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EFFECT OF COPPER, CADMIUM AND Cu.Cd MIXTURE ON AMINO ACID CONTENT IN THE POSTLARVAE OF PENAEID SHRIMP, *PENAEUS MONODON* AND *P. PENICILLATUS*

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ABSTRACT: This study document effects of short-term (96h) sublethal levels of copper, cadmium and their mixture on the amino acid composition of postlarvae of the penaeid shrimp, *P.monodon* and *P.penicillatus* . All experimental conditions were kept constant , temperature between 25-27°C and salinity 21-22 ppt . The estimated LD50 for Cu was 200 ug/L, for Cd 177.5 ug/L and for Cu.Cd mixture 250ug/L. In *P.penicillatus* at the same concentration of each metal, there was significant reduction in amino acid content, which was 8.01% higher than the control. Almost similar reduction in some amino acids was observed in *P.monodon*. At the maximum concentration of 400 ug/L, cadmium caused higher reduction in amino acid composition than did copper. Thus, amino acid composition may be regarded as a sensitive biochemical indicator of Cu and Cd toxicity because of the effect of these metals on protein synthesis, a signal of physiological stress in marine organisms subjected to heavy metal pollution.

KEY WORDS : Penaeid shrimp, amino acids, heavy metal

INTRODUCTION

Presently considerable attention is being paid to the study of protein metabolism becasuse of its importance in metabolic activities of marine organisms. Protein metabolism has been investigated in crustaceans and the occurrence of metallothione (MT) mainly in cytoplasmic protein was characterized by a high content of metal binding sulphydryl groups (Scholz, 1980). In the case of an essential metal like copper, body concentrations of metabolically available copper must also reach a minimum concentration because of its role in shrimp biochemistry (Harrison, 1977). The levels of essential and non-essential metals in marine environment play an important role in regulation of metals in marine organisms. They are also essential factors for specific enzymes in enzyme systems (Kohler and Reisgard, 1982).

Short term experiments of metal exposure effects can provide useful information about their relative toxicity on amino acid profiles. It is now well understood that the mode of action of heavy metals in marine organisms is in the form of metallothionine (Kohler and Reisgard, 1982; Scholz, 1980).

The aim of the present study was to quantify the impact of copper, cadmium and their mixture (1: 1) on the amino acid profiles of postlarvae in two species of shrimp, *Penaeus monodon* (Fabricius, 1798) and *P. penicillatus* (Alcock, 1905) under laboratory condition. Postlarval stages of the life cycle seem to be the most sensitive and may extend to physiological changes as seen in the crab *Paragrapus quadridentus* caused

by sublethal concentration of Copper, Cadmium and Zinc (Ahsanullah and Arnott, 1978). These are relatively large postlarvae and contain high levels of amino acids and total protein (Ronnestad and Fyhn, 1992). According to the literature, amino acid contents are supposed to be important potential indicators of the toxic effects of copper and cadmium. The study of effects of heavy metals on amino acid profiles may be an important aspect for future research.

MATERIALS AND METHODS

Identified samples of postlarval shrimp were procured from the culture facility of Xiamon University. In the laboratory these postlarvae were exposed to different concentrations of Cu,Cd separately (10, 100, 200, 400 ug/L) and a mixture of Cu.Cd (1:1). Experiments were conducted using 600ml beakers each containing 500 ml of sand-filtered (0.45 um) seawater and 20 individuals six concentrations of metals were applied. A static culture system at 30°C was used in the experiment to prevent precocious development of juvenile shrimp as had been observed in flow through systems (Hamilton, 1984).

TEST SOLUTION AND METAL ION(S) MEASUREMENT

Metal solutions were prepared from stock solutions (10,000ug/L) of $CdCl_2$ and $CuSO_4$ $5H_2O$ at 10 ug/L to 400 ug/L (a series of 4 or 6 dilutions) with deionized water. Five replicates and a control were used for each dilution. Each experiment was continued for 4d (96h), with pH and salinity of the culture water noted daily; fresh solutions were used for each experiment. All glasswares were washed with 5.5M HCl and rinsed with deionized water. After exposure, all specimens were dried to a constant weight in a drying oven at 70-80°C. The dried material was ground into a homogenous powder.

