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Assessment of lead toxicity in red tilapia *Oreochromis* sp. through hematological parameters

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Abstract

Fish is one of the reliable biomonitoring used to assess the levels of heavy metal pollution in the aquatic environment. Analyzing the biological parameters of fish provides a predictable transformation of the underwater ecosystem and the current state of nursery water. Blood directly or indirectly reacts to changes in the environment, objectively reflects the physiological state and allows predicting the direction of adaptive responses in the body. According to the blood indicators and proper generalization of the blood system, we can estimate the physiological and pathological processes inside the body. This study aimed to estimate the effects of lead on hematological parameters in red tilapia. Fish were investigated for the effects of the lead after one week. Fish blood is taken from the tail vein by suction injection. Hematological parameters were determined by standard methods. The number of WBC is counted indirectly through blood smears stained Giemsa. The result of the study shows when the concentration of lead for infection increase from $0 mgL^{-1}$ (control) to $0.5 mgL^{-1}$, $1.0 mgL^{-1}$ and $1.5 mgL^{-1}$, there is a decrease in the hematocrit, hemoglobin, RBC and increases of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, WBC. RBC and WBC have observed the shape. Blood cells of red tilapia *Oriochromis* sp. living in a lead polluted environment can be affected and result in many different values of hematological parameters. This is an adaptive reaction of the body in response to harmful factors at the cellular level.

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Introduction

Environmental pollution from industrial complexes and livestock waste sources is a serious threat to various aquatic ecosystems. In particular, heavy metal pollution is being concerned by researchers. The studies about the heavy metal pollutant factors have shown that Pb, As, Cu, Pb, Cr, Ni and Hg regularly exceeds the permissible standards in water and soil environments (Hou et al., 2013; Mansour and Sidky, 2002). Lead (Pb) is one of the most highly toxic metals (Mansour and Sidky, 2002). Lead toxicity causes serious damages to fish organs such as the liver, kidney and muscle (Al-Balawi et al., 2013) and

changes hematological parameters (Ergönül et al., 2012; Shah et al., 2020; Tulaby et al., 2020; Verma et al., 2020).

Fishes are considered the most sensitive aquatic animals to toxic ingredients present in water (Hemmadi, 2017). Fish is one of the reliable biomonitoring used to assess the levels of heavy metal pollution in the aquatic environment. Analyzing the biological parameters of fish provides a predictable transformation of the underwater ecosystem and the current state of nursery water (Authman et al., 2015; Lamas et al., 2007; Mohamed and Osman, 2014; Rashed, 2001). Blood directly or indirectly reacts to changes in the environment, objectively reflects the physiological state and allows predicting the direction of adaptive responses in the body (Nussey et al., 1995). Copper, cadmium, and mercury cause red blood cells (RBC) resolution of animals (Kotsanis et al., 2000), as well as impacting the environment that alters the morphology and size of RBC in many studies (Chernyavskikh et al., 2018; Yang and Chen, 2003). According to the blood indicators and proper generalization of the blood system, we can estimate the physiological and pathological processes inside the body (Nussey et al., 1995). Changes in blood physiological parameters (red blood cell count, white blood cell count, hematocrit, hemoglobin) are the most apparent manifestations of physiological reactions of blood with the influence of the environment. They are the helpful diagnosis of the health status of fish (Dethloff et al., 2001). Many studies have shown the effect of heavy metals on the hematological parameters of fish: decreasing in the amount of hematocrit (HCT), hemoglobin (Hb), RBC and increasing in the number of white blood cells (WBC) (Kori-Siakpere and Ubogu, 2008; Nussey et al., 1995; Sharma and Langer, 2014).

Additionally, the study of Alkahem (1994) about the effect of nickel (Ni) on *Oreochromis niloticus* showed the opposite potential: increasing in the value of HCT, Hb, RBC and decreasing in the number of WBC (Alkahem, 1994). Although many studies of the effect of lead on hematological parameters in fish, there are no studies to mention the changes in hematological parameters and blood cell shapes of the red tilapia exposed to lead. Hence, this study aimed to estimate the effects of lead (Pb) on hematological parameters and the change of blood cell shapes in red tilapia (*Oreochromis* sp).

