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Iron overload consequences for submerged plants stoichiometry, homeostasis and performance

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Abstract Accelerated lakes eutrophication is one of the greatest challenges nowadays. To counteract its negative effects, large-scale restoration treatments are carried out worldwide. However, research in this field is mainly focused on the process effectiveness and there is a scarcity of studies concerning the impact of restoration treatments on water organisms and ecosystem homeostatsis. Our microcosm study presents the effects of a phosphorus coagulant (iron [III] chloride) on functional traits changes, oxidative stress and macro- and microelement stoichiometry disturbances in macrophyte *Myriophyllum spicatum*, a model species inhabiting eutrophic waters. Application of the coagulant to experimental vessels influenced the physicochemical and optical parameters of water and led to significant changes in biogeochemistry. Stoichiometric alterations were reflected by disturbances in the relative contents of macro- (C, N, P, Ca, Mg) and microelements (Fe, Zn, Cu, Co) and induced luxury consumption of available ions. Physicochemical and stoichiometric changes mutually exerted negative influence on *M. spicatum* functional traits. The parameters of oxidative stress remained at low levels, comparable to the untreated control whereas stoichiometric analysis revealed the activation of mechanisms responsible for minimizing low light stress. The ability of *M. spicatum* to maintain homeostasis of Cu and Co under simulated chemical water restoration

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was closely related to high concentrations of Fe and Zn ions, which simultaneously were not subjected to homeostasis control. Thus, chemical lake restoration treatments based on phosphorus coagulants are not as environmentally safe as previously considered and may have far-reaching consequences for the biogeochemical cycle and food web functioning.

Keywords Ecological stoichiometry · Biogeochemical networks · Eutrophication · Chemical restoration · *Myriophyllum spicatum*

Introduction

Despite ongoing efforts to reduce anthropogenic nutrient inputs, eutrophication remains one of the most serious threats to the sustainable functioning of lake ecosystems (Klimaszyk and Gołdyn 2020; Woolway et al. 2021), with far-reaching consequences manifested as biogeochemical and trophic disturbances to the lacustrine reservoirs themselves as well as the adjacent terrestrial ecosystems (Sardans et al. 2012; Le Moal et al. 2019; Su et al. 2019). These in turn impact negatively on the provision of ecosystem goods and services, as well as human health and economic activity (Pretty et al. 2003; Von Blottnitz et al. 2006).

The chemical variability in aquatic ecosystems is incredibly high and results from many abiotic reactions and metabolic pathways. Thus, biogeochemical diversity can affect biodiversity and vice versa (Welti et al. 2017; Rojo et al. 2020). Any interference in these pathways causes disturbances in the natural cycles in water ecosystems, and restoration treatments are carried out to intensify positive mechanisms and limit negative ones (Cooke et al. 2005; Rosińska et al. 2018). One of the most commonly used restoration methods is chemical phosphate inactivation, which provides almost immediate improvement in water quality at a relatively low cost (Jančula and Maršálek 2011; Zamparas and Zacharias 2014). This involves the introduction of inorganic coagulants to the ecosystem, i.e. acidic solutions of iron or aluminium salts, which bind phosphates into complexes and remove them to the bottom sediments (Sobczyński and Joniak 2009; Lürling and Oosterhout 2013; Dunalska et al. 2015). The technique is also employed to control eutrophication and effectively remove arsenic from natural waters (Chen et al. 2015; Wang and Jiang 2016). However, chemical intervention causes rearrangements of trophic networks and disturbances in natural biogeochemical cycles, leading to changes in the stoichiometry of macroelements in the entire ecosystem (Kritzberg and Ekström 2012; Rybak et al. 2020b; Zhan et al. 2021).

A helpful concept exploring ecosystem structure and function related to the environment and organisms' elemental ratios is called ecological stoichiometry (Elser et al. 2000). It provides a framework for understanding elemental balance in ecological interactions and environmental processes (Sterner and Elser 2002). The degree to which organisms maintain a constant elemental composition in response to their availability from environmental resources is referred to as "stoichiometric homeostasis". This reflects a physiological and biochemical adaptation of the organism to the surrounding environment (Sterner and Elser 2002; Hessen et al. 2004), and can be regulated by organisms dependently on the limitation of resources, physiological constraints and growth rate potential (Sistla et al. 2015).

During the application of coagulants, high doses of bioavailable metals are delivered and accompanied by high acidification of the environment with simultaneous removal of phosphorus. Thus, elemental homeostasis of the ecosystem can be easily disturbed, leading to rearrangements of biogeochemical networks. Studies on method effectiveness, development and improvement from an anthropogenic point of view are dominant, while biotic components of the ecosystem are mostly ignored even though chemical substances influence them directly. This is extremely relevant to submerged macrophytes—key producers able to stabilize freshwater ecosystems (Van Donk and Van de Bund 2002; Hilt et al. 2006). Available studies revealed significant changes in plant communities (Rosińska et al. 2017; Hilt et al. 2018) or the response of selected charophyte species to chemical restoration treatments (Immers et al. 2013; Rybak et al. 2017, 2020a). However, how submerged plants regulate their multi-elemental stoichiometry in response to chemical restoration treatments is completely unknown for many macrophyte species. This raises the crucial question of whether lake chemical restoration is as safe as previously assumed. Therefore, the aim of our study was to: (i) evaluate the functional traits and physiological response of *Myriophyllum spicatum* following changes in the abiotic environment during iron application, (ii) define stoichiometric features of plant adaptation to iron coagulant, (iii) assess the micronutrient stoichiometry adjustment as a response to changes in their availability. The classical C, N, P approach was extended in our study to other macronutrients, i.e. calcium (Ca) and magnesium (Mg), as well as microelements: iron (Fe), zinc (Zn), copper (Cu) and cobalt (Co).

Materials and methods

Study species, cultivation and experimental set-up

Myriophyllum spicatum, a rooted, perennial submerged aquatic plant was used as a model species because it tolerates eutrophic conditions, is a very common in both clear and turbid state waters, and has worldwide distribution (Song et al. 2017). Due to the habitat requirements, *M. spicatum* is highly vulnerable to restoration treatments, which also makes it an excellent and universal research object.

