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TOXICITY AND BIOACCUMULATION OF CU^{2+} AND ZN^{2+} IN TWO ISOPOD SPECIES (CRUSTACEA)

BY

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ABSTRACT

Toxicity experiments were conducted to study the influence of different concentrations of Cu^{2+} (as $CuSO_4$) and Zn^{2+} (as $ZnSO_4$) on the survival of both the isopods ***Cirolana bovina*** *and* ***Idotea baltica****, and to determine the LC_{50} and the bioaccumulation of both metals during the 96 hours exposure period. The experiments revealed that the LC_{50} of Cu^{2+} was 8.106 and 1.2 mg. l^{-1} for* ***C. bovina*** *and* ***I. baltica****, respectively, while the LC_{50} of Zn^{2+} was 32.5 mg.l^{-1} and 1.95 mg. l^{-1} for* ***C. bovina*** *and* ***I. baltica****, respectively. In General* ***C. bovina*** *was less sensitive to both toxicants than* ***I. baltica****. Copper was more toxic for both species than Zinc. The bioaccumulation of Cu^{2+} in* ***C. bovina*** *increased from 83.89 mg. kg^{-1} dry wt. in the blank to 377.9 mg.kg^{-1} dry wt. in 12.606 mg Cu^{2+}. l^{-1} seawater and in* ***I. baltica*** *it increased from 115.58 mg. kg^{-1} in the blank to 905.2 mg. kg^{-1} dry wt in 10.106 mg Cu^{2+}. l^{-1} seawater. The bioaccumulation of Zn^{2+} in* ***C. bovina*** *increased from 193.2 mg. kg^{-1} dry wt in the blank to 1153 mg. kg^{-1} dry wt in 40.144 mg Zn^{2+}. l^{-1} seawater and in* ***I. baltica*** *it increased from 45.6 mg. kg^{-1} in the blank to 1223.2 mg. kg^{-1} dry wt in 30.144 mg Zn^{2+}. l^{-1} seawater.*

INTRODUCTION

Copper and Zinc are considered as essential constituents of some proteins and enzymes, and they increase the activity of many other enzymes (Vallee, 1978; Weser ***et al.***, 1979). They are required for normal growth and development of many marine invertebrates (Eisler, 1979). Zinc is found mainly in the plasma and is thought to be associated with Copper-protein hemocyanin (Bryan, 1964). It was found that variations of levels of zinc in the blood from different lobsters were related to concentrations of copper in the blood (Bryan ***et al.***, 1986). At higher concentration, however, copper is toxic and may be used as biocide (Effler ***et al.***, 1980). Brown and Newell (1972) and Scott and major (1972) found that copper at higher concentrations depressed oxygen consumption in ***Mytilus edulis***.

Several authors (Adema ***et al.***, 1972; Stebbing, 1976; Hodson ***et al.***, 1979; Neuhoff and Theede, 1983) indicated that concentrations of Cu^{2+} above 0.01 mg. l^{-1} often exceed the regulating capacity of marine invertebrates and result in various chronic effects. On the other hand, Wolf ***et al.*** (1972) and Neuhoff and Theede (1983) mentioned that a persistent long term contamination with low copper concentrations may result in reduced reproduction and increased mortality of benthic macrofauna.

Studies on the toxicity and accumulation of the essential metals copper and zinc in isopods are lacking. The present study therefore concerns with the toxicity and bioaccumulation of Cu^{2-} and Zn^{2+} in ***C. bovina*** and ***I. baltica*** under identical physico-chemical conditions.

MATERIAL AND METHODS

Collection of samples:

Adult specimens of ***Cirolana bovina*** and ***Idotea baltica*** were picked up from fouling samples collected from the Eastern Harbour of Alexandria (E.H.), Egypt during August 1995.

Bioassay technique:

In the laboratory, the animals were kept in seawater and fed on green algae and Bryozoa. Four days prior to bioassay tests, isopods were acclimatized in aerated filtered (using Watman F.P.1) seawater (E.H. origin) in acid-washed glass containers. Animals were starved for 24 hours before the experiment and throughout the experiment. Bioassay tests were carried out in glass jars to determine the 96 hours LC_{50} of either Cu^{2+} or Zn^{2+}. In each jar 10 individuals of the same size were placed in 750 ml of filtered aerated seawater containing known concentrations of $CuSO_4$ or $ZnSO_4$. Stock solutions of $CuSO_4$ and $ZnSO_4$ were prepared in deionized distilled water (DDW) and diluted to the appropriate concentration. The $CuSO_4$ solution was acidified with a drop of 6N HCl to prevent precipitation (Mckim and Benoit, 1971).In addition to the concentration of the metal in the blank, the experienced concentrations of Cu^{2+} were 1, 1.5, 2, 2.5, 3, 4, 5, 6.5, 8, 10 and 12.5 mg. l^{-1} and of Zn^{2+} were 2,4, 6, 8, 10, 12, 16, 20, 24, 28, 30 and 40 mg. l^{-1}. Two replicas for each concentration of each metal were examined, as well as a control test was performed in which no metal was added.

