Intrapopulational variability and biochemistry of the globally rare species Coleanthus subtilis in response to the sediment parameters

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Abstract

Aims

Coleanthus subtilis is a globally rare or threatened, ephemeral therophyte of exposed bottoms of water reservoirs and riverbanks. Nevertheless, it shows a potential to conquer new areas and dominate in phytocoenoses showing morphological differentiation. The main aim of the study was to reveal the intrapopulational variability of the species in relation to sediment characteristics, ecological preferences in microscale, and allelopathic potential in the context of biochemical composition of its biomass.

Methods

A fishpond with a stable population was selected and data were collected from 107 plots distributed in transects perpendicular to the pond banks; in each plot following parameters were determined: pH, macroelement content in sediments, morphotypes of *C. subtilis* specimens, species composition of vegetation patches, and ecological indicator values. Collected specimens were used to determine the chemical composition and ecotoxicity.

Results

Morphological variability of *C. subtilis* is determined by pH and macroelement content of the sediments. Those parameters and values of ecological indicators (moisture, temperature and light) show a link to the composition of vegetation patches and the phytocoenoses with the high abundance of *C. subtilis*. Capability of *C. subtilis* to inhibit germination and growth of other species may account for its prevalence in phytocoenoses. Benzoxazinone derivatives, phenolic compounds and carboxylic acids known to be allelochemicals were identified in the species.

Conclusions

Morphological variability of *C. subtilis* is controlled by pH and nutrient content in sediments and species composition of vegetation patches; allelopathic effect found experimentally may be conditioned by chemical compounds determined in its specimens.

1. Introduction

Plant species, whose habitats disappear in the recent decades due to anthropogenic pressure but which have the ability to adapt to changing conditions, are of special interest in the context of plant species conservation. Such abilities are known in numerous plant species, representing different taxonomic and ecological groups (Adamowski 2006; Nowak and Nowak 2006; Zalewska-Gałosz et al. 2012; Böckelmann et al. 2017; Otto 2018; Rewicz et al. 2018; Fekete et al. 2019r et al. 2021). Among wetland plants, a

specific group constitutes the species associated with periodically exposed bottoms of water courses, which lose their habitats (Poschlod and Rosbakh 2018) as a result of the adaptation of river valleys for navigation or water damming to maintain its constant level. However, representatives of this group of species appear in substitute habitats, such as man-made ponds and water reservoirs, where conditions favorable for their complete life cycle occur periodically (Zając and Zając 1988; Deil 2005; Nowak and Nowak 2006; Spałek and Nowak 2006; Šumberová et al. 2006, 2012; Popiela et al. 2010; Francová et al. 2019). One of the most interesting species in this group is the moss grass *Coleanthus subtilis* (Tratt.) Seidl, classified as a globally rare vascular plant (Richert et al. 2014, 2016). It is a small annual grass from the family Poaceae, reaching 5–10 cm in height. The species is known for its disjunctive geographic range, occurring in Asia, North America and Europe. In Asia, it grows primarily along large rivers in Russia, such as Amur (Nečajev and Nečajev 1972) or Ob (Taran 2001), as well as in China (Liu et al. 2022). In North America, it is present mostly in the western part, on the border of the USA and Canada (Catling 2009). In Europe, the largest number of sites of *C. subtilis* is currently known in the Czech Republic, while the species localities (present or historical) have also been reported from Germany, France, Austria, Poland, Slovakia, Italy, Norway and Russia. Its populations have lost most of their natural sites in river valleys, and disappeared (Woike 1969; Bensettiti et al. 2002; Bernhardt 2005; Lacroix et al. 2006; Richert et al. 2014, 2016), but, importantly, the species has also a potential to colonize new areas. Thus, populations of *C. subtilis* are known primarily from banks or periodically dried bottoms of anthropogenic reservoirs, mainly fishponds. An example of species expansion into new locality is a location in SW Poland, where this species has been known for only two decades, initially from fishponds (Fabiszewski and Cebrat 2003). Starting from four ponds, where patches of vegetation with its participation were discovered, the population of this species has been gradually increasing in the region, and currently its specimens are being found in 19 ponds (personal observations). Because *C. subtilis* is listed in Annexes II and IV of the Habitat Directive and in Annex I of the Bern Convention, as well as due to the status of a legally protected plant in Poland (Dajdok 2012), its selected sites are subject to environmental monitoring. The results of the monitoring showed that in some reservoirs the number of *C. subtilis* specimens per 1 m^2 exceeds 6,000 and the entire population of this species in some ponds is estimated at millions of individuals (Leśniański et al. 2022). However, it should be stressed here that within the same pond, the frequency of *C. subtilis* phytocoenoses developing on exposed bottoms is diversified, and the specimens can form compact patches, in which they absolutely dominate, as well as patches (located in the neighborhood) with only sporadic participation. This may suggest that various factors, including bottom sediment characteristics, ecological conditions, plant-plant interactions, determine the quantitative participation of *C. subtilis* in patches of vegetation developing in similar conditions at the bottom of a periodically dried water reservoir.

Potential threats to *C. subtilis* populations are quite well recognized in a global (IUCN 2015) and European scale (Bilz et al. 2011; Richert et al. 2016), as well as in the countries where it occurs, e.g., in France (Lacroix et al. 2006), the Czech Republic (Danihelka et al. 2012; Šumberová 2013), Germany (John 2011; Richert et al. 2014), and Poland (Fabiszewski and Dajdok 2014). The habitat criteria, influencing the density of specimens, have been indicated (Dajdok et al. 2017) and conditions for maintaining it in ex

situ collections (Navrátilová and Navrátil 2022) have recently been published. However, little is known about factors determining the species success in newly inhabited areas. For example, the biochemical composition of the individuals and the species potential allelopathic capacity which may account for creating patches with its absolute dominance (Inderjit and Callaway, 2003), have not been recognized yet. Also, although, it is known that *Coleanthus* specimens can form compact rosettes or be slender and elongated and a few morphotypes were distinguished by Hejny (1969), the species intrapopulational morphological variability and its relationship to the habitat conditions have not been analyzed in detail.

Our main aim was to fill the gaps mentioned above. Therefore, we performed comprehensive analysis of the intrapopulational variability of the species in relation to bottom sediment characteristics, ecological preferences, phytocoenosis composition and assessed its allelopathic potential. In particular, we aimed at addressing the following questions:

1. what is intrapopulational variation of the main morphological features in *C. subtilis*?

2. how are the habitat conditions diversified in terms of pH and contents of macroelements in bottom sediments and Ellenberg indicators within one pond and how does this variation (or lack thereof) translate into the morphology of *C. subtilis* individuals and the composition of phytocoenoses with its participation?

3. what is the biochemical composition of *C. subtilis* specimens and are there any compounds which could be responsible for allelopathic interactions?

2. Material and Methods

2.1. Data sampling

Since its discovery at the end of the 1990s (Fabiszewski and Cebrat 2003), *C. subtilis* has been occurring in the Staw Borowski fishpond near Borowa Oleśnicka (SW Poland) regularly, in a great abundance, occupying a comparable area every year. As this particular population seems to be stable as well as the fishery management of the pond is, it could be treated as a model population. To eliminate the impact of the differences between ponds, the study was limited to one reservoir only.

