CMFRI
bulletin 42
Part Two

DECEMBER 1988

NATIONAL SEMINAR ON SHELLFISH RESOURCES AND FARMING

TUTICORIN
19-21 January, 1987

Sessions II-VI

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
P. B. No. 2704, E. R. G. Road, Cochin-682 031, India

79. COMBINED TOXICITY STUDIES ON *PERNA* SPP.

N. R. Menon, K. N. Prabhudeva and K. V. Baby
School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin-682 016.

ABSTRACT

Knowledge of the interaction of various pollutants in the environment is necessary to set water quality standards. The water body receiving the effluents can have pollutants of varied nature resulting in a conglom eration of toxic components. Measurements of toxic effects of pollutants on sessile marine bivalves provide a valuable indication of environmental impact since the resident time of such animals in any locality follows a specific pattern In this connection a multiple factor approach to study toxic effects is warranted. With this view in mind combined toxicity studies employing heavy metals, crude and pesticides were undertaken with mussels as target organisms. In the present document one of such investigations is reported. The joint action of silver (unvarying) + copper (varying) and copper (unvarying) + silver (varying) mixtures were delineated. In the case of *Perna viridis* these combinations produced less than additive reaction whereas in *Perna indica* the reaction was more than additive, species specific dependence in toxicity. Silver was proved to be highly toxic when present alone in the test medium The results clearly show that the interaction between two or more pollutants should be considered while estimating environmentally 'safe' levels of pollutants.

INTRODUCTION

It is konwn that marine animals in the field are exposed to many different combinations of environmental conditions and toxicants. Most data on acute toxicity experiments deal only with the effects of individual variables. Since metals usually occur as mixtures rather than singly in estuarine and coastal regions, information on their interactions might help a more realistic assessment of their toxicity to estuarine organisms. Bliss (1939) suggested that toxicant mixtures may have the following pattern of actions on a biological system (a) independent joint action (where each toxicant acts on a different site), (b) similar joint action (where each toxicant acts on the same site), or (c) synergistic action. Our previous studies (Prabhudeva 1983; Prabhudeva and Menon 1986) have shown that silver and copper, when tested individually have deleterious effects on the life of *Perna viridis* and *P. indica*. Recent studies have shown that metals, depending on their effective concentration level maintained may interact synergistically or antagonistically with reference to a biological reaction (Mac Innes 1981; Negilski et al 1981; Mathew and Menon 1983; Prabhudeva and Menon 1986; Mohan et al 1986).

In the present study an attempt was made to study the toxic effects of copper (unvarying) + silver (varying), and silver, unvarying) + copper (varying) mixtures on survival of *Perna viridis* and *P. indica*.

MATERIAL AND METHODS

The green mussel, *P. viridis* from Someshwara beach (12°47'N; 74°51'E) and the brown mussel *P. indica* from Shakthikulangara beach (8°56'N; 76°35'E) were transported to the laboratory in polythene containers. The animals were maintained under laboratory conditions (water temperature 30 ± 1°C, salinity 32 ± 0.5 ppt, pH 8.2 - 8.4) for a period of 48 h before the commencement of the experiment. Mussels of 15 - 20 mm shell length were used for the experiments.

Copper sulphate ($CuSO_4.5H_2O$) and silver sulphate ($AgSO_4$) were the source of copper and silver respectively. The experimental vessels were cylindrical glass troughs of 5t capacity containing 4 t test media prepared in aged seawater, which was aerated to saturation before use. Mortality test was performed for 96 h. The inability to close the valves upon mechanical stimulation and/or valve gaping of 5 mm were the criteria used to define the death of the test organisms. The media were replenished with fresh ones at every 24 h and the dead animals were removed from the experimental media at 12 h intervals. Ten mussels were exposed to each metal mixtures, along with a control group.

All concentrations reported in this paper are the calculated levels of metal ions added at the start of the test and do not include background levels. Actual concentrations of each metal in the test cultures were not determined. The test containers were not aerated but dissolved oxygen level in the water was monitored. The lines of best fit were drawn after linear regression analysis. Additive toxicity index developed by Marking and Dawson (1975) was used to determine the toxicity of metal mixtures.

