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Effect of lead on the morphology of erythrocytes in red tilapia (*Oreochromis* sp.)

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Abstract

The study investigated the effect of lead at concentrations of 0.5 mgL^{-1}, 1.0 mgL^{-1}, 1.5 mgL^{-1} on the morphology of red blood cells in *Oreochromis* sp. Experimental results showed that at lead concentrations of 0.5 mgL^{-1}, 1.0 mgL^{-1}, 1.5 mgL^{-1}, the size of the major axis of erythrocytes decreases gradually compared to the control, decreased from 10.177±0.782 µm to 7.989±0.862 µm. The size of minor axis of erythrocytes gradually increases from 6.052±0.787 µm (control) to 7.458±0.801 µm (concentration 1.0mgL^{-1}), but the size of minor axis decreases from 7.458±0.801 µm (concentration 1.0mgL^{-1}) to 7.312±0.885 µm (concentration 1.5mgL^{-1}). Perimeter and area of erythrocytes increase due to the emergence of more premature red blood cells.

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Introduction

Hematological parameters are important for the diagnosis of the structural and functional state of fish exposed to toxicants. Blood directly or indirectly reacts to changes in the environment, objectively reflects the physiological state and may forecast the direction of the body's adaptive response (Nussey *et al.*, 1995b, 1995a). The release of heavy metals into the aquatic environment causes many deviations in the physiology of fish and serious impacts on hematological parameters (Tomova *et al.*, 2008). Red blood cells are the most abundant cells in peripheral blood and perform many functions in the whole blood system. Many studies show that heavy metal ions such as copper, cadmium and mercury ions cause red blood cells to destruction in animals (Adams *et al.*, 1979; Ichikawa *et al.*, 1987; Kori-Siakpere and Ubogu, 2008; Ribarov and Benov, 1981). Studies of the shape and size of blood cells were mentioned, for example, the study of Panigrahi and Misra about the effect of mercury on the shape of red blood cells in *Anabas scandens* (Panigrahi and Misra, 1979); study of Yang and Chen (2003) about the effect of gallium on the changes in carp *Cyprinus carpio* erythrocyte morphology (Yang and Chen, 2003); study about the effect of temperature on red blood cell shape of *Hypophthalmichthys molitrix* of Frolova *et al.* (2017) (Frolova *et al.*, 2017) and study about impacts on *Carassius gibelio* (Bloch) of Chernyavskikh *et al.* (2018) (Chernyavskikh *et al.*, 2018). All the above studies give the result that the shape of erythrocytes is deformed upon impact. However, studies on the effect of lead at various concentrations on the morphology and size of red blood cells are still limited.

This study aimed to investigate the effect of lead (Pb) on the morphology of red blood cells in red tilapia *Oreochromis* sp.

Materials and methods

The experiment is conducted on red tilapia with an average weight of 158.25±9.31g/fish and an average length of 20.68±2.21 cm purchased at the National Center for Southern Freshwater Aquaculture under the Research Institute of Aquaculture No.2, Vietnam. During the experiment, the fishes were fed three times a day. We used Tilatech commercial feeds of Cargil Vietnam Co., Ltd.

Red tilapia was raised in the tanks, with the size of each tank is 90 cm × 80 cm × 60 cm. The fish were randomly divided into four treatments of 15 individuals per treatment, infected with lead, in turn, with concentrations 0.5 mgL^{-1}, 1.0 mgL^{-1}, 1.5 mgL^{-1}, control treatment with non-infected lead. Before infection, the fish are raised under the same conditions for 14 days.

Blood samples were collected ten days after infection, taken with a 3 mL syringe from the tail vein, and pumped into a unique blood tube coated with the anticoagulant heparin. Blood smears were prepared and stained according to Hrubec *et al.* (2000) (Hrubec *et al.*, 2000). Each fish made five blood smears. The smears were subsequently examined and photographed under a microscope Olympus, Japan. The samples were observed and the size of 150 erythrocytes per smear was randomly measured. The size of the major (a) and minor (b) axes was measured for each erythrocyte. Perimeter (P) and area (S) are calculated from the sizes of the major and minor axes using the following formula (Chernyavskikh *et al.*, 2018; Dethloff *et al.*, 2001):

$P = 2\pi\sqrt{\frac{\left(\frac{a}{2}\right)^2+\left(\frac{b}{2}\right)^2}{2}}$ and $S = \pi ab$, where P – the perimeter of erythrocyte, S – the area of erythrocyte, a – the size of the major axis of erythrocyte, b – the size of the minor axis of the erythrocyte.

