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Freshwater Palaemonid Prawn, *Macrobrachium Kistnensis* (Tiwari)-Effect of Heavy Metal Pollutants*

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The effects of heavy metal pollutants like $CuSO_4$ and $ZnSO_4$ on a freshwater prawn, *Macrobrachium kistnensis*, have been studied. $CuSO_4$ and $ZnSO_4$ were lethal at 2.25 ppm and 17.5 ppm respectively, after 24 hr exposure. Oxygen consumption increased significantly ($P < 0.05$) from 1.5 to 2.5 ppm of $CuSO_4$ and 5.0 to 15.0 ppm of $ZnSO_4$ as compared to control but it slashed severely at slightly higher concentrations of both pollutants. The carbohydrates metabolites like haemolymph glucose and midgut gland glycogen showed an inverse relationship in their changes up to 72 hr of exposure to sub Lc_{50} concentrations of $CuSO_4$ (1.0 ppm) and $ZnSO_4$ (5.0 ppm) but both metabolites decreased remarkably after prolonged (120 hr) exposure. The ecophysiological significance of these findings is discussed in brief.

Key Words: Heavy metals, CuSO , $ZnSO_4$, Pollutants, Survival, Respiration, Carbohydrates

Introduction

Heavy metal salts constitute a very serious type of pollution in freshwater because they are stable compounds and are not readily removed by oxidation, precipitation or other means and affect the activity of the animals (Costa 1965). During the last decade or so, much of the work on prawn pollution has been confined to the laboratory studies assessing direct toxicity of commonly occurring poisons under various environmental conditions (Costa 1965, Chinnayya 1971, Nagabhushanam & Diwan 1972, Thurberg et al. 1973). Eisler (1973) has extensively elaborated the biological effects of metals on aquatic organisms and contended that metals along with other substances are most toxic pollutants of river system. Some of the heavy metals bioassayed include copper (Goettl & Nehring 1972, Brown et al. 1974), zinc (Nehring & Goettl 1974, Solbe & Flook 1975) and their mixtures (Eaton 1973). So far, no report has come to our notice regarding the impact of the heavy metal pollutants like copper (Cu^{++}) and zinc

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(Zn^{++}) on the biological activities of freshwater prawns, barring a report on histological changes in the gills of the prawns, *Macrobrachium* and *Caridina* exposed to copper sulphate (Ghate & Mulherkar 1978). In the current investigation, efforts have been mede to aquire the information on the effects of these two pollutants on (a) survival, (b) respiration, and (c) carbohydrate metabolism of a freshwater prawn, *Macrobrachium kistnensis*.

Material and Methods

Adult and intermoult prawns, *M. kistnensis*, of both sexes having approximately equal size were collected from Sukna river near Chikalthana Industrial Area, Aurangabad. They were brought to the laboratory and held in glass aquaria containing continuously aerated fresh water (temperature 23–24°C) and kept under normal day/night illumination. There are about 100 major and minor industries manufacturing a variety of products including chemicals, drugs, food products and machinery, etc., which generally discharge their effluents in this river. For the current investigation, toxicity of two commonly occurring heavy metal pollutants of the river in the form of copper sulphate (0.5 to 0.8 ppm) and zinc sulphate (2.0 to 3.0 ppm) have been tested by static bioassay (table 1). Temperature by thermometer, dissolved oxygen (DO) by standard Winkler's method (Welsh & Smith 1961) and *p*H by photoelectric *p*H meter of both control and experimental media were measured. Along with each experimental group, a corresponding control unit was also set up for the experimental period in non-polluted freshwater aquarium to compare the results.

In order to ascertain the lethal concentrations (Lc 50) of the pollutants, groups of 10 prawns were held in aquaria separately containing 5 litres of test solutions having different concentrations (ppm) of $CuSO_4$ (0.5; 1.0; 1.5; 2.0; 2.5 & 3.0) and $ZnSO_4$ (1.0; 5.0; 10.0; 15.0; 20.0 & 25.0). The desired concentrations were obtained by dissolving appropriate quantity of the respective pollutant in 5 litres of freshwater. The above groups of prawns were observed for 24 hr under different concentrations of pollutants, during which mortality was noted, prawns were declared dead when their pleopods did not respond to mild probing.

