



Accumulation of potentially toxic elements by plants of North Caucasian *Alyssum* species and their molecular phylogenetic analysis

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Abstract Accumulations of potentially toxic metals were investigated in soils and five North Caucasian *Alyssum* species from metalliferous areas and non-metalliferous areas in Karachay-Cherkessia, Kabardino-Balkaria, Dagestan and the Krasnodar region. Analyses of field samples showed that chemical features of the soils significantly affected the concentrations of Ni, Co, Zn, but had less effect on Cu and Pb concentrations in the shoots of *Alyssum*. Variations in the degree of accumulating ability were found in the studied species, including hyperaccumulation of Ni in *Alyssum murale* (up to 12,100 mg kg⁻¹), and significant accumulation of Zn in *A. gehamense* (up to 1700 mg kg⁻¹). A comparative molecular genetic analysis of two *A. murale* populations, both Ni-hyperaccumulating population from Karachay-

Cherkessia and non-hyperaccumulating population from Dagestan, indicated considerable genetic difference between them. This result supports the hypothesis that the selection of metal hyperaccumulator species with enhanced phytoremediation efficiency should be considered at the population level.

Keywords *Alyssum* species · Population · Zinc · Nickel · Copper · Cadmium · Lead · Molecular phylogenetic analysis

Introduction

Metalliferous soils, owing to their extreme chemical characteristics, are hostile habitats and often unfavourable to normal plant growth. However, plants growing on naturally metal-enriched soils are adapted to cope with bioavailable levels of potentially toxic metals. Many of these plants have metal content higher than for plants on non-metalliferous soils. A small number of these metallophytes are hyperaccumulators. Hyperaccumulators are unusual plants that accumulate particular metals or metalloids in their living tissues to levels that may be hundreds or thousands of times greater than is normal for most plants (van der Ent et al. 2013). The criterion for hyperaccumulation varies for different metals and represents a leaf concentration > 100 mg kg⁻¹ for Cd, > 1000 mg kg⁻¹ for Ni, Co, Cu,

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Pb, > 3000 mg kg⁻¹ for Zn, and > 10,000 mg kg⁻¹ for Mn (Reeves et al. 2018).

The phenomenon of metal hyperaccumulation by plants aroused much interest because its biochemical and physiological uniqueness may be exploitable economically in the clean-up of metal-contaminated soils. Soil decontamination by hyperaccumulator cropping might represent a low-cost, environmentally friendly technique of detoxifying soils contaminated with metals (Baker et al. 1994). Hyperaccumulator plants often appear to be restricted in their distribution to metalliferous soils, from which they always exhibit hyperaccumulation of some element; these are described as ‘obligate’ hyperaccumulators (Pollard et al. 2002). However, some are more widespread, with populations that hyperaccumulate from metalliferous soils, and other populations, from non-metalliferous soils, that do not show unusual accumulation; these are ‘facultative’ hyperaccumulators (Pollard et al. 2014).

Among hyperaccumulators, the largest number of species possessing the ability to hyperaccumulate Ni. Species of Euphorbiaceae and Brassicaceae families include about 25% of the known Ni hyperaccumulators (Krämer 2010). Most of these species are almost completely restricted to soils enriched with Ni, Cr and sometimes Co derived from ultramafic (serpentine) rocks. The genus *Alyssum* (Brassicaceae) has attracted the interest of botanists because of the association of many of its species with substrata with potentially toxic metals (Baker and Brooks 1989). Species of this genus found on soils derived from ultramafic rocks in southern Europe can accumulate concentration of Ni in excess of 2% of dry weight (Bani et al. 2010). Among them, *Alyssum murale* is a well-known Ni hyperaccumulator that has been developed as a commercial crop for phytoremediation/phytomining Ni from metal-enriched soils (Tappero et al. 2007; Bani et al. 2015; Osmani and Bani 2018; Saad et al. 2018).

