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ACUTE TOXICITY OF VARIOUS METALS TO *MOINA MACROCOPA*

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ABSTRACT

The toxicities of various metals to *Moina macrocopa* were investigated by the static bioassay method. The 24-h and 48-h LC_{50} for the metals were as follows: nickel nitrate 2.201 and 0.461 mg/l, lead nitrate 1.120 and 0.755 mg/l, zinc sulphate 0.246 and 0.094 mg/l, cadmium nitrate 0.218 and 0.013 mg/l, cupric nitrate 0.019 and 0.017 mg/l, and mercuric nitrate 0.003 and 0.001 mg/l, respectively. The yeast-algal method was successfully used in culturing *Moina*. The data may be useful for the planning of water quality criteria and bioassay toxicity tests of industrial wastes in the regions where *M. macrocopa* is found abundantly.

INTRODUCTION

Freshwater Cladocera, especially the genus *Daphnia,* are frequently recommended for aquatic toxicity bioassays (BEISINGER & CHRISTENSEN, 1972; BUIKEMA et al., 1976; BAUDOIN & SCOPPA 1974). BUIKEMA et al. (1980) reviewed literature on its biology and discussed the use of *Daphnia* in toxicity tests and how to culture *Daphnia* in the laboratory.

The advantages of using daphnids as test organisms were noted by ANDERSON (1944). ANDERSON (1950) compared 64-h apparent-threshold metals' concentrations between daphnids and fish, and concluded that *Daphnia* and related forms were more susceptible to cations than fish. DANIELL & DAVIS (1951) suggested that metal ions exerted their toxic influence by covalent binding at cell surfaces, and that the difference in electronegativity of the various ions is a toxicity-determining factor. In addition, daphnids are cultured more easily and have a shorter generation time than fish.

Four basic types of tests with *Daphnia* have been conducted. These are: (1) acute lethality tests conducted over a defined period of time, usually 24-48 h; (2) 21-day chronic tests; (3) lifetime chronic tests; and (4) multigeneration chronic tests to determine possible effects of toxicants on the physiology of *Daphnia* (BUIKEMA et al., 1980). *Daphnia* species have been used to measure acute toxicity of insecticides and refinery wastes (DORRIS et al., 1974), aquatic herbicides (CROSBY & TUCKER, 1966), and metals (BEISINGER & CHRISTENSEN, 1972). Generally, the results obtained by using *Daphnia* species as test organisms have been less variable than those using fish

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(CROSBY & TUCKER, 1966; MACEK & SANDERS, 1970; GILDERHUS, 1967). Although *D. magna* and *D. pulex* have been widely used in bioassay toxicity of heavy metals, *Moina macrocopa*, a species also belonging to the family Daphnidae, has not yet been studied.

M. macrocopa is very common in Thailand. Its life history and culture have been studied in Thailand, but have never been reported in English (DUANGSAWASDI et al., 1981). *M. macrocopa* plays a very important role in natural food chains because it serves as food for larval fish of many species in rivers and streams. The major difference between *Moina* and *Daphnia* is that *Moina* has a placenta which is absent in *Daphnia* (GOULDEN, 1968).

This paper reports on a practical method for using *M. macrocopa* as a bioassay animal for toxicity testing, and on the determination of the degree of toxicity of various metal salts to *M. macrocopa*.

MATERIALS AND METHODS

Food and Feeding

It is generally assumed that cloned or parthenogenetically reproducing organisms are genetically uniform (MANNING et al., 1978). *Moina macrocopa* reproduces by parthenogenesis, which is one reason why it is useful in toxicity tests. While genetic uniformity is expected within parthenogenetic clones derived from a single female, BUIKEMA et al., (1980) reported that genetic variations can be observed in *Daphnia*.

