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Impact of Heavy Metals on Physiological and Histopathological Parameters in the Catfish *Clarias gariepinus* From Lake Maryout, Alexandria, Egypt

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ABSTRACT
This study was carried out to assess the accumulation and distribution of heavy metals (Pb, Hg, Cd, and As) in Lake Maryout. The impact of heavy metal contaminations was also assessed in different target tissues (gills, liver, kidney, and muscles) of *Clarias gariepinus* (*C. gariepinus*) using physiological and histopathological parameters in the Main Basin and a relatively clean area (South-west Basin). Heavy metals concentration in water showed that Main Basin had the highest levels of Pb, Hg, As and Cd metals. The abundance of heavy metals in different fish organs; gills, livers, kidney, and muscles followed the order: Pb> Cd >As >Hg. Fish muscle compared to other tissues; usually, contain the lowest level of metals. Physiological alterations showed a significant severe anaemia, leukocytosis, neutrophilia, lymphopenia, and monocytosis was detected in fish collected from Main Basin in comparison to South-west Basin. Moreover, a significant increase in serum ALT, AST and creatinine were observed, while serum urea showed insignificant decreased in fish collected from Main Basin in comparison to South-west Basin. Histopathological alterations of gills showed lamellar fusion and hypertrophy of epithelial cells. Liver samples included necrosis of hepatocytes. Finally, kidney pathologies demonstrated the glomeruli appeared shrunken, desquamated, and vacuolated and often destroyed tubular epithelium. The mean fish intake of adults provides exposure to metals that fall within the accepted standards for the safe intake. It is recommended that the situation need a scientific method of detoxification to improve the health of this Lake and economic fish.

INTRODUCTION

Lake Maryout is highly polluted among the Egyptian Lakes (Arafa and Ali, 2008). It is situated south to the city of Alexandria and separated from the Mediterranean Sea by the narrow isthmus on which the city of Alexandria was built. It is a hypertrophic Lake which is heavily polluted with industrial, domestic and agricultural untreated discharges (El-Bestawy. 2014). Adham *et al*. (2001) reported that the Lake area was considerably reduced from 243 km^2 (60 000 acres) to 69 km^2 (17 000 acres) at present during the last 50 years due to these several urbanization and reclamation schemes. The leftover area was again split apart into five principal Basins. The presence of heavy metals in the environment is consequently accumulated in fish tissues due to metabolic activities and bio-absorption process (Ahmed *et al*., 2012).

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Fish may concentrate large amounts of some metals from the water and transfer throughout the web chain into the human (Abdel-Mohsien and Mahmoud, 2015).

The effects of Pd, Hg, Cd and Co concentrations in water and tissues of *Oreochromis niloticus* were reported from some Egyptian fish farms by Kaoud and El-Dahshan (2010). These effects include increases of Pb, Cd and Co concentrations in liver and decrease in kidney tissue. Meanwhile, the Hg concentrations were increases in muscles and decreases in kidney tissue.

Also, Siraj *et al*. (2014) showed the effects of Pb, Zn, Cu, Ni and Cr in the liver, muscle, gills, intestine, and skin of two freshwater species of *Aorichthys seenghala* (carnivorous) *Ompok bimaculatous* (omnivorous). They demonstrated the highest concentrations of heavy metals were recorded in Omnivorous fish, *Ompok bimaculatous* than carnivorous fish, *Aorichthys seenghala*. Moreover, Fábio *et al*. (2016) showed increases in the concentration of Pb, Zn, Cd, Hg and Cr in muscle, liver and spleen tissues of a large commercially valuable catfish species from Brazil.

Accumulation of metals may lead to a high mortality rate or cause many haematological, biochemical (Mahmoud *et al*., 2013) and histological alterations in the survived fish (Abdel-Baki *et al*., 2011). These studies are being used as indicators in the measurement of health conditions and toxicological symptoms of organisms and abnormal environmental conditions (Shivakumar *et al*., 2014). Abdel-Moneium *et al*. (2008) studied the haematological, biochemical and histopathological changes in Catfish *C. gariepineus* exposed to dyestuff and chemical wastewater showed that stressed fish exhibited a severe anaemia and increased in leucocytes count. Moreover, Al-Balawi *et al*. (2013) showed necrosis and degeneration in the kidney, liver, gills, and muscle of *C. gariepinus* after the exposure to lead acetate as histopathological alterations.

The objective of this study was to determine some physiological and histopathological alterations in *C. gariepinus* collected from different locations from Lake Maryout. Accordingly, this will clarify the potential of public health risk that could be associated with the current dietary intake of fish.