Sampling of the bottom sediments, specimen collection and floristic lists were done between May 21 and 28, 2019, in the Staw Borowski fishpond (Borowa Oleśnicka village, Oleśnica Plateau, SW Poland). Staw Borowski belongs to the complex of fishponds which are a Special Area of Habitat Protection of Natura 2000 Stawy w Borowej PLH020045. Condition of *C. subtilis* population here is monitored every three years in the program of State Environmental Monitoring supervised by the Chief Inspectorate of Environmental Protection (CIEP) in Warsaw (Dajdok 2012). Collection of the specimens of *C. subtilis* was conducted with the permission of Regional Director for Environmental Protection in Wrocław (No. WPN.6400.202019.AR.1.).

Data collection was performed along 1 m wide transects perpendicular to the northern and western pond shores, where phytocoenoses with *C. subtilis* develop each year. During sampling, the pond was already partially filled with water thus transects were established between the water surface and the border between the slit and rush phytocoenoses at the foot of the inner slope of the pond dike. Each transect was divided into 1 m x 1 m squares. Depending on the distance between the bank of the pond and water transects contained from three to 25 squares; in total, 107 squares were analyzed. Within each square, a smaller plot of 10 cm x 10 cm was selected at the central part of the square, from which all *C. subtilis* specimens and the bottom sediments of upper layer (5 cm depth) were collected. Collected plants were further used to evaluate their biomass, selected morphological features, and to analyze the biochemical composition of the species and its allelopathic properties, whereas bottom sediments were used for chemical analyses.

2.2. Morphology

Specimens of *C. subtilis* collected within all the smaller plots (10 cm x 10 cm) were gently cleaned from soil; then, selected morphological features were measured in 30 randomly taken individuals or in all of them if there were less than 30 specimens per plot. Measurements were performed with an accuracy of 1 mm and presented as mean values for the feature per plot. The following lengths were measured (Fig. 1): the highest culm (a), the inflorescence (b), the uppermost leaf blade (c), and the uppermost leaf sheath, from the node to the leaf blade (d). Finally, the plants were dried at 70°C to a constant weight and weighted to calculate the total biomass of the individuals growing in each plot.

For further analyses, the specimens were classified into three groups, according to the size of the culm: 1) class I contained the specimens of height < 20 mm (below the minimal length cited in the literature), 2) class II the individuals of height between 20.1 mm and up to 50.0 mm (the average size range given in species descriptions), and 3) class III the above average size individuals > 50.1 mm (above the maximal length cited in the literature (Czarna et al., 2013; Dajdok, 2012; Fabiszewski and Dajdok, 2014; *Flora of North America North of Mexico [Online]*, 1993+; Hejný, 1969; John, 2011; Richert et al., 2014).

2.3. Ecology

2.3.1. Floristic survey

In each square within the transect, the phytosociological relevé was made according to the standardized methodology; all plant species were listed and their frequencies (in scale from one to seven) established (Westhoff and van der Maarel 1978). Seedlings and not fully developed plants, if the species was not possible to identify, were assigned to the genus level. Relevés were uploaded to the database Turboveg for Windows (Hennekens and Schaminée 2001) and analyzed using the Juice program (Tichý 2002). For each relevé, the mean values of Ellenberg ecological indicator (Ellenberg et al. 1992) in modification for Central European species by Tichý et al. (2023) were calculated; for further analyses following indicators were selected: light (L), temperature (T), moisture (M), nitrogen (N), and soil reaction (R). The average weighted values of the mentioned indicators were calculated for each of the 107 samples of the 1 m^2

area, using the values of these indicators assigned to the species found within the sample. The species names are given after the study by Mirek et al. (2020).

2.3.2. Analyses of bottom sediment

Prior to analyses, all plant residues, stones and objects were removed from the samples. Then sediments were air dried (at room temperature), sieved through a 2 mm diameter sieve and grinded to fine powder in a Mortar Grinder (PULVERISETTE 2, Fritsch). The reaction of soils was determined potentiometrically (soil/distilled water ratio 1:2.5) (Mikroprozessor-pH-meter HI9107, Hanna instruments). Content of total sulphur and carbon was determined with infrared absorption method using SC-144DR Sulfur/Carbon Determinator (LECO Corporation) and total nitrogen was analyzed with the Kjeldahl method using a Vapodest 40 automatic steam distillation apparatus (Gerhardt GmbH & Co. KG). The bioavailable fraction of metals (Ca, Cu, Fe, K, Mg, Mn, Zn) was determined in DTPA-extracts. The extracts were prepared by shaking sediments for 2 hours with extracting solution (0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M TEA adjusted to pH 7.3) and filtered through a filter paper (Benton Jones 2001). Finally, extracts were analyzed for Ca, Cu, K, Mg, Fe, Mn and Zn using flame atomic absorption spectrophotometry (FAAS) (AVANTA PM AAS, GBC Scientific Equipment). In order to measure P content, sediments were extracted in 0.03 M acetic acid and analyzed in flow injection analyzer (FIA compact, MLE GmbH).

Analyses were done in duplicate, and all results were calculated on a dry weight basis. To assure the high quality of analyses, Sigma Chemical Co. standards for all elements and blank samples containing the same matrix as the samples were used during all procedures. The Certified Reference Materials INCT-OBTL-5 (Oriental Basma Tobacco Leaves, Institute of Nuclear Chemistry and Technology Warsaw, Poland) were analyzed along with samples to check the reproducibility of the element determination. The calculated recovery rates were 97% (± 5 SD).

2.3. Allelopathic potential and biochemical composition

2.3.1. Testing of phytotoxic potential

To assess the allelopathic potential of *C. subtilis*, the impact of aquatic extracts on an indicator species (white mustard *Sinapis alba* L.) was studied (Kruse et al. 2000). For this purpose, different concentrations (0.5, 1, 2, 4, 6, 8%) of aqueous extract of *C. subtilis* were prepared by adding dried grinned plant material to distilled water, shaking the mixture for 4 hours, and then percolating through filter paper.

The impact of *C. subtilis* on germination was tested on seeds of *S. alba* (experiment I), while its impact on growth (visualized by radicle and hypocotyl elongation) on one-day-old seedlings, which were germinated the day before (experiment II). In both experiments, Petri dishes lined with filter paper were used for plant cultivation to avoid chemical and microbiological interactions which may occur in soil (Lipińska and Lipiński 2009; Vidotto et al. 2013). In each dish, 25 seeds or seedlings were arranged evenly and watered with 10 mL of extract or distilled water (in control). Each concentration of extract and control were tested in 4 dishes with seeds (experiment I) and in 4 dishes with seedlings (experiment II); in total, 100 seeds and 100 seedlings for each treatment were analyzed. Experiments were conducted for 8 days, in a growth

chamber at temperature 20 ± 2°C and a light intensity of 4000 lux with a photoperiod of 14:10h (light:dark) (Chon et al. 2002; Vidotto et al. 2013).

In experiment I, germinated seeds were counted every day and the germination speed was presented as the ratio of the number of living seedlings each day to the number of sown seeds*100%. In experiment II, to evaluate the seedling growth, lengths of roots and hypocotyls were measured on the last day of experiment.

