Fe (µg/L)	Zn (µg/L)	Mn (µg/L)	Cu (µg/L)	Pb (µg/L)	Ni(µg/L)	Cd (µg/L)
Area A	Summer	0.46± 0.01^{c}	0.01± 0.01^{c}	0.16± 0.01^{d}	0.03± 0.01^{a}	0.04± 0.01^{b}	BDL	BDL
	Autumn	0.11 ±0.01^{a}	0.03± 0.01^{a}	0.002± 0.01^{ab}	BDL	BDL	BDL	BDL
	Spring	2.29± 0.06^{e}	0.05± 0.01^{e}	0.03± 0.01^{c}	0.01± 0.01^{c}	0.03± 0.004^{b}	0.14± 0.00	0.01± 0.001
	Winter	0.16± 0.01^{a}	0.02± 0.01^{a}	0.01± 0.01^{a}	BDL	BDL	BDL	BDL
Area B	Summer	0.63± 0.01^{d}	0.02± 0.01^{d}	0.17± 0.01^{e}	0.04± 0.00^{b}	0.03± 0.002^{b}	BDL	BDL
	Autumn	0.36± 0.02^{b}	0.01± 0.01^{b}	0.01± 0.001^{b}	BDL	0.02± 0.01^{a}	BDL	BDL
	Spring	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Winter	0.28± 0.03^{b}	0.01± 0.003^{b}	0.36± 0.004^{c}	BDL	BDL	BDL	BDL

The average of monthly samples (3 samples each month; n=9 for each season). Data presented as mean ± S.E. Different letter superscript is significant $p< 0.05$. BDL – below the detection limit.

Heavy metal in fish muscle

Comparing the seasonal average levels of heavy metals in the muscle of *Tilapia* fish species and muscle of *clarias* fish species in the two different study sites, area A and B, (Tables 3 and 4) showed variations between heavy metal levels in fish muscle. The concentrations of heavy metals in the muscle of *Tilapia* fish species had the order: Fe> Zn > Mn > Pb > Cu during the autumn for the area A. The heavy metals in the muscle of *Tilapia* fish species had the order: Fe> Zn > Cu during the same season for area B (Table 3). However, the heavy metals in the muscle of *clarias* fish species had the order: Fe> Zn > Mn for the two areas during Spring.

Oxidative stress biomarker in the muscles of fish species

The LPO biomarker of oxidative stress and activities of antioxidant in the muscle tissues of *O. niloticus* and *C. gariepinus* are shown in Tables 5 and 6. The seasonal variations in the level of LPO in muscle tissue of *O. niloticus* were in the following order; winter> spring> summer> autumn for the area A (Table 5). However, the level of LPO decreased as the following order; autumn, summer, spring, winter for the area B. There was an irreversible relationship between the level of LPO and all the antioxidants (CAT, SOD, and GSH). The same relationship was observed in area B with the same sequence for the LPO level for *C.*

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gariepinus (Table 6). However, the maximum level of LPO was observed during winter in the muscles of *C. gariepinus* that collected from area B with increasing the level of GSH.

Table 3: Seasonal Concentration of Heavy Metals in the Muscles of *Oreochromis nilotica* Harvested from the Two Areas

	Season	Fe (µg/g)	Zn (µg/g)	Mn (µg/g)	Cu (µg/g)	Pb (µg/g)
Area A	Summer	10.94±0.6^{a}	3.50±0.28^{ab}	173.71±5.1^{b}	BDL^{a}	22.17±0.3^{b}
	Autumn	44.60±1.9^{d}	3.69±0.92^{b}	0.35±0.07^{a}	0.010±0.01^{a}	0.010±0.0^{a}
	Spring	145.21±2.0^{f}	3.33±0.08^{ab}	1.78±0.03^{a}	BDL^{a}	BDL^{a}
	Winter	36.13±1.04c	1.89±0.2^{a}	BDL^{a}	BDL^{a}	0.01±0.01^{a}
Area B	Summer	14.57±0.6^{a}	18.64±0.8^{e}	223.14±2.8^{b}	BDL^{a}	23.93±0.6^{c}
	Autumn	41.42±1.7^{d}	10.84±1.07^{d}	BDL^{a}	0.044±0.01^{b}	BDL^{a}
	Spring	117.15±1.9^{e}	3.99±0.29^{b}	1.49±0.13^{a}	BDL^{a}	BDL^{a}
	Winter	28.12±0.69^{b}	6.99±0.42^{c}	BDL^{a}	BDL^{a}	BDL^{a}