The rate of oxygen consumption in the above mentioned six concentrations of $CuSO_4$ was measured separately using two experimental sets, each having six groups, of prawns and each group containing 10 individuals, by standard Winkler's method (C. opp). The quantity of oxygen consumption was calculated in relation to unit wet weight of prawns and the values thus obtained were expressed as the rate of O_2 consumed ml/hr/g/1 of wet weight.

To assess the effect of pollutants on carbohydrate metabolism prawns were acclimatised to the sublethal concentrations of $CuSO_4$ (1.0 ppm) and $ZnSO_4$ (5.0 ppm) for 24, 48, 72 and 120 hr. To avoid any change in the toxicity, temperature, DO_2 and *p*H, renewal of the test solution was made at every 12 hr. After the respective acclimation period, hemolymph glucose levels of both control and experimental groups were estimated by Nelson and Somogy's method as given by Oser (1976). Glycogen content of midgut gland was quantified by the method of Kulkarni and Nagabhushanam (1977). Hemolymph glucose and tissue glycogen levels were expressed as mg/100 ml of hemolymph and mg/g dry weight of tissue, respectively. Significance of the data obtained during all experiments was analysed using student 't' test (Ostle 1966).

Table 1 *Bioassay tests and physical conditions of the test media*

Pollutants	Concentration used (ppm)	No. of test prawns	Avg. body length (mm)	Avg. weight (g)	Effect end point	Physical condition of the test solutions: Fresh solution			At the end of 24 hr of exposure		
						Temperature °C	*p*H	DO (ppm)	Temperature °C	*p*H	DO_2 (ppm)
Copper sulphate	0.5	20	16	1.5	Lc50	26.0	7.2	7.4	26.0	6.9	7.2
	1.0	20	15	1.5	Lc50	26.3	8.1	7.3	26.3	7.1	7.3
	1.5	20	16	1.4	Lc50	26.2	7.0	7.3	26.1	7.0	7.0
	2.0	20	15	1.5	Lc50	26.2	7.1	7.2	26.0	7.1	7.2
	2.5	20	15	1.4	Lc50	26.1	7.2	6.9	26.2	6.9	7.0
	3.0	20	16	1.6	Lc50	26.0	7.0	7.1	26.1	7.1	7.1
Zinc sulphate	1.0	20	15	1.6	Lc50	26.1	7.1	7.3	26.0	7.0	7.2
	5.0	20	16	1.5	Lc50	26.0	7.0	7.2	26.1	7.1	7.0
	10.0	20	15	1.4	Lc50	26.0	7.0	7.2	26.0	7.2	7.2
	15.0	20	15	1.5	Lc50	26.0	7.1	7.2	26.1	7.1	7.1
	20.0	20	15	1.5	Lc50	26.1	7.1	7.1	26.1	7.2	7.1
	25.0	20	16	1.5	Lc50	26.0	7.2	7.2	26.1	7.0	7.2
Control freshwater	0.0	20	15	1.5	—	26.1	7.1	7.0	26.0	7.0	7.1

Results

Effect of various concentrations of Copper and Zinc sulphates on survival of M. kistnensis.

Data obtained in this study obviously reveal that the prawns survived well from 0.5 to 2.0 ppm of $CuSO_4$ and 1.0 to 15.0 ppm of $ZnSO_4$ for the period of 24 hr. The survival of the prawns gradually decreased with an increase in the concentration of pollutants, and 100% mortality was found in 3.0 ppm of $CuSO_4$ and 25 ppm of $ZnSO_4$. In 2.5 ppm of $CuSO_4$ and 20.0 of $ZnSO_4$ only 48% and 42% of prawns survived respectively, after 24 hrs. The concentrations of $CuSO_4$ and $ZnSO_4$ tolerance of *M. kistnensis* were found to be 2.25 ppm and 17.5 ppm respectively, when 50% survival is the criterion designated for ascertaining the lethal concentrations.

Effect of different concentrations of copper and zinc sulphates on oxygen consumption of M. kistnensis.

The results presented in figure 1 clearly show that the rate of oxygen consumption of *M. kistnensis* increased significantly ($P< 0.05$) from 1.5 ppm to 2.5 ppm of $CuSO_4$ and 5.0 ppm to 15.0 ppm of $ZnSO_4$ as compared to control. The rate of oxygen consumption decreased severely in 3.0 ppm of $CuSO_4$ and 25.0 ppm of $ZnSO_4$.

Changes in the hemolymph glucose and midgut gland glycogen levels after exposing M. kistnensis to sublethal concentrations of Copper Sulphate and Zinc Sulphate for different periods.

