Acute Toxicity of Various Metals to Freshwater Zooplankton[1]

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The discharge of heavy metals by industry represents a serious water pollution problem due to the toxic properties of these metals and their adverse effects on water quality.
Studies on the toxicity of heavy metals to freshwater organisms have concentrated on fish and have yielded only limited information concerning invertebrates. However, it has been shown that some zooplanktonic species are more susceptible to cations than fish are (ANDERSON, 1950; FREEMAN and FOWLER, 1953; BIESINGER and CHRISTENSEN, 1972). Since zooplankton is the main source of food for several species of fish, its destruction by heavy metals may result in the disappearence of some fish even though these latter may not be affected directly.
Therefore, more thorough knowledge of the effect of heavy metals on zooplankton would be very useful in two rather practical applications: 1) the establishment of water quality criteria that could make possible the conservation of aquatic life and 2) the use of zooplanktonic organisms in the bioassay of industrial wastes.
The present investigation was undertaken as a first step in the development of these studies, in order to determine the degree of toxicity of various metal salts to three species of freshwater zooplankton.

Material and Methods

Three of the most representative species of freshwater zooplankton were used: Cyclops abyssorum prealpinus (Einsle), Eudiaptomus padanus padanus (Burkhard) and Daphnia hyalina (Leydig). Planktonic material was collected in Lake Monate (Lago di Monate), a small unpolluted subalpine lake of Italy extensively studied for several ecological aspects (BAUDOUIN, 1971). Single species were isolated from mixed planktonic populations as described elsewhere (BAUDOUIN and SCOPPA, 1973).
Reagent-grade chemicals were used: $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $SrCl_2 \cdot 6H_2O$, $CsCl$, $CoCl_2 \cdot 6H_2O$, $NiCl_2$, $CuCl_2 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $3CdSO_4 \cdot 8H_2O$, $HgCl_2$, $Pb(CH_3COO)_2 \cdot 3H_2O$, K_2CrO_4 .

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Fresh stock solutions were prepared in lake water for each experiment. All concentrations given in this paper are amounts added, expressed as metal.

All tests were carried out in Lake Monate water, filtered through 5 μm porosity cellulose acetate and air saturated. Water characteristics: pH 7.2, conductivity 75 μS, alkalinity 0.58 meq/l, calcium 0.46 meq/l, magnesium 0.20 meq/l, sodium + + potassium 0.16 meq/l, sulphate 0.18 meq/l, chloride 0.06 meq/l.

Test organisms

Test animals were adult individuals acclimated to the laboratory environment for at least 24 hr. The average size of copepods, i.e. the length of cephalothorax, was 0.62 mm for *Cyclops* and 0.43 mm for *Eudiaptomus*; mean size of *Daphnia*, i.e. the distance between the top of the head and the base of the shell spine, was 1.27 mm .

Daphnia (15 to 20 individuals) were exposed to serial dilutions of the chemical under test in cylindrical glass tubes (volume: 300 ml). Particular care was necessary to avoid the presence of air bubbles. Dead animals settled on the bottom of the tube where they could be counted with a reversed microscope by placing the test tube on a glass plate marked in squares.
For copepods, to avoid cannibalism, single animals were exposed to metal solutions in Carrel flasks (volume: 20 ml) and periodically examined under a magnifying lens. Five to twenty individuals were used to test each solution.
Complete immobilization was taken as the end point. Mortality was recorded frequently during the first 48 hr of testing, then the observation became daily.
For each pair metal - species of zooplankton, preliminary experiments in a wide range of concentrations allowed determination of the most suitable concentration interval for acute toxicity tests.

Control mortality

A series of controls for each species showed that less than 1% mortality occurred in *Cyclops* after 15 days, 9.8% in *Eudiaptomus* after 10 days, and 11.2% in *Daphnia* after 5 days.
Attempts to improve holding conditions for *Daphnia* did not give positive results. Therefore, observation periods were fixed to a maximum of 15 days for *Cyclops*, 10 days for *Eudiaptomus*, and 5 days for *Daphnia*.
Observed mortalities were corrected for mortality in controls.

