

**IMPACT OF SOME TRACE METALS POLLUTION IN
THE RIVER NILE WATER ON MUSCLES OF
CLARIAS GARIEPINUS INHABITING
EL-KANATER EL-KHYRIA AND
HELWAN SITES**

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ABSTRACT: This study was conducted to assess the effect of some trace metals (Fe, Cu, Pb and Cd) on the muscles of *Clarias gariepinus* in some areas of River Nile. Concentrations of these metals in water and their accumulation in muscles of *Clarias gariepinus* were investigated during winter and spring 2004 at El-Kanater El-Khyria and Helwan regions. Also, the histological changes of fish muscles were studied. The present results show that trace metals concentration in water and their accumulation in fish muscles at the two regions during two consecutive seasons were detected in the following descending order : Fe > Pb > Cu > Cd. Histological study indicated that the muscles of *Clarias gariepinus* living in the two regions suffered from several pathological alterations including: degeneration, necrosis, hemorrhage, hemolysis and hemosidrin. It was noticed that the muscles of the fish collected from Helwan region showed much more damages than that collected from El-Kanater region as the first site receives more drainage water loaded with industrial wastes than the latter.

Key words: Trace metals, *Clarias gariepinus*, River Nile, histopathology, necrosis, hyperplasia, hypertrophy.

INTRODUCTION

The River Nile is the life artery of Egypt. In the past, the flood water was the main characteristic of the River Nile regime which cleaned the river every year. After the construction of the High Dam, the Nile lost its capacity for self purification. It becomes also, subjected to different types of pollutions ; chemical (industrial), biological (domestic and agricultural) and thermal pollution Meanwhile, the raw industrial wastes (untreated) are drained from many factories along the Nile.

River Nile extends through several hundred kilometers from Aswan till Cairo, then it bifurcates into two branches at El-Kanater El-Khyria, north Cairo to Damietta and Rosetta branches. The two branches extend to pour into the Mediterranean Sea.

Several works were conducted to explore the effect of water pollutants on fish organs and to estimate the amount of heavy metals on different fish organs (Abdel-Satar, 1998; Yacoub, 1999; Abdel-Satar and Shehata, 2000; Abdel-Satar and Elewa, 2001; Elewa *et al.*, 2001; Mahmoud, 2002; El-Serafy *et al.*, 2005;

Ibrahim and Mahmoud, 2005 and Ibrahim and Tayel, 2005).

Some heavy metals such as Zn, Cu, Mn and Fe are essential for growth and well being of living organisms including man. However, they likely show toxic effects when organisms are exposed to level of doses higher than those of the normally required. Other elements such as Pb and Cd are not essential for metabolic activities and exhibit toxic properties even with trace levels. Many heavy metals including Hg, Cd, Pb, As and Cu, inhibit photosynthesis and phytoplankton growth. At higher trophic levels, they have delayed embryonic development, causing malformation and reduced growth of adult fish, molluscs and crustaceans (FAO, 1992).

Fish which live in polluted water may accumulate toxic trace elements via their food chain, thus possibly endangering human health (Mahmoud, 2002).

In regard to metals accumulation in aquatic organisms, Saleh *et al.*, (1988) studied the accumulation of heavy metals in body tissue and organs of *Tilapia zillii*, but they didn't find any change with the seasons of collection or with the age of the fish except for lead.

Abdel-Baky *et al.* (1998), studied the heavy metals concentration in some organs of *Oreochromis niloticus* and reported that, the concentration of heavy metals (Cd, Zn, Cu and Pb) in different organs and tissues of these fish varied considerably with regards to seasons and sites.

Phillips, (1980), reported that many factors affect the rate of heavy metals accumulation including seasonal variation (temperature) . Weakly bound metals in soft organs (liver) may be more easily influenced by seasonal changes than those strongly bound in flesh (Gomaa, 1995). The distribution of heavy metals among the major components of aquatic ecosystem may be affected by sampling area (PNUE, 1984).

Yacoub, (1999) studied the heavy metals concentration in the organs of *Clarias gariepinus* and in water of the River Nile between Helwan and El-Kanater El-Khyria, reporting that, concentration of the trace metals (Fe, Mn, Pb and Cd) were found in the order of $Fe > Mn > Pb > Cd$. The increase of heavy metals in Helwan site was due to the industrial wastes discharged in the River Nile from the Iron and Steel Factory. He added that, the accumulation of

heavy metals in fish are varying according to the locality and tissue type.