Three shoots of *M. spicatum* were planted per one pot filled with prepared substrate (Rodrigo 2021). Each pot was placed in a cylindrical glass microcosm filled with 2 L of water. A set of 30 microcosms were placed in a controllable cultivation room. After the acclimatisation period, baseline conditions were determined, and iron (III) chloride ($FeCl_3$) was applied (time “Apl”). Due to its strong coagulation and flocculation features, it is widely used in lake restoration processes. The coagulant is characterised by very low pH (<1.0), high density (1.44 g cm^{-3}) and dark, strong brown colour. To mimic the effects of iron coagulant addition in lake restoration treatments, it was applied once in the following concentrations: C1—0.02, C2—0.05, C3—0.09, C4—0.18 and C5—0.37 g Fe dm^{-3}. Employed doses exceed concentrations commonly dispensed into degraded lakes; however, they still correspond to so-called “aggressive restoration” and are applied commercially (Orihel et al. 2016; Bakker et al. 2016). A microcosm without coagulant addition was used as a control. The entire system was replicated five times and the study was carried out for 14 days starting from the application time (Supplementary Materials). Coagulant doses are predominantly reported as the amount dosed per square meter. However, when the effects of the coagulants on the organisms are being measured, the concentration of the substance in water is much more informative than the carrier’s dose per surface area. Therefore, we decided to report the concentration of iron per liter rather than the amount dosed.

Physicochemical analysis of water, sampling and plant functional traits

Physicochemical measurements were performed at the same time of the day as follows: 5 and 1 days before Apl (-5, -1, respectively), immediately before Apl, 4 h and 1 day after Apl, and repeated every 2 days until the end of the experiment (Fig. 1). The following parameters were measured: water and substrate pH, oxygen concentration, electric conductivity, turbidity and water colour. Substrate and plant sampling for chemical analyses and morphological traits measurements was performed at the end of the experiment.

Morphological traits of each specimen were measured at the endpoint of the experiment as follows: main shoot length, root length, eventual side-axis length and their number, total fresh mass, root, stem and leaf mass fraction, total dry mass and the relative growth rate (RGR) according to Hunt formula (Hunt 1990). More details and procedure descriptions are attached in the Supplementary Materials.

Elemental analyses, pigment content and parameters of oxidative stress

Nutrient concentrations in water were analysed as dissolved inorganic phosphorus ($DIP = PO_4^{3-}$) and dissolved inorganic nitrogen ($DIN = NO_2^- + NO_3^- + NH_4^+$). Further calculations were performed based on DIP and DIN since only dissolved forms can be taken up by macrophytes and thus better reflect stoichiometric reliance than total forms (Reynolds and Davies 2007; Glibert et al. 2011). In addition, the concentrations of Fe, Ca, Mg, Zn, Cu, Co and Cl^- ions in water samples were analysed. In plants and bottom substrates were analysed following elements: C, N, P, Fe, Ca, Mg, Zn, Cu and Co.

Analysis of chlorophylls (Chl-*a*, Chl-*b*) and carotenoids (Caro) was conducted using the spectrophotometric method after methanol extraction (Lichtenthaler and Buschmann 2005). The total phenolic content

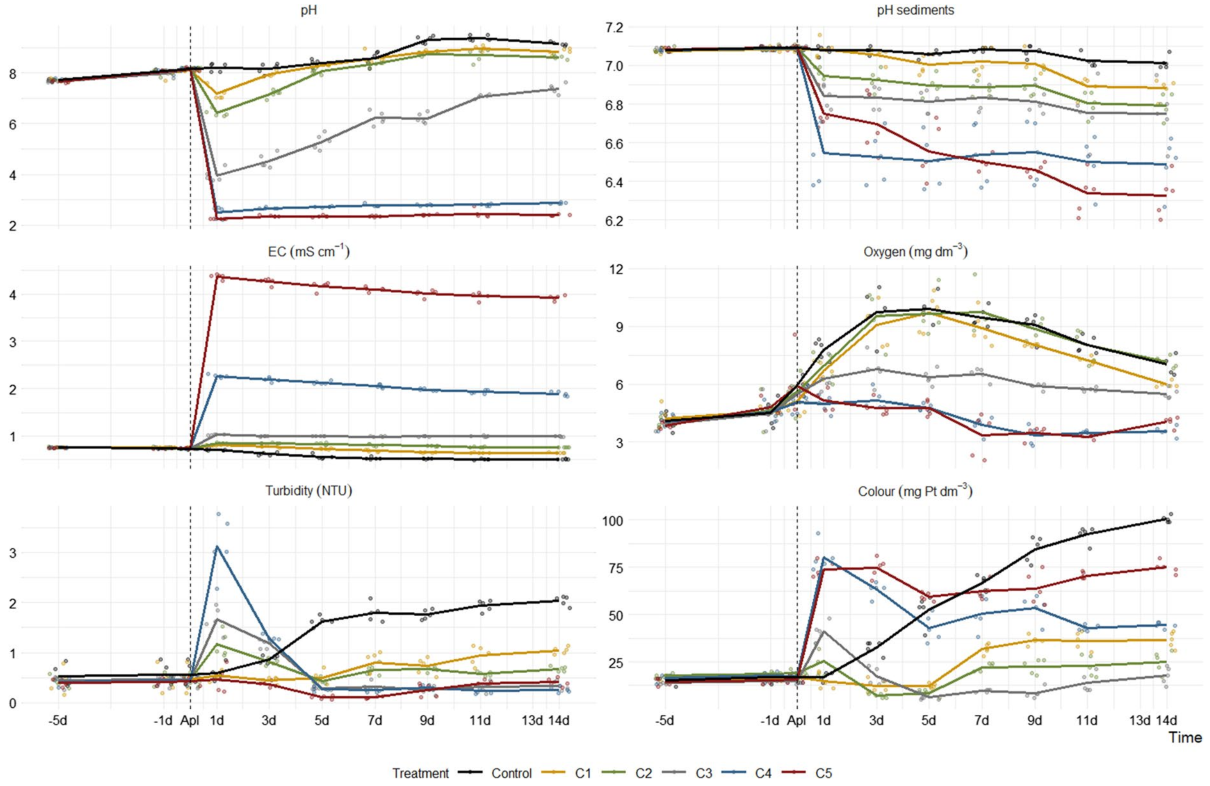

Fig. 1 Physicochemical parameters of water during the experiment; Apl abbreviation and dashed line indicates coagulant application time

(TPC) was determined by the Folin-Ciocalteu method (Singleton et al. 1999) and expressed in mg of gallic acid equivalents per g of fresh tissue (mg GAeq g^{-1} fresh weight, fw). Total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method (Woisky and Salatino 1998) and expressed in mg of quercetin equivalents per g of fresh tissue (mg Qeq g^{-1} fw). The content of free salicylic acid (SA) was assayed according to the method of Yalpani et al. (1991). The equipment used, descriptions of procedures and standards applied are detailed in the Supplementary Materials.