Material and Methods

The experiment was conducted on red tilapia having an average weight of 158.25 ± 9.31 g/fish and an average length of 18.68 ± 2.21 cm. These fish were purchased at the National Center for Aquaculture Breeding Southern Vietnam of the Southern Research Institute for Aquaculture No. 2 in Tien Giang province (Vietnam). Fish were reared in the same conditions for 14 days. During the experiment, fish were fed three times/day, ensured a 36% crude protein, using Tilatech commercial feed of Cargil Vietnam Co., Ltd.

Red tilapia were reared in tanks with a size of 90cm × 70cm × 60cm. The fish were randomly divided into four groups with 15 individuals/group: one control and three experimental. Experimental groups infected with lead at concentrations of 0.5 mgL^{-1}, 1.0 mgL^{-1}, 1.5 mgL^{-1}, control group was not lead infected (0 mgL^{-1}). Fish were investigated for the effects of the lead after one week (seven days). Fish blood is taken from the tail vein by suction injection. The needles were impregnated with heparin at a concentration of 10IU/mL to prevent blood clotting. Blood was contained in heparin-impregnated specialized blood storage tubes. Blood smears were prepared according to Hrubec et al. (Hrubec et al., 2000) and Giemsa stained.

HCT (%) was determined by the centrifugation method. Hb (g%) was estimated by using Sahli's Hbometer (Dethloff et al., 2001). The number of RBC ($\times 10^6$/mm^3) was counted by the Neubauer chamber. Because RBC of fish have a nucleus preventing the count of WBC directly by the Neubauer chamber, so the number of WBC ($\times 10^3$/mm^3) was counted indirectly through blood smears stained Giemsa. Calculation of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were carried out by using standard formulae:

MCV (μm^3) = HCT (%)/RBC ($\times 10^6$/mm^3) $\times$ 10;
MCH (pg) = Hb (g%) / RBC ($\times 10^6$/mm^3) $\times$ 10;
MCHC (g/dL) = Hb (g%) / HCT (%) $\times$ 100.

Statistical analysis

Statistical analyses were performed using Minitab 18 software and the data were presented as Mean $\pm$ SD. The difference was considered to be statistically significant with $p \leq 0.05$.

Results and Discussion

The following table presents the result of experiments about the changes in hematological parameters of red tilapia (HCT, Hb, RBC, WBC, MCV, MCH, MCHC) undergone the infection of lead with the concentrations: $0.5 mgL^{-1}$, $1.0 mgL^{-1}$ and $1.5 mgL^{-1}$ after seven days of infection.

Comparative analysis of the control group's data with that of the experimental groups clearly showed a marked decline in RBC, Hb, HCT and increasing WBC at the end of the experimental period in lead-infected fish, that is shown in table 1.

Table-1: Hematological parameters of *Oreochromis* sp. exposed to lead

Hematological parameters	Control	Concentration of Lead (mgL^{-1})		
		0.5	1.0	1.5
HCT (%)	40.76±3.21[a]	25.30±5.72[b]	23.97±5.21[bc]	21.80±3.18[c]
Hb (g%)	7.96±0.69[a]	5.92±0.47[b]	5.57±0.58[b]	5.04±0.52[c]
RBC (×10^6/mm³)	2.52±0.22[a]	1.50±0.32[b]	1.43±0.35[b]	1.22±0.19[c]
MCV (μm³)	161.81±1.63[c]	168.67±2.75[b]	167.15±3.52[b]	178.98±2.18[a]
MCH (pg)	31.60±0.27[d]	39.47±0.48[b]	38.86±0.75[c]	41.38±0.93[a]
MCHC (g/dl)	19.53±0.18[c]	23.40±0.24[a]	23.25±0.34[b]	23.12±0.29[b]
WBC (×10³/mm³)	60.95±2.57[d]	70.23±2.28[c]	80.03±2.28[b]	86.05±2.22[a]

Notes: a, b, c, d – The difference is statistically significant ($p<0.05$).

