Chapter 12

The Effect of Lead and Zeolite on Hematological and Some Biochemical Parameters in Nile Fish (*Oreochromis niloticus*)

Hikmet Y. Çoğun and Mehmet Şahin

Additional information is available at the end of the chapter

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1. Introduction

The elemental lead (Pb) occurs naturally in the environment as well as being produced by mining and manufacturing activities [1]. Lead and its compounds are serious pollutants of the aquatic environment. Moreover, several authors also agree that toxic and non-biodegradable metals such as lead accumulate in many fish species, causing various diseases such as renal [2, 3], hepatic lesions [4], endocrine impairment [5] and effect of cell membrane lipids in cells of the central nervous system.

Zeolites are used in industry, agriculture, environment protection and even in medicine. Zeolites have a relatively high Si/Al compositional ratio which gives it is special ion-exchange selectivity for large monovalent cations. Natural or synthetic zeolites (sodium aluminum silicates) are known to easy adsorb metal ions by exchange reactions [6].

Oreochromis niloticus is a widely used species in aquaculture for food supply and as a bioindicator of water contamination [7]. Fish hematological parameters are often determined as an index of their health status [8].

Hematological indices such as hematocrit (Hct), hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs) and plasma enzyme activities such as AST (aspartate aminotransferase), ALT (alanine aminotransferase), stress hormone cortisol and choline esterase have been used as an indicator of metal pollution in the aquatic environment [9-11].

There is a strong correlation between lead and zeolite that have been found in aquatic organisms and several authors have demonstrated that zeolite protects against lead and also other heavy metals in the experiment [12, 13]. There are numerous biological mechanisms

between lead and zeolite. These mechanisms are (I) ion-exchange and (II) adsorb metal ions by exchange reactions [6, 14]. This effects decreases homeostatic mechanisms on fish.

The aim of this study was to determine the effects on hematological and some biochemical parameters on adult *Oreochromis niloticus* exposed to sublethal concentration of lead and zeolite for 10 and 20 days.

2. Materials and methods

Freshwater fish *O. niloticus* were obtained from pools and acclimatized in the laboratory for two months at 25±1°C. After this period the mean body size and body mass of the animals were 15.9±1.87 cm and 56.5±2.15 gr., respectively. We used fish Nile tilapia, *Oreochromis niloticus*, is a teleost widely distributed around the world with economic importance for fisheries [15].

Water quality characteristics in tanks;

- Temperature: 25±1°C
- pH: 8.4±0.8
- Dissolved Oxygen: 7.6±0.5 mg/L
- Total Hardness: 184.5±4.38 $CaCO_3$ mg/L
- Total Alkalinity: 278.2±8.7 $CaCO_3$mg/L

The effects of lead and zeolite were shown as 0.1 mg/L Lead (Pb), 0.1 mg/L Lead+0.1 g/L Zeolite (PbZ1), 0.1 mg/L Lead+0.2 g/L Zeolite (PbZ2) and Control (C). A total of 4 aquariums sized 40x100x40 cm in height were divided into two groups. These were filled with 100 L. aerated class aquarium tanks. Nine fish were put in each aquarium (3 repetition x 3 fish). One aquariums of the first group contained 0.1 mg Pb/L (PbCl2, 2 H2O) solutions and two aquariums of the second group contained 0.1 mg/L Lead+0.1 g/L Zeolite and 0.1 mg/L Lead +0.2 g/L Zeolite solutions and one aquarium was used as a control. All fish were fed with Pinar Yem at a concentration of 1 % of their body mass per day.

Fish were anaesthetized with MS-222 blood was collected from each fish by cutting the caudal peduncle. Fish blood was collected for hematological parameters. The blood was centrifuged at 4000 rpm over 10 min at 15 ^{0}C to obtain the serum. Blood samples were sent to Cukurova University (Balcalı Hastahanesi, Merkez laboratory) for hematological analysis. The serum was divided in two portions from the ephandof tubes, first portion for cortisol and cholineesterase, second portion for ALT and for AST, ALT and AST activities. Those analyses were determined using UV test technique [16]. The serum samples were frozen and stored -20^{0}C until required for assays. Cortisol, ALT, AST and cholinesterase were determined by ROCHE Hitachi E-170 and DPP.

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Data are presented as mean ± standard error. For the statistical analysis, it was used one-way analysis of variance (ANOVA) followed by Student Newman–Keul's test using SPSS 10.0 statistical software (SPSS Inc., Chicago, IL). Differences were considered significant if $P < 0.05$.

3. Results and discussion

No mortality was observed at concentrations of the lead and its zeolite mixtures studied during the experiments. The statistical analysis was done with "SNK" differences among groups were measured to be significant at p<0.05 and showed Table 1 and 2.