RESULTS

Perna viridis

The progressive rate of mortality of *P. viridis* exposed to a mixture of copper and silver (ie. 50 ppb Cu + 30 to 65 ppb Ag) is shown in Fig 1. The 96 h LC 50 value calculated was 51.7 ppb silver with 50 ppb copper (95% confidence limits : 50.8 to 52.5 ppb Ag + 50 ppb Cu). The additive index computed for joint action of copper + silver mixtures on mortality of *P. virids*, was -0.124.

The progressive rate of mortality of *P. viridis* exposed to a mixture of silver and copper (ie. 40 ppb Ag + 40 to 80 ppb Cu) is shown in Fig 2. The 96 h LC 50 estimated was 64.0 ppb (95% confidence limits: 63 to 65 ppb) of copper with 40.0 ppb silver. The additive index of silver + copper mixtures was -0.111.

Perna indica

Fig 3 shows the progress of mortality of *P. indica* exposed to a mixture of copper and silver (9.0 ppb Cu + 3 to 12 ppb Ag). The 96 h LC50 worked out was 6.35 pph (95% confidence limits : 9.81 to 5.36 ppb) of silver + 9 ppb copper. The additive index was +1.02.

Fig 4 indicates the progress of mortality of *P. indica* exposed to a mixture of silver and copper (9 ppb Ag + 3 to 12 ppb Cu). The 96 h LC 50 was 7.3 ppb (95% confidence limits: 12.28 to 4.33 ppb); of copper with 9 ppb silver. The additive index was +0.76.

DISCUSSION

Assessment of the toxicity of chemical combinations is done by adding the concerned chemicals in a definite ratio decided by the chemicals individual toxicity. In practice, one of the chemicals will be retained at a fixed