3.2.2. Sample preparation and UHPLC/UV/MS analysis

To identify the compounds which could be responsible for allelopathic potential of the species the biochemical composition of *C. subtilis* specimens was determined (Inderjit and Callaway 2003). To this aim, the powdered plant material (8.9 g) was extracted by stirring with 900 ml of deionized water, at room temperature for 4 hours. The extract was filtered through the Whatman paper and evaporated under reduced pressure, yielded approximately a 0.29 g of dry residue. Then, 10 mg of the residue was solved in 10 mL of deionized water for mass spectrometry and passed through 0.22 µm filter before chromatography. The analyses were carried out by UHPLC/UV/MS (ultrahigh performance liquid chromatography with diode array detection coupled with electrospray time-of-flight mass spectrometry) in the negative ion mode. The Thermo Scientific UHPLC Ultimate 3000 system (Thermo Fisher Scientific, Waltham, USA) coupled with the ESI-QTOF compact TM (Bruker Daltonics, Bremen, Germany) mass spectrometer was used. Separation was achieved on a Kinetex C18 column (100 x 2.1 mm) of 2.6 µm core-shell particle size (Phenomenex, Torrance, USA). The gradient elution system consisted of 0.1% HCOOH in water (mobile phase A) and 0.1% HCOOH in acetonitrile (mobile phase B). The following elution program was used at a flow rate of 0.3 mL/min: 0 → 30 min (5 → 95% B), 30→40 min (95% B), 40→45 min (95→5% B), 45→50 min (5% B). The injection volume for samples solutions was 3 µL. All analyses were carried out isothermally at 30°C.

2.4. Statistical analyses

The normality of the data was checked using Shapiro–Wilk's W test and the homogeneity of variances by Levene's test, and as the distribution of the data deviated from normality and variances were not homogenous, non-parametric tests were chosen for all analyses. The relationship between morphological parameters of plants and habitat conditions (chemical parameters of bottom sediments and mean Ellenberg indicator values) was evaluated using Spearman's rank correlation. Then, three groups of specimens (classes I–III) were compared to check the differences between habitat conditions at sites in which they grew using Kruskal-Wallis ANOVA and Median Test with post-hoc Dunn's test. In the allelopathic experiments, differences in root and hypocotyl lengths between treatments with increasing concentration of the extract and the control were checked using the analysis of variance Kruskal-Wallis ANOVA and Median Test. Multiple comparisons of mean ranks (Dunn's test) were also calculated. Data concerning treatment with 8% extract were excluded from analysis because only 5% of all plants were alive at the end of the experiment, so the results were not representative. The probability level was set at 0.05 for all tests. All calculations were carried out using Statistica 13 (StatSoft Inc. 2013; Dell Inc. 2016).

3. RESULTS

3.1. Ecology

In total, in 107 analyzed plots, 4,367 specimens of *C. subtilis* were collected, and 2,685 of them were used for the measurements. The number of individuals per small plot (10 cm x 10 cm) ranged from 4 to 186, and on average counted 41 plants. The total dry biomass of *C. subtilis* specimens in one plot ranged from 29.4 to 1035 mg. Analyzed specimens showed the wide range of morphological variability (Fig. 2) with three distinct phenotypes and a gradual transition between them.

Some plants, which represented class II, formed dense, compact tufts, with numerous and relatively short culms. Leaf blades in these plants were relatively short, wide and conspicuously curved, their leaf sheaths were strongly inflated and with purple-reddish coloring. The inflorescences were also short, with densely packed branches and groups of spikelets (Fig. 2A). Plants representing the opposite phenotype (class III), were elongated, with a few slender culms. Their leaves were long and narrow, without the pronounced inflation of leaf sheaths and discernible coloration. Clusters of the spikelets in the inflorescences were distantly arranged (Fig. 2B). In addition, some plants formed secondary inflorescences in the axils of the uppermost leaf. *Coleanthus subtilis* specimens, which were placed in class I, were characterized by much reduced growth, slender stems and delicate, short leaves; some of them did not flower (Fig. 2C). All of these three phenotypes corresponded well to the morphotypes distinguished in the literature (Hejný 1969; John 2011; Richert et al. 2014); the first one to the forma *typica* (class II of analyzed specimens, Fig. 2A); the second form to the forma *laxa* (class III, Fig. 2B), although some more elongated specimens exceeded the height range for this morphotype and thus could represent the forma *luxurians;* the last class of specimens (class I, Fig. 2C) was categorized as the forma *nana.* Differences between distinguished classes were significant in respect of morphological selected features, as shown by Kruskal-Wallis ANOVA ($p < 0.05$) (Fig. 3A-D).

Floristic relevés taken in transects showed little variation among vegetation patches in terms of the number of species they contained. In total, 32 vascular plant taxa were recorded in all 107 samples, while from 7 to 17 species (mean: 12.1) were recorded in each individual 1 m^2 (Appendix A). As many as 11 of them were recorded in more than half of all samples. The other group included sporadic species, which occurred in less than 10% of the relevés (11 taxa). Four of all component species of the phytocoenoses deserve special attention, as occurred in all patches (100% of samples); in addition to *C. subtilis* these were *Polygonum lapathifolium* subsp. *lapathifolium*, *Polygonum hydropiper* and *Veronica peregrina*. At the same time, these species prevailed in patches, covering more than 25% in most of them (range 3–5 of the cover-abundance scale). In this regard, *Ranunculus (Batrachium) trichophyllus* for. *terrestre* and *Myosurus minimus* also played an important role, although their presence was recorded in a slightly smaller number of relevés, accounting for more than 90% of all samples. Taking into account the latest classification of ephemeral plant communities in Poland (Kącki et al. 2021) the patches of vegetation were classified as a community with *Coleanthus subtilis.*

The habitat conditions, as determined by average values of the Ellenberg ecological indicators in Tichý's modification (Tichý et al. 2023), also showed little variation between samples. The light indicator (L) was the least variable (min. 6.9 - max. 7.7; mean 7.4) and the values pointed to the conditions intermediate between semi-shade and full light. Temperature indicator (T) reached average values on a 9-point scale (min. 5.0 - max. 6.4; mean 5.6). The achieved values of moisture indicator (M), for individual 1 m^2 squares were close to the range of higher values (min. 7.7 - max. 10.3; mean 8.8), and placed them between intermediate moist and wet, to aquatic conditions. The middle values on the 9-point scale, referring to moderately acid to neutral soil conditions, were also reached by the indicator of soil reaction (R) (min. 4.8 - max. 6.2; mean 5.5). Similarly, in the case of nitrogen (productivity) indicator (N), values were in the range from min. 4.5 to max. 6.5 (mean 5.5), corresponding to the average level of soil richness of N (productivity) conditions. On the other hand, the values of pH and the content of macro- and trace elements in bottom sediments were within wide ranges (Table 1). The biomass of *C. subtilis* specimens and morphological features of plants correlated with chemical parameters of bottom sediments and mean Ellenberg indicator values. In particular, significant correlations were noted between soil reaction index (R) and the total biomass (R=-0.50); soil reaction index (R) and the length of the highest culm (R=-0.56); pH of sediments and the total biomass (R=-0.50); pH and the length of the highest culm (R=-0.59) (Spearman's rank correlation, $p < 0.05$).