Mahmoud, (2002) studied the effect of trace metals in the River Nile (iron, zinc, copper and lead) in water and fish. He deduced that, the heavy metals concentration of water and fish flesh were found in the order of $Fe > Mn > Pb > Cu$. He added that, the increase in heavy metals may be due to the industrial discharge into the River Nile.

Ibrahim and Mahmoud, (2005) studied the heavy metals (Fe, Mn, Zn, Cu, Pb and Cd) in the liver tissues of the three fish species (*Tilapia zillii*, *Clarias gariepinus* and *Mugil cephalous*) caught from River Nile water at El-Kanater El-Khyria region . They reported that the high concentration of iron was recorded in *Tilapia zillii* followed by *Clarias gariepinus* and *Mugil cephalous*. The increase of Fe may be related to the increase of total dissolved iron in River Nile water and consequently increases the free metals iron concentration and thereby leads to an increase in metals uptake by different organs. Also, Mn was accumulated by the following order $Tilapia zillii > Mugil cephalous > Clarias gariepinus$. For all fish species, Zn,

Cu, Pb and Cd concentrations were higher than the permissible limit of WHO and NHMRC (National Health and Medical Research Council in Australia).

Al-Afify, (2006) studied trace elements concentration (Fe, Mn, Zn, Cu, Pb and Cd) in water and in two common fish species (*Oreochromis niloticus* and *Tilapia zillii*) in the River Nile at Damietta branch and he found such metals in this order Fe > Mn > Zn > Pb > Cu > Cd and Fe > Zn > Pb > Mn > Cd > Cu. In these fish species, respectively.

Although muscle is the most edible part of fish body, it is the most exposed part to be damaged by several types of pollution (Mahmoud, 2002; Tayel, 2003 and El-Serafy *et al.*, 2005).

The present study aims to investigate the effect of heavy metals on *Clarias gariepinus* muscle. The study is essentially focusing on the histopathological lesions of muscles and accumulation of heavy metals in those muscles. In addition, concentration of these trace metals in surrounding water was also considered.

MATERIALS AND METHODS

Water and fish samples were collected from two stations, the

first was El-Kanater El-Khyria City while the second was Helwan City during winter and spring seasons, 2004.

Water Samples:

Twenty ml of concentrated nitric acid was added to 500 ml of water sample in a beaker and boiled on a hot plate until complete digestion. The remaining volume was made up to 100 ml with deionized distilled water. A portion of this solution was used for the determination of heavy metals. The metals (Fe, Cu, Pb, and Cd) were determined using Graphite Atomizer (G.A.Z) atomic absorption spectrophotometer (Hitachi model 170-30) and the readings were compared to a standard curve. The results are expressed in $\mu\text{g l}^{-1}$.

Fish Samples:

Fish samples of *Clarias gariepinus* were collected and the muscles were carefully removed and fixed; half of each was placed in 10 % formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin, sectioned (μS), stained with hematoxylin and eosin (H&E) and examined microscopically. Another half of muscle of each

sample was transferred to a weighing beaker and placed in a drying oven thermostatically regulated at 105 °C overnight. Dried sample (1gm) was taken and digested according to the method described by Goldberg *et al.*, (1963) in which concentrated nitric and perchloric acids with ratio of 5 ml + 5 ml were used. The digested solutions were cooled and made up to 25 ml using deionized water. The concentration of trace elements (Fe, Cu, Pb and Cd) in solution were determined using Graphite Atomizer (G.A.Z) atomic absorption spectrophotometer (Hitachi model 170-30) and the readings were compared to a standard curve. The results were expressed in µg dry weight/g body weight.

RESULTS AND DISCUSSION

Trace Metals in Water:

The River Nile is the main source for potable water and as a result of human activities in and on the River body, it became loaded by metal pollution (Abdo, 2004; Abdel-Satar, 2005 and Ibrahim and Tayel, 2005).

Table 1 shows water concentration of trace metals viz: Fe, Cu, Pb and Cd at El-Kanater El-khyria and Helwan regions during winter and spring, 2004.

