Dept. of Biochemistry
Animal Health Research Institute
Mansoura Vet. Lab.

THE INFLUENCE OF SOME HEAVY METALS ON CLARIAS GARIEPINUS IN POLLUTED WATER WITH SPECIAL STRESS ON SOME PHYSIOLOGICAL AND PATHOLOGICAL CHANGES ON THESE FISHES

"With 9 tables and 16 figures"

BY

S.A. El-Dessouky; Rawia S.M. Adawy[*] and H.A. Shalaby[**]

[*]Fish and [**]Biochemistry Deptrtments

تأثير بعض المعادن الثقيلة على أسماك القرموط الأفريقي في المياه الملوثة مع التركيز علي بعض التغيرات الفسيولوجية والباثولوجية على هذه الأسماك

صبري عبده الدسوقي ، راوية سعد محمد عدوي ، حامد عبد المجيد شلبي

تم إجراء هذه الدراسة لبيان مدى خطورة المعادن الثقيلة الموجودة في المصارف والترع والبحيرات نتيجة الصرف الزراعي والصحي والصناعي في هذه المناطق الطبيعية للمياه ومدى تأثيرها الضار على صحة الكائنات الحية والأسماك وأيضا الإنسان المستهلك لها. وأوضحت الدراسة على أسماك القرموط الأفريقي المجمعة من أربعة مناطق مختلفة في محافظة الدقهلية وهي نهر النيل بمدينة المنصورة، بحر البقر، مصرف نمرة ٧ وبحيرة المنزلة وجود أخطر المعادن الثقيلة وهي النحاس، الكادميوم والرصاص الذي يؤثر مباشرة على أسماك القرموط الأفريقي فيفقد شهيته وتزيد لزوجة الإفرازات المغطاة لجلد السمك والخياشيم وتسبب ضيق في التنفس ويحدث نفوق للأسماك مع وجود احتقان شديد في الأعضاء الداخلية (خياشيم، كبد، كلى، طحال) كما تم عمل تحليل لعينات المياه المجمعة من هذه المناطق فتبين ارتفاع نسبة الملوحة وعسر المياه وزيادة معدل تركيز المعادن الثقيلة في المناطق الأكثر تلوثا وهي بحر البقر، مصرف نمرة ٧ وبحيرة المنزلة عن الحد المسموح به بينما كانت مكونات المياه ونسبة المعادن الثقيلة أقل من المسموح به في نهر النيل بمدينة المنصورة.

وقد أدت زيادة معدلات المعادن الثقيلة (نحاس وكادميوم ورصاص) إلي وجود تغيرات معنوية في صورة ومصل الدم في أسماك المناطق الملوثة (بحر البقر، مصرف نمرة ٧، بحيرة المنزلة) ، فبالنسبة لصورة الدم اتضح وجود نقص معنوي واضح في العد الكلي لكرات الدم الحمراء، تركيز الهيموجلوبين وحجم الخلايا المضغوطة وعلى النقيض وجدت زيادة معنوية واضحة في العد الكلي لكرات الدم البيضاء بينما كانت صورة الدم طبيعية في عينات دم الأسماك المجمعة من نهر النيل بالمنصورة أما بالنسبة لمصل الدم فقد ثبت وجود نقص معنوي في قيم المتوسطات لكل من البروتين الكلي، الألبيومين، الجلوبيولين الكلي، الكوليستيرول وهرمون الثيرونين (T4).

وعلي الجانب الآخر وجدت زيادة معنوية في قيم متوسطات كل من سكر الدم، هرمون الكورتيزون، أنزيمات الكبد وتشمل S.AST ، S.ALT ، S.ACP وكذلك مستوي الكرياتينين وبولينا

الدم والذي تم تأكيده بواسطة الفحص الباثولوجي للكبد والكلي. وقد أوضحت دراسة التغيرات الباثولوجية التي سببتها سموم هذه المعادن الثقيلة للأعضاء الداخلية (الكبد – الكلي – الخياشيم - العضلات) ، ففي الكبد نرى وجود فجوات في الخلايا الكبدية مع ارتشاح في الأوعية الدموية الكبدية، تنكرز شديد في معظم جدار الكبد وجود تنكرز وتحلل في خلايا جدار القنوات المرارية مع تكون قنوات صغيرة جدا وتليف جدارها مع انتشار خلايا الميكروفاج بين أنسجة الكبد المتحللة.

أما التغيرات الباثولوجية في الكلى فأوضحت الدراسة وجود التهابات في الخلايا المبطنة لأنابيب الكلى ومع وجود تحلل في الخلايا وتحطمها وظهور خلايا الميكروفاج مع وجود احتقان ، خلايا ميتة وظهور حبيبات بنية الشكل وسط مناطق ميتة في نسيج الكلى ووجود تليف حول الجلوميريول وزيادة سمك الغلاف المحيط بالكلى مع وجود أنزفة.

أما التغيرات الباثولوجية في أنسجة الخياشيم فكانت واضحة حيث يظهر تحطم الجزء الطرفي من الخياشيم مع وجود تضخم بخلاياها وضمور الخلايا في الجزء الثاني المبطن للخياشيم مكونة شريط مع زيادة إفراز الخلايا المخاطية. التهاب القوس الخيشومي مع ظهور ارتشاح والخلايا الليمفاوية والإيزينوفيل مع الخلايا المتحطمة وأيضا تبين وجود احتقان وتجلط الدم في شعيرات الدموية الموجودة في الخلايا المبطنة للخياشيم وأيضا أنسجة العضلات ظهرت بها بعض التغيرات الهستوباثولوجية فنرى نسيج العضلات يفقد شكل التخطيط الطبيعي والفراغات التي بين ألياف العضلات مع وجود ارتشاح وتنكرز جزئي مع التهاب بعض الألياف المكونة للعضلات ، ظهور تنكرز وتحلل شديد لبعض أنسجة العضلات مع وجود خلايا ليمفاوية والميكروفاج.

SUMMARY

Pollution with heavy metals has become serious during recent years. Heavy metals such as copper, lead and cadmium when discharged into water can enter the food chain, bioaccumulate in fish tissues therefore they are highly toxic for consumers when exceeding the recommended safety levels. The concentration of heavy metals differed in four examined areas. The concentrations of heavy metals (copper, cadmium and lead) were in normal levels in water of River Nile in Mansoura city and in tissues of *Clarias gariepinus* fish collected from River Nile. But the concentrations of heavy metals in water samples and tissues of fish collected from (drainage canal No 7 then Bahr El-Bakar and Manzala Lake) were higher than the permissible limits as reported by ***FAO/WHO (1992) and WHO (1993).*** The bioaccumulation of the studied heavy metals in vital organs of *Clarias gariepinus* collected from studied locations were high concentrations in liver, gills, kidney then muscles. The haematological analysis of blood revealed presence of significant changes in blood and serum of *Clarias gariepinus* which collected from drainage canal No 7, Bahr El-Bakar and Manzala Lake. The total red blood cells, heamoglobin and backed cell volume levels were lower than fish collected from River Nile at Mansoura city which were in normal ranges. The

total leukocytes values nearly similar to normal ranges in Mansoura city, in contrary marked leucocytosis were observed in other three polluted areas.

Concerning the biochemical parameters, the results showed significant decrease for both of proteinogram parameters, cholesterol and thyronine (T4). On other side significant increase for both of glucose, cortisol hormone, serum AST and S.ALT, S.ACP, S. creatinine and S. blood urea were observed.

The clinical signs and gross lesions on *Clarias gariepinus* including respiratory manifestations and congestion of skin and fins with increase amount of slimy mucous secretion on skin and presence of congestion in gills, kidney and liver with enlargement in size.

The histopathological studies revealed the hepatic lesions of affected fish with heavy metals as vaculation of hepatic cells, edema portal blood vessels, hydropic degeneration of hepatic parenchyma, melanomacrophages among degenerated hepatic tissues. The lesions of kidneys were nephrosis in renal tubules with activation of haemopoietic elements, hyaline degeneration of epithelial lining of some renal tubules and death cells of other tubules with presence of hemorrhages with small brown granules among necrotic areas, periglomerular fibroblastic proliferation and roused renal capsule with subcapsular hemorrhages.

