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Effect of Copper on Hematological, Biochemical Changes and Reproductive Hormones of the Nile tilapia *Oreochromis niloticus*

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ABSTRACT

The effect of copper concentrations on alterations in hematological and biochemical parameters as well as reproductive hormones of the Nile tilapia *Oreochromis niloticus* was investigated. The copper sulfate at sub-chronic concentrations 25, 50 and 75 µg/L on *Oreochromis niloticus* for 90 days of exposure. Significant changes ($P < 0.05$) in almost all hematological aspects were found in the all groups exposed to different concentrations of copper 25, 50 and 75 µg/L for 90 days. Biochemical analysis revealed various significant ($P < 0.05$) differences among the all groups that exposed to 25, 50 and 75 µg/L of copper for 90 days of exposure. There was a significant difference in the copper concentrations in the tissues ($P < 0.05$) among all treated groups; in liver, gills and kidney. Glucose and cortisol in fish plasma increased significantly, while total protein and total lipids decreased significantly due to copper stress. The obtained results showed also that copper stress was harmful to the fish liver and kidney, where plasma aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, uric acid, and creatinine values were significantly increased with increasing exposure of copper on *Oreochromis niloticus* for 90 days compared to the control one. Plasma follicular stimulating hormones (FSH), 17ß estradiol (E2) and testosterone (T) were decreased significantly in fish with increasing exposure of copper concentrations. Among antioxidative enzymes, significant changes were revealed mainly in plasma ceruplasmin and glutathione reductase and glutathione peroxidase activity in liver and gills ($P < 0.05$. The results demonstrate the deleterious influence of copper on *Oreochromis niloticus*, even at low doses. So, it is recommended to not use copper too much during treatment of the water that used in growing the fish.

INTRODUCTION

Copper (Cu) is an essential micronutrient, with an either anti or pro oxidant properties **Majewski *et al.*, (2019).** Cu is able to regulate superoxide dismutase (SOD), lysyloxidase and monoamine oxidase and is engaged in tryptophan metabolism by regulating the activity of enzymes on the kynurenine pathway **Majewski, *et al.*, (2016),** which can generate toxic products when dysregulated (Majewski, *et al.*, 2018). Recent studies have questioned the safety of the standard dosage of Cu looking for a possible alternative. Meanwhile, the beneficial antidiabetic and cardioprotective role of Cu nanoparticles (NPs) with decreased production of inflammatory mediators has been reported **(Sharma, *et al.*, 2018a; Sharma, *et al.*, 2018b; Cholewińska, *et al.*, 2018; Majewski *et al.*, 2019; Sharma *et al.*, 2016).** copper can become highly toxic to living

organisms. Copper contamination of the aquatic environment is of particular concern because organisms are continuously exposed via the skin and respiratory surfaces, as well as via the diet, including ingestion of contaminated water or sediment **Wood *et al.*, (2012**). As a consequence of contemporary and historical mining activities, industrial processes and urban and agricultural runoff, copper concentrations currently reported in the aquatic environment often reach those known to be toxic to fish **Batty *et al.*, (2010).** Copper can adversely affect multiple processes including branchial ion transport, haematopoiesis and glycolytic enzymatic activity, and cause immune suppression and oxidative stress **Richards, (2009).** In addition, fish exposed to copper have been shown to lose the ability to sense environmental oxygen levels **Geest *et al.*, (2002).** This raises a significant concern as aquatic hypoxia and copper pollution often co-occur in the environment **Malekpouri *et al.*, (2016).** Other studies investigating the combined effects of copper and hypoxia demonstrated that copper toxicity changed dramatically under hypoxic conditions during zebrafish (*Danio rerio*) development (copper toxicity was reduced during early development but increased in hatched larvae) **Fitzgerald *et al.*, (2016),** with similar results observed for three-spined stickleback (*Gasterosteus aculeatus*) **Fitzgerald *et al.*, (2017).** Combined exposures to copper and hypoxia also increased oxidative stress and caused alterations in blood parameters in the killifish (*Fundulus heteroclitus*) **Ransberry *et al.*, (2016),** *pacu (Piaractus mesopotamicus*) **Sampaio *et al.*, (2008)** and carp (*Cyprinus carpio*) **Mustafa *et al.*, (2012)**. For mangrove rivulus (*Kryptolebias marmoratus*), exposure to copper induced hypoxia-like changes to gill morphology and increased the sensitivity of the hypoxia emersion response **Blewett *et al.*, (2017).** In addition, for the common carp, a reduction in standard metabolic rate and critical oxygen level (Pcrit) was seen after combined exposure to copper and hypoxia **Malekpouri *et al.*, (2016).** Copper is a transition metal with a high abundance in aquatic and terrestrial environments. Copper, as an essential nutrient, plays an important role in various functions in cellular biochemistry, especially as a cofactor for many enzymes and as a constituent of the non-enzymatic antioxidants ceruloplasmin and the metallothioneins **Amiard *et al.* (2006).** Through these mechanisms, copper participates in antioxidative defence against various deleterious substances **Pandey *et al.* (2001).** Copper at a dosage rate of 0.15–0.20 mg/L Cu^{2+} is effective for control of important fish reported by **Roy, (2010**). Copper naturally occurs in fresh water systems in the concentration range from 0.5 mg/l **Ruas *et al.* (2008).** One of the known mechanisms of copper toxicity to fish is the promotion of oxidative stress **Lushchak, (2011).** Direct oxidative damage to cells can be the result of the participation of copper in the production of reactive oxygen species through the Fenton reaction. Copper can interact with various antioxidant enzymes, particularly in acute exposures, or at the beginning of chronic exposure. Copper can also be bound to thiolcontaining molecules such as glutathione and metallothioneins **Amiard *et al.* (2006),** and thereby interfere with antioxidant defense. The aim of the present study was to investigate the impact of copper (copper sulphate) at slightly higher concentrations than environmental ones on *Oreochromis niloticus* for period 90 days of exposure. Furthermore, the study also focused on the difference in their effects on fish. The toxic impact was evaluated on the basis of results of hematological, biochemical and reproductive hormones and indices of oxidative stress.

