Establishment of *Bryum argenteum* and concentrations of elements in its biomass on soils contaminated by As, Cd, Pb and Zn

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ABSTRACT

Using a pot experiment with slightly acidic and alkaline soils anthropogenically contaminated by As, Cd, Pb, and Zn, we assessed how the establishment of *Bryum argenteum* and concentrations of elements (P, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in its biomass are affected by the pH of the substrate, mobility of trace elements, and by quick lime (CaO) and superphosphate (P) additives. Over one vegetation season, in pots naturally colonised by *B. argenteum*, a substantially higher cover of *B. argenteum* was recorded on acidic soil that was heavily contaminated with Cd, Pb, and Zn than on alkaline soil with higher As but lower Cd, Pb, and Zn mobility. In acidic soil, the establishment of *B. argenteum* was substantially improved by CaO additive, which reduced the mobility of Zn and Cd, and by P additive, which improved the P nutritional status and reduced the extremely high concentrations of many elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in its biomass. Although *B. argenteum* can be used for the monitoring of soil contamination, concentrations of trace elements in its biomass must be evaluated with caution as they can be affected by total and mobile concentrations of elements in the substrate, and by other soil chemical properties.

Keywords: silver or silvery-thread moss; metal toxicity and tolerance; arsenic; cadmium; lead; zinc

Bryum argenteum is one of the few species of bryophytes that can be found on all continents (Stark et al. 2010). This short and dioecious moss is typically found on disturbed substrates, such as arable land, frequently cut grasslands, margins of roads, mines, and waste areas. The species tolerates high N, P, and K application rates and can infest golf greens and nurseries where it is a serious weed (Post et al. 2011). A sufficiently high availability of nutrients in the substrate enables fast germination of its spores and completion of the last germination phase and protonemal growth in comparison to substrates with low nutrient availability (da Silva et al. 2010). Once established, *B. argenteum* reproduces by sexual and asexual means and spreads rapidly by wind, water, and human disturbance.

The species is known for its high tolerance to trace elements, particularly to Cd, Pb, Ni and Zn (Shaw and Albright 1990, Aceto et al. 2003, Cuny et al. 2004). Although the high tolerance of *B. argenteum* to trace elements is known (Sobovljević et al. 2007, Vukojević et al. 2009), no study has been performed to test the effect of different soil pH and mobility of metals on establishment of the species and concentration of elements in its biomass.

Using lime and superphosphate additives applied to slightly acidic Litavka and alkaline Malín soils contaminated by As, Cd, Pb, and Zn, we

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primarily aimed to investigate the effect of soils and additives on the emergence and survival of *Rumex obtusifolius*. In the pot experiment, higher and quicker emergence, together with substantially higher mortality of seedlings were recorded in Litavka than in Malín soil (Hejcman et al. 2012). A positive effect of the lime treatment on seedlings was recorded in Litavka soil. During this pot experiment, we recorded colonisation of the soil surface by *B. argenteum*. In contrast to *R. obtusifolius*, no symptoms of trace element toxicity were visually detected on plants of *B. argenteum* in any treatment. To our knowledge, there have not been any studies on the colonisation of *B. argenteum* on such heavily contaminated soils. In this study, we assessed how the establishment of *B. argenteum* in contaminated soils was affected by the pH of the substrate, metal mobility, and by lime and superphosphate application. In addition, we determined the concentration of elements (P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the biomass of *B. argenteum*, to evaluate to what extent *B. argenteum* is able to accumulate different elements and how its nutritional status is affected by soils and additives.

