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THE IMPACTS OF PHENOL AND MALATHION TOXICITY ON RED BLOOD CORPUSCLES AND BEHAVIOR OF *Oreochromis mossambicus*

[1]Feba Achu Andrews, [2]Anit Mani Thomas

[1]Department of Zoology, Catholicate College, Pathanamthitta, Kerala, India.

[2]Department of Zoology, Baselius College, Kottayam, Kerala, India.

Abstract: Toxicity is represented as a series of physical and behavioral responses evident in an organism upon the exposure to any harmful chemicals. Fishes are the group of organisms, which are affected to these kinds of toxicities to a great extent, as they breathe, live and eliminate wastes on to the same water in which they are in. Phenol, on the other hand, is a harmful chemical to living creatures in a variety of ways, but it is employed in hospitals for a range of purposes. Phenol and phenol-contaminated materials are disposed, in waterways and landfills near hospitals in some regions, where waste disposal is inefficient. As a result, phenol may reach nearby water bodies, posing a major threat to fishes and other living organisms out there. Malathion is an insecticide that is widely employed in agricultural fields, which eventually finds its way into waterbodies. The impact of both of these toxins on *Oreochromis mossambicus*, a renowned robust fish, which is a common inhabitant of freshwater and brackish water bodies, with reference to behavioral and hematological aspects, is the focus area of this study.

Keywords: Behavior, Hematology, Malathion, Phenol, Toxicity.

INTRODUCTION:

The health profile of the fishes is greatly influenced by hematological tests. This is a well-known fact nowadays. Blood-related biochemical, hematological, and immunological examinations are increasingly being employed to get preliminary conclusions prior to diagnostic follow-ups, which are held at a later stage of the relevant study. Fish aren't exempt to the widespread use of contemporary health and environmental management techniques at various levels. The hematological parameters indicate not only the inherent metabolic activities occurring inside the complete organism, as well as their requirements and products, but also the effect of the environment, or simply the ecosystem, on the fish community. Such long-term influences are mirrored in diverse elements of fish blood to some extent. As a result, it is clear that the circulatory system in most fishes responds substantially to their activity. The Red Blood Cells, or Erythrocytes, and their changes under diverse conditions are the most important of these. Fish have innate ('built-in') patterns of maturation (developmental changes) and learning processes that influence their behavior, (imprinting and trial-and-error learning). Innate behavioral tendencies are thought to be "hard-wired" and unchangeable. Fish, on the other hand, may adapt to environmental change by learning.

However, alterations in the habitat of the fish, brought on by chemical influences may lead to disastrous changes in the fishes' physical and behavioral state. Phenol and malathion are two such pollutants to which fishes of our waterbodies are frequently exposed. The fish will display various behavioral gestures in reaction to the conditions presented, such as hiding, battling, swimming erratically, listlessness, gasping for breath, and so on. We are also dealing with a side research of fish behavior in response to the many pressures being investigated as part of this project, along with the hematological examination. The more familiar you are with your fish's usual appearance and activity, the more likely you are to notice when one or more of your tank's inhabitants begins to act strangely.

REVIEW OF LITERATURE:

Mallatt (1985) reported that the pathological changes of fish gills are induced by elevated heavy metals and low oxygen content in water. Dawson (1993) classified immature erythrocytes into five categories according to their structure, distribution and quantity of basophilic substances within the cell. Mature erythrocytes contain abundant hemoglobin and are pink or yellowish on preparation stained with Giemsa. The number of erythrocytes in the blood varies according to the species as well as the age of the

individual fish, season and the environment in which it is bought up. The nucleus is centrally placed and round or oblong in shape. Aquatic organisms, like fish, accumulate pollutants directly from contaminated water and indirectly through the food chain (Sasaki et al., 1997; Nussey et al., 2000; Ashraf, 2005 and Riba et al., 2004). There are several reports on the effects of pesticides and heavy metals of the contaminated sediments on fish population (Wong et al., 2000; Amaraneni and Pillala, 2001; Wong et al., 2001; Mondon et al., 2001; Ramesh and Vijayalekshmi, 2002 and Moore et al., 2003).

