

## Effect of Lead on the expression of nutritional content in edible lobster, *Thenus orientalis* (Lund, 1793)

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**Abstract:** The edible lobster, *Thenus orientalis* is an important food of commercial interest in the parts of South India. Evaluation of toxic effect of lead on the chosen animal for the LC<sub>50</sub> value and effect of heavy metal lead on the nutritional status viz. protein, carbohydrate and lipid in ovary, spermatheca, hepatopancreas, muscle and haemolymph was made. The results assume greater interest as most water bodies are increasingly subjected to environmental pressure due to pollution.

**Keywords:** Lead toxicity, lobster, LC<sub>50</sub>, protein, pollution.

### Introduction

Our coastal water bodies are highly polluted due to industrial and urban wastes (Shanthi & Gajendran, 2009). Heavy metals such as copper, zinc and lead are now the normal constituents of marine and estuarine environments due to pollution. When additional quantities are introduced from industrial effluents, they enter into the biogeochemical cycle of organisms. All heavy metals become toxic at some concentration (Bryan, 1971). They are not only neurotoxic but also affect other systems and have shown a high degree of impact on metabolism by altering the carbohydrates, proteins and lipids (Mckee & Knowles, 1986; Nagabhushanam *et al.*, 1972). The concentration of heavy metals in seawater and in the flesh of crab and lobster has been observed. Accumulation of cadmium by the shrimp *Palaemon elegans* has been examined (White & Rainbow, 1986). The effect of heavy metal copper on the nutritive value of field crab *Spiralothelphusa hydrodroma* has been reported (Senthil kumaar *et al.*, 2007). Lethal effect of cadmium on the respiratory metabolism of crustacean *Leptomysis lingvura* was determined (Gaudy *et al.*, 1991). In the present study, the effect of lead on the nutritional content of edible lobster *Thenus orientalis* is reported.

### Materials and methods

#### Study area

The Nellore fish landing center (Andhrapradesh, India) is one of the major fishing grounds for fishes and crustaceans. The edible lobster *Thenus orientalis* on which the present investigation has been carried out was collected from this fishing center. This animal enjoys wide distribution in Red Sea, Gulf of Aden, East Africa, Madagascar, Mauritius, South Africa, Indian Seas, Sri Lanka, Andhra Pradesh Coast, Orissa coast,

Tamil Nadu coast, China, Singapore, Phillipines, West and North Australia and Kerama Islands.

#### Animal collection

Live specimens of edible lobster *Thenus orientalis* were collected from the commercial catches of Nellore fish landings, when fishing for other species of lobsters and fishes by mechanised trawlers. These incidental catches appear to be available in all seasons throughout the year. They were brought to the laboratory in a plastic bucket with seawater and maintained in the laboratory for various analyses.

The experimental animals were treated with the heavy metal lead in the form of lead acetate. Before sacrificing the animals, haemolymph was collected using a sterilized syringe and needle; and before collecting the haemolymph the needle was rinsed in 0.2% EDTA to avoid coagulation (Subhashini & Ravindranath, 1980). The tissues were stored in deep freezer for bio-chemical analysis. The control and experimental animals were dissected. Ovary, spermatheca, hepatopancreas, and muscle were taken and subjected to analysis for nutritional content.

Toxicity study carried out to determine the potency of lead for static but renewal type of bioassay was adopted in the present investigation to estimate the LC<sub>50</sub> values. The heavy metal lead as lead acetate, commercial grade was used as the test material since only commercial preparation is used in many dyeing industries. The experiment was performed to find out the range of concentration for confirmatory evaluation. The mortality was recorded for the lobster at 24hr, 48hr, 72hr, and 96hr exposure to lead; were corrected for natural response by Abbott's formula (Abbott, 1925). The LC<sub>50</sub> values for 24hr, 48hr, 72hr and 96hr exposure periods were estimated as 0.194, 0.169, 0.154 and 0.120ppm respectively (Table 1).