The highest values of copper, lead and cadmium were recorded during winter, while the lowest values were observed during spring. The levels of iron were lower winter during than those spring. Also, in the present study,

Table 1. Heavy metals concentration (µg/l) in the River Nile at El-Kanater El-Khyria and Helwan region during winter and spring seasons (2004)

Season Station Parameter	Winter		Spring	
	I	II	I	II
Fe	310.00	484.00	803.00	960.00
Cu	18.07	17.87	12.53	17.73
Pb	67.00	72.67	38.00	33.63
Cd	8.87	17.80	7.73	8.47

I: El-Kanater El-Khyria region

II: Helwan region

the level of trace metals was in the following descending order: Fe > Pb > Cu > Cd, which is in accordance with Al-Afify, (2006).

Iron is an abundant and important element, unsurpassed by any other heavy metal in the earth's crust. In the present study the iron concentrations in the water of El-Kanater and Helwan regions were 310 and 484 µg/l during winter and 803 and 960 µg/l during spring, respectively. The decrease in iron concentration during winter season may be due to the increase in dissolved oxygen resulting in the oxidation of iron and its precipitation under alkaline pH (Abdel-Satar, 2005). On the other hand, the relative increase in iron concentration at Helwan actually may be due to industrial wastes effluent to the River Nile from Iron and Steel Factory in this area.

Copper is a micronutrient, essential to all form of life, in enzyme activity or random rearrangement of natural proteins (Bower, 1979). The copper metals is insoluble in water but many of its salts are highly soluble (Anon, 1978). Also, detritus effects of free copper concentration on fish and zooplankton were greater in hard water than in soft one (Mount, 1968). In the present study, Cu

value varied between 17.87 and 18.07 µg/l during winter and between 12.53 and 17.73 µg/l during spring at El-Kanater and Helwan regions, respectively. The increase in water copper concentration is mainly due to domestic sewage discharge from boats and tourist ships, as the domestic sources were reported to be the major contributors of copper in the environment (Issa *et al.*, 1997 and Abdel-Satar, 2005).

Lead is a serious accumulative body poison. It acts by complexing the oxo-group in enzymes and affects virtually all steps in the presence of hemosynthesis. It also inhibits acetylcholine esterase, acid phosphatase, ATPase, carbonic anhydrase etc. and also inhibits protein synthesis by modifying transfer RNA. Typical symptoms of lead poisoning are colic, anaemia, headache, convulsion, chronic nephritis, brain damage and nervous system disorder (Greenwood and Earnshaw, 1985). The concentrations of water lead in the present study were 67.00 and 72.67 µg/l during winter but 38.00 and 33.63 µg/l during spring at El-Kanater and Helwan regions, respectively. The high concentration of lead may be attributed to the industrial wastes

inflow, atmospheric inflow of dust containing car exhaust and increase in density of boats and ships, which discharge their effluents directly to the Nile adding high amount of Pb in both the dissolved and particulate phases (Kadry *et al.*, 2003). The increase in Pb during winter season might be attributed to the decrease in water discharges during drought period, where the dilution and assimilative capacities of the Nile water are low as cited by Abdel-Satar, (2005).

Cadmium is used in various industrial processes, and as a product of zinc mining, fuel and base metal smelting. This biologically non-essential elements is highly toxicant (Ibrahim and Tayel, 2005). In the present study, the cadmium concentrations were 8.87 and 17.80 $\mu\text{g/l}$ during winter

and 7.73 and 8.47 $\mu\text{g/l}$ during spring at El-Kanater and Helwan, respectively. The high concentration of cadmium in the present study may be due to direct input of sewage, agricultural and industrial wastes (Abdel-Satar and Shehata, 2000; Ibrahim and Mahmoud, 2005).

Trace Metals Accumulation in *Clarias gariepinus* Muscles:

The accumulation of four selected trace metals: Fe, Cu, Pb and Cd in the flesh of *Clarias gariepinus* caught during winter and spring seasons, 2004, from the two selected stations at the River Nile (Barrage and Helwan) are presented in Table 2 .

Trace metals in the *Clarias gariepinus* flesh caught from

Table 2 . Heavy metals accumulation in muscles ($\mu\text{g/g}$ dry wt.) of *Clarias gariepinus* located at El-Kanater El-Khyria and Helwan regions during winter and spring seasons (2004) .