HCT is decreased from 40.76±3.21(%) to 21.80±3.18(%), statistically significant with $p<0.001$. Specifically: at the concentration of $0.5 mgL^{-1}$, it is lower 15.46% ($p<0.001$) than the control; at the concentration of $1.0 mgL^{-1}$, it decreased 1.33% ($p>0.05$) compared with group having the concentration at $0.5 mgL^{-1}$; at $1.5 mgL^{-1}$ concentration, HCT decreased 2.17% ($p>0.05$) compared with group of $1.0 mgL^{-1}$ concentration; HCT at $1.5 mgL^{-1}$ concentration decreased 3.5% ($p<0.001$) compared with the group of $0.5 mgL^{-1}$ concentration.

The Hb concentration collected after seven days in the blood of red tilapia decreased from 7.96±0.69g% in control to 5.04±0.52g% at a concentration of $1.5 mgL^{-1}$. The Hb concentration of red tilapia at $0.5 mgL^{-1}$ concentration decreased 2.04g% ($p<0.001$) compared with the control; at concentration of $1.0 mgL^{-1}$, it is decreased 0.35g% ($p>0.05$) compared with the concentration of $0.5 mgL^{-1}$, at concentration of $1.5 mgL^{-1}$, Hb decreased 0.53g% ($p<0.05$) compared with $1.0 mgL^{-1}$ concentration. Red tilapia lives in a

higher concentration of lead, Hb is more affected, the difference was statistically significant with $p<0.001$.

The number of RBC decreases from $2.52\pm0.22\times10^6/mm^3$ to $1.22\pm0.19\times10^6/mm^3$ according to the concentrations of the infected lead after seven days. Specifically, the number of RBC of red tilapia in the $0.5 mgL^{-1}$ concentration of lead decreased by 40.5%; it is the concentration of $1.0 mgL^{-1}$ decreased by 43.1%; in the concentration of $1.5 mgL^{-1}$ decreased by 51.6% compared with the control, all these differences were statistically significant with $p<0.001$. The number of RBC at the $0.5 mgL^{-1}$ concentration of lead and the $1.0 mgL^{-1}$ concentration was not significantly different with $p>0.05$.

MCV, MCH, MCHC depend on the change of HCT, Hb and RBC. Overall, MCV increased from $161.81\pm1.63\mu m^3$ to $178.98\pm2.18\mu m^3$ with $p<0.01$. The decreasing numbers of RBC confirmed MCV increasing because of the inversion of MCV and RBC. Increasing MCV is obviously due to enlarged RBC, possibly swelling of RBC (Sharma and Langer, 2014). Such change in MCH and MCHC may be due to increased lysis of RBC and reduction in cellular blood iron, which simply reflects a decline in Hb concentration due to metal toxicity (Sharma and Langer, 2014). That is the lack of peripheral blood in the body of poisoned fish.

Through the lead concentrations analyzed after seven days, the results indicate the decline of HCT, Hb and RBC compared to the control, consistent with similar trends reported about heavy metal effects with hematological parameters such as: copper (Cu) and hematological consistent in *Channa punctatus* (Singh et al., 2008); hematological parameters changes of *Oreochromis niloticus* exposed to cadmium (Cd) (Al-Attar, 2005) and continued with mercury (Hg) (Ishikawa et al., 2007). HCT is calculated as the ratio of the volume of RBC in a unit of blood. HCT, through decreased lead levels, shows the decreasing of the volume of RBC, which may be the reduction of the number of RBC and possibly the action of the circulatory system decreases when affected by lead (Kaya et al., 2013), by the reduction of Hb and RBC is anemia, not enough to response the amount of mature RBC in the peripheral blood system. Lead may have affected the blood transporters, dissolved blood, lysis pseudo-RBC, and released normal RBC in the blood circulation (Sharma and Langer, 2014). Blood cell-producing organs may have been affected by lead that reduces RBC production, leading to a sharp decline of RBC in the blood circulation when the fish live in an

