Variation of trace metal accumulation, major nutrient uptake and growth parameters and their correlations in 22 populations of *Noccaea caerulescens*

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Abstract

Background and aims Noccaea caerulescens is a model plant for the understanding of trace metal accumulation and a source of cultivars for phytoextraction. The aim of this study was to investigate natural variation for trace metal accumulation, major nutrient uptake and growth parameters in 22 populations. The correlations among these traits were particularly examined to better understand the eco-physiology and the phytoextraction potential of the species.

Methods Populations from three edaphic groups, i.e. calamine (CAL), serpentine (SERP) and non metalliferous (NMET) sites were grown in hydroponics for seven weeks at moderate trace metal exposure. Growth indicators, element contents and correlations between these variables were compared.

Results All the phenotypic characteristics showed a wide variability among groups and populations. The SERP populations showed a smaller plant size, higher cation contents and strong correlations between all element concentrations. NMET populations did not differ in plant size from the CAL ones, but had higher Zn and Ni contents. The CAL populations showed higher Cd and Mn accumulations and lower Ca contents. The trade-off between biomass production and Cd, Ni and Zn accumulation was high in SERP populations and low in the CAL and NMET ones.

Conclusions N. caerulescens is a genetically diverse species, showing specific features depending on the group and the population. These features may reflect the wide adaptive capacities of the species, and also reveal promising potential for phytoextraction of Cd, Ni and Zn.

Keywords Hyperaccumulation · Phenotyping · Genetic variability · Major element · Trace metal

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Introduction

Noccaea caerulescens (formerly *Thlaspi caerulescens*) is a pseudo-metallophyte growing both on unpolluted and trace metal enriched soils. This species is particularly known to accumulate some trace metals in its aboveground parts. These include zinc (Zn), cadmium (Cd) and nickel (Ni) (Meerts and Van Isacker 1997; Escarré et al. 2000; Escarré et al. 2013; Assunção et al. 2003; Visioli et al. 2011). In situ, the plant can

concentrate in its shoots up to 53,450 mg Zn kg^{-1}, 2,890 mg Cd kg^{-1} and 12,880 mg Ni kg^{-1} (Reeves et al. 2001). Previous studies have shown that this wild species has a good potential for remediation, through phytoextraction, of soils moderately contaminated with Cd (Schwartz et al. 2003; Zhao et al. 2003; Maxted et al. 2007; Koopmans et al. 2008). Feasible trace metal phytoextraction implies simultaneous improvement of several plant characteristics, such as metal uptake, biomass production and growth speed. The achievement of phytoextraction may thus imply genetic engineering, necessarily based on existing variation for parameters of interest in candidate species. To date, natural variation of Zn, Cd or Ni hyperaccumulation has been largely investigated in *N. caerulescens* (Meerts and Van Isacker 1997; Escarré et al. 2000; Escarré et al. 2013; Assunção et al. 2003; Keller et al. 2006; Visioli et al. 2011). In the field, the various studied populations have shown contrasting accumulation abilities, some of them being able to hyperaccumulate Cd, Ni and Zn in their shoots at higher concentrations than the others (Reeves et al. 2001; Molitor et al. 2005; Basic et al. 2006). In general, the highest concentrations of Cd and Zn have been measured in calamine sites and those of Ni in serpentine ones (Reeves et al. 2001; Chardot et al. 2007), leading to the idea that hyperaccumulation of trace elements could be mainly attributed to their high bioavailability in soils. However, when cultivated in controlled conditions, the calamine populations of *N. caerulescens* exhibited a lower ability to hyperaccumulate Zn in their shoots than the non-metallicolous ones (Escarré et al. 2000; Escarré et al. 2013). For Cd the reverse was found, with the calamine populations exhibiting the highest concentrations (Escarré et al. 2000), except in Belgian calamine populations which show no group difference with non-metallicolous populations from Luxembourg (Dechamps et al. 2005).

Although the exploration of natural variation of trace metal hyperaccumulation provides interesting phenotypic data, the underlying genetic mechanisms are still far from being uncovered, as hyperaccumulation would be a multigenic process (Clemens 2001; Verbruggen et al. 2009; Hanikenne and Nouet 2011), involving numerous genes from the homeostasis network (Van de Mortel et al. 2006; Van de Mortel et al. 2008; Halimaa et al. 2014; Milner et al. 2014). This renders highly improbable, on the medium term, the creation of transgenic cultivars for phytoextraction, for example by introducing Cd hyperaccumulation ability into a high dry mass yield

crop. That is why it seems more reasonable in a first phase to investigate natural variations both for trace metal accumulation and for nutritional and growth parameters, in order to exploit original genetic combinations potentially created in the wild. Therefore, what now appears essential is to estimate on a large scale the natural variability not only of trace metal accumulation, nutrient uptake, or plant growth taken separately, but also of the correlations between these parameters. A trade-off between plant dry biomass and Zn hyperaccumulation has already been demonstrated in some populations of *N. caerulescens* from Southern France, Belgium and Luxembourg (Dechamps et al. 2005; Escarré et al. 2000; Meerts and Van Isacker 1997). In these studies, metallicolous populations displayed higher plant biomass and lower Zn hyperaccumulation levels than non-metallicolous populations cultivated on metal-contaminated soils. On the contrary, a positive correlation between Cd hyperaccumulation and plant dry biomass was evidenced by Escarré et al. (2000), since metallicolous populations had higher Cd hyperaccumulation than non-metallicolous ones. Using F1 progenies within the same edaphic origin (i.e. among metallicolous or among non-metallicolous populations) or among different edaphic origins, Frérot et al. (2005) showed that a negative relationship between biomass production and Zn hyperaccumulation clearly appeared when plants expressed high levels of hyperaccumulation, that is to say, mostly among progenies from non-metallicolous origin. Therefore, the correlation between growth parameters and hyperaccumulation seems to be origin and metal-dependent, even metal concentration-dependent. This suggests the possibility of a large natural variation for correlation among these traits, to be exploited for agronomic purposes. On the other hand, very few studies reported the correlation between plant growth, metal hyperaccumulation and plant nutritional status. Meerts and Van Isacker (1997) estimated calcium, phosphorus, magnesium, and potassium shoot concentrations, and found no clear correlation between foliar concentrations of these elements and plant growth, but a highly positive correlation between Mg and Zn accumulation in non-metallicolous populations of *N. caerulescens*. In the present study, we investigated all the possible correlations among several nutritional and growth parameters in a large sampling of population to better understand eco-physiology and phytoextraction potential of the species. In Western Europe, and particularly in France, *N. caerulescens* is present in numerous

locations both on metalliferous and non-metalliferous sites (Basic et al. 2006; Molitor et al. 2005; Peer et al. 2006; Reeves et al. 2001). The various populations of *N. caerulescens* are usually classified into three main groups, corresponding to the three contrasting edaphic environments in which they live. Two edaphic groups from metalliferous soils can be distinguished. The calamine group (hereafter CAL) is composed of populations which grow in soils highly enriched with Zn, Cd, and lead (Pb), such as those of mining or smelting sites (Reeves et al. 2001; Escarré et al. 2011). These plants tolerate high amounts of Zn and Cd and hyperaccumulate these metals. The serpentine group (hereafter SERP) corresponds to populations which seem to be adapted to soils naturally enriched with Ni, such as those originating from ultramafic rocks, i.e. mainly rocks with serpentine in Western Europe. These populations are known to tolerate and accumulate higher amounts of Ni than the others (Assunção et al. 2003; Escarré et al. 2013). The last edaphic group corresponds to the populations developing in non-metalliferous soils (hereafter NMET), which have been generally found in mountain areas such as the Ardennes, Jura, Alps and Cévennes (Reeves et al. 2001; Molitor et al. 2005; Basic et al. 2006). However, some NMET populations also develop in the Vosges and Massif Central mountains, but these have yet to be investigated.

