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Effect of sub lethal concentrations of cadmium and lead on *Oreochromis niloticus.*

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Abstract

The aim of this study was to evaluate the effect of heavy metal on *Oreochromis niloticus* was exposed to different concentrations of cadmium chloride and lead acetate for 96 h. The effects of sub-lethal exposure on haematological parameters, liver functions, and kidney functions were also investigated in *O. niloticus*. There were significant decrease of cadmium or lead exposure on erythrocyte count, haemoglobin concentration, and haematocrit values. There were significant effects on other haematological parameters (MCV, MCH, and MCHC). Cadmium or lead exposures were time and dose increased the creatinine, uric acid levels, alanine transaminase (ALT) and aspartate transaminase (AST) activities. The present investigation indicates that the cadmium chloride and lead acetate are toxic to aquatic organisms.

Keywords: Cadmium chloride, Lead acetate, Haematological parameters, Liver function, Kidney function.

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Introduction

Heavy metal contamination is one of the greatest vital ecological complications today. Heavy metals may enter into aquatic ecosystems and induce stress symptoms in fish. Some metals are essential since they play an important role in biological systems, while some others are nonessential metals as they have no known role in biological systems [1]. Toxic metal contaminants in aquatic water are disposed to increase gradually thereby representative the greatest risk to human consumers of fish and other seafood. Inquiries on the toxic effect of metals upon fish are attended by the analysis of variations in some biochemical and haematological parameters in fish [2,3]. Cadmium (Cd) is reflected one of the greatest toxic contaminants present in polluted water and may cause kidney, liver and skeletal injuries [4,5]. Whereas, lead (Pb) is a toxic pollutant and continuous exposure to Pb even at low concentrations has been established as a risk factor causing hepatotoxicity, nephrotoxicity, and reproductive and behavioral dysfunctions [6]. In fish, even at sublethal meditations of Cd and Pb have a sensitive biochemical effect, causes serious physiological harmful effects [7].

Toxicity studies applying fish under controlled environments provide, through the evaluation of mortality, reproductive success, behavioral, damage to tissues, hematological changes, and important information corresponding to the effects of pollutants on the biota of a natural aquatic ecosystem [8]. Tilapia (*Oreochromis* sp.) is one of the most important freshwater used in the estimation of the quality of marine systems [9,10]. Serum enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) are important serum markers to study the health of animal species [1]. The present study was aimed to evaluate the effect of Cd and Pb at different concentrations on some haematological and biochemical parameters of *Oreochromis niloticus*.

Material and Methods

Experimental animals

The study was carried out on 80 *Oreochromis niloticus*. The body weight and total body length of the fish ranged from 50-100 g and 10-15 cm, respectively. Fish were collected from El-Abbassa Fish Farm and transferred alive in a large plastic container to the laboratory where they were distributed in well-

ventilated glass aquaria. The fish were acclimatized for 14 days before the onset of the experiment. Fish were fed commercial pellets and checked daily. The use of experimental animals in the study protocol was carried out in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University Appendix 1, (Guiding Principles for Biomedical Research Involving Animals, 2011).

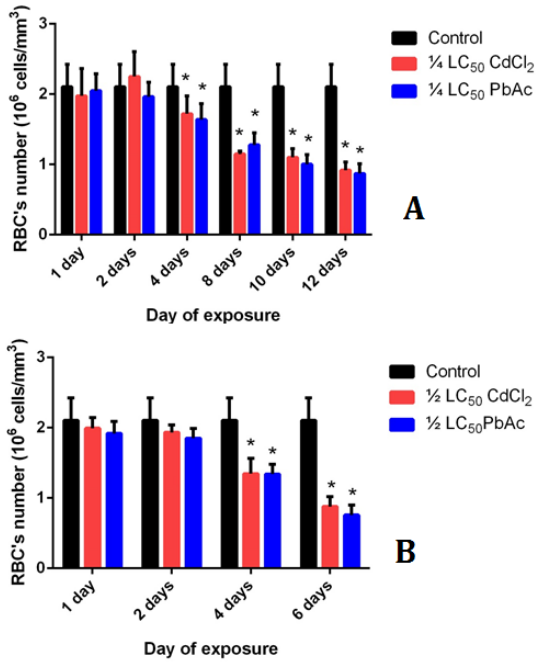

Figure 1. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on red blood cells (R.B.Cs; 10^6 cells/mm^3) of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to Control group.*

