Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **S-3** Year **2023** Pages **282:293***

Assessment of the Toxic Effect of Hexavalent Chromium on the Hematological Indices in Nile Tilapia, *Oreochromis Niloticus*

Debkanta Ghosh[1*], Samir Kumar Saha[2]

[1] Department of Zoology, West Bengal State University, Barasat, West Bengal, India
[1'] Department of Zoology, Vidyasagar college for Women, Kolkata, West Bengal, India.
2. Department of Zoology, West Bengal State University, Barasat, West Bengal, India
**Corresponding Author E-mail: ghoshdebkanta40@gmail.com*

Article History

Received: 26 March 2023
Revised: 12 August 2023
Accepted:29 Sept 2023

Abstract

The presence of heavy metals, which are typically released into water bodies as a result of fast industrialization, causes the aquatic environment to become extremely contaminated. Chromium is one of the most common heavy metals in the environment and is used in several types of industries. The effect of sublethal toxicity of hexavalent chromium Cr (VI) on hematological indices of the Nile tilapia, Oreochromis niloticus has been analyzed following exposure of concentration 9.349 mg/L and 18.698 mg/L (10% & 20% of LC_{50} value) of potassium dichromate ($K_2Cr_2O_7$) as hexavalent chromium for 7, 15, 30 days. Hematological parameters are the most important indicators of fish health status. The aim of this study was to determine the effect of Cr (VI) toxicity on hematological parameters in O. niloticus. The results show that hemoglobin percentage (Hb%), red blood cell (RBC), mean corpuscular volume (MCV), and platelet count level were significantly ($P > 0.05$) decreased and the total count of white blood cell (WBC) was significantly ($P > 0.05$) increased with increasing time of exposure at a concentration of 9.349 mg/L of $K_2Cr_2O_7$ in the test groups compared to the control group. At the concentration of 18.69 mg/L, Hb%, RBC, MCV, mean corpuscular hemoglobin concentration (MCHC), and platelet count level were significantly ($P > 0.05$) decreased and the total count of WBC and erythrocyte sedimentation rate (ESR) value were significantly ($P > 0.05$) increased with the increasing time of exposure in the test groups compared to the control group. To study the R^2 value of the linear regression equation, we found that the trend of the chronic toxic effect of hexavalent chromium at 60 and 90 days of both the sublethal concentrations 9.349 mg/L and 18.69 mg/L of $K_2Cr_2O_7$, where the value of Hb, RBC, packed volume cell (PCV), MCV are found to drastically decreased and the value ESR and WBC are found to drastically increased in the experimental groups compared to the control group. The investigation recommended that the presence of hexavalent chromium in the aquatic medium has a strong impact on the hematological indices in O. niloticus.

Keywords: *Hexavalent chromium, Oreochromis niloticus, Hematological parameters, LC_{50} of hexavalent chromium, Linear regression.*

1. Introduction

The Nile tilapia, *Oreochromis niloticus* is one of the most important freshwater fish in global aquaculture (Gretchen et al., 2012). It is popular for its high growth performance and high national and local market demand (M. Menaga and K. Fitzsimmons, 2017). This is one of the most consumed fish species in India after carp. After being introduced to India from Africa in 1987, it has been contributing a lot, estimated to be 22000 tones in 2020 annually to the aquaculture industry (M. Menaga and K. Fitzsimmons, 2017). This fish is omnivorous i.e they consume zooplankton, phytoplankton, and insects. The species has the ability to tolerate a wide range of environmental conditions and show satisfactory production. (Peterson et al., 2005; Singh et al., 2011; Chakraborty et al., 2009).

Heavy metals are a major aquatic environmental hazard, although they have biological importance as micronutrients. Over the previous few decades, the enormous expansion in the use of heavy metals has automatically resulted in an amplified inflow of heavy metals in the aquatic medium (Islam et al., 2021). The heavy metals have diversified effects and their different scales of concentration stimulated toxic

effects on aquatic organisms. Most heavy metal ions have toxic effects on nature and give rise to a threat to the environment and human health (Di-Giulio and Hinton, 2008; Farombi et al., 2007). An earlier study has revealed that heavy metal contamination decreased the production of fish (Lu et al., 2015). Therefore, it is necessary to control the heavy metal use in industry. Moreover, the toxic effect of heavy metals are hazardous impacts on aquatic organisms that ultimately lead the major human health concern (Ullah, et al., 2017; Rajeshkumar and Li, 2018; Maurya et al. 2019; Kortei et al. 2020).

Chromium enters the aquatic environment via the effluents discharged from different types of industries like tanneries, electroplating, textiles, dyeing, mining, and pharmaceutical industries (Monterio et al., 2002; Palanippan et al., 2009; Mohammed and Sahu, 2019). The most environmentally stable forms of chromium are trivalent Cr (III) and hexavalent Cr (VI). In the aquatic environment, the hexavalent form of chromium is reduced to the trivalent form (Mushtaq et al., 2022; Bashir et al., 2022). Chromium compounds have a biological function in lipid and glucose metabolism as important nutrients. The hexavalent form of chromium has a mutagenic and carcinogenic effect in a variety of aquatic organisms (Velma et al., 2009).

Fishes cannot avoid the negative effects of heavy metals contaminants that indicate fish is good bio-indicators of aquatic pollution, making their adoption as a model in ecotoxicological investigations significant. (Ahmad and Ahmad, 2015; Javed et al., 2016). The bioaccumulation of heavy metals in fish occurred via the food chain and they enter to the blood circulation and ultimately settled to organs (Javed and Usmani, 2012). Fish inhabit in close connection with their aquatic system; hence they are very sensitive to chemical and physical changes in water which may be expressed in their hematological indices (Wilson and Taylor, 1993). Hematological parameters of fish are being studied progressively for monitoring the aquatic environmental pollution and toxicological events as a strong indicator of physiological and pathological alterations in the disease examinations (Remyla et al., 2008, Maceda-Veiga et al., 2015). The chemically originated pollutants can influence the hematological parameters of fish. Furthermore, it is reported that hematological parameters are of different sensitivity to different types of chemicals and environmental factors (Sijm et al., 1991).

Hematological indices are considered physiological and pathological indexes of the entire body, hence they are significant in diagnosing the health condition of fish introduced to toxicants (Adhikari et al., 2004). Therefore, Blood parameters like hemoglobin (Hb), red blood cells (RBCs), white blood cell (WBC), platelet counts, packed volume cell (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and erythrocyte sedimentation rate (ESR) have been used as an important marker of heavy metals contamination in the water body (Shah and Altindag, 2004).