This is why this work was designed to characterize the populations of the three groups of *N. caerulescens* on a wider scale than previously undertaken. We assessed for the first time nine new NMET populations of *N. caerulescens* from the Massif Central mountains and one new SERP population from the Alps, thus extending knowledge about the species' abilities to accumulate trace metal and to grow in trace metal-contaminated environments. In particular, our main objectives are:

- To assess phenotypic variability for growth parameters, major nutrient uptake and trace metal accumulation among edaphic groups, among populations and within populations in controlled conditions, on a large number of populations (22) exposed to moderate metal concentrations,
- To search for significant correlations among nutritional traits, among growth traits, and between these two categories of traits,
- To detect those populations with a highest potential for use in Cd phytoextraction.

Materials and methods

Plant material

During the summer of 2011, seeds from three maternal plants (=family) of 22 populations of *N. caerulescens*, were collected by cutting the base of the inflorescence. Seeds were first stored in paper bags at room temperature for 2 months and then at 4 °C. The accessions studied included nine calamine (CAL) and 10 non-metallicolous (NMET) populations from the Massif Central (9) and Luxembourg (1) and three serpentine (SERP) populations from the Massif Central, the Vosges and the Alps (Table 1). Two species were used as controls, namely *Arabidopsis thaliana* (Col-0) and a population of *Noccaea montana* from an ultramafic soil in the Massif Central. *A. thaliana* was chosen because its ionic composition has been extensively studied (e.g. McDowell et al. 2013). *N. montana* was chosen because it belongs to the *Noccaea* genus as a non hyperaccumulating species (mean Ni concentration in shoots in situ: 334 mg kg^{-1}, standard deviation: 226 mg kg^{-1}; C. Gonneau, unpublished data). These controls allowed the toxicity of the nutrient solution to be tested and the hyperaccumulating status of the individuals in the studied populations to be assessed.

Plant cultivation

For each *Noccaea ssp* population, twelve plants (4 plants x 3 families) were randomly distributed in 12 polyethylene trays (400×300×220 mm) so that each tray contained 24 plants (i.e. one plant per population). They were grown hydroponically in a growth chamber for 49 days, each tray containing 17 L of nutrient solution. In each tray, a 2 cm thick polystyrene plate, floating on the nutrient solution was used to support the plants. Each plate contained 24 conical holes filled with 7 gL^{-1} agar as a stand for the seeds and the young seedlings after germination.

The composition of the nutrient solution was adapted from Küpper et al. (2007): 1 mM KNO$_3$, 1 mM Ca (NO$_3$)$_2$, 0.5 mM MgSO$_4$, 0.1 mM KH$_2$PO$_4$, 50 μM KCl, 20 μM NaFe (III) EDTA, 10 μM H$_3$BO$_3$, 10 μM ZnSO$_4$, 1 μM Cd (NO$_3$)$_2$, 1 μM MnCl$_2$, 0.7 μM NiSO$_4$, 0.2 μM Na$_2$MoO$_4$, 0.2 μM CuSO$_4$ in deionized water. The nutrient solution also contained 2 mM 2-(N-morpholino) ethanesulfonic acid (MES) to buffer the pH at 5.5, which was adjusted through the addition of

Table 1 Location and coding of the 22 *Noccaea caerulescens* populations and the two control species (*Arabidopsis thaliana* and *N. montana*) used in the experiments

Species	Edaphic group	Department (except for Luxembourg)	Location	Altitude (m)	Coordinate	Abbreviation
Noccaea caerulescens	CAL	Saône-et-Loire	Auxy	500	46°57′44.13″N 04°23′47.05″E	AUX
		Southern Ardèche	Largentière	264	44°32′26.33″N 04°18′18.44″E	LAR
		Southern Ardèche	Sainte Marguerite	741	44°27′29.13″N 04°00′31.32″E	SML
		Lozère	Ramponenche	698	44°20′15.85″N 03°40′00.31″E	RAM
		Gard	Saint Felix	349	44°02′36.21″N 03°56′11.27″E	SFP
		Gard	Durfort	195	43°59′56.70″N 03°57′08.54″E	DUR
		Gard	Malines	436	43°55′20.67″N 03°37′14.57″E	MAL
		Gard	Ganges	175	43°56′10.98″N 03°40′19.88″E	GA
		Aveyron	Viviez	202	44°33′34.16″N 02°13′01.39″E	VIV
	NMET	Southern Ardèche	Pic de Chenavari	460	44°35′58.80″N 04°41′04.20″E	PC
		Northern Ardèche	Mezilhac	1118	44°48′28.00″N 04°20′44.80″E	MEZ
		Northern Ardèche	Sainte Eulalie	1270	44°48′13.10″N 04°12′47.10″E	SEU
		Haute-loire	L'Herm	1111	44°54′24.20″N 03°49′10.10″E	HER
		Cantal	Nouvialle	1016	45°02′49.00″N 02°56′45.40″E	NOU
		Cantal	Salesses	1132	45°17′44.80″N 02°51′35.20″E	SAL
		Puy-de-Dôme	Chavignée	954	45°23′48.30″N 02°43′59.00″E	CHA
		Puy-de-Dôme	Besse	1036	45°30′25.50″N 02°56′26.30″E	BES
		Puy-de-Dôme	Laschamp	964	45°44′36.00″N 02°58′08.90″E	LAS
		Wiltz (Luxembourg)	Winseler	325	49°57′N 5°53′E	WIN
	SERP	Vosges	Bergenbach	810	47°54′22.90″N 06°57′25.50″E	BER
		Aveyron	Puy de Wolf	472	44°33′23.80″N 02°18′21.07″E	PW
		Hautes-Alpes	Montgenèvre	1860	44°55′33.49″N 06°43′08.02″E	MON
Arabidopsis thaliana	Controls	-	-	-	-	At
Noccaea montana		Northern Ardèche	Clava	586	45°18′39.10″N 04°38′46.00″E	Nm

CAL, Calamine; *NMET*, non-metalliferous; *SERP*, serpentine

20 ml of 0.34 mM KOH. The Cd concentration (1 μM) can be regarded as moderate, although it is higher than the majority of the concentrations of contaminated soil solutions which are generally around 0.1 μM. However, Cd concentrations above 4 μM can be found in highly contaminated soils (Maxted et al. 2007). In addition, the value of 1 μM is a bit above the *Km* value determined through Cd absorption kinetics with *N. caerulescens* (Redjala et al. 2009). The nutrient solution might therefore enable a high (even close to the maximum) root influx, without excessive root exposure and toxicity for the plant.

Six capillary tubes were placed at regular intervals through each tray and sunk into the nutrient solution to allow its oxygenation by bubbling with filtered air. The nutrient solutions were renewed once a week at the beginning of the cultivation, then twice a week. Cadmium concentration in the solution was monitored by sampling and analysing the nutrient solution before and after its renewal (Online Resource, Fig. S1). The photoperiod was set at 16 h day and 8 h night, with a light intensity of 196 μmol photon m^{-2} s^{-1}. The temperature was maintained at 23 °C during the day and 18 °C during the night and the relative humidity was set at 70 %. Twice a week, the trays were translated to the neighbouring position and individually rotated at 180°, in such a way that each of them thus held at least once, each of the positions available. This was done to reduce the effect of environmental differences that may exist in different points of the growth chamber.