The lesions of gills revealed that destroyed upper portion of gill filaments with hyperplasia of secondary lamellar epithelium with numerous secreting cells, edema in primary filaments, inflammatory cells in gill arch with edema and destruction of surface epithelium. Also gill rocker revealed edema and haemorrhages. Gill filaments had thrombosis in dilated capillaries of secondary lamellar epithelium.

The pathological changes in muscles including swollen with loss of their striation, partial and intense hyalinization with intermuscular edema, partial necrosis with myolysis of some muscle fibers with proliferation of fibroblasts and intense necrosis with few melanomarcrophages and lymphocytes. The study concluded that the heavy metals had dangerous effect on physiological parameters and vital organs of fishes exposed in it.

INTRODUCTION

Fish had long been regarded as a nutritious and highly food due to their contribution of high quality animal protein, richness in calcium and phosphorus and generous supply of vitamins. The pollution of the aquatic environment with heavy metals has become a serious health concern during years ***(Abdelhamid et al., 2006).*** Commercial farming of sharptooth catfish (*Clarias gariepinus*) has significantly increased in different areas of A.R.E over the past few year, *Clarias gariepinus* is widely accepted by consumer in Egypt as relatively cheaper choice of fish protein ***(Ahmed and El-Kamel, 2006).***

The environmental pollution represent a major problem in the world, specially in the less developed countries. Egypt is one of these countries which suffer from biospheres pollution (air and water) ***(Mona and Sawsan, 2004).***

Heavy metals have a great ecological significance due to their toxicity and accumulative behavior playing role in aquatic organisms, therefore the toxic heavy metals become hazard for man and mammals ***(De Gregori et al., 1994)***. The heavy metals introduced into aquaria from metal aquarium hoods, ceramic were that lead glaze and certain rocks, groundwater especially soft acid water, rain water may be a source to aluminum and algaecid or treat ectoparasites lead to poisoning with copper ***(Noga, 1996).*** The industrial and agricultural discharge, coal and oil combustion, chemical and chloride plants emissions, aerial all out phosphate fertilizers and sludge used on agricultural lands, sewage effluents, some types of plastics and pesticides are considered the primary sources of lead, cadmium and mercury pollutions in fish ***(WHO, 1992; Shibamoto and Bjeldames, 1993; Jehan et al., 1999 and Abdelhamid et al., 2006)***.

Many of heavy metals such as cadmium and lead have no nutritional importance and their presence in relatively high concentration in body tissues can result in health problems in human as well as in animals ***(Gold Frank et al., 2001)***. The impact of heavy metals on fish has been of great concern for many years. Bioaccumulation of heavy metals in fish many critically influence the growth rate, physiological and biochemical status and consequently the meat quality of fish ***(El-Naggar et al., 1998 and Haggag et al., 1999).*** The exposure of fish to different levels of individual heavy metals and to very lesser extent combined ones showed monotring alterations in metabolic aspects of the exposed fish where one of them increased plasma glucose in different fish species ***(Gill et al., 1992 and Dethloff et al., 1999a&b)***. Inorganic lead alters plasma glucose and cholesterol in Bayad ***(Hema et al., 1987).*** Cadmium also elevated blood glucose in tilapia ***(Almeid et al., 2001)*** and gold fish ***(Zikic et al., 2001).*** Elevated plasma protein concentration was also observed in catfish exposed to zinc ***(Hilmy et al., 1987).*** The objective of this work was planned to study the following:

(1) The clinical signs of some heavy metals pollution and its residual in water, muscle tissue and organs in *Clarias gariepinus*.

(2) The haematological aspects and physiological responses including some metabolic and enzymatic activities in *Clarias gariepinus* collected from polluted locations with heavy metals.

(3) The pathological changes in some organs in *Clarias gariepinus* exposed to heavy metals pollution at Dakahlia Governorate.

MATERIAL AND METHODS

(I) Fish:

120 of *Clarias gariepinus* were collected from four (4) different regions in Dakahlia Governorate from River Nile in Mansoura city, Bahr El-Bakar, drainage canal number (7) in Belkas region and Manzala Lake. The collected fishes were transported to laboratory and detected the clinical signs and abnormal symptoms on the fish. The blood samples were taken from all examined fishes. The tissue samples from liver, kidney, gills and muscles were taken from the dissected fish and stored in the deep freezer at -20°C for heavy metals evaluation.

(II) Residual analysis:

(1) Determination of copper (Cu), lead (Pb) and cadmium (Cd) in surface water was carried according to the methods prepared by ***Polprasert (1982) and Sprenger et al., (1987).***

(2) Determination of copper, lead and cadmium in fish tissues according to the method described by ***Heckman, (1970) and Greig et al., (1982).*** Tissue samples measured by using atomic absorption spectrophotometer according to ***Capar (1977).***

(IV) Haematological examination:

The collected blood samples for haematological techniques were carried according to ***Lucky (1977).*** Blood samples were taken from examined fishes from each region by section of the caudal peduncle for haematological and biochemical studies as following:

(1) Whole blood:

About 0.5 – 1.0 ml whole blood was collected in small plastic vials containing a suitable amount of dipotassium salt of Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant. These samples were used for, the evaluation of Total Erythrocytic Count (TEC), hemoglobin concentration (Hb), Packed Cell Volume (PCV) and Total Leucocytic Count (TLC) according to ***Stoskopf (1993).***

(2) Blood serum:

About 3-4 ml blood collected in plastic centrifuge tubes and left to clot for 3 hours at 4°C, after that the blood samples were centrifuged to separate the serum and kept in glass vials at –20°C until used.

The separated serum was used in biochemical studies, where serum total protein was measured according to ***Doumas et al., (1981),*** albumin ***(Frank, 1950),*** total globulin calculated mathematically by subtracting albumin from total protein, serum glucose ***(Trinder 1969),*** serum cholesterol ***(Allain et al., 1974)*** cortisol ***(Foster and Dum, 1974),*** serum triiodothyromine (T_3) and tetraiodthyronine (T_4) ***(Eastman et al., 1975 and Chopra 1972)*** respectively, serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) ***(Reitman and Frankel, 1957),*** acid phosphates (ACP) ***(Young 2001)***, blood urea ***(Patton and Crouch, 1977)*** and creatinine ***(Husden and Rapaport, 1968).***

(V) Histopathological examination:

Tissue specimen from muscles, liver, kidney and gills of examined fish were fixed in 10% phosphate buffer formalin. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin according to ***Roberts, (1989).***

(VI) Statistical analysis:

All data were statistically analyzed by the one-way analysis of variance (ANOVA) Technique using the linear models (GLM) procedures of SAS (SAS® ***Institute, 2001).*** Significant differences among treatment means were determined by Duncan's Multiple Range Test according to ***Duncan, (1955).***

RESULTS

Heavy metals are widely distribution in aquatic system due to wide use of chemicals in agriculture and industry in Egypt. The hazards of residual heavy metals on fish live in polluted water detected in clinical signs. The external signs on *Clarias gariepinus* including respiratory manifestation, congestion in fins and skin and increase amount of slimly mucous secretion covered skin and gills. The gross lesions of internal organs were liver enlargement, friable and very congested, distended in gall bladder and congestion in spleen and kidney (Fig 1).

Water quality:

Dealing with the water analysis (Table 1) showed that changes in the physicochemical properties of water samples collected from the four (4) different locations revealed that different variations in the water quality were the oxygen dissolved in water in normal range but high in River Nile than other (3) three locations. The pH values of water samples collected from studied locations were increase in drainage canal number (7), then Bahr El-Bakar and Manzala Lake, but in River Nile pH value in normal range. The water salinity increase in 3 highly polluted location arranged in the following drainage canal number (7), then Bahr El-Bakar then Manzala Lake while salinity degree of River Nile was in normal value.