The manual method such as the microhaematocrit centrifuge is the common method used to estimate hematocrit value (Bull et al., 2000). The use of automated hematology analyzers has rapidly got acceptance over the last three decades (Kaznowska and Bystryk , 2011; Chhabra, 2018). The automated hematology analyzer has better accuracy than the manual method (Gebretsadkan et al., 2015). Hence the automated hematology analyzer was used in the present hematological study. Using the linear regression analysis method we found the best-fitted line corresponding to sample values with time (Das, 2009). We used linear regression to predict the long-term effect of Cr (VI) on the hematological parameters of *O. niloticus*. The linear regression was used in fish toxicology analysis in lot of previous research (Suter et al.,1987).

Since the hematological indices used to assess mammalian health have not yet been sufficiently established for use in fish hematology examinations, advanced diagnostic approaches are necessary for fish health status. Hematological analysis can provide intrinsic diagnostic information after reference values are established. However, there are no critical experiments demonstrating the alteration in hematological indices of *Oreochromis niloticus* exposed to hexavalent chromium. Hence, the objective of this study was to assess the long-term impact of Cr (VI) sublethally on the hematological parameters of *O. niloticus*. We applied linear regression for this purpose.

2. Materials And Methods

The test specimen, Nile tilapia*, O. niloticus* were collected from Amda beel, (23º11'N, 88º32'E), Ranaghat, West Bengal, India. There are no industries around the fish collection region. Fishes were

acclimatized to the aquarium conditions for 21 days before the test in a dechlorinated water glass aquarium (80 L) with sufficient lighting arrangement. The aquarium water was exchanged daily basis and fish were properly fed twice a day. The fishes were maintained on a 14 hours light/10 hours dark photoperiod. *O. niloticus* measuring 13 to 15cm in length and 90 to 100 gms weight were used in the test. *O. niloticus* was used as the experimental animal in this test since it has rightly documented common biology, short-term development, easily cultured, and yearly reproduction features, hence *O. niloticus* was considered an appropriate model for the toxicity test.

Chemicals

Potassium dichromate ($K_2Cr_2O_7$) [Merck Life Science Private Limited, Vikhroli (East), Mumbai (No. DJOD701528)] was used for this test. The stock solution of potassium dichromate was prepared in distilled water. The toxicity of potassium dichromate to *O. niloticus* was measured using a static-renewal method (APHA, 1989). Required concentrations of potassium dichromate were made from potassium dichromate stock solution. The toxicant, potassium dichromate in the experimental glass aquarium was changed totally with new solution of the identical concentration every 24 hours.

Study Design

The physicochemical properties of the water were determined following the APHA method (APHA, 1989) and were as follows: water temperature 34.1±1.314°C, pH 6.67±0.170, total hardness (as $CaCo_3$) 160.52±25.564 mg/L, TDS 818.5±12.974, total alkalinity 68.42±9.340 mg/L, Free CO_2 13.92±3.045, and dissolved oxygen 7.82±0.736mg/L. Previously, we determined that the LC_{50} value of the Potassium dichromate as hexavalent chromium was 93.49 mg/l [Ghosh and Saha, 2022]. Chronic toxicity studies were conducted for 7, 15, and 30 days with five replicates of each treatment. All the glass aquariums were filled with 80L of water and 10% of the median lethal concentration (LC_{50}) of Potassium dichromate as hexavalent chromium i.e., 9.349 mg/l was added to each glass aquarium. Another experimental setup was all the glass aquariums were filled with 80L of water and 20% of the LC_{50} value of Potassium dichromate i.e., 18.698 mg/l was added. Ten fish were introduced into all glass aquariums. The Toxicant, $K_2Cr_2O_7$ was replaced daily basis in all the glass aquariums.

The blood was collected directly from the heart by cardiac puncture of 7-, 5-, and 30-days treatment for both 10% and 20% of LC_{50} value of $K_2Cr_2O_7$ as well as control live fish. The blood was taken in EDTA containing vial which was kept in an ice-cold medium (Mgbenka et al., 2003). The collected blood samples were used to evaluate the hematological indices. The hematological parameters such as RBC, Hb, WBC, PCV, MCV, MCH, MCHC, platelet counts, and ESR value were estimated by Mindray BC-2800Vet automated hematology analyzer following the manufacturer's instructions (Ode et al., 2017). In the last three decades, the use of automated hematology analyzers progressively achieved acceptance in the research field (Kaznowska and Bystryk , 2011). The automated hematology analyzer has provided better precision than the manual methods (Gebretsadkan, 2015).

Statistical Analysis

1. ANOVA is used to analyze the data.
2. Linear Regression is used to analyze the trend of toxicity on different hematology parameters.

Equation of Linear Regression

Let there be n-data points having values (xi,yi), i=1,2,...n. The based line, $\hat{y} = \hat{m}x_i + \hat{b}$, to fit the scatter plot as best as possible and then use this line to predict values of y for given values of x_i. Estimate the values of m and b by using the least-square criterion, specially, find numbers $\hat{m}$ and $\hat{b}$ which minimize sum of squared residuals, distances betwen observed y_i and the line, $y_{i-}\hat{y}_i$,

$$S = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2 = \sum_{i=1}^{n}\left(y_i - (\hat{m}x_i + \hat{b})\right)^2.$$

Take the derivative of S with respect to both $\hat{m}$ and $\hat{b}$, set them equal them equal to 0, and solve for $\hat{m}$ and $\hat{b}$ in terms of the x and y coordinates:

$$\frac{dS}{d\hat{m}} = \sum 2(y_i - \hat{m}x_i - \hat{b})(-x_i) = 0$$

$$\frac{dS}{d\hat{b}} = \sum 2\left(y_i - \hat{m}x_i - \hat{b}\right)(-1) = 0$$

Solving the above two relations we obtain

$$\hat{m} = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{n\sum x_i^2 - (\sum x_i)^2}, \quad \hat{b} = \bar{y} - \hat{m}\bar{x}.$$

The corresponding regression coefficient (R) is defined by $\frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2}\sqrt{\sum(y_i-\bar{y})^2}}$ for the best fitted line the value of regression coefficient satisfies the relation **$0.5<R^2<1$**. [Das (2009)]

3. Results and Discussion

Hb, RBC, WBC, platelet counts, PCV, MCV, MCH, MCHC, and ESR in Nile tilapia, *O. niloticus* exposed to 10% (9.349 mg/l) and 20% (18.698 mg/l) of the LC_{50} value of 96 hours of hexavalent chromium as Potassium dichromate for 7, 15, and 30 days are given **in (Table 1 and 2). The** results revealed that values of Hb%, RBC, MCV, and platelet count levels were significantly (P > 0.05) decreased and the total count of WBC was significantly (P > 0.05) increased with increasing time of exposure at a concentration of 9.349 mg/L of $K_2Cr_2O_7$ in the test groups compared to the control group. At the concentration of 18.69 mg/L, Hb%, RBC, MCV, mean corpuscular haemoglobin concentration (MCHC), and platelet count level were significantly (P > 0.05) decreased and the total count of WBC and erythrocyte sedimentation rate (ESR) value were significantly (P > 0.05) increased with the increasing time of exposure in the test groups compared to the control group.

