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Effect of cadmium and LC50 values of WBCs of Labeo rohita, a freshwater fish (Hamilton, 1822)

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ABSTRACT

Heavy metal-containing industrial effluents can enter aquatic systems by direct discharge or surface runoff, causing harm to aquatic creatures' immune systems and health. As a result, the current study was conducted to investigate the effects of cadmium on the WBCs of Labeo rohita, a freshwater fish. WBCs serve a critical part in the fish's immunological response. Healthy fish were submitted to static bioassays to determine acute toxicity. The LC50 values after 12 hours, 36 hours, 48 hours, and 96 hours were 52.54, 46.32, 26.43,and18.55ppm,respectively.For 20 days, the fish were exposed to 0.5, 1, 1.5, and 2 ppm. For a total of twenty days, the differential count of WBCs and the total WBC count were determined every five days. Two way analysis of variance was used to analyse the data.

1. INTRODUCTION

The most significant biomonitoring agents for assessing hazardous components accumulating in contaminated aquatic environments are fish. They aid in the effective understanding of the nature and changes of aquatic ecosystems. When compared to other aquatic animals, they are extremely susceptible to environmental changes, particularly aquatic pollution. Heavy metals have a significant influence on the physiology and immunology of fish. Heavy metal deposition inside the fish body causes chronic or acute disorders in humans who consume the m (Raja *et al*., 2009; Biswas *et al* 2011; and Hongium *et al* 2013) As a result, a thorough understanding of fish physiology and immunology is extremely useful when dealing with the effects of heavy metals and aquatic pollution.

1.1. Labeo rohita biological characteristics Body is bilaterally symmetrical, fairly elongated, and has a more arched dorsal shape than ventral profile; Snout depressed, projecting beyond mouth, without lateral lobe; eyes dorsolateral in position, not visible from outside of head; body with cycloid scales, head without scales; snout fairly depressed, projecting beyond mouth, without lateral lobe; eyes dorsolateral in position, not visible from outside of head; Lips thick and fringed with a distinct inner fold to each lip, lobate or entire; mouth tiny and inferior; lips thick and fringed with a distinct inner fold to each lip, lobate or entire; in the lateral groove, a pair of tiny maxillary barbells; Jaws have no teeth; Three rows of pharyngeal teeth; upper jaw does not extend to the front margin of the eye; Three to four simple (unbranched) dorsal fin rays, 12 to 14 branched dorsal fin rays; dorsal fin placed halfway between snout tip and base of caudal fin; pectoral and pelvic fins inserted laterally.

The Rohu prefers zooplankton, mostly rotifers and cladocerans, in its early life stages, with phytoplankton serving as an emergency food source. Adults, on the other hand, demonstrate substantial positive selection for the majority of phytoplankton. Rohu is primarily a herbivorous column feeder in its juvenile and adult stages, favouring algae and submerged plants. Furthermore, the presence of degraded organic debris, sand, and mud in its stomach indicates that it is a bottom feeder. The fish's nibbling mouth, which has soft fringed lips, sharp cutting edges, and no teeth in the buck-pharyngeal area, allows it to graze on soft aquatic vegetation that does not need seizing or crushing. The fish's modified thin, hair-like gill rake’s further indicate that they feed on minute plankton by sifting water. In ponds, fry and fingerlings engage in schooling behaviour primarily for the purpose of eating; however, adults do not engage in this behaviour. Under regular cultivation circumstances, it grows to a total length of 35-45 cm and a weight of 700to800 g in a year. Both sexes acquire initial maturity at the age of two years, whereas total maturity takes four y ears for males and five years for females. Spawning takes place in the shallow and marginal p arts of flooded rivers in nature.

The Rohu's spawning season, which runs from April to September, usually corresponds with the southwest monsoon. The species reaches adulthood in captivity with good nutrition near the end. Due to industrial and agricultural activities, heavy metal contamination is one of the world's most serious issues. By triggering free radicals/

reactive oxygen species, they are well recognised for producing oxidative stress and/or carcinogenesis. The most important aspect in the amount of toxicity to fish is the form in which heavy metals are contained in water. When heavy metal concentrations are high, toxicity increases, paving the path for a variety of ailments in the future. Heavy metal has an impact on the physiology and metabolic activity of fish, which are not only an important component of the ecosystem but also a food source. Fish and shellfish have been shown in previous research to be substantial contributors to consumer consumption of certain pollutants due to their high toxicity. Earlier studies showed that fish and shellfish are the important contributors to consumer intake of some contaminants due to their presence in the aquatic environment and their accumulation in the flesh of fish and shellfish (Victor 2017, Rajasulochana and Preethy,2016, and Authman 2015).

2. MATERIALS AND METHODS

Labio rohita is a herbivore fish, It is a herbivore, and in Tamil Nadu, Orissa, Bihar, and Uttar Pradesh, it is considered a delicacy. A local fish farm in Cudalore, Tamil Nadu, and, India provided the stock of fish. They were acclimatised to laboratory conditions in well water for about two weeks. They were given algal and artificial fish feed during the acclimatisation phase. For the studies, only fish of the same size and weight (20 to 25g) were chosen.

