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Effect of heavy metal salts on the life history of *Daphnia similis* Claus (Crustacea: Cladocera)

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Abstract. The acute toxicity of heavy metal salts on *Daphnia similis* Claus is studied in terms of LC_{50}. Copper is found to be the most toxic and zinc the least. Toxicity of the heavy metals is studied by observing changes in the longevity, body length, fecundity and moulting frequency of the animal. The decreasing order of toxicity of heavy metals on the longevity, body length and fecundity is indicated by Zn > Pb > Cu > Hg, Cu > Zn > Hg > Pb and Pb > Cu > Zn > Hg respectively. The average number of instars increased with the increase of the concentration of metals in the medium. The order of accumulation pattern is Zn > Pb > Cu for 24 h and Zn > Cu > Pb for 48 h. On doubling the concentration of metal to whioh *Daphnia similis* is exposed and fed to the fish *Saratherodon mossambicus*, the biomagnification increases for copper and zinc while it decreases for lead.

Keywords. Heavy metal salts; acute and chronic studies; *Daphnia similis*.

1. Introduction

As most waters from industrial wastes that contain heavy metals are released into continental waters, studies on the effect of these metals on the aquatic life have in recent years attracted the attention of many ecologists. Cladocerans constitute one of the dominant zooplankton of the aquatic ecosystems experimenting varied polluted conditions. Some of them exist even in highly polluted waters serving as indicators of water pollution. Various species of *Daphnia* have been used as test organisms to study the effect of heavy metals in temperate regions, while the information is lacking in tropics (Eaton 1973; Winner and Farrel 1976; Leeuwangh 1977; Chen *et al* 1980; Winner 1981). As *Daphnia* forms an important source of food for many aquatic organisms, it has been used to study the effect of heavy metals through food chain. However, the metal content in this crustacean after exposure is not known. The present study is intended to ascertain the effect of various sublethal concentrations of some heavy metal salts on the life of *Daphnia similis* Claus, their accumulation pattern and biomagnification when they are used as food.

2. Materials and methods

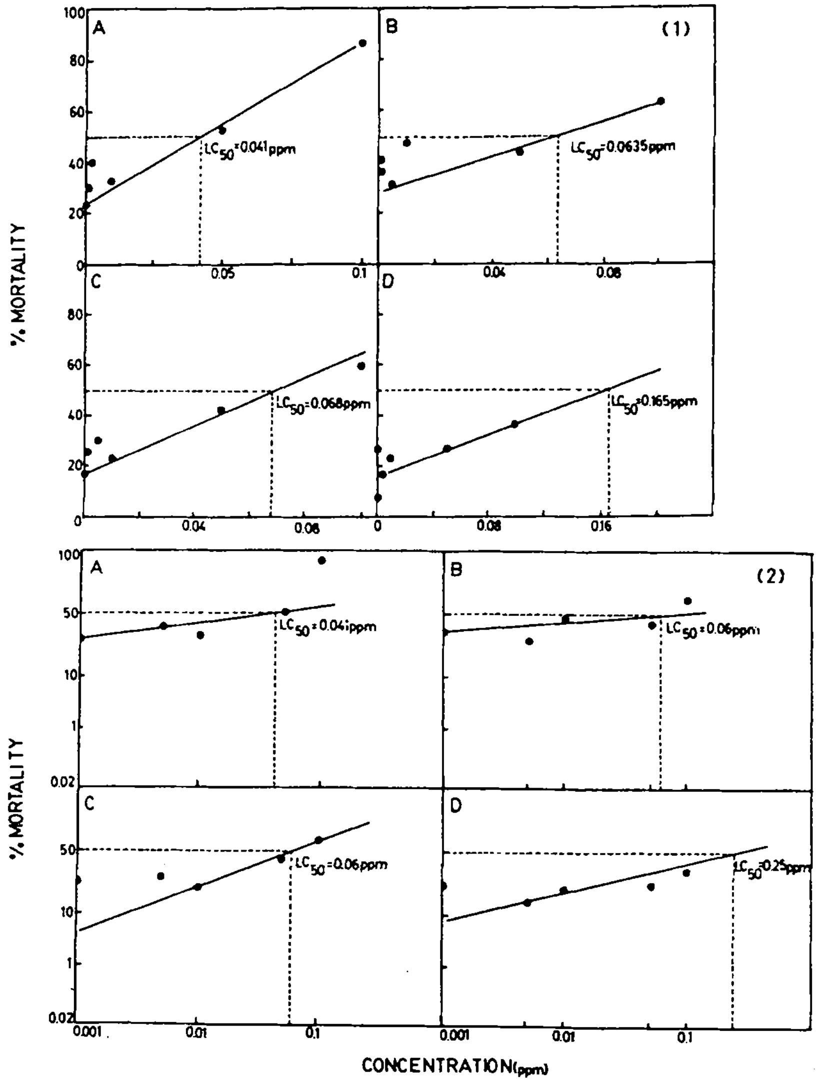
Heavy metal solutions were prepared using $CuSO_4$, $5H_2O$; $Pb(CH_3COO)_2$, $2H_2O$; $HgCl_2$ and $ZnSO_4$, $7H_2O$ salts, the concentrations being 0·1, 0·05, 0·01, 0·005 and 0·001 ppm. The test organisms of *D. similis* were collected from an artificial tank set under laboratory conditions (pH 8·15 and DO 2 ppm) and the animals fed with organic matter from the cow dung slurry from a biogas plant. LC_{50} fresh samples of adult parthenogenetic females of *D. similis* are maintained in 40 ml of each of the heavy metal solutions and examined daily for mortality during a period of 96 h. LC_{50} values were calculated by graphical and probit-graph methods. Tests were conducted in triplicate under laboratory conditions (28°–30° C).

