EFFECT OF SOME HEAVY METAL POLLUTANTS ON SOME BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN BLUE TILAPIA; *OREOCHROMIS AUREUS*

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Abstract

Copper and lead are among the most widely common pollutants distributed in the aquatic environment. The 96-hr LC_{50} bioassays were carried out to determine the toxicity of these two heavy metals (cu &pb) on the blue tilapia; *Oreochromis aureus*. The results showed that the 96-hr LC_{50} of copper and lead were 8.17 and 25.86 mg/L, respectively .Groups of adult and healthy *O.aureus* were subjected to the acute (lethal) concentrattion (96-LC_{50})of each metal and examined after 24 and 96 hours. Other groups were subjected to the chronic (sublethal) concentration (1/10LC_{50}) of each metal and examined after 6 and 12 weeks .

The lethal and sublethal concentrations of copper and lead altered the behavioural activities of the treated fish. The gills, liver, kidney and spleen tissues were irritated and injured causing dysfunction in these organs. Haemoglobin contents were increased in fish toxicated by copper and decreased in those toxicated by lead . Serum glucose was increased in all groups treated with copper or lead. The serm AST and ALT recorded the same trend of increase . Moreover, the creatinine and the uric acid levels were elevated .

INTRODUCTION

The aquatic environment has been regionally contaminated, sometimes heavily with a variety of dangerous heavy metals coming from both natural and artificial sources causing serious healthy hazards in human (Mohamed and Saleh, 1996) .Heavy metals are considered as one of the most important factors which affect fish population, reducing their growth, reproduction and /or survival rate (Mohamed and Saleh, 1996 and Saeed,1999) Moreover, it has been recognized that environmental changes can contribute factors in the development of stress in fish (Saeed,1999). The toxic levels of the different metals differ and depend on factors such as susceptability of fish species, pH, temperature , salinity or chemical speciation of the metal . The most sublethal

effects are biochemical in origin and that are expressed as histological ,morphological or behavioural response (Shaker *et al* ., 2000) .

Copper is essential as micronutrient for fish and aquatic life, widely used as a very effective algicide and molluscicide (Saeed, 1999 and Shaker *et al* , 2000) On the other hand, poisoning on man was described (Saeed,1999) .

The permissible levels of copper and lead in water are 1.0 and 0.05 ppm, respectively, while, in fish tissues, they are 20 and 2 ppm, respectively , according, to WHO (1984).

The objective of the present study was to determine the toxicological effects of copper and lead on *Oreochromois aureus* .

Tilapia (*Oreochromois aureus)* were selected as a research fish model because these fish were easily produced and economically important ,fishes are known by their tendency to localize significant amounts of metals. They absorp metals from water through gills, skin and digestive tract. Bioconcentration and biomagnification for heavy metals were previously reported by many authers (Saeed 1999 and El –Bagori, 2001) .

MATERIALS AND METHODS

The present study was carried out on the blue tilapia *(O.aureus)* . The body weight and total body length were ranging from 40-60 g and 13-15 cm, respectively . Fish were collected from El- Abbassa Fish farm and transferred alive to the laboratory where they were distributed in glass aquaria (75x50x50cm). The fish were acclimatized for 14 days before the onset of the experiment. During the acclimatization period ,bacteriological , mycological and parasitological examinations were carried out to exclude any disease interference according to Schaperclaus *et al* . (1992). Fish were fed commercial pellets and checked daily. Copper sulphate and lead acetate (Merck Chemical Co.) were mixed individually into solution to provide the required concentration .The determination of the 96-hr half lethal concentrations (LC_{50}) for copper and lead were determined by exposing *O.aureus* to different concentrations separately (Table 1) by the method of Litchfield and Wilcoxon (1949) . Quality criteria of the water used in the experiment is shown in Table 2.

In the short term exposure experiment, the groups of blue tilapia were exposed separately to the 96-hr (LC_{50}) of copper or lead determined previously. Eight fish were randomly selected and were scarificed after 24 and 96 h of exposure to the metals for analysis and examinations .

In the long term exposure experiment, the fish were exposed to the 1/10 of the 96-hr (LC_{50}) of each metal for 12 weeks. Fish were scarificed after 6 and 12 weeks of exposure to each metal. Control non-exposed fish group was also included in the experiment. Water in all experimental aquaria were changed every 3 days .

The clinical examination and post-mortem findings were performed according to the method described by Schaperclaus *et al.* (1992).

Blood samples were withdrawn from the caudal vein. Serum was obtained by centrifugation at 5000 rpm for 12 minutes and stored in deep freezer (-20) till the time of biochemical analysis.