In fact, hyperaccumulators can be exploited not only as natural remediation species but also as sources of genes for improvement of other remediation species (Galardi et al. 2007a). Many studies are devoted to assessing the genetic diversity of populations of plant species native to areas where soils are naturally enriched with chemical elements (Deng et al. 2007; Galardi et al. 2007a). The estimation of genetic variation between plants in their ability to accumulate

metals is of both practical and theoretical importance (Pollard et al. 2002). Genetic study of *A. bertolonii* populations showed that they are strongly genetically distinct from each other and that a relatively high genetic heterogeneity does exist within the same population (Galardi et al. 2007a). Moreover, in the same study a clear relationship between geographical isolation and genetic differentiation of populations has been found. A field study at the population level can join the theoretical research on the estimation of differences between plants in levels of metal concentration to the practical advantage of being a possible first step towards the selection of populations with the highest extraction ability (Pollard et al. 2002). Recently, Bani et al. (2010) demonstrated that different populations of *A. murale* living in their natural habitats, present differences in Ni hyperaccumulation related to soil Ni concentration. Intra-specific variation in Ni hyperaccumulation have presented also for other Ni-hyperaccumulating species, e.g. *A. lesbiacum* (Adamidis et al. 2014). In this context, the most effective populations could be used both directly as phytoremediation species themselves (Bani et al. 2010) and indirectly for the improvement of plant traits through selective breeding or as sources of genes for improvement of other remediation crops (Pollard et al. 2002).

Russia has a number of biodiversity centres of Brassicaceae species in the North Caucasus mountain areas. North Caucasus mountain *Alyssum* populations can occupy much smaller areas than in the case of plains. This is primarily due to the diversity of environmental conditions. Altitudinal zonation, exposure, markedly more pronounced erosion, diversity of edaphic conditions, including those due to differences in parent rocks, not only reduce the population area, but also create the prerequisites for the genetic variability at the population level.

In mountainous areas of the North Caucasus, many outcrops of ultramafic and mafic rocks give rise to soils with a wide range of compositions and concentrations of potentially toxic metals. However, very little information is available on metal uptake by natural North Caucasus vegetation in naturally mineralized soils. Investigations on metal-accumulating ability of plants and phylogenetic relationships among species and populations of plants growing on naturally metal-enriched soils can be important for the fundamental knowledge on metal uptake and tolerance

mechanisms for phytoremediation of large areas of soils around the world. Therefore, the objectives of this study were (1) to determine the metal concentrations and their variability in metalliferous and non-metalliferous soils, (2) to assess the metal-accumulating ability of the North Caucasian *Alyssum* species and populations and (3) to analyse phylogenetic relationships in order to evaluate their potential for phytoremediation purpose.

Materials and methods

Site description and field sampling

The study was conducted in four North Caucasus mountain regions of Russia: Krasnodar region (site 1), Dagestan (sites 2–4), Karachay-Cherkessia (site 5), Kabardino-Balkaria (site 6) and at altitude from 900 to 2600 m above sea level (Fig. 1). These sites with different characteristics were selected at metalliferous (naturally mineralized) and non-metalliferous localities (Table 1). Soil forming calcareous rocks of Krasnodar region (site 1) and Dagestan (site 2) areas are mostly not metalliferous. The mafic rocks of Dagestan areas (sites 3, 4) are mostly diabase, which have mineralization in Fe, Cu, Zn, and Pb sulphides (Palivoda 2008). The geological substrata of Karachay-Cherkessia and Kabardino-Balkaria areas (sites

5, 6) consist of the ultramafic rocks—serpentinous harzburgites, lherzolites, dunites, and pyroxenites, which contain minerals with high concentration of Ni, Co, and Cr (Potapenko 2004; Markin 2011). The mountain skeletal soils of studied areas are developed mainly directly on eluvium or eluvium-deluvium materials and everywhere were closely related to the bedrock. Their soil profile is not differentiated, highly stoniness, and minor amounts of fine earth. The climatic conditions of the studied areas are distinguished primarily by the degree of moisture. The climate in the western part of North Caucasus region is the wet sub boreal (site 1, 5, 6), the eastern part is more arid and continental (site 2–4).