Chemical analysis to water:

The concentration of heavy metals in water samples collected from (4) four locations (River Nile in Mansoura city, Bahr El-Bakar, Drainage canal number (7) and Manzala Lake) showed in Table (2). Copper, cadmium and lead concentrations in water samples collected from three polluted locations were higher than these concentrations in River Nile in Mansoura city. However copper, cadmium and lead concentrations in the three locations (drainage canal number (7), Bahr EL-Bakar and Manzala Lake) were higher concentrations than permissible levels as reported by ***WHO (1993)*** .

Residual heavy metals in fish tissues:

Heavy metals as copper, cadmium and lead were accumulated in fish tissues as gills, muscles, liver and kidney. The concentrations of the studied heavy metals (Cu, Cd and Pb) in the vital organs and muscles of *Clarias gariepinus* were highly significant different among different studied locations showed in Tables (3, 4, 5 and 6). The highest concentrations of copper in muscles and kidney of fish in region (3), then in liver of fish in region (2) and liver of fish in region (4). The concentrations of cadmium were highest in liver and muscles of fish in region (3 and 4) respectively. The higher concentrations of lead were detected in liver of fish in region (3 and 4) respectively then muscles of fish in region (3) the bioaccumulation of the studied heavy metals in vital organs of *Clarias gariepinus* were collected from studied locations were in the following order: liver tissue> gills> kidney> muscles were higher concentrations than permissible limits as reported by ***FAO/WHO, (1992)***.

Blood parameters:

Hematological analysis of blood samples collected from *Clarias gariepinus* in different studied locations showed that blood parameters of fish collected from River Nile in Mansoura city were in the normal ranges, but the blood parameters of fish collected from Bahr EL- Bakar, drainage canal No (7) and Manzala Lake showed high significant and significant ($p < 0.01$), ($p < 0.05$) decrease respectively in the mean values of total RBCs, haemoglobin content and packed cell volume. The total W.B.Cs of fish collected from River Nile was in normal range but in fishes collected from other polluted locations showed very highly significant ($p < 0.001$) increase in total WBCs (Table 7).

Serum biochemical parameters:

Fish collected from polluted regions (Bahr EL-Bakar, drainage canal No (7) and Manzala Lake) showed significant ($p < 0.05$) decrease in total protein mean values, also occur significant ($p < 0.01$), ($p < 0.001$) and ($p < 0.05$) decrease in albumin levels in the other three polluted locations respectively were observed. The total globulin levels of fish in Bahr EL-Bakar, drainage canal No (7) and Manzala Lake present significant ($p < 0.01$) and ($p < 0.05$) decrease in the mean values respectively when compared with the normal level of fish collected from River Nile in Mansoura city (Table 8).

Regarding the glucose mean values (Table 8) showed significant ($p < 0.01$), ($p < 0.001$) and ($p < 0.05$) increase in blood serum of fish collected from polluted regions Bahr EL-Bakar, drainage canal No (7) and Manzala Lake respectively in comparison with those of River Nile locations.

Serum cholesterol results revealed significant ($p < 0.01$), ($p < 0.001$) and ($p < 0.05$) decrease in fish collected from the three polluted locations respectively (Bahr EL-Bakar, drainage canal No (7) and Manzala Lake) in comparison with fish collected from River Nile in Mansoura city (Table 8).

The result given in Table (8) declared that level of cortisol hormone significantly ($p < 0.01$) and ($p < 0.05$) increase in Bahr EL-Bakar, drainage canal No (7) and Manzala Lake respectively when compared with the level of those fish in River Nile location.

Sera analysis of fish collected from heavy metals polluted water localities which tabulated in table (8) showed that serum levels of triiodothyronine (T_3) were not affected, but serum levels of thyronine (T_4) showed significant ($p < 0.05$) decrease in collected fish from Bahr EL-Bakar and Manzala Lake respectively, while the fish collected from drainage canal No (7) showed high significant ($p < 0.01$) decrease.

Concerning liver enzymes (Table 9) revealed that significant ($p < 0.01$), ($p < 0.001$) and ($p < 0.05$) increase in serum levels of AST in fishes collected from the three polluted localities (Bahr EL-Bakar, drainage canal No (7) and Manzala Lake) respectively, while serum level of ALT showed significant ($p < 0.01$) increase in regions (Bahr EL-Bakar and drainage canal No 7), while in Manzala Lake the level of significance was ($p < 0.05$) increase. The level of (ACP) showed significant ($p < 0.001$) and ($p < 0.05$) increase in (Bahr EL-Bakar , drainage canal No 7 and Manzala Lake respectively.

Dealing with kidney function (Table 9) showed that both of serum creatinine and blood urea levels significantly ($p < 0.001$) increased in fish collected from Bahr EL-Bakar and drainage canal No (7) but serum creatinine significantly ($p < 0.05$) increased in Manzala Lake. Also serum blood urea significantly ($p < 0.01$) increased in fish collected from Manzala Lake.

Results of histopathology:

The morphological changes among examined organs of different localities varied in intensity according to the type and amount of detectable heavy metal and their toxicity.

The histopathological changes in liver included that the hepatic lesions of fish among different areas were sever and represented by vaculation of the hepatic cells beside edema in portal blood vessel and mild inflammatory cells (Fig. 2). Sometimes, the lesions become more wide and represented by intense hydropic degeneration in the majority of the hepatic parenchyma (Fig. 3). Areas of coagulative necrosis with proliferation of bile duct epithelium forming numerous bile ductules with periductular fibrosis could be seen (Fig. 4). Melanomacrophages could be seen scattered among degenerated hepatic tissues. Hepatopancrease revealed inactivation with depletion of their zymogenic granules with congestion of their blood vessels.

On the histopathological findings in kidneys were the renal lesions in posterior kidney were sever mainly in areas Bahr El-Bakar and drainage canal No. (7) and become moderate in area of Manzala Lake. Nephrosis of all renal tubular epithelium with activation of haemopoietic elements and a few scattered melanomacrophages were seen (Fig. 5). The epithelial lining of some renal tubules became disassociated from their basement membrane and undergo hyaline degeneration could be seen (Fig. 6). The other tubules suffered from either accidental or programmed cell death with haemorhages and presence of small scattered brown granules necrotic areas (Fig. 7).

The contracted glomerular tufts with periglomerular fibroblastic proliferation could be seen (Fig. 8) and thickened renal capsule by fibrous tissue with subscapular haemorrhages was common.

The pathological changes in gills of *Claria gariepinus* revealed that lesions in gills were severe of drainage canal No. (7) and moderate in area of Bahr El-Bakar and Manzala Lake. Distorded upper portions of gill filaments with hyperplasia and hypertrophy of their secondary lamellar epithelium forming sheets containing numerous mucus secreting cells were seen (Fig. 9). The forementioned lesions were extended to involve whole length of some primary filaments beside presence of edema (Fig. 10). The gill arch revealed intense inflammatory cell mainly lymphocytes and eosinophil granular cells and edema with destruction of their surface epithelium (Fig. 11). The gill racker revealed edema, haemorrhages and presence of numerous mucous secreting cells in their coxexing. Some gill filaments had telangectiasis or thrombosis in the dilated capillaries of secondary lamellar epithelium with haemorrhages and edema (Fig. 12).

The pathological changes in muscles including moderate muscular lesions were seen in Bahr El-Bakar and drainage anal No. (7) and become sever lesion in fish muscles of Manzala Lake. The muscle fibers became swollen with loss of their striation and absence intermuscular spaces and partially hyalinized (Fig. 13). Intense hyalinization and intermuscular edema were also detected (Fig. 14). Partial necrosis with mymyolysis of some muscle fibers and proliferation of fibroblasts could be seen (Fig. 15). Intense necrosis in the majority of muscles with presence of a few melanomacrophages and lymphocytes could be seen (Fig. 16).