Experimental design

For the experiments, first the 96 h LC_{50} (50% lethal concentration) of cadmium and lead was determined. 40 fish were used for each metals. Four concentrations of cadmium in the form of cadmium chloride ($CdCl_2$) were used (10, 20, 30, 40 mg/L), and for lead, lead acetate (PbAc) at 50, 100, 150, 200 mg/L were used. Each concentration was added into an aquarium containing 10 fish.

Fish in each aquarium were continuously observed during the 96 h experiment period. Mortalities and time of death were recorded during the experiment period. These data were entered into SPSS (version 8.0) and probit analysis was used to determine the 96 h LC_{50} value for $CdCl_2$ and PbAc [11].

Thereafter, the fish were classified into three groups as follows:

Group 1: Served as control group where fish in this group was received zero treatment.

Group 2: This group studied the effect of ¼ LC_{50} of each $CdCl_2$ and PbAc on some biological parameters after 1, 2, 4, 8, 10 and 12 days.

Group 3: This group studied the effect of ½ LC50 of each $CdCl_2$ and PbAc on some biological parameters after 1, 2, 4, and 6 days.

The water of treated groups was changed every 2 days to minimize metal loss and maintain the concentration of examined metal.

Tissue collection

Liver was weighed and homogenized immediately to give a 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose. The homogenate was centrifuged at 500 g for 10 min at 4°C. The supernatant (10%) was used for biochemical determinations.

Blood sampling

The blood samples were taken from caudal vein by sterilized syringe or cutting the tail into sterile heparinized tube for haematological studies and other portion of blood was kept it for half an hour in test tube and then centrifuged at 500 g for 15 min at 4°C to separate serum and stored at -70°C for biochemical studies.

Haematological analysis

Red blood cell counts were carried out using the improved Neubauer hemocytometer, hemoglobin content was estimated using the cyanmethaemoglobin method described by Cooper et al. [12] (Biodiagnostic Kit, Giza, Egypt). Briefly, a known volume of fully oxygenated, uncoagulated blood was centrifuged at 3000 rpm for 10 minutes in a microhaematocrit capillary tube until cells were packed down to constant volume (Britton, 1963). The percentage of relative volume of erythrocytes to the whole blood volume was determined, mean cellular volume (MCV), mean cellular haemoglobin (MCH) and mean cellular hemoglobin concentration (MCHC) were determined. The three parameters were calculated using the following formulas.

MCV (PL)=(packed cell volume as percentage/RBC in millions) × 10

MCH (pg)=(Hb in g/RBC in millions) × 10

MCHC (g/dL)=(Hb in g/packed cell volume) × 100.

Biochemical analysis

Liver function test: Colorimetric determination of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine, according to the method of Reitman and Frankel [13]. The color of which was measured at 546 nm, using kits provided from Biodiagnostic Co. (Giza, Egypt).

Kidney function test: Serum creatinine (Cr), uric acid (UA), were assayed in serum, using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the methods that were

described by Bartels et al. [14] and Sanders et al. [15], respectively.

Statistical analysis

The results were expressed as the mean ± standard error of means (SEM). Data were statistically analyzed using Student's t-test with the program Statistical Package for Social Sciences (SPSS) version 0.8. The means of the data were considered significantly different at $p<0.05$.