Harvest and analyses

At the end of the cultivation, namely 49 days after germination for each plant, the roots were separated from the leaves at the crown. Leaf surface area (LA, cm^2) was measured by scanning using WinRhizo® software. The total number of leaves (NL) and the largest diameter of the rosette (RD, mm) were also measured. Root and leaf dry biomass (RDM and LDM respectively, g) were weighed after drying for 24 h at 70 °C.

The plant material was finely ground using an agate mortar before successive digestion of 0.5 g with 8 ml 65 % HNO_3 for 12 h and 4 ml 30 % H_2O_2 during 3.5 h at 95 °C using the DigiPREP® system (SCP Science, Baie-d'Urfé, QC, Canada). After filtration of the extract at 0.45 μm, the samples were adjusted to 25 mL with ultrapure water (18 MΩ).

Major elements (Ca, Fe, K, Mg, Na) and minor elements (Cd, Cu, Mn, Ni and Zn) in the nutrient solution and plant extracts were determined by induced coupled plasma emission spectrometry (ICP-AES, iCAP 6000 Series, Thermo Scientific, Cambridge, UK). Control material from N. caerulescens with known compositions (internal analyses carried out by INRA-USRAVE, Villenave d'Ornon, France), as well as a certified solution (EU-H₂, SCP Science, Courtaboeuf, France) were included in all analyses for quantitative verification of the results. All elements concentrations were expressed in mmol kg^{-1}.

Data processing

The translocation factor was calculated as $[Element]_{shoot}/[Element]_{root}$. The allocation factor corresponded to the ratio of the element quantity in the shoot to its quantity in root of each plant: $([Element]_{shoot} \times LDM)/([Element]_{root} \times RDM)$.

All statistical analyses were performed using R v2.10.14. Before analysis, all data were log-transformed in order to improve normality and variance homogeneity of residues. Variance analyses for biomass production and element content in N. caerulescens were analysed by a hierarchical mixed model analysis using the lmer function inside the lme4 package. For this analysis, the edaphic group was treated as a fixed factor, whereas the population nested within the group and the family nested within the population nested within the group were treated as random factors. The significance of the fixed effect was determined using type III F-statistics. Log-likelihood

ratios corresponding to comparisons between the likelihoods of the models with and without the random effects were calculated. For significance tests of random effects, log-likelihood ratios were compared to a χ^2 distribution. Then, within each edaphic group, variance components were estimated for each random factor using the restricted maximum likelihood (REML) method. Multiple correlation tests were performed using the Pearson method and FDR control for type I errors.

Results

Growth parameters and their correlations

At the end of cultivation, that is 49 days after seed germination, N. caerulescens and N. montana were at the rosette stage whereas A. thaliana was at the flowering stage which started after 20 days of cultivation. No plants presented any symptoms of toxicity (e.g. chlorosis). Mean values and variation coefficients for LDM, RDM, LDM/RDM, LA, LN and RD at the population level are given in Table S1 (Online Resource).

Only LN and the LDM/RDM ratio were not significantly different between groups (Table 2). Considering LDM and RDM, mean values showed no significant differences between CAL and NMET, whereas SERP exhibited significantly lower mean values by a factor from 1.5 to 1.9 (Fig. 1; Table S1 in Online Resource). As for dry mass, CAL and NMET showed similar mean LN and LA. Unlike LN, LA was significantly lower in SERP populations. Finally, mean RD per group varied from 93.4 mm to 132 mm and was significantly different between groups in the order CAL > NMET > SERP. Among populations within groups, the variation factors for both LDM and RDM reached 2.5, 3 and 2 for CAL, NMET and SERP respectively (Online Resource, Table S1). Among individuals, LDM and RDM ranged respectively from 32 to 860 mg and from 10 to 873 mg. The highest and lowest total biomasses were found respectively in a non metallicolous (BES) population and a serpentine (MON) one (Fig. 1). Populations of LAR and VIV showed the highest LDM and RDM among calamine populations (Fig. 1). Within the CAL and NMET groups, a factor of two was found between maximum and minimum values for LDM/RDM.

Most of the biomass parameters were significantly positively correlated ($p=0.05$). In order to describe the strongest correlations, only |r| values above 0.57 were

Table 2 Analyses of variance for growth parameters accounted for edaphic group (group), population within group (pop (group)) and family within population within group (fam {pop (group)})

Source	ddl	LDM[a]	RDM[b]	LDM/RDM	LA[c]	LN[d]	RD[e]
group	2	3.49*	7.97***	0.58	3.45*	1.12	4.49*
pop (group)	19	69.1 ***	8.48*	4.50	42.4***	9.50**	10.4**
fam {pop (group)} residues	44	32.3 ***	6.61*	1.78	70.7***	40.2***	35.1***
	187						

[a] Leaf dry matter (g); [b] Root dry matter (g); [c] Leaf area (cm²); [d] Number of leaves from the rosette; [e] Diameter of the rosette (mm)

The table shows test statistics with F-ratios for fixed effects and $\chi 2$ for random effects. Group is considered as a fixed factor, whereas pop (group) and fam {pop (group)} are random factors. Significant level: *$p<0.05$; **$p<0.01$; ****$p<0.001$

taken into account. On this basis, Table S2 (Online Resource) shows very strong positive correlations between LDM and LA ($r>0.9$), between RD and LA ($0.63>r>0.76$), and between LDM and RD ($0.59<r<0.73$) whatever the group considered. The LDM/RDM ratio was also strongly negatively correlated ($r<-0.62$) to RDM for NMET and SERP. Lastly, some correlations were observed only in the NMET group, namely RDM, which was positively correlated to LDM and LA ($r=0.66$).

Major element contents and their correlations

Only Ca and Na contents in shoots showed some significant differences between groups (Table 3; Online Resource, Table S3). Indeed, the CAL group had a significantly lower mean shoot concentration and translocation factor of Ca than the NMET and SERP ones, the highest values being observed for the SERP group. A quite similar behaviour was found for Na with the CAL and NMET groups showing significantly lower mean shoot concentrations and translocation factors than the SERP one. At the population level, there were significant differences for all major elements when considering their concentrations in shoots and their translocation factors, except for the shoot Ca concentration (Table 3). However in roots, the population effect was significant only for K. At this level, the variation coefficients for major element concentrations in shoots and in roots were similar for a given element, but differed between elements. These coefficients varied from 7.3 % for Ca to 149.0 % for Na (Online Resource, Table S3). The MON serpentine population clearly showed higher concentrations in major elements than the other populations did, with however a high variation coefficient (Online Resource, Table S3). The family factor caused significant differences in shoots for Ca, Fe, K and Mg but none in roots. The translocation factor of Ca only was significant at the family level (Table 3). In each edaphic group, most of the phenotypic variance was residual (from 36.9 to 85.7 %), particularly

Fig. 1 Roots (*grey bars*) and shoot (*white bars*) mean biomass in 22 populations of *Noccaea caerulescens* after 49 days of hydroponic cultivation. Mean per edaphic group (CAL: calamine; NMET: non metalliferous; SERP: serpentine). *Vertical bars* represent standard errors. For population code, see Table 1
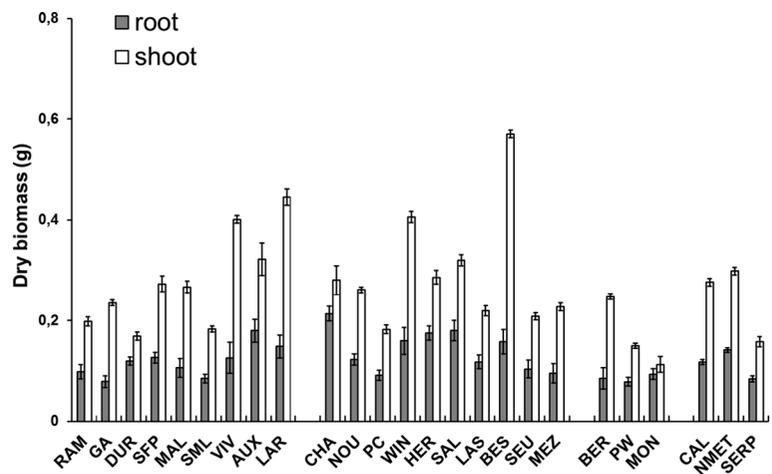
Table 3 Analyses of variance for element concentrations in shoot, in roots and translocation factor accounted for edaphic group (group), population within group (pop (group)) and family within population within group (fam {pop (group)})