Table (1): **Physical analysis of water collected from different localities**

Items / Location	*Temperature ^oC*	*Oxygen ppm*	*pH value*	*Salinity g/L*
1- River Nile	20 ± 2	8.35	7.51	0.13
2- Bahr El-Bakar	18 ± 2	6.01	8.61	1.452
3- Drainage canal (7)	22 ± 2	4.21	9.51	2.11
4- Manzala Lake	16 ± 2	6.2	8.5	1.322

Table (2): **Heavy metal concentration (ppm) of water were collected from different localities**

Heavy metal / Location	*Copper*	*Cadmium*	*Lead*
1- River Nile (Mansoura city)	0.058 ± .0.02	0.075 ± 0.01	0.007 ± 0.002
2- Bahr El-Bakar	1.35 ± 0.20	0.059 ± 0.01	0.24 ± 0.09
3- Drainage canal No. (7)	1.37 ± 0.21	0.072 ± 0.02	1.27 ± 0.17
4- Manzala Lake	1.27 ± 0.20	0.081 ± 0.01	0.68 ± 0.11

*** permissible limit of heavy metals in water according to WHO (1993) cadmium 0.003 mg/l, lead 0.01 mg/l, copper 2.00 mg/l.**

Table (3): **Heavy metal concentrations in some organs in *Clarias gariepinus* collected from River Nile**

Organ / Heavy metal	*Muscles*	*Gills*	*Liver*	*Kidney*
Copper (Cu) ppm	0.371 ± 0.015	0.312 ± 0.022	0.241 ± 0.026	0.165 ± 0.02
Cadmium (Cd) ppm	0.011 ± .002	0.021 ± 0.005	0.035 ± 0.013	0.012 ± 0.004
Lead (Pb) ppm	0.021 ± 0.005	0.032 ± 0.007	0.043 ± 0.01	0.039 ± 0.009

Table (4): **Heavy metal concentrations in some organs in *Clarias gariepinus* collected from Bahr El-Bakar**

Organ / Heavy metal	*Muscles*	*Gills*	*Liver*	*Kidney*
Copper (Cu) ppm	0.878± 0.007	0.475± 0.006	1.045±0.011	0.546±0.008
Cadmium (Cd) ppm	0.180± 0.006	0.095± 0.003	0.075±0.006	0.041±0.004
Lead (Pb) ppm	0.035± 0.005	1.010± 0.003	1.033±0.014	0.601±0.033

*** permissible limit of heavy metals in organs of fish according to WHO (1992), copper 0.5 ppm, cadmium 0.05 ppm and lead 0.5 ppm.**

Table (5): **Heavy metal concentrations in some organs in *Clarias gariepinus* collected from drainage canal No (7)**

Organ / *Heavy metal*	*Muscles*	*Gills*	*Liver*	*Kidney*
Copper (Cu) ppm Mean	2.525 ± 0.095	0. 763 ± 0.011	0.903 ± 0.056	1.39 ± 0.175
Cadmium (Cd) ppm Mean	0.118 ± 0.021	0.095 ± 0.015	0.145 ± 0.017	0.110 ± 0.017
Lead (Pb) ppm Mean	1.440 ± 0.032	1.280 ± 0.017	1.840 ± 0.05	1.130 ± 0.035

Table (6): **Heavy metal concentrations in some organs in *Clarias gariepinus* collected from Manzala Lake**

Organ / *Heavy metal*	*Muscles*	*Gills*	*Liver*	*Kidney*
Copper (Cu) ppm Mean	0.743 ± 0.20	0.610 ± 0.039	1.210 ± 0.053	0.491 ± 0.032
Cadmium (Cd) ppm Mean	0.145 ± 0.017	0.120 ± 0.041	0.128 ± 0.034	0.66 ± 0.005
Lead (Pb) ppm Mean	0.765 ± 0.038	1.158 ± 0.022	1.720 ± 0.017	0.023 ± 0.006

Table (7): **Mean values of some blood parameters of fish from different locations**

Location / *Blood parameters*	*River Nile (Mansoura city)*	*Bahr El-Bakar*	*Drainage canal No (7)*	*Manzala Lake*
Total R.B.Cs ($\times 10^6/ \mu l$)	1.92 ± 0.06^a	1.57 ± 0.07^c	1.48 ± 0.11^c	1.62 ± 0.12^b
Haemoglobin (G / dl)	7.84 ± 0.40^a	5.22 ± 0.48^c	5.55 ± 0.49^c	6.24 ± 42^b
Packed cell volume (%)	21.55 ± 0.90^a	16.90 ± 0.82^c	17.45 ± 0.77^c	18.80 ± 0.90^b
Total W.B.Cs ($\times 10^3/ \mu l$)	30.76 ± 1.14^d	38.6 ± 0.83^a	39.10 ± 0.86^a	37.10 ± 0.95^a

*Means with the same letter in the same row are not significantly different.

Table (8): **Mean values of some serum biochemical parameters of fish from different locations**

Serum blood parameters / *Location*	*River Nile (Mansoura city)*	*Bahr El-Baker*	*Drainage canal No (7)*	*Manzala Lake*
Total protein (G/dl)	4.41 ± 0.33[a]	3.15 ± 0.44[b]	3.10 ± 0.35[b]	3.33 ± 0.38[b]
Albumin (G/dl)	2.11 ± 0.05[a]	1.73 ± 0.09[c]	1.65 ± 0.08[d]	1.85 ± 0.11[b]
Total globulin (G/dl)	2.30 ± 0.18[a]	1.42 ± 0.15[c]	1.45 ± 0.13[c]	1.48 ± 0.27[b]
Glucose (mg/dl)	87.60 ± 1.57[d]	96.81 ± 1.85[b]	99.10 ± 1.45[a]	92.80 ± 1.50[c]
Cholesterol (mg/dl)	126.28 ± 1.96[a]	115.94 ± 2.05[c]	114.23 ± 2.23[d]	119.80 ± 2.20[b]
Cortisol (ng/ml)	3.41 ± 0.12[c]	4.21 ± 0.17[a]	4.36 ± 0.23[a]	3.96 ± 0.22[b]
Thyronine (T4) (ng/ml)	13.42 ± 0.39[a]	11.67 ± 0.49[b]	10.88 ± 0.66[c]	11.92 ± 0.55[b]
Triiodothyronine (T_3) (ng/ml)	3.53 ± 0.11[a]	3.27 ± 0.17[a]	3.31 ± 0.14[a]	3.45 ± 0.15[a]

* Means with the same letter in the same row are not significantly different.

Table (9): **Mean values of serum parameters of liver and kidney functions of fish from different locations**

Serum blood parameters / *Location*	*River Nile (Mansoura city)*	*Bahr El-Baker*	*Drainage canal No (7)*	*Manzala Lake*
AST (µ / dl)	284.11 ± 1.76[d]	293.21 ± 2.01[c]	296.80 ± 1.67[a]	289.77 ± 1.73[b]
ALT (µ / dl)	8.23 ± 0.70[c]	12.13 ± 0.75[a]	11.33 ± 0.74[a]	10.71 ± 0.78[b]
ACP (µ / dl)	2.23 ± 0.14[c]	3.33 ± 0.15[a]	3.75 ± 0.17[a]	2.85 ± 0.23[b]
Creatinine (mg /dl)	1.47 ± 0.05[c]	2.10 ± 0.08[a]	2.40 ± 0.09[a]	1.85 ± 0.15[b]
Blood urea (mg /dl)	3.51 ± 0.14[d]	7.10 ± 0.29[a]	8.35 ± 0.35[a]	4.60 ± 0.32[c]

* Means with the same letter in the same row are not significantly different.

Plate (I):

Fig. (1): ***Clarias gariepinus*** **collected from polluted water showing congestion in gills, liver, kidney and spleen.**

Fig. (2): Liver showing mild vaculation of hepatic cells and odema in blood vessel wall (H & E × 150).

Fig. (3): Liver showing hydropic degeneration of the hepatic cells (H & E × 150).

Fig. (4): Liver showing periductuoeular fibrosis (H & E × 150).

Plate (II):

Fig. (5): Kidney showing nephrosis and activation of hemopoietic elements (H & E × 150)

Fig. (6): Kidney showing hyaline degeneration of some tubular epithelia (H & E × 300).

Fig. (7): Kidney showing accidental or programmed cell death of some renal tubules (H & E × 300).

Fig. (8): Kidney showing contracted glomerular tuft and periglomerular fibrosis (H & E × 300).

Plate (III):

Fig. (9): Gills showing hyperplasia and hypertrophy of secondary lamellar epithelium on the tips of primary filaments (H & E × 150).