Table 1
Mean and ranges of pH and element concentration (mg/kg) in bottom sediments

Element	Mean	Minimum	Maximum	SD
C	49617	10726	162300	33583
Ca	3649	535	18773	3233
Cu	9.01	2.04	35.9	6.64
Fe	1165	377	2544	463
K	110	35.7	247	48.0
Mg	180	29.0	489	101
Mn	62.7	10.7	355	57.2
N	4668	1148	15666	2987
P	19.7	3.46	69.1	12.5
S	2550	320	9943	2073
Zn	18.6	3.19	96.9	19.8
pH		3.68	7.64	

The contents of N and P, and pH of sediments differed significantly between plots overgrown by *C. subtilis* specimens belonging to three different classes (Fig. 3E-G) (Kruskal-Wallis ANOVA, $p < 0.05$). In particular, the content of N was significantly higher in stands with the specimens of the class III comparing to the class II, and the content of P and pH were significantly higher in plots with specimens of classes I and II than in those of the class III (Dunn's test, $p < 0.05$). The concentrations of other studied

elements did not differ significantly between plots related to size classes of *C. subtilis* individuals (Kruskal-Wallis ANOVA, $p < 0.05$). Significant differences in habitat conditions were, however, expressed by the values of Ellenberg ecological indicators: light (L) and temperature (T) indicators were higher for classes I and II than for the class III; moisture (M) was higher in the class III than in the class I, and acidity (R) was higher in the class I comparing to the class III (Kruskal-Wallis ANOVA and Dunn's test, $p < 0.05$).

3.2. Allelopathic potential and biochemical composition

The exposure to the extract of *C. subtilis* adversely affected the germination of *S. alba* seeds (Fig. 4A). The significant reduction in the percentage of germinated seeds, as compared to that in control, occurred only in treatments when the extract concentration was even or higher than 2% and almost complete inhibition occurred at high concentrations of 4, 6 and 8%. The total number of germinated seeds in treatments with 0.5% and 1% extracts were similar to that in control. Nevertheless, the treatment with 1% extract resulted in a clear delay in germination; the maximal number of seedlings occurred 2 days later than in 0.5% extract treatment and control.

The length of roots of *S. alba* seedlings was adversely affected by 4 and 6% extract concentrations of *C. subtilis* (Fig. 4B). Plants had significantly shorter roots than in control and other treatments (Kruskal-Wallis ANOVA, $p < 0.05$). Seedlings grown in 0.5% extract had slightly longer roots than plants in control, but the difference was not significant. Treatments with 1 or 2% extracts caused slight inhibition of growth, but again, the root lengths were not significantly different from the control. The impact of *C. subtilis* on hypocotyl growth (Fig. 4C) was positive in treatments with concentrations up to 4% and manifested by significantly longer hypocotyls than in control (Kruskal-Wallis ANOVA, $p < 0.05$); in treatments with 6% extracts, the hypocotyl lengths did not differ from those of control (Kruskal-Wallis ANOVA, $p > 0.05$).

When analyzing the water extract of *C. subtilis* by UHPLC/UV/MS in the negative ion mode, a number of compounds was identified (Fig. 5). The main peaks were tentatively characterized based on the MS spectra as follow:

Peak 1: consist of two compounds: benzoic acid $C_7H_6O_2$ (cald. m/z 121.0295; observed m/z 121.0296; mass error 0.82) and unidentified compound at m/z 129.0558;

Peak 2: HMBOA (2-hydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one) $C_9H_9NO_4$, (cald. m/z 194.0459; observed m/z 194.0458; mass error − 0.51). The fragment ions at m/z 122 and m/z 123;

Peak 3: coumaric acid $C_9H_8O_3$ (cald. m/z 163.0401; observed m/z 163.0403; mass error 1.22). The fragment ion at m/z 119;

Peak 4: ferulic acid derivative (m/z 545.2234). The fragment ions at m/z 337 and 193;

Peak 5: MBOA (6-methoxybenzoxazolin-2-one) $C_8H_7NO_3$, (cald. m/z 164.0353; observed m/z 164.0355; mass error 1.21). The fragment ions at m/z 149 and m/z 121;

Peak 6: vitexin/isovitexin $C_{21}H_{20}O_{10}$ (cald. m/z 431.0984; observed m/z 431.0977; mass error 1.62) The fragment ions at m/z 311 and m/z 341;

Peak 7: the compound presented a molecular ion at m/z 305.1066 $[M-H]^-$ m/z (not determined);

Peak 8: azelaic acid $C_9H_{16}O_4$ (cald. m/z 187.0976; observed m/z 187.0978; mass error 1.06). The fragment ions at m/z 142, 125 and m/z 97;

Peak 9: the compound presented a molecular ion at m/z 303.0909 $[M-H]^-$ m/z (not determined);

Peak 10: trihydroxyoctadecadienoic acid $C_{18}H_{32}O_5$ (cald. m/z 327.2176; m/z 327.2184; mass error 2.14). The fragment ions at m/z 229, 211 and m/z 171;

Peak 11: trihydroxyoctadecenoic acid $C_{18}H_{34}O_5$ (cald m/z 329.2333; observed m/z 329.2332; mass error − 0.44). The fragment ions at m/z 229, 211 and m/z 171.

4. Discussion

Coleanthus subtilis is characterized by quite a wide intrapopulation morphological variability in response to the environmental conditions, however this relationship has not been analyzed in detail so far. Therefore, we aimed to link plant performance in the model population to abiotic (bottom sediment pH and fertility) and biotic (co-occurring and potentially competing plant species) conditions of growth. We showed that the morphology of *C. subtilis* specimens was diversified in terms of both the size of individuals and their phenotypes and proved that this variability was related to the pH and macroelement content in the sediment of the site as well as it could be related to the microrelief of the site, which diversified the most such factors as moisture and light availability.

It is already known that the morphotypes of *C. subtilis* develop in relation to the macroelement contents in the sediment and moisture of the site, with typical forms present in places with organic material deposition and moisture maintained during the life of a plant; on the contrary, when the site is easily drying off (mostly sandy substrate) plants are much smaller (forma *nana*) (Hejný 1969; John 2011). The latter situation corresponds well to class I of specimens (Figs. 2 and 3). Importantly, a dwarfy phenotype of plants correlated with increased pH and the content of P, as well as the decreased content of N, shown in analyses of bottom sediments (Fig. 3) and by Ellenberg ecological indicators. This further indicates that such conditions are suboptimal for *C. subtilis* development and growth, what is in agreement with previously published data (Richert et al. 2014; Dajdok et al. 2017). On the other hand, low reaction of bottom sediments (expressed as both pH and the values of Ellenberg ecological indicator of soil reaction, R) as well as the high content of N, favored the bigger size and biomass of *C. subtilis* (Fig. 3). Moreover, plots with high values of Ellenberg ecological indicators for moisture (M) as well as low values for light indicator (L) and temperature (T) were dominated by bigger specimens of *C. subtilis* (classes II and III). It is in line with the data of Šumberová et al. (2006) that high temperature, drought and high pH of substrate are the main limiting factors for *C. subtilis*. It is important to stress here that the light

