ACCUMULATION AND ELIMINATION OF COPPER AND LEAD FROM *O. NILOTICUS* FINGERLINGS AND CONSEQUENT INFLUENCE ON THEIR TISSUE RESIDUES AND SOME BIOCHEMICAL PARAMETERS

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Abstract

Oreochromis niloticus fingerlings (27.5 ± 2.5 g) raised in either copper (2 and 1 mg/l) or lead (12 and 6 mg/l) polluted water (with an average pH value of 7.4 ± 0.09 and an average total hardness of 191.5 ± 15.85 mg/l as $CaCO_3$ for 30 days and then transferred to non-polluted water for 15 days. Copper and lead residues in the muscles and gills of experimented fish were measured together with some biochemical parameters throughout the study period. It was observed that, the accumulation of both metals was higher in the gills than in the muscles. Accumulation rate increased with the increasing of exposure concentration or period. Two weeks of elimination significantly decreased the levels of both copper and lead. All investigated biochemical parameters increased significantly than normal during the accumulation period and returned back to the normal values after elimination period.

INTRODUCTION

Out of the 5.5 billion cubic meters of water released from the Aswan High Dam, about 50 percent ends up in the drainage system where Egypt possesses approximately 50000 Km of irrigation and drainage canals (Redding and Midlen, 1990). Drainage water above the Delta is returned to the Nile River, recycled downstream and reused. Drainage water, however, consists not only of irrigation return water but in many cases industrial and domestic wastewater where huge volumes of untreated wastewater are discharged into agricultural drains daily. Drainage water is therefore contaminated with salts, agricultural chemicals such as Heavy metals, and other pollutants as pathogens from the domestic sewage and industrial discharge.

Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Accumulation levels vary considerably among metals and species (Heath, 1987). Toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer able to counter uptake. This capacity, however, also varies between different species and different metals (Langston, 1990 and Heath, 1987).

The presence of copper (II) ions, cause serious toxicological concerns, it is usually known to deposits in brain, skin, liver, pancreas and myocardium. Most organic and inorganic copper complexes and precipitates appear to be much less toxic than

free cupric ion and tend to reduce toxicity attributable to total copper (Borgmann and Ralph, 1983). Davis *et al* (2000) indicated that, the occurrence of copper in large amounts is extremely toxic to living organisms.

Lead, the most toxic metal, is detectable in practically all phases of the inert environment and all biological systems, because it is toxic to most living things at high exposure levels. Lead is non-essential element and it is a bone-seeking element, it is processed along with calcium because of its chemical resemblance to calcium. However, tissues other than bone are considered to be storage sites for lead in fish (Sorensen, 1991).

Fish exposed to Cu showed hematological changes in numbers of blood cells, hemoglobin levels, hematocrit values, protein concentrations, ammonia levels, and as well as changes in concentrations of other constituents. Sometimes changes are transient; other times changes seem to be long-lived (Sorensen, 1991).

MATERIALS AND METHODS

Aim of the study: the present study aims to monitor the residues of both copper and lead in *O. niloticus* fingerlings muscles and gills after one month of exposure period and the effect of 15 days elimination period on the levels of these residues. The present study aims also to investigate the effect of both accumulation and elimination periods on some biochemical parameters.

Healthy Nile tilapia (*O. niloticus*) fingerlings weighing 27.5 ± 2.5 g acclimated in an indoor tanks for 2 weeks to laboratory conditions. 180 of the acclimated fish were distributed randomly in fifteen 100 liters glass aquaria (40 × 50 × 50 cm), at a rate of 12 fish per aquarium that containing aerated tap water. The tap water used for the study had an average pH value of 7.4 ± 0.09 and an average total hardness of 191.5 ± 15.85. mg/l as $CaCO_3$. Pb-polluted water was prepared by using Pb $(NO_3)_2$, while Cu-polluted water was prepared by using $CuSO_4$. $5H_2O$.

Acclimated fish were divided into 5 treatments with three replicates for each as follow:

- Fish raised in 2 mg Cu/l polluted water.
- Fish raised in 1 mg Cu/l polluted water.
- Fish raised in 12 mg Pb/l polluted water.
- Fish raised in 6 mg Pb/l polluted water.
- Fish raised in non polluted tap water.

Fish were fed 25% protein fish feed, with the rate of 3 % of total bio mass 6 days/week. Lost water during siphon process was compensated with water having the same concentration of copper or leads to keep their concentrations alongside the study period.

After 30 days exposure period, fish were transferred to another aquaria filled with non-polluted tap water and kept for one month elimination period.