M. macrocopa from a laboratory clone was used. They were originally purchased from an aquarium fish shop and were cloned in the laboratory. They were cultured in 5-l aquaria filled with soft, dechlorinated tap water, at 29° ± 1°C, placed near the windows for natural photoperiod. *M. macrocopa* cultures were fed with 2 drops of Fleishman's yeast solution (prepared from 2 teaspoons of yeast dissolved in 200 ml water) per litre of cultured water, 3 times a week (BOND, 1934), and one drop of green algae *(Chlorella)* cultured in the laboratory, once a week (PAYER, 1970). The density of *M. macrocopa* was kept at about 20 individuals per litre. No aeration was required to acclimatize the organisms to test conditions because *Moina* can live in low dissolved oxygen concentration. Moreover, air bubbles might cause *M. macrocopa* to come to the water surface and might affect the chemical state of metals. The presence of air bubbles also decreased the organism's effective density. *Moina* exposed to air bubbles while being drawn up by dropper usually got air bubbles beneath the carapace (BUIKEMA et al., 1980).

Test Methods

The test organisms were transferred from culture aquaria to the test chambers (300-ml beakers) with a medicine dropper.

In the tests, 20 *Moina,* 12 ± 2 h old, were placed in beakers in duplicate and

subjected to the test conditions for 24 h to acclimatize them to the test conditions. The tests were run without providing food to the organisms as the food could change the physical properties and toxicity values (BEISINGER & CHRISTENSEN, 1972). Death defined as immobility of the organisms was used as the endpoint. The number of live organisms in each concentration was recorded at 0, 24, and 48 h.

All bioassay tests were conducted without aeration or renewal of water maintained at 29°C ±1°C in a water bath with thermostat. The test chambers were placed near the windows for natural daylight.

Reagent-grade chemicals were used: $CdNO_3$, $Ni(NO_3)_2$, $CuNO_3$, $ZnSO_4.7H_20$, $HgNO_3$, and $Pb(NO_3)_2$. The experimental water used was dechlorinated tap water, aerated until saturation and left without aeration for 24 h prior to use in experiments. Its mean pH and dissolved oxygen were, respectively, 7.0 (range: 6.8 – 7.2) and 4.2 mg/l (range: 3.9 – 4.8 mg/l).

Data Analysis

A computer programme based on probit analysis was used to find the median lethal concentration (LC_{50}) values for 24-h and 96-h exposure times (FINNEY, 1971). The median lethal concentration was defined as the concentration at which 50% of the experimental animals died during the exposure time.

RESULTS

The results of acute toxicity tests are shown in Table 1. The 24-h and 48-h LC_{50} values estimated from graphs and calculated by probit analysis, and the slopes of the log-probit lines are shown in Figs. 1 – 6. It should be emphasized that all LC_{50} values were based on the initial concentration of metals, and there were no change in concentrations of metals within 48 h.

The 24-h and 48-h LC_{50} for the metals were as follows: nickel nitrate 2.201 and 0.461 mg/l, lead nitrate 1.120 and 0.755 mg/l, zinc sulphate 0.246 and 0.094 mg/l, cadmium nitrate 0.218 and 0.013 mg/l, cupric nitrate 0.019 and 0.017 mg/l, and mercuric nitrate 0.003 and 0.001 mg/l, respectively.

The toxicities of heavy metals, from the most toxic to the least toxic, are as follows: mercury, copper, cadmium, zinc, lead, and nickel. In Table 2, acute toxicities of the 6 metals to *Moina macrocopa* was compared to those reported for *Daphnia magna*. The 48-h LC_{50} results obtained from *M. macrocopa* were in agreement with *D. magna* for nickel, zinc, and copper. There is no report on lead toxicity to *D. magna*. However, the magnitudes of toxicity of the metals to *M. macrocopa* and *D. magna* are of the same order, from the most toxic to the least toxic, that is, from mercury to copper, cadmium, zinc, and lead.

The results of 24-h and 48-h LC_{50} of nickel to *M. macrocopa* were interesting because nickel was more toxic than lead as the exposure time increased (see Table 1).

Table 1 Acute toxicities (24-h and 48-h LC_{50}) of various metal ions (mg/l) to *Moina macrocopa* (C.L. = confidence limit).