Plants of five *Alyssum* species were sampled from soil derived from different rock types (Table 1). The studied species are common in the sparse grass communities of open stony and clayey habitats of the middle mountain zone of the North Caucasus. However, the number of samples depends on the occurrence in each studied site and is primarily associated with the difficult conditions of the mountainous terrain. Soils and plants were sampled in representative sub-sites (≈ 30 m² each) from each studied site. A total five of *Alyssum alyssoides*, six of *A. daghestanicum*, three of *A. gehamense*, eleven of *A. murale* (six—in Karachay-Cherkessia and five—in Dagestan), and five samples of *A. tortuosum* were collected. Each sample consists of at least five adult



Fig. 1 Localization of the studied sampling sites (1–6) of the North Caucasus region

Table 1 Characterization of sampling sites from the North Caucasus region

Site number	Species	Location	GPS coordinates	Rocks
1	<i>Alyssum tortuosum</i>	Krasnodar Territory	44°03'11.97" N 43°06'37.19" E	Calcareous
2	<i>Alyssum daghestanicum</i>	Dagestan	42°24'06.90" N 46°55'09.21" E	Calcareous
3	<i>Alyssum gehamense</i>	Dagestan	41°17'05.70" N 47°50'12.66" E	Mafic
4	<i>Alyssum murale</i>	Dagestan	41°22'47.79" N 47°26'04.48" E	Mafic
5		Karachay-Cherkessia	43°54'57.27" N 40°57'40.51" E	Ultramafic
6	<i>Alyssum alyssoides</i>	Kabardino-Balkaria	43°43'22.02" N	Ultramafic

plants of about the same size, which were randomly chosen over the entire spatial distribution of each sampling sub-site. The samples for chemical and molecular genetic analysis were collected simultaneously. The shoots of plants, which were flowering and fruiting, were analysed. Soil samples of 0–10 cm depth (below the surface layer of stone in debris areas) were taken from around the roots of all field collected plants. Combined samples consisted of three-point samples. In total, eighteen soil samples were collected.

Chemical analyses

The collected samples were transferred at ambient temperature to the laboratory and air-dried. For subsequent analysis, the soil samples were ground in a porcelain mortar and then sieved through a 1-mm screen. Soil pH was determined in a 1:2.5 soil/deionised water suspension using a glass pH electrode, Model I-160. Soil available fraction of the potentially toxic metals were extracted by shaking 3 g of air-dry soil with 30 ml 1 M ammonium acetate adjusted to pH 4.8, and Ca was extracted by shaking 1 g of air-dry soil with 40 ml 1 M ammonium acetate adjusted to pH 6.5 (Vorobyeva 1998). After shaking, the soil suspension was filtered using slow-filtering paper for the finest deposits.

For the analyses of the total Ni, Co, Zn, Cu and Pb concentrations in the plant samples, 2 g of oven-dried plant material was weighed into quartz cups, ashed at 450 °C in a muffle furnace, and then transferred into a desiccator for cooling. The ash was taken up in 6 ml mixture of 1.5 M HNO₃ and 3.71 M HCl then filtered using slow-filtering paper in a volumetric flask, and

the digest made up to 25 ml volume with deionized water.

Plant and soil extracts were analysed by atomic absorption spectrometry. Performance of the instrument was checked by analysing the reference standard material solutions: State Standard Samples GSO 7272–96, and GSO 7325–96. All analyses were carried out in duplicate.

The statistical analyses were in order to identify potential differences between plant species. Data were analysed nonparametrically using Kruskal–Wallis ANOVA by Ranks test ($p < 0.05$). A Bivariate Spearman correlation was used to correlate chemical element concentrations in soils and plants ($p < 0.05$). All statistical evaluations were performed using Statistica 7.0 software.