Haemoglobin content was estimated by using the cyano-methomoglobin method described by Boehringer Mannheim Kit according to Van Kampen and Zijistro (1961)

The concentration of serum glucose was measured by using the GOD-PAP method (Enzymatic colorimetric method) according to Trinder (1969). The serum aspartate aminotransferase (AST) and Alanine amino-transferase (ALT) activities were measured as described by Reitman and Frankel (1957). The serum creatinine was measured according to Henery (1974). The serum uric acid was determined as mentioned by Barhan and Trinder (1972) .Tissue specimens from liver, kidney and spleen from exposed and control fish groups were fixed in 10% phosphate buffer formalin, 5μ thick paraffin sections were prepared and stained with hematoxylin and eosin (H&E) and examined microscopically (Roberts, 1989)

RESULTS AND DISCUSSION

Heavy metals are the most widely common pollutants distributed in the aquatic environment. The toxic effects of different metals are usually associated with biochemical abnormalities manifested in histological, morphological, and physiological or behavioural responses that may lead to direct mortality.Ingesion of such contaminated fish

can be considered a public healthy hazards (Mousa , 1999 and El –Bagori, 2001).

The 96-hr LC_{50} bioassays were carried-out to determine the toxicity of the two studied heavy metals on *O. aureus.* The results showed that the 96-hr LC_{50} of copper and lead were 8.17 and 25.86 mg/L, respectively (Table 1) .

However El- Bagori,. (2001) recorded that there is no mortalities among *O.niloticus* and *Cyprinus carpio* exposed to lead up to concentration of 14 mg/L. They mentioned that lead is relatively insoluble but its soluble forms such as lead acetate change into the insoluble lead sulfate in the gastrointestinal tract. Also, they added that, protein and vitamin C reduce metal absorption. The environment under which the experiments were carried out also affects the values of LC_{50}.

Copper and lead altered the behaviour of the previously acclimatized fish. The clinical signs of the intoxicated fish were sluggish movements, loss of escape reflex, loss of appetite and respiratory distress. Also, excessive secretion and accumulation of mucus on gills and skin and mottled skin were recorded . Similar signs were previously described by Mohamed and Saleh (1996) and Mousa (1999) . The black spots and mucus secretion may be attributed to the melanosis due to the estimulation of α- melanin stimulating hormone secretin in the fish subjected to stress (Shaker *et al.* 2000). Accumulatins of mucus on gills were due to irritation of gills by pollutants (El-Bagori, 2001). Shaker, *et al*, (2000) mentioned that, after the first 24 h of exposure to the copper, the percentage of oxygen utilization increased eight times in fish held in 3 ppm than those in 0.5 ppm and twelve times higher in fish held in 5 ppm than those in 0.5 ppm . On the other hand, the postmortem findings of the polluted fish showed pale gills, skin abrasions, congestion of the internal organs and accumulation of ascitic fluid in the abdominal cavity. Similar findings were observed by Mousa (1999) in case of *O. niloticus* when exposed to gramoxone and stomp herbicide.

Pathological changes in fish are powerful indicator of exposure to environmental stress such as chemical pollutants. Gills of tilapia exposed to the copper showed hyperplastic changes in the epithelial cells of secondary lamellae (Fig. 1), while, gills of tilapia exposed to the lead showed partial unilateral sloughing of epithelium of secondary lamellae and congestion of branchial blood vessels (Fig. 2). These results agree with those of Marie *et al.* (1998) and Mousa (1999). The sloughing of lamellae epithelial

cells and hyperplasia induced by any pollutant may be due to the simple response to cellular necrosis as previously mentioned by Marie *et al.* (1998). Morever, Shaker *et al.* (2000) reported that, the epithelial hyperplasia are known as a protective and defence mechanism of fish gills. The congestion in branchial blood vessels may be due to the counter irritation and paralysis of vasovonstrictors of stimulating vasodilators (Marie *et al.* 1998). Liver of *O. aureus* toxicated with copper showed numerous bile ductules besides few lymphocytes in the portal areas and degenerative chanes of the hepatocytes (Fig. 3). Moreover, *O. aureus* exposed to lead showed degenerative changes and focal coagulative necrosis represented by pyknosis of the nuclei (Fig. 4). These results were previously recorded by El-Bagori, (2001) who attributed these lesions to the severe toxic effect. Kidny showed degeneration and coagulative necrosis of some tubular epithelial cells (Fig. 5). In addition, few scattered melanomacrophages (Fig, 6) were detected. These findings coincided with those of Mousa (1999) and they were attributed to the impairment of the electrolyte exchanges between the intracelluar and the extracelluar fluids Marie *et al.* (1998).

The presence of melanomacrophages may be due to the increase of the body catabolism, or may be due to the anorexia (Mousa, 1999). The spleen of the toxicated *O. aureus* showed congestion besides numerous melanomacrophages and depletion of lymphocytes (Figs. 7 & 8).

