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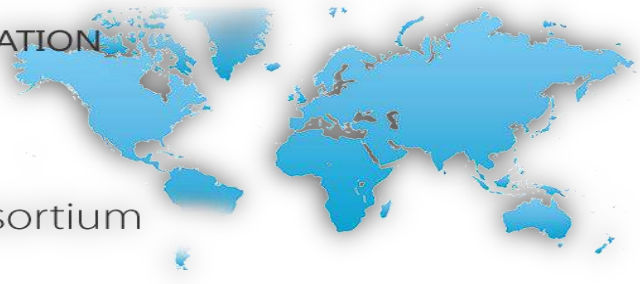

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TOXICITY AND BIOACCUMULATION OF CADMIUM IN THE CRAB BARYTELPHUSA-CUNICULARIS

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ABSTRACT:

Heavy metals are ingredients of liquid wastes discharged from a number of industries. The common metal ground in industrial waste water is cadmium, since cadmium is of great concern since it is more toxic to aquatic organisms such as crabs etc. The toxicity of cadmium to the crab Barytelphusa-cunicularis for 24h was tested according to the OECD (1992) guidelines No.203. 24h $L_{c50\ of}$ cadmium chloride ($CdCl_2$, 2 ½ H_2O) was found to be 7.7ppm. Bioaccumulation of cadmium in grills, intestine, hepatopancreas and muscles of B. cunicularis was analyzed using PC based AAS. The crabs were exposed to sub lethal concentration to (5ppm) of cadmium chloride for 24h, 48h, 72h, and 96h. It was observed that the trend of degree of accumulation cadmium different tissues was as follows as gills>Muscles>hepatopancreas>intestine.

Keywords- ***Crab, cadmium, toxicity, bioaccumulation.***

INTRODUCTION:

Rapid industrialization and consequent discharge of effluents into fresh water bodies made heavy metals a major pollutant of aquatic ecosystem. Heavy metals are important contaminants of liquid wastes discharged from a number of industries such as electroplating, dyes and dye intermediates, textiles tanneries, oil refineries, pesticides, mining's, smelters etc. the common toxic metals found in industrial effluents are cadmium, chromium, nickel, manganese etc. though a number of factors govern the toxicity of any compound, the relationship between the dose of a compound and its toxicity is important in toxicology as it is from these studies the arbitrary lethal concentrations are arrived at. Thus in bioassaying a living organism becomes an agent for the evaluation of potency of toxic substances and these experiments were reported to be more sensitive and reliable than physical and chemical investigations (Warren 1971).

Metals exist in variety of state. The toxicity of metals depends on its nature and chemical form whether it is in ionic form or in an oxidized reduced state in combination with organic substances and other metals.

Contamination of the aquatic environment with the cadmium is a matter of concern because this heavy metals can enter the food chain an as a result of bioaccumulation can cause serious health problems to human, (Freiberg et.al., 1973 Piscater, 1980) large amount of cadmium are used for electroplating and in the manufacture of pigments, plastics, stabilizers and batteries (Eisler 1985). Cadmium concentrations are highest near smelters and industrialized areas.

Industrial evolution and growing population has created main problem of heavy metals pollution to aquatic life because of their toxicity, resistance, tendency to accumulate in organisms and food chain amplification (Weis and weis, 1977).

Bioaccumulation is the ability of an organism to concentrate elements or a compound form food and water to a level higher than that of its environment (Menjer and Nelson, 1980). Bioaccumulation is the resultant process of many interactions within the compartment of the organisms. Thus bioaccumulation studies are important in the estimation of potential Pierson (1981) studied effects of chronic zinc exposure on the growth, sexual maturity, reproduction and bioaccumulation in the guppy Paecilia – recticulata. Lomteet-al (1994) reported on bioaccumulation of mercury in the freshwater snail, Thiara – tuberculata. Sultana and Arumugam (1989) studied nature and distribution of zinc in the marine crab, ScyllaserrataRadgakrishanaiahet.al (1992) studied on size and sex related cadmium accumulation in different organs of the freshwater field crab, oziotelphusasenexsenex. Satya (2000) reported on bioaccumulation of copper and chromium in functionally different tissues of a bivalve, Lamellidens – marginalis.