Molecular genetic analysis methods

DNA was extracted from dry leaves (≈ 100 mg) with CTAB protocol (Doyle & Doyle, 1987). The primers ITS-A (5'-GGAAGGAGAAGTCGTAACAAGG-3') and ITS-B (5'-CTTTCCTCCGCTTATTGATATG-3') (Blattner 1999) were used for the amplification of ITS1-5.8S-ITS2 region of nuclear rDNA. Polymerase chain reaction amplifications were performed in a total volume of 20 μ l containing 1 \times Phire Plant PCR Master Mix F160L (Thermo Fisher Scientific) and 4 ng of template DNA according to the manufacturer's recommended protocol: initial denaturation 98 °C 5 min; 35 cycles (denaturation 98 °C 5 s; annealing 56 °C 5 s; extension 72 °C 20 s); final extension 72 °C 1 min. Reactions were performed in a Bio-Rad T100 thermocycler (Bio-Rad Laboratories,

Inc.). Automated DNA sequencing was performed on both strands directly from the ITS-A and ITS-B primers on the purified PCR products using BigDye Terminator v.3.1 chemistry and an ABI 3130 sequencer (Applied Biosystems, Norwalk, CT, USA) according to the manufacturer's recommendations. The alignment was slightly modified manually.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura–Nei model (Tamura and Nei 1993). The bootstrap consensus tree inferred from 500 replicates (Felsenstein 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein 1985). The initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 44 nucleotide sequences and 641 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). Parsimony informative nucleotide substitutions and variable sites are indicated according to the reference sample *Alyssum tortuosum* (EF514625). Variable sites were evaluated according to the trace data in abi format produced by sequencers. The site was considered as variable if in the forward and reverse sequence the variant peaks differ by no more than 60%. Single-letter codes: Y (T or C), R (G or A), S (G or C), M (A or C), N (A or T or G or C). Parsimony informative and variable sites were sorted by phylogenetic tree with OpenOffice 4 software (The Apache Software Foundation).

Results and discussion

Soil characterization

Soil from the sampling sites had a neutral to alkaline pH 7.23 to 7.95. Many studies have analysed metal-liferous areas with moderately acid and acid soils and native plants, which can grow on these soils (Abreu et al. 2012; Bech et al. 2012a). Much less information is available on metal uptake by natural vegetation in neutral to alkaline soils with high metal burdens. However, investigation on natural vegetation

simultaneously adapted to high Ca content in soils and high metal availability can be important for the fundamental knowledge on metal uptake and tolerance mechanism (Bech et al. 2012b). According to our data, extractable Ca varied fairly highly between studied soils (Table 2). Soils from Dagestan derived from calcareous and mafic rocks (site 2 and 3, respectively) presented significant differences from the other studied soils; moreover, Ca concentration in soils of site 2 reached the highest levels (31.6 g kg^{-1}). Calcium is one of the main antagonistic elements in relation to the absorption and metabolism of many trace elements (Kabata-Pendias and Pendias 2001). This is confirmed by the results of this study in which significant relation between Ca soil concentrations and plant concentrations of Ni ($r = -0.50$), Co ($r = -0.60$), Zn ($r = -0.65$), Cu ($r = -0.53$) and Pb ($r = -0.42$) were shown.

Potentially toxic metal concentrations (available fraction extracted with ammonium acetate) in soils, in which *Alyssum* plants grew, are shown in Table 2. In this study, we used data on available fraction of metals; since this represents the most mobile and readily plant-available fraction, which corresponds to the best correlation values between the trace element concentration in soils and plants (Kumar et al. 2011; Santos et al. 2012). For example, Abreu et al. (2012) reported that Pb tolerance of *Cistus salvifolius* plants, which were growing on soils presenting a wide range of Pb concentration, is not related to the total concentration but to the available fraction of metal.

Soils from Karachay-Cherkessia and Kabardino-Balkaria (sites 5, 6) presented greater concentrations of Ni and Co in the available fraction than other studied soils. High levels of these elements in soils may generate from ultramafic parent rocks, which are associated with Ni and Co minerals. A large number of chemical analyses have been made for soils over ultramafic rocks. They show that most of these soils have the available concentrations of Ni and Co at much higher levels than other soil types (Proctor and Nagy 1992; Oze et al. 2008). In this study, the mean level of Ni in the soils derived from ultramafic rocks ranged from 9.33 to 15.9 mg kg^{-1} and Co from 0.70 to 0.86 mg kg^{-1} . For the soils derived from mafic and calcareous rocks the corresponding values were much lower (0.41 – 0.67 mg kg^{-1} and below the detection limit, respectively). Thus, soils from two ultramafic areas (sites 5, 6) presented concentration of Ni that