MATERIALS AND METHODS

Sampling and test animals:

During (May) 2017, fifteen specimens of Catfish *Clarias gariepinus* were collected alive from various fishermen from a polluted location Main Basin and a relatively clean area South-west Basin in Lake Maryout. The fish sizes were (23.0 ± 5.0 cm) and an average weights of (115.0 ± 5.0gm). Fish were trapped in closed meshed nets, maintained alive and quickly transported to the laboratory At the Physiology lab of the National Institute of Oceanography and Fisheries in large vessels filled with aerated Lake Water. Tanks were maintained within an appropriate range of temperature (22.0°C±1.0°C), pH (8.0±0.1), color (dark brown), hardness (49.0-63.0 NTU), and alkalinity (6.0-7.0 mg $CaCO_3$/L).

Determination of heavy metals in Lake Water:

Concentrations of heavy metals were measured in water according to APHA (2005).

Determination of heavy metals in tissue samples:

Concentrations of heavy metals were measured according to Finerty *et al*. (1990). The concentrations of (Pb, Hg, Cd and As) in the gills, livers, kidney and muscles were measured by Perkin Elmer 3110 Atomic Absorption Spectrophotometer and the results were expressed in (μg/g) of the dry' weight of the tissue, while blood samples measured according to Selander and Cramer (I968).

Haematological Examinations: These examinations were measured according to Dacie and Lewis (1975). Blood samples were taken from the caudal vein. Haematological parameters consist of the total number of erythrocytes, the packed-cell volume (%), and the haemoglobin concentration (g/dL). The erythrocyte indices including the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration were examined. Also, the total number of leukocytes was counted and a differential white blood cell count was performed according to Dacie and Lewis (1975).

Human risk assessment: The human risk assessment has been estimated according to (Joint FAO/WHO Expert Committee on Food Additives, 2004). Weakly exposure to given heavy metal was determined according to this formula:

Weakly exposure to a given heavy metal = concentration of heavy metal in fish (µg/g wet weight) x mean fish intake/week (g/person/week).

According to GAFRD (2015), the mean consumption of fish/capita = 351 g/w.

Biochemical Determination: Serum liver function enzymes were determined according to Reitman and Frankel (1957). Serum kidney function parameters were determined according to Henry *et al* (1974). Meanwhile, Urea was determined according to Tietz (1995).

Histopathological Examinations: The light microscopic observations were recorded according to Bancroft and Gamble (2002).

Statistical analysis: Data are expressed as means ± SD. The results were computed statistically (SPSS software package, version 20) using one-way analysis of variance (ANOVA). Means in a row with no common superscripts are significantly different (P<0.05).

RESULTS AND DISCUSSION

The Concentration of heavy metals in water:

Heavy metals concentrations in water collected from Main Basin and South-west Basin from Lake Maryout is shown in Table. 1. It was clear that the Main Basin had the highest levels of metals ranged from 2.00 to 4.60 mg/l while the ranges of metals in South-west Basin were 1.00 to 1.26 mg/l.

Moreover, the result of this study showed that Cd recorded the lowest concentration in South-west Basin (1.00 mg/l) while Pb was recording the highest concentration (4.60 mg/l) among the tested metals in the Main Basin. By comparing the levels of Cd with recommended levels of World Health Organization was 0.01 mg/l (WHO, 2003); the levels Cd appear higher than that in both basins. In comparison to The Egyptian Standards of the Environmental Laws no. 48/1982 and 4/1994 state that the permissible Pb concentration in water is 0.05 mg/l. This means that Pb concentration in the examined basin not allowable. The concentration of Pb is dependent on the natural organic matter content of the water and the absorption into the sediments as well as the alkalinity, hardness, and pH (Palaniappan, and Vijayasundaram, 2009). Adham (2002); Abdel-Moneim and Abdel- Mohsen (2010) reported that fish in Main Basin were more susceptible to stress which caused adverse effect resulting in retarded growth and impaired function of the liver, kidney, and heart of C. *gariepinus.*

Table 1: Heavy metals concentrations (mg/l) in water from south-west South-west Basin and Main Basin of Lake Maryout

Metals	South-west Basin	Main Basin
Lead (Pb)	1.26 ± 0.3[a]	4.60 ± 0.14 [b]
Mercury (Hg)	1.24 ± 0.2[a]	2.00. ± 0.5[b]
Cadmium (Cd)	1.00 ± 0.5 [a]	4.25 ± 0.1[b]
Arsenic (As)	1.25 ± 0.3[a]	2.25 ±0.5[b]

Values are expressed as mean ± SD, for five water samples in each group, means in a row with no common superscripts are significantly different (p<0.05)

Accumulation of heavy metals in fish organs:

In this study, the concentrations of heavy metals in different organs of C. *gariepinus* collected from Main Basin and South-west Basin are given in Table 2.

