Bull. Environ. Contam. Toxicol. (1997) 59:956-962

Bulletin of Environmental Contamination and Toxicology

Sensitivity of *Chironomus plumosus* Larvae to V^{5+}, Mo^{6+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, and Cu^{+} Metal Ions and Their Combinations

A. Fargašová

Slovak University of Technology, Faculty of Chemical Technology, Department of Environmental Sciences, Radlinského 9, SK-812 37 Bratislava, Slovak Republic

Received: 25 July 1997/Accepted: 15 September 1997

The introduction of heavy metals into the aquatic environment has increased parallel with the rapid industrialization of our world during the past 150 years. While many heavy metals are required micronutrients for biological systems, they become toxic to most aquatic lifeforms at only slightly higher concentrations than that minimum requirement. Because of precipitation, sorption and particulate-settling processes, many of these anthropogenically mobilized metals such as copper are deposited in the sediments of lakes, rivers, and oceans. Binding to and burial in sediments may be one of the safest fates for metallic pollutants due to imposed limitations in the metal's mobility and potential for biological uptake. However, changes in environmental conditions may facilitate the metals' release to the overlying water. For instance, changing environmental conditions such as Eh, pH, temperature, salinity, biological activity, and ligand types and concentrations may affect the speciation of the metals, their complexation and, consequently, mobility, bioavailability, and toxicity (Lion et al. 1982).

Heavy metal concentration in surface waters is generally assumed to be controlled by scavenging particles of abiotic or biotic origin. The similarity between the distribution of some metals (Cd, Cu, Ni, Zn) and nutrients suggests that phytoplankton and benthos play a key role in the water environment (Martin and Windom 1991). For freshwaters, a biogenic flux of trace metals to the sediments has been demonstrated for deep stratified lakes (Baccini 1984) as well as for shallow and well-mixed eutrophic lakes (Van der Meent et al. 1985).

A huge number of studies has oriented at the biological activity of metal ions and their effects on flora and fauna. The studies involve models from viruses and procaryotic organisms to the human beings; much less is known on metal - metal interactions in biological systems. These interactions can produce either synergistic or antagonistic effects. Intensive studies on various models resulted in numerous reports and some reviews (Chang and Sibley 1993; Tomasik et al. 1995a; Tomasik et al. 1995b; Jaworska et al. 1996). Dirilgen and Inel (1994) and Lee et al. (1994) attempted to quantify predictions of the toxicity resulting from metal-metal interactions in several biological systems. Heavy metal pollutants are known to be quite toxic to a wide variety of aquatic organisms. In water bodies, metals are always present in combinations. However, there are few studies on the effects of metal pair interactions on benthos.

The purpose of this laboratory study was to investigate the effects on mortality of *Chironomus plumosus* larvae of V^{5+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, Cu^{2+}, Cu^{+} and their combinations in concentrations corresponding with LC_{50} values after 96 hr exposure. Manganese, Mo and Cu were chosen since they are known to be essential trace elements up to a certain concentration, above which inhibitory or lethal effects might occur. Vanadium and Ni were chosen because they are often environmental pollutants.

MATERIALS AND METHODS

The chironomid test provides information on the bioavailability and adverse effects of contaminants associated with whole sediment. Chironomus larvae have been used successfully in freshwater toxicity tests because the individuals are fairly large midges with a short generation time, are easily collected, and live in direct contact with the sediment. They have been shown to be sensitive to many contaminants associated with sediments. Methods for culture and testing are summarized in the ASTM (1992).

Chironomus plumosus larvae were acquired from natural water sediments. For the tests larvae 25 mm long were used. The tests were carried out in boiled and cooled tap water using metal solutions in concentrations corresponding with LC_{50} values for 96 hr exposure. These were estimated by the method described by Fargašová (1994). Duration of the exposure was 96 hr. Ambient temperature was 25 ± 1 °C, while water temperature was 20 °C. Specimens were kept out of direct sunlight, solutions were not aerated and organisms were not fed during the tests. The tests were carried out in 80 mm Petri dishes. The volume of solution used per dish was 20 mL. Each concentration was tested in triplicate with 10 organisms (Fargašová 1994). Survival was compared with that of control after 24, 48 and 96 hr. Only replicates in which 10% or less of control organisms died were considered for evaluation.

