



**Accumulation of some heavy metals and biochemical alterations in muscles of *Oreochromis niloticus* from the River Nile in Upper Egypt.**

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**ABSTRACT**

The River Nile is the life artery of Egypt. It is the main source of drinking, irrigation, natural fertilization of fields, fishing, and navigation. It receives continuously waste water discharge from agricultural, sewage and industrial sources. The present study aimed to evaluate levels of some heavy metals (Cu, Mn, Pb and Zn) in certain tissues (gills, intestine and muscles) of *Oreochromis niloticus* collected from different sites of the River Nile at Upper Egypt during winter and summer (2009). Moreover the effect of accumulated heavy metals on total proteins, total lipids and the activities of transaminase enzymes (ALT and AST) in the fish muscles were studied. The obtained results revealed that the abundance of heavy metals in fish organs followed the order: Mn>Zn>Pb>Cu. The highest levels of the heavy metals was recorded in the intestine and the lowest was recorded in the muscles. The concentrations of Cu in the fish muscle were below the maximum permissible limit, however, Mn, Pb and Zn exceeded the permissible limits. Total proteins, Total lipids and activities of ALT and AST were significantly lower in the muscles of the studied fish.

**1. INTRODUCTION**

Nowadays, pollution of the aquatic environment is a serious and growing problem throughout the world. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment have led to various deleterious effects on the aquatic organisms, including fish. Heavy metals contamination of aquatic system has attracted the attention of several investigators in both the developed and developing countries of the world.

The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment make these toxicants deleterious to the aquatic environment and consequently, to man.

Rivers represent the most complex aquatic systems in terms of transport and interactions of heavy metals with geochemical and biological processes. The River Nile nowadays suffers from several environmental problems.

According to the National Water Research Center (NWRC, 2000), the River Nile from Aswan to Al-Kanater Barrage receives wastewater discharge from 124 point source, of which 67 are agricultural drains and the remainders are industrial sources.

Bio-monitoring of hazardous substances in tissues of aquatic organisms has been successfully applied during recent years for heavy metals pollution (Lamas *et al.*, 2007).

Fish are often at the top of the aquatic food chain and metals are accumulated in it to concentrations much time higher than that present in water and sediment. Fish can absorb heavy metals through epithelial or mucosal surface of the skin, gills and gastrointestinal tract (Jovanovic *et al.*, 2011). Fish in the River Nile was used as a biological marker for its pollution. The impact of heavy metals on fish affects directly or indirectly human health (NWQCU, 1995). *Oreochromis niloticus* is one of the aquatic organisms affected by heavy metals, so it was frequently used as a metal biological marker in toxicological studies (Rashed, 2001 a&b). Studies from the field and laboratory experiments showed that accumulation of heavy metals in fish tissues is mainly dependent on concentrations of metals in water and exposure period. The bioaccumulation of heavy metals in the different fish tissues had been studied by several investigators (Gad and Yacoub 2009; Cao *et al.*, 2010; Malik *et al.*, 2010; Jovanovic *et al.*, 2011; Ebrahim and Taherianfard, 2011). Biochemical profile in fish has proved to be a sensitive index for evaluation of the fish metabolism under metallic stress. Studies proved that, fish subjected to metals showed reduced levels of proteins, lipids, ALT and AST activities in the muscles (Almeida *et al.*, 2001; Atli and Canli, 2007; Mohamed and Gad, 2008).

The aim of the present study was to investigate the distribution of some heavy metals (Cu, Mn, Pb and Zn) in certain tissues (gills, intestine and muscles) of *O. niloticus*

inhabiting seven locations in the River Nile during winter and summer seasons (2009). Also the effect of the pollution of the River Nile on some biochemical parameters and enzyme activities in the muscles of fish were studied.

## 2. MATERIAL AND METHODS

### 2.1 Sampling:

Samples of *Oreochromis niloticus* (L.) fish were collected during winter (Joinery) and summer (August) 2009 from seven stations along the main channel of the River Nile at Upper Egypt covering different environmental conditions. The sampling stations included Aswan reservoir, Chema, Com Ombo, Naga Hammadi, Qus, El Menia and El Hawamdia. The weights of the fish samples ranged from 77 to 125g and the lengths from 15 to 21.5 cm.

### 2.2 Determination of heavy metals

A representative sample of 1g dry weight of each tissue (gills, intestine and muscles) was taken from 8 fish specimens of *Oreochromis niloticus*. The samples were digested according to the method described by Goldberg *et al.* (1993) in which concentrated nitric and perchloric acids with ratios of 5ml + 5ml were used in Teflon beakers on a hot plate at 50 °C for about 5 hours till complete decomposition of organic matter. The digested solutions were cooled to room temperature, filtered and diluted to a final volume of 50 ml using deionized distilled water. The concentrations of copper, manganese, lead and zinc in the gills, intestine and muscles of *O. niloticus* were measured by SHIMADZU atomic absorption spectrophotometer model AA-6800 equipped with Flame Unit and auto-sampler SHIMADZU ASC-6100 in Mediterranean Sea branch of National Institute of Oceanography & Fisheries. Results were expressed in mg/Kg dry weight of the tissue.

