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Respiratory Response and Hemolymph Sugar Level of the Crab *Barytelphusa gureini* Exposed to Heavy Metals

Akhter Ali Siddiqui, Arif Ahmad, Nadeem Fatima Ansari

Abstract*: To explore the respiratory and metabolic responses, the fresh water crabs Barytelphusa gureini, were exposed to sublethal concentrations (2.0 & 2.25 µg/L) of cadmium chloride and copper sulphate respectively. Oxygen consumption and hemolymph sugar level of the animals were estimated to study the stress caused by these heavy metal toxicants. These animals after acclimatization to the laboratory conditions exposed for 0, 24, 48, 72 and 96 hrs to the toxicants. 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. These results showed that cadmium was found to be more toxic than copper metal.

Keywords*: Barytelphusa gureini, Cadmium Chloride, Copper Sulphate, Oxygen Consumption, Hemolymph Sugar, Sublethal Concentration.

I. INTRODUCTION

Cadmium toxicity has become the focus of intense research globally next to mercury as the most notorious heavy metal pollutant. It becomes toxic when it is not metabolized by the body and accumulates in soft tissues, liver, and kidneys mostly as metalloprotein. Cadmium toxicity to aquatic animals depends on complex biochemical interactions and imbalance between rates of absorption, detoxification, and excretion. In aquatic animals (e.g. crabs, shrimps, oysters, and mussels) heavy metals enter into various compartments of the body in different ways such as respiratory tract, digestive tract, surface penetration, etc. [1]-[3]. They are seriously harmful to the growth of aquatic life and survival, resulting in decline in their population. At the same time, as aquatic food products, these animals exposed to cadmium might threaten human health.

Respiration is the most vital process of life for the derivation of energy in the form of ATP to perform different biological and physiological functions like 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 uniquely [4]. However, it is well known that respiration is a vital phenomenon of life & the rate of oxygen consumption in turn controls metabolic activities and acts as a measure of the intensity of metabolism.

The 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 respiratory activities has been rightfully used as an indicator of stress in general and toxicant-induced change in the exposed animal in particular [5],[6].

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 a pivotal role in carbohydrate metabolism such as gluconeogenesis, glycogenesis, and glycolysis. Thus, the assessment of toxic heavy metals in aquatic animals can serve as a bio-indicator of their impacts on these organisms. It also gives an insight into the degree of pollution of the water body and the health status of the aquatic population [7]. The *Barytelphusa gureini* is well known for its high nutritive value and is commonly cultured by local farmers. Cadmium causes instantaneous respiratory impairment and alteration in the pathways of carbohydrate metabolism. In the present investigation, the rate of oxygen consumption and hemolymph sugar level is considered a tool to evaluate the toxic effect of heavy metals as the salt of cadmium chloride.

II. MATERIALS AND METHODS

Experimentation

A. Acclimatization

The adult specimens of freshwater field crab *Barytelphusa gureini*, were collected from the outskirt of paddy fields of Rangareddy district (Telangana), 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.

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* Correspondence Author
Akhter Ali Siddiqui*, Anwarul Uloom College, Hyderabad (Telangana), India. Email: akhter_1956@yahoo.in
Arif Ahmad, Zoology, School of Sciences, Maulana Azad National Urdu University, Hyderabad (Telangana), India. Email: arifamu@gmail.com
Nadeem Fatime Ansari, Department of Biotechnology, Anwarul Uloom College Hyderabad (Telangana), India. Email: mailtonfa@gmail.com

Toxicity bioassay

The aqueous stock solutions of cadmium chloride and copper sulphate were 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. The mortality rate was noted up to 96 hrs, and the test medium and dead crabs were removed at the interval of 24 hrs immediately. The **LC_{50}** was calculated by using the Probit analysis method [16]. After finding the **LC_{50}**, one set of ten crabs was treated with a sub-lethal concentration of cadmium chloride (2.0 µg/L) and (2.25 µg/L) of copper sulphate for 0-96 hrs respectively. The other set of ten crabs was kept as control under similar conditions without toxicants. (Table. 1)

B. Physicochemical parameters:

The physicochemical parameters of water were estimated as follows: dissolved oxygen: 7.2- 7.4 ppm, pH: 7.0-7.2, temperature: 29±20 c, salinity: 0.4-0.5 µg/ml and the total hardness: 280-288 mg/L.