Concerning the effects of copper and lead on haemoglobin content of the exposed *O. aureus* (Table 3), the results showed a significant increase in haemoglobin content of fish exposed to copper. These results were in agreement with those of Mousa (1999), who attributed it to the dehydration of the blood and/or hypermetabolic state of fish due to the toxic stress. On the contrary, the haemoglobin content in fish exposed to lead was decreased in the lethal and sublethal concentration .Similar findings were met with by Marie *et al.* (1998), who recorded that this decrease could be attributed to ALA-D inhibition , impairment of gas exchanges by gills, hemolysis of erythrocytes and /or spleen dysfunction .

Serum biochemical profiles are fundamental tools used in human and veterinary medicine and in diagnosis of disease and other pathological conditions. In the present study (Table 4), there is a significant increase in the serum glucose of fish exposed to

lethal and sublethal concentrations of copper and lead throughout the exposure period. This view was in agreement with Marie *et al.* (1998) and Mousa (1999) who reported that increasing of blood sugar level revealed a stress susceptability of fish against stress .

The effect of toxicant on the enzyme activity of fresh water fish had been observed by many investigators. In the present study (Tables 5 & 6) , serm AST and ALT in the examined *O. aureus* fish showed a general trend of increase when exposed to lethal and sublethal concerntrations of copper and lead. Marie *et al.* (1999) observed elevation of serum AST and ALT in *Heteropneustes fossilis* after being exposed to congo red. They mentioned that increase in serum AST is an indicator of liver dysfunction and might be due to leakage of these enzymes from the injured tissue into the blood. This is confirmed in the present study by liver histological investigations. Marie *et al.* (1998) reported a significant elevation in serum AST and ALT of *O.niloticus* exposed to mercury and zinc individually and in mixture .They attributed this elevation to the damage of liver and kidney cells that affect membrane permeability which, in-turn, liberates the enzymes to the extracellular fluid and blood .

The creatinine and uric acid levels may be an indicator of the kidney dysfunction and important in predicting disease in which the kidney is adversely affected (Mousa, 1999). In the present study, (Tables 7 & 8) showed significant increase in serum creatinin and uric acid concentration of fish exposed to toxic metals compared with the control group . This may be attributed to the action of the metals on glomeruli filteration rate which causes pathological changes of the kidney. This assumption is highly supported by the work conducted by Mousa (1999) and EL- Bagori (2001) and confirmed in this work by histopathological investigations of the kidneys of toxicated *O. aureus*. Therefore , it could be concluded that copper and lead have harmful effects on the behavioural, histological, physiological and biochemical responses of fish which, in-turn, affect the growth rate, production and reproduction of fish. Also, heavy metal residues in fish muscles can be hazardous to human health .

Table 1. LC_{50}- 96 hour (95% confidence limit) and 1/10 96 hours LC_{50} of copper and lead in *Oreochromus aureus*.

Metals	96 hrs LC_{50} (mg/L) & 95% c.l.	1/10 (96 hrs- LC_{50}) (mg/L)
Copper	8.17	0.82
Lead	25.86	2.59

Table 2. Quality criteria of the water used in the experiment.

Parameter	Value
PH	7.83±0.039
Dissolved Oxygen (DO)	5.93±0.08 mg/l
Temperature	24.76±0.14°C
Total hardness	138.0±3.96 mg/L as $CaCO_3$
Total alkalinity	216.0±8.48 mg/L as $CaCO_3$
Electric conductivity	0.362±0.014 Mmohs
Salinity	0.10±0.00 mg/l
NH_4^+	0.79±0.059 mg/l
NH_3 (Ammonia)	0.041±0.004 mg/l
NO_2^- (Nitrite)	0.023±0.002 mg/l
NO_3^- (Nitrate)	Nil mg/l
Total dissolved solids	184.92±24.52 mg/l

Table 3. Changes in hemoglobin content (g/100ml) of *O.aureus* exposed to copper and lead.

Acute (96-hr -LC_{50}			Chronic (1/10 LC_{50}		
	Copper	Lead		Copper	Lead
Control	11.04 ± 0.76^{c}	11.04± 0.76^{a}	Control	10.56± 0.36^{c}	10.56± 0.36^{a}
24-hrs	13.22 ± 0.62^{b}	8.26 ± 0.29^{b}	6 weeks	11.03± 0.32^{b}	10.87± 0.32^{a}
96-hrs	14.22 ± 0.83^{a}	6.05 ± 0.91^{c}	12 weeks	12.34±0.41^{a}	9.74±0.22^{b}

Data are represented as mean ± S.E. - Total No. of fish used/interval = 8

Means with the same letters in the same collumn are not significantly different.

Table 4. Changes in serum glucose (mg/dl) of *O.aures* exposed to copper and lead.

Acute (96-hr -LC_{50}			Chronic (1/10 LC_{50}		
	Copper	Lead		Copper	Lead
Control	54.60 ± 2.12^{c}	54.60± 2.12^{c}	Control	58.19±2.94^{c}	58.19± 2.94^{c}
24-hrs	80.53 ± 1.99^{b}	83.61±2.43^{b}	6 weeks	69.71± 1.91^{b}	69.71±2.99^{b}
96-hrs	102.80± 3.8^{a}	97.36±3.89^{a}	12 weeks	81.44±4.72^{a}	76.95±2.27^{a}

Data are represented as mean ± S.E. - Total No. of fish used/interval = 8

Means with the same letters in the same collumn are not significantly different.

