IMPACT OF HEAVY METALS ON *OREOCHROMIS NILOTICUS* FISH AND USING ELECTROPHORESIS AS BIO-INDICATOR FOR ENVIRONMENTAL POLLUTION OF ROSETTA BRANCH, RIVER NILE, EGYPT

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In the present work, samples of water and fish were collected from the river Nile at El- Qanater El- Khyria as non-polluted site and from downstream of El-Rahawy drain (El-Qatta) as a polluted drain during different seasons of 2016. The concentration of metals (Fe, Zn, Cu, Pb, Mn and Cd) in water and their accumulations in fish muscles were measured. Electrophoresis pattern of *Oreochromis niloticus* including protein pattern, calcium pattern and *β*-esterase have been determined. In the present study, results revealed increased concentrations of studied metals in water and fish samples mainly during the winter season. The values of Fe, Cu, Pb, Mn, Zn and Cd were higher than the permissible limits in water, while Fe, Pb and Zn exceeded the permissible limits in fish muscles, especially those of El-Qatta station. Results of electrophoretic protein pattern showed similarities in arrangement of the bands in liver and muscles tissues in fishes taken from both locations (A and B). The cumulative risk impacts have been discussed.

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INTRODUCTION

The river Nile is one of the longest rivers in the world (its mainstream is about 6,740 km in length). The total area of its basin is about 2.9 million km^2. 22 % of the Nile's course runs through Egypt. At the north of Cairo, the Nile divides into two branches, the Rosetta branch to the west and the Damietta to the east.[1] Rosetta branch represents the area of investigation and its length is about 225 km. The width of the branch varies from 150 to 200 m and its average depth varies from 2 to 3.5 m. Nowadays, Rosetta branch suffers from several environmental problems. It receives pollutants from three main sources, the first source is El-Rahawy drain which receives domestic and agriculture wastes from Giza city and pours more than 1,900,000 m^3 day^{-1} of its effluents into Rosetta branch. The second source results from Kafr El-Zayat industrial area and the third source of pollution is several small agricultural drains that discharge their wastes into the branch in addition to sewage discharged from several cities. [2]

Metals are non-biodegradable and once discharged into water, they precipitated on sediment particles and accumulated in the living aquatic organisms. So, fishes absorb these metals from the surrounding water, sediment and food, which may accumulate in their tissues in significant amounts,[3] therefore, fish can be used as a bioindicator for detecting metals contaminating the freshwater ecosystems.

The heavy metals contamination is a very serious threat due to their toxicity, bioaccumulation and biomagnifications in the food web.[4] Also, metals are regarded as dangerous pollutants of the aquatic ecosystem due to their toxicity impact on living organisms.[5,6] While, the essential metals such as Cu, Zn and Fe have normal physiological regulatory functions.[7] They may accumulate and reach toxic levels in living organisms. Furthermore, the non-essential metals are usually strong toxic chemicals and their accumulation in organism's tissues may lead to intoxication, infertility, tissue damage and dysfunction of a variety of organs.[8] Concern about the impact of anthropogenic pollution on aquatic ecosystems is growing, where metals from man-made pollution sources are continuously released into both aquatic and terrestrial ecosystems. Heavy metals may accumulate in fish tissues from the surrounding water and/or food to be a very serious threat for fish and human health.[3,9,10]

An electrophoretic method has been described for distinguishing between fish slices according to their protein constituent. Reproducible electrophoretic patterns were obtained for different samples and sizes of the same fish type, but small differences were shown for fish of widely different origin.[11] A comparative study of fish species correspondence was accomplished using three different electrophoretic methods. Sarcoplasmic proteins were extracted from three related fish species and exposed to gel isoelectrofocusing, two-dimensional polyacrylamide gel electrophoresis and capillary zone electrophoresis.[12]

The present work was conducted to explore the effect of water pollutants of the river Nile (Rosetta branch) from El-Qanater El-Khyria to El-Rahawy drain (El-Qatta) on fish organs and estimate the amount of heavy metals in water and fish muscles.

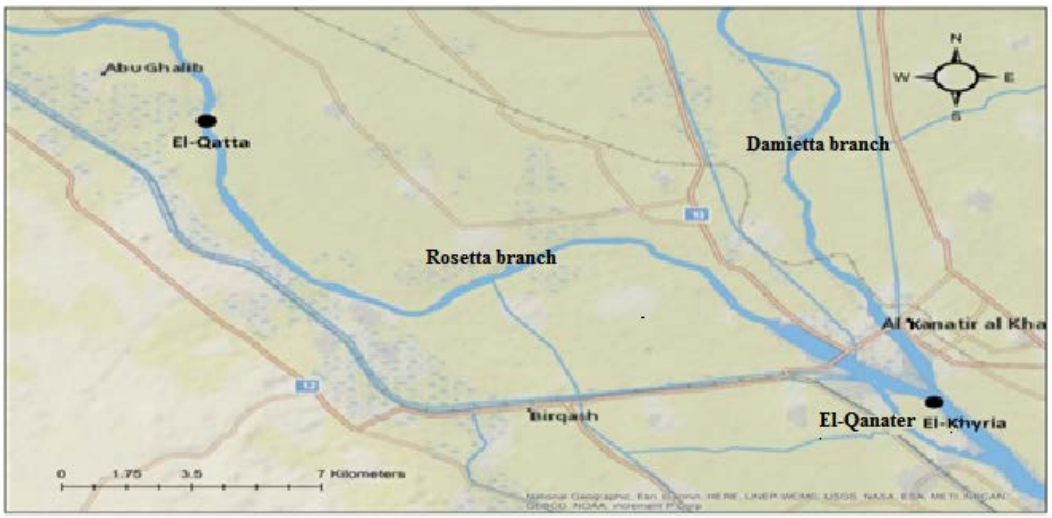

Figure 1. Map of northern Egypt showing the area of study and sampling stations on the Rosetta branch.

Furthermore, the study was concerned with revealing the adverse effect of these heavy metals on the biomacromolecules (proteins and calcium pattern and enzymes *β*-esterase) that were separated and identified electrophoretically and use of electrophoresis as an effective tool in revealing the adverse effect of heavy metals which accumulate in different fish organs when exposed to toxic concentrations.

EXPERIMENTAL

The studied area is located at the beginning of Rosetta Branch, about 25 km downstream of Cairo. Seasonal sampling of water and fish was performed from winter 2016 to autumn 2016. Two stations were chosen for this study. The first was El-Qanater El-Khyria city, while the second was El-Qatta (after El-Rahawy drain 7 Km). El-Rahawy drain is a huge drain, which receives domestic and agricultural wastes from Giza city and pours its effluents into the Rosetta branch (Figure 1).

Heavy metals in water

20 mL of conc. nitric acid were added to 500 mL of water sample in a beaker and boiled on a hotplate until complete digestion of suspended material. The remaining volume was made up to 100 mL with deionized distilled water. A portion of this solution was used for the quantitative determination of heavy metals (iron, copper, lead, manganese, zinc and cadmium) using atomic absorption model (Perkin Elmer 3110, USA) with graphite atomizer HGA-600, according to the reported method.[13] The results are expressed in mg L^{-1}.

Heavy metals in fish

Fish samples were transferred to weighing beakers and placed overnight in a drying oven thermostatically regulated at 105 ^{0}C. Dried samples (1 g) were taken and digested,[14] where 5 mL each of conc. perchloric and nitric acids were used. The digested solutions were cooled and made up to 25 mL using deionized water, the concentration of trace elements (Fe, Cu, Pb, Mn, Zn and Cd) in solution were determined using atomic absorption model (Perkin Elmer 3110, USA) with graphite atomizer HGA-600, according to the reported method.[13] The results are expressed in mg kg^{-1} of dry weight and then converted to mg kg^{-1} of wet weight basis.

Electrophoretic study

Preparation of the tissue homogenates

Liver and muscle tissues were excised from fishes (*O. niloticus*) caught from the two different areas. The tissues were washed with cold phosphate buffered saline, frozen rapidly with liquid nitrogen, ground then homogenized in 0.05 M Tris-HCl buffer (pH 7.4). The homogenates were left in refrigerator overnight and shaken using vortex for 15 sec and then centrifuged at 10,000 rpm at 4˚C for 15 min. The supernatants including water-soluble proteins were transferred to Eppendorf tubes and stored at the deepfreezer until electrophoretic analysis.

Electrophoretic protein and calcium patterns

The native electrophoretic patterns were carried out through vertical slab polyacrylamide Gel Electrophoresis using Mini-gel electrophoresis (Biorad, USA) according to a reported method[15] and its recent modification[16] The resolving gel was prepared at the concentration 8 % from stock solution consisting of acrylamide: bis-acrylamide (30 % T, 2.67 % C) (acrylamide/bis = 29.2:0.8) and 10 % glycerol.

After the electrophoretic run, the native bands were stained by Commassie Brilliant Blue G-250 for visualization. The relative mobility (*RF*) and band percent (*B*, %) of the isolated proteins were determined in addition to the molecular weights (MWs) that estimated in comparison to marker of standard molecular weights (ranging from 6.458

to 195.755 KDa). Moreover, lipid and calcium moieties of the native proteins were stained by mean of isoelectrophoresis using Sudan Black B and Alizarin Red S respectively as suggested earlier.[17,18]

Electrophoretic localization of in-gel enzyme activity:

The electrophoretic *β*-esterase in the native gel was stained using benzidine stain prepared as per a reported method.[19] After developing the colored bands of enzyme activity, the gel was fixed in 7 % glacial acetic acid for 30 min, then it was preserved in 5 % acetic acid prepared in 10 % methanol.

