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Effects of zinc and copper on development and survival of the moss *Atrichum undulatum* in controlled conditions

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ABSTRACT: We studied the effects of zinc and copper on gametophyte development and survival of the moss *Atrichum undulatum* in axenic and controlled conditions. Various salts of these two essential elements were applied in different ways in order to document effects dependent ion binding of zinc and copper to this moss. It can be concluded that zinc is less toxic than copper in higher concentrations. Survival is better and the recovery rate faster after exposure to zinc. Both metals are more harmful to the moss when applied in an aqueous solution.

Keywords: bryophytes, essential elements, growth, harmfulness, *Atrichum undulatum*

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INTRODUCTION

Bryophytes are widely used as biomonitors and indicator organisms due to their specific features of cuticle absence and uptake of water by the whole body or ectohydric nature. They were among the first terrestrial plants. In settling land habitats, they faced a very harsh environment and developed many different survival mechanisms to cope with various problems.

However, bryophytes (sub-kingdom Bryobiotina within Plantae), the second biggest group of plants after angiosperms, are very diverse and very little is known about their biology apart from data on a few model organisms. Chen *et al.* (2015) noted that physiological parameters which can indicate metal contamination quickly and non-invasively are unknown. Thus, there are reports indicating high sensitivity of moss to heavy metals (Chen *et al.* 2015), but also some species are highly dependent on heavy metals at sites loaded with them (Stanković *et al.* 2018) and are known as bryo-metalophytes [e.g., the copper moss *Scopelophila cataractae* (Mitt.) Broth. or lead moss *Ditrichum plumbicola* Crundw.].

Only a few moss species have been used to study accumulation of various pollutants such as nitrogen (Berisha *et al.* 2017), palycyclic aromatic hydrocarbons (Foan *et al.* 2015), and heavy metals (Figueira *et al.* 2002; Sabovljević *et al.* 2009) or in transplantation studies (Aničić Urosević *et al.* 2017). These are mainly pleurocarp moss species, like those used in the national pollutant deposition survey (Sabovljević *et al.* 2014). Although some studies have also been carried out on acrocarp mosses, these are rather rare (Schintu *et al.* 2005).

Since both copper and zinc are essential for plant development and play significant roles in it, these two were chosen for study, along with different anions.

Copper activates some plant enzymes involved in lignin synthesis and is essential in several other enzyme systems as well. It is also required in the process of photosynthesis (it has a key role in chlorophyll formation). Moreover, copper is essential in plant respiration and assists in plant metabolism of carbohydrates and proteins. As a micronutrient, it is needed in very small quantities by plants. The normal range in the growth medium is

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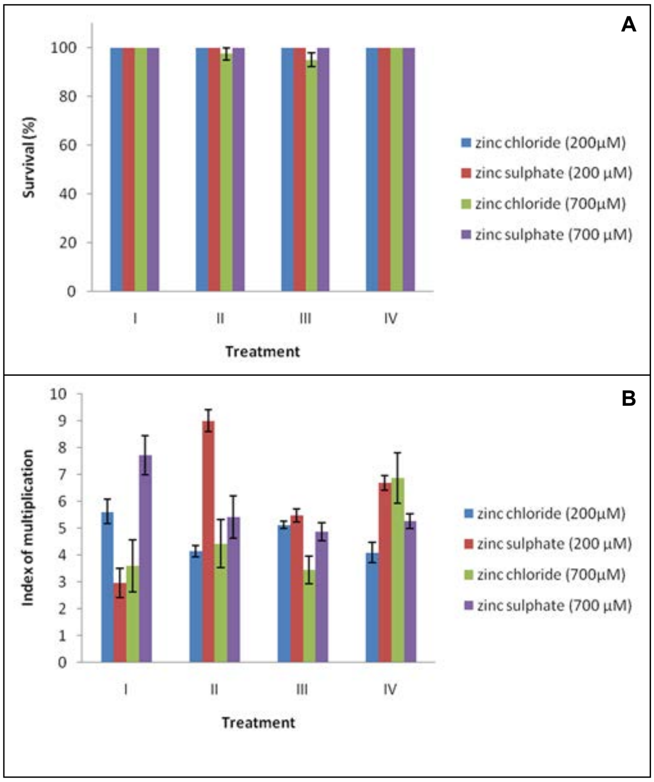

Fig. 1. Effect of zinc salts on the moss *Atrichum undulatum*. A) survival rate; B) development (index of multiplication).

