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Effect of Heavy Metal Copper on the Nutritive Value in a Freshwater Field Crab, *Spiralothelphusa hydrodroma*

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Abstract: The fresh water field crab, *Spiralothelphusa hydrodroma* is an important human food source in parts of South India and the crab is constantly exposed to pesticides, which are used extensively to control agricultural pests. Evaluation of the toxic effect of copper on the experimental crab for the LC_{50} value was carried out. Effect of copper on the quantitative study of nutritive value viz. protein, carbohydrate and lipid in ovary, spermatheca, hepatopancreas, muscle, gills, haemolymph, brain, thoracic ganglia and eyestalk was observed.

Key words: Protein, carbohydrate, lipid, $LC_{50,}$ Copper, *Spiralothelphusa hydrodroma*

INTRODUCTION

The pesticides include insecticides, herbicides, fungicides, molluscides and nematicides and heavy metals like copper, zinc, arsenic, lead, cadmium, mercury etc.[8]. These pesticides are non-biodegradable and accumulate in the food chain. Mostly they are prone to affect the nervous system causing tumors in living organisms. They are not only neurotoxic but also affect other systems and have shown a high degree of impact on metabolism by altering the proteins, carbohydrate and lipids[16,13]. The trace metal concentration in Queensland estuarine crabs, *Australoplax tridentate* and *Scylla serrata* has been observed[14]. The present work was that the effect of heavy metal copper on the nutritive value *Spiralothelphusa hydrodroma*.

MATERIALS AND METHODS

The fresh water field crabs were collected from, in and around the irrigating channels and paddy fields. The crabs were maintained in normal daylight illumination in the laboratory thereby providing normal acclimatization. The crabs were fed with uncooked oats. For all experiments, the crabs were used with carapace length ranging from 3.27 cm to 4.86 cm. The water level was maintained carefully so that the crabs were partially immersed.

Acute toxicity study was carried out to determine the potency of copper for static but renewal type of bioassay was adopted in the present investigation to estimate the LC_{50} values. The heavy metal copper as copper sulphate, commercial grade was used as the test material since only commercial preparation is used in agriculture.

The experiment was carried out to find the range of concentrations for confirmatory evaluation. The mortality was recorded for the crab at 24 hr, 48 hr, 72 hr and 96 hr exposure to copper; were corrected for natural response by Abbott's formula[1]. The LC_{50} values for 24 hr, 48 hr, 72 hr and 96 hr of exposure periods were estimated as 271.01, 265.46, 257.03 and 254.68 ppm respectively (Table: 1).

Design of Sublethal Toxic Study: Chronic time course study on the effect of copper on the crab was conducted by exposing to two sublethal, safe concentrations for 15 days and 30 days. According to[12,21], $1/3^{rd}$ and $1/10^{th}$ of the 96 hr LC_{50} value represent higher and lower sublethal concentrations respectively. Hence lower (25.46 ppm) and higher (84.66 ppm) sublethal concentrations of the insecticide were arbitrarily used. At the end of the treatment period, the control and treated crabs were dissected and the above said tissues were collected to analyse the nutritive value.

Biochemical Analysis: Protein, Carbohydrate and Lipid estimations were studied following the techniques adopted[2,27,4]

Statistical Analysis: One-way Analysis of Variance (ANOVA) was performed based on the methods of[40]

RESULTS AND DISCUSSIONS

Effect of Copper on Protein: Data in Table 2 showed that the maximum decrease of protein content was observed in 30 days of treatment at both concentrations in all the tissues.

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Table 1: The LC_{50} values and regression equations for *S. hydrodroma* treated with heavy metal copper

Exposure periods (hours)	LC_{50} (ppm)	Upper Confidence limits (ppm)	Lower Confidence limits (ppm)	Regression results	Slope function (SF)	r^2
24	271.01	297.29	248.24	Y = - 143.77 X + 61.06	1.040	0.99
48	265.46	288.60	244.17	Y = - 116.02 X + 49.91	1.042	0.99
72	257.03	281.06	235.06	Y = - 143.36 X + 61.42	1.044	0.98
96	254.68	277.12	234.25	Y = - 121.88 X + 52.72	1.050	0.98

Table 2: Effect of Sublethal concentrations of copper on PROTEIN content in different tissues of *S.hydrodroma*

