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Research note

Environmental Impact of Zinc on hematological, Biochemical and Immunological Parameters in Caspian Roach (*Rutilus caspicus*)

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A B S T R A C T

The aim of study was to investigate the toxicity effect of zinc at low concentration (10% LC50) and high doses (50%LC50) on roach (*Rutilus caspicus*). Roach with an average weight of 3.3 g and lengths of 4 cm were used in this work. Acclimatized fish were fed daily with a formulated feed.Caspian Roach were exposed to a low concentration of 10% LC50 and high concentration of 50% LC50 ofzinc for a period of control (0), 24, 48 and 96h static toxicity examination, operated within the tank of 400L. In 10% LC50 concentration zinc (96h) immune parameters neutrophil, monocyte and lymphocyte are not shown significant change toward control group ($P>0.05$) and in 50% LC50 concentration zinc (96h), immune factors lym showed significant decrease toward control but neu factor significantly increase toward control group ($P<0.05$). Eosino and mono did not showed significant correlation with zinc exposure ($P> 0.05$). In low and high concentrations of zinc, results showed zinc can cause changes in hematological, biochemical and immunological factors in Caspian roach; therefore, assessment of these indicators, could be useful to detect pollution of aquatic ecosystems.

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INTRODUCTION

Zinc is essential for wellhealth, but excess zinc can be harmful. Excessive absorption of zinc represses Cu and Fe absorption [1]. The zinc heavy metal in solution is very toxic toinvertebrates, plants, and even vertebrate fish [2]. Zinc toxicity is a medical situation involved in an overdose on, or toxic overexposure to zinc. Such toxicity levels have been seen to be happened at ingestion of greater than 225 mg of zinc [1]. Hematology, also spelled haematology is the tributary of medicine concerned with the study, treatment, diagnosis, and curb of sickness depends on blood. Hematology includes the study of blood cells. It involves treating diseases that affect the production of blood and its components, such as hemoglobin, blood cells, bone marrow, blood proteins, platelets, blood spleen, vessels, and the mechanism of coagulation.

Zinc is an important metal in industrial activities and energy consumption. More than half of metallic zinc ions used for galvanizing alloy steel. However, it is also important in the procurement of certain alloys that required less consumption of energy than pure metals. Zinc is used for the negative electrodes in some electric batteries; also for roofing and gutters in building construction for the purpose of saving energy. Zinc is used in plastics and cosmetics, while in rubber material; it has significant role as a catalyst during production and as a heat disperser in the production of desired products [1].

The environmental impact of zinc cannot be evaluated like man-made compounds; because of zinc occurs naturally in environment; also, its elimination from the ecosystem would not be possible. However, because of zinc is an essential metal, achieving such a goal would ultimately lead to detrimental effects throughout an environment. For essential elements environmental impacts should be considered within the context of fish natural ability to regulate (excretion and uptake) and keep a certain level of homeostasis [2]. Also, environments containing zinc at different concentrations may produce undesirable effects; Therefore, it should be

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assessed for optimal and lethal concentrations of zinc on valuable aquatic species.

The Caspian roach (*Rutilus rutilus*), also known as the common roach, is a fresh and brackish water of the *Cyprinidae* family, native to the most of western Asia and Europe. The name "roach" is not inimitable, but fishes called roach can be any species of the genera *Rutilus* and *Hesperoleucus*, depending on locality. The plural of the term is also roach [3]. Toxicology is a part of biology, medicine, and chemistry (more specifically pharmacology) concerned with the study of the harmful effects of chemicals on water organisms.

The best predictive models were identified to be deep neural networks, support vector machines and random forest, which can reach the performance of in vitro experiments [4]. Biochemical factors which used in fish and other aquatic organisms exposure to heavy metal stress could be used as the important bio-indicators for monitoring aquatic environment [5].

Therefore, a studywas conducted to investigate the toxicity effect of several zinc at low concentration (10% LC50) and high doses (50%LC50) in roach (*Rutilus rutilus*).

MATERIAL AND METHODS

Caspian Roach (*Rutilus rutilus*) with an average weight of 3.3 g and lengths of 4 cm were used in this present study. Acclimatized fish were fed daily with a formulated feed. Dead fish were directly removed with special plastic forceps to elude possible deterioration of the water quality [6]. Fish were exposed to a low concentration of 10% LC50 and high concentration of 50% LC50 zinc for a control period of 24, 48 and 96h static toxicity examination, introduced to the tank of 400L, each including fifteen pieces of fishes. One group control was conserved in a fiberglass tank without accessing toxicant. The experiments were carried out in triplicates. In the course of experiment, pH, dissolved oxygen, temperature and conductivity were fixed for monitoring purposes [7].

