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Effect of Hexavalent Chromium on the RBCs of the Fresh Water Fish, *Labeo rohita*

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ABSTRACT

Industrial effluents when reach aquatic systems become hazardous to aquatic fauna. Heavy metals present in such effluents do not degrade but affect the physiology of fish when present in excess concentrations. The present study has been designed to determine the acute toxicity of hexavalent chromium to Labeo rohita. 24, 48, 72 and 96 hr LC_{50} values were estimated using probit analysis. Effect of sublethal concentrations of hexavalent chromium on the RBC count and indices were analysed for 20 days and the results are discussed.

Keywords: *Labeo rohita, Chromium, Hematology, RBC count, Fish.*

INTRODUCTION

The world population is increasing day by day and this has led to increase in industrialization, urbanization and intensification of agriculture. Development of industries led to the release of several wastes into the environment. These waste materials containing pollutants cause many harmful effects on living things. The toxic effects of these pollutants not only cause alterations or disturbances in the structure and function of organisms but also lead to death of organisms. Pollutants discharged into environment are in the form of pesticides, fertilizers, heavy metals and oxidants. These pollutants or toxicants are released by refineries, tanneries, plating industries, agrochemical industries, textile industries, chemical manufacturing units and heavy industries. Environmental degradation has become a new addition due to the development of industrial sectors. There are about three million chemical substances known and we are adding a thousand new ones every year. The nature's self purification capacity is diminishing (McDonough and Braungart, 2017; Manahan, 2017; Victor, 2017).

Water is one of the most important natural resources. Clean water is essential for the survival of the living organisms. Unfortunately the quality of the water is deteriorated immensely because of various types of pollution. They are mainly non-degradable and biodegradable pollutants. The pollutants which cannot be decomposed, once they fall in an environment, they remain as such. Hence continuous use of these substances leads to their accumulation in the environment and in the organisms through biogeo chemical cycles and food chain. This accumulation of pollutants in the organisms leads to biological magnification. Biodegradable pollutants can be decomposed by natural process (Wang and Yang, 2016; Health, 2018).

Heavy metals are normally non-degradable, hence they accumulate in the environment and cause disturbances to the organisms and interfere with man's own use of his environment. Toxicity and physiological impacts of heavy metals have been well studied in fishes and other organisms. The toxicity of metal depends on the inherent capacity of a metal to affect adversely any biological structure and system into inflexible and irreversible conformations, leading to deformity in the body and finally death. Accumulation of heavy metals has already been reported by several investigators which in turn disturbs metabolism and restricts development and growth in animals (Izah and Angaye, 2016; Rajasulochana and Preethy, 2016).

Fishes are one of the major sources of nutrition for human beings. Efforts are being made all over the world to exploit both the marine and fresh water bodies for fish production. Fishes are very excellent indicator organisms for the assessment of impacts of toxicants like pesticides, effluents and heavy metals in aquatic ecosystems. Chromium is a micronutrient essential for normal metabolic activities. But in higher concentration, the heavy metal, chromium may affect the hematological parameters. Chromium finds widespread use in industries and it is used widely in dyes, paints, pigments, ceramics, pesticides and some therapeutical preparations as well. It is necessary for the normal biological activity, RBC synthesis and its deficiency is characterized by hypochromic microcytic anemia which develops as a consequence of defective hemoglobin synthesis. However the effect of chromium on lower organisms like fish with higher concentration may cause some haematological changes (Silva et al., 2016; Poon et al., 2016). Hence, the present study was undertaken to study the effect of hexavalent chromium on the RBCs of fresh water fish, *Labeo rohita*.

Materials And Methods

The Indian major carp, *Labeo rohita* is a fish of the carp family Cyprinidae, found commonly in rivers and freshwater lakes in and around the South Asia and South-East Asia. It is a herbivore and is treated as a delicacy in Orissa, Bihar and Uttar Pradesh. In fact, the Kayastha community of Uttar Pradesh treats it as one of their most sacred foods: to be eaten on all auspicious occasions.

