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Original Paper

Alyssum montanum subsp. *gmelinii*, a rare plant species from coastal sand dunes, as a potential Ni accumulator: comparison with *Alyssum murale*

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Gederts Ievinsh*, Una Andersone-Ozola, Ineta Samsone

Department of Plant Physiology, Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV–1004, Latvia

*Corresponding author, E-mail: gederts.ievins@lu.lv

Abstract

Plants from a separated population of *Alyssum montanum* subsp. *gmelinii* with a coastal-specific distribution on dune habitats of the Eastern Baltic Sea in territory of Lithuania and Latvia were used in the present study to assess Ni tolerance and its accumulation potential in comparison to a well-known Ni hyperaccumulator, *Alyssum murale*. *A. montanum* plants showed moderate tolerance to Ni, with pronounced negative effects on growth and physiological status from 0.5 g Ni L^{-1}. No *A. montanum* plants survived in 4 g L^{-1} Ni longer than for two weeks. In contrast, growth of *A. murale* was stimulated at 2 and 4 g L^{-1} Ni. Cultivation in presence of increasing concentration of Ni in substrate resulted in a linear increase of Ni concentration in leaf and stem tissues of both species. Leaves of *A. montanum* accumulated up to 0.626 g kg^{-1} Ni, compared to 21.976 g kg^{-1} in leaves of *A. murale*. Accumulation capacity of Ni in roots was less pronounced, with a clear saturation effect for both species. Ni treatment induced changes in concentration of different mineral nutrients and nonessential elements. Among the most pronounced stimulative effects, Co concentration increased in leaves and roots of both species, Cd concentration in leaves of both species, Cu concentration increased in stems of both species, and Fe concentration in stems of both species. It is concluded that the accession of *A. montanum* subsp. *gmeliniii* from coastal sand dunes of the Baltic Sea has good tolerance to increased substrate Ni concentration and exceptional Ni accumulation potential.

Key words: *Alyssum montanum* subsp. *gmelinii, Alyssum murale*, coastal dunes, mineral nutrition, Ni accumulation potential, Ni tolerance.

Introduction

Metal tolerant plant species and their metal accumulation capacity have raised significant scientific attention during the last 30 years, regarding elucidation of physiological and molecular mechanisms of the phenomenon as well as due to possible practical use of plants metallophytes in environmental technologies (Clemens et al. 2002; Bothe 2011; van der Ent et al. 2018). Plant nickel (Ni) tolerance and accumulation potential has received special attention, being most widely studied among different heavy metals, mostly because of its importance as a component of environmental pollution as well as discovery of a large number of Ni-tolerant plant species native to metalliferous soils (Chen et al. 2009; van der Pas, Ingle 2019).

Ni is an integral component of metalloenzyme urease, which catalyzes hydrolysis of urea to ammonia and CO_2 (Sirko, Brodzik 2000). It is a functionally important enzyme in bacteria and other groups of organisms, and is present also in plants, but its role has been confirmed only for certain groups of plants, such as in legume species with active rhizobial symbiosis (Dalton et al. 1985), in specific situations like mobilization of organic nitrogen compounds during seed germination (Zonia et al. 1995) and for plants with urea as the only nitrogen source (Gerendas, Sattelmacher 1999). Therefore, a role of Ni as a plant mineral nutrient has been proposed (Eskew et al. 1983; Brown et al. 1987). As a heavy metal, Ni is toxic for plants at relatively low soil concentration due to accumulation of a critical concentration of the metal in plant tissues. For the majority of relatively Ni-sensitive plants, the threshold toxic Ni concentration in tissues is 10 mg kg^{-1} DM, increasing to 50 mg kg^{-1} in tolerant species (Krämer 2010). Similar to other heavy metals, plant species can be either excluders, indicators or hyperaccumulators of Ni. Typical excluders (*Triticum aestivum*) accumulated up to 11 mg kg^{-1} Ni, indicators (legume *Trifolium pratense* for which Ni is essential element) up to 40 mg kg^{-1} Ni, and hyperaccumulators (*Alyssum murale*) up to 39 000 mg kg^{-1} Ni in shoots when cultivated in soil with increased Ni concentration (Massoura et al. 2004).

The majority of known Ni hyperaccumulating species are tropical trees from New Caledonia, Cuba and other regions with ultramafic soils (Reeves et al. 2018). In the

temperate zone, the genus *Alyssum* (Brassicaceae) seems to be exceptional among vascular plants, as there are more than 50 Ni hyperaccumulator species, mostly endemics on ultrabasic serpentine soils in mountainous parts of Europe (Brooks, Bradford 1978; Brooks et al. 1979). Therefore, use of different *Alyssum* species in phytomining of Ni has been proposed and many field trials have been set up (Nkrumah et al. 2016).