RESULTS AND DISCUSSION

Heavy metals in water

Heavy metals concentrations in the water samples at the two localities of the River Nile, at El- Qanater El- Khyria as non-polluted site and from downstream of El-Rahawy drain (El-Qatta) as a polluted site, during the period of study are presented in Figures 2 to 7.

The concentration of iron in the water at the two stations varied from the maximum value of 0.760 mg L^{-1} at El-Qatta during winter to a minimum value of 0.126 mg L^{-1} at El-Qanater El-Khyria during spring. The concentration at El-Qatta station is higher than the permissible level of 0.5 mg L^{-1}, according to the Egyptian Organization for Standardization,[20] (Figure 2). The low iron content in spring is possibly due to the consumption of iron by phytoplankton[21] and oxidation of Fe^{2+} to Fe^{3+} and subsequent precipitation as hydroxide at high dissolved oxygen content,[22] whereas, the high value of iron concentration at station II during winter may be ascribed to the breakdown of organic matter and dead microorganisms that releases the metal into water.[23] Finally, the increasing of iron may be due to the small water level during the drought period and discharge of effluent from El-Rahawy drain which is loaded with agriculture and domestic sewage.[24]

Copper values in the investigated area varied in the range of 0.016 – 0.021, 0.010-0.017, 0.009-0.018 and 0.010 -0.020 mg L^{-1} during winter season, spring season, summer season and autumn season, respectively, (Figure 3), which are higher than the permissible level (0.010 mg L^{-1}) according to Egyptian Standards of the Environmental Laws no. 48/1982 decree 92/2013.[25] Winter recorded the highest values (0.021 mg L^{-1}) of (Cu) at El-Qatta station may be due to decrease water level in the River Nile and increase domestic sewage at El-Rahawy drain. While summer recorded the lowest values, the decrease (0.009 mg L^{-1}) in Cu-concentration in water is probably due to its tendency to form complex with organic ligands and humic matter, which leads to lessening the penetration of free ions into water, where 90 % of (Cu) in water is complexed by dissolved organic and suspended matters.[26,27]

The concentrations of lead in water are 0.017-0.059, 0.017-0.052, 0.015-0.038 and 0.020-0.055 mg L^{-1} during winter, spring, summer and autumn, respectively (Figure 4). The increase in lead during winter (0.059 mg L^{-1}) at El-Qatta station might be ascribed to the reduction in water discharges during drought period, whereas the highest value of Pb may be resulted from heavy metals in agricultural waste runoff containing fertilizers, agrochemicals, or pesticides at El-Rahawy drain. Thus, El-Qatta station showed a higher level of lead than the permissible value[20] (0.050 mg L^{-1}) except for summer season.

The present results showed that manganese values in the investigated areas range between 0.048-0.135, 0.048-0.085, 0.030-0.055 and 0.045-0.123 mg L^{-1} during winter, spring, summer and autumn, respectively. The low concentration of manganese may be attributed to oxidation of Mn^{2+} to solid MnO_2 which precipitates to the sediment layer.[23] El-Qatta station showed higher value than the permissible[20] level (0.050 mg L^{-1}) as observed in Figure 5. The seasonal average values of zinc concentrations in water vary between the maximum value of 0.155 mg L^{-1} at El-Qatta during winter and a minimum value of 0.059 mg L^{-1} at El-Qanater El-Khyria during summer. Low Zn concentration may be related to the contribution of phytoplankton, pH and dissolved oxygen concentration.[2] Zinc in the River Nile exceeded the permissible levels at El-Qatta station,[28] (Figure 6).

Cadmium concentrations of the two stations in the water range between (0.0030-0.011 mg L^{-1}) and are higher than the permissible level (0.001 mg L^{-1}) recommended by Egyptian Standards of the Environmental Laws no. 48/1982 decree 92/2013,[25] (Figure 7). The maximum concentration of cadmium during winter at El-Qatta station might be attributed to the effect of pollution sources in that sites, as sewage and domestic wastes at El-Rahawy drain.

Generally, the concentrations of heavy metals in water samples were in the of Fe > Zn > Mn > Pb > Cu > Cd. The concentration of heavy metals in water samples showed seasonal variations, elevated in winter seasons, may be due to decreased level of water during drought period which results elevation of concentration of the metals,[29,24] and due to increase of the amount of discharge of agricultural, sewage waste water and industrial wastes into water of the River Nile in winter season.[30] While decreased in summer seasons may be attributed to phytoplankton growth which can absorb large quantity of heavy metals from water and also due to increase the water level during the summer season in the River Nile.[31]

Accumulation of heavy metals in muscles of *O. niloticus* fish

Metal concentrations in the muscle samples of *O. niloticus* at the two localities of the River Nile at El- Qanater El-Khyria as non-polluted site and from downstream of El-Rahawy drain (El-Qatta) as a polluted site during the study period are illustrated in (Figure 8 to 13). The annual average of metal concentrations in the muscle samples were ranked in the order of Fe > Zn > Mn > Cu > Pb> Cd.

The concentration of iron in fish muscles varies between 14.455 and 36.763 mg kg^{-1} at El-Qanater El-Khyria during summer and El-Qatta during winter. Iron was the most abundant metal in the tissues studied.

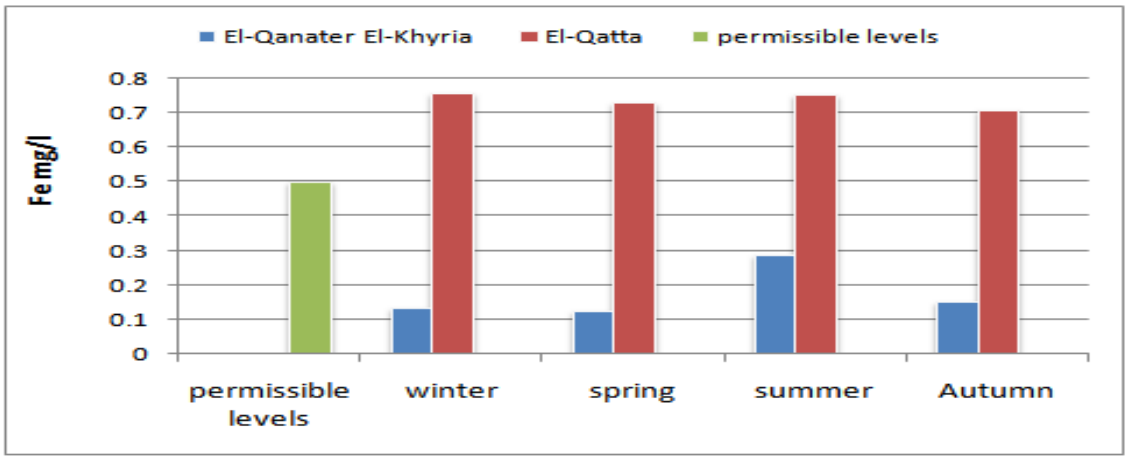

Figure 2. Seasonal variation of iron concentrations in water at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 20.

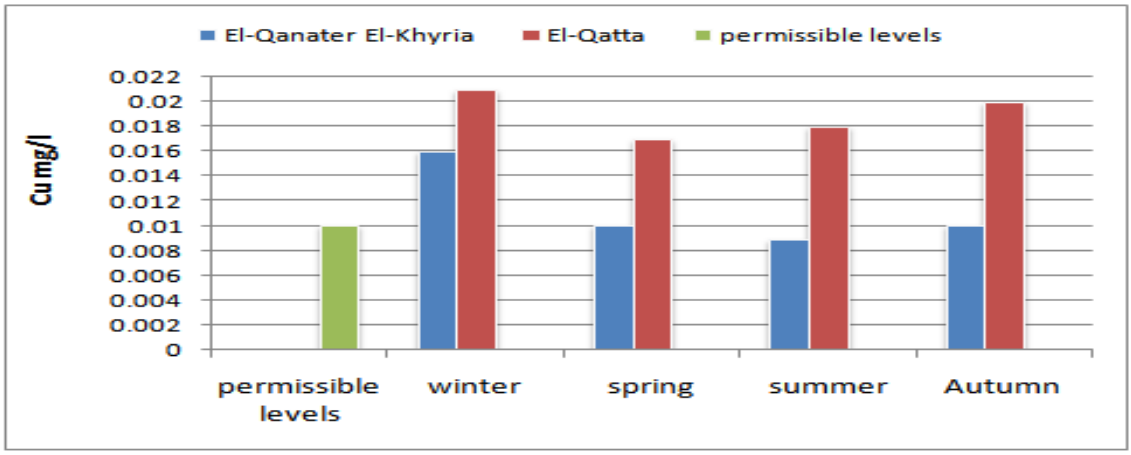

Figure 3. Seasonal variation of copper concentrations in water at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 25**.**

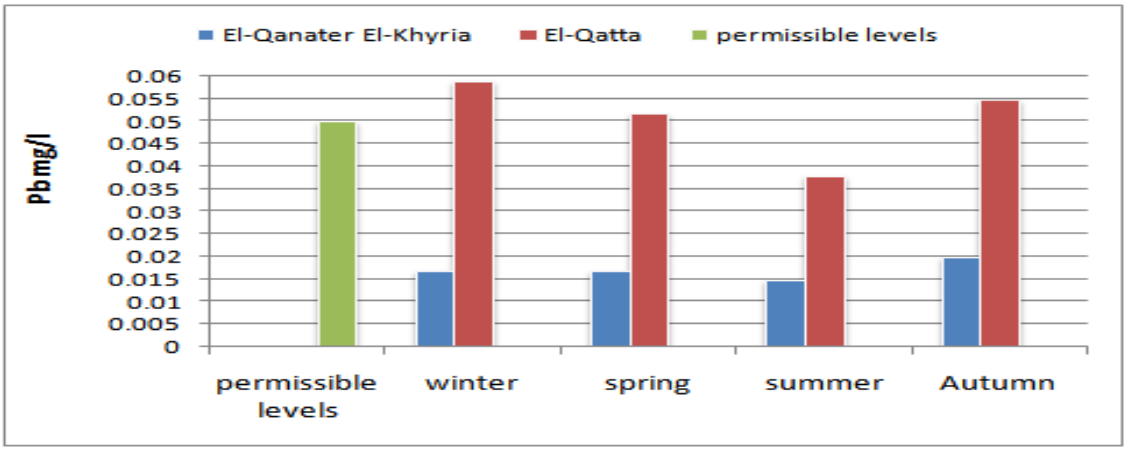

Figure 4. Seasonal variation of lead concentrations in water at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 20.