The average of monthly samples (3 samples each month; n=9 for each season). Data presented as mean ± S.E. Different letter superscript is significant $p< 0.05$. BDL – below the detection limit.

Table 4: Seasonal Concentration of Heavy Metals in the Muscles of *Clarias gariepinus* Harvested from the Two Areas

	Season	Fe (µg/g)	Zn (µg/g)	Mn (µg/g)	Cu (µg/g)	Pb (µg/g)
Area A	Summer	2.11±0.11^{a}	2.01±0.09^{a}	34.02±1.6^{b}	BDL	3.30±0.30^{a}
	Autumn	30.55±3.23^{bc}	2.30±01^{a}	BDL	BDL	BDL
	Spring	193.80±9.94^{e}	3.92±0.17^{d}	0.84±0.05^{a}	BDL	BDL
	Winter	139.62±2.65^{d}	2.54±0.19^{a}	1.23±0.17^{a}	BDL	BDL
Area B	Summer	10.40±0.58^{ab}	7.70±0.49^{e}	214.56±9.1^{c}	BDL	21.47±1.43^{b}
	Autumn	50.40±4.16^{c}	3.22±0.75^{cd}	0.34±0.05^{a}	BDL	BDL
	Spring	216.07±21.3^{e}	2.84±0.21^{bcd}	1.60±0.16^{a}	BDL	BDL
	Winter	52.27±0.66	3.46±0.35^{cd}	0.45±0.02^{a}	BDL	BDL

Data presented as mean ± S.E of the average of monthly samples(3 samples each month; n=9 for each season). Different letter superscript is significant $p< 0.05$. BDL – below the detection limit.

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Table 5: Seasonal Oxidative Stress Biomarkers in the Muscles of *Clarias gariepinus* Harvested from the Two Areas

	Season	MDA (µM/mg protein)	CAT (µM/mg protein)	GSH (µM/mg protein)	SOD (µM/mg protein)
Area A	Summer	2.73 ± 0.05^{b}	6.57 ± 1.03	0.16 ± 0.004^{a}	268.52 ± 6.7^{bc}
	Autumn	0.33 ± 0.01^{a}	0.26 ± 0.01	10.99 ± 0.5^{c}	137.27 ± 2.7^{a}
	Spring	2.97 ± 0.02^{b}	0.40 ± 0.02^{a}	12.47 ± 0.1^{c}	492.40 ± 5.05^{d}
	Winter	8.30 ± 0.3^{c}	0.40 ± 0.02^{a}	6.11 ± 0.3^{cb}	232.19 ± 0.31^{b}
Area B	Summer	14.99 ± 0.39^{d}	2.33 ± 0.3^{b}	0.47 ± 0.02^{a}	296.4 ± 15.2^{c}
	Autumn	15.01 ± 0.39^{d}	0.33 ± 0.01^{a}	0.47 ± 0.02^{a}	296.4 ± 15.2^{c}
	Spring	13.81 ± 1.3^{d}	0.33 ± 0.01^{a}	1.84 ± 1.3^{a}	267.27 ± 30.49^{bc}
	Winter	8.67 ± 12^{c}	0.48 ± 0.03^{a}	7.29 ± 13^{b}	163.82 ± 29.1^{a}

Data presented as mean ± S.E. of the average of monthly samples (3 samples each month; n=9 for each season). Different letter superscript is significant $p < 0.05$.