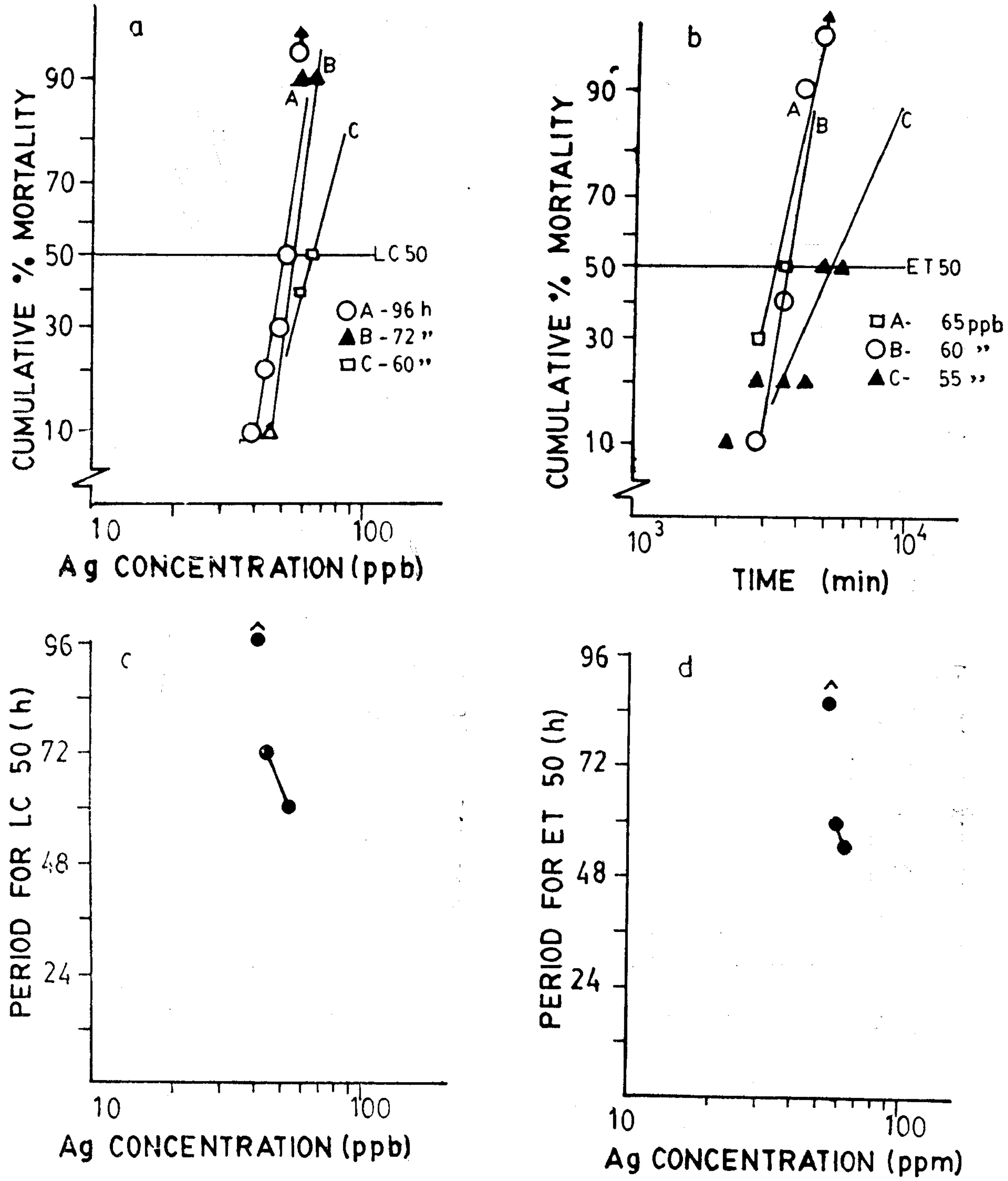

Fig. 1. Lethal effects of 50 ppb copper along with varying concentrations of silver on *Perna viridis* a. Progress of mortality against concentration. b. Progress of mortality against time. c & d. Toxicity curves.

concentration and the other treated as a variable. Although, such a procedure is possible under controlled laboratory conditions, it is quite unlikely that such a situation exists in nature. Similarly, in the present work four sets of experiments were performed where copper and/or silver was in combination, either as a constant or as a variable.