The salinity of seawater was measured at the beginning of the experiments and the deionized water was used to adjust it during the experiment. The aeration rate was maintained at the same level throughout the experiment. The level of Cu^{2+} and Zn^{2+} in seawater used at the beginning of the experiments, as well as in the isopods, in the blank and around the LC_{50} was measured. The number of dead and surviving isopods was recorded at 12 hours intervals for over 96 hours period. Live organisms from each concentration were rinsed in blank seawater and moved to new jar containing the same experiment concentration of the metal. Organisms that died during the experiment were removed from the jars. Isopods were considered dead when no movement of pleopods was observed. After 96h isopods from the blank and from each concentration were separately rinsed in deionized distilled water and kept in a deep freezer until the measurement of the heavy metal level.

Determination of Heavy metals in seawater and organism:

Dissolved and particulate heavy metals in seawater were measured according to Riely and Taylor (1968), and heavy metals in the organisms were measured according to Weeks and Rainbow (1991).

RESULTS

Effect of Cu^{2+} on survival of *C. bovina* :

Adult individuals of ***C. bovina*** were subjected to concentrations of Cu^{2+} solution ranging from 1.106 to 12.606 mg. l^{-1}. Surviving animals were counted every 12h for a period of 96h. The results obtained (Table 1 and Fig. 1) were as follows:

1. the concentrations 1.106 and 1.606 mg Cu^{2+}. l^{-1} showed no effect on the survival of ***C. bovina*** within the first 36h exposure while survival was 95% after 96h at both concentrations;
2. throughout the used range of concentrations, mortality rate did not exceed 10% after 12h;
3. mortality increased more or less gradually with increasing concentration and exposure period, reaching 95% in the highest concentration (*i.e.* 12.606 mg Cu^{2+} . l^{-1}) after 96h exposure, and
4. the LC_{50} was 8.106 mg Cu^{2+}. l^{-1} after 84h and 96h; 10.106 mg Cu^{2+}. l^{-1} at 60h and 12.606 mg Cu^{2+}. l^{-1} at 36h exposure.

Bioaccumulation of Cu^{2+} in *C. bovina* after 96h exposure:

As shown in table (2), ***C. bovina*** exposed to the blank seawater with a concentration of 0.106 mg.Cu^{2+}.l^{-1} for 96h contained 83.89 mg Cu^{2+}. kg^{-1}. On increasing the concentration of Cu^{2+} in sea water by 4 mg.Cu^{2+}.l^{-1}, the accumulation of Cu^{2+} in the organisms increased sharply (Fig. 2) to about four folds its concentration in the blank (Table 2) and the concentration factor (concentration of metal in organism/concentration of metal in seawater) decreased to about one tenth of its value in the blank (Table 2). In the higher concentration of Cu^{2+} (8.106 mg. l^{-1} which is the LC_{50}) the ability of the animal to accumulate the metal decreased (Fig. 2) and the concentration factor markedly decreased (Table 2). More concentrations of the metal (10.106 and 12.606 mg Cu^{2+}. l^{-1}) lead to more decrease in the rate of bioaccumulation and concentration factor (Table 2). As shown in figure (2) the concentration of Cu^{2+} in the animals remained almost stable within the range 4.106 to 10.106 mg Cu^{2+}. l^{-1}, although the mortality rate increased from 20% in the first concentration to 70% in the latter one. The significant rise in the concentration of Cu^{2+} in the animals at concentration 12.606 mg Cu^{2+}. l^{-1} is noteworthy and was accompanied by the highest rate of mortality (95%).

Table (1). Survival of ***Cirolona bovina*** in different concentrations of copper ions.

Conc. (mg. l^{-1})	Survival of ***Cirolana bovina*** after different exposure periods									% survival
	0h	12h	24h	36h	48h	60h	72h	84h	96h	96h
1.106	20	20	20	20	20	20	19	19	19	95
1.606	20	20	20	20	19	19	19	19	19	95
2.106	20	19	19	18	18	18	18	18	18	90
2.606	20	19	19	18	18	18	17	17	17	85
3.106	20	19	19	18	17	17	17	16	16	80
4.106	20	18	18	18	17	17	17	16	16	80
5.106	20	20	19	18	18	17	17	16	15	75
6.606	20	20	18	18	17	16	15	15	14	70
8.106	20	19	17	16	15	13	12	10	10	50
10.106	20	20	17	15	11	10	9	7	6	30
12.606	20	20	16	10	7	7	4	4	1	5
Blank 0.106	20	20	20	20	20	20	20	20	20	100

Table (2): Bioaccumulation of Cu^{2+} in ***Cirolana bovina*** after 96h exposure to different concentrations of Cu^{2+} solution (B= blank).