### 2.3 Biochemical analysis:

Samples from muscles of the studied fish were subjected for calorimetric analysis of total proteins content (Henry, 1964) (SPECTRUM kit, Egypt), total lipids

(Knight *et al.*, 1972). ALT and AST activities were calorimetrically assayed following the method of Reitman and Frankel (1957), using SPECTRUM kit.

**2.4 Statistical analysis:** The data were computed, expressed as means  $\pm$  standard error and statistically analyzed (Snedecor, 1962).

**3. RESULTS**

**3.1 Metals Concentration in fish Tissues**

Tables (1-4) show the concentrations of the analyzed metals in the tissues (gills, intestine and muscles) of *O. niloticus* from seven

stations of the River Nile during winter and summer seasons 2009. The lowest accumulation levels of the tested metals were recorded in the muscle, while the highest ones were recorded in the intestine.

**3.2 Copper (Cu):**

Table (1) shows the concentrations of Cu in the studied tissues of *O. niloticus* from the seven stations of River Nile. Cu accumulation in the studied tissues of fish followed the order: intestine>gills>muscles for all stations in winter and summer seasons. The highest level of Cu in different fish organs was recorded in winter season.

Table 1: Concentrations of copper (mg/kg dry wt.) in different organs of *O. niloticus* collected from River Nile during summer and winter (means  $\pm$ SD)

Stations	Gills		Intestine		Muscles	
	1	2	1	2	1	2
Aswan Reservoir	4.0 $\pm$ 0.01	5 $\pm$ 0.03.	7.3 $\pm$ 0.01	9.7 $\pm$ 0.03	2.1 $\pm$ 0.01	14.8 $\pm$ 0.03
Chema	10. $\pm$ 0.03	11. $\pm$ 0.04	19 $\pm$ 0.01	35.2 $\pm$ 0.04	2.3 $\pm$ 0.02	4.0 $\pm$ 0.01
Com Ombo	5.4 $\pm$ 0.03	6.3 $\pm$ 0.02	27 $\pm$ 0.04	28 $\pm$ 0.02	3.2 $\pm$ 0.01	4.5 $\pm$ 0.04
Naga Hammadi	5.2 $\pm$ 0.02	6.7 $\pm$ 0.03	30.6 $\pm$ 0.03	26 $\pm$ 0.03	3 $\pm$ 0.01	3.7 $\pm$ 0.02
Qus	8.3 $\pm$ 0.04	9.6 $\pm$ 0.02	30 $\pm$ 0.05	74 $\pm$ 0.02	3.8 $\pm$ 0.04	5.4 $\pm$ 0.01
El-Menia	4.9 $\pm$ 0.01	5.5 $\pm$ 0.01	17 $\pm$ 0.04	23.6 $\pm$ 0.01	3.9 $\pm$ 0.01	4.7 $\pm$ 0.03
ElHawamdia	6.8 $\pm$ 0.03	8.0 $\pm$ 0.01	13 $\pm$ 0.01	20 $\pm$ 0.01	3.0 $\pm$ 0.02	3.8 $\pm$ 0.01

1-summer samples      2- winter samples

**3.3 Manganese (Mn)**

Table (2) shows the concentrations of Mn in the studied tissues of *O. niloticus*. Mn accumulation in tissues of fish was in the following order: intestine>gills>muscles for

all studied stations in winter and summer seasons. The highest accumulation of Mn in different fish organs was recorded in summer season.

Table 2: Concentrations of manganese (mg/kg dry wt.) in different organs of *O. niloticus* collected from River Nile during summer and winter (means  $\pm$ SD)

Stations	Gills		Intestine		Muscles	
	1	2	1	2	1	2
Aswan Reservoir	32.1 $\pm$ 1.0	6 $\pm$ 3.0	19.3 $\pm$ 5	10 $\pm$ 0.3	9.9 $\pm$ 0.1	2.8 $\pm$ 0.7
Chema	86.7 $\pm$ 0.3	8.5 $\pm$ 4.5	316 $\pm$ 6	25.2 $\pm$ 44	38 $\pm$ 2.9	34 $\pm$ 0.5
Com Ombo	111 $\pm$ 13.0	53 $\pm$ 2.9	549 $\pm$ 24	165 $\pm$ 21	22 $\pm$ 0.1	3.8 $\pm$ 0.9
Naga Hammadi	54 $\pm$ 2.0	50 $\pm$ 3.6	371 $\pm$ 33	235 $\pm$ 16	13 $\pm$ 0.6	6.7 $\pm$ 0.7
Qus	127 $\pm$ 14.0	67 $\pm$ 11	729 $\pm$ 51	336 $\pm$ 25	33 $\pm$ 0.64	23 $\pm$ 0.9
El-Menia	61.3 $\pm$ 12	61 $\pm$ 6.0	710 $\pm$ 34	288 $\pm$ 5.0	30 $\pm$ 0.21	12 $\pm$ 0.4
El Hawamdia	114 $\pm$ 3.9	32 $\pm$ 8	290 $\pm$ 14	90 $\pm$ 7.0	12 $\pm$ 0.72	7.8 $\pm$ 0.21

1-summer samples      2- winter samples

**3.4 Lead (Pb)**

Table (3) shows the concentrations of Pb in the studied tissues of *O. niloticus*. The concentrations of Pb followed the order:

intestine>gills>muscles in all stations during winter and summer seasons for all studied stations.