The highest value of Hb% (12.6±0.812), RBC (1.6±0.070), PCV (28.8±5.863), MCV (174±10.690), MCHC (39.9±5.739), Platelet count (2.4±0.412) were found in control group and highest the value of MCH (43.1±7.605), ESR (27±5.431), WBC (42600±1978.635) were recorded after 30 days of treatment of $K_2Cr_2O_7$ at the concentration of 9.349 mg/L. The minimum value of WBC (16500±1124.722), ESR (16±3.741) were found in the control group. The minimum value of MCH (39.3±9.267), MCHC (29.6±5.863) after 7 days of treatment and Hb% (8.2±0.628), RBC (1.1±0.1), PCV (20.1±3.546), MCV (136.8±12.9), Platelet count (0.31±0.041) after 30 days of treatment were recorded in exposed fish at the concentration of 9.349 mg/L.

The results revealed that Hb%, RBC, PCV, MCV, and Platelet count values decreased gradually from the control medium to 30 days of treatment. ESR and total count of WBC increased gradually from the control medium to 30 days of treatment when fish is exposed at the concentration of 9.349 mg/L. The highest the value of ESR (30±5.431), WBC (34020±5179.479) were recorded after 30 days of treatment of $K_2Cr_2O_7$ at the concentration of 18.698 mg/L. The minimum value of Hb% (7.72±0.465), RBC (1.02±0.083), PCV (20.24±2.076), MCV (132.92±12.584), MCH (35.66±8.619), MCHC (26.54±3.673), Plateletlet (0.828±0.218) after 30 days of treatment were recorded in exposed fish at the concentration of 18.698 mg/L. The results revealed that Hb%, RBC, PCV, MCV, and MCHC, Platelet count values decreased gradually from the control medium to 30 days of treatment. ESR and total count of WBC increased gradually from the control medium to 30 days of treatment when fish is exposed at the concentration of 18.698.

Table-1: Effect of 9.349 mg/L concentration of Potassium dichromate on haematological parameters of Oreochromis niloticus at different days of exposure

Hematological Parameters	Control Medium	On 7 Days Treatment	On 15 Days Treatment	On 30 Days Treatment
HB (%)	12.6±0.812	9.5±1.286	8.8±0.681	8.2±0.628
RBC (10^6mm^3)	1.6±0.070	1.4±0.1	1.2±0.158	1.1±0.1
PCV (%)	28.8±5.863	23.8±5.440	22.26±3.907	20.1±3.546
MCV (fL)	174±10.690	149.1±15.057	138.1±11.782	136.8±12.9
MCH (pg)	40.4±12.430	39.3±9.267	41.9±10.657	43.1±7.605
MCHC (g/L)	39.9±5.739	29.6±5.863	30.3±9.069	31.5±4.904
PLATELET COUNT (10^6mm^3)	2.4±0.412	1.6±0.608	1.1±0.447	0.31±0.041
ESR (mm/1st hr)	16±3.741	20±5.477	22±4.898	27±5.431
WBC (per Cumm)	16500±1124.722	21930±1091.787	31300±3265.731	42600±1978.635

Table-2: Effect of 18.698 mg/L concentration of Potassium dichromate on haematological parameters of Oreochromis niloticus at different days of exposure

Hematological Parameters	Control Medium	On 7 Days Treatment	On 15 Days Treatment	On 30 Days Treatment
HB (%)	12.6±0.812	10.3±0.777	9.02±0.952	7.72±0.465
RBC (10^6mm^3)	1.6±0.070	1.36±0.114	1.12±0.130	1.02±0.083
PCV (%)	28.8±5.863	25.16±4.824	23.44±4.104	20.24±2.076
MCV (fL)	174±10.690	142.3±16.374	139.72±7.298	132.92±12.584
MCH (pg)	40.4±12.430	41.2±9.959	37.48±8.436	35.66±8.619
MCHC (g/L)	39.9±5.739	31.74±7.266	28.52±4.473	26.54±3.673
PLATELET COUNT (10^6mm^3)	2.4±0.412	1.52±0.389	1.24±0.433	0.828±0.218
ESR ($mm/1^{st}$ hr)	16±3.741	22±7.6485	25±4.743	30±5.431
WBC (per Cumm)	16500±1124.722	20080±2015.44	26780±4813.211	34020±5179.479

Trend Analysis

In this part of the paper, we shall relate different blood parameters with the effect of hexavalent chromium at different sublethal concentration of different days on Nile tilapia, *O. niloticus* with Linear Regression analysis. (Table-3 & 4) First, we consider the value of HB, RBC, PCV, MCH, MCHC, Platelet count, ESR, and WBC with sublethal concentration of hexavalent chromium, 9.43 mg/L (10% of LC_{50} of 96 hour**)** and 18.698 mg/L (20% of LC_{50} of 96 hour**)** for 7 days, 15 days, 30 days treatment, and control medium.

Let us denote the characteristic Hematological parameter as Y(x) and the number of days by *x* then the corresponding best fitted line can be presented as Y=f(x). We have presented the best fitted line corresponding to different Hematological parameters with 7, 15, 30 days at the 10% and 20% of LC_{50} of 96 hour of Hexavalent chromium concentration in Table-3 & 4 (the corresponding best fitted lines in each case is given in Fig: A-R) using the said notation.