MATERIALS AND METHODS

Experimental Design:

Oreochromis niloticus body weight (50 ± 2 g) were obtained from Abbasa fish farm. They were randomly distributed into twelve glass aquaria; each aquarium has 10 fish. Each treated group, including the control, was tested in triplicate. The control groups were subjected to dechlorinated tap water. The actual copper concentrations were measured on three different sampling days during the experiment, each time before and after bath renewal. The copper concentrations determined by atomic absorption spectrometry were 25.5 ± 0.5, 50.3 ± 0.7 and 75.6 ± 0.9 µg/l for the groups exposed to copper sulphate (mean ± SEM). The experiment was conducted and siphoned every day. The control groups were subjected to dechlorinated tap water. For all groups.

Measurement of water quality:

Water samples were collected at 20 cm depth from each aquarium. Dissolved oxygen and temperature were measured daily in site using a portable DO meter (Jenway, London, UK). The pH values were measured using a Digital Mini-pH Meter (model 55, Fisher Scientific, Denver, USA). The electric water conductivity was measured using a Portable Conductivity Meter (Jenway, London, UK). The unionized ammonia (NH3) concentration was measured using a Multiparameter Ion Analyzer (HANNA Instruments, Rhodes Island, USA). Total alkalinity and total hardness were measured by titration method according to **Boyd (1984).** In all treatments, water temperature ranged from 28 to 30 °C, pH ranged from 7.5 to 7.6, conductivity ranged from 51 to 52 uS/cm, and unionized ammonia concentrations ranged from 0.10 to 0.12 mg/L. Total alkalinity and total hardness ranges were 115–116 and 240–241 mg/L as CaCO3, respectively. All the previous water quality parameters are within the acceptable range for fish growth. Tissue samples were removed (liver and gills) and stored at –85 °C until further analyses were performed. Whole livers were weighed prior to freezing. Fish growth were calculated as follows: Weight gain (g) = final weight (g) - initial weight (g), Weight gain % = 100 [final weight (g) - initial weight (g)]/initial weight (g), Condition factor [CF = (weight × 100)/ length3] and Hepatosomatic index [HSI = (liver weight/body weight) × 100]. Tissue samples (liver, gills and muscle) were homogenized in 50mM potassium phosphate buffer with 1mM EDTA (pH 7.4). The homogenate was centrifuged at 5000 rpm for 20 min at 4 °C and the supernatant was used for the determination of GR (glutathione reductase) and GPx (glutathione peroxidase) using a Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) with bovine serum albumin as a standard The catalytic concentration of GR was determined by measuring NADPH (nicotinamidad-eninedinucleotide phosphate-reduced) oxidation at 340 nm **(Carlberg and Mannervik 1975).** The catalytic concentration of GPx was calculated from the rate of NADPH oxidation in the reaction with GR at 340 nm **(Flohe and Gunzler 1984).** All these spectrophotometric methods were performed using a Varioskan Flash Spectral Scanning Multimode Reader (Thermo Scientific). Samples were measured using high performance liquid chromatography with electrochemical detection. The system consisted of two solvent delivery pumps operating in the range of 0.001–9.999 ml/min (Model 582 ESA Inc., Chelmsford, MA), a Zorbax eclipse AAA C18 (150 × 4.6; 3.5 µm particle size; Agilent Technologies, USA), and a CoulArray elec- trochemical detector (Model 5600A, ESA, USA).