Table 3: Concentrations of lead (mg/kg dry wt.) in different organs of *O. niloticus* collected from River Nile during summer and winter (means  $\pm$ SD)

Stations	Gills		Intestine		Muscles	
	1	2	1	2	1	2
Aswan Reservoir	13.8 $\pm$ 0.1	10 $\pm$ 0.3	7 $\pm$ 0.1	12 $\pm$ 0.3	11.5 $\pm$ 0.1	9.7 $\pm$ 0.3
Chema	22.8 $\pm$ 0.3	25 $\pm$ 0.4	17 $\pm$ 0.8	13.2 $\pm$ 0.4	14 $\pm$ 0.2	13 $\pm$ 0.1
Com Ombo	15 $\pm$ 0.3	15 $\pm$ 0.2	14 $\pm$ 0.3	18 $\pm$ 0.2	20 $\pm$ 0.1	12 $\pm$ 0.4
Naga Hammadi	21 $\pm$ 0.2	12 $\pm$ 0.3	12.6 $\pm$ 0.4	17 $\pm$ 0.3	19 $\pm$ 0.1	9 $\pm$ 0.2
Qus	21 $\pm$ 0.4	16 $\pm$ 0.2	22 $\pm$ 0.5	42 $\pm$ 0.2	18 $\pm$ 0.4	9 $\pm$ 0.1
El-Menia	22 $\pm$ 0.1	15 $\pm$ 0.1	21 $\pm$ 0.4	13.6 $\pm$ 0.1	17 $\pm$ 0.1	11 $\pm$ 0.3
ElHawamdia	20 $\pm$ 0.3	13 $\pm$ 0.1	22 $\pm$ 0.7	14 $\pm$ 0.1	19 $\pm$ 0.2	14 $\pm$ 0.1

1-summer samples 2- winter samples

**3.5 Zinc (Zn)**

In Table (4) the concentrations of Zn in the studied tissues of *O. niloticus* showed the following order: intestine > gills > muscles

in all studied stations except in station (4) where, the concentrations of Pb followed the order: gills > intestine > muscle.

Table 4: Concentrations of zinc (mg/kg dry wt.) in different organs of *O. niloticus* collected from River Nile during summer and winter (means  $\pm$ SD)

Stations	Gills		Intestine		Muscles	
	1	2	1	2	1	2
Aswan Reservoir	62.8 $\pm$ 2.0	13 $\pm$ 3.0	30 $\pm$ 1.8	22 $\pm$ 3.0	44 $\pm$ 1	45 $\pm$ 3.8
Chema	72 $\pm$ 3.0	68 $\pm$ 4.5	94 $\pm$ 8.1	87.2 $\pm$ 4.4	70 $\pm$ 2	62 $\pm$ 1.3
Com Ombo	81 $\pm$ 3.8	13 $\pm$ 2.2	89 $\pm$ 3.4	47 $\pm$ 2.5	53 $\pm$ 1.5	11 $\pm$ 4.0
Naga Hammadi	84 $\pm$ 2.6	59 $\pm$ 3.6	70.6 $\pm$ 3.5	78 $\pm$ 3.2	66 $\pm$ 1.7	32 $\pm$ 2.2
Qus	99 $\pm$ 6.0	76 $\pm$ 2.7	94 $\pm$ 5.6	122 $\pm$ 2.4	62 $\pm$ 4.3	37 $\pm$ 1.2
El-Menia	72 $\pm$ 5.0	52 $\pm$ 1.4	77 $\pm$ 4.6	63.6 $\pm$ 1.4	53 $\pm$ 1.2	62 $\pm$ 3.2
ElHawamdia	74 $\pm$ 3.0	25 $\pm$ 1.8	40 $\pm$ 7.1	22 $\pm$ 1.5	60 $\pm$ 2.2	18 $\pm$ 1.5

1-summer samples 2- winter samples

**3.6 Biochemical parameters:**

Table (5) presents the changes in total proteins and lipids contents in the

muscle of *O. niloticus* collected from the seven stations on the River Nile during winter and summer seasons.

