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Toxicity and Bioaccumulation of Copper, Zinc, and Cadmium in Some Aquatic Organisms

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Coastal inland water receive discharges from different sources including industrial plants, sewage drainage, atmospheric pollution and other natural sources which lead to heavy metal pollution (Bryan, 1971). Heavy metals can affect the aquatic organisms as toxic substances in water and sediment or as a toxicant in the food chain (Zyadah, 1995). Cichlidae and Mugilidae are widely distributed in the Egyptian inland waters. *They are* the main source of fish food and are economically important in inland fisheries and aquaculture resources in Egypt. *Mysis sp.* is considered a main source of fish food. The objective of this study is to measure the toxicity of Cu, Zn & Cd in *T. zillii, M. cephalus* and *Mysis sp.,* to determine the bioaccumulation rate in the fish, as well as LC_{50} and total mortality of each species.

MATERIALS AND METHODS

Water samples for heavy metal measurements were collected with fry and juveniles of *Mugil cephalus, Tilapia zillii* and crustacean *Mysis sp.* from River Nile Estuary at Damietta. Fish fry and crustaceans were transported in aerated containers to large aquarium in the laboratory, where stocked and adapted 5 days to the laboratory condition in mixed water and aerated continuously with air pumps. Water quality was determined twice a week and the physico-chemical parameters were measured, water was renewed every 72 h. $Cu\ SO_4$, Cd Cl2 and Zn Cl2 were used for the preparation of stock solutions of heavy metals for bioassays. Experiments were run in a series of 101 glass Aquarium, percentage of mortalities was recorded at the time intervals. Both safe and toxic concentrations were measured and back calculated using a formulae of Bahren and Karber (1953) the modification introduced by Bailey (198 1) via regression analysis, showing the correlation between actual LC_{50} and exposure periods. Correlation coefficient (r) was calculated at probability 0.05.

The experiment was performed with apparent healthy organisms after being acclimatized to the laboratory conditions. Tested fishes were fed during test periods; the experiments were conducted between 10/8/1997 to 6/10/1997. A 96 hr bioassays were performed to determine LC_{50} values. Series of heavy metal concentrations were used as follow: (Cu: 0.5,2, 5 and 10 mg/l) and (Zn:

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20, 30, 40 and 60 mg/l) for *M. cephalus;* (Cu: 0.5,2, 5, 10, 15 and 20 mg/l), (Zn: 30, 60, 80 and 100 mg/l) and (Cd: 0.05, 0.1, 0.5, 1, 2, 5 and 10 mg/l) for *T. zillii;* (Cu: 0.5, 2, 5, 10, 15 and 20 mg/l) and (Cd: 0.05, 0.1, 0.5, 1, 2, 5 and 10 mg/l) for *Mysis sp.* and coupled treatment (Cu and Zn: 0.25+5, 1+10, 2.5+20, and 5+30 mg/l, respectively) for *M. cephalus.* After different times of heavy metal exposures, samples of *M. cephalus* and *Mysis sp.* were separated for digestion as whole organism. Head and body of *T. zillii* were digested. The residues of heavy metals were determined by flame atomic absorption spectrometry Daziel & Baker (1983).

RESULTS AND DISCUSSION

M. cephalus never reach LC50 after 168 hr exposure to 0.5 mg Cu/l, but LC_{50} appeared at 5 and 2 mg Cu/l after 96 and 120 hr exposure periods, respectively, (Table 1A). At 10 mg/l concentration, 100% mortality was re corded after 24h. Strong negative significant correlation ($P<0.05$) existed between LC_{50} and exposure time. The actual 96hr LC_{50} (4.35 mg/l) was almost close to the back calculated value (4.12 mg/l). 96 h LC_{50} on *T. zillii* fry was 3.3 mg Cu/l, it was slightly lower than the back calculated value (4.6 mg Cu/l). Acute mortality appeared at 10 mg/l after 48h exposure, and 48 hr LC_{50} (5.5 mg/l) was close to the back calculated value (6.1 mg/l). *Mysis sp.* was exposed to four Cu concentrations Actual 96hr LC_{50} was 1.44 mg Cu/l; this value is less than LC_{50} values of both *M. cephalus* and *T. zillii,* because *Mysis sp.* is more susceptible to Cu toxicity than the fish species.