Stock of fish was procured from a local fish farm in Madurai, Tamil Nadu, India. They were acclimatized to laboratory conditions for about two weeks in well water. During the period of acclimation they were fed on algal and artificial fish feed. Only fish of equal size and weight (20 to 25g) were selected for the experiments.

Chromium

Heavy metal chromium can be obtained from different salts. For this study, crystals of Potassium dichromate ($K_2Cr_2O_7$) were used as a source of chromium. 2.8g of $K_2Cr_2O_7$ was dissolved in 1000 ml of distilled water to get 1 ppt solution and it was kept as a stock solution for the future study.

Test Medium

The test medium was the bore well water into which the stock solution of chromium can be mixed in varying volumes in order to get different experimental concentrations and these were used for the estimation of LC_{50} values for 24, 48, 72 and 96 hours. The temperature of the water used in this study ranged between 25 and 30 ^{0}C and pH of the water ranged between 7.5 and 8.5. For the experimental purpose stock solution was used to prepare different working ppm concentration by dissolving desired volume of ppt solution in 1000 ml of bore well water as regularly till the end of experiment.

Experimental Design

After the completion of acclimation, all the healthy fishes were selected for experimental purpose. The acute toxicity of hexavalent chromium was assessed by determining the LC_{50} value with triplicate sets. In each set, different concentrations of chromium were used to estimate the LC_{50} value by observing the mortality of fish for different exposure periods.

Estimation of LC_{50}

The acute toxicity of chromium was estimated with static renewal bioassay procedure (Eaton et al, 2005). Different concentrations of chromium were selected and in each concentration, ten fishes were introduced to find out the percent mortality. The mortality in all the concentrations was recorded after 24, 48, 72 and 96 hours of exposure. The percent mortality was recorded in wide and then narrow ranges of different concentrations of chromium for 24 48, 72 and 96 hours of exposure. The LC_{50}value for different exposure periods was obtained by applying probit analysis.

Selection of Sublethal Concentrations

From the obtained 96 hr. LC_{50} value of chromium, four sublethal concentrations namely 1/40th, 1/20 th, 1/15 th and 1/10 th of 96 hr LC_{50} value were selected tor long term exposure to study the haematological changes in the fish, *Labeo rohita*. By this method 0.5, 1.0, 1.5 and 2 ppm concentrations of hexavalent chromium were selected as sublethal concentrations. The test media were daily changed. Along with these sublethal concentrations control group of fishes were also maintained side by side throughout the period of experiment In all these control and sublethal concentrations (0.5, 1, 1.5 and ppm), ten fishes were introduced for the experiment.

Haematological Studies

From the control and four sublethal concentrations, each fish was sacrificed and blood samples were taken for the estimation of haematological parameters by using standard procedures (Fazio, 2019; Duman and Sahan, 2017; Kandeepan, 2014; Maheswaran et al., 2008).

Results

The percent mortality of *Labeo rohita* exposed to different concentrations of chromium was noted. No mortality was noted in 5ppm concentration till 96 hr, whereas 100% mortality occurred in 80 ppm within 24 hr of exposure. The LC_{50} values (graphical values) for 24, 48, 72 and 96 hr are 20, 30, 45 and 50 ppm respectively (Fig. 1). The LC_{50} values observed decreased with increase in the duration of exposure.

RBC count of *Labeo rohita* exposed to different concentrations of hexavalent chromium is presented in Fig. 2. The normal RBC count showed 3.9 million cells per cubic mm. The effect of hexavalent chromium was more in higher concentration of prolonged exposure when compared with the least concentration of chromium. An increase in the duration of exposure caused a decrease in the RBC count and increase in the chromium concentration resulted in a decrease in RBC count.

