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SOME HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN TILAPIA, *Oreochromis niloticus* And *Tilapia zillii* EXPOSED TO MIXTURE OF COPPER AND LEAD.

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ABSTRACT

A hematological and biochemical study was conducted on the freshwater fish *Oreochromis niloticus* and *Tilapia zillii*. The fish were acclimated to laboratory conditions for two weeks before use. Then the fish were exposed for 12 weeks to a mixture of copper and lead at a ratio 1:1 of the LC₅₀ of both metals. Hematological and biochemical analyses were carried out every two weeks during the exposure period. Results indicated the following:

In the hematological study, there was a significant increase in the total erythrocytes count (RBCs), as well as the hematocrite (Ht) and mean corpuscular hemoglobin concentration (MCHC) in *O. niloticus* upon exposure to the heavy metals mixture. This increase was observed after 10 weeks for RBCs count, from the 6th week of exposure till the end of the experiment for Ht and throughout the whole time of exposure for MCHC. For *T. zillii*, the increase in the RBCs count was noticed at weeks 4, 6 and 12; the increase in Ht occurred at weeks 10 and 12; while a decrease in MCHC was noticed during the entire period of exposure. On the other hand, hemoglobin content (Hb) decreased significantly starting from the second week of exposure till the end of the experiment for *O. niloticus*. Similar decrease was noticed for *T. zillii* (except the value at 8 weeks of exposure) and continued till the end of the exposure period.

Non significant changes were observed in the serum glucose concentration for *O. niloticus*, while serum glucose was significantly increased in metals treated *T. zillii*. For both species, non significant changes were noticed in Liver and muscle's glycogen levels. However, serum protein levels were significantly increased in *O. niloticus*, but changes were non significant in *T. zillii*. On the other hand, muscle total protein showed a significant decrease after 10 weeks of exposure in *O. niloticus*. Such significant decrease in muscle total protein of *T. zillii* appeared only during the 12th week of exposure. Changes in serum creatinine and uric acid levels in *O. niloticus* starting from the 8th week of exposure till the end of the experiment.

Key words: Tilapia – Copper – Lead – Blood – Muscles – Liver .

INTRODUCTION

In recent years, heavy metals are widely distributed in aquatic systems due to industrial development and the wide use of chemicals in agriculture as well as

the lack of environmental regulations (El-Nabawi, et al., 1987; Calamari and Nave, 1994; Abdelhamid and El-Zareef, 1996; Zaghloul, 1997 and Abdelhamid and Gawish, 1998). The passage of different environmental pollutants to the aquatic system demonstrates the need for a comprehensive study for their effects on the living resources. Waste discharges never occur singly but always as a mixture (Abdelhamid et al., 1997; 2000 and 2006 a). A knowledge of mixture toxicity is important in determining water quality criteria since interaction may increase or decrease toxicity depending upon the nature of the individual pollutants (Sprague and Fogles, 1977).

Copper and lead are considered as the most toxic among heavy metals, which are detectable practically in aquatic environment and in all biological systems (Haux and Larsson, 1982 and Ghazaly and Said, 1995).

Copper and lead toxicity depends on many factors such as species differences, life stage, concentration in food and water, food consumption rate, water quality, time of exposure and metal speciation in water (Elsa, 1991 and Ghazaly and Said, 1995).

Hematological and biochemical measurements have been used as good indicators of the state of fish health condition in addition to the detection and diagnosis of metabolic processes (Heath, 1987).

The toxicity of heavy metals to different fish species have been reported by many authors (Radhakrishnan et al., 1992; Ghazaly and Said, 1995; James and Sanpath, 1995; El-Sabbagh, 1996 and Rizkalla et al., 1999). However, information about the subchronic toxicity of copper and lead mixture are not currently available.

The aim of the present study was to examine and evaluate the magnitude of hematological and biochemical alterations in blue tilapia *O. niloticus* and *Tilapia zillii* after exposure to chronic concentration of copper and lead mixture in a concentration of 1:1 of LC₅₀ copper and lead for 12 weeks.

