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Effects of Sub-lethal Lead Nitrate and Copper Sulfate Concentrations on Hematological Parameters During Long-term Exposure in Nile tilapia (*Oreochromis niloticus)*

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Nile tilapia (*Oreochromis niloticus*) weighing 51.66 ± 2.42 g were exposed to 0%, 20%, 40%, and 60% of LC_{50} to either lead nitrate ($Pb(NO_3)_2$) or copper sulfate ($CuSO_4$) for 30 days. The $Pb(NO_3)_2$ and $CuSO_4$ concentrations employed in the treatments of this study were 8.8, 17.6, and 26.4 mg/L and 2.57, 5.14, and 7.71 mg/l, respectively, and multiple hematological variables were evaluated. The red blood cell (RBC) count for the control group was 2.41 ± 0.13 while those of the treatment groups exposed to 8.8, 17.6, and 26.4 mg/L of $Pb(NO_3)_2$ were 2.21 ± 0.10, 1.94 ± 0.16, and 1.36 ± 0.10 × 10^6/µl, respectively, at the end of the study. Similarly, the hemoglobin (Hb), hematocrit (Hct), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and platelet (PLT) levels significantly decreased as the $Pb(NO_3)_2$ concentration increased ($p < 0.05$), while white blood cell (WBC) and mean cell volume (MCV) levels significantly increased. However, results of fish exposed to $CuSO_4$, showed decrease in the levels of RBC, Hb, Hct, WBC, and PLT when the concentration of $CuSO_4$ increased, while the MCHC, MCH, and MCV levels significantly increased.

Keywords: *Oreochromis niloticus*, Lead nitrate, Copper sulfate, Hematology

Introduction

Heavy metals change or alter the environmental balance by affecting different physiological, biochemical, and cellular processes within organisms.[1,2] The toxic impacts of different heavy metals have been found to result in low growth rates, impaired physiological functions, low reproductive success, and high mortality in various fish species.[3] Multiple studies have reported that fish exposed to heavy metals present deficient immune systems and functions and are thus at greater risk of mortality.[4] In particular, lead is a heavy metal that has been found to injuriously impact aquatic organisms.[5]

Previous studies have presented a hematological preference for the evaluation of heavy metals in fish because lead toxicity is most evident in the blood, which may be sampled multiple times from the same fish without sacrificing the fish itself.[6] As such, hematological components are important in that they can be used in nondestructive methods to evaluate the effects of heavy metal pollutants.[7,1] In addition, many hematological variables in fish have been identified as potential toxic effect biomarkers, including hemoglobin, hematocrit, protein, and glucose levels.[7,1]

Copper sulfate is a known inorganic fungicide and algaecide worldwide and has been used to control the size of algae populations in lakes, ponds, and reservoirs.[8] In addition, copper is found in most aquatic ecosystems. Given its ubiquity in aquatic habitat, copper is also toxic for fish and its toxicity has been studied in most fish species.

Tilapia (*Tilapia spp.*) constitute one of the most economically important fish resources worldwide and are farmed in numerous countries. Tilapia species are geographically dispersed across all continents in about 97 countries and are present as either local or introduced species, which largely depends on the aquaculture activities of the given region.[9]

The aim of this study was to test the sub-lethal toxicity of both $Pb(NO_3)_2$ and $CuSO_4$ in the *Oreochromis niloticus* on long-term exposure by

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evaluating effects on hematological parameters as indicators of the physiological state.

Materials and Methods

Experimental fish

Nile tilapiawere obtained from fish hatcheries of the King Abdulaziz City for Science and Technology in Mozahmiya, Saudi Arabia, and the fish were transported to the laboratory in tanks supplied with aeration units. After arriving in the laboratory, the fish underwent an acclimation period lasting a fortnight prior to beginning the experiment.

Experimental design

Three hundred and twenty fish (51.66 ± 2.42 g, mean ± SD) were randomly divided into two experimental groups. A control group (n = 40) was comprised of fish not exposed to either lead nitrate or copper sulfate in each experiment. Glass aquaria (100 ×60 ×50 cm) with a capacity of 100 L were used in all experiments, and 20 fish for each aquarium were used as a duplicate group. The first experiment was comprised of three treatment groups (n = 120) that were exposed to 20%, 40%, and 60% of the LC_{50} of $Pb(NO_3)_2$, respectively. The $Pb(NO_3)_2$ concentrations were based on the 96-h LC_{50} of $Pb(NO_3)_2$ for *O. niloticus* that was previously determined to be 44 mg/L according to Ullah *et al.*[10] Thus, the $Pb(NO_3)_2$ concentrations were determined to be 8.8, 17.6, and 26.4 mg/l respectively, this experiment was carried out for 30 days.