	24-h LC_{50}				48-h LC_{50}			
Metal ions	Conc. (mg/l)	Slope	Intercept	95% C.L.	Conc. (mg/l)	Slope	Intercept	95% C.L.
1. Nickel	2.201	2.106	4.278	1.473 – 3.289	0.461	2.517	5.847	0.288 – 0.738
2. Lead	1.120	4.343	4.786	0.471 – 2.667	0.755	2.884	5.353	0.543 – 1.048
3. Zinc	0.246	2.921	6.779	0.189 – 0.321	0.094	4.231	9.348	0.078 – 0.113
4. Cadmium	0.218	0.979	5.648	0.100 – 0.473	0.013	0.786	6.475	0.033 – 0.052
5. Copper	0.019	6.770	16.590	0.018 – 0.021	0.017	5.770	15.270	0.015 – 0.019
6. Mercury	0.003	2.515	11.300	0.003 – 0.004	0.001	4.218	17.060	0.001 – 0.002

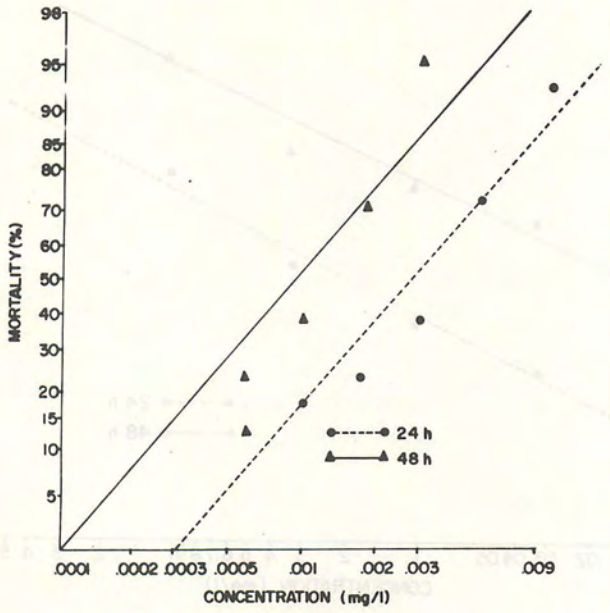

Figure 1. The percentage mortality (probit scale) of *Moina macrocopa* in relation to concentration of mercury.

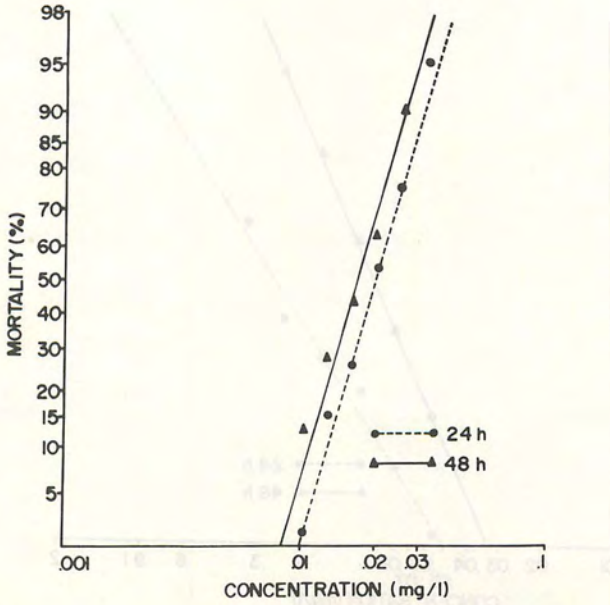

Figure 2. The percentage mortality (probit scale) of *Moina macrocopa* in relation to concentration of copper.

