Respiratory response and hemolymph sugar level of the crab, *Barytelphusa gureini* exposed to cadmium chloride.

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ABSTRACT

To explore the respiratory and metabolic responses of the freshwater crab Barytelphusa gureini, were exposed to sub lethal concentration (2.0 µg/L) of cadmium chloride. Oxygen consumption and hemolymph sugar level of the animal were estimated to study the stress caused by this heavy metal toxicant. These animals after acclimatization to the laboratory conditions exposed for 0, 24, 48, 72 and 96 hrs to the toxicant. Total oxygen consumption was studied by Winkler's method and the hemolymph sugar level was estimated by Anthrone method. In the present study, total oxygen consumption showed a gradual decline trend from 0 to 96 hrs in the experimental animals exposed to cadmium chloride, while the hemolymph sugar level recorded an elevation, with maximum increase at 96 hrs.

Key words: *Barytelphusa gureini,* cadmium chloride, oxygen consumption, hemolymph sugar,

Sub lethal concentration

.INTRODUCATION

Cadmium toxicity has become the focus of intense research globally next to mercury as the most notorious of heavy metal pollutant. It becomes toxic when it is not metabolized by the body and accumulates in soft tissues, liver, kidney mostly as metalloprotein. Cadmium toxicity to aquatic animals depends on complex biochemical interaction and imbalance between rates of absorption, detoxification and excretion. In aquatic animals (e.g. crabs, shrimps, oysters and mussels) heavy metals enter in to various compartments of body through different way such as respiratory tract, digestive tract, surface penetration etc., [16, 17, 18]. They are seriously harmful to the growth of aquatic life and survival, resulting in decline of their population. At the same time, as aquatic food product, these animals exposed to cadmium might threaten human health.

Respiration is the most important and vital process of life for the derivation of energy in the form of ATP to perform different biological and physiological functions likes locomotion, feeding, reproduction, muscular contraction etc. Metabolic processes are the most sensitive parameters of stress as all enzymatic reactions on the substances and physiological responses are incorporated in a unique manner

[4]. However, it is well known that respiration is a vital phenomenon of life & rate of oxygen consumption in turn control the metabolic activities and act as a measure of the intensity of metabolism. Metabolic response of an organism to a changing or stressful environment is an overall indicator of its adaptive ability. Different species of crustaceans vary in their ability to reduce metabolism. Therefore any change in the respiratory activities has been rightfully used as an indicator of stress in general and toxicant induced change in exposed animal in particular [1,6,13, and 14].

It has been found that cadmium could change glycogen reserves and serum glucose levels in aquatic animals by affecting the activities of liver enzymes that have pivotal role in the carbohydrate metabolism such as gluconeogenesis, glycogenesis and glycolysis. Thus, the assessment of toxic heavy metals in aquatic animals can serve as bio-indicator of their impacts on these organisms. It also gives an insight to the degree of pollution of the water body and the health status of aquatic population [11]. The *Barytelphusa gureini* is well known for its high nutritive value and is commonly cultured by the local farmers. Cadmium causes instantaneous respiratory impairment and alteration in the pathways of carbohydrate metabolism. In present investigation, rate of oxygen consumption and hemolymph sugar level is considered as tool to evaluate the toxic effect of heavy metal as salt of cadmium chloride.

MATERIALS AND METHODS

Experimentation:

Acclimatization:

The adult specimens of fresh water field crab *Barytelphusa gureini,* were collected from the out skirt of paddy fields of Pune district (Maharashtra), and were brought to the laboratory. They were acclimatized in the laboratory for seven days before they were used for experiments. Only healthy crabs weighing between 30-40 gram were selected for experimentation to avoid problems of sex and size. The animals were fed with small pieces of goat flesh and uncooked oats.

Toxicity bioassay:

The aqueous stock solution of cadmium chloride was used to test the toxicity with appropriate dilution by tap water. A group of 10 crabs was exposed to eight different concentrations ranging from (1 - 2.75 µg/L) of cadmium chloride. The mortality rate was noted up to 96 hrs, the test medium and dead crabs were removed with the interval of 24 hrs immediately. The LC_{50} was calculated by using probit analysis method [5]. After finding the $LC_{50,}$ one set of ten crabs treated with a sub lethal concentration of cadmium chloride (2.0 µg/L) for 0-96 hrs respectively. The other set of ten crabs kept as control under the similar conditions without toxicant (Table. 1 & Fig. 1).

