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Alteration In Haematological Indices In Abramis Brama Juveniles Induced By Sublethal Concentration Of Zinc

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Abstract: ***In the present study acute toxicity of zinc to Abramis brama juveniles and its haematological responses were conducted. Fish were exposed to 25%, 50% and 70% concentrations of*** $Zncl_2$ ***and blood samples were collected after 7days of exposure. the levels of heamoglobin (Hb), hematocrit(HCt), mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin concentration (MCHC), red blood cell (RBC) count and total white blood cell (WBC) count was mentioned. The(Hb), (HCt), (MCH), (RBC) values decreased significantly lower (p<0.05) compare with control group, for (WBC) and (MCHC) there was a downward trend but did not differ significantly, the levels of (MCV), neutrophils and monocytes were increasd during the experiment but no significant alteration were observed.***

Keywords: Haematological parameters; Heavy metal; sublethal concentration; zinc; Abramis brama

I. INTRODUCTION

Heavy metals contamination Due to their toxicity and long persistence is a serious threat to aquatic environment. These pollutants are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals, these metals have been reported to exert a wide range of metabolic, physiological, ecological and behavioral effects on fish (Lal Sah, 2009).

Among the metals, zinc plays an important role in the biology of living organisms. Zn in certain concentration is desirable for the growth of animals, normal development and metabolism of organisms and also required for the function of various enzymes, but become toxic at increased waterborne levels (Srivastava and Verma, 2009). Most trace metals like zinc are important for the functioning of the physiological processes in fish, Uncommonly high concentration of these metals can, however cause cellular and histological changesand so may exert adverse effect in fish causing structural damage, which affect the growth, development and survival of fish (Tuurala and Soivio, 1982). Annune et al. (1994b) reported that zinc could cause sub-acute effects that change fish behaviors, Such observed behaviors include lack of balance since most fins are motionless in the affected fish, agitated swimming, air gulping and death (Kori-Siakpere et al, 2008).

Fishes have widely been used as effective bioindicators of environmental pollution for assessing the quality of aquatic environment (Vinodhini and Narayanan, 2010). Because they are sensitive to changes in the surrounding water and readily absorb dissolved metals, Early toxic effects of pollution may be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. *Abramis brama* is a widely used species in aquaculture for food supply and as a bioindicator of water contamination, Fish hematological parameters are often determined as an index of their health status (Blaxhall and Daisley, 1973). haematological parameters depend on the magnitude of impact of concentration, the duration of exposure besides fish species, their age and health status. Alteration in white blood cells may be regarded as a prognostic tool as well as early warning signal of the disturbance in homeostatic defense abilities of

fish (Sharma and Langer, 2014). Haematological patterns appear very quickly and precede changes in fish behavior and visible lesions (Brucka-jastrzebska and Protasowiecki, 2005). The blood parameters of the fish have been selected for the study because ambient contaminants often produce rapid changes in the blood characteristics (Carvalho and Fernandes, 2006). Stress response involves various physiological changes including alteration in blood composition and immune mechanisms (Gabriel et al., 2004). Stress is a general and non-specific response to any factors disturbing homeostasis. Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Witeska, 2003). It has also been as a one major factor of disease indication, low productivity and mortality in aquaculture. The most common hematological variables measured during stress included red and white blood cells count, hemoglobin content, and hematocrit value and red blood cells indices. Fish hematological parameters are often determined as an index of their health status (Oshode et al., 2008).

McLeay (1975) reports high mortality of juvenile fish and reduced breeding potentiality of adults after long term exposure to heavy metals. Well documented reports can be found in organs of fish exposed to heavy metals, chemical pollutants and microorganisms (Vinodhini and Narayanan, 2010).

The purpose of this study was to evaluate the effects caused by sub lethal concentrations of zinc on hematological parameters as a bio marker to assess environmental stress.

II. METHODOLOGY

EXPERIMENTAL FISH

In the present study, *Abramis brama* juveniles (one hundred and fifty) were obtained from Shahid ansari fish farm, then fishes were transferred to the Sefidrood research center. Prior to the experiment the fishes were acclimated for one week.

EXPERIMENTAL DESIGN

The total number of fishes was grouped into four groups and each tub containing ten fishes; each group was treated with sub lethal concentration (25, 50 and 70%) of ZnCl respectively, an unexposed group served as the controls and each treatment had three replicate. The numbers of dead fishes were counted daily. The water quality parameters were measured during the experiment to estimate various factors such as water temperature, dissolved oxygen, PH and hardness. The exposure period lasted seven days, at the end of the experiment the blood samples were collected from the caudal vessels of fish using the method of (caltolidi et al., 1999). The zinc treatment level in *Abramis brama* was based on the LC50(96 h)of $ZnCl_2$with hardness (320 mg/L),to be 17 mg/L which was taken as the LC50 value determined and calculated by (Ministry of Technology, U.K., 1996).