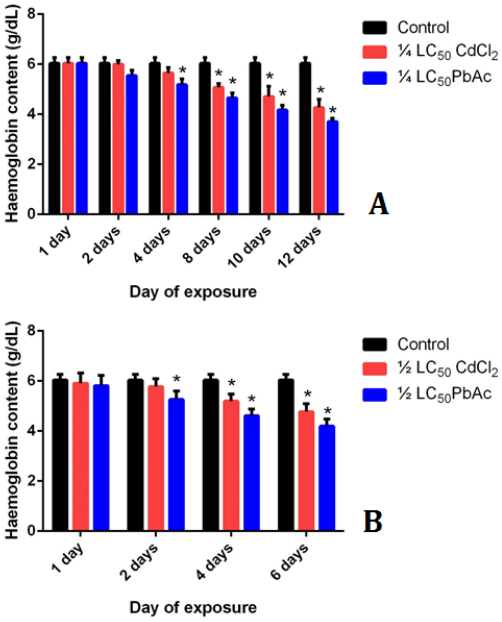

Figure 2. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on haemoglobin (Hb) contents of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

Results

Change in haematological parameters

As shown in Figure 1a, the control value of red blood cell count in *Oreochromis niloticus* was 1.88 ± 0.15 × 10^6 cells/ mm^3. Red blood cells count in fish that exposed to ¼ LC_{50} of $CdCl_2$ and PbAc individually for 1, 2, 4, 8, 10 and 12 days showed significant decrease after the 8th day (-40.43% and -38.30%), 10th day (-46.28% and -51,60%) and 12th day (-55.32% and -59.04%), respectively. Also, Figure 1b represent the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 6 days on R.B.Cs counts of *Oreochromis niloticus*. R.B.Cs counts showed significant decrease occurred after the 4th day (-36.70% and -34.04%) and 6th day (-58.52% and -64.89%), respectively. As illustrated in Figure 2a, the control value of Hb in *Oreochromis niloticus* was 5.88 ± 0.15 g/dL. The Hb content in fishes exposed to ¼ LC_{50} of $CdCl_2$ showed significant decrease after the 8th day (-15.31%), 10th day (-24.83%) and 12th day (-31.29%) while, the administration of ¼ LC_{50} of PbAc showed significant decrease after the 4th day (-14.63%), 8th day (-23.13%), 10th day (-31.29%) and 12th day (-38.44%). Figure 2b represent the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 6 days on Hb content of *Oreochromis niloticus* showed significant decrease after the 4th day (-14.96% and -24.83%) and 6th day (-22.45% and -31.97%), respectively.

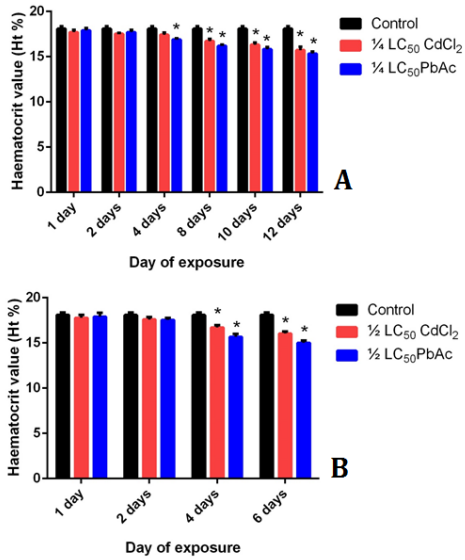

Figure 3. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on haematocrit value (Ht %) of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

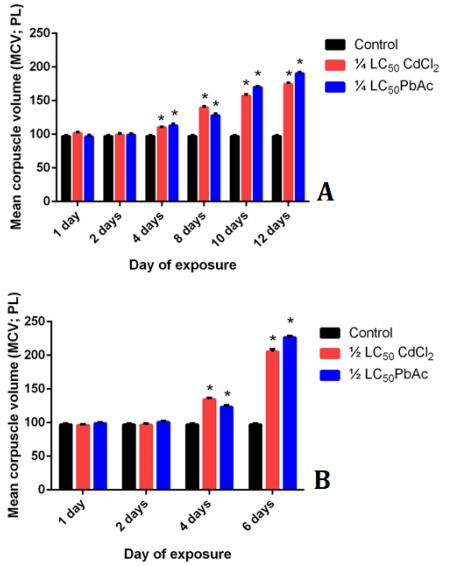

Figure 4. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on mean corpuscle volume (MCV) of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

As shown in Figure 3a, the control value of Ht% in *Oreochromis niloticus* was 17.90 ± 0.21%. The Ht% in fishes exposed of ¼ LC_{50} of $CdCl_2$ and PbAc individually for 12 days showed significant decrease after the 4th day (-7.54% and

-10.17%), 8th day (-7.54% and -10.17%), 10th day (-9.83% and -12.51%), and 12th day (-13.63% and -15.20%), respectively.