Alyssum murale is a plant native to Mediterranean serpentine soils with high Ni concentration, and it is recognized as both a Ni and Co hyperaccumulator (Tappero et al. 2007). When grown in controlled conditions in soil spiked with 40 mmol kg^{-1} (2.4 g kg^{-1}) Ni, *A. murale* can accumulate about 15 g kg^{-1} Ni in leaves (Broadhurst et al. 2009). In native conditions, *A. murale* plants accumulated up to 5885 mg kg^{-1} in roots, 7410 mg kg^{-1} in stem and 10 552 mg kg^{-1} Ni in leaves (Stamenković et al. 2017). The phytoextraction potential of *A. murale* in field conditions with additional fertilization reached 25 kg Ni ha^{-1}, compared with only 3 kg ha^{-1} without fertilization (Bani et al. 2007), or even 112 kg ha^{-1} with higher plant densities (Bani et al. 2015).

Another species of the genus, *Alyssum montanum*, is characterized by extremely complex taxonomy and wide geographical distribution (Španiel et al. 2012). A separated population of *Alyssum montanum* subsp. *gmelinii* has a coastal-specific distribution on dune habitats of the Eastern Baltic Sea in territory of Lithuania and Latvia and is known locally by a synonym *Alyssum gmelinii*. The plant is an umbrella species of the European protected habitats EUH 1230 "Vegetated sea cliffs of the Atlantic and Baltic coasts" (Rēriha 2013) and EUH 2130* "Fixed coastal dunes with herbaceous vegetation (grey dunes)" (Laime 2013). This species is extremely tolerant to moderate sand burial by means of induced clonality (Samsone et al. 2009), pointing to existence of a locally-adapted ecotype of *A. montanum* subsp. *gmelinii*.

Metal tolerance and their accumulation potential have not been studied so far for a dune ecotype of *A. montanum* subsp. *gmelinii*. However, in a co-cropping experiment using natural serpentine soil (Ni concentration 4.71 g kg^{-1}) with *A. murale* and ornamental variety of A. montanum it was established that irrespective of cropping, *A. montanum* accumulated only 50 to 71 mg Ni kg^{-1} in shoots, but the level for *A. murale* was 3320 to 4030 mg kg^{-1} (Broadhurst, Chaney 2016). *A. montanum* showed good tolerance but was outcompeted by *A. murale*. Therefore, it is reasonable to suggest a relatively good Ni tolerance level also for other species of the genus besides these native to metalliferous soils.

The aim of the present study was to evaluate effect of increased substrate Ni concentration on growth of plants from a coastal accession of *A. montanum* subsp. *gmelinii* and to compare Ni accumulation potential with that of Ni hyperaccumulator, *A. murale*.

Materials and methods

Seeds of *A. montanum* subsp. *gmelinii* (further indicated as *A. montanum*) was collected in July 2016 from plants natively growing in dune habitat in Uzava, Latvia. Seeds of *A. murale* were received from the Botanical Garden of the University of Bonn, Germany. Initially seeds were surface sterilized with sodium hypochlorite and germinated aseptically on filter paper in Petri dishes with deionized water at thermoperiod (10/16 °C). Germinated seeds were transferred to a mixture of autoclaved garden soil (Biolan, Finland) and quartz sand (1:3, v/v) in closed tissue culture containers and further cultivated in a growth cabinet with photosynthetically active radiation photon flux density 50 μmol m^{-2} s^{-1}, 18 °C.

When seedlings reached second true leaf stage, they were replanted to individual 200 mL containers with the same substrate mix and placed in 48 L plastic boxes in greenhouse for acclimatization and further used for experiments. Plants were cultivated in an experimental automated greenhouse (HortiMax, Netherlands) with supplemented light from Master SON-TPIA Green Power CG T 400 W (Philips, Netherlands) and Powerstar HQI-BT 400 W/D PRO (Osram, Germany) lamps (380 μmol m^{-2} s^{-1} at the plant level), 16 h photoperiod, day/night temperature 23/15 °C, relative air humidity 60 to 70%.

Two separate experiments were performed. Only *A. montanum* was used for the first experiment. Plants were transplanted to Ni-containing substrate (0, 300, 500 and 700 mg Ni L^{-1} as $NiSO_4$) after two weeks of acclimatization in a greenhouse. Six plants (three larger and three smaller) per treatment were used. Plants were cultivated for three weeks after transplanting.