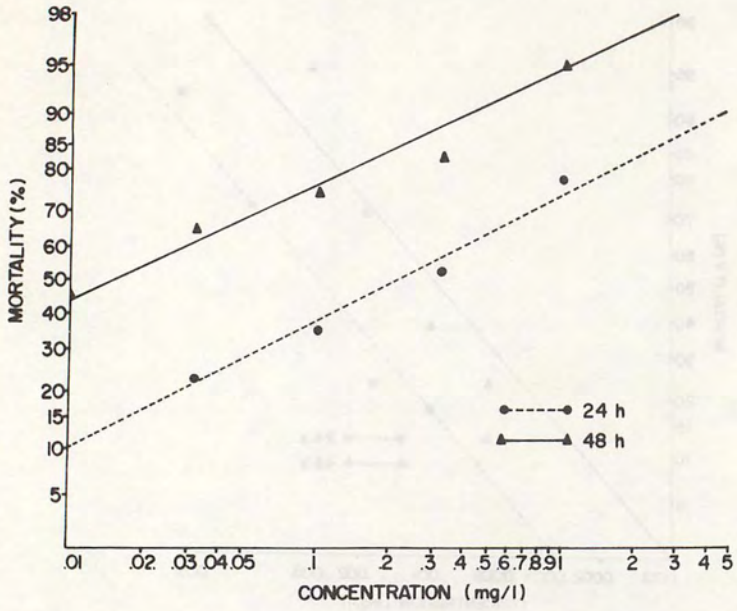

Figure 3. The percentage mortality (probit scale) of *Moina macrocopa* in relation to concentration of cadmium.

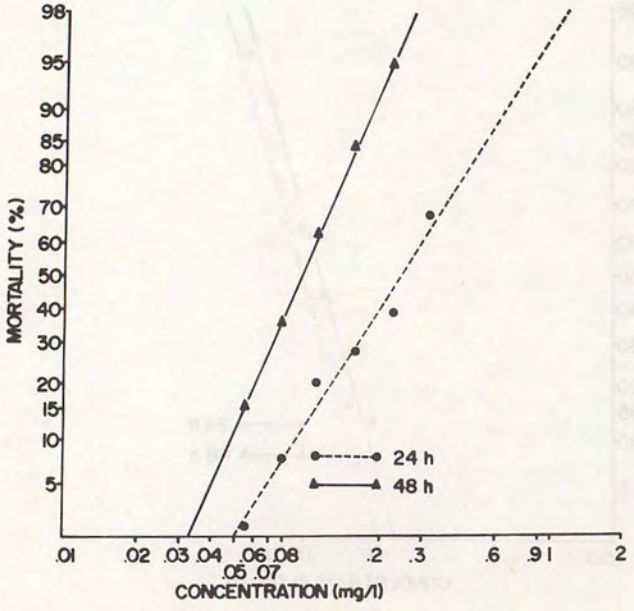

Figure 4. The percentage mortality (probit scale) of *Moina macrocopa* in relation to concentration of zinc.

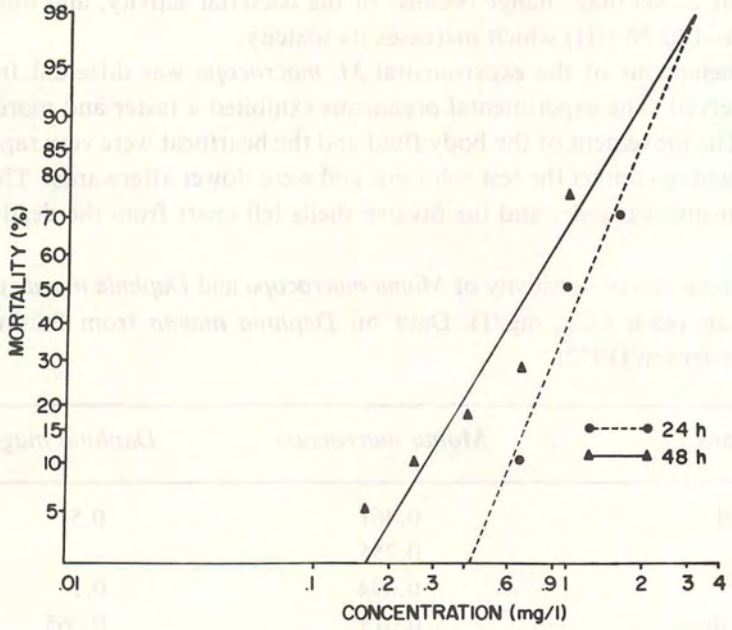

Figure 5. The percentage mortality (probit scale) of *Moina macrocopa* in relation to concentration of lead.