Even though the fishes can tolerate the adverse effects of pollution stress to a certain extent by some physiological mechanism, a decline of fish species with respect to pollution load was observed (Saravanan et al., 2003). Once the toxicant enters the body of the fishes, they may affect the organs leading to physiological and pathological disorders. An array of histopathological fluctuations are observed among fishes exposed to pollutants both in field and in laboratory conditions (Abdallah, 2008; Amundsen et al., 1997 and Andres et al., 2000). Fishes are considered as one of the most significant indicators in freshwater systems for the evaluation of metal pollution (Rashed, 2001). Several commercial and edible species have been widely investigated to detect the presence of hazardous chemicals (Begum et al., 2005). Extreme increase and decrease in pH value in an aquatic medium are reported to cause disturbance in acid – base and iron regulation, fish growth and reproduction and sometimes leads to mortality (Evans et al., 2005 and Zanibomi-Filho et al., 2009).

OBJECTIVES:

1. To study the effect of Phenol over the Fish population.
2. To study the effect of Malathion over Fish Population.
3. To study the change in the characteristics of the Red Blood Corpuscles of fishes in accordance with the exposure to Phenol.
4. To observe and study the behavioral changes of fishes in accordance with the exposure to Phenol.
5. To observe and study the behavioral changes of fishes in accordance with the exposure to Malathion.

METHODOLOGY:

A. Collection of *Oreochromis mossambicus.*

Figure 1: Collected fishes in tanks.

Oreochromis mossambicus was the experimental organism employed throughout the investigation. On the 11th of March 2018, the experimental organism, *Oreochromis mossambicus*, was taken from the Government Fisheries Farm and Hatchery, Panniveilchirra, Kozhencherry, Pathanamthitta. The average weight of the fish was 20.0±0.10g. They were raised in a tank, at the Department of Zoology of Catholicate College, in Pathanamthitta. Oreochromis mossambicus is a tilapiine cichlid fish native to southern Africa. It is a popular fish for aquaculture. Dull coloured, Oreochromis mossambicus often lives up to a decade in its native habitats. Due to human introductions, it is now found in many tropical and subtropical habitats around the globe, where it can become an invasive species because of its robust nature. These same features make it a good species for aquaculture because it readily adapts to new situations.

B. Acclimatisation.

The experimental organisms were first acclimatised to the laboratory settings in the Zoology Laboratory of Catholicate College, Pathanamthitta. Acclimatisation is the process by which an organism adapts to a variations in its environment, allowing it to retain performance under a variety of conditions. The fish were acclimatised to laboratory settings for about 60 days. To avoid dissolved oxygen depletion, the water was replaced and oxygenated on a regular basis. The fish were observed to ensure that they were pleased with the laboratory environment and stayed healthy.

C. Maintaining a Control Population.

A healthy group of fishes were taken out from the acclimatised population, for maintaining them as the control population. The red blood corpuscles and behavior of the controlled population was then used to compare with those of the experimental

population of *Oreochromis mossambicus.* This control population was ensured with comfortable external conditions, for making them sound in health.

D. Exposing fishes to Phenol and Malathion.

The fishes were then treated to the predetermined experimental setups as the following sequence in the research. Chemical pollutants such as, Phenol and Malathion were among the concerns considered during the study. The experimental organisms were moved to different tanks, for being exposed to the experimental circumstances. The various stressors were applied in a predetermined quantity and to a predetermined extent on separate tanks, based on information gathered from prior studies in this area about the lethal concentrations of Phenol and Malathion. During the study, the fishes' movements and mannerisms were also examined time to time. When the fishes appear to be lethargic or have less energy after being exposed to various stresses, their blood was extracted and employed to prepare a blood smear, which was then stained and analysed for further investigations and findings.