Both *A. montanum* and *A. murale* plants were used for the second experiment. Plants were transplanted to Ni-containing substrate (0, 0.25, 0.5, 2.0 and 4.0 g Ni L^{-1} as $NiSO_4$) after four weeks of acclimatization in greenhouse. Five plants per treatment per species were used. Plants were cultivated for 16 weeks after transplanting.

Plants were watered with deionized water keeping substrate moisture at 40 to 50%. Fertilization was performed once a week with Kristalon Blue soluble fertilizer (Yara International, The Netherlands; 0.5 g L^{-1}), 50 mL per plant.

Nondestructive analysis of chlorophyll a fluorescence was performed for leaves dark-adapted for at least 20 min by a Handy PEA fluorometer (Hansatech Instruments, UK). Chlorophyll a fluorescence parameters F_v/F_0 and Performance Index Total was used for characterization of photochemical activity. F_V/F_0 characterizes apparent activity of photosystem II, and Performance Index Total is a complex indicator of photochemical efficiency combining three function-related (trapping of absorbed excitons, electron transport between the photosystems, reduction of end-electron acceptors) and structure-related (antenna chlorophyll per reaction center chlorophyll) parameters

Fig. 1. Typical morphology of *Alyssum montanum* subsp. *gmelinii* plants cultivated in presence of increasing concentration of Ni in substrate for three weeks. A, smaller plants; B, larger plants. From left to right: control, 300, 500, 700 mg Ni L^{-1}.

(Strasser et al. 2000). Three fluorescence measurements per individual plant were performed, in six replications per treatment.

Peroxidase actvity in leaf and root tissues of A. montanum was performed spectrophotometrically as described previously (Andersone, Ievinsh 2002) with guaiacol and H_2O_2 as substrates.

Statistical significance of differences between treatments was evaluated by *t* test using Prism (GraphPad Software, USA).

Analysis of chemical elements was performed at the Faculty of Chemistry, University of Latvia. Concentration of chemical elements was measured by inductively coupled plasma mass spectrometry. Plant samples were dried in an oven at 60 °C for 72 h to achieve stable mass. A single sample for leaves, stems and roots each was made using all individual plants from the respective treatment. Samples were homogenized and ground using a laboratory mill. A sample of 0.3 g was mineralized by microwave digestion in 8 mL 65% HNO_3 with 4 mL 30% H_2O_2. An Agilent 7700X ICP-MS was used for estimation of Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, and Zn using appropriate standard solutions.

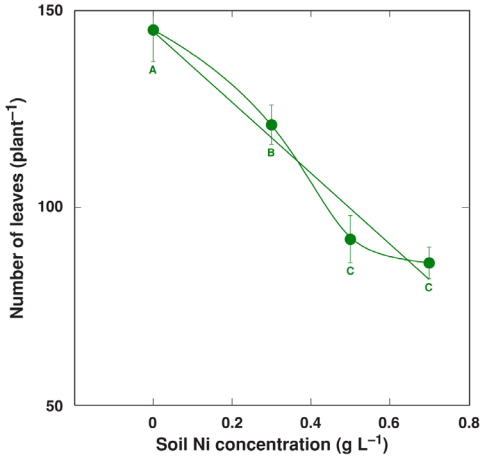

Fig. 2. Effect of increasing concentration of Ni in substrate on number of leaves of *Alyssum montanum* subsp. *gmelinii* plants after three weeks of treatment. Results are means ± SE from five individual plants. Means with identical letters are not statistically significantly different ($P < 0.05$).

Results

Experiment 1: short-term effect of Ni on A. montanum

In the short-term experiment, *A. montanum* plants were planted in soil with Ni concentration 0, 300, 500, and 700 mg L^{-1} and cultivated for three weeks. Growth of *A. montanum* plants was negatively affected by increasing soil Ni concentration, and the effect was larger in younger/smaller plants (Fig. 1). A group of smaller plants replanted in Ni-containing soil (Fig. 1A) had more pronounced negative effect of Ni at high concentration, observed as overall chlorosis and necrosis of older leaves, in comparison to the group of larger plants (Fig. 1B) with just a few toxicity symptoms visible. Increasing concentration of Ni in substrate resulted in near-linear decrease of number of leaves (Fig. 2).

Nondestructive analysis of chlorophyll *a* fluorescence in leaves was used to assess physiological status of *A. montanum* plants during cultivation after one, two, and three weeks (Fig. 3). While the indicator of apparent photochemical activity of photosystem II, F_v/F_0, was largely uninformative, changes in the indicator of plant photochemical efficiency, Performance Index Total, clearly showed development of negative physiological effect of increasing substrate Ni concentration.