AMINO ACID DETERMINATIONS

Approximately 8-10 mg of samples were placed in ampules and hydrolyzed with 3ml HCl (6M). Oxygen was removed from the ampules by passing nitrogen and then each ampule was sealed. For a minimum, the samples were allowed to hydrolyze for 24h at 120°C (± 1) .

The digested amino acid samples were removed from HCl and washed with distilled water and 0.1N HCl at a concentration of 1mg/ml. Each sample was then analyzed with an Amino acid analyzer (Hitachi, Model 835-50) .

DATA ANALYSIS

Two way analysis of variance (ANOVA) was used as the variable. The main factors were concentration of each metal and number of postlarvae. The duration period of exposure was used as variable.

RESULTS

Two way analysis of variance indicated that the dry weight of postlarvae decreased with increasing concentrations of metals; all values were divided by the respective of log weights of the controls, and the data were reanalyzed. An almost linear regression of

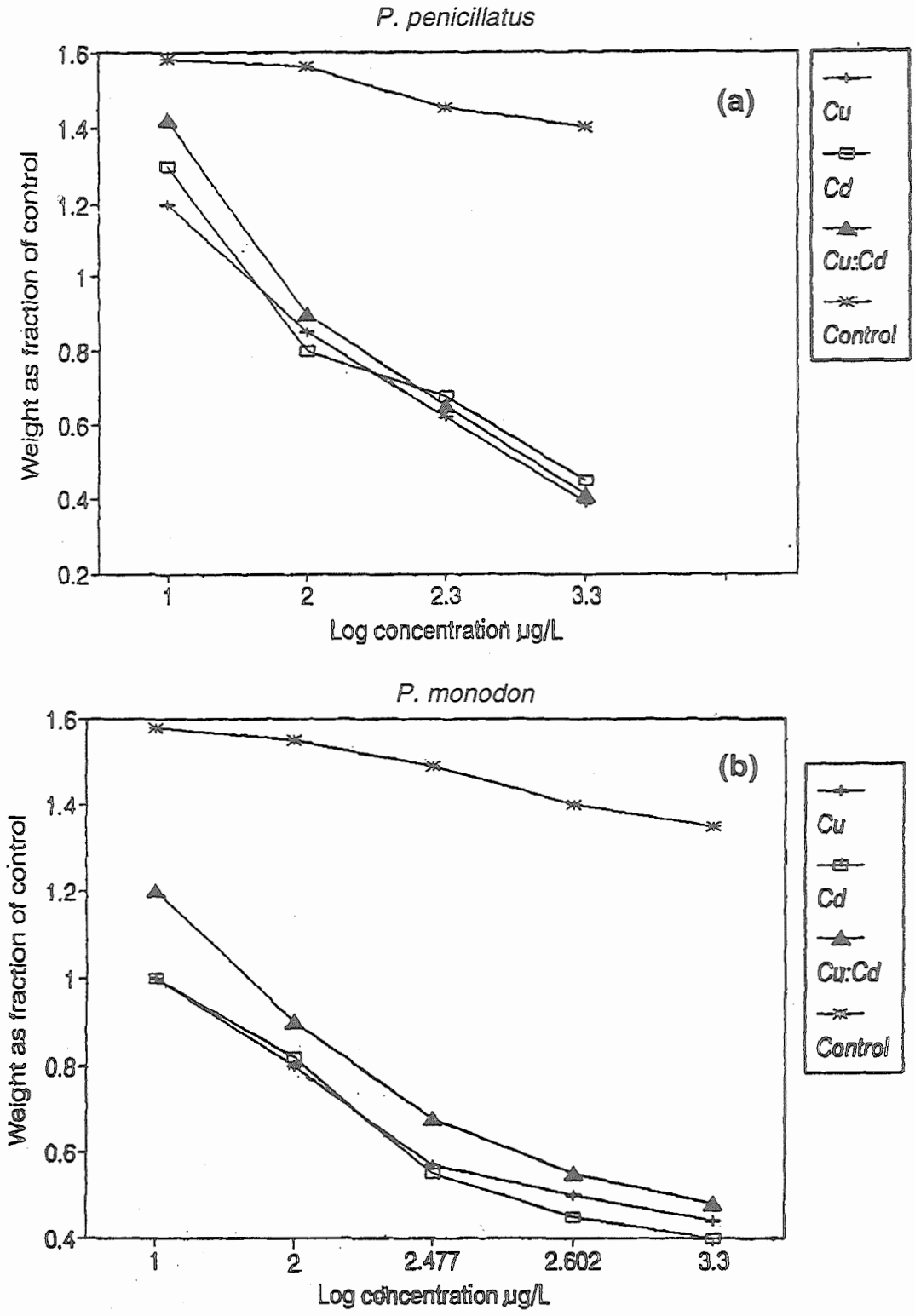

Fig.1. Effect of heavy metals on dry weight at 24-96h exposure at laboratory conditions, values are showing ±SD of mean based 20.

corrected weights of exposed postlarvae and concentration of metals were observed (Fig.1). By calculating the standard error of estimates, the differences between controls and tests, and the weights of exposed larvae were also significantly lower than those of the controls.

The total concentrations represented the sum of various amino acids along with a significant difference between these values. The total concentration of amino acid decreased as concentration of each metal increased (Fig.2). In all exposed samples the total concentration of essential amino acids was higher than non-essential amino acids except in the control of *P.penicillatus*, where the total of essential amino acid was determined to be 363.34 ug/mg of dry weight and the non-essentials were found to be 398.65ug/mg of dry weight. Most values of the essential amino acids found were higher than for the controls at different concentrations of each metal but values of non-essential amino acids were lower than those of the control of *P.penicillatus* except for aspartic acid,which was not same as that of *P.monodon* except for isoleucine.

