ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XXIX, Fasc. 2 Szczecin 1999

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Fish physiology

PHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN *TILAPIA ZILLII* (GERV.) EXPOSED TO SUBLETHAL CONCENTRATIONS OF THE EFFLUENT OF THE EGYPTIAN COPPER WORKS

FIZJOLOGICZNE I HISTOLOGICZNE ZMIANY U TILAPII (*TILAPIA ZILLII* GERV.) POD WPŁYWEM SUBLETALNEJ KONCENTRACJI WODY ZRZUTOWEJ Z EGIPSKICH ZAKŁADÓW MIEDZIOWYCH

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The physiological and histological changes in *Tilapia zillii* (Gerv.) after exposure to sublethal concentrations of the effluent of the Egyptian Copper Works have been investigated. The results of acute toxicity test showed that the LC_{50} was 25 cm^3/dm^3, which means that this waste water is highly toxic. The results of physiological and histological changes in *Tilapia zillii* (Gerv.) showed that, fish were under considerable stress during exposure to sublethal doses of this waste water. Physiological response of fish revealed a significant disturbances in respiratory system, fish metabolism, and ionic osmoregulation. Pathological changes attributed to heavy metals were observed in the gills, liver, and kidney. Bioaccumulation of copper was highest in the liver, followed by the gills, and flesh. It is concluded that the waste water of the Egyptian Copper Works was not acceptable to discharge to drainage canal. Moreover, this plant should institute appropriate in-plant control to reduce emission of heavy metals.

INTRODUCTION

Uncontrolled discharge of industrial effluent in Alexandria has led to severe impact on ecological balance and appreciable environmental deterioration. Heavy metals are among the pollutants which can cause pollution to aquatic organisms. Their concentrations usually show a considerable rise in the waters receiving industrial wastes.

The Egyptian Copper Works is considered to be an important source of pollution with heavy metals (Mourad 1984). This factory is located in Hagger El-Newatya and discharges about 70 000 m^3/day. The waste water of this factory is collected in municipal sewer system and discharged to Lake Mariut.

Although there are several papers available on the effects of the effluent of the Egyptian Copper Works to aquatic organisms (Hamza et al. 1985; Mourad 1995; Aboul-Naga and Allam 1996), yet no information has been published about physiological and histological changes following the exposure to this waste water. The measurement of these changes may provide a sensitive method for predicting the effect of chronic exposure on survival, reproduction and growth.

Therefore, the main objective of this study is to assess the physiological and histological changes in *Tilapia zillii* (Gerv.) after exposure to sublethal concentrations of the effluent of the Egyptian Copper Works.

MATERIALS AND METHODS

Specimens of *Tilapia zillii* (Gerv.) obtained from El-Mex Fish Farm, weighing 11.9 ±1.2 g and measuring 9.1 ±1.2 cm were brought to the laboratory and acclimated under appropriate experimental conditions for two weeks. The fish were fed on a diet containing 35% of protein. The water temperature ranged from 22 to 24°C. The dissolved oxygen concentration was maintained at, or close to 100% of air saturation by vigorous aeration.

Representative large samples of the effluent of the Egyptian Copper Works were brought to the laboratory in plastic containers.

Acute toxicity test was carried out according to Standard Methods for the Examination of Water and Wastewater (1975).

To study the effects of sublethal concentrations of this waste water, ten fish were introduced to each aquarium containing 50 litres of different dilutions (2.5; 5.0 cm^3/dm^3). The time of experiment was 4 weeks. To determine the effects of sublethal concentrations on some haematological parameters of fish, the blood was collected directly from the caudal artery into heparinized capillary tubes. Plasma protein and glucose were measured using standard kits (Modern Laboratory Chemicals). Plasma ion concentrations of sodium and potassium were measured using Gallenkamp flame analyser. Hematocrit was determined using microhematocrit tubes. Muscle protein concentration was measured using the method of Biuret (Gornall et al. 1949). Muscle lipid concentration was measured using the method of Knight et al. (1972). Moisture was determined by drying at 125°C for 3 hours and ash was measured by heating at 550°C for 3 hours.