Table 6: Seasonal Oxidative Stress Biomarker in the Muscles of *Oreochromis nilotica* Harvested from the Two Areas

	Season	MDA (µM/mg protein)	Catalase (µM/mg protein)	GSH reduced (µM/mg protein)	SOD (µM/mg protein)
Area A	Summer	0.78 ± 0.02^{b}	0.19 ± 0.001^{d}	0.56 ± 0.002^{a}	97.72 ± 1.15^{d}
	Autumn	0.25 ± 0.01^{a}	0.14 ± 0.001^{b}	0.46 ± 0.01^{a}	12.32 ± 014^{a}
	Spring	1.02 ± 0.02^{c}	0.15 ± 0.001^{b}	0.62 ± 0.02^{a}	130.91 ± 0.03^{e}
	Winter	1.34 ± 0.06^{d}	0.26 ± 0.008^{e}	0.90 ± 0.01^{b}	73.28 ± 9.5^{c}
Area B	Summer	0.16 ± 0.01^{a}	0.06 ± 0.004^{a}	4.78 ± 0.25^{c}	48.09 ± 1.04^{b}
	Autumn	1.16 ± 0.12^{c}	0.17 ± 0.005^{c}	0.73 ± 0.01^{b}	97.30 ± 0.97^{d}
	Spring	1.60 ± 0.02^{e}	0.18 ± 0.005^{c}	0.56 ± 0.02^{a}	92.52 ± 2.2^{d}
	Winter	6.73 ± 0.16^{f}	0.75 ± 0.005^{f}	0.47 ± 0.01^{a}	145.28 ± 0.3^{f}

Data presented as mean ± S.E. of the average of monthly samples(3 samples each month; n=9 for each season). Different letter superscript is significant $p< 0.05$.

Histopathological changes in muscle

In this present study, the muscles of *Oreochromis niloticus* that collected from area A showed parasitic infection sampling in summer season surrounded by infiltration of inflammatory cells, destruction of the surrounding area (Plate 1A). The sampling during the other season (autumn, spring, and winter), the histopathological damage is infiltration of inflammatory cells followed by degeneration of muscle fiber (Plate 1B-1D). However, in some places, broken myofibrils are seen. The muscle seems to have lost the myoseptum that separates each myotome. Focal areas of myolysis in the muscles also observed (Plate 1A-1D).

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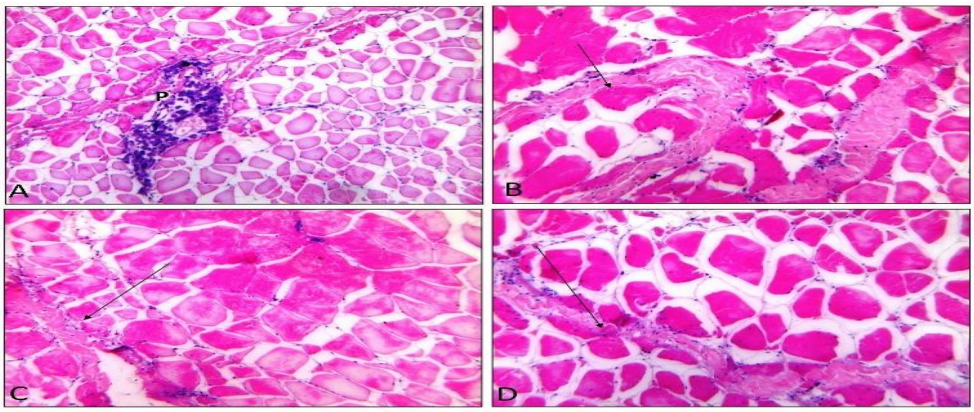

Plate 1: Photomicrograph of the muscles of *Oreochromis nilotica* collected in area A. In the summer season (A) showing parasitic tissue surrounded by infiltration of inflammatory cells with erosion. In autumn (B) showing degenerative inflammation of the muscle fiber (black arrow) and myolysis moreover, the muscles shrinkage away from surrounding perimysium. In spring (C) showing degenerative inflammation of the muscle fiber. In winter (D) showing degenerative of the muscle fibers and myolysis with edema (H & E, x200).

