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The inhibition of growth and oospores production in *Chara hispida* L. as an effect of iron sulphate addition: Conclusions for the use of iron coagulants in lake restoration

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ABSTRACT

Charophytes, as a group of algae inhabiting waters characterized by varied fertility levels, represent highly critical attributes which are important for ecosystem services. One of the popular methods adopted for lake restoration is chemical inactivation of phosphates using iron coagulants. This paper presents the findings of laboratory experiments on the effects of iron sulphates on the growth and production of oospores in the charophyte *Chara hispida* L. In the course of the investigations, responses of this species to three dosages (Low, Medium, High) of iron (III) sulphate corresponding to the Fe concentrations of: 5.4, 10.8 and 21.6 g m^{-3} Fe were analysed. The results demonstrated there was a decrease in the speed of growth of the major axis and reduced production of oospores on the one hand (differences were statistically significant only between High vs. Control), and stimulation of the development and growth of side branches of the first order on the other. Several factors that cause disorders in oospore growth and production were encountered, with a wide spectrum of physico-chemical changes in the water, which resulted in the charophyte thallus being coated by a brown colloid film (limiting access of light) and lowered pH level. The study showed that the use of iron coagulants for lake restoration poses a threat to the development (growth) of charophytes.

1. Introduction

The pollution of freshwaters with biogenic compounds, despite remedial treatments, is still a major threat to the ecological integrity and biodiversity of aquatic ecosystems (Hilt et al., 2006; Xu et al., 2014). Chemical restoration treatments consisting of the inactivation of phosphates using iron coagulants are usually undertaken prior to or complementary to active biological and physical methods (e.g. wind aeration) (Sobczyński et al., 2012; Gołdyn et al., 2014). Ad hoc use of treatments aiming at precipitation of phosphates produces only a short-term improvement in water transparency and penetration depth of photosynthetically active radiation (Joniak et al., 2013; Sobczyński and Joniak, 2013).

Phosphate coagulants are acidic solutions of inorganic salts of iron or aluminium in oxidized forms. Combined with phosphates, the ions of these metals create sparing salts which undergo precipitation and sedimentation. At the same time aluminium and iron salts hydrolyse rapidly and in an uncontrolled manner, forming a range of metal hydrolysis species. Finally, as an effect of post-coagulation/flocculation processes, aggregate-flocs are formed with a large specific surface area. Due to the large absorptive area, the uptake of contaminants is very effective and the resulting large mass prompts gravity sedimentation to the bottom sediments. A major problem when using such chemical substances is their low pH (<1.0), which has proved to be dangerous for lake ecosystems, especially in weakly buffered waters (Persson, 2008). As a result of the high concentration of iron, coagulants have a dark brown colour and persist in the water until the precipitation of phosphates has terminated, after which sedimentation of new-created aggregate-flocs occur (Cooke et al., 2005).

The effects of iron impacts on aquatic plants are characterized by varying degrees of severity depending on particular species (Lucassen et al., 2000). Experimental studies have shown that iron causes changes in growth and reproduction, induces oxidative

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stress on a cellular level and disrupts cell membranes, pigments and even DNA damages, leading to death of the organism. The main changes are chlorosis and necrosis, reduction of the leaf surface area, general flaccidity and reduction of tillering (Wheeler et al., 1985; Linton et al., 2007; Van der Welle et al., 2007; Bakker et al., 2016). A high concentration of iron in the water reduces the availability of many essential nutrients and deteriorates light conditions (Wheeler et al., 1985; Gerhardt and Westermann, 1995).

Charophytes are an important component of freshwater ecosystems due to their ability to maintain good water quality (Van den Berg and Coops, 1999). By forming dense underwater beds they provide ecosystem services that rank among the most valuable assets in all freshwater ecosystems (Blindow et al., 2002; Kufel and Kufel, 2002). Charophytes significantly contribute to the physical stabilization of bottom sediments (Søndergaard et al., 2007) and play a refugial role for zooplankton against predation (Kuczyńska-Kippen et al., 2009; Liu et al., 2014). Many authors have remarked on the potential role of charophyte vegetation as a nutrient sink in water bodies as a result of their incorporation in biomass and carbonates or co-precipitation with calcite (McConnaughey and Whelan, 1997). The allelopathic activity of charophytes leads to phytoplankton growth reduction at a rate comparable to that of competition for nutrients (van Donk and van de Bund, 2002).

The impact of lake restoration using the iron coagulant-based method is still poorly understood in terms of its effect on macrophytes. Moreover, in spite of research on the effects of iron on macrophytes (Wheeler et al., 1985; Bakker et al., 2016) no specific mechanism explaining its toxicity can be said to have been identified. Literature concerning the impact of coagulants on charophytes is very scarce, although limited growth and biomass under the influence of high doses of iron such as iron (III) chloride has been demonstrated (Immers et al., 2013). In this study, the authors made use of an annual charophyte species of smaller size, without a stem cortex (*Chara virgata*, *Chara globularis*). Coagulant doses were of atypically high concentration, also applied to sediments and they frequently exceeded the quantity commonly used in restoration (Gołdyn et al., 2014; Kozak et al., 2015). To fulfil this gap we developed a laboratory experiment in which we tested the effect of iron sulphate on the growth and reproduction of *Chara hispida* L. It was assumed that the application of iron coagulant will cause the deterioration of abiotic conditions at an intensity proportional to the dose. Accordingly, energy will be directed to surviving adverse changes, which will result in growth inhibition and/or reduction of reproductive ability expressed by the number of oospores.