MATERIAL AND METHODS

Design of the pot experiment, data collection and analytical procedure. In May 2011, we established the pot experiment in an outdoor weather-controlled vegetation hall in Prague-Suchdol with natural temperature and light conditions. We used (1) a slightly acidic Fluvisol termed Litavka contaminated by Cd, Zn, and Pb due to waste from smelter setting pits; and (2) an alkaline Luvisol termed Malín contaminated by As, Cd, and Zn due to the tailings of silver mining in the 13th–16th centuries (Vondráčková et al. 2013). The chemical properties of Litavka and Malín soils before the establishment of the experiment were following: pH_{CaCl_2} = 5.8 and 7.2; P = 9 and 56 mg/kg (the same units were used for all elements); K = 192 and 234; Ca = 1856 and 8914; Cd = 54 and 11; Zn = 6172 and 1022; Pb = 3305 and 98; As = 354 and 688. Values for P, K and Ca are plant available concentrations extracted by Mehlich III solution and values for Cd, Zn, Pb and As are pseudo-total concentrations extracted by *aqua regia*.

In addition to the effect of soil type, the effect of Ca (CaO – quick lime) and P ($Ca(H_2PO_4)_2$ – superphosphate) additives on *B. argenteum* cover and the concentration of elements in its biomass were investigated. Additives were applied to pots in the following amounts: 7.3 g CaO per 1 kg of soil and 1.3 g $Ca(H_2PO_4)_2$ per 1 kg of soil. The pot experiment was, therefore, composed of six treatments replicated five times (30 pots altogether): LC – Litavka soil without any additive, as the control; LCa – Litavka soil with Ca additive; LP – Litavka soil with P additive; MC – Malín soil without any additive, as the control; MCa – Malín soil with Ca additive; and MP – Malín soil with P additive. Concentrations of mobile elements in the soil, extracted by 0.01 mol/L $CaCl_2$ at the end of the experiment (Vondráčková et al. 2014), are given in Table 1. We used 5 L pots filled with 5 kg of air-dried soil sieved through a 10 mm sieve. We then applied the following nutrients: 0.5 g N (in the form of NH_4NO_3), 0.16 g P, and 0.4 g K (in the form of K_2HPO_4) to each pot and mixed with the soil to make conditions adequate for plant growth in all treatments. The additives were mixed with the soil after the application of N, P, and K fertilizers and then all pots were watered. The nutrients and additives were applied on the morning of 3rd May 2011. In the evening of the same day, we sowed 100 seeds of *R. obtusifolius* in each pot. Three of the most vital seedlings were left in each pot after thinning on 9th June 2011, in order to determine the effect of soils and Ca and P additives on the growth of this species (Hejcman et al. 2012). The pots were regularly watered if necessary to maintain optimal growth conditions during the course of the experiment. They were naturally colonised by spores and fragments of *B. argenteum* as this species was freely and commonly growing in their surroundings. The first green plants of *B. argenteum* were recorded in pots one month after establishment of the experiment. *Bryum argenteum* cover was visually estimated directly in percentages, as a proportion of the area of each pot covered by this species in October 2011. In order to determine the relationship between the aboveground biomass of *R. obtusifolius* and *B. argenteum* cover, we cut the aboveground biomass of *R. obtusifolius*, dried it, and determined the dry matter aboveground biomass per individual plant (Vondráčková et al. 2014). Biomass samples of *B. argenteum* used for chemical analysis were collected from the soil surface of each pot using pincers in October 2011. We carefully selected only clean biomass to avoid any bias in concentrations of elements caused by

Table 1. Effect of treatment on mean mobile (extracted by $CaCl_2$) concentrations of elements (± standard error of the mean) in soil and in dry matter biomass of *Bryum argenteum* at the end of the experiment