Table 5. Changes in serum GOT (U/L) of *O.aures* exposed to copper and lead.

Acute (96-hr -LC_{50}			Chronic (1/10 LC_{50}		
	Copper	Lead		Copper	Lead
Control	20.86 ± 1.92^{c}	20.86± 1.92^{c}	Control	21.43±1.71^{b}	21.43±1.71^{c}
24-hrs	25.91 ±0.85^{b}	32.27±2.o3 ^{a}	6 weeks	27.o9±2.41^{a}	26.22±o.98^{b}
96-hrs	27.o6± 1.76^{a}	26.41±1.74^{b}	12 weeks	25.61±1.77^{a}	30.43±1.22^{a}

Data are represented as mean ± S.E. - Total No. of fish used/interval = 8

Means with the same letters in the same collumn are not significantly different.

Table 6. Changes in serum GPT (U/L) of *O.aures* exposed to copper and lead.

Acute (96-hr -LC_{50}			Chronic (1/10 LC_{50}		
	Copper	Lead		Copper	Lead
Control	8.62 ± 1.02^{b}	8.62± 1.02^{b}	Control	8.47±o64^{a}	8.47±o.764^{b}
24-hrs	10.21 ±0.77^{a}	10.04±0.72 ^{a}	6 weeks	9.o2±o.76^{a}	9.23±o.91^{b}
96-hrs	5.92±0.88^{c}	7.22±0.13^{b}	12 weeks	10.38±o.18^{a}	11.09±0.62^{a}

Data are represented as mean ± S.E. - Total No. of fish used/interval = 8

Means with the same letters in the same collumn are not significantly different.

Table 7. Changes in serum Creatinine (mg/100ml) of *O.aures* exposed to copper and lead.

Acute (96-hr -LC_{50}			Chronic (1/10 LC_{50}		
	Copper	Lead		Copper	Lead
Control	0.93 ± 0.03^{c}	0.93± 0.04^{c}	Control	0.91±0.04^{c}	0.91±0.04^{c}
24-hrs	1.36 ±0.04^{b}	1.45±0.04 ^{b}	6 weeks	0.99±0.04 ^{b}	1.02±0.03^{b}
96-hrs	1.86± 0.03^{a}	1.88±0.03^{a}	12 weeks	1.13±0.05^{a}	1.16±0.02^{a}

Data are represented as mean ± S.E. - Total No. of fish used/interval = 8

Means with the same letters in the same collumn are not significantly different.

Table 8. Changes in serum Uric acid (mg/100 ml) of *O.aures* exposed to copper and lead.

Acute (96-hr -LC_{50}			Chronic (1/10 LC_{50}		
	Copper	Lead		Copper	Lead
Control	11.67 ±1.13^{c}	11.67±1.13^{c}	Control	11.27±0.99^{c}	11.27±0.99^{c}
24-hrs	17.02 ±0.98^{b}	15.85±0.74 ^{b}	6 weeks	13.62±0.32 ^{b}	13.81±0.61^{b}
96-hrs	21.32± 1.26^{a}	23.09±2.02^{a}	12 weeks	16.75±0.49^{a}	16.29±0.35^{a}

Data are represented as mean ± S.E. - Total No. of fish used/interval = 8

Means with the same letters in the same collumn are not significantly different.