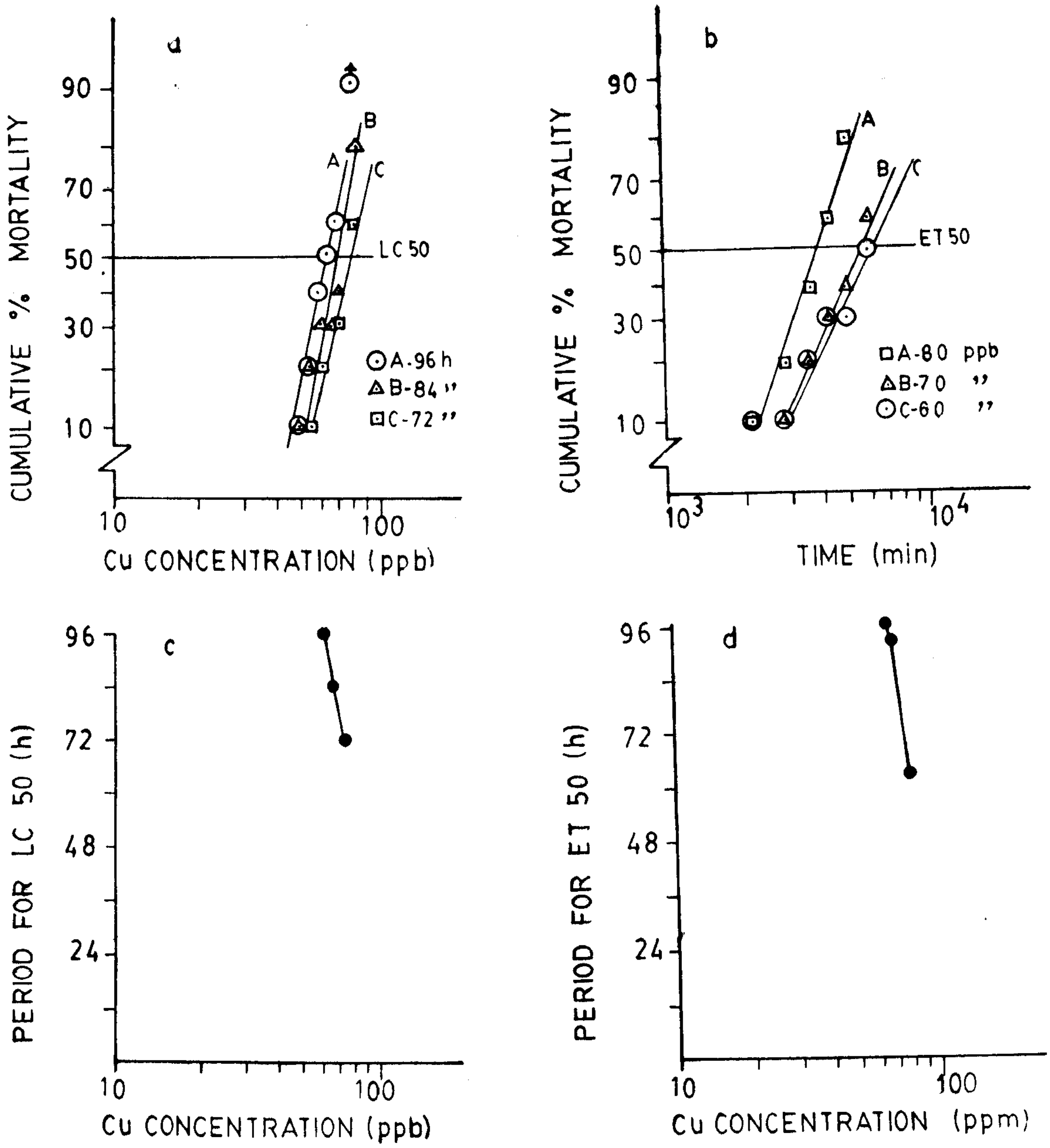

Fig. 2. Lethal effects of 40 ppb silver along with varying concentrations of copper on *Perna viridis* a. Progress of mortality against concentration b. Progress of mortality against time, c & d. Toxicity curves.

Working on combined toxicity, Mathew and Menon (1983) found that a mixture of 31.0 ppb copper and 31.0 ppb silver caused 50% mortality of *P. viridis* in 96 h. They further found that silver in combination with copper becomes more toxic. Similarly, Mac Innes and Calabrese (1978) reported less than additive interaction of mercury + silver and zinc + copper on the embroys of *Crassostrea virginica*. Discussing on the combined toxicity of copper and zinc on *P. viridis,* Prabhudeva and Menon (1986) reported less than additivity at lower concentrations and more than additivity at higher concentrations of these two metal mixtures. They also found that zinc in combination with copper becomes more toxic. Pavicic (1977) concluded that a combination of cadmium and zinc increased the toxic resistance of the embroys of *Mytilus galloprovincialis*.