In area B, muscles of *O. nilotica* sampling during the four seasons showed infiltration of inflammatory cells, destruction of surround area, the large separation between the muscle, intracellular degeneration of the muscle (Plate 2A-2D). However, in some places, broken myofibrils are seen. It seems that the muscle has lost the myoseptum separating each myotome. Focal areas of myolysis in the muscles also observed.

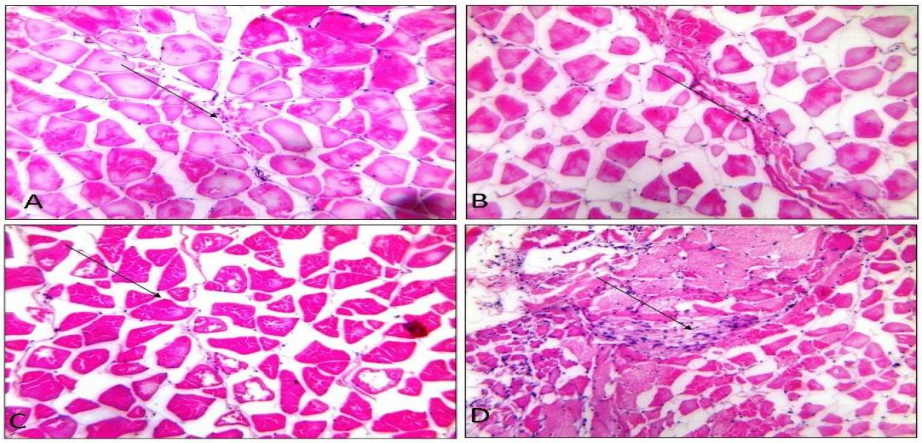

Plate 2: Photomicrograph of the muscles of *Oreochromis nilotica* collected in area B. In the summer season (A) showing degenerative and inflammation of the muscle fiber (black arrow) with myolysis. In autumn (B) the packed bundles of muscles shrinkage and degradation of the muscle fiber arrow and myolysis. In spring (C) showing spaces between muscle bundles are remarkable (edema) and intracellular degeneration of the muscle. In winter (D) showing degenerative and inflammation of the muscle fiber and myolysis (H & E, x200).

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Muscles of *Clarias gariepinus* sampling in the four-season from areas A and B showed cysts of the parasite with a thin wall of fibrous tissue surrounded by infiltration of inflammatory cells and destruction of the surrounding area. In addition, the large separation between the muscle, intracellular degeneration of the muscle, fatty degeneration, edema, hyaline degeneration, focal of granulosa cells, splitting of muscle bundles and focal areas of myolysis in the muscles also observed (Plate 3A-3D and 4A-4D).