Fishes without delay were anesthetized with 200 ppm clove power. Blood samples rapidly were taken from tail blood vessel by heparinized syringes. Blood factors were carried out on fresh blood. Amounts of blood erythrocytes and leukocytes were carried out at 1:30 dilution by dilute heparinized blood with giemsa stain. Blood cells were counted using a hemocytometer-neubauer below the optical microscope [8]. By measuring the formation of cyanmethemoglobin were found hemoglobin levels (Hb mg/l). Erythrocytes indices (mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean corpuscular volume (MCV)) were computed from Hb, RBC, and Ht [9].

RESULTS AND DISCUSSION

In this study, we investigated effects of 50% LC50 and 10% LC50 zinc on a series of Immunological, biochemical and hematological parameters of roach for 0, 24, 48 and 96 h. No mortality observed in the control group. Fish exposed to zinc for 96h, a significant change in hematological (Ht, Hb, MCHC, RBC, MCV, MCH), biochemical (cortisol, glucose) and immunological (eosinophil, neutrophil and lymphocyte) factors concentrations compare to the control groups ($p<0/05$), whereas among significant indices, Hb, RBC, MCV, Ht lymphocyte in fish exposed to zinc for 96h were significantly lower than control groups ($P< 0.05$) and MCH, WBC, glucose, MCHC, neutrophil, eosinophil, cortisol were significant ($P< 0.05$).

Table 1 summarized hematological parameters of *Rutilus rutilus* exposed to 10% LC50 and 50% LC50 zinc concentration.

At duration 96 h(10% LC50 zinc), RBC and WBC parameters did not show significant correlation with zinc exposure ($P> 0.05$). RBC and Hb parameters had significant decrease compare to the control group (Table 1) ($P<0.05$). At duration 96 h (50% LC50 zinc), RBC, WBC, Hct and Hb parameters had significant decrease compare to the control group ($P<0.05$) (Table 1). In addition MCHC, MCV and MCH factors did not show significant reduction in compare to control group ($P<0.05$) (see Table 1). In 10% LC50 concentration zinc (96h) immune parametersneu,mono and lym did not show significant change toward control group ($P>0.05$). In 50% LC50 concentration zinc (96h), immune factors lym showed significant decrease toward control but Neu factor significantly increase toward control group ($P<0.05$). Eosino and mono did not show significant correlation with zinc exposure ($P> 0.05$)(see Table 2).

In the present study, cortisol and glucose levels in concentration of 50 % LC50 for 96 h significantly increased toward control ($P <0.05$). Cortisol and glucose significantly decreased in compare to control group ($P <0.05$). Blood factors in low concentrations (10%LC50 zinc) in different time 0, 24, 48, and 96 h (Figs. 1 and 2)

In 10% LC50 and 50% LC50 concentration zinc, glucose level did not show significant increasecompare to control group ($P <0.05$).

In 10% LC50concentration zinc, glucose level significantly increased in comparison to control group ($P <0.05$).But in 50 % LC50 cortisol did not show significant correlation with zinc exposure ($P> 0.05$).

Excess of contaminant are depleted into the environment in daily bases. Heavy metals are regarded as one of the most earnest pollutants of the aquatic environment because of their environmental durability and orientation to accumulate in fish and aquatic organisms [10]. In the present study, cortisol and glucose

TABLE 1. The hematological parameters *Rutilus rutilus* in the face of 10% LC50 and 50% LC50 zinc concentration in different time

Blood parameter	Time 0 (Control)	Time 24	Time 48	Time 96
RBC (10^3mm^3) (10% LC50)	2.43±0.7	2.29±0.3	2.18±0.6	2.03±0.6
Hb (gdl^{-1})(10% LC50)	7.30±0.8	6.33±0.8	5.81±0.7	5.42±0.5
Hct (%)(10% LC50)	23.49±7	19.28±2	16.89±2	18.65±3
MCV (fl)(10% LC50)	101.2±44	84.4±3	81.4±23	93.6±11
MCH (pg)(10% LC50)	32.39±11	27.78±11	27.81±6	28.06±7
MCHC (gdl^{-1})(10% LC50)	3.43±1.5	3.28±0.1	3.44±0.2	2.95±0.4
RBC (10^3mm^3)(50% LC50)	2.43±0.7	1.62±0.4	1.50±0.3	1.21±0.3
Hb (gdl^{-1})(50% LC50)	7.30±0.8	6.07±0.2	5.58±0.5	4.49±1.4
Hct (%)(50% LC50)	23.49±7	16.09±5	15.31±1.4	14.20±2
MCV (fl)(50% LC50)	101.2±44	100.01±18	110.2±23	118.7±11
MCH (pg)(50% LC50)	32.39±11	39.75±12	38.18±9	37.19±9
MCHC (gdl^{-1})(50% LC50)	3.43±1.5	3.98±1.0	3.67±0.6	3.12±0.6