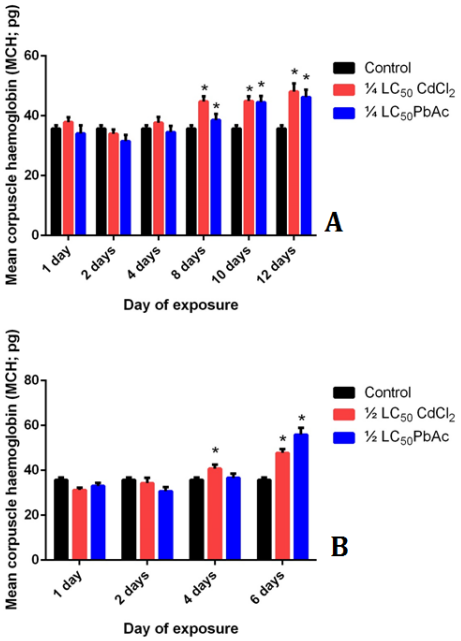

Figure 5. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on mean corpuscle haemoglobin (MCH) of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

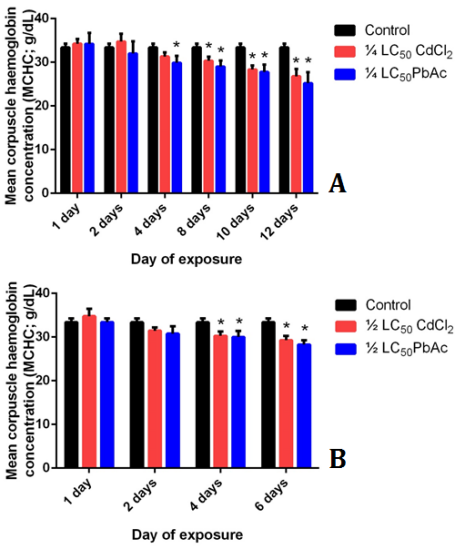

Figure 6. *Effect of Concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on mean corpuscle haemoglobin concentration (MCHC) of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

Figure 3b illustrated the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 6 days on Ht % of *Oreochromis niloticus*. The administration of ½ LC_{50} of $CdCl_2$ significant decrease after the 2nd day (-5.44%), 4th day (-7.71%) and 6th day (-11.28%), while the administration of ½ LC_{50} of PbAc significant decrease after the 4th and 6th day (- 13.63% and -17.21%, respectively). As illustrated in Figure 4a, the control value of MCV in *Oreochromis niloticus* was 96.24 ± 0.60 PL. The MCV in fishes exposed to ¼ LC_{50} of $CdCl_2$ showed significant increase after the 1st day (+4.45%), 3rd day (+13.99%), 8th day (+43.56%), 10th (+61.26%), and 12th (+80.28%), while the administration of ¼ LC_{50} of PbAc showed significant increase after the 4th day (+15.50%), 8th day (+31.12%), 10th day (+75.19%) and 12th day (+96.38%). Figure 4b illustrated the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 6 days on MCV of *Oreochromis niloticus*. The administration of ½ LC_{50} of $CdCl_2$ showed significant increase after the 4th day (+38.58%) and 6th day (+110.59%), while the administration of ½ LC_{50} of PbAc showed significant increase after the 2nd, 4th and 6th day (+3.43%, +26.31%, and +133.96%, respectively).