Analytical techniques

Resediual analysis

Water sample preparation for heavy metal measurements

Water samples were prepared according to (Parker, 1972) where Five cm^3 of concentrated hydrochloric acid was added to 250 cm^3 of water sample at the time of analysis and evaporated to 25 cm^3. The concentrate was transferred to a 50 ml measuring flask and dilute to the mark with distilled de-ionized water**.**

Measuring residues in fish tissues

Fish were dissected to get muscles and gills for residual analysis. Fish tissues were prepared for residual analysis according to the method described by Official Methods of Analysis (1980) where fish samples were burned in a muffle furnace for 16 hours at 550°C to be ached, to the ached sample, add 2 ml HNO_3 were added, evaporated carefully just to dryness on hot plate, transferred to cooled furnace, temp., slowly raised to 450 ºC-500 ºC and holed at this temp. for 1 hour, 10 ml of 1N HCl were added and ash were dissolved by heating cautiously on a hot plate, transferred to 25 ml volumetric flask, cooled and diluted to volume. Cu and Pb residues were measured using atomic absorption spectrophotometer (model: Perkin Elmer, 2280).

Accumulation factor (AF)

AF was calculated according to Aboul Ezz and Abdel-Razek (1991) using the following equation:

$$\text{Accumulation Factor (AF)} = \frac{\text{Pollutant concentration in fish organ (mg / Kg)}}{\text{Pollutant in water (mg / L)}}$$

Blood sampling

Blood samples were withdrawn from the caudal vein. The needle is run quite deep, as such as possible through the middle line just behind the anal fin in a dorso-cranial direction. By drawing the needle gently backward, blood is usually sucked into the syringe.

Biochemical analysis

Blood was allowed to clot then centrifuged (at 3000 rpm for 15 minutes) to obtain the serum samples for the following analysis:

Serum glucose: was determined by GOD-pap-method, mentioned by Trinder (1969)**.**

Serum total protein: was determined by colorimetric method previously mentioned by Henry (1964).

Serum ASAT: serum aspartate amino transferase (ASAT) was determined according to the method described by Reitman and Frankel (1957).

Serum ALAT: serum alanine–aminotransferase (ALAT) was determined according to the method described by Reitman and Frankel, (1957).

Serum uric acid: the serum uric acid was measured using enzymatic determination according to Barham and Trinder (1972).

Statistical analysis of the results

The obtained data were statistically analyzed according to Analysis of variance (ANOVA). It was carried out following the method described for one way classification by Snedecor and Cochran (1989) and the different mean values were compared with Duncan's multiple range test by Duncan (1955).

RESULTS AND DISCUSSION

Heavy metals residues

As shown in Fig. (1), the highest copper concentration (187.84 ± 8.34 mg/kg) was recorded after 4 weeks in the muscles of tilapia fingerlings raised in 2 mg Cu/l polluted water. However two weeks of elimination resulted in a significant decrease of copper residues in the gills of fingerlings raised in 2 and 1 mg Cu/l to values of 90.4 ± 4.77 and 39.42 ± 2.19 mg/kg respectively.

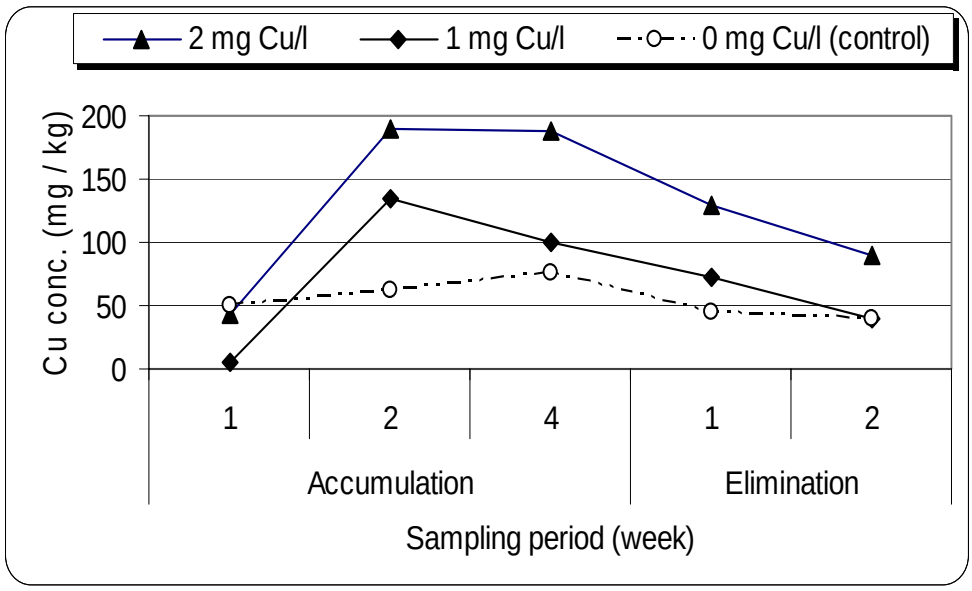

Fig. 1. Cu levels in the muscles of *O. niloticus* Fingerlings raised in Cu-polluted water (2 and 1 mg/l) for 4 weeks and then transferred into un-polluted water for 2 weeks.