Table 2: Metal concentration (µg/ gm) in different organs of *Clarias gariepinus* from south-west and main Basin of Lake Maryout

Organs	Pb south-west	Pb main Basin	Hg south-west	Hg main Basin	Cd south-west	Cd Main Basin	As south-west	As main Basin
Gills								
Group mean ± SD	2.43 ± 0.4[a]	4.76 ± 0.9 [b]	0.93 ± 0.1[a]	0.9 ± 0.53 [b]	1.92 ± 0.3[a]	3.31 ± 0.7 [b]	0.63 ± 0.1[a]	1.10 ± 0.35 [b]
Maximum value	2.30	6.20	0.8	3.10	2.1	4.4	0.6	1.9
Minimum value	1.95	4.40	0.7	1.80	1.3	2.5	0.4	0.9
Blood								
Group mean ± SD	0.70 ± 0.19[a]	2.06 ± 0.8 [b]	0.30 ± 0.07[a]	0.64 ± 0.2 [b]	0.70± 0.18[a]	2.56 ± 0.71 [b]	0.40 ± 0.15[a]	0.7 ± 0. 18 [b]
Maximum value	0.9	3.9	0.9	3.9	0.9	3.9	0.9	3.9
Minimum value	0.4	1.9	0.4	1.9	0.4	1.9	0.4	1.9
Liver	0.86 ± 0.2[a]	*2.35* ± 0.81[b]	0.32 ± 0.06[a]	*0.8*5 ± 0.1[b]				
Group mean ± SD	1.1	3.4	0.2	0.7	1.90 ± 0.3[a]	*2.91* ± 0.3[b]	0. 62± 0.17[a]	*1.04* ± 0.13[b]
Maximum value	0.5	1.9	0.3	0.3	2.3	3.4	0.6	3.4
Minimum value					1.5	2.5	0.4	1.9
kidney				2.5 ± 0.9 [b]	0.78 ± 0.3[a]	2.55 ± 0.9 [b]	0.7 ± 0.1[a]	1.44 ± 0.2 [b]
Group mean ± SD	0.58 ± 0.1[a]	1.64 ± 0.51 [b]	1.99 ± 0.3[a]	0.2	2.4	3.5	0.9	1.3
Maximum value	0.7	2.3	1.9	0.1	1.6	1.4	0.4	0.9
Minimum value	0.4	1.4	0.8					
Muscle								
Group mean ± SD	0.25± 0.05[a]	0.8± 0.3 [b]	0.2± 0.07[a]	0.54± 0.14 [b]	0.79± 0.2[a]	1.90± 0.28 [b]	0.47± 0.09[a]	1.00± 0.2 [b]
Maximum value	0.2	1.3	0.3	0.7	0.7	2.3	0.4	1.5
Minimum value	0.1	0.9	0.1	0.3	0.5	1.6	0.3	0.9

Values are expressed as mean ± SD, for five animals in each group, means in a row with no common superscripts are significantly different (p<0.05)

Lead (Pb):

The results indicated that the mean Pb concentrations in organs showed a highly significant increase (p<0.05) of the studied fish in Main Basin compared to South-west Basin ranged between (0.80 to 4.76 µg/gm) and (0.25 to 2.43 µg/gm) respectively. Lead accumulations in the studied organs were recorced in the following order: muscle< kidney< liver< blood<gills in the two Basins. Gill tissues are considered as the main site for heavy metal uptake and its excessive intake can easily become the cause of fish death by causing the precipitation of mucous on the gill surface membrane (Siraj *et al.*, 2014). Fish muscles accumulated from 0.25to1.9 µg/gm compared to the other tissues from 0.3to 4.76 µg/gm; usually contain the lowest levels of lead. These results were in agreement with Abumourad *et al.* (2013) who recorded that the exposure to Pb may lead to high accumulation in the gills of Tilapia from three different Egyptian fish farms: Kafr El- Al-Abbasa, Sheikh and El-Fayoum, that causes a structural damage and a sharp reduction in the metabolic rate of fish due to the decrease in oxygen consumption and consequently a reduction in protein contents in tissues. Abdel- Baki *et al.* (2011) showed that tilapia fish collected from Wadi Hanifah, Saudi Arabia were in the safety permissible level for the use of the human which disagreed with this study that showed Pb concentration in *C. gariepinus* tissues was exceeding the permissible limits according to WHO (2003) 2.00 µg/gm dry wt. It is in the unsafely permissible level for the use of the human.

Mercury (Hg):

The results showed that the concentration of Hg ranged between (0.20 to 1.99 µg/gm) in South-west Basin and (0.54to2.50 µg/gm) in Main Basin, respectively. These results recorded a significant increase ($p<0.05$) in the concentration of Main Basin. The concentrations of Hg in the studied organs were in the following order: muscles <liver<blood<gill<kidney in two Basins. It is well known that the kidney is the gateway for heavy metal detoxification in the body (Vinodhini and Narayanan, 2008). The obtained results were in agreement with Asante et al. (2014) who showed that the muscles of *Sarotherodon galilaeus* accumulated the lowest concentration of Hg while *Labeo senegalensis* were accumulated the highest concentration of the same metal.