Copper concentration in the liver, gills, and flesh was determined according to Standard Methods for the Examination of Water and Wastewater (1975) using Atomic Absorption Spectrophotometer (Spectr AA 10 plus). Copper was chosen in this study since it represent the highest metal concentration recorded in this waste water (Mourad 1995). Also, the toxic effects of mixture of heavy metals on the physiological parameters were mainly attributed to copper (Hilmy et al. 1987).

Biopsy specimens were collected for microscopic investigation from the gills, liver, and kidney of control and treated fish. Fixed in saline formalin solution, dehydrated, cleared, and embedded in paraffin wax, sectioned at 5-μm-thick sections, stained using Ehrlish haematoxylin and eosin, mounted for microscopically examination. Statistical test (t-test) was made to evaluate the significant changes caused by this waste water. Significance of differences was accepted at 5% level.

RESULTS AND DISCUSSION

The results of acute toxicity test for *Tilapia zillii* (Gerv.) exposed to different concentrations of the effluent of the Egyptian Copper Works showed that the LC_{50} was 25 cm^3/dm^3, which means that this waste water is highly toxic. The toxicity of this waste water is attributed mainly to combination of several synergistic factors e.g. high concentration of heavy metals and solids besides low pH and dissolved oxygen (Mourad 1995).

Tab. 1 represents the physiological response of *Tilapia zillii* (Gerv.) after exposure to sublethal concentrations of the effluent of the Egyptian Copper Works (2.5; 5.0 cm^3/dm^3) for 28 days. A significant increase in hematocrit from 25.88% to 30.23 and 31.81% was observed after exposure to this waste water which may be attributed to gill damage or increased demand for oxygen by certain tissues (Andersson et al. 1988). Hematocrit value increase was also observed by several authors after exposure to heavy metals (McKim et al. 1970; Hilmy et al. 1987).

A significant hyperglycaemia was also recorded after exposure to this waste water e.g. control fish had a mean plasma glucose of 56.80 mg/100 cm^3 while the-treated fish exhibited an increase in the levels of plasma glucose to 65.30 and 81.00 mg/100 cm^3, respectively. This means that the fish were subjected to some sort of hypertoxic stress. It is well known that stressful stimuli elicit rapid secretion of both glucocorticoids (Wedemeyer 1969) and catecholamines (Nakano and Tomlinson 1967) from the adrenal tissues of fish and both of these hormones produced hyperglycaemia (Oguri and Nace 1966). The obtained results are in agreement with Dange (1986) and Benson et al. (1987) who recorded an increase in plasma glucose levels after exposure to heavy metals.

In this study, tissue and plasma total protein were generally influenced by this waste water which may be attributed to the relative changes in the mobilisation of protein. Changes in the plasma protein concentrations may be a result of increased production of metallothionein which is a sequestering agent (Cousins 1982). The synthesis of these protein was found to be induced by several factors mainly the heavy metals (Oh et al.1978). On the other hand, the elevation of plasma glucose which runs parallel to a decrease in muscle protein content may be on indication of a gluconeogenetic response. This additional source of glucose may support the fish with the required energy highly demanded to cope with the presence of a potentially harmful substances such as heavy metals.

Table 1

Physiological response of *Tilapia zillii* (Gerv.) ** exposed to sublethal concentrations of the effluent of the Egyptian Copper Works for 28 days