Higher copper quantities were accumulated in the gills than muscles. Cu levels in the gills of fingerlings were 653.36 ± 37.48 and 364.26 ± 19.55 g/kg for 4 weeks in 2 and 1 mg Cu/l respectively. These values significantly decreased to values of 162.81 ± 19.28 and 225.52 ± 24.38 mg/kg after 2 weeks elimination period (as shown in Fig. 2).

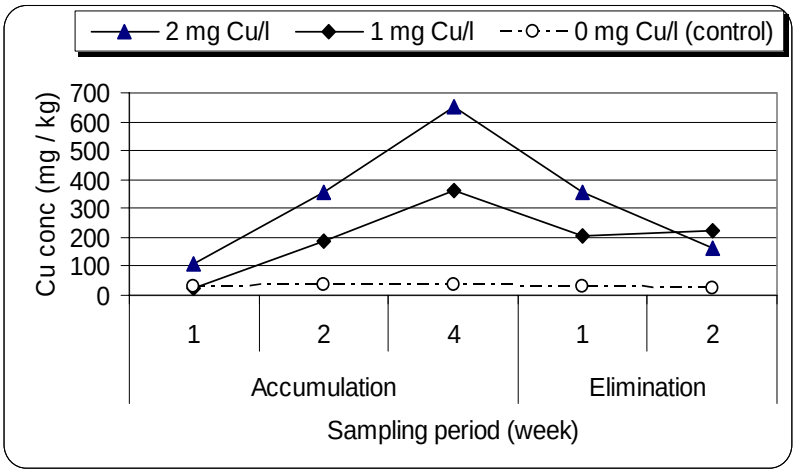

Fig. 2. Cu levels in the gills of *O. niloticus* Fingerlings raised in Cu-polluted water (2 and 1 mg/l) for 4 weeks and then transferred into un-polluted water for 2 weeks.

Lead residues in the muscles, as shown in Fig. (3), recorded a significant increase than control in fingerlings exposed to both lead concentrations (12 and 6 mg/l) during accumulation period. Highest lead concentration (132.42 ± 7.62 mg/kg) was recorded after 4 weeks of exposure to 12 mg Pb/l. An elimination period of 2 weeks was sufficient for Pb residues in muscles to be significantly decreased to a value of 51.50 ± 3.34 mg/kg.

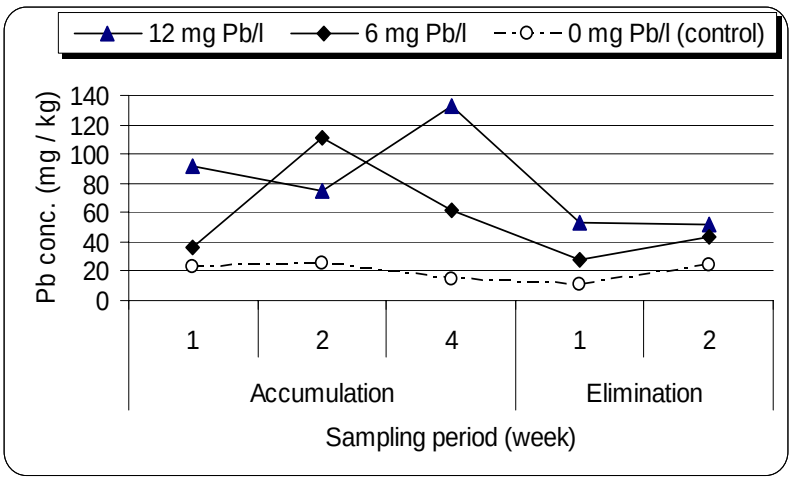

Fig. 3. Pb levels in the muscles of *O. niloticus* Fingerlings raised in Pb-polluted water (12 and 6 mg/l) for 4 weeks and then transferred into un-polluted water for 2 weeks.

Higher quantities of lead were accumulated in the gills of *O. niloticus* fingerlings than those accumulated with muscles. Highest gills lead (1367.28 ± 125.69 mg/kg) was recorded after 4 weeks of exposure to 12 mg Pb/l (as shown in Fig. 4). Despite lead value in the gills of fingerlings raised in 12 mg Pb/l significantly decreased (363.76 ± 14.39 mg/kg) after 2 weeks of elimination, but this value still significantly higher than control.