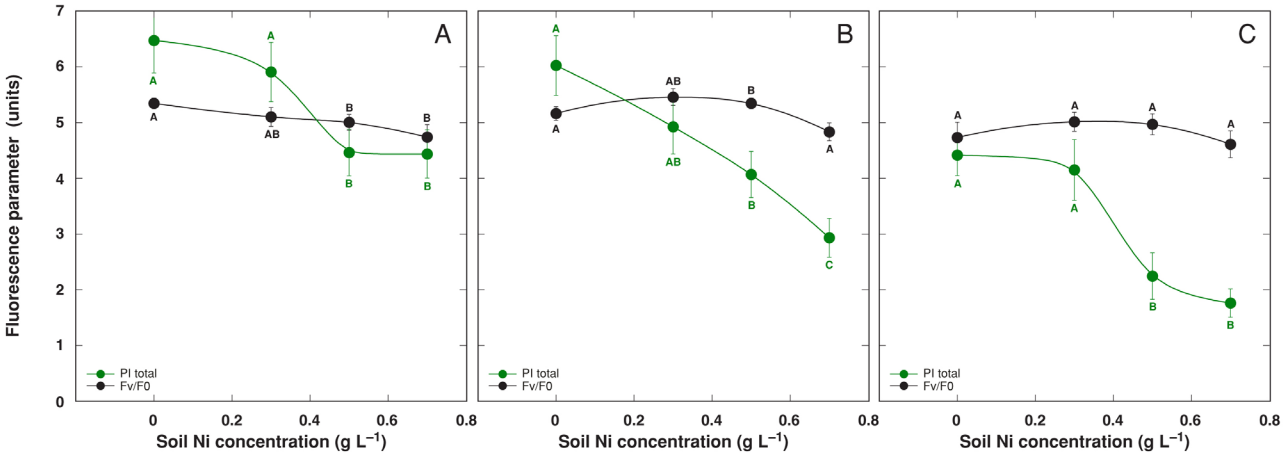

Fig.3. Effect of increasing concentration of Ni in substrate on chlorophyll a fluorescence parameters in leaves of *Alyssum montanum* subsp. *gmelinii* plants after 1 (A), 2 (B) and 3 (C) weeks of treatment. Results are means ± SE from five individual plants with five measurements each. Means for each parameter with identical letters are not statistically significantly different ($P < 0.05$).

Tissue peroxidase activity was used as an indicator of stress-related changes, showing an opposite trend in changes of the activity in leaves and roots: Ni at 300 mg L^{-1} had no effect on peroxidase activity in both leaves and roots, but a significant increase of the activity was evident for leaves and decrease in roots, with further increase in substrate Ni concentration (Fig. 4).

Experiment 2: comparison of long-term effect of Ni on A. montanum *and* A. murale

During a long-term experiment, both *A. montanum* and *A. murale* plants were grown in a substrate with increasing concentration of Ni. As significant older plants were transplanted to Ni-containing substrate, morphological signs of Ni toxicity were visible in lower amounts and

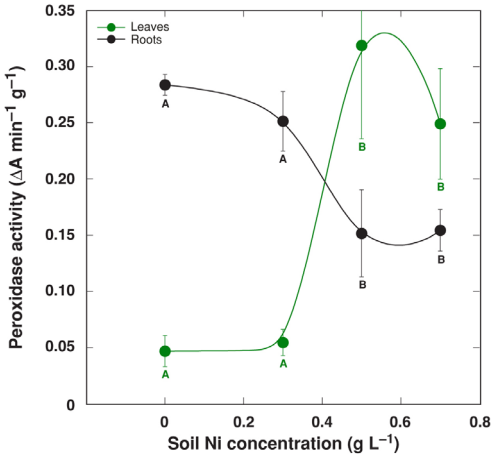

Fig. 4. Effect of increasing concentration of Ni in substrate on peroxidase activity in leaves and roots of *Alyssum montanum* subsp. *gmelinii* plants after three weeks of treatment. Results are means ± SE from three individual plants. Means for each plant part with identical letters are not statistically significantly different ($P < 0.05$).

were associated with induced senescence of older leaves of *A. montanum* at 0.5 and 2.0 g L^{-1} Ni (Fig. 5A). Plants transplanted to soil with 4 g kg^{-1} Ni died after two weeks of cultivation. A higher range of Ni concentration (2 and 4 mg L^{-1}) was used for treatment of *A. murale* plants, and no signs of toxicity were visible after 16 weeks (Fig. 5B).