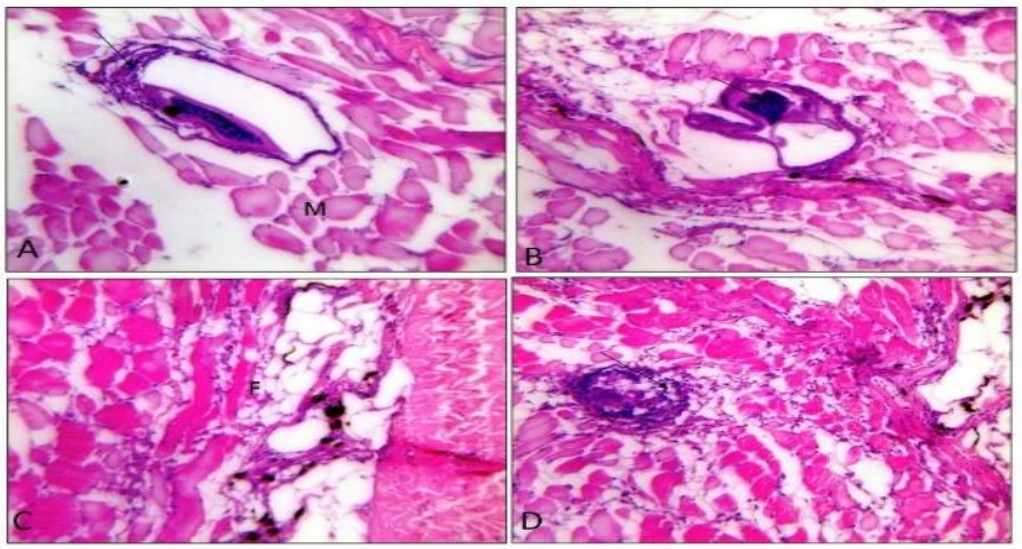

Plate 3: P**hotomicrograph of the muscles of *Clarias gariepinus* collected in area (A).** In the summer season (A) showing parasite tissue with fibrous coat surrounded with degenerative inflammation of the muscle fiber (arrow) and myolysis with erosion. In autumn (B) showing parasite tissue with a fibrous coat (fibrosis) with degeneration and inflammation of the muscle fiber (arrow) and myolysis. In spring (C) showing degenerative inflammation of the muscle fiber with fibrosis and fatty degeneration. In winter (D) showing infiltration of the muscle fiber (arrow) with myolysis (H & E, x200).

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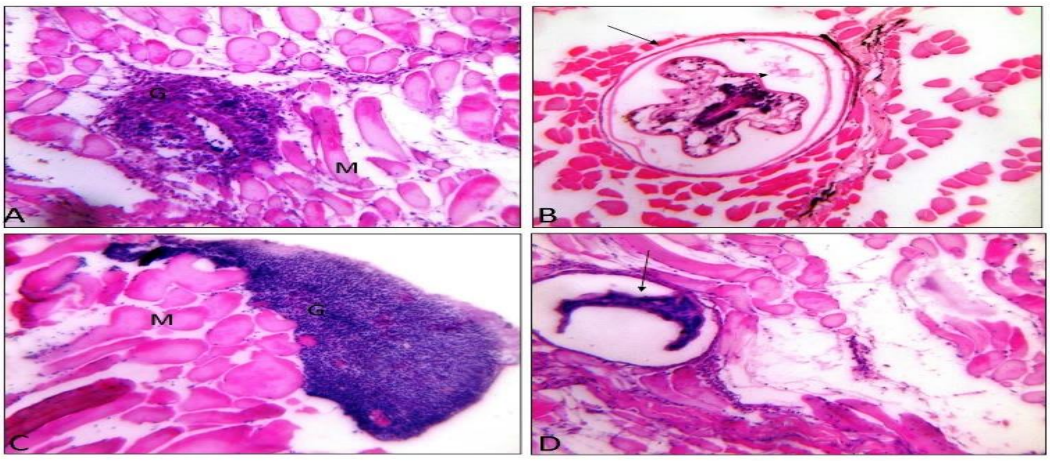

Plate 4: Photomicrograph of the muscles of *Clarias gariepinus* collected in area (B). In the summer season (A) showing fibrosis surrounded with granulosa cell (G) (arrow) and myolysis (M). In autumn (B) showing the parasite surrounded with a fibrous coat (arrow) and integrity of muscle bundles are exhibited as well as edema between muscle fibers. In spring (C) showing disintegration of the muscle fiber and myolysis (M) with focal area of granulosa cell (G). In winter (D) showing cyst of the parasite with a thin coat (arrow) surrounded with degenerative inflammatory muscle fiber (arrow) that appear to be loosely packed with myolysis, the space between muscle bundles are large (H & E, x200).

Discussion

In this study, the levels of heavy metals have been determined in water and the fish' muscles. Different factors such as physical and chemical properties of water as well as seasonal changes of heavy metals are the reason for the significant accumulation of metals in the muscle of fish. Water physicochemical analysis, trace metals, major anions, and cations were compared to the permissible limits of the Egyptian law 48/1982 regarding the protection of the River Nile and waterways from pollution.

