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ABSTRACT Fish is one of the major sources of protein which meets the demand for higher food production and thus aquaculture plays an important role in food production. But the addition of untreated industrial, agricultural and domestic wastes to the water bodies introduces stress to the aquatic organisms including fishes. Among the pollutants the heavy metal introduced into water bodies have detrimental effect on the physiological characters of the fishes. In the present study effect of nickel has been studied on *Channa punctatus*. The results obtained during the present investigation shows that there was significant changes in blood parameters on exposure to acute and chronic sub-lethal concentration of nickel. The haemoglobin percentage and the haematocrit percentage showed a steady decrease with an increase in the concentration of nickel. The total WBC count showed slight increase at lower concentration and then decreased drastically. The MCV, MCH and MCHC in the present investigation decreased steadily with an increase in the concentration of nickel.

KEYWORDS : Nickel, Haemoglobin percentage, haemacrit percentage, MCV, MCH and MCHC

# INTRODUCTION

Fresh water aquaculture plays an important role to meet the demand for a higher rate of food production. Fishes constitute a major source of protein. Ponds, rivers and lakes constitute the major sources of fresh and brackish water animals and plants, which are highly conditioned to dissolve oxygen, hydrological cycles, pH, temperature and nutrient concentration. The tropic structure and functions of fresh water ecosystem are highly sensitive to introduction of organic and inorganic pollutants. The untreated water drained from industrial, agricultural and domestic sources are usually released into rivers, streams and lakes. These contain a lot of toxic substances, some of them in lethal concentrations. The aquatic environment, where fish and other aquatic organisms live, is subjected to different types of pollutants which enter water bodies through industrial, domestic and agricultural discharge systems thereby introducing stress to living creatures. Heavy metals are nonbio degradable and once discharged into the water bodies accumulate in the aquatic organisms, including fish causing an adverse effect on them. Fish are widely used to evaluate the health of aquatic ecosystem as the physiological changes can serve as biomarkers of environmental pollution. The effects of different pollutants on the aquatic organisms have been studied by many workers.

# MATERIAL AND METHODS

*Channa punctatus* is the commonly available fresh water fish locally known as "Khabsi". It is also called spotted snake head due to presence a no. of spots on its head It is plently available in Sambalpur area which is an air breathing fish. Due to this reason they can be easily kept in the laboratory for a long period of time and effects of Nickel can be studied in different tissues. The specimen weight varies from 30 to 35 gram and length about 10-15 cm.

Prior to experiment specimen were disinfected in 0.01% KMnO<sub>4</sub> and acclimatized to laboratory conditions for 15 days in many large glass aquaria of 40 liters capacity. The water used in aquaria was chlorine free and changed every alternate day. They were fed daily with chopped earthworm but no food was given during experimental period to determine  $LC_{50}$  dose.

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determine LC<sub>50</sub> dose.

## **Haematological Changes**

The experimental fishes Channa punctatus were divided into several batches of 10 each, irrespective of sex and a body weight of 30-35g. Various physiological and biochemical changes in the blood were observed. Four groups of fishes were treated with 10, 20, 30 and 40 ppm Nickel for 30 days and a fifth group of fishes were kept as control at a sub lethal dose of 10 ppm Nickel for 60 days and alternations in the blood parameters were analysed at 15, 30, 45 and 60 days of exposure. The alternations were subjected to ANOVA to ascertain, whether the changes were significant or not.

## **Blood Sampling**

Blood was collected directly from the heart using a well heparnised disposable syringe. That blood was kept in 2 vials, one coat with EDTA and the other uncoated. 1" one was used for estimation of Haemolobin, RBC count and WBC count. Standard haematological methods as described by Dacie and Lewis (2001) were adopted to study the different blood parameters.

# a. Red Blood Count (TEC)

The total count was done by Neubauer's improved double haematocytometer using Hayem's solution as dilution fluid for RB.

#### b. White blood cell count (TLC)

This was done by using Turk's fluid instead of Hayem's fluid.