Table-3: Regression analysis between Hematological parameters with 7, 15, 30 Days at the 10% of LC50 of 96 hour of Hexavalent chromium concentration

Parameters	Linear Regression Equation
HB	$f(x)= -0.1089x + 10.9009$; $R^2 = 0.9716$
RBC	$f(x)= -0.0137x + 1.4048$; $R^2 = 0.8431$
PCV	$f(x)= -0.2138x + 26.6532$; $R^2 = 1$
MCV	$f(x)= -0.4137x + 145.4836$; $R^2 = 0.9936$
MCH	$f(x)= -0.2255x + 42.0224$; $R^2 = 0.8696$
MCHC	$f(x)= -0.214x + 32.6428$; $R^2 = 0.9065$
PLATELET COUNT	$f(x)= -0.0298x + 1.7117$; $R^2 = 0.9959$
WBC	$f(x)= 590.2445x + 1.67291E + 04$; $R^2 = 0.9772$
ESR	$f(x)= 0.346x + 19.6699$; $R^2 = 0.9991$

Table-4: Regression analysis between Hematological parameters with 7, 15, 30 Days at the 20% of LC50 of 96 hour of Hexavalent chromium concentration

Parameters	Linear Regression Equation
HB	$f(x)= -0.0544x + 9.7763$; $R^2 = 0.9531$
RBC	$f(x)= -0.0122x + 1.4452$; $R^2 = 0.8732$
PCV	$f(x)= - 0.1587x + 24.8042$; $R^2 = 0.994$
MCV	$f(x)= -0.4773x + 149.6059$; $R^2 = 0.6801$
MCH	$f(x)= 0.1543x + 38.7592$; $R^2 = 0.86$
MCHC	$f(x)= 0.0823x + 29.0406$; $R^2 = 0.9995$
PLATELET COUNT	$f(x)= -0.0556x + 1.9679$; $R^2 = 0.998$

WBC	f(x)= 880.0367x + 1.6689E + 04; R^2=0.9856
ESR	f(x)= 0.3081x + 17.6601; R^2 = 0.9953

It is clear from the values of R^2 from Table-3 and Table-4 (where regression lines for hematological parameters with 7, 15, 30 Days at the 10% and 20% of LC_{50} of 96 hour of hexavalent chromium concentration are presented) that the least lines are highly fitted with the data points. To study the R^2 value of the linear regression equation, we can predict the value of Hb%, RBC, PCV, MCV, MCH, MCHC, Platelet count, ESR, and WBC with linear regression graph at 60 and 90 days of both the 10% and 20% of LC50 of 96 hours of hexavalent chromium concentration (Figs: A-R).

It is clear from the Fig: A-I that the value of HB, RBC, PCV, MCV drastically decreased and MCH, ESR and WBC drastically increased at the concentration of 9.349 mg/L in 90 day's experimental group compared to control group. Platelet count drastically reduced to 0 at 35 days treatment at the concentration of 9.349 mg/L. It is also clear from the Fig: J-R that the value of HB, RBC, PCV, MCV, MCH, MCHC drastically decreased and ESR, WBC drastically increased at the concentration of 18.698 mg/L in 90 day's experimental group compared to control group. Platelet count drastically reduced to 0 at 57 days treatment at the concentration of 18.698 mg/L mg/L. Only MCH value shows different behaviour as its value increased at the concentration of 9.349 mg/L and decreased at the concentration of 18.698 mg/L at 90 day's experimental group compared to control group.

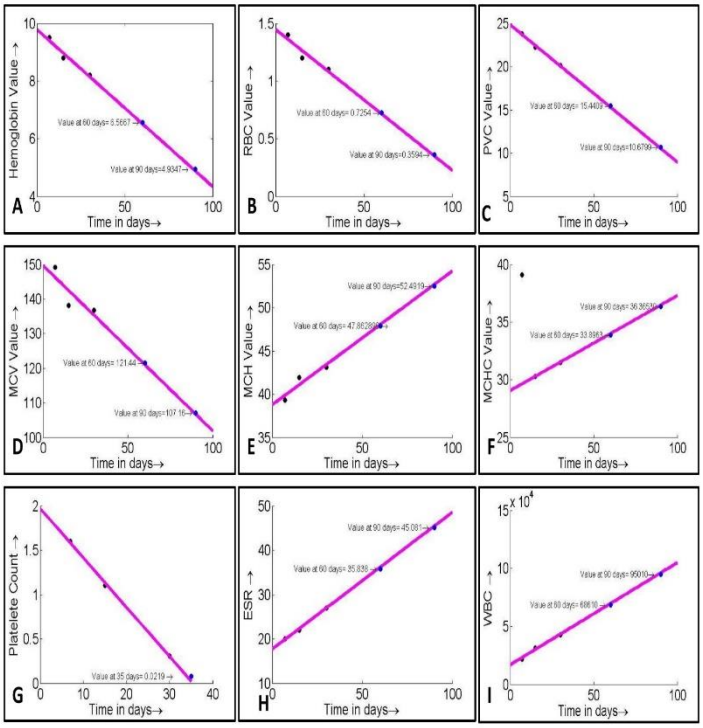

Fig: (A- I): Least square Regression line at 60 and 90 days of the 10% of LC_{50} of 96 hours of hexavalent chromium concentration; (A) Haemoglobin value-Time, (B) RBC vaule-Time, (C) PCV value-Time, (D) MCV value- Time, (E) MCH value-Time, (F) MCH value-Time, (G) Platelet count value – Time, (H) ESR value-Time, (I) WBC value – Time.

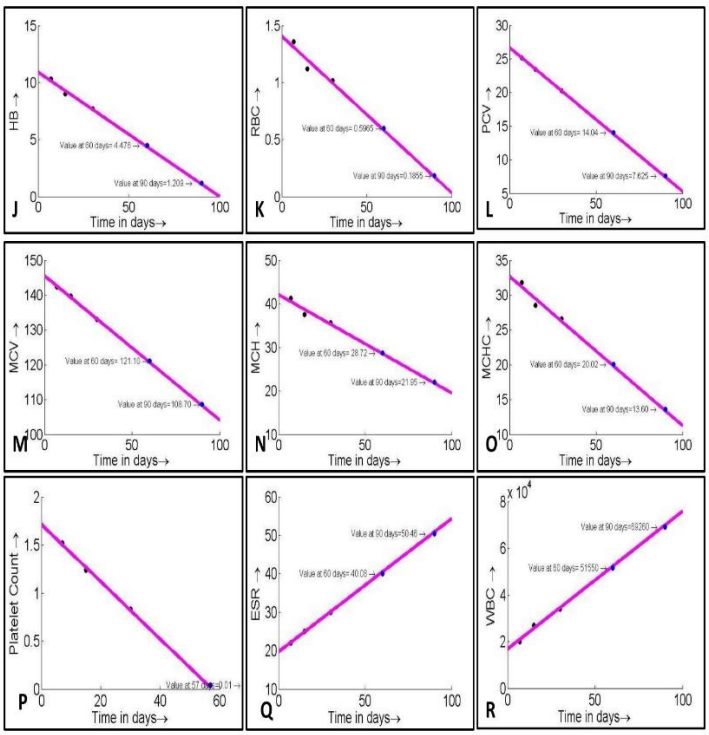

Fig: (J- R): Least square Regression line at 60 and 90 days of the 20% of LC_{50} of 96 hours of hexavalent chromium concentration; (J) Haemoglobin value-Time, (K) RBC vaule-Time, (L) PCV value-Time, (M) MCV value- Time, (N) MCH value-Time, (O) MCHC value-Time, (P) Platelet count value – Time, (Q) ESR value-Time, (R) WBC value – Time.