environment contaminated with lead. In 1985, Fänge & Nilsson suggested that the spleen is an organ that stores blood, creates blood and destroys old blood cells and produces other mature cells (Fänge and Nilsson, 1985). The spleen is one of the organs sensitive to infectious agents from the surrounding environment. The lead acts on and disrupts the spleen's function, reducing the number of RBC circulating in the blood of red tilapia. This result is consistent with Zapata (1980), it was suggested that environmental impact conditions on the spleen caused microscopic blood loss and resulted in hemolysis (Zapata, 1980). Besides, lead causes anomalies in the shape of RBC, there are many irregularly shaped RBC (Fig. 1).
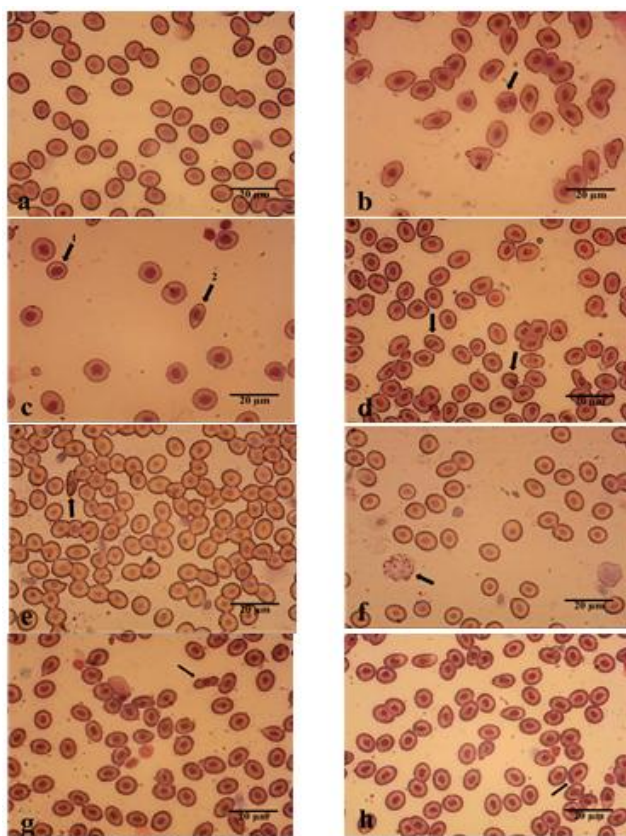

Figure-1: Red blood cells of red tilapia *Oreochromis* sp.: a – normal RBCs; b – polynuclear RBC; c: c1– premature RBC, c2 –water drop RBC; d – skewed RBC; e –crescent shaped RBC; f – reticulocytes; g –dumbbell RBC; h – micronuclei

According to Clauss et al. (2008), abnormal hematological morphology of fish related to RBC include polynuclear erythrocytes, deformed erythrocyte morphology, erythrocyte without nucleus, or RBC that remain alone nucleus without cytoplasm (Clauss et al., 2008). Reticulocytes appear, unknown causes, have not been mentioned, many studies have observed reticulocytes in their experiments (Hrubec et al., 2000). Brenden and Huizinga (1986) suggest that the presence of premature erythrocytes is often a symptom of anemia, the number of premature erythrocytes increases when the fishes are infected (Brenden and Huizinga, 1986). However, according to Clauss et al., the presence of premature erythrocytes in the blood circulation does not always indicate anemia but the presence of premature erythrocytes responses to the application of toxicants from the environment (Clauss et al., 2008).

Premature erythrocytes often have a more rounded shape and more swelling, which may cause the increase of MCV. Fish being infected by heavy metal for a long time can increase the occurrence of premature erythrocytes and malformed erythrocytes in the peripheral blood, which is a manifestation of the blood disease of fish (Vosylienė, 1999). The shape of the red cells is elliptical, the cell surface is convex. The shape and size of erythrocytes are determined by the cell membrane. Depending on the types of effects that lead to the changes in shape, which are suitable for environmental conditions, the impact at different environmental conditions changes the convex surface of RBC (Chernyavskikh et al., 2016). Ribarov and Benov (1981) suggest that peroxidation of membrane lipids is also a mechanism that can alter erythrocyte shape (Ribarov and Benov, 1981).

