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Chemical Toxicity of a Bipartite Mixture of Copper and Cadmium

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Toxicity of a mixture containing varying ratios of copper and cadmium to a widely distributed estuarine clam *Villorita cyprinodies* var. *cochinensis* has been studied and the effects quantified using the models proposed by Marking and Dawson and by Koneman. Accumulation of copper over a period of 96 h has also been studied. An attempt has been made to explain the observed toxic effects on the basis of the Ligand Field Splitting Energies and Hard and Soft Acid and Base concept.

INTRODUCTION

The Cochin Estuarine System, (CES), on the southwest coast of India (9°50′ N and 76°20′ E), is burdened with industrial and agricultural wastes that contain a variety of pollutants like trace metals, polyaromatic hydrocarbons, polychlorinated biphenyls, petroleum hydrocarbons etc. Bivalves, being widely distributed and being capable of reflecting even minute changes in the environmental water quality, have been globally recognized as effective agents for monitoring pollution levels[1, 2].

There has been a pertinent and growing emphasis on the need to quantify toxicity of mistures of pollutants in preference to individual pollutants in views of the reality that natural waters are exposed to various permutations and combinations of pollutants[3–5]. Murakami *et. al.*[6] reported that additive toxic effects were noticed when maturing sea-urchin eggs were maintained in seawater dosed with a combination of chlorides of copper and mercury. Reeve *et. al.*[7] reported a synergistic action of mercury to copper whereas equal concentrations of copper and mercury showed only a simply additive effect in combination.

Studies on the distribution of pollutants in the environment as well as their toxic effects on organisms are necessary to clearly identify the potential hazardous effects of pollutants. The present investigation, meant to assess to toxicity of two metals-copper and cadmium—applied both individually and in combination to a typical estuarine calm *V cyprinodies* var. *cohinenis* forms the continuation of our earlier reported works on the distribution of trace metals in the environment[8–10].

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EXPERIMENTAL

Materials and Methods

The bivalves *V. cyprinoides* var. *cochinesis* were collected from a site in the cochin estuary (CES) about 8 km southeast of Cochin barmouth. In the laboratory the calms were reared in the habitat environmental conditions to acclimatize them to the test conditions (salinity 20%, pH 7.5 ± 0.3, temp. 30 ± 2°C).

The metal solutions were prepared from 99.9% pure metals. Stock solutions (1000 ppm) of the metals were prepared by dissolving 1 g each of the metal in nitric acid (BDH-AR) and making upto one litre using de-ionised double distilled water. These solutions were later diluted to obtain the required concentrations for the experimental studies.

Acute toxicity tests were done by determining the 96 h LC_{50} values. Ten calms of the size group 20 ± 2 mm were maintained in 51 of filtered seawater (salinity 20%) in 10 1 troughts. Calculated volumes of metal solutions were added to maintain the required concentration of the toxicants. The mortality of the organisms were noted every 12 h; the calms were considered dead when the valve gap was atleast 5 mm and when they showed no response to external stimulii. The 96 h LC_{50} values were calculated from the cumulative mortality in each concentration using the log-probit method[11]. Thus, LC_{50} values of combinations of cadmium (concentrations 1 to 5 ppm) and copper (constant concentration of 0.5 ppm) as well as of copper (concentrations 0.1 to 0.5 ppm) and cadmium (constant concentration of 2.5 ppm) were determined.

Accumulation studies were carried out at different time intervals for assessing the effect of copper applied singly as well as in presence of cadmium. Clams were exposed to different levels of Cu-Cd concentrations. At the experimental concentrations of the metals selected for the accumulation studies, the clams were found to be actively filtering. Separate sets of controls were kept for each experiment. Groups of five clams were withdrawn at regular intervals of 24, 48, 72 and 96 h and the soft tissues of these clams were pooled together, dried, weighed and analysed for copper on an atomic absorption spectrometer (Perkin-Elmer 2380) following the method of Martincic *et.al.*[12]. The values of the metals in the control animals and the back ground levels at the sampling site are given in Table 1.

