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Toxicological Effects of Cobalt on Freshwater Fish *Channa punctatus (*Bloch.)

Saroj Kumari

Research Scholar Department of Zoology Jai Prakash University Chapra Saran, Bihar, India
Email ID: *ranjansaroj2012[at]gmail.com*

Abstract: ***Cobalt serves as a crucial micronutrient for diverse organisms; however, an overabundance of cobalt can have detrimental effects on aquatic life, particularly fish. Channa punctatus, commonly referred to as the "Northern snakehead" or "Spotted snakehead, " is a freshwater fish species inhabiting various aquatic environments in India. Cobalt is a transition metal widely used in various industrial applications, and its presence in aquatic environments poses a potential threat to aquatic organisms. This paper investigates the toxicological effects of cobalt exposure on Channa punctatus (Bloch.), and understanding the toxicological effects of cobalt for effective environmental management and conservation efforts. The study investigated the impact of different concentrations of cobalt chloride (30, 60, 90, 120, and 150 ppm) on freshwater fish Channa punctatus (Bloch.) over a 96 - hour period. The lethal concentration LC50 was determined to be 340 ppm. Micronuclei (MN) in fish erythrocytes were observed to detect cytogenetic abnormalities, with MN frequencies showing significant increases across varying dose levels. These alterations have the potential to disrupt vital physiological functions such as respiration, osmotic and ionic regulation, nitrogenous waste excretion and absorption, as well as storage and secretion of various enzymes, ultimately impacting the survival and growth of Channa punctatus. The findings strongly indicate that even low concentrations of cobalt chloride (in ppm) are toxic and significantly disrupt major hematological, biochemical, and tissue structures in fishes. Therefore, proactive measures should be taken to prevent cobalt chloride from exceeding permissible limits in aquatic environments to mitigate its detrimental effects.***

Keywords: Cobalt, *Channa punctatus*, Freshwater fish, Toxicity, Oxidative stress, Histopathology, Biomarkers

1. Introduction

Cobalt is an essential micronutrient for various organisms, but excessive exposure to cobalt can lead to adverse effects on aquatic organisms, including fish. Channa punctatus, commonly known as the "Northern snakehead" or "Spotted snakehead. " freshwater fish species found in various aquatic habitats in India. Due to its ecological importance and widespread distribution, *Channa punctatus* serves as an excellent model organism for studying the toxicological effects of environmental contaminants like cobalt. Natural aquatic systems serve as the ultimate recipients of pollutants, a growing concern documented over recent decades. Contamination of aquatic ecosystems with a diverse array of pollutants, stemming from both natural processes like weathering of geological matrices and anthropogenic sources such as industrial effluents and mining wastes, poses a significant threat to biological life. Aquatic animals, particularly fish, play pivotal roles as keystone species in many ecosystems, making them prime indicators of environmental pollution. The global attention of researchers has been drawn to the contamination of aquatic systems. Cobalt, the 33rd most abundant oligo - element, is crucial for the synthesis of vitamin B12 and other cobalamines. It occurs naturally in low concentrations in aquatic environments. Permissible limits for cobalt in various water sources have been established, reflecting its widespread use in alloy metals, paints, glass, ceramics, agricultural additives, and medical products. Tilapia (*Channa punctatus*) stands out as one of the most popular freshwater fish consumed worldwide, renowned for its ability to withstand environmental stressors. Its resilience, disease resistance, and adaptability make it an excellent biological model for toxicological studies. Blood parameters offer valuable insights into the physiological and chemical changes in organisms subjected to treatments. Monitoring blood parameters serves as a diagnostic tool for detecting early signs of pesticide poisoning and assessing the overall health status of fish exposed to toxicants. The erythrocyte micronucleus assay serves as a vital biomarker for genotoxic pollutants, facilitating field surveys, monitoring studies, and comparisons of pollutant levels. It is a preferred bioindicator for environmental mutagenicity studies due to its ability to detect both clastogenic and aneugenic effects. Biochemical changes represent sensitive indicators of an organism's stress response, with high concentrations of biochemical molecules indicating stress and intensive energy utilization. Histopathological studies provide crucial insights into the effects of toxicants on organ health. They serve as cost - effective tools for assessing the health of fish populations and elucidating the functional responses of organisms to sub - lethal concentrations of toxicants. Gills and liver are key organs in assessing the impact of aquatic pollutants on freshwater habitats. The liver, in particular, plays a central role in metabolism, accumulation, biotransformation, and excretion of contaminants in fish. The binding of Co_2+ with gills disrupts various physiological processes such as ionic regulation, acid - base balance, gas transfer, and nitrogenous waste excretion.