Source	ddl	Major elements					Trace elements				
		Ca	Fe	K	Mg	Na	Cd	Cu	Mn	Ni	Zn
Shoot											
group	2	5.00**	1.19	0.839	0.516	4.12*	9.61***	5.31*	20.0***	4.97*	7.65***
pop (group)	19	10.6**	22.1***	62.4***	37.6***	19.0***	114***	61.9***	98.9***	154***	40.1***
fam {pop (group)}	44	5.03*	10.2**	14.2***	11.9***	0.317	10.1**	24.7***	13.5***	28.9***	20.0***
residues	187										
Roots											
group	2	0.318	0.202	0.315	0.401	0.384	7.69***	3.14*	4.60*	3.19*	4.43*
pop (group)	19	0	0.478	3.51*	0.044	0.081	19.9***	7.96*	24.8***	41.4***	60.6***
fam {pop (group)}	44	0	0.485	0.03	0.042	0.071	9.87**	0.298	0	6.34*	2.29
residues	187										
Translocation factor											
group	2	1.38	0.405	0.740	0.584	1.80	1.37	5.68**	2.22	3.14*	6.95**
pop (group)	19	49.3***	3.67*	5.08*	3.79*	6.64*	43.7***	19.2***	11.1**	24.7***	68.5***
fam {pop (group)}	44	44.4***	0	1.49	0.514	2.37	17.9***	0.039	1.10	0.072	2.25
residues	187										

The table shows test statistics with F-ratios for fixed effects and $\chi 2$ for random effects. Group is considered as a fixed factor, whereas pop (group) and fam {pop (group)} are random factors. Significant level: $*p<0.05$; $**p<0.01$; $****p<0.001$

in the SERP group (Table 4). In CAL and NMET groups, the population factor explained 40.3 and 44.4 % of the variability for K, respectively, and 48.7 and 20.1 % for Mg. The family factor was mainly explicative only for

Mg in the NMET group (41 %). All major elements, except Na, were positively correlated to each other ($p=0.05$, Table S2 in Online Resource). As previously indicated, to better describe the correlations between the

Table 4 Percentage of total variance of major and trace elements concentrations in shoots for each edaphic group accounted for population within population (pop) and family within population (fam (pop))

Edaphic group	Source	Major elements					Trace elements				
		Ca	Fe	K	Mg	Na	Cd	Cu	Mn	Ni	Zn
CAL	pop	31.0	14.2	40.3	48.7	0	42.3	12.0	44.3	0	1.1
	fam (pop)	11.4	22.6	12.0	0	17.2	8.1	29.7	21.0	41.3	62.1
	residues	57.6	63.1	47.7	51.3	82.8	49.6	58.2	34.7	58.7	36.8
NMET	pop	10.6	22.2	44.4	20.1	25.9	63.9	39.2	41.1	76.9	41.0
	fam (pop)	17.6	3.1	18.7	41.0	0	8.6	15.9	9.9	11.4	0.1
	residues	71.8	74.7	36.9	38.6	74.1	27.5	44.8	49.0	11.7	52.9
SERP	pop	0	0	0	0	25.0	14.3	0	22.0	32.4	0
	fam (pop)	14.3	38.5	29.0	30.1	16.3	27.3	44.4	22.0	6.13	16.0
	residues	85.7	61.5	71.0	69.9	58.7	58.3	55.6	56.0	61.4	84.0

major elements in *N. caerulescens*, only |r| values above 0.57 have been considered. On this basis, Table S2 (Online Resource) showed an increasing number of significant correlations in the order NMET (1:2:2) < CAL (1:5:1) < SERP (9:6:4), with values in parentheses corresponding respectively to the number of correlations for the concentrations of major elements in shoots and in roots and for the translocation factors (shoot/root). The most significant correlations were thus found in roots of the CAL group and in shoots of the SERP group. Moreover, the strength of the correlations decreased in the order SERP > CAL > NMET. For instance the correlation between Ca and Mg was the only one found for all groups and all parameters with the correlation coefficient ranges: 0.75-0.95 for SERP, 0.81-0.83 for CAL and 0.57-0.80 for NMET.

Trace element contents and their correlations

The mean shoot concentrations of Cd exceeded the hyperaccumulation threshold (Van der Ent et al. 2013) in all *N. caerulescens* populations and in *A. thaliana*. Significant differences were observed between groups with increasing mean Cd concentrations as follows (Fig. 2, Table 3, Table S4 in Online Resource): SERP (4.4 mmol kg^{-1}) < NMET (5.7 mmol kg^{-1}) < CAL (11.5 mmol kg^{-1}). A similar pattern was observed for Cd concentrations in roots. Furthermore, Cd concentrations in shoots appeared as a continuum among populations, with six populations from the NMET group showing Cd concentrations close to those of the three lowest CAL populations (Cd range: 6 to 9 mmol kg^{-1}, Fig. 2). The variation factors for Cd concentrations in shoots reached 6.4 among populations and 2.2, 3.2 and 1.4 within groups for CAL, NMET and SERP respectively. The population factor mainly explained a large part of the total variance with 42.3, 63.9 % within CAL and NMET groups respectively, but only 14.3 % in SERP groups since most of the variance was residual in this group (Table 4).

With 0.113 mmol kg^{-1} on average (Online Resource, Table S4), Cu was not accumulated in the shoots of *N. caerulescens*. Considering the new threshold value for Cu hyperaccumulation (4.72 mmol kg^{-1}; Van der Ent et al. 2013), all populations had Cu concentrations in shoots from 30 to 67 times below this level. However, significant differences were found at the group level, the NMET group having Cu concentrations lower in shoots but higher in roots, than the two others (Table 3, Table S4 in Online Resource). For this element, a large part of total

variance was residual whatever the group (58.2, 44.8 and 55.6 % for CAL, NMET and SERP respectively). In SERP group, 44.4 % of total variance was nevertheless attributed to families within population, with no variance left for the population factor. Manganese concentrations in shoots and roots showed the same pattern as Cd (Fig. 2, Table S4 in Online Resource). The Mn concentrations in shoots were more than twice as high in the CAL group (4.26 mmol kg^{-1}) than in the NMET (1.53 mmol kg^{-1}) and SERP (2.06 mmol kg^{-1}). The variation factor for Mn concentrations in shoots was similar to that observed for Cd. It reached 6.4 among populations and about 2 within the group, whatever the group considered. Concerning the control *A. thaliana*, Mn shoot concentrations fell between those of CAL and NMET (Fig. 2). For Mn, most of the variance was attributed to populations (from 22.0 to 44.3 %) and/or to residues (from 34.7 to 56.0 %) whatever the group.