The degree of stoichiometric homeostasis of *M. spicatum* was presented using the homeostasis coefficient *H* (eta): $\mathrm{H} = \frac{\log_{10}(x)}{\log_{10}(y)-\log_{10}(c)}$ where x is the resource nutrient stoichiometry, y is the organism's nutrient stoichiometry (the same units as a resource) and c is a constant (Sterner and Elser 2002). Consequently, *1/H* is the slope of the regression between log(x) and log(y) and was expected to be greater than or equal to zero (homeostatic) and one (plastic). If the relationship was non-significant at $p > 0.05$, *1/H* was considered as strict homeostasis (Persson et al. 2010).

Statistical analysis

The differences between treatments with respect to the physicochemical and optical properties of water were assessed by within-subject analysis of variance. Elements' concentration in water, substrates and plants, morphological traits of *M. spicatum*, pigment concentration, and oxidative stress parameters were analysed by one-way analysis of variance. Linear mixed-effects models (Bates et al. 2015) were used to test relation between element concentration in a plant and the environment (Supplementary Materials). The elements below the detection limit were not included (N, P, Fe). Analyses were performed using the R software (ver. 3.5; R Development Core Team).

Results

Physiochemical properties and elemental characteristics of water and substrates

Water treatment with iron coagulant significantly altered its physicochemical properties (Fig. 1). After 4 h of coagulant application, water pH decreased from initial 8.14 ± 0.02 in the control to 6.63 ± 0.01 in C1 and 2.02 ± 0.00 in C5. Neutralization was observed within 24 h in C1, 3 days in C2 and after 11 days in C3. In C4 and C5 treatments water pH slightly increased, but did not reach pH 3.0 at the end of the experiment. It was due to hydrolysis processes that occurred intensively only when the acidic coagulant was added to slightly alkaline water. Throughout acidifying the entire microenvironment, these processes were suppressed, resulting in a lack of alkalinization. The substrates in treatments C3–C5 were also acidified ($pH_{C3} = 6.8$, $pH_{C4} = 6.5$ and $pH_{C5} = 6.3$) and their pH remained constant until the end of the experiment (Fig. 1). After coagulant application, the EC values increased significantly compared to the control, reaching values from 793 ± 5 $\mu S\ cm^{-1}$ in C1 to 3556 ± 18 $\mu S\ cm^{-1}$ in C5 (Fig. 1, Table 1). Differences in oxygen concentration were observed between particular treatments providing three homogeneous groups: 1—Control, C1, C2; 2—C3 and 3—C4, C5 (Fig. 1). Iron coagulant addition caused an increase of turbidity and water colour at the Apl timepoint lasting for ~ 24 h. Afterwards, the turbidity and water colour decreased to a value comparable to the control system, for which an increased level of both parameters was observed until the end of the experiment (Fig. 1, Table 1). The decrease was caused by the coagulant reactions and generated large amounts of a rust-like (orange-red) coloured suspension, which sedimented to the bottom and covered the plants (Fig. S1).

The coagulant application resulted in significant changes in macronutrient and microelement levels in water. DIN in control, C1 and C2 treatments remained at a similar level (~ 0.2 mg dm^{-3}), whereas in C3, C4 and C5 it increased significantly to 2.7 ± 0.3, 5.4 ± 0.4 and 5.9 ± 0.2 mg dm^{-3}, respectively ($F_{5,\ 24} = 620.1$, $p < 0.001$). DIP showed the opposite pattern and was present only in the control while in other treatments it was successfully removed ($F_{5,\ 24} = 89.0$, $p < 0.001$; Fig. 2). Relatively to the coagulant dose, concentrations of Zn and Co increased (from 0.000 ± 0.001 in the control to 19.230 ± 0.339 and 0.061 ± 0.005 mg dm^{-3} in C5, respectively), while Cu concentration significantly increased in C4 and C5 treatments compared to the control (Fig. 2). Despite the large amount of Fe supplied along with the phosphate coagulant, Fe ions were detected only in C4 and C5 treatments at the end of the experiment (Fig. 2). Coagulant application increased Ca concentration from 6.60 ± 0.29 in the control to 30.39 ± 4.34 mg dm^{-3} in C5, excluding C3 treatment, where Ca concentration was similar as in the control. Mg concentration was comparable for control, C1 and C2 treatments (15.51 ± 0.23 mg dm^{-3}), while in C3 it decreased to 12.83 ± 0.11 mg dm^{-3}, and the highest values were noted in C4 and C5 ($F_{5,\ 24} = 329.40$, $p < 0.001$). Chloride ions were added along with the coagulant;

Table 1 Results of within-subjects ANOVA for abiotic parameters of water with treatment as a fixed factor and time as the repeated factor

Parameter	df	Treatment		df	Time		df	Treatment × Time	
		F	p		*F*	p		*F*	p
pH_{water}	5, 24	4952.7	< 0.001	5, 264	3295.5	< 0.001	55, 264	724.9	< 0.001
$pH_{substrates}$	5, 24	30.9	< 0.001	8, 192	126.0	< 0.001	40, 192	10.7	< 0.001
EC	5, 24	9054.1	< 0.001	11, 286	8713.6	< 0.001	55, 286	3960.8	< 0.001
Oxygen	5, 24	198.5	< 0.001	9, 45	93.2	< 0.001	45, 234	16.6	< 0.001
Turbidity	5, 24	179.1	< 0.001	8, 192	101.0	< 0.001	40, 192	76.0	< 0.001
Water colour	5, 24	147.5	< 0.001	8, 192	60.5	< 0.001	40, 192	21.7	< 0.001