Hexavalent chromium is one of the most harmful pollutants to aquatic life. Hence, we aimed to investigate the chronic toxicity of hexavalent chromium considering its effects on hematological parameters in Nile tilapia, *O. niloticus*. Several changes were observed in hematological parameters at different concentrations of hexavalent chromium of different periods of exposures, which indicates its stressful effects on physiology in the *O. niloticus*. There were Several studies on the evaluation of the acute toxicity of chromium in different kinds of fish species. In the current study, the LC_{50} value of 96 hours of $K_2Cr_2O_7$ was 93.49 mg/L in *O. niloticus* which is more or less similar to some previous studies (Krumschnabel and Nawaz, 2004; Velma et al., 2009). The toxicity range of chemicals depends on the physiological conditions of the experimental fish exposed in chemicals, habitat, and the purity of the chemicals (Sial et al., 2009).

In the current experiment, significant alterations of the haematological parameters of *O. niloticus* were observed. Generally, toxicants exposure exerts an adverse effect on the hematopoietic organs which in turn alters blood parameters. Due to their relationship with the body's defense mechanisms and respiration, haematological indices serve as physiological indicators of an environment that is changing (Nwani et al., 2014; Beyea et al., 2005). The hematological indices, like Hb, RBC, PCV and total WBC counts and other parameters like MCH, MCV, and MCHC may be sensitive to various types of pollutants because they are closely interacting with the external environment and they are used to measure the physiological condition of animals (Adhikari et al., 2004).

Previous studies have shown that heavy metals that enter the aquatic environment have a unique harmful effect on fish haematological parameters, which is consistent with our current findings (Javed, et al., 2016; Chaudhary, et al., 2023). Our results are in strong acceptance with earlier research that revealed a significant reduction in the value of Hb, RBC, and PVC of freshwater fish introduced to heavy metals (Vutkuru, 2005; Majharul Islam et al., 2020; Sen et al., 1992). The reduction in RBC value may be due to hemolysis or a low rate of RBC production (Zutshi, et al., 2010; Shaheen and Akhtar, 2012). The alteration of hematological parameters may be a result of erythropoiesis being triggered as a defense

mechanism against the toxicity of heavy metals. The decline in haematological parameters proved the toxicity of Cr (VI) that affects the hemopoietic activities of *O. niloticus.*

A significant decrease in Hb concentrations of *Tilapia sparrmanii* after Chronic exposures of 28 days at the concentration of 0.098 and 3.2 mg/l of hexavalent chromium (Remyla et al., 2008). It was reported that a reduction of Hb level indicates the anemic state of the fish, because of iron deficiency and the following declined used for Hb synthesis (Vosylienė, 1999). The decline in Hb value seems to rely on both concentration and duration and could be caused by changes in haemoglobin synthesis. However, hemolysis or the inhibition of the hemoglobin-producing enzyme, which results in the interaction of Hb with plasma haptoglobins, was the likely cause of the drop in total RBC count and Hb concentration in the heavy metal-exposed fish. The harmful effects of heavy metals on the gills and a decrease in oxygen value are two typical factors that contribute to the decrease in Hb concentration (Vinodhini and Narayanan, 2009).

A report revealed that PVC value decreased in *Tilapia sparrmanii* due to chronic exposure of hexavalent chromium (Remyla et al., 2008). A decline in the PVC level indicates the weakening of an organism condition and create an anemic condition (Gill and Pant, 1985). After exposure to cadmium for 15 and 45 days, it was discovered that the MCHC level of *O. niloticus* had significantly decreased (Koprucu et al., 2006). The decreased value of MCH and MCHC is positive evidence of faulty Hb synthesis in the fish. Alteration in MCH, MCHC, and MCV value due to heavy metal exposure that is directly linked to the structural degradation of the RBC membrane, resulting in hemolysis and impeding Hb production. (Wiersma et.al., 1998).

In the present study, the total WBC count increased after exposure to Cr (VI) may be due to stimulation for its defense from diseases (Singh and Tandon, 2009). The rise in total WBC count seen in the current study was also documented in the blood of fish exposed to heavy metals, suggesting that the bone marrow may have been affected by the toxicity of heavy metals (Murugesen and Haniffa, 1985). In the present study, with the increasing concentration of Cr (VI) and experiment time, the total WBC count increased. An increase in total WBC value following exposure to Cr (VI) appears to be concentration and time-dependent and may indicate a stress response that causes an increase in splenic cell production. The significant increases in total WBC counts might be due to the increased production of antibody (Anandkumar, et al., 2001; Raphael and Kuttan, 2003), which helped the organism in healing and survival during exposure to toxic contaminants (Begg and Pankhurst, 2004). This increase in WBC also might be due to leukocytosis under chemical stress caused by Cr., resulting from stimulation of immunological resistance (Marti et al., 1996).

The ESR increased significantly in the exposed fish with increasing time and this may conclude the concept that immature RBC are being released into the blood circulation after chronic Cr (VI) exposure, an increase in serum globulins or fibrinogen or a decrease in serum albumin, may also cause an increase in the ESR (Singh and Tandon, 2009). Increase in ESR and total WBC count recommended that the anaemia was of macrocytic type (Sampath et al., 2011; Kumari et al., 2016). The decrease in the level of Hb, PCV, and platelet count in the present investigation indicated a hemodilution mechanism probably due to gill damage. It was reported that Similar results with a significant decline of RBC, Hb, PCV, and platelet counts in fishes which were exposed to different types of heavy metals (Allin and Wilson, 2000; Singh, D., Nath, 2008). In this study, haematological parameters appeared to be decreased due to the low cell count in the blood after Cr (VI) exposure.