#### Design of sublethal toxic study

Chronic study on the effect of lead on the lobster was

Table 1. The LC<sub>50</sub> values and regression equations for *T. orientalis* treated with lead

Exposure periods (hours)	LC <sub>50</sub> (ppm)	Upper Confidence limits (ppm)	Lower Confidence limits (ppm)	Regression results	Slope function (SF)	r <sup>2</sup>
24	0.194	0.230	0.163	Y = - 5.832 X + 7.285	1.355	0.992
48	0.169	0.200	0.142	Y = - 4.959 X + 7.939	1.353	0.966
72	0.154	0.246	0.127	Y = - 3.997 X + 7.563	1.381	0.982
96	0.120	0.249	0.081	Y = - 2.167 X + 6.394	0.772	0.988

conducted by exposing to two sublethal safe concentrations for 15days and 30days. According to Matsumura (1975), Ramana Rao and Ramamurthy (1980), 1/3<sup>rd</sup> and 1/10<sup>th</sup> of the 96hr LC<sub>50</sub> value represent higher and lower sublethal concentrations, respectively. Hence lower (0.012 ppm) and higher (0.04 ppm) sublethal concentrations of the pollutant were arbitrarily used. At the end of the treatment period, the control and treated lobsters were dissected and the above said tissues were collected to analyse the nutritive value.

#### Biochemical analysis

Protein, Carbohydrate and Lipid estimations were carried out by following the methods of (Bradford, 1976; Reddy *et al.*, 1989; Folch *et al.*, 1957).

#### Statistical analysis:

One way Analysis of Variance (ANOVA) was performed based on the methods of Winer (1971).

Table 2. Effect of sublethal concentrations of lead on protein content in different tissues of *T. orientalis*

Exposure Period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15	Ovary	79.48 ± 3.20	76.06 ± 4.00	72.90 ± 3.51	4.2	0.0413
	Spermatheca	71.31 ± 3.88	69.09 ± 2.45	57.52 ± 3.90	22.59	<0.001
	Hepatopancreas	79.05 ± 4.43	69.01 ± 2.64	61.62 ± 3.00	32.14	<0.001
	Muscle	96.63 ± 1.53	87.48 ± 1.58	81.29 ± 3.34	55.65	<0.001
	Haemolymph	80.95 ± 3.64	74.42 ± 3.10	69.53 ± 3.12	15.07	<0.001
30	Ovary	83.22 ± 2.40	73.09 ± 3.59	63.37 ± 3.46	48.12	<0.001
	Spermatheca	81.20 ± 2.97	67.19 ± 6.86	54.82 ± 3.68	37.57	<0.001
	Hepatopancreas	82.53 ± 3.01	66.79 ± 3.37	60.46 ± 3.23	62.68	<0.001
	Muscle	109.80 ± 1.08	86.49 ± 1.82	78.92 ± 2.06	141.07	<0.001
	Haemolymph	79.58 ± 3.87	65.41 ± 3.17	56.60 ± 3.22	56.79	<0.001

Mean ± SD of six individual observations; Values are expressed mg/g wet tissue and mg/ml haemolymph; Statistically significant (By Tukey's multiple comparison test).

## Results and discussion

### Effect of lead on proteins:

Data presented in Table 2 reveal that the maximum reduction of protein content was observed in 30days of exposure in all the tissues that were taken for study.

### Effect of lead on carbohydrates

Decrease of total sugars was maximum in 30days of treatment at both lower sublethal and higher sublethal concentration in all the tissues that were investigated. The corresponding data is depicted in Table 3.

### Effect of lead on lipids

The decrease in the lipid content was to the maximum in 30days of treatment at both experimental concentrations in all the investigated tissues. The corresponding data is provided in Table 4.

Proteins play a crucial role in virtually all-biological processes. Enzymes catalyze nearly all-chemical reactions in biological systems (Stryer, 1980). Under extreme stress conditions, proteins supply energy in metabolic pathways and biochemical reactions (Yerragi *et al.*, 2000). In our experiment of lead exposure, the

protein content was decreased in all the tissues such as ovary, spermatheca, hepatopancreas, muscle and haemolymph. The decrease was drastic in higher sublethal (0.04 ppm) concentration of lead exposure for 30days.

The decrease of protein content in the prawn *Penaeus indicus* post larvae after exposure to sublethal concentration of lead was recorded (Sathyavathi & Prabhakara Rao, 2002). Decrease in protein content was also found in prawn *Penaeus indicus* after exposure to the pesticides phosphomidon and methyl parathion (Reddy *et al.*, 1988). Similar observations were also found in the prawn *Metapenaeus monoceros* due to phosphomidon exposure (Vijayalakshmi & Rao, 1985).