Cadmium (Cd):

The results indicated that the mean Cd concentrations in organs from South-west Basin to Main Basin ranged between (0.70to1.92 µg/gm) and (1.90to3.31 µg/gm), respectively. The results showed significant increase ($p<0.05$) of the studied fish in Main Basin compared to South-west Basin. Cadmium accumulations in the studied organs were in the following order: muscle< kidney< liver< blood< gills for the studied fish in two Basins. Gill tissues play an important role in the interface with the environment in performing its functions in gas exchange, ion regulation, acid balance and waste excretion while the muscle is not an active tissue in bioaccumulation (Al-Balawi *et al.*, 2013). In this study, the muscles of C. *gariepinus* in South-west Basin contained the lowest concentrations of Cd (0.70 µg/gm dry wt.) followed by C. *gariepinus* in Main Basin (3.31 µg/gm dry wt.). These results were in agreement with Murtala *et al.* (2012) who examined *Hydrocynus forskahlii*, *Hyperopisus bebe occidentalis* and *Clarias gariepinus* organs collected from the downstream Ogun coastal water. In this study, the concentrations of Cd in the muscles of the studied fish from South-west Basin are still below WHO permissible level for Cd 2.0 mg/kg reported by FAO (2003) while the concentrations of Main Basin were higher than permissible levels.

Arsenic (As):

The results indicated that the mean As concentrations in organs from South-west Basin to Main Basin ranged between (0.40 to 0.70 µg/ gm) and (0.70 to 1.44 µg/ gm), respectively. The results showed a highly significant increase ($p<0.05$) of the studied fish in Main Basin compared to South-west Basin. Arsenic accumulations in the studied organs and blood were in the following order: blood< muscle < liver < gill < kidney for the studied fish in South-west Basin and Main Basin. The same results were recorded by Koch *et al.* (2001) demonstrated that total arsenic in freshwater fish ranged from (0.28 to 3.1ppm) for whitefish *Coregonus clupeaformis*, (0.98 to 1.24 ppm) for sucker *Catostomus commersoni*, (0.46 to 0.85 ppm) for walleye Stizostedion vitreum, and (1.30 to 1.40 ppm) for pike Esox Lucius. The records exceed the permissible limit recommended by FAO/WHO (2004) 2.0 mg/kg.

The enhancement of heavy metals may be due to the presence of industrial, sewage and agricultural discharge (Kaoud and El-Dahshan, 2010). Most of these metals accumulate mainly in kidney, gills and liver. Moreover, the present results indicated that the concentrations of heavy metals in the organs of the studied fish depended mainly on the metal, organ and species. This is in agreement with that reported by (Fábio *et al.*, 2016) in muscle, liver, and spleen tissues of large commercially valuable catfish species from Brazil after exposure to Hg , Cd, Zn, Cr, and Pb which pose a serious risk to ecosystems and human health.

Human risk assessments.

The mean weekly intake of Pb, Hg, Cd and As were 87.75, 70.20, 273.78 and 164.97µg/week, respectively for consuming fish from South-west Basin, while the weekly intake of these metals was 280.80, 189.54, 666.9, and 351.00 µg/week, respectively for consuming fish from the Main Basin. Therefore, the estimated intake of Pb, Hg, Cd and from weekly consumption of fish didn't pose a risk of adverse effects (Table 3). This result is in agreement with Shreadah *et al.* (2015) showed the concentration of Cd, Zn, Cu and Hg in different fish species *Sardinella, Pagellus, Solea vulgaris, Mugilcephilus, Scomber japonicas, Donax Sp and Moron labrox* collected from several locations along the Mediterranean coast of Egypt are much lower than PTWI values with no risk for the consumption of the human.

Table 3: Estimated dietary exposure of *Clarias gariepinus* – consuming adult's population to metals from Lake Maryout

Metal	Estimated dietary exposure (µg/w)		PTWI (µg/w)*
Pb	SW.B:	87.75	1750
	M.B:	280.80	
Hg	SW.B:	70.20	350
	M.B:	189.54	
Cd	SW.B:	273.78	490
	M.B:	666.9	
As	SW.B:	164.97	1050
	M.B:	351.00	

* A reference weight of 70 Kg is used by both the United States Food and Drug Administration

Haematological Studies

A highly significant ($p<0.05$) decrease in haemoglobin concentration (Hb) from 10.74 to 7.16 g /dL, red blood cell count (RBC) from 3.67 to 2.84 $10^6/mm^3$ and haematocrit value count (Hct %) from 36.8 to 29.4 % in fish collected from South-west Basin when compared to Main Basin. Also, a significant ($p<0.05$) decrease in mean cell volume (MCV) from 29.4 to 25.1 pg and mean cell haemoglobin (MCH) from 29.2 to 28.7% while mean cell haemoglobin concentration (MCHC) did not change in collected fish from different Basins (Table 4).