To study the R^2 value of the linear regression equation, we found that the trend of the chronic toxic effect of Cr (VI) at 60 and 90 days of both the sublethal concentrations 9.349 mg/l and 18.69 mg/l of potassium dichromate, where the value of Hb, RBC, PCV, MCV, platelet count are found to reduce drastically and the value ESR and WBC are found to reduce drastically at both the sublethal concentrations. We also found the platelet count reduced drastically to 0 at concentration of 9.349 mg/L on 35 days and at concentration of 18.69 mg/l on 57 days. To analysis the linear regression equations, we predict that hexavalent chromium has the chronic effect on hematological parameters of *O. niloticus*. Present the study clearly shows that the alterations in hematological parameters depend on the concentration of hexavalent chromium and duration of exposure to *O. niloticus*. The toxicant entering

into fish body are slowly eliminated (James et al., 1996), and hence the hematological profiles got effected on account of pollutant toxicity.

4. Conclusion

The toxicity of hexavalent chromium was assessed using the hematological status of *Oreochromis niloticus*. Chronic exposure of hexavalent chromium confirmed to be highly toxic to *Oreochromis niloticus* and induced cumulative deleterious effects at haematological profiles. From the current study, we confirmed that hematological parameters are highly sensitive indicators for determining the toxicity of hexavalent chromium to freshwater fish. The negative consequences of hexavalent chromium reveal the anaemia of fish species. We presume that alterations in hematological parameters may be a defensive action against the toxicity of hexavalent chromium through the triggering of leucopoiesis. The results of the current study reveal the knowledge about hexavalent chromium toxicity in aquatic organisms and also found that hematological parameters are sensitive marker to evaluate the toxicity test and importance to show the awareness of the pollution in aquatic ecosystem.

Ethical Statement

Fish samples were handled as per the animal care protocol of the West Bengal State University and the methods used to analyse Cr toxicity in fish blood was carried out in accordance with relevant national and international guidelines and regulations.

Declaration of Competing Interest

The authors declare no competing interest.

Acknowledgment

The authors are thankful to Head, the Department of Zoology, West Bengal State University, for providing the necessary facilities for this research and thankful to Dr. Uttam Ghosh, Associate Professor, Department of Applied Mathematics, University of Calcutta for his help to develop the trend analysis using linear regression.

References:

K.M.A. Ullah, M.A. Maksud, S.R. Khan, L.N. Lutfa, S.B. Quraishi, Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh, Toxicol. Rep. 4 (2017) 574–579.

Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S. 2004. Effects of cypermethrin and carbofuran on haematological parameters and prediction of their recovery in a freshwater teleost, Labeorohita (Hamilton, 1849). Ecotoxicology and Environmental Safety 58: 220–226. DOI: 10.1016/j.ecoenv.2003.12.003

Ahmad I, Ahmad M (2015) Freshwater fish, Channa punctatus, as a model for pendimethalin genotoxicity testing: a new approach toward aquatic environmental contaminants. Environ Toxicol. doi:10.1002/tox.22156

Allin. C.J., Wilson. R.W. 2000. Effects of pre-acclimation to aluminium on the physiology and swimming behavior of juvenile rainbow trout (Oncorhynchus mykiss) during a pulsed exposure. Aquat Toxicol 51(2):213–224, DOI: 10.1016/s0166-445x(00)00111-9

Anandkumar, A., Tripathy, A.P., Tripathy, N.K. 2001. Effect of dimecron on the blood parameters of Heteropneustes fossilis, J. Environ. Biol. 22 (2001) 297e299.

APHA (1989) Standard Methods for Examination of Water and Wastewater. 17th Edition, American Public Health Association, Washington DC.

Bashir, M.S., Ramzan, N., Najam, T., Abbas, G., Gu, X., M. Arif, M. Qasim, H. Bashir, S.S.A. Shah, M. Sillanp. 2022. Metallic nanoparticles for catalytic reduction of toxic € hexavalent chromium from aqueous medium: a state-of-the-art review, Sci. Total Environ. (2022), 154475, https://doi.org/10.1016/j.scitotenv.2022.154475.

Begg, K., Pankhurst, N.W., 2004. Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish Acanthochromis polyacanthus, J. Fish Biol. 64 (2004) 133–145. https://doi.org/10.1111/j.1095-8649.2004.00290.x

Beyea, M.M., Benfey, T.J., Kieffer, J.D. 2005. Hematology and stress physiology of juvenile diploid and triploid shortnose sturgeon (Acipenser brevirostrum), Fish Physiol. Biochem. 31, 303–313.

Bull, BS, Koepke, JA, Simson E & van Assendelft OW (2000). Procedure for Determining Packed Cell Volume by Microhematocrit Method; Approved Standard, third edition. Clinical and laboratory standards institute. Document, NCCLS 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087- 1898, USA. Vol 20(18): 1-7, https://clsi.org/media/2392/h07a3e_sample.pdf

Chakraborty. S. B. and S. Banerjee, 2009. Culture of monosex Nile tilapia under difference traditional and non-traditional methods in India. World Journal of Fish and Marine Sciences 1(3):212-217.

Chaudhary, A., Javaid, G. K., Bughio , E., Faisal, N. 2023. Study on histo-biochemical biomarkers of chromium induced toxicity in Labeo rohita, Emerging Contaminants, 9(2023) 100204, 2405-6650, doi: https://doi.org/10.1016/j.emcon.2023.100204

Chhabra, G. 2018. Automated hematology analyzers: Recent trends and applications, J Lab Physicians. 2018 Jan-Mar; 10(1): 15–16. doi: 10.4103/JLP.JLP_124_17

Das, N. G. "Statistical Methods (Volume I & II)." 2009. 978-0. https://www.springerprofessional.de/en/representation-of-geographical-data-using-graphs/19912042

Farombi, E., Adelowo, O., Ajimoko, Y. 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River, Int. J. Environ. Res. Public Health 4 (2007) 158–165.

Gebretsadkan G, Tessema K, Ambachew H, Birhaneselassie M. 2015. The Comparison between Microhematocrit and Automated Methods for Hematocrit Determination. Int J Blood Res Disord 2:012, https://clinmedjournals.org/articles/ijbrd/ijbrd-2-012.pdf

Ghosh, D., Saha, S.K. 2022. Determination of the Lethal Concentration 50% (LC50) of Hexavalent Chromium in Nile Tilapia (Oreochromis niloticus). Advances in Zoology and Botany, 10(4), 123 - 131. DOI: 10.13189/azb.2022.100406.