Table 5: Total proteins and lipids contents in muscles of *Oreochromis niloticus* from River Nile (means  $\pm$ SD)

Stations	Total proteins		Total lipids	
	1	2	1	2
control	15.9 $\pm$ 0.07	16 $\pm$ 0.6	0.67 $\pm$ 1.8	0.70 $\pm$ 0.5
Aswan reservoir	13.1 $\pm$ 0.05 (-16.9)	14 $\pm$ 3.2 (-12.5)	0.82 $\pm$ 6.3 (-22.3)	0.79 $\pm$ 0.06 (+12.8)
Chema	9.6 $\pm$ 0.04 (-39.6)*	8 $\pm$ 0.03 (-50.0)**	0.51 $\pm$ 0.02 (-23.8)	0.50 $\pm$ 0.04 (-28)*
Com Ombo	7.4 $\pm$ 0.03 (-53.4)**	6.0 $\pm$ 0.02 (-62.5)**	0.64 $\pm$ 3.4 (-4.4)	0.45 $\pm$ 0.06 (-35.7)*
Naga Hammadi	7.8 $\pm$ 0.02 (-50.9)**	7.5 $\pm$ 0.03 (-53.1)**	0.53 $\pm$ 0.05 (-20.8)	0.46 $\pm$ 0.04 (-34.2)*
Qus	8.1 $\pm$ 0.04 (-49.0)**	10.1 $\pm$ 0.02 (-37.5)*	0.8 $\pm$ 0.05 (+19.4)	0.82 $\pm$ 0.05 (-14.28)
El-Menia	9.6 $\pm$ 0.06 (-39.6)*	4.2 $\pm$ 0.05 (-73.7)**	0.86 $\pm$ 0.07 (-4.0)	0.32 $\pm$ 0.06 (-54.2)**
ElHawamdia	8.5 $\pm$ 0.06 (-46.5)**	6.3 $\pm$ 0.04 (60.6)**	0.62 $\pm$ 0.01 (-5.0)	0.55 $\pm$ 0.03 (-21.4)

Data are represented as means  $\pm$ S.E of 6 fish

\*Significantly different from control (P $\leq$ 0.05)

\*\*Highly significant different from control (P $\leq$ 0.01)

Data in parentheses are percent of change from control

1-summer samples 2- winter samples

The results indicated that there was a highly significant ( $P \leq 0.01$ ) decrease in the total proteins in muscle of the studied fish compared to the control except in station (1) where, there was a decrease in total proteins in muscles of *O. niloticus* but not statically significant during summer season. On the other, hand there was a highly significant decrease in the total lipids in the muscles of the studied fish during winter season ( $P \leq 0.01$ ).

Table (6) presents the changes in the activities of ALT and AST in the muscle of *O. niloticus* collected from River Nile during winter and summer seasons. The results indicated that there was a highly significant ( $P \leq 0.01$ ) decrease in the activities of ALT and AST in the muscle of the studied fish compared to the control during winter season except in station (1). In summer season the activities of ALT and AST were decreased but statistically not significant.

Table 6: Alanine amino transferase (ALT) and aspartateaminotranseferase (AST) in muscles of *Oreochromis niloticus* from River Nile (means $\pm$ SD)

Stations	ALT		AST	
	1	2	1	2
control	15.7 $\pm$ 0.2	15 $\pm$ 0.2	195 $\pm$ 8	190 $\pm$ 9
Aswan reservoir	15 $\pm$ 0.21 (-3.1)	14 $\pm$ 0.01 (-6.6)	185 $\pm$ 5.5 (-5.1)	93 $\pm$ 0.06 (-51.0)**
Chema	10. $\pm$ 0.04 (-33.7)*	8.5 $\pm$ 0.01 (-42.0)*	124 $\pm$ 3.4 (-36.4)*	119 $\pm$ 5.4 (-37.3)*
Com Ombo	11 $\pm$ 0.02 (-28.4)	8.2 $\pm$ 0.02 (-45.3)**	122 $\pm$ 5 (-37.4)*	97 $\pm$ 6 (-48.9)*
Naga Hammadi	11.6 $\pm$ 0.02 (-29.9)*	5.2 $\pm$ .31 (-65.3)**	148 $\pm$ 8 (-24.1)	66 $\pm$ 3.4 (-65.2)**
Qus	11 $\pm$ 0.02 (-29.0)*	8 $\pm$ 0.04 (-46.6)*	136 $\pm$ 0.05 (-30.2)*	67 $\pm$ 5 (-63.6)**
El-Menia	11 $\pm$ 0.05 (-29.6)*	6.0 $\pm$ 0.04 (-54.7)**	145 $\pm$ 3.5 (-25.6)	65.4 $\pm$ 8 (-65.7)**
ElHawamdia	11.8 $\pm$ 0.05 (-25.4)	9.1 $\pm$ 0.32 (-39.3)*	147 $\pm$ 7.2 (-24.6)	71 $\pm$ 6.8 (-62.6)**

Data are represented as means  $\pm$  S.E of 6 fish  
 \*Significantly different from control ( $P \leq 0.05$ )  
 \*\*Highly significant different from control ( $P \leq 0.01$ )  
 Data in parentheses are percent of change from control  
 1-summer samples 2- winter samples

**4. DISCUSSION**

Fish are notorious for their ability to concentrate heavy metals in their tissues .The metals exist most probably as cationic complexes and accumulate in the internal organs of fish.