MATERIALS AND METHODS

Sample collection and treatment manipulation:

Specimens of the tilapia, *Oreochromis niloticus* and *Tilapia zillii* 36.38 ± 0.77 g in weight and 12.93 ± 0.88 cm in length were caught from Tawarga pond and transported immediately to the fish laboratory at Biology Department, Faculty of Science, 7 October University. The experimental fishes were reared in aerated glass aquaria (75 x 50 x 50 cm), each of about 187 L capacity and acclimatized for two weeks before being used in the experimental study. The studied fishes were fed 25% CP commercial fish diet at a ratio of 3% of their body weight /day. During this acclimation period the mortality was less than 2%. Fishes were not fed for 48 hours prior to the experiments. Water temperature, pH, dissolved oxygen, copper and lead (for the control group) concentrations were 26.07 ± 1.17 °C (measured three times daily), 6.81 ± 0.05 (measured daily), 6.02 ± 0.02 ppm (measured daily) and 0.00 ppm (Measured weekly) respectively.

At the end of the acclimation period individuals of *O. niloticus* and *T. zillii* were divided randomly into six experimental groups (20 fish each) and reared separately in aquaria containing copper and lead mixture. The aquaria were provided with air pumps for aeration during the entire exposure period. Another

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group (from each fish species) was kept in an aerated aquarium containing natural water and used as a control.

Stock solutions (1000 ppm) of copper as copper sulphate $\{Cu SO_4 \cdot 5H_2O\}$ Analar (Merk) and of lead as lead nitrate $\{Pb (NO_3)_2\}$ Analar (Merk) were prepared separately and used in preparing the experimental water (to which fish were exposed) containing the mixture of the two metals at a ratio 1:1 of the 96 hours LC_{50} values of copper and lead (LC_{50} is the concentration of toxicant causing 50% mortality). The 96 hr LC_{50} values in both fish species previously determined according to the method described by **Litchfield and Wilcoxon (1949)**. These values were: 8.15 and 25.88 ppm copper and lead, respectively for *O. niloticus*, while they were 6.95 and 17.44 ppm copper and lead, respectively for *T. zillii*.

For each fish species, five individuals from the treated groups (and five control fishes) were taken after 2, 4, 6, 8, 10 and 12 weeks of exposure to the metals mixture. Blood and tissue samples were obtained for hematological and biochemical analysis.

Blood sampling:

Blood samples were withdrawn from the arteria caudalis. The needle (heparinized glass pipette) was run quite deep through the middle line just behind the anal fin in a dorso-cranial direction.

Serum was obtained by centrifugation (at 5000 rpm for 12 minutes) and was stored at -20 degrees centigrade for further analysis.

Muscle and Liver sampling:

After decapitation of fish, pieces of white apaxial muscle and liver were taken. The muscle and liver samples were transferred directly into weighing jars and accurately weighed. Then, the jars were placed in a drying oven which is thermostatically controlled at 105 degrees centigrade for 72 hours.

Hematological analysis:

Erythrocytes (RBCs) and leukocytes (WBCs) count was carried out using the improved Neubauer Hematocytometer (**Maxine and Benjamine, 1985**). Hemoglobin content (Hb) was measured as described by **Zulsira (1961)**. The Hematocrite volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by **Maxine and Benjamine (1985)**.

Biochemical analysis:

Commercial diagnostic kits from Bio-Merieux chemicals (France) were used for the following biochemical assays.

The concentration of serum glucose was measured by the GOD-PAD method (Enzymatic Colorimetric method) according to **Trinder (1969)**. Total protein levels were determined by biuret test according to **Henry (1964)**. Serum creatinine was measured colorimetrically as described by **Henry (1974)**. Serum uric acid was determined according to **Barham and Trinder (1972)**.

Tissue analysis:

Liver and muscle glycogen levels were determined using the anthrone reagent according to the method of **Handle and Van (1965)**. Muscle total protein was estimated as mentioned by **Josylne (1950)**.

