EFFECTS OF COPPER AND LEAD ON SOME HEMATOLOGICAL PARAMETERS OF *Oreochromis niloticus*

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ABSTRACT

Effects of sub-lethal concentrations of copper and lead on hematocrit and mean cell volume (MCV) levels and on erythrocyte numbers, erythrocyte and erythrocyte nucleus areas were determined after exposing *Oreochromis niloticus* to 4 ppm Cu and 0.2 ppm Pb over 7, 15 and 30 days.

Micro-hematocrit methods were adopted in determining hematocrit levels and microscopic methods were used to determine MCV, erythrocyte numbers, erythrocyte and erythrocyte nucleus areas.

Hematocrit and MCV levels and erythrocyte and nucleus areas increased significantly ($P<0.05$), whereas the erythrocyte numbers decreased under the effect of both metals at the exposure periods tested, except at 30 days of copper exposure.

There was an increase in MCV levels under the effect of lead and an increase in erythrocyte and nucleus areas under the effect of both metals with increasing exposure periods. Prolonged exposure periods decreased other hematological parameters studied.

Hematological parameters studied were higher in fish exposed to lead compared to copper during the experiments, except the erythrocyte area.

It was concluded that both copper and lead caused significant changes in hematological parameters of *O. niloticus* which, in turn, affected the physiological conditions of fish.

KEYWORDS:
O. niloticus, copper, lead, hematological parameters

1. INTRODUCTION

Heavy metals are natural constituents of the earth crust. Their levels in aquatic environments, however, increase mainly due to increasing agricultural and industrial activities causing environmental and health problems [1-3]. Acute elevated aquatic pollution results in mortality in flora and fauna which, in turn, cause permanent changes in structural components of the environment [4]. Prolonged exposure to low levels of metals results in tissue accumulation in aquatic organisms, and is transferred to higher trophic levels through the food chain [5].

Copper is present as a structural component of living organisms at low concentrations. It acts as a prosthetic group in various proteins and as a cofactor in enzymes, and also has functions in the formation of connection and bone tissues, impulse transmission, and the antioxidant defence system. Erythrocuprein, a copper-containing metallothionein, is required for hemoglobin synthesis [6].

Copper was used in making various kitchen and ornamental materials over long periods [7]. Today, it is widely used in electric, electronic, construction and chemistry industries, algaecide, detergent, dye, insecticide and artificial agricultural fertilizer production and in strengthening of cement and reinforced concrete beams. Both production and usage of wastes of these activities constitute the main source of copper in aquatic environments.

Lead is a toxic heavy metal which has no biological function in animals. It is used in accumulator production, isolation of underground cables, and in steel constructions as a corrosion preventer; its tetraethyl and tetramethyl complexes are used in fuels as octane adjuster, and in nuclear energy plants against radioactive emittance [8]. Studies carried out with various fish species showed that lead has a neurotoxic effect, since it passes the blood-brain barrier, it causes changes in hematologic parameters, and causes structural deformations in various tissues including bones [9, 10].

Heavy metals up-taken through gills and the digestive system are firstly bound to carrier proteins in the blood stream; hence, their first effects can be observed on hematological parameters. Hematological parameters, such as hematocrit and hemoglobin levels and erythrocyte numbers, not only reflect the oxygen carrying capacity to various tissues but also allow to determine the changes in organ and organ systems [11].

Studies carried out with various fish species have shown that heavy metals can cause polycythemia, anemia,

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morphological changes in erythrocytes, and inclusions in nucleus or cytoplasm [12-14].

Experimental animal *O. niloticus* is an economically important fish species that is widely consumed in the equatorial region as a protein source. The reproduction of the species is easy, it is resistant to diseases and is used for plantation of inland water resources. Predetermination of heavy metal toxicity, in aquatic organisms, especially in economically important species, is important as far as environmental and human health is concerned. Hence, the present study was undertaken to find out the effects of 4.0 ppm Cu and 0.2 ppm Pb, which are 10% of the LC_{50} level of each metal, on hematocrit and MCV levels, erythrocyte number, erythrocyte and nucleus areas of *O. niloticus* after exposing the animals to these metals over 7, 15 and 30 days.