Copper is commonly a natural element in water and sediment. The metal is insoluble in water, but many of its salts are highly soluble. Copper is a fundamental micronutrient to all forms of life, in enzyme activity and random rearrangement of natural proteins (Bower, 1979). At slightly higher

but sublethal concentrations, it causes chronic toxicity to aquatic life. Gainey and Kenyom (1990) mentioned that exposure of fishes to sublethal concentrations of copper leads to cardiac activity and reduction in heart rate. Dietary Cu level of 20 mg/Kg significantly reduced the weight gain of growing tilapia (Shiau and Nig, 2003).Chronic toxic effects may induce poor growth, decreased immune response, shortened life span, reproductive problems, low fertility and changes in appearance and behavior (Choongo *et al.*, 2005).

In the present study the concentrations of copper were measured in the gills, intestine and muscles of *Oreochromis niloticus* fish from seven stations in the River Nile. The mean values of copper in the fish organs followed the order: intestine > gills > muscles. The mean values of copper in winter were higher than in summer in all the studied fish organs (Table 1). This may be attributed to the drought period (winter blockage) which causes the metal salts to concentrate in water and ultimately accumulate in fish.

In the present study, the concentrations of Cu in the intestine of *O. niloticus* were higher than the standard concentration, however its concentrations in the gills and muscles of the studied fish were lower than the permissible limit of NHMRC. Gills accumulated lesser copper than intestine, indicating that the food is the primary pathway for uptake of copper. Similar results were observed in different fish species from El Max Bay Alexandria (Khaled, 2004). In the present study the lowest concentrations of Cu were observed in the muscles of studied fish, similar to results recorded by Khaled, (2004), Abdo & Yacoub (2005) and Yacoub (2007).

Manganese is an essential constituent for bone structure, reproduction and normal functioning of the enzyme system (Fleck, 1976). It is toxic only when present in higher amounts, but at low levels is considered as micronutrient (Sarkkaet *al.*, 1978).

In the present study the mean values of manganese in intestine were higher than those in gills and muscles (Table 2). Values of manganese in the fish organs were higher in summer. Rising water temperature results in increasing gills ventilation rate due to higher oxygen demand for metabolic requirements and decreased oxygen concentration in the water (Joyeux *et al.*, 2004) leading to higher volume of water passing through the gills and increased uptake of metals from the water. During winter, elimination exceeds the uptake of metals, leading to decreasing metal concentrations (EL Bialy *et al.*, 2005).

Lead is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. It is toxic even at low concentrations and has no known function in biochemical processes (Burden *et al.*, 1998). It is known to inhibit active transport mechanisms, involving ATP, to depress cellular oxidation reduction reactions and to inhibit protein synthesis (Waldron and Stofen, 1974). Lead was found to inhibit the impulse conductivity by inhibiting the activities of monoaminoxidase and acetylcholine esterase, to cause pathological changes in tissue and organs (Rubio *et al.*, 1991) and to impair the embryonic and larval development of fish species (Dave and Xiu, 1991).

The mean values of lead followed the same trend as copper: intestine > gills > muscles. Also, these values were higher in summer than in winter in all the fish organs.

The results showed that all the studied fish organs were heavily loaded with lead, whereas their levels were more than the permissible limits of FAO(1983) and Australian NHMRC (1987) (0.5 ug/g dry wt.) for the first and (0.2 ug/g dry wt.) for the second. The concentrations of Pb in intestine reflect their concentrations in fish food (phytoplankton) and their concentrations in gills reflect their concentrations in water (Romeo *et al.*, 1999). The high concentrations of Pb in muscles of *O. niloticus* leads to hazardous effects due to human consumption (Greenwood and Earnshaw, 1985). The high concentrations of Pb may be attributed to industrial wastes inflow and the use of leaded gasoline in boats.

Zinc is an essential element for normal growth, reproduction and longevity of animals (Sultana and Roa, 1998). Mining, smelting and sewage disposal are the major sources of zinc pollution (Skidmore, 1964).

The mean values of zinc in fish intestine were higher than those in gills and muscles. In summer, the mean values of zinc in the gills, intestine and muscles were higher than their values in winter.

The maximum permissible level (MPL) of zinc is 50 ug/g dry wt. according to Australian NHMRC (Bebbington *et al.*, 1977) and 40 ug/g dry wt. according to Food and Agriculture Organization (FAO, 1983). The mean values of zinc in the gills, intestine and muscles of *O. niloticus* were significantly higher than (MPL) except in fish muscles in winter. The concentrations of heavy metals (Cu, Mn, Pb and Zn) varied according to season, locality and tissue type (Yacoub, 2007). Metal accumulation in tissues of fish is dependent upon environmental factors such as temperature, size and age of fish and processes of biotransformation and excretion (Zhou *et al.*, 2001).

Studies proved that, fish subjected to metals showed reduced levels of proteins, lipids, ALT and AST activities in the muscles (Almeida *et al.*, 2001; Atli and Canli, 2007; Mohamed and Gad, 2008).

In various fish species, proteins are of importance as structural compounds, biocatalysts and hormones for control of growth and differentiations. So variation in fish proteins could be used as bioindicator for monitoring physiological status of the tested fish (Begum and vijayaraghavan 1996).