Gill, T.S., Pant, J.C. 1985. Erythrocytic and leukocytic responses to cadmium poisoning in a freshwater fish, Puntius conchonius Ham. Environmental Research. 36 (2):327 -37. https://doi.org/10.1016/0013-9351(85)90028-

Gretchen L. Grammer1 *, William T. Slack2 , Mark S. Peterson3 and Mark A. Dugo, Nile tilapia Oreochromis niloticus (Linnaeus, 1758) establishment in temperate Mississippi, USA: multi-year survival confirmed by otolith ages, Aquatic Invasions (2012) Volume 7, Issue 3: 367–376 doi: http://dx.doi.org/10.3391/ai.2012.7.3.008

James, R., Sampath, K. and Alagurathinam, S. 1996. Effects of lead on respiratory enzyme activity glycogen and blood sugar levels of the teleost, Oreochromis mossambicus (Peters) during accumulation and depuration. Asian Fish. Sci., 9:87-100. https://doi.org/10.33997/j.asf.1996.9.2.001

Javed M, Ahmad I, Usmani N, Ahmad M (2016) Bioaccumulation, oxidative stress and genotoxicity in fish (Channa punctatus) exposed to a thermal power plant effluent. Ecotoxicol Environ Saf 127:163–16, DOI: 10.1016/j.ecoenv.2016.01.007

Javed M, Usmani N (2012) Toxic effects of heavy metals (Cu, Ni, Fe Co, Mn, Cr, Zn) to the haematology of Mastacembelus armatus thriving in Harduaganj Reservoir, Aligarh, India. Glob J Med Res 12:59–64

Javed, M., Ahmad, I., Ahmad, A., Usmani, N., Ahmad, M. 2016. Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in Channa punctatus (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent, SpringerPlus (2016) 5:761 DOI 10.1186/s40064-016-2478-9

Kaznowska and Bystryk. 2011. The automated hematology analyzers. Annales Universitatis Mariae Curie-Slodowska Lublin, Polania, 24 (3): 7.

Köprücü, S.Ş., Köprücü, K., Ural, M.Ş., İspir, Ü., Pala, M. 2006. Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (Silurus glanis L.), Pesticide Biochemistry and Physiology, Volume 86, Issue 2, 2006, Pages 99-105, ISSN 0048-3575, https://doi.org/10.1016/j.pestbp.2006.02.001.

Krumschnabel, G., Nawaz, M. 2004. Acute toxicity of hexavalent chromium in isolated teleost hepatocytes, Aquat. Toxicol. 70 (2004) 159–167.

Kumari, B., Kumar, V., Sinha, A,K., Ahsan, J., Ghosh, A.K., Wang, H , DeBoeck, G. 2016. Toxicology of arsenic in fish and aquatic systems, Environ Chem Lett, DOI 10.1007/s10311-016-0588-9

Maceda-Veiga, A., Figuerola, J., Martínez-Silvestre, A., Viscor, G., Ferrari, N., Pacheco, M. 2015. Inside the Redbox: applications of haematology in wildlife monitoring and ecosystem health assessment, Sci. Total Environ. 514 (2015) 322e332, https://doi.org/10.1016/j.scitotenv.2015.02.004

Marti, H.H., Wenger, R.H., Rivas, L.A. 1996. Erythropoietin gene expression in human, monkey and murine brain, Eur. J. Neurosci. 8, 666–676. DOI: 10.1111/j.1460-9568.1996.tb01252.x

Menaga M. Fitzsimmons, K. 2017. Growth of the Tilapia Industry in India, WORLD AQUACULTURE, 2017, p. 49-52

Mgbenka, BO. and Oluah NS et al, 2003. Effect of Gammalin 20 (Lindane) on differential white blood cell counts of the African catfish, Clarias albopunctatus. Bull of Environmental Contamination Toxicol. 71: 248 – 254. DOI: 10.1007/s00128-003-0157-3

Mohammed, K., Sahu, O. 2019. Recovery of chromium from tannery industry waste water by membrane separation technology: health and engineering aspects, Sci. Afr. 4, e00096, https://doi.org/10.1016/j.sciaf.2019.e00096.

Monterio, M.I.C., Fraga, I.C.S., Yallouz, A.V., de Oliveria, N.M.M., Riberio, S.H. 2002. Determination of total chromium traces in tannery effluents by electrothermal atomic absorption spectrometry, flame atomic absorption spectrometry and UV visible spectrophotometric methods, Talanta 58 (2002) 629–633.

Murugesan, A & A, Ramathilaga & Haniffa, Mohammed. (2013). Haematotoxicity of Integrated Textile Mill Effluent to an Air-Breathing Fish Heteropneustes fossilis (Bloch). Bulletin of environmental contamination and toxicology. 90. 10.1007/s00128-012-0946-7.

Mushtaq, F., Chen, X., Veciana, A., Hoop, M., Nelson, B.J., Pane, S. 2022. Magnetoelectric reduction of chromium (VI) to chromium (III), Appl. Mater. Today 26 (2022), 101339, https://doi.org/10.1016/j.apmt.2021.101339.

N.K. Kortei, M.E. Heymann, E.K. Essuman, F.M. Kpodo, P.T. Akonor, S.Y. Lokpo, N.O. Boadi, M. Ayim-Akonor, C. Tettey, Health risk assessment and levels of toxic metals in fishes (Oreochromis noliticus and Clarias anguillaris) from Ankobrah and Pra basins: Impact of illegal mining activities on food safety, Toxicol. Rep. 7 (2020) 360–369.

Nwani CD, Mkpadobi BN, Onyishi G, Echi PC, Chukwuka CO, Olua SN, Ivoke N. 2014. Changes in behavior and hematological parameters of freshwater African catfish Clarias gariepinus (Burchell 1822) following sublethal exposure to chloramphenicol. Drug and Chemical Toxicology 37: 107–130. DOI: 10.3109/01480545.2013.834348

P.K. Maurya, D.S. Malik, K.K. Yadav, A. Kumar, S. Kumar, H. Kamyab, Bioaccumulation and potential sources of heavy metal contamination in fish species in river Ganga basin: possible human health risks evaluation, Toxicol. Rep. 6 (2019) 472–481.