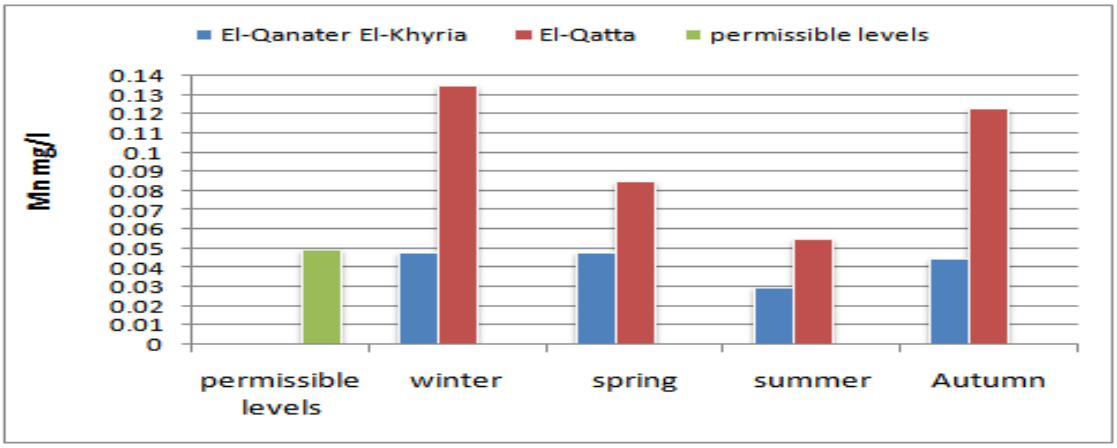

Figure 5. Seasonal variation of manganese concentrations in water at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 20.

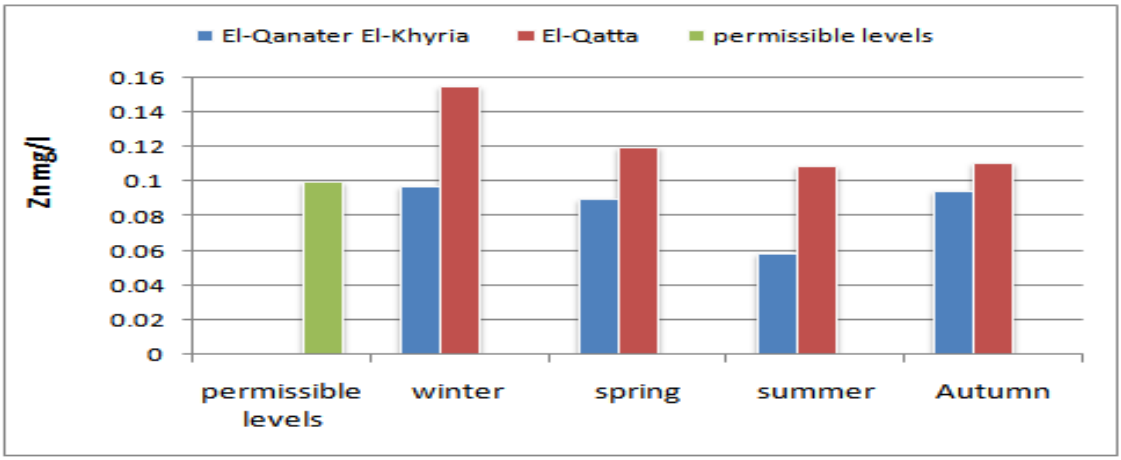

Figure 6. Seasonal variation of zinc concentrations in water at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 28.

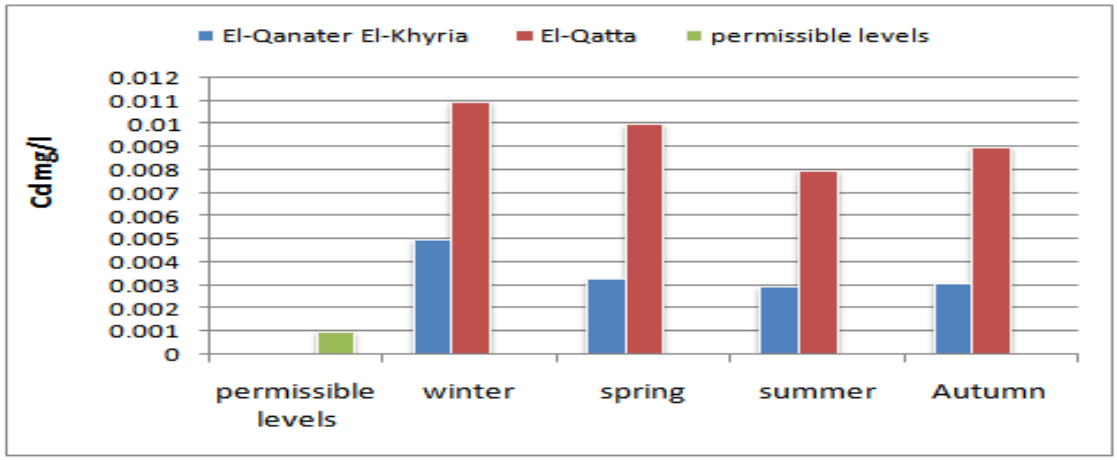

Figure 7. Seasonal variation of cadmium concentrations in water at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 25.

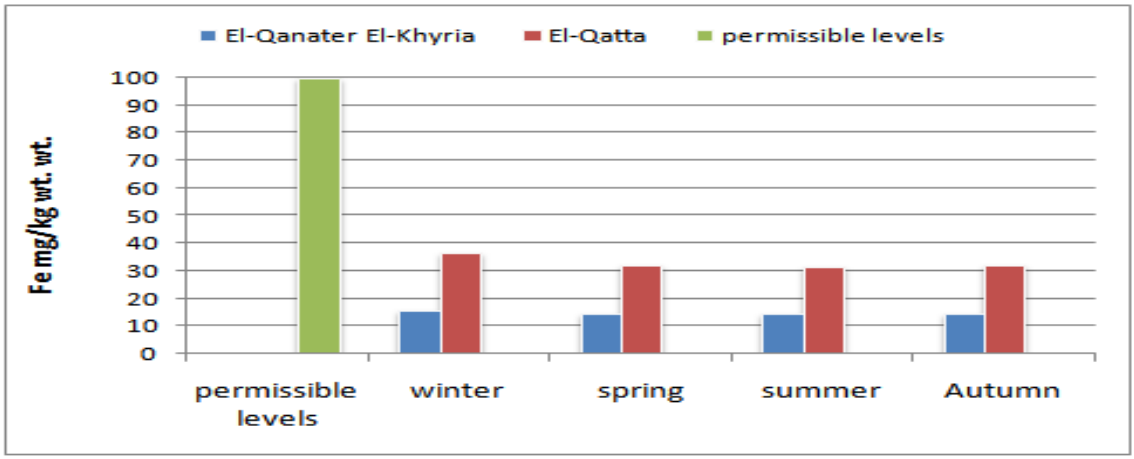

Figure 8. Seasonal variation of iron concentrations in the muscles of *O. niloticus* at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 32.

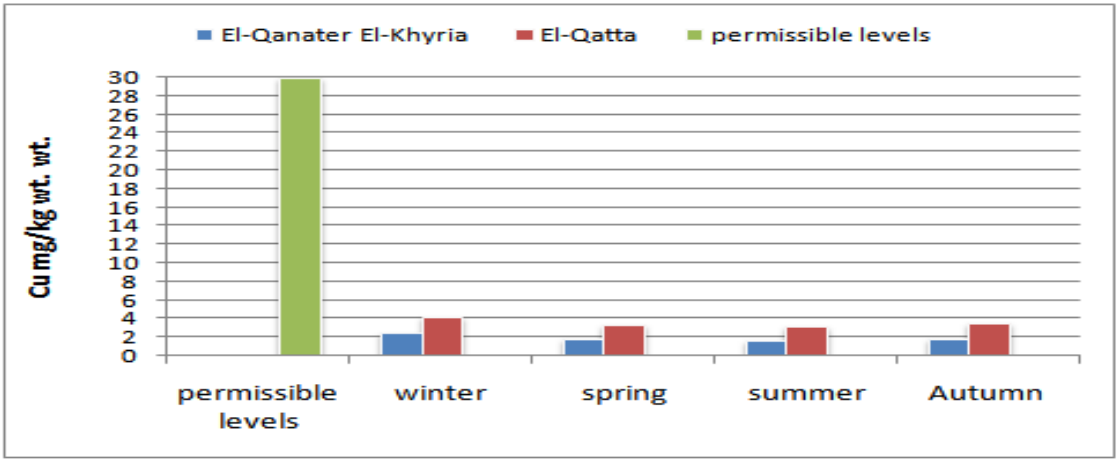

Figure 9. Seasonal variation of copper concentrations in the muscles of *O. niloticus* at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 32.