In the present study, the examined *O. niloticus* collected from different stations of the River Nile exhibited lowered protein value in muscles during winter season, which was suggested as a metabolic adaptation to food shortage in the environment (White *et al.*, 1986). During this period of inadequate food supply, energy required for metabolic maintenance may be provided from utilization of protein reserves which mainly accumulate in the muscle tissues (Haggag *et al.*, 1999). Besides, this protein depletion could be attributed to change in the water quality of River Nile as a result of the discharged effluents from different sources, including hydrocarbons found in sewage wastes and heavy metals in the industrial and agricultural drainage. (Zaghloul, 2000). This may be explained as the exposure to metals (as Cu and Zn) may lead to high

accumulation in gills that cause a structural damage and a reduction in oxygen consumption causing sharp reduction in the metabolic rate of fish and consequently decrease protein contents in tissues. Moreover, decreased tissues protein in fish living in polluted environment may be a result of decreased insulin level caused by metal toxicity (Zaghloul, 2001). Insulin is known to have profound effects on the proteogenic pathways in fish. It stimulates the inward cellular transport of amino acids, particularly in muscle, leading to intracellular accumulation of amino acid with subsequent decrease of protein contents (Reda *et al.*, 2002).

Concerning the effects of different pollutant in the River Nile on muscle lipids contents of the studied fish the obtained data showed highly significant decrease during winter season but in summer the decrease in total lipids in muscles was statistically non significant. The decrease in total lipids contents in muscle of the studied fish may be due to secretion of catecholamine and corticosteroids in the blood after the toxicant stress that produces an enhanced metabolic rate which in turn reduces the metabolic reserves (as proteins and lipids) (Marie, 1994). Also, the decrease in muscles total lipids may be due to the use of energy-rich lipids for energy production during toxic stress as previously reported by Sancho *et al.*, (1998). The decreased lipids content observed in the present investigation agrees with that recorded in muscle of fish exposed to different pollutants (Chandra *et al.*, 2004 and Blaner *et al.*, 2005).

The environmental pollution in the River Nile induced a marked decrease in the muscle ALT and AST activities. The results indicate that under the influence of different heavy metals or in a state of stress, the damage of muscle tissues may occur with concomitant liberation of transaminase into the circulation (Allen, 1995). The decreased activities of ALT and AST indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system (Humtsoe *et*

*al.*, 2007). Marie (1994) stated that the reduction in ALT and AST activities in fish exposed to metals could be attributed to the high accumulation of metals in fish tissues. Therefore, the depletion of enzymes in the fish tissues observed in the present study can be attributed to the increased metals accumulation in the tissues. The reduction in the muscle ALT and AST activities in the fish exposed to various pollutants and heavy metals, in particular have been reported by Rao (2006); Humtsoe *et al.* (2007) and Mohamed and Gad (2008).

In conclusion, the results of the present study revealed that the abundance of heavy metals in fish organs followed the order: Mn>Zn>Pb and Cu. The highest accumulation of the heavy metals was recorded in the intestine and the lowest values was recorded in the muscles. The concentrations of Cu in the fish muscle were below the maximum permissible limit, while Mn, Pb and Zn in the muscles exceeded the permissible limit. It was found that the environmental pollution in the River Nile induced significant changes in the proteins lipids and activities of ALT and AST of the muscles of the studied fish.

It is recommended that treatment of drainage industrial wastes water, sewage and agricultural wastes must be conducted before discharge into the River Nile besides washing of the river by elevation of water level once a year in the flood period. Also, enforcement of all articles of laws 48/ 1982 and 4/ 1994 regarding the protection of River Nile and the environment must be taken into consideration.