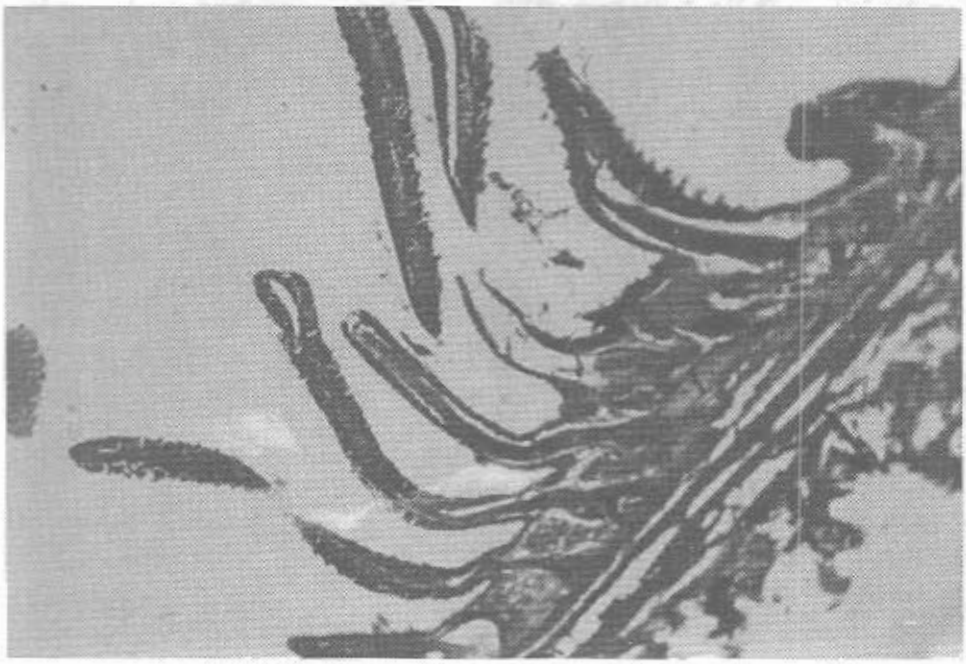

Fig. 1. Gills of *O.aures* exposed to 8.17 mg/l of copper for 96-hour showing hyperplastic of the epithelial cells of secondary lamellae . (H & E X 300) .

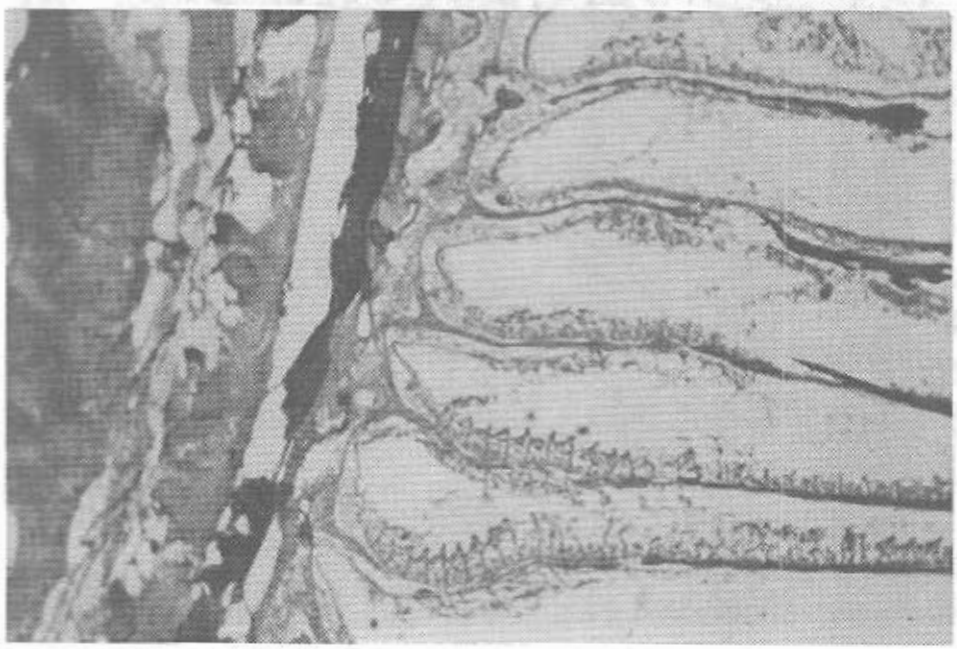

Fig. 2. Gills of *O.aures* exposed to 25.86 mg/L of lead for 96- hr showing partial unilateral sloughing of the epithelium of secondary lamellae and congestion of branchial blood vessels . (H & EX300).

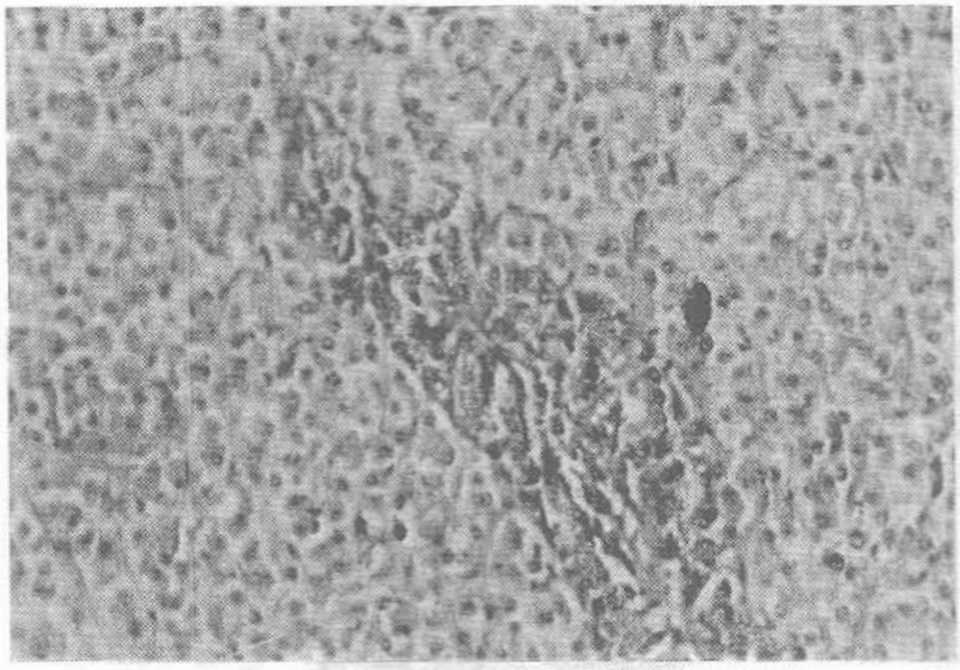

Fig. 3. Liver of *O.aures* exposed to 8.17 mg/L of copper for 96-hr showing numerous bile ductules beside few changes of the hepatocytes. (H&E X 300).

