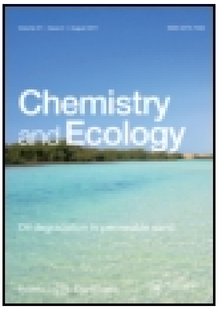

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Effects of polyaluminium chloride on the freshwater invertebrate *Daphnia magna*

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In a number of countries across the world, aluminium in the form of polyaluminium chloride has been used in the treatment of freshwaters for the direct removal of cyanobacteria, or phosphorus removal, but knowledge about its effect on zooplankton species is poor. In our study, polyaluminium chloride toxicity was tested on both artificial and natural freshwaters for a better understanding and prediction of effects in real ecosystems. Our results indicate that prediction of effects in a real ecosystem based on standard ISO methods is insufficient, and tests with nontarget species (including invertebrates) should be done before each treatment using the water samples from the treated location. Effective concentrations of polyaluminium chloride can differ markedly according to the type of water composition used in the assay. Our experiments proved that EC_{50} values can fluctuate between 9.89 and 54.29 $mg \cdot L^{-1}$ of Al^{3+}, and the toxicity is dominantly dependent on the treated water conductivity. This parameter seems to be the dominant source of different effects on zooplankton species after treatment and thus should be properly tested before each use of polyaluminium chloride as a treatment compound.

Keywords: polyaluminium chloride; PAX18; removal; cyanobacteria; bloom

1. Introduction

The occurrence of toxic freshwater cyanobacterial blooms and their toxins has been reported across the world in many studies [1–3]. Cyanobacterial toxins (microcystins, cylindospermopsin and many others) present serious problems for both aquatic and terrestrial organisms, including humans [4,5]. Many methods have been developed for the management of cyanobacterial blooms in dams, reservoirs and lakes. All these methods can be classified by several criteria according to: (1) application site (water column, sediment); (2) purpose (decrease of nutrient bioavailability, inhibition of cyanobacterial growth, mineralisation of sediments); and (3) character of method (physical, biological, chemical).

The use of chemical algicides as the most common method in controlling of noxious phytoplankton may cause toxicity towards nontarget species, the accumulation of metals in sediments, cell lysis and a release of intracellular toxins. One of the most promising control strategies is coagulation of blooming phytoplankton species by different types of compounds; clays, aluminium sulfate, polyaluminium chloride, ferric chloride, slaked lime and others. The main objective of

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these applications is to immobilise cyanobacterial cells by creating physico-chemical interactions between phytoplankton species and coagulant. It is assumed that the cell life cycle will be disturbed and cyanobacteria will be slowly decomposed at the surface layer of sediments as a result of the coagulation process. Aluminium salts and polymers are used to help remove nutrients and insoluble materials (including phytoplankton species) from waters during drinking water production [6], industrial and domestic wastewater treatment [7,8], sludge treatment [9], etc. In a number of countries across the world, aluminium in the form of polyaluminium chloride has been used in the treatment of freshwaters for the direct removal of cyanobactrial cells without detailed knowledge of its effect on invertebrates. Although aluminium occurs in natural waters, its concentration is very low (because of the low solubility of aluminium in the pH range 6–8). By contrast, introducing a high dosage of aluminium into water as a treatment agent might have toxic effects on aquatic nontarget species.

The aim of this study was to evaluate the potential toxic effects of aluminium in the form of polyaluminium chloride on freshwater invertebrates (daphnids) as representatives of sensitive nontarget aquatic organisms. For this study, polyaluminium chloride toxicity was tested in both artificial and natural freshwaters for a better understanding and prediction of the effects in real ecosystems.

2. Materials and methods

2.1. *Tested chemical*

Polyaluminium chloride (PAX18) (Kemifloc a.s., Prerov, Czech Republic) is a yellowish liquid coagulant freely miscible with water. Polyaluminium chloride contains $9.0 \pm 0.2\%$ of aluminium, its pH is 0.9 ± 0.3 and basicity is $42 \pm 2\%$.

2.2. *Standard bioassay with* Daphnia magna

Daphnia magna bioassays were performed according to ISO 6341 [10], modified in the application of toxicant (polyaluminium chloride). Juveniles of *D. magna* (continuous laboratory breeding, juveniles < 24 h old) were randomly transferred into separate polystyrene plates with standard exposure solution. Each concentration and control was tested in four replicates. Temperature was maintained at 20 ± 2 °C during exposure and the test was performed in the dark. Daphnids were inspected after 24 and 48 h of exposure. Acute toxicity was expressed as the average effective concentration (EC_{50}) for immobilisation. According to the ISO test, transfer of the larvae to the multiwell plate is accomplished in two steps: (1) transferring the larvae from the hatching dish to the rinsing wells, and (2) transferring the larvae from the rinsing wells to the test wells. This transfer though rinsing wells minimises the dilution of the toxicant solutions in the test wells. However, because polyaluminium chloride is a rapidly reacting chemical in neutral waters (immediately causing floccules to settle to the bottom), in our tests, polyaluminium chloride was applied directly to the wells with tested fresh water and daphnids. This application should have no influence on the outcome of the assay because the amount of chemical was always $< 0.5\%$ v/v.

