Acute Toxicity of Copper Cadmium and their Mixture to Tilapia (*Oreochromis mossambicus*)

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Summary: Acute toxicity of copper and cadmium including median lethal concentration (LC_{50}) of tilapia species (*Oreochromis mossambicus*) in static condition for a definite period of exposure was determined. The LC_{50} for 24 hours exposure to Cd and Cu and their mixture estimated graphically was found to be 1mg, 2mg and 3 mg/l, respectively. The LC_{50} of tilapia exposed for 72 hours exposure was found to be 2 mg/l where as for the same period to Cd it reached 4mg/l. Unlikely for Cu prolong exposure the LC_{50} values decreased proportionally with the exposure period. The uptake amount of metals was found to increase gradually with exposure concentration as well as period of exposure but accumulation did not follow the same pattern. The accumulated amounts were determined by Atomic Absorption Spectroscopy.

Introduction

Whether essential or not all trace metals are potentially toxic at a threshold of bioavailability. The concentration of trace metals in the marine environment has been widely reported [1]. No matter how low the concentration of trace metals in the ocean, marine organisms absorbs and accumulate them in soft tissues up to a magnitude above ambient environmental level. Results of a number of experiments in oceanic waters have shown increased level of heavy metals that gradually accumulates in marine organisms. Among metals Cu, Cd mixture have been reported by a number of workers [2].

During previous studies 100% mortality was observed in shrimp larvae at higher concentration. All ecological factors were playing definite role in uptake of Cu and Cd. [3]. Laboratory experiments on marine organisms exposed to heavy metals showed that sensitivity to heavy metals is a direct function of size. Among all creatures of oceanic world, smallest organism face the marine pollution hazard [4]. Marine organisms can tolerate comparatively higher concentration of copper than fresh water organisms [5].

Present studies were undertaken on brackish water fish Tilapia (*Oreochromis mossambicus*). This fish is a major source of protein in many developing countries. They possess an impressive range of attributes. They show excellent growth rate on low protein diets. They can tolerate wide range of environmental conditions, show little susceptibility to diseases and can be handled in captivity. They have a short generation time and an important food fish. Considering all these attributes this fish was selected for experimental studies.

Results and Discussion

Relationship between accumulated metal contents (Cu, Cd and Cu: Cd mixture) and dry weights of small fishes were determined under lower to higher exposed concentration of metals. Mortality was studied against exposure of different concentration of Cu and Cd and Cu: Cd mixture. Time duration for LC_{50} varied with each metal and concentration tested. The copper and cadmium of 50, 100 and 150 mg/l was not harmful for fishes, but 200 mg/l caused 20% mortality in case of Cu, 25% for Cd and 30% for Cu: Cd mixture (table-1). The collective mortality was measured by rate of mortality of each size of fish depending upon variation of concentration. The LC_{50} for Cu was 1000mg/l and for Cd 2000 mg/l for tilapia. The 50% mortality occurred at 2500mg/l in case of mixture (Cu:Cd) and the 100% mortality at 5000mg/l in all three cases. This may be due to different mode of action of both metals. (table-1)

All fishes showed an inverse relationship between survival rate and uptake amount of metals. Fishes were exposed to higher concentration (5000 mg/l) for Cu:Cd and their mixture, all the larvae died within 24 hours. At the exposed concentration of Cu 50 µg/l accumulated amount of Cu was estimated 30.01ug/g of body weight of fishes and Cd was found 0.015 µg/g which is just a negligible amount (table-2). However the same pattern was not observed for

Table-1: Mortality of Tilapia exposed to various concentration of Cu, Cd and Cu: Cd mixture. (an average of 24-96 hours)

S. No.	Exposed conc. (mg/l)	Cu* (mg/l)	Cd* (mg/l)	Cu:Cd mixture*
1.	50	5 ±0.5	7±0.6	4±21.2
2.	100	10 ±1.25	10±1.21	7±6.21
3.	150	15±10.06	20±12.65	18±12.22
4.	200	20 ±5.20	25±17.21	30±16.56
5.	250	25 ±7.20	28±16.16	32±21.29
6.	300	30 ±4.14	29±19.21	34±6.72
7.	350	35 ±12.15	32±12.41	35±17.5
8.	400	40 ±15.20	40±8.21	38±6.25
9.	500	45 ±16.41	46±9.21	40±25.21
10.	1000	50 ±19.21	48±6.75	42±12.95
11.	2000	75 ±10.25	50±2.15	45±9.01
12.	2500	80 ±9.16	85±6.25	50±10.21
13.	3000	95 ±15.25	96±9.85	95±16.21
14.	5000	100 ±2.45	100±8.20	100±9.21
15.	control	.3 ±2.01	0.2±1.21	0.5±6.21

* data is in mean percentage

higher concentration of copper. In control the concentration of Cu and Cd was observed 1.7 and 0.014 µg/g respectively. (table-2)

These results showed that accumulated amount of copper was not increased with exposed concentration (table-2), while Cd was accumulated comparatively at lower concentration and found at minimum lesser amount in all samples of exposed concentration of either Cu or Cd.

