

Original Research

# Experimental Investigation into Disturbance of Ca-Mg Equilibrium and Consequences for Charophytes after Iron and Aluminium Coagulants Application

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## Abstract

Iron sulphate and polyaluminum chloride are commonly used in water restoration to eliminate cyanobacteria bloom and improve water quality. Nevertheless, the influence of coagulants on water organisms remains insufficiently studied. The study involves the analysis of phosphate coagulants' impact on calcium and magnesium concentrations in the *Chara hispida* community. The experiments were carried out in field mesocosms. Both coagulants were applied once in three different doses: 50.0, 100.0, and 200.0 cm<sup>3</sup>·m<sup>-3</sup>. The application of coagulants caused a decrease of pH and calcium carbonate and magnesium carbonate dissolution. Although the changes were proportional to the coagulant concentrations, the aluminum coagulant triggered more considerable disturbances. The highest dose of iron sulphate caused the precipitation of hardly soluble calcium sulphate and the elimination of part of calcium from biological circulation. The concentrations of magnesium in water increased only at pH <4.5 following the application of the highest dose of polyaluminum chloride. Shifts in the Ca-Mg equilibrium, which result in the disturbance of biogenic calcification, may affect charophyte metabolism and lead to the elimination of charophyte communities. Therefore, inactivation treatments using acidic coagulants in lakes with charophyte communities ought to be preceded by preliminary studies in order to determine the least harmful dosage for the ecosystem.

**Keywords:** charophyte encrustation, Ca-Mg equilibrium, chemical phosphate coagulants, acidification, lake restoration

## Introduction

Charophytes contribute to maintaining a clear water state by producing large quantities of biomass and accumulating large concentrations of nutrients on the one hand, and competing with phytoplankton in resources on the other [1-2]. They stabilize bottom sediments, limit the migration of nutrients, and create refugium and food base for animals [3]. Moreover, the water decalcification process is followed by co-precipitation of phosphates with calcite, resulting in reduced phosphorus bioavailability in water [4-5].

Despite the fact that charophytes are supposed to be a typical component of clear, alkaline, and nutrient-poor lakes, some species also inhabit meso-eutrophic and eutrophic water bodies [6-7]. These types of lakes are more vulnerable to restoration treatments when taking into account an urgent need to implement the guidelines of the Water Framework Directive. Furthermore, occasionally some restoration treatments are carried out in lakes with temporary trophic disturbances, which is most often correlated with phytoplankton blooms and shifts in physicochemical features of water [8-9]. One of the most common methods of restoration is the chemical inactivation of phosphates. Usually this is undertaken prior to or complementary to active biological and physical methods, e.g., wind aeration [10-11]. This method is based on the precipitation of phosphates to the bottom sediments after binding with iron or aluminium acid coagulant [12]. These metals, added to water, create with mineral phosphates salts, which undergo precipitation and sedimentation. Finally, aggregate-flocs are formed with a large absorptive surface as a result of coagulation and flocculation processes [13]. Although this process is very effective, acidification poses a threat to hydrobiota [14].

The hardness of water mainly depends on the presence of bivalent cations that are naturally present in water and follows features of the catchment as well the chemical composition of watercourses in the case of lakes [15]. Calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ) are the main elements that influence water hardness as well as affect the buffer capacity of lakes and regulate their biological productivity. A higher concentration of calcium and magnesium cations is vital for many organisms, especially the algae from the Chlorophyta, Rhodophyta, Phaeophyceae, and Chrysophyceae [16]. Thalli of these organisms is covered by calcium and/or magnesium carbonate, which is related to photosynthetic activity. This is a consequence of changes in pH caused by the use of hydrogencarbonate ( $\text{HCO}_3^-$ ) as an alternative to carbon dioxide ( $\text{CO}_2$ ) during photosynthesis. As a result, insoluble calcium and/or magnesium carbonate is deposited on the surface thalli, producing encrustation [16]. In this way, easily soluble calcium hydrocarbonate is transformed into hardly soluble calcium carbonate (1). An analogous process occurs during the transformation of magnesium hydrocarbonate into magnesium carbonate [17-18].