Similar to the first experiment, growth of *A. montanum* plants was inhibited by increasing soil Ni concentration, and the decrease in shoot fresh and dry mass was near-linear (Fig. 6A, B). In contrast, growth was stimulated by Ni treatment in *A. murale* plants, with statistically significant increase of shoot fresh mass at 2 and 4 g Ni kg^{-1}, and significant increase of shoot dry mass at 4 g Ni kg^{-1} (Fig. 6A, B). When relative effect of Ni treatment on fresh mass of different plant parts was evaluated, it appeared that only leaf and root growth of *A. montanum* was constantly inhibited, while stem growth was even stimulated for plants in 0.25 and 0.5 g Ni L^{-1} treatments (Fig. 6C). It is interesting to note that stem growth was the least stimulated part for *A. murale* plants by Ni, and was even inhibited at 4 g Ni kg^{-1} (Fig. 6C). Increase in root biomass was most pronounced for *A. murale*, with nearly 4-fold increase in 2 g Ni L^{-1} treated plants.

Ni-induced concentration-dependent accumulation of Co was evident for both *Alyssum* species (Fig. 8). For *A. murale*, leaves accumulated up to 50 mg Co kg^{-1}, while Co concentration in other parts was within the same range as that for *A. montanum*, 1.0 to 1.75 mg kg^{-1}.

Concentration of other mineral elements in different parts of both *Alyssum* species was affected by Ni treatment, as summarized for control and 2 g kg^{-1} Ni treated plants in Table 1. Among the most pronounced stimulative effects, there was an increase in Cd concentration in leaves of both species, increase of Cu concentration in stems of both species, and increase in Fe concentration in stems of both species. In addition, Fe, K, Mg, Mn concentration increased in leaves of *A. montanum*, Na increased in all parts of *A.*

Fig. 5. Typical morphology of *Alyssum montanum* subsp. *gmelinii* (A) and *Alyssum murale* (B) plants cultivated in presence of increasing concentration of Ni in substrate for 16 weeks. A; from left to right: control, 0.25, 0.5, 2.0 g Ni L^{-1}. B, from left to right: control, 2.0, 4.0 g Ni L^{-1}.

montanum, and Pb increased in stems of *A. montanum*. The most pronounced decrease was evident for Ba (all parts of both species), and for *A. murale*, concentrations decreased for all parts also for Ca, K, Mn, Na, Sr, and Zn.

Discussion

Negative effect of excess Ni on plants is usually associated with reduced plant growth. Nickel-intolerant plants (i.e. *Triticum aestivum*) suffered from excess metal at concentration as low as 25 to 50 µg L^{-1} in hydroponics, showing significantly inhibited growth and reduced plant vigour, accumulating up to 18 and 13 mg kg^{-1} Ni in roots and leaves, respectively (Parlak 2016). For relatively Ni-tolerant plants, i.e. *Brassica juncea* and *Mesembryanthemum crystallinum*, growth reduction by 70 and 66% was evident, respectively, at 5.9 mg L^{-1} Ni in hydroponics (Amari et al. 2014). The plants accumulated 1300 and 1900 mg kg^{-1} Ni in roots and 550 and 480 mg kg^{-1} Ni in leaves.

Leaf chlorosis and necrosis are common morphological phytotoxicity symptoms of Ni, appearing on young and old leaves, respectively (Amari et al. 2014). Similar toxicity