At four concentrations of Zn, it is shown that, 96h LC_{50} was 26 mg Zn/l. The back calculated values were very close to the actual ones (table 1B). The acute mortality of *M. cephalus* fry appeared at 60 mg Zn/l after 24hr exposure. Toxicity of Zn on *T. zillii* fry during different exposure periods showed that 96hr LC_{50} did not appear at any tested concentration. The actual and back calculated 168hr LC_{50} were appeared at 78.5 and 80.1 mg Zn/l concentrations, respectively. Acute mortality at 100 mg/l after 168h exposure was detected Back calculated 96hr LC_{50} could be inferred as 91.2 mg Zn/l (Table 3). 96hr LC_{50} of Cd in *T. zillii* did not appear but LC_{50} was obtained after 332hr exposure at 5.66 mg Cd/l concentration, and acute mortality was delayed at 4.47 mg/l after 380hr exposure, it was very close to the back calculated value (4.62 mg Cd/l). The prospective back calculated 96hr LC_{50} value was determined as 13.1 mg Cd/l (Table 3). Acute toxicity of *Mysis sp.* appeared at concentration 1 mg/l after 48hr exposure period, 96hr LC_{50} of Cd in *Mysis sp.* was 0.31 mg/l (table 1C).

The combined Cu and Zn for 96hr LC_{50} on *M. cephalus* were 1.1 and 10.8 mg/l Cu and Zn, respectively (Table 2). Acute mortality appeared after 96hr exposure period. Rate of sensitivity of fish fry increased after coupled treatment. Table 3 showed the actual and back calculated LC_{50} values of Cu, Zn and Cd concentrations of *T. zillii, M. cephalus* and *Mysis sp.* during different exposure periods. The actual LC_{50} values of heavy metals tested showed a remarkable concordance with the comparable back calculated ones for all the tested organisms during the exposure periods (r ranged between 0.95-0.99 & p<0.01-0.001).

Table 1. Mortality (%) of *M. cephalus* fry, *T. zillii* and *Mysis sp.* at different copper concentrations (A), *M. cephalus* fry, and *T. zillii* at different zinc concentrations (B) and *T. zillii* and Mysis sp at different cadmium concentrations (C) (mg/l) during exposure periods.

Aquatic organisms	Exposure period (hr)	copper conc. (A) 0.5	2	5	10	LC50 Actual	*C
M.cephalus	24	0	0	30	100	6.3	6.28
	48	10	20	40	100	5.33	5.56
	96	10	30	60	100	4.35	4.12
	120	10	60	70	100	3.58	3.40
	144	10	70	80	100	2.65	2.68
	168	40	80	90	100	1.80	1.96
T. zillii	24	0	0	20.8	60.4	9.2	8.7
	48	5.26	11.1	43.9	100	5.5	6.1
	72	6.56	14.1	51.6	100	4.5	5.5
	96	8.44	20.0	55.4	100	3.3	4.6
	12	0	0	33.3	35	8.5	-
Mysis sp.	24	0	33.3	66.7	82.7	4.86	4.56
	48	0	46.7	90	100	2.89	3.52
	72	16.7	60	100	100	2.04	2.52
	96	33.3	80	100	100	1.44	1.14

Aquatic Organisms	Exposure period (hr)	Zn conc. (B) 20	30	40	60	80	100	LC50 A`	*C
M. cephalus	24	10	50	50	100			37.0	36.7
	48	20	60	70	100			32.5	33.1
	72	30	70	80	100			29.5	29.5
	96	50	80	90	100			26.0	25.9
T. zillii	168		33.3		22.2	11.1	100	78.5	80.1
	216		44.4		22.2	22.2	100	75.0	72.8
	264		44.4		44.4	44.4	100	66.0	65.4
	312		44.4		55.6	55.6	100	57.0	58.1