Parameter	Concentration (vol./vol.)		
	Control	2.5 cm^3/dm^3	5.0 cm^3/dm^3
Hematocrit (%)	25.88 ±1.08	30.23 ±1.19*	31.81 ±0.98*
Plasma protein (mg/100 cm^3)	4.63 ±0.13	4.85± 0.13*	4.93 ±0.21*
Plasma glucose (mg/100 cm^3)	56.80 ±4.34	65.30 ±4.13*	81.00 ±6.38*
Plasma sodium (mmol/dm^3)	140.00 ±3.79	144.50 ±3.08*	146.50 ±4.78*
Plasma potassium (mmol/dm^3)	14.50 ±1.10	15.60 ±1.70	16.40 ±1.80*
Muscle protein (mg/100 mg)	21.60 ±1.20	20.20 ±1.08*	18.30 ±1.27*
Muscle lipid (mg/100 mg)	1.68 ±0.62	1.72 ±0.48	1.69 ±0.52
Water content (%)	75.20 ±1.84	74.10 ±1.78	73.70 ±1.86
Ash content(%)	7.10 ±0.85	7.30 ±0.94	7.60 ±0.59
Fish condition (Kf)	1.51 ±0.03	1.47 ±0.03*	1.47 ±0.02*

* Significant difference in comparison to control group.
** Average of 10 fish ±standard deviation.

An increase in the levels of plasma sodium and potassium concentrations were also observed after exposure to this waste water. This may be attributed to the changes in the permeability to sodium and potassium at the branchial site. The obtained results are in accordance with Stagg and Shuttleworth (1982) who found disturbances in plasma electrolyte concentrations after exposure of the fish to copper.

The condition of fish exhibited a significant depression after exposure to this waste water which might be a result of elevation of the fish metabolic rate and cessation of feeding. Buckley et al. (1982) showed also a decrease in the condition of fish after exposure to copper. Changes in the muscle lipid, ash, and water content were statistically insignificant.

Tab. 2 represents bioaccumulation of copper in different organs of fish after exposure to sublethal concentrations of the waste water. It is evident that, bioaccumulation of copper in fish organs increased as the concentration of copper in the exhibited water increased. Also, the copper levels were elevated in the liver, gills, and flesh of the exposed fish compared to control with the liver tissue accumulating a much larger amount of the metal than any other tissue. This is a common phenomenon, since liver plays a major role in the protection against chronic heavy metals exposure both by producing metallothioniens and by

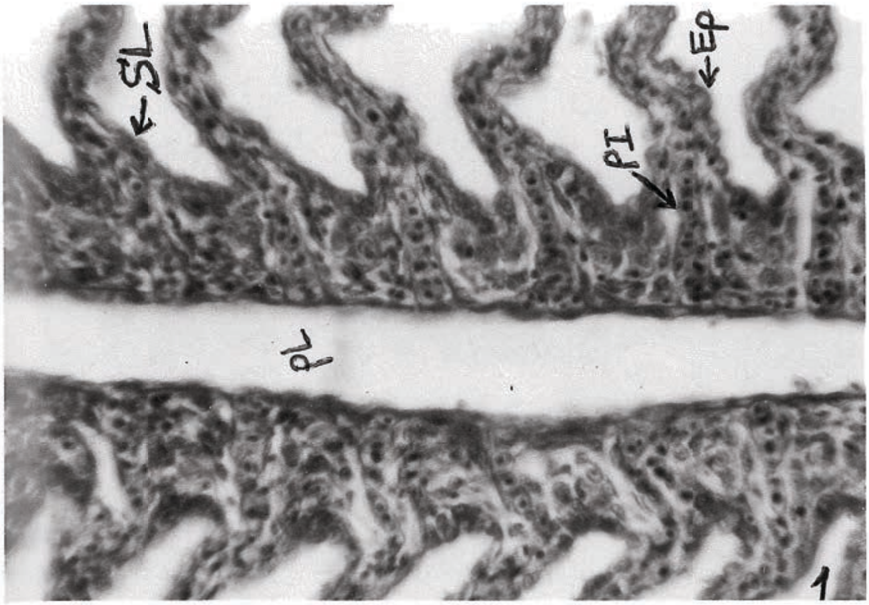

Fig. 1. Frontal section in gill filament of control *Tilapia zillii* showing secondary lamellae (SL) orginated on either side of primary lamella (PL), wide interlamellar space (see arrow). H & E x400

