

TOXICITY BIOASSAY OF UREA ON TWO FISHES

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ABSTRACT

Observations on the toxicity of urea to *Ambassis gymnocephalus* and *Puntius bimaculatus* were made by employing the bioassay technique. Dose mortality responses for 6 to 8 different concentrations of urea were recorded. The concentrations fatal to 50% of the test animals were determined for the two fresh water species. The 96 hr median lethal concentration (LC_{50}) of urea was found to be 18.5 g/l for *Ambassis* and 22.5 g/l for *Puntius*.

INTRODUCTION

Fertilizer production in India has recorded a considerable increase in recent years in view of the urgent need for a greater food production in the country. Like any other industry, the fertilizer industry also discharges liquid, gaseous and solid wastes whose disposal becomes a problem. Ammonia, urea, arsenic, phosphate, fluoride, oil and greases form some of the major constituents of the waste material produced. Very often, the discharge of effluents from the fertilizer factories into the sea are primarily responsible for fish mortality and also affect the nearby wells, which, probably form the source of public water supply. It has been reported that upto 2% urea and 0.5-1% ammonia, produced by the major fertilizer industries in India, are thrown away as wastes (Alagarwamy *et al.*, 1973).

Our knowledge of the toxicity of urea to fish is currently limited and hence static bioassays were conducted to determine the relative toxicity of urea on two species of fish, viz. *Ambassis gymnocephalus* and *Puntius bimaculatus*. Toxic effects were measured by determining the median lethal concentration (LC_{50}) for 24, 48 and 96 hrs.

MATERIALS AND METHODS

Experimental fishes were collected from the fresh water ponds at Velsao and Colva (Goa) and maintained for 14 days at room temperatures (27-30°C) in aerated water. Aliquots of urea were added to the bioassay containers to achieve the desired urea concentrations in 2 litres of fresh water before the test animals were introduced. All the bioassays were conducted at room temperature. The acclimatized animals were

TABLE I

Number, length and weight of the two species of fish used during experiments

Species	No. of specimens used per container	Average length (mm)	Average weight (mg)
<i>Ambassis gymnocephalus</i>	10	20.2*	115*
<i>Puntius bimaculatus</i>	8	31.6*	655*

* In both the species the specimens used were of similar size and weight.

not fed for 48 hr prior to the experiment. The numbers, lengths and weights of the fishes used in each container are shown in Table I. All the animals were approximately of the same size and weight. Total mortality in all the jars was recorded at 24 hr interval over a 96 hr period, with some observations on the behaviour of the fishes in between.

RESULTS

Concentrations of urea tolerated by 50% of the test specimens of each species were determined by plotting the cumulative percentage mortality after 24, 48 and 96 hr against the varying concentrations of urea. The estimated concentrations lethal to 50% of the fishes were then read off from the graph as shown in Figs. 1 and 2. The 96 hr LC₅₀ value has been taken as a convenient index of toxicity of the pollutant (Davis and Hoos, 1975).

The percentage mortality for varying concentrations of urea for the two species of fish has been given in Table II. The criteria for determining death were

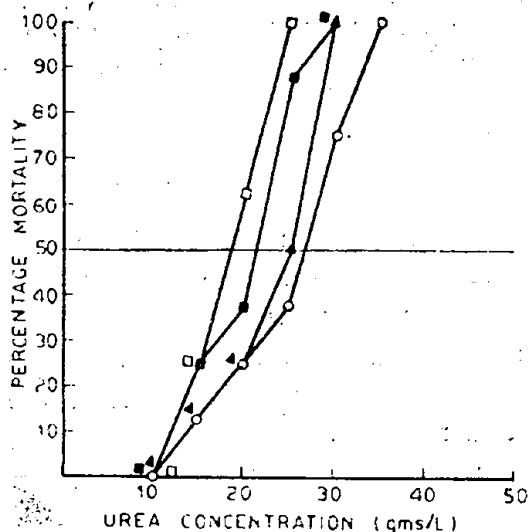


Fig. 1 *Ambassis gymnocephalus* : Cumulative percentage mortalities plotted against concentrations of urea ○, 24 hr; △, 48 hr; ◻, 72 hr; and ◊, 96 hr.

the lack of respiratory movements and no response to prodding for a period of 2-3 minutes.