Encrustation is affected by various factors, such as pH of water, temperature, calcium, magnesium, and carbonate ions concentration as well as by plant age and photosynthetic activity [19].



Charophytes (*Characeae*, *Chlorophyta*) as the most encrusted group of algae, produce internal encrustation and external encrustations on the surfaces of cell walls and, in some species, organic-matrix-mediated calcification within the walls of the oogonium [5, 20]. Due to tight adherence to the thalli (unlike vascular plants), the encrustation fortifies the cell structure, increasing the resistance to mechanical injuries and protecting it from UV radiation [16, 21]. In the case of the most common genus, *Chara*, it is supposed that the increase of photosynthetic activity results in an increase in water decalcification [22].

Due to the chemical features of coagulants, their introduction to a lake causes changes in the physical and chemical properties of water. These disturbances may directly and indirectly influence the macrophyte and charophyte communities [23-24]. It has been hypothesized that chemical coagulants cause water acidification, which changes alkaline conditions preferred by charophytes and dissolves charophyte encrustation. The aim of the study was an analysis of the scale and direction of changes in calcium and magnesium concentrations under the influence of different doses of iron and aluminium coagulants in charophyte meadows, which was tested during mesocosm field experiments.

## Methods

The field experiment was conducted in a shallow lake (area 13.3 ha, max depth 4.0 m) located in Wielkopolska National Park (N 52°17'43", E 16°40'5"). This lake is characterized by a dominance of charophyte meadows and a wide belt of helophytes. Eight steel constructions (open to the sediments and atmosphere; dimensions 1×1×2 m) with walls made of polyethylene foil, transparent to sunlight, used as mesocosms. The mesocosms were placed in the gyttja bottom sediments in the littoral zone (Fig. 1) and each of them had about 1 m<sup>3</sup> volume. This site was densely inhabited by only *Chara hispida* L. (approx. 50 shoots per m<sup>2</sup>). The embedding of mesocosm in the lake sediments (to a depth of 30 cm) limited the infiltration of water to the inside and walls, reaching 20 cm above the water level excluding water inflow by waves. After inserting the chambers in the habitat, they were left for 1 month to allow to stabilize. Subsequently, baseline conditions were determined (day 0). The study was carried out in the peak of vegetation season (July) separately for iron coagulant – iron sulphate (2014) and aluminium coagulant – polyaluminium chloride (2015).

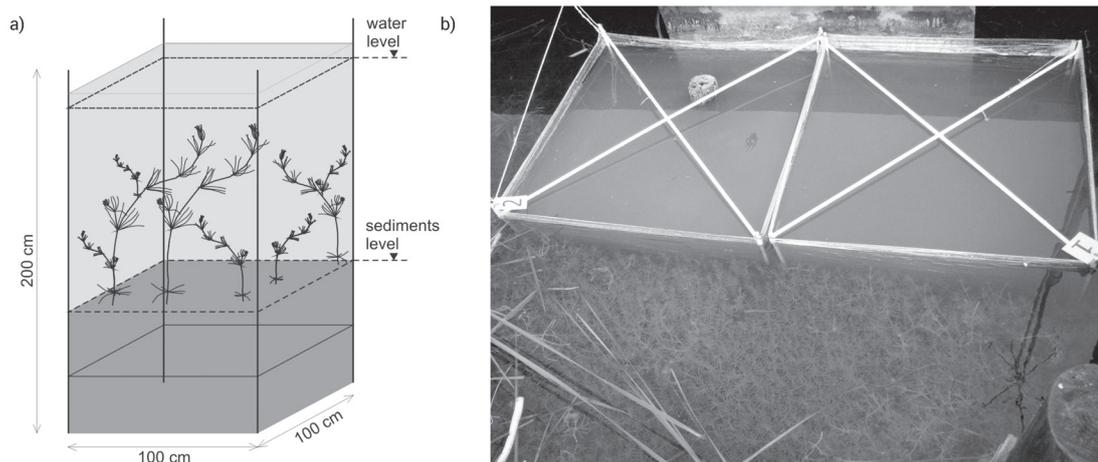


Fig. 1. Scheme of mesocosm construction a) and mesocosms in the field, immediately after coagulant application b).