TABLE 2. The immunological parameters of*Rutilus rutilus* in the face to10% LC50 and 50% LC50 zinc concentration at different time

Blood parameter	Time 0(Control)	Time 24	Time 48	Time 96
Neu (%)(10% LC50)	5.0±2	6.33±2	7.33±1	5.00±1
Lym (%)(10% LC50)	94.6±2	93.6±2	92.0±1	94.3±1.5
Eosino (%)(10% LC50)	0.33±0.5	0.00±0.0	0.66±0.5	0.41±0.5
Mono (%)(10% LC50)	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
WBC (10^6. µl)(10% LC50)	8200.0±1578	36216.6±46275	10790.0±814	10297.3±1216
WBC (10^6. µl)(50% LC50)	8200.0±1578	10987.3±1937	11887.3±2473	13721.6±885
Neu (%)(50% LC50)	4.00±2	8.00±1.0	9.00±1	8.66±1
Lym (%)(50% LC50)	93.3±1.5	90.3±0.5	90.0±1	90.6±0.5
Eosino (%)(50% LC50)	2.66±1.5	1.33±1.5	1.00±0.0	0.66±0.5
Mono (%)(50% LC50)	0.00±0.0	0.66±0.5	0.66±1.1	1.00±1.7

levels in concentration of 50 % LC50 for 96 h significantly increased in compare to control group. Blood factors in low concentrations (10%LC50 zinc) at different time 0, 24, 48, and 96 h. Glucose and cortisol significantly decreased in compare to control group.

Respectively, gill, thews and liver reposition of metal were Zn > Fe > Si > Al >Mn>Pb> Cr > V, Si > Fe > Zn > Al >Pb> V > Cr >Mn and Fe > Zn > Al > Si >Pb>Mn> V > Cr > Cu were reported in literature [11]. Charbonneau and Nash [12] reported that, the concentration of element iron ranged from 0.68 to 12.65 µg/g. The highest concentration of 12.65 µg/g was reported in gill of *Synodentis budgetti*, while the lowest valence of 0.68 µg/g was reported in the meat of *Oreochronmis niloticus*. Iron element is a necessary in organism meal. It forms section of haemoglobin, which permits oxygen to be carried from the respiratory organs to the tissues. The highest concentration observed in the Charbonneau and Nash [12] study was below the high

remainder concentrations of Fe (34 - 107 ppm) in fish samples on MNW refuge.

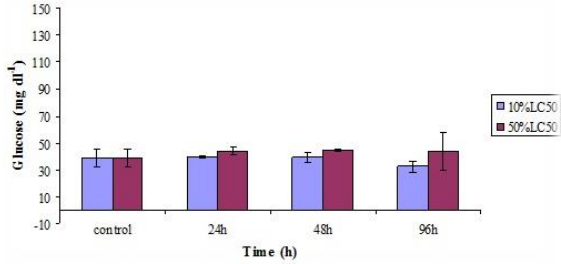

Figure 1. Glucose changes of Roach during exposure to Zn in low and high concentrations

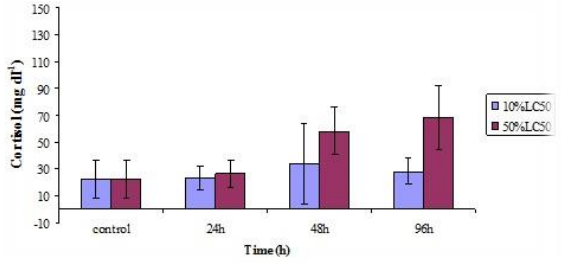

Figure 2. Cortisol changes of Roach during exposure to Zn in low and high concentrations

Jahanbakhshi et al. [13] evaluated the $LC50_{96h}$ values of CuO nanoparticles was 2.19±0.003 mg/l. *Rutilus rutilus* showed significantly lower in RBC count, Hct and Hb values and a significant increase in the MCV numbers, MCHC, MCH and WBC indices ($p<0.05$). Low glucose and higher cortisol content in blood were observed in the *Rutilus rutilus* exposed to CuONPs than those in control group ($p<0.05$) [13]. The immunological (neutrophil, lymphocyte), hematological (Hct, Hb, MCH, RBC, WBC, MCHC) and biochemical (cortisol, glucose) indices of *Hypophthalmichthys molitrix*, silver carp were assessment at low and high (10%LC50 and 50%LC50) concentrations of CuSO4 for (0-96h). At both concentrations, Hb, Hct, RBC, lymphocyte, and MCVwere significant ($P<0.05$, $P<0.01$) lower than control group whereas MCHC, MCH, glucose, WBC, neutrophil, cortisol were significant ($P<0.05$, $P<0.01$) higher in compare to the corresponding control group [14].