Concentration of Cu^{2-} in sea water (mg. l^{-1})	Concentration of Cu^{2-} in organism (mg. kg^{-1})	concentration factor
(B) 00.106	083.89	791.40
04.106	333.20	081.15
08.	337.90	041.68
10.106	339.80	033.62
12.606	377.90	029.98

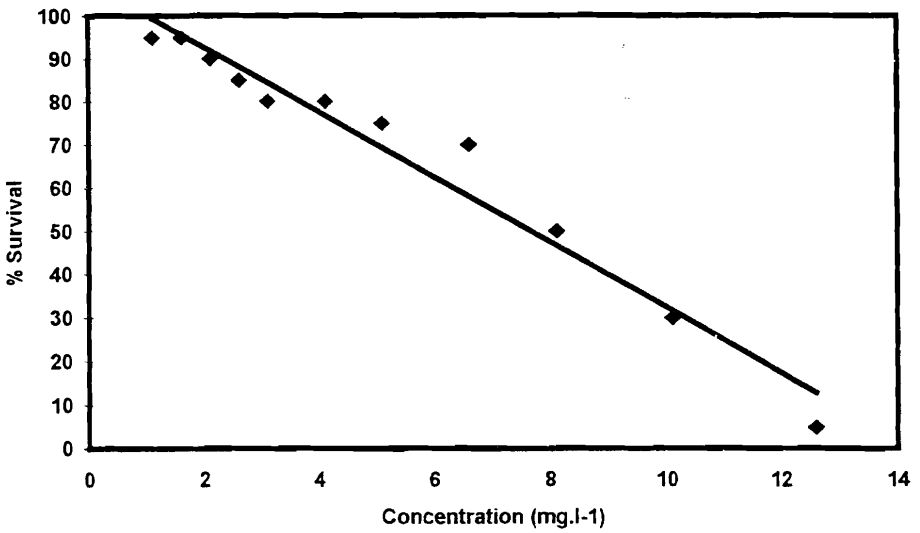

Fig. (1): Survival of ***Cirolana bovina*** in different concentrations of copper after 96h exposure.

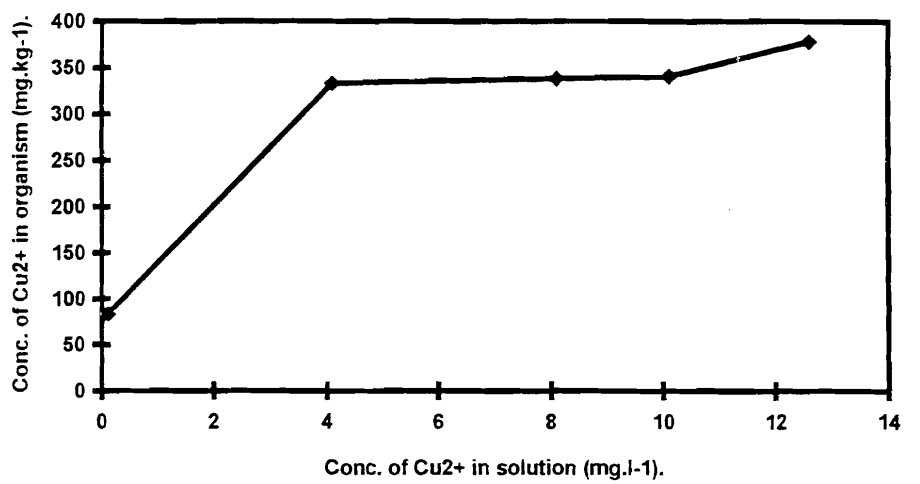

Fig. (2): Bioaccumulation of Cu^{2+} in ***Cirolana bovina*** after 96h in different concentrations of Cu^{2+} solution.