The chemical characteristics of the iron coagulant,  $\text{Fe}_2(\text{SO}_4)_3$  (trade name PIX 113) is:  $\text{pH} < 1.0$ , density 1500-1570  $\text{kg m}^{-3}$ , base substance: sulphuric acid ( $\text{H}_2\text{SO}_4$ ), colour dark brown. In the case of aluminium coagulant  $[\text{Al}_n(\text{OH})_m\text{Cl}_{(3n-m)}]_x$  (trade name PAX 18),  $\text{pH} = 1.0$ , density 1350-1370  $\text{kg m}^{-3}$ , base substance hydrochloric acid (HCl), colour light yellow.

The charophyte species involved in the experiment was *C. hispida* L. The species is widely distributed in Europe (frequent in Poland) as well as in North Africa and Asia. This is one of the largest representatives of the genus – its stem-like length can reach up to 200 cm [6]. In Central and Eastern Europe, it inhabits shallow eutrophic lakes and waters with slightly acidic pH, such as peatland exploitation ponds or humic waterbodies [25-26]. *C. hispida* prefers alkaline and neutral waters with the calcium content between 17.0 and 167.0  $\text{mg Ca}^{2+} \text{ dm}^{-3}$  [6], and proves capable of growing with a wide range of available light radiation [27]. Due to habitat requirements, which are characteristic for eutrophic lakes, *C. hispida* communities are strongly vulnerable to restoration treatments.

The coagulants were applied one-off at the beginning of the experiment (time  $T_0$ ) in three doses: low (L), medium (M), and high (H), in turn 50.0, 100.0, and 200.0  $\text{cm}^3 \cdot \text{m}^{-3}$ , respectively. Concentrations of coagulants were determined in the laboratory experiment, where it was assumed that the lowest dose should lead to the total precipitation of suspension. Higher concentrations were a multiple of the lowest dose. To mesocosms chemicals were slowly added by a pipette (semiautomatic pipettor) and gently stirred by a hand mixer. Six of the mesocosms were used for the treatments (two for each concentration) and two of them were the control (C) trial.

The measurements and samples collection for chemical analysis were performed every day (for 3 days) and repeated after 1, 2, and 3 weeks. Measurements were done consistently at the same time of day (11:00). Dissolved oxygen (Pro Plus, Yellow Spring Instruments)

and pH (HI 98129, Hanna Instruments) were measured in the field. The water samples for chemical analyses were preserved with sulphuric acid or nitric acid, depending on the method, directly after the collection. The following were analysed in a laboratory: total calcium and total magnesium (EDTA titrimetric method, ISO 6058:1984), sulphate ( $\text{SO}_4^{2-}$ , gravimetric method using barium chloride, ISO 9280:1990), and chloride ( $\text{Cl}^-$ , silver nitrate titration with chromate indicator, ISO 9297:1989).

Dry mass of charophytes was investigated to determine a potential loss of encrustation. For this purpose, 4 individuals of *C. hispida* from each mesocosm were collected at the same time as chemical samples. After transportation to the laboratory (in closed plastic bags) they were analyzed after drying in a dryer (80°C) and weighted with accuracy to 0.0001 g.

The Shapiro-Wilk test was used to assess the normal distribution. The Levene test was applied to assess the equality of variances for groups. A two-way analysis of variance with dose and time as fixed factors (followed by Tukey multiple comparisons test) was used to check the differences between the examined elements and dry mass. All the statistical analyses were performed using Statistica 12.5 software.

## Results

In both experiments, at the start the pH of water in mesocosms was slightly alkaline. The application of coagulants caused a temporary decrease in pH appropriate to the dose. The addition of iron coagulant caused a small pH decrease in the L dose ( $0.88 \pm 0.01$ , mean  $\pm$  SD), and a large one in the H dose ( $1.45 \pm 0.18$ ). Meanwhile, the aluminum coagulant caused much higher reductions of pH – about  $2.61 \pm 0.57$  in L dose and  $4.89 \pm 0.18$  in H dose. Irrespective of the dose, the negative effect of acidity was reduced to the initial values 24 h after coagulant application (Fig. 2a-b).