In the present study the toxicity of cadmium chloride to a freshwater crab Barytelphusa-cunicularis is determined along with bioaccumulation of cadmium in the gills, intestine, hepatopancreas muscle.

GiamberiniL (2003) reported on lysosomal in the digestive gland of the freshwater mussel, Dreisenapolymorpha, experimental exposed to cadmium. Schill RO (2003) reported on laboratory simulation of a mining accident: acute toxicity, hsc/hsp70 response, and recovery from stress in Gammarusfossarum (Crustacea, Amphipoda) exposed to a pulse of cadmium. Siekerska, E., and Urbanska-Jasik, D. (2002) reported on cadmium effect on the ovarian structure in earthworm Dendrobaenaveneta. (Rosa).

MATERIAL AND METHODS:

Healthy intermoult stage (C_3) freshwater crabs Barytelphusa-cunicularis Westwood (1836) were collected from the Godavari River at Paithan situated 55Kms South of Aurangabad City (Maharashtra State) The collected animals were quickly transferred to the laboratory. These were maintain $30^0C \pm 0.5^0C$ under normal day/night illumination (13L: 11D). The crabs were kept separately in different plastic troughs (18" diameter) 10 crabs per trough just submerged in water. The water was changed after every 12h. The crabs ware fed ad libitum with earthworms thrice a week. The crabs approximately of the same C_3 were sorted from the collected animals.

The aqueous stock solution of cadmium chloride ($Cdcl_2$ 2 ½ H_2O) was used in the toxicity testing with appropriate dilution by tap water. Static biossay tests were performed according to understandized procedures of Sprague (1973) and OECD (1992) guidelines no. 203 for the period of 24h. Initially several exploratory tests with random concentrations were conducted. Toxicity of cadmium as a cadmium chloride salt has been tested. For this purpose, a series of static biossays were conducted under laboratory conditions. A group of 10 crabs were exposed to 4 different concentration (ppm) ranging from 5 to 8 ppm of cadmium chloride. Dissolved oxygen was recorded by standard winklers method (Welsh et. at 1968). The temperature was recorded with thermometer and the p^H of water was checked using p^H meter. A control set of 10 crabs was maintained in non contaminated freshwater medium to compare results. The resulting mortabity was noted in each concentration for the duration of 24h. No aeration and foo was provided during the exposure period. Each experiment was repeated twice to secure constant results. The data collected were then extrapolated statistically by means of the probits method and transforming the toxicity curve (% mortality /concentration) into regression lines(mortality in probits / concentration i.e. probit kill/ concentration) according to the method Finery (1952) which allows the calculation of average lethal concentration (L_{c50}) for 24h.

DETERMINATION OF CADMIUM BIOACCUMULATION:

The crabs were exposed to 5ppm sub lethal concentration of cadmium chloride for the period of 24, 48, 72, and 96h to determine the bioaccumulate of cadmium in different tissues like muscle, hepatopancreas intestine and gills using the procedure of APHA (1985) using PC based AAS Geffard, A., et.al. (2002) reported on Relationships between metal bioaccumulation and metallothionein levels of Mytilusgalloprovincialis exposed to contaminated estuarine sediment elutriate.

The tissues were dissected out after the en of each exposure period and tissues were dried in oven 70^0C for 3 days. The dried tissues was powdered using mortal and pestle and 500mg dried tissue powder was taken in beaker and 10ml of concentration nitric acid and 2ml of 5N perchloric acid. Mixed well and kept on hot plate and waited till the solution evaporates and solution becomes colourless. Then third time added again 10ml of concentrated nitric acid mixed the contents well and kept on hot plate for digestion till 5ml remain in the beaker. It was cooled and made upto 25ml with 2M solution of concentrated nitric acid. This 25ml sample solution was used for metal estimation directly following the nitric acid digestion method of APHA (1985) and using the PC-Based Atomic Absorption Spectrophotometer (AA1275 BD Varian Techtron, USA) at 228.9nm wavelength. The unit given is ug cadmium/g of tissue. The data were statistically analyzed using student‘t’ test (Mungikar 1997)

RESULTS:

The observed lethal concentration of cadmium chloride for 50% mortality of the crab, Barytelphusacunicularis for 24h was found to be 7.7ppm whereas the statistically calculated Lc50 value with the help of regression analysis was found to be 7.649ppm as shown in table 1 and 2.