Environmental conditions

Toxicity tests were carried out in water baths thermostated at 10 ± 0.5°C. To avoid adsorption and/or chemical interactions of metal ions, no food was added during the experiments. There was no artificial aeration because air bubbling makes *Daphnia* come to the water surface and may affect the physico-chemical state

TABLE 1

Acute toxicity of various metals (mg/1) to freshwater zooplankton

METAL	CYCLOPS ABYSSORUM			EUDIAPTOMUS PADANUS			DAPHNIA HYALINA		
	48h-LC_{50}	Conf. Limit	S	48h-LC_{50}	Conf. Limit	S	48h-LC_{50}	Conf. Limit	S
Calcium	7000	9242-5302	1.73	4000	5455-2933	1.85	3000	3948-2280	2.15
Magnesium	280	429-183	2.33	180	342-95	2.45	32.0	37.4-27.4	1.75
Strontium	300	354-254	1.39	180	255-127	2.22	75.0	91.1-61.8	1.87
Cesium	400	569-381	2.45	135	186-98	1.89	7.4	8.7-6.3	1.58
Chromium(VI)	10.0	12.0-8.0	1.62	10.1	11.9-8.6	1.56	0.022	0.032-0.015	4.05
Cobalt(II)	15.5	18.8-12.8	1.55	4.0	8.0-2.0	3.04	1.32	1.63-1.07	1.79
Nickel(II)	15.0	25.5-8.8	2.86	3.6	4.6-2.8	1.88	1.90	2.48-1.45	2.11
Lead(II)	5.5	7.7-4.0	2.12	4.0	6.4-2.5	2.98	0.60	0.89-0.41	4.13
Mercury(II)	2.2	3.3-1.5	2.19	0.85	1.02-0.71	1.94	0.0055	0.0098-0.0031	5.05
Zinc(II)	5.5	6.8-4.5	1.60	0.50	0.72-0.35	2.06	0.040	0.051-0.031	2.19
Cadmium(II)	3.8	6.3-2.3	2.67	0.55	0.77-0.39	1.98	0.055	0.095-0.032	5.84
Copper(II)	2.5	3.0-2.1	1.67	0.50	0.65-0.38	2.11	0.005	0.006-0.004	1.74

of some metals. Wide spectrum fluorescent tubes provided light of approximatively 70 lux at the water's surface. Light was automatically controlled and the daily photoperiod was 12 hr. The volume of testwater was sufficiently large to prevent oxygen depletion and minimize changes of water quality caused by metabolic activities of zooplanktonic organisms. Dissolved oxygen, pH, conductivity and alkalinity did not show significant changes after the test period.

Evaluation of data

Observed mortalities were plotted on log-probit paper and LC_{50} (median lethal concentration) was calculated. The series of LC_{50}'s obtained at different observation times were used to construct toxicity curves from which ET_{50}'s (median effective times) could be derived (SPRAGUE, 1969).
Confidence limits of LC_{50} and slope function of the log-probit line were calculated by a desk-top calculator Hewlett-Packard, using a program derived from the equations which served as the basis of nomographic method (LICHTFIELD and WILCOXON, 1949).

Results and Discussion

A summary of the acute toxicity results is given in the Table. The 48hr-LC_{50} values and 95% confidence limits are reported, as well as the slope (S) of the log-probit lines. These key data allow the probit lines to be reconstructed if desired. It must be emphasized that all LC_{50}'s are based on the initial amount of metal added to the dilution water and that after 48 hr concentrations may have been somewhat less than those indicated.

Toxicity data for manganese are not included in the table because of the very poor reproducibility of experimental results. The presence in dilution water of bacteria able to oxidize divalent manganese (COLLINS, 1969) could be responsible for these unreliable data. According to this hypothesis, variations in the type, number and metabolic activity of such bacteria in test water should have serious influence on the concentration of manganous ions and, therefore, on the toxic response.