The same results were reported by Abdel-Moneium *et al.* (2008) showed that these results are symptoms of anaemia after exposure *C. gariepinus* to dyestuff and chemical wastewater or confirms the toxic impact of lead in *C. gariepinus* (Adeyemo, 2008). Senthamilselvan *et al.* (2012) supposed that cadmium and mercury might alter the properties of haemoglobin of *Lates calcarifer* by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more permeable and fragile, which might result in damage and cell swelling deformation.

Table 4: Changes in erythrocyte values in C. *gariepinus* from south-west and main Basin of Lake Maryout

Parameters	South-west Basin	Main Basin
Haemoglobin content (g/dL)	**$10.74±0.8^a$**	**$7.16±1.3^b$**
Red blood cell count ($10^6/mm^3$)	**$3.67±0.4^a$**	**$2.84±0.2^b$**
Haematocrit value (%)	**$36.8±10.3^a$**	**$29.4±3.1^b$**
Mean cell volume (fl)	**$100.5±4.6^a$**	**$87.3±7.5^b$**
Mean cell haemoglobin (Pg)	**$29. 4±1.7^a$**	**$25.1±3.4^b$**
Mean cell haemoglobin concentration (%)	**$29.2±0.8^a$**	**$28.7±2.3^a$**

Values are expressed as mean ± SD, (n=5) in each group. Means in a row with no common superscripts are significantly different ($P<0.05$).

These alterations may be due to direct or feedback responses of RBC membranes structural damage resulting in stress-related, release of RBC from the spleen, impairment and haemolysis in haemoglobin synthesis, and hypoxia, induced by exposure to lead (Shah 2006).

Leucocytes count showed highly significant increased from 30000.0 to 74400.0 $10^3/mm^3$ in fish collected from South-west Basin compared to Main Basin (Table 5). These results came in accordance with Mayilathal and Thamizhselv (2014) who recorded that there was a significant leucocytosis in the fish *C. gariepinus* exposed to lead poison. This increase in WBC may be attributed to lymphocytosis and immune response in lead-exposed fish (Altindag, 2005). Also, fish from Main Basin showed significant ($p<0.05$) elevation in the relative percentage of the circulating neutrophils and Stab forms. Furthermore, reading of the differential white blood cell count demonstrated a highly significant ($p<0.05$) lower percentage of lymphocytes in fish from Main Basin Moreover, monocytosis was increased at collected fish from Main Basin compared to South-west Basin. The eosinophils percentages did not reveal any significant changes in leucocytic parameters (Table 5). The decreases in the percentage of lymphocytes attributed to immunosuppression of fish stressed. So, the reduction in antibody titer also observed by Abdel-Moneium *et al.* (2008) in catfish *Clarias gariepineus* exposed to Dyestuff and Chemical wastewater. The concurrent stimulation of neutrophils and monocytes might be a response to the destruction of phagocytic cells by contaminants such as metals (Adeyemo, 2008).

Table 5: Changes in leucocytes values in C. *gariepinus* from south-west and main Basin of Lake Maryout

Parameters	South-west Basin	Main Basin
Total leucocyte count ($10^3/mm^3$)	**30000 ± 10025.0^a**	**74400.0 ± 14117.36519^b**
Neutrophils	**53.2 ± 3.2^a**	**57.6 ± 4.3^b**
Stab forms	**5.1 ± 1.3^a**	**7.0 ± 1.581139^b**
Eosinophils	**1.0 ± 0.7^a**	**1.0 ± 0.5^a**
Basophils	**0.00**	**0.00**
Lymphocytes	**37.0 ± 3.2^a**	**26.2 ± 3.701351^b**
Monocytes	**5.6 ± 1.5^a**	**8.8 ± 1.923538^b**

Values are expressed as mean ± SD, for five animals in each group, means in a row with no common superscripts are significantly different ($p<0.05$)

Biochemical parameters:

A significant ($p<0.05$) increase in ALT 70.80 and 48.60 U/ml and AST 173.60 and 123.20 U/ml in fish collected from South-west Basin to Main Basin, respectively (Table.6). Our result in accordance with Abdel-Moneim *et al.* (2008) who recorded increases in ALT and AST levels after 28 days of exposure of *C. gariepinus* to dyestuff and chemical effluent indicate liver damage. In the same aspect, Olojo *et al.* (2012) found increases in AST and ALT levels of *C. gariepinus* after exposure to lead. The increases in the activities of blood transaminase have been attributed to tissue damage, particularly the liver that increase of these enzyme activities is a sensitive indicator of even minor cellular damage (Palanivelu *et al.*, 2005).