2. Literature Review

Numerous studies have evaluated the effects of various chemicals on the behaviors and hematological responses of different fish species. This study focuses on exploring the effects of $CoCl_2$ on *Channa punctatus* (Bloch.) a freshwater fish species.

As highlighted by Ramesh et al. (2009), biochemical changes serve as the most sensitive indicators of an organism's stress state. Elevated concentrations of biochemical molecules in the blood suggest that the fish is experiencing stress and is utilizing its energy reserves intensively, including glycogen stored in the liver and muscles, as discussed by Vijayavel et al. (2006).

Histopathological studies serve as highly sensitive and crucial parameters that reflect the effects of toxicants on organs, as indicated by Abdel - Warith et al. (2011). Historically, histological analysis has been a cost - effective tool for assessing the health of fish populations. Changes in tissues observed in test organisms exposed to sub - lethal concentrations of toxicants represent the functional responses of organisms, offering valuable insights into the nature of the toxicant, as discussed by Das and Mukherjee (2000). The binding of CO_2+ with gills can impede ionic regulation, disrupt acid - base balance, hinder gas transfer, and interfere with nitrogenous waste excretion, as outlined by Evans (1987) and Wood (1992). Various researchers have examined the effects of different chemicals on the behaviors and hematological responses of various fish species, as demonstrated by Benarji and Rajendranath (1990) and Svoboda et al. (2001).

Previous studies have documented the toxic effects of cobalt on various aquatic organisms, including fish. Cobalt toxicity in fish is primarily attributed to its ability to disrupt essential biological processes, such as oxidative stress, ion transport, enzyme inhibition, and DNA damage. The uptake of cobalt by fish occurs primarily through the gills and gastrointestinal tract, leading to systemic distribution and accumulation in various tissues. Histopathological examinations have revealed tissue damage, including alterations in the liver, gills, kidney, and brain, following cobalt exposure in fish species.

3. Methodology

To investigate the toxicological effects of cobalt on Channa punctatus, experimental exposure studies were conducted in controlled laboratory conditions. Fish were exposed to varying concentrations of cobalt solution in tanks equipped with aeration and temperature control. Physiological parameters, such as oxygen consumption, ammonia excretion, and antioxidant enzyme activities, were measured to assess the impact of cobalt exposure on fish metabolism and oxidative stress. Histopathological analysis of fish tissues was performed to evaluate morphological changes associated with cobalt toxicity.

Fish maintenance

Fresh water fishes *Channa punctatus* (Bloch.) were collected from the Jai Prakash University campus pond (lat.25.7797° N'; long.84.7533° E), Chapra Saran District, (North Bihar). Fishes were acclimatized to laboratory conditions in Environmental Research Laboratory, Jai Prakash University Chapra Saran Bihar After two weeks, the fishes were divided into groups (n=10) and kept in aquaria (30 L) under light - dark (12: 12 hrs) cycle. As per the APHA (1998), the water parameters were maintained throughout the experiment (Table 1) and aerated. Fish food pellets were provided ad libitum (Affonso et al., 2002), but the fishes were fastened for at least 24 hrs prior to the experiments.

Acute lethal studies

The chemical cobalt chloride purchased from nice chemicals Pvt. Ltd. (Kochi, India). Based on the literature, the different concentrations of cobalt chloride (50, 100, 200, 300, 400, 500 and 600 ppm) were exposed to seven groups and one group maintained as control, the results observed at 24, 48, 72, 96 hrs time intervals. For confirmatory results, twelve groups of fishes were exposed to twelve concentrations (280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, ppm) of Cobalt Chloride.

Experimental design

The five groups were exposed to 30, 60, 90, 120 and 150 ppm concentrations of cobalt chloride. Control group was maintained separately. After 96 hrs, blood and organs were collected from control and treated fishes.

Micronuclei (MN) assay

Fish peripheral blood smear is fixed in methanol for 10 min and left to air - dry at room temperature and finally stained with 5% Giemsa in Sorenson buffer (pH 6.9) for 20 min. The slides were randomly selected and total of 1100 erythrocytes were examined for MN in each group under the light microscope (Leica microscope, Switzerland) (Fenech et al., 2003). Only the cells clearly isolated from the surrounding cells were scored (Cavas et al.).