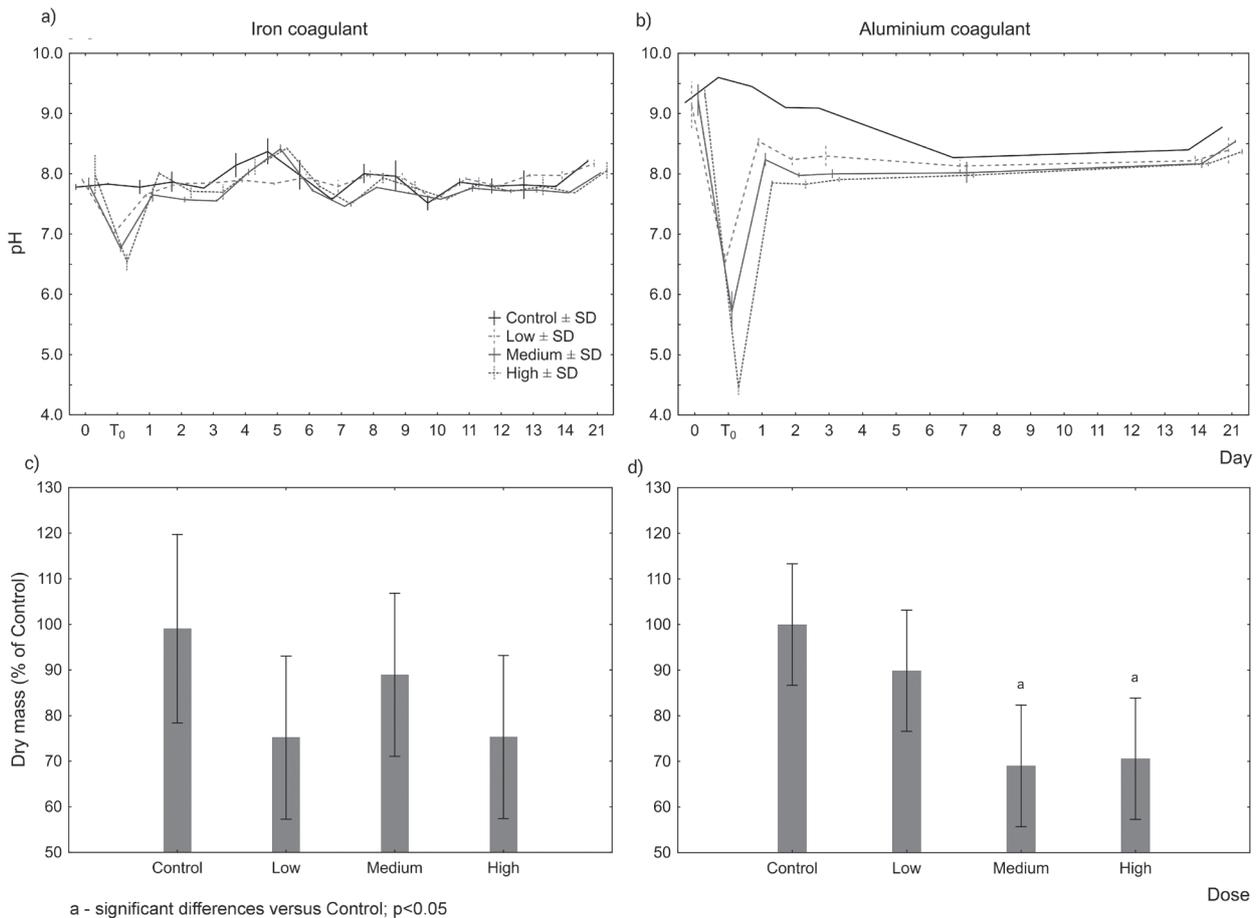


Fig. 2. Changeability of pH and dry mass of charophytes (as percent of control concentration) before and after application of iron coagulant (a, c) and aluminium coagulant (b, d).