The tested metal ions were used in the following compounds and LC_{50} concentrations (mg/L): V^{5+} [V^2O_5] 0.24; Ni^{2+} [$NiSO_4.7H_2O$] 0.25; Mn^{2+} [$MnSO_4.H_2O$] 0.055; Mo^{6+} [$(NH_4)_6Mo_7O_{24}.4H_2O$] 0.36; Cu^{2+} [$CuSO_4.5H_2O$] 0.000091; Cu^{+} [Cu_2Cl_2] 0.00083. The following metal combinations were tested: $V^{5+} + Ni^{2+}$; $V^{5+} + Mo^{6+}$; $V^{5+} + Cu^{2+}$; $V^{5+} + Cu^{+}$; $V^{5+} + Mn^{2+}$; $Ni^{2+} + Mo^{6+}$; $Ni^{2+} + Cu^{2+}$; $Ni^{2+} + Cu^{+}$; $Ni^{2+} + Mn^{2+}$; $Mo^{6+} + Cu^{2+}$; $Mn^{2+} + Cu^{+}$; $Mo^{2+} + Mn^{2+}$; $Mn^{2+} + Cu^{2+}$; $Mn^{2+} + Cu^{+}$.

RESULTS AND DISCUSSION

It is important to have an agreement about what is meant by the term “interaction". A task group of metal interaction has discussed and formulated a definition as follows: "Interaction is a process by which metals in their various forms change the critical concentration or a critical effect of a metal under consideration"

(Nordberg 1976). Such changes are regarded as interactions only, if they deviate from single additivity. In other words, interactions should be called positive, if they cause overadditive (synergistic) effects, and negative, if they cause less than additive (antagonistic) effects.

Table 1. Influence of individual metals and their combinations on mortality of Chironomus plumosus larvae

			Mortality (%)		
Metal ions	Conc. (mg/L)	pH	24 h	48 h	96 h
V^{5+}	0.24	7.715	3.3	13.3	53.3
$V^{5+} + Mn^{2+}$	0.24 + 0.055	7.384	3.3	3.3	3.3
$V^{5+} + Mo^{6+}$	0.24 + 0.36	7.611	0	3.3	30
$V^{5+} + Ni^{2+}$	0.24 + 0.25	7.641	6.7	16.7	33.3
$V^{5+} + Cu^{2+}$	0.24 + 0.000091	7.628	0	3.3	20
$V^{5+} + Cu^{+}$	0.24 + 0.00083	7.643	0	0	6.7
Mn^{2+}	0.055	7.716	3.3	26.7	43.3
$Mn^{2+} + Mo^{6+}$	0.055 + 0.36	7.439	0	0	13.3
$Mn^{2+} + Ni^{2+}$	0.055 + 0.25	7.521	0	3.3	30
$Mn^{2+} + Cu^{2+}$	0.055 + 0.000091	7.666	0	0	20
$Mn^{2+} + Cu^{+}$	0.055 + 0.00083	7.698	0	0	6.7
Mo^{6+}	0.36	7.859	10	46.7	50
$Mo^{6+} + Ni^{2+}$	0.36 + 0.25	7.518	0	3.3	13.3
$Mo^{6+} + Cu^{2+}$	0.36 + 0.000091	7.558	0	3.3	13.3
$Mo^{6+} + Cu^{+}$	0.36 + 0.00083	7.52	3.3	3.3	36.7
Ni^{2+}	0.25	7.868	10	16.7	43.3
$Ni^{2+} + Cu^{2+}$	0.25 + 0.000091	7.622	3.3	6.7	53.3
$Ni^{2+} + Cu^{+}$	0.25 + 0.00083	7.521	0	3.3	30
Cu^{2+}	0.000091	7.707	3.3	13.3	46.7
Cu^{+}	0.00083	7.711	3.3	13.3	40

Table 1. presents the results of studies on the toxicity of metal ions alone and in combinations. Control larvae remained active during the whole test period, clustering at the bottom of the test dishes with typical movement like tubificid worms. Test animals remained separated at the beginning of the experiment and showed rapid twisting movement. In later phases of intoxication they showed reduced tactile movement. Necrosis and disintegration of the body as described by Khangarot (1991), appeared with no other noticeable signs. Their hemoglobin completely disappeared and the rear part of the body became white, and disintegrated. Similar changes in movement activity of juvenile nematodes treated with single and paired metal ions was reported by Jaworska et al. (1994). The

most toxic ions for Ch. plumosus larvae were Cu^{2+} and Cu^{+}. Cu^{2+} was 10 times and both were 100 to 10 000 times more toxic than the other metal ions. The high acute toxicity of Cu in comparison to Mo, Mn, and Ni was also noted against Tubifex tubifex (Khangarot 1991). In this case EC_{50} values for Cu ion were 10 000 times lower than those for Mo, Mn, and Ni. High toxicity of Cu is also known for other aquatic organisms such as Daphnia magna (Khangarot and Ray 1989; Tomasik et al. 1995a) and the fresh water snails Bulinus globosus (Tomasik et al. 1995b) and Lymnea luteola (Khangarot and Ray 1988). For B. globosus Cu^{2+} was the most toxic among 14 tested metals.