## 5. REFERENCES

- Abdo, M. H. and Yacoub, A. M. (2005). Determination of some heavy metals in water and fish flesh of common species in Bardawil Lagoon, Egypt. *Egypt. J. Anal. Chem.*, 14: 65-76.
- Allen, P. (1995). Accumulation profiles of lead and cadmium in the edible tissues of *Oreochromis aureus* during acute exposure. *J. Fish Biol.*, 47: 559
- Almeida, J.E.; Novelli, M. Dal Pai Silva and Junior, R. (2001). Environmental cadmium exposure and metabolic responses of the Nile tilapia *Oreochromis niloticus*. *Environ. Pollut.*, 114:169-175.
- Atli, G. and Canli, M. (2007). Enzymatic responses to metal exposures in a freshwater *Oreochromis niloticus*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 145:282-287.
- Australian NH and MRC (1987): Food Standards code – 1987. Australian Government Publishing Service, Canberra.
- Bebbington, G. N.; Mackey, N.J.; Chvoike, R.; William, R. J.; Dunn, A. and Auty, E. H. (1977). Heavy metals (selenium and arsenic) in nine species of Australian Commercial Fish. *Aust. J. Mar. Freshwater Res.*, 28: 277-280.
- Begum, G. and Vijayaraghavan, S. (1996). Alterations in protein metabolisms of muscle tissues in the fish *Clarias batrachus* by commercial grade Dimethioate. *Bullet. Environ. Contamination. Toxicol.*, 57: 223-228.
- Blaner, C.; Curitis, M. and Chan, H. (2005). Growth, nutritional composition and hematology of Artic chair (*Salvelinus alpinus*) exposed to toxaphene and tapeworm (*Diphyllobothrium dendritium*). *Archive of Environmental Contamination and Toxicology*, 48: 397-404.
- Bower, J. J. M. (1979). Environmental chemistry of the elements. Academic Press, London.
- Burden, V. M.; Sandheinrich, M. B. and Caldwell, C. A. (1998). Effects of lead on the growth and aminolvolulinic acid dehydratase activity of juvenile rainbow trout, *Oncorhynchus mykiss*. *Environ. Poll.*, 101: 285-289.
- Cao, L.; Huang, W.; Liu, J.; Yin, X. and Dou, S. (2010). Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure. *Comp. Biochem. Physiol. Toxicol. Pharmacol.*, 151(30):386-392
- Chandra, S.; R. Ram and Singh, I. (2004). First ovarian maturity and recovery response in common carp *Cyprinus carpio* after exposure to carbofuran. *Journal of Environmental Biology* 25:239-249.
- Choongo, K. C.; Syakalina, M. S. and Mwase, M. (2005). Coefficient of condition in relation to copper levels in muscle of *Serronochromis* fish and sediment from the Kafue River, Zambia. *Bull. Environ. Contam. Toxicol.*, 75: 645-651.



- Dave, G. and Xiu, R. (1991). Toxicity of mercury, copper, nickel, lead and cobalt to embryos and larvae of zebrafish *Brachydaniorerio*. Arch. Environ. Contam. Toxicol., 21: 126- 134.
- Ebrahim, M. and Taherianfard, M. (2011). The effects of heavy metals on reproductive system of cyprinid fish from Kor River. Iranian J. of Fish. Science. 10(1):13-24.
- El Bialy, A. B.; Hamed, S. S.; Mousa, W. M. and Abd El Hameed, R. K. (2005). Spectroscopic determination of some trace elements as pollutants in fish. Egypt. J. Solids, 281(1): 151- 161.
- FAO (1983). Compilation of legal limits for hazardous substances in fish and fishery products. FAO, Fishery circular. 464: 5- 100.
- Fleck, H. (1976). Introduction to Nutrition, 3<sup>rd</sup> edn. Mac Milan Publishing Co., Inc., New York, 552pp.
- Gad, N. S. and Yacoub, A. M. (2009). Antioxidant defense agents and physiological responses of fish to pollution of Rosetta Branch of the River Nile, Egypt. Egypt. J. Aquat. Biol. & Fish., 13(4): 109- 128.
- Gainey, L. F. and Kenyom, J. R. (1990). The effects of reserpine on copper induced cardiac inhibition in *Mytilusedulis*. COMP. Biochem. Physiol., 95(2): 177- 179.
- Goldberg, E. D.; Koide, M.; Hodge, V.; Flegel, A. R. and Martin, J. (1993). U. S. mussel watch: 1977-1978 results on trace metals and radionuclides. Estuar. Coastal Shelf Sci., 16: 69- 93.
- Greenwood, N. N. and Earnshaw, A. (1985). Chemistry of the elements. Pergaman Press Ltd., Headington Hill, Oxford OX3OBW.
- Haggag, A. M; Mohamed, A. S. and Zaghoul, K.H. (1999). Seasonal effects of industrial effects on the Nile cat fish *Clariasgaripepinus*. J. Egyptian Germany Soc. Zool. (Comparative physiology) 28: 365-391.
- Henry, R. J.(1964).Clinical chemistry (principles and techniques),Harper and Row, New York, pp:1128s
- Humtsoe, N.; Dawoodi, R.; Kulkarni, B. and Chavan, B. (2007). Effect of arsenic on the enzymes of the Rohu carp, *Labeorohita* (Hamilton, 1822).Raffles Bull Zool., 14: 17-19.
- Jovanovic B.; Mihaljev, E.; Maletin S. and Palic, D. (2011). Assessment of heavy metal load in chub liver (Cyprinida: *Leuciscuscephalus*) from the Nisava River (Serbia), Biologica Nyssana ., 2(1):1-7
- Joyeux, J. C.; Filho, E. A. C. and de Jesus, H. C. (2004). Trace metals contamination in estuarine fishes from Vitoria Bay, Es, Brazil. Brazil. Arch. Biol. Tech., 47(5): 765- 774.
- Khaled, A. (2004). Heavy metals concentrations in certain tissues of five commercially important fishes from El Max Bay, Alexandria, Egypt. Egypt. J. Aquatic Biol. & Fish., 8(1): 51- 64.
- Knight, A.; Anderson, S. and Rowle, J. M. (1972). Chemical basis of the sulfophosphovanillin reaction of estimating of total serum lipids. Clin. Chem., 18(3):199.
- Lamas,S., Fernandez, J.A., Aboal, J.R. and Carballeira, A.(2007). Testing the use of juvenile *Salmotrutta* L. as biomonitors of heavy metals pollution in fresh water, Chemosphere, 67(2):221-228.
- Malik,N.;Biswas,A.; Qureshi,T.;Borana ,K. and Virha ,R.(2010). Bioaccumulation of heavy metals in fish tissues of a freshwater Lake of Bhopal .Environ.Monit. Assesss ., 160(1-4): 267-276
- Marie, M. A. 1994.Toxic effects of aluminum on blood parameters and liver function of Nil cat fish *ClariasLazera* , J. Egyptian German SocZool ., 13:279-294.
- Mohamed ,F.A. and Gad , N. S. (2008). Environmental pollution induced biochemical changes in tissues of *Tilapia zillii*, *Solea vulgaris* and *Mugilcapitof* from Lake Qarun , Egypt .Global Veterinaria 2(6) : 327-336
- NWQCU (National Water Quality Conservation Unit) (1995). Assessment of water quality hazards in Egypt. 2<sup>nd</sup> Advisory Committee Workshop, National Water Quality C0nservation Program, 24- 25 March, 1995.
- NWRC (National Water Research Centre), WL/DELFT Hydrolics (2000). (National Water Resources Plan for Egypt, Water Quality and Pollution Control). Technical Report No. 5.
- Rashed, M. N. (2001a). Cadmium and lead levels in fish (*Tilapanilotica*) tissues as biological indicator for lake water pollution. Environ. Monit. Assess. 68: 75- 89.
- Rashed, M. N. (2001b). Monitoring of environmental heavy metals in fish from Nasser Lake. Environ. Inter. 27-33.
- Rao, J. (2006). Biochemical alterations in euryhaline fish, *Oreochromis mosambicus* exposed to sublethal concentrations of