Table 2 Mean concentration of chemical elements in soils (available fraction^a) from sampling sites of the North Caucasus region

Sites	Soils	Element concentration ^a					
		Ca	Zn	Ni	Co	Cu	Pb
1	Non-metalliferous	5.64 ± 0.73 ^a	3.35 ± 1.11 ^a	0.61 ± 0.33 ^a	< 0.40 ^a	0.31 ± 0.12 ^a	2.97 ± 0.57 ^{ac}
2	Non-metalliferous	31.6 ± 1.08 ^b	0.70 ± 0.14 ^b	0.41 ± 0.07 ^a	< 0.40 ^b	< 0.15 ^b	3.88 ± 0.18 ^a
3	Metalliferous (Pb, Zn)	1.47 ± 0.25 ^c	97.7 ± 23.5 ^c	0.43 ± 0.11 ^a	0.40 ± 0.11 ^b	0.40 ± 0.15 ^{ac}	36.4 ± 11.7 ^b
4	Metalliferous (Ni, Co)	4.95 ± 0.67 ^a	0.76 ± 0.26 ^{bd}	0.67 ± 0.23 ^a	0.53 ± 0.27 ^b	1.32 ± 0.22 ^d	0.97 ± 0.32 ^c
5	Metalliferous (Cu)	10.2 ± 1.38 ^d	1.35 ± 0.36 ^{ad}	15.9 ± 4.56 ^b	0.86 ± 0.36 ^c	0.17 ± 0.09 ^{ab}	< 0.50 ^d
6	metalliferous (Ni, Co)	5.28 ± 0.67 ^a	0.60 ± 0.21 ^b	9.33 ± 3.31 ^b	0.70 ± 0.27 ^c	0.50 ± 0.13 ^c	2.07 ± 0.26 ^c

Values are mean ± SD, $n = 18$. Concentrations in mg kg^{-1} DW, except for Ca in g kg^{-1}

^aExtracted with ammonium acetate; n —number of samples; DW = dry weight. In each column, mean ± SD followed by different letters are statistically different ($p < 0.05$)

exceeded the Russian maximum permissible value (MPV) for acetate ammonium-extractable fraction of this element in the soils (5.0 mg kg^{-1} , MPV 2006).

The Dagestan soils of site 3 were extreme in their high available concentration of Zn and Pb (97.7 mg kg^{-1} and 36.4 mg kg^{-1} , respectively), exceeding MPV values (23 mg kg^{-1} and 6.0 mg kg^{-1} , respectively), while other studied soils had significantly lower concentrations of Zn and Pb (Table 2). High concentrations of these metals in the site 3 due to the parent material of these soils that was Zn and Pb-enriched diabasic eluvium. Ghaderian et al. (2007) have been reported by the high soil concentrations of Pb (20 mg kg^{-1}) and Zn (30 mg kg^{-1}) in the available fraction in the Irankouh area (Central Iran), which is a vast mountainous region with naturally mineralized soils. Copper concentration in the skeletal soil derived from Cu enriched Dagestan mafic rocks (site 4) was relatively low, but considerably (3–8 times) higher in this soil than at other sites (Table 2).

A high proportion of total metals are an immobile fraction not immediately bioavailable for plants and therefore are not directly toxic (Pollard et al. 2002; McLaughlin et al. 2008). However potentially toxic metals in the available fraction may be directly absorbed by plants from the soils. Thus, the extreme concentration of Ni and Pb–Zn in the available fraction in soils of sites 1, 5 and site 3, respectively, suggest high toxicity.

Metal accumulation in plants

According to our results, Ca in *Alyssum* plants was very high (Table 3) and was not statistically correlated with Ca in soils ($r = 0.29$). *Alyssum* species have an effective Ca absorption system, regardless of the element concentration in the soil, as reported by Tumi et al. (2012) for *A. murale*, which contained up to $35,350 \text{ mg Ca kg}^{-1}$ in the leaves when growing on serpentine soils in Serbia with low Ca level (310 mg kg^{-1}). Several studies have reported exceptional concentrations of Ca in leaves *A. murale*, predominantly in the CaCO_3 nodules on the surface of the trichomes (Tappero et al. 2007; Broadhurst and Chaney 2016).