The effect of Cu , Cd and the Cu.Cd mixture on each specific amino acid was found to be variable in the two species of penaeid shrimp. In *P.monodon* the content of leucine was lower at 10ug/L of copper than at 100 and 200ug/L; the concentration was even lower in the control i.e. 88.45ug/L of dry wt; but under the toxic influence of cadmium its concentration was lower than that of the control (Table 1) . In *P.penicillatus* the concentration of leucine was found to be more than in the control and was different in each exposed concentration of the three tests . The value of lysine was found to be 25% higher than that of the control when larvae of *P.penicillatus* were exposed to copper at a concentration of 10-100 ug/L (Table 2) . However, in *P.monodon* the level of lysine was found to be 58.97ug/mg of the dry weight at the 100 ug/L concentration of copper. The value of lysine was observed 67.3 ug/mg of dry weight, cadmium at the concentration of 400 ug/L was exposed. In exposure experiments of Cu.Cd mixture the same results were observed, and all values of amino acid gradually decreased as mixture concentration increased. In almost all samples the value of valine was found to be higher than that of the control being 28.52 ug/mg in *P.penicillatus*, in *P.monodon* these values were lower than in the control.

The value of isoleucine gradually decreased as the concentration of copper and cadmium increased in *P.monodon*, but in the case of the Cu.Cd mixture its value at 100ug/L concentration was found to be 35.1ug/mg of dry weight, which was more than that at 10ug/L concentration. In *P.penicillatus*, isoleucine was higher than in the control at concentration of 10ug/L of Cu, Cd, and mixture (Table 2). The amino acid composition of both species was found to be different when exposed to each concentration of Cu, Cd, and mixture. Threonine was higher than in the control in *P.monodon* but lower in *P.penicillatus*. There is a gradual decrease in phenylalanine and methionine content with increased concentration of metals in both species.

Alanine gradually decreased as the concentration of Cd increased upto 400 ug/L in *P.monodon* but the value was still lower than the control. In case of *P.penicillatus* (Table 2) there was a systematic decrease in alanine with increased concentration of each metal, except 400 ug/L of Cu and 100ug/L of Cd at which alanine was found to be 70% and 80% more than the control. The decrease in the serine of both species was not shared equally among the individual metal concentration, resulting in more amount of it i.e. 116.65 ug/mg of dry. wt. at 10ug/L of Cd in *P.penicillatus* (Table 2). Glutamic acid was

