

sinuses where they are useful in fighting the microbial infection entering through the skin.

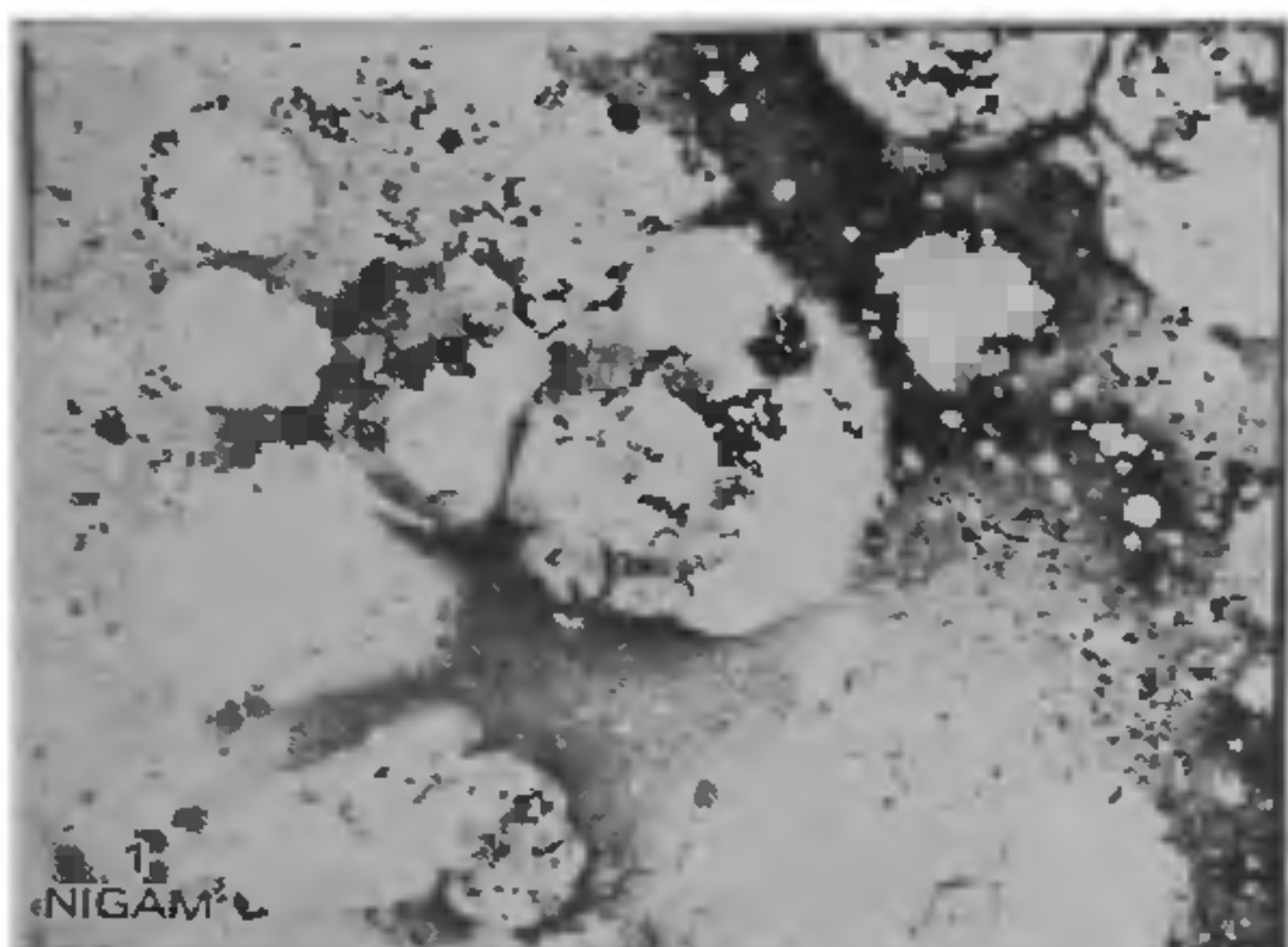


PLATE I. Photomicrograph of the leucocytopoietic tissue of the hibernation period, $\times 300$.