The dry biomass of the charophytes in mesocosms with iron coagulant was lower than in control mesocosms (Fig. 2c). Values varied between 75% in L and H dose and 90% in M dose compared to C. There were no significant differences between particular treatments ( $F_{3,2} = 1.75$ ;  $p = 0.21$ ). In the case of aluminium coagulant the lowest decrease was observed in L dose (90% compare to control). In treatments with M and H coagulant concentration dry mass was reduced to 70%. Statistical analyses revealed differences ( $F_{3,2} = 6.11$ ;  $p < 0.01$ ) between C chambers and M as well as H dose of coagulant (Fig. 2d). The changes were accompanied by bubbles of gases, which were tightly adhered to the charophytes thalli. In the case of M and H doses of both coagulants the amount of bubbles was so high that charophytes were even elevated above water level. As a result of bubble release, the water surface was covered by foam for 5 days.

At the beginning of each experiment the oxygen saturation of water in mesocosms was 100%  $O_2$ . After the addition of iron coagulant the oxygenation increased to max. 120%  $O_2$  in L dose, min. 102%  $O_2$  in H dose. In the experiment with aluminium coagulant there were no differences in oxygen saturation of water between the doses and control mesocosms.

The analysis of  $Ca^{2+}$  concentrations under the influence of iron coagulant showed the greatest increase in the case of M dose, where an increase by 15% was observed, in comparison with C (Fig. 3a). In H dose, despite higher water acidification, the value of calcium concentration was comparable to C and L. Hence statistical differences were shown between M dose and the other doses. The application of aluminium coagulant caused an increase in calcium concentration – linear in relation to the dose – by 40% in M dose and by 70% in H dose in comparison to control (Fig. 3b). A two-way ANOVA revealed that both the coagulant dose and time had a significant effect on  $Ca^{2+}$  concentration differences (Table 1). In the case of  $Mg^{2+}$  there were no statistically significant differences indicating the relationship between the dose and concentration of the element resulting from the application of iron coagulant (Fig. 3c). However, time was the key factor of this experiment (Table 1). Following the application of aluminium coagulant, magnesium concentrations increased significantly only in H dose (Fig. 3d). The dose as well as time were the differentiating factors, and to a lesser extent the interaction of both (Table 1).

The application of coagulants also caused an increase in concentration of ions constituting the base substance

of sulphates and chlorides. An interesting fact is that the highest concentrations of  $\text{SO}_4^{2-}$  were found in M dose, where they were 70% higher compared with control and 40% higher than in H dose (Fig. 3e). In the case of aluminum coagulant, the increase in the concentrations of chlorides in L and M doses was similar (60% in comparison to control), and noticeably higher in H dose (over 200%; Fig. 3f). A statistical analysis revealed that the dose of coagulant and time were the sources of variability of  $\text{SO}_4^{2-}$  concentrations, while the variability of  $\text{Cl}^-$  concentrations was due to the dose size, time, and interaction of both (Table 1).

### Discussion

The coagulants applied in the experiment belong to the most common ones used in restoration treatments. Doses applied to mesocosms corresponded with the so-called ‘aggressive restoration.’ This type of restoration treatment results in the total elimination of phosphates, total suspended solids, and water colour after single, high-dose coagulant application [24]. This is the opposite method to ‘sustainable restoration’ consisting of low concentrations of coagulant application several times a year [28]. Although