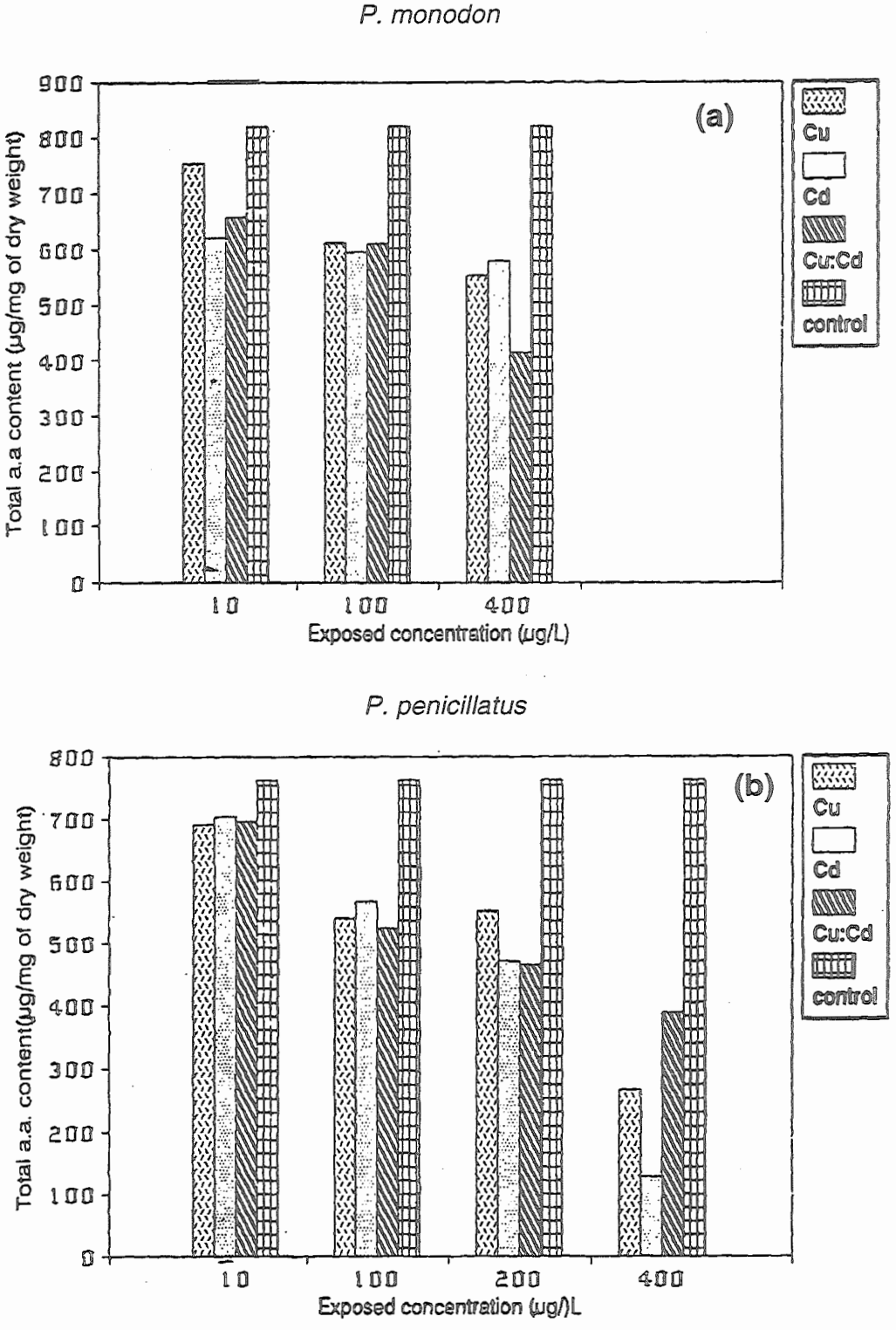

Fig.2. The comparison of total amino acid contents between heavy metal treated and control specimen.

Table.1. Contents of amino acid of *Peneaus monodon* under toxic effect of copper, cadmium and Cu.Cd mixture in comparision to control. Data presented as mean (± SD) of 2 to 3 samples (ug/mg dry weight).