Element (mg/kg)	Concentration of elements in soils (n = 5)					
	LC	LCa	LP	MC	MCa	MP
P**	1.0 ± 0.2^{c}	1.1 ± 0.2^{bc}	3.4 ± 0.6^{ab}	1.5 ± 0.1^{abc}	1.9 ± 0.2^{abc}	3.3 ± 0.1^{a}
K**	246 ± 7^{a}	44 ± 8^{b}	205 ± 17^{a}	122 ± 4^{ab}	90 ± 2^{b}	111 ± 3^{ab}
Mg**	54 ± 2^{abc}	23.5 ± 1^{c}	50 ± 3^{abc}	67 ± 2.5^{a}	42 ± 1^{b}	67 ± 2.5^{a}
As**	0.35 ± 0.04^{bc}	0.33 ± 0.1^{c}	0.4 ± 0.1^{bc}	1.1 ± 0.1^{abc}	1.4 ± 0.1^{ab}	1.8 ± 0.1^{a}
Cd**	4.2 ± 0.2^{a}	0.1 ± 0.01^{ab}	3.8 ± 0.2^{ab}	0.02 ± 0.002^{b}	0.03 ± 0.01^{b}	0.04 ± 0.01^{b}
Cr^{ns}	0.02 ± 0.004^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.003^{a}
Cu**	0.1 ± 0.01^{ab}	0.3 ± 0.02^{a}	0.1 ± 0.01^{ab}	0.1 ± 0.004^{b}	0.2 ± 0.005^{ab}	0.1 ± 0.01^{b}
Fe**	7.5 ± 0.9^{b}	6.3 ± 1.6^{b}	7.8 ± 1.2^{b}	11.2 ± 1.4^{ab}	18.1 ± 1.4^{a}	13.3 ± 1.2^{ab}
Mn**	9.1 ± 0.5^{a}	0.3 ± 0.03^{abc}	7.8 ± 0.5^{ab}	0.2 ± 0.02^{c}	0.2 ± 0.02^{bc}	0.2 ± 0.03^{bc}
Ni**	0.1 ± 0.003^{a}	0.04 ± 0.01^{ab}	0.1 ± 0.01^{a}	0.03 ± 0.01^{b}	0.04 ± 0.01^{ab}	0.05 ± 0.01^{ab}
Pb**	0.65 ± 0.1^{a}	0.5 ± 0.1^{a}	0.65 ± 0.1^{a}	0.04 ± 0.01^{ab}	0.02 ± 0.002^{b}	0.06 ± 0.01^{ab}
Zn**	179 ± 7^{a}	3.7 ± 0.7^{abc}	174 ± 7^{ab}	0.6 ± 0.1^{c}	1.25 ± 0.3^{abc}	1.4 ± 0.6^{bc}
Element	concentration of elements in biomass					
	LC (n = 5)	LCa (n = 2)	LP (n = 4)	MC (n = 1)	MCa (n = 3)	MP
P (g/kg)*	1.2 ± 0.05^{a}	1.1 ± 0.15^{a}	1.5 ± 0.1^{a}	2.4^{a}	1.5 ± 0.1^{a}	–
K (g/kg)^{ns}	7.5 ± 0.8^{a}	7.5 ± 0.8^{a}	9.6 ± 0.3^{a}	9.8^{a}	8.2 ± 1.2^{a}	–
Ca (g/kg)**	8.5 ± 0.8^{b}	14.7 ± 2.2^{ab}	18.3 ± 1.9^{ab}	2.7^{ab}	38.5 ± 1.6^{a}	–
Mg (g/kg)*	2.2 ± 0.1^{ab}	2.9 ± 0.1^{a}	1.8 ± 0.2^{b}	2.6^{ab}	2.4 ± 0.1^{ab}	–
As (mg/kg)*	84.5 ± 25^{a}	199 ± 15^{a}	16 ± 9^{a}	171^{a}	405 ± 294^{a}	–
Cd (mg/kg)*	35 ± 6^{ab}	56 ± 13.5^{a}	22 ± 4^{ab}	9^{ab}	6 ± 1^{b}	–
Cr (mg/kg)^{ns}	13 ± 4^{a}	30 ± 5^{a}	2.8 ± 1.8^{a}	7.5^{a}	5.7 ± 1.1^{a}	–
Cu (mg/kg)*	36 ± 7^{ab}	71 ± 8^{a}	13 ± 2^{b}	45^{ab}	33 ± 2^{ab}	–
Fe (mg/kg)^{ns}	7454 ± 2117^{a}	15717 ± 1497^{a}	1749 ± 944^{a}	5618^{a}	5347 ± 1645^{a}	–
Mn (mg/kg)^{ns}	1139 ± 316.5^{a}	2631 ± 725.5^{a}	184 ± 113^{a}	84^{a}	153 ± 84^{a}	–
Ni (mg/kg)^{ns}	7.5 ± 1.6^{a}	12.6 ± 0.9^{a}	3.1 ± 0.7^{a}	8.1^{a}	5.5 ± 0.6^{a}	–
Pb (mg/kg)*	702 ± 210^{a}	1724 ± 260^{a}	113 ± 61^{a}	16^{a}	27 ± 9^{a}	–
Zn (mg/kg)*	2668 ± 331^{a}	3562 ± 731^{ab}	1771 ± 217^{ab}	497^{ab}	364 ± 62^{b}	–