Exposure Period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-Valuein days	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15	Ovary	85.11 ± 0.69	71.74 ± 2.12	68.23 ±1.32	116.24	<0.001*
	Spermatheca	67.84 ± 0.72	65.85 ±1.24	63.88 ± 1.01	32.44	<0.001*
	Hepatopancreas	71.16 ± 1.11	68.49 ± 1.84	65.73 ± 1.09	26.48	<0.001*
	Muscle	54.32 ± 0.70	51.29 ± 1.23	48.82 ± 1.09	49.91	<0.001*
	Gills	58.49 ± 1.35	55.40 ± 1.19	51.09 ± 1.45	55.87	<0.001*
	Haemolymph	83.38 ± 1.29	82.03 ± 1.15	79.87 ± 1.79	12.71	<0.001*
	Brain	62.24 ± 1.71	59.11 ± 1.27	56.52 ± 1.09	36.71	<0.001*
	Thoracic ganglia	65.10 ± 1.37	62.19 ± 1.83	58.40 ± 1.32	37.73	<0.001*
	Eyestalk	27.38 ± 0.97	25.24 ± 2.39	21.50 ± 1.03	22.28	<0.001*
30	Ovary	85.77 ±2.65	69.48 ± 1.03	65.51 ± 0.97	770.39	<0.001*
	Spermatheca	68.45 ± 0.58	64.37 ± 1.34	61.94 ± 1.34	46.09	<0.001*
	Hepatopancreas	71.39 ± 0.91	65.32 ± 1.16	63.99 ± 1.10	68.66	<0.001*
	Muscle	54.65 ± 0.61	50.87 ± 1.34	47.70 ± 1.01	59.25	<0.001*
	Gills	58.66 ± 1.05	54.19 ± 1.25	48.33 ± 0.54	157.21	<0.001*
	Haemolymph	83.68 ± 0.84	81.08 ± 1.23	77.45 ± 1.22	34.21	<0.001*
	Brain	62.63 ± 1.33	56.64 ± 0.81	54.36 ± 1.08	63.84	<0.001*
	Thoracic ganglia	65.43 ± 0.88	60.19 ± 0.67	57.21 ± 0.92	88.88	<0.001*
	Eyestalk	27.84 ± 1.17	24.76 ± 0.90	20.40 ± 1.06	76.74	<0.001*

Mean ± SD of six individual observations.
Values are expressed mg/g wet tissue and mg/ml haemolymph.
* Statistically significant (By one-way analysis of variance)
Statistically significant (By Tukey's multiple comparison test).
NS – Not Significant.

Effect of Copper on Carbohydrate: Maximum decrease of carbohydrate content was identified in 30 days of treatment at both the concentration in all tissues. The data was given in Table 3.

Effect of Copper on Lipid: The decrease in the lipid content was to the maximum in 30 days of treatment at both the concentration in all tissues as the data provided in Table 3.

Proteins are important organic substances required in tissue building and repair. Under extreme stress conditions, proteins suppliers energy in metabolic pathways and biochemical reactions[41]. In all the experimental tissues such as ovary, spermatheca, hepatopancreas, muscle, gill, haemolymph, brain, thoracic ganglia and eyestalk the protein content was decreased. The decrease in protein content was drastic in higher (84.66 ppm) sublethal concentration of copper for 30 days.

Table 3: Effect of Sublethal concentrations of copper on Carbohydrates content in different tissues of *S.hydrodroma*

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15	Ovary	18.41 ±1.04	17.84 ±0.88	16.31 ± 0.91	11.45	<0.001*
	Spermatheca	12.03 ± 1.17	11.44 ± 1.04	10.28 ± 1.58	4.24	0.0347*
	Hepatopancreas	16.33 ± 1.04	15.95 ± 0.82	14.39 ± 1.45	6.82	0.0078*
	Muscle	10.36 ± 0.86	9.81 ± 1.00	8.79 ± 0.93	4.77	0.025*
	Gills	8.87 ± 0.54	8.09 ± 0.59	7.40 ± 0.57	7.13	0.0067*
	Haemolymph	7.96 ± 0.65	7.54 ± 0.93	6.88 ± 0.65	4.84	0.0239*
	Brain	11.38 ± 0.78	9.77 ± 0.96	8.20 ± 0.67	22.42	<0.001*
	Thoracic ganglia	11.85 ± 0.90	10.42 ± 0.87	9.56 ± 0.79	19.11	<0.001*
	Eyestalk	5.24 ± 0.87	4.83 ± 0.78	3.96 ± 0.48	5.11	0.0204
30	Ovary	18.63 ± 0.76	16.36 ± 0.78	15.59 ± 0.84	15.7	<0.001*
	Spermatheca	12.23 ± 0.70	10.42 ± 0.77	9.68 ± 1.28	6.83	0.0078*
	Hepatopancreas	16.61 ± 0.79	14.86 ± 1.47	13.90 ± 0.70	7.21	0.0064*
	Muscle	10.58 ± 1.07	8.63 ± 0.87	7.87 ± 0.40	17.44	<0.001*
	Gills	8.94 ± 0.90	7.49 ± 0.84	6.43 ± 0.61	19.45	<0.001*
	Haemolymph	8.24 ± 0.69	6.99 ± 0.41	5.82 ± 1.02	12.51	<0.001*
	Brain	11.28 ± 0.72	9.55 ± 0.94	8.08 ± 0.62	25.65	<0.001*
	Thoracic ganglia	11.59 ± 0.80	9.61 ± 0.83	8.52 ± 0.75	24.75	<0.001*
	Eyestalk	5.18 ± 0.73	4.38 ± 0.58	3.67 ± 0.63	5.67	0.0146*