2. MATERIALS AND METHODS

Experimental material *O. niloticus* was obtained from the implementation unit of Aquaculture Faculty of Mersin University. The total length and weight of the fish used in experiments were 18.58±0.91 cm and 110.27±3.29 g, respectively. Fish were placed in 6 glass aquaria, 40x100x 40 cm in size, in the basic sciences laboratory under 24± 1 °C constant temperature conditions, and 12-h light and 12-h dark illumination period was applied. Experiments were started after two weeks of adaptation period.

Four glass aquaria of the same size were used in the experiments. Experimental solutions were prepared from water-soluble compounds of copper ($CuSO_4$) and lead ($(Pb(NO_3)_2$). Tri-sodium citrate was used in the preparation of stock solutions in order to prevent precipitation of metal salts. 120 L of 4.0 ppm Cu and 0.2 ppm Pb, which are the 10% of LC_{50} levels of each metal, were added in the first two aquaria. The same amount of metal-free tap water was added to the third and the fourth aquaria, and were used as controls.

Experiments were run in triplicate, being 3 fish in each replicate. Hence, 9 fish were placed in each aquarium taking the 1, 7 and 15 days of experimental periods into account. Some physical and chemical characteristics of experimental aquaria are given in Table 1.

TABLE 1 - Some physical and chemical parameters of experimental water.

Temperature (°C)	24.0 ± 1.0
pH	8.62 ± 0.16
Dissolved oxygen (mg L^{-1})	5.29 ± 0.7
Total Hardness (mg L^{-1} $CaCO_3$)	227 ± 0.48
Alkalinity (mg L^{-1} $CaCO_3$)	332 ± 0.50

Fish were fed once a day with ready fish feed (Pınar, Bream feed, Pellet No:2) at amounts of 2% of total biomass. Central aeration system was used to aerate experimental tanks. Experimental waters were changed once in two days to prevent possible alterations in experimental solutions due to evaporation, precipitation and adsorption.

Three fish were removed from each aquarium at the end of experimental periods and were anaesthetized using anesthetic ethylene glycol monophenyl ether (= phenoxiethanol, $C_8H_{10}O_2$). After washing the fish with tap water to remove metal residues, they were dried with Whatman drying paper and prepared for sampling.

Blood samples to be used in determining the blood parameters were obtained by cutting the caudal peduncle vertically. Among the hematological parameters to be determined, heparinized capillary hematocrit pipettes were used in determining hematocrit levels according to Wedemayer and Yasutake [15]; erythrocyte numbers were determined using EDTA (ethylene-diamine tetraacetic acid)-containing tubes, and spread slides were used to determine erythrocyte and erythrocyte areas.

Erythrocyte numbers of blood samples were determined by counting on thoma slides using a light microscope (Leica, Model CME) [15]. Erythrocyte numbers and MCV levels were determined according to the formulas given below:

$$Erythrocyte\ numbers = \frac{Erythrocyte\ number \times Dilution\ rate}{Number\ squares\ counted} \times 100$$

$$MCV = \frac{Hematocrit\ level}{Erythrocyte\ number} \times 10$$

Erythrocyte and nucleus areas were determined by morphometric measurements of dyed spread slides under microscope. Giemsa method was adopted in preparing dyed spread slides [16]. Erythrocyte and nucleus areas were calculated according to the following formulas after measuring the length and width of at least 150 erythrocyte and their nucleus under a microscope (Nicon, H550-L) [17].

$$Erythrocyte\ area = \pi \frac{Erythrocyte\ lenght \times Erythrocyte\ width}{2}$$

$$Erythrocyte\ nucleus\ area = \pi \frac{Nucleus\ lenght \times Nucleus\ width}{2}$$

Data were analyzed by a series of variance analysis and Student Newman Keuls test (SNK) using SPSS statistical package. Arcsine transformation was applied on hematocrit levels, since they were expressed in percentages.