No *N. caerulescens* population reached the hyperaccumulation threshold for Ni (17 mmol kg^{-1}) with an average of 1.73 mmol kg^{-1} in the shoots. Significant differences were observed at the group level (Table 3), with increasing Ni concentrations in shoots as follows: CAL (1.13 mmol kg^{-1}) < SERP (1.86 mmol kg^{-1}) ≤ NMET (2.25 mmol kg^{-1}). Thus, three NMET populations showed higher Ni shoot concentrations than PW, a typical SERP population (Fig. 2, Table S4 in Online Resource). Some CAL and other NMET populations also showed similar Ni concentrations to the two SERP populations BER and MON. In the roots, the concentrations of Ni were relatively close for the three groups. However, Ni root concentration was higher for NMET than that of SERP, the CAL group being intermediate (Online Resource, Table S4). For this element, phenotypic variance was mainly residual in CAL and SERP groups (58.7 and 61.4 %, respectively), but was largely due to populations in NMET group (76.9 %).

For Zn concentrations in shoots, twelve populations exceeded the hyperaccumulation threshold (46 mmol kg^{-1}), including nine NMET and two SERP (BER and MON) populations (Fig. 2, Table S4 in Online Resource). Significant differences were observed between groups (Table 3) with increasing mean Zn concentrations in shoots as follows: CAL (38.2 mmol kg^{-1}) < SERP (55.5 mmol kg^{-1}) < NMET (63.7 mmol kg^{-1}). An opposite trend was observed for Zn concentrations in roots, the CAL group having a significantly higher value than those of NMET and SERP, which were close together (Online Resource, Table S4). Phenotypic variance was mainly
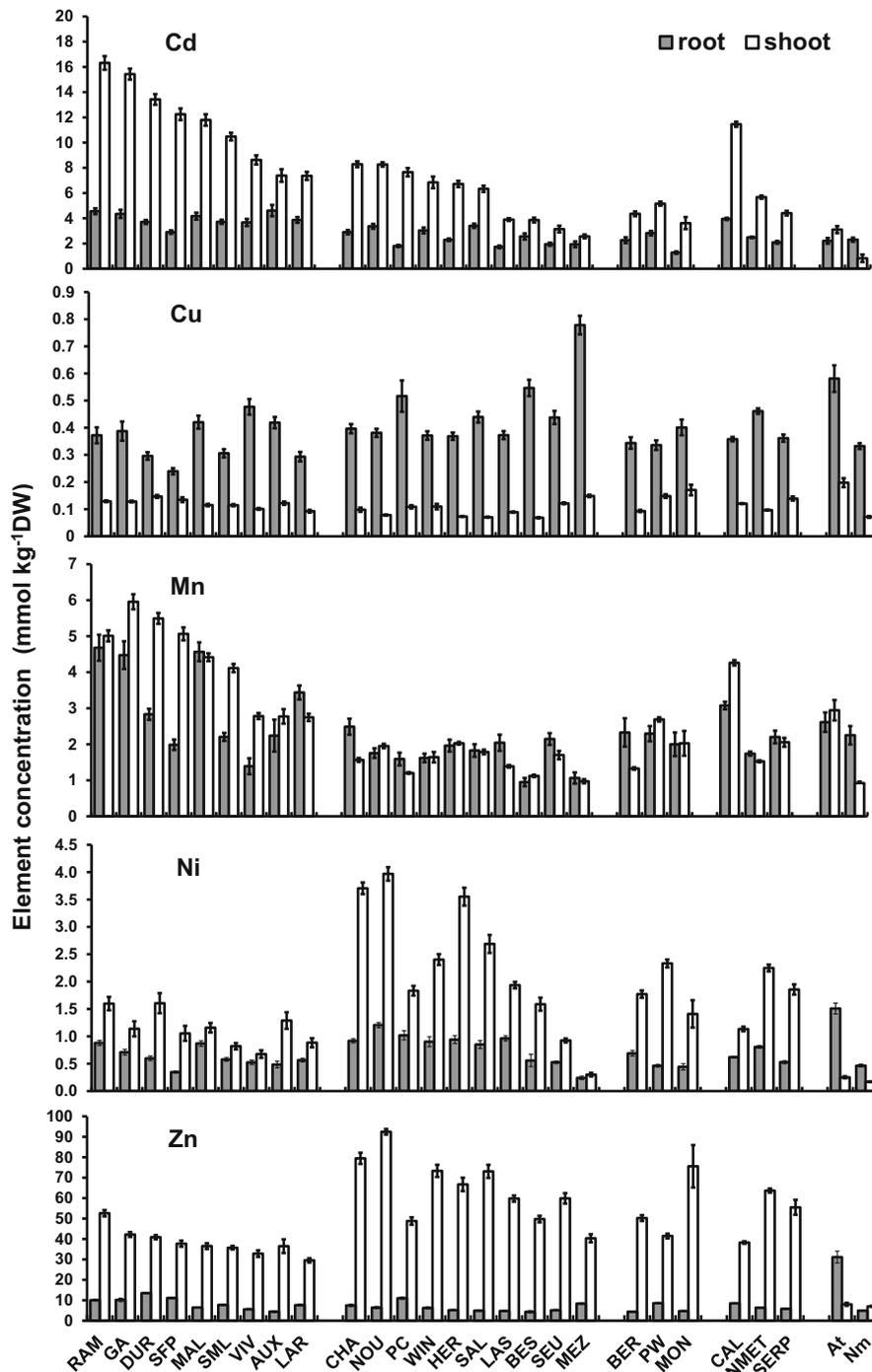
Fig. 2 Cadmium, Cu, Mn, Ni and Zn mean concentrations in roots (*grey bars*) and shoot (*white bars*) in 22 populations of *Noccaea caerulescens* after 49 days of hydroponic cultivation. Mean per edaphic group (*CAL*, calamine; *NMET*, non metalliferous; *SERP*: serpentine) and control plant (At: *Arabidopsis thaliana* Col-0; Nm: *N. montana*). *Vertical bars* represent standard errors. For population code, see Table 1

explained by the population factor for the NMET group (41.0 %), whereas it was largely explained by families

within population for the CAL group (62.1 %). For SERP group, most of the variance was residual (84.0 %).

All *N. caerulescens* populations presented Cd, Ni and Zn translocation factors greater than 1, as is consistent with one of the hyperaccumulation criteria (Fig. 2, Table S4 in Online Resource). All populations combined, the mean values reached 3 for Cd and Ni and 13 for Zn. For Cu, an inverse pattern was found with concentrations in roots about three times higher than those in shoots, whereas concentrations in shoots and in roots were close for Mn. Even if *A. thaliana* is not a hyperaccumulator, it exhibited a translocation factor of 2.29 for Cd and < 1 for Zn. Inversely, *N. montana* showed a translocation factor <1 for Cd and of 1.60 for Zn. At the group level, the translocation factors of Cd, Mn and Zn were significantly greater in CAL than in NMET and similar to SERP (Online Resource, Table S4). For Ni, the translocation factor significantly increased from 2.1 to 5.9 in the order CAL < NMET < SERP. In the case of Cu, the lowest translocation factor was found in NMET, while it was similar for CAL and SERP.

Correlations between trace elements varied widely between groups (Online Resource, Table S2). When considering only |r| values above 0.57, there was an increasing number of significant correlations in the order NMET (4:0:0) < CAL (5:0:0) < SERP (9:9:1), with values in parentheses corresponding respectively to the number of correlations for the concentrations of trace elements in shoots and in roots and for the translocation factors. In contrast to major elements, the most significant correlations were always found in shoots, whatever the group. Moreover, as observed with the major elements, the strength of the correlations was higher in the SERP group. This group had a particular behaviour, as all the elements were strongly correlated both in the shoots and in the roots. In the shoots, three strong positive correlations were found for all groups: Cd/Mn (0.56<*r*<0.86), Mn/Ni (0.58<*r*<0.88) and Ni/Zn (0.63 <*r*<0.69). Three other weaker correlations were also outstanding: Cd/Ni (0.46<*r*<0.85), Cd/Zn (0.50<*r*< 0.81) and Mn/Zn (0.41<*r*<0.77).