2.3. Daphnia magna *bioassay with natural waters*

Three natural water samples for polyaluminium chloride toxicity testing with daphnids were obtained from the Brno reservoir (Brno, Czech Republic), the Splaviska pond (Brno-Chrlice, Czech Republic) and the Novovesky pond (Nova Ves, Czech Republic). Values of pH and conductivity were as follows: Brno reservoir water (pH, 6.1; conductivity, 317 $\mu S \cdot cm^{-1}$), Splaviska pond water (pH, 6.4; conductivity, 725 $\mu S \cdot cm^{-1}$) and Novovesky pond (pH, 7.4; conductivity, 1044 $\mu S \cdot cm^{-1}$). In these bioassays, ISO standard fresh water was replaced by natural water samples from real aquatic ecosystems to compare the effects of PAX-18 between real samples and ISO fresh water. The bioassay experiments were conducted in the same way as in the modified bioassay described above (Standard bioassay with *Daphnia magna*).

2.4. *Aluminium (Al^{3+}) concentrations tested*

For each type of freshwater used in the study (i.e. standard ISO freshwater, water from the Brno reservoir, Splaviska pond and Novovesky pond), different series of concentrations were used. On the basis of the first screening test (1000, 100, 10 and 1 $mg \cdot L^{-1}$ of Al^{3+}), concentrations for the final toxicity assessment were made. Final-concentration solutions were prepared as follows: ISO freshwater (50, 25, 12.5, 6.25, 3.125 $mg \cdot L^{-1}$ of Al^{3+}), Brno reservoir water (100, 50, 25, 12.5, 6.25 $mg \cdot L^{-1}$ of Al^{3+}), Splaviska pond (500, 250, 125, 62.5, 31.25 $mg \cdot L^{-1}$ of Al^{3+}) and Novovesky pond (250, 125, 62.5, 31.25, 15.56 $mg \cdot L^{-1}$ of Al^{3+}). Different concentrations for each water sample were prepared to reach the optimal dilution row according to which it was possible to calculate EC_{50} values. Values of pH and conductivity were measured during the tests using WTW Multiline P4 (Weilheim, Germany) electrode sensors.

3. Results

The toxic effects of polyaluminium chloride are shown in Table 1 as EC_{50} values after 24 and 48 h of exposure. EC_{50} values are always lower after 48 h of incubation than after 24 h of incubation, which obviously relates to the longer contact between the chemical and the tested organism. Effective concentrations were found to be 10.71 and 10.33 $mg \cdot L^{-1}$ of Al^{3+} for standard ISO freshwater, 54.29 and 43.47 $mg \cdot L^{-1}$ for Novovesky pond water, 11.52 and 9.89 $mg \cdot L^{-1}$ for Brno reservoir water and 15.8 and 12.18 $mg \cdot L^{-1}$ of Al^{3+} for Splaviska pond water. The percentage of immobilised daphnids in various concentrations is shown in Figure 1.

Application of polyaluminium chloride was connected with a decrease in pH values during toxicity testing. The evolution of pH changes over time is depicted in Figure 2 for all types of waters tested. At the end of testing, the conductivity of all waters used was measured as well. The conductivity of standard ISO freshwater changed from 648 $\mu S \cdot cm^{-1}$ (control) to 937 $\mu S \cdot cm^{-1}$ (in highest concentration tested) at the end of the test. A similar increase in conductivity was also observed in other waters tested as follows: from 317 to 2066 $\mu S \cdot cm^{-1}$ for Brno reservoir water, from 1044 to 2285 $\mu S \cdot cm^{-1}$ for Novovesky pond water and from 725 to 1234 $\mu S \cdot cm^{-1}$ for Splaviska pond water.

Table 1. Comparison of EC_{50} ($mg \cdot L^{-1}$) values and standard deviations for Al^{3+} in different types of fresh water.