The second experiment was carryout comprised of three treatment groups (n = 120) that were exposed to 20%, 40%, and 60% of the LC_{50} of $CuSO_4$. The $CuSO_4$ concentrations were based on the 96-h LC_{50} of $CuSO_4$ for *O. niloticus* that was previously determined to be 12.85 mg/L by Mutlu *et al.*[11] Thus, the determined $CuSO_4$ concentrations were 2.57, 5.14, and 7.71 mg/L, respectively. This experiment was also, carried out for 30 days.

Supplemental aeration was provided to all aquaria to maintain dissolved oxygen levels near saturation, and the water temperature was maintained at 28 ± 1ºC. The fish were fed twice a day at the rate of 2% of the fish weight using a commercial diet from Maram Feed Factory that contained approximately 32% crude protein.

Hematological analyses

At the end of the experimental period, food was withheld for 1 day before the fish anesthetized by buffered MS222 (50 mg/L). Blood samples were collected from six fish of each group in both experiments. Blood was collected from the caudal vein with a 3-mL sterile syringe using heparin as an anti-coagulant. Then, the Mindray BC-2800Vet Auto Hematology Analyzer (Shenzhen Mindray, Bio-Medical Electronics Co, Ltd. China) was used to determine the complete blood count (CBC), the red blood cell count (RBC × 10^6/µl) according to the methods described by Dacie and Lewis[12], white blood cell count (WBC × 10^3/µl) following the methods of Stoskopf[13], and estimated hemoglobin (Hb) according to the methods described by Van Kampen and Zijlstra.[14] The hematocrit percentage (Hct) was calculated based on the formulas described by Britton.[15] Finally, the mean cell hemoglobin (MCH), MCH concentration (MCHC), mean cell volume (MCV), and Platelets PLT (× 10^3/µl) were determined.

Statistical analysis

A one-way analysis of variance (ANOVA) using the Minitab software program and the Tukey's least significance difference post-hoc test were used to compare the variation among the treatments. Differences were considered to be statistically significant at $P < 0.05$.

Results and Discussion

Experiment 1: $Pb(NO_3)_2$

The results of the 30-d exposure experiment to sub-lethal $Pb(NO_3)_2$ concentrations in *O. niloticus* indicated that significant differences ($p < 0.05$) in the hematological variables were found among the treatment and control groups (Table 1). The values of the hematological variables decreased significantly when the concentration of $Pb(NO_3)_2$ increased ($p < 0.05$). For example, the RBC count for the control group was 2.41 ± 0.13 while those of the treatment groups exposed to 8.8, 17.6, and 26.4 mg/L $Pb(NO_3)_2$ were 2.21 ± 0.10, 1.94 ± 0.16, and 1.36 ± 0.10 × 10^6/µl, respectively.

Similarly, Hb and Hct levels decreased as the $Pb(NO_3)_2$ level increased. A similar trend was also observed for the red blood indices, such as MCHC (g/dl) and MCH (pg), which agrees with data obtained by Abdel-Warith *et al.*[16] In that study, *Clarias gariepinus* exposed to $Pb(NO_3)_2$ for 20 days showed significantly decreased of RBCs, Hb, Hct, MCH and MCV when levels of metals increased. In addition, Adhim *et al.*[17] and Çiftçi *et al.*[18] found that lead-

Table 1 — Hematological parameters of *Oreochromis niloticus* exposed to sublethal concentration of $Pb(NO_3)_2$ for 30 days (Mean ± SE n = 6)

Parameters	Treatments			
	Control	20% LC_{50}	40% LC_{50}	60%LC_{50}
RBC ($\times 10^6$/ µl)	2.41 ± 0.13^a	2.21 ± 0.10^{ab}	1.94 ± 0.16^b	1.36 ± 0.10^c
Hb (g/dl)	16.23 ± 1.01^a	14.57 ± 0.59^a	11.87 ± 1.12^b	7.52 ± 0.85^c
Hct (%)	30.40 ± 1.52^a	29.93 ± 1.15^a	27.35 ± 2.20^a	19.32 ± 1.23^b
MCHC (g/dl)	53.52 ± 2.84^a	48.69 ± 0.98^a	43.20 ± 1.21^b	38.42 ± 2.36^b
MCH (pg)	68.52 ± 3.44^a	66.41 ± 2.35^a	61.12 ± 2.71^{ab}	54.52 ± 2.77^b
MCV (fl)	128.17 ± 1.61^b	136.26 ± 2.37^a	141.52 ± 3.47^a	142.84 ± 3.75^a
WBCs ($\times 10^3$/µl)	89.28 ± 5.17^{bc}	82.83± 3.61^c	106.13± 11.64^b	133.72± 9.57^a
Lymphocyte (%)	8.92± 0.37^b	8.87± 0.18^b	9.47 ± 0.79^b	16.12 ± 2.26^a
Monocyte (%)	2.30 ± 0.03^b	2.30 ± 0.03^b	2.25 ± 0.08^b	3.70 ± 0.54^a
Granulocyte (%)	88.78 ± 0.40^a	88.83 ± 0.19^a	87.87 ± 1.69^a	80.18 ± 2.79^b
PLT (10^3/µl)	67.80 ± 14.17^a	39.75 ± 5.59^{ab}	32.80 ± 2.13^b	38.75 ± 1.80^b