Season Station Parameter	Winter		Spring	
	I M \pm SD	II M \pm SD	I M \pm SD	I M \pm SD
Fe	40.96 \pm 0.91	50.75 \pm 0.67	59.25 \pm 1.12	64.45 \pm 0.66
Cu	2.11 \pm 0.53	3.25 \pm 0.96	1.90 \pm 0.23	2.79 \pm 0.32
Pb	7.79 \pm 0.70	8.92 \pm 0.95	8.75 \pm 0.98	10.93 \pm 1.13
Cu	1.20 \pm 0.33	1.04 \pm 0.28	1.10 \pm 0.23	1.30 \pm 0.80

I: El-Kanater El-Khyria region

II: Helwan region

Helwan region were higher than those of the same fish caught from El-Kanater region during the two selected seasons. This may be due to the large amount of industrial, agricultural run-off and sewage discharging at Helwan water region as recorded by WHO, (1977); El-Serafy *et al.*, (2005) and Al-Afify, (2006).

On the other hand, the accumulation of trace metals in flesh of *Clarias gariepinus* was higher during spring than that during winter for the two stations which may be due to heavy loaded industrial, agricultural and sewage wastes as cited by Ibrahim and Tayel, (2005).

The highest value ($64.45 \pm 0.566 \mu\text{g/g}$) of iron accumulation in flesh of *Clarias gariepinus* was recorded during spring at Helwan region, while the lowest one ($40.96 \pm 0.907 \mu\text{g/g}$) was recorded during winter at El-Kanater El-khyria region. The muscles Fe level was higher than the permissible limit ($30.0 \mu\text{g/g}$). These increases of iron concentration in flesh may be related to increase of total dissolved iron in Nile water and consequently increase the free metal iron concentration and accordingly increased iron uptake by different organs (Carbonell *et al.*, 1998 and Ibrahim and Mahmoud, 2005).

Copper concentration in muscles of *Clarias gariepinus* in the present study was in the level of $1.90 \pm 0.235 \mu\text{g/g}$ during spring at El-kanater El-khyria. These results agree with the results of El-Serafy *et al.*, (2005). Increased Cu during winter may be due to the decrease in water discharging during this season, as recorded by Abdel-Satar and Shehata, (2000).

Lead concentration in flesh of *Clarias gariepinus* ranged between $7.79 \pm 0.678 \mu\text{g/g}$ during winter at Barrage and $10.93 \pm 1.13 \mu\text{g/g}$ during spring at Helwan region. These values are considered high because the recommendations of National Health and Medical Research Council in Australia (NHMRC), which imply that the concentration of Pb in the edible part of fish should not exceed $2.0 \mu\text{g/g}$ (Bebbington *et al.*, 1977). In present study the high concentration of lead may be attributed to the industrial waste inflow as recorded by Kadry *et al.*, (2003).

Cadmium concentration in flesh of *Clarias gariepinus* in the present study ranged between $1.046 \pm 0.278 \mu\text{g/g}$ during winter at Helwan region and $1.3 \pm 0.786 \mu\text{g/g}$ during spring at the same station. Cadmium levels in the investigated fish muscles were

generally higher than permissible limit (0.5 µg/g) of National Academy of Science (1972). The high concentration of cadmium may be due to direct input of sewage, agricultural and industrial wastes (Ibrahim and Tayel, 2005).

Histopathological Changes in the Muscles;

The muscles are composed chiefly of segmental myomeres. Each myomere is regarded as apparent muscle and its fibers are parallel to the long axis of the body. This muscle layer covered with skin which formed of three layers (epidermis, dermis and hypodermis). Also, this skin is covered with an epithelial layer.

In present study, the major malformation observed in skin and muscle of *Clarias gariepinus* caught from the two regions (Helwan and El-Kanater El-khyria) during the two seasons (winter and spring) were:

- Absence (A) of epithelial layer (Fig. I, a).
- Hypertrophy (Ht) and hyperplasia (Hy) of mucous cells of epidermal layer (Fig. I, b, c).
- Degeneration (D) of collagen bundle of dermal layer (Fig. I, a, d).
- Necrosis (N), Dilation (Di) and hemosidrin (Hn) in blood vessel and connective tissue of

hypodermal layer (Fig. I, e, f).