Fig. (10): Gills showing sheescs along the length of some gill filaments (H & E × 150).

Fig. (11): Gills showing intense inflammatory cells, odema and haemorrhages in gill arch (H & E × 150).

Fig. (12): Gills showing telangiectiasis and odema of secondary lamellae (H & E × 300).

Plate (IV):

Fig. (13): Skeletal muscles showing loss of striation and absence of intermuscular spaces (H & E × 300).

Fig. (14): Skeletal muscles showing partial hyalinization and inter muscular odema (H & E × 300).

Fig. (15): Skeletal muscles showing partial myolsis of some muscle fibers (H & E × 300).

Fig. (16): Skeletal muscles showing intense necrosis and presence of a few melanomacrophages (H & E × 300).

DISCUSSION

The higher concentration of heavy metals beyond the tolerance limit of fishes affect fish populations, reducing their growth, reproduction and/or survival and may even kill fishes ***(Schreck & Iorz, 1978 and Zaghloul 1997&2000).***

The main clinical signs observed were similar to that mentioned by ***Elsa (1991), El-Shaieb et al., (2001) and Hafez et al., (2003).*** The postmortem examination revealed congestion in internal organs (gills, liver, kidney, spleen) and enlargement in liver. Similar lesions were previously mentioned by ***Sorensen (1991), El-Shaieb et al., (2001) and Hafez et al., (2003).***

The changes in the physico-chemical properties of water samples collected from the different studied localities reflect the differences in the effluents that discharged to each locality (Industrial effluents, agricultural drainage and waste municipal).

Dissolved oxygen levels decreased in water of polluted localities and similar to result of ***Boyd, (1990)*** were dissolved oxygen content in industrial effluents, agricultural and sewage drainage varies widely and water becomes unsuitable for fish if the concentration falls to 1ppm**.** Also reduction in oxygen content to 4ppm increases the toxicity of heavy metals ***(Skidmore, 1974).*** The fish were exposed to low levels of oxygen in pumping more water through the gills in order to obtain sufficient oxygen and thereby exposed to addition poisons ***(Zaghloul et al., 2001).***

Water samples collected from polluted localities were increased in salinity compared with water samples from River Nile due to the high levels of dissolved salts in water that discharged directly to this locality as reported by ***Zaghloul et al., (2000).***

Water pH values of polluted water localities in normal range were 6.5 to 9 ***(Boyd, 1990).*** However, slight increase in pH of water in drainage canal No (7) due to agriculture drainage water and changes in alkalinity due to uptake of free carbon dioxide from water and precipitation of calcium carbonate ***(Boyd, 1990 and Saeed, 2000).***

Heavy metal pollution in water is generally associated with industrial, agricultural and municipal discharges ***(Haggag, et al., 1999, Salah El-Deen, et al., 1999 and Zaghloul, 2000).*** Once metals are in the water column, they may be taken up by living organisms, deposited in the sediments or remain for some period in the water itself ***(Salah El-Deen, et al., 1999).*** The highly significant differences in the studied heavy metals (copper, cadmium and lead) among the water samples collected from the different locations could be attributed to the differences in the effluents (waste municipal, agricultural drainage water and/or other urban activities discharged direly to the studied locations. This is in agreement with ***Nagdi and Shaker (1998) and Zaghloul (2000)*** who attributed the increase of heavy metals in drainage water to the decomposition of the organic matter and / or the use of fertilizers and other chemicals in agriculture.

Heavy metals such as copper, lead and cadmium when discharged into the water can enter the food chain, bioaccumulate in fish tissue and reached to man ***(Ajmal et al., 1985).*** Bioaccumulation of heavy metals in fish may critically influence the growth rate, physiological and biochemical status and consequently the meat quality of fish ***(Salah El-Deen, et al., 1996&1999, El-Naggar, et al., 1998, Haggag, et al., 1999 and Zahra et al., 2001).*** The concentrations of studied heavy metals (Cu, Cd and Pb) in vital organs and muscles of *Clarias gariepinus* were highly significant among different studied locations showed in tables (3, 4, 5 and 6). However, highest concentrations of copper in muscles and kidney of fish collected from drainage canal No (7), then liver in fish collected from Bahar El-Bakar and Manzala Lake respectively. The concentrations of cadmium were highest in liver and muscles of fish collected from drainage canal No (7) and Manzala Lake. Also the higher concentrations of lead were detected in liver of fish collected from drainage canal No (7) and Manzala Lake respectively then lead concentration in muscles of fish collected from drainage canal No (7). In the present investigation the high concentrations of the various heavy metals in different vital organs of *Clarias gariepinus* collected from different locations may be attributed to their exposure to high concentration of water heavy metals and the specific effluents discharged to each location.

Bioaccumulation of either of the studied heavy metals in vital organs of *Clarias gariepinus* were collected from studied locations were in the following order: liver tissues > gills > kidney > muscles. Heavy metals were significantly higher in fish viscera, including liver tissue, than in muscle tissues as previously shown ***(Shereif & Moaty, 1995; Khalil & Hussie, 1996 and Zahra et al., 2001).***

Accumulation of heavy metals in the vital organs of fish is correlated with metabolic rate ***(Sorensen 1991).*** The highest concentrations of the studied heavy metals in liver may be attributed to the major role of liver in the detoxification and protection from heavy metals exposure both by producing metallothionein and by acting as storage site for bound metals ***(Pratap, et al., 1989).***

Fish accumulate the heavy metals from the water primarily through the gills ***(Eisler 1967).*** Its uptake could be controlled by the amount of water passing through the gills. The obtained results showed highly concentration of copper in gills of fish collected from drainage canal No (7), but highly concentration of lead in fish gills collected from drainage canal No (7), these results agreement with ***Hilmy et al., (1987).:***

Haematological studies have been developed for evaluation blood parameters as indicator of general health conditions of animal body. Subsequently, haematological studies are promising tools for investigating physiological changes caused by environmental pollutants ***(Ghazaly & Said, 1995, El-Naggar et al., 1998 and Haggag et al., 1999).***

The haematological analysis of blood samples from *Clarias gariepinus* in the different studied locations tabulated previously in table (7) showed significant differences between polluted areas and those of River Nile at Mansoura city. These findings coincide with those reported by ***El-Naggar et al., (1998) and Haggag et al., (1999) and Zahra et al., (2001)*** who attributed the decrease in RBCs, Hb and PCV values to the reduction in red blood corpuscles production in the haematopoietic organs under the action of heavy metals accumulated at high concentrations in the various tissues and to intrahepatic and intrasplenic haemmorrhage induced by action of accumulated heavy metals.

Also the decrease in RBCs in *Clarias gariepinus* collected from Manzala Lake may attribute to the high concentrations of lead in water. This may be due to reduction in red blood corpuscles production in the bone marrow under the action of mentioned heavy metals as well as intrahepatic and intrasplenic haemarrhage by the action of accumulated heavy metals in the present study. These results agreed with ***(Marie, 1990 and Abbas, 1994).*** The observed leucocytosis could be attributed to the release of corticoids into circulation under the stress effect of heavy metals accumulation. These results agreed with ***(Hafez et al., 2003).***

Fish collected from polluted location (Bahr El-Bakar, drainage canal No (7) and Manzala Lake) showed significantly decrease in total protein, albumin and globulin mean values respectively when compared with normal values in fish collected from River Nile (Table 8). These reductions may be attributed to liver damage. The obtained results were similar to those of ***(Al-Ekel 1994).*** The protein analysis showed an opposite trend where ***(Abd Allh et al., 2002)*** reported increase in plasma protein with a constant albumin level and that is may related to their role as a transport vehicle for xenobiotics ***(Ghosh and Bhattacharya, 1992 and De Smet et al., 2001).***

Regarding the glucose mean values (Table 8) showed significant increae in fish collected from (Bahr El baker, drainage canal No (7) and Manzala Lake). The noticed hyperglycaemia might be related to the stress response in which fish show increased concentrations of cortisol ***(Dethloff et al., 1999a)*** and adrenaline ***(Mazeaud and Mazeaud 1981)*** where these hormones could cause a direct inhibition of eating or they might even do it indirectly and produce changes in the blood glucose level by mobilizing of liver glycogen into blood glucose. Our results were in agreement with the findings of ***Abd-Allh et al., (2002) and Metwally et al., (2002).***

The data illustrated in table (8) revealed significant decrease in cholesterol levels in three polluted locations (Bahr El-Bakar, drainage canal No (7) and Manzala Lake). The result in the current work is also similar to that obtained by ***Ferranda and Anderew, (1991)*** and ***Ali et al., (2003),*** where they said that such decrease in cholesterol level could be attributed to its greater level of utilization during corticosteroidogenesis as it is precursor for steroid hormones.

