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Effects of Organic and Inorganic Lead on the Oxygen Equilibrium Curves of the Fresh Water Field Crab, *Barytelphusa guerini*

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Haemocyanin serves as normal transporter of oxygen in many Arthropods. The oxygen equilibrium curves have been described for the haemocyanins of many Arthropods and Molluscs (Redmond 1971; Padmanabha Naidu 1966; Manwell 1960). Oxygen equilibrium curves of the blood reveal the relationship between the oxygen tension and the percentage satuaration of the haemocyanin. The shape of the oxygen equilibrium curves vary in position from sigmoid to hyperbolic in different animals or even undulatory as shown in some chitons (Manwell 1960; Redmond 1962). Oxygen equilibrium curves are known to be influenced by pH, temperature and inorganic ions (Manwell 1960; Redmond 1955; Redfield 1934; Jones 1972; Prosser 1973). Oxygen equilibrium curves in relation to environmental stress conditions like aestivation in snails and starvation in crabs has been reported (Venugopala Reddy et al 1980; Venkat Reddy et al 1983). But the effect of environmental pollutants like the heavy metals on the oxygen equilibrium curves of the fresh water crab has not been previously reported.

One of the toxic heavy metals with regard to aquatic organisms is lead (Holcombe et al 1976; Reichert et al 1979; Hosdon et al 1984). Effluents containing lead are discharged during the manufacture of many industrial products, including paints, pigments, dyes and in match industry (Hodson et al 1984). The wide spread use of lead salts and their subsequent introduction in water bodies is becoming a lethal factor to various aquatic organisms. Hence the present study was designed to determine the effect of organic and inorganic lead on the oxygen equilibrium curve of the fresh water crab, Barytelphusa guerini.

MATERIALS AND METHODS

Crabs were collected from the local paddy fields. They were then transported to the laboratory. On arrival, only healthy male adult crabs in intermoult condition were selected and acclimatized to laboratory conditions for 2 weeks in round plastic tubs containing 8 L of water. They were fed with fish meat daily during this period. After two weeks of acclimatization

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they were exposed to different concentrations of lead nitrate and lead acetate and LC 50 values were determined by probit method (Finney 1964). The details of the LC 50 values are given elsewhere (Tulasi et al 1985). The crabs were exposed to a sublethal concentration of 0.5 mg/L for a period of 30 days and the oxygen equilibrium curves were determined on different days (1, 4, 7, 15 and 30) of exposure in the normal and experimental crabs.

The blood was collected by inserting a hypodermic syringe through the second arthrodial membrane. The pH of the blood of normal and experimental animals were measured on a pH meter. The difference spectra and the oxygen equilibrium curves of the blood of normal and experimental animals were determined by the spectrophotometric methods as described by Redmond 1955, with certain modifications according to Padmanabha Naidu 1966.

RESULTS AND DISCUSSION

The pH of the normal and experimental crabs are given in Table 1. Marginal increase in blood pH was noted on 1st and 4th days of exposure. (Maximum increase was from 7.49 in normals to 7.63 in lead nitrate exposed and 7.66 in lead acetate exposed crabs). On 7th, 15th and 30th day of exposure a significant decrease was observed (Maximum decrease observed was from 7.43 in normals to 6.91 in lead nitrate exposed and 6.72 in lead acetate exposed crabs).

Table 1. pH levels, P 50 Values and the Degree of interaction 'n' of the Haemocyanin of the blood of <u>Barytelphusa guerini</u> on exposure to lead nitrate and lead acetate.

Exposure period in days	Normal pH	Normal P 50	Normal 'n'		Experimental pH	Experimental P 50	Experimental 'n'
1	7.49	11.50	1.63	(A)	7.63	9.00	1.57
				(B)	7.66	8.25	1.54
4	7.48	11.50	1.63	(A)	7.59	10.50	1.60
				(B)	7.57	9.75	1.63
7	7.43	11.50	1.63	(A)	7.28	13.00	1.77
				(B)	7.16	13.50	1.54
15	7.35	11.50	1.63	(A)	7.09	15.00	1.73
				(B)	7.07	15.50	1.70
30	7.43	11.50	1.63	(A)	6.91	16.25	1.95
				(B)	6.72	16.00	1.60

(A) Values of Lead Nitrate (B) Values of Lead Acetate (Each value is the mean of five samples).