The obtained data were statistically processed using computer software Minitab 18, and the difference was statistically significant with $p \leq 0.05$.

Results and discussion

The experiment studied the change in the size of red blood cells in red tilapia *Oreochromis* sp. under the influence of lead of various infected concentrations: 0

mgL^{-1}, 0.5 mgL^{-1}, 1.0 mgL^{-1} and 1.5 mgL^{-1} after 10 days of infection. The results are shown in the following Table 1.

The results of the current study show that the erythrocyte size of red tilapia raised in non-infected with lead condition (10.177×6.052 µm) is lower than erythrocyte size of red tilapia obtained by Hrubec *et al.* (2000) (12.9×7 µm) (Hrubec *et al.*, 2000) and of *Oreochromis aureus* obtained Silveira-Coffigny *et al.* (13.05x7.25 µm) (Silveira-Coffigny *et al.*, 2004). Differences in erythrocyte size may be influenced by growing conditions, food source and fish seed source (Affonso *et al.*, 2002; Benfey and Biron, 2000). In addition, the swimming speed, activity, shape and age of fish can affect the size of red blood cells in the peripheral blood of fish (Satheeshkumar *et al.*, 2011, 2012).

Table 1. Erythrocyte red tilapia sizes at various concentration of lead.

	Concentration of lead (mgL^{-1})			
	Control (0)	0.5	1.0	1.5
Major axis size (µm)	10.177 ± 0.782^{a}	9.662 ± 0.687^{b}	8.676 ± 0.507^{c}	7.989 ± 0.862^{d}
Minor axis size (µm)	6.052 ± 0.787^{a}	6.884 ± 0.877^{b}	7.458 ± 0.801^{c}	7.312 ± 0.885^{c}
Perimeter (µm)	26.34 ± 1.80^{a}	26.40 ± 1.67^{a}	25.45 ± 1.45^{b}	24.13 ± 1.91^{c}
Area (μm^2)	48.39 ± 7.64^{a}	52.20 ± 7.61^{b}	50.80 ± 6.27^{b}	45.84 ± 7.40^{c}

Note: a, b, c, d- the difference is statically significant with $p<0.05$.

Results from table show that the size of major axis of erythrocytes gradually decreases at various lead concentrations of 0.5 mgL^{-1}, 1.0 mgL^{-1}, 1.5 mgL^{-1}: from 10.177±0.782 µm to 7.989±0.862 µm. The size of major axis at 0.5 mgL^{-1} concentration decreases 5.06% compared to the control; at 1.0 mgL^{-1} concentration decreases 10.2% compared to 0.5 mgL^{-1} concentration; at 1.5 mgL^{-1} concentration decreases 7.92% compared to 1.0 mgL^{-1} concentration. All those differences are statistically significant with $p<0.001$.

The tendency to change the size of the minor axis of erythrocytes is the opposite of the tendency to change the size of the major axis. At lead concentrations of 0.5 mgL^{-1} and 1.0 mgL^{-1}, the size of minor axis increases from 6.052±0.787 µm to 7.458±0.801 µm, specially: at a lead concentration of 0.5 mgL^{-1}, it increases by 13.75% compared to the control; at a lead concentration of 1.0 mgL^{-1}, it increases by 8.34% compared to a concentration of 0.5 mgL^{-1}. All those differences are statistically significant, with $p<0.001$. The size of the minor axis of erythrocytes tends to decrease with increasing lead concentration from 1.0 mgL^{-1} to 1.5 mgL^{-1}, and the difference is not statistically significant with $p>0.05$. The tendency to change the perimeter and area of erythrocytes is relatively similar. Details of the change are as follows: increasing at a lead concentration of 0.5 mgL^{-1} compared to the control and decreasing gradually at lead concentrations of 1.0 mgL^{-1} and 1.5 mgL^{-1} compared to a lead concentration of 0.5 mgL^{-1}. Perimeter of erythrocytes at a lead concentration of 0.5 mgL^{-1} increases by 0.23% ($p>0.05$) compared to the control; at a lead concentration of 1.0 mgL^{-1}, it decreases by 3.6% compared to a lead concentration of 0.5 mgL^{-1} and at a lead concentration of 1.5 mgL^{-1} – decreases by 5.19% compared to a concentration of 1.0 mgL^{-1}. Both differences are highly statistically significant with $p<0.001$. The area of erythrocytes increases at a lead concentration of 0.5mgL-1 by 7.87% ($p<0.01$) compared to the control and decreases at a lead concentration of 1.0 mgL^{-1} by 2.68% ($p>0.05$) compared to the lead concentration of 0.5 mgL^{-1}, at a lead concentration of 1.5 mgL^{-1} by 9.76% ($p<0.001$) compared to a lead concentration of 1.0 mgL^{-1}.