Ratios between elements in shoots presented high coefficients of variation at both group (from 37.9 to 47.3 %) and population levels (from 9.3 to 127.0 %; Online Resource, Table S5). All *N. caerulescens* plants showed higher average trace element ratios in shoots than those of the hydroponic solution, except for Zn/Cd ratio which was the same in shoot as in the nutrient solution (Online Resource, Table S5). The CAL group showed lower Zn/Cd and Zn/Mn ratios and higher Cd/ Ni and Mn/Ni ratios (by a factor of about four) than

SERP and NMET groups, which presented similar values. Only Cd/Mn and Ni/Zn ratios did not vary between groups.

Correlations between growth parameters and element contents

In the principal component analysis performed on all the plant parameters, the first axis explained 23.1 % of the variation; it was positively correlated to Cd and Mn concentrations in shoots and negatively to Zn and Na concentrations in shoots (Fig. 3a). This axis allowed separating populations into two sets: all CAL populations had positive values, whereas all NMET + SERP populations all had negative values (Fig. 3b). The second axis, which opposed dry mass production to major element concentrations (in particular shoot Na and Mg concentrations), explained 19.3 % of the variance (Fig. 3a), and mostly discriminated the MON population from the others due to its low dry mass production. In general, the interpretation of these two first axes showed that growth parameters were negatively correlated to major element uptake but mostly uncorrelated to trace element accumulation. The third axis explained 14.2 % of the variance (data not shown) and was linked to Ni concentrations in shoots and roots. This last axis did not discriminate any set of populations.

When considering the relationships between trace and major elements in the shoots and the biomass production, a general trend in negative correlations was observed, whatever the element and the growth parameter considered (Online Resource, Table S2 and Fig. S2). Although mostly weak, the strength of the correlations was higher for major elements than for trace elements. Only K in CAL and NMET groups and Cu in SERP group were strongly correlated with LDM (|r|>0.57). In the roots, the significant correlations between dry mass and element content were also negative. They were stronger in the roots of SERP than in those of the two other groups (Online Resource, Table S2).

Correlations between major and trace elements

The two first axes of the PCA showed that accumulation of major and trace elements were mostly uncorrelated traits (Fig. 3a). However, when looking into detail, these correlations seemed to depend on the group and the plant part (Online Resource, Table S2). As a general trend, a decrease in the strength of correlations was observed
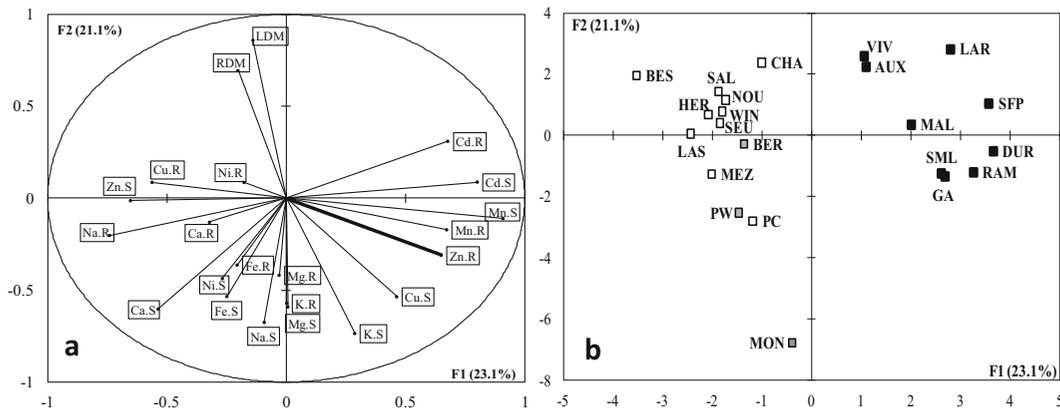
Fig. 3 Principal component analysis on all data including biomass production, elemental concentrations in shoots and in roots: **a** Correlation circle on the axis F1 and F2; **b** distribution of the population according to the correlation circle on axis F1 and F2 with the 22 *Noccaea caerulescens* populations. For population code, see Table 1

whatever the element considered both at the group (SERP > CAL > NMET) and the plant part (shoots > roots) levels. In the SERP group, the trace element concentrations in shoots were strongly positively correlated (i.e. $|r| > 0.57$) with those of all the measured major elements except Na. In the CAL group, trace elements (Cd, Cu, Ni, Zn) concentrations in shoots were significantly, positively but weakly correlated with those of Ca, K and Mg (Online Resource, Table S2). Only three close correlations (i.e. $|r| > 0.57$) were found in CAL shoots: Mn/Ca, Cu/Fe and Mn/K. In NMET, the only relevant correlation was Zn/Ca. Furthermore, even if the correlations were weak, Fe and Na appeared negatively correlated with Cd, Mn, Ni and Zn, but positively correlated with Cu in the shoots of NMET and CAL groups. A similar pattern was observed in the roots.

Sum and proportions of cations

Sum of cation concentrations in whole plants differed between groups, populations and families and also between the three species (Table 5, Table S6 in Online Resource). Plants from the SERP populations showed a higher cation content than the other two groups, which were not significantly different on this point. The same ranking applied when comparing the major cation contents, while the NMET population appeared to accumulate more trace cations than CAL populations, the SERP ones being intermediate (Online Resource, Table S6).

Major elements represented from 97.5 to 98.0 % of the whole plant cation content in the three edaphic groups. In the shoots, values were slightly lower (96.4 to 97.1 %), because of much lower Fe concentrations than in the roots. The variation of the Ca proportion was compensated by that of K, the proportions of the other major cations (Fe, Mg, Na) being only slightly variable between groups. This was particularly visible in the CAL group, which had the lowest Ca proportion (Online Resource, Table S7).

The proportion of trace elements was very low compared to that of the major elements, as it varied between 1.97 % (SERP) to 2.55 % (NMET) for the whole plant. However, it was higher in *N. caerulescens* than in *A. thaliana* (1.05 %) and *N. montana* (0.66 %).

Quantity of trace elements taken up

The quantities of accumulated Cd, Cu and Mn (concentration x dry mass) decreased significantly in the order

Table 5 Analysis of variance of the sum of cations and percentage of total variance accounted for by sink, edaphic group (group), population within group (pop (group)) and family within population within group (fam {pop (group)})

Source	Sum of cations	Sum of major cations	Sum of trace cations
group	3.28*	4.08*	9.36
pop (eco)	14.3***	13.7**	48.3***
fam {pop (group)} residues	14.1***	4.54*	15.2***

The table showed test statistics with *F*-ratios for fixed effects and χ^2 for random effects. Group is considered as a fixed factor, whereas pop (group) and fam {pop (group)} are random factors. Significant level: *$p < 0.05$; **$p < 0.01$; ****$p < 0.001$

CAL > NMET > SERP, whereas Ni and Zn amounts were significantly higher in the NMET group compared than in CAL and SERP, which had similar values (Fig. 4, Table S8 in Online Resource). Six CAL populations showed the greatest quantities of Cd in shoots. This was explained either by a high biomass production for VIV and LAR populations, or a high shoot concentration for RAM, GA, MAL and SML. Then, similar quantities were observed in the other CAL populations and the six NMET populations with the highest Cd uptake (Fig. c4). These NMET populations also took up the highest Ni and Zn quantities. In contrast, two NMET populations, SEU and MEZ, geographically very close, extracted the lowest quantities for all elements (Online Resource, Table S8). Lastly, two serpentine populations showed similar Ni quantities, but with opposing behaviours i.e. one by producing higher biomass (BER) and the other by a higher shoot Ni concentration (PW).