Difference spectra of the exyhaemocyanin was determined in the visible range of the spectrum. Maximum absorption spectra was found to be at 580 nm for normal and experimental crabs (Figure 1 and 2). No change in the absorption spectra of the oxyhaemocyanin was noted during exposure. But the optical density values

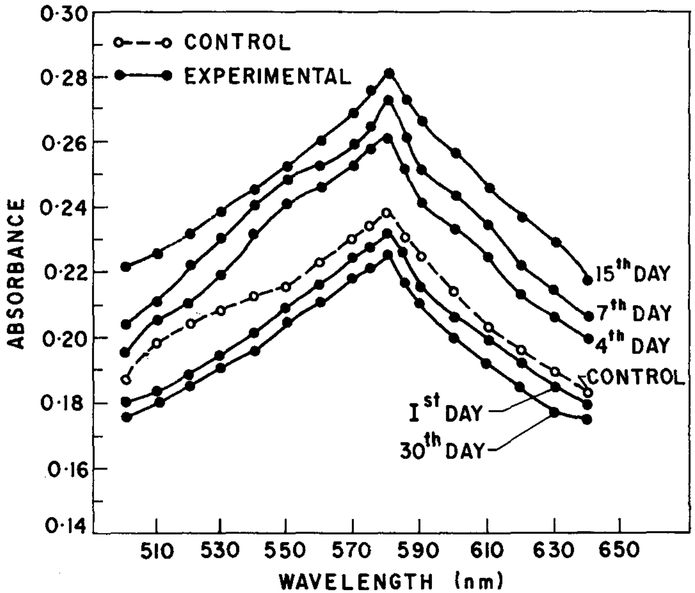

Figure 1. ABSORPTION SPECTRA OF THE OXYHAEMOCYANIN OF B. GUERINI ON EXPOSURE TO LEAD NITRATE (EACH POINT IS THE MEAN OF FIVE SAMPLES)

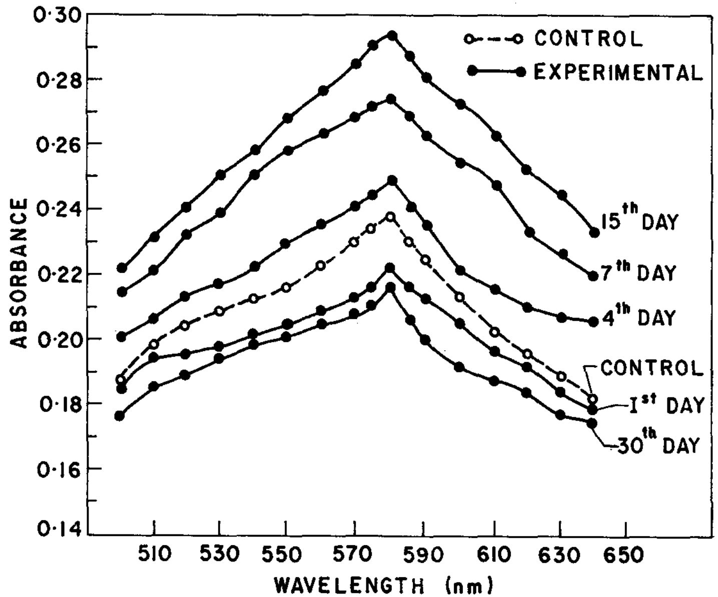

Figure 2. ABSORPTION SPECTRA OF THE OXYHAEMOCYANIN OF B. GUERINI ON EXPOSURE TO LEAD ACETATE (EACH POINT IS THE MEAN OF FIVE SAMPLES.

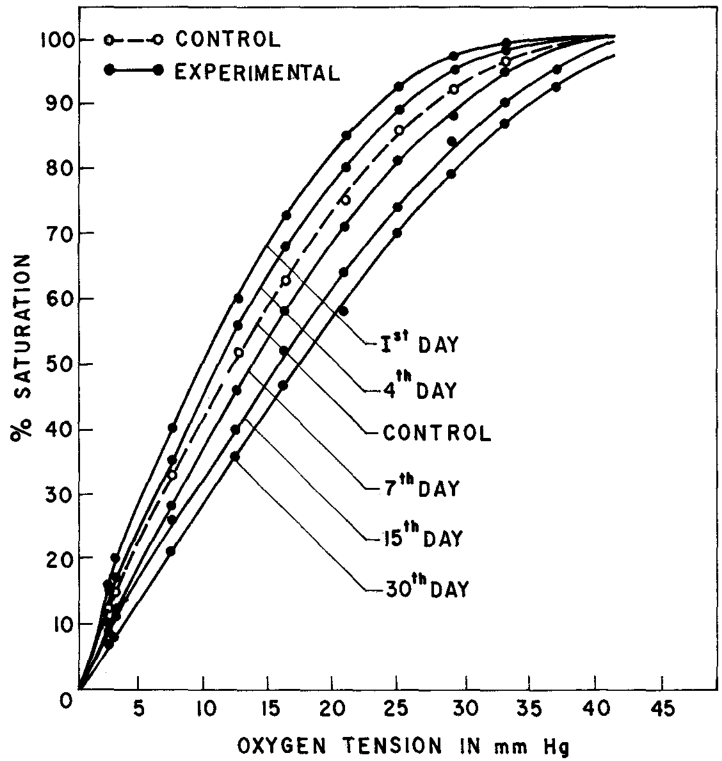

Figure 3. OXYGEN EQUILIBRIUM CURVES OF THE BLOOD OF B. GUERINI ON EXPOSURE TO LEAD NITRATE (EACH POINT IS THE MEAN OF FIVE SAMPLES)