Table 1: Physiochemical parameters maintained in aqueous medium

Parameters	Values
Temperature (1°C)	25–32
Salinity (ppt)	1.3
Total Hardness (mg/L)	254.0
Ph	8.1
Nitrate (mg/L)	1.5
Chloride (mg/L)	26.0
Ammonia (mg/L)	0.048
Dissolved Oxygen (DO) (mg/L)	6.5
Biological Oxygen Demand (BOD) (mg/L)	5.6
Chemical Oxygen Demand (COD) (mg/L)	14.4
Total solids (g/L)	1.3

The criteria for the identification of micronuclei were as follows:

1) MN must be smaller than one - third of the main nuclei
2) MN must be separated from the main nuclei or marginally overlap with main nuclei
3) MN must be same plane of focus and have same colour (stain)

$$\text{MN (\%)} = \frac{\text{Number of cells containing micronuclei} \times 100}{\text{Total number of cells counted}}$$

4. Results

The experimental results revealed dose - dependent effects of cobalt exposure on Channa punctatus. Fish exposed to higher concentrations of cobalt exhibited decreased oxygen consumption rates, increased ammonia excretion, and alterations in antioxidant enzyme activities compared to control groups. Histopathological examinations indicated tissue damage, including necrosis, vacuolization, and inflammation, in the liver, gills, kidney, and brain of cobalt - exposed fish.

Acute lethal studies

The Cobalt Chloride exposure with high intervals of concentrations (50, 100, 200, 300, 400, 500 and 600 ppm) showed mortality between 300 - 400 ppm. In confirmatory studies, the lethal concentration (LC50, 96 hrs) of cobalt chloride in *Channa punctatus* (Bloch.) fishes were attained at 340 ppm in three replicates respectively (Figure 1).

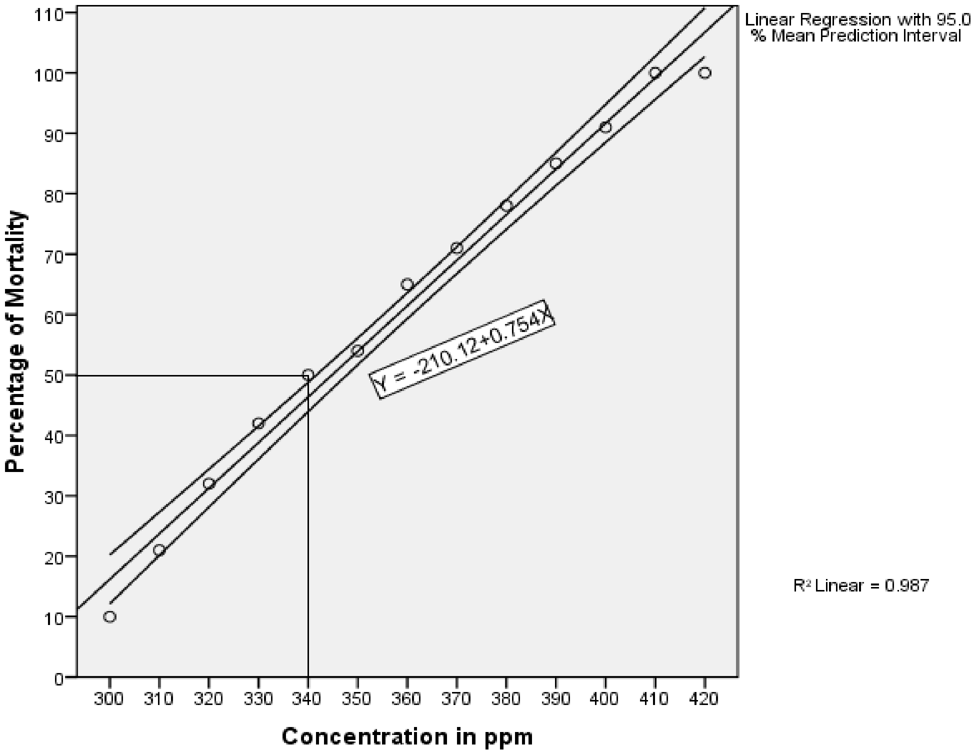

Figure 1: The acute lethal concentrations of cobalt chloride in *Channa punctatus (Bloch.)* for 96hrs

3.2 Micronuclei assay

Frequency of micronuclei in control group was recorded as 1.98 ± 0.11 (Table 2).

Micronuclei frequencies revealed (96hrs) that higher values were obtained in 150 ppm (9.06 ±0.15) > 120 ppm (7.43 ± 0.20) > 90 ppm (5.80 ± 0.39) > 60ppm (4.32 ± 0.32) > 30 ppm (3.17 ± 0.16).