Concentration ug/l	Copper																	
	Essential									Non essential								
	Leu	Lys	Val	Ile	Thr	Arg	Phe	His	Met	Ala	Ser	Glu	Gly	Tyr	Asp	Pro	Cys	Trp
10	12.11	-	107.07	33.72	66.69	123.12	72.42	23.83	25.48	110.07	58.59	21.75	13.87	31.19	10.65	43.48	-	-
100	58.93	58.97	45.62	33.24	29.52	61.28	54.97	12.32	0.957	52.92	31.07	11.03	72.51	24.22	70.23	3.06	-	-
400	54.45	63.784	11.96	27.76	10.33	67.20	37.73	15.85	15.96	84.75	34.34	6.86	14.27	22.46	24.41	62.01	-	-
	Cadmium																	
10	55.5	44.2	46.1	87.6	25.03	44.9	27.1	11.2	16.68	40.7	28.5	19.03	68.97	20.97	59.2	26.3	-	-
100	52.98	83.6	56.7	28.3	23.57	57.4	32.6	13.71	28.88	46.1	27.2	10.07	58.3	2.78	10.23	45.0	-	-
400	60.5	67.3	78.6	32.5	38.35	59.3	4.00	1.72	16.00	55.8	39.9	12.75	57.9	25.3	8.78	41.5	-	-
	Cu . Cd mixture																	
10	66.1	66.4	67.44	30.9	6.79	59.5	39.85	15.66	42.3	61.4	42.69	13.20	73.1	28.86	8.916	5.47	-	-
100	74.1	67.1	53.7	35.1	29.50	59.3	42.4	17.2	26.50	63.7	38.9	14.28	39.4	32.7	10.01	3.06	-	-
400	34.38	33.99	42.04	17.71	55.87	36.26	20.89	8.29	25.20	33.45	5.7	7.93	39.56	-	48.10	7.64	-	-
Control	88.45	38.89	9.023	54.05	7.84	95.69	50.48	18.73	9.13	88.79	35.86	12.79	113.2	37.62	77.84	83.38	-	-

Table.2. Contents of amino acid of *Peneaus penicillatus* under toxic effect of copper, cadmium and Cu.Cd mixture in comparision to control. Data presented as mean (± SD) of 2 to 3 samples (ug/mg dry weight).

Concentration ug/l	Copper																	
	Essential									Non essential								
	Leu	Lys	Val	Ile	Thr	Arg	Phe	His	Met	Ala	Ser	Glu	Gly	Tyr	Asp	Pro	Cys	Trp
10	68.56	70.5	51.8	38.4	26.4	6.99	50.8	16.8	93.37	63.85	38.6	13.33	16.29	32.40	87.6	17.80	-.	-
100	75.4	75.2	59.9	30.6	40.5	8.55	49.1	19.0	3.50	6.87	48.1	4.26	10.89	3.61	84.44	10.59	-	-
200	76.3	56.2	59.9	27.4	30.9	52.8	34.0	13.9	9.40	9.10	31.9	9.24	5.189	24.7	74.0	11.92	-	-
400	-	23.2	47.0	22.9	58.0	21.5	2.16	5.3	9.40	15.5	5.80	9.40	-	21.5	34.5	-	-	-
	Cadmium																	
10	20.49	20.49	14.97	91.80	86.55	18.18	12.04	51.32	70.42	17.43	116.65	39.55	13.24	88.9	25.64	16.58	-	-
100	54.33	56.02	58.32	32 40	27.99	58.85	33.78	17.55	26.7	5.18	27.44	10.22	7.37	17.15	75.84	8.10	-	-
200	37.30	37.30	22.10	20.70	32.50	22.60	9.70	55.58	76.50	29.30	24.20	5.28	23.2	16.5	50.69	7.12	-	-
400	33.00	44.20	52.50	19.30	-	27.80	17.20	8.90	4.70	4.80	-	27.5	33.36	2.30	55.96	-	-	-
	Cu . Cd mixture																	
10	13.53	13.33	93.73	88.49	9.52	14.41	75.20	25.00	25.25	8.82	47.45	26.35	15.83	58.85	28.19	26.53	-	-
100	63.3	67.70	5.45	34.50	32.90	58.30	39.60	14.90	33.40	5.82	3.15	11.92	7.64	38.7	75.9	20.9	-	-
200	11.20	11.45	14.50	57.20	60.10	12.98	68.40	29.44	79.40	3.61	6.98	22.47	18.96	43.70	13.58	10.83	-	-
400	16.70	12.71	23.50	10.67	13.86	11.49	96.60	42.10	11.40	2.06	17.22	38.93	23.30	11.50	28.55	27.99	-	-
Control	45.68	45.32	28.52	26.19	35.65	51.06	24.75	11.37	94.80	48.77	50.24	90.91	51.19	22.23	71.44	63.87	-	-

