Probit Analysis Approach on Performance of Toxicity in Freshwater Fish

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Abstract

The probit analysis approach was used to perform the acute toxicity bioassay on textile industrial effluent (TIE). The LC50 values were found to be 56.23 percent, 28.84 percent, 22.38 percent, and 16.59 percent after 12, 24, 48, 72, and 96 hours. There was a considerable reduction in LC50 readings as effluent concentration rose. A 32.88 percent safety margin was determined. With increasing toxin concentration, the experimental fish C. batrachus displayed more stressed behavioural patterns. The acute toxicity of textile-based industrial effluent caused the experimental fish C. Batrachia to exhibit excessive mucus production, uncoordinated and tailfin movement, surface, loss of buoyancy, escape propensity, hyperactivity, and discoloration of the skin. All treatments had some level of mortality, with the exception of the control. C.batrachus experimental fish exposed to textile-dyeing effluents were also subjected to a hematological examination. Hb, RBCs, WBCs, PCV and MCHC of the test species were analysed using hematological data. It's been brought out that these settings might be tweaked. As a result, it may be determined that the TIE is harmful to fish and other aquatic life.

Keywords: *Acute Toxicity, Industrial Effluents, Biochemical Composition, Total Organic Carbon, Fish.*

1. INTRODUCTION

Anthropogenic activity alters the water quality in aquatic habitats. In addition, the textile sector and its effluents constitute a major cause of pollution across the globe, particularly in developing countries. Organics, dye, and toxicants make up the bulk of textile wastewater contaminants. Chemicals from printing, scouring, bleaching, and dyeing are found in the effluent, which is then disposed away. It has been explained by Berardi et al. (2019) how various types of dyeing and printing pigments are employed. A large amount of Pali's manufacturing sector is dedicated to printing and dyeing textiles. As a result, the textile effluents produced by these enterprises are hazardous. The discharge of wastewater from Pali's textile and dyeing businesses has a negative impact on the quality of the groundwater and the surrounding surroundings. Toxic textile effluents released into the Bandi River, Pali city, change river water quality and harm aquatic life, making the river unsafe for swimming. pH exceeds the permitted level in river water, but other measures like BOD and total hardness are substantially higher, according to the research. The outflow sample has higher TDS, chloride, and sulphate contents than the intake sample. Pollutants emitted during the wet process of textiles are exactly as expected. Chemical oxygen demand and biochemical oxygen demand in textile dye effluents are high because of the high pH of the effluent and the number of suspended particles in it. Before being released into the environment, textile effluent from a factory on Oba Akran Road (Ikeja) undergoes some kind of treatment, according to research by Taiwo et al. (2018), who examined samples from the industry. A variety of health and environmental issues may result from textile effluent including allergies, dermatitis and skin conditions. Toxic pollutants from textile dyes may be found in the environment and stored in aquatic creatures due to the widespread usage of these colours. In order to determine a chemical's relative strength, a bioassay compares its effects on animals to those of standard preparations.

2. LITERATURE REVIEW

Xinmiao Luan et. al, (2021): During the current coronavirus disease 2019 (COVID-19) epidemic in China, wastewater treatment has gotten a lot of attention because of the risk of fecal–oral transmission. Ecological safety is at stake since disinfection decreases the danger of waterborne diseases. Contaminants including disinfection byproducts (DBPs) and residual disinfectants in disinfected wastewater effluents harm aquatic species and disrupt the ecology of the water body they are discharged into. Bioassays of disinfected wastewater effluents on aquatic life at various trophic levels, including freshwater and marine creatures, were evaluated in this study. The outcomes of these in vivo toxicity bioassays may be greatly influenced by associated factors, such as total suspended solids (TSSs), ammonia nitrogen (NH_3-N), residual disinfectants, and effluent characteristics (water temperature and sampling season). When conducting ecotoxicological investigations, it is important to take into consideration the advantages and disadvantages of each typical test organism, as well as the species type, life stages, and test endpoints. Ecotoxicological effects of disinfected wastewater effluent should be better mimicked by conducting bioassays that take into account actual recipient water body scenarios; extended exposure times should be considered in order to gain additional insights into long term or pass-generation ecotoxicological effects, which are closer to the true levels in the water body.