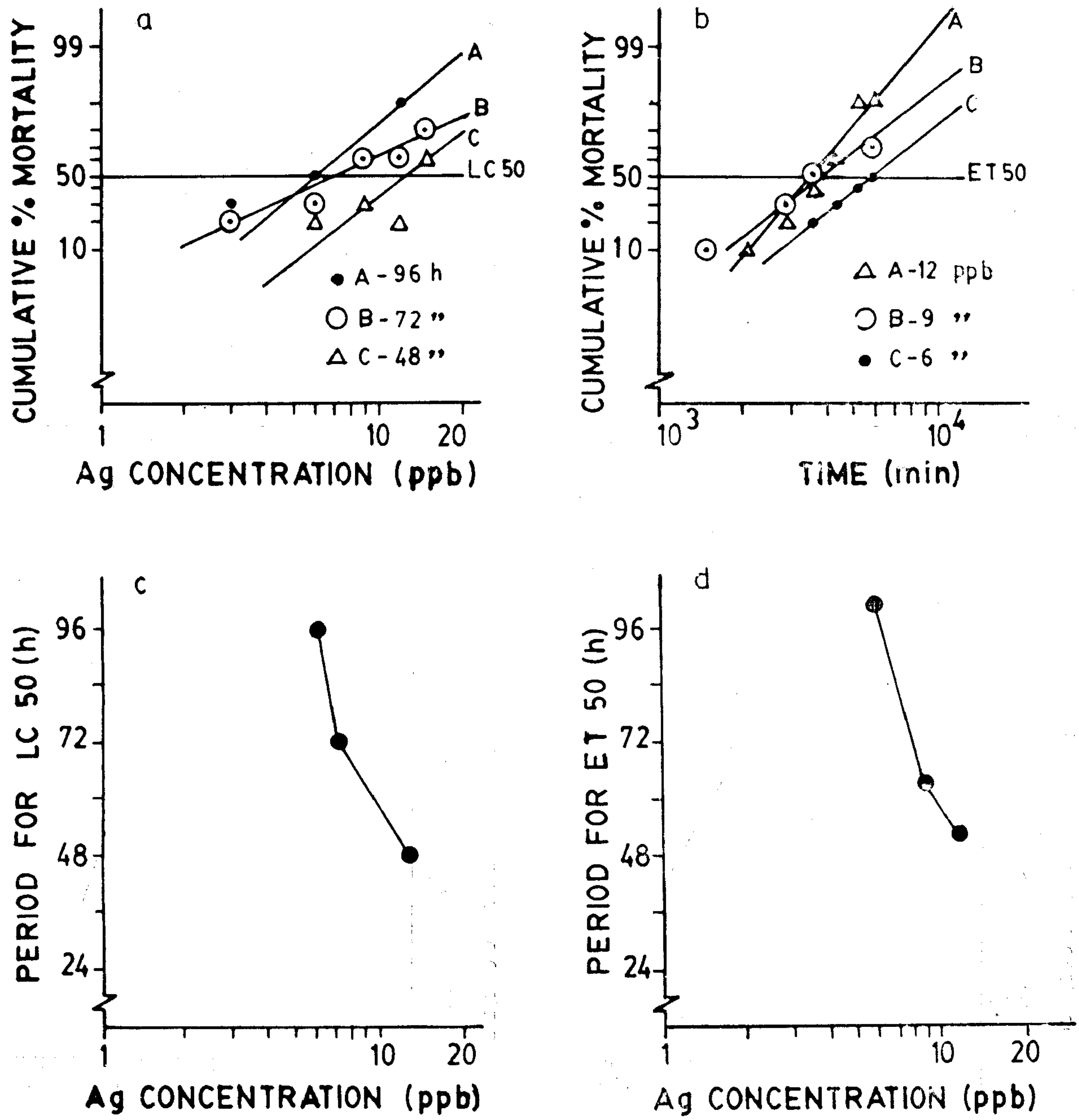

Fig. 3. Lethal effects of 9.0 ppb copper along with varying concentrations of silver on *Perna indica* a. Progress of mortality against concentration, b. Progaess of mortality against time. c & d. Toxicity curves.

In the present study the 96 h LC 50 with reference to copper + silver and silver + copper on *P. viridis* was 51.7 ppb Ag + 50.0 ppb Cu and 64.0 ppb Cu + 40 0 ppb Ag respectively. The additive indices of -0.124 and –0.111 respectively indicate less than additivity in both the cases. However, the individual 96 h LC50 for *P. viridis* was 79.8 ppb silver and 105.0 ppb copper. In the case of *P. indica* the 96 h LC50 was 6.35 ppb Ag + 9 ppb Cu + 9 ppb Ag respectively for copper + silver and silver + copper mixtures. The additive indices + 1.02 and + 0.76 indicate that these two mixtures interact more than additivity in causing lethal toxicity to *P. indica*. She 96 h LC50 was 24.0 ppb in the case of silver and 38.0 ppb in the case of copper. Here also the individual toxicity of the two metals was relatively high. Mac

