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Comparative Toxicity of Five Metals on Various Biological Subjects

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Environmental contaminants, including metals, can have toxic effects on many different organisms and affect biological processes at cellular, population, community and ecosystem levels of organization. Metals are of particular interest because they may have a positive effect as micronutrients at low concentrations as well as a toxic effect at higher levels.

Numerous efforts have been made to correlate and predict metal-ion toxicities on various aquatic and terrestrial species, according to the physical and chemical properties of the metals (Kaiser 1980). The acute and chronic toxicities of metals have been studied on many freshwater organisms (Khangarot and Ray 1989) and also on plants (Koeppe 1977; Outridge 1992). The water flea, Daphnia magna, an important freshwater zooplankter, is a useful test species (Khangarot and Ray 1989) to study sensitivity to environmental toxicants and has been recognized as a general representative for other freshwater animals (Baudouin and Scoppa 1974).

As Khangarot (1991) described, little information is available on the effects of metals to tubificid worms, which are widely distributed in the aquatic environment and are useful indicators of varying degrees of aquatic pollution.

Most recent hazard evaluation programs recommend algal toxicity tests for initial evaluations of chemicals that are expected to reach surface waters and that have suspected or unknown phytotoxic properties. For higher plants, the accumulation of metals, especially cadmium, was tested when plants grew in sewage sludge-amended soils or in soils of cadmium residues from phosphate fertilizers (Adema and Henzen 1989). No reports were available indicating the effects of these metals on seed germination and root growth.

The present study was conducted to determine the acute toxicological effects of some metals on four biological subjects and to compare the subjects' sensitivity to individual test metals.

MATERIALS AND METHODS

Tubificid worms (Tubifex tubifex), 20 mm in length, were used. Daphnia magna sp. were cultured from parthenogenetic females maintained in the laboratory. In the tests, 6- to 24-hr old daphnids were used. Before the tests, both organisms were adapted to test conditions for 24 hr in distilled water containing 44.0 mg/L $CaCl_2.2H_2O$, 123.25 mg/L $MgSO_4.7H_2O$, 62.5 mg/L $NaHCO_3$ and 5.75 mg/L KCl. Water temperature was 20 ^oC and pH 6.67. For T. tubifex the amount of solution per dish was 20 mL, and for D. magna it was 50 mL. During the tests, the solutions were not aerated, organisms were not fed nor situated in direct sunlight. Each metal-ion was tested in ten concentrations for T. tubifex ranging from 0.024-1.50 mg/L for Hg^{2+}, 0.50-4.05 mg/L for Cd^{2+}, 2.07-26.90 mg/L for Pb^{2+}, 56.19-179.80 mg/L for As^{5+}, 0.52-9.41 mg/L for Cr^{6+}(1), 0.98-7.79 mg/L for Cr^{6+}(2); for D. magna the test concentrations ranged from 0.01-0.40 mg/L for Hg^{2+}, 0.09-0.92 mg/L for Cd^{2+}, 0.41-10.34 mg/L for Pb^{2+}, 29.82-115.38 mg/L for As^{5+}, 0.05-1.14 mg/L for Cr^{6+}(1) and 0.05-1.04 mg/L for Cr^{6+}(2).

Scenedesmus quadricauda /TURP./BRÉB. strain Greifswald 15 was supplied by the Department of Botany, Třeboň, Czech Republic. During the tests, the culture was incubated under continuous light at 25±1 ^oC and a light intensity produced by three 40 W white fluorescent lamps. The culture was maintained in a liquid medium (Báslerová and Dvořáková 1962) containing 0.1 g/L KNO_3, 0.01 g/L $K_2HPO_4.7H_2O$, 0.001 g/L $MgSO_4.7H_2O$, 0.001 g/L $FeCl_3.6H_2O$, soil extract 50 mL, at pH 7.18 and supplemented with various concentrations of metal ions. Each metal ion was tested in six concentrations ranging from 0.15-0.6 mg/L for Hg^{2+}, 0.005-0.25 mg/L for Cd^{2+}, 5.5-33 mg/L for Pb^{2+}, 24-144 mg/L for As^{5+}, 0.26-5.12 mg/L for Cr^{6+}(1) and 0.17-3.4 mg/L for Cr^{6+}(2). Approximately 25,000 coenobia were inoculated into 100-mL Erlenmayer flasks with 50 mL of cultivation media. Growth was monitored by hemocytometer every 48 hr during a 20-d period.