Effect of Zn^{2+} on survival of *C. bovina* :

Adult individuals of ***C. bovina*** were subjected to concentrations of Zn^{2+} within the range 2.144 to 40.144 mg. l^{-1} and the surviving animals were recorded every 12h during 96h exposure period in each concentration. The following results (Table 3 and Fig. 3) were obtained:

1. the concentration 2.144 mg Zn^{2+}. l^{-1} had no effect on the survival of animals within 96h exposure;
2. the survival of ***C. bovina*** exposed for 96h to Zn^{2+} concentrations from 4.144 to 24.144 mg. l^{-1} varied between 85% and 75% ;
3. apart from a few irregularities, survival rates of the species decreased with the increase of both concentration and exposure period, and
4. as deducted from figure (3) the LC_{50} was 32.5 mg Zn^{2+}. l^{-1} after 96h exposure, while at a concentration of 40.144 mg Zn^{+2}. l^{-1},50% of the animals died after 72h (Table 3).

Bioaccumulation of Zn^{2+} in *C. bovina* after 96h exposure:

The concentration of Zn^{2+} in ***C. bovina*** exposed for 96h to the blank concentration (0.144 mg Zn^{2+}. l^{-1}) was 193.2 mg. kg^{-1}, indicating a concentration factor of 1341.67. As shown in figure (4) the rate of bioaccumulation increased by increasing the ambient concentration of Zn^{2+}. However, this increase was not steady but, instead it took the form of sudden rise after a nearly steady state concentration. Thus at ambient concentrations of 8.144 and 20.144 mg Zn^{2+}. l^{-1}, the concentration of Zn^{+2} in the animals was 491.005 and 497 mg. kg^{-1}, respectively, which suddenly increased to 1126.7 mg.$kg.^{-1}$ under the ambient concentration of 30.144 mg Zn^{2+}. l^{-1}.On the other hand, the concentration factor decreased from a maximum of about 1342 at the lowest ambient concentration (0.144 mg Zn^{2+}. l^{-1}) to a minimum of about 29 at the highest ambient concentration (Table 4).

Effect of Cu^{2+} on survival of *I. baltica* :

Adult individuals of ***I. baltica*** were subjected to concentrations of Cu^{2+} solution varying from 1.106 mg. l^{-1} to 10.106 mg. l^{-1}. Surviving animals were counted every 12h for a period of 96h. The results (Table 5 and Fig. 5) indicated that:

1. ***I. baltica*** was able to withstand concentrations of Cu^{2+} up to 10.106 mg. l^{-1} for the first 12h exposure without any mortality ;

Table (3). Survival of ***Cirolana bovina*** in different concentrations of zinc ions.

Conc. (mg. l^{-1})	Survival of ***Cirolana bovina*** after different exposure periods									% survival
	0h	12h	24h	36h	48h	60h	72h	84h	96h	96h
2.144	20	20	20	20	20	20	20	20	20	100
4.144	20	20	20	19	19	18	18	17	16	80
6.144	20	20	19	19	19	17	17	16	16	80
8.144	20	20	20	19	19	19	17	17	17	85
10.144	20	20	18	18	18	16	16	16	16	80
12.144	20	20	18	17	17	17	17	17	17	85
16.144	20	20	18	18	18	18	17	16	16	80
20.144	20	20	19	18	18	16	16	16	15	75
24.144	20	19	17	17	16	15	15	15	15	75
28.144	20	19	15	14	14	13	13	13	12	60
30.144	20	18	14	14	13	12	12	11	11	55
40.144	20	18	15	14	13	12	10	8	5	25
Biank 0.144	20	20	20	20	20	20	20	20	20	100

Table (4): Bioaccumulation of Zn^{2+} in ***Cirolana bovina*** after 96h exposure to different concentrations of Zn^{2+} solution (B= blank).

Concentration of Zn^{2-} in sea water (mg. l^{-1})	Concentration of Zn^{2-} in organism (mg. kg^{-1})	concentration factor
(B) 00.144	0193.200	1341.67
08.144	0491.005	0060.29
20.144	0497.300	0024.69
30.144	1126.700	0037.38
40.144	1153.100	0028.72