Cadmium: Cadmium may be found in a variety of salts. Cadmium (Cd) crystals were employed as the cadmium source in this work.

Test medium: The water utilised in this investigation had a temperature of between 25 and 30 degree Celsius and a pH of between 7.5 and 8.5. Stock solution was used to produce different working ppm concentrations for the experiment by dissolving necessary volume of ppt solution in 1000 ml of bore well water on a daily basis until the experiment was completed. Designing Experiments: All of the healthy fishes were chosen for the experiment after they had completed their acclimatisation. The acute toxicity of hexavalent chromium was evaluated using triplicate sets of the LC50 value. Different doses of cadmium were employed in each set to estimate the LC50 value by measuring fish death during various exposure times. A static renewal bioassay approach was used to calculate the LC50 of chromium's acute toxicity (Eaton *et al* 2005).

Various cadmium concentrations were chosen, and 10 fish were placed into each concentration to determine the % mortality. After 12, 24, 36, and 48 hours of exposure, death was measured in all concentrations. Profit analysis was used to determine the LC50 value for various exposure intervals. Sub lethal Concentration Selection Four sub lethal doses of cadmium, namely 1/40th, 1/20th, 1/15th, and /10th of the 96 hour LC50 value, were chosen for long term exposure to evaluate the haematological l alterations in the fish L. rohita.

Sub lethal cadmium doses of 0.5, 1.0, 1.5, and 2 ppm were chosen using this procedure. The test media were replaced on a daily basis. In addition to these subordinates.

3. RESULTS AND DISCUSSION

There was no mortality in the 5ppm concentration until 96 hours, while 100 percent mortality occurred in the 80 ppm concentration within 24 hours of exposure.

Table 1. Shows the LC50 values for 12, 24, 36, 48, and 96 hours.

Exposure Periods (hr)	LC50 (ppm)	95% fiducial limits		Slope Function (S)	Chi square test result (0.05 level)
		Lower(ppm)	Upper(ppm)		
12	52.54	47.43	53.19	1.43	Not significant
24	46.32	36.52	41.78	1.58	Not significant
36	26.43	25.61	30.41	2.43	Not significant
48	18.55	16.56	22.45	2.69	Not significant

Table 1 shows the total WBC count of L. rohita subjected to various cadmium concentrations. During all of the exposure periods, 6900 cells/mm3 were detected in the control group of fish. Changes were detected in toxicant, exposed fishes. At comparison to lower concentrations of cadmium, the effect of cadmium was greater in higher concentrations of extended exposure.

Table 2. shows the neutrophil count of L. rohita subjected to various cadmium doses.

Table 2. Effect of cadmium on the different types of WBCs (%) of L. rohita

WBC Count (%)	Cd Concentration (ppm)	Exposure Period (days)				
		0	5	10	15	20
Basophil	0.5	0	0	0	1	1
	1.0	0	0	0	1	1
	1.5	0	0	0	1	1
	2.0	0	0	0	1	2
Eosinophil	0.5	3	3	3	3	3
	1.0	3	3	4	3	3
	1.5	3	3	4	4	4
	2.0	3	4	4	4	4
Neutrophil	0.5	60	61	61	62	70
	1.0	61	63	64	69	73
	1.5	63	65	69	71	75
	2.0	65	58	72	74	79
Monocytes	0.5	0	0	0	1	1
	1.0	0	0	0	1	1
	1.5	0	0	0	1	1
	2.0	0	0	0	1	1
Lymphocytes	0.5	27	30	29	24	20
	1.0	30	29	26	21	17
	1.5	30	27	24	20	14
	2.0	30	25	22	15	10

The neutrophil count increased as the concentration increased, and the number of neutrophils increased as the exposure period increased. Table 2 shows the lymphocyte count of L. rohita subjected to various cadmium concentrations. The lymphocyte count was reduced as cadmium concentrations increased. At the same time, as the exposure period was lengthened, the lymphocyte count decreased. Higher concentrations and longer exposure time s resulted in more lymphocyte depletion. L. rohita eosinophil count after exposure. There were no changes in all of the cadmium concentrations during the initial period. The effects were only noticed after a 20, day exposure period. Cadmium concentration had no influence on monocyte count.

Changes in exposure period were only observed after 15 and 20 days of exposure, but not before that. Table 2 shows the basophil count of L. rohita subject ed to cadmium. Only at higher concentrations and for longer periods of time did the basophil count change. During the initial exposure periods, no alterations were noticed. A significant rise in basophil count was seen after extended exposure at higher concentrations. The two-way ANOVA for the factors is shown in Table 3.