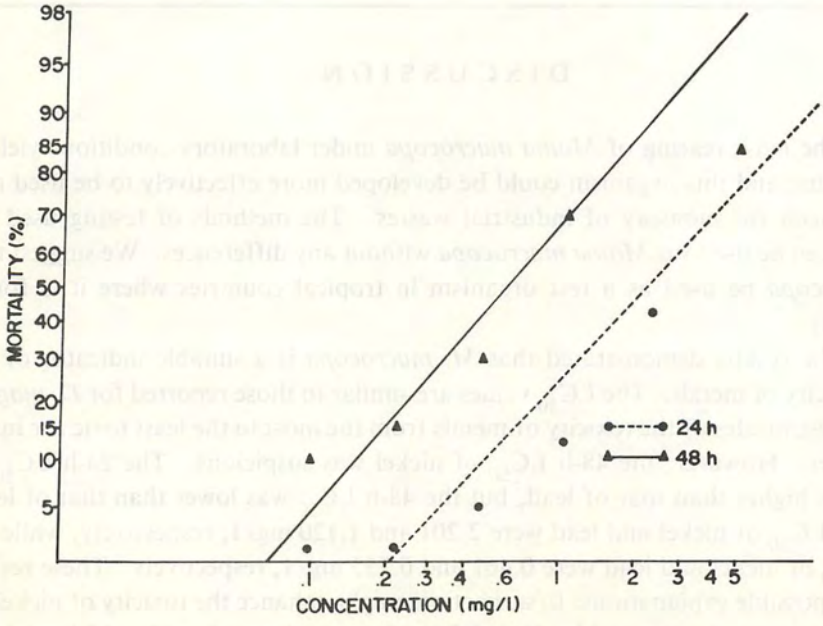

Figure 6. The percentage mortality (probit scale) of *Moina macrocopa* in relation to concentration of nickel.

The toxicity of nickel may change because of the bacterial activity, and thus Ni (II) may be oxidized to Ni (III) which increases its toxicity.

The behaviour of the experimental *M. macrocopa* was different from that normally observed. The experimental organisms exhibited a faster and more jerking movement. The movement of the body fluid and the heartbeat were very rapid as the organisms began to contact the test solutions and were slower afterwards. The colour of dead organisms was pale, and the bivalve shells fell apart from the dead bodies.

Table 2. Comparison of sensitivity of *Moina macrocopa* and *Daphnia magna* to heavy metals (48-h LC_{50} mg/1). Data on *Daphnia magna* from Beisinger and Christensen (1972).

Metal ions	*Moina macrocopa*	*Daphnia magna*
1. Nickel	0.461	0.51
2. Lead	0.755	—
3. Zinc	0.094	0.1
4. Cadmium	0.013	0.065
5. Copper	0.017	0.0098
6. Mercury	0.001	0.005

DISCUSSION

The mass rearing of *Moina macrocopa* under laboratory conditions yielded good results, and this organism could be developed more effectively to be used as a test organism for bioassay of industrial wastes. The methods of testing used for *Daphnia* can be used for *Moina macrocopa* without any differences. We suggest that *M. macrocopa* be used as a test organism in tropical countries where it is found abundantly.

The results demonstrated that *M. macrocopa* is a suitable indicator of the acute toxicity of metals. The LC_{50} values are similar to those reported for *D. magna,* and the magnitudes of the toxicity of metals from the most to the least toxic are in the same order. However, the 48-h LC_{50} of nickel was suspicious. The 24-h LC_{50} of nickel was higher than that of lead, but the 48-h LC_{50} was lower than that of lead. The 24-h LC_{50} of nickel and lead were 2.201 and 1.120 mg/1, respectively, while the 48-h LC_{50} of nickel and lead were 0.461 and 0.755 mg/1, respectively. These results have two possible explanations: first, bacteria might enhance the toxicity of nickel by oxidation; second, the algae which were fed to *Moina* might produce O_2 which might be responsible for the oxidation of nickel. *M. macrocopa,* similar to other cladocerans,

molts periodically. This might change the susceptibility of the organisms during the molt cycle and could bias the bioassay results. LEE & BUIKEMA (1979) reported increased sensitivity of organisms to toxicants during molting. In our experiment, we observed that molting organisms were frequently the first to die. However, no experiment was done on the effects of metals during the molt period.

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