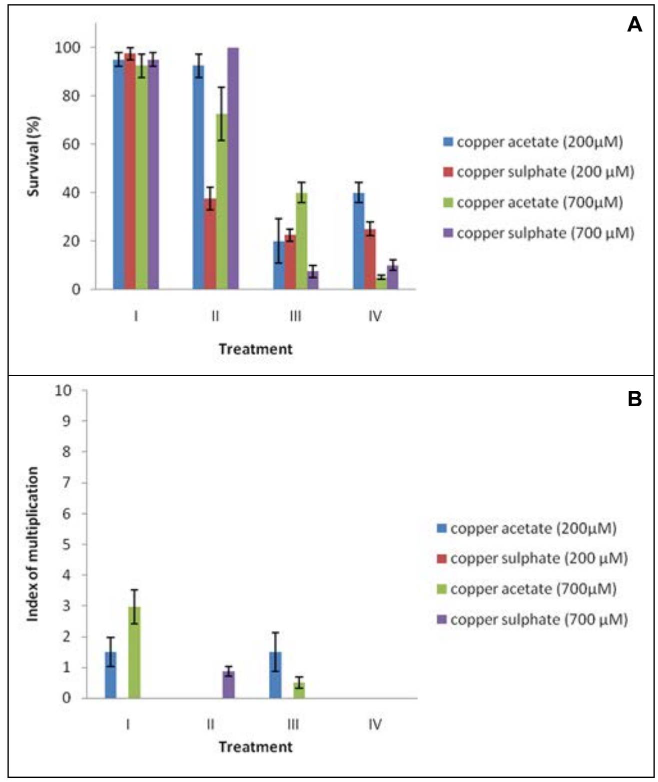

Fig. 2. Effect of copper salts on the moss *Atrichum undulatum*. A) survival rate; B) development (index of multiplication).

0.05-0.5 ppm, while in most tissues it is between 3 and 10 ppm. In comparison, the ideal range for iron in plant tissue is 20 times higher than that of copper. Although copper deficiencies or toxicities rarely occur, it is best to avoid either extreme since both can have a negative impact on crop growth and quality (Yruela 2005).

Another plant micronutrient, zinc is an important constituent of several enzymes and proteins. This micronutrient is needed by plants in only small quantities, but it is crucial to plant development, as it plays a significant part in a wide range of processes (Tsonev & Cebola Lidon 2012). The normal range for zinc is 15-60 ppm in plant tissue and between 0.10 and 2.0 ppm in the growth medium. Zinc deficiency or toxicity does not occur often; however, they both have a negative impact on crop growth and quality. Zinc activates enzymes that are responsible for the synthesis of certain proteins. It is used in the formation of chlorophyll and some carbohydrates, as well as in conversion of starches to sugars, and its presence in plant tissue helps the plant to withstand cold temperatures. Zinc is essential in the formation of auxins, regulators of plant growth and stem elongation.

In this study, we established an *in vitro* culture of the woodland moss *Atrichum undulatum* (Hedw.) P. Beauv. in order to control environmental factors and study the effects of various zinc and copper salts on its development.

MATERIAL AND METHODS

The procedure of Sabovljević *et al.* (2006) was followed for establishment of axenic *in vitro* culture. Methods previously described by Bijelović *et al.* (2004), Sabovljević *et al.* (2005), and Cvetić *et al.* (2009) were used to optimise growth, obtain fully developed gametophores, and achieve massive production. The material was collected on Mt. Avala near Belgrade in May of 2008, and the sporophytes obtained at that time were used to establish new culture. All the plantlets used in these experiments were ones that were laboratory produced, to avoid remnants or artifacts of the plants collected from nature.

The conducted experiments included four different salts ($ZnSO_4$, $ZnCl_2$, $CuSO_4$, and Cu-acetate) applied as 200 and 700 µM solutions in four different treatments. Gametophores measuring 10 mm in length were used as the experimental material and included 40 plants per treatment in four replicates.

Treatment 1 involved five days of growth on the base medium supplemented with the selected salt in one of the two concentrations and then transferred for up to five weeks to the base medium (KNOP medium with salt content of 250 mg/L KH_2PO_4, 250 mg/L KCl, 250 mg/L $MgSO_4$, 1000 mg/L $Ca(NO_3)_2$, and 12.5 mg/L $FeSO_4$).