BLOOD ANALYSES

Blood samples of the control and treated fish added to the heparin solution as anticoagulant. These blood samples were used for determining RBC and WBC count. Red and white blood cell counts were counted under microscope after dilution with an improved haemocytometer also differential leucocyte counts were made from Giemsa stained blood smears. Haematocrit percentage was determined with microhaematocrit way. The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae.

STATISTICAL ANALYSIS

The mean values of the various haematological parameters for the control and experimental fish were analysed with Shapiro-wilk test to be sured for normal data. Because of the normal data statistical significance among treatment group were done by one-way ANOVA then used Tukey test to sepration between groups and the results were expressed by using SPSS version 13 and Excel 2003.

III. RESULTS AND DISCUSSION

	control	t_1	t_2	t_3
HB g/dl	[b] 35/0 ± 75/6	[a] 07/0 ± 05/5	[a] 07/0 ± 45/4	[a] 07/0 ± 35/4
H.Ct%	[b] 12/2 ± 5/33	[a] 71/0 ± 5/28	[a] 0 ± 28	[a] 71/0 ± 5/27
MCV	[a] 4/13 ± 5/236	[b] 89/9 ± 307	[b] 71/0 ± 5/312	[b] 71/0 ± 5/314
MCH	[a] 54/3 ± 5/47	[a] 41/1 ± 54	[a] 0 ± 50	[a] 71/0 ± 5/48
MCH C %	[c] 0/0 ± 20	[b] 21/0 ± 65/17	[ab] 71/0 ± 5/16	[a] 21/0 ± 85/15
NOTRO	[a] 12/2 ± 5/39	[a] 54/3 ± 5/42	[a] 41/1 ± 45	[a] 71/0 ± 5/48
LANFO	[b] 71/0 ± 5/58	[ab] 83/2 ± 53	[a] 71/0 ± 5/50	[a] 41/1 ± 49
MONO	[a] 71/0 ± 5/1	[a] 71/0 ± 5/3	[a] 71/0 ± 5/3	[a] 0 ± 3
RBCs	[b] 6/169705 ± 1420000	[a] 9/6363 ± 926500	[a] 6/4242 ± 890000	[a] 5/3535 ± 887500
WBC	[a] 98/494 ± 12850	[a] 8/282 ± 12000	[a] 3/424 ± 10500	[a] 4/1131 ± 10900

*T_1: treatment (25%) t_2: treatment (50%) t_3: treatment (75%) * Significant at p<0.01 ** Non-significant*

Table 1: Effect of zinc on some haematological parameters in Abramis brama after 7 days experiment (mean ± SE)

The mean values of haematological parameters of control and treatment group after 7 days, exposed to zinc chloride are shown in Table 1. In this study, haematological parameters of *Abramis brama* were evaluated. In treatments group the levels of heamoglobin (Hb), hematocrit, mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin concentration (MCHC), red blood cell (RBC) count and total white blood cell (WBC) count was mentioned as compared to the controls. The haemoglobin

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concentration decreased ($p<0.05$) in the treatment groups differed significantly ($p<0.05$) with test concentrations. The haematocrit of *Abramis brama* to the zinc-exposed fish were generally lower than in the control, therfore the haematocrit of the fish treated with zinc decreased with test concentration. As seen in table1, a significant alteration were observed in MCV among control and each of the treatment group ($p<0.05$). The MCH values in the treatment groups were higher ($p<0.05$) than the control and it also increased with increasing zinc concentration during the exposure. The MCHC values also decreased with increasing test concentrations. The MCHC values were significantly different in the treatment groups ($p<0.05$).The lymphocyte counts indicate significant differences ($P < 0.05$) from control, but the levels of monocytes and neutrophils did not differ significantly. The increase was observed in the number of monocytes and neutrophils and while decrease was noticed in the number of lymphocytes. In control fishes, the number of RBC was observed to be 6/169705 ± 1420000, the red blood cell showed a considerable decrease in the treatment groups than in the control ($p<0.05$) R.B.Cs count significantly decreased than that of control fish after 7 days of exposure to sub lethal concentration of zinc and for WBC no significant alteration exposed to zinc after 7 day was observed ($p>0.05$).

Haematological alterations are biomarkers of effect exposure to environmental stressors, these alteration are non specific to a wide range of substance, some of these changes are the result of activation the protective mechanisms, they can reflect the poor condition of fish more quickly than other commonly measured parameters and they respond quickly to changes in environmental conditions also they have been widely used for the description of healthy fish Blaxhall and Daisley (1973). In the present study after 7 days, haemoglobin level decreased significantly above the controls. Similar finding was observed by the Cyriac et al. (1989), They considered decreases in haemoglobin concentration as a contribution to haemodilution, which is a mechanism that reduce the concentration of the pollutant in the circulatory system. Vasem and Banerjee (2012) menshioned that the significant decrease in the haemoglobin concentrations of fishes might be due to increase in the rate of haemoglobin destruction or decrease in the rate of haemoglobin synthesis. These data, similarly as the result of the study was done by the Dongre et al. (2011) they reported that pb can reduces the hemoglobin synthesis, it might be due to decreased heme and globin synthesis or erythrocyte formation and function, but this occurs only with high levels of exposure. Ololade and Oginni (2010) Noted that observe depression in hematocrit and hemoglobin values coupled with decrease and deform erythrocytes are obvious signs of anemia. Blaxhall and Daisley (1973) mentioned that the determination of haemoglobin concentration can be a good indicator of anemic conditions in fish.