	Enzymatic parameters			
Treateds	Cortisol (ug/dL)	ALT (uL)	AST (uL)	Cholinesterase (uL)
C	5.76±0.03 **a**	14.33±1.20 **a**	126.0±2.64 **a**	776.3±5.78 **a**
Pb	6.73±0.08 **a**	18.00±0.01 **b**	121.1±0.57 **b**	649.0±6.11 **b**
PbZ1	4.30±0.13 **b**	9.66±0.66 **c**	98.00±1.00 **c**	313.6±3.17 **c**
PbZ2	3.12±0.30 **c**	8.66±0.65 **a**	88.0±0.57 **b**	214.6±0.66 **c**

Letters a, b,and c show the differences between groups (P < 0.05).

Table 1. Enzymatic changes Mean±SE (N=6) of the fish *O. niloticus* exposed to Pb, PbZ1 and PbZ2 over 10 days

	Enzymatic parameters			
Treateds	Cortisol (ug/dL)	ALT (uL)	AST (uL)	Cholinesterase (uL)
C	5.77±0.03 **a**	15.66±0.88 **a**	126.3±0.88 **a**	790.3±5.17 **a**
Pb	7.28±0.09 **b**	20.33±0.87 **b**	132.6±0.88 **b**	529.0±3.21 **b**
PbZ1	3.81±0.18 **c**	11.66±0.66 **c**	112.6±1.20 **c**	431.3±3.84 **c**
PbZ2	3.26±0.17 **c**	7.33±0.66 **b**	109.0±0.57 **c**	302.0±1.52 **c**

Letters a, b,andc show the differences between groups (P < 0.05).

Table 2. Enzymatic changes Mean±SE (N=6) of the fish *O. niloticus* exposed to Pb, PbZ1 and PbZ2 over 20 days

Zeolite may decrease the toxicity of lead in water and Pb may form a complex with Zeolite. Hb, Hct, RBCc and WBCc levels decreased Pb, PbZ1 and PbZ2 exposed fish at both exposure periods (Fig 1-4). The exposures of Pb, PbZ1 and PbZ2 did not cause any significant changes in RBCc and WBCc levels of fish at 10 days while they caused a decrease in its levels at the end of the exposure period.

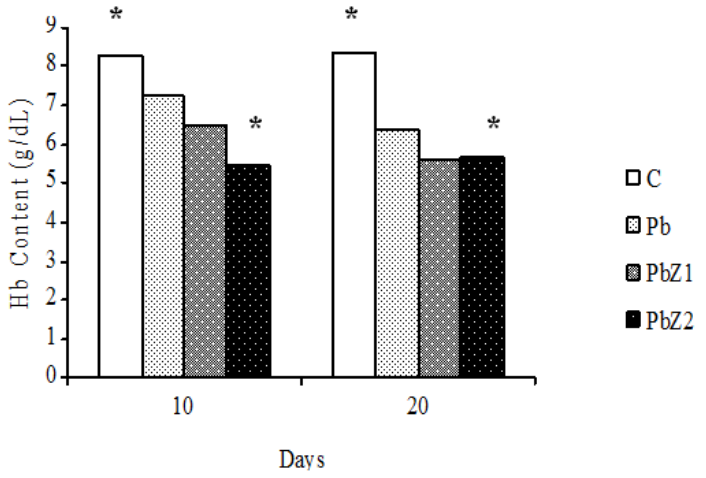

Figure 1. Serum Hemoglobin levels in *O. niloticus* to lead and its zeolite mixtures for 10 and 20 days. Data are expressed as mean ± standard error (N = 6). * shows significant differences between time for the same exposure group ($P < 0.05$).

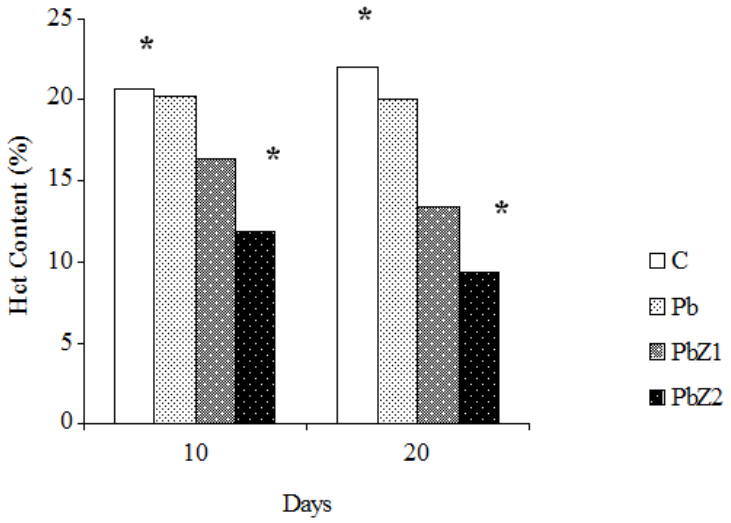

Figure 2. Serum Hemotocrit levels in *O. niloticus* to lead and its zeolite mixtures for 10 and 20 days. Data are expressed as mean ± standard error (N = 6). * shows significant differences between time for the same exposure group ($P < 0.05$).