Water used	24 h EC_{50}	48 h EC_{50}
Standart ISO freshwater	10.71 (±0.51)	10.33 (±0.26)
Novovesky pond water	54.29 (±2.56)	43.47 (±1.25)
Brno reservoir water	11.52 (±0.63)	9.89 (±0.68)
Splaviska pond water	15.80 (±1.94)	12.18 (±0.54)

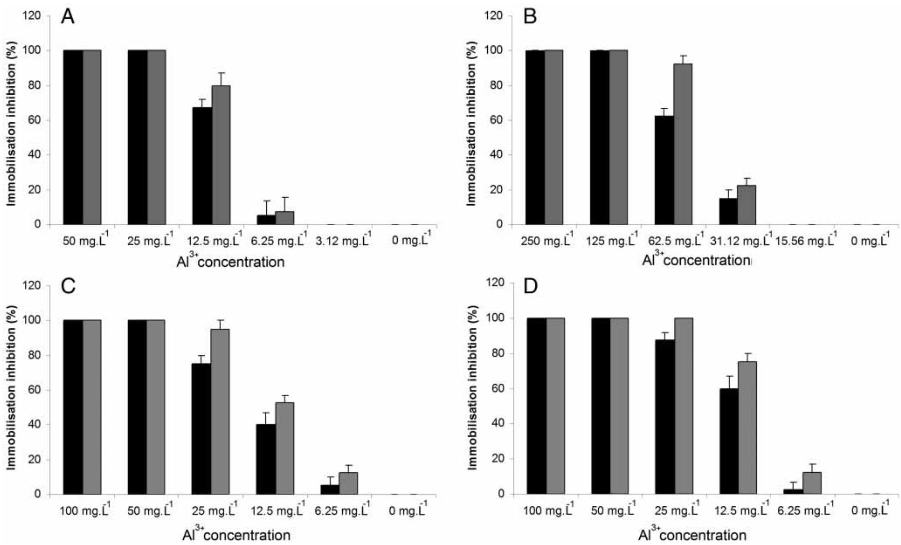

Figure 1. Percentage of immobilised daphnids in different freshwaters after application of aluminium (A, standard ISO medium; B, Novovesky pond; C, Splaviska pond; D, Brno reservoir) after 24 h of exposure (black columns) and 48 h of exposure (grey columns).

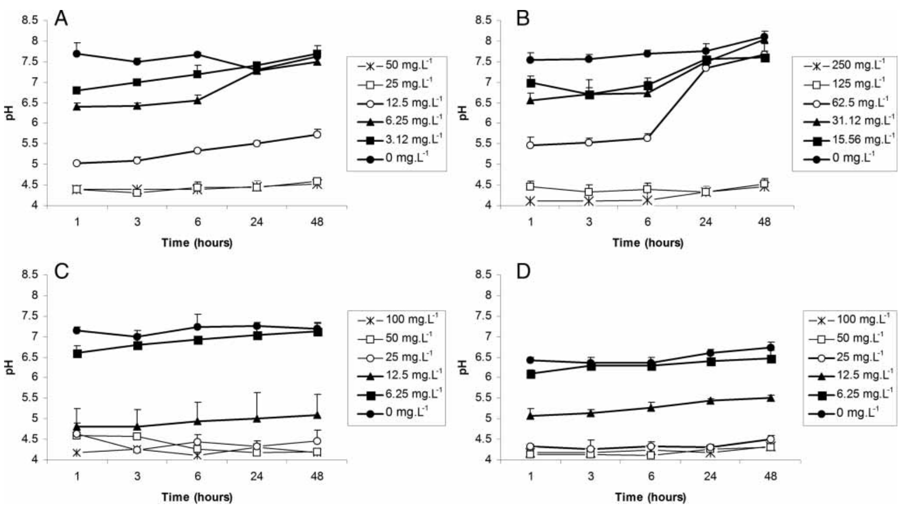

Figure 2. Changes in pH during immobilisation tests in different freshwaters after application of aluminium (A, standard ISO medium; B, Novovesky pond; C, Splaviska pond; D, Brno reservoir).

4. Discussion

An increasing dosage of aluminium in an individual test was connected with a decrease in pH, which is obviously caused by the addition of polyaluminium chloride into the water (the pH of PAX-18 was found to be ~ 1). It is known that aluminium solubility increases with declining water pH [11] and thus its toxicity takes effect. This has been confirmed in other studies assessing the toxic effect of aluminium on not only crustaceans, but also fish [12] and phytoplankton [13] species. The fact that a higher solubility of aluminium, as a result of lower pH values in water, occurred during toxicity tests was proved by the higher conductivity measured at the end of assays.

Conductivity was found to be two to six times higher in comparison with controls in all treated waters.

Because treatment of medium or water samples with polyaluminium chloride was followed by a dose-dependent decrease in pH after the preparation was applied, we are not able to clearly determine whether the final toxic effect of polyaluminium chloride on daphnids was due to the increasing concentrations of aluminium itself or because of increasing solubility in water.