Pradip Kumar Maurya et. al, (2018): In this research, the effects of varying quantities of industrial waste water on the hematologic parameters and histology of the fish Heteropneustes fossilis were examined. They penetrate into the fish body in a variety of ways, including inhalation and dermal oral contact. On the first, fifth, tenth, and twenty-first days of the trial, hematologic fluctuation was seen in RBC, WBC, MCV, MCH, and MCHC count in the fish H. fossilis control group and treatment. When pesticide accumulation was compared to histopathologic alterations in the same tissues in the liver, colon, gills, muscle, and heart, the latter revealed a normal architecture. In contrast, the former indicated increasing degrees of tissue damage as pesticide accumulation increased. Pesticides have distinct toxic effects on the fish H. fossilis notable in different organs. Uncoordinated behavioural responses, such as erratic and jerky swimming, physiological, malformation, histologic and biochemical changes as well as frequent surfacing and gulping of mucus and an increase in opercular movement, were observed in fish exposed to higher concentrations of the drug. The fish also secreted more mucus throughout the body.

D. Selvaraj et. al, (2015): Aquatic life has been shown to be negatively affected by textile wastewater. A textile industry effluent was tested using a toxicity bioassay approach on the freshwater fish Poecilia reticulata in order to identify the fatal concentration. Six, 12, 24, 48, 72, and 96-hour effluent LC50 values were 73.081, 67.030, 55.521, 49.211 and 39.726 percent for each time point. In addition to irregular swimming, hyper-excitation, fast opercular movement, and thick mucus coating, the fish displayed atypical behaviours that suggested the effluent's toxicity. Histopathological alterations were also caused by the effluent. The main gill bar was expanded, and the secondary gill bar was removed from the creature. Of the liver, cytoplasmic vacuolation and nuclei clustering were seen, whilst intestinal villi were disintegrated and hemoglobin was infiltrated into the lumen in the gut.

Mehmet Emin Aydin et. al, (2015): A primary goal of this work was to develop and use acceptable biotests that were highly sensitive, convenient to execute and affordable enough to replace fish toxicology tests owing to ethical concerns about animal welfare. Lebistes reticulatus, Vibrio fischeri, Thamnocephalids platyurus, Daphnia magna, Lepidium sativum and Lepidium sativum were substituted for the fish toxicity test in order to undertake an ecotoxicological evaluation of industrial wastewaters, which were characterised by several micro biotests. It was also tested with Pseudokirchneriella subcapittata and Protox FTM with Tetrahymena thermophila. However, the wastewater samples could not be properly applied to them. It was determined that wastewater samples from seven distinct industrial zones, each representing a different industry, were tested for toxicity against the species indicated above. T. platyurus, D. magna, and L. reticulatus were shown to be the most sensitive test organisms for the wastewaters that were studied. Thamnotox FTM, Daphtox FTM, and fish toxicity testing found that the majority of wastewater samples were harmful. Daphtox FTM and Thamnotox FTM were shown to be a viable alternative to the fish toxicity test, which is now the only one recognised by Turkish Water Pollution Control Regulation. This is a significant finding.

3. METHODOLOGY

In the current research, fingerlings of the Catla catla species (average weight: 6-7 grammes) were taken from a local fish farm in Nandivelugu, Guntur Dt., Andhra Pradesh, India, and kept in plastic pools with a capacity of 50 litres. Acidity, dissolved oxygen, and bicarbonate concentrations in the ground water used to maintain the fish tanks were all within acceptable ranges. According to Finney (1971), LC50 was measured using the Renewal bioassay.

These five biochemical components were measured using normal protocols in healthy fish (Control) and fish exposed to sub-lethal and fatal concentrations of Cadmium chloride in the muscles, gills, livers, hearts, kidneys, and kidneys of the fish (Sub-Lethal) and fish (Lethal) subjected (Merck). The fish were subjected to a sub-lethal dosage of the fatal concentration for seven days before being sacrificed for biochemical investigation. Carbohydrates, proteins, lipids, and amino acids free of charge were all determined to be acceptable.