Reddy and Rao (1991) found decrease in the protein level in the marine prawn *Metapenaeus monoceros* after the prawn was exposed to the pesticide methyl parathion.

Geraldine *et al.*, (1999) reported protein depletion in the freshwater prawn *Macrobrachium malcolmsonii* in response to dichlorvos exposure. Zhao and Yang (2008) noticed that metabolic enzymes significantly decreased in accordance with increase in Cu<sup>2+</sup> concentration in giant fresh water

prawn *Macrobrachium rosenbergii*.

Marked decrease in protein content was found in the freshwater field crab *Paratelphusa hydrodromous* in response to the pesticide Malathion toxicity (Singaraju *et al.*, 1991). Senthil kumar *et al.* (2007) observed decrease in protein content in all the experimental tissues of field crab *Spiralothelphusa hydrodroma* due to the effect of heavy metal copper. Studies about the depletion of protein content in the marine edible crab *Scylla serrata* due to organochlorine pesticide dimecron toxicity were recorded (Sambasiva Rao *et al.*, 1987). The effect of endosulfan in various tissues of the freshwater field crab *Barytelphusa guerini* showed the decrease in protein level (Reddy *et al.*, 1989).

Ramana Rao and Ramamurthy (1980) noted decrease in protein content in the freshwater snail *Pila globosa* because of the sublethal effect of the pesticide sumithion. Shivaprasad *et al.*, (1981) studied the effect of the pesticide methyl parathion in the tissue proteins and secretory products of the snail *Pila globosa* and reported that the depletion of protein content in treated animal may

be due to enhanced proteolytic activity. Patil (1986) found decrease in protein level in the worm *Mythima sepearata* after it was exposed to different pesticides.

and Lead acetate bring changes in the glucose level in the fresh water prawn *Macrobrachium kistensis* (Fawade *et al.*, 1983). The activity of the enzyme phosphorylase in

Table 3. Effect of sublethal concentrations of lead on carbohydrate content in tissues of *T.orientalis*

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-Value	P-Value
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
15	Ovary	21.37 $\pm$ 2.83	16.18 $\pm$ 3.49	12.66 $\pm$ 1.62	12.59	<0.001
	Spermatheca	21.17 $\pm$ 2.78	17.79 $\pm$ 2.50	14.36 $\pm$ 2.37	8.86	0.0043
	Hepatopancreas	17.96 $\pm$ 1.65	15.89 $\pm$ 3.56	13.11 $\pm$ 1.08	5.35	0.0219
	Muscle	17.30 $\pm$ 2.51	14.95 $\pm$ 1.57	11.96 $\pm$ 1.97	8.41	0.0052
	Haemolymph	12.12 $\pm$ 1.91	9.82 $\pm$ 1.26	8.89 $\pm$ 1.22	6.12	0.0147
30	Ovary	22.38 $\pm$ 3.36	16.49 $\pm$ 2.73	11.09 $\pm$ 0.77	24.75	<0.001
	Spermatheca	22.78 $\pm$ 1.47	15.38 $\pm$ 2.51	11.39 $\pm$ 1.05	52.23	<0.001
	Hepatopancreas	18.76 $\pm$ 2.16	13.69 $\pm$ 2.67	11.71 $\pm$ 1.21	14.86	<0.001
	Muscle	18.50 $\pm$ 1.77	14.43 $\pm$ 1.55	11.06 $\pm$ 0.77	33.7	<0.001
	Haemolymph	12.37 $\pm$ 1.86	8.74 $\pm$ 1.19	7.50 $\pm$ 1.11	24.3	<0.001

Mean  $\pm$  SD of six individual observations; Values are expressed mg/g wet tissue and mg/ml haemolymph; Statistically significant (By Tukey's multiple comparison test)