Hematological and Biochemical parameters:

Blood samples were taken at the end of experiment (90 days) from caudal vein and were stabilized with sodium heparin (50 IU/ml of blood) for control fish and treated with copper sulfate at concentrations 25, 50 and 75 µg/l for 90 days according to **(Lebrun *et al.* 2014 and Roy, 2010).** Heparinized blood samples were used for the evaluation of

hematological indicators, including erythrocyte count (RBCs), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocyte count (WBCs), and differential leukocyte count. Samples were processed according to **Svobodova *et al.* (2012).** The total leukocytes count were performed by the diluent/dye direct method outlined by (**Natt and Herrick, 1952)** in a Neubauer chamber at a dilution of 1:100. Following the total cell count of nucleated cells (leukocytes) in the Neubauer chamber. The packed cell volume was determined by the microhematocrit technique described by (**Soivio and Oikari, 1976 and Jain 1986).** Blood was used for erythrocyte count by (Dacie and Lewis 1984), hemoglobin content by **Vankampen, (1961).** Plasma samples were obtained after the centrifugation of blood (10000 rpm for 10 min at 4 °C) and stored at –85 °C until further analyses were performed. The analysed biochemical parameters included lactate dehydrogenase (LDH) and Plasma Ceruloplasmin was measured by **(Ceron and Martinez 2004).** The analysis was performed using a Varioskan Flash Spectral Scanning Multimode Reader (Thermo Scientific). Plasma cortisol levels were determined by radioimmunoassay by kits obtained from Bayer for assay, reagents and protocols which were described by (**Pankhurst and Sharples 1992**) were used. Plasma glucose concentration was measured according to **Trinder, (1969)**, using Boehring Mannheium kits. Plasma protein content was determined by the Biuret method described by **Wootton, (1964).** Total lipids, cholesterol and triglycerides were determined calorimetrically using a kit supplied by El Nasr Pharmaceutical Chemical Co., according to **Knight *et al.*, (1972).** Electrolytes using spectrophotometers. Creatinine, uric acid, ALP using methods of **Britton, (1963)**. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically using kits supplied by Diamond Diagnostics, according to (**Reitman and Frankel, 1975).** Determination of copper concentration in liver, gills, and muscle. 10 mg of tissues were placed into glass vials and 700 µl of nitric acid (65%, w/w) and 300 µl of hydrogen peroxide (30%, w/w) were added. The digestion of samples took place in a microwave system Multiwave 3000 (Anton-Paar GmbH, Austria). Sample preparation for subsequent electrochemical measurements was as follows: 100 µl mineralised sample were pipetted into Eppendorf tubes with 1900 µl acetate buffer (pH = 5.00). A blank digestion was simultaneously carried out in the same way. The determination of copper concentration by differential pulse voltammetry was performed with a 797 VA Computrace instrument connected to an 813 Compact Autosampler (Metrohm, Switzerland) using a standard cell with three electrodes. A hanging mercury drop electrode with a drop area of 0.4 mm^2 was used as the working electrode. An Ag/AgCl/3M KCl electrode was used as the reference and a platinum electrode as the auxiliary. The copper concentrations in fish tissues are given as µg per g of wet weight.

Statistical analysis:

The obtained data in this study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to **(Snedecor and Cochran, 1982).** Differences among treatment means were compared using Duncan's multiple range tests **(Duncan, (1995)**. Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

RESULTS

Growth changes in *Oreochromis niloticus* exposed to copper sulfate at concentrations 25, 50 and 75 µg/L for 90 days were observed at the highest concentration at the end of the experiment. Abnormal behavior included reduced food intake and reduced swimming activity. The mean values of growth indices are presented in Table 1. A significant increase ($P < 0.05$) in HSI was found in fish exposed to a copper concentration of 25, 50 and 75 µg/L.

Condition factor, body weight, and Weight gain (g) were significantly decreased ($P < 0.05$) at the copper concentrations of 25, 50 and 75 µg/L compared to control groups.