The increased MN frequency of erythrocytesisdose dependent (Figure2).

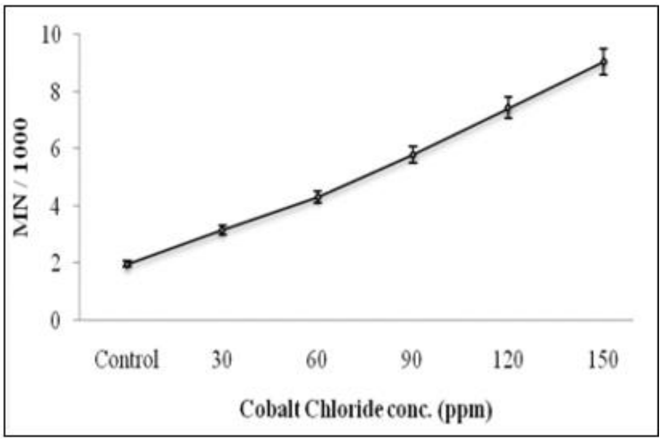

Figure 2: Micronuclei frequency comparison in cobalt chloride exposed groups with control in 96 hrs after exposure.

Table 2: Frequency of micronuclei in peripheralery throcytes of *Channa punctatus (Bloch.)* exposedto cobaltchloride (n= 10)

Concentration of CoCl2 (ppm)	Total no. of Counted cell	MN/1000 (mean±SD)
Control	5333	1.97±0.10
30	5190	3.16±0.15
60	5221	4.31±0.31
90	5217	5.79±0.38
120	5430	7.42±0.19
150	5161	9.05±0.14

5. Discussion

The findings of this study highlight the potential toxic effects of cobalt on *Channa punctatus*and underscore the importance of monitoring cobalt levels in aquatic ecosystems. The observed physiological and histopathological alterations indicate the need for regulatory measures to limit cobalt contamination and mitigate its impact on freshwater fish populations. Additionally, the identification of potential biomarkers of cobalt exposure in *Channa punctatus* could aid in the early detection of environmental contamination and facilitate risk assessment strategies. Mortality (LC50) and impaired hatching were observed in $CoCl_2$ exposed common carp embryo at 96 mg/L (96 hrs). The adult giant Gouramis exposed to 232.8 mg/L (96 hrs), fathead minnow 48 mg/L (96 hrs) and Olyzias latipes to 620 ppm (48 hrs) were evidenced LC50 for $CoCl_2$ (DOSE, 1993; NPIS, 1998) in other species. The LC50 (96 hrs) for gold fish exposed to cobalt chloride were determined at 333 ppm (Das and Kaviraj, 1994); for

Cyprinus carpio at 327 ppm and 328 ppm in two replicates respectively (Naji et al., 2007).

The MN frequency shows increased mean value at 96 hrs exposure period in all treated groups, but in control group it was found to be lesser. Yadav and Trivedi (2009) reported gradual increase of MN frequency upto 96 hrs (15 ± 1.414) in *Channa punctatus* exposed to AS_2O_3. Hooftman and Raat (1982) concluded that based on sampling time, there was a time - dependent increase in MN induction in Ethyl Methane sulfonate exposed peripheral blood of Umbra pygmaea, an effect corroborated by the present work.

6. Conclusion

Cobalt exposure poses a significant risk to freshwater fish species like Channa punctatus, leading to physiological disruptions and histopathological alterations. Continued research efforts are warranted to further elucidate the mechanisms of cobalt toxicity and develop effective mitigation strategies to protect aquatic ecosystems and biodiversity. Exposure of freshwater fishes Channa punctatus, to varying concentrations of Cobalt Chloride resulted in diverse hematological, biochemical, and histological irregularities. The RBC levels notably decreased in the 150 ppm exposed group compared to the control. Elevated WBC and Hb levels indicated the development of toxicant - induced stress and cobalt's interaction in hemoglobin synthesis. Additionally, cytogenetic abnormalities such as micronuclei frequency were observed. In response to toxicant stress, the fish utilized stored energy reserves in the form of proteins, carbohydrates, and lipids from vital organs. Consequently, the examined organs (muscle, liver, and gills) of exposed groups exhibited lower levels of biomolecules compared to the control group. The toxicity of cobalt chloride also disrupted the normal histology of gill and liver tissues. Therefore, it is imperative to implement all possible remedial measures to prevent cobalt chloride from exceeding permissible limits in the aquatic environment.

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