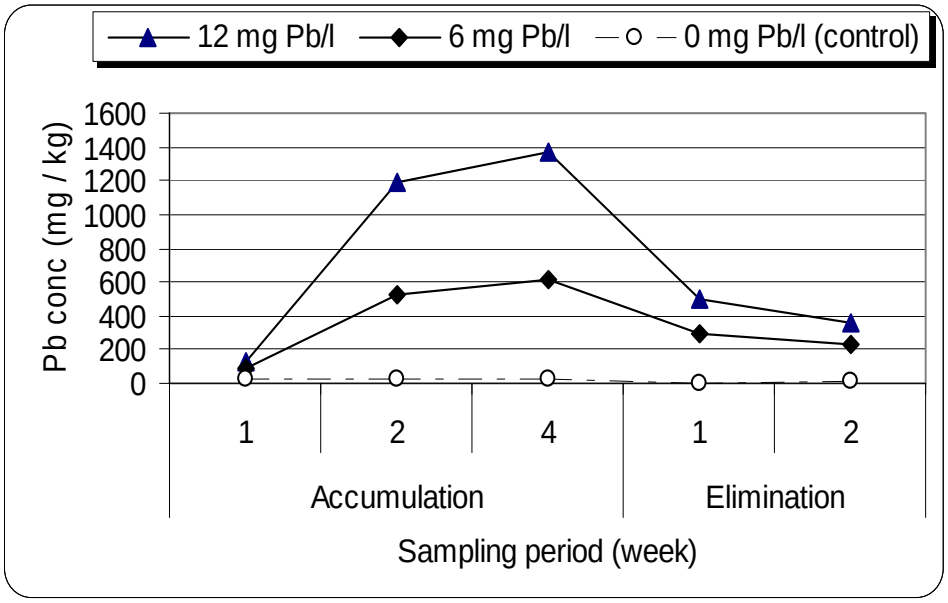

Fig. 4. Pb levels in the gills of *O. niloticus* Fingerlings raised in Pb-polluted water (12 and 6 mg/l) for 4 weeks and then transferred into un-polluted water for 2 weeks.

It is well documented that pollutants, such as metals and organic compounds, can be accumulated by aquatic biota (USEPA, 1991). Bioaccumulation measurements refer to studies or methods monitoring the uptake and retention of pollutants like metals or biocides in organs and/or tissues of organisms, such as fish (Roux, 1994). This can only take place if the rate of uptake by the organism exceeds the rate of elimination (Spacie and Hamelink, 1985). Fish can regulate metal concentrations to a certain extent, after bioaccumulation occur (Heath, 1991). Therefore, the ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulated in that specific tissue. Furthermore, physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal (Kotze, 1997).

As indicated in Table (1), bioaccumulation factor increased as exposure concentration increased. Metal uptake is dependent upon the exposure concentration and period as previously mentioned by Canli and Furness (1993) and Allen (1995).

The obtained results revealed that, gills accumulated both copper and lead higher than muscle. Nussey, *et al*. (2000) mentioned that gills generally had the highest metal concentrations, due to their intimate contact with the environment and their importance as an effectors of ionic and osmotic regulation.

Its revealed from Table (1) also that copper bioaccumulation factor is lower than that of lead. Similar results previously mentioned by Heath (1987) who mentioned that the accumulation of essential metals is normally smaller than the accumulation of non-essential metals.

Table 1. Bio accumulation factor of Cu and Pb in the muscles and gills of *O. niloticus* fingerlings after 4 weeks accumulation period

Organ	Treatments					
	Cu in 2 mg Cu/l polluted-water	Cu in 1 mg Cu/l polluted-water	Cu in Non-contaminated water (control)	Pb in 12 mg Pb/l polluted-water	Pb in 6 mg Pb/l polluted-water	Pb in Non-contaminated water (control)
Muscles	408.35	250.98	184.9	1018.62	471.38	112.46
Gills	1416	910.65	91.35	10517.54	12178	245.92

Concerning elimination efficiency as shown in Table (2), it could be observed that elimination capacity from the gills was higher than muscles in the higher exposure concentrations of both copper and lead (2 mg Cu/l and 12 mg Pb/l), while in case of the lower exposure concentrations of both Cu and Pb (1 mg Cu/l and 6 mg Pb/l) elimination capacity from the gills was lower than in muscles. This result could be explained by that previously mentioned by Kalay and Canli (2000) who stated that the elimination levels of metals from the gills were also the highest among other investigated organs, suggesting that metals in the gills might be a combination of metals adsorbed onto the gill surface and metals in the circulation system. As a result of this, metals accumulated in the gills removed from the first day of the cleansing period to the last day, especially metals adsorbed on the gills probably returned to the water, and metals in the circulation system moved to other parts of the body.

Table 2. Elimination capacity (%) of Cu and Pb from the muscles and gills of *O. niloticus* fingerlings after 2 weeks of the elimination period.

Organ	Treatments			
	Cu (mg/kg) in 2 mg Cu/l polluted-water	Cu (mg/kg) in 1 mg Cu/l polluted-water	Pb (mg/kg) in 12 mg Pb/l polluted-water	Pb (mg/kg) in 6 mg Pb/l polluted-water
Muscles	51.87	60.73	61.11	53.86
Gills	75.01	38.09	73.40	52.06

It could be observed, as shown in Table (3), that 2 weeks of elimination resulted in a significant decrease in muscle copper to be lower than the maximum permissible limit mentioned by BOE (1991). However, muscle lead concentration still higher than the maximum permissible limit mentioned by BOE (1991) after 2 weeks elimination period.