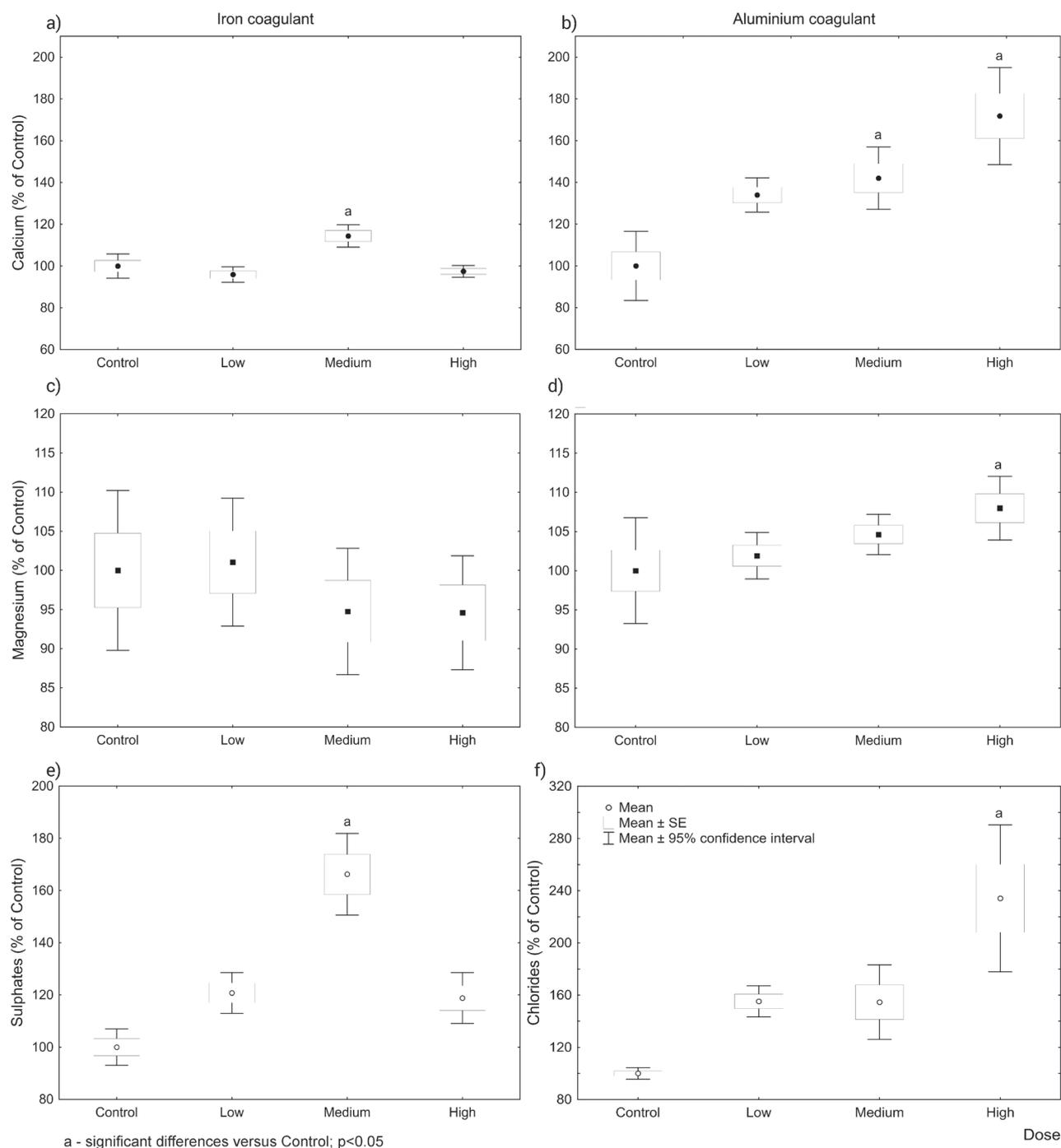


Fig. 3. Differences of ions concentrations between iron (a, c, e) and aluminium coagulant (b, d, f) doses.

Table 1. Results of the two-way ANOVA (*F*-ratios and *p* values) to test for the effects of coagulant dose, experiment time, and their interaction on elements concentration.

Iron coagulant						
Variable	Ca <sup>2+</sup>		Mg <sup>2+</sup>		SO <sub>4</sub> <sup>2-</sup>	
	F	p	F	p	F	p
Dose	42.97	<0.000	1.20	0.322	56.80	<0.000
Time	7.55	<0.000	4.57	<0.000	5.13	<0.000
Dose × Time	1.84	<0.03	1.37	0.154	2.41	<0.002
Aluminium coagulant						
Variable	Ca <sup>2+</sup>		Mg <sup>2+</sup>		Cl <sup>-</sup>	
	F	p	F	p	F	p
Dose	110.94	<0.000	4.48	<0.02	37.01	<0.000
Time	20.34	<0.000	4.35	<0.02	7.95	<0.000
Dose × Time	7.71	<0.000	2.91	<0.03	2.23	0.053

concentrations used in the experiment seem to be high, all could be classified as commonly used in lake restoration [29, 30].

