Ecotoxicology of Metals Related to Freshwater Benthos

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Abstract. The toxicity of Cu, Mn, Mo, Ni, V, As, Pb, Cr, Hg and Sn on the behaviour and survival of benthic worms Tubifex tubifex was studied. All tested metals were dissolved and determined in tap water under standardized conditions. The adverse effects of the metals were evaluated as acute toxicological effects upon exposure expressed as LC50 value with 95 % confidence interval. On the basis of the LC50 values, the toxicity of the metals after an incubation for 96 h was ranked as follows: Cu(II)>Cu(I)>V>Hg>Mn>Ni>Cd>Cr>Mo>Pb>Sn(IV)=Sn(II)>As. From this sequence it is evident that copper was the most toxic metal ion. In addition, differences were found between Cu(II) and Cu(I) ions toxicity, the former being 2.5 times more toxic. In contrast, no differences could be confirmed between acute toxic effects of Sn(II) and Sn(IV). Arsenic showed the weakest toxicity of the tested metals. The LC50 value for As was 10,000 times higher than those for both copper ions.

Key words: Acute toxicity — Benthos — Tubifex tubifex — Inorganic metal compounds

Introduction

The pollution of aquatic ecosystems with metals is a serious environmental problem. Aquatic ecosystems are monitored for the pollution by heavy metals using chemical or biological assays (Wong and Dixon 1995). Although precise, chemical determinations of heavy metals concentrations in water do not provide information about the biotoxicity of a heavy metal. The biological impact of heavy metals on aquatic invertebrates has been extensively studied under natural and laboratory conditions (Dallinger 1994). Aquatic invertebrate organisms accumulate heavy metals and have long been used as bioindicator species of environmental pollution and for bioassays of pollutants (Hare 1992). Benthic macroinvertebrates occupy various niches and functional feeding groups (Merrit and Cummins 1978), and their behavior can suggest the level of metal contamination (Timmermans et al. 1989). The species composition of benthic communities has often been related to water
quality (Battegazzore and Renoldi 1995). An accurate laboratory assessment of the toxic potential of a given contaminant also requires the selection of appropriate test organisms. Species such as *Tubifex tubifex* have been perceived to be sensitive enough to various contaminants (Khangarot 1991). Besides mortality, exposure of aquatic invertebrates to metals can result in deviations of exclusion rates, locomotion, behavior, oviposition, and mating (Rayms-Keller et al. 1998).

The endpoints for detecting biotoxicity used in this study were mortality and the body burden. Concurrently, the acute toxicity of inorganic metal substances was tested on commonly used ecotoxicological model *Tubifex tubifex* worms and expressed as LC50 value and its 95% confidence interval.

**Materials and Methods**

The worms *Tubifex tubifex* were collected from natural water sediments. Before testing they were washed up with tap water for 24 h. Only mature individuals, 20 mm long, were included in the test procedures. The tests were carried out in boiled tap water (80 mg/l for Ca2+, 27 mg/l for Mg2+, pH 7.8 ± 0.5). After the range-finding preliminary tests, the stock solutions of the metals were prepared in order to obtain six concentrations at the same common logarithmic intervals. The metals were added to the boiled tap water to obtain specific metal concentrations. At the start of each test, organisms (n=10) were added to Petri dishes with a diameter of 80 mm containing 20 ml of tested metal solution each. After incubation for 24, 48 or 96 h in the contaminated water the toxic effect was recorded and compared with the control. Animals which (when gently prodded with a spatula) were showing no movement of the body, were considered dead. During the tests performed under static conditions, without aeration and under a normal daylight photoperiod cycle, the animals were not fed and, like in all handling and rearing operations, the temperature of the water was kept at 20 ± 1°C. Details on these toxicological endpoints can be found in ASTM (1992).

The number of dead organisms in each particular Petri dish of all triplicates representing a total of 30 animals (3 x 10) did not differ by more than ± 2 individuals. This split of readings constituted a maximum experimental error of 10%. The toxicological potential of the tested compounds was expressed in terms of the LC50 value (or 50% lethal concentration), which is the concentration of a compound required to induce 50% mortality. For each substance and for each assay, the LC50 value was calculated by the probit analysis of the data expressed as percentages of the control response versus the concentrations of the substances tested (Stephan 1977). The LC50 values and their 95% confidence intervals were expressed as nominal concentrations of toxic metals. T-test was used to evaluate the differences in worms survival between the control and the treated groups. The 5% alpha probability level was used in all statistical analyses.