As shown in Figure 5a, the control value of MCH in *Oreochromis niloticus* was 34.97 ± 0.46 pg. The MCH in fishes exposed to ¼ LC_{50} of $CdCl_2$ showed significant increase after the 1st day (+5.15%), 8th day (+24.48%), 10th (+25.25%), and 12th (+32.11%), on other hand the administration of ¼ LC_{50} of PbAc showed significant increase after the 10th day (+22.76 %) and 12th day (+26.97%). Figure 5b illustrated the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 6 days on MCH of *Oreochromis niloticus*. The administration of ½ LC_{50} of $CdCl_2$ showed significant increase after the 4th day and 6th day (+13.15% and +33.23%, respectively), while The administration of ½ LC_{50} of PbAc showed non-significant change after the 4th day, significant increase after the 6th day (+53.76%) and significant decrease after the 1st and 2nd day (-8.49% and -15.93%, respectively).

As illustrated in Figure 6a, the control value of MCHC in *Oreochromis niloticus* was 32.74 ± 0.55 g/dL. The MCHC in fishes exposed of ¼ LC_{50} of $CdCl_2$ for 12 days showed significant decrease after the 4th day (-6.41%), 8th day (-9.16%), 10th day (-15.36%) and 12th day (-21.81%). The MCHC in fishes exposed of ¼ LC_{50} of PbAc for 12 days showed non-significant change after 1st day and significant decrease after the 2nd, 4th, 8th, 10th and 12th day (-8.4%, -12.03%, -14.51%, -18.75%, and -28.53%, respectively). The effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 6 days on MCHC of *Oreochromis niloticus* demonstrated in Figure 6b. Significant decrease after the 2nd day (-5.74%, -9.41%), 4th day (-9.65%, -11.42%) and 6th day (-12.65%, -15.70%), respectively, were occurred.

Change in biochemical parameters

The control value of serum creatinine level in *Oreochromis niloticus* was 1.34 ± 0.10 mg/dL. The creatinine level in fishes exposed to ¼ LC_{50} of $CdCl_2$ for 12 days showed significant increase after the 4th, 8th, 10th and 12th day (+55.96%, + 98.51%, + 117.16%, and 135.07%, respectively) (Figure 7a). Figure 7b illustrated the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 6 days on creatinine of *Oreochromis niloticus*. The administration of 1/2 LC_{50} of $CdCl_2$ showed significant increase after the 2nd day (+76.21%), 4th day (+108.21%) and 6th day (+127.61%), while the administration of ½ LC_{50} of

PbAc showed significant increase after the 4th and 6th day (+66.42%, and +96.27%, respectively).

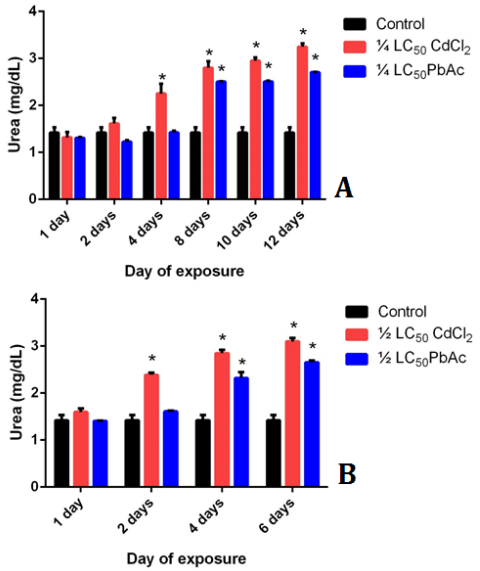

Figure 7. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on creatinine of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

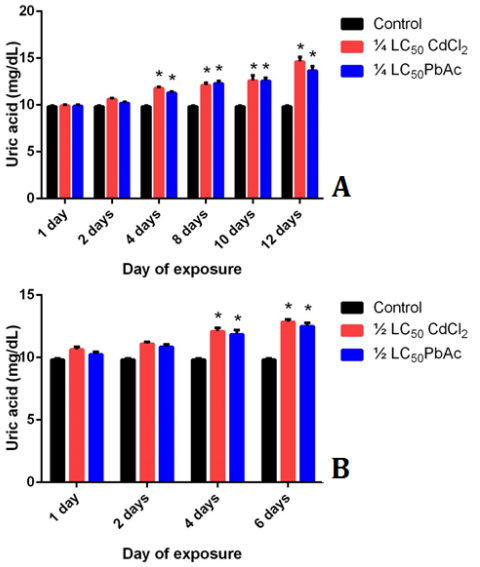

Figure 8. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on uric acid of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to Control group.*