Similar results have been published previously by Macova et al. [14] who investigated the toxic effects of the preparation PAX-18 on different developmental stages of the common carp (*Cyprinus carpio*). Macova et al. reported that whereas the 96 h LC_{50} value observed in an acute toxicity test on juvenile stages of the fish was $753 \pm 24.3\,mg{\cdot}L^{-1}$ (i.e. $67.8\,mg{\cdot}L^{-1}$ of aluminium), the effect on early developmental stage expressed as the 'no observed effect' concentration was $10\,mg{\cdot}L^{-1}$ (i.e. $0.9\,mg{\cdot}L^{-1}$ of aluminium).

The toxic effects of aluminium on daphnids have also been studied previously by Strigul et al. [15] who evaluated acute toxicity for four different types of nanoparticles (boron, titanium dioxide, and two types of aluminium nanoparticles differed by surface coating). As Strigul et al. stated, particles coated with a thin layer of aluminium oxide, which are not hydrophobic, showed a 24 h LD_{50} value of $219.6\,mg{\cdot}L^{-1}$, whereas the 48 h LD_{50} value was $7.483\,mg{\cdot}L^{-1}$. However, hydrophobic aluminium nanoparticles coated with carboxylate ligand showed a 48 h LD_{50} value of $107.588\,mg{\cdot}L^{-1}$. Strigul et al. also mentioned that the actual concentration of hydrophobic nanoparticles in suspension is lower than declared, because some particles float on the water surface.

According to our results, the composition of the freshwater used in the test can strongly influence the toxicity of polyaluminium chloride on crustaceans. For example, 24 h EC_{50} for aluminium in water from the Novovesky pond was five times higher than the same endpoint realised in standard ISO freshwater. A lower toxic effect was also found in another water sample, the Splaviska pond water. The different toxicity results for different water samples can be explained as the presence/absence of chelators, buffering capacity, concentrations of humic acids or the initial pH of the water.

As a trivalent metal ion, aluminium can bind strongly to oxygen donor ligands such as hydroxide, carboxylic acids or phosphates. When associated with low pH, these chelators can strongly influence aluminium solubility, leading to higher availability (absorption) for cells [16]. Nevertheless, in standard ISO freshwater there are no such compounds and thus, the high toxic effects cannot be explained by the presence of chlelators. Moreover, the presence of sodium bicarbonate may decrease the toxicity of some heavy metals, which could mainly be attributed to the formation of complexes [17]. Carbonates also play important role in water ecosystems. Their concentrations are responsible for the buffering capacity of waters and thus guard against sudden variations in pH. Thus, the buffering capacity of natural waters (especially those from the Novovesky pond and the Splaviska pond) seems to be important for the development of pH values after aluminium treatment.

In natural waters, aluminium occurs commonly in the form of soluble salts, namely chlorides, sulfates and nitrates. Nevertheless, these different forms of aluminium should not alter the toxic effects on water organisms, as published by Sauvant et al. [18]. However, other metals, such as copper or manganese with aluminium, may cooperate in waters and influence toxicity through synergistic, as well as antagonistic, processes [19]. But since we did not determine all metals, herbicides, toxic residues and other possible materials which could affect aluminium toxicity, speculations would be ineffective here.

The presence of humic acids might be another parameter that affects the toxicity of aluminium in waters from real ecosystems, because humic acids have a significant impact on the bioavailability and toxicity of aluminium to freshwater biota through their ability to form complexes that affect aluminium speciation [20]. This was confirmed in a study by Dobranskyte et al. [21] where the behavourial toxicity of Al was reduced by humic acid. This might partly explain differences between the toxicity in real ecosystem water treated with alum and artificial ISO water, because no humic substances are present in ISO water.

According to our observations, the imobilisation of daphnids can be caused by both the toxic effect of aluminium, and daphnids being trapped by forming floccules of polyaluminium chloride following water treatment. This usually occurs when a large number of insoluble particles are present in the water and thus the formation of large floccules is connected with the trapping of dapnids. Daphnids immobilised in this way are usually easy to identify because they settle at the bottom encapsulated by floccule and move by antennae. In our tests, this occurred twice in Splaviska water and water fleas were counted as immobilised according to the ISO norm.

In general, we may summarise that the toxic effects of polyaluminium chloride are connected with water conductivity and the decrease in pH after application of polyaluminium chloride to the water. Application of polyaluminium chloride may have lethal effects on daphnids, especially if the concentration of aluminium is $> 9\,\mathrm{mg{\cdot}L^{-1}}$, and because similar concentrations of aluminium (typically $3 - 20\,\mathrm{mg{\cdot}L^{-1}}$) are used for water treatment in reservoirs, invertebrates in such water may be harmed. The reaction of natural water to the addition of polyaluminium chloride differs markedly. Prospective assessment of the effects on freshwater invertebrates is difficult, because the response of zooplankton to polyaluminium treatment can be affected by water composition, and therefore a screening ecotoxicological biotest with daphnids should be carried out to predict the negative effects on nontarget invertebrate organisms before each treatment.

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