Based on the current observations, the tested metal ions can be divided into two groups: (1) metals very toxic at concentration levels which can naturally occur with eventual involvement of environmental pollution, and (2) metals toxic at concentrations that are naturally unrealistic. The first group includes only Cu^{2+} and Cu^{+} ions which occur naturally in the hydrosphere and rivers at 10^{-3} mg/L. The estimated LC_{50} values, which exterminated half of the population of Ch. plumosus in 96 hr, achieved the levels found in the environment (Tölgyessy 1993). The second group of ions consists of Ni, Mo, Mn, and V. Among them the possible LC_{50} for V, Mo, and Ni is in the order of 10^{-1} mg/L, and for Mn, 10^{-2} mg/L, indicating that these metals are virtually non-toxic (Tomasik et al. 1995b). Their LC_{50} values do not exceed the levels found in the environment.

Table 2. The effect of metal-metal interactions in Chironomus plumosus larvae

Metal	Effect of interaction [a]	Interacting ions	Metal	Effect of interaction [a]	Interacting ions
V^{5+}	A	Mn, Mo, Ni, Cu^{2+}, Cu^{+}	Ni^{2+}	A	Cu^{+}, Mo, Mn, V
	S			S	Cu^{2+}
	I			I	
Mn^{2+}	A	Mo, Ni, Cu^{2+}, Cu^{+}, V	Cu^{2+}	A	Mo, Mn, V
	S			S	Ni
	I			I	
Mo^{6+}	A	Ni, Cu^{2+}, Mn, V	Cu^{+}	A	Ni, Mo, Mn, V
	S			S	
	I	Cu^{+}		I	

A - antagonistic effect; S - synergistic effect; I - indifferent effect

We next tried to determine how metals influence each other in their toxicity to *Ch. plumosus* larvae. The results of these studies are shown in Table 1. It is evident that the toxicity of metals in combination is different from that of individual metals, either because of antagonism or synergism. A third alternative is indifference. On

the basis of these terms, the results are summarized in Table 2. From this table it can be concluded that in metal-metal combinations the prevailing action was antagonism. Synergism occurred only in the combination of Ni^{2+}+ Cu^{2+} in which the 96 hr mortality of chironomid larvae increased when compared comparison with that observed with Ni^{2+} and Cu^{2+} alone - about 10 % and 6.6 % higher, respectively. Indifference was observed after 96 hr treatment only in the combination Mo^{6+} Cu^{+} when compared to toxicity of Cu^{+} alone. In this case it can be concluded that Mo^{6+} ion is indifferent to Cu^{+} ion but Cu^{+} ion has an antagonistic effect to Mo^{6}+ ion (decreasing mortality about 13.3 %). When the intensity of antagonistic effects in combinations after 96 hr exposure was compared, the possibility to divide combinations into two groups became apparent, with one group including those combinations with very strong antagonistic effect (mortality between 3.3 - 20 % only); and another group including those combinations with gentle antagonistic effects (decreasing mortality about 20 %: V^{5+}+ Mo^{6+}, V^{5+}+ Ni^{2+}, Mn^{6+}+ Ni^{2+}, Mo^{6+}+ Cu^{+}, and Ni^{2+}+ Cu^{+}) (Tab. 1.).

It is commonly known that the resistance of organisms to toxic metals depends on the metal, its valence and concentration as well as on the organism and its physiological conditions. Chironomid larvae are considered as very sensitive benthic organisms (Fargašová 1997), which during our tests confirmed. They were sensitive to extremely low concentrations of copper ions (10^{-4} mg/L of Cu^{2+} and 10^{-3} mg/L of Cu^{+}). The LC_{50} values for other tested metals did not exceeded 10^{-1} mg/L. Attention should be directed especially to copper. Copper is biologically available as Cu^{+} or Cu^{2+} inorganic salts and in organic complexes. Water organisms take in high amounts (Chang and Sibley 1993; Fargašová et al. 1997) and interactions with other metals have been observed (Dirilgen and Inel 1994; Tomasik et al. 1995a; Jaworska et al. 1996).Toxicity of other tested metals (Mo, Mn, and Ni) is several times lower compared with Cu (Lee et al. 1994) and toxicity of V is seen only rarely (Tomasik et al. 1995b; Jaworska et al. 1996). Metal-metal combinations can decrease the toxic effects of metals by decreasing their accumulated amounts (Fargašová et al. 1997). Molybdenum is also considered to be a micronutrient. Nickel and vanadium are reported to be highly toxic (Hetmánska and Tomasik 1994), and vanadium highly (Patel et al. 1988) or slightly toxic (Hetmanska and Tomasik 1994), in our experiments V^{5+} was only slightly toxic. In combination with other tested metals vanadium greatly decreased their toxic effects extremely high especially for Mn^{2+} and Cu^{+} (Tab. 1.). We have early reported that V also inhibited the bioaccumulation of Mn and Ni by the alga *Scenedesmus quadricauda* and it has a neutral effect on Mo accumulation (Fargašová et al. 1997). All above mentioned metals entered into the interactions decreasing their toxic effects.