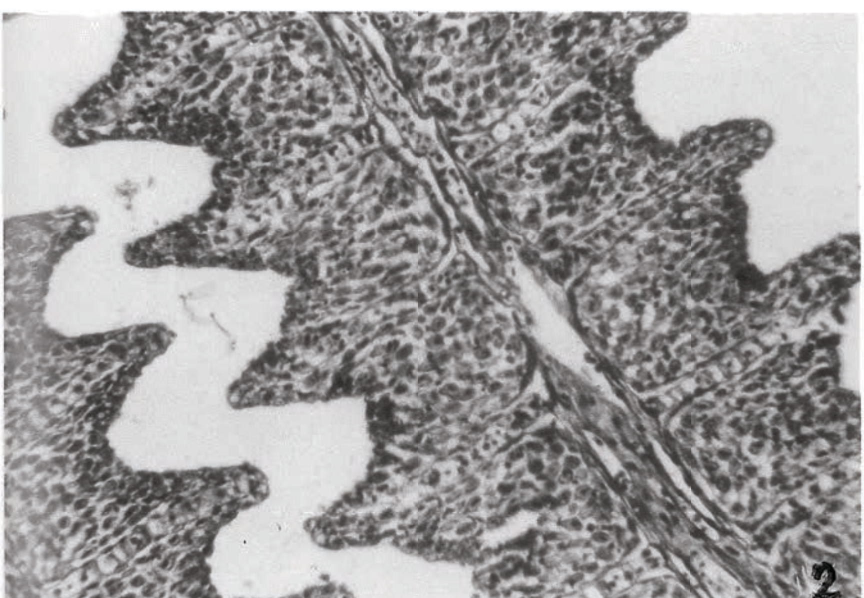

Fig. 2. Frontal section in gill filament of *Tilapia zillii* exposed to 2.5 cm^3/dm^3 of effluent showing hyperplasia of primary epithelial cells leading to partial blocking of interlamellar space. H & E x300

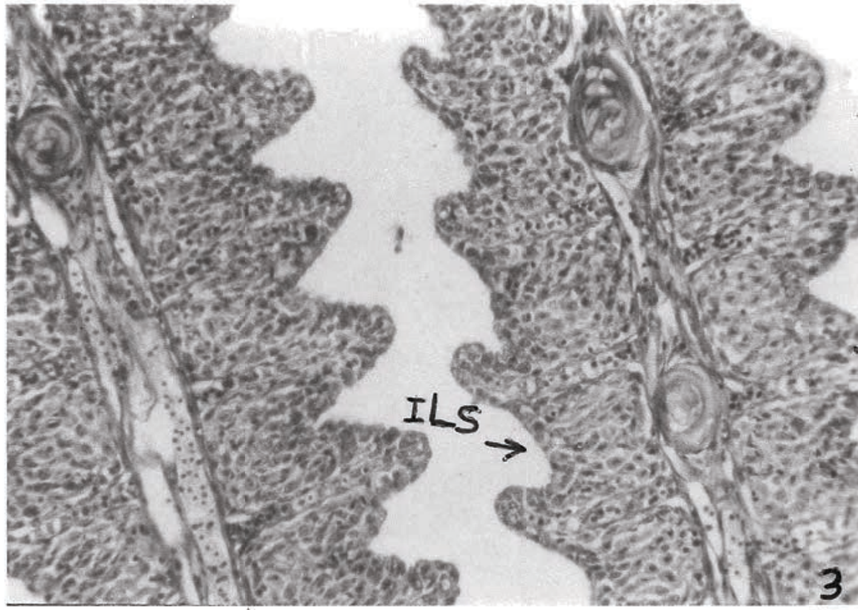

Fig. 3. Frontal section in gill filament of control *Tilapia zillii* exposed to 5.0 cm^3/dm^3 of effluent showing extensive hyperplasia of primary epithelium cells and blocking of interlamellar space. H & E x300
(PL) primary lamella, (SL) secondary lamella, (PI) pillar cell, (EP) epithelial cell, (ILS) interlamellar space