2. Material and methods

Charophytes as well as the water used in the experiment were collected at the end of May from the natural charophyte Wielkowiejskie Lake (N 52°17′43”, E 16°40′05”), a mesotrophic lake of good ecological state (Joniak and Kuczyńska-Kippen, 2008). The Charophyte species, *Chara hispida* from the genus *Chara* (Characeae, Charophyta), a species widely distributed in Europe as well as in North Africa and Asia (Gąbka, 2009), was used in the study. This charophyte creates monospecific meadows in lakes up to a depth of 3 m, being one of the largest representatives of the genus – its stem-like length can reach up to 200 cm. It is a monoecious alga producing single oogonia, and prefers neutral and alkaline waters with a wide range of calcium concentrations (Urbaniak and Gąbka, 2014).

The experiments were performed in June 2015 in 12 glass cylinders (2.3 dm^3 volume) which were placed in a cultivation room. Temperature was kept constant at 25.5 °C, while the light regime was set at 14 h light and 10 h darkness with a light intensity at the water surface of 160.6 $\mu mol\ m^{-2}\ s^{-1}$. Four apical shoots of *C. hispida*, each 20 cm in length, were placed in sediment-free cylinders which were then filled with 2.0 dm^3 of lake water (height of water column 24 cm). After 72 h (stabilization of the environment) iron (III) sulphate at doses of: 5.4 $g\ m^{-3}$ Fe (Low), 10.8 $g\ m^{-3}$ Fe (Medium) and 21.6 $g\ m^{-3}$ Fe (High) were added to the cylinders once. Cylinders with no addition of iron sulphate served as controls. Although the applied doses were high such amounts are commonly used in restoration practise in so-called “aggressive restoration”, which involves precipitation of phosphates, total suspended solids (including phytoplankton) and water colour (caused by high concentration of dissolved organic matter). “Aggressive restoration” is used to generate immediate improvement in the visual features of the water (after the elimination of the phytoplankton blooms). Doses were determined experimentally with the assumption that the lowest dose should be sufficient for the complete precipitation of suspension. Higher doses were a multiplication of the lowest dose, although all doses could be classified as used in lake restoration (Orihel et al., 2016). During application, the water was subjected to a gentle swirling for better dispersion of the coagulant. All four combinations were tested with three replications (Fig. 1). The cylinders were covered with LDPE foil containing holes (gas exchange). Biometric measurements of each specimen were made prior to the experiment and upon its termination (at an interval of 30 days), according to the following scheme: lateral shoot lengths, longest leaf in the nodes (nodes 1–3, counted from the top) and internode lengths (up to the 3rd node). The number of oospores on each branch was also determined. Each branch was labelled, hence measurements always related to the same specimen. Water temperature, pH, electric conductivity (HI 98129, Hanna Instruments), turbidity (using the TN-100 nephelometer, Eutech), colour (visual method in platinum-cobalt scale) and sulphate concentrations (gravimetric method, with barium sulphate precipitating) were measured every 3 days in each sample. Phosphates were analysed spectrophotometrically before application, after 3 days and at the end of the experiment using the molybdate method with the detection limit of 0.002 mg $PO_4^{3-}\ dm^{-3}$ (APHA, 1998). Photosynthetically active radiation was measured at the water surface using a quantum meter with a spherical sensor (LI-COR Biosciences).

Statistical tests were used to analyse the differences between the control and experimental treatments with respect to the algae features, the different relative growth rate (RGR) mean values and environmental conditions: an ANOVA with repeated measures with time as a fixed factor or one-way ANOVA followed by Tukey post-hoc tests (when ANOVA with repeated measures proved unworkable). The Shapiro-Wilk test was used to assess normal distribution. The Levene test was applied in order to assess the equality of variances for groups. All analyses were performed using Statistica 12.0 software.

The relative growth rate was calculated at the end of the experiment by the formula: $RGR = (\ln X_2 - \ln X_1)/(t_2 - t_1)$, where X_1 and X_2 were the algae mean lengths, at time t_1 and t_2, (start and end of experiment, respectively) (Hunt, 1990). The difference between the growth curve and treatments was tested with ANCOVA with the sampling day as a covariate and iron concentrations as fixed factors.

3. Results

For all specimens an increase in overall length (mean ± SD; 9.0 ± 3.2 cm) was recorded. Individuals subjected to the influence of the highest dose of coagulant were characterised by a significantly lower RGR versus the control (Table 1, Appendix A in the Supplementary material). A regression curve revealed a significant overall decrease in the average length of *Chara hispida* along the gradient of concentration of iron (based on coagulant doses) and accounted for 76% of the variance (Fig. 2). There were no signif-