Table 1. Effect of different copper concentrations on growth parameters of *Oreochromis niloticus*

Parameters	Doses of copper sulfate			
	Control	25 µg/L	50 µg/L	75 µg/L
Initial weight (g)	50 ± 3.2^{a}	$50 \pm 3.5^{a,}$	$50 \pm 2.2^{a,}$	$50 \pm 1.2^{a,}$
Final weight (g)	74.0 ± 2.2^{d}	73.0 ± 4.2^{c}	$68.7 \pm 2.3^{,b}$	$64 \pm 3.2^{a,}$
Weight gain (g)	24.0 ± 2.4^{d}	23 ± 2.3^{c}	18 ± 1.6^{b}	14 ± 6.0^{a}
Weight gain %	48 ± 2.18^{d}	46 ± 3.17^{c}	36 ± 1.21^{b}	28 ± 1.17^{a}
Hepatosomatic index	3.77 ± 0.09^{d}	3.40 ± 0.08^{c}	2.89 ± 0.08^{b}	2.41 ± 0.11^{a}
Condition factor	2.32 ± 0.03^{a}	2.23 ± 0.04^{a}	2.2 ± 0.04^{a}	2.2 ± 0.03^{a}

Data expressed as means ± SE, means with the same letter in the rows is not significant at p<0.05

Hematological parameters

Hematological parameters of blood samples are presented in Table 2. It is obvious that *Oreochromis niloticus* exposed to copper sulfate at concentrations 25, 50 and 75 µg/L for 90 days resulted in significant changes in almost all hematological aspects, especially in the group exposed to the highest concentration of copper. Erythrocyte count increased with copper concentration, with a significant ($P < 0.05$) difference between the highest concentration and control; hematocrit values exhibited a similar tendency. Leukocyte count decreased non-significantly with increasing copper concentration. Significantly less ($P < 0.05$) MCV were found at the highest copper concentrations 25, 50 and 75 µg/L compared with the control and with the 75 µg/L concentration. On the other hand, a significantly higher amount of MCH, and MCHC at the 25, 50 and 75 µg/L concentrations ($P < 0.05$) compared with the control.

Table 2. Effect of different copper concentrations on hematological changes of *O. niloticus*

Parameters	Doses of copper sulfate			
	Control	25 µg/L	50 µg/L	75 µg/L
RBCs (10^6/mm^3)	1.66 ± 0.05^{a}	$1.83 \pm 0.04^{a,b}$	$1.97 \pm 0.05^{b,c}$	2.22 ± 0.05^{c}
Hb (g/dl)	$7.13 \pm 2.46^{a,b}$	$8.14 \pm 1.98^{a,b}$	$8.\ 9 \pm 1.49^{a,b}$	9.5 ± 4.72^{b}
PCV (%)	28 ± 0.01^{a}	$29 \pm 0.01^{a,b}$	31 ± 0.01^{b}	34 ± 0.01^{c}
MCV (µm3)	122.3 ± 3.9^{a}	$114.63 \pm 4.2^{,b}$	104.2 ± 2.8^{c}	103.4 ± 5.1^{d}
MCH (pg)	42.3 ± 2.96^{a}	$45.6 \pm 1.4^{,b}$	46.8 ± 0.4^{c}	48.4 ± 1.1^{d}
MCHC (%)	29.2 ± 7.6^{a}	30 ± 3.0^{b}	32 ± 3.0^{c}	34 ± 1.3^{d}
WBCs (10^3/mm^3)	3.7 ± 0.7^{d}	3.0 ± 0.7^{b}	2.2 ± 0.8^{c}	2 ± 0.5^{d}

Data expressed as means ± SE, means with the same letter in the rows is not significant at p<0.05

Biochemical aspects

The results of blood plasma biochemical indices are shown in Table 3 Significant ($P < 0.05$) differences in total protein, phosphorus, calcium, glucose, triacylglycerol's, cholesterol,

LDH and ALT were found among the tested groups. The activities of AST and ALP were significantly affected by copper exposure. Also, there were increased in plasma glucose, cortisol, cholesterol, triglycerides and LDH due to exposure to copper compared to control one as shown in Table 3.