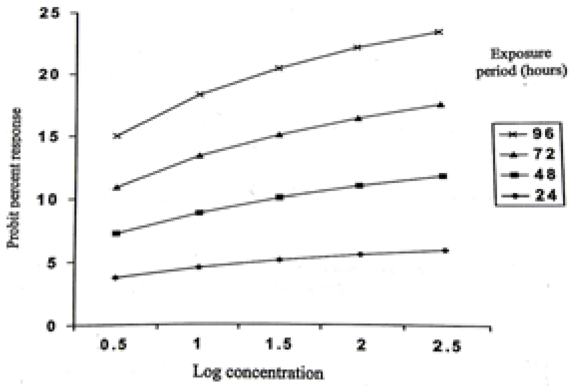

Fig1. *Probit percentage response of Labeo rohita exposed to hexavalent chromium for 24, 48, 72 and 96*

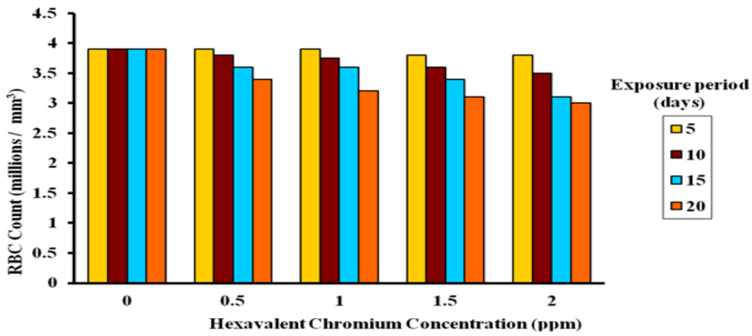

Fig2. *Effect of hexavalent chromium on the RBC count (millions/mm^3 blood) of Labeo rohita*

Haemoglobin content of *Labeo rohita* exposed to different concentration of hexavalent chromium for different exposure periods is shown in Fig. 3. Decreased level of haemoglobin content was observed in chromium exposed fishes. The depletion was noted to be more in higher concentrations of chromium than control and lower concentrations of chromium. The depletion was more in long term exposure than that of control and short term exposure. The effect of chromium was more in long term exposure and higher concentration of chromium.

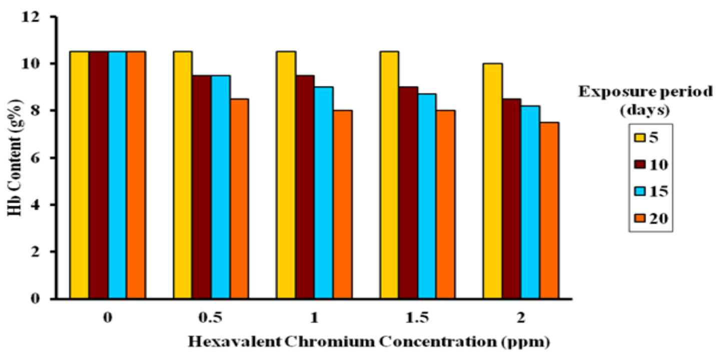

Fig3. *Effect of hexavalent chromium on the Haemoglobin content (g / 100 ml blood) of Labeo rohita*

Figure 4 shows the PCV values of *Labeo rohita* exposed to different concentrations of hexavalent chromium. The effect of chromium was more in long term exposure and higher concentrations when compared to control and lower concentrations of chromium. Increase in the concentration of chromium caused a decrease in the PCV level of fish, at the same time increase in exposure period also resulted in a decrease in the PCV level of fish. MCV of *Labeo rohita* exposed to different concentrations of hexavalent chromium is exhibited in Fig. 5. The effect of chromium on MCV was found to alter normal values. As the duration and chromium concentration increased the MCV also showed an increase. But the level of increase was not uniform in all the concentrations of chromium and in all exposure periods.