The phosphorus coagulants influenced calcium and magnesium concentrations, which affected *Chara hispida* communities. The main factor causing the changes was the decrease in pH determined by the coagulant concentration applied to the mesocosms. Lower pH caused a dissolution of charophyte encrustation, therefore releasing calcium and magnesium ions into the water. This was confirmed by decreasing the dry mass of charophytes, since encrustation can exceed even 80% of their dry weight [31]. An additional aspect causing changes in the abiotic environment was the increased concentration of ions constituting the coagulant. In the conducted experiments, acidification was neutralized within the first 24 h due to the reaction of hydrogen ions with calcium carbonate. Neutralization was supported by the photosynthesis process due to the increase in concentration of hydrogen carbonate ions originating from the dissolved encrustation. This was indicated by water oversaturation when pH changes were highest [32]. The slower pace of neutralization observed in the experiment involving polyaluminum chloride resulted from the more intensive acidification of the environment by hydrochloric acid. In natural conditions the encrustation is created as a result of CO<sub>2</sub> uptake from bicarbonates during photosynthesis [33]. The acidification caused the dissolution of CaCO<sub>3</sub> (2) and/or MgCO<sub>3</sub> (3), and a Ca<sup>2+</sup> and Mg<sup>2+</sup> ions were released into the water. In this process CO<sub>2</sub> is also released, which was visible as bubbles on charophytes thalli and was the reason of foam generation on water surface.



The difference in the concentration of released calcium ions, observed in the case of iron coagulant application between M and H dose, was contrary to expectations. Higher acidification was obtained in H dose, and therefore the highest calcium concentrations were also expected in this dose (such an effect was obtained in the case of aluminum coagulant). An analogous situation with the concentration of SO<sub>4</sub><sup>2-</sup> suggests that the solubility equilibrium of calcium sulphate, CaSO<sub>4</sub> ( $K_{sp} = 6.3 \cdot 10^{-5}$ ), was exceeded in H dose of coagulant. As a consequence, the precipitation of excess calcium ions occurred due to a high supply of sulphate ions. The product of solubility calculated on the basis of concentrations of Ca<sup>2+</sup> and SO<sub>4</sub><sup>2-</sup> ions occurring in Medium dose after 24 h was lower by one order of magnitude ( $K_{sp} = 2.15 \cdot 10^{-6}$ ). It should be underlined that a 2-fold lower dose of coagulant was used here and, consequently, lower acidification of water affecting the dissolution of encrustation occurred. As a result of weak solubility of calcium sulphate (CaSO<sub>4</sub>), a part of the calcium budget is permanently removed from biological circulation. This type of decalcification may result in a negative calcium balance in the lake ecosystem and, as a consequence, in the disturbance or elimination of charophyte communities. In the case of aluminum coagulant we observed the increase in calcium concentration proportional to acidification. The precipitation of calcium chloride was not found due to the high solubility of the compound. Calcium ions are vital for regulating various levels of functions in plants and algae, such as electric balance between the extracellular and intracellular spaces as well as metabolic activities [34]. Apart from these functions, calcium is an essential cation, which affects chlorophyll accumulation. However, extremely increased calcium content in water (when it does not precipitate) may be a disturbance factor in the photosynthesis process for charophytes [35]. As Pelechaty et al. indicated [31],

an average  $\text{CaCO}_3$  precipitation can be estimated as  $167 \text{ g m}^{-2}$ . This implies that in a hypothetical situation when only 60% of encrustation is dissolved, an additional  $40 \text{ g}$  of  $\text{Ca}^{2+}$  is released into the water. However, the data indicate that the values for precipitated  $\text{CaCO}_3$  may actually be higher than the above mean and exceed  $1500 \text{ g CaCO}_3 \text{ m}^{-2}$  [36]. A larger than required amount of  $\text{Ca}^{2+}$  results in chlorophyll *b* reduction and changes the chlorophyll *a:b* ratio together with decreasing photosynthesis efficiency [2, 37]. Moreover, thalli damage that occurred under coagulant influence decreased photosynthetic pigment concentration and impaired recovery significantly. This is particularly dangerous in the case of aluminium coagulant. Acidification leads to increased toxicity of aluminium ions, which penetrated to charophyte cells and caused severe impairment [38-39].