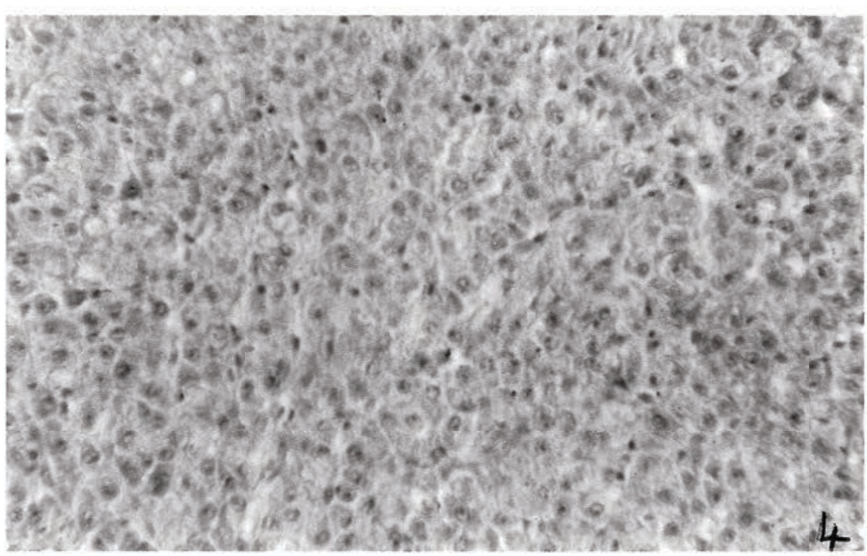

Fig. 4. Cross-section in liver of control *Tilapia zillii* showing uniform cells and well organized liver cords alternating with sinusoids. H & E x300

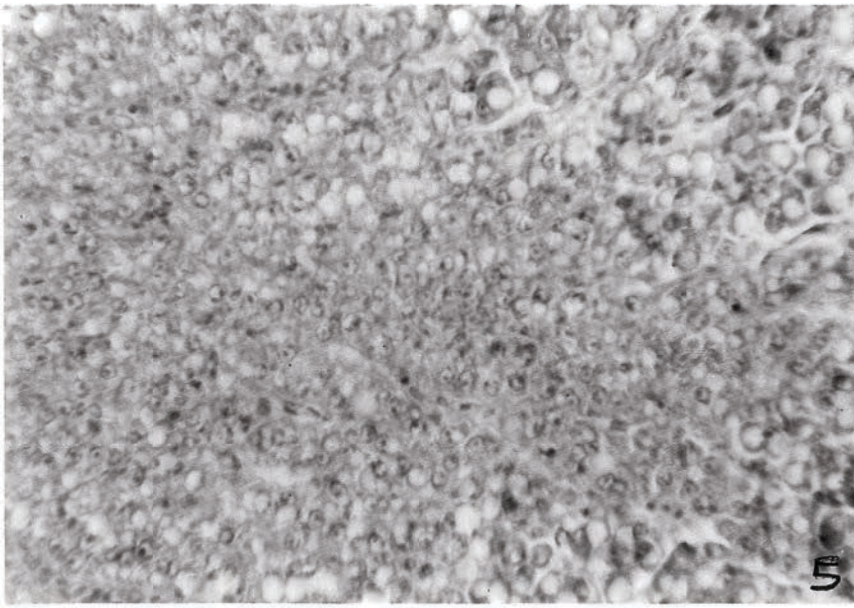

Fig. 5. Cross-section in liver of *Tilapia zillii* exposed to 2.5 cm^3/dm^3 of effluent notice hepatic cells deposite large amount of fat-inducet by clear area.
H & E x300