Table 3. Effect of different Copper Concentrations on Biochemical Changes of *Oreochromis niloticus*

Parameters	Doses of copper sulfate			
	Control	25 µg/L	50 µg/L	75 µg/L
Glucose (gl/l)	63.51 ± 0.13^{a}	74.44 ± 0.21^{b}	85.12 ± 0.29^{c}	87.97 ± 0.74^{d}
Cortisol (g/dl)	14.2 ± 0.01^{a}	$15.4 \pm 0.01^{,b}$	25.6 ± 0.01^{c}	28.3 ± 0.01^{d}
Total protein (g/l)	1.32 ± 0.01^{a}	$1.24 \pm 0.01^{a,b}$	1.16 ± 0.01^{bc}	1.15 ± 0.01^{bc}
ALT (u/l)	37 ± 0.04^{d}	43 ± 0.04^{b}	49 ± 0.05^{c}	52 ± 0.08^{d}
AST (u/l)	27 ± 0.13^{a}	34 ± 0.07^{b}	49 ± 0.08^{c}	51 ± 0.10^{d}
ALP (ul/l)	51 ± 0.09^{a}	55 ± 0.08^{b}	63 ± 0.11^{c}	71 ± 0.20^{d}
Uric acid (mg/dl)	9 ± 1.4^{a}	10 ± 1.2^{b}	11 ± 1.4^{c}	12 ± 1.1^{d}
Creatinine (mg/dl)	0.25 ± 0.1^{a}	0.27 ± 0.1^{b}	0.29 ± 0.1^{c}	0.34 ± 0.2^{d}
Cholesterol (mmol/l)	3.54 ± 0.15^{a}	$3.89 \pm 0.11^{a,b}$	4.87 ± 0.17^{c}	4.91 ± 0.18^{c}
Triacylglycerols (mmol/l)	1.38 ± 0.10^{a}	$1.41 \pm 0.09^{a,b}$	$2.1 \pm 0.1^{a,b}$	$2.2 \pm 0.3^{a,b}$
LDH (mmol/l)	32 ± 0.06^{a}	42 ± 0.05^{b}	56 ± 0.07^{c}	78 ± 0.08^{d}
Lactate (mmol/l)	1.6 ± 0.09^{a}	2.1 ± 0.09^{b}	2.4 ± 0.07^{c}	2.6 ± 0.08^{d}
Phosphorus (mmol/l)	2.0 ± 0.09^{a}	2.2 ± 0.09^{ab}	2.4 ± 0.07^{b}	2.6 ± 0.08^{c}
Calcium (mmol/l)	2.2 ± 0.05^{a}	2.5 ± 0.03^{ab}	2.9 ± 0.04^{b}	3.5 ± 0.13^{c}

Data expressed as means ± SE, means with the same letter in the rows is not significant at $p<0.05$

Accumulation of Copper concentration in tissues

Accumulation of copper distribution in *Oreochromis niloticus* exposed to copper sulfate at concentrations 25, 50 and 75 µg/L for 90 days tissues is shown in Table 4. The highest copper content was measured in liver and the lowest in muscle in all tested groups, including the control. Significant differences ($P < 0.05$) between groups were found with respect to copper concentrations in liver, gills and kidney. Also, a significant change was found in muscle.

Table 4. Effect of different Copper sulfate on accumulation of copper in tissues of *Oreochromis niloticus*

Parameters	Doses of copper sulfate			
	Control	25 µg/L	50 µg/L	75 µg/L
Liver	144.4 ± 4.2^{a}	166.2 ± 3.4^{b}	182.1 ± 2.2^{c}	221.7 ± 11.8^{d}
Gills	44 ± 3.1^{a}	67.0 ± 1.0^{b}	73.2 ± 2.1^{c}	75.8 ± 3.2^{d}
Kidney	27.1 ± 2.0^{a}	44.4 ± 4.3^{b}	55.9 ± 1.1^{c}	53.9 ± 3.1^{c}
Muscle	14.8 ± 0.2^{a}	31.1 ± 1.5^{b}	37.4 ± 1.2^{c}	36 ± 3.1^{c}

Data expressed as means ± SE, means with the same letter in the rows is not significant at p<0.05

Measurement of antioxidants enzymes

Plasma ceruloplasmin activity (A) (increase in absorbance per min ×10 000) in *Oreochromis niloticus* exposed to copper sulfate at concentrations 25, 50 and 75 µg/L for 90 days was significantly increased with increasing copper concentrations. The enzymatic activities of glutathione reductase (GR) and glutathione peroxidase (GPx) are shown in Table 5. GR and GPx activity in liver and gill were changed significantly after copper exposure. Where, GR and GPx increased significantly ($P < 0.05$) with increasing copper concentration compared to control group.