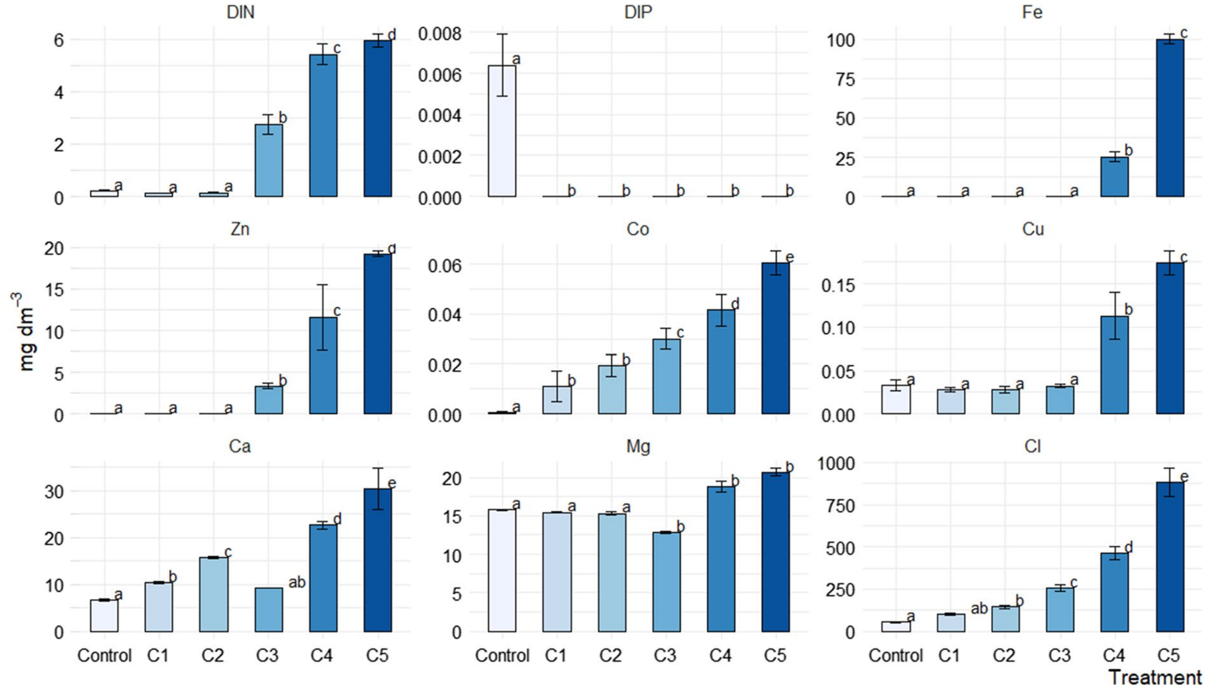

Fig. 2 Effect of coagulant application on element concentrations in the water at the end of the experiment (mean value ± standard error); different letters denote significant differences at $p < 0.05$

thus their concentration significantly increased from 52.0 ± 1.3 in the control to 883.2 ± 81.8 mg dm^{-3} in C5 ($F_{5, 24} = 335.1$, $p < 0.001$; Fig. 2).

Water elemental composition was significantly altered as a result of coagulant application, however, substrates composition remained undisturbed (Fig. S2).

Growth, biochemical response and stoichiometric adjustment of *M. spicatum*

Changes in physicochemical parameters of water following coagulant application, mainly strong and persisting acidification in C4 and C5 treatments, caused specimens' death, and only individuals from the remaining treatments were analysed. However, the very fact of their deaths should be considered an important result.

Specimens of *M. spicatum* in the coagulant treatments differed considerably in their functional traits from control plants. Stem elongation was inhibited by ~ 20% for all treatments, however only in C3 was a 26% reduction found significant ($F_{3, 16} = 4.26$, $p < 0.02$). Growth retardation was reflected by the RGR factor, which decreased in all treatments by ~ 28, 24 and 34% for C1, C2 and C3, respectively, compared to the control ($F_{3, 16} = 3.91$, $p < 0.03$), with statistical significance in C3 only (Fig. 3). Despite the reduction of stem elongation growth, stem dry mass remained unchanged. Coagulant application caused root length reduction, most pronounced in C3 (by ~ 57%; $F_{3, 16} = 24.17$, $p < 0.001$). In remaining treatments root length was comparable to the control, but their dry mass was reduced by ~ 78% ($F_{3, 16} = 3.84$, $p < 0.01$). Growth disturbances were reflected in leaf dry mass, which was lower by ~ 45, 55% and 60% in C1, C2 and C3, respectively, with significant differences for C2 and C3 versus the control ($F_{3, 16} = 5.36$, $p < 0.01$). An analogous trend was noted for total dry mass, which was lowered in all treatments, but a significant reduction was observed only in C2 and C3 (~ 54% and 51%, respectively; $F_{3, 16} = 4.23$, $p < 0.05$). Non-significant differences were noted for side-axis length and side-axis number (Fig. 3).

Coagulant application caused an increase in Chl-*a*, Chl-*b* and Caro contents in *M. spicatum* tissues; however, these changes were not significant compared to the control. In contrast to chlorophylls, TPC

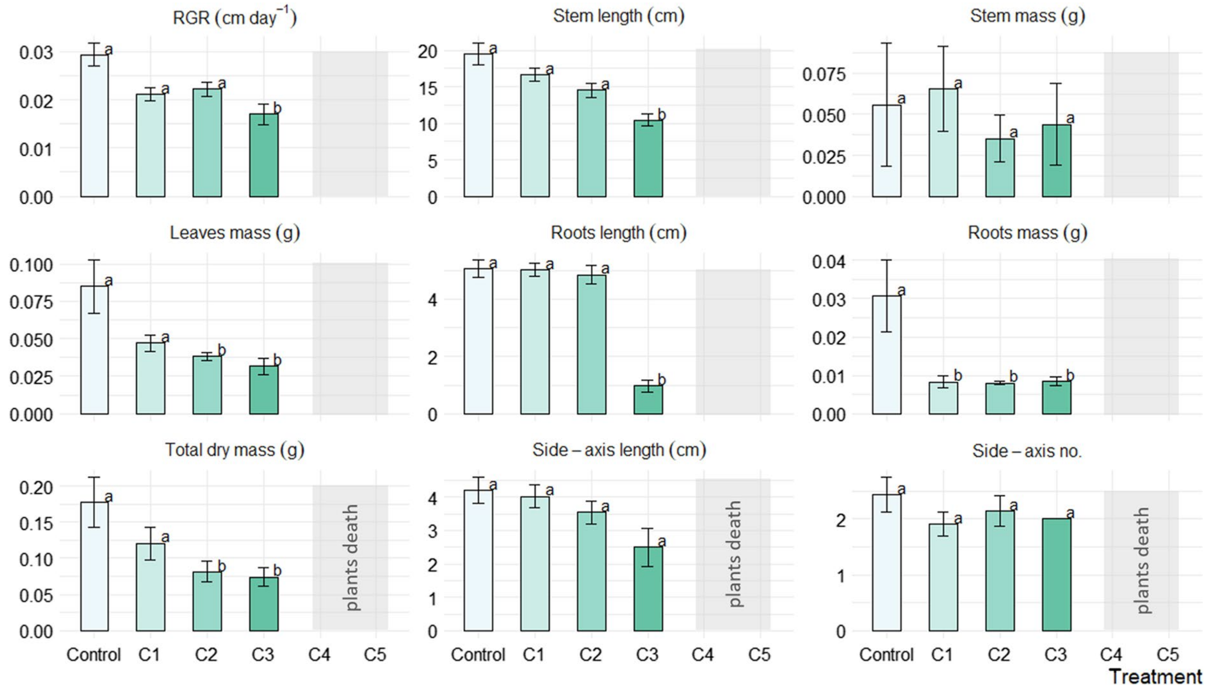

Fig. 3 Effect of coagulant application on biometric traits of *M. spicatum* at the end of the experiment (mean value ± standard error); mass of particular fraction refers to dry mass; different letters denote significant differences at $p < 0.05$

decreased in the coagulant treated group and TFC was characterised by fluctuation between treatments. Nonetheless, both TPC and TFC changes were not statistically different from the control or between treatments (Table S1). Similarly, SA content remained at a similar level at the experiment endpoint.