Umminger (1970) observed decrease in the protein content in the fish *Fundulus heteroclitus* and stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source. Ramalingam and Ramalingam (1982) also reported that the protein level in liver and muscle of *Sarotherodon mossambicus* decreased because of the exposure to the pesticide DDT. Borah and Yadav (1985) studied the effect of the insecticide rogor in the muscle and gill of *Heteropneustes fossilis* and found a decrease in protein content. Malla reddy and Mohideen (1988) recorded the reduction of protein content in the branchial tissue of the *Cyprinus carpio* because of the toxic impact of fenvalerate. Susan *et al.*, (1999) also reported a significant decrease in protein content under sub lethal concentration of pyrethroid fenvalerate in the gill of the fish *Catla catla*. In the present investigation, reduction in total protein content was noted in the tissues of the test lobster exposed to lead. This was possibly due to the direct effect of the toxicant on protein metabolic demands following exposure to the toxic stress of lead. Reduction of protein content in the hepatopancreas and haemolymph of *Thenus orientalis* indicates the possibility of gluconeogenesis to meet the energy budget.

Carbohydrate typically contributes to structural support and protection, and serves as nutrient and energy stores to be increased or decreased according to organismal need. The results obtained in the present study showed that the carbohydrate content decreased significantly in both the lower (0.012 ppm) and higher (0.04 ppm) sublethal concentration for 30days. Changes in the energy level of *Penaeus indicus* post larvae after exposure to sub-lethal concentration of lead has been reported (Sathyavathi & Prabhakara Rao, 2002). DDT

the hepatopancreas and muscle has been found to reduce the carbohydrate level in the crab *Oziotelphusa senex senex* (Ramamurthi & Venkataramaniah, 1982). Lorenzon *et al.*, (2000) reported the changes in the haemolymph glucose level in the shrimp *Palaemon elegans* due to heavy

metal toxicity. Decrease in carbohydrate content was observed in the tissues of the marine prawn *Metapenaeus* following exposure to the pesticide methylparathion (Reddy & Rao, 1990). Stimulation of glycogenolysis was observed in the crab *Oziotelphusa senex senex* on exposure to cadmium (Radhakrishnan & Busappa, 1986). Jabakumar *et al.*, (1990) studied the sublethal exposure of the cypermethrine in the organic constituents of the freshwater fish *Labeo thermilis* and reported decrease in the carbohydrate content. Somanath (1991) reported reduction of carbohydrate level in the fish *Labeo rohita* due to the effect of sublethal concentration of tannic acid toxicity. Significant decrease in glucose and glycogen levels has been reported in the abdominal muscle and hepatopancreas of the cray fish *Porcamarus clarkia* following exposure to cadmium toxicity (Torreblanca *et al.*, 1991). Reddy *et al.*, (1988) reported decreased carbohydrate level in the brain of the teleost fish *Channa punctatus* exposed to chlorocyclohexane stress. Murthy and Devi (1982) observed decreased glycogen content in the fish *Channa punctatus* after the exposure of endosulfan.

The depletion of carbohydrate in the edible lobster may be due to its rapid utilization to meet the energy demands under the impact of heavy metal lead. Reduction of carbohydrates in the reproductive and other tissues indicated the possibility of active glycogenolysis. Akhter Ali Siddiqui and Siddiqui Afsheen (2007) reported a declining trend in the oxygen consumption after exposure to copper sulfate in the crab *Barytelphusa gureini*. Tissue acidosis due to reduced oxygen transport must have also favoured the process of glycogenolysis in the tissues of *Thenus orientalis*. Further, the decrease in carbohydrate may also be due to hypoxia, since hypoxia

increases carbohydrate consumption. Hypoxic condition of the lobster may be due to anaerobic breakdown of glucose, which is available to the cells by increased glycogenolysis.

In *Thenus orientalis*, the lipid content decreased in all the tested tissues namely ovary, spermatheca, hepatopancreas, muscle and haemolymph when treated with lead. The decrease of lipid content was high in the higher (0.04 ppm) sublethal concentration, when exposed for 30 days to lead.