The seeds of Sinapis alba were placed in 14-cm diameter Petri dishes with filter paper on the bottom. In each Petri dish 50 seeds were evenly distributed on the surface of the filter paper and the amount of solution used was 10 mL per dish. The dishes were not situated in direct sunlight. The test concentrations for germination ranged from 36.10-300.88 mg/L for Hg^{2+}, 213.56-1686.00 mg/L for Cd^{2+}, 580.13-1989.00 mg/L for Pb^{2+}, 11.98-112.38 mg/L for As^{5+}, 25.99-389.97 mg/L for Cr^{6+}(1) and 39.00-311.97 mg/L for Cr^{6+}(2) and for root-growth inhibition 2.00-52.15 mg/L for Hg^{2+}, 12.4-213.56 mg/L for Cd^{2+}, 125.75-1077.38 mg/L for Pb^{2+}, 1.98-45.70 mg/L for As^{5+}, 0.50-51.00 mg/L for Cr^{6+}(1) and 5.00-79.80 mg/L for Cr^{6+}(2). For each metal-ion, ten concentrations were tested.

Each concentration was duplicated three times for each case study.

For T. tubifex the exposure lasted 96 hr, for D. magna 48 hr, for S. quadricauda 12 d and for S. alba 72 hr. Then the survival (%) [T. tubifex, D. magna], growth rates (number of coenobia) [S. quadricauda], germination (%) and root growth (cm) [S. alba] were determined. The LC50 and EC50 values and their 95 % confidence limits were calculated by using probit analysis for mortality (T. tubifex, D. magna and S. alba seed germination) and least squares regression for growth rate inhibition (S. quadricauda) and root growth inhibition (S.alba). Differences were considered significant at $P<0.05$ (Harris 1959).

RESULTS AND DISCUSSION

There were statistically significant differences between the sensitivity of T. tubifex and D. magna. D. magna was, in all tests, more sensitive to metal ions than T. tubifex. The most notable were differences between LC50 values for T. tubifex and D. magna with Cr^{6+}(1) and the lowest with As^{5+} and Cd^{2+}. The rank order of toxicity for the metals tested was for T. tubifex: $Hg^{2+}>Cd^{2+}>Cr^{6+}(1)>Cr^{6+}(2)>Pb^{2+}>As^{5+}$, and for D. magna: $Hg^{2+}>Cr^{6+}(1)>Cd^{2+}=Cr^{6+}(2)>Pb^{2+}>As^{5+}$. Both rank orders of toxicity differed only in position of Cd^{2+} and Cr^{6+}(1).

For S. quadricauda, the most toxic metal was Cd^{2+}. It was nearly 9000 times more toxic than As^{5+}, which was the least toxic. The rank order of toxicity for S. quadricauda was $Cd^{2+}>Hg^{2+}\geq Cr^{6+}(2)>Cr^{6+}(1)>Pb^{2+}>As^{5+}$. Also for S. quadricauda, As^{5+} the least toxic metal, was the same as that for D. magna and T. tubifex. The results of this study indicate that Pb^{2+} and As^{5+} ions are low in toxicity to the aquatic organisms T. tubifex, D. magna and S. quadricauda.