conditions, created locally by the microrelief of the site and shading by co-occurring plants affected strongly *C. subtilis* morphology. Elongated plants, with limpy stems and leaves (forma *laxa*) developed, when individuals of *C. subtilis* were rapidly overgrown by taller plants (e.g., *Polygonum* spp.) as shown also in other populations of the species (Richert et al. 2014). However, our research showed that such elongated forms could also be observed in patches with high density of individuals, which results from the numerous seeds produced in previous seasons and deposited in bottom sediments. When the conditions are suitable, seeds germinate in a great abundance, as shown by Bernhardt et al. (2013). Our analyses showed that preferences of the model population of *C. subtilis* (in one water reservoir) are consistent with the general trends discovered previously when the relationship of the *C. subtilis* abundance and habitat conditions were analyzed in the regional scale (pond complex in SW Poland; Dajdok et al., 2017)). This species was shown to prefer substrates with lower pH and P, and at the same time rich in N, K, Ca, Mg and Na. In the present study, we have found less correlations between element contents in sediment and the size of *C. subtilis* individuals, which may be related to the lower diversity of habitats due to the smaller scale, and thus somewhat limited ranges of element concentrations in bottom sediments, which were wider (especially for Ca, Mg, P) in the regional scale (Dajdok et al. 2017). Similar relationships were already indicated by many authors (e.g., Hejný and Husák, 1978; Šumberová et al., 2006; Šumberová and Hrivnák, 2013). Also, Hrivnák et al. (2010) showed that the impact of P and nitrate content on macrophyte species composition was greater in a large scale, while in a microscale the correlations weakened. Although differences in the macroelement concentrations in sediments within one pond were less pronounced than in the pond complex studied before (Dajdok et al. 2017), the phytosociological results showed comparable patch diversity in terms of the *C. subtilis* quantity. Many patches with high cover values (3–5) of this species were observed, but phytocoenoses with only sporadic participation of *C. subtilis* specimens were also found (Appendix A). This quantitative diversity further suggests that other factors, such as, e.g., the microrelief affecting the humidity and light conditions and, indirectly, the time of the exposure of the bottom part of reservoir enabling seed germination and influencing plant developmental stage, have the impact on the occurrence of *C. subtilis* specimens.

The characteristic feature of vegetation patches with *C. subtilis* analyzed in this study, was their low diversity and low species richness, which can result from the relatively uniform substrate conditions, as indicated by the moderately diverse content of basic elements, and the type of the pond's fishery management (which in this case was nursery fishponds). The low species richness of the vegetation that develops on exposed bottoms of nursery fishponds was also previously pointed by Šumberová et al. (2006), who related this feature to the relatively short period of time when the bottom is available for plants, and which also favors the species of the extremely short life cycle (Šumberová and Hrivnák 2013).

Phytosociologically, such patches of vegetation with abundant participation of *C. subtilis* are usually included into the association *Polygono-Eleocharitetum ovatae* Eggler 1933, within the alliance *Elatini-Eleocharition ovatae* Philippi 1968 and the class *Isoëto-Nanojuncetea* Br.-Bl. et R. Tx. 1943 (Šumberová 2011; Šumberová and Hrivnák 2013; Richert et al. 2016). However, there is a disagreement about the rank and syntaxonomy of the community with *C. subtilis*, thus some authors classified it as a separate

association *Coleantho-Spergularietum echinospermae* (Vicherek 1972) Hejný 1978 (Hejný and Husák 1978) or subassociation within *Cypero-Limoselletum* (Oberdorfer 1957) Korneck 1965 as Taran (1995) did in relation to data from eastern Siberia, *C.-L. coleanthetosum* Taran 1994. In the present study, we classified the analyzed patches of vegetation as a community with *C. subtilis* (within the alliance *Eleocharition soloniensis* Philippi 1968, class *Isoëto-Nanojuncetea*), as was done in the recently published comprehensive classification of ephemeral vegetation in Poland (Kącki et al. 2021). The high values of the substrate moisture index (M), shown in our analysis of the model population, confirmed that patches with *C. subtilis* have the highest demands on moisture and light in comparison with other plant communities of the *Isoëto-Nanojuncetea* class as suggested earlier by Šumberová and Hrivnák (2013).

In the framework of the study discussed here, quite a noticeable variation of vegetation patches was detected, affected by the site microrelief. Namely, places which were earlier boar-rooted (during the period when the pond was empty, in November-March) were dominated by *Callitriche palustris*, the species which is able to develop in shallow water (Šumberová et al. 2006), while *C. subtilis* and other species were more numerous on the elevations, in places subjected to more rapid exposure as the pond was drying off. However, the role of microrelief in shaping vegetation patches requires more detailed research. Likely, it can account for the development of patches with the absolute dominance of *C. subtilis*, as while habitats are homogenous in terms of trophic and moisture conditions, then even the subtle advantage as, e.g., an earlier exposed bottom of the reservoir (micro-elevation) speeds up seed germination and seedling growth, and in turn may enable individuals of *C. subtilis* to outcompete the other plants. On the other hand, lagging biomass of undecomposed plant debris from the previous season may be a factor limiting germination. Such a situation was observed during monitoring of this model population, in the years before the current study was undertaken (Dajdok, Klink & Bartosz, *not published*). Within the pond, where the present study was conducted, significantly fewer individuals of *C. subtilis* were recorded on the sites with left biomass of *Polygonum lapathifolium* individuals than in parts of the pond characterized by an exposed bottom surface. The slower growth rate of seedlings in sites covered by the biomass layer compared to the exposed substrate has also recently been shown by Navratilová and Navratil (2022).

The other important factor determining the composition of phytocoenoses and biomass of *C. subtilis* can be the allelopathic potential of this species (Inderjit and Callaway 2003). Allelopathy is defined as any process where secondary metabolites of plants, fungi, or microorganisms have the impact (positive or negative) on the growth and/or development of biological systems (excluding animals). It is a form of competitive plant-plant interactions that may increase the ability of a plant to colonize and establish in new ecosystems (Kruse et al. 2000). Experiments performed in this study showed negative allelopathic effects of *C. subtilis* on germination and initial root growth of *Sinapis alba* at high concentrations of extract (Fig. 4). Interestingly, the allelopathic effect was positive for hypocotyl growth of *S. alba* at low concentrations. Positive allelopathy at low concentrations is not very common but was observed previously (Sinkkonen 2006). The allelopathic inhibition may lead to a homogenic composition of phytocoenoses due to selective pressure on susceptible species (Weidenhamer 2006) and facilitate maintaining high density of *C. subtilis*, primarily conditioned by the number of seeds deposited in the substrate in the previous growing seasons (Dajdok 2012; Münzbergová 2012). The allelopathic potential

may be especially important in the early stages of *C. subtilis* growth and the formation of communities with the species, as it may delay and/or reduce the germination of other species and thus their competition (Sicker et al. 2003). It was shown that even very low concentrations of allelochemicals, if constantly delivered to the habitat, may affect the diversity of plant communities and the distribution pattern of some species (Kruse et al. 2000).

Under natural conditions, the negative effects of allelochemicals usually result from combined impact of several compounds (Kruse et al. 2000). The study of An et al. (2001) showed that the phytotoxic effect is dependent on the proper proportion of compounds. The specific compounds, which could be responsible for allelopathic potential of *C. subtilis*, were identified for the first time.