The decline in lipid content was observed in *Macrobrachium idella* due to cadmium toxicity (Villalan *et al.*, 1990). Nagabhushanam *et al.*, (1972) reported decrease in lipid level in the hepatopancreas of the freshwater prawn *Macrobrachium kistensis* in response to pesticide exposure. Saravana Bhavan and Geraldine (1997) reported the reduction in lipid content in the prawn *Macrobrachium malcolmsonii* when the prawn was exposed to chlorpyrifos and suggested that the accelerated hydrolysis of lipid might be to cope up with the increased energy demand occurring due to the pesticide toxicity. Manohar Patil and Kulkarni (1995) found the reduction in lipid content in the freshwater fish *Channa punctatus* when it was exposed to the pesticide summach. Tazeen *et al.*, (1996) observed decline in the total lipid content when the catfish *Mystus vittatus* exposed to the pesticide nuvan. Lomte and Muley (1993) reported the decrease in lipid level in the freshwater snails *Thaira tuberculata* and *Parresia corrugata* exposed to copper toxicity. In stress condition induced by pesticide or heavy metal, the lipid content depleted to meet the energy demand. In the present investigation, stress imposed by sublethal doses of lead to *Thenus orientalis* resulted decrease in lipid content in the reproductive and other tissues, there by indicating high-energy demand.

suitable biomarkers of environmental  $\text{Cu}^{2+}$  stress in *M. rosenbergii* (Zhao & Yang, 2008). Histopathological changes in the hepatopancreas of *Palaemonetes turcorum* after exposure to lead acetate were recorded (Kutlu *et al.*, 2005). Hepatopancreas in Crustacean is a potential indicator organ for heavy metal toxicity. These reports suggests that hepatopancreas is an organ which is actively involved in lessening the tone of toxicity by altering the metabolic processes through glycogenolysis and gluconeogenesis. Similar observation was found in the present study.

### Conclusion

In biological systems, the biocatalysts play a vital role in the metabolic pathways. Animal exposed to stress conditions alter their physiological status with the help of enzymes. Toxicants like heavy metals are known to inhibit enzyme action and this result in decreased calorie concentration. Elumalai *et al.*, (2007) suggested an interference with energy production pathways in the tissues of crab *Carcinus maenas* due to heavy metal toxicity.

To meet the energy demand the lobster might have adopted glycogenolysis and gluconeogenesis. It is exhibited in the results as the protein and lipid content demonstrated a considerable decrease on exposure to sublethal concentrations of lead for 15days and 30days but the carbohydrate content showed a slight decrease on 15days and 30days indicating more utilization of protein and lipids to meet the calorie demand. Lead as an environmental toxicant affects the nutritive value of the edible lobster *Thenus orientalis* and the changes could also adversely affect the taste, texture and in turn the marketability of this export oriented edible lobster.

Table 4. Effect of sublethal concentrations of lead on lipid content in different tissues of *T. orientalis*

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-Value	P-Value
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
15	Ovary	73.62 $\pm$ 3.11	70.61 $\pm$ 3.4	66.37 $\pm$ 3.03	6.53	0.012
	Spermatheca	54.06 $\pm$ 2.87	48.59 $\pm$ 3.56	44.18 $\pm$ 2.56	13.33	<0.001
	Hepatopancreas	72.61 $\pm$ 3.34	67.32 $\pm$ 5.0	63.85 $\pm$ 4.41	5.31	0.0223
	Muscle	50.89 $\pm$ 2.04	45.25 $\pm$ 0.70	43.65 $\pm$ 4.24	9.58	0.0033
	Haemolymph	52.61 $\pm$ 1.65	48.91 $\pm$ 2.43	43.62 $\pm$ 4.05	12.2	0.0013
30	Ovary	79.31 $\pm$ 2.47	70.47 $\pm$ 3.15	65.18 $\pm$ 3.62	26.1	<0.001
	Spermatheca	57.26 $\pm$ 5.61	45.04 $\pm$ 3.69	42.73 $\pm$ 2.38	17.97	<0.001
	Hepatopancreas	78.31 $\pm$ 2.28	63.9 $\pm$ 3.70	53.43 $\pm$ 8.89	23.86	<0.001
	Muscle	54.53 $\pm$ 2.48	44.25 $\pm$ 2.47	35.50 $\pm$ 5.16	34.93	<0.001
	Haemolymph	53.41 $\pm$ 0.93	46.30 $\pm$ 3.61	40.61 $\pm$ 2.43	30.98	<0.001

Mean  $\pm$  SD of six individual observations; Values are expressed mg/g wet tissue and mg/ml haemolymph; Statistically significant (By Tukey's multiple comparison test).

The responses of the metabolic and digestive enzymes in the hepatopancreas of *M. rosenbergii* to water-borne copper contamination were reported. Enzymes of hepatopancreas were found to be most

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