# c. Determination of mean cell diameter (MCD) and differential count (DC)

The blood smear was examined for measurement of mean cell diameter and differential count. Blood cells were observed under oil immersion magnification in a research microscope. Fifty mature erythrocytes of each blood film were measured and their mean values for longitudinal and transverse diameters were calculated using an eye piece micrometer calibrated with a stage micrometer in relation to the objective (40×) and eye piece (10×) used. The differential count of leucocytes was done by counting 500 cells of uniform blood smears avoiding extreme edges of the slides. The percentage of leucocytes was calculated for such observation.

## d. Determination of Haemoglobin percentage

Sahil's haemoglobinometer was used for the estimation of haemoglobin.

### e. Determination of Haematocrit value

The haematocrit value or PCV was estimated by microhaematocrit method or Wintrobe's tube method (Wintrobe, 1967).

#### f. Measurement of absolute corpuscular values

The mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) are reffered to as 'absolute' values. These values are necessary for classification of anaemia and are calculated from TEC, Hb% and Ht%.

1.MCV=<u>Haematocritic%×10</u> TEC×10<sup>3</sup>/mm<sup>3</sup>

The average volume single red cell is expressed in femolitre (fl)

2. MCH=<u>Haemoglobin %×10</u> TEC×10<sup>3</sup>/mm<sup>3</sup>

The average Hb content in a single red cell and expressed in picogram (pg)

2. MCHC=<u>Haemoglobin%×10</u>

Ht value%

The average Hb conc. In each Red cell expressed as g/I

## RESULT

#### Table 1: Effect of nickel on hematological parameters

		15	30	45	60
Paramet	Contol(0	days(10pp	days(10pp	days(10pp	days(10pp
es	ppm)	m)	m)	m)	m)
TEC	3.1	3.21	2.9	2.86	2.5
Hb %	9.42	9.5	9.5	8.43	7.97
<b>PCV</b> %	23.46	22.98	20.5	19.68	19.06
<b>MCV</b> %	96.51	95.31	94.37	87.31	86.31
MCH %	33.03	32.91	30.73	28.16	26.3
MCHC	6.51	5.98	4.47	3.92	3.4
TLC	6.93	6.51	6.11	5.36	4.78

The results obtained during the present investigation shows that the blood parameters altered on exposure to acute and chronic sublethal concentration of nickel, haemoglobin percentage and the haematocrit percentage showed a steady decrease with an increase in the concentration of nickel (Table 1). The total WBC count showed slight increase at lower concentration and then decreased drastically. The MCV, MCH and MCHC in the present investigation decreased steadily with an increase in the concentration of nickel.

#### DISCUSSION

The haemoglobin percentage and haematocrit values significantly deceased (at 0.05 level) with an increase in the exposure time to a sub-lethal chronic dose of 10ppm nickel, whereas TEC increased upto some days but thereafter decreased at 10 ppm nickel concentration for 60 days. This decrease in RBC count and haemoglobin percentage was due to acute erythroblastic anaemia associated with erythropenia (Behera, 1997). The decrease in PCV as observed during this present investigation was due to the decrease in the RBC count and haemoglobin percentage.

Bagdonas and Vosyliene (2006) have reported that copper and zinc applied separately did not have any effect on RBC and Hb%. But when used together brought about an increase in RBC count in Rainbow trout. Milda and Audrone (2006) were of the opinion that metal model mixture decreased the erythrocyte count in Rainbow trout. Similar results were also reported in other fishes, when the fishes were exposed to various metals like mercury, cadmium, zinc, copper, lead etc. (Panigrahi and Mishra, 1978; Mishra and Srivastava, 1980; Goel and Kalpana, 1985; Tyagi and Srivastava, 2005). steadily with an increase in nickel concentration may be due to the effect of nickel on the haematopoetic organs of mice (Machalinska et.al, 2002). Adeyemo (2007) reported on increase MCV, MCH and MCHC in *Clarias gariepinus* treated with lead. But Vutukuru (2005) reported a decrease in MCH in *Labeo rohita* treated with chromium, which indicates anemia.

## CONCLUSION

The nickel toxicity may also vary significantly among fish species due to other factors such as exposure dose and time (De Boeck et al., 2004) and physiological conditions of the individuals and also water physicochemical parameters. It can be concluded that the hematological parameters are the most sensitive parameters in monitoring the toxicity of nickel especially at sublethal concentrations.

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MCV, MCH and MCHC value decreased significantly (at 0.001 level)