Analysed by Kruskal-Wallis test, differences between treatments were either not statistically significant (^{ns}) or significant at the 0.01 (**) probability level. Using the multiple comparisons of mean ranks, treatments denoted with the same letter were not statistically significant. LC – Litavka soil without any additive, as the control; LCa – Litavka soil with Ca additive; LP – Litavka soil with P additive; MC – Malín soil without any additive, as the control; MCa – Malín soil with Ca additive; MP – Malín soil with P additive; n value – number of replications for analyses of *Bryum argenteum* biomass; – not determined

the contamination of biomass by soil particles and additives. In addition, all biomass samples were cleaned by ultrasound before their chemical analysis.

Total concentrations of P, K, As, Cd, Pb, and Zn in the plants were determined in the digests obtained by the following decomposition procedure: 0.5 g of dried and powdered plant matter was decomposed in a digestion vessel with a mixture of 8 mL concentrated nitric acid and 2 mL hydrogen peroxide. The mixture was heated in an Ethos

1 (MLS GmbH, Leutkirch im Allgäu, Germany) microwave-assisted wet digestion system for 33 min at 210°C. The digest was then transferred into a 20 mL glass tube, filled with deionised water, and kept at laboratory temperature until measurement. Concentrations of P, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in the extracts were determined by ICP-OES (Varian Vista Pro, Mulgrave, Australia) and K concentration was determined by FAAS (Varian SpectrAA-280, Mulgrave, Australia).

Data analysis. Factorial ANOVA followed by comparison using Tukey *HSD* test was applied to determine the effect of soil and additives on *B. argenteum* cover. Kruskal-Wallis test followed by multiple comparisons of mean ranks was used to determine the effect of soil and treatment on concentrations of mobile elements and concentrations of elements in the biomass of *B. argenteum*. The relationship between *B. argenteum* cover and aboveground biomass production of *R. obtusifolius* was evaluated using linear least squares regression. All statistical analyses were performed using the Statistica 8.0 program (Statsoft, Tulsa, USA).

RESULTS

Bryum argenteum cover was significantly affected by the soil used (Figures 1a and 2). Substantially higher cover was recorded on the acidic and heavily Cd-, Pb- and Zn-contaminated Litavka soil than on the less contaminated, alkaline Malín soil. The mean *B. argenteum* cover over all treatments was 81% and 17% in Litavka and Malín soils, respectively. *Bryum argenteum* cover was significantly positively affected by the application of Ca and P additives in Litavka soil, but no effects of Ca or P additives were recorded in naturally alkaline Malín soil. In P treatment in Litavka soil, *B. argenteum* was able to fully cover the soil surface over the period of only one vegetation season. *Bryum argenteum* cover was not significantly affected by biomass production of *R. obtusifolius* in experimental pots (Figure 1b).

Total concentrations of elements in the biomass of *B. argenteum* were not determined in MCa treatment as an insufficient amount of biomass was available for the chemical analysis. With the other treatments, we analysed between one to five replicates, according to the amount of biomass available (Table 1). With the exception of K, Cr, Fe, Mn and Ni, concentrations of all other elements were significantly affected by the treatment.