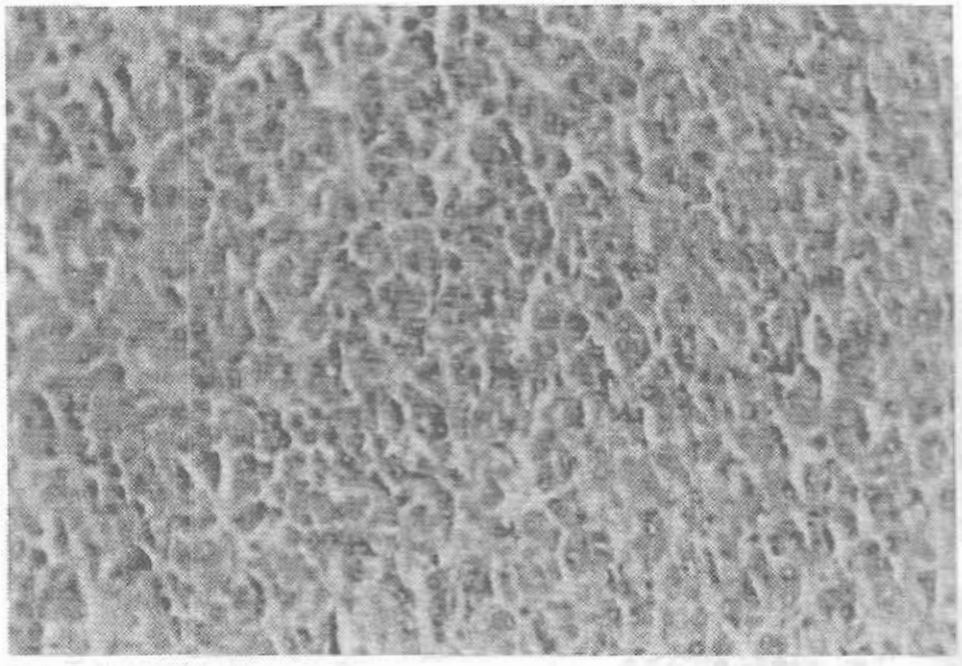

Fig. 4. Liver of *O.aures* exposed to 25.86 mg/L of lead for 96-hr showing degenerative changes and focal coagulative necrosis represented by pyknosis of the nuclei. (H & E X 300) .

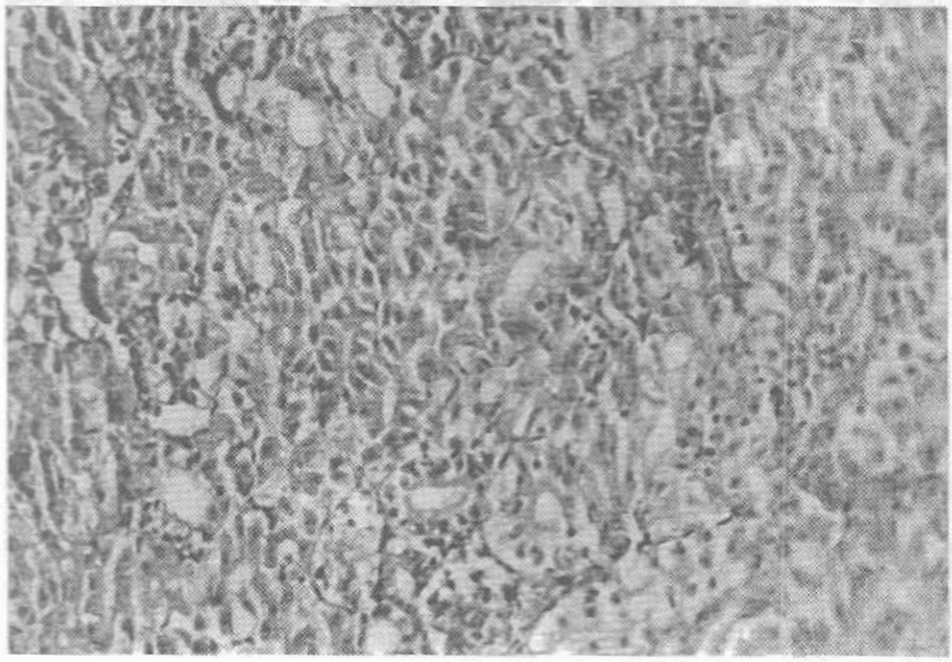

Fig. 5. Kidney of *O.aures* exposed to 8.17 mg/L of copper for 96- hr showing degeneration and coagulative necrosis of some tubular epithelial cells (H & E X 300) .