4. ANALYSIS

At varied cadmium chloride concentrations, the percentage of death is shown in Figure 1. There are three techniques used to evaluate the fish C. catla's LC50 cadmium chloride exposure level, and the results are 4.57 mg/L, 4.89 mg/L, and 4.13 mg/L, respectively (Figs. 2 and 3), according to Finney (1971). 4.533 mg/L is the average determined LC50. Y = 2.65X + 3.368 was discovered to be the equation for the dose-mortality regression line.

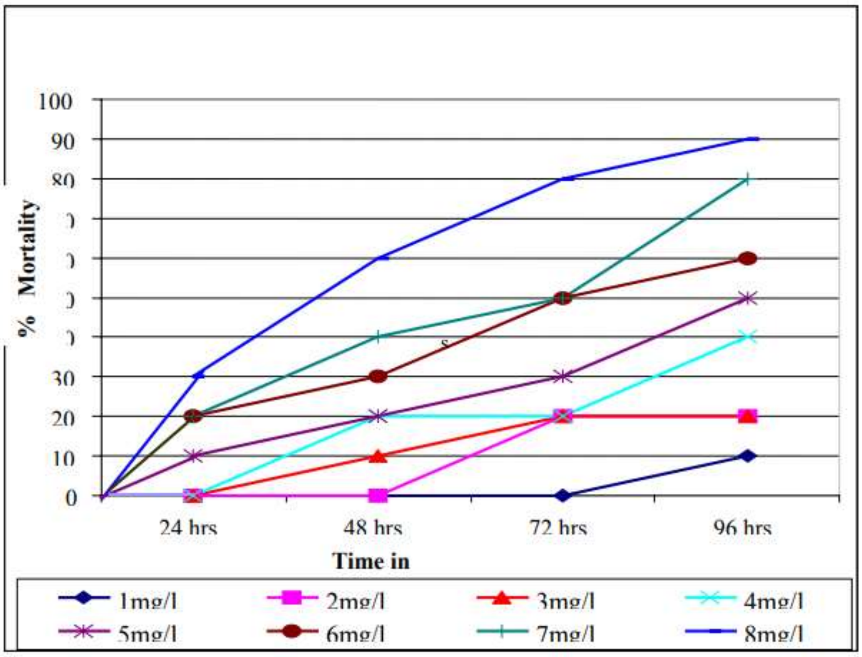


Figure 1: % Mortality Vs Exposure time

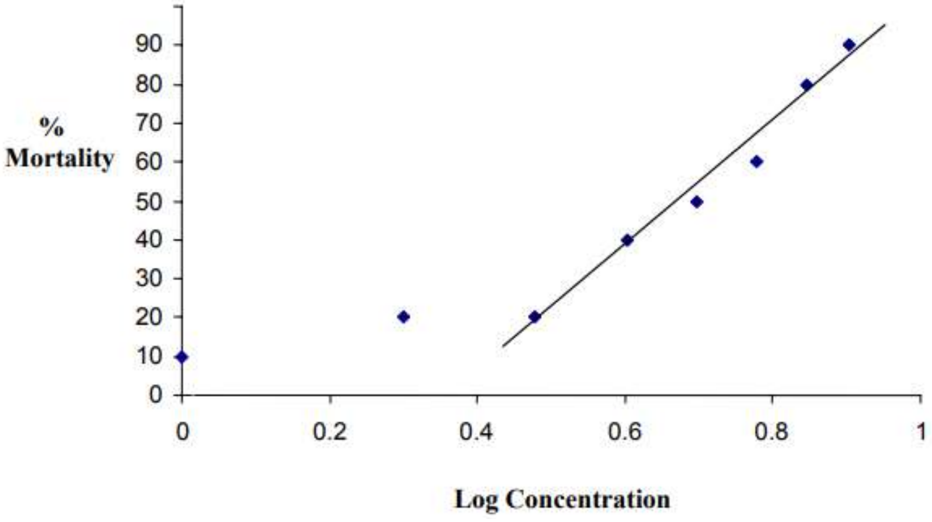


Figure 2: Simple graphic method (Log conc. Vs % mortality)