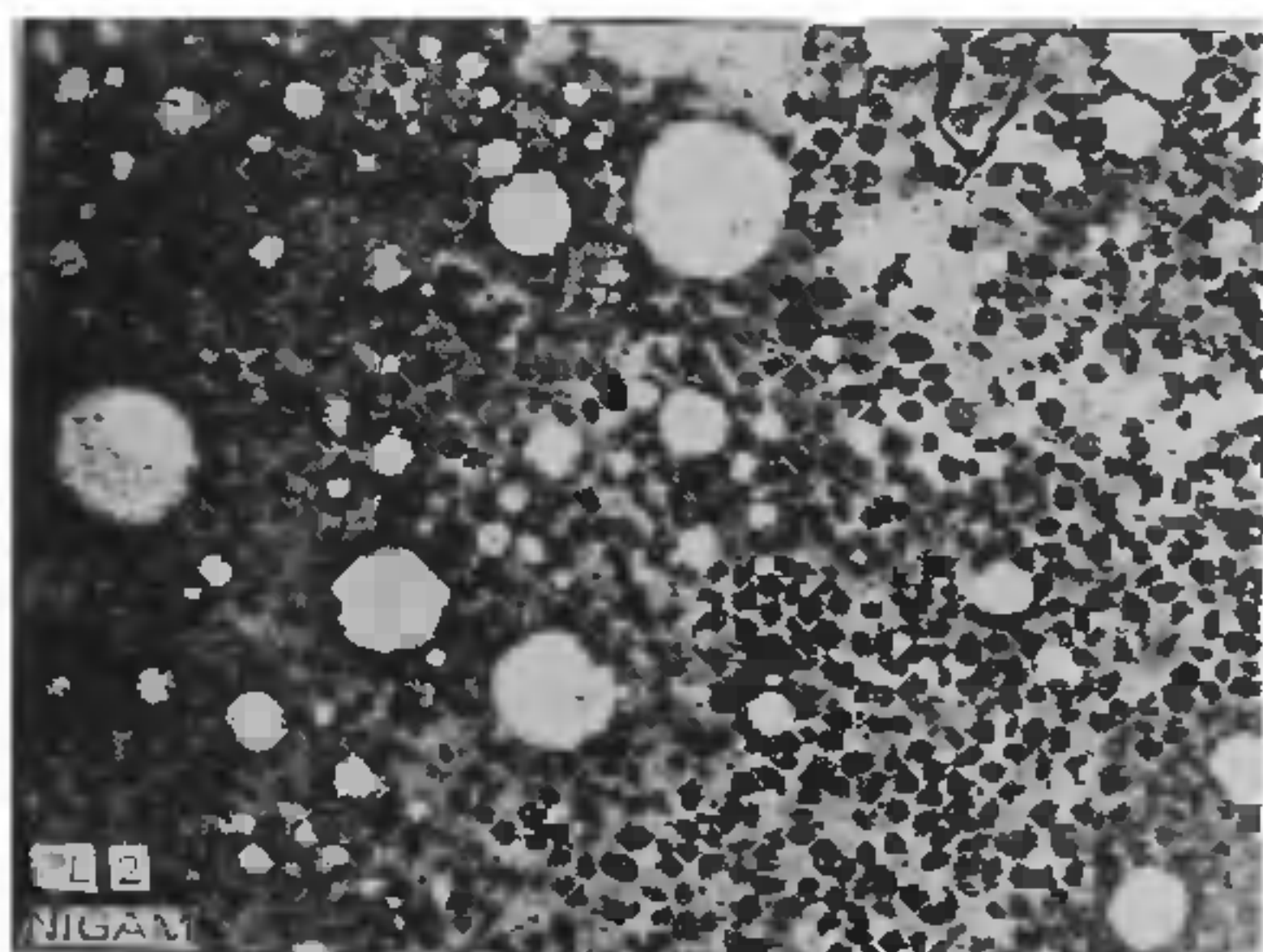


PLATE II. Photomicrograph of the leucocytopoietic tissue of the posthibernation period, $\times 300$.

Absence of the leucocytopoietic centres in the pelvic region of *Rana cyanophlyctis*, contrary to

Rana tigrina, may be accounted for, by the small size and slender body of *Rana cyanophlyctis*, which is roughly 1/3rd the dimensions of the bullfrog *Rana tigrina*. It is, therefore, concluded that the leucocytopoietic centres of *Rana cyanophlyctis* are also defence stations from which hosts of phagocytic cells are mobilized for attack against bacterial and protozoan invaders with the food and air reaching the mouth and lungs and also those coming with water diffusing through the skin.