The inorganic metal substances tested included Cu(I) (as Cu2Cl2 salt), Cu(II) (CuSO4 5H2O), Ni (NiSO4 7H2O), V (V2O5), Mn (MnSO4 H2O), Mo ((NH4)6Mo7O24 4H2O), As (Na2HAsO4 7H2O), Pb (Pb(CH3COOH)2 3H2O), Cr ((NH4)2CrO4), Hg (HgCl2), Cd (CdCl2 2.5H2O), Sn(II) (SnCl2 2H2O) and Sn(IV) (Na2SnO3). All substances were of analytical grade, and were purchased from Merck (Darmstadt, Germany), except SnCl2 2H2O and Na2SnO3 which were purchased from Lachema (Brno, Czech Republic). Each metal ion was tested in ten concentrations (mg/l) 0.02 - 1.5 Hg, 0.5 - 4.0 Cd, 2.0 - 30.0 Pb, 50.0 - 200.0 As, 1.0 - 10.0 Cr, 0.0003 - 0.03 Cu(I), 0.0001 - 0.01 Cu(II), 0.05 - 1.0 Mn, 1.0 - 10.0 Mo, 0.1 - 1.0 Ni, 0.1 - 1.0 V, 5.0 - 150.0 Sn(II), 5.0 - 150.0 Sn(II).
Table 1. LC50 values (mg/l) for the tested metal ions and their corresponding 95% confidence intervals (CI) for T tubifex

<table>
<thead>
<tr>
<th>Metal</th>
<th>LC50 ± 95% CI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Cu(I)</td>
<td>0.021 (0.017-0.028)</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>0.005 (0.003-0.006)</td>
</tr>
<tr>
<td>Mn</td>
<td>0.768 (0.735-0.827)</td>
</tr>
<tr>
<td>Mo</td>
<td>60.57 (52.36-73.82)</td>
</tr>
<tr>
<td>Ni</td>
<td>0.669 (0.640-0.729)</td>
</tr>
<tr>
<td>V</td>
<td>0.578 (0.506-0.671)</td>
</tr>
<tr>
<td>As</td>
<td>398.11 (382.4-443.1)</td>
</tr>
<tr>
<td>Cr</td>
<td>4.36 (3.60-8.67)</td>
</tr>
<tr>
<td>Hg</td>
<td>0.51 (0.48-0.55)</td>
</tr>
<tr>
<td>Cd</td>
<td>3.09 (2.59-3.67)</td>
</tr>
<tr>
<td>Sn(II)</td>
<td>125.8 (118.7-132.5)</td>
</tr>
<tr>
<td>Sn(IV)</td>
<td>120.0 (117.0-132.0)</td>
</tr>
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</table>

Results and Discussion

The toxicological potential of the inorganic metal compounds tested, expressed as their LC50 values and 95% confidence intervals (CI) are summarized in Table 1. The survival of the control organisms in all experiments ranged from 91 to 100%. As in all treatments T tubifex responses to metal substances were strongest after 96 h, and significantly different from those obtained after 24 and 48 h, LC50 values were only compared for this time interval. All data obtained from the experiments after 96 h passed the normality and homogeneity test at the 0.01 level, but the lower concentrations for some experiments, especially in the case of Hg (0.2 mg/l) and Pb (2.0 mg/l), yielded data not significantly different from the controls at the 0.05 level.

After 96 h of treatment the LC50 values for metals varied from less than 20
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μg/l for Cu(II) to more than 100 mg/l for As. The LC₅₀ values allowed the substances to be arbitrarily ranked in five levels of toxicity (increasing one logarithm at each level): "very high" toxicity LC₅₀<8.0 μg/l (Cu(I), Cu(II)); "high" toxicity 0.1<LC₅₀<0.6 mg/l (V, Hg, Mn, Ni); "moderate" toxicity 0.7<LC₅₀<5.0 mg/l (Cd, Cr, Mo); "low" toxicity 8.0<LC₅₀<33.0 mg/l (Pb, Sn); and "very low" toxicity LC₅₀<140.0 mg/l (As). The sequence of toxicity of the metals after 96 h incubation was as follows: Cu(II)>Cu(I)>V>Hg>Mn>Ni>Cd>Cr>Mo>Pb>Sn(IV)=Sn(II)>As. On the basis of this toxicity sequence it can be concluded that copper (both univalent and bivalent) was the most toxic ion to *T. tubifex* worms. The LC₅₀ values for these ions were 10² – 10⁴ times smaller than those for other metal ions. Cu(II) ion was 2.5 times more toxic than Cu(I). The high acute toxicity of copper to *T. tubifex* has also been confirmed by Khangarot (1991). In this case the value of EC₅₀ (e.g. the effective concentration at which 50 % immobilization response was recorded) for Cu ions was 1000 times smaller than that for Mn, and 100 times smaller than those for Sn, Mo, Cd and Ni. High Cu toxicity has also been observed for other aquatic organisms (Khangarot and Ray 1989; Tomasik et al. 1995). Copper, as an essential biological element, interferes with numerous physiological functions. It is a constituent micronutrient of the protein component of several enzymes, mainly of those participating in electron flow, catalysing redox reaction in mitochondria, cell wall and in the cytoplasm of cells (Murthy and Mohanty 1995). The key role of copper to animals is mediated through specific copper proteins. Cytochrome oxidase, the most important copper protein, is probably directly associated with the most dramatic form of copper deficiency in animals (Buck 1977). The evolutionary process during which copper was selected to carry out specific physiological roles has avoided its toxic potential in three ways: (1) by developing an active process to eliminate any excess copper ingested in the diet; (2) by reducing the thermodynamic activity of copper ions virtually to zero by utilizing the metal only as a prosthetic element tightly bound to specific copper proteins; and (3) by an interaction between zinc and copper (Scheinberg and Sternlieb 1984). Copper is probably only toxic to man and animals when one of these mechanisms is defective, either because of genetic or acquired reasons. Then, ionic copper accumulates in excess of that which can be incorporated into normally occurring copper proteins. Copper toxicity seems entirely or in large part to be due to free copper ions combining with new copper proteins and altering their physiological functions (Aaseth and Norseth 1986).