Results presented in table 3 shows the bioaccumulation of cadmium in different tissues like gill, intestine, hepatopancreas, and muscle of the crab, Barytelphusacunicularis exposed to sublethal concentration (5ppm) of cadmium chloride for 24, 48, 72, and 96h. Gills showed maximum accumulation where as minimum accumulation was found in intestine. Values of accumulation were found in hepatopancreas and muscle as compared to controls. The degree of cadmium accumulation was found to be as gills>muscle>hepatopancreas>intestine. Based on the values of Lc50 and bioaccumulation cadmium was found to be more toxic to crab.

Results presented in fig.1 shows that the treated crabs Barytelphusacunicularis when returned to freshwater; they started loosing the accumulated concentration of cadmium in gills, muscle, hepatopancreas and intestine. The concentration of cadmium in all tissues started decreasing when kept in freshwater. The values of experimental crabs were not significantly different from control indicating the attainment of almost normalcy. Sri Lakshmi P, Prabhakar Rao Y (2002) reported on Evolution of cadmium toxicity on survival, accumulation and depuration in an intertidal gastropod, Turbo intercostals.

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Table: 1

Calculation of probit regression line for some experiments in which the crab, Barytelphusacunicularis were exposed to different concentration of cadmium chloride for 24h

I	II	III	IV	V	VI		VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Sr. No.	Conc. Of (ppm)	Log of Conc	No.of Animals Exposed	Mortality for 24hrs	% Mortality $p=100_{xy/n}$		Empirical probit value	Expected Probit	Weight Coefficient	Weight Probit	Working $y=y_o+kp$						Improved expected probit
		X	n	y	p			Y	w	W=nw	y	Wx	Wy	Wx^2	Wy^2	Wxy	Y^1
1	6	0.7782	10	1	10		3.7184	3.72	0.33589	3.3589	3.719	2.6139	12.4918	2.0342	46.4571	9.7211	3.5346
2	6.7	0.8261	10	2	20		4.1584	4.2	0.50266	5.026	4.16	4.1520	20.9032	3.4300	86.9365	17.2682	4.1976
3	7	0.8451	10	3	30		4.4756	4.4	0.55788	5.5788	4.480	4.7147	24.9763	3.9844	111.8189	21.1078	4.4636
4	7.7	0.8865	10	4	40		5.0000	4.8	0.62742	6.2742	5.003	5.5621	31.3899	4.9309	157.04337	27.8272	4.2186
5	8	0.9031	10	5	50		5.2533	5.0	0.6662	0.3662	5.251	5.1494	33.4299	5.1923	175.5405	30.1901	5.9866
		Total								**SW=26.6041**		**SWx=22.7921**	**SWy=123.1911**	**SWx²=19.5718**	**SWy²=557.7967**	**SWxy=106.1744**	

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Table: 2

Calculation of LC_{50} values using probit analysis for the freshwater crab, Barytelphusacunicularis after exposure to cadmium chloride for 24, 48, 72 and 96h.