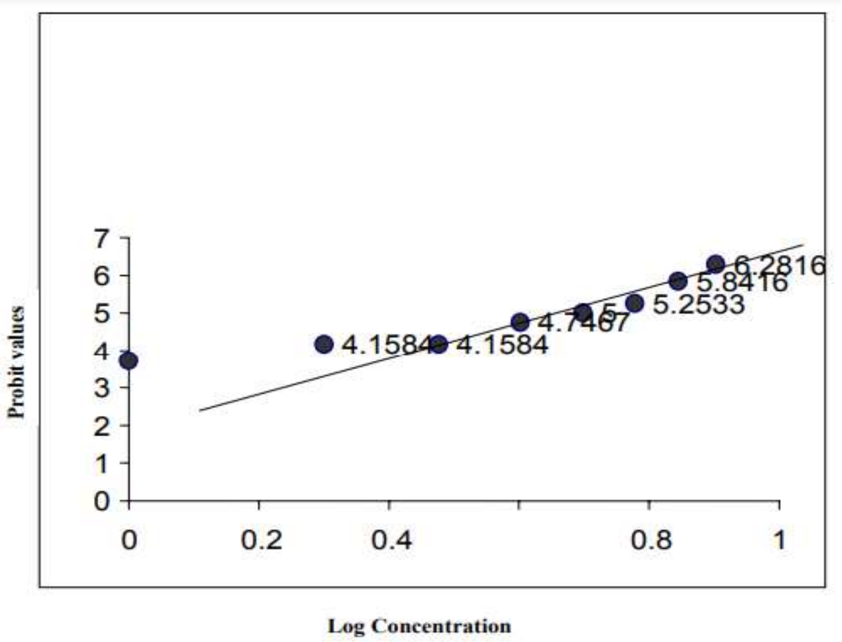


Figure 3: Probit Graphic Method

At the time of our experiment, we discovered that the behaviour and death rate of C. catla were dependent on the amount of toxicant and how long it was exposed for. C. catla are more vulnerable than other fish and crustaceans to environmental stressors. This is shown by the 96-hour LC50 value of 30.4 mg/L for Poecili reticulata, 43 mg/L for Uca rapax, and 25 mg/L for scorpion fish, Scorpaena guttata, in a static bioassay test setup. The size of the animal, the salinity of the water, the temperature, and the kind of animal all influence the action of the metal. In spite of the fact that the organism's defenses are strong in the beginning, the cumulative effects of exposure to even modest doses of toxins/pollutants will present themselves later on as the organism's resistance begins to decline as it matures. It is also influenced by the state and reaction of test organisms to the quantity of metal infiltrating into their bodies, how much is retained, and how quickly it is excreted.

Figure 4: Behavioral changes in the exposed fish. Curling of Spine and vertical movement of the fish due to loss of equilibrium is depicted. Due to complete loss of equilibrium, fish turned upside down and finally died as can be noticed in the picture.

