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Bioaccumulation and toxicity studies of macroalgae (Charophyceae) treated with aluminium: Experimental studies in the context of lake restoration

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ABSTRACT

The objective of this study was to examine the impact of aluminium on the perennial macroalgae *Chara hispida* L. and its bioaccumulation capacities. Aluminium (Al) was introduced into the environment in the form of polyaluminium chloride, an agent utilized in the restoration of waterbodies. Research was conducted in an experimental setting using *meso*cosms (volume 0.8 m^3) placed in the littoral zone of a lake with *C. hispida.* Three doses of the coagulant were applied, each with a different volume: low – 6.1 g Al m^{-3}, medium – 12.2 g m^{-3} and high – 24.5 g Al m^{-3}. A significant acidification of environment was determined, which would imply the presence of toxic Al^{3+} ions. It has been demonstrated that aluminium penetrates and accumulates in the cells of the charophyte. This caused damage to the thalli, which manifested itself in chloroses, necroses, flaking of the cortex cells and softening of the thallus, whose severity was proportionate to the dose of the coagulant. The first negative signs were observed after 24 h. The study shows that *C. hispida* is a poor accumulator of aluminium (bioconcentration factor < 200), while bioaccumulation capacity was inhibited at the concentration of approx. 2.0 mg Al g^{-1} d.w. Accumulation in the thalli of the charophytes accounted for 58% of variation following removal of aluminium from the environment. The results of the experiment demonstrate a negative impact of aluminium on charophytes at concentrations used in aggressive restoration of lakes.

1. Introduction

Macroalgae are characterized by a capacity to accumulate toxic metals, and numerous species of this organisms are considered to be effective biomonitors and bioindicators (e.g. Murphy et al., 2007; Rybak et al., 2012; Clabeaux et al., 2013). Thus far, aluminium (Al) has not been taken into account in studies on metal accumulation, despite the fact that in acidic conditions it proves toxic to plant and animal life as reviewed by Gensemer and Playle (1999). One of the routes through which aluminium is introduced into an environment is application of chemical phosphorus coagulants in the course of surface water restoration (Lewandowski et al., 2003; Cooke et al., 2005; Sobczyński et al., 2012) and wastewater processing (Aguilar et al., 2002). Utilization of aluminium salts (e.g. alum – $Al_2[SO_4]_3{\cdot}14H_2O$) in lake restoration dates back to the early 1970s (Smeltzer, 1990). They have since been in widespread use as flocculation agents in Europe and United States (Cooke et al., 2005; Zamparas and Zacharias, 2014). At present, new generations coagulants are employed, e.g. pre-hydrated and polymerized products (containing polyaluminium chloride) which are more efficient than conventional aluminium coagulants (Grochowska et al., 2015). The principal advantage of aluminium-based coagulants is the durability of aluminium-phosphate bonds in anaerobic conditions, which inhibits release of bound phosphates in deep-water zones (Burley et al., 2001). Another major asset of polyaluminium chloride is that sulphates are not introduced into the environment. Sulphate contamination boosts eutrophication processes, leading to the formation of toxic sulphides in anoxic conditions and reduce iron availability to aquatic plants (Van Der Welle et al., 2007). Acidic pH is the parameter which restricts their application, as it can substantially affect abiotic conditions, especially in shallow and poorly buffered lakes (Łopata et al., 2013; Immers et al., 2015). Acidification of the environment causes the release of aluminium ions from mineral structures (e.g. aluminosilicates, alunite) and formation of aluminium hydroxide which then transitions into the active hexa-aqua-aluminium complex $Al(H_2O)_6{}^{3+}$, usually abbreviated as Al^{3+}. The resulting molecule is highly reactive, mobile, and easily absorbed by plants (Martin, 1986; Driscoll

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and Schecher, 1990). The mechanism of action of aluminium-based coagulants in aquatic environments owes to the synergistic effect of acidification and toxicity of aluminium in the first phase and, in the second phase, to the effect of Al ions in plant cells.

Application of coagulants without prior determination of the existing problem and mechanisms behind eutrophication in particular waterbodies is an ill-advised approach. Regrettably, coagulants are used increasingly often to achieve short-term, provisional improvements in water quality e.g. in case of seasonal fluctuation of trophic level or incidental inflow of biogens from the catchment area (Joniak et al., 2013). Temporary trophic disturbances result most often in phytoplanktonic blooms and shifts in physicochemical properties of water (Rosińska et al., 2017). Consequently, one observes increased turbidity combined with unpleasant odour of the water, as well as associated nocturnal oxygen deficiency which could lead to fish kills (Paerl and Huisman, 2008). Such procedures are also motivated by the need to restore hampered recreational functions of the affected waterbodies (Brooks et al., 2016). In either case, the changes which occur reduce their utilization capacity and lead to financial losses (Pretty et al., 2003). However, restoration treatments must only take place under strict supervision in lakes which constitute important habitats of plants, e.g. stonewort communities, which play an important role in maintaining good water quality (e.g. Van den Berg and Coops, 1999; Kufel and Kufel, 2002).

