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Lethal and sub-lethal impacts of lead on some hematological, biochemical and immunological indices in Caspian roach (*Rutilus rutilus*)

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ABSTRACT: The present study has been conducted on Caspian Roach (*Rutilus rutilus*) so that the impact of different concentrations lead on blood hematological, biochemical, and immunological indicators could be investigated. The roach (*Rutilus rutilus*), used in this study, was 3.3±0.3 g heavy and 4±0.80 cm long in average. The fish were exposed to a low concentration of 10% LC50 and high concentration of 50% LC50 lead for a period of 0, 24, 48, and 96 h. The experiment was done in a static toxicity condition, within tanks of 400L, each including 14 fish. In the 96-hour period (10% LC50 Lead), the parameters of WBC and RBC dropped significantly in comparison to the control group (0 h) ($P<0.05$). MCV and MCH elevated as the exposure time ascended up to 96 h ($P<0.05$), but the cortisol decreased in the meantime. In the 96-hour period (50% LC50 Lead) RBC, Hb and Hct parameters plummeted towards the control group ($P<0.05$). Heavy metals can change physiological and biochemical parameters in fish blood. The results show that lead (10% LC50 and 50% LC50 concentration Lead) may poison the fish, causing their death. The major toxicity belongs to the 50% LC50 concentration Lead.

Keywords: blood parameters, Caspian Roach, lead, physiological, toxicity.

INTRODUCTION

Water sources (groundwater, lakes, streams, and rivers) can be polluted with heavy metals that leach from wastewaters; and acid rain can exacerbate this process by releasing the heavy metals, trapped in soils. Plants are exposed to heavy metals through their water uptake and animals through their consumption of such plants. Consequently, ingestion of plant and animal-based foods is the largest source of heavy metal intake in humans. Another potential source of heavy metal contamination is absorption through skin contact, e.g. from contact with soil (Qu et al., 2014). Blood factors are beneficial criteria to show physiological perturbation in anxiously-farmed fish and can provide important information to forecast and assess diseases.

Hematological indicators have also been used to indicate physiological changes in fish (Satheeshkumar et al., 2011). A common source of confusion in fish cell nomenclature is in the varied names given to the granulocytes, varied from species to species without any clear mammalian or avian/reptilian counterpart. B and T lymphocytes bearing immunoglobulins and T cell receptors, respectively, are found in all jawed fishes. Indeed, the adaptive immune system as a whole evolved in an

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ancestor of all jawed vertebrate (Flajnik and Kasahara, 2009). Lead is a chemical element in the carbon group, represented with the symbol Pb (from Latin: plumbum), having an atomic number of 82. A soft, malleable, and heavy post-transition metal, it is used in building construction, lead-acid batteries, bullets and shot, weights, as part of solders, pewters, fusible alloys, and as a radiation shield.

This oxidation state is not specifically stable, as the ion of lead (III) (also along with larger complexes containing it) is a radical substance. The same goes for lead (I), which can also be found in such species (Chia et al., 2013). Long-term exposure can result in decreased performance in some tests that measure functions of the nervous system. The roach (*Rutilus rutilus*), also known as the common roach, is a fresh and brackish water fish of the *Cyprinidae* family, native to most regions of Europe and western Asia. The name "roach" is not unique, though; as any type of fish, called roach, may fall under any species of the genera Rutilus and Hesperoleucus, depending on the locality. The plural form of the term is also roach (Kottelat and Freyhof, 2007).

Toxicology is a branch of biology, chemistry, and medicine (more specifically pharmacology), concerned with the study of the adverse effects of chemicals on living organisms (Unterthiner et al., 2015).