The detected compounds in the analyzed plant extract belong to different classes of natural substances including benzoxazinone derivatives (HMBOA, MBOA), phenolic acids (benzoic acid, coumaric acid), as well as dicarboxylic acids (azelaic acid). All of them could be involved in allelopathy either alone or as a complex mixture. Phenolic compounds are known to be of great significance in allelopathy (Inderjit 1996), they are one of the main groups of phytotoxic substances associated with wheat allelopathy (Guenzi and McCalla 1966). Phenolic acids like benzoic acid and coumaric acid are common and widespread phytotoxic agents that are able to affect growth at various stages of plant development (Blum 1996; Chung et al. 2001; Bouhaouel et al. 2019). Likewise, benzoxazinones and their metabolites from corn, wheat, and rye are of great importance in the contest of overall phytotoxicity. Ferulic acid was identified in *Medicago sativa, Triticum aestivum* and *Secale cereale* as one of allelochemicals responsible for weed suppressing ability (Kohli et al. 2006). Vitexin was proven to inhibit germination and growth of *Tortula muralis* and *Raphanus sativus* (Basile et al. 2003). Phytotoxic activity of these compounds is also considered with respect to allelopathic interaction with other plants (Friebe 2001; Gierl and Frey 2001; Huang et al. 2003; La Hovary et al. 2016). In the study by Ma et al. (2011), the authors determined the main allelopathic substance of *Jatropha curcas* leaves and roots as azelaic acid. This compound is also mentioned as a one of the allelochemicals in rice *Oryza sativa* (Rimando et al. 2001; Ma et al. 2011).

To sum up, we may conclude that the compounds mentioned above are responsible for the perceived allelopathic activity of the investigated *C. subtilis* water extract. On the other hand, the regulation of the expression of biochemical responses is complicated involving external factors and the biological state of plants. In particular, stress, including harsh environmental conditions, competition, and nutrient limitation, may induce qualitative changes in secondary metabolites (Kruse et al. 2000; Siemens et al. 2002; Gawrońska and Golisz 2006) as well as the increase in concentrations of phytotoxins (Tang et al. 1995; Weidenhamer 2006), which may enhance the allelopathic activity. Also, the actual effects of allelochemicals in natural environment are influenced by habitat conditions, such as soil reaction, organic matter, nutrient and moisture content and presence of xenobiotics, and may differ from observed in laboratory conditions (Kruse et al. 2000; Hoagland and Williams 2003; Scavo et al. 2019). Moreover, in the phytocoenoses with *C. subtilis*, the competition with other species with the allelopathic potential is also possible and could complicate the interspecific interactions (Weidenhamer et al. 2023). In particular, *Veronica peregrina* which reached coverage in the range of 25–50% in some patches (Appendix A), was

showed to have allelopathic potential (Polechońska et al. 2020). However, these issues require further research.

5. Summary and conclusions

In conclusion, in our study on the model population within the analyzed pond we proved that:

1. General uniformity of habitat conditions, expressed by relatively narrow ranges of macronutrient contents, pH of bottom sediments and illustrated by values of Ellenberg ecological indicators, limits the diversity of vegetation patches and favors phytocoenoses with the high abundance and density of *C. subtilis*;

2. Intrapopulation morphological variability of *C. subtilis* is a complex effect, related to local micro-conditions created by the species composition of vegetation patches, patch cover and species density, pH and nutrient content in bottom sediments, and other factors such as probably microrelief of the site;

3. Ecological tendencies and preferences of *C. subtilis* are similar and comparable both in a model population (microscale analysis of the single pond with relatively unified conditions) and in a complex of ponds differing in habitat conditions (macroscale analysis);

4. The extract of *C. subtilis* inhibits seed germination and impairs root and hypocotyl growth of the test species, indicating an allelopathic potential of *C. subtilis.* The main compounds that may influence the allopathic potential of *C. subtilis* were identified as benzoxazinone derivatives, phenolics and carboxylic acids.

Declarations

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Figures

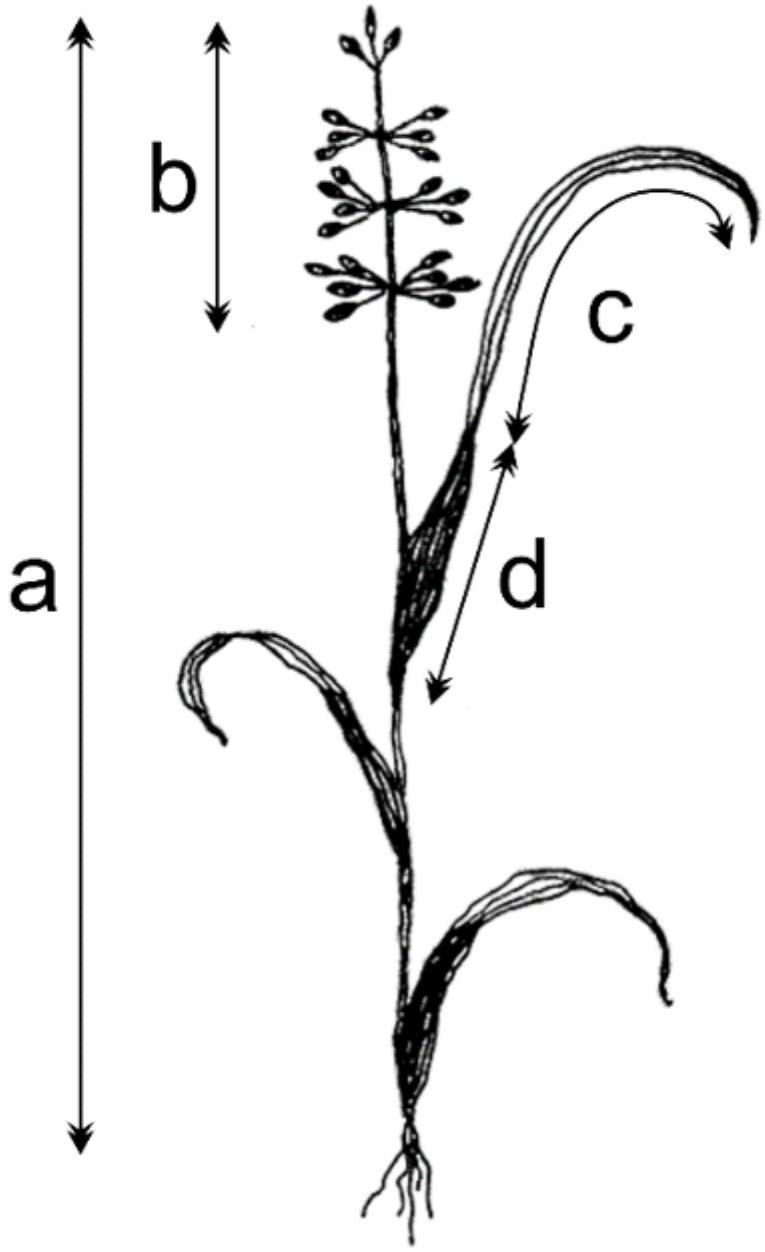

Figure 1

A scheme of *Coleanthus subtilis* showing measured traits: a. culm length, b. inflorescence length, c. leaf blade length, and d. leaf sheath length.