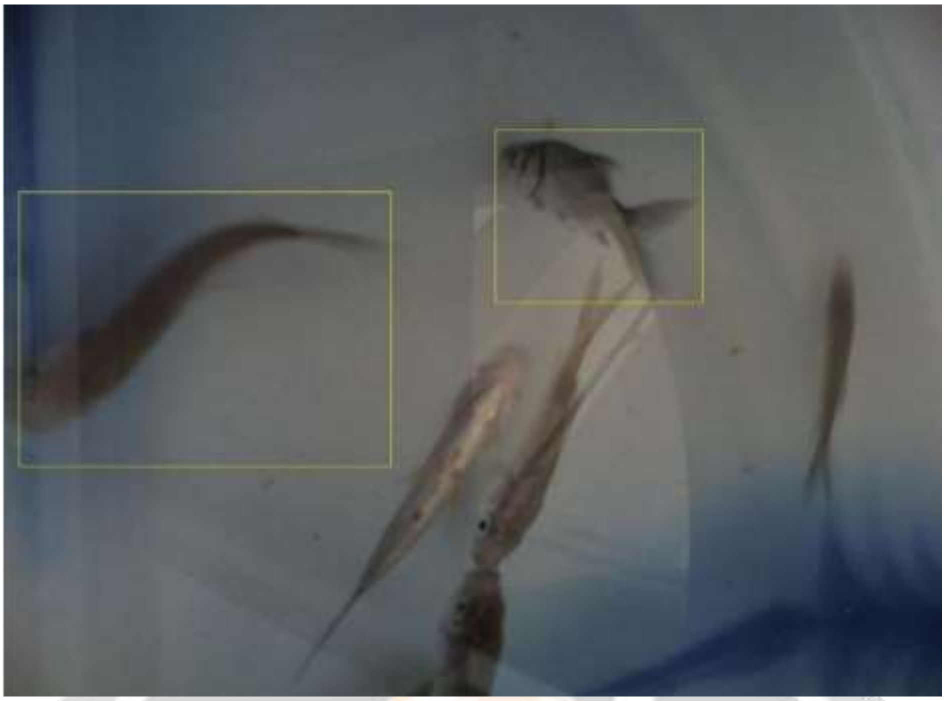
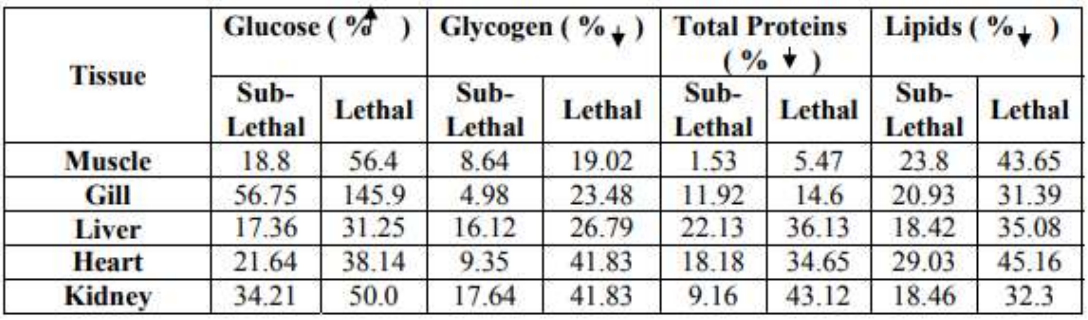


Figure 5: Hemorrhage in the head region of dead fish exposed to cadmium chloride. When this fish was dissected, hemorrhage was observed around eye orbits.

As shown in Table 1 and Table 2, the amounts of several biochemical components in mg/g of the wet tissue in control fish and fish subjected to sub-fatal and lethal dosages of cadmium chloride are shown. All tissues revealed a significant rise after 96 hours and seven days of exposure to sub-lethal concentrations; however, this increase was not uniformly distributed across tissues. Total protein, free amino acids and lipids of the 5 tissues of the control fish are in this order: There are four types of carbohydrates: glucose (L), glycogen (L), total proteins (M), free amino acids (L=M=G=H=K), and lipids (M>L>G>K>H). The rise in tissue glucose while the decrement in tissue glycogen in exposed fish demonstrates that the glycogen stores are being depleted to satisfy the stress induced by the environment. Stress may lead to an increase in fish's blood glucose levels. The specific activity of several enzymes, such as phosphofructokinase, lactate dehydrogenase, and citrate kinase, has decreased, which reduces glycolysis' capability.