The results illustrated in figure 2 indicate an inverse relationship between the content of hemolymph glucose and midgut gland glycogen from 24 hr to 72 hr of exposures to both $CuSO_4$ and $ZnSO_4$ solutions. The hemolymph glucose level

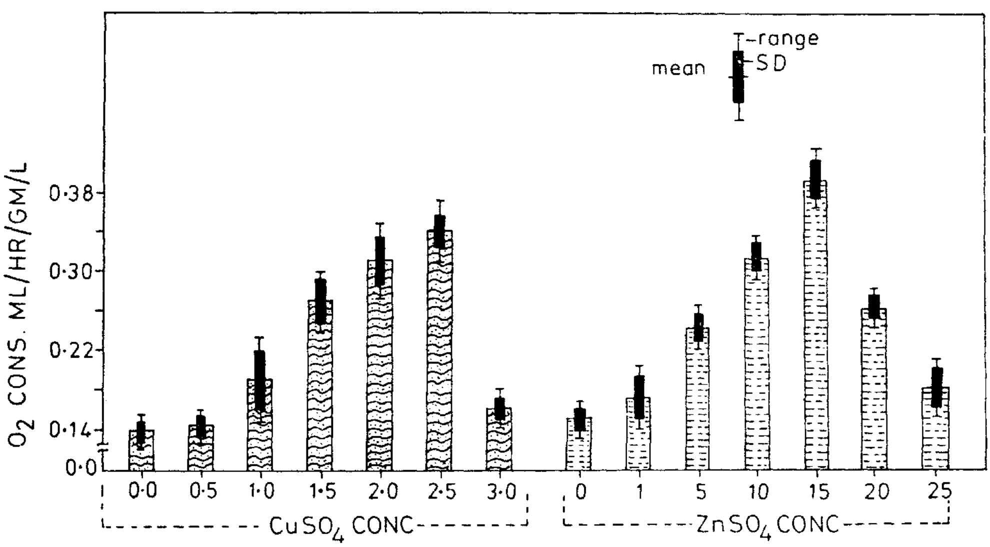

Figure 1 Oxygen consumption of *M. kistnensis* in different concentrations of $CuSO_4$ and $ZnSO_4$

increased considerably after exposure for 48 hr to $CuSO_4$ and 72 hr to $ZnSO_4$ whereas noticeable decrease was found after 120 hr of exposure to both pollutants. The midgut gland glycogen also decreased significantly ($P < 0.05$) after acclimation for 48 and 72 hr to $CuSO_4$ and $ZnSO_4$ respectively, but slashed more severely ($P < 0.01$) after 120 hr of exposure to both pollutants.

Discussion

The main water pollutants of the river system are metals, pesticides, detergents, chemicals, industrial and domestic wastes including organic substances. One of the harmful effects of these pollutants in water system is on prawns. The present study obviously indicates that as the concentrations of copper and zinc sulphates in the media increased the % survival of *M. kistnensis* decreased, when the physical and chemical conditions of the test solutions (table 1) were kept constant. If the mortality rate of the prawn in the two pollutants is compared, it becomes higher in $CuSO_4$ than that in $ZnSO_4$. This is apparent from the 50% mortality occurring within 24 hr in 2.5 ppm of $CuSO_4$ whereas 17.5 ppm of $ZnSO_4$ is required to cause the same mortality. Hence, in order to achieve 50% mortality within 24 hr (24 hr Lc50), the concentration of $ZnSO_4$ required is more than 7 times higher than that of $CuSO_4$. On the basis of mortality of the prawns, $CuSO_4$ appears to be more lethal toxicant than $ZnSO_4$. The causes of death owing to lethal action of metals may be due to damage of the respiratory surface or the formation of mucus film over the gills and body surface. Except for a study on *Macrobrachium* and *Caridina*, in which gill surfaces were damaged after prolonged exposure to copper sulphate (Ghate & Mulherkar 1978), no other report is available but similar reasons have been forwarded for fishes (Aronson 1971, Skidmore & Tovell 1972).