Each sublethal toxicity test was initiated with 10 samples of *D. similis* (approximately 24 h old) reared in 40 ml of metal salt solutions of various concentrations prepared in tank water. Animals were changed every day to freshly prepared metal concentrations and the young ones observed through a compound microscope. Mortality and reproductive activities were determined. The number of instars and the young ones produced in each instar were noted (Venkataraman 1981). Body length was measured using an ocular micrometer. Mean body lengths and brood sizes were compared among metal concentrations by analysis of variance. Longevities were compared by percentage survivors.

A known amount of adult *D. similis* was exposed to selected metal concentrations in triplicate for 24 and 48 h. The material was filtered, dried and weighed to the nearest 0·001 mg to study bio-accumulation. It was later digested in 0·5 ml of nitric acid at 60°C and diluted to 6 ml. The metal concentrations were determined by Perkin Elmer 372 atomic absorption spectrophotometer. Metal loads were expressed as bioconcentration factor (BCF) mg metal g^{-1} daphnid/mg metal g^{-1} water. The concentration of copper, lead and zinc in *D. similis* before exposure was 0·02, 0·06 and 0·05 ppm, respectively. Adult daphnids exposed to metal concentrations in triplicate for 24 and 48 h were filtered, rinsed with water and fed to a fish fry of *Sarotherodon mossambicus* (approximately 2 cm). The fish was dried, powdered and digested in a triplicate acid medium. The digest was diluted and analysed for metal load by atomic absorption spectrophotometer. Biomagnification has been expressed as mg metal g^{-1} fish/mg metal g^{-1} daphnid.

3. Results

The 96 h LC_{50} values obtained by graphic and probit methods are shown in figures 1 and 2. The concentration at which 50% mortality occurs is highest for zinc and lowest for copper, the decreasing order of their sensitivity to heavy metals being Cu > Pb > Hg > Zn. The longevity of *D. similis* exposed to different concentrations of metals is estimated and expressed as the mean duration of life. The stress of mercury became very pronounced only in 0·05 and 0·1 ppm, while animals exposed to copper, lead and zinc suffered a severe decrease in longevity, almost equally in all concentrations (figure 3). The order of decreasing toxicity of heavy metals on longevity of *D. similis* is Zn > Pb > Cu > Hg. The effect of heavy metals assessed on the growth of *D. similis* is shown graphically (figure 4) and by analysis of variance. The variation in growth in lead solutions is not significant, while in all other metal solutions it exhibited statistically significant results (table 1). The decreasing order of toxicity of heavy metals on the growth of *D. similis* is Cu > Zn > Hg > Pb. Compared to other metals, the mercury stress on fecundity is less prominent (figure 5). Considering the average number of eggs laid by an animal per day, the decreasing order of toxicity of heavy metals on egg production is Pb > Cu > Zn > Hg (table 1). For any specific concentration, the order of bioaccumulation is Zn > Pb > Cu during 24 h and Zn > Cu > Pb during 48 h exposure (table 2). On doubling the concentration of metal to which *D. similis* is exposed and fed to the fish, the biomagnification increased for copper and zinc, while it decreased for lead



Figures 1 and 2. Acute toxicity of (A) copper, (B) lead, (C) mercury and (D) zinc salts on *D. similis*; 96 h LC_{50} determined by (1) graphical and (2) probit-graph methods.

in the order of Pb>Cu>Zn in 0·05 ppm and Cu>Zn>Pb in 0·1 ppm concentration (table 3). The average number of instars increased with increasing concentration of heavy metals than in the control medium (table 4).