Haematocrit is defined as the volume occupied by erythrocytes in a given volume of blood and used to determine the oxygen carrying capacity (Vasem and Banerjee, 2012). The significant decline in the Hct levels in the present study is in agreement with the previous reports such as Magdy and Salah el-Deen et al. (2001) they reported a significant decrease in Hb content and Hct values. They suggested that this decrease could be attributed to many factors such as impairment of gas exchange by the gills, disequilibrium of the osmotic pressure, haemolysis of erythrocytes and dysfunction of the spleen and destruction of large number of erythrocytes. Similar finding was observed by moosavi et al. (2015) in Hct and Hb level. Chandanshive et al. (2012) reported changes in blood parameters (erythrocyte count, haematocrit level, leukocyte count), Erythrocyte count decreased significantly in the blood of fish exposed to almost all Heavy metal model mixture concentrations studied while alterations of haematocrit level depended on Heavy metal model mixture concentration.

Results of the haematological indices showed a significant increase for MCV level but no significant alterations were observed in the MCH of juvenile fish treated with $ZnCl_2$ but In the case of MCHC there was a significant decline between control and treatment group. Dongre et al. (2011) note that significantly decreased may be due to decreased heme concentration or decreased erythropoietin hormone or decreased maturation of RBC by metal. Ololade and Oginni (2010) Reported that the derived hematological indices of mean corpuscular volume (MCV), (MCH) and (MCHC) were equally lowered. Kavitha et al. (2010) observed decrease in various haematological parameters (Hb, Ht, RBC, WBC, plasma glucose, plasma protein) in arsenate treated Indian major carp, *Catla catla*, they however observed increased corpuscular indices like MCV, MCH and MCHC in the same fish. moosavi et al. (2015) noted that no significant change was recorded in MCV and MCHC but a significant change in the MCH especially at higher concentrations they suggested that it may be due to decrease in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis.

Red blood cells count decreased significantly in the fish exposed to Zn. Annue et al. (1994) reported a significant increase in RBC count of *C. gariepinus* when subjected to Zn treatment. This different results may be explained by the fact that decrease or increase in certain blood parameters can be associated with the nature of species and the toxicants in different studies (Ololade and Oginni, 2010). Few studies are concerned with study of the morphological changes in red blood cells of fishes upon exposure to heavy metals or pollutants. yamamato (1988) reported the increased level of stress hormones causes drop in erythrocytes and leukocytes and plays important role in impairing blood parameters.

The number of White blood cells did not significantly decreased in exposed fish but there was a downward trend with increase in the concentration of the metal. Similar finding was observed by moosavi et al. (2015) in *Carassius auratus* after exposure to nickel. The decrease in WBC count of the treatment groups may be due to the release of epinephrine during stress which is capable of causing the contraction of spleen (Svoboda, 2001). This finding disagrees with the results reported by Vosyliene et al. (2010) who observed an increase in the WBC concentration and in *Orechromis aureus* after mercury exposure. Ray et al. (2014) have reported that the number of white blood cells can be affected by physiological and environmental factors. Snyder (1999) found that there was an adverse alterations in the level of white blood cells depending on the concentrations of metals and

duration of exposure in the fishs. An increase in the total number of neutrophils and monocytes and a decrease in lymphocytes in the treated group were observed, although no significant differences between control and exposed group were observed for the neutrophils and monocytes, but for lymphocytes a significant reduction was observed. A decrease in the percentage of lymphocytes is one of the most common responses seen in fish exposed to toxic substances (Witeska, 2003). Some behavioral changes such as fast swimming, erratic movement and greater surfacing accompanied by loss of equilibrium and turning the body upside down was observed by (Lal shah, 2010). Hyperactivity, erratic swimming and and loss of equilibrium have been reported in *Salvalinus fontinalis* when exposed to lead (Holcombe, 1976) this is a consequence of nerves impairment or paralysis and depression of respiratory center in fishes were exposed to heavy metals.

IV. SUMMARY AND CONCLUSION

This study shows that almost all the blood parameters decreased with increasing concentration of toxicant (with an exception for MCV, neutrophils and monocytes) and become significantly lower ($P < 0.05$) at higher concentration when compared with the control, (in the number of White blood cells and MCHC No significant alterations were observed in exposed fish, but there was a fall with increase in the concentration of the metal.

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