found to gradually decrease as concentration of mixture increased in *P.monodon* (Table 1). But, if compared to the results of *P.pencillatus* at 200 ug/L and 400 ug/L of the mixture it was almost 50% and 25% respectively of that at 100 ug/L of mixture. A similar pattern of glutamic acid was observed at concentrations of 100 and 400 ug/L of Cu and Cd in *P.monodon*. In *P.penicillatus* at 100ug/L concentration of Cd , 200 ug/L and 400 ug/L concentration of 1:1 of the mixture; the content of glycine decreased because of higher concentrations of any metal(Table 2); whereas, in *P.monodon* glycine was 12% more than that of the control. Tyrosine varied on exposure to Cu, Cd and mixture was found to be more than that of the control but this variation was not systematic because in *P.penicillatus* at 400 ug/L of mixture it is almost 50% lower than that of the control; however, a similar trend was noted in case of *P. monodon*. In *P.monodon* the maximum decrease of tyrosine was found at an exposure of 100ug/L of Cd that is 70% lower than that of the control (Table 1). Aspartic acid content increased gradually as concentrations of Cd increased as compared to that of 10ug/L but in exposure to Cu there was a decrease as concentrations increased. At 10 ug/L of Cu 22.62% more aspartic acid was found in *P.penicillatus* than in the control; however, the minimum content of aspartic acid was found at exposure of 200ug/L of mixture, 19.08% of the control. In *P.monodon* aspartic acid gradually decreased by increasing concentrations of each metal except at 100ug/L of Cu and at 400 ug/L of the mixture. There was a very sytstematic decrease in proline content as concentrations of each metal increased in *P.penicillatus* (Table 2) but was found to be lower than in the control. In case of *P.monodon*, proline was found variable and it was lower than the control at all concentrations of Cu, Cd and mixture; at 100ug/L of Cu and Cd, proline was slightly higher than at 10ug/L of the mixture. The total amino acid content of both species did not change significantly during lower concentration exposure of each metal.

DISCUSSION

The initial effect of copper, cadmium and Cu:Cd mixture on postlarvae was that of a simultaneous decrease in dry weight and mean weight significantly lower than in the controls. Similar findings have been reported in studies on echinoderm and molluscan larvae (Lee and Xu, 1984; Nacci *et al.*, 1986). Heavy metals being attached to active enzyme sites may ultimately reduce body weight and thus appear to be responsible for delay in development of shrimp. Total amino acid content at every test concentration of the metal found to vary in both species. In *P.monodon* total amino acids were lower than those of *P.penicillatus* and may be the result of specific variation in selective uptake of individual amino acid by each species (Ronnested, 1992). It may be because *P.penicillatus* is not an indigenous species and having been imported from Singapore, may have a different intake of nutrients in a different environment. Some metals, including cadmium, are known to accumulate in decapod crustaceans in proportion to environmental exposure (Jennings and Rainbow, 1979). In Crustacea, the relationship of larvae with environmental exposure is seriously complicated by presence of fluctuating Cu, Zn haemocyanine and methionine level during the moult cycle. However, the general concept, that aquatic organisms synthesize metallothionine as a defence against toxic metal influence laboratory trials where exposure conditions can differ greatly from the natural environment (Yues *et al.*, 1993). Studies have shown that serine content

decreased along with increased concentration of cadmium with interruption in oxidase activity since serine oxidase is the only amino oxidase that has been found in Crustacea (Sisini,1969). During the present study tryptophane and cysteine were not detected because in acid hydrolysis several amino acids are altered but tryptophane can be determined after alkaline hydrolysis of the protein with 5N sodium hydroxide which was not done during present work.