As showed in Figure 8a, the control value of serum uric acid in *Oreochromis niloticus* was 9.77 ± 0.15 mg/dL. The uric acid in fishes exposed to ¼ LC_{50} of $CdCl_2$ for 12 days showed significant increase after the 2nd, 4th, 8th, 10th and 12th day (+7.88 %, + 19.65 %, + 22.21%, +24.97%, and +46.37%, respectively), while The administration of ¼ LC_{50} of PbAc showed significant increase after the 4th, 8th, 10th and 12th day (+14.26%, +24.53%, +26.31% and +36.54%, respectively).

Figure 8b illustrated the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 12 days on uric acid of *Oreochromis niloticus*. The administration of 1/2 LC_{50} of $CdCl_2$ showed significant increase after the 1st day (+7.57%), 2nd day (+12.79%), 4th day (+21.60%) and 6th day (+29.58%), while the administration of ½ LC_{50} of PbAc showed significant increase after the 2nd, 4th and 6th day (+9.72%, +19.04%, and +30.40%, respectively).

As showed in Figure 9a, the control value of serum AST in *Oreochromis niloticus* was 14.75 ± 0.39 U/L. The AST in fishes exposed to ¼ LC_{50} of $CdCl_2$ for 12 days showed significant increase after the 4th (+9.76%), 8th (+34.17%), 10th (+36.34%) and 12th day (+59.80%), while The administration of ¼ LC_{50} of PbAc showed significant increase after the 2nd, 4th, 8th, 10th and 12th day (+8.41%,+18.58%, +39.59%, +45.02% and +70.64%, respectively). Figure 9b illustrated the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 12 days on AST of *oreochromis niloticus*. The administration of ½ LC_{50} of $CdCl_2$ showed significant increase after the 2nd day (+11.12%), 4th day (+38.24%) and 6th day (+65.36%), while the administration of ½ LC_{50} of PbAc showed significant increase after the 2nd, 4th and 6th day (+22.58%, +51.80%, and +78.78%, respectively).

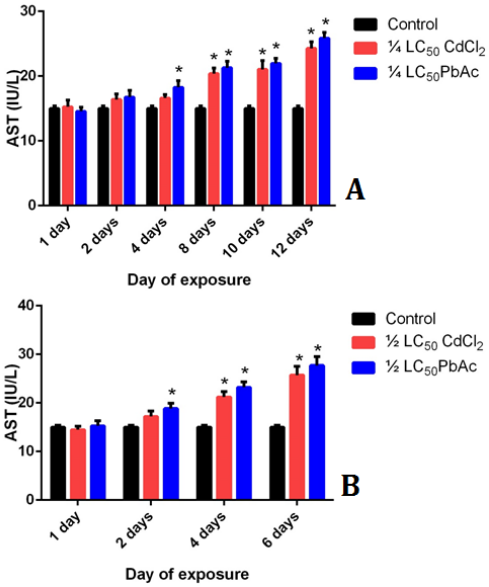

Figure 9. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on serum AST of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

As illustrated in Figure 10a, the control value of serum ALT in *Oreochromis niloticus* was 6.58 ± 0.40 U/L. The ALT in fishes exposed to ¼ LC_{50} of $CdCl_2$ for 12 days showed significant increase after the 2nd (+21.28%), 4th (+56.38%), 8th (+90.58%), 10th (+133.59%) and 12th day (+169.45%), while The administration of ¼ LC_{50} of PbAc showed significant increase after the 4th, 8th, 10th and 12th day (+63.68%, +100.15%, +149.24% and +181.61%, respectively). Figure 10b illustrated the effect of ½ LC_{50} of $CdCl_2$ and PbAc individually for 12 days on ALT of *Oreochromis niloticus*. The administration of

1/2 LC_{50} of $CdCl_2$ showed significant increase after the 2nd day (+56.38%), 4th day (+96.66%) and 6th day (+127.36%), while the administration of ½ LC_{50} of PbAc showed significant increase after the 2nd, 4th and 6th day (+79.33%, +106.32%, and +158.36%, respectively).