Table 1: Variations in the levels of biochemical constituents in terms of % increase (↑) or % decrease (↓) in C. catla exposed to sub-lethal and lethal doses of cadmium chloride as compared to control fish



Tissue	Glucose (%↑)		Glycogen (%↓)		Total Proteins (% ↓)		Lipids (%↓)	
	Sub-Lethal	Lethal	Sub-Lethal	Lethal	Sub-Lethal	Lethal	Sub-Lethal	Lethal
Muscle	18.8	56.4	8.64	19.02	1.53	5.47	23.8	43.65
Gill	56.75	145.9	4.98	23.48	11.92	14.6	20.93	31.39
Liver	17.36	31.25	16.12	26.79	22.13	36.13	18.42	35.08
Heart	21.64	38.14	9.35	41.83	18.18	34.65	29.03	45.16
Kidney	34.21	50.0	17.64	41.83	9.16	43.12	18.46	32.3

Among mammals, the liver has the largest quantities of glycogen, since it is the organ most responsible for glucose metabolism. It is important to note that the storage and export of hexose units for blood glucose maintenance is handled by liver glycogen, whereas muscle glycogen is used to provide glucose for glycolysis inside the muscle. Fish exposed to toxicant through glycolysis or the Hexose Monophosphate route will show a decrease in glycogen levels, indicating that their bodies are rapidly using it to fulfil their increased energy needs. As a result of the suppression of hormones that promote glycogen production, it is hypothesized that glycogen content decreases. Decreases in glycogen stores in the liver and muscle are consistent with previous findings. In fish exposed to a sublethal dosage of the toxicant, glycogen stores are depleted in the following order:

K>L>H>M>G>K>L>H>G>M. Due to the quick use of glycogen stores by the gills when exposed to fatal concentrations, this may explain why. Protein-rich muscles, which are primarily used for movement, do not engage in metabolic processes.

In addition to serving as a hub for several metabolic processes, the liver is an excellent source of protein. The amount of total protein in the exposed fish's tissues was found to be lowered in all of them. It was shown that when a sub-lethal dosage was used, the order of decline in various organs was L > H > G > K > M, but exposure to a deadly concentration reversed this order. This shows that the kidneys are affected by larger quantities of the toxicant, whereas the liver is affected by lower levels. Due to the metabolic use of the ketoacids to the gluconeogenesis pathway for glucose synthesis, or because the free amino acids are directed to proteins or osmoregulation, the protein content of most fish tissues decreased in this research. Alternatively, it might be a side effect of heavy metal-induced apoptosis, which results in the formation of free radicals and heat shock proteins. Fish subjected to sub-lethal concentrations of free amino acids had no free amino acids found in their tissue. However, they were only found in the muscles, liver, and kidneys of fish that had been exposed to the deadly quantity. The acute impact of the fatal quantity of cadmium on these tissues may be inferred. A study by De smet and Blust (2007) found that proteolysis is used to boost the involvement of proteins in the generation of energy under cadmium stress.

Glycogen and lipids can serve as energy storage mechanisms. Both fatal and sublethal cadmium chloride concentrations reduced lipid content in fish tissues. Previous studies have shown that cadmium has an effect on the lipid content. In fish exposed to sub-lethal doses, the sequence of lipid loss is H>M>G>K>L, but in fish subjected to deadly doses, the order is H>M>L>K>G.

5. CONCLUSION

When Cadmium was tested on edible carp, the results showed a considerable alteration in the fish's biochemical components such glucose and glycogen as well as their total protein content, lipid content, and free amino acids. The cadmium poisoning of the deceased fish is plainly evident by the presence of hemorrhagic conditions. Fisheries with a history of exposure to low concentrations of heavy metals are better able to withstand larger concentrations of the same heavy metals. An adaptive reaction, which is typical of vertebrates, may be to blame for this phenomenon. Adults, on the other hand, are shown to be more vulnerable to the toxicant. Biodegradation is not an option for these heavy metals, as opposed to other types of contaminants. Some micro-organisms can be employed for biosorption of these metals, however the greater the concentration is, the more hazardous they are to those micro-organisms. The microorganism's cell wall composition dictates the specifics of this procedure. In order to properly use farmed fish with high nutritional value for human consumption, further effort must be done to find and apply appropriate strains of microorganisms for efficient removal of heavy metal toxicants.

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