Analysed elements were characterized by increasing concentration in *M. spicatum* with increasing coagulant application. Carbon content significantly enhanced its share in the tissue between treatments from 310 ± 19 in the control to 385 ± 3 mg g^{-1} dry weight (dw) in C3 ($F_{3,\ 16} = 50.7$, $p < 0.001$). Likewise, N concentration increased in plants following coagulant addition, i.e. from 28 ± 3 in the control, through 34 ± 2 (by ~ 22%) in C1 to 43 ± 1 mg g^{-1} dw in C3 (~ 53%; $F_{3,\ 16} = 40.9$, $p < 0.001$). These changes led to a decreased C:N ratio, from 11 in the control, through 10 in C1 and C2 to 9 g g^{-1} in C3 ($F_{3,\ 16} = 17.5$, $p < 0.001$; control, C1, vs C2, C3, $p < 0.05$). Simultaneously, P concentration was comparable in all treatments and fluctuated at the level of 2.4 ± 1 mg g^{-1} dw ($F_{3,\ 16} = 1.1$, $p = 0.39$). Further, non-significant differences were noted for C:P ratio with the average level of ~ 148 g g^{-1}. Ca concentration significantly decreased with coagulant dose ($F_{3,\ 16} = 40.7$, $p < 0.001$) from 90.45 ± 0.01 in the control to 12.22 ± 0.02 mg g^{-1} dw in C3. Unlike Ca, Mg content significantly increased ($F_{3,\ 16} = 10.3$, $p < 0.001$), mainly in C2 (120.8 ± 11.1) and C3 (111.3 ± 11.0 mg g^{-1} dw) treatments, changing Ca:Mg ratio from 1.1 in the control to 0.1 g g^{-1} in C3. Fluctuations in Ca concentration significantly ($F_{3,\ 16} = 44.5$, $p < 0.001$) altered C:Ca ratio from 4 in the control, through 7 in C1, 16 in C2 to 30 g g^{-1} in C3, whereas C:Mg ratio remained undisturbed (~ 4 g g^{-1}).

In the case of micronutrients, a similar trend was observed as for macronutrients. Zn content in *M. spicatum* tissues increased from 0.16 ± 0.01 in the control to 0.31 ± 0.04 in C1 (by ~ 97%) and to 1.06 ± 0.2 mg g^{-1} dw in C3 (by ~ 550%; $F_{3,\ 16} = 38.4$, $p < 0.001$). For Cu a significant increase was noted in all treated plants ($F_{3,\ 16} = 58.4$, $p < 0.001$) from ~ 50% (up to 0.19 ± 0.02 mg g^{-1} dw) in C1 to ~ 270% (up to 0.45 ± 0.06 mg g^{-1} dw) in C3. Co content displayed relatively minor fluctuations in the tissue, but significant differences ($F_{3,\ 16} = 154.3$, $p < 0.001$) were observed between the control and

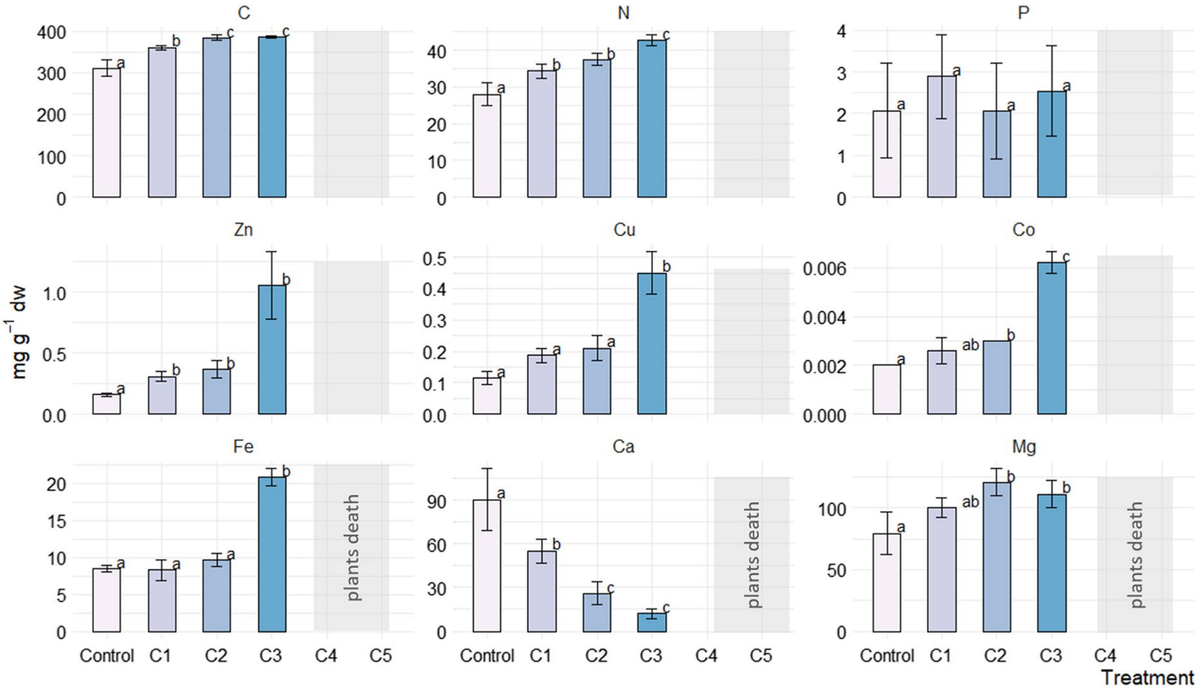

Fig. 4 Effect of coagulant application on element concentrations in the *M. spicatum* tissues at the end of the experiment (mean value ± standard error); different letters denote significant differences at $p < 0.05$

C2 (0.003 ± 0.000 mg g^{-1} dw) with the increase of ~ 50% and C3 (0.006 ± 0.000 mg g^{-1} dw) with the increase of ~ 200% (Fig. 4). Despite Fe influx as a coagulant component, its content in the tissue was constant for the control, C1 and C2 treatments (~ 8.8 ± 1.1 mg g^{-1} dw), and a significant increase was noted in C3 (20.8 ± 1.1 mg g^{-1} dw) ($F_{3,\,16} = 173.8$, $p < 0.001$).