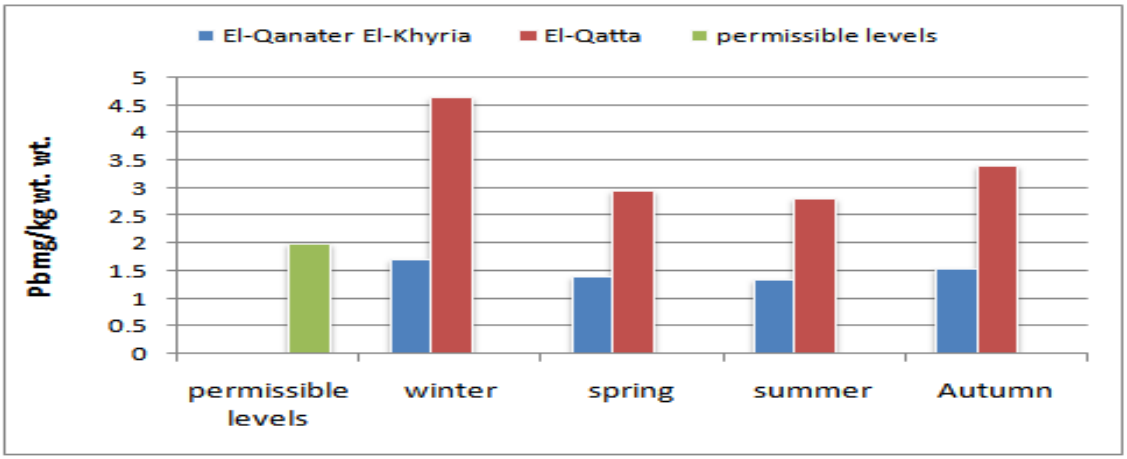

Figure 10. Seasonal variation of lead concentrations in the muscles of *O. niloticus* at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 32.

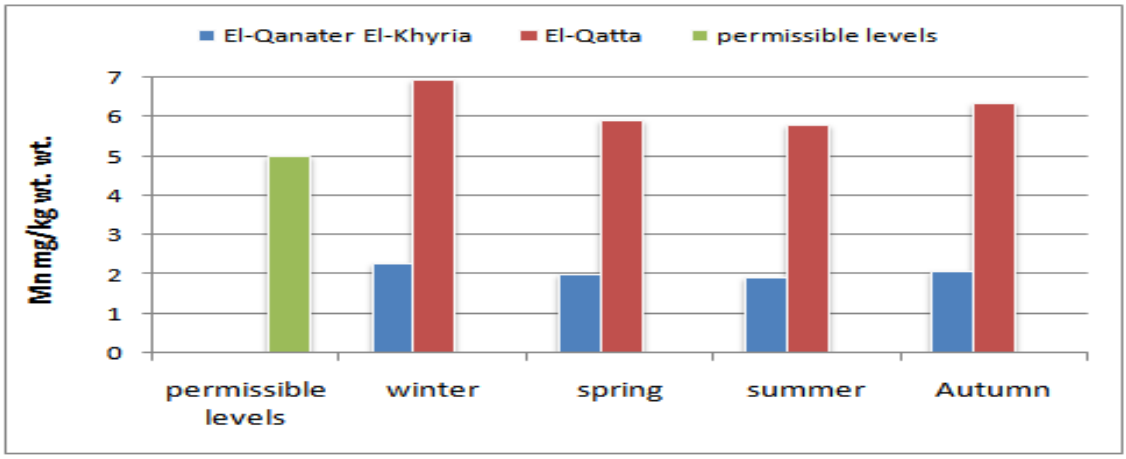

Figure 11. Seasonal variation of manganese concentrations in the muscles of *O. niloticus* at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 32.

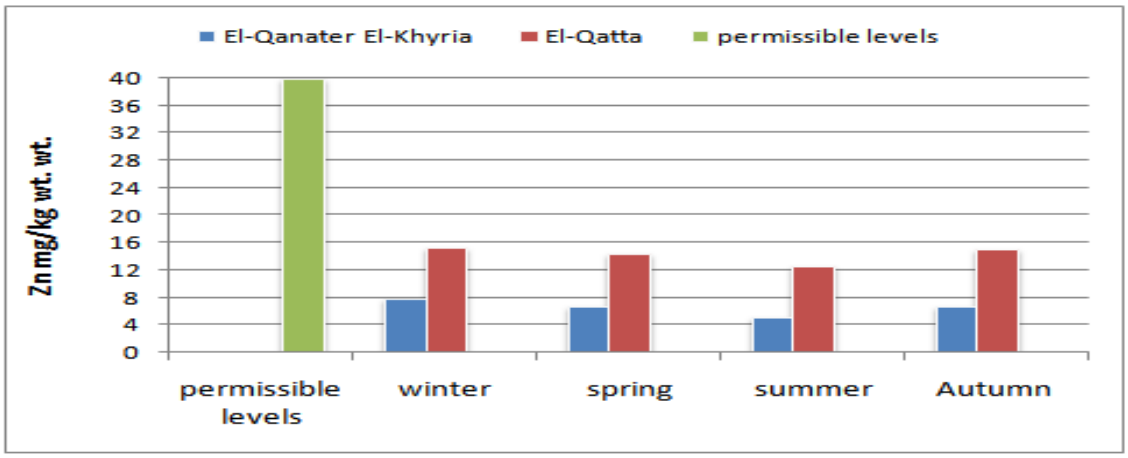

Figure 12. Seasonal variation of zinc concentrations in the muscles of *O. niloticus* at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 32.

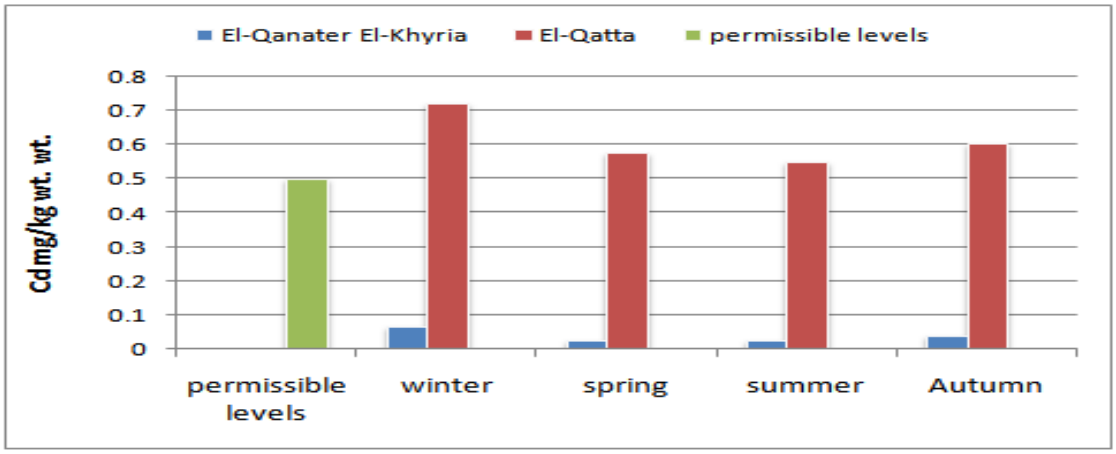

Figure 13. Seasonal variation of cadmium in the muscles of *O. niloticus* at El-Qanater El-Khyria and El-Qatta of the River Nile. Permissible limit is according to ref. 32.

The high accumulation of Fe in fish muscles can be attributed to the large amount of Fe detected in water. Higher Fe content in the fish muscles at El-Qatta station agrees with earlier finding.[30] The present data (Figure 8) showed that iron concentrations in the fish muscles were less than permissible level.[32] The highest accumulation of iron in fish muscles may be attributed to the large amount of iron in water of the River Nile and domestic sewage at El-Rahawy drain, however the minimum values at El-Qanater may be due to oxidation of Fe^{2+} to Fe^{3+} which remains as $Fe(OH)_3$ in the sediment of the oxygenated water.

Copper concentration of the muscles of the fish varies between 1.675 to 2.566 mg kg^{-1} wt. wt. at El-Qanater El-Khyria and 3.235 to 4.226 mg kg^{-1} wt. wt. at El-Qatta station during summer and winter season, respectively. Concentrations of copper (Figure 9) in the fish muscles are still less than the permissible level of 30 mg kg^{-1} wt. wt. [32] Decreased copper concentration in the muscles of *O. niloticus* fish may well be due to the decrease in Cu concentration in water because of the decrease in water discharge during cold seasons and increase of domestic waste at El-Rahawy drain.[33,24] On the other hand an increase in Cu concentration in muscles of *O. niloticus* fish may be due to the increment of copper in water because in flow of large quantity of sewage waste in studied area.