4. Discussion

Chronic effects of metals may be detectable more quickly with smaller species of *Daphnia* which have shorter life spans (Winner and Farrell 1976). The significant reduction in longevity in lead, zinc and copper solutions in the present study confirms *D. similis* as a smaller sized species with shorter span of life showing more

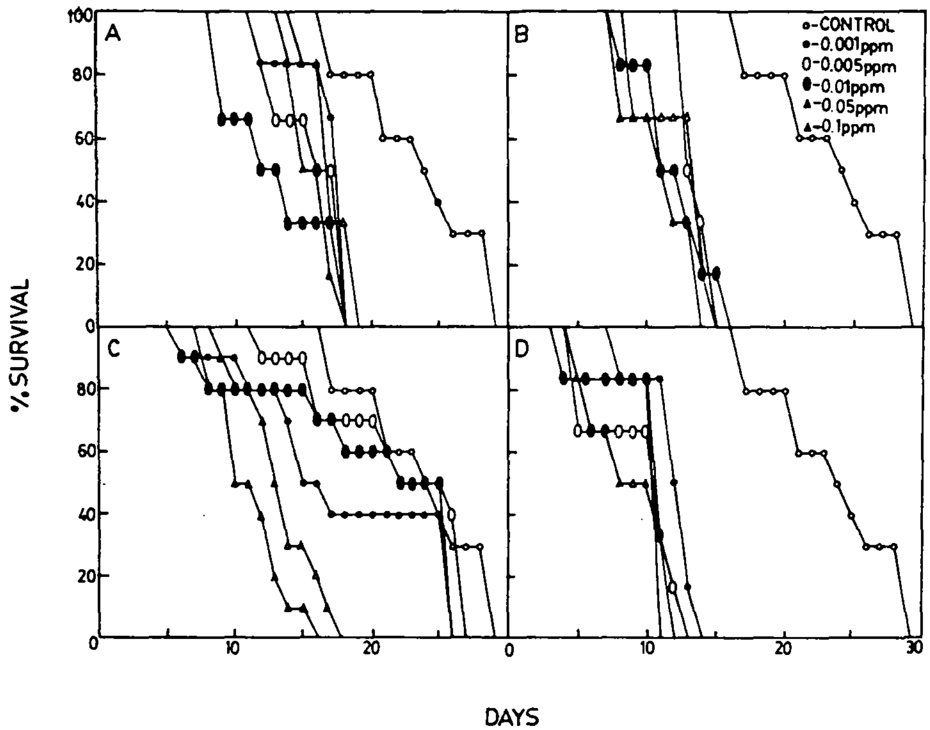


Figure 3. Survivorship curve of *D. similis* exposed to different concentrations of (A) copper, (B) lead, (C) mercury and (D) zinc salts.

detectable chronic effects. However, mercury does not decrease the longevity of *D. similis* to a large extent at lower concentrations probably due to its use in inorganic form.

For all the 4 metals investigated, concentration above 0·005 ppm causes reduction in body length of *D. similis*. Reduction in body size of *D. magna* has also been observed due to copper and zinc stresses (Winner 1981). The growth curves showed a steep rise in the initial stages, while it was gradual after the attainment of primiparous stage. Thus, there does not seem to exist any distinct relationship between concentration and growth (figure 4). The stress of heavy metals was reflected in the average number of instars per day. In 0·1 and 0·05 ppm, the number of instars were more than in any other concentration showing an increased number of instars (table 4). The average number of instars in the present study increased with the increasing concentration, which may facilitate the excretion of metal load as suggested by Dethlefsen (1978) and Bertram (1980).