E. Extraction of Fish Blood.

The fish were isolated and brought out of the tanks alive, where they had been treated to Phenol, Malathion or controlled settings. Blood was drawn from the veins around the fish's caudal fin with a clinical syringe. After that, the blood samples were placed in vials containing 1.2 percent sodium citrate as an anti-clotting agent. In the bottles, the samples were labelled. The sodium citrate, at 1.2 percent, is a well-known anti-clotting chemical that inhibits blood from clotting, rendering the blood samples unfit for research.

F. Preparation of Blood smear and Staining.

Clean slides were taken out, washed, and dried using a clean cloth to make the smears. The blood smear was made on clean, dry slides that were free of oil. A drop of blood is extracted from each vial and placed on the glass slides with anti-coagulating agent. The amount of blood drawn will decide whether or not a nice blood smear will be obtained. The slide containing the blood drop is held in one hand while the edge of the coverslip on the other hand is put on it at an angle of 45° with the other hand. After that a thin smear was drawn out. It is Leishman's stain, which is used here to stain the blood cells for further analysis. The Leishman's stain is added to the air-dried blood smear and left for one minute after which the slide is washed with distilled water, to clear off the stain from the slide. The glass slides are them air dried and labelled appropriately for the following studies.

G. Examination of Blood Smears.

The blood smears were then examined and analysed under the high power of the microscope for observing the stained blood cells. Morphological changes in the RBC of control and experimental fishes were observed. The slides were seen under oil immersion for a thorough examination, in order to analyse them in the context of our experimental conditions and to draw useful conclusions. The glass slides were then kept in slide boxes. Photographs were taken using OLYMPUS CH 20i microscope with Olympus E420 camera.

RESULTS:

a) Control Population

The Red Blood Corpuscles in the blood smear of controlled population was found to be normocytic. RBCs are biconcave, disc-shaped cells in their mature state. The word "normocytic" is used to designate RBCs of normal size. The standard rule of thumb for determining red cell size on a blood smear is to compare them to the nucleus of a tiny normal lymphocyte, which has an approximate diameter of 8 microns. During the project's work, however, the blood smear of the control population of fishes revealed some normal RBC characteristics. The red blood cells were normochromic and normocytic, which means that their color and size were both normal. The behavior of the fishes under controlled condition was found to be normal. The fishes were active and healthy throughout the experimental tenure.

b) Fishes under Phenol Toxicity

Phenol, also known as phenolic acid, is an aromatic organic compound with the molecular formula C6H5OH. It is a white crystalline solid that is volatile.The molecule consists of a phenyl group (−C6H5) bonded to a hydroxyl group (−OH). It is mildlyacidic and requires careful handling due to its propensity to cause chemical burns. Phenolic wastes are common water pollutants generated from a variety of industrial processes used in oil refineries, gas operation, coke ovens, coal gasification and by natural processes such as the decomposition of plant matter (Buikema et al., 1979). Relatively high concentrations of phenol are found in rivers near the outlets of channels where industrial wastewaters have been discharged (Buikema et al., 1979; Loh et al., 2000). Phenol is thus major pollutant for aquatic life in different ecosystems. The major outlets through which phenols or phenolic compounds reach the surface of water so as to make an encounter with the environment of fishes or the ecosystem in which fish population thrives is of great importance when we are discussing the impact of phenolic compounds on the fish community.

RBC under the effect of phenolic compounds shows a major and significant changes. The cells appear to be crenated in nature. It is the condition of a rounded projection, as on the margin of a shell. The condition or state of being crenated is a process resulting from osmosis in which red blood cells, in a hypertonic solution, undergo shrinkage and acquire a notched or scalloped surface. Red blood cells are prone to undergoing crenation as either a response to ionic changes in the blood or abnormalities in the cell membrane, disrupting the cell's ability to maintain an isotonic state. There are two different types of crenated red blood cells: echinocytes and acanthocytes. Instead of the usual rounded biconcave shape, both these cells appear with a rounder form

and spiny projections on the cell surface. Occurrence of crenation suggests an underlying disease is present. This type of crenation is usually reversible and can be caused by either ionic imbalances, such as the presence of high pH or high calcium concentrations.