Table 3. Copper and lead concentrations (mg/kg) in muscles and gills of *O. niloticus* fingerlings raised in either Pb (12 and 6 mg/l) or Cu (2 and 1 mg/l) at the end of the study (after 4 weeks of accumulation and 2 weeks of elimination) in comparison to the maximum permissible limits.

Organ	Cu concentration (mg/kg)				Pb concentration (mg/kg)			
	Cu conc. mg/l of the surrounded water			Cu max. acceptable limits (µg/g dry wet)	Pb conc. mg/l of the surrounded water			Pb max. acceptable limits (µg/g dry wet)
	2	1	0	100.00 according to BOE 1991	12	6	0	25.00 according to BOE 1991
Muscles	90.4	39.42	19.81		53.47	28.27	54.53	
Gills	162.81	225.52	31.55		363.76	231	31.37	

Serum constituents

Results concerning biochemical investigation obtained along the period of the study were illustrated in figures 5-9. All investigated parameters recorded a significant variation in comparison to the control as a result of exposure to either Cu or lead. How ever these parameters start to be closer to control values after 2 weeks elimination period.

Serum glucose: in comparison to a value of 50.5 ± 4 mg/dl which recorded in control, 4 weeks exposure of *O. niloticus* fingerlings to Cu (2 and 1 mg/l) or Pb (12 and 6 mg/l) raised their serum glucose significantly (76.3 ± 3.3 , 53.3 ± 3, 78.4 ± 4.4 and 77.1 ± 3.1 mg/dl respectively). Abou El-Naga *et al.* (2005) reported that plasma glucose of *Mugil Seheli* was increased after exposure to 0.5 mg Cu/l. The rise of glucose level indicates the presence of stressful stimuli eliciting rapid secretion of both glucorticoids and catecholamines from the adrenal tissue (Mazeaud *et al*., 1977). Hyperglycemia was previously reported by Larsson *et al*. (1985) in case of freshwater species exposed to lead and cadmium. However, 2 weeks elimination period resulted in significant decrease in serum glucose in all treatments to normal values.

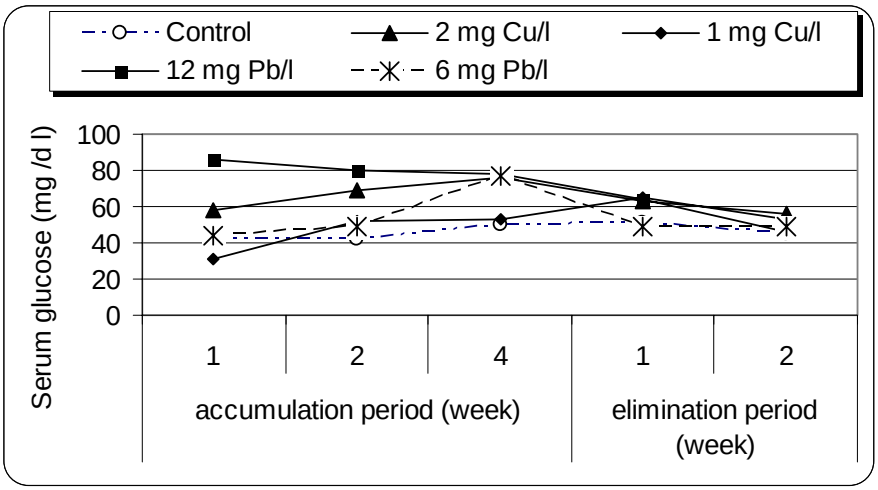

Fig. 5. Serum glucose (mg/dl) in *O. niloticus* fingerlings raised in either Cu polluted-Water (2 and 1 mg/l) or Pb polluted water (12 and 6 mg/l) for 4 weeks and transferred into non-polluted water for 2 weeks.

Serum total protein: a significant increase in *O. niloticus* serum total protein fingerlings after rising in Cu-polluted water (2 and 1 mg/l) or Pb-polluted water (12 and 6 mg/l) where the recorded values of serum total protein were 4.36 ± 0.36, 3.88 ± 0.5, 3.32 ± 0.4 and 4.36 ± 0.6 g/dl respectively, while the control value was 3.27 ± 0.23 g/dl. Elevated serum total protein may be attributed to the damage of kidney and gills as a result of exposure to copper and lead, which in turn leads to disturbance in osmoregulation **(**Gluth and Hanke, 1985). The increase in serum total protein in fishes exposed to lead may also be due to impaired water balance (Wedemeyer and Yasutaka, 1977). Serum total protein values returned to their normal levels in most treatments (except in case of fish raised in 6 mg Pb/l polluted-water, where their serum total protein still higher than control value) after 2 weeks elimination period.