The results given in table (8) declared that the levels of cortisol hormone in the three polluted locations significantly increased when compared with those levels of River Nile fishes. In respect our data come in accordance with those of ***Ali et al., (2003)***, they attributed such increase to stress factors and the intoxication of fish exposed to heavy metals pollution specially lead. ***Heakal et al., (1999)*** said that the exposure to cadmium elevate serum cortisol level, he explained this elevation by the fact that cadmium is a chemical stressor that able to activate the hypothalama- pituitary- internal (HPI) axis as has been previously reported by ***James and Wigham, (1986).***

The sera analysis of heavy metals of fish collected from polluted locations showed that the serum levels of triiodothyronine (T_3) were not affected; while serum levels of thyronine (T_4) were decreased as showed in table (8). It has been suggested that most of heavy metals specially cadmium induced decrease in serum (T_4) and this may be due to direct inhibition of hormone synthesis in the gland by the metal ***(Gupta et al., 1995).***

Concerning liver enzymes (Table 9) revealed significant increase in serum levels of AST and ALT in fish collected from polluted locations (Bahr El-Bakar, drainage canal No (7) and Manzala Lake) The level of acid phosphatase (ACP) showed also significant increase in its level as showed in table (9). The increased activity of liver enzymes in the current work may reflect the hepatocellular damage, and to a lesser extent other tissues ***(Whitby et al., 1989 and Srivas Tava et al., 1998).*** The obtained data support the suggestions by ***Hema et al., (1987)*** and ***Zikic et al., (2001)*** that cadmium, besides most heavy metals causes oxidative stress and tissue damage in exposed fish to pollution.

Dealing with kidney function table (9) showed that both of serum creatinine and blood urea levels increased as in previously results. The obtained results in the present work agreed with those of ***El-Naggar et al., (1998)*** and ***Hafez et al., (2003)***, they attributed such increases in blood urea and creatinine levels in fish collected from polluted location to gills and kidney damage.

The main lesions were found in liver represented by vaculation of the hepatic cells beside edema in portal blood vessel and mild inflammatory cells (Fig 2). Sometimes the lesions become more wide and represented by intense hydropic degeneration in the majority of hepatic parenchyma (Fig 3). Areas of coagulative necrosis with proliferation of duct epithelium forming numerous bile ductules with periductular fibrosis could be seen in (Fig 4). These data were in agreement with ***El- Shaieb, et al., (2001).*** These findings reflect the role of the liver in detoxification of heavy metals. ***Klaverkamp et al., (1984)*** suggested that the liver plays a major role in the protection of body against the heavy metals toxicosis. ***Abouzaid et al., (1992)*** add that the liver is a target organ for heavy metal toxicity in fish. The wide spread necrosis of hepatocytes was confirmed with ***El-Naggar et al., (1998) and Hafez et al., (2003).***

Melanomacrophages could be seen scattered among degenerated hepatic tissues. These results are in agreement with ***Fantin et al., (1992).*** Hepatopancreas revealed inactivation with depletion of their zymogenic granules with congestion of their blood vessels. These histopathological findings were in agreement with ***Roberts (1989).*** The ultastructural changes in the liver due to toxic effect of heavy metals were in agreement with ***Al-Ekel., (1994).***

The histopathological changes in kidneys were sever in areas (Bahr El-Bakar and Drainage canal number [7]) and moderate in (Manzala Lake area). Nephrosis of renal tubular epithelium with activation haemopoietic elements and melanomacrophages were scattered and epithelial lining of some renal tubules become disassociated from their basement membrane and undergo hyaline degeneration could be seen (Fig 7). These results were in agreement with ***Fantin et al., (1992); El-Shaieb et al., (2001) and Hafez et al., (2003).*** The contracted glomerular tufts with periglomerular fibroblastic proliferation could be seen (Fig 8) and thickened renal capsule by fibrous tissue with subcapsular haemorrhages was common. This picture was in agreement with those reported by ***El- Naggar et al., (1998)*** and the accumulate of metabolic waste products could induce erythrocytes hemolysis and depression in the production of erythrocytes by hamatopoietic organs. These data in agreement with ***Tewari et al., (1987); Tabache et al., (1990) and Hafez et al., (2003).***

The gills showed distorded upper portions of gill filaments with hyperplasia and hypertrophy of their secondary lamellar epithelium forming sheets containing numerous mucus secreting cells were seen (Fig 9). The forementioned lesions were extended to involve whole length of some primary filaments beside presence of edema (Fig 10). These results were similar to ***Crespo and Karnaky (1983) and El- Shaieb et al., (2001)*** and these findings could be attributed to heavy metal potent inhibition on chloride transport across the opercular epithelium. They inhibit No, K-ATpase and may interact, as well, with the coupled NaCl carrier.

These features lead to the magnification of the chloride cells volume due to NaCl accumulation. The gill racker revealed edema, haemorrhages and presence of numerous mucous secreting cells in their coxexing. Some gill filaments had tetangetiasis or thrombosis in the dilated capillaries of secondary lamellar epithelium with haemorrhages and edema (Fig 12). These results could be due to partial disturbance of gas exchange and tissue hypoxia which may increase the flow of blood to the gills ***(El-Shaieb et al., 2001).*** The gill and kidney dysfunction that resulted from histopathological alterations lead to increase in urea and creatinine ***(El-Naggar et al., 1998 and Hafez et al., 2003).***

The pathological changes in muscles including moderate muscular lesions were seen in Bahr El-Bakar and drainage canal No (7) and sever lesion in fish muscles of Manzala Lake. The muscle fibers became swollen with loss of their striation and absence intermuscular spaces and partially hyalinized (Fig 13). Intense hyalinization and intermuscular edema were also detected (Fig 14). Partial necrosis with myolysis of some muscle fibers necrosis with myolysis of some muscle fibers and proliferation of fibroblasts could be seen (Fig 15). These results were in agreement with ***El-Shaieb et al., (2001).***

In conclusion based on the physico-chemical properties of water, fish health, agricultural drainage and sewage water were considered the lowest water quality among other sources where it contains high concentrations of heavy metals that implicate fish tissues affecting its quality and hence become a threat to man so, treatment of agricultural drainage and sewage water should be carried out before draining in natural water areas e.g. small tributaries of River Nile and lakes in Egypt for increase fish production and muscles of fish become good in quality and not hazard on human public health.

Acknowledgment:

It is great pleasure to us to record our independence to ***Prof. Dr. Abd El-Moniem A. Ali***, Professor of Pathology, Faculty of Veterinary Medicine, Zagazig University for his great help throughout the period of this study reading and comment on the histopatholagical specimens.

REFERENCES

Abbas, H.H. (1994): Effect of lead on some physiological and biochemical aspect of Nile tilapia; *Oreochromis niloticus*. M.Sc. Thesis, Faculty of Science, Cairo University, Egypt.

Abd-Allh, G.A.; Mansy, S.E.; Khider, A.A.; Hassan, S.H. and El-Fayoumi, R.I. (2002): Evaluation of physiological changes in canal catfish during exposure to different levels of metal pollution. Proc. 2^{nd} Conf. Food Borne Contamination and Egyptians Health, 23-24 April, El-Mansoura, Egypt. p. 201-211.

Abdelhamid, A.M.; Maha, M.M. Gawish and Soryal, K.A. (2006): Comparative study between desert cultivated and natural fisheries of Mullet in Egypt: 1- concerning heavy metals. J. Agric. Sci., Mansoura Univ., 31 (9): 5665-5680.