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ON THE UPTAKE OF COPPER(II) BY *MERETRIX CASTA* (CHEMINITZ), AN INDICATOR SPECIES OF METAL POLLUTION

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ABSTRACT

The bivalve, *Meretrix casta* (Chemnitz), when maintained in sea water containing various concentrations of copper, was found to take up large amounts of the metal; a linear relation between rate of uptake and copper (II) concentration was observed. Gill tissues form the major site of accumulation. Mortality was comparatively low, even in 10 ppm solutions.

INTRODUCTION

IN recent years, many papers on the toxicity of heavy metals on marine and estuarine organisms have been published¹⁻¹⁰. It is generally observed that the metals at lethal and sub-lethal levels are accumulated to very high levels, often depending

on the animals and environmental parameters^{2,5,6,9,10}. However, the mechanism of such accumulation is not well understood.

Molluscs form a particularly important group of animals noted for their ability to take up large amounts of metal ions from solutions. The

trace metal accumulation by estuarine molluscs has been studied by various workers¹¹⁻¹⁵. Bivalves have also been considered as possible indicators of marine pollution by non-radioactive trace metals^{9, 12, 14}.

The enrichment of trace metals within certain animals and their transfer through food chain to higher animals constitute a danger to man. High amounts of zinc, cadmium and copper would cause symptoms of nausea and vomiting; the toxic effects of mercury are well known¹⁵.

The present work is on the toxicity and uptake of copper(II) on the back-water clam, *Meretrix casta*, which is abundantly found in the Cochin estuary. Estuary is an important part of marine environment and owing to increased industrial activities and failure to take sufficient safety measures, it is becoming polluted by various chemicals. This study was undertaken to know the toxic effect of copper salt on the animal and to find out whether it could be used for monitoring the heavy metal pollution. The uptake of Cu by the clam, *Meretrix casta*, exposed to different concentrations of Cu was investigated.

MATERIALS AND METHODS

The clams were collected from a place about two kilometers south-east of the Cochin bar-mouth. Animals of length 30.0 ± 0.5 mm were taken. They were acclimatised to the laboratory conditions by maintaining in millipore filtered sea water in large polythene vessels; the salinity was ca. 10‰ which corresponds to the natural habitat salinity of the experimental clams. The water was aerated daily. Two series of tests were conducted.

In case 1, thirty animals each were kept in five litres of sea water containing 0.5, 1.0, 5.0 and 10.0 ppm Cu(II) as copper sulphate. Temperature was $27.0 \pm 2^\circ$ C and oxygen content was $95 \pm 10\%$ saturation. The medium was changed once every two days. The pH was maintained at 6.5 ± 0.1 . The mortality was noted every twenty-four hours. After a period of 20 days, six animals from each vessel were analysed for the total copper content of the whole soft tissues (*vide infra*).

In the second series of experiments the animals were treated similarly. At definite intervals (as shown in Figs. 3-5) samples of five animals each were dissected into different component organs, viz., adductor muscle, gills, mantle and foot.

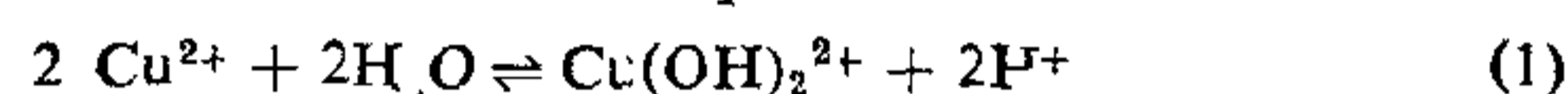
The various tissue samples were dried to constant weight at 80° C under vacuum. The dry tissue was homogenised into a fine powder, and ashed at $450-500^\circ$ C. The ash was extracted with

1:1 HCl and the copper content of the sample was determined spectrophotometrically by the IUPAC carbamate method¹⁷.