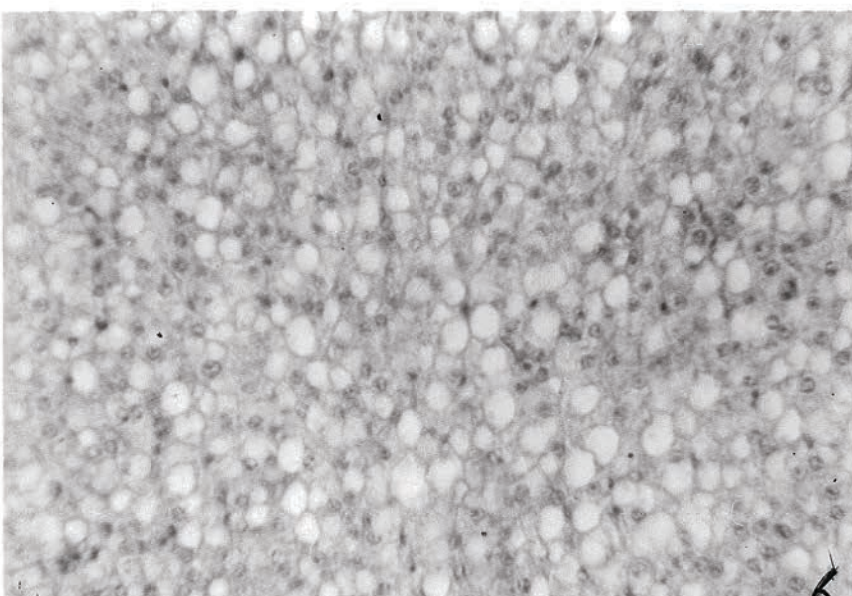

Fig. 6. Cross-section in liver of *Tilapia zillii* exposed to 5.0 cm^3/dm^3 of effluent showing severe infiltration and necrosis of nuclei. H & E x300

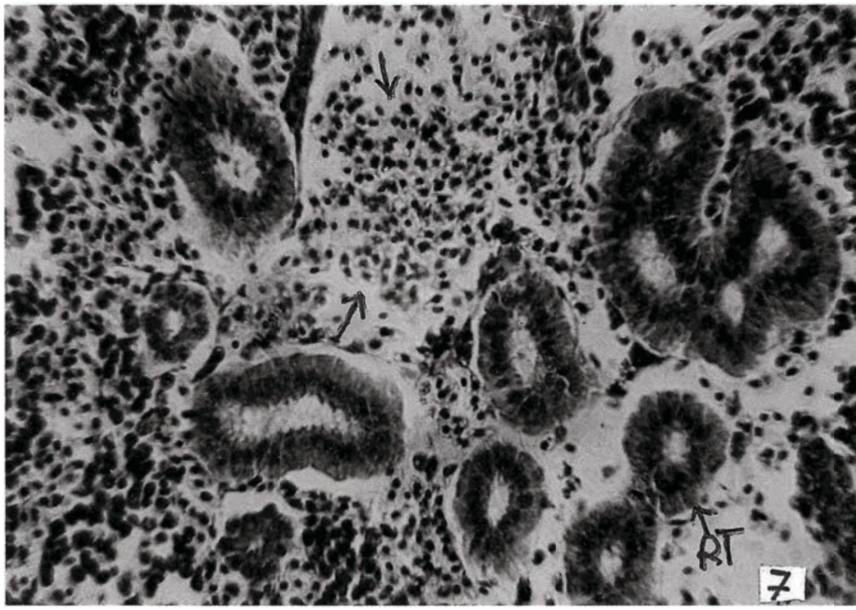

Fig. 7. Cross-section in kidney of control *Tilapia zillii* showing normal structure of tubules and haemapoietic tissue in between tubules. H & E x300