Sr. No.	Time of exposure hours	Regression Equation $y^1=(y^-bx^-)+bx$	LC_{50} value(ppm)	Chisquare (c^2)	Variance	Fuducial limit upto 95% limit confidence	
						M_1	M_2
1	24	$y^1=(4.6306-3.974x0.85672)+13.974x$ $y^1=7.3413+13.974x$	7.7 ppm	3.8003	0.000183	0.821247	0.87525
2	48	$y^1=44.6481+85.87x$	3.8 ppm	175.7299	0.0000046	0.569035	0.577485
3	72	$y^1=4.8506+0.486x$	1.9 ppm	5.1378	0.142634	0.493874	0.98659
4	96	$y^1=2.86036+1.23x$	0.9 ppm	3.3742	0.025969	1.459825	2.091536

Table 3:

Bioaccumulation of Cadmium chloride in different tissues of the crab, Barytelphusacunicularis exposed to sublethal concentration (5ppm) of cadmium chloride for different periods (Mean cadmium concentration in the habitat water: 0.017 µg/ml ±0.003)

Tissue		Mean concentration of cadmium (µg/g) ±SEM after ---------h			
		24	48	72	96
Gill	Control	03.5 ± 0.5	03.7 ±0.8	03.5 ±0.5	03.8 ±0.7
	Experimental	15.6 ±1.4	27.2 ±1.8	45.8 ±1.2	56.3 ±1.7
Hapatopancreas	Control	02.3 ± 0.7	02.4 ± 0.6	02.4 ±0.6	02.6 ± 0.9
	Experimental	10.2 ± 1.1	17.8 ± 1.2	30.5 ± 1.5	40.7 ± 1.3
Muscle	Control	03.1 ± 0.9	03.3 ± 0.7	03.4 ± 0.8	03.4 ± 0.8
	Experimental	13.3 ± 1.2	22.7 ± 1.3	36.7 ± 1.8	44.9 ± 1.6
Intenstine	Control	02.1 ±0.4	02.2 ± 0.6	02.5 ± 0.5	02.6 ± 0.4
	Experimental	05.2 ± 0.3	08.3 ±0.3	10.8 ± 0.7	13.7 ± 0.8

DISCUSSION:

The toxicity is defined as harmful effect of a chemical or a pesticide on target OECD (1992) defined acute toxicities as “the adverse effect occurring within a short time of total administration of a single dose of a substances are multiple doses given within 24h. Statistically derived single dose of substance that can be exposed to cause death in 50% of the animals. The Lc50 in its simplest form is dose of a compound that causes 50% mortality in a population. As per the opinion of the panel of experts of OECD (1992) the statistically calculated values of 50% mortality (Lc50) of the crab, Barytelphusacunicularisdue to cadmium chloride after 24h of exposure was 7.749ppm against observed experimental values of 7.7ppm which are not significantly different. In this crab the effect lethal concentration of cadmium chloride, prior to death are shown by erratic swimming, difficulty with respiration, loss of balance and convulsions. The mortality of the crab increased with an increase in the concentration of cadmium chloride. The relationships between the observed toxicity values and statistically

ascertained values have been varying in the same directions. This implies that there is a positive correlation between experimentally observed and statistically calculated values which is desirable as shown in table 1 and 2. it was observed that the degree of bioaccumulation of cadmium in various tissues of Barytelphusacunicularis was as follows : gills>muscles hepatopancreas>intestine. Based on the values of Lc50 and Bioaccumulation cadmium was found to be more toxic to crab. Canliet. al (2001) reported on cadmium accumulation in tissues of Sardinavilchardus and found increased levels in gills, liver and pancreas. Shukla et.al (2001) reported on Effect to toxicants on the intestine transport in fishes Channapunctatus and Heteropneustesfossilis exposed to sublethal concentration of cadmium and zinc (1.12 and 4.0ppm, respectively) showed decrease in the rate of transport of glucose and fructose, which was more marked after 30 days as compared to 15 days in the two fishes. Sinha AK et al (2002) reported on Bioaccumulation of heavy metals in different organs of some of the common edible fishes of Kharkai River, Jamshedpur. Gill, liver, kidney, intestine and muscle of some of the common edible fishes captured from Kharkai river were analyzed for their iron, zinc, nickel, lead, copper, manganese, chromium and cobalt contents.

It appears that Barytelphusacunicularishas efficient depuration mechanism perhaps through the process of depuration. Crabs appeared normal just within 48h after returning freshwater.

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