Puntius was found to be more resistant than *Ambassis* with no death occurring after 96 hr in concentrations upto 15 g/l. *Ambassis* showed no mortality at 10 g/l concentration of urea. The

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TABLE 2

Dose mortality response of the two species of fish to urea

Species	Conc. of urea (g/l)	% Mortality after :			
		24 hr	48 hr	72 hr	96 hr
<i>Ambassis gymnocephalus</i>	nil (control)	0	0	0	0
	10	0	0	0	0
	15	12.5	12.5	25	25.5
	20	25	25	37.5	62.5
	25	37.5	50	75	100
	30	75	100	100	100
	35	100	100	100	100
<i>Puntius bimaculatus</i>	nil (control)	0	0	0	0
	10	0	0	0	0
	15	0	0	0	0
	20	0	0	0	12.5
	25	12.5	37.5	75.0	100
	30	25	100	100	100
	35	100	100	100	100

LC₅₀ values for 24, 48 72 and 96 hrs for the species are presented in Table III. The 96 hr LC₅₀ value for *Ambassis* was 18.5 g/l and for *Puntius* it was 22.5 g/l.

The specimens revealed no evidence of injury or parasitic disease, which could have been the cause of their death. In all the specimens, the gills looked normal, thus suggesting that the fishes have not died as a result of suffocation. The exact mechanism of the toxicity of urea leading to the mortality of fish has

not been established. In each species, the LC₅₀ values decreased with an increase in the time of exposure, or in other words, the resistance of the fish decreased with the experimental time. It is worth-while to mention here that LC₅₀ values cannot be considered safe for very long exposures, because continued action of less-toxic or non-toxic chemicals may eliminate fish species indirectly (Toor and Kaur, 1974). Similarly, if the same stock of fishes were immediately retested under identical

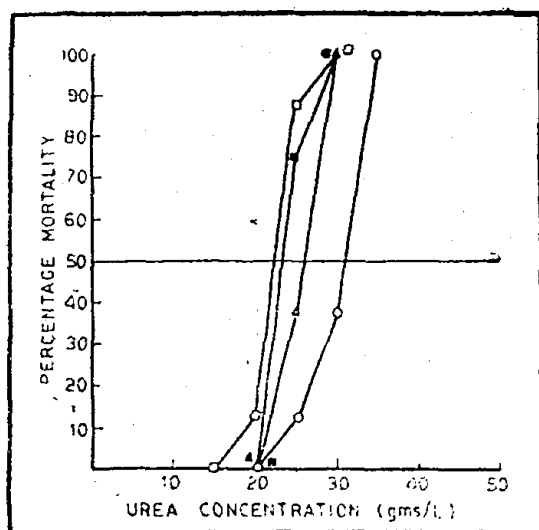


Fig. 2 *Puntius bimaculatus*: Cumulative percentage mortalities plotted against concentrations of urea O, 24 hr; Δ 48 hr; ■, 72 hr; and □, 96 hr.

conditions, or if the bioassays were conducted with similar animals at different times of the year, it could possibly give somewhat different LC₅₀ values (Anderson *et al.*, 1974).

DISCUSSION

Almost all the components present in the waste water of a fertilizer factory

can affect the environment in one way or the other. Acids and alkalis can destroy the normal aquatic organisms of a stream and inhibit or prevent its self-purification. They can also be lethal to fish and other aquatic organisms. Sudden changes in the concentrations of the components of the same waste can also affect the fish adversely (Chakrabarty and Arceivala, 1971). The waste material of fertilizer factories is usually alkaline which is primarily attributed to the presence of ammonia, urea and other alkalis.