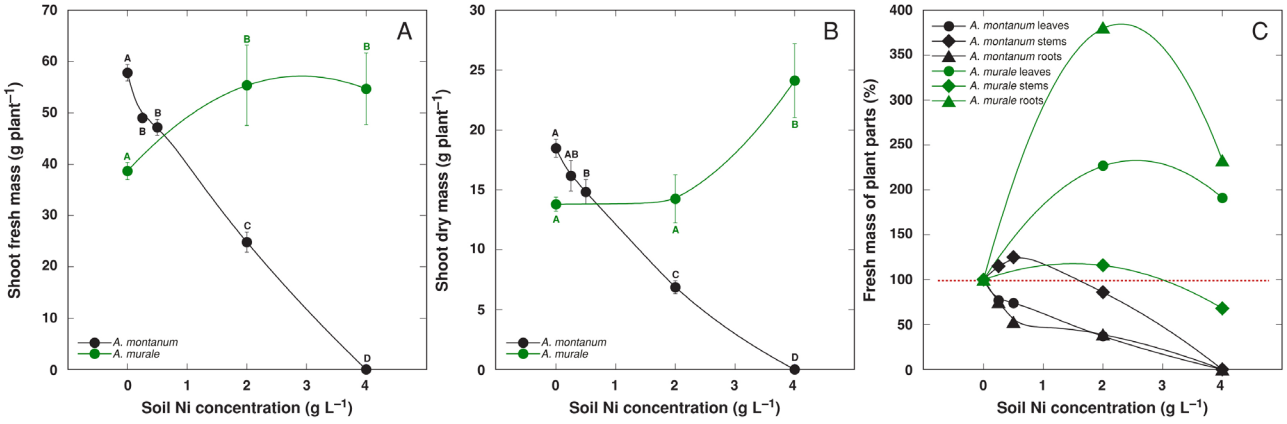

Fig. 6. Effect of increasing concentration of Ni in substrate on morphological parameters of *Alyssum montanum* subsp. *gmelinii* and *Alyssum murale* plants cultivated for 16 weeks. A, shoot fresh mass; B, shoot dry mass; C, relative effect of treatment on distribution of fresh mass in plant parts

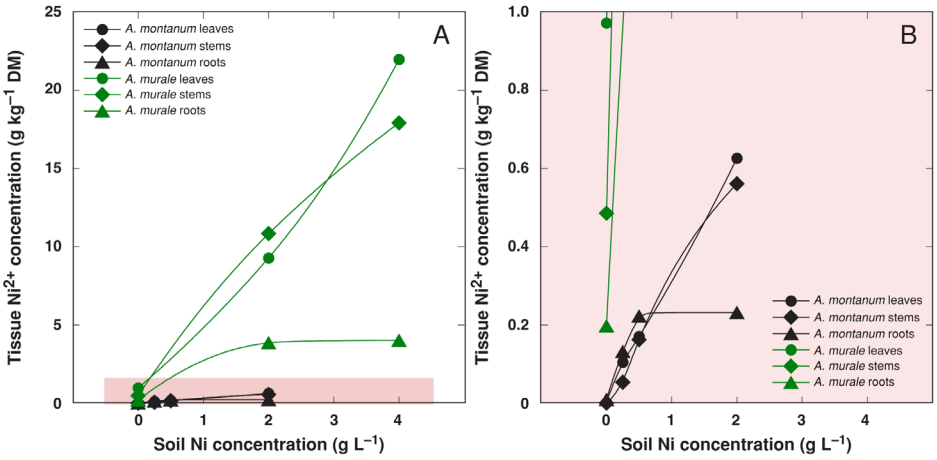

Fig. 7. Effect of increasing concentration of Ni in substrate on Ni concentration in different parts of *Alyssum montanum* subsp. *gmelinii* and *Alyssum murale* plants cultivated for 16 weeks. A, full concentration range; B, fragment of the same graph at the lower part of the concentration range indicated by shading.

symptoms were evident also for *A. montanum* in the present study (Fig. 1).

There is continuous scientific debate on whether metal hyperaccumulation has evolved as a microevolutionary response to metalliferous soils resulting in formation of hyperaccumulating ecotypes as was initially established by Antonovics et al. (1971) and later confirmed in numerous studies, or that innate metal tolerance and high accumulation capacity are species-wide characteristics (Boyd, Martens 1998). The second concept assumes that metal hyperaccumulation ability has adaptive value also for plants growing in non-metalliferous soil, possibly, associated with more efficient mineral nutrient uptake system. It is evident that Ni tolerance and hyperaccumulation ability explored so far in *Alyssum* species is a constitutive property (Broadhurst, Chaney 2016).

A significant characteristic of plants-metal accumulators is predominant buildup of metals in aboveground parts (Bothe 2011; Reeves et al. 2018). This criterion was met for Ni in both *Alyssum* species tested. In addition, root accumulation capacity was clearly saturable in response to increasing substrate Ni concentration. While Ni accumulation in stems and leaves of *A. murale* was within a range reported in other studies in controlled conditions (Broadhurst et al. 2009; Broadhurst, Chaney 2016), accumulation potential in stems and leaves of *A. montanum* (about 600 mg kg^{-1}) was significantly higher than that reported in other studies in controlled conditions (50 to 71 mg kg^{-1}; Broadhurst, Chaney 2016). Ni concentration was determined in a total of 22 *A. montanum* leaf samples in the initial study of Brooks and Radford (1978), but no particular results were reported as concentration was less than the defined threshold limit, 1000 mg kg^{-1}. In the following study, Ni concentrations of < 1, < 1, 2, 2, and 5 mg kg^{-1} were reported for *A. montanum* specimens (Brookes et al. 1979). Only one leaf sample of *A. montanum*, growing