The RBC also appear as hypochromic in nature. Hypochromic means that the red blood cells have less hemoglobin than normal. Low levels of hemoglobin in the red blood cells leads to their appearance in pale color. The red blood cells also shows nuclear pyknosis . Pyknosis, or karyopyknosis, is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis. It is followed by karyorrhexis, or fragmentation of the nucleus. Intense nuclear condensation with intense refractivity is also seen at chronic conditions. The cell nucleus also shows degeneration or significant changes in the cell and nuclear structure. Rupture of the cell wall is the rather noticeable change in this stage. The rupture of the cell wall is then being followed by a series of deleterious alterations resulting in significant abnormalities in the cell structure and thereby it is deleterious to the function of the cell also.

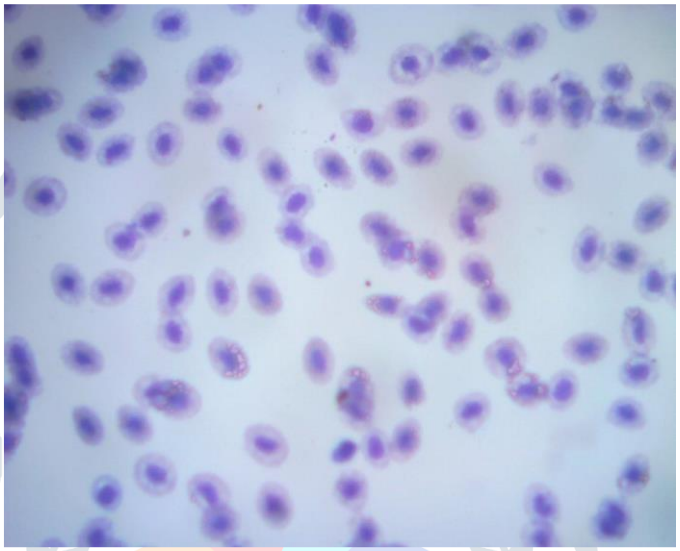

Figure 2: Microscopic view of RBC's under Phenolic toxicity.

The exposure to phenol resulted in sudden and fearful changes in the behavior and mannerisms of the fish. Fish changed it's swimming pattern and swims along a linear manner. The fishes occasionally showed a criminal behavior. During the preliminary exposure the fishes made a vigorous attempt to jump and cross the tank and thereby to escape from the stress. But later on during prolonged exposure to phenol, the fishes began to swim along the margin of the tank and that too by a typical waving of the fin.

c) **Fishes under Malathion Toxicity**

Malathion is a pesticide that is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication. Upon uptake into the target organism, it binds irreversibly to several, random serine residues on the cholinesterase enzyme, with a peroxide as the leaving group. The resultant phosphoester group is strongly bound to the cholinesterase, and irreversibly deactivates the enzyme which leads to rapid build-up of acetylcholine at the synapse. Malathion, a widely used insecticide is known to cause serious metabolic disturbances in non-target species, like fish and fresh water mussels. With the increasing industrialization human beings are continuously disturbing the delicate ecological balance in aquatic ecosystems. Pesticides are mainly synthetic organic compounds that are deliberately introduced into the environment to control selected organisms. Organophosphorous insecticides are employed in medicine and industry, because of their relatively low persistence due to biodegradability. So is the effect of malathion also. Thus the fish red blood corpuscles get seriously affected over the stress of malathion.

Moderate hypochromic red blood cells are been observed under acute conditions of the stress. Loose vesicular nucleus is observed under a high power microscope in oil immersion. The red cell cytoplasm also shows mild vacuolation. Vacuolization implies the process by which the cells becomes vacuolated. It can also refer to the vacuolation of the adjacent cells and the changes bought to the observed cells. The cells at an advancement of the stress condition shows cellular and nuclear degeneration. This will eventually lead to the cell death. The loss of the number of red blood cells in the blood will however lead to anaemia also.