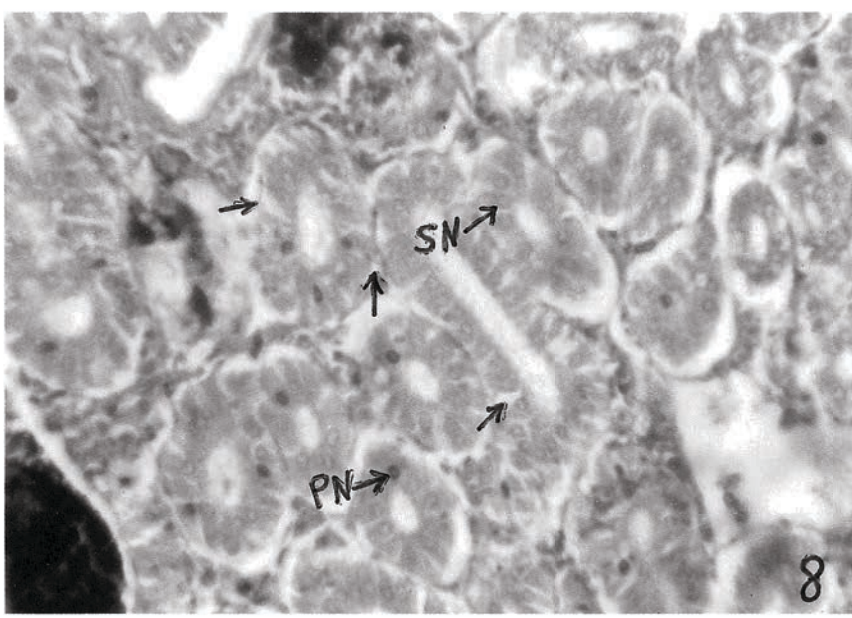

Fig. 8. Cross-section in kidney of *Tilapia zillii* exposed to 2.5 cm^3/dm^3 of effluent showing beginning of tubules destruction and pycnosis of nuclei. Notice deposition of dark granules in glomeruli. H & E x300

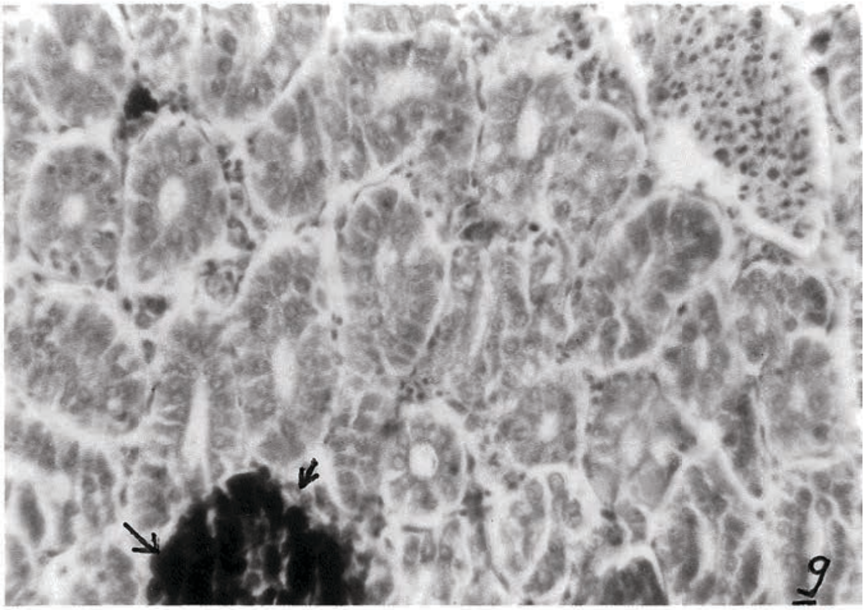

Fig. 9. Cross-section in kidney of *Tilapia zillii* exposed to 5.0 cm^3/dm^3 of effluent showing great destruction of tubules, nuclei pycnosis, darkly staining glomerulus and reduction of haemapoietic tissue in between tubules.
H & E x300
(RT) renal tubule, (PN) pycnotic nucleus, (SN) swelling of nucleus

acting as a storage site of the bound metal (Webb 1979). The obtained results are in agreement with Shakweer and Abbas (1997).