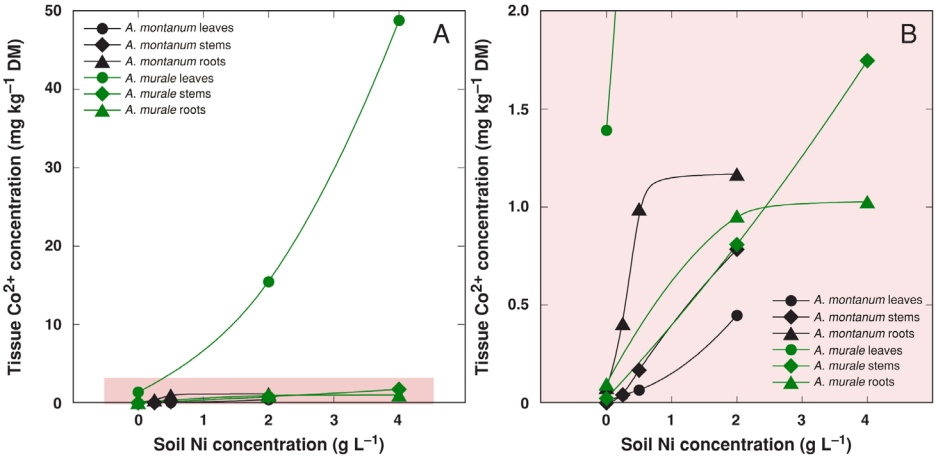

Fig. 8. Effect of increasing concentration of Ni in substrate on Co concentration in different parts of *Alyssum montanum* subsp. *gmelinii* and *Alyssum murale* plants cultivated for 16 weeks. A, full concentration range; B, fragment of the same graph at the lower part of the concentration range indicated by shading.

Table 3. Effect of Ni treatment on mineral element concentration in different parts of *Alyssum montanum* subsp. *gmelinii* and *Alyssum murale* plants after 16 weeks of cultivation in comparison to cotrol plants. Data are means from a combined sample from 5 plants

Element	Treatment	*Alyssum montanum* subsp. *gmelinii*			*Alyssum murale*		
		Leaves	Stems	Roots	Leaves	Stems	Roots
Al	Control (mg kg^{-1})	9	6	155	9	4	85
	Ni 2 g L^{-1} (±%)	+42	0	−26	−29	−16	+55
Ba	Control (mg kg^{-1})	9	43	20	15	21	17
	Ni 2 g L^{-1} (±%)	−10	−52	−17	−66	−31	−23
Ca	Control (mg kg^{-1})	48 142	20 484	8521	58 895	11 886	9631
	Ni 2 g L^{-1} (±%)	−11	+33	−4	−53	−33	−13
Cd	Control (mg kg^{-1})	0.03	0.07	0.19	0.12	0.20	0.30
	Ni 2 g L^{-1} (±%)	+153	+71	+63	+167	−40	−60
Cr	Control (mg kg^{-1})	0.03	0.06	0.33	0.14	0.04	0.19
	Ni 2 g L^{-1} (±%)	+73	−63	−26	−88	0	+71
Cu	Control (mg kg^{-1})	9	3	46	9	3	40
	Ni 2 g L^{-1} (±%)	−29	+354	+224	−29	+300	+5
Fe	Control (mg kg^{-1})	48	39	513	167	39	372
	Ni 2 g L^{-1} (±%)	+338	+356	−46	−36	+339	+48
K	Control (mg kg^{-1})	12 980	24 047	1580	30 767	18 845	3649
	Ni 2 g L^{-1} (±%)	+101	+24	−73	−58	−12	−5
Mn	Control (mg kg^{-1})	36	48	8	176	36	15
	Ni 2 g L^{-1} (±%)	+133	−1	0	−55	−35	−36
Mg	Control (mg kg^{-1})	4874	2274	1211	2657	915	1053
	Ni 2 g L^{-1} (±%)	+250	+160	−14	−42	+55	0
Na	Control (mg kg^{-1})	56	16	87	233	178	258
	Ni 2 g L^{-1} (±%)	+147	+254	+300	−54	−62	−32
Pb	Control (mg kg^{-1})	0.56	0.03	1.30	0.58	0.04	1.48
	Ni 2 g L^{-1} (±%)	−66	+800	+136	+21	+50	+4
Sr	Control (mg kg^{-1})	31	23	21	38	18	22
	Ni 2 g L^{-1} (±%)	+8	+42	+14	−51	−17	−23
Zn	Control (mg kg^{-1})	22	40	38	72	144	125
	Ni 2 g L^{-1} (±%)	0	+70	+4	−61	−34	−40

on serpentine soils, showed accumulation of 22 mg kg^{-1} Ni (Reeves et al. 1983). Consequently, the accession of *A. montanum* subsp. *gmelinii* from coastal dunes of the Baltic Sea shows exceptional Ni accumulation potential.