Physicochemical parameters:

The physicochemical parameters of water were estimated and were as follows: dissolved oxygen: 7.2-7.4 ppm, pH: 7.0-7.2, temperature: 29 ± 2^0c, salinity: 0.4-0.5 µg/ml and the total hardness: 280-288 mg/L.

Oxygen estimation:

To avoid the effect of starvation, the animals were fed with small pieces of goat flesh. The experimental animals were subjected to sub lethal concentration of cadmium. Oxygen consumption was studied after 0, 24, 48, 72 and 96 hours of exposure. Modified Winkler's method was used to estimate total oxygen consumption.

A specialized respiratory chamber was used to estimate oxygen consumption, in the form of a black colored bottle having inlet, outlet and control openings. The animal was kept in the airtight respiratory chamber, and initial water sample collected after taking all precaution. They animal were allowed to stay in the chamber for one hour at the end of which the final sample was collected. By this method, oxygen consumption the initial and final water samples were determined, and the differences between the two readings constituted the amount of oxygen consumed by the animal during one hour (Table. 2 & Fig.2).

Sugar estimation:

The hemolymph was extracted from the thigh region of the crab with the help of syringe and turned in to powder form by keeping it in an oven at 40^0_C for a long period. The sugar level was estimated by the Anthrone method after 0, 24, 48, 72 and 96 hrs (Table.3 & Fig.3).

RESULTS AND DISCUSSION

Present study reveals that the total oxygen consumption of *Barytelphusa gureini* decreased gradually when exposed to 2.0 µg/L concentration of cadmium chloride solution. The control set of experimental animals showed maximum respiratory metabolism. Oxygen consumption is one of the most important physiological phenomena, which controls all metabolic activities. It is the most important indicator of metabolic rate and status of the stress condition of exposed animals [8]. Since cellular and sub-cellular functions form the basis of all disorders, the toxic effects of xenobiotics mainly influence the cellular responses. The injury caused by the foreign compounds may be direct or indirect. Direct cell injury occurs when a toxicant interacts with one or more cell components. In indirect cell injury, the effect is due to disturbance in the microenvironment of the cell. For example, when tissues have insufficient supply of oxygen during hypoxia or anoxia, the energy metabolism is disturbed leading to damage to the cellular metabolism. The increased glycolytic activity during oxygen deficiency cannot meet the energy requirements of the cell. As a result, the energy requiring processes such as protein synthesis, and glycolytic activity during oxygen deficiency cannot meet the energy requirements of the cell. As a result, the energy requiring processes such as protein synthesis, phospholipids metabolism, and membrane transport processes are inhibited.

The rate of oxygen consumption is influenced by size, activity, stage in the life cycle of the animal and different environmental factors such as, oxygen, pH, oxygen content of water etc. The factors have well pronounced effect on oxygen consumption of freshwater poikilotherms, since they have to live under the influence of natural fluctuations of these parameters. The responses of different animal to the environmental factor are different and even within the same species, the rate of oxygen consumption

may be different. Considerable amount of literature is available showing relationship between respiratory activity and pollution stress in aquatic animals [2].

Many investigators have demonstrated harmful effects of heavy metals on histological structures of gills of crustaceans [9, 10, and 12]. The decline in oxygen consumption may be the result of hyperplasia and formation of coagulated mucus over the gills and body surface of the crab. Similar changes were observed and reported by many investigators [7]. The inhibition in oxygen consumption may be due to disintegration or rupture of respiratory epithelium and coagulation of mucus film over the gills surface. As a result, the absorption of oxygen by the gills is adversely affected [3]. The prominent mucus secretion on the gills and body surface was also observed during experimentation. In the present investigation, the decline of oxygen consumption in Barytelphusa *gureini* may be due to the onset of poisoning, gill damage, formation of mucus film over the gill and on the body surface. In nutshell these activities are helpful to minimize the toxic effect of toxicant on the body and reduce the efficiency of oxygen uptake.