Aquatic organisms	Exposure period (hr)	Cd Conc (C) 0.05	0.1	0.5	1	2	5	10	LC50 A	*C
T. zillii	192	0	0	0	0	25	22.2	11.1		10.2
	332	0	0	22.2	20	50	44.4	66.7	5.66	5.97
	380	0	0	33.3	40	50	44.4	100	4.47	4.62
	428	0	0	44.4	50	50	55.6	100	3.55	3.18
	476	0	0	66.7	80	87	100	100	0.78	0.74
	514	0	0	77.8	80	100	100	100	0.54	0.30
Mysis sp.	24	0	26.3	26.3	80.8	100	100	100	0.8	0.86
	48	0	36.8	47.4	100	100	100	100	0.47	0.56
	72	0	36.8	68.4	100	100	100	100	0.36	0.41
	96	0	52.6	68.4	100	100	100	100	0.31	0.25

* (r = - 0.99: - 0.95 and P < 0.001:0.01; C: calculated)

Table 2. Mortality (%) of *Mugil cephalus* fry at different concentrations (mg/l) of copper and zinc (coupled treatment) during the exposure periods.

Exposure period (hr)	Cu	and	Zn	Conc.	LC50	
	0.25 + 5	1 + 10	2.5 + 20	5 + 30	Actual	*C
24	0	0	55.6	70		2.4+18.4
48	0	0	66.7	90		2+15.9
72	20	25	77.8	100	1.7+14.3	1.6+13.7
96	20	50	100	100	1.1+10.8	1.2+11.3
120	30	75	100	100	0.8+8.3	0.9+8.9
144	40	90	100	100	0.6+7.3	0.5+6.6
168	60	100	100	100		

(r = - 0.99: - 0.95 and P < 0.001:0.01; C: calculated)

Table 3. LC50 of copper, cadmium and zinc concentration (mg/l) in *T. zillii* , and) in *Mysis Sp.* and *M. cephalus*, actually (A) b y Behren and Karber (1953), and back (B) calculated by regression analysis (Bailey, 1982).

Aquatic organisms	Exposure period (hr)	Metal		conc.			
		Cu A	B	Cd A	B	Zn A	B
T. zillii	24	8.7	8.2	-	15.3	-	102.2
	48	6.1	6.9	-	14.6	-	98.5
	72	5.5	5.6	-	13.9	-	94.8
	96	4.6	4.3	-	13.1	-	91.2
	168	-	0.4	-	10.9	78.5	80.1
	216	-	-	-	9.5	75	72.8
	264	-	-	-	8	66	65.4
	312	-	-	-	6.6	57	58.1
	560	-	-	4.5	4.6	-	50.8
	408	-	-	3.6	3.2	-	43.4
	456	-	-	0.79	0.7	-	36.1
	504	-	-	0.54	0.3	-	28.7
Mysis sp.	24	4.86	4.56	0.80	0.86		
	48	2.89	3.52	0.47	0.56		
	72	2.04	2.25	0.36	0.41		
	96	1.44	1.14	0.31	0.25		
M. cephalus	24	6.3	6.3			37.0	36.7
	48	5.3	5.6			32.5	33.1
	72	-	-			29.5	29.5
	96	4.4	4.1			26.0	25.9
	120	3.6	3.4				
	144	2.7	2.7				
	168	1.8	2.0				

Table (4A) demonstrates the bioaccumulation of Cd in both *T. zillii* and *Mysis sp* during 428h exposures to seven Cd concentrations ranging from 0.05 to 10 mg/l. The bioaccumulation of Cd in tested fish is usually increased with the increase of both exposure time and Cd concentration. At 356 hr exposure, the bioaccumulation rate of *T. zillii* increased to about 858 folds (60.1 µg/g wet wt) more than the control concentration (0.07 µg/g wet wt). In *Mysis sp.*,the bioaccumulation of Cd (48.6 µg/g fresh wt) increased 1215 folds more than the control concentration (0.04 µg/g fresh wt) during the exposure conc. (1.0 mg/l) after 48h exposure. Table 4B shows the bioaccumulation of Cu in *T. zillii, M. cephalus* and *Mysis sp.* during different exposure periods. The maximum bioaccumulation rate of *T. zillii* (5.6 µg/g wet wt) was about ten times more than control values at 0.5 mg Cu/l. The bioaccumulation rate of Cu in *M. cephalus* increased slowly with increase of both concentration of Cu and exposure periods. The bioaccumulation of Zn by *M. cephalus* and *T. zilli* increased with increasing Zn concentration and exposure time except in *T. zillii* **at** 30 mg Zn/l, which showed a remarkable decrease in bioaccumulation at 120 and 144 hr exposure periods (table 4C). In *T. zillii,* Cu and Cd accumulation in body is nearly higher than the concentration in head.