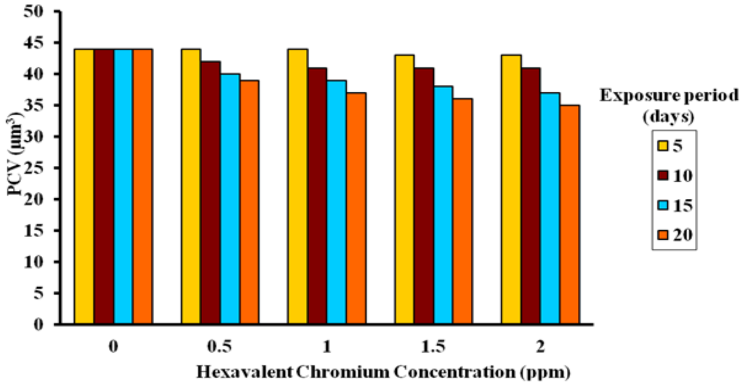

Fig4. *Effect of hexavalent chromium on PCV (µm^3) of Labeo rohita*

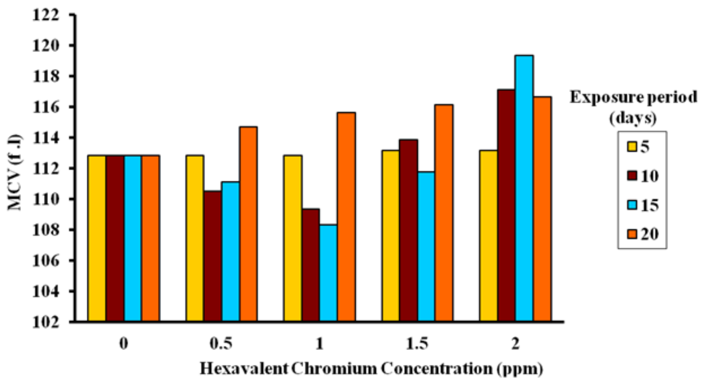

Fig5. *Effect of hexavalent chromium on MCV (femto litre) of Labeo rohita*

MCH of *Labeo rohita* exposed to different concentrations of hexavalent chromium for different exposure periods is presented in Fig. 6. The effect of chromium was found to influence MCH values. As the duration of exposure increased, decrease in the MCH level was found out. Regarding concentration, there were no remarkable changes. However the duration and concentration caused a change in the MCH levels. MCHC of *Labeo rohita* exposed to different concentrations of hexavalent chromium for different exposure periods is exhibited in Fig. 7. The effect of chromium on MCHC was found with decreased level with the increase in chromium concentration and exposure period. More depletion was noted in higher concentration of chromium and long term exposure when compared with the control group.