Because of the very limited literature on the toxicity to benthic organisms of metal-metal interaction of V, Ni, Mo, Mn and Cu there is no possibility to compare our results with Ch. plumosus to that of other authors. It can be concluded that metals in combinations very often decrease their individual toxicities. The interaction between ions of particular atoms can be caused by competition for the same reaction centers. A comparison of the lethality of metals and their combinations for Ch. plumosus and other water biota mentioned in the literature (Tomasik et al. 1995a, Tomasik et al. 1995b) reveals that various aquatic biota react in different ways to metals. Some combinations of metal ions synergistic in one organism are antagonistic in another.

REFERENCES

ASTM (1992) Standard guide for collection, storage, characterization, and manipulation of sediment for toxicological testing, Method E1391-90, Annual Book of ASTM Standards, Water and Environmental Technology, Vol. 11.04, American Society for Testing and Materials, Philadelphia, PA

Baccini P (1984) Metal transport and metal biota interactions in lakes. In: Proc Symp “Micro-pollutants in sediment water systems", Delft, The Netherlands, pp. 15-22

Chang C, Sibley TH (1993) Accumulation and transfer of copper by Oocystis pusilla. Bull Environ Contam Toxicol 50:689-695

Dirilgen N, Inel Y (1994) Effects of zinc and cooper on growth and metal accumulation in duckweed Lemna minor. Bull Environ Contam Toxicol 53: 442-449

Fargašová A (1994) Toxicity of metal on Daphnia magna and Tubifex tubifex. Ecotox Environ Saf 27:210-213

Fargašová A (1997) Comparative study of ecotoxicological effect of triorganotin compounds on various biological subjects. Ecotoxicol Environ Saf 36:38-42

Fargšová A, Bumbálová A, Havránek E (1997) Radionuclide X-ray fluorescence analysis of metal bioaccumulation by freshwater alga Scenedesmus quadricauda. J Radioanal Nucl Chem 218: 107-110

Hetmánska B, Tomasik P (1994) The metal-metal interactions in biological systems. Part II. Saccharomyces cerevisiae. Wat Air Soil Pollut 74: 281-288

Jaworska M, Sepiol J, Tomasik P (1996) Effect of metal ions under laboratory conditions on the entomopathogenic Steinernema carpocarsae (Rhabditida: Steinernematidae). Wat Air Soil Pollut 88:331-341

Khangarot BS (1991) Toxicity of metals to a freshwater tubificid worm, Tubifex tubifex (Müller). Bull Environ Contam Toxicol 46:906-912

Khangarot BS, Ray PK (1988) Sensitivity of freshwater pulmonate snails, Lymnea lutiola L. to heavy metals. Bull Environ Contam Toxicol 41:208-213

Khangarot BS, Ray PK (1989) Investigation of correlation between physico-chemical properties of metals and their toxicity to the water flea Daphnia magna Straus. Ecotoxicol Environ Saf 18: 109-120

Lee LH, Lustigman B, Dandorf D (1994) Effect of manganese and zinc on the growth of Anacystis nidulans. Bull Environ Contam Toxicol 53: 158-165

Lion LW, Altmann RS, Leckie JO (1982) Trace metal adsorption characteristics of estuarine particulate matter: Evaluation of contributions of Fe/Mn oxide and organic surface coatings. Environ Sci Technol 16:660-666

Martin JM, Windom HL (1991) Present and future roles of ocean margins in regulating marine biogeochemical cycles of trace elements. In: Ocean Margin Processes in Global Changes. Martoura RFC, Martin JM, Wollast RW (eds.), pp. 45-67. Wiley, Oxford

Nordberg GF (1976) Effects and dose response relationships of toxic metals. Elsevier, Amsterdam

Pate1 B, Haswell S, Grzeskowiak R (1988) Heavy metal hydrological cycle. Astruue M, Lesster JN (eds.), Selfer Ltd., London. p. 199

Tomasik P, Magadza ChHD, Mhizha S, Chirum A (1995a) The metal-metal interactions in biological systems. Part III. Daphnia magna. Wat Air Soil Pollut 82: 695-711

Tomasik P, Magadza ChHD, Mhizha S, Chirum A, Zaranyika MF, Muchiriri S (1995b) Metal-metal interactions in biological systems. Part IV. Bulinus globosus. Water, Air and Soil Pollut 83:123-145

Tölgyesssy J (ed.) (1993) Chemistry and biology of water, air and soil. Environmental aspects. Elsevier, Amsterdam - London - New York -Tokyo

Van der Meent D, De Leeuw JW, Schenck PA, Salomons W (1985) Geochemistry of suspended particulate matter in two natural sedimentation basins of the river Rhine. Wat Res 19:1333-1340