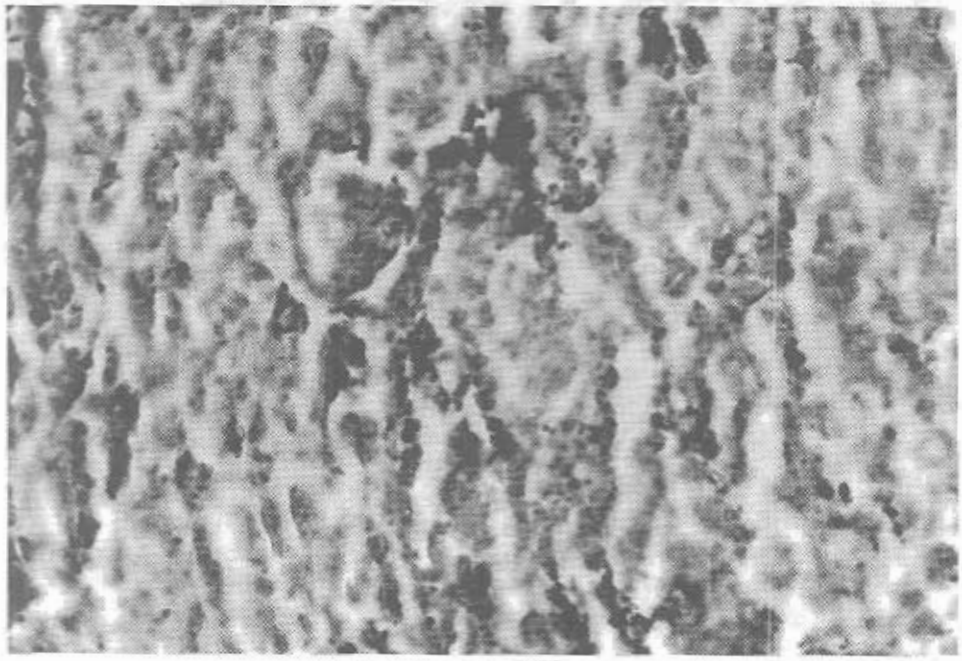

Fig. 6. Kidney of *O.aures* exposed to 25.86 mg/L of lead for 96-hr . showing few scattered melanomacrophages . (H & E X 300) .

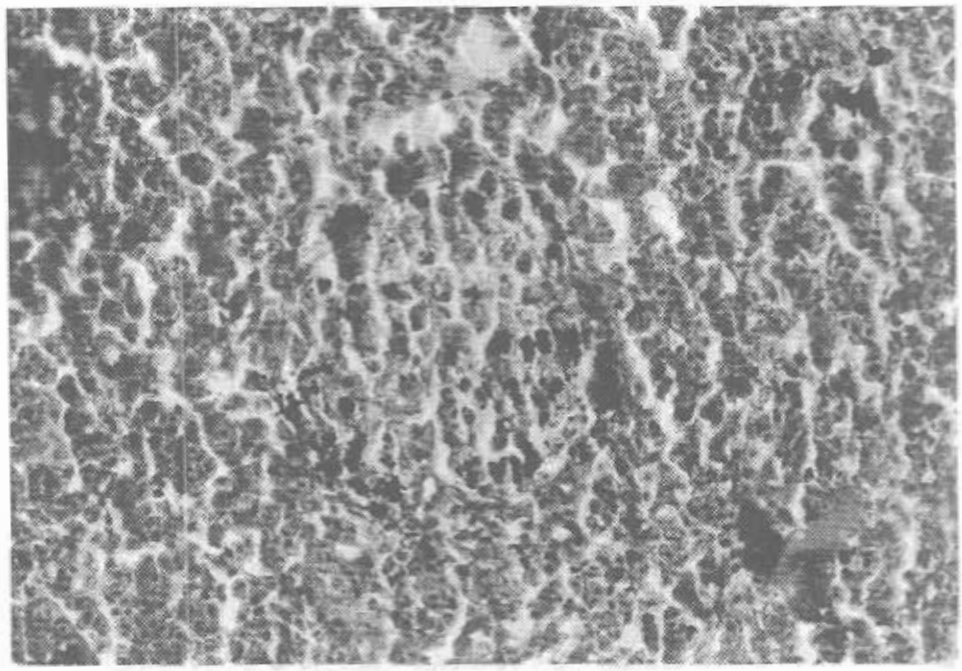

Fig. 7. Spleen of *O.aures* exposed to 8.17 mg/L of copper for 96- hr showing congestion beside numerous melanomacrophages (H & E X 300)