Regarding kidney function test, creatinine significantly increased ($p<0.05$) from 0.22 mg/dL to 0.43 mg/dL in fish collected from South-west Basin to Main Basin, respectively (Table.6). Our result in accordance with Abdel-Moneium *et al.* (2008) who showed the increased serum creatinine of C. *gariepinus* after exposure to the dyestuff and chemical wastewater indicating kidney failure. In the same line, Mahmoud *et al.* (2013) recorded increased in the creatinine in *C. gariepinus* after exposure to lead. Similarly, Zaki *et al.* (2014) reported that long- term exposure of

C. gariepinus to metals caused gradual elevation of serum creatinine that might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrates metabolism. Urea insignificantly (p>0.05) decreased from 21.00 to 20.20 mg/dL in fish from South-west Basin and Main Basin, respectively (Table 6). National Kidney Foundation (2002) reported that creatinine was a more accurate marker of kidney disease than Urea.

Table 6: Changes in serum biochemical parameters indicative of liver and kidney functions in C. *gariepinus* from Lake Maryout:

Parameter	South-west Basin	Main Basin
ALT (U/mL)	**70.80±15.945[a]**	**173.60±57.91[b]**
AST (U/mL)	**48.60±11.19[a]**	**123.20±33.64[b]**
Urea (mg/dL)	**21.00±3.67[a]**	**20.20±6.69[a]**
Creatinine (mg/dL)	**0.22±0.16[a]**	**0.43±0.17[b]**

Values are expressed as mean ± SD, for five animals in each group, means in a row with no common superscripts are significantly different (p<0.05).

Histological examination

In this study, gill tissue of C. *gariepinus* collected from South-west Basin showed gill filaments had the almost normal architecture with two raw of secondary lamellae that were perpendicular to each filament which surrounded with blood vessels that darkly stained and sinusoidal space (Fig. 1). The normal architecture of gill filaments maintain the physiological functions such as the acid-base balance, Gas exchange, and osmoregulation occur in the secondary lamellae (Sayed *et al.*, 2012). Gills have enhanced the absorption of toxic chemicals by inhibition of ions exchange activity of the chloride cells and increasing the permeability to ions and water of gill epithelium (Mokhtarand Abd-Elhafeez, 2013). The slight hypertrophied epithelium of secondary lamellae and mild hypertrophy of interlamellar epithelium were recorded (Fig. 2). On the other hand, fish collected from Main Basin displayed hemorrhagic foci in the pillar cell system. Extensive aneurism (swollen lamellae packed with erythrocytes) and dilation of blood capillaries were observed (Fig. 4). Secondary lamellae were recorded necrotic changes with rupture in the epithelial cells of lamellae (Fig. 3). The fusion of the secondary lamellae commonly occurred along the entire length due to epithelial hyperplasia and hypertrophy as well as simple apposition (Fig. 4). These results came in accordance with Mohamed (2009) who showed edema in secondary lamellae, dilation, and congestion in blood vessels of gill filaments and mucous cells proliferation. The same results were recorded by Sayed *et al.* (2012) and Al-Balawi *et al.* (2013) in *Clarias gariepinus* exposed to 4-Nonylphenol and lead acetate, respectively.

The present microscopic picture of liver from South-west Basin showed less organized hepatic cord (Fig. 5) forming an irregular clump, and sinusoid filled with red blood cells. The liver from Main Basin showed hepatic cords disorganization with the destruction of the cell membrane, and extensive congestion of hepatocytes was observed. The cellular structure was totally obscured. Sinusoids with massive congestion. The liver showed a spongy appearance due to severe lesions (Fig.6). The same histological examinations were observed by Chavan and Muley (2014) in the liver of *Cirrhinus mrigala* which collected from Kalambe village reservoir near Kolhapur treated with mercury and lead. The sections showed damage of cellular architecture, destruction of erythrocytes in dilated blood vessels and haemolysis and vacuole appearance. The same results were reported in the liver of *Oreochromis niloticus* fish exposed to heavy metals (Kaoud and El-Dahshan, 2010).