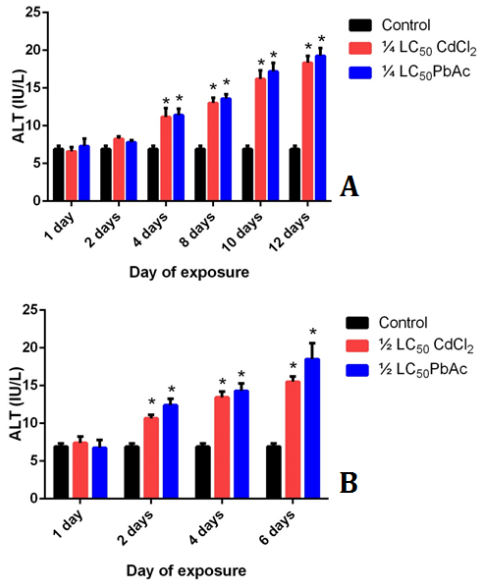

Figure 10. *Effect of concentration of cadmium chloride ($CdCl_2$) or lead acetate (PbAc) on serum ALT of Oreochromis niloticus; A) ¼ LC_{50}; B) ½ LC_{50}. Values are mean ± SEM (n=5); *p<0.05, significant change with respect to control group.*

Discussion

Cadmium is one of these heavy metals that have become widely distributed in the aquatic environment as an industrial waste produced from electroplating and plastic industry [16]. Lead has been recognized as the major heavy metal pollution in some areas because of its wide distribution in the human environment [17]. Blood parameters are often measured, when clinical diagnosis of fish physiology is applied to determine the sub-chronic effects of pollutants. The use of haematological variables, such as haemoglobin and haematocrit indicate a physiological response to a contaminated environment [18]. The count of red blood cells is quite a stable index and the animal body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation [19]. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters [20].

This study, therefore, assessed the haematological profile of *O. niloticus* exposed to ¼ and ½ of LC_{50} for PbAc and $CdCl_2$ individually; this profile indicated that decrease in red blood cells count, Hb content and Ht%. These results were supported by Alkahemal-Balawi et al. [20], they reported that sub-chronic exposure to Pb in the Clarias gariepinus, affects blood parameters and plasma chemistry. Adeyemo [21] reported decreased haemoglobin, RBC count and haematorit values in *C. gariepinus* exposed to lead nitrate. Enuneku and Ezemonye [22] indicated the administration of Cd to *H. occipitalis* and *B. maculatus* induced declines in TEC (total erythrocytes count), Ht% and Hb levels with increase in the concentration of Cd. The observed decrease in these blood indices is indicative of Cd-induced anaemia and this is consistent with previous studies of anemia in fish, rats and rabbits exposed to cadmium, mercury, lead, nickel, copper and zinc [23]. Various mechanisms have been proposed for Cd induced anaemia. These include iron deficiency anemia due to competition of Cd with iron in the iron transfer system of the intestinal mucosa, hypoplastic anaemia derived from the inhibitory effect of Cd on the growth of erythroid progenitor cells, or bone marrow cells and haemolytic anemia due to red cell sequestration in the spleen [22].

Among the heavy metals causing anaemia, the mechanism of Pb-induced anaemia is well known to involve the inhibition of red cell delta aminolevulinate hydrogenase [24], but cadmium does not have any effect on the activity of this enzyme [22]. Pb is known to inhibit the activities of three enzymes in heme biosynthesis viz aminolaevulinic acid dehydratase, coproporphyrin oxidase and ferrochelatase. Therefore, the formation and excretion of heme precursors such as aminolevulinic acid, porphobilinogen, coproporphyrin and zinc protoporphyrin is increases by Pb exposure. Pb inhibits ferrochelatase enzyme that incorporates iron into protoporphyrin-IX to form heme [25]. Enuneku and Ezemonye reported that the decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity [22]. The significant decrease in the haemoglobin concentrations may also be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis. The prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be described as pathological conditions in fishes exposed to toxicants.