Heavy metals are a group of main pollutants in the aquatic environment, entering it through natural processes, not to mention human activities. (Humtsoe et al., 2007). In addition to haematological and biochemical factors, already used to prepare data about the health situation of fish and quality of the water in which they live (Fazio et al., 2012), alterations in the levels of stress oxidative factors have been proposed as biomarkers in this fish (Ben Ameur et al., 2012; Yonar et al., 2012). Biochemical factors used in the fish and other aquatic organisms when facing heavy metal stress as the important bio-indicators for monitoring aquatic environment (Shalaby et al., 2005). Oxidative stress might happen when the antioxidant advocacy system is overwhelmed with either an increased oxidant burden or a decrease antioxidant reserve (Kirschvink et al., 2008). This study aims to investigate the impact of different lead concentrations on blood hematological, biochemical and immunological indicators in caspian roach (*Rutilus rutilus*).

MATERIALS AND METHODS

The present study used roach (*Rutilus rutilus*) with an average weight of 3.3±0.3 g and length of 4±0.80 cm. Acclimatized fish were fed daily with a formulated feed. Dead roach were immediately removed with special plastic forceps to avoid possible deterioration of the water quality (Gooley et al., 2000). Roach Caspian fish were exposed to a low concentration of 10% LC50 and high concentration of 50% LC50 lead for the control period as well as 24, 48, and 96 h the static toxicity examination, presented in tank of 400L, each including 14 fish. The group control was conserved in a fiberglass tank with no access to any toxicant. Each treatment was repeated three times. pH, dissolved oxygen, temperature, and conductivity were monitored during the experiment (Hedayati et al., 2010).

Fish were immediately anesthetized with 200 ppm clove powder. The required blood samples were rapidly taken from tail blood vessels by heparinized syringes and varied parameters of such fresh blood went under study, wherein differing amounts of blood leukocytes and erythrocytes were analyzed at a dilution of 1:30 by attenuate heparinized blood with Giemsa stain. Cells were enumerated using a hemocytometer Neubauer, below the light microscope (Stevens, 1997). Measuring the formation of cyanmethemoglobin, led to hemoglobin levels (Hb mg/l). Erythrocytes Indices, namely Mean Corpuscular Hemoglobin (MCH), Mean Cell Hemoglobin

Concentration (MCHC), and Mean Corpuscular Volume (MCV), were computed from RBC, Ht, and Hb (Lee et al., 1998).

RESULTS AND DISCUSSION

In this study, we investigated effects of 50% LC50 and 10% LC50 lead on a series of Immunological, biochemical, and hematological parameters of roach for 0, 24, 48, and 96 h. There was no mortality in the control group (0h). Yet the fish, exposure to lead for 96h, showed significant change in hematological (Hb, Ht, MCH, MCHC, MCV, and RBC), biochemical (glucose and cortisol), and immunological (neutrophil, lymphocyte, and eosinophil) parameters, compared to the control groups ($P<0.05$). After the exposure, MCV, RBC, Ht, Hb, and Lymphocyte were significantly lower ($P< 0.05$), whereas WBC, MCH, MCHC, glucose, cortisol, neutrophil, and eosinophil stood considerably higher than the control group ($P< 0.05$).

During the 96 h period (10% LC50 Lead), WBC and RBC parameters descended significantly ($P<0.05$). On the other hand, Hb and Hct did not show any significant correlation with lead exposure ($P> 0.05$). The level of MCV and MCH increased at 96 h ($P< 0.05$), while cortisol decreased, in comparison to the control group (0h). During the 96 h exposure (50% LC50 Lead), RBC, Hb, and Hct plummeted greatly, compared to the control group ($P <0.05$); however, MCV, MCH, and MCHC showed no significant reduction whatsoever (Table 1).

At 10% LC50 of lead concentration, Neu and Mono levels significantly increased, compared to the control group. Our findings showed that Lym level decreased in the fish, exposed to 10% of LC50 lead, being exposed for 96 h. Similarly, in 50% LC50 concentration of lead, the Lym factor decreased and Neu factor increased significantly, compared to the control group ($P <0.05$) (Table 2).