Statistical analysis:

The results were statistically analyzed using the analysis of variance (ANOVA) and Duncan's multiple comparison test to compare between means at $P < 0.05$ (Steel and Torrie, 1980).

RESULTS**Hematological analysis:**

The results of hematological studies on *Oreochromis niloticus* are illustrated in Table (1). The exposure of fish to 1:1 of the LC_{50} of copper and lead showed a gradual non significant increase in the total RBCs count. After 10 weeks of exposure, a significant increase was noticed ($P < 0.05$). However, significant decrease ($P < 0.01$) in the total RBCs count of *Tilapia zillii* was noticed during the 10th week of exposure, while an increase was noticed at weeks 4, 6 and 12. Non significant change ($P > 0.05$) in the total WBCs count was recorded in both species.

Hemoglobin content was decreased significantly ($P < 0.01$) starting from the second week of exposure till the end of the experiment for *Oreochromis niloticus*. Similar decrease ($P < 0.01$) was noticed in *Tilapia zillii* during the entire exposure period (except the value at eight weeks of exposure).

Table (1): Changes of blood pictures in *Oreochromis niloticus* and *Tilapia zillii* exposed to 1:1 of LC_{50} of copper and lead mixture for 12 weeks (w).

Time (W)	RBCs $10^6/\text{mm}^3$	WBCs $10^6/\text{mm}^3$	Hb g/dl	Ht %	MCV FL	MCH Mg/100ml	MCHC %
Control	0.91 ± 0.01	80.07 ± 0.90	10.83 ± 1.35	16.32 ± 0.08	181.45 ± 0.63	113.11 ± 1.70	52.27 ± 1.11
<i>O. niloticus</i>	2	0.90 ± 0.01	80.07 ± 0.12	9.96 ± 0.04	16.35 ± 0.07	$181.39 \pm .51$	110.47 ± 0.94
	4	0.92 ± 0.10	79.93 ± 0.90	10.07 ± 0.09	16.42 ± 0.06	179.38 ± 1.35	109.99 ± 1.23
	6	0.92 ± 0.10	80.55 ± 0.17	10.35 ± 0.10	16.82 ± 0.19	183.99 ± 2.60	114.19 ± 2.05
	8	0.92 ± 0.10	79.16 ± 0.31	10.19 ± 0.19	16.78 ± 0.10	182.71 ± 1.60	113.14 ± 2.59
	10	0.94 ± 0.10	79.65 ± 0.35	10.01 ± 0.08	16.94 ± 0.17	174.55 ± 6.05	107.83 ± 1.77
	12	0.92 ± 0.10	80.07 ± 0.90	10.35 ± 0.11	16.91 ± 0.22	184.71 ± 0.05	115.04 ± 1.76
	F value	1.74	3.27**	37.06**	3.94**	1.25	1.86**
Control	0.96 ± 0.01	80.07 ± 0.9	11.03 ± 1.25	16.12 ± 0.07	171.62 ± 0.53	119.01 ± 1.7	62.29 ± 0.19
<i>T. zillii</i>	2	0.95 ± 0.01	80.07 ± 0.12	10.06 ± 0.08	16.05 ± 0.07	177.39 ± 1.11	113.57 ± 0.95
	4	0.99 ± 0.1	80.03 ± 0.9	10.07 ± 0.09	16.10 ± 0.06	179.18 ± 1.25	119.99 ± 1.13
	6	0.98 ± 0.1	80.15 ± 0.13	10.35 ± 0.10	16.18 ± 0.19	183.01 ± 2.2	114.19 ± 2.05
	8	0.96 ± 0.1	79.16 ± 0.11	11.09 ± 0.29	16.11 ± 0.7	181.72 ± 1.61	123.18 ± 2.29
	10	0.94 ± 0.1	79.65 ± 0.35	10.08 ± 0.09	16.93 ± 0.17	171.55 ± 2.05	117.53 ± 1.79
	12	0.97 ± 0.1	80.07 ± 0.9	10.65 ± 0.15	16.81 ± 0.20	187.11 ± 1.15	125.14 ± 1.96
	F value	1.81**	3.02	39.25**	3.74**	1.55	1.92

Data are represented as means $\pm$ S.E.



total protein during the first 10 weeks of exposure while a significant decrease was noticed at 12 weeks of exposure.