With few exceptions the results obtained on Daphnia hyalina are in good agreement with those reported for Daphnia magna (BIESINGER and CHRISTENSEN, 1972). Daphnia hyalina is more sensitive to magnesium, strontium, zinc; less sensitive to calcium and niçkel.
The figures reported in the Table can be represented in a graphic form (Figure 1), from which some conclusions may be drawn directly. Calcium is practically non-toxic. Magnesium, strontium and cesium have a low toxicity. Divalent heavy metals and chromate are very toxic. Only minor differences occur in the sequence of metal toxicities to each planktonic species. Chromate is much more toxic to Daphnia than to Cyclops or Eudiaptomus, probably because copepods do not absorb chromate to a significant extent (BAUDOUIN et al., 1972).
Cyclops abyssorum is the most resistant of the three species

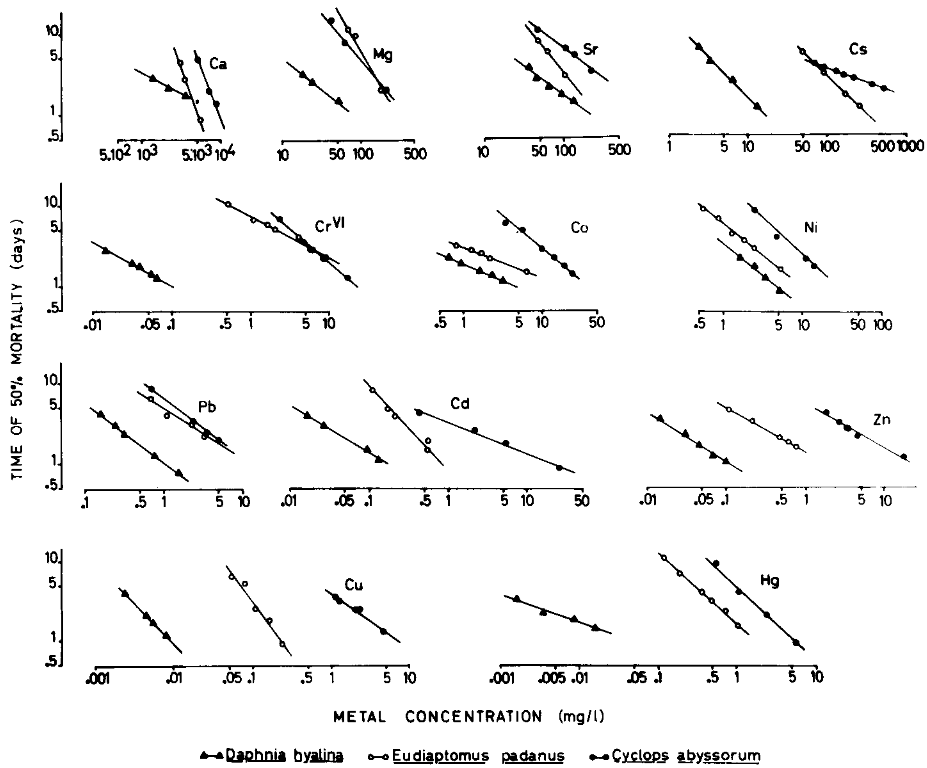

Figure 1 - Logarithmic toxicity curves.

examined, followed by Eudiaptomus padanus. Daphnia hyalina is considerably more sensitive than copepods.

Studies to correlate the toxicity of metals with some of their physico-chemical characteristics showed significant correlations ($P < 0.05$) between median lethal concentration to Cyclops or Daphnia and the solubility product of metal sulfide. In the case of Eudiaptomus the correlation coefficient was not statistically significant. Other correlations were found between toxicity to the three zooplanktonic species and metal electronegativity or equilibrium constant for the metal-adenosine triphosphate complex. When less toxic metals (alkaline-earth and alkaline elements) were not included in the calculations, no significant correlation was found.
Therefore, these studies did not provide any useful information on the mechanism of toxic action of heavy metal pollutants to zooplankton.