The pH values for all sampling locations ranged between 7.07 – 7.25, where it falls within the permissible limits (7.0 – 8.5) (WHO, 2004). In general, tilapias can survive at pH ranging between 5 and 10, while they can live in a pH range of 6 to 9 [23,24]. Found the best survival rate of *C. gariepinus* pH range of 6 to 9.

Depending on the amount and sort of dissolved substances, water can differ considerably in performance [25]. Salt originates primarily from rocks and soil weathering, including gypsum dissolution, lime, and other rapidly dissolved soil minerals [26]. As water evaporates, the crystals are deposited in the soil [27]. Water with TDS of less than 500 mg/L is regarded great, whereas for drinking and irrigation, while water with more than 2000 mg/L is unfit for these purposes [28]. TDS drops below 500 mg/L for all sampling places. According to Umar et al. [29], the low level of TDS in summer, autumn, and spring samples may be due to low pollution sources around the location area, while the rise in winter concentration may be due to increased pollution operation.

The information revealed that levels of SO_4^{2-} and Cl^- varied from 0.15 to 0.9 mg/L^{-1} and from 0.10 to 1.60 mg/L, respectively. All registered values were within allowed boundaries. SO_4^{2-} levels show that the anal is not extremely affected by human activities or point sources of

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pollution. However, Cl^- levels show water quality [30].

Major accessible information indicated that Na^+, K^+, Ca^{2+}, and Mg^{2+} levels were actually variable during different seasons based on modifications in physicochemical parameters. These levels permissible under Egypt and WHO in distinct seasons and regions as recorded before by [23].

The water Mg^{2+} level is one of the most critical variables in agriculture, irrigation, and drinking water quality determination [29]. Ca^{2+} and Mn^{2+} sustain water balance [29]. The existence of more or fewer Mn^{2+} in water increase alkalinity of sediment and may influence the fish biota [31].

The magnesium contents of the water in the two locations varies from 0.05 to 0.2 mg/L In the current study. The high or low sodium level in water has a negative impact on fish development [32]. In the present study, the Na level is low in the samples for two locations.

For most heavy metals in the water in the two location areas, the maximum concentrations were observed in spring. However, the minimum values were detected in summer and winter that may probably due to a decrease in both temperature and pH which favors the increased mobilization of metals from sediment to water as described before by Abdel-Satar [33].

Moreover, the highest levels of all heavy metals were detected in water samples collected is Fe. This result is different from El-Sayed et al. [34], that found the highest concentration is zinc in water sampling from Sharkia province. The water content of heavy metal may be attributed to wastes of industrial activities and the nature of water properties changed by pollution according to El-Sayed et al., [34]. The increased human activities and fishery boats may be a condition have to be considered with the seasonal variation of heavy metal [29].

The selection of fish muscles as a goal for this research as it has been approved to be the primary consumed part of fish so their impact effect is directly be reflected on human health [35]. The presence of trace metals in fish muscle is an alarming problem worldwide since fish occupy high trophic food levels and are a significant food source [36].

In the current research, the levels of four trace element (Cu, Fe, Pb, and Zn) identified in water analysis were determined in muscles of fish specimens. The presence of trace metals in the fish tissues is often affected by many external and internal factors [37]. Metals levels are correlated with ambient metals concentration in the surrounding environment, the available metal form in water, the structure of the target organ as well as the interaction between the metal and this organ [38].

Gbem et al., [39] revealed the presence of heavy metals in the fish muscle may be connected to the low levels of binding protein in fish muscles. In view of the previous findings, the presence of Fe and Zn in both muscles of two fish species could be anticipated to the increase in total dissolved Fe and Zn in the water and consequently increase in metal availability and uptake by different fish organs [40]. Comparative data for both metal content of water and fish tissues suggested the strong link between water characteristics and fish quality.