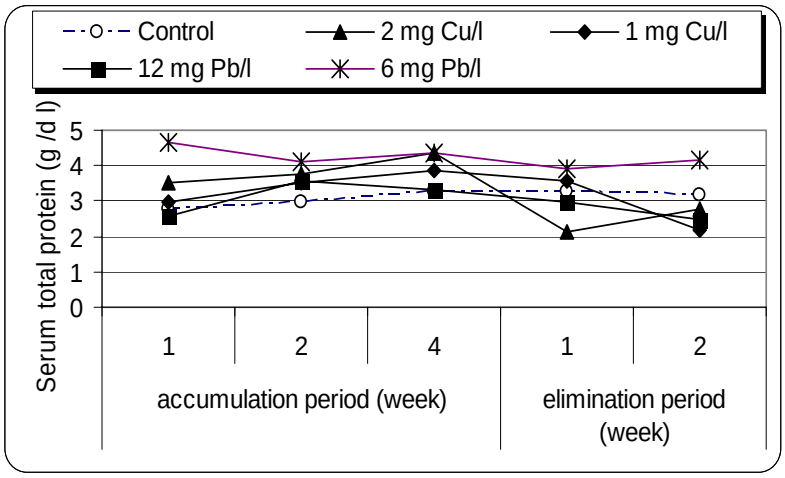

Fig. 6. Serum total protein (g/dl) in *O. niloticus* fingerlings raised in either Cu polluted- Water (2 and 1 mg/l) or Pb polluted water (12 and 6 mg/l) for 4 weeks and transferred into non-polluted water for 2 weeks.

Aspartate and alanine transaminase enzymes: both ASAT and ALAT enzymes increased significantly after exposure of fingerlings to sub-lethal concentrations of either copper or lead. Serum ASAT values recorded after 4 weeks of exposure to Cu-polluted water (2 and 1 mg/l) and Pb-polluted water (12 and 6 mg/l) were 127 ± 12, 59 ± 6, 101 ± 4 and 83 ± 5 U/l respectively. Serum ALAT values recorded in the mentioned treatments after 4 weeks of exposure were, 54 ± 3, 103 ± 6, 56 ± 6 and 152 ± 4 U/l respectively. Elimination period caused both enzymes values to be decreased significantly in comparison to their values at the end of accumulation period, but these values still significantly higher than control in most cases. Serum ASAT values recorded after 2 weeks of elimination were 45 ± 3, 31 ± 2.8, 55 ± 3.7 and 56 ± 3.2 U/l in fingerlings exposed to Cu-polluted water (2 and 1 mg/l) or Pb-polluted water (12 and 6 mg/l) respectively. These values as previously mentioned, are significantly higher than control (11 ± 1.0 U/l). Concerning serum ALAT, in spite of its values recorded at the end of the elimination period were significantly lower than its values at the end of accumulation period, but its values recorded in fishes raised in 1 mg Cu/l and 6 mg Pb/l (14 ± 2.1 and 40 ± 3.4 U/l respectively) were significantly higher than control value (9 ± 1.9 U/l). Its suggestible that exposure to copper or lead may causing partial irreversible damage in some organs such as liver, the case in which the increase in enzymes levels hardly return to normal values. Gluth and Hanke (1985) reported that, liver damage due to accumulation of lead elevate the levels of GOT and GPT activities. Abbas (1994) recorded an increase in both aspartate and alanine transaminases of *O. niloticus* serum after exposure to 7, 14 and 21 mg Pb/l. Burtis and Ashwood (1996) reported that cell injury of certain organs leads to the release of tissue-specific enzymes into the blood stream.

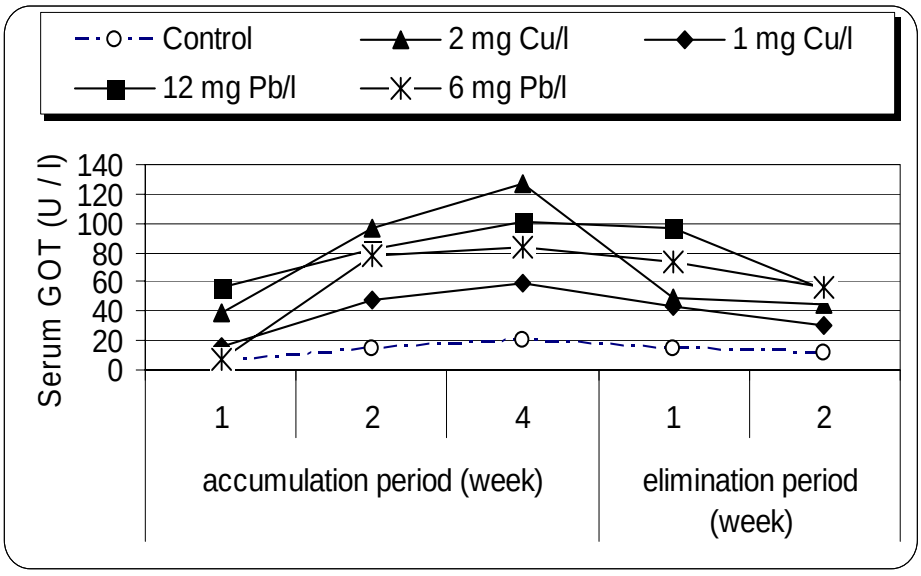

Fig. 7. Serum ASAT (U / l) in *O. niloticus* fingerlings raised in either Cu polluted- Water (2 and 1 mg/l) or Pb polluted water (12 and 6 mg/l) for 4 weeks and transferred into non-polluted water for 2 weeks.