DISCUSSION

Bryum argenteum performs better on slightly acidic soil than on naturally alkaline soil, although the slightly acidic soil was heavily contaminated

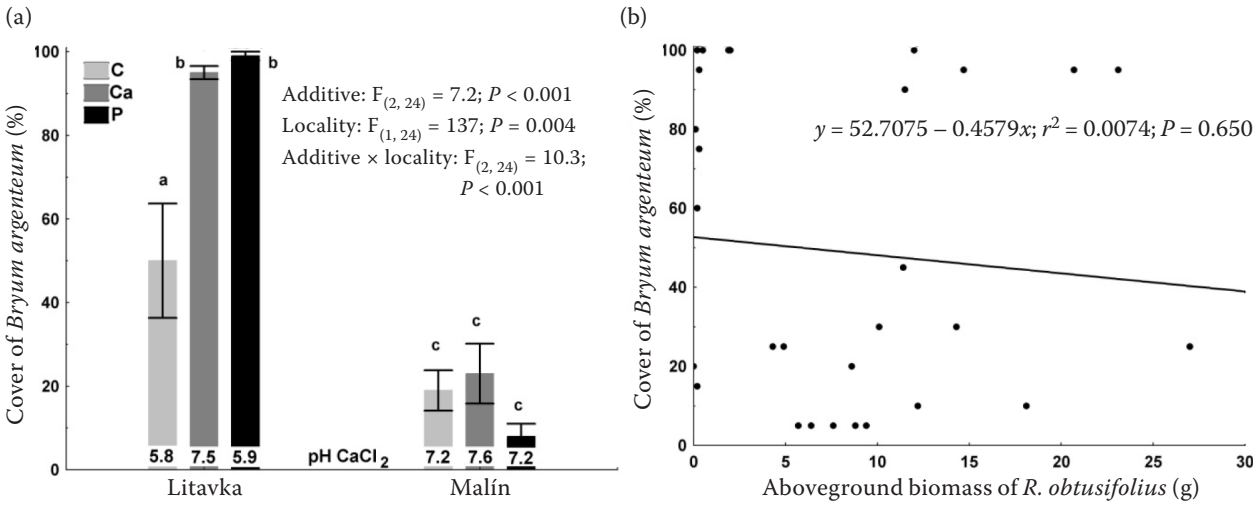

Figure 1. (a) Effect of soils (Litavka and Malín) and applied treatments (C – control; Ca – application of quick lime; P – application of superphosphate) on *Bryum argenteum* cover in experimental pots. Bars represent mean values and error bars represent standard error of the mean. Results of factorial ANOVA are provided on the graph. Using Tukey *HSD* test, treatments with the same letter were not significantly different. Numbers in the bottom part of each bar represent pH_{CaCl_2} in soils in particular treatments in October 2011. (b) Effect of dry matter aboveground biomass per individual plant of *Rumex obtusifolius* on *B. argenteum* cover in experimental pots