C. 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 hrs 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 precautions. 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. 1).

Table 1: Percentage mortality of Barytelphus gureini exposed to cadmium chloride and copper sulphate.

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 toabove				
Control		----	----	----	----	----	----	00	00	00
$Cd\ Cl_2$ (1.00)	10	----	1	----	----	----	1	10	0.000	3.72
$Cu\ So_4$ (1.00)	10	----	----	----	----	----	----	00	00	00
$Cd\ Cl_2$ (1.25)	10	----	----	2	----	----	2	20	0.096	4.16
$Cu\ So_4$ (1.25)	10	----	----	----	----	1	1	10	0.000	3.72
$Cd\ Cl_2$(1.50)	10	1	1	----	1	----	3	30	0.176	4.48
$Cu\ So_4$(1.50)	10	----	----	----	1	1	2	20	0.096	4.16
$Cd\ Cl_2$(1.75)	10	----	----	2	1	1	4	40	0.243	4.75
$Cu\ So_4$(1.75)	10	----	---	----	----	1	2	30	0.176	4.48
$Cd\ Cl_2$(2.00)	10	1	1	----	2	1	5	50	0.301	5.00
$Cu\ So_4$(2.00)	10	----	1	1	1	1	4	40	0.243	4.75
$Cd\ Cl_2$(2.25)	10	1	----	2	2	1	6	60	0.352	5.25
$Cu\ So_4$(2.25)	10	----	----	1	2	2	5	50	0.301	5.00
$Cd\ Cl_2$(2.50)	10	1	2	2	2	1	8	80	0.397	5.84
$Cu\ So_4$(2.50)	10	----	1	1	3	2	7	70	0.372	5.60
$Cd\ Cl_2$(2.75)	10	----	2	2	5	1	10	100	0.439	8.09
$Cu\ So_4$(2.75)	10	----	1	1	6	2	10	100	0.439	8.09
Total ($Cd\ Cl_2$)	90	4	7	10	13	5	39	----	----	----

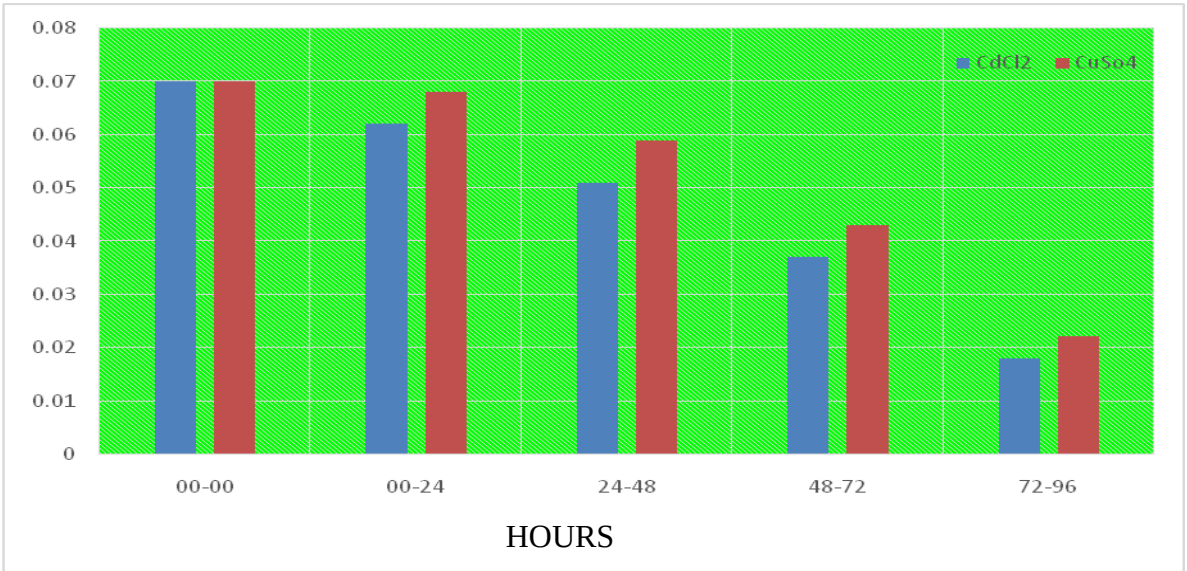

Fig:1. Effect of Sublethal Conc of cadmium chloride and copper sulphate on oxygen consumption in Barytelphus gureini (mg/l/hr/gm wet weight)