Blood cortisol is an important corticosteroid hormone in fish. In the present study, cortisol level in concentration of 10% LC50 significantly increased in the 96 h group, compared to the control one ($P <0.05$), though it did not show any outstanding correlation with lead exposure ($P > 0.05$). Glucose and cortisol ascended considerably, compared to the control group ($P <0.05$; Fig .1).

Table 1. The hematological parameters of *Rutilus rutilus* in the face to 10% LC50 and 50% LC50 of lead concentration in different times

Blood parameter	Time 0 (Control)	Time 24	Time 48	Time 96
RBC (10^3 mm^3) (10% LC50)	2.43±0.7	2.26±0.8	1.57±0.4	1.15±0.1
Hb (g/dl) (10% LC50)	7.30±0.8	6.51±0.3	6.27±0.2	6.81±0.5
Hct (%) (10% LC50)	23.49±7.6	20.35±1.9	13.38±1.8	18.43±2.2
MCV (fl) (10% LC50)	101.2±44	97.69±33	87.51±15.2	160.1±3
MCH (pg) (10% LC50)	32.39±11	31.49±12	42.37±14	59.46±5
MCHC (g/dl) (10% LC50)	3.43±1.5	3.22±0.4	4.76±0.8	3.71±0.3
RBC (10^3 mm^3) (50% LC50)	2.43±0.7	2.10±0.2	1.27±0.1	0.95±0.1
Hb (g/dl) (50% LC50)	7.30±0.8	6.71±1.6	5.17±0.7	4.39±1.2
Hct (%) (50% LC50)	23.4±7	20.9±4	14.9±3.8	11.8±3.5
MCV (fl) (50% LC50)	101.2±44	100.2±23	116.4±18	122.8±21
MCH (pg) (50% LC50)	32.3±11	31.8±7.4	40.6±0.6	45.7±9
MCHC (g/dl) (50% LC50)	3.43±1.5	3.39±1.5	3.54±0.5	3.7±0.3

Table 2. The blood immunological parameters of *Rutilus rutilus* in the face to 10% LC50 and 50% LC50 of lead concentration in different times

Blood parameter	Time 0(Control)	Time 24	Time 48	Time 96
Neu (%) (10% LC50)	4.66±2.5	8.00±1	7.66±1.1	9.66±1.5
Lym (%) (10% LC50)	95.3±2.5	91.3±1.1	91.66±1.5	88.00±1.7
Eosino (%) (10% LC50)	0.00±0.0	0.00±0.0	0.33±0.5	1.00±1.0
Mono (%) (10% LC50)	0.00±0.0	0.6±0.5	0.66±0.5	1.33±0.5
WBC (10^6. µl) (10% LC50)	8200.0±1578	9133.3±1582	14761.6±2441	14578.3±2499
WBC (10^6. µl) (50% LC50)	8200.0±1578	44564.0±59870	76383.3±103418	23695.6±4744
Neu (%) (50% LC50)	5.0±2.6	9.3±1.5	10.66±1.5	12.00±1.7
Lym (%) (50% LC50)	93.33±0.5	89.6±1.5	87.66±1.5	86.00±3.0
Eosino (%) (50% LC50)	1.66±2	0.33±0.5	0.66±0.5	0.33±0.5
Mono (%) (50% LC50)	0.00±0.0	0.66±0.5	1.00±1	1.66±1.5

Table 3. The biochemical parameters *Rutilus rutilus* when exposed to 10% LC50 and 50% LC50 of lead concentration in different times

Blood parameter	Time 0(Control)	Time 24	Time 48	Time 96
Glucose (mg/dl) (10% LC50)	38.6±7	51.33±5.8	73.33±5	74.6±13
Cortisol (mg/dl) (10% LC50)	22.06±14	24.3±8	12.7±6	31.46±17
Glucose (mg/dl) (50% LC50)	38.6±7.0	55.24±11	100.3±12	176.05±44
Cortisol (mg/dl) (50% LC50)	22.06±14	32.9±14	55.09±28	165.9±129