Table 4. Bioaccumulation of metal (µg/g wet wt) in *T. zillii* and *Mysis sp.* (A); in *T. zilli, M. cephalus* and *Mysis sp.* (B) and in *T. zillii* and *M. cephalus (C)* exposed to different concentrations of cadmium, copper and zinc during the exposure periods.* H &B: head and body of fish and Exp. Per.: exposure period.

exposure period (hr)	Species	(A) Cd Conc. ppm							
		0.05	0.1	0.5	1.0	2.0	5.0	10.0	Control
12	*Mysis sp.*	-	-	14.9	4.32	5.12	-	-	0.04
24	*Mysis sp.*	-	1.06	2.56	5.28	-	-	-	0.04
48	*T.zilli* B	-	-	-	-	10.6	-	-	0.07
	Mysis sp.	-	1.77	12.3	48.6	-	-	-	0.04
120	*Mysis sp.*	-	4.95	9.91	-	-	-	-	0.04
168	*T.zilli* B	0.49	1.06	3.89	2.48	-	-	-	0.07
192	*T.zilli* B	-	-	-	-	13.8	-	-	0.07
228	*T.zilli* B	-	-	-	16.5	6.02	-	-	0.07
356	H	-	-	-	-	7.43	-	26.2	0.05
	T.zilli B	9.63	6.37	-	-	59.1	-	60.1	0.07
380	H	-	-	-	-	-	5.3	-	0.05
	T.zilli B	-	-	-	10.6	-	30.4	-	0.07
428	H	-	-	1.49	-	-	-	5.2	0.05
	T.zilli B	-	-	4.82	-	-	-	57.3	0.07

Exp. per (hr)	Species	(B) Cu Conc. ppm						
		0.5	2	5	10	15	20	Control
12	*H					4.5	4.1	0.7
	T.zilli B			9.4	7.3	5.9	6.4	1.1
	Mysis sp.			9.7	7.4	9.7	9.5	3.7
18	*Mysis sp.*			6.5	9.7			3.7
24	H						7.3	0.7
	T.zilli B			4.4	4.5		5.9	1.1
	M.cephalus			1.6	7.5			1.6
	Mysis sp.			5.9	7.8			3.7
36	H	5.6	9.9		11.4			0.7
	T.zilli B	4.1	1.1	4.6	3.2			
	Mysis sp.		-	7.5	5.9			3.1
96	*M.cephalus*	1.5						
168		2.5						
192		3.9	2.9	3.1				1.6

Table 4. Continued.

Exp per. * (hr)	Species	(C) Zn Conc. ppm						Control
		20	30	40	60	80	100	
24	*M.cephalus*	-	-	30.8	33.1	-	-	8.6
	T.zillii	-	-	-	24.6	-	29.3	12.4
48	*M.cephalus*	-	28.1	23.1		-	-	8.6
	*H	-	-	-	42.7	-	-	12.4
	T.zillii B	-	-	-	25.0	-	-	-
72	*M.cephalus*	51.3	33.3	-	-	-		8.6
	H	-	-	-	-	-	48.1	12.4
	T.zillii B	-	-	-	-	-	29.2	-
	H	-	45.9	-	-	-	-	8.6
120	*T.zillii* B	-	35.2	-	-	-	-	12.4
	H	-	15.4	-	33.8	33.1	88.9	12.4
	T.zillii B	-	13.1	-	21.8	30.3	75.4	-
144	*M.cephalus*	-	73.5	-	-	-	-	8.6
	T.zillii H	-	-	-	-	49.8	61.1	12.4
	B	-	-	-	-	39.1	48.8	-