3. RESULTS

No mortality was observed during the 30 days of exposure to the metal concentrations tested. The same behavioral and morphological changes were observed at the beginning of experiments as indicated by Cicik [18], except for darkening of the skin in fish exposed to Cu continued throughout the experiments.

Hematocrit levels increased compared to control in fish exposed to metals up to 7 days of exposure which then decreased on the 30 days of exposure, compared with the 7th day (P<0.05) (Table 2). The decrease in hematocrit levels was higher in fish exposed to Pb than to Cu (P<0.05).

Erythrocyte numbers decreased compared to control with increasing exposure periods under the effect of both metals (P<0.05). The decrease in erythrocyte numbers was higher in fish exposed to copper than to Pb (P<0.05) (Table 2).

Exposure to copper increased MCV compared to control, except on 30 days of exposure (P<0.05). There was also an exposure period-dependent increase in MCV in fish exposed to lead (P<0.05). Lead had a greater effect on MCV, compared to copper (P<0.05) (Table 2).

Erythrocyte and erythrocyte nucleus areas increased significantly with increasing exposure periods under the effect of both metals (P<0.05). Copper had a more pronounced effect on erythrocyte and nucleus areas than lead (P<0.05), except on day 30 (P>0.05) (Table 2).

4. DISCUSSION AND CONCLUSION

Effects of heavy metals on fish mortality depend on the metal concentration, exposure period, environmental factors, species, developmental stage and sex. Mortality increases rapidly over a given concentration [19, 20]. No mortality was observed in *O. niloticus* exposed to 4.0 ppm Cu and 0.2 ppm Pb over 7, 15 and 30 days which might be due to tested concentrations of these metals not being lethal to this species at exposure periods tested, or detoxification mechanisms might be stimulated against metal toxicity.

Toxicants, such as heavy metals, may directly cause structural changes, or they may cause stress which, in turn, results in behavioral changes in fish. Same behavioral changes were observed in *O. niloticus* under the effect of Cu [21] and Pb [22], at the beginning of experiments as mentioned in previous studies.

Hematologic parameters, such as hematocrit and hemoglobin levels, MCV, erythrocyte numbers, erythrocyte and erythrocyte nucleus areas, leucocyte and thrombocyte numbers are normally kept at given levels by homeostatic mechanisms. They, however, instantly change under the effect of chemical toxicants, infection or other stress factors [19, 23].

Studies carried out with various fish species, hypoxic conditions, and metal effects demonstrate increase of mucus secretion which covers gill surface area and increase oxygen diffusion distance. The resulting inhaling difficulties increase adrenalin secretion and cause contraction in spleen and increase in erythropoiesis [12]. Sub-lethal concentrations of copper increase hematocrit and hemoglobin levels and erythrocyte numbers in *Ichtalurus nebulosus* [24], *Scyliorhinus canicula* [25], *Oreochromis mosambicus* [26], *Heteropneustes fossilis* [27], *Oncorhynchus mykiss* [28], *Clarias gariepinus* [29] and *Prochilodus scrofa* [30]. This increase was reported to be a result of hemo-concentration due to osmoregulation failure. The increase in hematocrit levels, MCV, erythrocyte and erythrocyte nucleus areas in *O. niloticus* exposed to Cu and Pb might be either hemo-concentration or stimulation of erythropoiesis by feedback mechanisms.