The maximum value of lead (4.658 mg kg^{-1} wt. wt.) was recorded during winter at El-Qatta station, while the lowest value (1.351 mg kg^{-1} wt. wt.) was registered during summer at El-Qanater El-Khyria. The study revealed (Figure 10) that the lead concentration in muscles of *O. niloticus* fish of the studied area was higher than the permissible limit of 2.0 mg kg^{-1} at El-Qatta station.[32] Figure 11 shows observed concentrations of Mn in the muscles which ranged from 1.945 to 6.954 mg kg^{-1} wt. wt., these results, at El-Qatta, were higher than permissible level[32] of 5.0 mg kg^{-1}. The highest value of zinc (Figure 12) was recorded in the fish muscles at El-Qatta station during winter (15.417 mg kg^{-1} wt. wt.), while the lowest value (5.211 mg kg^{-1} wt. wt.) recorded in El-Qanater El-Khyria station during summer. The values are lower than permissible level 40.0 mg kg^{-1} wt. wt.[32]

The increase of Pb and Mn concentration in muscles of *O. niloticus* may be due to high concentration of the metal in water brought about by large amount of sewage discharged at El-Rahawy drain. The increase of zinc may be attributed to the increase in metabolic rates which result in increased heavy metals uptake as has been previously indicated.[34] On the other hand, it may be due to the increase of Zn in water at El-Qatta station because of decrease in water discharge during winter season and increase domestic waste at El-Rahawy drain. The results (Figure 13) showed that cadmium values in the investigated area in the range between 0.0675-0.721, 0.027-0.578, 0.027-0.551 and 0.0405-0.605 mg kg^{-1} wt. wt. during winter, spring, summer and autumn, respectively. The values were lower than permissible level[32] of 0.5 mg kg^{-1} at El-Qanater El-Khyria station, while higher than permissible level[32] at El-Qatta station during all seasons. The high level of cadmium accumulation in the fish muscles may well be due to its strong binding with cystine residue of metalothionein as suggested earlier.[31,35] Also, the high levels of cadmium may be attributed to waste from sewage, industrial and mining operations as well as from the phosphate fertilizer at El-Rahawy drain station which accumulates most of the cadmium in the environment.[36]

Generally, the maximum values of accumulation of metals in fish muscles of *O. niloticus* at El-Qatta station during winter season may be attributed to their increase in water of the Rosetta branch of the river Nile because of domestic sewage at El-Rahawy drain and lower level water during the drought period.

Hazard quotient (HQ)

The Hazard Quotient is a ratio of estimated dosage of polluted to a reference dose level.[37] It is calculated for the individual heavy metals using the following Eqn. (1).

$$HQ = \frac{EF \times ED \times FI \times MCf}{RfD_0 \times BW \times AT} \times 10^{-3} \qquad (1)$$

where, *EF* is the exposure amount (365 days $year^{-1}$), *ED* is the life time exposure period (70 years), *FI* is mass of the fish ingested by person per day (57 mg) in Egypt,[38] *MCf* is the metal concentration in fish (in mg kg^{-1}), *BW* is the body weight, a body weight of 70 kg is recommended[39] as a default value for the adult. *AT* is average time for non-carcinogens (365 days $year^{-1}$ × ED), RFD_0 is the reference dosage by mouth (ppm day^{-1}).

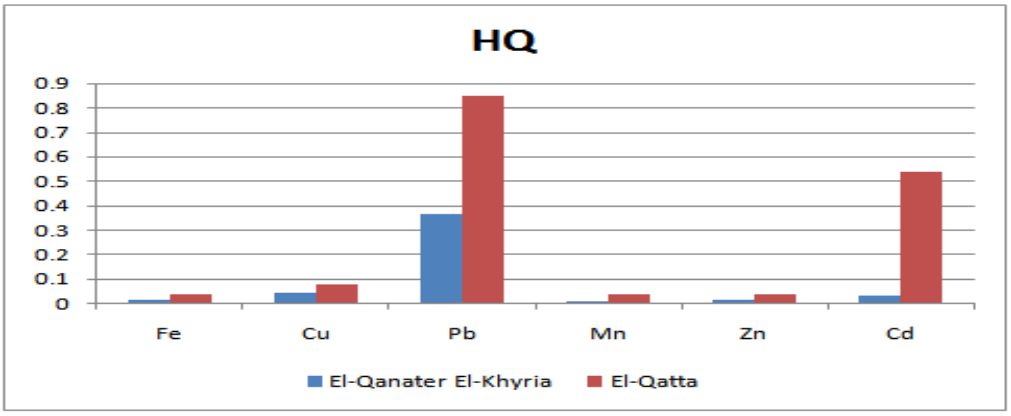

Figure 14. Hazard Quotient (HQ) to humans due to individual metal through the muscles of *O. niloticus* at El-Qanater El-Khyria and El-Qatta station of the River Nile.

Table 1. HQ and HI to human population from metals through muscle of *O. niloticus* Rosetta branch.

Metal	*CMF* (mg kg^{-1} wt. wt.) El-Qanater	*CMF* (mg kg^{-1} wt. wt.) El-Qatta	*RfD*$_0$ (mg kg^{-1} per day)	*HQ* El-Qanater	*HQ* El-Qatta
Fe	14.89375	33.20863	0.7	0.018684	0.04166
Cu	1.96525	3.60025	0.04	0.043144	0.079038
Pb	1.50625	3.46725	0.0036	0.367415	0.845756
Mn	2.08	6.2675	0.14	0.013047	0.039312
Zn	6.645375	14.418	0.3	0.019452	0.042203
Cd	0.0405	0.61375	0.001	0.035565	0.538957
HI	∑			***0.497307***	***1.586926***

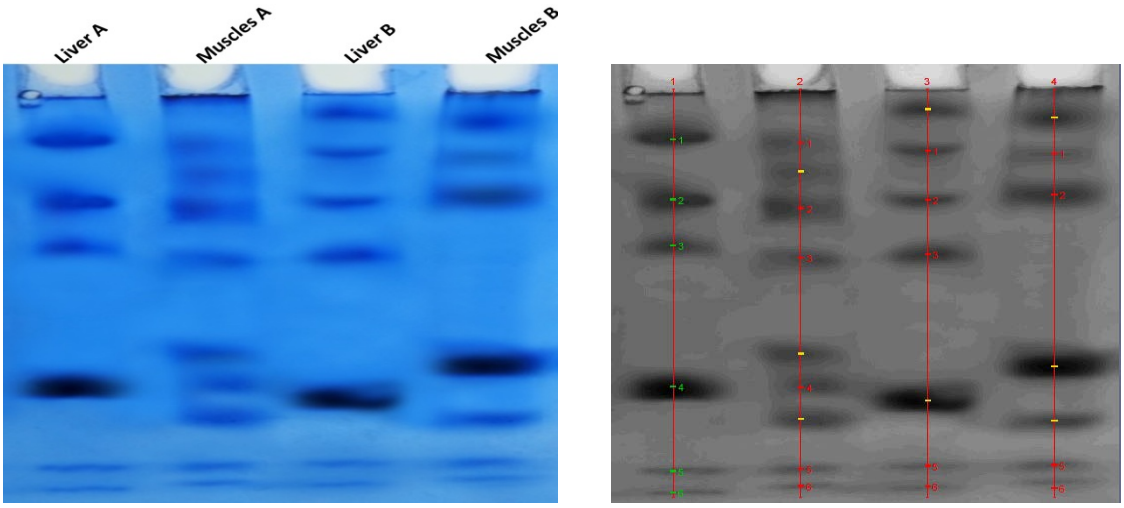

Figure 15. Native electrophoretic protein pattern showing the variations in number and arrangement of protein bands in liver and kidney tissues isolated from two different locations (A and B).

Table 2a. Data of the electrophoretic protein pattern in liver of fishes living in different contaminated areas with different enviromental conditions.

El-Qanater				El-Qatta			
Rf	**Band intensity**	***B*, %**	**Qty**	***Rf***	**Band intensity**	***B*, %**	**Qty**
0.125	188.258	17.478	4.457	0.051	174.669	14.636	8.221
0.272	185.678	17.239	11.202	0.152	174.693	14.638	5.296
0.385	180.856	16.791	8.011	0.274	173.965	14.577	6.245
0.730	210.465	19.540	8.679	0.406	176.846	14.819	8.324
0.937	156.804	14.558	3.592	0.765	194.946	16.336	13.064
0.990	155.039	14.394	2.013	0.925	151.675	12.710	3.267

Table 2b. Data of the electrophoretic protein pattern in muscles of fishes living in different contaminated areas with different enviromental conditions.

El-Qanater				El-Qatta			
Rf	**Band intensity**	***B*, %**	***Qty***	***Rf***	**Band intensity**	***B*, %**	***Qty***
0.134	171.222	11.264	4.217	0.072	185.165	15.703	8.456
0.203	178.818	11.764	3.028	0.160	169.296	14.357	1.600
0.293	181.541	11.943	7.686	0.260	175.980	14.924	10.254
0.415	170.667	11.228	5.255	0.681	204.329	17.328	12.710
0.650	176.669	11.623	6.391	0.814	156.977	13.312	7.787
0.733	169.296	11.138	3.909	0.922	148.433	12.588	4.558
0.932	155.644	10.240	4.073				
0.974	146.064	9.609	3.710				

Rf. = Relative Mobility, *B* % = Band percent, *Qty* = Band Quantity.