Analysis of relation between element concentrations in water, substrates and *M. spicatum* tissues indicated that in all cases substrates have a negligible effect on tissue content. In the case of the Zn water-tissue relation, there was a significant increase of tissue content within an elevated concentration of Zn in water (t-value = 7.40; $p = 0.02$; $R^2_{model} = 0.93$) and return $1/H_{Zn} = 0.22$. Neither the Cu nor the Co relation model demonstrated a significant impact of the explanatory variable (t-value = 0.97; $p = 0.35$; $R^2 = 0.93$ and t-value = − 0.17; $p = 0.87$; $R^2 = 0.91$, respectively) while maintaining a high level of fitness and return $1/H$ values – $1/H_{Cu} = 0.26$ and $1/H_{Co} = 0.02$. In the case of Ca and Mg, exponents were characterised by a negative value, but neither macronutrient in *M. spicatum* tissue was affected by its concentration in water (t-value = − 0.72; $p = 0.47$; $R^2 = 0.93$ and t-value = − 0.94; $p = 0.68$; $R^2 = 0.70$, respectively; Fig. 5) and return negative value of $1/H$ – $1/H_{Ca} = − 0.91$ and $1/H_{Mg} = − 1.14$.

Discussion

Abiotic parameters of water

Phosphate coagulant, iron (III) chloride, caused marked changes in water parameters and significantly influenced the functional traits and ecological stoichiometry of *M. spicatum*. The observed water environment transformations were specific for chemical lake restoration methods (Immers et al. 2014; Rybak et al. 2020a). At the first stage, high acidification and

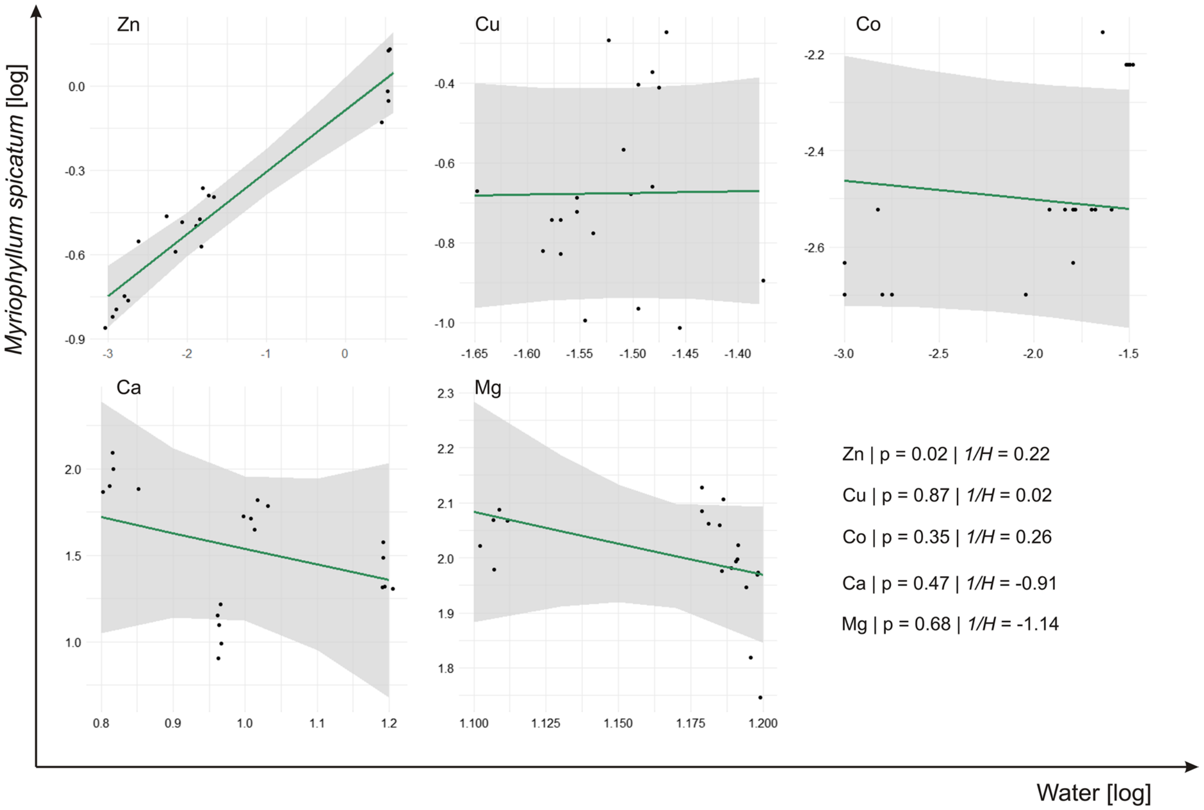

Fig. 5 Linear mixed-effects models' analysis of the element content in *M. spicatum* in relation to water concentration in log–log values with homeostasis coefficient (*1/H*) values

increase of turbidity and water colour occurred. The alterations of pH and water conductivity were neutralized within time in a dose-dependent manner with the exception of the highest treatments, which they remained unaltered through the entire experiment. This result suggests a risk posed by high concentrations of coagulants, especially in shallow or poorly buffered water bodies (Rybak et al. 2019). The effects of environment acidification are multidimensional. They are mainly related to increased solubility and toxicity of metals followed by their release from bottom sediments and carbon balance shifting towards carbon dioxide (Brouwer et al. 2002).

The deterioration of water optical properties resulted from the coagulant's intense brown colour and immediate initiation of complexation processes, leading to precipitation, flocculation and sedimentation of aggregate-flocs (Pizarro et al. 1995). In this way, specimens were covered with a large amount of orange-red sediment, which limited light availability (Rybak et al. 2020a). In control, increased turbidity and water colour resulted from developing of phytoplankton cells. The availability of light and nutrients is a major factor determining the growth and fitness of submerged plants (Barko and Smart 1981). However, this is a principle of coagulant operation in lake restoration—phosphate elimination, creation of flocks and moving them together with suspensions to sediments (Cooke et al. 2005; Dunalska and Wiśniewski 2016; Orihel et al. 2016).