In all experiments, the animals were fed on the blue-green algae, *Synnechocystis* grown in a metal free medium; 5 ml of the suspension containing ca. 30,000 cells/ml were added to each tank, once every 24 hours. Blanks were run concurrently. Analysis on the composition of the medium used was also carried out. All chemicals used were of analytical reagent grade.

RESULTS AND DISCUSSION

Results are presented in Table I and Figs 1 to 5. Table I gives the data on the water quality. The important reactions which reduce the free metal-ion concentration in aquatic medium are^{18,19}:



and



TABLE I
Water characteristics
S‰ = 10.5

Na	—	3800 ppm
K	—	150 ppm
Ca	—	560 ppm
Mg	—	242 ppm
Fe	—	1.1 $\mu\text{g at. l}^{-1}$
Cu	—	Nil
Cl ⁻	—	5820 ppm
SO ₄ ²⁻	—	820 ppm
NO ₂ —N	—	3.29 $\mu\text{g at. l}^{-1}$
NO ₃ —N	—	4.16 $\mu\text{g at. l}^{-1}$
PO ₄ —P	—	Traces
SiO ₃ —Si	—	61.5 $\mu\text{g at. l}^{-1}$
pH	—	6.5 ± 0.1
Temperature	—	$27 \pm 2^\circ$ C

Adsorption of cupric ions also decreases the amount of copper in solution. All these processes are, however, significant only at higher pH values.

Figure 1 gives % mortality *versus* time (in number of days) of exposure to Cu(II) salt solutions. The mortality observed in the initial stage stopped after a couple of days, the animals developing some resistance. The animals were found to survive even upto 2 months in an unbuffered medium of 10 ppm Cu(II). Earlier experiments on the effect of copper (II) on the black clam, *Villorita cyprinoides* var. *cochinensis* have shown that a concentration above 0.75 ppm is toxic and an LC₅₀ value for a 10-day period was found to be 2 ppm¹⁵.

The determination of copper content of the whole soft tissues of the clam reveals that rate of uptake is linearly dependent on the Cu(II) concentration of the medium. The least square plot of the rate of uptake (along with the actual

values obtained) against concentration of Cu(II) is given in Fig. 2.