Our data clearly demonstrates that concentrations of chemical elements in available fraction of the soils (Table 2) significantly affected the concentrations of Ni ($r = 0.63$), Zn ($r = 0.48$), Co ($r = 0.86$), but not significantly on Cu ($r = 0.33$) and Pb ($r = 0.31$) concentrations in the shoots of *Alyssum* plants (Table 3). *Alyssum daghestanicum* from non-metalliferous soils derived from calcareous rocks (site 2) with low potentially toxic metal contents (Table 2) had shoot concentrations of Zn and Cu significantly lower than other studied plants (Table 3). *Alyssum gehamense* and *A. murale* population from metalliferous Karachay-Cherkessian soil had the highest concentrations of Zn and Ni–Co, respectively, which could be a consequence of these high metal concentrations in the geological material and soil (Table 2).

Table 3 Mean concentrations of chemical elements in plant shoots of *Alyssum* populations from sampling sites of the North Caucasus region. Values are mean ± SD

Species	Sites	n	Element concentration (mg kg ⁻¹ dry weight)					
			Ca	Zn	Ni	Co	Cu	Pb
<i>Alyssum tortuosum</i>	1	5	6.02 ± 0.12 ^a	24.5 ± 3.72 ^a	2.08 ± 0.3 ^{ab}	0.50 ± 0.02 ^{ac}	2.43 ± 0.25 ^a	2.29 ± 0.44 ^a
<i>Alyssum daghestanicum</i>	2	6	8.88 ± 0.30 ^b	10.2 ± 2.78 ^b	0.65 ± 0.09 ^a	< 0.40 ^c	1.84 ± 0.07 ^b	1.61 ± 0.15 ^a
<i>Alyssum gehamense</i>	3	3	5.84 ± 0.28 ^a	1640 ± 100 ^c	2.21 ± 0.19 ^b	0.80 ± 0.05 ^{bc}	3.25 ± 0.38 ^{ac}	15.7 ± 1.62 ^b
<i>Alyssum murale</i>	4	5	8.52 ± 0.29 ^b	18.9 ± 3.31 ^a	20.0 ± 3.09 ^c	1.36 ± 0.17 ^b	2.82 ± 0.45 ^{ac}	1.95 ± 0.10 ^a
	5	6	8.81 ± 1.28 ^{ab}	30.7 ± 2.69 ^d	6805 ± 182 ^d	33.1 ± 13.6 ^a	3.96 ± 0.44 ^c	3.12 ± 0.74 ^{ac}
<i>Alyssum alyssoides</i>	6	5	5.61 ± 0.23 ^a	23.5 ± 9.29 ^{ad}	24.4 ± 11.6 ^c	1.52 ± 0.99 ^b	3.85 ± 0.49 ^c	3.75 ± 0.71 ^c

n—number of samples. In each column, mean ± SD followed by different letters are statistically different (*p* < 0.05)

The highest mean concentration of Zn (1640 ± 100 mg kg⁻¹) was in the shoots of *A. gehamense* from the Dagestan soils derived from mafic rocks (site 3). Total Zn shoot concentration in the other studied plants ranges between 10 and 31 mg kg⁻¹. These values do not excessively exceed the metabolic needs (< 10 mg kg⁻¹) common of non-accumulator plants. Among all the plant species studied, none was close to the minimum concentration criteria for being considered a Zn hyperaccumulator plant. However, *A. gehamense* had an ability to accumulate Zn in their shoots. Shoots of *A. gehamense* also showed higher Pb concentrations (Table 3). However, the Pb bioaccumulation factor for this plant was low—0.43. According to the study by Szczygłowska et al. (2011), *Brassica* species could accumulate lower amounts of Pb than other metals. Moreover, Pb is neither essential nor beneficial to plant nutrition; it is usually quite immobile and poorly accumulated in upper plant parts.