Table 5. Effect of different Copper Concentrations on antioxidant enzymes of *Oreochromis niloticus*

Parameters	Doses of copper sulfate			
	Control	25 µg/L	50 µg/L	75 µg/L
Ceruloplasmin activity nmol/mg	38.1 ± 1.2^{a}	49.3 ± 1.2^{b}	55.5 ± 1.2^{c}	67 ± 1.1^{d}
Liver GR nmol/mg	7 ± 0.4^{a}	7.5 ± 0.1^{a,b}	8 ± 0.7^{a,b}	9 ± 0.6^{b}
Gills GRnmol/mg	11 ± 1.0^{a}	11.5 ± 1.3^{a,b}	12.5 ± 1.2^{b}	13 ± 1.1^{b}
Liver GPx nmol/mg	325.0 ± 4.4^{a}	367.0 ± 5.1^{b}	387 ± 4.4^{c}	399 ± 3.6^{d}
Gills GPx nmol/mg	77.1 ± 4.0^{a}	97.1 ± 2.3^{b}	115.7 ± 2.2^{c}	129.9 ± 4.1^{d}

Data expressed as means ± SE, means with the same letter in the rows is not significant at p<0.05

Reproductive hormones

Plasma levels of follicular stimulating hormone (FSH), 17β-estradiol (E2) and testosterone (T) of Nile tilapia decreased significantly with the increase of copper concentrations levels lower than these of the control fish group (Table 6).

Table 6. Effect of different copper concentrations on reproductive hormones of *Oreochromis niloticus*

Parameters	Doses of copper sulfate			
	Control	**25 µg/L**	**50 µg/L**	**75 µg/L**
FSH ng/ml	9.3 ± 21.8^{b}	7.8 ± 0.2^{ab}	6 ± 0.2^{a}	6 ± 0.8^{a}
E2 ng/ml	17.0 ± 0.4^{c}	$12.0 \pm 0.1^{a,b}$	$11.7 \pm 0.7^{a,b}$	10.4 ± 0.6^{a}
T ng/ml	0.83 ± 0.1^{c}	0.65 ± 0.3^{bc}	0.55 ± 0.2^{ab}	0.49 ± 0.1^{a}

Data expressed as means ± SE, means with the same letter in the rows is not significant at $p<0.05$.

DISCUSSION

The copper is important to biological functions; copper can be harmful to fish. The present study showed that chronic waterborne exposure to slightly higher copper concentrations than environmental ones could have negative effects on the health status of *Oreochromis niloticus*. Such effects include behavioral changes, the impairment of hematological and biochemical indices, an imbalance in antioxidant defense, and also pathological lesions in tissues. In connection with reduced food intake, in our study both body weight, hepatosomatic index and condition factor were reduced in fish exposed to copper at concentrations of 25, 50 and 75 µg/L for 90 days. Lower body weight together with unchanged liver weight led to a reduced at the highest copper concentrations at 25, 50 and 75 µg/L for 90 days. The liver is the main organ responsible for the maintenance of copper homeostasis and is also a possible target for copper induced damage in fish **(Kamunde and McPhail 2008).** the accumulation of copper in liver tissue is related to its concentration in the environment and the exposure time **(Jezierska and Witeska 2001).** Quantitatively speaking, food taken up through the intestine is the most important source of copper in fish **Grosell, (2012).** We registered a concentration dependent increase in copper content in liver, gills, and kidney. In contrast, copper content in muscle was not affected by exposure time; also, the copper concentration was lowest in muscle tissue compared with the other samples. Overall, we observed a copper distribution in tissues comparable to the findings of other studies **(De Boeck *et al.* 2004; Celechovska *et al*. 2007; Kandemir *et al.* 2010)**. In general, hematological indices can be influenced by a wide range of factors, both endogenous and exogenous **(Nespolo and Rosenmann 2002).** The hematological response is nonspecific towards chemical stressors; however, it can indicate that fish are exposed to environmental stress **(Cazenave *et al.* 2005 and shokr, 2015).** Changes in red blood cell profile are probably an adaptive response to the impairment of gas exchange in copper exposed gills and increased energetic demands on the fish **Witeska *et al.* (2010).** In the blood of fish under stress, an increase in erythrocyte counts, hemoglobin concentrations, and hematocrit levels are frequently observed.