Comparisons of the LC50(G) values for seed germination of S. alba and EC50(I) values for inhibition of S. alba root-growth indicated that there were statistically significant differences. For inhibition of root-growth, the EC50(I) values were, in some cases, nearly 25 times [Cr^{6+}(1)] lower than were the LC50(G) values for germination. The most toxic metal tested was As^{5+} for both germination as well as for root-growth inhibition. The rank order of toxicity of the metals tested was for

Table 1. LC50 and EC50 values (mg/L) for metals and their corresponding 95 % confidence limits (CL).

Metal	pH	Organism	LC50, EC50 + 95 % CL
	7.40	T. tubifex[a1]	127.36 (108.76-134.29)
	7.30	D. magna[b1]	44.66 (35.22-50.93)
As^{5+}	8.02	S. quadricauda[c2]	61.00 (59.25-70.14)
	7.28	S. alba (G)[d1]	30.20 (25.71-37.12)
	7.25	S. alba (I)[d2]	5.49 (2.89-6.58)
	6.00	T. tubifex	14.62 (8.53-17.52)
	7.20	D. magna	3.73 (2.71-5.28)
Pb^{2+}	6.48	S. quadricauda	12.16 (10.63-15.71)
	5.10	S. alba (G)	1148.15 (960.89-1298.12)
	5.80	S. alba (I)	263.03 (250.41-289.45)
	6.15	T. tubifex	2.91 (2.07-3.20)
	7.96	D. magna	0.16 (0.15-0.19)
Cr^{6+}(1)	6.98	S. quadricauda	0.54 (0.48-0.66)
	2.46	S. alba (G)	123.00 (119.28-152.43)
	4.20	S. alba (I)	5.01 (4.58-7.02)
	7.70	T. tubifex	3.31 (2.78-3.74)
	7.84	D. magna	0.36 (0.31-0.50)
Cr^{6+}(2)	7.06	S. quadricauda	0.19 (0.14-0.28)
	7.25	S. alba (G)	100.00 (93.76-115.00)
	7.32	S. alba (I)	45.71 (36.25-47.89)
	7.86	T. tubifex	0.28 (0.22-0.36)
	7.87	D. magna	0.02 (0.01-0.02)
Hg^{2+}	7.10	S. quadricauda	0.24 (0.17-0.27)
	7.09	S. alba (G)	128.82 (119.81-139.49)
	7.70	S. alba (I)	9.33 (7.52-11.00)
	7.85	T. tubifex	1.03 (0.68-1.23)
	7.95	D. magna	0.36 (0.32-0.86)
Cd^{2+}	7.15	S. quadricauda	0.008 (0.006-0.012)
	6.16	S. alba (G)	691.83 (676.53-715.33)
	6.90	S. alba (I)	47.86 (42.76-56.30)

[a] - after 96 hr, [b] - after 48 hr, [c] - after 12 d
[d] - after 72 hr, [1] - LC50 value, [2] - EC50 value

S.alba seed germination: $As^{5+}>Cr^{6+}(2)>Cr^{6+}(1)=Hg^{2+}>Cd^{2+}>Pb^{2+}$ and for root-growth inhibition: $Cr^{6+}(1)=As^{5+}>Hg^{2+}>Cr^{6+}(2)=Cd^{2+}>Pb^{2+}$. Both rank orders of toxicity were different only in the position of Cr^{6+}(1) and Cr^{6+}(2). Differences were also noted in the position of Cd^{2+} and Hg^{2+} in comparison with the rank orders of toxicity for the previous three biological subjects. For them, Cd^{2+}

and Hg^{2+} were the most toxic but for S. alba seed germination and root growth inhibition their toxicity was relatively low, especially for germination. From both rank orders of toxicity to plants, the conclusion is that the most toxic metal ion for plant seeds (their germination and root growth) in our tests was As^{5+} and the least toxic was Pb^{2+}.

The rank order of toxicity for T. tubifex was different from that reported by Khangarot (1991), who found that Pb and Hg were the most toxic and As and Cd the least toxic ions. In the present study, the LC50 values for As^{5+}, Pb^{2+} and Hg^{2+} were higher and for Cd^{2+} were lower than Khangarot (1991) described.