The study of Sharma and Langer (2014) also points out the effects of heavy metals that affect and alter the erythrocyte shape of fish (Sharma and Langer, 2014). The deformed erythrocyte morphology can lead to the loss of the primary function of RBC, such as oxygen transport to tissues, as well as deformed erythrocytes that clog blood vessels (Sharma and Langer, 2014; Yang and Chen, 2003). Therefore, premature erythrocytes and malformed erythrocytes do not guarantee the function of RBC in the blood system. In this study, the change in the shape and size of erythrocytes may be due to the destruction of mature erythrocytes and the appearance of more malformed and premature erythrocytes in peripheral blood. The red blood cell shape of red tilapia living in lead polluted environment becomes more rounded and bulging. Stress in the organism is stimulated by the effects of toxicants that have not compensated for sufficiently mature RBC, this lead to many different types of premature RBC in peripheral blood (Tomova et al., 2008). This is also the evidence of a shortened

cycle of erythrocytes as well as detection of errors in hematopoiesis (Witeska and Kościuk, 2003).
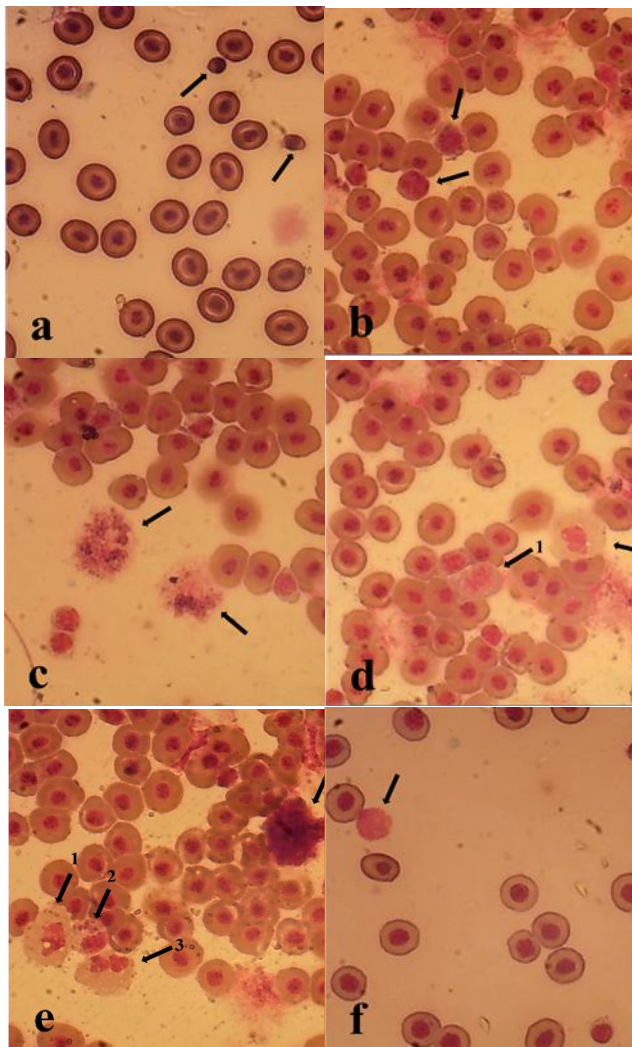

Figure 2. White blood cells of red tilapia (*Oreochromis* sp.): a – small lymphocyte; b, d-1 – large lymphocyte; c – Eosinophilic leukocyte; f – mono leukocyte; d-2, d-3, d-4, d-5, e-1, e-2, e-3 – neutrophil; e-4 – basophilic leukocyte

The blood cells and Hb suffer from damages and dysfunction due to stress anemia caused by heavy metals (McKim et al., 1970). The decrease in Hb concentration is due to the heavy metal impacting directly on the enzyme system, which have functions to synthesize and regulate Hb concentration (Pamila et al., 1991). In addition, heavy metal effects reduce iron absorption leading to heme nucleus not forming and reducing Hb concentration (Joshi et al., 2002). This anemia is thought to occur because of δ-amino levulinic acid dehydratase (ALA-D) inhibition by lead and thus causing severe damage in Hb synthesis. In heavy metals such as Pb, Zn, Cd, Hg, Cu, only lead (Pb) causes inhibition of ALA-D activity (Hodson et al., 1977).