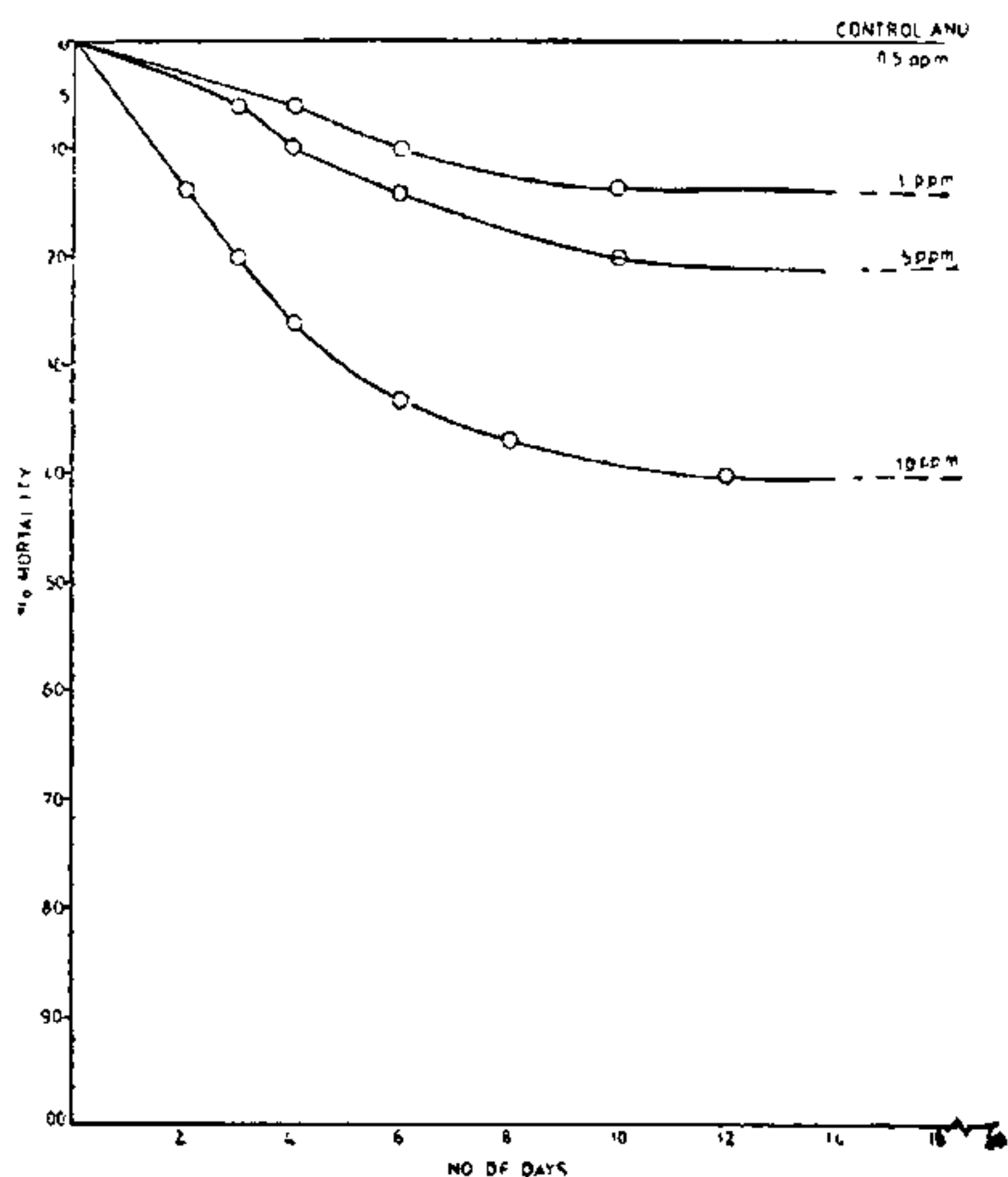


FIG. 1. Mortality (%) of *M. casta* exposed to various concentrations of Cu(II) solutions.