When the rank order of toxicity for D. magna was compared with other toxicity sequences for this organism or other Crustacea, mercury was the most toxic metal in all cases (Baudouin and Scoppa 1974). The rank orders of other metals used here varied from case to case, but the toxicity sequence for metal ions for D. magna was usually Hg>Cd>Pb>Cr>As (Khangarot and Ray 1989). There results are in agreement with these data, except for the position of Cr.

Many aquatic organisms have the capacity to accumulate toxic metals from polluted water. The uptake is influenced by concentration, time, pH and temperature. For S. quadricauda, pH values were between 7.0 and 7.5 and at these pHs it has been reported that a hydroxylated form of cadmium ($CdOH^{+}$) starts to form (Seyfried and Horgan 1983). This form was more toxic than bivalent cadmium ions, suggesting an explanation as to why the toxicity of Cd in this case was many times higher than the toxicity of other metals. Mercury is one of the most toxic metals to algae, with lethal levels ranging from 10 to 50 ppb for most species (Berland et al. 1976). By comparison, lethal levels in this study are higher. These differences could result from the different cultivation conditions and test organism. In my case, the values for S. quadricauda, for Pb^{2+} as well as for Cr^{6+}, were lower than what Rai and Raizada (1989) described; Cr^{6+} was more toxic than Pb^{2+}. From the available literature it appears that Pb is usually more toxic than Cr (Khangarot and Ray 1989), but there are also reports in which the toxicity orders are changed (Baudouin and Scoppa 1974). Few reports on the toxicity of arsenic are available. Its toxicity, compared with other metals, is not very high (Khangarot 1991). Compared with other metals used in there tests, arsenic was the least toxic. This low toxicity can be attributed to the arsenic valency (Williams and Silver 1984). Since algae are the primary producers in many aquatic ecosystems, their susceptibility to metal toxicity has been the subject of numerous reports.

Because of the scarcity of literature on metal toxicity to higher plants, including seed germination and root-growth inhibition, there is no possibility to compare rank orders of toxicity for S. alba and LC50(G) and EC50(I) values with those of other plant species. The results of the present study indicate that metal ions had very low toxic effects on seed germination; they are more likely to damage root growth, which is necessary for nutrient absorption and plant growth.

Two hexavalent chromium salts were compared on the survival of aquatic subjects, seed germination and root growth, and in three cases (for survival of T. tubifex, D. magna and root-growth inhibition of S. alba seeds) Cr^{6+}(1) (as CrO_3 salt) was more toxic than Cr^{6+}(2) (as $(NH_4)_2CrO_4$ salt). For the coenobia growth rate of S. quadricauda and seed germination of S. alba seeds, chromium salt $(NH_4)_2CrO_4$ was more toxic than CrO_3. These differences may have been the result of pH which regulates Cr uptake (Tripathi and Chandra 1991).

The rank orders of metal toxicity in this study were:

Hg^{2+}, Pb^{2+}, Cr^{6+}(1):
D. magna>S. quadricauda>T. tubifex>S. alba(I)>S. alba(G)

Cr^{6+}(2):
S. quadricauda>D. magna>T. tubifex>S. alba(I)>S. alba(G)

Cd^{2+}:
S. quadricauda>D. magna>T. tubifex>S. alba(I)>S. alba(G)

As^{5+}:
S. alba(I)>S. alba(G)>D. magna>S. quadricauda>T. tubifex

Based on these rank orders of sensitivity, the most sensitive aquatic organism is usually D. magna. This means that D. magna is a good biological subject for testing metal-ion toxicity. The toxicity of metals to various biological subjects and comparisons of their sensitivity stimulated this work. The presence of metals, often in large amounts in the environment, interferes with various ecosystems. These ecosystems consist of various numbers of living organisms of different sensitivities, which are interconnected through complicated food chains. Thus, it is important to understand the toxic effects of key metals on many members of these ecosystems.

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