P.L.R.M. Palanippan, S. Karthikeyan, Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish Cirrhinus mrigala individually and in binary solutions with nickel, J. Environ. Sci. China 21 (2009) 229–236

Peterson MS, Slack WT, Woodley CM (2005) The occurrence of nonindigenous Nile Tilapia, Oreochromis niloticus (Linnaeus) in coastal Mississippi: ties to aquaculture and thermal effluent. Wetlands 25: 112-121, http://dx.doi.org/ 10.1672/0277-5212(2005)025[0112:TOONNT]2.0.CO;2

R.T. Di-Giulio, D.E. Hinton, The Toxicology of Fishes, Taylor and Francis, 2008, pp. 319–884

Raphael, I.J., Kuttan, G. 2003. Immunomodulatory activity of naturally occurring monoterpenes carvone, limonene, and perillic acid, Immunopharmacol. Immunotoxicol. 25 (2003) 285–294. DOI: 10.1081/iph-120020476

Remyla, S.R., M. Ramesh, K.S. Sajwan and K.S. Kumar. 2008. Influence of zinc on cadmium induced hematological and biochemical responses in a freshwater teleost fish Catla Catla. Fish Physiol. Biochem. 34:169- 174. DOI: 10.1007/s10695-007-9157-2

S M Majharul Islam, Md. Fazle Rohani, Seyed Akib Zabed, Md. Tarikul Islam, Rayeda Jannat, Yeasmin Akter, Md. Shahjahan, Acute effects of chromium on hemato-biochemical parameters and morphology of erythrocytes in striped catfish Pangasianodon hypophthalmus, Toxicology Reports, Volume 7, 2020, Pages 664-670, ISSN 2214-7500, https://doi.org/10.1016/j.toxrep.2020.04.016.

S. Rajeshkumar, X. Li, Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China, Toxicol. Rep. 5 (2018) 288–295.

SA Ode , M Adamu & DI Saror; Determination of haematocrit using Mindray BC-2800Vet® automated haematology analyser and microhaematocrit method: A correlation study, Sokoto Journal of Veterinary Sciences, Volume 15 (Number 2). August, 2017, (P-ISSN 1595-093X/ E-ISSN 2315-6201). DOI: 10.4314/sokjvs.v15i2.9.

Sampath, K., James, R., & Ali, K. M. A. (2011). Effects of copper and zinc on blood parameters and prediction of their recovery in Oreochromis mossambicus (Pisces : Cichlidae). Indian Journal of Fisheries, 45(2), 129–139. Retrieved from https://epubs.icar.org.in/index.php/IJF/article/view/8735

Sen, G., Behera, M.K., Patel, P.N. 1992. Effect of zinc on hemato-biochemcical parameters of Channa punctatus, J. Ecotoxicol. Environ. Monito 2, 89–92.

Shah SL and Altindağ A (2004a) Hematological parameters of tench (Tinca tinca L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull. Environ. Contam. Toxicol. 73: 911–918. DOI: 10.1007/s00128-004-0513-y

Shaheen, T., Akhtar, T. 2012. Assessment of chromium toxicity in Cyprinus carpio through hematological and biochemical blood markers. Turk J Zool 36:682–690. doi:10.3906/zoo-1102-21

Sial, I.M., Kazmi, M.A., Kazmi, Q.B., Naqvi, S.N.H. 2009. Toxicity of biosal (Phytopesticide) and permethrin (Pyrethroid) against common carp, Cyprinus carpio, Pak. J. Zool. 41, 235–238.

Sijm DT, Flenner CK, Opperhuizen A. The influence of biochemical species differences on acute fish toxicity of organic chemicals. Comp Biochem Physiol C Comp Pharmacol Toxicol. 1991;100(1-2):33-5. doi: 10.1016/0742-8413(91)90117-c.

Singh, A. and W. Lakra. 2011. Risk and benefit assessment of alien fish species of the aquaculture and aquarium trade into India. Reviews in Aquaculture 3:3-18.

Singh, B.P., Tandon, P.K. 2009. Effect of river water pollution on hematological parameters of fish, Wallago attu, Res. Environ. Life Sci. ISSN: 0974-4908 page- 211-214

Singh, D., Nath, K., Trivedi, S.P., Sharma, Y.K. 2008. Impact of copper on haematological profile of freshwater fish, Channa punctatus, J. Environ. Biol. ISSN : 0254-8704 29(2), 253-257

Suter, G.W., Vaughan, D.S., Gardner, R.H. 1987. Risk assessment by analysis of extrapolation error: A demonstration for effects of pollutants on fish, Environmental Toxicology and Chemistry, 1552-8618, Volume -2, issue-3, p-369-378, https://doi.org/10.1002/etc.5620020313

Venkatramreddy Velma, S.S. Vutukuru, and Paul B. Tchounwou, Ecotoxicology of Hexavalent Chromium in Freshwater Fish: A Critical Review, Rev Environ Health. 2009 Apr–Jun; 24(2): 129–145. doi: 10.1515/reveh.2009.24.2.129

Vinodhini, M. and Narayanan, P. 2009. Th e impact of toxic heavy metals on the hematological parameters in common carp (Cyprinus Carpio L.) Iranian J. Environ. Health and Sci. 6(1): 23-28.

Vosylienė, M. Z. 1999. The Effect of Heavy Metals on Haematological Indices of Fish (Survey), Acta Zoologica Lituanica, 9:2, 76-82, DOI: 10.1080/13921657.1999.10512290

Vutukuru SS. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, Labeo rohita. Int J Environ Res Public Health. 2005 Dec;2(3-4):456-62. doi: 10.3390/ijerph2005030010. PMID: 16819101.

Wiersma, EJ., Collins, C., Fazel, S., Shulman, MJ. 1998. Structural and functional analysis of J chain -deficient IgM. The Journal of Immunology. 160 (12):5979 -89 . PMID: 9637512

Wilson, R.W. and Taylor, E.W. 1993. The physiological responses of freshwater rainbow trout, Onchorynchus mykiss, during acute exposure. J. Comp. Physiol., 163b: 38-47. DOI: https://doi.org/10.1007/BF00309663

Y. Lu, S. Song, R. Wang, Z. Liu, J. Meng, J.A. Sweetman, A. Jenkins, R.C. Ferrier, H. Li, W. Lou, T. Wang, Impacts of soil and water pollution on food safety and health risks in China, Environ. Int. 77 (2015) 5–15

Zutshi, B., Prasad, S.G.R., Nagaraja, R. 2010. Alteration in hematology of *Labeo rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India. *Environ Monit Assess* 168, 11–19 (2010). https://doi.org/10.1007/s10661-009-1087-2