The present investigation showed big differences of both toxicity and bioaccumulation rate among the aquatic organisms. A clear variation in LC_{50} and acute toxicity in tested organisms were evident. 96hr LC_{50} of Cu in *T. zillii* was 3.3 mg Cu/l, while in *Mysis sp., it* was 1.4 mg Cu/l. Acute toxicity was observed after 24hr and 48 hr exposure of 10 mg Cu/l to *M. cephalus* and *T. zillii,* respectively, and 5 mg Cu/l after 72hr exposure to *Mysis sp.* It is obvious that *Mysis sp* is more sensitive to Cu toxicity than the tested fish species. Other research reported lower Cu concentrations 96hr-LC_{50} for marine crustaceans as; 0.017 mg Cu/l for *Acartice tansa;* 0.049 mg Cu/l for *Cancer magister* and 0.1 mg Cu/l for *Homarus americanus* (Martin et al.,1981 and Mance, 1987).

96hr LC_{50} of Zn in *M. cephalus* appeared at 26 mg / 1, but in *T. zillii* appeared later after after 168 hr exposure at 78.5 mg Zn / 1, Acute toxicity was found for *M. cephalus* and *T. zillii* of 60 and 100 mg Zn/l after 24 and 168 hr, respectively. The obtained results confirmed that *M. cephulus* is more sensitive to Zn toxicity than *T. zillii,* this may be attributed to the lower salinity of the estuarine water or to the younger life stage of *M. cephalus* fry (Mance,1987). The present results showed less sensitivity of *T. zillii* and increase sensitivity of *Mysis sp* to Cd toxicity, this result is in agreement with finding of Martin et al. (1981), who found that plankton crustaceans are very sensitive to Cd, while some fish species are relatively less resistant to Cd.

Coupling concentrations of Cu and Zn during exposure periods have been experimented on *M. cephalus.* 96hr, LC_{50} were 1.1 and 10.8 mg/l for Cu and Zn, respectively. Rate of sensitivity of fish fry increased after coupled treatment. The binary combination (coupling) of Cu & Zn in different concentrations above and below the 96hr, LC50 showed that both metals behave antagonistically as shown in table 8. The effect produced by both metals coupled is less than the effects produced by individual metal. this may attributed to substitution and competition between Cu and Zn for available sites during protein synthesis as suggested by Bryan (1971) and Abdel-Moati & Farag (1991).

The variety degree is related to kind of species, its sensitivity and physiological responses to pollutants. and their uptake and depuration rate of heavy metals (Salanki and V. -Balogh 1985; Salanki & V. -Balogh 1989). El-Gindy, et. al (1991) recorded 24h LC_{50} for mollusks *Biomphalaria alexandrina* and *Bulinus truncatus* of Cu and Zn toxicity as 1.38, 0.99 & 54, 40 ppm, respectively. The 96hr LC_{50} value for Cu in *L,. balteni* was 0.9 ppm (Abdel-Moati & Farag 1991), but in *Mugil* fry was 1.3 ppm (El-Rayis & Ezzat 1984). The 96h LC_{50} of Zn *in L. bolteni* was 58 ppm (Abdel-Moati and Farag 1991), while that for *Portunus pelagicus* was 100 ppm, (El-Rayis & Ezzat (1984). However *T. zillii* have the ability to live in polluted areas for long time than other species of fish (Zyadah.1999). The actual and back calculated LC_{50} of Cu, Zn. and Cd values for the experimental species during the exposure periods showed a close concordance. Other results in the world showed different LC_{50} of Cu, Zn, and Cd values, where flounder fish exposed to 0.1 to 10 mg Cd/l for 15d (Larsson et al. 1976); Juvenile striped bass was exposed to 0.01 mg Cd/l for 120d (Dawson et al., 1977) and juvenile of shrimps *Penaeus duorarum* exposed to 5 mg Cd/l for 96hr (Nimmo et al.. 1977).

The rate of bioaccumulation of heavy metals by fish and shrimp appeared within a wide range. The bioaccumulation factor of Cd by *Mysis sp.* was 1215 times more than control concentration after 48hr exposure, and reaches 858 times in *T. zillii* after 356hr exposure. Other studies in USA showed the average residues of Cd in some invertebrate species to reach approximately 1000 to 9000 times greater than correspond control concentration after 28d exposure (Spehar et al. 1978).