As shown in Table (4), the level of serum creatinine in both *Oreochromis niloticus* and *Tilapia zillii* exposed to copper and lead mixture showed non-significant changes compared with values of the control fish. Values of uric acid in both species showed non-significant changes ($P>0.05$) during the first 8 weeks of exposure, while they increased significantly ($P<0.05$) at weeks 10 and 12.

Table (3): Changes of serum total protein (g/dl) in *Oreochromis niloticus* and *Tilapia zillii* exposed to 1:1 of LC₅₀ of copper and lead mixture for 12 weeks (w).

Time (w)	Serum total protein		Muscle total protein	
	<i>O. niloticus</i>	<i>T. zillii</i>	<i>O. niloticus</i>	<i>T. zillii</i>
Control	5.84± 0.21	6.06±0.19	16.79± 0.07	14.62± 1.11
2	6.23± 0.10	5.69± 0.22	16.78± 0.06	14.59± 0.09
4	6.33± 0.10	5.63± 0.20	16.81± 0.04	14.51± 0.07
6	6.35± 0.10	5.56± 0.18	16.72± 0.05	14.51± 0.05
8	6.35± 0.10	5.49± 0.19	16.73± 0.05	14.66± 0.06
10	6.39± 0.07	5.41± 0.19	16.60± 0.06	14.46± 0.08
12	6.50± 0.07	5.34± 0.18	16.41± 0.02	14.28± 0.04
F value	1.25**	1.52	7.4 **	3.14 **

Data are represented as means ±S.E.

Table (4): Changes of serum creatinine (mg/dl) and serum uric acid (mg/dl) in *Oreochromis niloticus* and *Tilapia zillii* exposed to 1:1 of LC₅₀ of copper and lead mixture for 12 weeks (w).

Time (w)	Serum creatinine		Serum uric acid	
	<i>O. niloticus</i>	<i>T. zillii</i>	<i>O. niloticus</i>	<i>T. zillii</i>
Control	1.01± 0.05	0.97± 0.05	14.4± 0.24	15.96± 0.36
2	0.91± 0.03	0.97± 0.05	14.21± 0.23	15.53± 0.29
4	0.91± 0.03	0.97± 0.05	14.45± 0.21	15.75± 0.29
6	0.92± 0.03	0.95± 0.05	14.70± 0.19	16.06± 0.31
8	0.93± 0.04	0.94± 0.05	15.09± 0.22	16.31± 0.27
10	0.93± 0.04	0.98± 0.04	15.26± 0.23	16.63± 0.25
12	0.94± 0.04	0.99± 0.03	15.51± 0.28	16.85± 0.25
F value	0.82	0.12	4.54**	2.63**

Data are represented as means ±S.E.

DISCUSSION

Many hematological, biochemical and physiological variables show specific responses to certain types of environmental stressors such as heavy metals (Abu-El Ella, 1996 and Rizkalla et al., 1999). This feature, of such responses, makes them particularly useful in fisheries management and resources protection as a mean of identifying possible courses of environmental

deterioration. However, the possibility to standard as diagnostic tool is still not definitive.

The present investigation dealt with hematological changes induced in *Oreochromis niloticus* and *Tilapia zillii* when both species were exposed individually to 1:1 of the LC₅₀ of copper and lead for 12 weeks.

In the hematological study, there was a significant increase in the total RBC's count, the Ht and MCHC in *Oreochromis niloticus* during the 10th week of exposure for RBCs, from the 6th week of exposure till the end of the experiment for Ht and throughout the whole time of exposure for MCHC. While such increase in the RBCs count, Ht and MCHC values was noticed in case of *Tilapia zillii* at the 12th week of exposure.