Besides the release of Fe, significant amounts of additional ions are introduced with coagulant (mainly Cl^- or SO_4^{2-}) related to the acid used for coagulant production as well as metallic impurities related to industrial production (e.g. Zn, Co, Cu). It was reflected by the persistent increase in EC and chloride concentration observed despite the precipitation and neutralization processes. Weakly reactive chlorides remain fully dissolved in water, and only a small amount of them is subject to sorption by bottom sediments. Meanwhile, they are the primary mineral anion for plants (Parihar et al. 2015).

Macrophyte growth and biochemical response

The observed responses of *M. spicatum* to iron coagulant application is generally in line with the previous studies on other macrophytes (Lin et al. 2021). Experiments carried out by Immers et al. (2014) demonstrated that *Potamogeton pectinatus* growth significantly decreased with increasing iron (III) chloride concentration. Nevertheless, the functional traits response can vary even within the genus (Liu et al. 2021). Studies on *Chara* macroalgae demonstrated a significant inhibition of growth in the case of *Chara globularis* and *C. tomentosa* (Immers et al. 2013; Rybak et al. 2020a), while *C. hispida* showed intensified development of rosettes at the expense of reproduction as an increase in the photosynthetic surface in response to coagulant application (Rybak et al. 2017, 2020b). In the present study, significant reduction of growth parameters was noted mainly for C3 treatment, but growth retardation was also noted at lower coagulant doses. *M. spicatum* did not exhibit a typical mechanism of morphological response to shadow, i.e. elongation to a better-illuminated water surface, yet only a slight increase in photosynthetic pigments that could increase the efficiency of photosynthesis was noted (Kirk 2010; Hussner and Jahns 2015). That gives a false picture of *M. spicatum* resistance, since the analysis of stoichiometric changes indicated the activation of mechanisms aimed at minimizing stress associated with shading.

Metal toxicity symptoms in plants include retardation of the overall plant growth, reduction in the leaf surface, formation of local discolouration of leaves (chlorosis, necrotic spots) with the possibility of total necrosis and plant death (Küpper et al. 2007; Decou et al. 2019). Additionally, iron toxicity manifests as blackening of roots, a loss of their flaccidity and reduced branching (Lucassen et al. 2000; Van Der Welle et al. 2006), and they were observed for *M. spicatum*. Besides direct toxicity (e.g. by displacing Mg in the chlorophyll molecule by Cu or Zn), excessive metals cause oxidative stress (Andresen et al. 2018) and damage to the photosynthetic apparatus (Küpper et al. 2007). Thus, no change in the photosynthetic pigment concentration could be linked with shading stress accompanied by the antagonistic effect of Zn and Cu toxicity.

Despite high concentrations of metal ions in water, their intense accumulation and observed toxicity symptoms, the accumulation of phenolic metabolites (well-known parameter of oxidative stress) was not observed in *M. spicatum*. However, numerous studies have reported metal-induced accumulation of phenolics in aquatic plants (Sinha et al. 2009; Decou et al. 2019), acting as free radical scavengers, metal chelating agents and signalling molecules. Intense biosynthesis of phenolic metabolites is observed in plants challenged with biotic or abiotic stressors (Kartal et al. 2010), and was documented in previous studies on plant response to oxidative stress inducers (Waśkiewicz et al. 2014; Drzewiecka and Mleczek 2017). It can be assumed that within two weeks after coagulant application *M. spicatum* overcame the oxidative stress, neutralized excessive metal ions and recovered the oxido-reductive balance. It was confirmed by the elevation of TPC and the antioxidant activity towards DPPH radical detected in water in the case of C4 and C5 treatments (results not shown). As assumed, antioxidants produced under the influence of the coagulant were released into the water environment due to plant death and leakage of intercellular content.

Stoichiometric adjustment

The coagulant application significantly changed the water chemistry features; however, it did not affect substrates chemistry despite its slight acidification. The model analysis revealed the ecological stoichiometry changes in *M. spicatum* resulting from the plant-water interactions, while the plant-substrates relationship was marginal, which is in line with previous studies (Xing et al. 2013). Changes in nutrient level, form or ratio lead to cellular adjustments in acquisition efficiency and can alter the proportions of ribosomes, enzyme activities, gene regulation, cellular pigmentation complement, and cellular elemental composition. These features have implications for competition and species fitness, changing species dominance and biodiversity. The environmental changes were reflected in plants' elemental composition, placing themselves very strongly in the luxury consumption hypothesis, which assumes maximized nutrient uptake during occurrence of different limiting growth factors (e.g. light, nutrient). Luxury consumption is reflected in unbalanced growth and variable chemical composition (Sterner and Elser 2002). This reduces the carbon: nutrients ratio, thus increasing the nutritional quality of plants and influencing the rebuilding of the food web (Küpper and Andresen 2016; Velthuis et al. 2017). Although the structural role of C is independent of the biochemical roles played by N and P, both C and N concentration significantly increased in plants within the coagulant dose. This, together with inhibited growth and development indicated an increase in nutrient accumulation. Plants adjust C and N metabolism by the synthesis of the required metabolites such as soluble carbohydrate, starch and free amino acid and maintain metabolic homeostasis to facilitate the tolerance to low light stress and, instead of growth, investing in the survival of unfavourable conditions (Middelboe and Markager 1997; Yuan et al. 2017). Acidification influences the proportion of C forms (towards CO_2), affecting the photosynthesis rate, which was demonstrated by increased oxygen concentration (Maberly and Madsen 1998). The above explanation is confirmed by the concentrations of nutrients in water; N was depleted in treatments with a lower coagulant dose; therefore with a lower stress intensity, P in plants did not change, since it was removed from the water, and Fe toxicity resulted in impaired root uptake (Sperotto et al. 2010). Limited P leads to a low RNA content and reduced growth rate (Elser et al. 2003). Additionally, the C:N ratio in plants decreased with increasing coagulant concentration, which means that the more growth was inhibited, the greater accumulation of nutrients occurred. Moreover, an excessive N absorption by submerged plants and the following internal physiological damages and growth inhibition may lead to degradation of submerged vegetation (Dai et al. 2022). The high content of N in water in C4 and C5 treatments was due to the plants' death and the leakage of the cell contents into the water environment. Fe concentrations showed a similar pattern, although its availability in water was short-lasting and pH-dependent (Pizarro et al. 1995). This explains the similar accumulation of Fe observed for C1 and C2 treatments. The long-lasting environment acidification in C3 extended the availability of Fe ions and increased their uptake by plants. Along with pH neutralization, soluble Fe decreased due to the precipitation process. These mechanisms were reflected in the absence of Fe in water at the end of the experiment in C1-C3 treatments and its continued presence in C4 and C5.