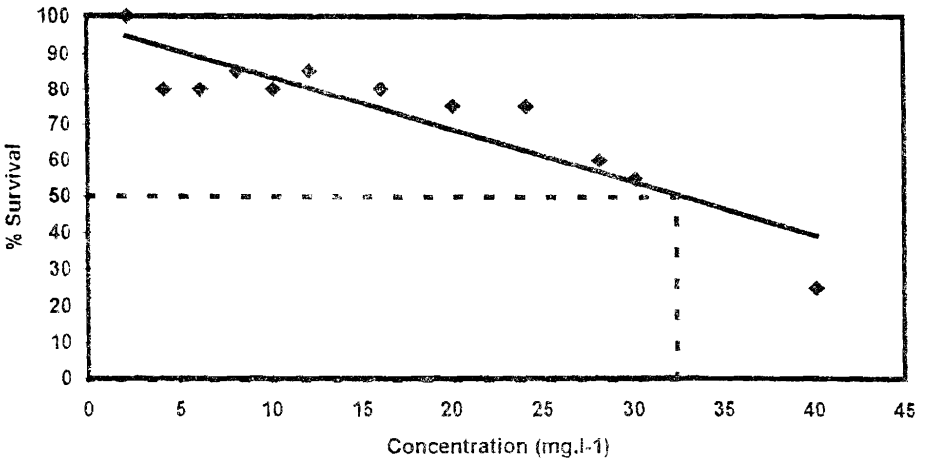

Fig. (3): Survival of ***Cirolana bovina*** in different concentrations of zinc after 96h exposure.

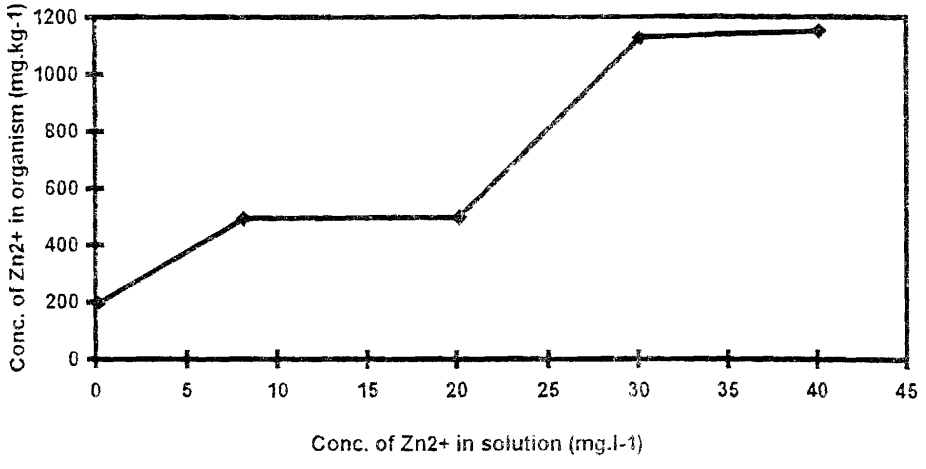

Fig. (4): Bioaccumulation of Zn^{2+} in ***Cirolana bovina*** after 96h in different concentrations of Zn^{2+} solution.

2. at the concentration of 0.606 mg.Cu^{2+}. l^{-1}, the mortality formed 30% of the individuals after 96h. As extrapolated from figure (5) the LC_{50} for 96h exposure was 1.2 mg Cu^{2+}. l^{-1}.
3. in concentrations of 1.106 and 1.606 mg Cu^{2+}. l^{-1}, 50% of the animals died after 84h exposure period. The exposure time corresponding to LC_{50} decreased by increasing the ambient concentration of Cu^{2+} being 72h at 2.606 mg.l^{-1} and 60h at concentration of 5.106 mg. l^{-1} (Table 5), and
4. apart from a few irregularities, survival rates of the species decreased with the increase of both concentration and exposure period. At the highest concentration used (*i.e.* 10.106 mg Cu^{2+}. l^{-1}) the survival rate reached 30% after 96h exposure.

Bioaccumulation of Cu^{2+} in *I. baltica* after 96h exposure:

The concentration of Cu^{2+} in ***I. baltica*** lived in the blank solution (seawater containing 0.106 mg Cu^{2+}. l^{-1}) for 96h was 115.58 mg. kg^{-1} giving a concentration factor of 1090.38 (Table 6). The uptake of Cu^{2+} by ***I. baltia*** increased with increasing the concentration of the metal in solution (Fig. 6), while the concentration factor decreased progressively from 1090.38 to 89.57. At LC_{50}, the concentration of Cu^{2+} in the organisms was estimated to be about 496 mg. kg^{-1} (Fig 6).

Effect of Zn^{2+} on *I. baltica* :

Adult individuals of ***I. baltica*** were subjected to different concentrations of Zn^{2+} ranging from 1.144 to 30.144 mg. l^{-1} and the survival of animals was recorded every 12h for 96h exposure in each concentration. The results (Table 7 and Fig. 7) indicated that:

1. there was no effect on the survival of organisms within the first 12h;
2. mortality of animals exposed for 96h increased more or less gradually with increase of the metal concentration and reached 100 percent at Zn^{2+} concentration of 28.1mg. l^{-1} (Fig. 7);
3. the LC_{50} as extrapolated from figure (7) was 1.95mg. l^{-1} after 96h and it was 3.144 mg. l^{-1} after 72h and 84h (Table 7), and
4. apart from a few irregularities mortality rates of the species increased with increasing of both concentration and exposure period.