-Hemorrhage (Hr) and degeneration (D) in myomeres of muscle layer (Fig. II, a, d).

-Necrosis (N) in myomeres of muscle layer (Fig. II, a, d).

-Dilation (Di) in blood vessel at muscle layer (Fig. II, c).

-Odema (O) in myomeres of muscle layer (Fig. II, b, e).

Fig. (I): a)): V. S. of skin of *Clarias gariepinus* obtained from El-Kanater El- Khyria region during winter season, showing : degeneration(D) in collagen bundle layer.(Bouin's H&E) x400 .

b)): V.S. of skin of *Clarias gariepinus* obtained from El-Kanater El-Khyria region during spring season, showing : Hypertrophy (Ht) in mucous cells.(Bouin's H&E) x 400 .

c)): V.S. of skin of *Clarias gariepinus* obtained from Helwan region during spring season , showing: Hyperplasia (Hy) of mucous cells (Bouin's H&E)x400 .

d)): V. S. of skin of *Clarias gariepinus* obtained from El-Kanater El-Khyria region during spring season, showing : Degeneration (D) in collagen bundle layer (Bouin's H&E) x 400

e)): V.S. of skin of *Clarias gariepinus* obtained from Helwan region during spring season, showing: Necrosis (N) in

connective tissue.(Bouin's H&E) x 400 .

f)):V. S. of skin of *Clarias gariepinus* obtained from Helwan region during spring season showing: Hemorrhage (Hr) ,hemosidrin (Hn) and Dilation (Di) in connective tissue and blood vessel . (Bouin's H&E) x 400 .

Fig. (II), a)):V. S. of muscles of *Clarias gariepinus* obtained from El- Kanater El-Khyria region during winter season, showing: Hemorrhage (Hr) and degeneration (D) in muscle fibers .(Bouin's H&E)x 400 .

b)):V. S. of muscles of *Clarias gariepinus* obtained from Helwan region during spring season, showing: Necrosis (N) and Edema (E) in muscle fibers layer.(Bouin's H&E)x 400 .

c)):V. S. of muscles of *Clarias gariepinus* obtained from Helwan region during spring season, showing: Dilation (Di) in blood vessel.(Bouin's H&E) x 400

d)):V. S. of muscles of *Clarias gariepinus* obtained from Helwan region during spring season, showing: Necrosis (N) and Hemorrhage (Hr) in muscle layer (Bouin's H&E)x 400 .

e)):V. S. of muscles of *Clarias gariepinus* obtained from Helwan region during spring season, showing: Edema (E) in

muscle fibers.(Bouin's H&E)x400

f)):V.S. of muscles of *Clarias gariepinus* obtained from El- Kanater El- Khyria region during winter season, showing : Necrosis (N) in muscle fibers. (Bouin's H&E) x 400 .

These histological changes of muscle specimens obtained from *Clarias gariepinus* living in Helwan region were of more and higher degree than those obtained from the same fish species living in El-Kanater region. Also, these changes appeared during spring at the two regions than those obtained during winter.

This may be due to high heavy metal concentrations in water and their accumulation in fish muscles. These results agree with that obtained by Yacoub, (1999);Mabrouk, (2004) and El-Serafy *et al.*, (2005).

Finally, it can be concluded that the muscles of *Clarias gariepinus* were contaminated with heavy metals arising from elevated discharges of different wastes into the River Nile at El-Kanater El-Khyria and Iron and Steel Factory at Helwan region. The results indicated that the examined heavy metals accumulated in the fish muscle lead to several histopathological alternation in these muscles .