The concentrations of Cu in the shoots of plants found in the present study were in the range of 1.84 to 3.96 mg kg⁻¹. The highest value was recorded in the shoots of *A. murale* (site 5). The relatively low shoot Cu accumulation can be attributed to both low concentrations of this metal in the available fraction in studied soils (Table 2) and taxon-specific features in the accumulation of this element. Ghasemi et al. (2015) have discussed the tolerance, uptake, accumulation and interactions of Ni and Cu in a range of *Alyssum* species from the Mediterranean region and have concluded that no tolerance to high concentrations of Cu was observed in any of the species tested.

However, in the presence of Ni, an increased Cu concentration was observed in both roots and shoots of the Ni hyperaccumulator plants, but not in the non-accumulators. According to Broadhurst and Chaney (2016) Ni hyperaccumulators, including *A. murale*, are very specific to Ni and do non-selectively accumulate/hyperaccumulate other transition metals such as Fe, Cr, or Cu.

Among studied plant species, only *A. murale* includes both Ni-accumulating and non-accumulating populations growing on soils derived from ultramafic and non-ultramafic rocks, respectively. The plants from the nickel-rich Karachay-Cherkessia soils (site 5) had Ni concentrations in the shoots within ranges 4280–12,130 mg kg⁻¹. This is in agreement with other published values for Ni hyperaccumulation in this and some other European *A. murale* populations (Bani et al. 2010; Tang et al. 2012). *Alyssum murale* emerging as Ni hyperaccumulator from this study would be the first reported from the North Caucasus.

The shoot Co concentration of Ni-accumulating population in this study was 33.1 mg kg⁻¹ with average values for other studied species from below the detection limit to 1.52 mg kg⁻¹ (Table 3). The bioconcentration factor shown in Table 4, based on ammonium acetate extractable soil concentration, revealed the expected accumulation of Ni and Co in the shoots of population *A. murale* from Karachay-Cherkessia was greater than in plants of other species. The fairly high bioaccumulation of Ni in the Dagestan *A. murale* population that grows in the soils with low Ni concentration seems to suggest that it also has an effective Ni absorption system. The ability of *A.*

Table 4 Accumulation factor^a of potentially toxic elements in plant shoots of *Alyssum* populations from sampling sites of the North Caucasus region

Species	Sites	Zn	Ni	Co	Cu	Pb
<i>Alyssum tortuosum</i>	1	7.31	3.41	–	7.84	0.77
<i>Alyssum daghestanicum</i>	2	14.6	1.59	–	–	0.42
<i>Alyssum gehamense</i>	3	16.8	5.14	2.00	8.13	0.43
<i>Alyssum murale</i>	4	24.9	29.9	2.57	2.14	2.01
	5	22.7	428	38.5	23.3	–
<i>Alyssum alyssoides</i>	6	39.2	2.62	2.17	7.70	1.81

^aDefined as (mean plant concentration)/(mean extractable soil concentration)

murale to accumulate Ni and Co has been studied in detail under controlled-environmental conditions, particularly in relation to tolerance to these respective metals (Tappero et al. 2007). These findings suggest exceptionally high uptakes of Ni and Co, but specialized biochemical processes linked with Ni high tolerance in *A. murale* do not confer high tolerance to Co. Malik et al. (2000) reported that *A. murale* hyperaccumulated Co from a low Ni sandy soil to which Co salt had been added. Nevertheless, in a greenhouse experiment with two Ni hyperaccumulator *A. murale* and *A. corsicum* grown on non-serpentine Ni-contaminated soils, showed that both species hyperaccumulated Ni, but not Co (Li et al. 2003).

The mean Ni concentrations of *A. murale* population from Dagestan were much lower than the Karachay-Cherkessia (Table 3). Thus, Ni concentrations of studied *A. murale* populations ranged from 20 to 12,000 mg kg⁻¹. According to this result, *A. murale* are classed as ‘facultative’ Ni hyperaccumulator. Analyses of the *A. murale* populations, which are found both on and off the soils derived from ultramafic rocks (Reeves et al. 1997) showed a range of Ni concentrations from < 30 mg kg⁻¹ to > 1000 mg kg⁻¹, according to the substrata. Kazakou et al. (2010) considered *A. lesbiacum* is as a ‘micro edaphic’ endemic Ni hyperaccumulator, since Ni hyperaccumulation varies considerably among different natural populations, depending on soil Ni concentration. Leaf analysis of the widespread *Rinorea bengalensis*, for example, includes 23 specimens showing Ni concentrations of 1000–17,750 mg kg⁻¹ and another 77 specimens with 1–300 mg kg⁻¹ according to sampling sites (from soils derived from ultramafic and non-ultramafic rocks, respectively),

and this species is hence a typical facultative hyperaccumulator (van der Ent et al. 2013).