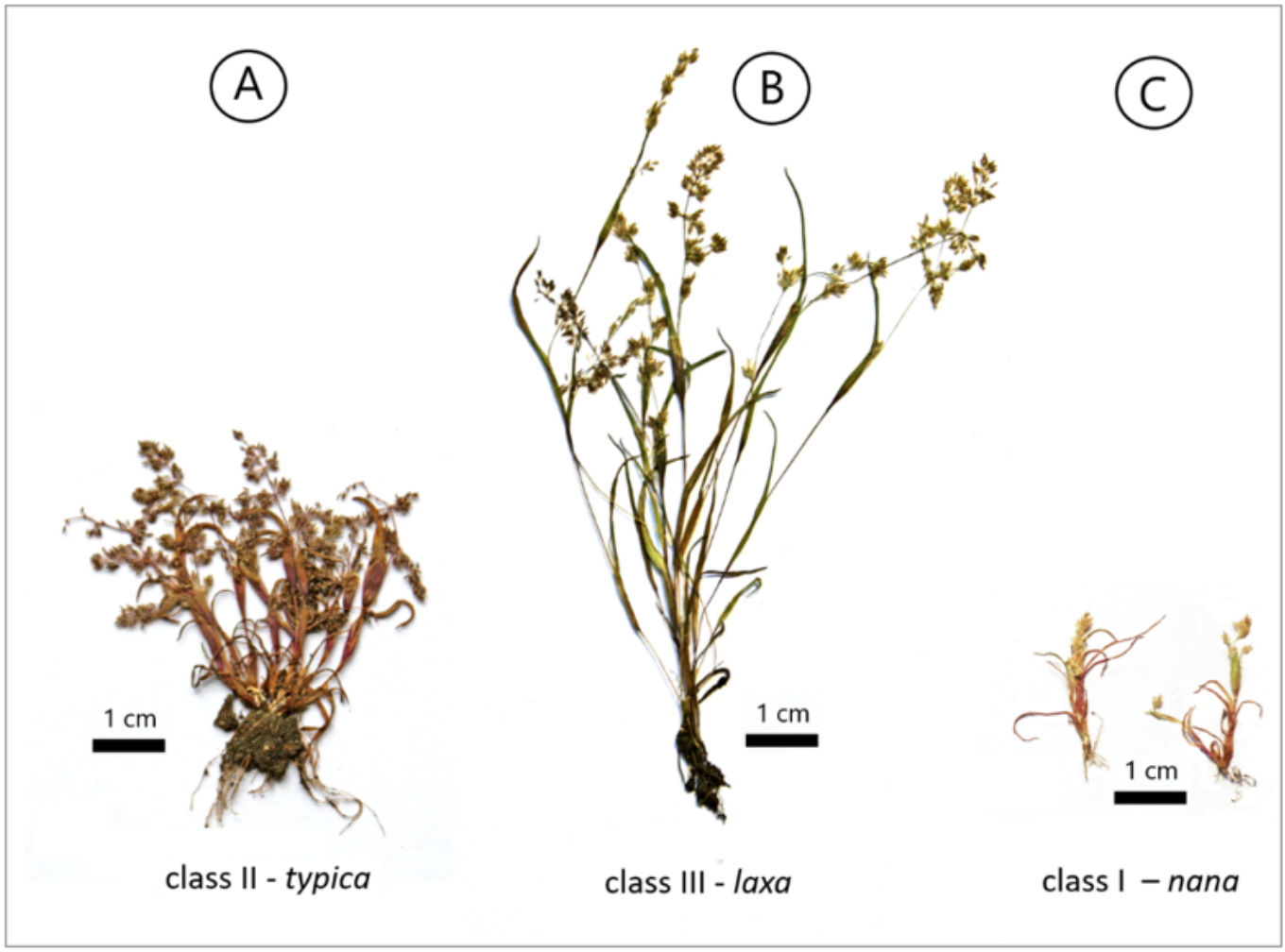

Figure 2

Three morphotypes of *Coleanthus subtilis* specimens: A – *typica* (class II), B – *laxa* (class III), C – *nana* (class I). Scale bar = 1 cm

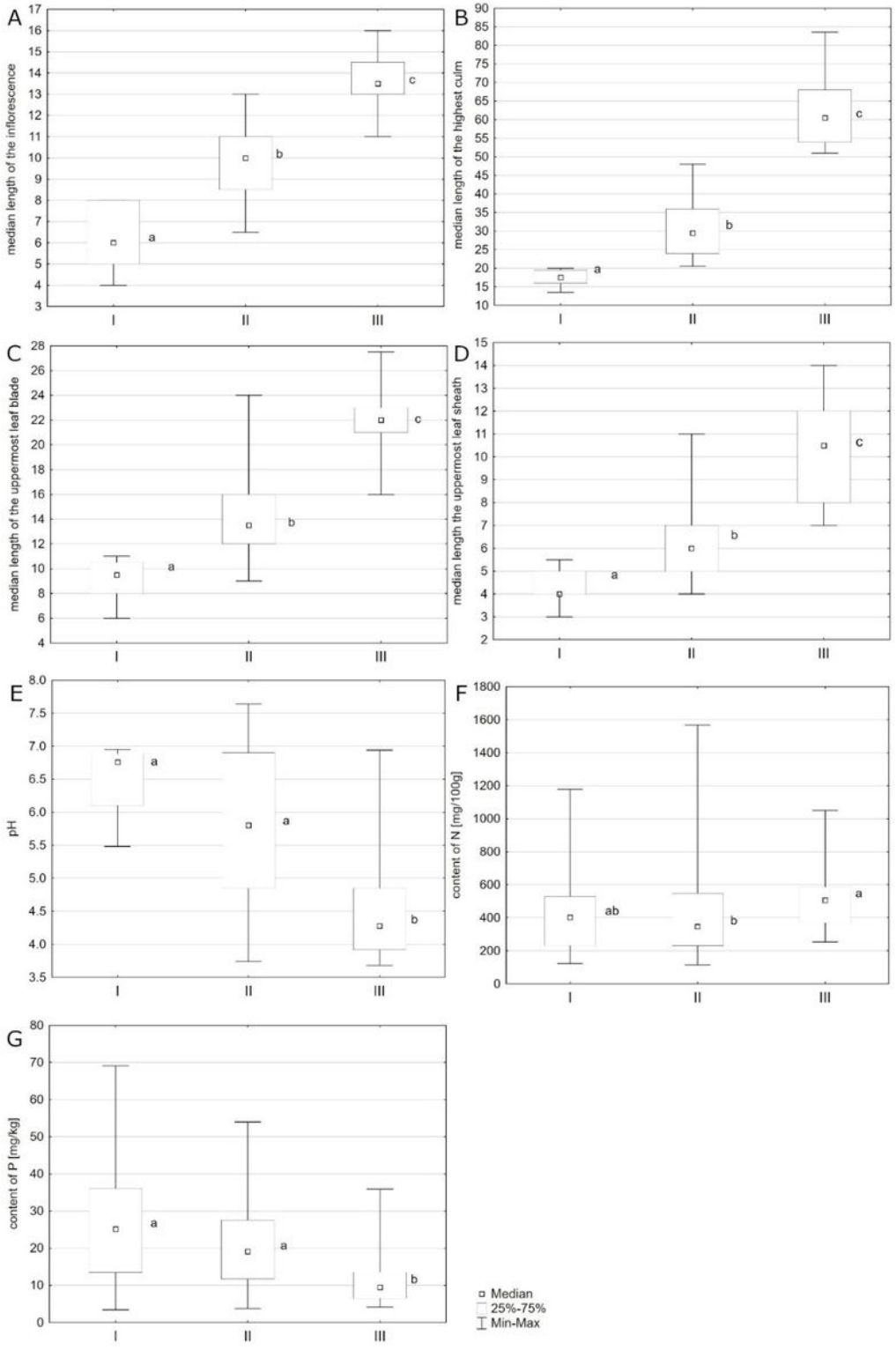

Figure 3

The morphological parameters of three classes of specimens of *Coleanthus subtilis (*A-D) and the differences between pH and content of N and P in bottom sediments at sites grown by three classes of *C. subtilis* specimens (E-G). Different letters indicate statistically significant differences (Kruskal-Wallis ANOVA and Median Test with post-hoc Dunn's test, $p<0.05$).