Values in the same row with the same superscript are not significantly different ($p>0.05$)

exposed *O. niloticus* presented clear differences in hematological variables when compared to those of the respective control groups.

The lead-exposed fish in this study showed lower RBC and Hct values and higher WBC levels when compared with those of the fish of the control group. However, MCV (fl) increased significantly as the concentration of $Pb(NO_3)_2$ increased, with values ranging between 128.17 ± 1.61 to 142.84 ± 3.75 (Table 1). The data in this study agree with those of Çiftçi *et al.*[18] who reported that MCV increased when *O. niloticus* was exposed to lead for 7, 15, and 30 days. Also, WBC levels showed significant differences among the treatment and control groups. Furthermore, WBC levels increased as the $Pb(NO_3)_2$ concentration increased and ranged between 89.28 ± 5.17 to 133.72 ± 9.57 $\times 10^3$/µl in the same manner as to what was observed for the lymphocyte and monocyte (%) levels.

However, granulocyte levels decreased when the concentration of $Pb(NO_3)_2$ increased. These results agree with those of Adhim *et al.*[16] who reported that *O. niloticus* exposed to lead showed lower WBC values when compared with those of fish in the control group. In addition, all hematological data in this study completely agree with the data obtained by Palipoch *et al.*[19] who studied *O. niloticus* exposed to $Pb(NO_3)_2$ for 28 days. In that study, PLT (10^3/µl) values were found to significantly decrease when *O. niloticus* were exposed to high levels of $Pb(NO_3)_2$. These findings agree with data obtained by Abdel-Warith *et al.*[16] who demonstrated that PLT decreased when the concentration of $Pb(NO_3)_2$ increased in *C. gariepinus.*

The differences in hematological variables observed in this study were directly caused by structural damage to RBC membranes, resulting in hemolysis and the deterioration of Hb structures, the stress-related release of RBCs from the spleen, and hypoxia, which were induced when the fish were exposed to lead.[20]

Experiment 2: $CuSO_4$

The hematological changes in *O. niloticus* resulting from exposure to different sub-lethal $CuSO_4$ concentrations for 30 days indicated a significant decrease in RBC ($\times 10^6$/µl), Hct (%), and WBC ($\times 10^3$/µl) levels at the end of the experiment when compared to those of the control group. However, Hb levels did not show any significant differences among the treatment and control groups. The RBC level in the control group was 2.32 ± 0.09$\times 10^6$/µl, whereas RBC levels in fish in the treatment groups exposed to 2.57, 5.14, and 7.71 mg/L $CuSO_4$ decreased and were 2.15 ± 0.11, 1.84 ± 0.04, and 1.77 ± 0.06 $\times 10^6$/µl, respectively, at the end of the experiment (Table 2). Similarly, Hct (%) decreased when the sublethal concentration of $CuSO_4$ increased. These results agree with those of Srivastava and Punia[21] and Thangam *et al.*[22] who reported that *C. carpio* exposed to various levels of zinc sulphate after 10, 20, and 30 days presented significantly reduced RBC, Hct(%), and leucocyte levels. Furthermore, the results of the current study fully agree with those obtained by Çiftçi *et al.*[18] who found that erythrocyte numbers (RBC) decreased when *O. niloticus* was exposed to both

Table 2 — Hematological parameters of *Oreochromis niloticus* exposed to sublethal concentration of ($CuSO_4$) for 30 days (Mean ± SE n = 6)