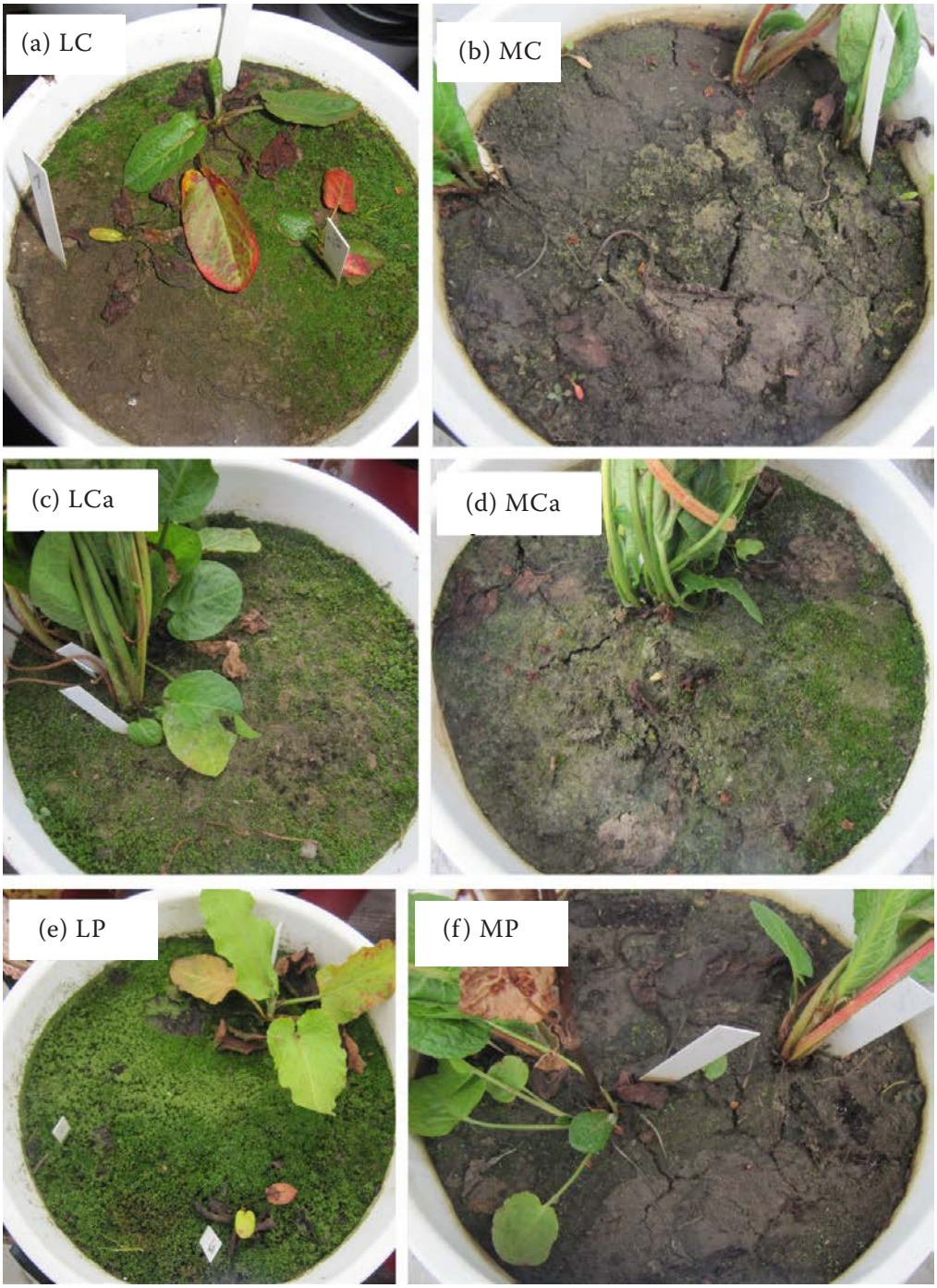

Figure 2. Photographs of *Bryum argenteum* in investigated treatments at the end of the experiment in October 2011. (a) LC – Litavka soil control treatment; (b) MC – Malín soil control treatment; (c) LCa – Litavka soil with quick lime; (d) MCa – Malín soil with quick lime; (e) LP – Litavka soil with superphosphate; (f) MP – Malín soil with superphosphate

by Cd, Pb, and Zn. The preference of *B. argenteum* for slightly acidic soil rather than alkaline soil is in accordance with field observations (Cuny et al. 2004). According to Ellenberg et al. (1991) and Stark et al. (2010), *B. argenteum* performs the best on substrates with a pH ranging from 5 to 7. Although better establishment of *B. argenteum* was recorded on the slightly acidic Litavka soil, the pH_{CaCl_2} recorded for Litavka and Malín soils with a Ca additive (7.5 and 7.6, respectively) was not out of the ecological tolerance of the species. The species can, therefore, also colonise naturally

alkaline soils, but probably with a reduced growth rate.