In this study, the kidney from South-west Basin showed less deformed renal tubules in which the tubular segment renal corpuscles with well-developed glomeruli (Fig. 7). Healthy glomeruli maintains the homeostasis and receives the largest proportion of the post-brachial blood and therefore renal lesions might be good indicators of environmental pollution (Ebrahimi and Taherianfard, 2011). The Kidney from Main Basin was showing extensive damage of renal tubules and the glomeruli, which led to the occasional replacement by interstitial lymphoid tissue and the gradual disappearance of the renal tubules (Fig. 8). Shrinkage of The glomeruli and increased space in the Bowman's capsule followed by necrotic tubular cells includes complete lysis of the cytoplasm were recorded. So, the tubular system of the mesonephros incapable of functioning properly (Fig. 8). Our result in accordance with Sirimongkolvorakul *et al*. (2012) who showed cloudy swelling, tubular narrowing, and hyaline droplet. Also, vacuolar degeneration in the epithelium of renal tubules, focal areas of necrosis, haemorrhage between the renal tubules and edema in Bowman's capsules with atrophy in the glomeruli of *Puntius altus* exposed to lead. Also, in *cyprinid* fish collected from Kor River reported by Ebrahimi and Taherianfard (2011).

CONCLUSION

Although, the current study indicated that the main basin of lake maryout showed significant accumulation of heavy metals which in turn showed some physiological and histological alterations in the target tissues we studied, the estimated Pb,Hg,Cd and As weekly consumption was not considered risky for human.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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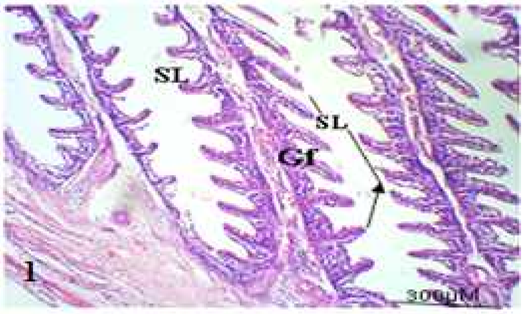

Fig. 1: Gill tissue of *C. gariepinus* from South-west Basin showed gill filament (GF), secondary lamellae (SL), interlamellar epithelium (ILE), surrounded with darkly stained blood vessels and sinusoidal space (10X H&E stains).

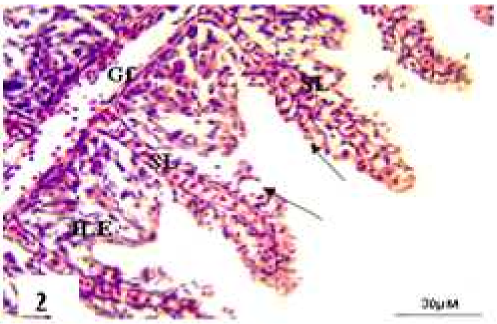

Fig. 2: Gill tissues of *C. gariepinus* from South-west Basin showed slight hypertrophied epithelium (arrows) of secondary lamellae (SL) with organized pillar cell system and mild hypertrophy of interlamellar epithelium (ILE) (40X H&E stains).

Fig. 3: Gill tissues of C. gariepinus from Main Basin showed lamellar aneurism (arrow) with clubbing at the tips of the secondary lamellae or grossly curled (blue arrow), Disappearance of the normal architecture by complete fusion of adjacent secondary lamellae (asterisks) (10X. H&E stains).

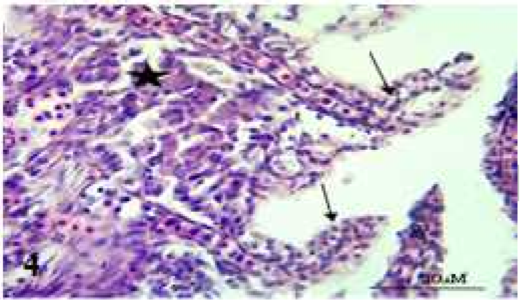

Fig. 4: Gill tissues of C. gariepinus from Main Basin showed disappearance of the normal architecture by complete fusion of adjacent secondary lamellae (asterisk), curling secondary lamella (arrows) with deformed capillary channels (40X. H&E stains).

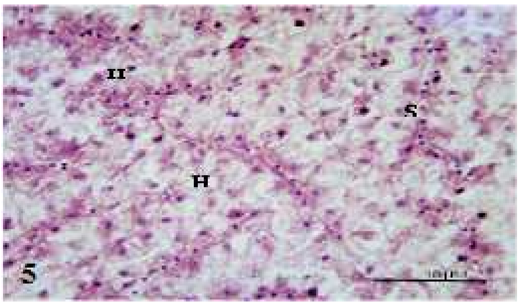

Fig. 5: Liver of *C. gariepinus* from South-west Basin showed less organized hepatic cord forming an irregular clump, and sinusoid (S) filled with red blood cells (10X. HE stains).

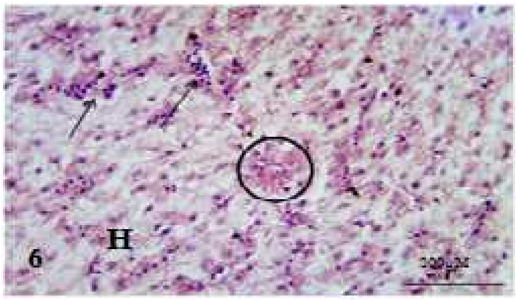

Fig. 6: The liver of *C. gariepinus* from Main Basin showed severe disorganization of the hepatic cords, damaged cell membrane (H), and extensive congestion (circle) of hepatocytes were observed. The cellular structure was totally obscured. Sinusoids in most cases were distended with massive congestion (arrows) .The liver displayed a spongy appearance due to severe lesions. (10X. HE stains).