Almost all fish have nuclear erythrocytes. Mature erythrocytes are oval or elliptical with a central oval or round nucleus, dark in color with Giemsa dye containing a dense chromosphere, and a lighter erythrocyte cytoplasm surrounding the nucleus.

Mature erythrocytes have major and minor axes and their nuclei occupy a small area on the cytoplasm. Premature erythrocytes are malformed erythrocytes that usually occur when the body is anemic (Barham *et al.*, 1980). Premature erythrocytes are produced to replace mature and old decomposed erythrocytes. Premature erythrocytes have a more rounded shape; their sizes of the major axis and minor axis are almost equal. The nuclei of premature erythrocytes are bigger and occupy more area than nuclei of mature erythrocytes. Premature erythrocytes usually appear when the fish's environmental conditions change unfavorably (Barham *et al.*, 1980; Brenden and Huizinga, 1986; Vosylienė, 1999). The results of the current study show a change in the morphology of the red tilapia erythrocyte. The measuring the size of erythrocytes showed a noticeable change in the shape of the cytoplasm of erythrocytes and a change in the size of nuclei compared to normal erythrocytes. That is why erythrocyte size measurements tend to be shorter on the major axis and longer on the minor axis. The largest perimeter and area at a lead concentration of 0.5 mgL^{-1} indicates that the highest occurrence of premature erythrocytes is observed at this concentration of lead.

The presence of premature erythrocytes is usually anemia, and the number of premature erythrocytes increases with infection (Barham *et al.*, 1980; Brenden and Huizinga, 1986). The production of premature erythrocytes may increase in fish that have lived for a long time in water bodies contaminated with high metal content. And an increase in these cells in the peripheral blood is a pathological manifestation of fish blood (Vosylienė, 1999). The cell membrane determines the shape and size of erythrocytes (Chernyavskikh *et al.*, 2016). Depending on the type of impact that leads to a change in shape following environmental conditions, a change in the shape of cells occurs by a change in the protrusions on the surface of erythrocytes (Frolova *et al.*, 2017; Chernyavskikh *et al.*, 2016, 2018). Heavy metals such as lead can penetrate the cell membrane, oxidize and denature hemoglobin by inhibiting energy metabolism (glycolysis), slow down the metabolism of substances in red blood cells. The long-term toxicity of heavy metals can lead to a change in the morphology of normal red blood cells or their easy degradation in the peripheral blood (Mehjbeen and Nazura, 2014). The study of Sharma and Langer (2014) also mentioned the effects of heavy metal that influence and change the shape of erythrocytes (Sharma and Langer, 2014).

The deformed morphology of erythrocytes leads to the loss of basic functions such as transporting oxygen to tissues and blockage of blood vessels (Gill and Pant, 1985; Adeyemo, 2007). Therefore, premature and malformed erythrocytes cannot guarantee the function of erythrocytes in blood. In this study, the change in the shape and size may be due to the destruction of normal erythrocytes and more malformed and premature red blood cells.

Nilsson and Holmgren (1976) demonstrated that erythrocytes enter the circulatory system from the spleen (Nilsson and Holmgren, 1976). Lead acts on the spleen and loses its function, causing large numbers of abnormal red blood cells to circulate in the blood (Zapata, 1980). Lead affects the blood storage function of the spleen and destroys mature red blood cells and reduces their number in the blood system, causing the development of premature red blood cells (Tomova *et al.*, 2008).

There is also evidence of a shortening of the red blood cell cycle and errors in the hematopoietic process (Witeska and Kościuk, 2003). However, Clauss *et al.* (2008) suggested that premature red blood cells in the circulation do not always indicate anemia, but sometimes the presence of premature red blood cells in response to environmental toxicants (Clauss *et al.*, 2008).

Conclusion

In this research, red blood cells of red tilapia *Oriochromis* sp. living in a lead-contaminated environment can be affected, resulting in changes in morphological parameters. After ten days of lead infection, at lead concentrations of 0.5 mgL^{-1}, 1.0

mgL^{-1} and 1.5 mgL^{-1}, erythrocyte size measurements tend to be shorter on the major axis and longer on the minor axis. This is because premature erythrocytes are produced to replace malformed and old decomposed erythrocytes. The increase in perimeter and area is due to more premature red blood cells in the peripheral blood. The largest perimeter and area at a lead concentration of 0.5 mgL^{-1} indicates that the highest occurrence of premature erythrocytes is observed at this concentration of lead.

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