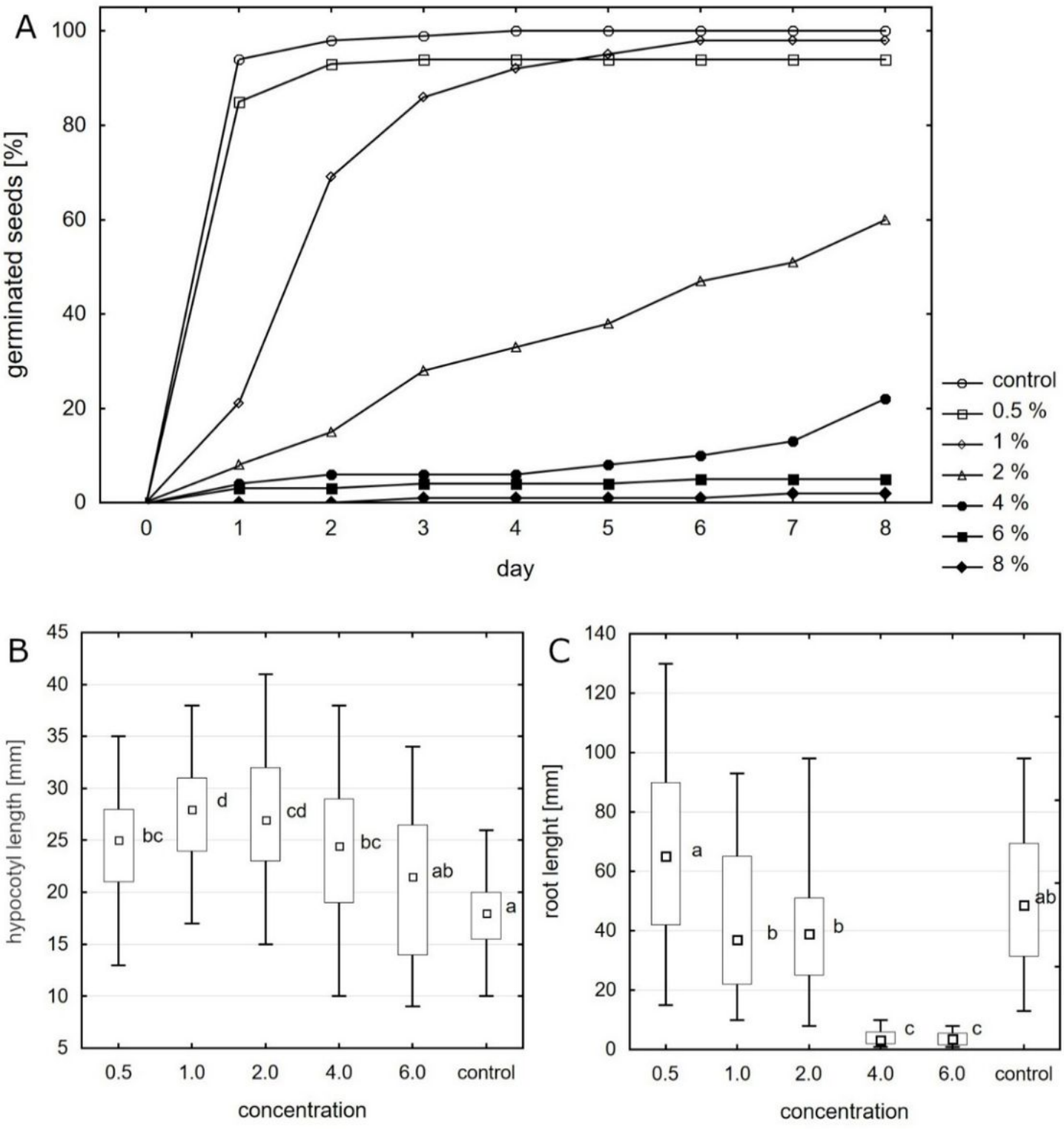

Figure 4

Effect of different concentrations of extracts of *Coleanthus subtilis* on germination of *Sinapis alba* seeds (A) and on hypocotyl (B) and root (C) length of *S. alba* seedlings. Different letters indicate statistically significant differences (Kruskal-Wallis ANOVA and Median Test with post-hoc Dunn's test, $p<0.05$)

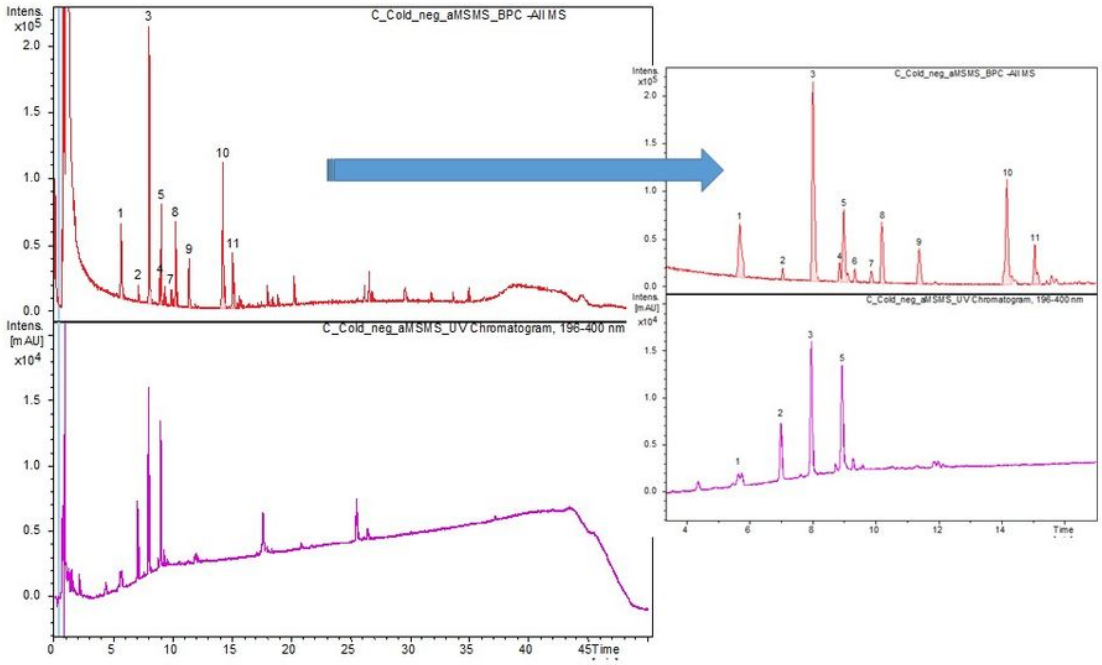

Figure 5

UHPLC/UV/MS chromatograms of the water extract from *Coleanthus subtilis*. The upper chromatogram (red) is recorded with MS detector. The lower chromatogram (purple) is recorded with UV detector. For the peaks identification - see the text

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DajdoketalColeanthussubtilisAppendixA.xlsx