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Static Bioassay with *Liza parsia* Exposed to Copper Sulphate, Zinc Sulphate and Lead Nitrate

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Short-term static bioassays were conducted on the brackishwater mullet, *Liza parsia* to determine the combined effect of copper sulphate, zinc sulphate and lead nitrate in the ratio 1:1:1. From probit analyses the LC_{50} values for 12, 24, 72 and 96 hours were found to be 160.5, 152.2, 135.6, 117.1 and 106.7 ppm, respectively. The toxicities of these elements were found to be "additive" in nature.

Key words : Bioassay, heavy metals, toxicity.

In recent years, biological monitoring has become a useful tool for assessing water quality and the toxic effects of pollutants on aquatic biota (Buikema *et al.*, 1982). The criteria for the determination of toxicity in the above experiments is mortality at fixed intervals of time. The toxicological hazards measured by bioassay procedures are considered more realistic than those predicted from the results of chemical analyses and the available information on the toxicity of the compounds detected (Koeman *et al.*, 1978; Genjatulin, 1990). As per the recommendations of National Academy of Science and National Academy of Engineering (NSA/NAE, 1973) the "Application Factors (AF)" can be used along with 96 h LC_{50}s for calculating "safe limits" for environments. The AF could be empirically derived by determining the ratio of the maximum concentration of a material not having an effect on growth and reproduction to the LC_{50} (Mount & Stephan, 1967). The 48 or 96 h LC_{50} is usually used for calculation. According to Mohapatra (1994) for similar purposes an AF of 0.001 or 0.0001 may be used for copper, zinc and lead.

In the present investigation an attempt has been made to find out the median lethal concentration i.e., LC_{50}s of the toxic metals copper, zinc and lead in combination on a economically important and widely available fish *Liza parsia* (Hamilton-Buchanan), commonly known as the grey mullet.

Live *Liza parsia* (75 to 105 mm total length and 15.0 to 30.0 g weight) were collected from brackishwaters of Puduvypeen near Cochin. Fishes were acclimatized to laboratory conditions for one week by maintaining in brackishwater having salinity of 9.8±0.76‰, pH 7.2±0.12, temperature 28.0±1.5°C and total hardness 2956.0±142.2 ppm. To avoid fungal attack the medium was treated with 11 mg of malachite green per 100 litres of water (Mohapatra & Noble, 1991). The fish were fed once a day with pellet feed.

Copper sulphate ($CuSO_4.5H_2O$), zinc sulphate ($ZnSO_4.7H_2O$) and lead nitrate $Pb(NO_3)_2$ were used to prepare stock solutions in the ratio 1:1:1.

"Range-finding bioassay" was conducted prior to bioassay experiments exposing the test animals to concentrations in logarithmic scale such as 1.0, 10, 100 and 1000 ppm of copper sulphate, zinc sulphate

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and lead nitrate in the ratio 1:1:1. After 12 h of exposure 0, 10 and 100% mortalities were recorded in 10, 100 and 1000 ppm, respectively. 75, 100, 115, 135 and 210 ppm concentrations were selected for the bioassay based on APHA AWWA - WPCA (1976).

The range finding bioassay as well as the main bioassay experiments were conducted in 40 l fibre glass tanks holding 35 l of dilution water and toxicants. The water quality was similar to that maintained in acclimatization tanks. No feed was given to the animals during the bioassay experiments. All the tanks were aerated and no change of water was done during the test period (Reish & Oshida, 1987).

Each bioassay consisted a series of 5 test concentrations and a control, in duplicate. Twenty animals were exposed to each concentration and control. The cumulative percentage of death of every 12, 24, 48, 72 and 96 h was noted. The data were processed graphically as well as mathematically using probit analysis (Reish & Oshida, 1987; Mohapatra & Noble, 1991; Mohapatra & Renagarajan, MS) for determination of LC_{50} and 95% fiducial limits (upper and lower). The median lethal concentrations and the 95% fiducial limits for each concentrations were plotted against time on nomograph paper to obtain the toxicity curve.

The availability of Cu, Zn and Pb from $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$ and Pb $(NO_3)_2$ were calculated using the formula (Reish & Oshida, 1987) and joint toxicity was determined as suggested by Brown (1968) and Sprague (1970).

1 g of $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$ and $Pb(NO_3)_2$ contain 0.2545, 0.2274 and 0.6256 g of Cu, Zn and Pb, respectively. The ratio 1:1:1 of $CuSO_4.5H_2O$: $ZnSO_4.7H_2O$: $Pb(NO_3)_2$ became 23:20:57 in converting to elementary form i.e., Cu:Zn:Pb.

$$\text{Toxic units} = \frac{\text{Actual concentration in solution}}{\text{Lethal threshold concentration}}$$

As suggested by Ward & Parish (1982) the 96 h LC_{50} was substituted as equivalent to lethal threshold concentration for calculation.

Table 1. Toxicity of copper sulphate, zinc sulphate and lead nitrate combination in the ratio 1:1:1 to *Liza parsia*

Exposure period, h	LC_{50}, ppm	95% Fiducial limits Upper, ppm	Lower, ppm
12	160.5	180.6	142.6
24	152.2	188.4	122.9
48	135.6	160.0	114.9
72	117.1	128.8	106.5
96	106.7	118.6	95.9

The results of acute toxicity studies expressed in terms of LC_{50} values along with its fiducial limits obtained from probit analysis are given in Table 1. The 12, 24, 48, 72 and 96 h LC_{50} values calculated for elementary Cu, Zn and Pb at a ratio of 23:20:57 were 59.3, 56.1, 50.1, 43.2 and 39.5 ppm, respectively.

Table 2. Toxic units of compounds and their elements calculated from 96 h LC_{50}

	96 h LC_{50} ppm	Toxic Unit
Copper sulphate	85.6*	0.4155
Zinc sulphate	60.3*	0.5899
Lead nitrate	103.5*	0.3437
Copper	21.8**	0.4155
Zinc	13.7**	0.5899
Lead	64.7**	0.3437

* Mohapatra & Rengarajan, MS; ** Mohapatra, 1994

The median lethal concentrations (LC_{50}) decreased gradually with the increase in exposure time. The toxic units of the toxic metals calculated from the 96 h LC_{50} of 1:1:1 combination (106.7 ppm, individual contribution = 35.57 ppm) is given Table 2.

The sum of toxic units i.e., 0.4155+0.5898+0.3436 = 1.3489 was found to be greater than unity. The synergism of copper and zinc to aquatic organisms is given by Waldichuk (1974) Pant *et al.* (1980) and Hilmy *et al.* (1987). The toxicity of these compounds is said to be "additive" in nature (Laws, 1981).

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