Discussion

Particular phenotypic characteristics observed in groups and populations

First, our results highlighted specific characteristics of *N. caerulescens* populations from the SERP group. They had lower shoot and root dry mass, and smaller leaf surface area and rosette diameter compared to the two other groups, while CAL and NMET groups were not clearly different in terms of biomass production. SERP populations also exhibited the highest cation contents, while they were similar in the CAL and NMET populations. The strongest differences among groups were the number and the strength of positive correlations between major element contents: the SERP populations clearly showed many strongly positive correlations between major and trace element contents in roots and shoots and also between shoot/root concentration ratios. Such strong correlations between major elements in shoots was also reported by Lee et al. (1977) in the Ni hyperaccumulating plant *Homalium kanaliense* developed on a serpentine soil of New Caledonia. In contrast, the Ni hyperaccumulator *Hybanthus austrocaledonicus* developed on less hostile soils (higher Ca, K and P content) of the same region did not show such correlations. Later, Kazakou et al. (2010) compared the plant composition of 21 species that developed on both

serpentine and non-serpentine soils from Lesbos Island (Greece). They showed that serpentine populations exhibited much more strong positive correlations between major and trace elements in shoots than non-serpentine ones. Moreover, in both studies, plants from serpentine soils showed both high K content and Ca/Mg ratio despite the very great deficiency of K and Ca in soils. These characteristics may emphasize the particular homeostasis of SERP populations, which grow in soils with particularly low cation contents and Ca/Mg ratios (Brady et al. 2005; Kazakou et al. 2008). It can be hypothesised that these plants have adapted to nutritionally poor environments by increasing their cation uptake, allocating more energy to cation absorption (see below) and Mg detoxification, the excess of this element being toxic in serpentine soils (Kruckeberg 1954; Walker 1954; Proctor 1971). As it is well established that the energy cost of ion uptake is high (Tinker and Nye 2000), the supplementary uptake of cations could cause a trade-off with plant growth. This is also consistent with the study of Adamidis et al. (2014) on the variation of leaf traits of 17 species, all of them occurring on both serpentine and non-serpentine soils. They determined that non-serpentine populations are associated with a resource exploitative strategy where plants tend to acquire resources rapidly with high relative growth and photosynthesis rates whereas serpentine populations are associated with a resource conservative strategy where plants tend to invest more resources to structural compounds and have a low relative growth. In addition, Cd concentration in roots and shoots clearly discriminated the groups, as it increased according the following order: SERP < NMET < CAL. This result was also found in previous studies, but with higher Cd concentrations in solutions (Roosens et al. 2003). However, if Cd shoot concentrations were clearly higher in the CAL group than in the two other groups, this was not true for all the CAL populations. Indeed, only few of them showed remarkably high Cd concentrations: the Ganges (GA) population and the other geographically close populations (RAM, DUR, SFP, MAL, SML). More distant CAL populations from Ganges (VIV, AUX, LAR) accumulated Cd at the same level as the NMET did. These results confirm the specific trait of *N. caerulescens* from the Ganges region, i.e. the highest ability to accumulate Cd, which had already been observed (Escarré et al. 2000; Escarré et al. 2013; Roosens et al. 2003). It is interesting to note that manganese contents varied in a similar way to that of Cd, the
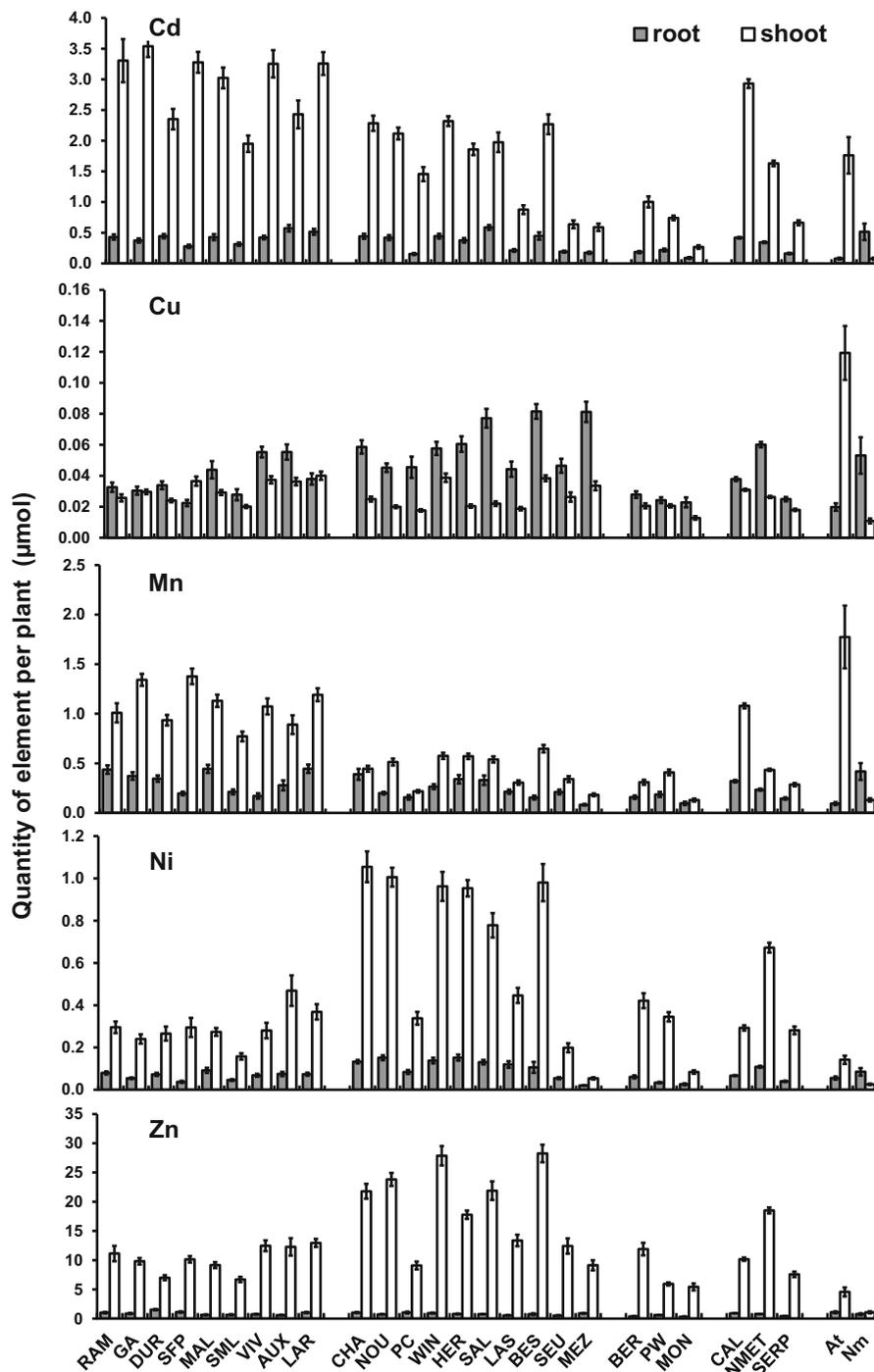
Fig. 4 Cadmium, Cu, Mn, Ni and Zn mean quantities in roots (*grey bars*) and shoot (*white bars*) in 22 populations of *Noccaea caerulescens* after 49 days of hydroponic cultivation. Mean per edaphic group (*CAL*, calamine; *NMET*, non metalliferous; *SERP*, serpentine) and control plant (At: *Arabidopsis thaliana* Col-0; Nm: *N. montana*). *Vertical bars* represent standard errors. For population code, see Table 1

CAL populations showing much higher Mn contents than the NMET and the SERP ones.