Hazard Index (HI)

Hazard Index (HI) and Hazard Quotient (HQ) are used to estimate the health risk for humans from fish.[37] Eqn. (2) is used to calculate both HI and HQ.

$$HI = \sum n_i = 1HQ_i \qquad (2)$$

Total *HQ* is expressed as the *HI*.[40] When *HQ* is equal to or lower than one, it signify no appreciable health risk, while if *HQ*>1, then it specify a reason for health concern.[39] Greater is the values of *HQ* and *HI* (above 1), the greater is the level of risk associated with fish consumption. Hence, *HI*<1means no hazard, 1>*HI*<10 means moderate hazard while greater than 10 means high hazard risk.[41] The *HQ* (Figure 14) due to intake of the studied metals through *O. niloticus* from the El Qanater and El-Qatta station are in the range for Fe (0.01868 – 0.04166), Cu (0.04314– 0.07904), Pb (0.3675 – 0.84576), Mn (0.01305 –0.03931), Zn (0.01945 –0.04221) and Cd (0.03556 –0.53896).

HI values (Table 1) because of consumption of *O. niloticus* from the two stations are different. *HI* = 0.4973 for El-Qanater El-Khyria, thus the *HI* of total for analyzed non-carcinogenic metals are less than the acceptable limit (*HI* = 1) and thus do not have human health risk concern, while *HI* for the analyzed heavy metals were higher than the acceptable limit (*HI*<1) at El-Qatta station (*HI* =1.5869) and therefore, the cumulative metals risk impact is alarming particularly at high rates of fish consumption.[39]

Electrophoretic study

It is well known that the rate of accumulation of metals in an organism's body vary from organ to organ. In an earlier study there was an equilibrium between concentricity of the metals in an organism's environment and its rate of ingestion and accumulation in muscles.[42]

The native protein pattern showed effect of aquatic toxic substances on the protein molecules totally. As recorded in Tables 2a and 2b and illustrated graphically in Figure 15, the native electrophoretic protein pattern revealed that there were four common bands noticed for both liver and muscle tissues at *Rf*s 0.125, 0.272, 0.937 and 0.990 (*B*, %: 17.478, 17.239, 14.558 and 14.394; Quant. 4.457, 11.202, 3.592 and 2.013, respectively). There were no unique or characteristic bands. With respect to liver of fishes taken from location A, as match to the electrophoretic protein pattern in liver of fishes living at Qanater El-Kahayria water (Figure 15), elevation of the heavy metals at El-Rahawy water caused qualitative mutagenecity, it was found that there are 2 abnormal bands identified in liver of fishes taken from location B at *Rf*s 0.051 and 0.765 (*B*, %:14.363 and 16.336; Quant. 8.221 and 13.064, respectively). On the other hand, as compared to muscles of fishes taken from location A, it was noticed that there was qualitative variations represented by appearance of unique band identified at *Rf* 0.072 (*B*, %: 15.703; Quant. 8.456) with disappearance of 3 bands identified in muscles A (location A) at *Rf*s 0.203, 0.650 and 0.809 (*B*, %:11.764, 11.623 and 11.191; Quant. 3.028, 6.391 and 7.594, respectively). As revealed in Table 3, the native protein showed physiological alterations in liver and muscles of fishes living at El-Qatta water severely more than at El-Qanater El-Khyria. Results showed a significant difference arrived to 45.4 % in the liver and 48.1 % in the muscle. This might be attributed to ability of the metals to change activities of the hepatic enzymes leading to histopathology hepatic changes. Furthermore, the deleterious effects of heavy metals depend on the metal type and concentration and length of exposure.[43]

Table 3. Data of the similarity index (*SI*) and genetic distance (GD) of the electrophoretic patternof protein in liver and muscle tissues of fishes living in different contaminated areas with different enviromental conditions.

		Liver A	**Muscles A**	**Liver B**	**Muscles B**
		Similarity Index			
Liver A	Genetic distance	100	66.1	54.6	37.8
Muscles A		33.9	100	43.3	51.9
Liver B		45.4	56.7	100	37.3
Muscles B		62.2	48.1	62.7	100

The electrophoretic alterations in the native protein patterns may refer to effect of the heavy metals especially Pb and Cd, turn proteins and peptides susceptible to structural modifications in sub-cellular compartments and tissues.[44]

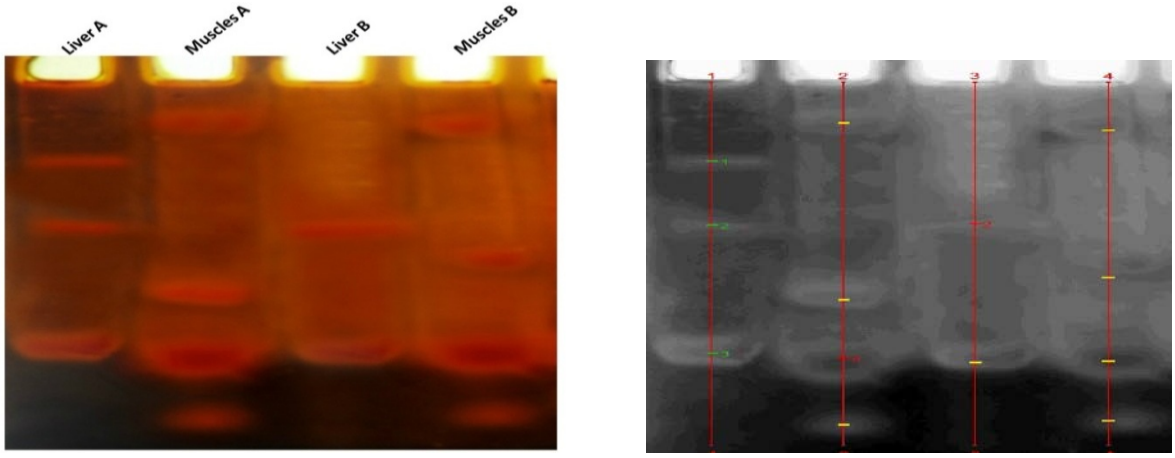

Figure 16. Native electrophoretic patterns of calcium moieties of native protein pattern showing the variations in number and arrangement of protein bands in liver and muscles tissues isolated from two different locations (A and B).

Table 4a. Data of the electrophoretic patterns of calcium in liver of fishes living in different contaminated areas with different enviromental conditions.

El-Qanater				El-Qatta			
Rf	**Band intensity**	***B*, %**	**Qty**	**Rf**	**Band intensity**	***B*, %**	***Qty***
0.218	98.816	30.348	7.162	0.391	128.638	58.428	3.272
0.395	109.094	33.504	6.046	0.774	91.527	41.572	4.967
0.748	117.701	36.148	2.760				

Table 4b. Data of the electrophoretic pattern of calcium in muscles of fishes living in different contaminated areas with different enviromental conditions.

El-Qanater				El-Qatta			
Rf	**Band intensity**	***B*, %**	***Qty***	***Rf***	**Band intensity**	***B*, %**	***Qty***
0.113	120.796	29.545	3.191	0.133	125.493	30.502	2.280
0.601	117.804	28.814	6.002	0.540	121.753	29.593	2.212
0.762	90.623	22.165	1.881	0.770	90.163	21.915	1.042
0.944	79.626	19.476	7.513	0.935	74.013	17.990	6.723

The electrophoretic calcium moieties of native protein pattern showed effect of the aquatic toxicity on the protein portion linked to calcium portion inside cells. As recorded in Table 4a and 4b and illustrated graphically in Figure 16**,** the native electrophoretic calcium moieties of native protein pattern presented that there was one common band in both of liver and muscle tissues identified at *Rf* 0.748 (*B*, % 117.701; Quant. 2.760). There were no unique or characteristic bands. With respect to liver of fishes taken from location A, it was found that the 1st normal band disappeared without appearance of any abnormal bands. On the other hand, as compared to muscles of fishes taken from location A, it was noticed that there was qualitative variations represented by deviation of the 2nd normal band to be appeared at *Rf* 0.540 (*B*, % 29.593, Quant. 2.212).

Table 5. Data of SI and GD of the electrophoretic pattern of calcium in liver and muscles tissues of fishes living in different contaminated areas with different enviromental conditions.

		Liver A	**Muscles A**	**Liver B**	**Muscles B**
		Similarity Index			
Liver A	Genetic Distance	100	66.1	54.6	37.8
Muscles A		33.9	100	43.3	51.9
Liver B		45.4	56.7	100	37.3
Muscles B		62.2	48.1	62.7	100

Data of the similarity indices and genetic distances compiled in table 5, number and arrangement of the bands in liver tissues in fishes taken from both locations (A and B) are similar by 33.9 %; while those in the muscle tissues are similar by 38.4 %. The similarity indices, there were similarity and physiological relationships among all the groups depending on the electrophoretic calcium moieties of native protein, results showed a significant difference arrived to 66.1 % in the liver and 61.6 % in the muscle. The native electrophoretic pattern showed physiological alterations in muscles and liver of fishes living in El-Rahawy drain water severely more than in El-Qanater El-Khyria water. Alterations in the electrophoretic calcium pattern of native protein pattern in liver and muscles tissues. This may be related to contamination with Cd that alters calcium homeostasis.[45]

The electrophoretic *β*-esterase showed that the aquatic toxicity exerted adverse effect leading to alterations in expression of these enzymes. As recorded in Table 6 a and 6b and illustrated graphically in Figure 17, the native electrophoretic *β*-esterase pattern revealed that *β*-esterase enzyme expressed as 2 types in liver tissue and 4 types in the muscle tissues. There were no common or characteristic bands. As compared to liver of fishes isolated from location A, there was qualitative difference represented by deviation of the 2nd band to be identified at *Rf* 0.784 (*B*, % 56.837 and Quant. 13.040). On the other hand, as compared to muscles tissues of fishes isolated from location A with those isolated from location B, it was found that there are no physiological or qualitative variations in number and arrangement of bands of native *β*-esterase pattern.