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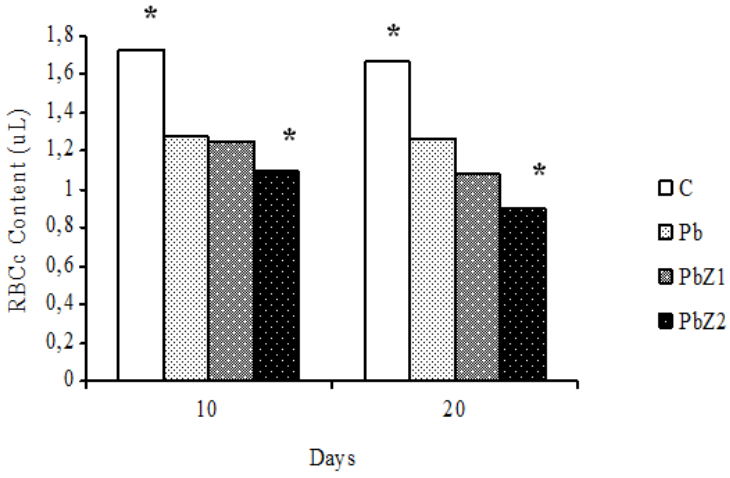

Figure 3. Serum RBCc levels in *O. niloticus* to lead and its zeolite mixtures for 10 and 20 days. Data are expressed as mean ± standard error (N = 6). * shows significant differences between time for the same exposure group (P < 0.05).

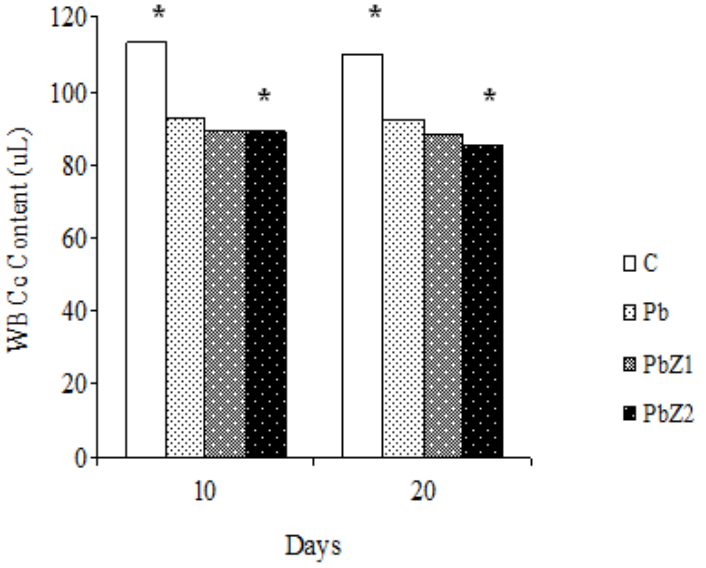

Figure 4. Serum WBCc levels in *O. niloticus* to lead and its zeolite mixtures for 10 and 20 days. Data are expressed as mean ± standard error (N = 6). * shows significant differences between time for the same exposure group (P < 0.05).

Serum Cortisol, ALT, AST and Cholinesterase activities of *O. niloticus* decreased in response to Pb, PbZ1 and PbZ2 exposures when compared to control during 10 and 20 days (Table 1 and 2). At the end of the exposure period elevations in Cortisol, ALT and AST enzyme activities of fish exposed to concentrations of Pb, PbZ1 and PbZ2 compared with metal-treated groups. While the cholinesterase activities of *O. niloticus* decreased to Pb, PbZ1 and PbZ2 at both exposure periods, an increase in AST activities 0.1 mg L^{-1} Pb exposure was observed at 10 days (Table 1 and 2).

4. Hematological parameters

Fig. 1-4 show distribution of hematological (Hb, Hct, RBCc and WBCc) parameters Pb and Pb+Ze mixtures exposed to *O. niloticus* over 10 and 20 days. In blood parameters notable declines were observed at all Pb, PbZ1 and PbZ2 exposure periods (Fig. 1-4). The maximum decrease of 41% Hct and 35 % RBCc were observed in Pb+Ze at 10 days and 55% Hct and 41% RBCc at 20 days.

Lead was classified as a toxic substance for fish. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage. Hematological indices are commonly used as indicators of metal pollution in fish [17, 18]. This index reflects respiratory status of animals. In addition to these status, infectious and stress have been shown to influence the fish hematology.