The values worked in the present experiment as safe concentrations of Cu, Zn and Cd to reach LC_{50} concentration and total mortality dose to aquatic organisms, these are of great practical utility for regulating and controlling the pollution limits in the water resources by those pollutants and to regulate their discharge to near-by water for protect the life within the aquatic environment.

REFERENCES

Abdel-Moati A., Farag E.(1991) Toxically and bioaccumulation studies of Cu, Zn and Pb in the fresh water gastropods, *Lanistes bolteni Chemnitz*, 1786.(Gastropoda:Ampullaridae). J Egyptian Germany Soc Zool 4: 289-299.

Bailey N (198 1) Statistical methods in biology. 2nd Ed.. Edward Arnold. London p 216.

Behrens , Karber (1953) Arch. fur Exp. Path. Pharm, 177: 372. (cited by El-Awady, M., 1976: Biochemical studies on the effect of a new long acting local anesthetic carbocaine.M.Sc. Th., Fac. Sci., Ain Shams Univ.,Cairo, Egypt.

Bryan GW (1971) The effects of heavy metals (other than mercury) on marine and estuarine organisms. Royal Soc London B 177 : 389 - 410.

Daziel J., Baker C.(1983) Analytical methods for measuring metals by atomic absorption spectrometry. FAO Fish Tech 212: 14-21.

Dawson M, Gould E, Thurberg F, Calabrese A (1977) physiological response of Juvenile Stiped bajj, *Morone saxatilis* to low levels of cadmium and mercury. Chesapeake Sci 18: 353-359.

El-Gindy H, Rawi S, Abo-el-Hassan A, Abdel-Kader A (1991) Effect of fresh water pollutants on the control of *Biomphalaria alexandrina* and *Bulinus truncatus.* J. Egyptian Germany Soc Zool 6: 297-312.

El-Rayis O, Ezzat A (1984) Bioaccumulation of some heavy metals in coastal marine animals in vicinity of Alexandria. Part I. Bioassay Meeting on the Toxicol and Bioaccumulation of selected subst. in marine organisms, Rovinji 1: 5-9.

Larsson A , Bengtsson B, Svanberg O (1976) Some haematological and biochemical effects of cadmium in fish. In effects of pollutants on aquatic organisms, ed.by Lockwood, A: 35-45. Soc. Exper. Biol., Seminar Ser. no. 11, Cambridge Univ. Press.

Mance G, (1987) Pollution threat of heavy metals in aquatic environments. Elsevier Applied Science Publishers LTD., 372pp.

Martin M, Osborn K, Billig P and Glickstein N (198 1) Toxicities of ten metals *To Crossostrea* and *Mytilus edulis embryos* and *Cancer magister* larvae. Mar. Pollut. Bull. 12: 305-308.

Nimmo D, Lighter D, Bahner L (1977) Effects of cadmium on the shrimps, *Penaeus duorarum, Palaemonetes pugio and Palaemonetes rulgaris.* In physiological responses of marine biota to pollutants, ed. by Vernberg, F.; Calaberse, A.; Thurberg, F. & Vernberg, W., 131-183, New York, Academic Press.

Salanki J, V.- Balogh, K (1985) Uptake and release of mercury and cadmium In various organs of mussels *(Anodonta cygnea L.)* In: Heavy metals in water, organisms (Ed by Salanki, J) Akademia Kiado Budapest Symposia .Biologica, Hungarica, 29: 325-342.

Salanki J, V- Balogh K (1989) Physiological background for using freshwater mussels in monitoring copper and lead pollution. Hydrobiologia, 188/189: 445-454.

Spehar R,Anderson R, Fiandt J (1978) Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environ Pollut1 5: 195- 208.

Zyadah, M (1995) Environmental impact assessment of pollution in Lake Manzalah and its effect on fishes. Ph.D.Thesis, Faculty of Science, Mansura University, Egypt, p 127.

Zyadah M (1999) Accumulation of some heavy metals in *Tilapia zillii* organs from Lake Manzalah, Egyptian Turkish J. of Zool., 23: 365-372.