Abou-Zaid F.A., Shenouda, T.S. and Abouda, A.E. (1992): Electron microscopal observations on hepatotoxic effects of zinc on Nile fish, *Oreochromis niloticus*. Delta J. Sci., 16 (3): 707-722.

Ahmed, Sh.M. and El-Kamel, A.A. (2006): *Proteous vulgaris*, an emerging fish pathogen in Egypt. Assiut Vet. Med. J., 52 (111): 36-50.

Ajmal M.; Khan M.A. and Nomani A.A. (1985): Distribution of heavy metals in plant and fish of the Yamuna River (India). Environ. Monit. Assess., (5): 361-367.

Al-Ekel, A. (1994): Change in behaviour, tissue glycogen and blood chemistry of freshwater carp, *Cyprinus carpio* in relation to toxicity of lead. Zeits-chrift-fur-Angewandte-Zoologie, 80 (1): 87-96

Ali, S.M.; Mona S. Zaki and Hala M. El-Genaidy (2003): Pathological, Biochemical, Haematological and Hormonal changes in catfish (*Clarias gariepinus*) exposed to lead pollution. J. Egypt. Vet. Med. Asso., 63 (1): 331-342.

Allain, C.C.; Poon, L.S.; Chan, C.S.G.; Richmond, W. and Fu, P.C. (1974): Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Almeida, J.A.; Novelli, E.L.; Dalpai Silva, M. and Junior, R.A. (2001): Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. Environ. Pollute., 114 (2): 169-175.

Boyd, C.E. (1990): Water quality in ponds for aquaculture in warm water fishponds. Aubmn Univ., Alabama, U.S.A.

Capar, M.S.G. (1977): Metals and other elements. J. of A.O.A.C., 60: 1401-1408.

Chopra, I.J. (1972): A radioimmunoassay for measurement of thyroxine in unextracted sera. J. of Clin. Endocrinol. Metabol., 34: 938.

Crespo, S. and Karnaky, K.J. (1983): Copper and zinc inhibit chloride transport a cross the opercular epithelium of seawater adapted killifish *(fundulus heterclitus)* short communications. J. Exp. Biol., 102: 337-341.

De Gregori, L.; Pinochet, H.; Delgado, D.; Gras, N. and Munoz, L. (1994): Heavy metals in *Bivalve mussels* and their habitats from different sites along the children coast Bull. Environ. Contam. Toxicol., 52: 261-268.

De Smet, H.; Blust, R. and Moens, L. (2001): Cadmium-binding to transferrin in the plasma of the common carp *Cyprinus carpio*. Comp. Biochem. Physiol., 128 (1): 45 - 53.

Dethloff, G.M.; Schlenk, D.; Hamm, J.T. and Bailey, H.C. (1999a): Alterations in physiological parameters of rainbow trout (*Oncorhynchus mykiss*) with exposure to copper and copper/zinc mixtures. Ecotoxicol. Environ., Saf. 24 (3): 253-264.

Dethloff, G.M.; Schlenk, D.; Khan, S. and Bailey, H.C. (1999b): The effect of copper on blood and biochemical parameters of rain bow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol., 36 (4): 415-423.

Doumas, B.; Bayso, D.; Crter, R.; Peters, T. and Schaffer, R. (1981): Determination of serum total protein. Clin. Chem., 27: 1642.

Duncan, D.B. (1955): Multiple ranges and multiple F test. Biometrics, 11: 1-42.

Eastman, C.J.; Corcoran, J.M.; Ekins, R.P.; Williams, E.S. and Nabarro, J.D. (1975): The radioimmunoassay of triiodothyronine and its clinical application. J. of Clin. Path., 28: 225.

Eisler, R. (1967): Acute toxicity of zinc to the killifish, *Fundulus heteroclius*. Chesapeake Sci. (8): 262-264.

El-Naggar, G.O.; Zaghloul, K.H.; Salah El-Deen, M.A. and Abo-Hegab, S. (1998): Studies on the effect of industrial water pollution along different sites of the River Nile on some physiological and biochemical parameters of *Nile tilapia*, *Oreochromis niloticus*, 4th Vet. Med. Zag. Congress (26-28 August, 1998 in Hurghada): 713-735.

Elsa, M. (1991): Metal poisoning in Fish. CRC Press Boea Raton, Boston.

El-Shaieb Ahmed, F.; Viola H. Zaki and Ahmed M. El-Ashram (2001): Pathological studies on the side effects of some environmental pollutants in *Tilapia Zilli.* The Second International Scientific Conference. The role of veterinary medicine for community. Faculty of Veterinary Medicine, Mansoura University, Mansoura. 8-9 April 2001, pp. 599-636.

Fantin, B.; Antonel, L.; Pierluigi, T. and Pederzoli, A. (1992): Histomorphological and cytochemical changes induced in the liver of goldfish, *(Carassius carassius)* var Auronls by short term exposure to lead. Acta Histochemical Biol., 92: 228-235.

FAO/WHO (1992): Codex alimentarius commission, standard programme codex committee on food additives and contaminates. 24th Session, Hague, 23-28 March, 1992.

Ferranda, M. and Andrew, M. (1991): Effect of lindane on the blood of fresh water fish. Bull. Environ. Contam. Toxicol., 97: 465-470.

Foster, L. and Dunn, R. (1974): Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clin. Chem., 20: 365.

Frank, H.M. (1950): Photometric analysis of albumin-globulins in serum. Dtsch. Arch. Klin. Med., 197: 181.

Ghazally, K.S. and Said, K.M. (1995): Physiological characteristics of *Tilapia nilotica* under acute stress of copper. J. Egypt. Ger. Soc. Zool., (16A): 287 – 301.

Ghosh, S. and Bhattacharya, S. (1992): Elevation of C-reactive protein in serum of *Channa punctatus* as an indicator of water pollution. Indian J. Exp. Biol., 30 (8): 736-737.

Gill, T.S.; Tewari, H. and Pande, J. (1992): Short and long term effects of copper on the rosy barb *(puntius conchoius Ham)*. Ecotoxicol Environ. Saf., 23 (3): 294-306.

Gold Frank, L.; Fomenbaum, N.; Lewin, N.; Weisman, R. and Howland, M. (2001): Glod Frank's Toxicological Emergenices. 5th Ed. Prentice-Hall International, INC., New Jersey, USA.

Greig, R.A.; Sawyer, T.K.; Lewis, E.T. and Galasso, M.E. (1982): A study of metal concentrations in relation to gill color and pathology in rock carb. Arch. Anviron. Contam. Toxicol., (8): 539-697.

Gupta, P.; Kar, A.; Maiti, P.K. and Chaurasia, S.S. (1995): Chronic cadmium administration alters circulating thyroid hormone concentrations and the status of reproductive organs in male mouse fresen. Environ. Bull. 4: 333 – 335.

Hafez, M.H.; Anisa M. Moustafa; Aly, S.M.; El-Matbuli, M.A. and Nouh, W. (2003): Pathological and electeron microscopic evaluation of the effect of water pollution with lead acetate in the health status and immune response of catfish (*Clarias gariepinus*) SCVM J. VI (1) 2003.

Haggag, A.M.; Mohamed, A.S.M. and Khaled, H.Z. (1999): Seasonal effects of the industrial effluents on the Nile catfish; *Clarias gariepinus*. J. Egy. Ger. Soc. Zool., 28A: 365-391.

Heakel, N.A.S.A. and Shehata, R.E.E. (1999): Effect of subacute exposure to cadmium on serum glucose, insulin, cortisol and thyroid hormones in Nile catfish (Clarias lazera). SCVMJ, II (1): 115 – 123.

Heckman, M. (1970): Collaborative study of copper, sodium and potassium in feed by atomic Absorption Spectrophotometry and sodium and potassium by flame emission spectrophytometery. J. AOAC, (53): 923.

Hema, T.; Gill, T.S. and Pande, J. (1987): Impact of chronic lead poisoning on the hematological profiles of fish. Bull. Environ. Contam. Toxicol., 38: 748.