Bryum argenteum is highly tolerant to As, Zn, Cd, and Pb toxicity, much more than *R. obtusifolius* directly planted with *B. argenteum* in the same soils. In Litavka soil, the mobility of Zn, Cd, and Pb was very high in the control, but no symptoms of metal toxicity in *B. argenteum* plants were visually recorded, in contrast to *R. obtusifolius* whose plants suffered from obvious metal toxicity in all Litavka treatments (Vondráčková et al. 2014). Although we recorded no visual symptoms of metal toxicity in *B. argenteum* plants, a decrease in the Zn and Cd mobility by the application of Ca additive (Table 1) substantially increased its colonisation potential and cover in LCa treatment. The paradox is that the concentrations of many trace elements (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the biomass of *B. argenteum* were the highest in LCa treatment (Table 1). *Bryum argenteum* is able to withstand very high mobility of Zn, Cd, and Pb in the soil, but also very high concentrations of these elements in its biomass. In addition, the concentrations of elements extracted by $CaCl_2$ cannot correctly predict mobile fractions of elements that are available to *B. argenteum*.

The highest cover and colonisation were recorded in LP treatment. It seems that trace elements can affect the P metabolism of the species. The addition of P and high P mobility in the soil enabled *B. argenteum* to overcome the adverse effects of high Cd, Zn, and Pb mobility in the soil and also to eliminate P deficiency, as is evident from the higher P concentration in its biomass in LP than in LCa and LC treatments. It must also be noted that the high mobility of P in the LP treatment soil resulted in lower concentrations of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in the biomass of *B. argenteum* than in LC and LCa treatments. P additive had a highly positive effect on *B. argenteum* cover in Litavka soil, although all treatments received a starting amount of N, P, and K fertilizer to avoid growth limitation by these elements. This starting amount of P was probably not sufficient to overcome P limitation during the germination of spores or establishment of plants from fragments in Litavka soil. High P supply probably enabled fast germination of its spores and protonemal growth, as the duration of these life stages is dependent on nutrient availability (da Silva et al. 2010).

In naturally alkaline Malín soil, Ca or P additive had no positive effect on *B. argenteum* cover. It seems that the weak establishment of *B. argenteum* in this naturally alkaline soil was caused by different reasons than insufficient N, P, and K supply. In addition, concentrations of Cd, Mn, Pb, and Zn in the soil and also in the biomass of *B. argenteum* were substantially lower in Malín soil than in Litavka soil. Therefore, the low *B. argenteum* cover in Malín soil could not be connected to toxicity of these elements. The highest concentration of As in the biomass of *B. argenteum* in MCa treatment was probably connected to the mobilisation of As after Ca additive application, as can be deduced from increased concentrations of mobile As in MCa treatment in comparison to MC treatment (Table 1). A similar result, the highest As concentration with Ca additive treatment, was also recorded in Litavka soil (Vondráčková et al. 2013).

Reasons for the weak establishment of *B. argenteum* in naturally alkaline Malín soil, therefore, require further research in connection with the question of the calcifuge/calcicole concept.

Concentrations of P, K, Ca, and Mg were generally in the physiological range typically observed in common bryophyte species (Hejcman et al. 2010), but concentrations of As, Cd, Fe, Pb, and Zn were extraordinary high in comparison to studies where bryophytes were used to estimate aerial deposition of trace elements (Aceto et al. 2003, Bonanno et al. 2012). Such extraordinarily high concentrations of trace elements indicate that *B. argenteum* accumulates trace elements not only from air deposition, but also from the substrate, and can thus be used for the monitoring of soil contamination. Similarly high concentrations of trace elements in the biomass of *B. argenteum* without obvious symptoms of their toxicity were recorded on contaminated soil by Sobovljević et al. (2007) and Vukojević et al. (2006, 2009). The lack of effect of biomass production of *R. obtusifolius* on *B. argenteum* cover was probably due to the relatively low biomass of *R. obtusifolius* in the pots and, therefore, sufficient amount of light for *B. argenteum* on the soil surface.

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