Reproductive impairment due to copper stress have earlier been observed in *D. magna* (Dave 1984), *D. pulex* (Ingersoll and Winner 1982), the present study also confirming the copper concentration taken for the life history study resulting in decreased brood size (figure 5). As the administration of mercury was in the form of mercuric chloride, the accumulation of mercury in *D. similis* was perhaps less than that of other metals and hence their lower reduction in brood size and longevity. The demonstration of trophic transfer of mercury in the form of mercuric chloride from *C. vulgaris* to *D. magna* supports this view (Ribeyre 1981). Hall (1964) has

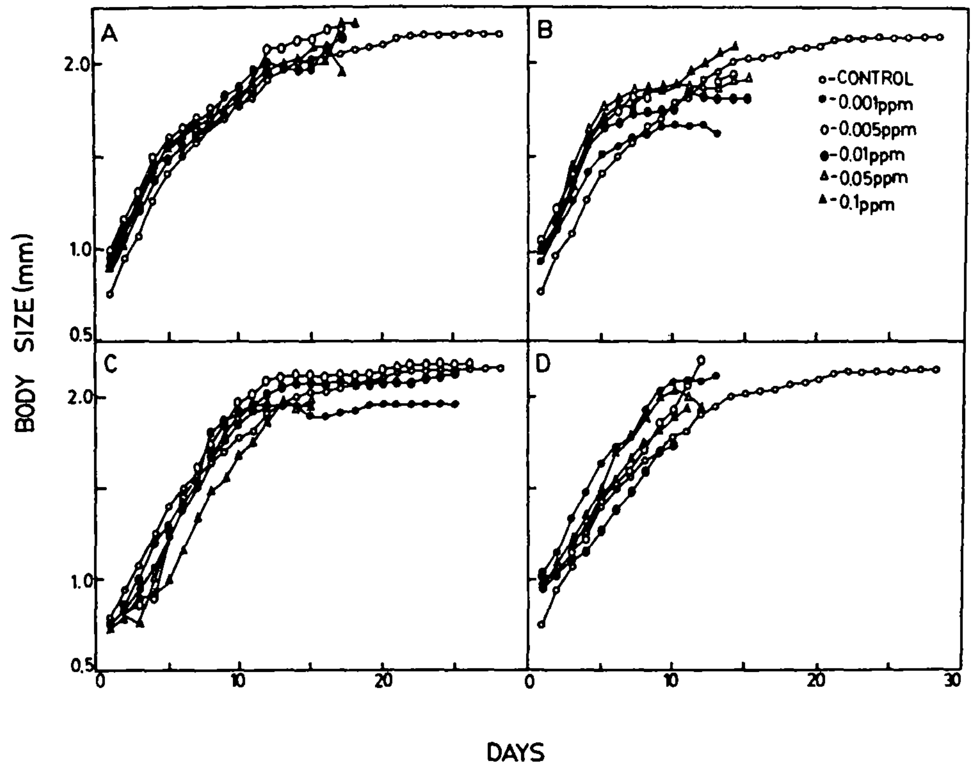


Figure 4. Growth curve of *D. similis* exposed to different concentrations of (A) copper, (B) lead, (C) mercury and (D) zinc salts.

Table 1. Differences in growth and fecundity of *D. similis* in relation to number of days, reared in 5 different concentrations of 4 different metals and in a control medium (ANOVA summary table).

Heavy metal	*F* value		*df*		*P*	
	Growth	Fecundity	Growth	Fecundity	Growth	Fecundity
Copper	5·998	11·170	5, 118	5, 108	0·001**	0·01**
Lead	2·050	9·850	5, 94	5, 93	0·1*	0·01**
Mercury	2·810	2·123	5, 128	5, 128	0·05**	0·1*
Zinc	3·033	4·760	5, 80	5, 80	0·025**	0·01**

*Not significant; **significant.

shown that brood sizes are determined by the nutritional state of the female during the period of egg development. However in the present study, the temperature and food resources remaining the same for all animals, the differences in reproductive performance were probably due to metal stress.

In 0·1 ppm concentrations of all the metals, the bioconcentration factor exhibited an increase when the duration of exposure was increased from 24–48 h. However, with 0·05 ppm concentration, the bioconcentration factor decreased with increase in exposure time. No such regular trend was noticed for other concentrations. Among

