

**EFFECTS OF NITRATE ON HEMOGLOBIN COUNT, HISTOPATHOLOGY AND
MICRONUCLEUS STUDY IN FRESH WATER FISH CATLA CATLA****¹*Subhashri Priyadarsini, ²Dr. T. Gayatri and ³Dr. Satya Narayan Sahoo**¹Research Scholar of Dept. of Zoology GIET University Gunupur Odisha.²Assistant Prof. GIETU.³HOD Dept. of Zoology Niali College, Cuttack.

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ABSTRACT

Water got contaminated by industrial, agricultural influence and city sewage water. Nitrate present in all industrial and mostly in agricultural waste and also city Sewage contain high level of nitrate which directly impact aquatuaquatic life. Nitrate is a major cause for the formation of met hemoglobin and shortage of hemoglobin which studied by cyanmethemoglobin method (1)20 ml of blood samples were taken in Thomas pipette at interval of 24, 48 and 72 hours. And nitrate cause stress environment which causes micronucleus and other genetic abnormalities and also a vital histological change in the gills of fish. 0, 0.5, 1.0 and 2.0 PPM nitrate dodge given to the fish and studied by conducting several tests with two way ANOVA significant variation.

KEYWORD: Nitrate, Hemoglobin, Histopathology, cyanmethemoglobin method
Micronucleus, Genetic modification.

INTRODUCTION

Nitrate is water soluble which highly found in pesticides, fertilizers and industrial waste and mostly in cities swages release high Nitrogen is water soluble substance which highly found in agricultural products level of nitrate into river waters which affect aquatic life. Which adversely change in the aquatic environment and cause a vital genetic modification and physical modification which also cause presence of met hemoglobin Nitrate compounds have been identified as major metabolic products in fish culture. Nitrite may reach toxic concentrations in high density aquaculture systems and in flowing waters due to industrial contamination and fertilizer use. It is an intermediate product in the bacterial oxidation of ammonia to nitrate in conditioned aquaculture systems (Collins, 1975). This nitrogen compound is highly toxic to aquatic organisms and posses a potential threat to cultured fish. Respiratory blood pigment hemoglobin manifests the transport of oxygen. Nitrite an intermediate product of ammonia nitrification, may reach toxic concentration in aquaculture systems when imbalances pour among species of nitrifying Bactria. Nitrite is present at unusually high concentrations in lakes (McCoy, 1972). One physiological response to nitrite is an increase in methemoglobin. The hemoglobin becomes oxidized fe., the ferrous ion (Fe') is oxidized to ferric ion (Fe) and unable to bind and carry molecules of oxygen. Hence, the toxicity of nitrite to fish received much attention in recent years (Russo and Thurston, 1977).

OBJECTIVES

- . To find out the effects of Nitrate on hemoglobin count.
- To find out the effects of Nitrate on gills of Labeo catla fish by Histopathology test.
- . To find out genetic modification and Micronucleus formation in stressful environment due to high nitrate content in water.

MATERIAL AND METHODS

- . 10-25 cm length with average 500gm weight fishes are used.
- . Nitrate with different concentrations which soluble in water.
- . 20ml of blood samples were taken in Thomas pipette and cyanmethemoglobin method used for hemoglobin count.
- . Histological study done by Microtome method to find out the effects of Nitrate on fish.
- . Giemsa stain used for staining in micronucleus Assay.
- . Labeo Catla. in the interval of 24, 48, 72hours. Fish blood was smeared on clean and oven dried microscopic slides. These blood smear slides were air dried at 25°C for two hours and then fixed in cold Cortney's fixative for five minutes and were again fixed in methanol for ten minutes and left to air dry at 25°C for 1 h. Slides were stained for M 30 min in 10% aqueous Giemsa and washed in double distilled water and again let them air dry. 35 fish specimens were analyzed for each experimental site for a total of 35,000 erythrocytes/fish sample. For positive control, blood Smith farmed

specimens was subjected to colchicine treatment. For each fish specimen five slides were prepared.

The frequencies of micronucleus induction in erythrocytes were scored.

RESULTS

Hemoglobin concentration (g/dl)

	A	B	C	D
24hr	5.34±0.02	6.36±0.00	5.36±0.03	6.22±0.01
48hr	5.32±0.01	7.01±0.01	7.14±0.01	5.55±0.13
72hr	5.36±0.02	5.75±0.02	7.18±0.05	5.25±0.01

Fish exposed to different dosage (0.5, 1.0, 2.0ppm)

Hemoglobin concentration of fish in different concentration.

Histopathology

	Chloride Cell	Epithelial cell	Lamellar Disruption	Hypertrophy of epithelium
24	00	01	00	01
48	01	00	01	01
72	00	02	01	02

Study showing different abnormal growth of abnormal cancerous cells in gills of fish due to concentration of Nitrate. Micronucleus concentration.

DOSE	24hr	24hr	48hr	48hr	72hr	72hr
	MN	NA	MN	NA	MN	NA
0	0.2	0.3	0.3	0.1	0.2	0.4
0.5ppm	0.3	0.6	0.5	1.1	0.6	0.9
1.0ppm	0.7	1.3	1.3	2.1	1.6	1.7
2.0ppm	1.5	2.1	2.9	3.0	3.3	3.9

MN-Micronucleus NA-Nuclear Anomaly

Frequency-(0/00) Effect of dose and time was studied by conducting the two way ANOVA significant variation between dose(0,0.5,1.0,2.0 ppm) was observed ($p < 0.01$) in micronucleus and nuclear anomaly induction.

RESULTS AND DISCUSSION

The aquatic environment makes up the major part of our environment and resources; therefore, its safety is directly related to the safety of our health and food security. Biomarkers and bio indicators using fish micronucleus assay, histopathology and hemoglobin count process which indicates that with the concentration of Nitrate the damage in fish body increases. Which shows a dangerous condition of aquatic ecosystem due to water pollution.

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