Table 2

Copper concentration (μg/g) in different organ of *Tilapia zillii* (Gerv.) exposed to sublethal concentrations of the effluent of the Egyptian Copper Works for 28 days

Organ	Concentration (vol./vol.)		
	Control	2.5 cm^3/dm^3	5.0 cm^3/dm^3
Liver	3.97 ±0.50	10.76 ±3.47 *	14.81 ±5.69*
Gills	3.52 ±0.39	4.26 ±0.32*	4.90 ±0.56*
Flesh	2.24 ±0.14	2.88 ±0.32*	3.30 ±0.56*

* Significant difference in comparison to control group.
** Average of 10 fish ± standard deviation.

Histological examination of the fish after exposure to sublethal concentrations of the effluent of the Egyptian Copper Works revealed serious disturbances in the gills, liver, and kidney tissues. As shown in Fig. 1, control gills had normal structure of lamellae and wide interlamellar space. The fish exposed to diluted effluent showed hyperplasia of primary lamellar epithelium leading to obstruction of the water passage and reduction of the respiratory surface area (Figs.2, 3). Similar results were recorded in other teleosts exposed to copper (Gardner and Roche 1973; Daoust 1981; Khadre 1990).

The liver of control fish group showed the usual polyhedral hepatocytes, homogeneous cytoplasm, and rounded nuclei (Fig. 4). The exposed fish showed fatty infiltration with nuclear pycnosis (Figs. 5, 6). This fatty infiltration may be due to metabolic disturbances in liver tissue that enhanced activity of biotransformation enzymes. Histological lesions involving extensive fatty infiltration due to abnormal lipid accumulation in present work is in agreement with those finding of Backer (1969) and Khadre (1991).

The kidney of control fish showed normal structure of renal tubules (RT) and haemapoietic tissue in between (Fig. 7). The exposed fish showed tubular destruction and epithelial cell oedema. This oedema caused detachment of the epithelial cells from the underling basement membrane, pycnosis of nuclei, and swelling of others. Deposition of dark granules inside glomeruli were also observed (Figs. 8, 9). This darkly stained granules may be a result of alteration in urinary pH or urinary stasis as a consequence of tubular destruction. Backer (1969) and Wahbi (1998) found the same results in winter flounder and *Solea vulgaris* due to copper and industrial effluent toxicity.

CONCLUSIONS

1. The median lethal concentration of the effluent of the Egyptian Copper Works for *Tilapia zillii* (Gerv.) was 25 cm^3/dm^3.
2. Significant changes in fish metabolism and osmoregulation were recorded during exposure to sublethal doses of this effluent (2.5; 5.0 cm^3/dm^3).
3. Histological examination showed serious disturbances in the gills, liver, and kidney.
4. The highest amount of copper was accumulated by the liver followed by the gills and flesh.
5. The effluent of the Egyptian Copper Works was not acceptable to discharge to aquatic system.

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FIZJOLOGICZNE I HISTOLOGICZNE ZMIANY U TILAPII (*TILAPIA ZILLII* GERV.) POD WPŁYWEM SUBLETALNEJ KONCENTRACJI WODY ZRZUTOWEJ Z EGIPSKICH ZAKŁADÓW MIEDZIOWYCH

STRESZCZENIE

Badano fizjologiczne i histologiczne zmiany u tilapii (*Tilapia zillii* Gerv.) powstałe w wyniku działania nieoczyszonych ścieków pochodzących z Egipskich Zakładów Miedziowych w Aleksandrii. Test toksyczności LC_{50}–96h wykazał, że koncentracja letalna ścieków dla tilapii wynosi 25 cm^3/dm^3 wody. W subletalnej koncentracji (2.5; 5.0 cm^3/dm^3) stwierdzono następujące zmiany we krwi – wzrost hematokrytu, białka, glukozy, jonów Na^+ i K^+. W mięśniach – spadek zawartości białka. Badania histologiczne wykazały: w skrzelach rozrost, zwiększenie liczby komórek; w wątrobie nacieczenie komórkami tłuszczowymi; w nerce kanalik nerkowy uległ destrukcji, wystąpił obrzęk. W wątrobie stwierdzono statystycznie wysoce istotny wzrost zawartości miedzi, natomiast w skrzelach i mięśniach umiarkowany.

Received: 1 March 1999

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