TABLE 1
CONTROL AND AMBIENT CONCENTRATIONS OF Cu AND Cd.

	Copper	Cadmium
Control Clams	10.99 ± 1.18 μg/g	7.44 ± 0.86 μg/g
Background level (Water)	7.04 ± 1.03 μg/l	1.76 ± 0.23 μg/l

RESULTS AND DISCUSSION

Several models have been proposed to explain and interpret the effects of toxicant mixtures on organisms. Loewe and Muischnek[12] displayed results on chemical mixtture's toxicity by using the isobole concept. The basic concept of joint action was proposed by Bliss[14] and further developed by later workers[15-22]. In this study we have chosen to apply the methods developed by Marking and Dawson[23] as well as that by Konemann[24] to study the toxic effects induced by the copper-cadmium combination to the clam *V. cyprinoides var. cochinensis.*

Although the method of Marking and Dawson was primarily based on *isoble* theory, it invoked the use of the toxic unit concept to sum the action of various components of a mixture, according to the equation

$$S = \frac{Am}{Ai} + \frac{Bm}{Bi}$$

where

S = Sum of biological activity; A and B are chemicals; 'i' and 'm' are toxicities (LC_{50} values) of individual metals and in combination respectively

Combined toxic action is considered to be

less than additive if, $S > 1$
more than additive if, $S < 1$
and simply additive if, $S = 1$.

In order to achieve linearity, they also introduced another system of calculating additive indices from S. In this system, the reference point is taken as zero and denotes a simply additive toxic action. Negative values indicate a less than additive (antagonistic) effect whereas positive values reflect a more than additive (synergistic) effect. The additive indices were then, derived from the values of S as follows:

$$\text{For } S \leq 1.0\text{, the additive index} = \frac{1}{S} - 1.0$$

$$\text{For } S \geq 1.0\text{, the additive index} = S(-1) + 1.0$$

From the Table 2, it can be seen that the sum of the biological activity for the cadmium (varying)-copper (constant) combination is 0.9966 and that for copper (varying)-cadmium (constant) combination is 0.808. The respective additive indices are +0.0034 and +0.24. Since additive indices not significantly different from zero, are deemed to indicate simply additive toxicity[25], the present Cu–Cd and Cd–Cu combinations are considered to be causing a simple additive effect on *V. cyprinoides* var. *cochinensis.*

TABLE 2
LETHAL TOXICITIES AND ADDITIVE INDICES

Combination	Individual LC_{50} (ppm)	Mixture LC_{50} (ppm)	S	Additive index
Cd (varying conc.) and	4.92	2.17		
Cu (constant)	0.90	0.50	0.9966	+0.0034
Cu (Varying conc.) and	0.90	0.27		
Cd (constant)	4.92	2.50	0.8080	+0.2400

Konemann[24] introduced the concept of mixture toxicity index (MTI) for quantifying the results of mixture toxicity experiments. According to him,

$$\text{MTI} = 1 - \frac{\log M}{\log n}$$

where n = no. of toxicants in the mixture

$M = f_i$;

$f_i = C_i/LC_{50}$

C_i = Concentration of the component 'i' in the mixture causing 50% mortality

MTI values of 0 and 1 represent 'no addition' and 'concentration addition' respectively. Values less than 0(–ve) show antagonistic effects, those between 0 and 1, partial addition and those above 1, supra addition (synergistic effect)[26].

The M values (Table 3) for the two combinations of toxicants (0.9966 and 0.808) and the corresponding MTI values (1.0049 and 1.3076), accordingly, suggest that both the combinations are concentration additive (or simply additive), with the Cu (varying)–Cd(constant) combination showing a slight supra-additive trend. Thus, using the schemes proposed by Marking and Dawson[23] and Konemann[24], it is observed that the Cd/Cu combination has a simply additive effect on *V. Cyprinoides* var. *cochinensis* at the experimental salinity (20%).