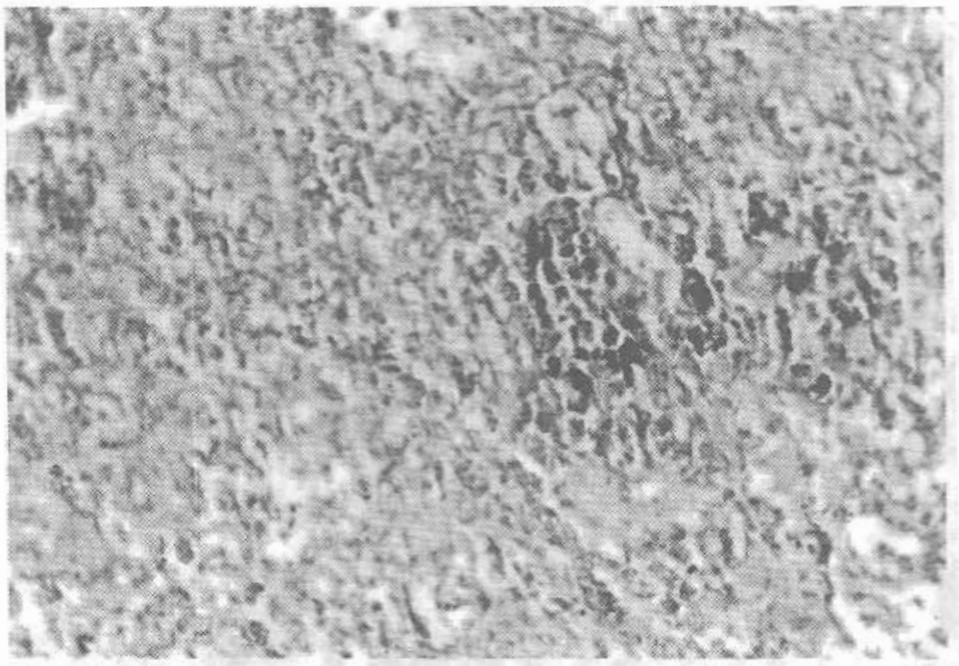

Fig. 8. Spleen of *O.aures* exposed to 25.86 mg/L of lead for 96-hr showing depletion of lymphocytes and numerous melanomacrphages (H & E X 300) .

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تأثيربعض الملوثات البيئية على بعض التغيرات البيوكيميائية والهستوباثولوجية فى سمكة البلطى الأزرق (اريوكرومس اوريس)

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١ *المعمل المركزى لبحوث الثروة السمكية بالعباسة – مركز البحوث الزراعية – وزارة الزراعة – الدقى – جيزة – مصر*

٢ *كلية العلوم – جامعة القاهرة – فرع الفيوم*

لما كان النحاس والرصاص من أكثر العناصر الثقيلة الشائعة الإنتشار فى البيئة المائية والتى تسبب أضرارا بالغة بالثروة السمكية كانت هذه الدراسة لتحديد التركيزات الميتة من هذين العنصرين وذلك بالنسبة لسمكة البلطى الأزرق (ألاوريا) الشائعة فى مياهنا المصرية والتى تستزرع على نطاق واسع فى مزارعنا . وقد وجد أن التركيز الميت للنصف خلال ٩٦ ساعة من النحاس يساوى ٨.١٧ مجم / لتر ومن الرصاص يساوى ٢٥.٨٦ مجم /لتر . وقد تم تعريض مجموعتين من البلطى الاوريا الناضجة والصحيحة لهذين التركيزين كل على حدة وفحصت الأسماك بعد مرور ٢٤ ساعة و٩٦ ساعة من التعرض، كما عرضت مجموعتين أُخريين من نفس الأسماك إلى ١٠/١ من هذين التركيزين وتم فحص الأسماك فيهما بعد ٦ ، ١٢ أسبوعاً . وقد وجد أن جميع التركيزات أحدثت اضطراباً فى سلوكيات الأسماك فى الأحواض وظهرت عليها أعراض عصبية وتغير فى الجلد ومخاط كثيف فوق الخياشيم . وقد أظهر الفحص الهستوباثولوجى حدوث أضرارٍ بالغة فى خلايا الخياشيم والكبد والكلى والطحال فى الأسماك التى تعرضت للتركيزات العالية بعد ٩٦ ساعة . كما وجد أن جميع التركيزات العالية والمنخفضة من النحاس قد أسفرت عن ارتفاع نسبة الهيموجلوبين فى الدم فى حين انخفضت فى الأسماك المعرضة للرصاص . كما سجلت النتائج زيادة كبيرة فى مستوى سكر الدم وكذلك زيادة نشاط الإنزيمات الناقلة لمجموعات الأمين وكذلك ارتفاع مستوى الكرياتينين وحمض اليوريك دلالة على الاضرار التى اصابت AST , ALT الكبد والكلى نتيجة سمية هذه العناصر .