Table 6a. Data of the electrophoretic pattern of *β*-esterase in liver of fishes living in different contaminated areas with different enviromental conditions.

El-Qanater				El-Qatta			
Rf	**Band intensity**	***B*, %**	***Qty***	***Rf***	**Band intensity**	***B*, %**	***Qty***
0.293	128.831	43.163	5.061	0.288	110.400	38.006	2.904
0.784	169.642	56.837	13.040	0.668	180.080	61.994	4.421

Table 6b. Data of the electrophoretic pattern of *β*-esterase in muscles of fishes living in different contaminated areas with different enviromental conditions

El-Qanater				El-Qatta			
Rf	**Band intensity**	**B %**	**Qty**	**Rf**	**Band intensity**	**B %**	**Qty**
0.168	114.768	22.471	4.630	0.159	115.602	23.298	10.225
0.454	131.571	25.761	6.996	0.434	129.127	26.024	10.232
0.657	128.520	25.164	11.076	0.616	124.242	25.040	2.518
0.734	135.869	26.603	4.235	0.707	127.213	25.638	3.048

Rf = Relative Mobility, *B*, % = Band Percent, *Qty* = Band Quantity

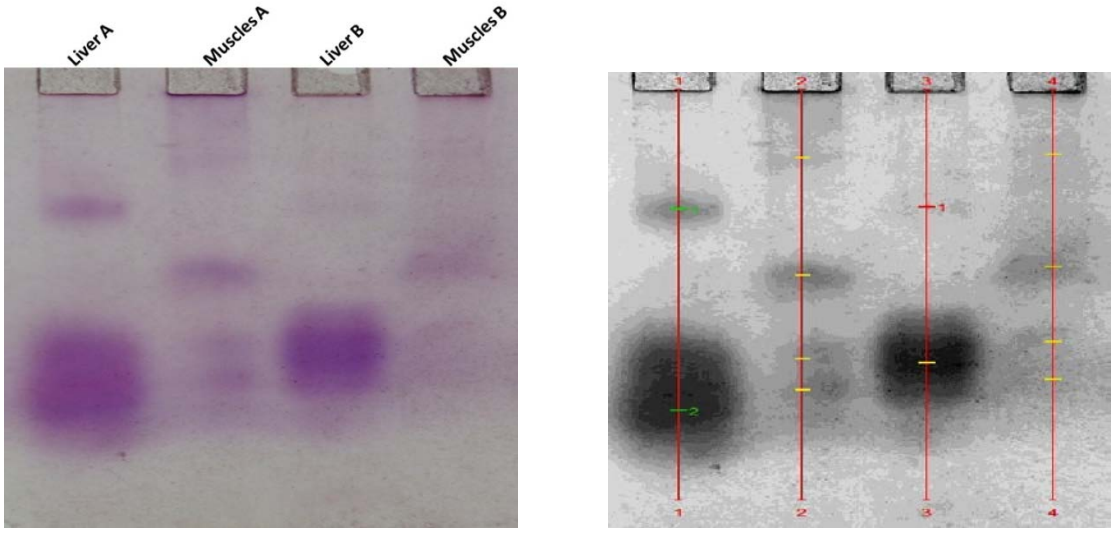

Figure 17. Native electrophoretic *β*-esterase pattern showing the variations in number and arrangement of enzyme types in liver and kidney tissues isolated from two different locations (A and B).

Data of the similarity indices and genetic distances documented in Table 7, number and arrangement of the bands in liver tissues in fishes taken from both locations (A and B) are similar by 25.5 %, while those in the muscle tissues are similar by 29 %. The similarity indices, results showed a significant difference arrived to 74.5 % in the liver and 70.0 % in the muscle, there were similarity and physiological relationships among all the groups depending on the electrophoretic *β*-esterase pattern. Alterations were detected in the electrophoretic *β*-esterase pattern which may be ascribe to increasing of heavy metals of the Nile River water.

Table 7. Data of SI and GD showing number and arrangement of the pattern of β-esterase bands in liver and muscles tissues isolated from two different locations (A and B).

		Liver A	**Muscles A**	**Liver B**	**Muscles B**
		Similarity Index			
Liver A	Genetic Distance	100	0	25.5	0
Muscles A		100	100	36.1	29
Liver B		74.5	63.9	100	0
Muscles B		100	71	100	100

A = El-Qanater, B = El-Qatta

During the study, he found that the heavy metals accumulate in the muscles fish with different concentrations. The fish muscle plays a key role in the metabolism and secretion of xenobiotic compound with morphological changes occurring in some toxic conditions.[46] During the study, electrophoretic alterations in the fish liver and muscles tissues were noticed. This might be attributed to ability of the metals to change activities of the hepatic enzymes leading to histopathological changes in the liver. Moreover, alterations were detected in the electrophoretic CAT and Gpx patterns. This might be caused due to presence of Cd that has the ability to alter the cell adhesion and the cellular antioxidant defence mechanisms.[47]

REFERENCES

[1]Helal, H. A., Studies on the Zooplankton of Dametta Branch of the River Nile North of El-Mansoura. Zoology Department, Mansoura Universisty, **1981**, p. 462.

[2]El Bouraie, M .M., Motawea, E. A., Mohamed, G. G., Yehia, M. M., Water quality of Rosetta branch in Nile delta, Egypt, *Suo*, **2011**, *62(1)*, 31–37.

[3]Eiman, M., Zamzam, H., Effect of selenium-mercury interaction on *Clarias lazera* fish. *Proc. 3rd Congr. Toxicol. Developing Countries*, Cairo, Egypt, 19-23 November **1996**, p. 379-392.

[4]Eisler, R., *Lead Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review*; US Fish and Wildlife Service, Patuxent Wildlife Research Center, **1988**.

[5]Khalil, M. K. H., Radwan, A. M. and El-Moselhy, K. H. M. Distribution of phosphorus fractions and some of heavy metals in surface sediments of Burullus lagoon and adjacent Mediterranean Sea. *Egypt. J. Aquat. Res*., **2007**, *33(1)*, 277–289.

[6]Ali, Z. Malik, R. N. and Qadir, A., Heavy metals distribution and risk assessment in soils affected by tannery effluents, *Chem. Ecol*., **2013**, 29, 676–692. https://doi.org/10.1080/02757540.2013.810728

[7]Hogstrand, C. and Haux, C., Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. Comparative, *Biochem. Physiol*., **2001**, *100*, 137-141. https://doi.org/10.1016/0742-8413(91)90140-O

[8]Damek-Proprawa, M. and Sawicka-Kapusta, K., Damage to the liver, kidney and testis with reference to burden of heavy metals in yellownecked mice from areas around steel works and zinc smelters in Poland, *Toxicology*, *2003*, **186,** 1-10. https://doi.org/10.1016/S0300-483X(02)00595-4

[9]Dautremepuits, C., Paris-Palacios, S., Betoulle, S., Vernet, G., Modulation in Hepatic and Head Kidney Parameters of Carp (Cyprinus Carpio L.) Induced by Copper and Chitosan. *Comp. Biochem. Physiol. Part C. Toxicol. Pharmacol.*, **2004**, *137*(4), 325–333. https://doi.org/10.1016/j.cca.2004.03.005

[10]Ahmed, M. K., Shaheen, N., Islam, M. S., Al-Mamun, M. H., Islam, S., Mohiduzzaman, M. and Bhattacharjee, L., Dietary intake of trace elements from highly consumed cultured fish (*Labeorohita*, *Pangasiu spangasius* and *Oreochromis mossambicus*) and human health risk implications in Bangladesh. *Chemosphere*, **2015**, *128*, 284–292. https://doi.org/10.1016/j.chemosphere.2015.02.016

[11]Manusu, H. P. and Wrigley, C. W., Identification of species of fish by gradient-gel electrophoresis. *Aust. Vet. J*., **1985**, *62(11)*, 372-6. https://doi.org/10.1111/j.1751-0813.1985.tb14212.x

[12]Valenzuela, M. A., Gamarra, N., Gómez, L., Kettlun, A. M., Sardón, M., Pérez, L. M., Vinagre, J., Guzman, N. A. A., Comparative Study of Fish Species Identification by Gel Isoelectrofocusing Two-Dimensional Gel Electrophoresis, and Capillary Zone Electrophoresis. *J. Capill. Electrophor. Microchip Technol*., **1999**, *6(3–4)*, 85–91.

[13]APHA, AWWA, WEF *Standard Methods for examination of water and wastewater*, American Public Health Association, American Water Works Association (AWWA) and Water Environment Federation (WEF), 22nd ed., Washington. **2012**

[14]Ghazaly, K. S., The Bioaccumulation of Potential Heavy Metals in the Tissues of the Egyptian Edible Marine Animals. 2.-Molluscs. *Bull. Natl. Inst. Oceanogr. Fish*., **1988**, *14(2)*, 79-86

[15]Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, **1970**, *227*, 680-685. https://doi.org/10.1038/227680a0

[16]Darwesh, O. M., Moawad, H., Barakat, O. S. and Abd El-Rahim, W. M., Bioremediation of textile reactive blue azo dye residues using nanobiotechnology approaches. *Res. J. Pharm., Biol. Chem. Sci*., **2015**, *6(1)*, 1202-1211.