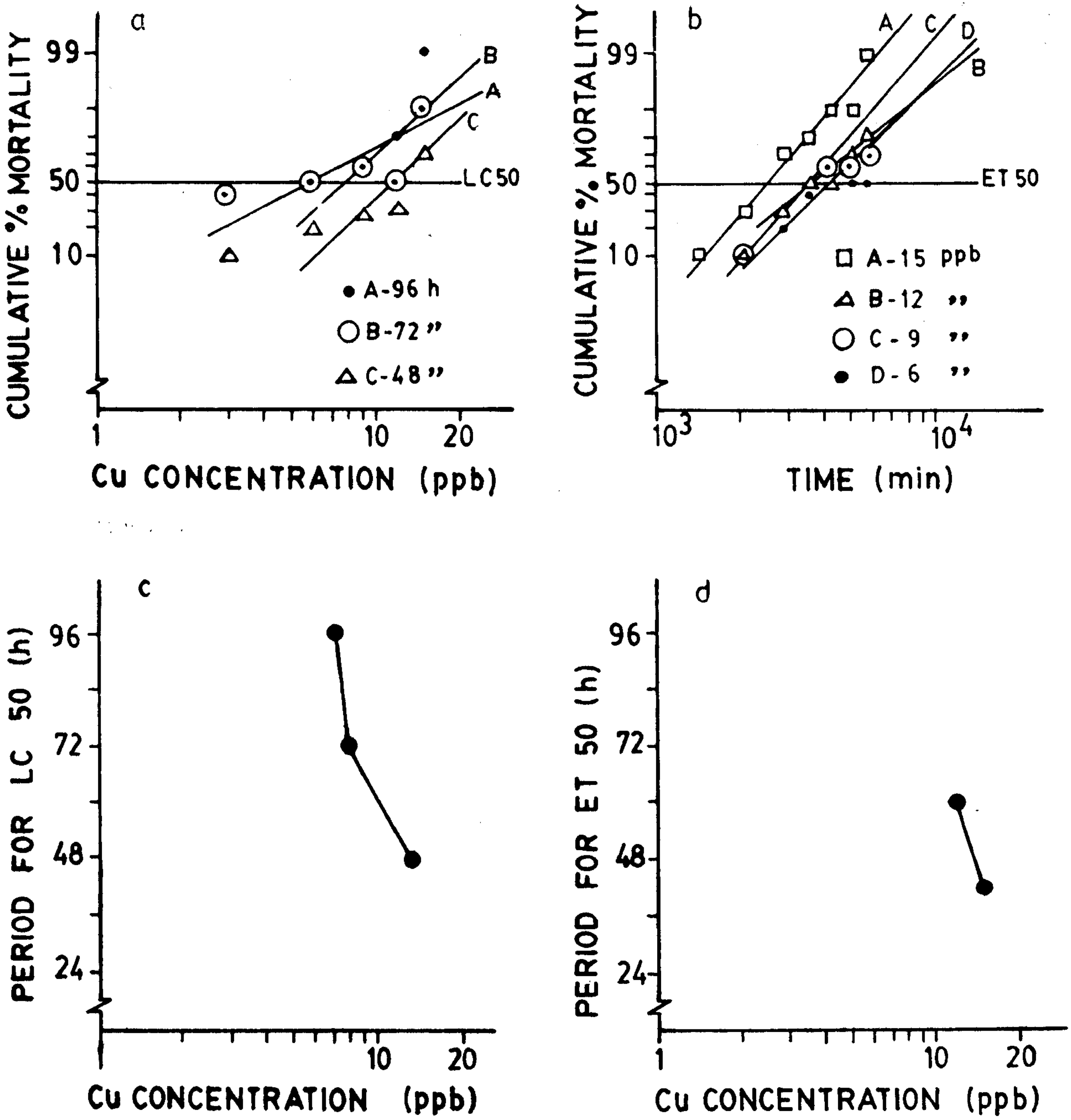

Fig. 4. Lethal effects of 9.0 ppb silver along with varying concentrations of copper on *Perna indica* a. Progress of mortality against concentration. b. Progress of mortality against time. c & d Toxicity curves.