Toxicity curves (Figure 2) show that for each pair metal-species of zooplankton there is a straight line relation between median effective time (ET_{50}) and metal concentration, without any evidence of a lethal threshold.
For most of the metals examined toxicity curves for the three zooplanktonic organisms are parallel, indicating a similar

mode of action but different sensitivity. When this does not occur, it can be suggested that there are qualitative and quantitative differences in the toxic action (SPRAGUE, 1969).

Another method of analysis, based on plots of the progress of mortality with time, for a single test concentration, did not provide more revealing information on the mechanism of action. No change in the slope of the straight lines was observed, indicating single mode of action and metal concentrations

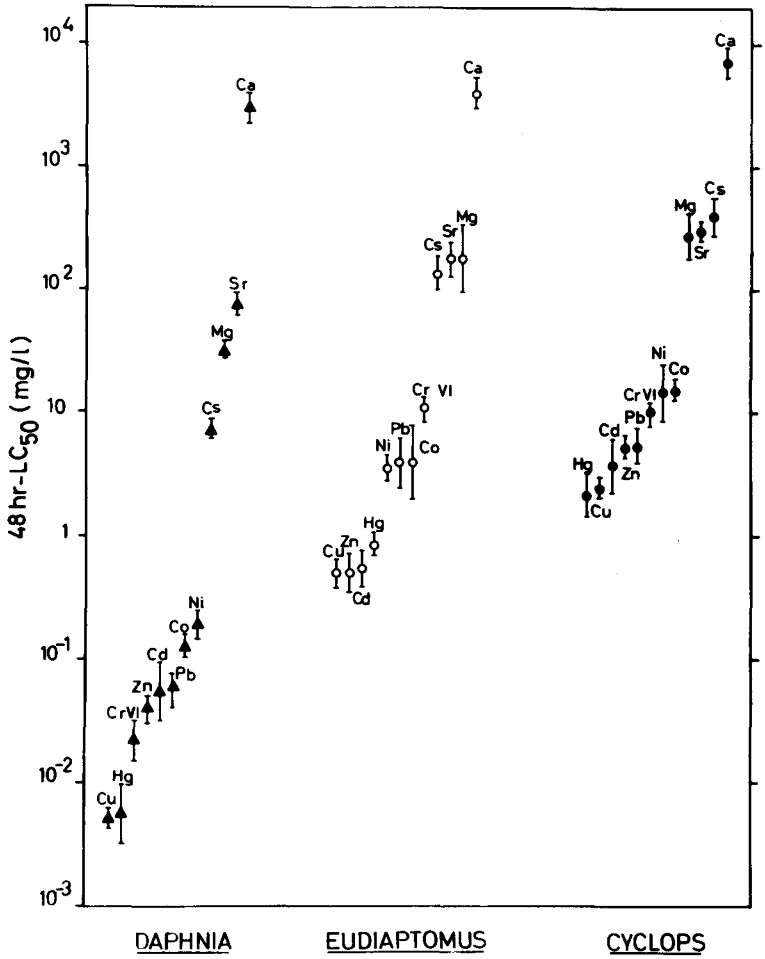

Figure 2 - Comparison of 48hr median lethal concentration of various metals for three zooplanktonic species.

not decreasing to a remarkable extent with time (SPRAGUE, 1969). Therefore, mathematical or graphical means may be used to extrapolate safe levels from lethal concentrations (SPRAGUE, 1971). Such parameters can be very useful in the establishment of water quality criteria which allow the conservation of aquatic life.

The high sensitivity of Daphnia and the linear relationship between metal concentration and median effective time make this invertebrate a useful test organism for heavy metal pollutants. Furthermore, Daphnia fulfils a whole series of requirements for an animal to be used in water pollution tests: a) easy to find everywhere; b) small size but not microscopic; c) simple level of organization to avoid secondary effects of toxic chemicals; d) rapid reproduction and easy to breed in the laboratory.

Further studies are in progress to evaluate the influence of physico-chemical characteristics of dilution water on the toxicity of heavy metals to zooplanktonic organisms.
To have a better insight into the mechanism of action, the variations in lethal concentration are examined taking into consideration absorption kinetics and the distribution of the chemical species of metal calculated on the basis of water composition (BAUDOUIN and SCOPPA, 1974).

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