The lowest concentration of 0.88 µg/g was reported in the meat of *Oreochronmis niloticus*, while the highest concentration of 3.45 µg/g Mn was found in gills of *Tilapia zilli*. Mn is necessary element for both animals, fish and plants [15]. Zinc is an essential metal for both under developed growth, loss of flavor and hypogonadism, leading to reduced fertility. Zn toxicity at concentrations in water up to 40 mg/kg, may induce toxicity, determined by signs of irritability, muscular stiffness and ache, loss of appetite [16]. The highest concentration of Zn (3.45 µg/g) was seen in the gill of *Tilapia zilli*, while the lowest amount of 0.06 µg/g was measured in the meat of *Clarias angullaris*.

The FAO most guide line for Zn is 30 µg/g [17]. The results showed at duration 96 h (10% LC50 zinc), RBC and WBC parameters did not show significant correlation with zinc exposure. Hemoglobin and RBC factors had significant reduction in compare to control group. MCV, MCH and MCHCparameters did not show significant reduction in compare to control group. At time 96 h (50% LC50 zinc),WBC, RBC, Hb and Hct parameters had significant reduction in compare to control group.

Heavy metals such as Zn, Cr, Ni and Pb might change the attributes of hemoglobin by subtractive their dependence towards oxygen binding capacity interpretation the erythrocytes more brittle and penetrable, which may be results in cell swelling metamorphose and damage [18]. Heavy metals induced a significant reductionin hematocrit level of the fresh water fish common carp during the exposure of 1, 8, 16 and 32 days [19]. Heavy metals increase glucose content in blood, because of severe glycogenolysis and the synthesis of glucose from extra hepatic tissue aminoacids and proteins [20].

CONCLUSION

In low and high zincconcentrations,results showed zinc ion can cause changes in hematological, biochemical and immunological factorsin Caspian roach, so assessmentof these indicators, could be useful fordetection of aquatic ecosystemspollution.The results showed heavy metal zinc (10% LC50 and 50% LC50 zinc concentration) can cause poisoning and death in fish. Most toxicity was related to the 50% LC50 concentration zinc.Zinc is essential for aquatics but can be harmful and reduces immune system in fish.

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Persian Abstract

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چکیده

هدف از مطالعه حاضر بررسی اثر سمیت روی در غلظت کم (LC50٪۱۰) و بالا (LC50٪۵۰) در ماهی کلمه خزری (Rutilus caspicus) بود. کلمه با وزن متوسط ۳/۳ گرم و طول ۴ سانتی متر مورد استفاده قرار گرفت. در دوره سازگاری، ماهی ها با غذای فرموله تغذیه شدند. ماهی کلمه در غلظت کم (LC50٪۱۰) و بالای (LC50٪۵۰) فلز روی برای یک دوره کنترل (۰)، ۲۴، ۴۸ و ۹۶ ساعت در تانک های ۴۰۰ لیتری تحت سمیت ثابت قرار گرفتند. در غلظت کم (LC50٪۱۰) روی در ۹۶ ساعت شاخص های ایمنی نوتروفیل، مونوسیت و لنفوسیت تغییر قابل توجهی نسبت به گروه شاهد نشان ندادند (P> 0.05) و در غلظت بالا (LC50٪۵۰ 96 ساعته شاخص ایمنی لمفوسیت کاهش قابل توجهی نسبت به گروه کنترل نشان داد، ولی نوتروفیل به طور قابل توجهی نسبت به گروه شاهد افزایش داشت(P<0.05). همچنین ائوزینوفیل و منوسیت تغییر معنی داری نشان نداند (P> 0.05). ، نتایج نشان داد روی می تواند در غلظت های پایین و بالا باعث تغییرات متعدد در شاخصهای خون شناسی، بیوشیمیایی و ایمونولوژیک ماهی کلمه خزری شود. بنابراین، ارزیابی این شاخص می تواند برای تشخیص آلودگی اکوسیستم های آبی مفید باشد.