In our study, the increased erythrocyte counts and leucocytes, hematocrit values, MCV, MCH and MCHC could be due to enhanced erythropoiesis as a result of chronic metal exposure **(Kondera and Witeska 2013).** The tendency towards a decreasing leukocyte count was not significant in the present study. The decrease in white blood cell count, as a sign of the suppression of nonspecific immunity, can be transient and gradually followed by a return to control levels, indicating an adaptive reaction of the organism. A decrease in lymphocytes together with a relative increase in neutrophil count is considered a common finding in the leukocyte profile of fish following exposure to copper and other metals **(Jezierska and Witeska 2001).** The plasma biochemical profile provides important information about the internal environment of the organism. An increased plasma glucose, cortisol, cholesterol, triglycerides, LDH and phosphorous and calcium concentrations, a commonly observed effect caused by various stress stimuli, was apparent in all tested copper concentrations. Initially, metals, including copper, activate glycogenolysis via increased secretion of catecholamines. Later, the gluconeogenic action of cortisol is apparent **(Takei and Loretz 2006)**. Reduced insulin secretion, causing hyperglycaemia, was also described in fish after longterm exposure to copper **Moon, (2001).** An increase in plasma glucose, cortisol, cholesterol, triglycerides, LDH and phosphorous and calcium concentrations were documented by several authors in similar studies after the chronic exposure of various fish species to low copper concentrations **(Heydarnejad *et al.*, 2013 and Pretto *et al.*, 2014).** Disturbances in carbohydrate metabolism caused by metal intoxication may also be represented by changes in the accumulation of lactate and LDH activity **(Teodorescu *et al.*, 2012 and Perumalsamy and Arumugam 2013).** Increased LDH activity was revealed at the highest copper concentrations 25, 50 and 75 µg/L for 90 days; however, it was not accompanied by an increase in lactate level. Increased LDH activity is known to be a sign of anaerobic metabolism and is often a consequence of slight tissue damage in my study, the consequence of liver damage. On the other hand, the liver still retains the ability to scavenge lactate from plasma **(Perumalsamy and Arumugam 2013).** Cortisol, produced in fish under stress, stimulates protein metabolism, resulting in increased its levels **De Boeck *et al.*, (2001).** Large amounts of ALT and AST are released into blood as a consequence of liver damage. In my study, the increase in ALT activity at the highest copper concentrations at 25, 50 and 75 µg/L for 90 days might have been due to hepatocellular damage, which was revealed by histopathological examination of the tissue. These results are in accordance with those reported by many authors **Shokr, (2015).** Hepatobiliary disorder may also lead to decreased cholesterol clearance from blood and its elevated concentration in plasma, as was revealed at copper concentrations of 25, 50 and 75 µg/L for 90 days. In fish, a unique glycoprotein, stanniocalcin, is produced by the so called Stannius

corpuscules, which participates in the regulation of calcium levels **Clark *et al.*, (2002).** The increased calcium levels observed in my study could be due to the activation of pituitary gland hormones initiated by copper exposure. Ceruloplasmin serves as a copper transporter in blood and represents 90% of the total amount of copper in plasma **(Di Giulio and Meyer 2008).** Besides its transport function, ceruloplasmin possesses antioxidant properties through its ferroxidase activity and is involved in the homeostasis of iron and modulates the coagulation cascade **Shukla *et al.*, (2006).** My findings on the dose dependent increase in ceruloplasmin levels confirmed the important role of ceruloplasmin in copper metabolism. My results are consistent with several other studies **Tang *et al.*, (2013).** In *Oreochromis niloticus*, antioxidant enzymes have been shown to be either activated. Decreased GPx activities in liver were found in common carp 24 and 48 h after the intraperitoneal injection of copper (10 mg/kg of body weight) **Varanka *et al.*, (2001).** In the present study, substantial changes were revealed in GR and GPx activities. A decline in GR and GPx activities at the highest copper concentration might be related to the inhibition of enzyme synthesis In my study, oxidative damage to lipids was probably a major cause of the morphological changes to hepatocytes. The present study revealed that decreased in the reproductive hormones in the fish exposed to different concentrations of copper may be due to decreased in food intake and decreased appetite of fish for food. Similar result reported by **(Sydney and Volkoff, 2019)** found larger eggs relative to body weight, compared to those with fewer or smaller eggs, suggesting that more mature females are more affected by fasting. **Ryo, *et al*., (2018).** confirmed that the variations in sex steroid hormone levels correlated with reproductive status in mature fish. Strongly suggest that E2 is an indicator for ovarian follicle development, and that T is a useful indicator for both the onset and end of the egg-laying period in fish. Also, **Lucas, *et al.*, (2019)** showed is the first investigation of concurrent changes in reproductive, thyroid and adrenal hormone concentrations in this endemic and physiologically unique South American lizard. Findings set the stage for future investigations to determine the extent to which these hormones influence activity and thermoregulation in *S. merianae*. Steroid hormones were extracted from blubber and testosterone and estradiol are associated reproductive patterns in fish. The present result showed decreased in FSH, estradiol and testosterone agreement Sydney and Volkoff, 2019 who showed that the average body length, body weight and the reproductive hormones in the fish had the trend of annual variation. the reproductive hormone levels and the migratory reproductive activities are synchronized.