In the present study, the seasonal variation played a major role in metal ion speciation and the comparative concentrations of each metal and thereby available for uptake by fish organs. No specific master season in the heavy metal accumulation in the muscle tissue of the two fish species. These results are not in harmony with those previously reported by Ibrahim and Mahmoud, [41]. The low concentrations of metals below the detection limit in fish

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suggested by Förstner and Wittmann, [11] that they are obtained primarily from agricultural wastes that reach the lake with chemical fertilizers and biocides, rather than heavy loads from industrial wastes. The increased human activities and fishery boats may also contribute to the seasonal variation in the water and fish heavy metal content [42].

Metals level in the muscles which is the main edible part in fish may threaten the public health. These results agree with those reported by Taweel et al. [43]. Moreover, it is obvious that the average heavy metal levels in different tissues of *C. gariepinus* were higher than those of *O. niloticus*. This may be due to the difference in the feeding habits of the two species. Where *O. niloticus* primarily feeds on phytoplankton Dempster et al., [44] which accumulate large quantity of heavy metals, living wanderings in water from surface to ground, are commonly in contact with soil particles, whereas, *C. gariepinus* lives mainly in muddy or semi-muddy bottom which is primarily omnivorous feeding on fish, insect larvae, mollusks, planktonic organisms and water weeds [45].

Due to greater metal levels in water and sediments, metals remaining tissues in the fish epithelium are severed [46]. On the contrary, heavy metals are of severe interest in this regard because they can readily be raised in the food chain owing to their bioaccumulation procedures [15]. Depending on distinct variables i.e. the behavioral and psychological conditions as well as the age of the fish, the pattern of heavy metal up taking in fish is distinct in different species.

The muscles tissues bioaccumulation of tilapia for Fe was higher than *C. gariepinus* during Autumn and Summer (177.1- and 20.6- fold, respectively) verse (159.6- and 11.5-fold, respectively), respectively. However, *C. gariepinus* accumulation factors were 447.3- and 131.1-fold for Fe during Winter and Spring, respectively. Also, Tilapia accumulated higher concentrations of Zn and Mn in their tissue than *C. gariepinus* during all seasons. The highest elevation of Pb was detected in both fishes during Summer only.

Induction of oxidative stress as seen in the elevated levels of malondialdehyde (MDA) and declined the antioxidant activities in the muscles of *O niloticus and* C. *gariepinus* from the two areas of Sharkia province. This observation can be related to the degradation of an environmental site due to a decrease in water quality and its pollution [47]. The highest MDA levels were found in the *C. gariepinus* muscles as compared with muscles of *O. niloticus* that may be due to the difference in the living mode of the two species. The marked disturbance in antioxidant and pro-oxidant balance in fish observed in the muscles may be due to the presence of some heavy metals in water samples and muscles as reported previously [48].

In the present study, histopathologically alteration of the muscles of *O. niloticus and C. gariepinus* collected from investigated areas A and B of Sharkia province were summarized in inflammation of the muscle's cells, fatty degeneration of muscle fiber, muscle fibers degeneration and focal area of accumulation granulosa cells. Alterations in muscles may be attributed to heavy metals accumulation or the parasitic infection which is a biomarker of heavy metal accumulation Sures, [49,50] or due to the changes in water quality. Therefore, underscores the need for managing waste discharges in these two lakes from the ongoing exploratory drilling activities in Sharkia province.

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Conclusion

Heavy metals levels changed seasonal and spatial in the two fished. The accumulation of heavy metals in the fish depend on its level in the water and the habitat of the fish. Heavy metal toxicity analysis, biochemistry and histopathology examination of fish's muscles can act as a biomonitoring model of the safety of fish habitats. The levels of elements in different fishes depend on the fish species, season, and place. However, fish muscles from Sharkia province City, Egypt are relatively not safe for human consumption from its bio-accumulative effects of heavy metals in muscles as confirmed by antioxidant alternation and histological changes. The aquatic pollution with anthropogenic activity is the main source of water loss and an imbalanced of the food chain.

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