[17]Subramaniam, H. N. and Chaubal, K. A., Evaluation of intracellular lipids by standardized staining with a Sudan black B fraction. *J. Biochem. Biophys. Methods*, **1990**, *21(1)*, 9-16. https://doi.org/10.1016/0165-022X(90)90040-J

[18]Abulyazid, I., Abd Elhalim, S. A., Sharada, H. M., Aboulthana, W. M. and Abd Elhalim, S. T., Hepatoprotective effect of carob pods extract (Ceratonia siliqua L.) against cyclophosphamide induced alterations in rats. *Int. J. Curr. Pharm. Rev. Res.*, **2017**, *8(2)*, 149-162. https://doi.org/10.25258/ijcprr.v8i02.9199

[19]Rescigno, A., Sanjust, E., Montanari, L., Sollai, F., Soddu, G., Rinaldi, A. C., Oliva, S. and Rinaldi, A., Detection of laccase, peroxidase and polyphenol oxidase on a single polyacrylamide gel electrophoresis. *Anal. Lett.*, **1997**, *30(12)*, 2211-2220.https://doi.org/10.1080/00032719708001733

[20]Egyptian Organization for Standardization, *Egyptian standard, maximum levels for heavy metal concentrations in food*. ES 2360-1993, UDC: 546.19: 815, Egypt. **1993**.

[21]Canli, M., Ay, Ö. and Kalay, M., Levels of heavy metals (Cd, Pb, Cu, Cr and Ni) in tissue of *Cyprinus Carpio*, *Barbus Capito* and *Chondrostoma Regium* from the Seyhan River. Turkey, *Turk. J. Zoology*, **1998**, *22*, 149-157.

[22]Ghallab, M. H., *Some physical and chemical changes on the River Nile downstream of Delta barrage at El-Rahawy drain*. M.Sc. Thesis. Fac. Sci. Ain Shams Univ., Egypt. **2000**.

[23]El-Sayed, M. and Salem, W. M., Hydrochemical assessments of surface Nile water and ground water in an industry area – South West Cairo, *Egypt. J. Petrol*., **2015**, *24, 277–288.*https://doi.org/10.1016/j.ejpe.2015.07.014

[24]Haggag, Y. H. M., *Environmental and chemical studies on the River Nile Pollutants and their relation to water quality at Rosetta branch, Egypt*. Ph. D. Thesis, Genetic Engineering & Biotechnology Institute, Sadat City University, **2017**.

[25]Egyptian Governmental Law No. 48/1982- Decision 92 (2013) The implementer regulations for Law 48/1982, 92/2013 regarding the protection of the River Nile and water ways from pollution. *Map Periodical Bull.*, **1982**, 21-30.

[26]Mantoura, R. F. C., Dickson, A. and Riley, J. P., The complexation of metals with humic materials in natural waters. *Estuarine Coastal Marine Sci*., **1978**, *6*, 382-409.https://doi.org/10.1016/0302-3524(78)90130-5

[27]Moustafa, S. S., *Alterations in antioxidant defense system and biochemical metabolites in fish from Rosetta branch of the River Nile as a bio-indicator of environmental pollution*. Ph.D. Thesis, Fac. of science, Ain Shams University, **2017**.

[28]*WHO Guidelines for drinking-water quality*. 2nded. Contents: V. I. Recommendations. WHO library cataloguing in publication date, Geneva. I-129, **1993**.

[29]Abdel-Moati, M. A. R. and El-Sammak, A. A., Man-made impact on the geochemistry of the Nile delta lakes: a study of metals concentrations in sediments. *Water Air Soil Contam.*, **1997**, *97*, 413-429. https://doi.org/10.1007/BF02407476

[30]Khalil, M., T.; Nahed, S. G., Nasr, A. M. A. and Sally, S. M., Antioxidant defense system alterations in fish as a bio-indicator of environmental pollution, *Egyp. J. Aquatic Biol. Fisheries*, **2017**, *21(3)*, 11-28. https://doi.org/10.21608/ejabf.2017.3536

[31]Tayel, S. I., Mahmoud, S. A., Ahmed, N. A. M. and Abdel Rahman A. A. S., Pathological impacts of environmental toxins on *Oreochromis niloticus* fish inhabiting the water of Damietta branch of the River Nile, Egypt. *Egypt. J. Aquatic Biol. Fisheries*, **2018**, *22(5)*, 309-321. https://doi.org/10.21608/ejabf.2018.25660

[32] WHO (World Health Organization), *Evaluation of Certain Food Additives and Contaminants* (Forty-first report of the joint FAO/ WHO Expert Committee on Food Additives). WHO, Geneva, Technical Report Series no. 837, **1993**.

[33]Ahmed, N. A., Biochemical studies on pollution of the River Nile at different station of Delta barrage, Egypt. Ph.D. Thesis, Fac. of Agric. Benha Univ., Egypt, **2012**.

[34]Sorensen, E. M. *Metal poisoning in fish*, Environmental and life sciences Associates. Austin. Texas. CRC Press Inc., Boston. **1991**.

[35]Abu El-Ella, S. M., *Studies on the toxicity and bioconcentration of cadmium on grass carp Ctenopharyngo donidella*. M. Sc. Thesis, Faculty of Science, Helwan University, Egypt., **1996**.

[36]Dimari, G. A., Abdulrahman, F. I., Akan, J. C. and Garba, S., Metals concentration in tissues of Tilapia gallier, Crariaslazera and OsteoglossidaeCanght from Alau Dam, Maiduguri, Borno State, Nigeria. *Am. J. Environ. Sci.*, **2008**, *4(4)*, 373-379. https://doi.org/10.3844/ajessp.2008.373.379

[37]Goher, M. E., Abdo, M. H., Mangood, A. H., Hussein, M. M., Water quality and potential health risk assessment for consumption of Oreochromisniloticus from El-Bahr El-

Pharaony Drain. Egypt., *Fresenius Environ. Bull.*, **2015**, *24*(11), 3590–3602.

[38]FAO *Report of fisheries and aquaculture markets in the Middle East*., **2014,** <http://www.thefishsite.com/articles/1870/fao report-fisheries-and-aquaculture markets-in-the-middle-east>

[39]USEPA (United States Environmental Protection Agency), Mid-Atlantic Risk Assessment. United States Environmental Protection Agency, Washington, **2013**, URL: <http://www.epa.gov/reg3hwmd/ risk/human/rb-concentration_table/users-guide. htm> (Accessed 26.03.14).

[40]Kumar, B., Virendra Kumar, V., Ashish Kumar, N., Paromita, C., Rita, S., Human health hazard due to metal uptake via fish consumption from coastal and fresh water waters in Eastern India along the Bay of Bengal. *J. Marine Biol. Oceanogr*., **2013**, *2(3)*, 7. https://doi.org/10.4172/2324-8661.1000115

[41]Ukoha, P. O., Ekere, N. R., Udeogu, U. V., Agbazue, V. E., Potential health risk assessment of heavy metals concentrations in some imported frozen fish species consumed in Nigeria. *Int. J. Chem. Sci.*, **2014**, *12(2)*, 366–374.

[42]Adeyeye E. I., Akinyugha N. J., Fesobi M. E. and Tenabe V. O., Determination of some metals in *Clarias gariepinus* (Cuvier and Valenciennes), *Cyprinocarpio* (L) and *Oreochromis niloticus* (L.) fishes in a polyculture freshwater pond and their environments. *Aquaculture*, **1996**, *147*, 205-214. https://doi.org/10.1016/S0044-8486(96)01376-2

[43]Paris-Palacios, S., Biagianti-Risbourg, S. and Vernet, G., Biochemical and (ultra) structural hepatic perturbation of *Brachydaniorerio* (Teleostei, Cyprinidae) exposed to two sublethal concentrations of copper sulphate. *Aquat. Toxicol.*, **2000**, *50*, 109-124. https://doi.org/10.1016/S0166-445X(99)00090-9

[44]Hechtenberg, S. and Beyersmann, D., Inhibition of sarcoplasmic reticulum Ca^{+2}-ATPase activity by cadmium, lead and mercury, *Enzyme*, **1991,** *45*, 109-115. https://doi.org/10.1159/000468875

[45]Pratap, H. B. and Wendelaar Bonga, S. E., Calcium homeostasis in low and high calcium water acclimatized *Oreochromismossambicus*exposed to ambient and dietary cadmium. *J. Environ. Biol.*, **2007**, *28*, 385-393.

[46]Rocha, E. and Monteiro, R. A. F., Histology and Cytology of Fish Liver: A Review. In: Ichthyology: Recent Research Advances, Saksena D.N. (Ed.). Science Publishers, Enfield, New Hampshire. ISBN: 1-57808-053-3, pp: 321-344, **1999**.

[47]Yano, C. L. and Marcondes, M. C. C. G., Cadmium chloride-induced oxidative stress in skeletal muscle cells *in vitro*. *Free Radicals Biol. Med.*, **2005**, *39*, 1378-1384. https://doi.org/10.1016/j.freeradbiomed.2005.07.001

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