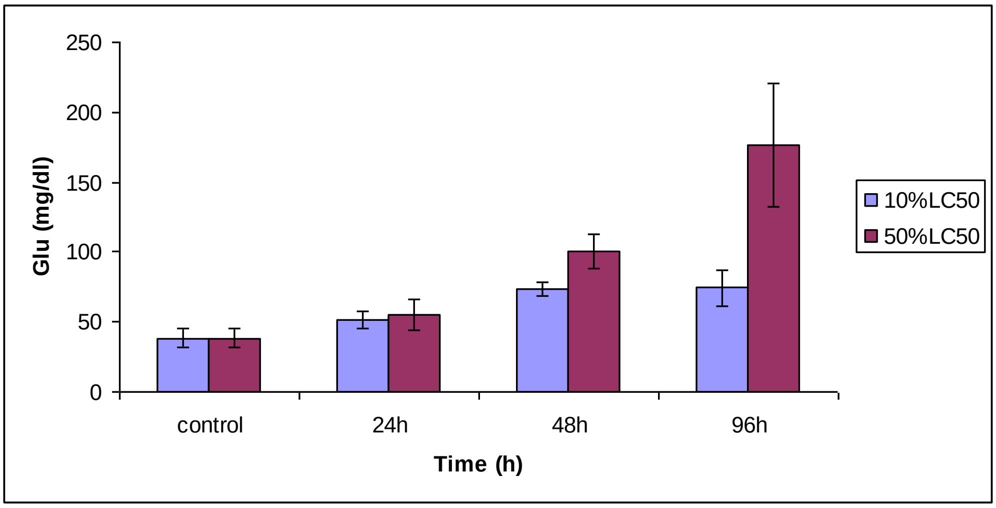

Fig. 1. Changes of glucose in the roach during their exposure to lead

In both 10% and 50% LC50 lead concentration, the level of glucose significantly increased in comparison to the control group (P <0.05).

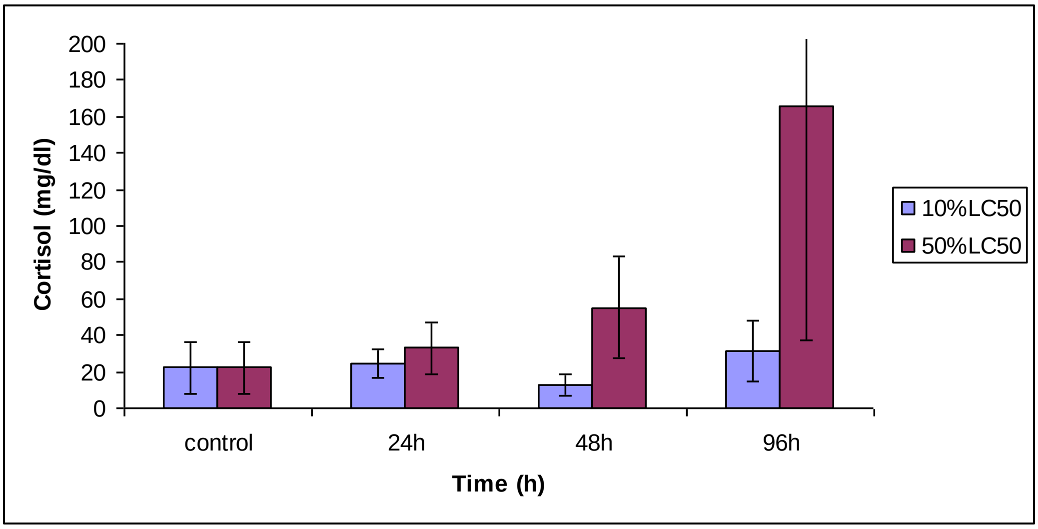

Fig. 1. Changes of cortisol in the roach during their exposure to lead

In both 10% and 50% LC50 lead concentration, the level of cortisol significantly increased in comparison to the control group (P <0.05).