Normal oxygen consumption was affected which has been discussed in the preceding section. Similarly, when the crabs were exposed to sub lethal concentration of the same toxicant for 0—96 hrs showed an elevation in the hemolymph sugar level with a maximum level with maximum increase at 96 hrs. Physiological processes are mostly coordinated by hormones and changes in hormone levels are expected to occur soon after exposure to environmental stress, such as pollutant, eventually acting as endocrine disruptors [8]. Hyperglycemia is a common stress response of many aquatic animals. In crustaceans it occurs following the involvement of the hyperglycemic hormone (cHH) produced in eyestalk, cHH mainly regulates glucose homeostasis. It belongs to the neuropeptide family synthesized in eyestalk by medulla terminalis x-organ, and is accumulated by and released from the sinus gland [8]. We are in agreement with the views of earlier researchers that an elevation in the hemolymph sugar level of the fresh water crab, Barytelphusa *gureini* can occur due to cadmium chloride, which may act on the neurotransmitter acting on cHH, a hemolymph sugar level regulating hormone [15].

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Conc in µg/L	No of crabs	Exposure time in hours					No of dead crabs	% Mortality	Log_{10} conc	Probit Mortality
		0 to 24	24 to 48	48 to 72	72 to 96	96				
Control	10	---	---	---	---	---	---	00	00	00
1.00	10	---	1	---	---	---	1	10	0.000	3.72
1.25	10	---	---	2	---	---	2	20	0.096	4.16
1.50	10	1	1	---	1	---	3	30	0.176	4.48
1.75	10	---	---	2	1	1	4	40	0.243	4.75
2.00	10	1	1	---	2	1	5	50	0.301	5.00
2.25	10	1	---	2	2	1	6	60	0.352	5.25
2.50	10	1	2	2	2	1	8	80	0.397	5.84
2.75	10	---	2	2	5	1	10	100	0.439	8.09
Total	90	4	7	10	12	6	39	----	----	----

Table 1: Percentage Mortality of *Barytelphusa gureini* exposed to cadmium chloride.

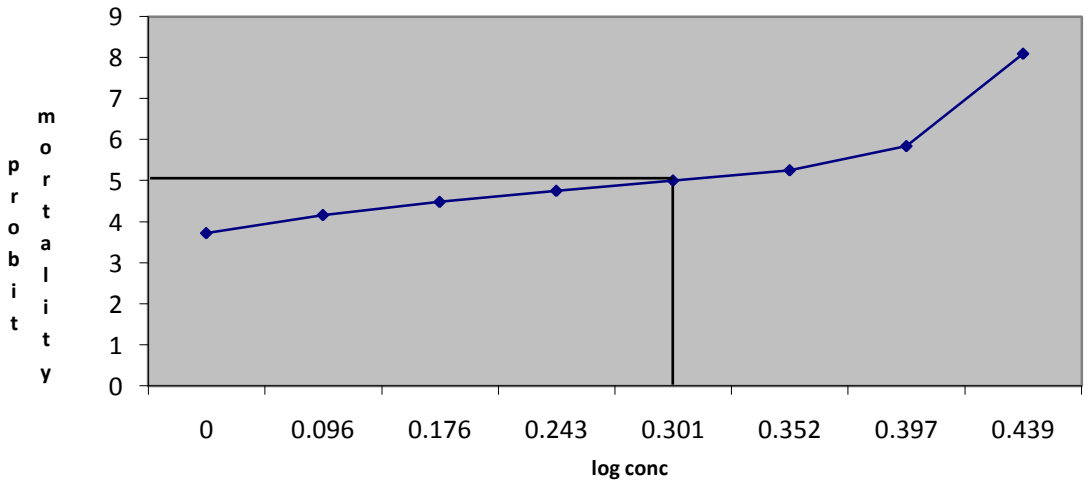

Fig 1: Lc 50 value of *Barytelphusa gurieni* exposed to cadmium chloride

Seiral number	Dose of Toxicant	Exposure period in hours	O_2 consumption mg/l/hr/gm wet weight
1	control	00-00	0.070 ± 0.008
2	2 µg/L	00-24	0.062 ± 0.006
3	2 µg/L	24-48	0.051 ± 0.03
4	2 µg/L	48-72	0.037 ± 0.002
5	2 µg/L	72-96	0.018 ± 0.001