The NMET populations showed the highest Ni contents and were followed by the SERP and CAL

populations, even though no population exceeded the Ni hyperaccumulation threshold (17 mmol kg^{-1}), probably because of a too low Ni content in the nutrient solution (0.7 μM). Ni hyperaccumulation by serpentine soils populations is well known (Assuncão et al. 2008; Escarré et al. 2013), but this work shows for the first time that NMET populations can accumulate more Ni than the SERP ones. This is a surprising result, as in the field (Reeves et al. 2001; Visioli et al. 2011) and in controlled conditions (soil and hydroponic cultivations) (Assunção et al. 2003; Escarré et al. 2013), SERP populations have always shown the highest Ni shoots concentrations. Assuncão et al. (2008) studied the effect of different ratios between Cd, Ni and Zn in hydroponics solutions on shoot trace element accumulation in four populations including two CAL and one NMET and SERP; they showed that Cd and Zn in solution inhibited shoots' Ni accumulation in each of three edaphic group, with the exception of the calamine population from La Calamine (Belgium). These results suggest that root absorption is controlled by a low-affinity transport system with Cd and Zn preference over Ni. It seems that in our results, the competition between Cd, Zn and Ni for root absorption is weaker in the NMET populations than in the two other groups.

Zinc content also enabled a clear discrimination between the three groups: CAL populations had lower Zn contents in shoots than SERP and NMET did, while the opposite was true for roots. A majority of the NMET populations exceeded the recently suggested hyperaccumulation threshold (3 000 mg kg^{-1}, i.e. 46 mmol kg^{-1}) (Van der Ent et al. 2013), as did two serpentines populations. These results were consistent with the high shoot concentrations measured in situ, mainly in NMET accessions growing in uncontaminated soils, with low Zn concentrations in soil solution (Basic et al. 2006).

Correlations between major and trace elements

According to the results presented in Meerts and Van Isacker (1997), major elements concentrations were poorly correlated to trace elements concentrations in *N. caerulescens*. However, these authors did not examine serpentine populations, which, in our study, displayed the most remarkable characteristics. Indeed, in the SERP group, as indicated above, trace element concentrations in shoots were strongly positively correlated with those of all the major elements measured,

except Na. It is not conceivable that the different cations are taken up by a unique poorly selective transporter. Therefore, these correlations suggest that SERP populations demonstrate a stronger sink for all cations, although they have a more or less specific transport system for each of them. This sink could result from an increase in the activity of proton pumps, particularly of the plasma membrane bound H$^+$-ATPase, which is responsible for the electrochemical gradient which drives root absorption and the vacuolization of most cations. Other ATPases are involved in cation uptake, such as HMA4, which controls the loading of xylem with trace metals (Verbruggen et al. 2009).

In CAL group, trace elements (Cd, Cu, Ni, Zn) concentrations in shoots were weakly correlated with those of Ca, K and Mg (Online Resource, Table S2). Only three close correlations were found in CAL shoots: Mn *vs* Ca, Cu *vs* Fe and Mn *vs* K. These indicate that, apart the specific transport system (e.g. ZIP, CAX, NRAMP transport systems), the trace metals might enter *N. caerulescens* taking the route of several major cations, which might be more or less specific according to the population.

In NMET, the only relevant correlation was Zn *vs* Ca, which suggests that Zn could take the Ca pathway to accumulate in the plant (Cheng et al. 2002; Cheng et al. 2005). The correlation between shoot Mg and Zn accumulation pointed in Meerts and Van Isacker (1997) was significant but quite weak in our study ($r=$ 0.367). Furthermore, even if the correlations were weak, Fe and Na were negatively correlated with Cd, Mn, Ni and Zn, which indicates that transporter(s) of Fe and Zn would also carry the listed trace elements, but with a lower affinity.

Sources of phenotypic variability

Our results showed a wide variability in biomass production and elemental composition among *N. caerulescens* plants. Nevertheless, most of the variability was attributed to residues and thus remained unexplained. This was particularly true for major elements (except for Mg and K concentrations in CAL and NMET groups). The same pattern was found for trace elements in the SERP group. On the contrary, in CAL and NMET groups, the population level was often a significant source of variability. An interesting exception was for Zn hyperaccumulation in CAL group, in which the responses of the different populations seemed

almost uniform. All these results suggest that in NMET populations, local selective pressures may act on (mainly trace) element shoot concentrations, creating strong among-population differentiation but low within-population variability. Among CAL populations, some shared selective pressures seem to widely act on Zn hyperaccumulation levels, creating low among-population differentiation but locally leaving the possibility of substantial within-population genetic variability.

Which populations for phytoextraction?

N. caerulescens is one of the plants which could be used for phytoextraction of Cd in moderately contaminated soils (Schwartz et al. 2003; Zhao et al. 2003; Maxted et al. 2007; Koopmans et al. 2008). To this end and based on the results obtained here, the CAL populations are the best candidates as they should extract more Cd than the others due to their concentrations and/or biomasses being among the highest. More particularly, the Ganges population, which shows Cd contents among the highest, also has the advantage of being highly tolerant of Cd and Zn (Assunção et al. 2003), thus allowing its use in more toxic soils. However, the use of NMET populations in phytoremediation strategy should not be excluded, as some of them can extract large amounts of Zn, while others can accumulate more Ni than the SERP populations. Some NMET populations can simultaneously extract significant amounts of Cd, Ni and Zn and this could be an advantage in case of polymetallic soil contamination, with the purpose of economic metal valorisation (phytomining). Moreover, NMET populations might be cultivated in climate and soil conditions to which the CAL populations from Southern France would not be well-adapted. However, as shown by Escarré et al. (2013), hydroponic cultivation might give different results to soil cultivation. As a consequence, the results found here have to be verified in soils. Noteworthy, it seems that SERP populations are not suitable for phytoextraction since they exhibited low biomass production and largely unexplained source of genetic variation. .

Finally, our results showed weak ($r<0.25$) but negative correlations between shoot or root biomass and cation contents. Consequently, they indicate a trend towards a trade-off between biomass production and

cation accumulation, which can be explained by the energetic cost of cation uptake (see above). This trade-off can be viewed as an obstacle in the search for naturally "high trace-metal- enriched biomass" genotypes. However, the fact that these correlations are weak and often non-significant for Cd, Ni and Zn in the shoots of the CAL and NMET populations indicates possibilities for associating by crossbreeding a high biomass yield with a high accumulation ability in a given cultivar.

Conclusions

Hydroponics enabled a characterisation of the biomass production and the cationic composition of 22 populations of *N. caerulescens*, with a realistic and controlled root exposure to trace metals. Our results suggest a wide genetic variability of the species, favouring their adaptation to specific environments. The populations from ultramafic soils showed particular features (low biomass, high major and trace element contents, strong correlations between all elements), suggesting a homeostasis adapted to their nutrient-poor environment. Calamine populations showed adaptive abilities (high biomass, high Cd and Mn accumulation) to their metal-contaminated environment. In particular, the strong accumulation of Cd of some populations from the calamine soils, particularly in the area of Ganges, confirms the potential of *N. caerulescens* for the production of phytoextraction cultivars. Non-metallicolous populations also showed interesting abilities (high biomass, high Ni and Zn accumulation) mostly related to each local condition. Our study showed for the first time, that the populations from non-metalliferous soils accumulated more Ni than those from Ni naturally enriched soils, when moderately exposed to this metal. Finally, the high genotypic variability of the species and the weak correlation between biomass production and Cd, Ni or Zn accumulation could be used to combine those traits favourable to the remediation of contaminated soils in a given cultivar.

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