Table (5). Survival of ***Idotea baltica*** in different concentrations of copper.

Conc. (mg. l^{-1})	Survival of ***Idotea baltica*** after different exposure periods									% survival
	0h	12h	24h	36h	48h	60h	72h	84h	96h	96h
0.606	20	20	20	19	17	14	14	14	14	70
1.106	20	20	20	18	15	14	12	10	9	45
1.606	20	20	19	17	15	13	11	10	9	45
2.106	20	20	19	17	15	13	11	9	8	40
2.606	20	20	19	17	15	12	10	8	8	40
5.106	20	20	18	17	14	10	9	7	7	35
8.106	20	20	17	15	13	9	9	8	7	35
10.106	20	20	17	14	12	9	8	7	6	30
Blank 0.106	20	20	20	20	20	20	20	20	20	100

Table (6): Bioaccumulation of Cu^{2+} in ***Idotea baltica*** after 96h exposure to different concentrations of Cu^{2+} solution (B= blank).

Concentration of Cu^{2+} in seawater (mg. l^{-1})	Concentration of Cu^{2+} in organism (mg. kg^{-1})	concentration factor
(B) 0.106	115.58	1090.38
0.606	324.73	0535.86
1.106	455.00	0411.39
1.606	675.00	0420.30
10.106	905.20	0089.57

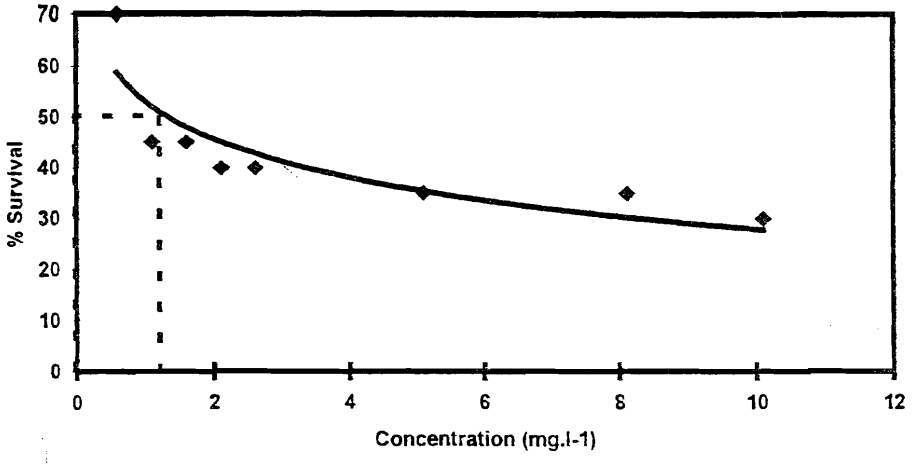

Fig. (5): Survival of ***Idotea baltica*** in different concentrations of copper after 96h exposure.

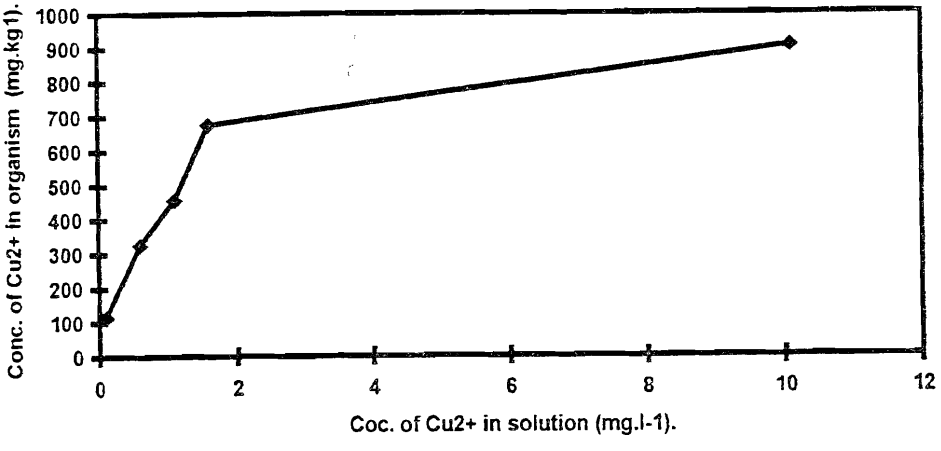

Fig. (6): Bioaccumulation of Cu^{2+} in ***Idotea baltica*** after 96h in different concentrations of Cu^{2+} solution.

Table (7). Survival of ***Idotea baltica*** in different concentrations of zinc.