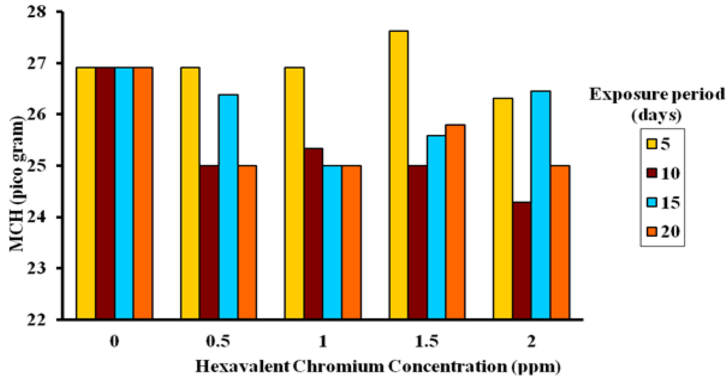

Fig6. *Effect of hexavalent chromium on MCH (pico gram) of Labeo rohita*

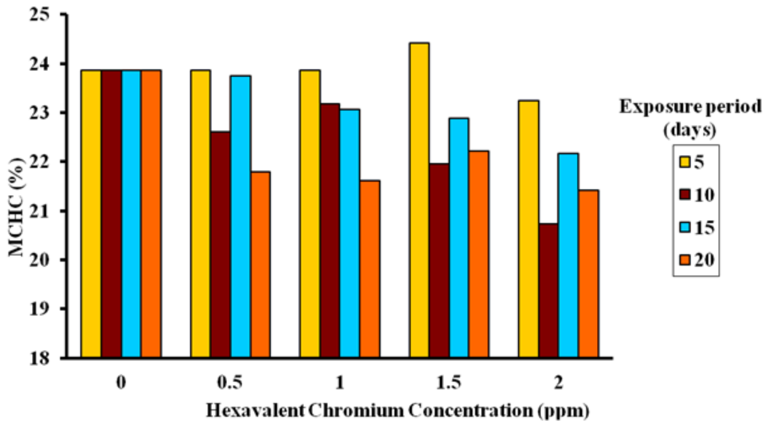

Fig7. *Effect of hexavalent chromium on MCHC (%) of Labeo rohita*

Discussion

The present study reveals that chromium is acutely toxic to the fish, *Labeo rohita* and the mortality rate increases with increasing concentration of chromium. The mortality rate also increases with increasing duration of exposure. The LC50 value of chromium for 96 hr is 20 mg/l. The LC50 value of chromium for 96 hr is about 6 times higher than that of Lepidocephalicthys thermalis (Rajakumar, 1992). Acute toxicity of mercury has been studied in Sarotherodon mossambicus (Naidu and Ramamurthy, 1984). The result indicated mercuric chloride impairing oxidative and transphosphorylative activities during acute mercury toxicosis in fish. Toxicity of mercuric chloride to Channa punctatus has been shown to depend upon the concentration and duration of exposure (Agarwal, 1991). When concentration and duration of exposure increased, the fish exhibited anamolous behaviour and a dose and time dependent mortality rate. The LC50Value for 96 hr of zinc observed in Oreochromis mossambicus was 2 times higher than that of the present study. But the range of toxicity varies for different species and for different toxicants. Furthermore, several factors like pH, hardness, alkalinity, equilibration and kinetics involved in the chemical reaction are detrimental in the toxic efficacy.

Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations (Mulcahy, 1975). The blood in the gill has direct contact with water medium and any unfavorable change in water could be reflected in the circulatory system. These studies could be used to indicate the health status of fish as well as the water quality.

Erythrocyte (or) Red Blood Cell count is very important in assessing the health condition of the organism. In an anaemic state, hepatic infection and few viral infections the total count will be far less than the expected. Hence the total count of RBCs assumes significance in diagnosis of some diseases. In the present investigation the level of total RBC count is decreased with increase in the concentration of chromium and also decreased with increase in the duration of exposure. The level of declined amount of RBC in millions was slightly higher in higher concentration of toxicant exposed fishes. This study was also supported by Dhanapakiam and Ramasamy (2001). According to their findings, the level of RBC also decreased in Cyprinus carpio exposed to copper and zinc mixture. They also found significant decrease in RBC count and haemoglobin content. But the level of the magnitude varied depending upon the exposure. Lead toxicity as lead nitrate on the fresh water cat fish, Heteropneustes fossilis at 2.25 mg/l and 2.85 mg/l concentrations caused adverse effect and showed fall in total erythrocyte concentrations (Neelima et al., 2002). It is also supported in the haematological response of carp to the acute effect of the deltamethrin based preparation at a 0.13mg/l concentration significantly decreasing the erythrocyte count (Suobodova et al., 2003).

The exposure of Oreochromis mossambicus to sublethal levels of copper resulted in a duration and concentration dependent decreases in the RBC count and increase in the WBC count. During exposure to copper for 30 days period RBC values decreased. During exposure to Lambda-cyhalothrin for 30 days period, RBC values decreased (Muthupandi, 2006). Haemoglobin is an indicator for the health of an organism. The changes in the haemoglobin levels indicate the disease (or) stress condition of such organisms. In the present study, the level of haemoglobin content of *Labeo rohita* exposed to 0.5 ppm of chromium exhibited less depletion when compared with 2 ppm of chromium. But increasing the duration of exposure to chromium decreased haemoglobin content.