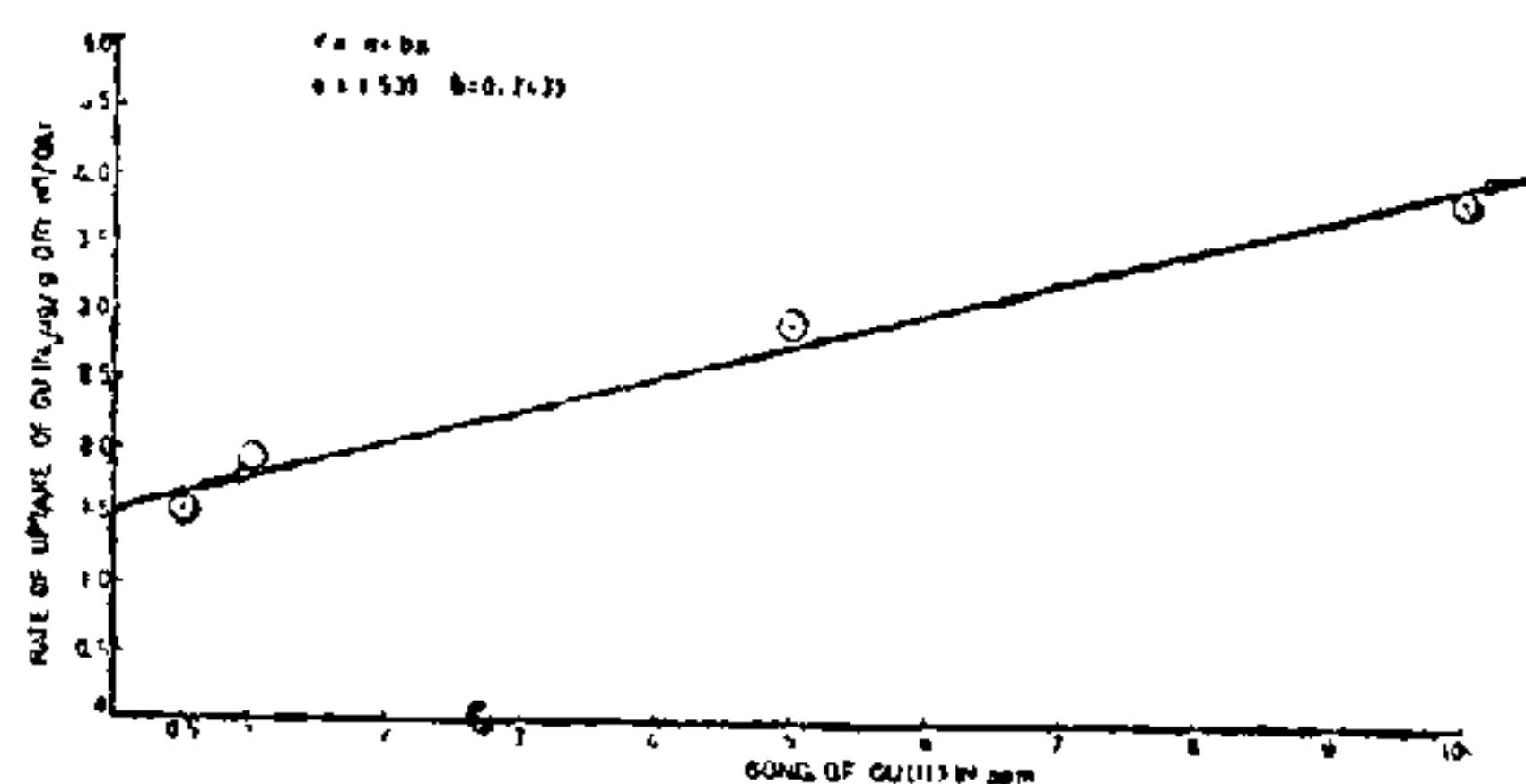
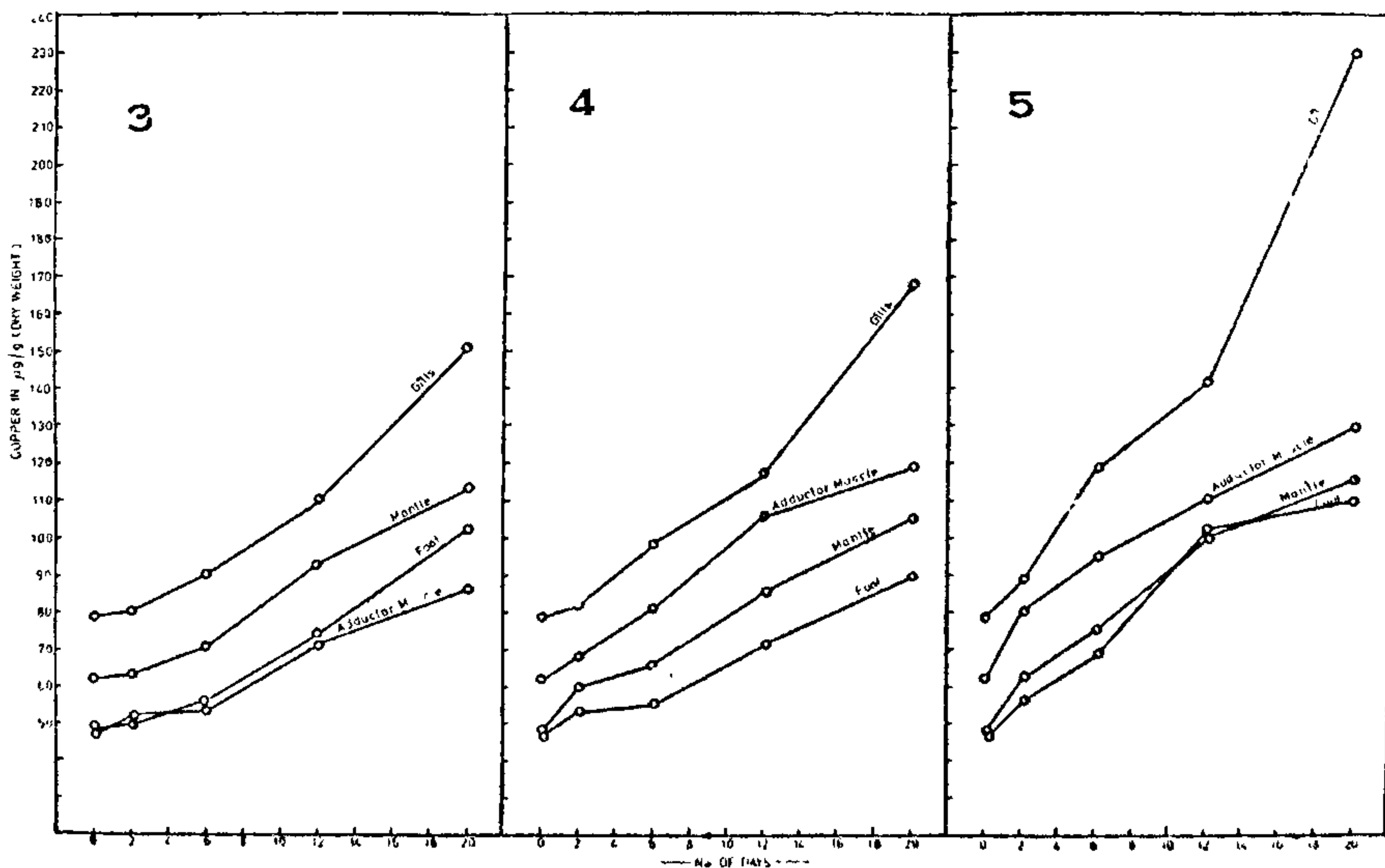