In this study, the decrease in hematological indices appeared to be due to the decreased cellular count in the blood of fish after Cd or Pb exposure. The PCV (Ht%) significantly decreased, while the MCV increased. This increase in MCV may be due to the swelling of the RBCs under heavy metal stress. Additionally, an increase in RBC size could be the result of a premature release of immature RBCs in response to anemia. Furthermore, previous work has shown that stress reaction causes an osmotic imbalance. This can diminish the pH of the blood and increase the volume of erythrocytes, subsequently affecting the hematocrit value. Stress reactions can also lead to epinephrine release, which may cause premature release of immature or deformed erythrocytes from the spleen and may affect the hematocrit value [26,27]. Indeed, the low values of PCV (Ht%) in fish exposed to stressors has been explained by a reduction in RBC volume caused by osmotic changes due to ion losses from the blood plasma and on the other hand by reduced number of RBC as a result of adrenergic-splinic expansion in hypoxic conditions [28].

Liver enzymes such as ALT and AST are marker enzymes for liver function and integrity [6]. These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication due to damage to the liver. Aminotransferases, ALT and AST, are an important class of enzymes linking carbohydrate and amino acid metabolism, the relationship between the intermediates of the citric acid cycle is well established. These enzymes are regarded as markers of liver injury [29]. Liver is a major target organ for Cd and Pb toxicities after acute exposure. Acute Cd exposure produces hepatocyte swelling and fatty change, as well as focal, zonal, and massive necrosis, resulting in marked elevation of serum enzymes [30]. Whereas, chronic Pb exposure causes alteration in liver functions and by binding to plasmatic proteins causes alterations in a high number of enzymes and may perturb protein synthesis in hepatocytes [29]. In the present study, serum AST and ALT were significantly high suggesting that such liver damage might have occurred and hence leading to the leakage of these enzymes into the blood [16]. Ajayi et al. showed that Pb is known to bind to the sulfydryl groups of enzymes containing cysteine, and found to form complexes with amino acids and protein [31]. Since AST, ALT is liver enzymes, Pb will alter the level of ALT activity in the tissues by disrupting their membrane. Consequently, there will be a discharge of the cell content into the blood stream and ALT activity is known to increase only in heavy metal poisoning, toxic hepatosis, and muscular dystrophy.

Creatinine and uric acid levels are indicators of kidney function [16]. Creatinine is the breakdown product of creatine, which is an important part of the muscle. The test is performed to evaluate the kidney function. If the kidney function is abnormal, the creatinine level will increase in the blood, due to decreased excretion of creatinine in the urine. Serum/plasma creatinine is a more sensitive indicator of renal function than the blood urea nitrogen. The presence of the increased level of urea and creatinine concentration in the blood suggests the inability of the kidney to excrete these products [32]. In the present study, creatinine, and uric acid showed a significant increase in fish exposed to $CdCl_2$. This result was supported by Abdel-Tawwab and Wafeek [16] reported that the action of heavy metal on glomeruli filtration rate and/or Cd may cause pathological changes to the kidney resulting in dysfunction. The changes in uric acid and creatinine level due to the severe injured effect of $CdCl_2$ on kidney. In the present study the creatinine level for all groups of *O. niloticus* exposed to PbAc increased that indicated kidney had been affected during PbAc administration. The present results have been supported by Khalil-Manesh et al. [33], who mentioned that lead acetate increased serum creatinine level as compared to the control group, where rats were intoxicated. Similar results have been reported by many researchers [34].

The present study showed that PbAc affected the level of uric acid. The uric acid level increased during PbAc exposure. The same results have been found by Abdel Moneim et al. [34] in rats, and have also been reported by Dioka et al. [35], who mentioned that exposure of human subjects to lead in petrol increased the concentrations of uric acid as compared to unexposed subjects. In conclusion, the obtained results in our study demonstrated that lead acetate and cadmium chloride are toxic to *O. niloticus* and exposures to lead acetate and cadmium chloride resulted in significant haematological and biochemical alterations. The present results clearly indicate that those metals may be a threat to aquatic fauna and flora as well as humans.

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