Changes in the element concentrations also concerned macronutrients such as Ca and Mg. The increased concentration of Ca in water was associated with a decrease in plants at higher coagulant doses accompanied by pH decline. It resulted from the dissolution of calcium carbonate ($CaCO_3$), covering plants and generated due to photosynthetic activity (Cicerone et al. 1999). This process was previously described for the chemical restoration method applied to the highly incrusted charophytes community (Rybak et al. 2019). A negative exponent in the analysed model also indicates Ca flow from plants to water. However, the homeostasis maintenance factor appears to be misguided because of the physical removal of Ca through the dissolution process, and not by the uptake restrictions. Although the *1/H* coefficient is commonly used, the obtained results confirm that it should be interpreted with care for both physiological and mathematical reasons (Persson et al. 2010). On the other hand, a negative *1/H* ratio was already noted for macrophytes, indicating strong Ca stoichiometric homeostasis (Xing et al. 2015). However, the total amount of Ca in plants decreases as a result of the iron coagulant application, which was reflected in the C:Ca ratio, and the reduction of the nutritional value. Although Mg exhibits similar chemical features, its changes were distinct from Ca. Due to the greater resistance of magnesium carbonate ($MgCO_3$) to dissolution (Müller et al. 1972), Mg concentration in water increased only in microcosms with the highest acidification (C4, C5), even though its amount increased in coagulant-treated plants compared to the control. This is related to similar mechanisms as for C and N, namely Mg uptake from the environment and growth inhibition resulting in an increase of Mg accumulation in tissues. Simultaneously, C accumulated at a similar rate caused that the C:Mg remained relatively stable which means that Chl *a*/*b* ratio that is depended on Ca:Mg (Vymazal 1995) was not disturbed.

Zinc concentration in *M. spicatum* tissues was significantly related to its concentration in water, and although *1/H* reached values indicating the ability to maintain homeostasis (Persson et al. 2010), it was significantly different from zero. This means a linear data system, confirmed by the high fit of the model, and despite the low exponent it was different from zero, which indicates the imperfection of this parameter. However, *M. spicatum* is not able to maintain Zn homeostasis. Zn limitation is more widespread in the environment than Zn toxicity, though anthropogenic activities can enhance its concentrations up to the toxic level (Küpper and Andresen 2016). The main symptoms of Zn toxicity involves chlorosis and necrosis of leaf blades (Broadley et al. 2007). Nevertheless, despite the high degree of accumulation (Samecka-Cymerman and Kempers 2004), minor toxicity symptoms were observed for *M. spicatum* plants. Moreover, the obtained results indicate that lake restoration processes can be another source of Zn pollution, unknown and not considered so far.

Both Cu and Co showed increased concentrations in plant tissue as a result of coagulant application. In relation to the environment, they showed strong (Co) or weak (Cu) ability to maintain homeostasis in analysed concentration intervals, and the *1/H* parameter did not differ significantly from zero. Although the level of accumulated microelements was relatively high, *M. spicatum* can be considered as a resistant species. Specimens collected in industrially polluted areas contained Co and Cu at much higher levels than those considered toxic for plants (Samecka-Cymerman and Kempers 2004). These results are at odds with the work of Xing et al. (2015) showing weak elemental stoichiometric homeostasis in macrophytes.

It should be emphasized that biogeochemical pathways and homeostatic control service constitute a comprehensive system whereby nutrient dynamics support the trophodynamic structure. Relations between biogeochemistry and homeostasis regulate the stable ecosystem state and cause shifts during disturbances to new ones—gradual or abrupt—and return to the original state may not occur even once the disturbance has subsided (Glibert 2012; Su et al. 2019). The homeostasis of trace elements is tightly regulated; however, even an excess of one element can cause disorder in these mechanisms. For example, Fe limitation symptoms are similar to Zn toxicity, Zn limitation similar to Cd toxicity, etc. (Grotz and Lou 2006). It results from shared ion transporters, since the same systems transport many bivalent ions, e.g. Fe transporters can also bind Zn. This means that the increased uptake of one element can lead to enhanced binding of another element, leading to its toxicity to the plant (Walker and Connolly 2008). Vice versa, under the high level of one element in the tissue, the other can alleviate its toxicity. It was demonstrated in Mg and Al for several plants (Bose et al. 2011) or Zn and Cd in *Ceratophyllum demersum* (Aravind and Prasad 2003). The mechanism controlling reduced toxicity results from competition between metal ions for binding sites of transporters. In this way, high Fe and Zn accumulation in plant tissue and occupation of the transporter binding site prevents further accumulation of Co and Cu and affects homeostasis maintenance of these elements. Moreover, the metabolic links between Cu and Fe homeostasis and mutual uptake regulation are well documented (Pätsikkä et al. 2002; Puig et al. 2007).

A parallel issue accompanying the use of coagulants in water restoration processes is high concentration of metallic impurities. Although the excess of Zn, Cu, Co did not show direct toxicity to *M. spicatum*, it is not excluded that it may pose a threat to other organisms, especially in the case of repeated treatments. Due to the form that is easily accessible for plants, accumulation in tissues and increasing share in the trophic chain, they may cause disturbances of the entire food network. Moreover, studies on the direct and negative effects of coagulants on animals are already available (see Bonisławska et al. 2021; Drewek et al. 2022). This may result in changes that are difficult to define from this perspective and certainly require increased investigation.

Conclusions

(i) Disturbances of the abiotic environment caused by iron chloride application were associated with environment acidification, reduced light availability and increased concentration of metals ions, including Fe, as well as coagulant impurities.

(ii) The growth and development of *M. spicatum* were significantly inhibited, and the changes were characteristic for iron toxicity.

(iii) Despite the lack of a functional traits response to low light and unaltered parameters of oxidative stress in *M. spicatum*, changes in ecological stoichiometry indicated the activation of defence mechanisms in response to coagulant application. It indicates that stoichiometric changes can reflect past stress when typical markers are no longer synthesized.

(iv) The high availability of both macro- and microelements in water induced luxury consumption and resulted in an increased concentration of these elements in the tissue, which, together with biomass decrease, can influence the entire food web.

(v) The relation between Cu and Co uptake by *M. spicatum* and their concentrations in water indicated a high ability of plants to maintain homeostasis under conditions characteristic for chemical water restoration related to the high concentration of Fe and Zn ions.

(vi) Despite the widespread use of iron as chemical methods in the restoration of surface waters, the application of phosphate coagulants may cause strong destabilization of ecosystems.

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Declarations

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