The estimated amount i.e. 71.85 µg/g of Cd and 3.45ug/g of Cu at exposed concentration of 5000mg/l was found unsatisfactory because according to literature [6] it must be higher than the value of lower exposed concentration that is 7.5 µg/g of Cd. (table-2)

In Cd exposure experiment cadmium was estimated as 0.02 µg/g,7.5µg/g and 71.85 µg/g at 3500, 2000 and 5000 µg/l exposed concentration of Cd respectively. As well as 3.45µg/g of Cu which was also estimated (table-2) which may be due to contamination otherwise the Cu concentration is depleted as compared to control. This may be due to different biological behaviour of both metals.

In case of mixture of Cu: Cd (1:1) exposed concentration of 100 and 200 mg/l the Cu was estimated as 12.39 and 29.35 µg/g respectively. (table -3) Cadmium was not found at exposed concentration of 200 mg/l. In mixture when exposed concentration was 100 mg/l of each metal the accumulated Cd was lower than Cu, while at the concentration of 200 mg/l the Cd was completely eliminated. This shows that Cu inhibits the absorption of Cd (table-3).

It was observed that mortality in small size fishes was higher than large size fishes. Metal toxicity for fishes was found to be different in shrimp [6] and Artemea species [7]. The pattern of accumulation of Cu and Cd was found similar to those for larvae of Dungenes crab cancer Magister [8]. It was also observed that in mixture, the exposure of metals and their penetration into specimen was not the same as in individual metal exposure. The study revealed that a period of 48-96 hours exposure was not enough to assess the acute toxicity of tilapia. It is

Table-2: Average dry weight of Tilapia species and the exposed concentration of metals & accumulated amount of metals determined by atomic absorption.

S.No	Metals Exposed	Exposed Concentration mg/l	Average Weight of fishes in (gm)	Accumulated Amount of Metals	
				Cu µg/g	Cd µg/g
1	Cu	50	1.376	30.01	0.015
2	*Cu*	100	1.39	8.12	0.01
3	Cu	150	1.172	10.05	0.002
4	Cu	2000	1.142	7.22	0.009
5	Control (Cu)	2500	1.026	1.700	0.014
6	Cu	3000	1.539	15.69	0.019
7	Cd	3500	0.452	1.69	0.02
8	Cu	4000	1.524	14.96	0.13
9	Control (Cd)	5000	0.985	1.70	1.21
10	Cu	1000	1.488	43.18	4.31
11	Cd	2000	0.876	1.23	7.5
12	Control	2500	0.949	1.01	1.025
13	Cu	3000	0.947	82.37	0.138
14	Cd	5000	0.87	3.45	71.85
15	Control	Control	1.203	0.99	0.001

Table-3: The relationship between exposed concentration of metals in a mixture Cu:Cd (1:1) and accumulated amount of metals (determined by AAS) in Tilapia fish.

Exposed conc. of Cu:Cd (mg/l)	Cu (µg/g)	Cd (µg/g)
200	12.39	4.65
200	29.35	Nil
250	69.85	25.65
500	70.25	60.16
1000	78.76	2.73
2000	90.20	65.01
5000	98.24	80.21

not the same as the findings of [7] who concluded that 96 hours exposure of metal is adequate to determine the acute toxicity for tilapia. [9].

Accumulated metals may be lost in solution or in particulate during the process of excretion via skin in granular form and with the cast molts of the crustaceans [7]. The regular mechanism usually occur via detoxification by marine organisms due to formation of metal rich granules and bindings by ligands, often protein with high metal affinity [4]. Heavy metal is known to be poisonous for small marine organisms.

Respective LC_{50} values caused by Cu and Cd and Cu: Cd mixture for tilapia can be used to assess the pollution status at Karachi coastal area. Metal binding protein immobilizes metals and effectively protects host organisms against toxicity but may increase metal accumulation in the host [8]. In our observation we did not find any case of high accumulation with lower mortality. It was confirmed that cadmium is more toxic than copper as reported by [6].

Experimental

Small fishes having size range of 10-15 mm with an average weight of 0.15-1.0 gm were obtained from a culture pond. Test specimens were isolated in large quantities in separate beakers. In each beaker 10 specimens were acclimatized for an hour prior to the experiment. All beakers were kept in open place at a temperature of 20-25°C for each metal treatment. Studies were made using the method described by [10]. When 50% fishes died all beakers were emptied and specimens were prepared for determination of uptake of metals by Atomic Absorption Spectroscopy.

Sample Analysis

All samples were prepared using the method of [9]. The metals were determined on Hitachi Model 2-800AAS equipped with Zeeman background corrector and a data processor. All parameters were set and followed strictly according to manufacturer's instructions using flame ionization. The samples were diluted such as to keep the concentration of metals within the linear range of absorbance.

The standard solution used was diluted from the stock solution (1000ppm). The results were analyzed graphically to calculate the median lethal concentration value at 95% confidence limit for each test using the statistical methods.

Conclusions

In conclusion, the uptake of copper increased with exposed concentration in the surrounding water medium as well as with the exposure of time. As far as cadmium was concerned it was observed that it only increased with the expose time period.

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