Innes (1981) noticed either antagonistic effect or simple additivity in lowest metal concentration of copper + mercury, copper + zinc and synergistic activity at high concentrations. Contrary to this, Prabhudeva and Menon (1986) reported more than additivity at lower concentrations and less than additivity at higher concentrations for copper and silver mixtures on *P. viridis*. Similarly, the present results showed more than additivity at lower concentrations on *P. indica* and less than additivity at higher concentrations on *P. viridis* Mohan and Menon (1986) found a more than additive interaction between mercury and cadmium on *P. viridis*. The alterations in toxicity recorded here could be due to the capacity of *P. viridis* and *P. indica* to selectively block the binding site of such metals to which they have high resistance.

The ecological implications of this study becomes significant only if the metal concentrations existing in the estuaries and coastal waters approach such high levels as used in the laboratory. However, with the ever-growing evidence of increasing pollution in certain estuaries and coastal waters the probability of attaining toxic concentration of these metals in certain areas increases with time. Evidences gathered on the sub-lethal effects of these two commercially important bivalves show that even at considerably low concentrations of these metals, the basic physiological activities are negatively affected. Therefore continued existence in the nature where the present metal levels are low, could lead to serious impairment of the 'scope for growth' of these bivalves although direct deleterious effect as indicated by mortality is not evident at majority of such localities. A realistic assessment of the effects of pollutants in marine animals, therefore, should be based on the study of the collective effects (antagonism, addition or synergism), as well as the mode of action of mixtures of pollutants, on these animals in future studies. Especially, the possibility of interaction between two or more compounds should always be considered or it could lead to the over-estimation of 'safe levels' for the environment.

REFERENCES

BLISS. 1939. The toxicity of poisons applied jointly. *Ann. Appl. Biol.* 26 : 585-615.

MAC INNES, J.R. 1981. Response of embryos of the American Oyster, *Crassostrea viriginica* to heavy metal mixtures. *Mar. Environ. Res.*, 4 : 217-227.

MAC INNES, J. R. AND CALABRESE A, 1978. Physiology and Behaviour of Marine Organisms. McLusky, D. S. and Berry, A. J. (ed). Pergamon Press, Oxford.

MATHEW, R. AND MENON, N. R. 1983. Effects of heavy metals on Byssogenesis in *Perna viridis* (L). *Indian J. Mar. Sci,* 12 : 125-127.

MARKETING, L. L. AND DAWSON. V. K. 1975. Method for assessment of toxicity or efficiency of mixtures of chemicals. *Investigations in fish control*, US Department of the Interior, Washington. 67 : 7.

MOHAN, C.V., GUPTA, T.R.C., H.P.C. SHETTY, AND MENON, N. R. 1986. Combined toxicity of mercury and cadmium on tropical green mussel *Perna viridis* (L). *Diseases of Aqua. Orga.*, 8 : 819-827.

NEGILSKI, D S, M. ASHANULLAH AND M. C. MOBLEY. 1981. Toxicity of Zinc, Cadmium, and Copper to the shrimp *Callianassa australiencis* II. Effects of paired and triad combinations of metals *Mar. Biol.* 64 : 305-309.

PAVICIC, J. 1977. In: Protection of the Meditarianean coast. Part I-III Study session on marine pollution. Publ by CIESM, 79-86.

PRABHUDEVA, K. N. 1983. Toxicology of *Perna viridis* exposed to metal-oil combinations, M. F. Sc. Thesis, University of Agricultural Sciences, Bangalore, 153 pp.

PRABHUDEVA, K N. AND N. R. MENON. 1986. Toxicity of metal mixtures on *Perna viridis* (L). (Mollusca, Pelecypoda). In : *The First Asian Fisheries Forum.* Asian Fisheries Society, Manila, Phillippies, 319-322 (Eds: J.L. Maclean L B. Dizon and L. V. Hosillos)