In the case of magnesium, the application of iron coagulant did not cause noticeable changes in its concentration since  $\text{MgCO}_3$  is more resistant to dissolution [40]. The acidification of water in the experiment was too weak to trigger the effect of the disintegration of  $\text{MgCO}_3$ . Therefore, the concentration changes in time were the result of natural and relatively small fluctuations. Much higher and statistically significant changes of  $\text{Mg}^{2+}$  ions concentrations were observed following the application of polyaluminum chloride, particularly in high doses. This indicated the existence of high potential dissolution of  $\text{MgCO}_3$  at  $\text{pH} < 4.5$ . The fluctuations of the element concentration observed during the experiment indicated small effectiveness of the process of its precipitation. High concentrations of magnesium inhibit the process of charophyte calcification by calcium carbonate [18]. This is the result of  $\text{Mg}^{2+}$  binding with hydrogen carbonate ions or getting onto the growing surface of the crystal, essentially preventing further precipitation of  $\text{CaCO}_3$  [41]. Magnesium is an essential constituent of chlorophylls and therefore is regarded as an absolute requirement for green algae [42]. Increased magnesium concentration aid shoot elongation and the development of primary, secondary, and tertiary branchlets in charophytes [35]. Moreover, with the increasing  $\text{Mg}^{2+}$  ions content in the water, Chl *b* might increase as well, changing the Chl *a:b* ratio in an opposite way from  $\text{Ca}^{2+}$  [2]. Thus, the presence of both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  resulted in no change in the Chl *a:b* ratio. This is caused by antagonistic interactions neutralizing the negative effect of one element in the presence of another, hence  $\text{Ca}^{2+}$  effects are reduced by  $\text{Mg}^{2+}$  and vice versa [2]. However, in conducted experiments the situation with increasing concentrations of both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  occurred only in the one particular treatment with the highest dose of aluminum coagulant. This may imply considerably greater calcium effect on charophytes under chemical restoration treatments.

Apart from iron and aluminum,  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  are also introduced to water with coagulants. In the experiment with iron coagulant, the highest concentration of  $\text{SO}_4^{2-}$

was observed in M dose, and not in H dose, where  $\text{CaSO}_4$  was precipitated (cf. above). In the oxygenic conditions sulphates do not have a negative influence on biota [43-44], but they intensify the secondary supply of water in phosphates from bottom sediments, and at the same time its eutrophication [45]. The content of  $\text{Cl}^-$  changed adequately to the dose of aluminum coagulant, reaching the maximum in H dose. Similar average concentration in L and M doses demonstrated in Fig. 3e) was a result of faster elimination of  $\text{Cl}^-$  in M than in L dose during the first week of the experiment. In the final phase, their concentrations in all mesocosms were similar to control. Chlorides, as weakly reactive, remain fully dissolved in water and only their small part is subject to sorption by bottom sediments. Meanwhile, they are the main mineral anion for plants [46]. Changes in their concentrations in conjunction with pH fluctuations may cause disturbances in  $\text{Cl}^-$  transport at the plasma membrane in charophyte cells [47].

## Conclusion

The coagulants based on strong acids, which are used in lake restoration, influence  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ion concentrations. These changes have an impact on *Chara hispida* communities, which inhabit eutrophic lakes. The main reason for changes is the pH drop, which significantly changes the abiotic environment preferred by charophytes and causes disturbances in the chemical equilibrium. It was shown that polyaluminum chloride causes stronger acidification of water than iron sulphate. A high dose of iron coagulant results in the elimination of a certain amount of calcium from biological circulation, while aluminum coagulant increases the mobility of magnesium. Both processes disturb the Ca-Mg equilibrium, which results in the disturbance of biogenic calcification and may affect charophyte metabolism and development. Treatments repeated in an amount that exceeds the environmental tolerance may disturb the whole ecosystem and cause profound changes in the abiotic environment. Therefore, applications of acid coagulants of phosphates for the purpose of improving a lake's ecological state may be dangerous for charophytes. In such a situation, the restoration effort may be diminished by a negative effect following the disappearance of underwater plant communities.

## Conflict of Interest

The authors declare no conflict of interest.

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