Table 2: Effect of sub-lethal conc of cadmium chloride and copper sulphate on hemolymph sugar in Barytelphus gureini

SNO	Dose of toxicants		Exposure period in hours	Estimated Hemolymph sugar mg/100ml
1	Control	$Cd\ Cl_2$	00--00	64.348 ± 1.12
	Control	$Cu\ So_4$	00--00	64.348 ± 1.12
2	2.00 µg/L	$Cd\ Cl_2$	00—24	67.971 ± 0.96
	2.25 µg/L	$Cu\ So_4$	00—24	70.571 ± 0.96
3	2.00 µg/L	$Cd\ Cl_2$	24—48	78.956 ± 0.89
	2.25 µg/L	$Cu\ So_4$	24—48	83.492 ± 0.92
4	2.00µg/L	$Cd\ Cl_2$	48---72	86.298 ± 0.76
	2.25 µg/L	$Cu\ So_4$	48---72	89.120 ± 0.83
5	2.00 µg/L	$Cd\ Cl_2$	72--96	93.391 ± 0.52
	2.25 µg/L	$Cu\ So_4$	72--96	97.453 ± 0.60

Table 3: Effect of sub lethal conc of cadmium chloride and copper sulphate on oxygen consumption in Barytelphus gureini

SNO	Dose of toxicants		Exposure period in hours	Oxygen consumption mg/l/hr/gm wet weight
1	Control	$Cd\ Cl_2$	00--00	0.070 ± 0.008
	Control	$Cu\ So_4$	00--00	0.070 ± 0.008
2	2.00 µg/L	$Cd\ Cl_2$	00—24	0.062± 0.006
	2.25 µg/L	$Cu\ So_4$	00—24	0.068 ± 0.005
3	2.00 µg/L	$Cd\ Cl_2$	24—48	0.051 ± 0.03
	2.25 µg/L	$Cu\ So_4$	24—48	0.059 ± 0.04
4	2.00 µg/L	$Cd\ Cl_2$	48---72	0.037 ± 0.002
	2.25 µg/L	$Cu\ So_4$	48---72	0.043 ±0.003
5	2.00 µg/L	$Cd\ Cl_2$	72--96	0.018 ± 0.001
	2.25 µg/L	$Cu\ So_4$	72--96	0.022 ± 0.002

D. Sugar estimation:

The hemolymph was extracted from the thigh region of the crab with the help of a syringe and turned into powder form by keeping it in an oven at 40^0c for a long period. The sugar level was estimated by the Anthrone method after 0, 24, 48, 72, and 96 hrs (Table. 3 & Fig. 2).

III. RESULTS AND DISCUSSION

The 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 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 an 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 can not meet the energy requirements of the cell. The energy-requiring processes such as protein synthesis, and glycolytic activity during oxygen deficiency can not meet the energy requirements of the cell.