The pH value of the medium was adjusted to 5.8 prior to sterilisation.

The next treatment (Treatment 2) involved five weeks of growth on the base medium supplemented with the selected zinc or copper salt.

Treatment 3 involved 0.5 hours in a solution of one of the selected zinc or copper salts, followed by 2 h of washing in distilled water and final transfer to solid KNOP base medium for up to five weeks. This treatment simulated the action of light rain, with collection of atmospheric heavy metal deposits during the first 0.5 h and their subsequent washing away.

The last treatment (Treatment 4) involved 2 hours in a solution of the selected zinc or copper salts, followed by 24 h of washing in distilled water and final transfer to solid KNOP base medium for up to five weeks. This treatment simulated the action of heavy rain, with collection of atmospheric heavy metal deposits during the first 2 h and their subsequent washing away.

After each treatment, the survival rate was recorded, together with the index of multiplication (the number of newly formed moss plants per explant).

RESULTS AND DISCUSSION

Survival on both zinc salts applied and in both concentrations in all treatments was high (Fig. 1A) if not complete (100%) compared to survival on the copper salts applied, where it was in some of the tests significantly lower. This implies that compared to copper, zinc is less harmful to *A. undulatum*. Interestingly, copper appears to be less harmful if added to solid media, while the same concentration in liquid solution even for a shorter period seems to be rather harmful (Fig. 2A). This can be explained by the fact that copper is very toxic and action of a liquid solution for even a short time can cause severe damage to the moss *A. undulatum*. Transport of copper from a solid medium is slow and difficult, while in liquid solution it is readily accessible to all moss cells, which are fully exposed to copper. The simulation of washing away by rain did not bring the expected results, suggesting that once copper binds to moss cells it is hard to unbind it, at least in a neutral aqueous solution (pH 7).

Multiplication was another parameter that revealed harmfulness of the two metals to this moss (Figs. 1B, 2B). Copper was clearly more toxic, while zinc proved to be less harmful. To judge from values of the index of multiplication, *A. undulatum* easily survives zinc stress and recovers after exposure at the tested concentrations, whereas it recovers with difficulty after exposure to copper stress. As expected, copper sulphate had stronger negative effects on multiplication compared to those of copper acetate. This was to be expected due to synergy of the toxicity of copper and the sulphate ion compared to the somewhat less harmful acetate ion.

Sabovljević *et al.* (2018) in a study of zinc binding by various bryophytes stated that they could not detect zinc bound to *A. undulatum* using fluoZin TM-3 dye, which is in accordance with the results obtained here. This suggests weaker toxicity, but also greater tolerance of *A. undulatum* to zinc. In contrast, *A. undulatum* is intolerant and sensitive to copper. Since copper and zinc are similar with respect to the range of pH values good for uptake by tracheophytes (slightly acidic conditions are good for uptake by the plants), it can be assumed that the difference in effects is due to moss species specificity as well characteristics of the heavy metals in question.

CONCLUSIONS

We can conclude that the moss *A. undulatum* copes with zinc contamination somewhat better than with copper contamination (Figs. 1, 2). It should also be noted that the presence of other ions can reduce or enhance the effects of trace metal ions. In addition, environmental conditions (e.g., occurrence of drought or wet periods) can influence the binding, uptake, and level of harmfulness to *A. undulatum* by changing the availability of ions, pH values, exposure of cells to contaminants, and other physiological parameters in mosses and/or ecological parameters in their immediate surroundings.

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REZIME

Uticaj cinka i bakra na razviće i preživljavanje mahovine *Atrichum undulatum* u kontrolisanim uslovima

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U ovom radu proučavan je uticaj cinka i bakra na preživljavanje i razviće mahovine *Atrichum undulatum* u akseničnim i kontrolisanim uslovima. Primenjivane su različite soli bakra i cinka u različitim tretmanima, sa ciljem da se dokumentuju efekti vezivanja pomenutih metala za mahovinu. Cink je manje toksičan od bakra za mahovinu *A. undulatum*. Preživljavanje je veće i oporavak od tretmana cinkom se odvija brže i bolje u poređenju sa bakrom. Oba metala pokazuju veći efekat na biljke kada se primenjuju u vodenom rastvoru u odnosu na tretmane kada se nalaze u čvrstoj hranljivoj podlozi.

Ključne reči: briofite, esencijalni elementi, rast, štetnost