In general, the limits of excessive and toxic Ni and Zn levels for most plant species range from 10 to 100 mg kg⁻¹ and from 100 to 400 mg kg⁻¹, respectively (Kabata-Pendias and Pendias 2001). Great Ni concentrations in the shoots of *A. murale* and Zn in *A. gehamense* are not accompanied by any toxicity symptoms and growth reduction. These plants are adapted to extreme concentrations of these metals in the soil and have a mechanism that allows them to bind the absorbed metals in inert compounds, avoiding damage to their own metabolism (Brooks, 1998; Olko et al. 2008).

In our earlier work (Drozdova et al. 2017) metalicolous population of *A. murale* from Karachay-Cherkessia under conditions of controlled-environment of sand culture, was studied. The tolerance of the seedlings of this species to Ni was examined by adding Ni sulphate to a nutrient solution with which the plants were watered. The experiment demonstrated that *A. murale* has an exceptional facility for uptake of and high tolerance to Ni. The plants in the high Ni growth medium accumulated the same aboveground biomass as the control plants, which was possible due to their preservation of a high level of hydration of the tissues, the content of photosynthetic pigments, and macronutrients. The experiment confirmed characteristic traits of *A. murale* such as hyper-tolerance to ions Ni, hyperaccumulation of this metal in the shoot, high bioconcentration factors and high shoot/root metal translocation. Thus, the ability to Ni accumulate and tolerance might be a constitutive property of this species.

character provides opportunities for further genetic and physiological dissection of the hyperaccumulation trait.

The genus *Alyssum* is a rather complex taxonomic formation with separate kinship groups. One is the monophyletic Odontarrhena section, which encompasses all the Ni accumulators, e.g. *A. murale* (Mengoni et al. 2003; Cecchi et al. 2010). According to our data, the Odontarrhena section also includes the *A. gehamense*, which is capable of high Zn accumulation (Table 3). Previously, the ability to accumulate high concentrations of other metals, in addition to Ni, in this section was not noted (Tappero et al. 2007; Verbruggen et al. 2009). More investigations of *A. gehamense*, which accumulate high metal concentrations, but at the same time not being a hyperaccumulator, can provide new molecular data of metal accumulation mechanisms in plants.

Conclusion

Our results indicated a high heterogeneity of all studied potentially toxic metal concentrations of North Caucasian soils and plants according to the type and degree of the substrata mineralized. There were close positive relationship between the concentrations of Zn, Ni and Co in the plants and in the soils in the available fraction, but not for Pb and Cu. The highest concentration of Ni- and Co and Zn had plants, as metallicolous population of *Alyssum murale* (up to 12,100 mg kg⁻¹) and *A. gehamense* (up to 1700 mg kg⁻¹), naturally growing on soils derived from ultramafic and mafic rocks, respectively. The concentrations of these metals in plant species, as *A. dagestanicum*, from non-metallicolous soils derived from calcareous rocks, was lowest.

Intra-specific variation in Ni-accumulating ability in *A. murale* was detected for Karachay-Cherkessia and Dagestan populations originating from different soil types. Nickel concentrations among these populations ranged in average from 20 to 6800 mg kg⁻¹. Hence, this species is a typical ‘facultative’ hyperaccumulator. The inter-population variations in Ni accumulation patterns in *A. murale* give support to the suggestion that the selection of metal hyperaccumulator species with enhanced phytoremediation efficiency should be considered at the population level. One of the potential directions of future research

is a search for correlations between the amounts of potentially toxic metals, that can accumulate and one or another *Alyssum* population, and its position in the phylogenetic system.

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