Charophytes (stoneworts, *Charophyta*) as "ecological engineers" are one of the major and most widespread groups of subaquatic plants (comprising around 400 species) found across a variety of waterbodies, from very shallow to deep. Nevertheless charophytes are threatened by direct anthropopressure in water ecosystems and increased pressure from the catchment area (Munsterhjelm, 2005; Torn et al., 2010). For this reason, hard oligo-mesotrophic waters with stonewort communities (code 3140) are encompassed by the European habitat conservation network Natura 2000 (Water Framework Directive, 2000). Stoneworts grow at substantial depths and form charophyte meadows which can cover extensive areas of the bottom. It should be noted, however, that stonewort species also inhabit eutrophic waters, where they may be exposed to pressure such as restoration treatments. Moreover, they are known for being pioneers of colonization, particularly in the waterbodies in which ecological balance has just been restored (Blindow, 1992; Van den Berg et al., 1998). Charophytes ensure shelter and source of nutrition to invertebrates, fish and waterfowl. They contribute to an improvement of water quality through physical stabilization and isolation of bottom sediment (Coops, 2002; Kufel and Kufel, 2002; John, 2003).

The administration of acid coagulants in natural waters may substantially and adversely affect the properties of the abiotic environment, rendering continued functioning of stoneworts and macrophytes impossible (Immers et al., 2013, 2014; Rybak et al., 2017). Previously, studies into bioaccumulation in charophytes have been undertaken sporadically, and even then they focused on toxic metals (Gomes and Asaeda, 2009). The literature concerning the impact of aluminium on stoneworts is equally scarce, and addresses only physiological response and the rates at which aluminium penetrates into isolated cells (Takano and Shimmen, 1999; Taylor et al., 2000). Thus, the aim of this study was to determine (1) the biological effect of polyaluminium chloride (types of lesions and their location) and (2) the accumulation capacity of evergreen *Chara hispida* L. for aluminium.

2. Materials and methods

The species selected for the experiment was *Chara hispida* L., a representative of the genus Chara (*Characeae*, *Charophyta*), widely distributed in Europe (frequent in Poland) and found in North Africa and Asia as well (Krause, 1997). The species prefers alkaline and neutral waters with the content of calcium compounds between 17.0 and 167.0 mg Ca dm^{-3} (Haas, 1994; Gąbka, 2009) and proves capable of growing with a wide range of available light radiation (Andrews et al., 1984; Menendez and Sanchez, 1998). In Central-Eastern Europe, it is encountered in shallow eutrophic lakes as well as in waters with slightly acidic pH, such as peatland exploitation ponds or humic waterbodies (Haas, 1994; Urbaniak and Gąbka, 2014). It is a monoecious macroalgae, one of the largest representatives of the genus: length of the stem ranges from 30 to 200 cm with axial diameters of 1–4 mm, internode length of 10–15 cm and 7–11 leaf-like structures, extending up to 8 cm in length (Krause, 1997; Urbaniak and Gąbka, 2014). It overgrows in highly hydrated, organic sediments, occurring less frequently on peat substrate and calcareous gyttja (Gąbka, 2009).

2.1. Experiment design

A *meso*cosm experiment was carried out over 3 days (beginning of August 2015), in the shallow Lake Wielkowiejskie (52°17′43″N; 16°40′5″E) where maximum depth reaches 4.0 m, located in Western Poland. The lake is characterized by the dominance of charophyte meadows and a wide belt of rushes. Eight open chambers (100 × 100 × 200 cm) were placed in the littoral zone, densely inhabited by *C. hispida* (approx. 50 shoots per m^2), separating the growing macroalgae into eight different treatment conditions. The chambers remained open both to the atmosphere and the sediments. Side walls were made of 2 layers of a transparent polyethylene foil, which allowed sunlight to infiltrate into the chambers from the sides as well. Water infiltration through sediments was limited by placing the chamber walls in semi-liquid bottom sediments at the depth of about 30 cm. Water depth in the chambers amounted to approximately 0.8 m. After securing the chambers in the studied habitat, they were left for 1 month to allow the separated, newly formed ecosystems to stabilize; subsequently, baseline conditions were determined.

2.2. Application and sampling

The chemical substance used in the experiment was polyaluminium chloride (pH < 1.0, density 1350–1370 kg m^{-3}), due to its strong flocculation/coagulation properties and widespread use in lake restoration treatments. Coagulant was added to the chambers in three dose volumes (two replicates per treatment): low at 6.1 g Al m^{-3} (680 kg per ha, referred to as Low), medium at 12.2 g Al m^{-3} (1360 kg per ha, referred to as Medium), and high at 24.5 g Al m^{-3} (2720 kg per ha, referred to as High). Two separate chambers served as controls (Control, 0.0 g Al m^{-3}). The doses administered in this study reflect the manner of "aggressive restoration" which would occur in a single application of the coagulant with resultant precipitation of phosphates, suspensions and reduction in water colour. Water and thalli samples were collected 24 h, 48 h and 72 h after Al application. Water samples (100 cm^3 each) were acquired by means of a pipette (semiautomatic pipettor by SwiftPet Pro) just above the community (without disturbing the sediment on the stoneworts), then transferred into glass bottles and fixed with concentrated nitric acid (Sigma-Aldrich). pH was measured each day at the same hour (Hanna Instruments HI 98129). Morphologically uniform thalli of *Chara hispida* (8–10 internodes, 40–50 cm length) were harvested, transferred into plastic bags (filled with a small amount of water) and transported to the cell biology laboratory at the Jagiellonian University in Cracow. Three replicates of each treatment were analyzed. Samples of fresh biomass of *C. hispida* were washed in distilled water.

2.3. Microscopic analysis and assessment of toxicity symptoms

To determine chloroplast reduction and metal bioaccumulation, the thinnest branches of the apical tips of thalli were mounted in a drop of water on microscope glass and closed with a cover slip (Martín-González et al., 2006). Images of the algal surfaces and internal parts of the thalli were obtained using a laser scanning confocal microscope