Table 3. Two way analysis of variance (ANOVA) for the factors with the variables, exposure period and Cadmium concentration

Factor	Source of Variation	SS	df	MS	Calculated F - value	Level of significance
Basophil	Exposure Period	0.15	2	0.05	1	Not Significant
	Cadmium Concentration	6.2	3	1.55	32	Significant
Eosinophil	Exposure Period	2	2	0.67	4.33	Significant
	Cadmium Concentration	1.3	3	0.33	2.6	Not Significant
Neutrophil	Exposure Period	80	2	27	10.57	Significant
	Cadmium Concentration	339.2	3	84.8	35.34	Significant
Monocytes	Exposure Period	8.77 E-16	2	2.96 E-16	- 4.0	Not Significant
	Cadmium Concentration	4.8	3	1.2	-1.6E+16	Not Significant
Lymphocytes	Exposure Period	113.6	2	38.2	10.54	Significant
	Cadmium Concentration	533.7	3	136.2	40.16	Significant
Total WBC	Exposure Period	1348 E3	3	337 E3	09.01	Significant
	Cadmium Concentration	15135 E2	2	5045 E2	13.99	Significant

As the period of exposure grew, so did the fatality rate. Cadmium has an LC50 value of 20 mg/l for 96 hours. Cadmium has an LC50 value for 96 hours that is roughly 6 times greater than Lepidocephalichthys thermals (Rajakumar 1992). The acute toxicity of mercury in Sarotherodon mossambicus has been investigated. During acute mercury toxicities in fish, cadmium chloride was found to impede oxidative and transphosphorylative activity.

Cadmium metal powder

Treated Labeo rohita fish

Fresh raw fish

The toxicity of mercuric chloride to Channa punctatus is dependent on the concentration and length of exposure (Agarwal 1991). Cadmium metal powder Albedo rohita fish Fresh raw fish. The fish displayed abnormal behaviour and a dose and time dependent mortality rate as the concentration and duration of exposure rose. The zinc LC50Value for Oreochromis mossambicus was 2 times greater after 96 hours than in this study. However, the range of toxicity varies depending on the species and the toxicant. Furthermore, the hazardous efficacy of a chemical reaction is determined by various parameters such as pH, hardness, alkalinity, equilibration, and kinetics.

The fate of substances inside the organism is one of the most current fields of toxicology research. A substance that enters an organism in a natural way must transit, though, the body. The blood in the gill comes into close touch with the aquatic medium, so any changes in the water could affect the circulatory system. These tests could be utilised to determine the health of fish as well as the quality of the water (Heath 2018). A toxicologist researching the kinetics or dynamics of a chemical in an organism is interested in learning about the chemical's entry points, translocation mechanisms, and metabolism, accumulation, and removal. Copper, iron, zinc, and lead contents in two significant period's prawns from Chilka were investigated.

This Total WBC Cadmium Concentration 15135 E2 2 5045 E2 13.99 Significant could be due to the role of leucocytes in phagocytosis, which involves their engulfing foreign items. In Heteropneustes fossils exposed to carbaryl and methyl parathion insecticides, the Leucocyte count increased with increasing concentrations and durations. (James, and Sampath (1996). The number of white blood cells (WBCs) increased as the concentration and time of copper exposure increased. WBCs increased as the concentration and duration of Lambda exposure increased (Muthupandi 2006). Increases in neutrophils, monocytes, and basophils were discovered.

The rise in level was steady as the concentration and duration of chromium exposure increase d. DDT caused haematotoxicity in Clarias batrachus, resulting in a steady decrease in total RBC, WBC count, haemoglobin content, and oxygen saturation. With increasing concentration and time of exposure, lymphocytes were shown to gradually drop in comparison to the control fish in the current investigation. Copper and zinc exposure in L. rohita at neutral and acidic pH produced similar outcomes (Nessy *et al* 2002). Increased TLC in the test fish could be related to increased lymphophoiesis and/or lymph myeloid tissue discharge of lymphocytes.

According to McLeay and Brown (Mused 2005), the increased lymphocyte population in treated fish is most likely due to the faster clearance of cellular debris from necrosis tissue. Even a t a low level, though, any change is noticeable. Chemical communication is disrupted by metals. After embryonic copper exposure, juvenile fathead minnows had a chemical alarm response. Excessive copper exposure during embryonic development is enough to damage chemosensory function during later life stages. In populations living in metal contaminated habitats, the inability to identify adjacent predators by olfaction may cause ecological disruption (Carreau and pyle 2005)..The fish's haematological characteristics are used to determine the water quality and health. Cadmium harmed the health of the fish and caused them to die. As a result, the fish becomes unfit for human consumption, and the water becomes unfit for drinking and recreation.

CONCLUSION

Cadmium the LC50 values after 12 hours, 36 hours, 48 hours, and 96 hours,were52.54,46.32,26.43,and18.55ppm,respectively.For 20 days, the fish were exposed to 0.5, 1, 1.5, and 2 ppm. The fish showed a decrease in lymphocytes after 20 days of exposure to sub lethal amounts of Cadmium, but an increase in other types of WBCs and total WBC count.

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