CONCLUSION

The present study provides further evidence that copper has a potentially deleterious effect on the fish organisms and is able to cause changes in biochemical and hematological aspects and enzymes of antioxidanta. The clearest changes were found

at highst concentrated groups. Liver was the most affected organ, with disturbances even at the morphological level. The most sensitive biochemical parameter in the present study appeared to be glucose, cortisol, cholesterol, LDH, triglycerides, phosphorus and calcium, which were already affected at the lowest concentration (25 µg/L), whereas hematological indices, copper level in kidney, and selected oxidative parameters were influenced at concentrations of 50 and 75 µg/L for 90 days. These results concluded that total copper concentration played a greater role on the changes of fish activities for decreasing fish reproduction. So, from this study, it is recommended not to increase copper concentration in fish farms water, that certainly will cause the deterioration of the Nile tilapia health, which leads to its death.

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ARABIC SUMMARY

تاثير النحاس على التغيرات الدموية و البيوكيميائية وهرمونات التكاثر للبلطى النيلى

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تم دراسة تأثير تركيزات النحاس على التغيرات في الدم والكيمياء الحيوية والهرمونات التناسلية للبلطي النيلي .تعرضت اسماك البلطي النيلي الي كبريتات النحاس بتركيزات ٢٥، ٥٠ و٧٥ميكروجرام / لتر لمدة ٩٠ يومًا. تم العثور على تغييرات كبيرة (في جميع الجوانب الدموية تقريبا وفي جميع المجموعات التي تعرضت الي كبريتات النحاس بتركيزات ٠. ٢٥، ٥٠ و٧٥ميكروجرام / لتر لمدة ٩٠ يومًا. كشفت التحليلات الكيميائي الحيوي عن اختلافات كبيرة) في جميع المجموعات التي تعرضت الي كبريتات النحاس بتركيزات٢٥، ٥٠ و٧٥ميكروجرام / لتر لمدة ٩٠ يومًا. كان هناك اختلاف كبير في تركيزات النحاس في الأنسجة بين جميع المجموعات المعالجة التي تم العثور عليها في الكبد والخياشيم والكلى. زاد الجلوكوز والكورتيزول في بلازما الأسماك بشكل كبير، في حين انخفض إجمالي البروتين والدهون الكلية بشكل كبير بسبب إلاجهاد تحت تاثير النحاس. أظهرت النتائج التي تم الحصول عليها أيضًا أن الإجهاد النحاسي كان ضارًا بالكبد والكلية، حيث زادت البلازما الأسبارتيز أمينوتيرفيراز ، ألانين أمينوترانسفيراز ، الفوسفاتيز القلوية ، حمض اليوريك ، والكرياتينين بشكل كبير مع زيادة التعرض للنحاس لمدة ٩٠ يومًا عن مجموعة السيطرة. انخفضت هرمونات التحفيز المسامي للبلازما(FSH) ، استراديول ١٧ ß (E2) وهرمون التستوستيرون (T) بشكل كبير في الأسماك مع زيادة التعرض لتركيزات النحاس لمدة ٩٠ يومًا. من بين الإنزيمات المضادة للأكسدة، تم الكشف عن تغييرات كبيرة بشكل رئيسي في سيروبلازمين البلازما وانزيم الجلوتاثيون المختزل ونشاط الجلوتاثيون بيروكسيديز في الكبد والخياشيم تم تسجيل تغير كبير في محتوى الجلوتاثيون الكلي في الكبد والخياشيم. زادت نسبة الدهون بشكل ملحوظ مع زيادة تركيز النحاس في الكبد والكلى، وأظهرت النتائج التأثير الضار للنحاس علي البلطي النيلي حتى في التركيزات المنخفضة ذات الصلة بالبيئة، لذلك ينصح بعلاج المياه المستخدمة في زراعة الأسماك من المعادن الثقيلة والتلوث.