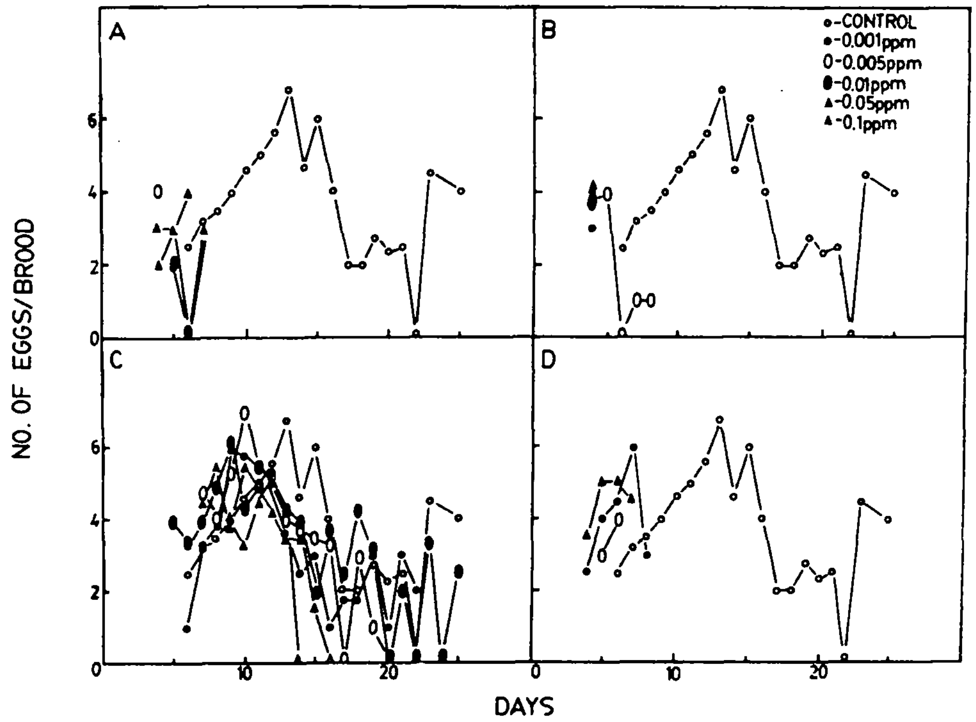


Figure 5. Fecundity of *D. similis* exposed to different concentrations of (A) copper, (B) lead, (C) mercury and (D) zinc salts.

Table 2. Bioaccumulation of heavy metal in *D. similis* after 24 and 48 h exposure.

Heavy metal	Conc. of metal in water (ppm)	Bioconcentration factor	
		24 h	48 h
Copper	0·001	4·1150	10·9900
	0·005	0·7338	0·5128
	0·01	0·2353	0·3333
	0·05	0·0741	Not read
	0·1	0·0330	0·0265
Lead	0·001	4·9260	5·5560
	0·005	0·8602	0·3243
	0·01	0·2500	0·4142
	0·05	0·0804	0·0860
	0·1	0·0513	0·2564
Zinc	0·001	24·7600	30·5600
	0·005	2·3000	2·0000
	0·01	2·2500	1·7200
	0·05	1·0000	0·3412
	0·1	0·1520	0·2037

solutions of the same metal, the bioconcentration factor decreased with increase in concentration. Bioaccumulation of copper by daphnids may also be affected by water hardness, humic acid concentration, age at which the animals were exposed

Table 3. Biomagnification of heavy metals in the fish *S. mossambicus* fed with *D. similis* exposed to heavy metals for a duration of 24 h.

Heavy metal	Conc. in water (ppm)	Conc. of metal in fish/wt. of fish	Conc. of metal/ wt. of *D. similis*	Biomagnification
Copper	0·05	1·3344	3·704	0·36
	0·1	1·7544	3·297	0·53
Lead	0·05	4·3230	4·020	1·08
	0·1	2·4096	5·128	0·47
Zinc	0·05	4·7770	50·000	0·10
	0·1	7·9125	15·240	0·52

Table 4. Average number of instars produced by each animal per day when exposed to different concentration of heavy metals.

	Concentration (ppm)				
Heavy metal	0·001	0·005	0·01	0·05	0·1
Copper	0·57	0·66	0·67	0·67	0·72
Lead	0·76	0·75	0·67	0·78	0·78
Mercury	0·69	0·68	0·68	0·80	0·77
Zinc	0·71	0·57	0·68	0·75	0·81

Number of instars per day in control medium = 0.67.

to metals and the total organic carbon present in the medium (Winner 1986). The lower accumulation of copper in *D. similis* in the present study was perhaps due to the nutritionally rich organic medium (biogas spent cowdung slurry) used.

Accumulation of zinc was more than that of copper or lead in *S. mossambicus*. In experiments demonstrated by Vighi (1981), lead accumulated in the trophic chain with a decreasing concentration factor from the lowest to the highest levels confirming with the observations made in the present study.

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