After seven days, red tilapia lived in a lead polluted environment at concentrations of $0.5 mgL^{-1}$, $1.0 mgL^{-1}$, $1.5 mgL^{-1}$, the number of WBC increases from $60.95 \pm 2.57 \times 10^3/mm^3$ (the control) to $86.05 \pm 2.22 \times 10^3/mm^3$ (at $1.5 mgL^{-1}$ concentration). The number of WBC of red tilapia in the concentration of $0.5 mgL^{-1}$ increased by $9.28 \times 10^3/mm^3$ ($p<0.001$) compared to the control; those of $1.0 mgL^{-1}$ concentration increased by $9.8 \times 10^3/mm^3$ ($p<0.001$) compared with those of the $0.5 mgL^{-1}$ concentration; Those of the concentration of $1.5 mgL^{-1}$ increased by $6.02 \times 10^3/mm^3$ ($p<0.001$) compared to those of the concentration of $1.0 mgL^{-1}$. The tendency to change the number of WBC is contrary to the changing trend of HCT, Hb, and the number of RBC. Changes in WBC can be considered a measurement tool as well as an early warning signal of disorders in immunity and fish balance (Brucka-Jastrzębska and Protasowicki, 2005). In this study, five types of WBC are detected: monocyte, lymphocyte, neutrophil, eosinophil, basophil (Fig. 2). Eosinophilic and basophilic leukocytes are two types of leukocytes rarely seen in previous studies. Most of the previous studies mainly found and observed three types: monocyte, lymphocyte, neutrophil (Bittencourt et al., 2003; Silveira-Coffigny et al., 2004). Several studies observed acidophil (Hrubec et al., 2000; Martins et al., 2008) but did not observe basophilic leukocytes. When the fish body is affected by harmful agents, the number of WBC increases significantly to counteract, the main function of WBC is generally destroying harmful agents invading the organism, creating immunity against pathogens (Martins et al., 2008; Sayed and Moneeb, 2015). The increase in the number of WBC undergone the effects of lead toxicity has shown an immune response in red tilapia. At lead concentrations of different infectious periods, the number of WBC increased in the reference range ($18.75-154.69 \times 10^3/mm^3$) (Hrubec et al., 2000; Mauel et al., 2007). This means the concentration of lead defined within the scope of the subject is still within the range of fish that can react to counteract toxicity.

Conclusion

In this study, blood cells of red tilapia *Oriochromis* sp. living in a lead polluted environment can be affected

and result in many different values of hematological parameters. The changes in HCT, Hb, RBC and the increasing potential of WBC and increased MCV are obviously observed. When the concentration of lead for infection increase from $0 mgL^{-1}$ to $0.5 mgL^{-1}$, $1.0 mgL^{-1}$ and $1.5 mgL^{-1}$after one week (7 days) of infection, there is mitigation of the number of red blood cells leading to a decrease in the HCT and hemoglobin level. The main reason for this phenomenon is lead toxicity directly causes damage to hematopoietic organs, reduces their function. The increase of the white blood cells is explained by the activation of the immune system under the effect of lead. This is an adaptive reaction of the body in response to harmful factors at the cellular level.

Acknowledgement

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Contribution of Authors

Vo VT: Designed experiment, helped in data analysis, write up and editing of the manuscript.
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Mai NAT: Performed experiments.
Huyen Nguyen Thi Thuong: Helped in data analysis, write up and editing of the manuscript.