Increase in RBCs count and Ht value of the two studied species may be attributed to impairment of gas exchange by the gills and a release of erythrocytes from the spleen to compensate impaired oxygen uptake, which resulted from disturbed gill function (Larsson *et al.*, 1980). It may also reflect hypoxic stress resulting in secondary polycythemia and/or a decrease in rate of erythrocyte destruction as previously reported by Salah El-Deen *et al.*, 1997. The increase in MCHC was also previously observed by Ahmed *et al.*, 1992 for *Oreochromis niloticus* that affected by ammonia.

On the other hand, Hb decreased significantly starting from the second week of exposure till the end of the experiment for *Oreochromis niloticus*. Such decrease was noticed for *Tilapia zillii* after 10 weeks of exposure. Similar results was obtained by Mukherjee and Sinha (1993) who found that exposure to 20 mg/L of CdCl₂ for 2 weeks caused a significant decrease in hemoglobin of *Labeo rohita*. Rizkalla *et al.*, 1999 also found such Hb decrease in *Tilapia zillii* exposed to a combination of copper and zinc for 30 and 90 days respectively. Such reduction in Hb level could be attributed to the intrahepatic and intrasplenic hemorrhage (Sinovec *et al.*, 1980).

Higher concentration of heavy metals beyond the tolerance limit may cause fluctuation in some blood parameters such as MCV, MCH and MCHC. These changes may include disturbances in the production of erythrocytes from haemopoietic tissue and changes in their volume (Marie, 1990 and 1992).

In the present study, MCV and MCH values of the exposed *Oreochromis niloticus* showed non-significant changes from the control mean value throughout the entire experimental period (except MCH value at weeks 10 and 12), while a significant increase in the values of those parameters in *Tilapia zillii* was recorded at 12 weeks of exposure. The increased MCV and MCH in *Tilapia zillii* could be attributed to hemolytic action of lead, which led to fluid loss from the tissues with subsequent decrease in plasma volume. This assumption is highly supported by Swift (1981).

It is assumed from the present results that the increase of the blood indices MCV, MCH and MCHC may be attributed to a defense reaction against the toxicity of heavy metal through stimulation of erythropoiesis. This is similarly observed by Rizkalla *et al.*, (1999) and supported by the opinion of Abd-Alla *et al.* (1992).

In biochemical studies, blood glucose measurements are known to be a sensitive indicator for environmental stress in fish. In the present study, serum

glucose as well as liver and muscle glycogen concentration of the two fish species showed non-significant changes when both species were exposed individually to 1:1 of the LC_{50} of copper and lead mixture throughout the whole period of exposure. Such non-significant changes could be explained by the antagonistic effect of copper and lead on the gluconeogenesis process. Several authors (Radhakrishnanalah *et al.*, 1992 and Ghazaly and Said, 1995) reported a direct correlation between copper treatment and substantial loss of liver and an increase in the activities of liver glycogen phosphorylase and glucose-6-phosphatase. They added that muscle glycogen was also decreased along with an increase in its glycogen phosphorylase activity accompanied by a considerable rise in the plasma glucose level. Such hyperglycemic effect was attributed to the increased secretion of catecholamines from adrenal medulla. Gluth and Hanke (1984) concluded that the reduction of the amount of liver and muscle glycogen is also a good indicator of toxicity.

The hyperglycemic effect of copper treatment was antagonized by the inhibitory effect of lead on the gluconeogenesis process suggested by Corell (1974). Similarly, several authors have also noticed the inhibitory effect of lead on serum glucose level in different fish species (Salmeron *et al.*, 1990 for blue tilapia (*Oreochromis aureus*) and Haux and Larsson, 1982 for Rainbow trout *Salmo gairdneri*). This antagonistic effect of copper and lead on the gluconeogenesis process may partially explain the immobilization of liver glycogen into blood glucose.

An important function of serum protein is the maintenance of osmotic balance between the circulating blood and the cell membrane (Harper *et al.*, 1977). In the present study, serum total protein of *Oreochromis niloticus* showed a pronounced significant increase throughout the whole period of exposure reaching its maximum value during the 12th week. This significant increase in total protein was only noticed in *Tilapia zillii* after 8 weeks of exposure.