- organo-phosphorus insecticides monocrotophos. *Chemosphere*, 65: 814-1820.
- Reitman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American J. Clin. Pathol.*, 28:56-63.
- Romeo, M.; Siau, Y.; Sidoumou, Z. and Gnassia-Barelli, M. (1999). Heavy metals distribution in different fish species from the Mauritania coast. *Sci. Total Environ.*, 232: 169- 175.
- Rubio, R.; Tineo, P.; Torreblanca, A.; Del-Romo, J. and Mayans, J. D. (1991). Histological and electron microscopical observations on the effects of lead on gills and midgut gland of *Procambarus clarkii*. *Toxicol. Environ. Chem.*, 31: 347- 352.
- Sancho, E. M.; Ferrando, C. Feranane and Andreu, E. (1998). Liver energy metabolisms of *Anguilla anguilla* after exposure to fenitration. *Eco-toxicol. Environ. Safety*, 41:168-175.
- Sarkka, J.; Hatulla, M. L.; Paasivirta, J. and Janatunem, J. (1978). Mercury and chlorinated hydrocarbons in food chain of Lake Paynma, Finland. *Holarctic Ecol.*, 1: 326- 332.
- Shiau, S. Y. and Nig, Y. C. (2003). Estimation of dietary copper requirements of juvenile tilapia, *Oreochromis niloticus* and *O. aureus*. *Anim. Sci.*, 77: 287- 292.
- Skidmore, J. T. (1964). Toxicity of zinc compounds to aquatic animals with special references to fish. *Quart. Rev. Biol.*, 39(3): 227- 248.
- Snedecor, G. W. (1962). *Statistical methods* 5<sup>th</sup> Edn. Iowa University Press. Ames, Iowa, USA.
- Sultana, R. and Roa, D. P. (1998): Bioaccumulation patterns of zinc, copper, lead and cadmium in grey mullet, *Mugilcephalus(L.)*, from Harbor waters of Visakhapatnam, India. *Bull. Environ. Contam. & Toxicol.*, 60: 949- 955.
- Waldron, H. A. and Stofen, S. (1974): *Subclinical Lead Poisoning*. Academic Press, New York, 1- 224.
- White, A.; T. C. Fletcher and Pope, J. A. (1986). Seasonal changes in serum lipid composition of the Plice *Pleuronectes spltesa* , L . *J. Fish Biol.*, 28: 595-606.
- Zaghloul, H. K. (2000). Effect of different water sources on some biological and biochemical aspect of the Nile tilapia *Oreochromis niloticus* and Nile cat fish *Clarias gariepinus*. *Egyptian J. Zool.*, 34:353-377.
- Zaghloul, H. K. (2001). Usage of zinc and calcium in inhibiting the toxic effect of copper on the African cat fish *Clarias gariepinus*. *J. Egyptian Germany Soc. Zool. (Comparative physiology)* 35: 99-120.
- Yacoub, A. M. (2007). Study on some heavy metals accumulated in some organs of three River Nile fishes from Cairo and Kalubia governorates. *African J. Biol. Sci.*, 3(3): 9- 21.
- Zhou, J. L.; Salvador, S. M.; Liu, Y. P. and Sequeria, M. (2001). Heavy metals in the tissues of common dolphin (*Delphinus delphis*) stranded on the Portugese coast. *Sci. Tot. Environ.*, 273: 61- 67.