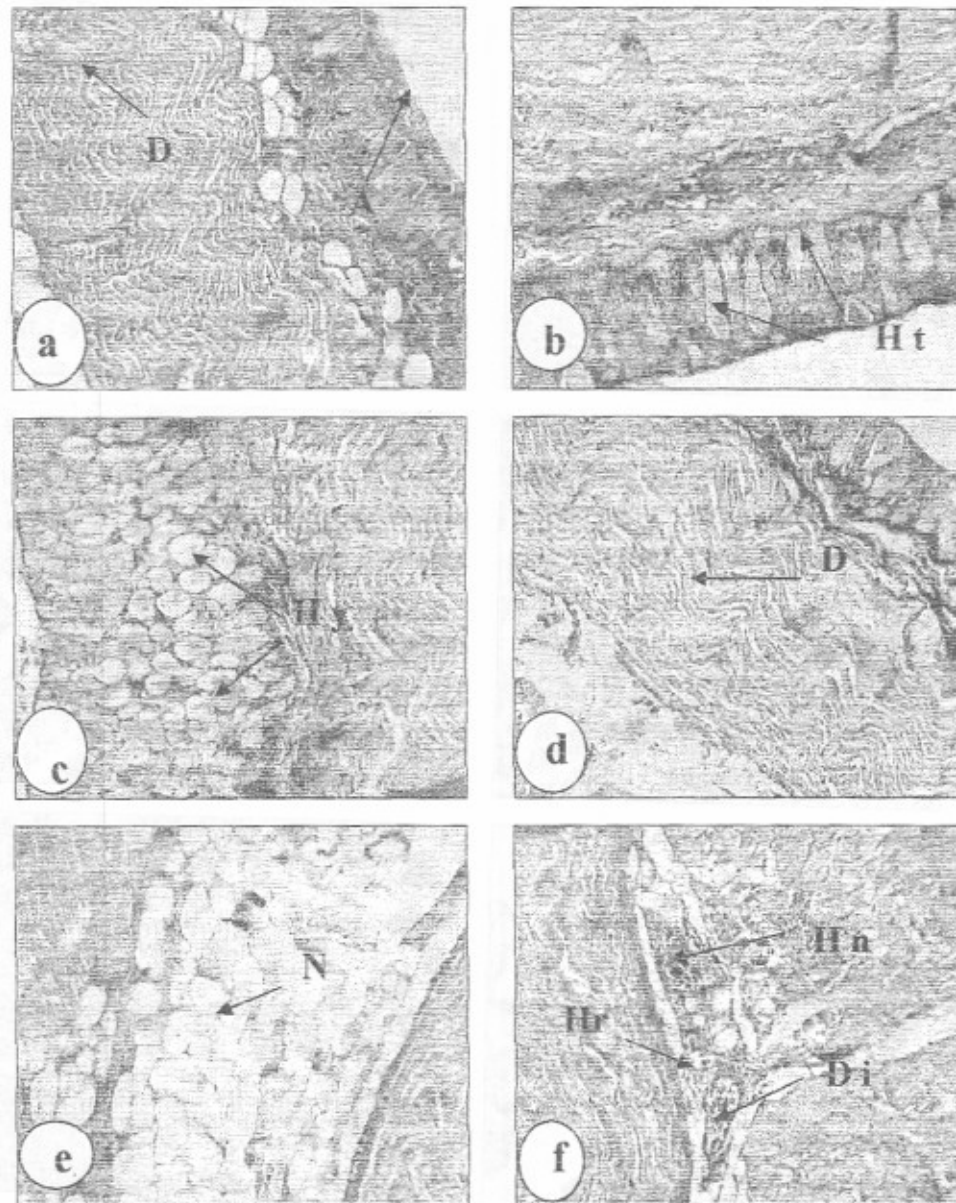


Figure 1

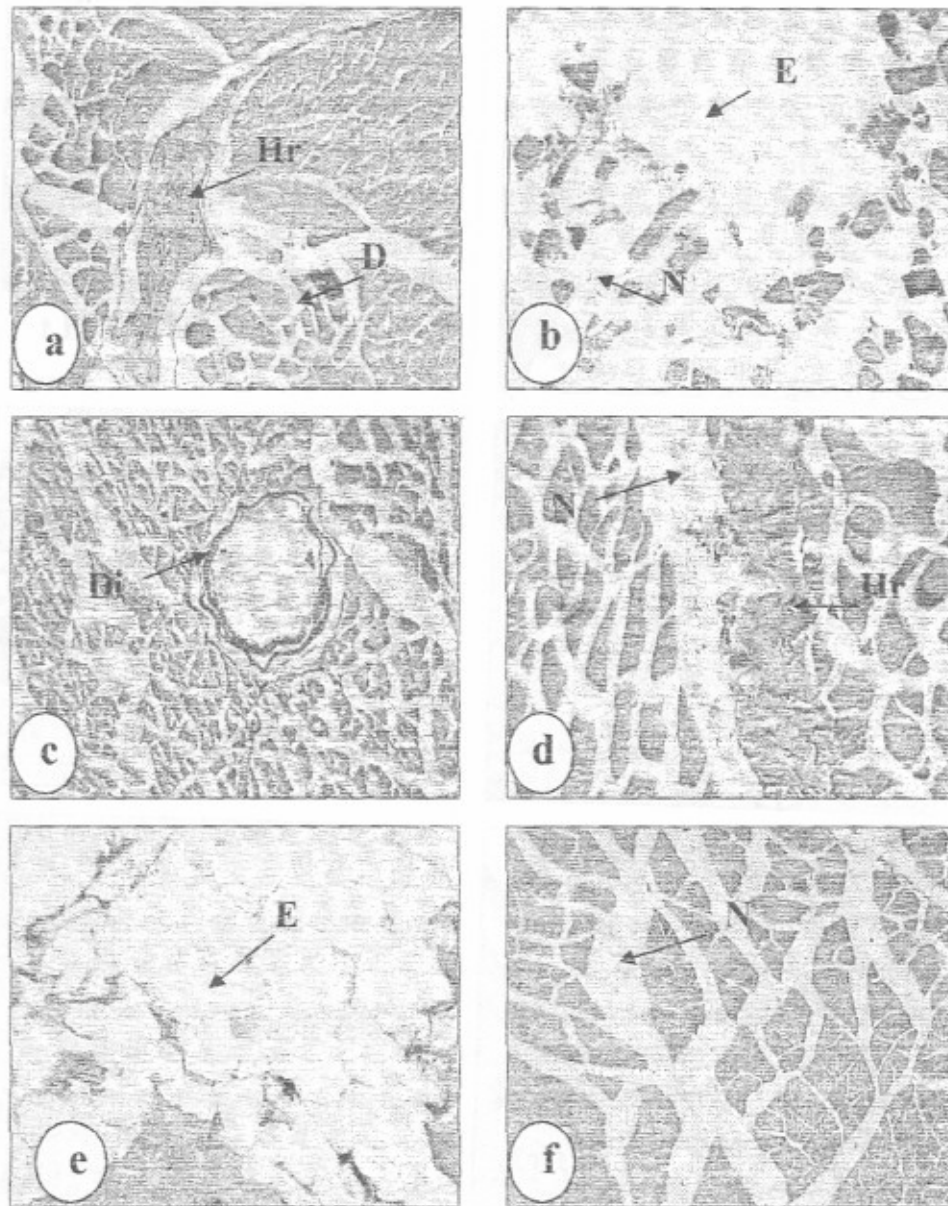


Figure II

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تأثير تلوث بعض المعادن الثقيلة على عضلات أسماك القراميط القاطنة لنهر النيل في منطقتي القناطر الحيرية وحلوان

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يهدف هذا البحث إلى تقدير تأثير بعض العناصر الثقيلة مثل الحديد، النحاس، الرصاص والكاديوم في بعض مناطق من نهر النيل على عضلات أسماك القراميط. وقد تم تقدير تركيز هذه العناصر وتراكمها في عضلات القراميط المصطادة من منطقتي القناطر الخيرية وحلوان خلال فصلي الشتاء والربيع. بالإضافة إلى ذلك، تم دراسة التغيرات النسيجية في عضلات أسماك القراميط. وقد أوضحت النتائج أن تركيز هذه العناصر في الماء وتراكمها في عضلات القراميط عند تلك المنطقتين أثناء فصلي الشتاء والربيع مرتبة تنازلياً كالاتي: الحديد، الرصاص، النحاس والكاديوم. كما أوضحت الدراسات النسيجية أن عضلات أسماك القراميط القاطنة لمنطقتي القناطر الخيرية وحلوان تعاني من تغيرات مرضية عديدة تشمل: تحلل وتحطم كامل ونزيف دموى وتحلل دموى مع وجود صبغة الهيموسدرين في العضلات. وقد لوحظ أن عضلات الأسماك المصطادة من منطقة حلوان كانت أشد تحطماً عن تلك المصطادة من منطقة القناطر الحيرية، وربما يرجع هذا إلى كثافة التلوث بمياه الصرف الصناعي في منطقة حلوان أعلى عنها في منطقة القناطر الحيرية.