Mean ± SD of six individual observations.
Values are expressed mg/g wet tissue and mg/ml haemolymph.
* Statistically significant (By one-way analysis of variance)
Statistically significant (By Tukey's multiple comparison test).
NS – Not Significant.

Reports about the depletion of protein content in the marine edible crab *Scylla serrata* due to organochlorine pesticide dimecron toxicity were recorded[28]. Marked decrease in protein content in the freshwater field crab *Paratelphusa hydrodromous* in response to the pesticide malathion toxicity[31]. The effect of endosulfan in various tissues of the freshwater field crabs *Barytelphusa guerini* were studied and noted the decrease in protein level[26].

Observations were made about the decrease in protein content in the prawn *Metapenaeus monoceros* due to phosphomidon exposure[37]. Decrease in protein content was noted in prawn *Penaeus indicus* after exposure to the pesticides phosphomidon and methyl-parathion[23,24] found a decrease in the protein level in the marine prawn *Metapenaeus monoceros* after the prawn was exposed to the pesticide methyl parathion.[5] reported protein depletion in the freshwater prawn *Macrobrachium malcolmsonii* in response to dichlorvos exposure.

[20]noted decrease in protein content in the freshwater snail *Pila globosa* because of the sublethal effect of the pesticide sumithion. [30] 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 pollutant treated animal may be due to enhanced proteolytic and decreased protein level in prosobranch snail *Bellamiya bengalasis* after treated with the pesticide malathion. [17] found decrease in protein level in the worm *Mythima seperata* after it was exposed to different pesticides. [36] observed decrease in the protein content in the fish *Fundulus beterolitus* and stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source.[18] have reported that the protein level in liver and muscle of *Sarotherodon mossambicus* decreased because of the exposure of the pesticide DDT. [3]studied the effect of the insecticide rogor in the muscle and gill of *Heteropneustes fossilis* and found a decrease

Table 4: Effect of Sublethal concentrations of copper on Lipid content in different tissues of ***S.hydrodroma***

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-Value	P-Value
		Mean ± SD	Mean ± SD	Mean ± SD		
15	Ovary	84.31.± 1.11	82.06 ± 1.59	79.96 ± 1.55	13.71	<0.001*
	Spermatheca	34.49 ± 0.54	32.68 ± 1.08	25.26 ± 0.99	182.18	<0.001*
	Hepatopancreas	91.08 ± 1.12	84.76 ± 1.35	68.67 ± 0.83	647.17	<0.001*
	Muscle	14.18 ± 1.10	13.29 ± 1.26	9.94 ± 0.98	50.19	<0.001*
	Gills	9.78 ± 1.18	9.05 ± 0.54	7.77 ± 0.51	13.32	<0.001*
	Haemolymph	19.51 ± 0.78	14.49 ± 1.09	9.87 ± 1.02	146.84	<0.001*
	Brain	29.29 ± 1.18	23.93 ± 1.13	19.16 ± 1.17	113.79	<0.001*
	Thoracic ganglia	26.63 ± 0.96	22.89 ± 1.12	19.75 ± 0.56	84.9	<0.001*
	Eyestalk	8.63 ± 0.51	5.89 ± 0.94	4.89 ± 0.87	35.46	<0.001*
30	Ovary	84.79 ± 1.31	79.95 ± 1.68	77.05 ± 2.01	31.94	<0.001*
	Spermatheca	34.75 ± 0.95	31.53 ± 0.72	21.31 ± 1.24	286.68	<0.001*
	Hepatopancreas	91.30 ± 1.11	81.21 ± 1.08	66.81 ± 1.05	757.15	<0.001*
	Muscle	14.54 ± 1.02	12.34 ± 1.01	7.50 ± 1.66	85.96	<0.001*
	Gills	10.15 ± 0.72	8.21 ± 0.54	6.80 ± 0.76	28.88	<0.001*
	Haemolymph	19.42 ± 0.80	11.14 ± 1.03	8.56 ± 1.07	201.65	<0.001*
	Brain	29.65 ± 1.21	21.99 ± 0.95	16.89 ± 1.19	234.34	<0.001*
	Thoracic ganglia	26.50 ± 0.82	20.65 ± 0.59	17.18 ± 0.39	334.84	<0.001*
	Eyestalk	8.35 ± 0.67	5.15 ± 0.61	4.32 ± 0.43	80.24	<0.001*