The increased serum protein in both *O. niloticus* and *T. zillii* exposed to sublethal concentrations of copper and lead mixture in the present study could be attributed to several pathological conditions such as damage of liver, kidneys and gills. This could be due to accumulation of metals in these organs and relative changes in the mobilization of serum protein (Reichert *et al.*, 1979). The increase in serum total protein in fishes exposed to metals may also be due to impaired water balance (Harper *et al.*, 1977).

In general, the changes in plasma total protein would seem to be of a great value for the additional expenditure of energy but of a limited value as potential specific indicators in fish exposed to pollutants. This conclusion was in agreement with the findings reported by Alam and Mahughan (1992).

Regarding the effect of exposure to copper and lead mixture on meat quality of the two studied species in the present work, it has been observed a decrease in the muscle total protein. Such decrease was noticed for *O. niloticus* and *T. zillii* after 10 and 12 weeks of exposure, respectively. The decrease in muscle total protein could be attributed to the reduction in food consumption and/or decrease in gross food conversion. It could be also related to the decrease in insulin level detected by the observed higher serum glucose level in copper and

lead exposed fish. Insulin has greater effect on protogenic and lipogenic pathways (Zaghloul, 1997).

Increasing levels of creatinine and uric acid above normal values indicate several disturbances in kidney function (Maxine and Benjamine, 1985). A significant increase in the uric acid level was noticed for *O. niloticus* starting from the 10th week of exposure till the end of the experiment. On the other hand, non-significant changes in the level of serum creatinine and uric acid were noticed in *Tilapia zillii* during the period of the experiment. Such increase in the uric acid level was noticed by other investigators (Zaghloul, 1997).

Recently, Abdelhamid *et al.* (2006 b) found that copper led to higher WBC's but lower RBC's, Ht, Hb, glucose and total protein of Nile tilapia plasma.

It could be concluded, that the combination of copper and lead induced different hematological and biochemical alterations which in turn may affect fish production and its meat quality. This may indicate the importance of further studies on the mode of action of different heavy metals both individually and as mixtures.

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ملاحظات هيماتولوجية وبيوكيميائية على

اسماك البلطي *Oreochromis niloticus* و *Tilapia zillii*

نتيجة التعرض لخليط من النحاس والرصاص

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تم فى هذا البحث دراسة بعض التغيرات الهيماتولوجية والبيوكيميائية فى الدم، العضلات والكبد لنوعين من اسماك المياه العذبة (*Oreochromis niloticus* و *Tilapia zillii*) تم جمعها من بحيرة تاورغاء بمصراتة بليبيا بعد تعرضهم لخليط من الرصاص والنحاس (بتراكيز تمثل القيم النصف المميتة لكلا المعدنين 96 hours LC₅₀) بنسبة ١:١ لفترة ١٢ اسبوع. وقد أظهرت النتائج الاتى: ظهور تغيرات فى عدد كرات الدم الحمراء والبيضاء والهيموجلوبين والهيماتوكريت عن مثيلاتها فى المجموعة الضابطة فى كل من البلطي تيلابيا أوريا و تيلابيا زيللى باختلاف مدة التعرض. بينما لم تظهر اختلافات معنوية فى كل من جلوكوز الدم وجليكوجين الكبد والعضلات فى كل من تيلابيا أوريا وتيلابيا زيللى. كما لوحظت زيادة معنوية فى البروتين الكلى فى دم اسماك البلطي تيلابيا أوريا فى حين ظهر نقص فى البروتين الكلى فى دم اسماك البلطي تيلابيا زيللى. أظهرت النتائج وجود زيادة تدريجية فى مستوى حمض اليوريك فى دم كل من البلطي تيلابيا أوريا و تيلابيا زيللى طيلة فترة التجربة (١٢ اسبوع) بينما لم تحدث تغيرات فى كرياتين الدم فى كلا النوعين من الأسماك خلال فترة التعرض.

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كلية الزراعة

رقم الإيداع

٢٠٠٣-١٢٢٩٣

مجلة الفيوم للبحوث والتنمية الزراعية