FIG. 2. Least square plot of copper uptake from aqueous solutions by *M. casta* as a function of Cu(II) concentration.

The results of analysis for the copper content of various organs (second experimental series) are presented in Figs. 3-5. In all components studied the copper content increased, but rates of uptake were different. Gills displayed by far the largest rate of uptake, the other organs showed a more or less equal rate of uptake. It may be mentioned here that a similar observation was made by Brookes and Rumsby¹¹ in the case of bivalves from New Zealand; Vernberg and Vernberg²⁰ have also observed a greater accumulation of metal in the gills of the Fiddler crab, *Uca pugilator*, exposed to metal salt solutions.



FIGS. 3 to 5. Copper in tissues of *M. casta* after exposure to various copper (II) concentrations in 10‰ sea water at $27 \pm 2^\circ \text{C}$ for varying lengths of time: Fig. 3—1 ppm; Fig. 4—5 ppm and Fig. 5—10 ppm.

It was observed on dissecting that the gills of all the clams maintained in 5 ppm and 10 ppm solutions had some deterioration; a change of colour from grey to yellow was observed. This may be due to the complexation of the copper in the gill. One of the suggested pathways¹³ of heavy metal accumulation is by coordination through organic molecules, presumably, using the -NH and or -SH groupings of the protein molecules. However, nothing much is known at present about the nature or composition of such complexes. The comparatively high amount of copper present in the gills may be because of the filter feeding nature of the animals. Other-workers have also made similar observations.

The present study indicates that *Meretrix casta* could be successfully used for monitoring the heavy metal pollution of the aquatic environment.

ACKNOWLEDGEMENT

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USE REMOTE SENSING OF ENVIRONMENT*

THE cataloguing of the natural resources of a country is essential for its economic growth. To do this by conventional means is an arduous and time-consuming task. Geologists have to travel through the length and breadth of the land exploring for minerals and ores; surveyors have to go to the most inhospitable places with their theodolites preparing maps; forest rangers have to wade through dense vegetation assessing the forest wealth; and agronomists have literally to move from field to field studying the conditions of the soil and crops and estimating the yield. All this will change even in developing countries when the science of remote sensing gets perfected and establishes itself as a new mode for natural resource survey.

* *Remote Sensing of Environment*. Edited by Joseph Lintz (Jr.) and David S. Simonett. (Addison Wesley Publishing Co., USA), 1976. Pp. xix + 694. Price \$27.50.

Remote sensing is based on the simple concept that each feature of a terrain emits or reflects electromagnetic energy at specific and distinguishable wavelengths. The spectrum may vary from the shortest wavelength corresponding to gamma rays, going up the scale through the ultraviolet, the visible, the near infrared, the thermal infrared reaching microwave regions. Remote sensing can also be done by the precise measurement of gravitational and magnetic fields.

The cameras or devices that sense the electromagnetic spectrum can be mounted on different 'platforms'. These could be kites, balloons, aeroplanes and satellites; they are stabilised against roll, pitch, yaw and insulated against vibrations.

The optical camera was invented more than a hundred years back. However if it is to be used for aerial photography the aberration of the lens must be a minimum and these photographs can be inter-