Mean ± SD of six individual observations.
Values are expressed mg/g wet tissue and mg/ml haemolymph.
* Statistically significant (By one-way analysis of variance)
Statistically significant (By Tukey's multiple comparison test).
NS – Not Significant.

in protein content. [10]have recorded the reduction of protein content in the branchial tissue of the *Cyprinus carpio* because of the toxic impact of fenvalerate. [33]have also reported a significant decrease in protein content under sublethal concentration of pyrethorid 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 crabs exposed to copper. This was possibly due to the direct effect of the insecticide on protein metabolic demands following exposure to the toxic stress of copper.

Carbohydrate is an important biochemical constituent of an animal tissue. They not only act as building blocks of the cells but also serve as a reservoir of chemical energy 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 (25.46 ppm) and higher (84.66 ppm) sublethal concentration for 30 days.

The activity of the enzyme phosphorylase in the hepatopancreas and muscle has been found to reduce the carbohydrate level in the crab *Oziotelphusa senex senex*[19]. Decrease in carbohydrate content was observed in the tissues of the marine prawn Metapenaeus following exposure to the pesticide methyl-parathion [22]. [7]studied the sublethal exposure of the cypermentrine in the organic constituents of the freshwater fish *Labeo thermilis* and reported decrease in the carbohydrate content. [32]reported reduction of carbohydrate level in the fish *Labeo rohita* due to the effect of sublethal concentration of tanic 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[35].

Fig. 1: *S. hydrodroma* (Dorsal view)

Fig. 2: *S. hydrodroma* (Ventral view)

[25]observed decreased carbohydrate level in the brain of the teleost fish *Channa punctatus* exposed to hemachlorocyclohexane stress. [15]observed decreased glycogen content in the fish *Channa punctatus* after the exposure of chlorpyrifos. [39]have also reported decreased glycogen content in the fish *Orechromis mossambicus* due to the exposure of tannic acid. [6]studied the effect of phosphomidon in *Gambusia affinis* and found decrease in carbohydrate level.

The depletion of carbohydrate may be due to its rapid utilization to meet the energy demands under the impact of heavy metal copper. Reduction of carbohydrate rates in the reproduction and other tissues indicated the possibility of active glycogenolysis. Tissue acidosis due to reduced oxygen transport must have also favored the process of glycogenolysis in tissues. Further, the decrease in carbohydrate may also due to hypoxia, since hypoxia increases carbohydrate consumption. Hypoxic condition of the crab must have derived its energy from anaerobic breakdown of glucose, which is available to the cells by increased glycogenolysis.

In *Spiralothelphusa hydrodroma* the crabs treated with copper, the lipid content decreased in all the tested tissues namely ovary, spermatheca, hepatopancreas, muscle, gill, haemolymph, brain, thoracic ganglia and eyestalk. The decrease of lipid content was high in the higher (85.66 ppm) sublethal concentration of chlorpyrifos for 30 days of exposure period.

The decline in lipid content was observed in *Macrobrachium idella* due to cadmium toxicity[38]. [16]reported decrease in lipid level in the hepatopancreas of the freshwater prawn *Macrobrachium kristensis* in response to pesticide exposure. [29]reported that the reduction in lipid content in the prawn *Macrobrachium malcolmsonii* when the prawn 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.

[11]found the reduction in lipid content in the freshwater fish *Channa punctatus* when it exposed to the pesticide summach. [34]observed decline in the total lipid content when the cat fish *Mystus vittatus* exposed to the pesticide nuvan. [9]reported the decrease in lipid level in the freshwater snails *Thaira tuberculata* and *Parresia corrugata* exposed to copper toxicity.

In stress condition induced by pesticides, the lipid content depleted to meet the energy demands. In the present investigation, stress imposed by sublethal doses of copper to *Spiralothelphusa hydrodroma* resulted in decrease in lipid content in the reproductive and other tissues, there by indicating high-energy demand.

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