The loss of membrane-selective permeability under the effect of metals results in hemo-dilution and osmotic hemo-

TABLE 2 - Effects of copper and lead on some hematological parameters of *O. niloticus.*

	Metal	Exposure Period (Days)			
		0 (Control)	7	15	30
		$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *
Hematocrit (%)	Cu	33,52±0,31 [a]	38,94±0,29 [b]	35,97±0,30 [c]	24,73±0,38 [d]
	Pb	31,25±0,31 [a]	41,27±0,29 [b]	40,69±0,29 [b]	39,52±0,30 [c]
Erythrocyte numbers (cell/mm^3)	Cu	2,58±0,002 [a]	1,27±0,01 [b]	1,14±0,02 [b]	0,80±0,13 [c]
	Pb	2,60±0,05 [a]	1,86±0,03 [b]	1,60±0,03 [c]	1,31±0,05 [d]
MCV (fl)**	Cu	166,24±2,78 [a]	331,06±3,33 [b]	292,44±6,73 [b]	78,29±1,71 [c]
	Pb	159,24±2,78 [a]	234,92±0,18 [b]	285,61±3,92 [c]	294,02±2,94 [d]
Erythrocyte Area (μm^3)	Cu	0,87±0,01 [a]	1,13±0,015 [b]	1,45±0,015 [c]	1,45±0,007 [c]
	Pb	0,90±0,009 [a]	0,94±0,009 [b]	1,23±0,015 [c]	1,68±0,003 [d]
Erythrocyte Nucleus Area (μm^3)	Cu	0,13±0,006 [a]	0,17±0,003 [b]	0,18±0,007 [b]	0,25±0,003 [c]
	Pb	0,15±0,007 [a]	0,20±0,006 [b]	0,23±0,006 [c]	0,26±0,006 [d]

*SNK; Letters a, b, c and d show differences between exposure periods. Data shown with different letters are significant at the P<0.05 level. $\overline{X} \pm S\overline{x}$: mean± standard error; **fl; femtoliter (10^{-15} L)

lysis of erythrocytes [31]. Acute effect of 3.2 mg L^{-1} Cu caused hemolysis and anemia in *Clarias lazera* [32]. Sub-lethal concentrations of Pb decreased erythrocyte numbers, hemoglobin and hematocrit levels in *O. mykiss* [33], *Barbus conchonius* [34] and *Oreochromis aureus* [35], which was related to hemolysis of erythrocytes under the effect of metals [35]. The tested concentrations of Cu and Pb decreased erythrocyte numbers and hematocrit levels in *O. niloticus* at the exposure periods tested. This might be either osmotic hemolysis depending upon deterioration of the erythrocyte membrane under the effect of metals, or functional disturbances caused by accumulation of metals in hematopoietic tissues, together with other tissues.

Uptake, tissue accumulation and excretion rates, biological functions and toxic effects of heavy metals differ between aquatic organisms [19]. Acute effects of mercury increased hematocrit and hemoglobin levels and erythrocyte numbers in *Tinca tinca* [36], whereas sub-lethal levels of copper decreased erythrocyte numbers in *Salmo gairdneri* [37]. Lead caused more changes in blood parameters of *O. niloticus* than copper. Since copper is an essential element, it might be less toxic than lead which has no biological function, or lead might stimulate erythropoiesis by inhibiting ALA-D (delta-aminolevulinic acid dehydratase) enzyme activity which plays a role in heme synthesis.

Witeska *et al.* [38] reported that lead caused morphological changes in erythrocyte nucleus, structural deformations and spreading in chromatin material. They concluded that lead might cause distortion in erythrocyte and erythrocyte nucleus membrane permeability or RNA synthesis.

In conclusion, hematocrit and MCV levels, erythrocyte numbers, erythrocyte and erythrocyte nucleus areas changed significantly in *O. niloticus* exposed to 4 ppm Cu and 0.2 ppm Pb over 7, 15 and 30 days. These changes in blood parameters under the effect of Cu and Pb might be due to the influence of metals on osmoregulation, and on membrane permeability or stimulation of feedback mechanisms under the effect of metals. Hence, changes in hematologic parameters under the effect of metals reflect the physiological conditions of organisms.

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