It is a well-known fact that the rate of oxygen consumption denotes the physiological state of metabolic activity in prawns. In this study, oxygen consumption by the prawns in normal and polluted waters have been measured and difference in the two conditions taken as a measure of toxic effect of the pollutants. The rate of oxygen consumption of *M. kistnensis* was continuously on ascending scale from 0.5 ppm to 2.5 ppm of $CuSO_4$ and 1.0 ppm to 15.0 ppm of $ZnSO_4$ but at 3.0 ppm of $CuSO_4$ and 25.0 ppm of $ZnSO_4$ it slashed severely. Therefore, it may be suggested that as concentrations of the media were increased there must be an increased osmotic work at the cellular level resulting in an enhanced oxygen consumption but at the lethal concentrations, the formation of coagulated mucus film over the body surface and gills must be taking place which interferes with the respiratory function and other vital processes of the gills resulting in lowering of respiration due to the moribund condition of prawns. Similar results were documented by Costa (1965) in *Gammarus*, Chinnayya (1971) in *Caridina rajadhari* and Nagabhushanam and Diwan (1972) in *B. cunicularis*. They reported a decreased oxygen consumption of these crustaceans in toxic substances like lead nitrate, copper sulphate, zinc sulphate and mercuric chloride. American workers on anti-fouling problems have published results supporting the view that copper retards vital processes through inactivation of essential enzymes (Clarke 1947).

The results of our third experiment (figure 2) clearly indicated that the hemolymph glucose and midgut gland glycogen undergo changes owing to the acclimation of prawns to the sub-lethal concentrations of $CuSO_4$, and $ZnSO_4$, for different periods. Variations in the hemolymph glucose and midgut gland glycogen were inversely proportional to each other and obviously show their interconversions in each other according to the energy demands for their metabolic activities up to 72 hr but both carbohydrate metabolites decrease ofter 120 hr of acclimation to both media. This fall in carbohydrate levels after prolonged exposure to the pollutted water may be expected due to the inactivation of the enxymes involved in the carbohydrate synthesis and metabolism owing to the effects of these heavy metals. To our knowledge, no report has been published so far, on this aspect for prawns, but similar observations have been recorded by Hodson (1976); Qayyum and Shaffi (1977) and Diwan et al. (1979) for fishes. As reported by earlier workers for the aquatic animals, in econclusion, it can be suggested for *M. kistnensis* also that at a low concentration of these pollutants, the only effect may be stimplatory, at a slightly higher concentration an enzyme system might be interfered and inactivated and at higher concentration the metals might interfere with respiration by decreasing blood pigment transport and at lethal concentration the pollurants might coagulate mucus or other vital secretions.

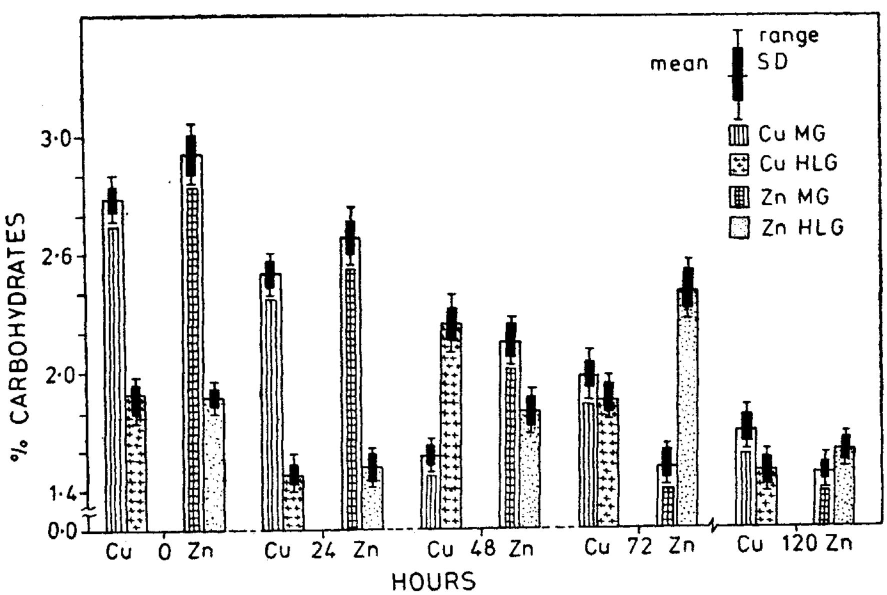

Figure 2 Percent change in hemolymph glucose (HLG) and midgut gland glycogen (MG) levels of *M. kistnensis* acclimated to sublethal concentrations of $CuSO_4$ and $ZnSO_4$ for different periods
O = Control

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