During exposure for 20 days period, haemoglobin content decreased in four concentrations and haemolysis was noticed in toxicant exposed fish. This was supported by Goel and Kalpana (1985) who reported that haemoglobin content significantly decreased resulting in macrocytic anaemia in Heteropneustes fossilis exposed to heavy metal zinc. Further the level of haemoglobin and RBC count decreased in Channa punctatus exposed to sublethal concentration (25mg/l) of cadmium (Karuppasamy et al., 2005). Effect of lead caused significant decrease in the level of the haemoglobin content of the fish, Tinca tinca (Shah, 2006). Decreased level of haemoglobin content was observed in neotropical fish, Hoplias malabaricus exposed to sub chronic and dietary doses of methyl mercury, inorganic lead and tributylin chloride (Ribeiro, et al., 2006). The level of decline of haemoglobin content may vary depending upon the proportion of those above toxicants.

Packed cell volume is an indicator for the amount of erythrocyte in the whole blood. As the level of PCV increases it will increase the RBC count and deplete the plasma level with the proportion to whole blood. The value of PCV is higher in male than female. In the present study the PCV values were found to decrease in higher concentration of chromium and increasing exposure period. This was also supported by Goel and Kalpana (1985) who reported that haematocrit level significantly decreased resulting in anaemia in Hetero-pneustes fossilis exposed to zinc. Decrease in erythrocyte count, Hb content and PCV reflects the anaemic state of fish (Bhatkar and Dhande, 2000). In the present study the percentage of PCV levels were found to decline with increasing duration of exposure as well as concentration of toxicant.

Mean Corpuscular Volume decreased at lower concentration of chromium in the beginning of exposure, But on the 10th day exposure, the level of MCV at lower concentration of chromium was slightly higher than that of higher concentration of chromium. After 20 days exposure increased volume was noted in 0.5 ppm concentration of chromium. But in 2 ppm concentration of chromium, MCV decreased. The magnitude of increase or decrease was not found as constant among the sublethal concentrations of chromium. Similar result was reported in Tinca tinca (Shah, 2006).

Mean Corpuscular Hemoglobin level was found to slightly increase at initial time of toxicant exposure. But with the increase in the duration of exposure, the same concentration depleted the MCH level with minute differences. But after 20 days exposure decreased levels were noted. Combined mixtures of copper and zinc caused toxic effects in Cyprinus carpio with significant increase in the level of MCH depending upon the exposure (Dhanapakiam and Ramasamy, 2001). Further it was supported by Shah (2006) who stated significant increase in the level of MCH in Tinca tinca exposed to lead. In the present study the level of decline of MCH was found to be very little and the values were very close to control fish.

Mean Corpuscular Haemoglobin Concentration level was found with fluctuations in the values at different exposure periods. In the present Study, it was observed that there was neither gradual increase nor a decrease. This may due to varying concentration of haemoglobin and haematocrit levels. However the chromium toxicity was found in fishes by observing different levels of MCHC in different exposure periods and concentrations. The levels of MCHC were found as not constant among the four sublethal concentrations of chromium. Haematological parameters of the fish are the indicators to find out the toxicity of the water and health of the fish. Hexavalent chromium caused remarkable changes in RBCs and affected the health of the fish, which led to the death of fish. There by the fish becomes unsafe for edible purposes and the water becomes unsuitable for potable and recreation purposes.

Conclusion

The 24, 48, 72, and 96 hr LC_{50} values of hexavalent chromium to the fish are 20, 30, 45 and 50 ppm respectively. Sublethal concentrations of hexavalent chromium caused decline in the RBC count and indices of *L.rohita.*

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