Hilmy, A.M.; Domiaty, N.A.; Daabees, A.Y. and Abdel Latife, H.A. (1987): Some physiological and biochemical indices of zinc toxicity in two fresh water fishes, *Clarias lazera* and *Tilapia zilli*. Comp. Biochem. Physiol., 87 (2): 297-301.

Husden, H. and Ropaport, A. (1968): Estimation of creatinine. Clin. Chemistry, 14: 222.

James, V.A. and Wigham, T. (1986): The effects of cadmium on prolactin cell activity and plasma cortisol levels in rainbow trout (*Salmo gairdneri*). Aquat. Toxicol., 8: 273.

Jehan R. Daoud; Aida M. Amin and Menha M. Abo El-Khalek (1999): Residual analysis of some heavy metals in water and *Oreochromis niloticus* fish from polluted areas. Vet. Med. J., Giza. 47 (3): 351-365.

Khalil, T.M. and Hussein, A.H. (1996): Recycling and reuse of wastewater for fish farming: An experimental field study at Sewage Treatment Plant, Egypt. J. Egypt. Ger. Soc. Zool., 19 (B): 59-79.

Klaverkamp, J.F.; MacDonald, W.A.; Duncan, D.A. and Wageman, R. (1984): Metallothionein and acclimation to heavy metals in fish are view. In contaminant effect on fisheries. (V.W. carirns, P.V. Hodson and J.O. Nriague, eds): Chi Chester and New York. John.

Lucky, Z. (1977): Methods for diagnosis of fish diseases. Glem, L. Hoffman (Editor). Fish and Wildlife Service, New Delhi, Bombay Calcutta, New York, USA. 69.

Marie, M.A.S. (1990): Effect of sublethal concentration of zinc on blood parameters of the Nile catfish; *Clarias lazera*. Proc. Zool. Soc. A.R.E. 18: 413 - 421.

Mazeaud, M.M. and Mazeaud, F. (1981): Adrenergic responses to stress in fish, In: Stress and Fish Pickering, A.D. (ed.), Academic Press, New York. P. 74.

Metwally, A.A.M.; El-Sayed, S.A.M.; Ali, A.M. and Abd Elnaser, G. (2002): Biochemical changes in serum of catfish; *(Clarias gariepinus)* exposed to some chemicals compounds. SCVM J. (2): 719-724.

Mona O. Abou El-Nile and Sawsan M. Arafa (2004): Incidence of heavy metals in local and imported fish. ISSN, 110-2047. Alex. J. Vet. Science, 20 (1): 7-19.

Nagdi, A.Z. and Shaker, I. (1998): Effect of water sources on some microelements in fish farm. Egypt. J. Agric. Res., 76 (1): 351-357.

Noga, E.J. (1996): Fish disease diagnosis and treatment. Mosby in Year Book, Inc. USA.

Patton, C.J. and Crouch, S.R. (1977): Determination of urea. Anal. Chem. 49: 464-469.

Polpraset, C. (1982): Heavy metal pollution in the Chaephraya River, Estuary, Thailand, Water, Res. (16): 775-784.

Pratap, H.B.; Lock, R.A.C. and Bonga, S.E.W. (1989): Effect of waterborne and dietary Cadmium on plasma ions of the telost *Oreochromis mossambicus* inrelation to water calcium levels. Aech, Environ. Contam. Toxicol., 18 (4): 568-575.

Reitman, A. and Frankel, S. (1957): Colorimetric method for determination of GOT and GPT. J. Clin. Path., 28: 56.

Robert, J.R. (1989): Fish pathology. 2nd ed. Bailliere Tindall. Pp. 391-392.

Saeed S.M. (2000): A study on factors affecting fish production from certain fish farms in the delta. M.Sc. Thesis. Department of Biological and Physical Sciences Institute of Environmental Studies and Research, Ain Shams University.

Salah El-Deen M.A.; Zaghloul, K.H.; El-Naggar, G.O. and Abo-Hegab, S. (1999): Concentrations of heavy metals in water, Sediment and fish in the River Nile in the industrial area of Helwan. Egypt. J. Zool., (32): 373-395.

Salah El-Deen, M.A.; Sharada, H.M. and Abu Ella, S.M. (1996): Some metabolic alterations in grass carp (Ctenopharyngodonidella) induced by exposure to cadmium. J. Egypt. Ger. Soc. Zool., 21 (A): Comparative Physiology, 441-457.

SAS (2001): SAS/STAT® User's Guide: Statistics. Ver. 8.2 SAS Institute Inc., Gary, NC.

Schreck, C.B. and Loz, H.W. (1978): Stress response of coho salmon; *Oncorhynchus kisutch* elicited by cadmium and copper stress. J. Fish. Res. Bd. Can., (35): 114-1129.

Shereif, M.M. and Moaty, M.A. (1995): Concentration levels and accumulation of heavy metals in Nile Tilapia grown in wastewater-fed fish pods. Sec. Midd. East. Conf. Waste Mang., Cairo, 284 - 298.

Shibamoto, T. and Bjeldanes, L.F. (1993): Introduction to Food Toxicology. Academic Press, Inc Harcourt Brace and Company. New York. Food Science and Technology International Series.

Skidmore, J.F. (1974): Factors affecting the toxicity of pollutants to fish. J. Vet. Res., 94: 456-458.

Sorensen, E. (1991): Metal poisoning in fish. Oxford and IBH Publishing Co., Bombay.

Sprenger, M.D.; McIntosh, A.W. and Lewis, T. (1987): Variability in concentrations of selected trace elements in water and sediments of six acidic lakes. Arch. Environ. Contam. Toxicol., (16): 383-390.

Stoskopf, M.K. (1993): Fish medicine. W.B. Saunders Comp. Philadelphia, pp. 113-131.

Tabche, L.; Martinez, C and Hidalgo, S. (1990): Comparative study of toxic lead effect on gill and hemoglobin of Tilapia fish. J. Appl. Toxicol., 10 (3): 193-195.

Tewari, H.; Gill, T. and Pont, J. (1987): Impact of chronic lead poisoning on the hematological and biochemical profiles of fish, *Barbus conchonius*. Bull. Environ. Contam. Toxicol., 38 (5): 748-752.

Trinder,P. (1969): Enzymatic determination of glucose. Ann. Clin. Biochem. 6 : 24 – 26.

Whitby, L.G.; Smith, A.F. and Beckett, G.J. (1989): Lecture Notes on Clinical Chemistry. 4th ed., Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Melbourne. P. 138.

World Health Organization (WHO) (1993): Guide lines for drinking water quality, 2nd, 1, WHO, Geneva.

WHO, (1992): Environmental Health Criteria. No. 134. Cadmium. WHO, Geneva.

Young, D.S. (2001): Effects of diseases on clinical lab. Tests, 4th Ed. AACC.

Zaghloul, K.H. (1997): Studies on the effect of water pollution along different sites of the River Nile on the survival and production of some freshwater fishes. Ph.D. Thesis., Zoology Dept., Fac. of Sci., Cairo Univ.

Zaghloul, K.H. (2000): Effect of different water sources on some biological biochemical aspects of the Nile tilapia, *Oreochromis niloticus* and the Nile catfish, *Clarias gariepinus*. Egypt. J. Zool., (34): 353-377.

Zaghloul, K.H.; Fayed, M.H. and Mohamed, A.H. (2001): Fish parasites as biological indicators of pollution with heavy metals. J. Union Arab Biol. (15A), Zoology, 533-561.

Zahra, M.H.; Elghobashy, A.H.; Zaied, A.F.; Diab, A.A. and Farg, M.E. (2001): Physiological studies on some fish collected from polluted locations. The Second International Scientific Conference. The role of veterinary medicine for community. Fac. Of Vet. Med., Mansoura University, Mansoura, 8-9, April, 2001. pp. 599-636.

Zikic, R.V.; Stajan, A.S.; Pavlovic, S.Z.; Ognjanovic, B.L. and Saicic, Z.S. (2001): Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (Carassius auratus gibelio Bloch.) exposed to cadmium. Physiol. Res., 5 (1): 105-111.