TABLE 3
M AND MTI VALUES FOR Cu-Cd COMBINATIONS

Combination	M	MTI
Cd + Cu (constant)	0.9966	1.0049
Cu + Cu (constant)	0.8080	1.3076

The results of the accumulation studies of copper in the presence and absence of cadmium at time intervals of 24, 48, 72 and 96 h are given in Tables 4 and 5. The values given are normalised values obtained after substracting the corresponding control values. Although, there is no significant change in the uptake of copper in both cases, a slight increase is observed in higher concentration as well as on longer exposure in the presence of cadmium. This result is in conformity with the slight synergistic trend of the Cu (varying)–Cd (constant) combination observed in acute toxicity studies. Reports of similar trends of copper and cadmium on administering in combinations to lower organisms are also available[27–29].

Negilski *et. al*[30] have reported a higher mortality than expected mortality for independent dissimilar action for *Callianasa australiensis* (Dana), when cadmium-copper mixture was administered. In a study by the same authors[31] on the accumulation effects,

TABLE 4
ACCUMULATION OF Cu BY *V. CYPRINOIDES* VAR. *COCHINENIS*

Conc. of Cu applied (ppm)	Conc. of Cu as μg/g dry wt. of whole tissue, n = 6			
	24 h	48 h	72 h	96 h
0.1	6.86 ± 0.26	9.93 ± 0.43	11.74 ± 0.56	12.99 ± 0.66
0.2	11.49 ± 0.35	13.61 ± 0.58	14.99 ± 0.64	15.74 ± 0.12
0.3	11.74 ± 0.75	13.99 ± 0.24	13.76 ± 0.48	16.61 ± 0.67
0.4	13.86 ± 0.62	14.41 ± 0.46	16.36 ± 0.69	17.86 ± 0.75

TABLE 5
ACCUMULATION OF Cu IN PRESENCE OF Cd

Conc. of Cd applied (ppm)	applied Cu (ppm)	Conc. of Cu as µg/g dry wt. of whole tissue, n = 6			
		24 h	48 h	72 h	96 h
2.5	0.1	6.74 ± 0.23	7.86 ± 0.74	11.36 ± 0.3	14.86 ± 0.24
2.0	0.2	7.45 ± 0.54	9.24 ± 0.53	13.61 ± 0.17	15.61 ± 0.43
1.5	0.3	9.25 ± 0.68	15.86 ± 0.42	16.49 ± 0.73	17.86 ± 0.52
1.0	0.4	14.86 ± 0.96	17.49 ± 0.36	19.74 ± 0.56	20.00 ± 0.26

however, cadmium was reported in inhibit copper uptake, whereas cadmium uptake was observed to be unaffected by the presence of copper. These two results seem to be at variance to each other, as well as to the fact enhanced toxic action is in direct proportion to the enhanced rate of accumulation.

Metal ions are incorporated into the biological system, through the formation of coordination compounds between the metal ions and the donor atoms of the ligand molecules (*viz.* N, O and S). In a ligand field, the Cu^{2+} ions (a $3d^9$ ion) would be conferred with an extra stabilisation-Ligand Field Stabilisation Energy (LFSE) which results from the ligand field splitting as well as from the Jahn-Teller effect, specific to a d^9 system. From the considerations of stabilization energetics, the Cu^{2+} ions would be expected to occupy a regular octahedral or a distorted octahedral environment. Cd^{2+} ions on the other hand, have a d^{10} electronic configuration and would prefer a tetrahedral configuration. Cu^{2+} ion can be considered as a border line acid while Cd^{2+} ion is a soft acid and N– and O– containing ligands are either hard bases or border line bases while S– containing ligands are regarded as soft bases.[32] Hence the Cd^{2+} ion would prefer to combine with soft bases (S– ligands), whereas the Cu^{2+} ions would form complexes with border line bases (N– and O– ligands). The slightly enhanced toxic action of the Cu^{2+} ion observed in these studies may be due to easy accumulation and direct access to biological systems and the higher stability of the Cu^{2+} complexes, that are a consequence of the smaller ionic radius of the Cu^{2+} ion and the LFSE available to the d^9 electronic configuration.

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