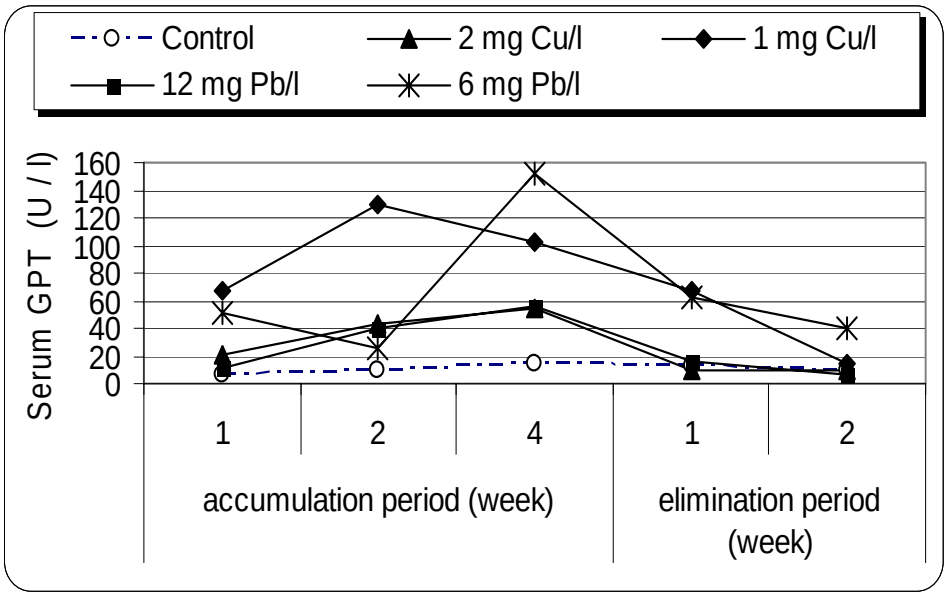

Fig. 8. Serum ALAT (U / l) in *O. niloticus* fingerlings raised in either Cu polluted-Water (2 and 1 mg/l) or Pb polluted water (12 and 6 mg/l) for 4 weeks and transferred into non-polluted water for 2 weeks.

Serum uric acid: As the other investigated serum constituents, exposure to Cu or Pb caused a significant increase in serum uric acid in all fingerlings, except in those raised in 1 mg Cu/l polluted-water, where there was no significant difference than control. Serum uric acid values recorded after 4 weeks in fingerlings rose in Cu-polluted water (2 mg/l) or Pb-polluted water (12 and 6 mg/l) were 1.88 ± 0.21, 1.68 ± 0.15 and 1.62 ± 0.01 mg/dl respectively. Two weeks of elimination resulted in normal serum uric acid values only in fingerlings exposed to lower doses of Cu (1 mg/l) or Pb (6 mg/l), while fish exposed to higher levels of Cu (2 mg/l) or Pb (12 mg/l) still had serum uric acid levels significantly higher than control. Serum uric acid values recorded at the end of elimination period in non-polluted water (control) or in polluted-water either with Cu (2 and 1 mg/l) or Pb (12 and 6 mg/l) were 1.13 ± 0.11, 1.35 ± 0.14, 14.04 ± 0.09, 1.26 ± 0.09 and 1.15 ± 0.05 mg/dl respectively.

Serum uric acid can be used as a rough index of the glomerular filtration rate (Hernandez and Coulson, 1967). Low values of uric acid have no significance but increasing values have a several disturbances in the kidney (Maxine and Benjamine, 1985). The elevation in uric acid due to the action of heavy metals causes pathological changes of the kidneys (Saad *et al*., 1973 and Oikari and Soivio, 1977). Similar results previously obtained by Shalaby (2000) who found that serum uric acid values in *O. niloticus* fingerlings exposed to sub-lethal concentrations of Cu or Pb were significantly higher than control values.