Ni accumulation in hyperaccumulating *Alyssum* species has been associated mainly with epidermal cell vacuoles and leaf trichomes (Broadhurst et al. 2004), as Ni concentration in the basal part of trichomes has been observed to reach 15 to 20% DM. In *A. murale*, Ni is transported from roots to shoots attached to histidine, and vacuolar storage in leaf epidermal cells is predominantly in the form of a Ni-malate complex (McNear et al. 2010).

In natural conditions *A. murale* accumulated up to 143 mg kg^{-1} Co in leaves (Stamenković et al. 2017). Stimulation of Co uptake by Ni was confirmed in the present study, where Ni treatment resulted in increased concentration of Co in all plant parts of both species growing in soil with no added Co in a Ni concentration-dependent manner. *A. murale* accumulated 50 mg kg^{-1} Co in leaves and 1.0 mg kg^{-1} in roots, and for *A. montanum* the respective values were 0.5 and 1.2 mg kg^{-1} (Fig. 8). It was found that in contrast to Ni, which accumulates in leaf epidermis, Co is localized in apoplasm of mesophyll cells at the tips and margins of leaves in *A. murale* (Tappero et al. 2007). As no Co was added to the substrate, the accumulated concentration of Co seems to be exceptionally high. For leaf samples from herbarium specimens collected in natural conditions, *A. murale* had Co concentration in the range 4 to 34 mg kg^{-1} (Brooks, Radford 1978).

Disruption of homeostasis of other essential plant metals by Ni has been associated with symptoms of Ni toxicity in plants. Thus, it was shown that for the Ni-tolerant hyperaccumulator species *Alyssum inflatum*, high Ni treatment resulted in increased sensitivity to and accumulation of Cu, but root-to shoot translocation of Fe was inhibited, resulting in appearance of chlorotic leaves (Ghasemi et al. 2009). Similarly, in the present study, Cu concentration was increased about three-fold in plant stems of both studied *Alyssum* species treated with 2 g L^{-1} Ni, but not in leaves (Table 1). However, in contrast to the above-mentioned study, Fe concentration also increased in Ni-treated plants of both species more than three-fold, and

a similar degree of increase was evident also in leaves of *A. montanum* (Table 1).

Photosynthesis-related parameters have been measured in *A. murale* grown in substrate with variable Ni concentration, and it was shown that photosynthetic functions of the plant were not affected by Ni concentration in substrate up to 1568 mg kg^{-1} DM, but this treatment resulted in decrease of shoot dry mass by 40% (Sellami et al. 2012). Photochemical capacity of *A. montanum* was negatively affected by increased Ni concentration in substrate, in parallel or even before development of visual toxicity symptoms (Fig. 3).

Ni-induced oxidative stress is suggested as one of the reasons for indirect negative effects of excess soil Ni concentration, even for plants hyperaccumulating Ni (Boominathan, Doran 2002). Peroxidase activity has not been studied in respect to Ni tolerance, but it was suggested that general differences in enzymatic antioxidative capacity can be important for metal tolerance, as the tolerant hyperaccumulating species *Alyssum bertolonii* had more than 500 times larger root catalase activity in comparison to that of intolerant species *Nicotiana tabacum* (Boominathan, Doran 2002).

Characteristic opposite changes in peroxidase activity found in leaves and roots of *A. montanum* under high Ni concentration indicate consequences of possible oxidative stress-associated metabolic toxicity of Ni (Fig. 4). Increase of peroxidase activity in plant tissues treated with heavy metals is usually associated with stress-induced tolerance responses (Van Assche et al. 1988; Verma, Dubey 2003; Sharma, Dietz 2009). Therefore, a significant increase in enzyme activity in leaves of *A montanum* could be associated with enzymatic defense responses involved in protection of mesophyll tissues important for maintenance of photosynthetic function, pointing to considerable heavy metal tolerance of the species. Thus, enzymatic activity of both ascorbate peroxidase and guaiacol peroxidase was highly elevated in the heavy metal hyperaccumulator *Arabidopsis halleri*, but not in non-accumulating related species *Arabidopsis thaliana* (Chiang et al. 2006).

The results of the present study indicate that the accession of *A. montanum* subsp. *gmeliniii* from coastal sand dunes of the Baltic Sea has good tolerance to increased substrate Ni concentration and exceptional Ni accumulation potential. This potential needs to be further explored regarding possible use of the particular accession in revegetation during restoration measures of degraded arid lands.

Acknowledgements

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