Parameters	Treatments			
	Control	20% LC_{50}	40% LC_{50}	60%LC_{50}
RBC ($\times10^6$/ µl)	2.32 ± 0.09[a]	2.15 ± 0.11[a]	1.84 ± 0.04[b]	1.77 ± 0.06[b]
Hb (g/dl)	15.40 ± 1.65[a]	13.35 ± 1.09[a]	16.37 ± 0.35[a]	14.90 ± 0.61[a]
Hct (%)	29.20± 2.07[a]	26.60 ± 1.86[ab]	24.00 ± 1.32[b]	22.96 ± 1.55[b]
MCHC (g/dl)	46.00 ± 4.75[b]	44.12 ± 2.42[b]	66.40 ± 2.28[a]	65.68 ± 3.85[a]
MCH (pg)	61.84 ± 6.67[b]	60.60 ± 3.88[b]	87.50 ± 4.53[a]	84.04 ± 1.66[a]
MCV (fl)	127.88 ± 7.35[b]	131.50 ± 3.35[ab]	141.12 ± 3.49[ab]	145.80 ± 4.92[b]
WBCs ($\times10^3$/µl)	236.20± 14.18[a]	210.72 ± 5.14[a]	110.22 ± 9.22[b]	99.44 ± 4.85[b]
Lymphocyte (%)	53.62 ± 18.23[ab]	82.16 ± 1.44[a]	38.42 ± 17.38[bc]	9.36 ± 0.20[c]
Monocyte (%)	3.44 ± 0.57[ab]	4.32 ± 0.45[a]	3.28 ± 0.60[ab]	2.32 ± 0.04[b]
Granulocyte (%)	42.94 ± 18.68[bc]	13.52 ± 1.02[c]	58.34 ± 17.91[ab]	88.32 ± 0.22[a]
PLT (10^9/l)	101.78 ± 6.23[a]	98.38 ± 6.87[a]	75.88 ± 4.01[b]	55.48 ± 7.91[c]

Values in the same row with the same superscript are not significantly different ($p>0.05$).

$CuSO_4$ and $Pb(NO_3)_2$. In this study, Hct (%) levels decreased when fish were exposed to $CuSO_4$ but increased when fish were exposed to $Pb(NO_3)_2$ for 7, 15, and 30 days compared to that of their respective control groups (Table 2). In addition, Al-Asgah *et al.*[1] found that RBC, Hb, and Hct values decreased when *O. niloticus* was exposed to various concentrations of cadmium chloride for 10, 20, and 30 days.

The results of the current study indicate that MCH, MCHC, and MCV levels significantly increased ($p < 0.05$) as the concentration of $CuSO_4$ increased. For example, the MCV value obtained for the control group was 127.88 ± 7.35 while the values obtained for fish exposed to 2.57, 5.14, and 7.71 mg/L of $CuSO_4$ were 131.50 ± 3.35, 141.12 ± 3.49, and 145.80 ± 4.92, respectively (Table 2). These results agree with those of Singh *et al.*[23] who found that MCHC, MCH, and MCV levels increased in the freshwater fish *Channa punctatus* when individuals were exposed to $CuSO_4$ for 15 and 30 days. However, the PLT results in this study indicated that PLT (10^3/µl) significantly decreased as the $CuSO_4$ concentration increased when compared to that of the control.

In general, exposure to lead resulted in more changes to hematological variables in *O. niloticus* than exposure to copper. Since copper is a major element, it may be less toxic than lead, which has no biological function.[18,24] Furthermore, lead may catalyze erythrocytes by inhibiting delta-aminolaevulinic acid dehydratase (ALA-D) activity, which plays a role in heme synthesis and shortens the lifespan of circulating red blood cells.[24] Witeska *et al.*[25] found that lead caused morphological alterations in the nucleus of red blood cells (RBCs) and structural distortion while being pervasive in chromatin material. They demonstrated that lead may cause deformations in red blood cells (RBCs), hindering nuclear membrane permeability and RNA synthesis. The toxicity caused by lead might also be related to its ability to create reactive oxygen species (ROS), which induce harmful oxidative effects in various tissues by improved lipid peroxidation through the Fenton reaction.[26–28]

Conclusions

In conclusion, this study was conducted to evaluate the long-term exposure (30 d) effects of $Pb(NO_3)_2$ and $CuSO_4$ at sublethal concentrations in *O. niloticus*. Pollution due to $Pb(NO_3)_2$ and $CuSO_4$ may produce significant changes in the physiology of *O. niloticus*, as evidenced by the observed changes in the hematological variables in this study. Additionally, profound impacts on hematological variables may cause a disruption in internal physiology. It is clear from the results of this study that water pollution due to $Pb(NO_3)_2$ and $CuSO_4$ has a deleterious influence on fish health, which is likely to be reflected both in terms of economics and with regard to human health. In addition, changes in hematological variables in the presence of $Pb(NO_3)_2$ and $CuSO_4$ may be attributed to the effect of heavy metals on osmoregulation, membrane permeability, and the stimulation of feedback mechanisms. As such, the effects of heavy metals may be reflected in the physiological conditions of the organisms that are exposed to them.

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