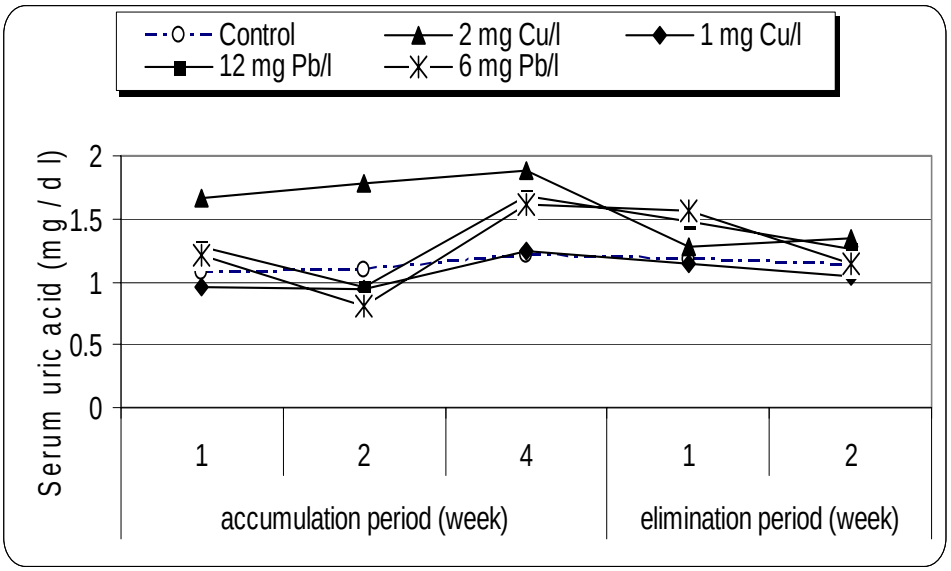

Fig. 9. Serum uric acid (mg/dl) in *O. niloticus* fingerlings raised in either Cu polluted-Water (2 and 1 mg/l) or Pb polluted water (12 and 6 mg/l) for 4 weeks and transferred into non-polluted water for 2 weeks.

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تراكم وإزالة كل من النحاس والرصاص من إصبعيات أسماك البلطي النيلي وتأثير ذلك علي متبقيات العنصران في الأنسجة وعلي بعض العوامل البيوكيميائية

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المعمل المركزي لبحوث الثروة السمكية – مركز البحوث الزراعية

أجريت هذه الدراسة لبيان تأثير تعرض إصبعيات البلطي النيلي لكل من النحاس والرصاص علي تراكم هذان العنصران بأنسجتها وعلي بعض الصفات البيوكيميائية لسيرم الدم. وكذلك بيان التغيرات الحادثة في متبقيات العنصران موضع الدراسة وفي العوامل البيوكيميائية لسيرم الدم كنتيجة لعملية التخلص من هذان العنصران.

إستخدم في هذه الدراسة إصبعيات البلطي النيلي (بمتوسط حجم ٢٧,٥ ± ٢,٥ جم) حيث قسمت هذه الأسماك إلي خمس مجموعات كالتالي:

- المجموعة الأولي عرضت لتركيز ٢ مجم نحاس / لتر
- المجموعة الثانية عرضت لتركيز ١ مجم نحاس / لتر
- المجموعة الثالثة عرضت لتركيز ١٢ مجم رصاص / لتر
- المجموعة الرابعة عرضت لتركيز ٦ مجم رصاص / لتر
- المجموعة الخامسة تم تربيتها في ماء غير ملوث (كمجموعة ضابطة)

وتم عمل ثلاث تكرارات لكل معاملة وكانت كثافة الأسماك ١٢ وحدة / ١٠٠ لتر وقد إستمر هذا الشق من التجربة لمدة ثلاثون يوما. تم نقل الأسماك بعد ذلك لأحواض بها ماء غير ملوث لمدة خمسة عشر يوما.تم قياس متبقيات كل من النحاس والرصاص في كل من الماء والعضلات والخياشيم وتم كذلك قياس بعض العوامل البيوكيميائية لسيرم الدم مثل الجلوكوز والبروتين الكلي وإنزيمات نشاط الكبد (ASAT& ALAT) بصفة دورية علي مدار التجربة خلال عمليتي المراكمة والإزالة. وقد أوضحت النتائج المتحصل عليها زيادة متبقيات كل من النحاس والرصاص كنتيجه للتعرض لهذان العنصران وأن معدل التراكم يزداد بزيادة كل من التركيز ومدة التعرض. وكانت نسبة التراكم في الخياشيم أعلي منها في العضلات. بالنسبة للعوامل البيوكيميائية ، بينت النتائج المتحصل عليها زيادة كل العوامل المختبرة زيادة كبيرة بالمقارنة بالقيم الطبيعية لإصبعيات المجموعة الضابطة كنتيجة للتعرض لأي من النحاس أو الرصاص، وأن معدل التغير في هذه العوامل يزداد بزيادة تركيز أي من العنصرين المختبرين وكذلك يزداد بزيادة فترة التعرض. أدت عملية الإزالة بتربية الأسماك في مياه غير ملوثة إلي تقليل متبقيات كل من النحاس والرصاص في كل من العضلات والخياشيم بصورة كبيرة وإن ظلت تركيزات الرصاص في كل من العضلات والخياشيم أعلي من الحد الأقصي المسموح به بينما كانت تركيزات النحاس في العضلات أقل من الحد الأقصي المسموح به كما أدت إلي تحسن قيم العوامل البيوكيميائية المختبرة وعودتها لقيم قريبة من قيم إصبعيات المجموعة الضابطة.