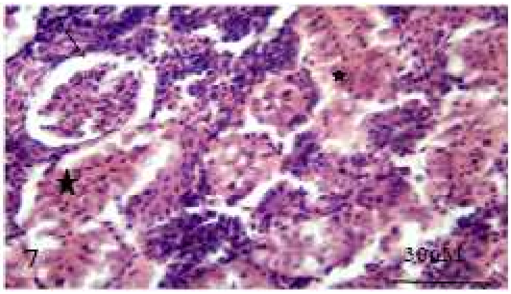

Fig. 7: Kidney of *C. gariepinus* from South-west Basin showed less deformed renal tubules (asterisk) in which the tubular segment renal corpuscles with well-developed glomeruli (arrow) (10X. HE stains).

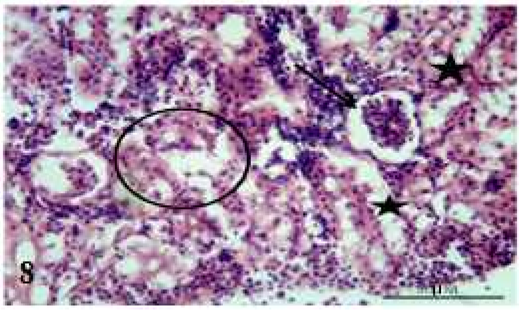

Fig. 8: Kidney of *C. gariepinus* from Main Basin complete necrosis of renal tubules (asterisk) and hyperplasia of interstitial tissues (blue arrow). Note, atrophied glomeruli (arrow) (10X. H&E stain).

ARABIC SUMMARY

تأثير المعادن الثقيلة على بعض العوامل الفسيولوجيه والنسيجية فى سمكه القرموط *Clarias gariepinus* من بحيره مريوط، الاسكندرية، مصر

هبة حافظ عبد القادر- محمد حسن مراد
المعهد القومى لعلوم البحار والمصايد – مصر

اجريت هذه الدراسة لتقييم تراكم وتوزيع المعادن الثقيلة مثل الحديد والزئبق والكادميوم و الزرنيخ في بحيرة مريوط كما تم تقييم تأثير تلوث المعادن الثقيلة في الأنسجة المختلفة مثل الخياشيم والكبد والكلى والعضلات من سمكه القرموط *Clarias gariepinus* باستخدام القياسات الفسيولوجية والنسيجية باستخدام (الحوض الرئيسي) ومنطقة نظيفة نسبيا (الحوض الجنوبي الغربي) .أظهر تركيز المعادن الثقيلة في الماء أن الحوض الرئيسي يحتوي على أعلى المستويات من معادن الحديد والزئبق والكادميوم و الزرنيخ. المعادن الثقيلة وجدت في مختلف أعضاء الأسماك بالترتيب التالى الخياشيم ثم الكبد ثم الكلى ثم العضلات.و العضلات السمكية مقارنة مع الأنسجة الأخرى عادة، تحتوي على أدنى مستوى من المعادن. أظهرت التغيرات الفسيولوجية وجود فقر دم حاد كبير ، زيادة في عدد الكريات البيضاء والخلايا اليمفاوية والالتهاب الأحادي في الأسماك التي تم جمعها من الحوض الرئيسي مقارنة بحوض الجنوبى الغربى. علاوة على ذلك ، لوحظت زيادة معنوية في مصل الدم ALT ، AST والكرياتينين ، في حين أن اليوريا في مصل الدم أظهرت تراجعا ضئيلا في الأسماك التي تم جمعها من الحوض الرئيسي بالمقارنة مع الحوض الجنوبي الغربى. أظهرت التغيرات النسيجية في الخياشيم اندماج فى الطبقة المبطنة للشعيرات الخيشومية. وشملت عينات الكبد نخر خلايا الكبد. وأخيرًا ، أظهرت أمراض الكلى ضمور الكبيبات الكلوية مما أدى الى أتساع الفراغ البولى مع زياده فى تحلل الانابيب الكلوية. واوضحت الدراسه ان متوسط استهلاك السمك للكبار المتراكم بالمعادن ضمن المعايير المقبولة للاستهلاك الآمن. وتوصى الدراسه أن الوضع الحالى للبحيره يحتاج إلى طريقة علمية لإزالة السموم لتحسين صحة البحيرة والأسماك الاقتصادية الكائنه بها.