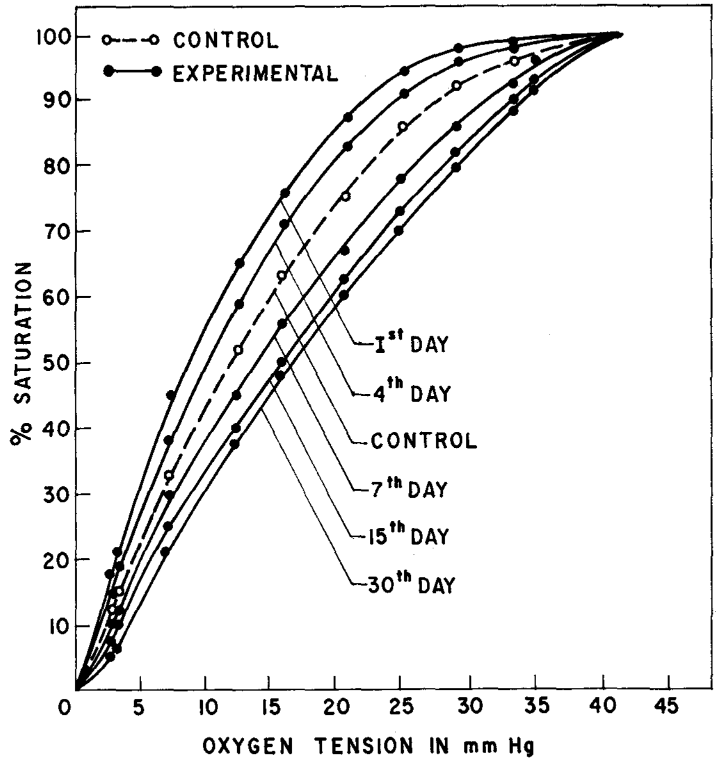

Figure 4. OXYGEN EQUILIBRIUM CURVES OF THE BLOOD OF B. GUERINI ON EXPOSURE TO LEAD ACETATE (EACH POINT IS THE MEAN OF FIVE SAMPLES)

increased with an increase in the exposure period upto a period of 15 days suggesting an increase in the concentration of haemocyanin. This may be due to stress and hypoxic conditions since these conditions are known to increase the haemocyanin concentration in the crab, Carcinus and in the lobster, Homarus americanus (Boone and Schoffeniels 1979; Senkbiel and Wriston 1981).

The oxygen equilibrium curves of the blood of the normal crabs are typically sigmoidal as in other decapod crustaceans (Redmond 1971) indicating a positive interaction among the oxygen combining sites of the haemocyanin molecule. The value of half saturation tension (P 50) for the normal crab is 11.50 mm Hg. Similar high P 50 values were reported for some crustaceans (Prosser 1973). The value of 'n' is more than "One" indicating a positive interaction among the oxygen combining sites of the haemocyanin molecule. (Table 1)

Exposure to subtoxic levels of organic and inorganic lead elicited two different types of responses. On 1st and 4th days of exposure there was a shift in the curves towards the left side of the normals. (Figure 3 and 4). There was a decrease in the P 50 values for haemocyanin indicating an increase in the affinity of haemocyanin to oxygen. On prolonged exposure the curves shifted towards the right side of the normals with an increase in the P 50 values suggesting a decrease in the affinity of haemocyanin for oxygen. The factors involved in the shifting of the oxygen equilibrium curves to the left on 1st and 4th days of exposure may be due to increase in blood pH. During prolonged exposure the curves shifted towards the right side of the normals which may be due to decrease in pH, suggesting an accumulation of acidic metabolites (Spry and Wood 1984). Experiments in our laboratory (Tulasi and Ramana Rao 1988) have also indicated high accumulation of lactic acid during lead toxicity. Shifting may be due to Normal or Negative Bohr effect. This is attributable to a decrease in the affinity of the respiratory pigments for oxygen under hypoxic conditions, since whole animal oxygen consumption of the fresh water crab was also decreased during lead toxicity (Tulasi and Ramana Rao in press).

Inorganic ions are known to play an important role in determining the position of oxygen equilibrium curves (Larimer and Riggs 1964; Prosser 1973). During lead toxicity a marginal increase in the monovalent cations like sodium and potassium and marginal fall in the divalent cations like calcium was observed (Tulasi 1986) which might have contributed for the shifting of the curves towards the right side of the normals. The response of the fresh water crab to lead nitrate and lead acetate was similar except for the fact that at the end of the exposure period shifting of the curves was more in the lead acetate exposed crabs. This may be due to the differences in the degree of toxicity induced by organic and inorganic lead and also due to difference in the accumulation of organic and inorganic lead (Tulasi et al 1985; 1987). Hence it is probable that the observed shift in the position of the curves may not be due to a single factor as all of them occur simultaneously on exposure to lead. The combination of all these factors might be responsible for the observed shifting of the oxygen equilibrium curves resulting in an increased or decreased affinity of the pigment for oxygen.

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