Table 2: Effect of sub lethal conc of cadmium chloride on oxygen consumption in *Barytelphusa gurieni*

Seiral number	Dose of Toxicant	Exposure period in hours	Estimated Hemolymph sugar mg/100ml
1	control	00-00	64.348 ± 1.12
2	2 µg/L	00-24	67.971 ± 0.96
3	2 µg/L	24-48	78.956 ± 0.89
4	2 µg/L	48-72	86.298 ± 0.76
5	2 µg/L	72-96	93.391 ± 0.52

Table 3: Effect of sub lethal conc of cadmium chloride on hemolymph sugar in *Barytelphusa gurieni*

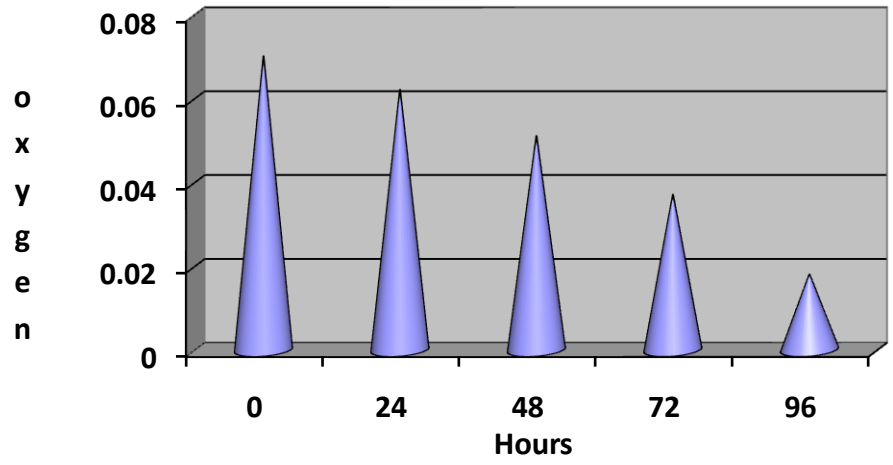

Fig 2: Oxygen consumption in *Barytelphusa gurieni*

(mg/liter/hour/gram wet weight)

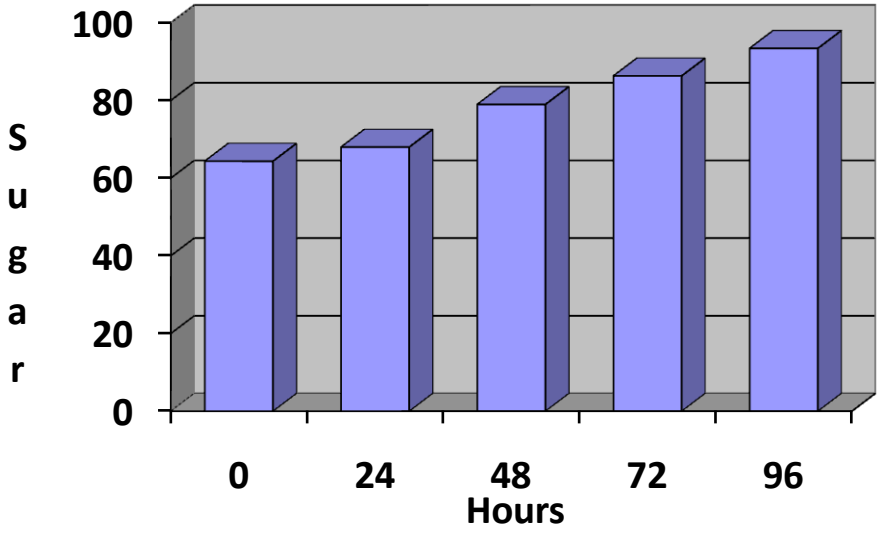

Fig 3: Hemolymph sugar level in *Barytelphusa gurieni*

(mg/100ml)