Metals in hematological parameters of fish generally occur due to the osmotic changes resulting in hemodilution (an increase in the volume of plasma, resulting in a reduced concentration of red blood cells in blood) or hemoconcentrations (an increase in the concentration of blood cells resulting from the loss of plasma or water from the bloodstream) [19]. Generally, spleen is responsible for this change. Because, spleen, serving as a potent blood storage organ in some teleost, sequestering blood cell under resting conditions and releasing them to circulating blood associated with various stress [20]. In [4] have been reported that histopathological lesions occur in spleen and intestine lead exposure on *Heteropneustes fossilis*. This also leads to changes in hematological parameters of fish. In this study, the effects of lead and zeolite mixtures decreased red blood cell, hemoglobin content and hematocrit values. Significantly depressed in this study blood parameters, an indicator of Pb, PbZ1 and PbZ2 intoxications, were observed in blood (RBCc and WBCc) ($P<0.005$) of fish at exposure time. This change was, however, not found in 0.1 mg L^{-1} Pb group. This may be zeolite is capable of ion exchange [6, 12].

5. Enzyme activity

In this study, at 10 and 20 days, compared with controls, ALT, AST, Cortisol and Cholinesterase were decreased PbZ1 and PbZ2 concentrations while ALT, AST and Cortisol in-

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creased only Pb concentrations. However, Cholinesterase decreased at not Pb, but PbZ1 and PbZ2 combinations (P<0.005) during 10 and 20 days.

Plasma cortisol level is widely used as a general indicator of stressful conditions in fish [21, 22]. Despite the interest in plasma cortisol measurement as an indicator of stress, few studies have actually measured the kinetics of cortisol in fish. A study [23], reported that during chronic stress, plasma cortisol back to the resting levels on *Salvelinus fontinalis*. In our study, cortisol levels of fish blood, compared with controls, increased both low and high concentration of lead (P<0.005). But, these levels were decreased with lead and zeolite combinations during exposure periods (Table 1 and 2). This may be zeolite is capable of ion exchange [6, 12]. The HPI (The hypothalamo-pituitary-interrenal) axis is activated to produce cortisol and other corticosteroid hormones for the maintenance of disturbed homeostasis [24]. The elevation of cortisol, in this study, it was noted that this may be a function of stimulation to the HPI axis in metal stress.

Serum ALT and AST activities used in diagnosis of damage fish tissues (i.e. gill, muscle, liver) [25]. Determinations of transaminases (AST and ALT) have been useful in the diagnosis of liver and kidney diseases in fish [26]. These enzymes of *O. niloticus* increased in response to lead exposures when compared to control during 10 and 20 days (Table 1 and 2). At the end of the exposure period, the activities of these enzymes in fish exposed to lead were higher when compared with PbZ1 and PbZ2 groups. While the ALT and AST activities increased in fish exposed to only lead concentrations at both exposure periods, an decreased in their activities following PbZ1 and PbZ2 exposure was observed at both exposure days (Table 1 and 2). There are numerous study in this serum activity of fish such as *Sparus aurata* [27] and *Cyprinus carpio* [28]. The researchers concluded that necrosis or disease of liver caused to leakage of this enzyme into blood stream might be responsible for increase of this enzyme in blood.

There are multiple forms of esterase in vertebrates' blood plasma [29]. However, acetyl cholinesterase content of fish blood is present in low concentration compared with other vertebrate [30]. One of the biomarkers most frequently used in fish for the diagnosis of exposure to pollutants is the measurement of the inhibition of the enzyme cholinesterase (ChE) [30]. Table 1 and 2 presents ChE activity in plasma of *O. niloticus* exposure lead and its zeolite combinations during 10 and 20 days. ChE activity was decreased significantly after exposure to Pb, PbZ1 and PbZ2 both days (P<0.05). Similar findings have been described by in reference [31]. The high levels of ChE activity found in control fish in this present study, as was previously described in [32].

In conclusion, the data from this investigation which is the blood-based enzymatic and hematological parameters responded to relatively Pb and its mixtures concentrations show useful for monitoring on fish [33, 34]. The hematological data, as well as gross observations from sample handing and fish necropsy, suggest that this data may have been related to erythrocyte fragility (erythrocyte easily broken, damaged, or destroyed) and hemorrhaging exposed to metals [20, 35]. It is possible mechanisms that the decrease in blood parameters may be hemolysis and damage to hematopoietic tissues by lead and its zeolite mixtures.

Further, the decrease of serum enzymatic mechanisms may be indicated liver damage [25, 28 and 36] and may be occurring from Pb and Ze mixtures form.

Author details

Hikmet Y. Çoğun and Mehmet Şahin

*Address all correspondence to: hcogun@kilis.edu.tr

Kilis 7 Aralik University, Faculty of Science and Letters, Department of Biology, Kilis, Turkey

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