V, Hg, Mn and Ni showed strong toxicity to the tested worm species but the toxicity of lead and tin was weak. Arsenic was shown to have the weakest toxicity of all the metals tested. There are many species and strain differences in the toxicity of arsenic compounds. The purity, physical form, and solubility of the compounds also influence toxicity. Arsenic toxicity is not related to water hardness, but is positively correlated with temperature. Lower pH also increases toxicity due to As³⁺ formation (US EPA 1980, 1981). Whitley (1967) has suggested that the toxic action is due to the formation of mucus-metal complex which precipitates on the body wall of worms and blocks the exchange of oxygen and carbon dioxide. By
Khangarot (1991) not only Hg but also Pb are introduced as very toxic to *T. tubifex*, while weak toxicity was confirmed for As and Cd. In our tests, LC$_{50}$ values for As, Pb, and Hg were higher and those for Cd were smaller than those reported by Khangarot (1991). The acute toxicity of metal ions varied with changes in water quality (Khangarot and Ray 1989) and some water parameters (e.g., pH value) (Whitley 1967). The acute toxicity of As was not strong similarly as the toxicity of lead and arsenic. Toxicity studies of inorganic Sn are lacking due to the opinion that inorganic Sn undergoes hydrolysis to form hydroxides or SnO$_2$ which are known to be non-toxic (McDonald and Trevors 1988). The small differences between Sn(II) and Sn(IV) compounds toxicity were insignificant. Differences between our results and those published by Khangarot (1991) for SnCl$_2$ 2H$_2$O were evident only after 48 h of incubation (in our study LC$_{50}$/48 h values were 2.5 times smaller for SnCl$_2$ 2H$_2$O and 5.0 times smaller for Na$_2$SnO$_3$).

Based on the determined mortality, the tested metal ions can be divided into two groups: (1) metals very toxic at the concentration levels that can occur in the nature, and (2) metals toxic at concentrations that are unrealistic in the environment. The first group includes only Cu(II), Cu(I) ions that occur naturally in the hydrosphere and rivers at $10^{-3}$ mg/l. Their estimated LC$_{50}$ values in *T. tubifex* after 96 h were similar to levels found in the environment (Tolgyessy 1993). Other tested metals represent the second group of ions. The LC$_{50}$ values for V, Mn, Hg, and Ni are in the order of $10^{-1}$ mg/l and $10^1$ mg/l for Mo, Cr, and Cd, $10^2$ mg/l for Pb, Sn(I) and Sn(II), or $10^3$ mg/l for As, indicate that these metals are virtually non-toxic. Their LC$_{50}$ values do not exceed the levels found in the environment (Tolgyessy 1993).

Furthermore, the metals tested evoked an uncoordinated writhing in the exposed worms. The control animals remained active throughout the test period. They were clustering at the bottom of the dishes with typical movements. Treatment with lower concentrations of metals had no effect on the exposed animals. In higher concentrations when a lethal effect appeared, the behavior and the appearance of the worms were observed to change. The treated animals remained separated at the beginning of the experiment and showed rapid twisting movements. In later phases of intoxication, they showed reduced tactile movement. Before their death, 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 have been reported for planarians (Calevro et al. 1998) treated with metals as well as for other larvae living in the benthos (Rayms-Keller et al. 1998).

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