Conc. (mg. l^{-1})	Survival of ***Idotea baltica*** after different exposure periods									% survival
	0h	12h	24h	36h	48h	60h	72h	84h	96h	96h
1.144	20	20	19	17	15	14	14	13	12	60
3.144	20	20	18	16	14	14	10	10	9	45
8.144	20	20	17	11	9	8	6	6	5	25
12.144	20	20	16	7	7	4	2	2	2	10
16.144	20	20	15	6	3	1	1	1	1	5
20.144	20	20	13	4	3	2	2	2	2	10
24.144	20	20	12	3	3	1	--	--	--	0
30.144	20	20	9	2	1	1	1	1	1	5
Blank 0.144	20	20	20	20	20	20	20	20	20	100

Table (8): Bioaccumulation of Zn^{2+} in ***Idotea baltica*** after 96h exposure to different concentrations of Zn^{2+} solution (B= blank).

Concentration of Zn^{2+} in sea water (mg. l^{-1})	Concentration of Zn^{2+} in organism (mg. kg^{-1})	concentration factor
(B) 00.144	0045.64	316.94
01.144	0337.80	234.58
03.144	0507.50	161.42
08.144	0675.67	082.96
30.144	1223.20	040.58

Fig

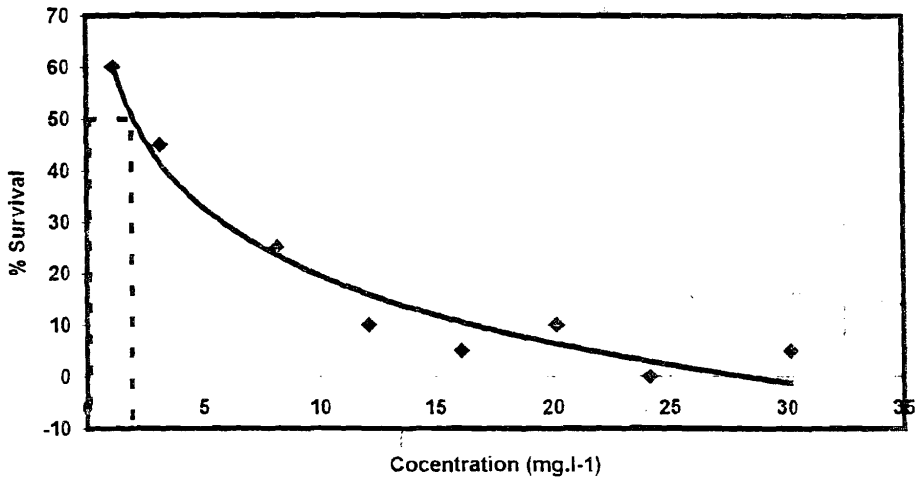

Fig. (7): Survival of ***Idotea baltica*** in different concentrations of zinc after 96h exposure.

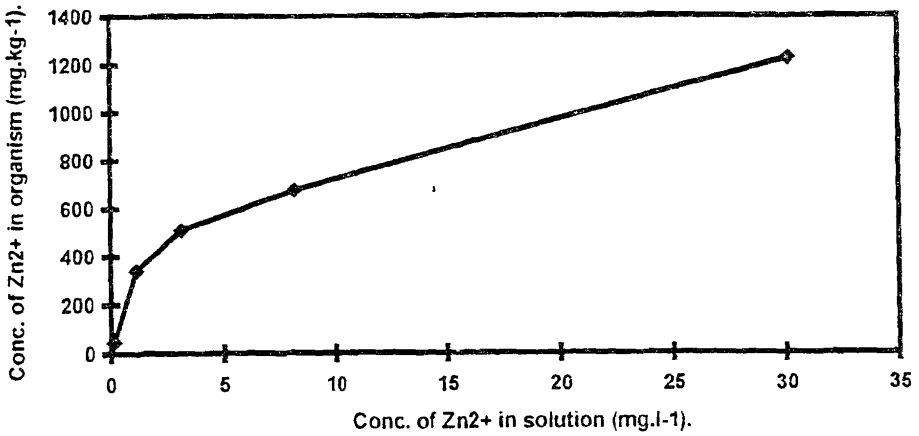

Fig. (8): Bioaccumulation of Zn^{2+} in ***Idotea baltica*** after 96h in different concentrations of Zn^{2+} solution.