Damaged metabolism in animals has been reported to repress their immune system, hence harming the blood cells (Witeska et al., 2006). The animals with higher metabolic activity need higher levels of oxygen and would have higher rates of breathing (Canli and Kargin, 1995), whereas metabolism degradation decrease animal activity, resulting in spoiled swimming ability and other depletory signs. In stressful conditions, the release of corticosteroids becomes a common event and is a non-specific answer any environmental stress (Wepener et al., 1992). Metal-based combinations that remain in the wastes can compile in various animals organs or make changes in plant and fish (Barman and Lal, 1994). Having dealt with the influence of 10% and 50% LC50 concentrations lead on hematological, biochemical, and immunological parameters of Roach (*Rutilus rutilus*), results showed that heavy metal lead is toxic and deadly for the fish.

During the 96 h exposure (10% LC50 Lead), WBC and RBC parameters had a noteworthy descend, compared to the control group; however, Hb and Htc did not show any significant correlation with lead exposure. Also, in comparison to the control group, levels of MCV and MCH increased at 96 h, though cortisol decreased. As for RBC, Hb, and Hct, there was a considerable reduction, compared to the control group, even though MCV, MCH, and MCHC did not undergo a similar drop.

Cicik and Engin (2005) showed a positive relationship between increasing concentrations of cadmium in the environment and blood glucose in *Cyprinus carpio*, which is followed by some changes in carbohydrate metabolism. A slight decrease of WBC was also reported in African catfish, subjected to very high lead levels (Olanike et al., 2008). Results showed that neu, mono, eosino, cortisol, and glucose increased, whereas Ht, Hb, lymphocyte, and RBC decreased at both concentrations. Changes in blood parameters are a suitable reflector of toxic stress, hence an analysis of hematological parameters in fish is extensively useful for the sake of evaluating toxic material impacts, on one hand, and animals' health, on the other. Throughout the study, the content of hemoglobin and hematocrit values reduced among the trial fish group with 96h of exposure, compared to the controls group (Banaee et al., 2008).

The transportation of oxygen and carbon dioxide within the blood are delicate and complex. The status acid-base and electrolyte depend on red blood cells (Narain and Srivastava, 1989). The reduction of RBC in blood causes respiratory limitations. In fact, one of the reasons for the changes of blood RBC is the accumulation of red blood cells in the gills of fish under pollution-related stress along with its reduction (Narain and Srivastava, 1989). On the other words, changes in hemoglobin and hematocrit are associated with RBC changes.

Singh and Sirvastava (2010) showed that changes in hemoglobin can be due to the changes in the number of red blood cells. Disorders of RBC, such as the rupture of red blood cells, can affect the hemoglobin in red blood cells. In this study, hemoglobin content in two concentrations increased at 96 h. The number of white blood cell in the blood may increase or decrease due to some disease or stimulants (Banaee et al., 2008). Remyla et al. (2008) showed that the immune system of fish would be adopted for any confrontation as long as the fish counteract environmental stress. Changes in the number of white blood cells might suggest some impairment of organs such as the kidney and spleen or may mark catching some infectious diseases. Banaee et al. (2008) reported that most infections accompanied with the increase in neutrophil and neutrophil blood.

CONCLUSION

The results showed heavy metal lead (10% LC50 and 50% LC50 concentration Lead) can cause poisoning and death in fish. Most toxicity was related to the 50% LC50 concentration Lead. Exposure to lead has negative effect on the blood biochemical and cells immune factors, decreasing the immune system and red blood cells. Results also showed that 50% LC50 lead has the most significant effect on biochemical parameters such as glucose and cortisol. Pollution-related stress in fish, as a result, can reduce the immune system. Lead is one of the heavy metal toxics that enter the fish body through food and water.

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