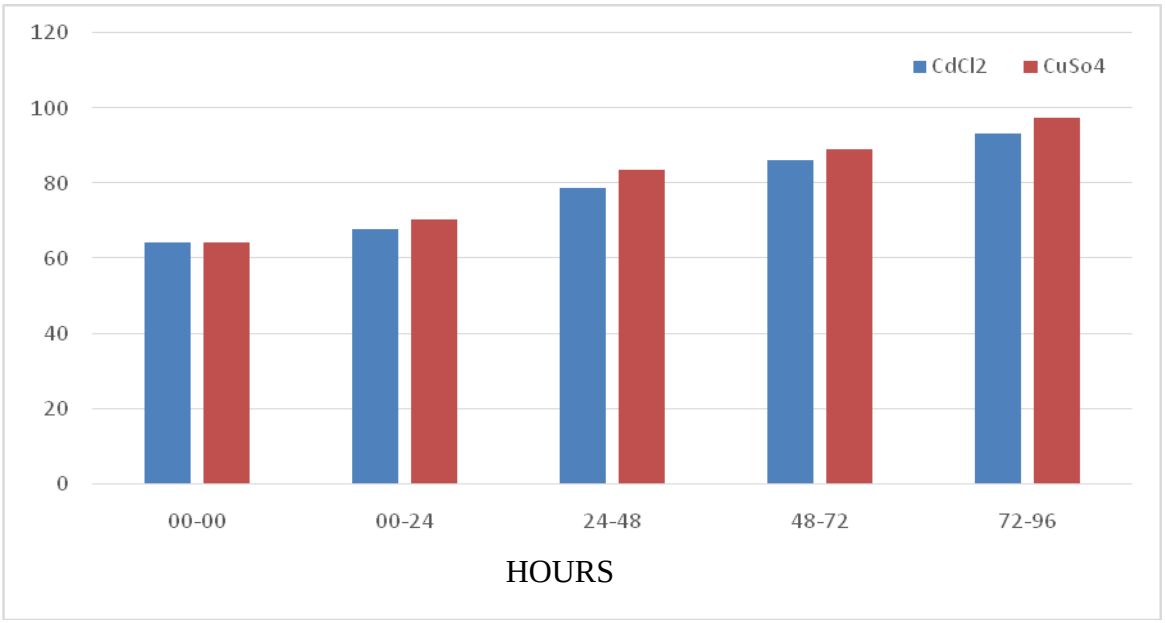

Fig: 2 Effect of sublethal conc cadmium chloride and copper sulphate on hemolymph sugar in *Barytelphus gureini* (mg/100ml)

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 the size, activity, stage in the life cycle of the animal, and different environmental factors such as, oxygen, pH, the oxygen content of water, etc. The factors have a 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 a different animal to the environmental factor are different and even within the same species, the rate of oxygen may be different. A considerable amount of literature is available showing the relationship between respiratory activity and pollution stress in aquatic animals [9]. Many investigators have demonstrated the harmful effects of heavy metals on histological structures of the gills of crustaceans [10]-[12]. The decline in oxygen consumption may be the result of hyperplasia and the formation of coagulated mucus over the gills and body surface of the crab. Similar changes were observed and reported by many investigators [13]. The inhibition in oxygen consumption may be due to disintegration or rupture of respiratory epithelium and coagulation of mucus film over the surface of the gills. As a result, the absorption of oxygen by the gills is adversely affected [14]. 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, and formation of mucus film over the gill and on the body surface. In nutshell, these activities are helpful to minimize the toxic effect of toxicants 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 a sub-lethal concentration of the same toxicant for 0—96 hrs showed an elevation in the hemolymph sugar level with a 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 pollutants, 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 freshwater 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].

IV. CONCLUSION

Based on the preliminary study, the authors concluded as follows: The toxicants may cause cellular injury which leads to disturbances in the microenvironment of cells. The insufficient supply of oxygen due to toxicants may lead to hypoxia or anoxia which may be the cause of the disturbances in cellular energy metabolism. Mucous secretion on the gills and body surface may be due to the disintegration of the respiratory epithelium. The presence of heavy mucous on the surfaces of gills and the body may be a resistance against the penetration of toxicants. The stress state of the experimental animals due to toxicant exposure may disrupt the physiological functions of their endocrine system which results in the elevation of hemolymph sugar levels. The toxicants in the brain and eyestalk may act as neurotransmitters, which may in turn influence the cHH resulting in increasing the hemolymph sugar levels of *Barytelphusa gureini*. Thus, the results obtained are in accordance with the earlier researchers.

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AUTHORS PROFILE

Akhter Ali Siddiqui, Working as associate professor, dept of Zoology, 32yrs of teaching experience at UG and PG level. 35 research papers and 3 books published 2 Ph.D. and 5 M.Phil. students awarded under my guidance. More than 80 conferences were attended and organized.

Arif Ahmed, working as a senior assistant professor, dept of Zoology, 12yrs of teaching experience, and research specialization molecular studies of HPV induced cervical cancer, Editor and author of two books, young scientist awarded by DST. Principal investigator of two research projects.

Nadeem Fatima Ansari, working as associate professor and Head dept of Biotechnology, 20 years of teaching experience at UG and PG level. 15 research papers published, more than 30 conferences and seminars attended and organized. Best teacher a warded at state level. At present working as controller of examination.