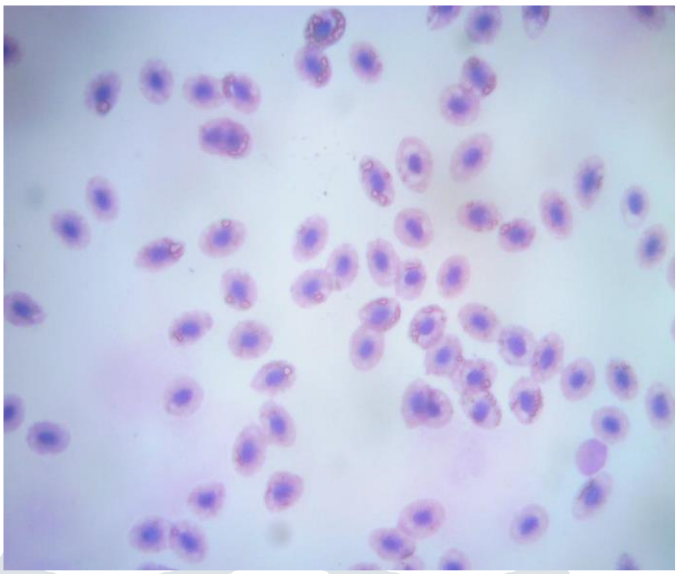

Figure 3: Microscopic view of RBC's of fishes under Malathion toxicity

Aggressive behavior is evident in the experimental fishes during the intercourse of this chemical stress.The fishes hits the tank glasses and always presses the nose against it. A chronic aggressive behavior has resulted in attacking the tank mates. This will lead to injury in fishes. Diarrhea of fishes is also seen following the primary exposure to malathion. But later on the fishes became tired and showed chronic lethargy and began to rely at the tank bottom.

DISCUSSION:

The effect of Phenol and Malathion on the RBC and ethology of *Oreochromis mossambicus* was studied. The specific mannerisms and changes in RBC identified in the experimental organism can be generalised as the impacts of these toxicants to the entire fish population. Although the experimental organism, *Oreochromis mossambicus* was an invasive and therefore a highly resistant species, the results of the study was really worth of significance. Thus it points out the effect of these stressors and pollutants under consideration, over the entire fish community with moderately resistant and sensitive populations.

During the course of study, the relevant and aggressive changes in RBC and ethology was seen during the exposure to Phenol and Malathion, when compared to that of the control population. According to Abernatty et al., (2003), even a low level of industrial effluent exposed for a period of time can reduce the erythrocyte production. The devastating effect of these chemicals is a point of discussion as the probability of the aquatic ecosystem to get contaminated by these chemicals are much more while considering the proximity to outlets of these chemicals, such as various industries and factories of detergents, insecticides etc. Both the parameters under consideration, RBC alterations and ethology was much evident and commendable and at varying degrees for different stresses.

CONCLUSION:

Effect of Phenol and Malathion was rapid and resulted in changes of swimming pattern and overall mannerisms. Diarrhea and fights among the tank mates were also observed. Fishes made attempts to jump out of the tank, to escape from the stress. These peculiar mannerisms pointed towards the degree in which these chemical stresses are affecting the fishes. Nuclear pyknosis, crenated RBC's and vacuolation of the cytoplasm were also observed during the exposure to chemical stresses. Major changes in the behaviour and mannerisms of the fishes was identified to be aggressiveness and fights among the tank mates. The present study also reveals the fact that Red Blood Corpuscles and Ethology, can be considered as ideal parameters for deriving superficial informations about the effect of various stresses and pollutants over the fish community. These parameters will serve as indicators of the stress condition, following which, detailed studies can be done.

Monitoring programs should be adopted by the governmental organizations, Environment protection teams and we the individuals ourselves, for the prevention of contamination of the freshwater ecosystems with various chemical stressors and pollutants. Otherwise, there is a chance that these pollutants would cause a bottom-up effect, that is into serious problematic situations such as Biomagnification. Laws should be implemented and proper execution of these laws should also be done, to act as a check, for controlling the flow of chemical pollutants and toxic substances from different chemical and industrial outlets into water bodies.

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