Small concentrations of urea, which is one of the major components of the effluent from the fertilizer factories, may not directly kill the fish as is evident from the tolerance experiments conducted with urea in which sufficiently high concentrations were found to be tolerated by the fish. However, under anaerobic conditions, even small concentrations of urea may be extremely toxic because of its dissociation into free ammonia and carbon dioxide. Free ammonia is solely responsible for the damage. The Indian Standards Institution (ISI) has put the limit of ammonical

TABLE 3
LC₅₀ values of urea for the two species of fish

Species	LC ₅₀ values (g/l) after :			
	24 hr	48 hr	72 hr	96 hr
<i>Ambassis gymnocephalus</i>	27.0	25.0	21.5	18.5
<i>Puntius bimaculatus</i>	31.0	26.0	23.5	22.5

nitrogen for fish culture as 1.2 mg/l (Verma *et al.*, 1974). The waste waters discharged from these factories are not only toxic to animals but also to plants. Moreover, these wastes are not readily amenable to any of the conventional biological treatments which are commonly adopted for the treatment of industrial wastes.

It should also be emphasized that bioassay results can be affected by many factors including size and age, photo-period, fish handling, exposure to visual stress, temperature, acclimatization, water hardness, pH, aeration and several other factors. It is however, difficult to expect that any two stocks or bioassay tests will yield identical results owing to large variations in the biological and environmental conditions (Davis and

Mason, 1973). Although low concentrations of urea may not damage the standing stock of fish, it can seriously injure or destroy the photosynthetic organisms upon which the estuarine food chain depends (Ciaccio, 1971). McCarthy and Eppley (1972) noted that the algal uptake of urea was inhibited by ammonia concentrations greater than about $7\mu\text{g N litre}^{-1}$. Hence the treatment and disposal of these wastes should be considered thoroughly and carefully before they are discharged from the factory premises.

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REFERENCES

- Alagarswamy, S. R., B. B. Bhalerao, and S. Rajgopalan, 1973. Treatment of wastes from fertilizer plants. *Indian J. Environ. Hlth.*, **15** : 52-71.
- Anderson, J. W., J. M. Neff, B. A. Cox, H. E. Tatem and G. M. Hightower, 1974. Characteristics of dispersions and water soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar. Biol.*, **27** : 75-88.
- Chakrabarty, R. N. and S. J. Arceivala, 1971. Disposal of wastes from fertilizer factories. *Indian J. Environ. Hlth.*, **13** : 1-8.
- Ciaccio, L. L., 1971. *Water and water pollution handbook*, Vol. 1. Marcel Dekker, New York.
- Davis, J. C. and J. B. Mason, 1973. Bioassay procedures to evaluate acute toxicity of neutralized bleached kraft pulp mill effluent to Pacific salmon. *J. Fish. Res. Board Can.*, **30** : 1565-1574.
- Davis, J. C. and R. A. W. Hoos, 1975. Use of sodium pentachlorophenate and dehydroabiatic acid as reference toxicants for salmonoid bioassays. *J. Fish. Res. Board Can.*, **32** : 411-416.

- McCarthy, J. J., 1970. A urease method for urea in sea water. *Limnol. Oceanogr.*, 15 : 309-313.
- McCarthy, J. J. and R. W. Eppley, 1972. A comparison of chemical, isotopic, and enzymatic methods for measuring nitrogen assimilation of marine phytoplankton. *Limnol. Oceanogr.*, 17 : 371-382.
- Toor, H. S. and K. Kaur, 1974. Toxicity of pesticides to the fish *Cyprinus carpio* Communis. Linn. *Indian J. Exp. Biol.*, 12 : 334-336.
- Verma, S. R., R. C. Dalela, M. P. Jyagi and S. P. Gupta, 1974. Studies on the characteristics and disposal of the industrial effluents with reference to ISI standards. Part I. *Indian J. Environ. Hlth.*, 16 : 289-299.