Bioaccumulation of Zn^{2+} in *I. baltica* after 96h exposure :

The Zn^{2+} concentration in the organisms in the blank (0.144 mg Zn^{2+} . l^{-1}) for 96h was 45.64 mg. kg^{-1} with a concentration factor of 316.94. By increasing the concentration of Zn^{2+} in the seawater, the accumulation of Zn^{2+} increases in the organisms (Fig. 8). In general, the bioaccumulation of Zn^{2+} by ***I. baltica*** was directly correlated with the ambient concentration of the metal. The concentration of Zn^{+2} in the animals at LC_{50} was about 400 mg. kg^{-1} (Fig. 8). On the other hand, the concentration factor decreased progressively by increasing the ambient concentration of Zinc. It decreased from 316.94 in the blank seawater to 40.58 in seawater containing 30.144 mg. l^{-1} (Table 8).

DISCUSSION

The present study revealed that the isopod species studied in the Eastern Harbour were able to live in Cu^{2+} concentration of 0.106 mg. l^{-1}. This agrees with Weser ***et al.***, (1979) who reported that marine organisms seem to be adapted to metal concentrations normally found in their natural environment. However, the organisms can tolerate higher concentrations of Cu^{2+} as shown in the Eastern Harbour of Alexandria where the LC_{50} of copper for ***C. bovina*** and ***I. baltica*** after 96h were estimated as 8.106 and 1.2 mg. l^{-1} respectively.

Table (9): LC_{50} of Cu^{2+} or Zn^{2+} ion recorded for some benthic crustaceans by different authors.

Author	Area	Species	LC_{50} after 96h	
			Cu^{2-}	Zn^{2-}
Portmann (1963)		Pink shrimp	<0.14 mg. l^{-1}	---
Abo-Nour (1984)	El-Maadia (Egypt)	***Callinectes sapedus***	0.18 and 0.17 mg. l^{-1} for males and females, respectively	5.6 and 5 mg. l^{-1} for males and females, respectively.
Hilmy ***et al.*** (1985)	El-Maadia (Egypt)	***Portunus pelagicus***	3.89 and 3.27 mg .l^{-1} for males and females respectively	22.38 and 18.62 mg .l^{-1} for males and females, respectively.
McLeese (1976)		***Homarus americanus***	---	13 mg .l^{-1}
Present study	Eastern Harbour	***Cirolana bovina***	8.106 mg. l^{-1}	32.5 mg. l^{-1}
	Eastern Harbour	***Idotea baltica***	1.2 mg. l^{-1}	1.95 mg. l^{-1}

Unfortunately no published bioassay data on Isopoda are available to compare with, however the data shown in table (9) indicate a wide difference in the LC_{50} of Cu^{2+} for the mentioned crustacean species. The present data indicate the high resistance capacity of ***C. bovina*** to Cu^{2+}.

For Zinc the 96h LC_{50} determined for ***C. bovina*** and ***I. baltica*** in the present study were respectively 32.5 mg. l^{-1} and 1.95 mg. l^{-1}.

As shown in table (9) the differences between the values of LC_{50} of either Cu^{2+} or Zn^{2+} for the different species agree with Karbe (1972); Theede ***et al.***, (1979); Wiederholm (1984) and Voshell ***et al.***, (1989) who stated that some marine organisms are very sensitive to heavy metals while others are resistant and can accumulate them to a high degree from seawater. Chapman ***et al.***, (1980) mentioned that invertebrate species vary widely in their tolerance of metals as well as in the metabolic strategy associated with that tolerance. On the other hand , the bioaccumulation and concentration factor of Cu^{2+} and Zn^{2+} in the normal population of both species in the natural seawater of the Eastern Harbour were pronouncedly high and differed widely (Tables 2,4,6 and 8). Several authors (Bryan, 1976; Rainbow, 1987; 1988; Rainbow and White, 1989; Rainbow ***et al.***, 1990. Augier ***et al.***, 1992) mentioned that the methods of heavy metal accumulation vary within the different species of Crustacea and with respect to metals. Furthermore, the present study revealed that the increase of either Cu^{2+} or Zn^{2+} concentration in the medium lead to increase their content in both species, but the concentration factors decreased progressively. This seems to be a common behaviour of many aquatic organisms towards the metal in the waters (Schulz-Baldes, 1974; Wolf, 1975; Phillips, 1977; Scholz, 1980; Taylor, 1983; Kay, 1985; Bryan ***et al.*** 1986; Weeks and Rainbow, 1991; Augier ***et al.***, 1992; Timmermans ***et al.***, 1992).

The present study has indicated that ***C. bovina*** has a higher capacity than ***I. baltica*** for regulating the bioconcentration of heavy metals in its body and keeping them at low level, as indicated by the higher value of LC_{50} in ***C. bovina*** than ***I. baltic.*** This may explain the observation of Elsonbaty (1997) that ***C. bovina*** has higher abundance in the Eastern Harbour than ***I. baltica.***

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