Toxic effect of copper, cadmium and Cu:Cd mixture may vary considerably with test conditions and life stage of each species. In the body of the marine organism, bioaccumulation of Cd is selective and localized in certain tissue, localization of Cd may vary from organism to organism as well as from that of copper. A number of scientists have speculated that cadmium in muscles is bound to a protein smaller than metallotheionine. (Kohler & Reisgard, 1982 and Scholz, 1980). According to Talbot and Magee (1978) Cd in gills and viscera of mussels was found as free amino acid fractions of high molecular weight proteins. The low molecular proteins, such as metallotheionin and free amino acid fractions. Cadmium and a mixture Cd:Cu may also cause an inhibitory effect, however, presently it is difficult to explain the variation of each amino acid due to different concentrations. There may be a disadvantage in the use of acid hydrolysis as the asparagine and glutamine are converted to aspartic and glutamic acid respectively. Cystine and other amino acids are either completely or partially destroyed during hydrolysis of proteins (Rudy and Avdrey, 1973). Therefore, it might be interpreted that Cu and Cd were chemically reactive in hydrolysis causing variation in individual amino acid contents in both species of the penaeid shrimp.

Results of the present study show that effect of each metal in each specific amino acid was not similar to the others. It was also found difficult to explain the sequential role of a typical amino acid in the development of each specific larval stage in all experimental exposures (especially at lower concentrations) considering the fact that the amount of essential amino acids content was higher in *P.penicillatus* than in *P.monodon.* This amount of essential amino acids were higher than non-essential amino acids; however, this is difficult to explain at this stage and one may only speculate that this is the result of the stimulative effect of heavy metals on protein synthesis (Lin and Li,1991).

The level of thyreonine at 100ug/L of cadmium is the same as that of copepod *Calanus sinicus* but higher than that of *C. finmarchicus* and *Pheaeodactylum triornutum* (Lin and Li, 1991).

REFERENCES

Ahsanullah, M and G.H. Arnott, 1978. Acute toxicity of copper, cadmium and zinc to larvae of the crab *Paragrapus quadridentus* and implications for water quality criteria. *Australian Journal of Marine and Freshwater Research* 29: 1-8.

Harrison, W.G., R.W. Eppley and E.H. Renger, 1977. Phytoplankton nitrogen metabolism, nitrogen budgets and observations on copper toxicity controlled stem pollution experiment. *Bulletin of Marine Science* 277:44-57.

Jenning, J.R and P.S. Rainbow, 1979. Studies on the uptake of cadmium by the crab *Carcinus maenas* in the labortory.1 Accumulation from sea water and a food source. *Marine Biology* 6: 50-131.

Kohler, K and H.U. Reisgard, 1983. Formations of metallotheionin in relation to accumulation of cadmium in common mussel *Mytillus edulis*. *Marine Biology* 66:53-62.

Lee, H.H. and C.H. Xu, 1984. Effects of metals on sea urchin development. *Marine Pollution Bulletin* 15 :18-21.

Nacci, D, E. Jackian and R. Walsh, 1986. Comparative evaluation of three rapid toxicity tests :Sea Urchin growth test, sperm cell and microtox. *Enviromental Toxicology and Chemistry* 5 :521-525.

Rudy, H. and E.V. Avdrey, 1973. *Entitled proteins.* Pub.A.Wiley Interscience Publication. U.S.A. Pp.68

Ronnested, I., E.P. Groot and H.J. Fyhn, 1992. Utilization of free amino acids related to energy metabolism of developing larvae of lemmon sole *Microstomus kitt* reared in the laboratory. *Marine Ecology Progress Series* 88:195-205.

Lin, R. and S.J. Li, 1991. An experimental study of effects of copper and cadmium on amino acid contents in a copepod *Calanus sinicus* Brosky. *Oceanologica et Limnologica Sinica* (22) 3:248.

Sisini, A. 1969. Metabolism of D and L-serine in marine invertebrates. Bulletin. Society. *Italian Biology* 39.

Scholz, N. 1980. Accumulation, loss and molecular distribution of cadmium in *Mytilus edulis*. *Helgolaender Meeresuntersuchungen* 33 :68-73.

Talbot,V. and R.J. Magee, 1978. Naturally occuring heavy metals binding proteins in invertebrates. *Archives of Environment Contamination and Toxicology* 17:73-89.

Yues C., P. G. Campbell and A. Tessier. 1993 . Response of metallothionine concentrations in fresh water bivalve (*Anadonta grandis*) along an environmental cadmium gradient. *Limnology and Oceanography* 38 (2) :299-313.

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