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Metal concentration and metal mass of metallicolous, non metallicolous and serpentine *Noccaea caerulea* populations, cultivated in different growth media

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Abstract

Aims Evaluate the genetic and environmental variability of metal concentration and metal mass of *Noccaea caerulea*, from metalliferous (MET), non metalliferous (NMET) and serpentine (SERP) soils.

Methods 18 populations were cultivated in 18 different growth conditions, such as a soil mine tailing, soils amended with zinc (Zn), cadmium (Cd) and nickel (Ni) salts (in mixtures or in monometallic salts) and a hydroponic solution with two Zn concentrations.

Results MET populations had Zn concentrations lower than NMET and SERP in the different soils but higher Cd mass (the product of aerial biomass and foliar metal concentration). SERP had the highest

Ni concentration and Ni mass values. The addition of Cd or Ni to a Zn-contaminated soil significantly decreases Zn concentration. In hydroponics, MET and NMET had equivalent Zn concentrations but these were three times higher than those obtained in soil experiments. Zn mass of NMET was significantly lower than MET with the latter having Zn mass values largely above those obtained in mine soil.

Conclusions Results showed a large heterogeneity of responses among populations depending on the substrate used, and it was not possible to correctly assign a single population to its accurate origin with only one experiment. Finally, data on metal concentration obtained in culture soils are closer to those in field soils than those from hydroponics so that they could give a more accurate information on the accumulating capacity of *Noccaea caerulea* and its use in phytoextraction of metals in field conditions.

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Keywords *Thlaspi caerulea* · Mine soil · Plant populations · Phytoremediation · Zn/Cd/Ni hyperaccumulation · Metal tolerance

Introduction

Natural soils with high metal concentrations, such as nickel-rich ultramafic soils or anthropogenic metal-contaminated soils from the mining industry, are the theatre of evolutionary processes resulting in genetic and physiological adaptations that enable organisms to

colonise these extreme environments (Antonovics et al. 1971). Most plant species growing on these sites show a tolerance to high metal concentrations due to a restriction of metal transport to the aerial tissues; however a limited number of these species, called hyperaccumulators, are able to accumulate metals at very high concentrations (e.g. 1 % of Zn) in aerial dry parts (Baker and Walker 1990; van der Ent et al. 2012).

Among these species, *Noccaea* (formerly *Thlaspi caerulescens*, a Brassicaceae that hyperaccumulates Zn, Cd and Ni, has been widely studied (Assunção et al. 2003b). The species shows a large climatic and geographical range in Europe. Populations are found from the lowlands up to 2000 m (Alpine climate) and from the North of Spain to Scandinavia (Tutin et al. 1964–1993).

N. caerulescens grows on soils contaminated by heavy metals (Zn, Cd, Pb), on serpentine soils (Ni) and is also present on non-contaminated soils (Reeves et al. 2001; Meyer 2006). Studies comparing plants from metal-contaminated soils (metallicolous populations, MET) and non-contaminated soils (non-metallicolous populations, NMET) showed that NMET plants often have a lower biomass than those in metal-contaminated soils. Interestingly, NMET individuals usually accumulate more Zn than MET plants when grown in the same soil (e.g. Meerts and Van Isacker 1997; Escarré et al. 2000; Meerts et al. 2003; Dechamps et al. 2007). Serpentine populations (SERP), that have been less studied, can also accumulate Zn even with low extractable soil Zn concentrations (Peer et al. 2003).

Despite the high number of papers concerning *N. caerulescens*, most experimental studies investigating metal concentration patterns have compared a very low number (usually 1 or 2) of MET populations (with the exception of Peer et al. 2003 and Roosens et al. 2003). Most experiments were performed with nutrient solutions (e.g. Brown et al. 1995; Assunção et al. 2003a) or with non-contaminated soils supplemented with metal salts (e.g. Meerts et al. 2003). Only a very few number included soils from contaminated sites (e.g. Meerts and Van Isacker 1997; Escarré et al. 2000; Lombi et al. 2001a). In addition, experimental studies comparing MET with NMET populations simultaneously are scarce and were mostly conducted by Belgian and French teams (Meerts and Van Isacker 1997; Escarré et al. 2000; Meerts et al. 2003; Noret et al. 2005, 2007; Dechamps et al. 2007, 2008b), and

even less common are studies that compared populations from metal-contaminated and serpentine soils (Peer et al. 2003; Roosens et al. 2003). Only two studies (Assunção et al. 2003a; 2008) compared one population of each of the three origins. However, when only one population per origin is used, generalisation is hazardous as population replicates are missing. To our knowledge there are no published experiments comparing several populations of plants from each origin, i.e. non-contaminated, metal-contaminated and serpentine soils.

If *N. caerulescens* must be used for the phytoremediation of moderate contaminated soils (Zhao et al. 2003), it would be necessary to conduct a large screening process to evaluate the among-population variability of metal accumulation with different metal concentrations and growth media, including contaminated soils from the field.

Here, several experiments are presented that compare leaf metal concentrations and metal masses (leaf metal concentration times aerial biomass, i.e. the amount of metal in the aerial parts) of *N. caerulescens* plants from the three origins that were cultivated in different substrata.

The following questions were addressed:

- 1) Do metallicolous, non-metallicolous and serpentine populations exhibit contrasting patterns of biomass, metal concentration and metal mass?
- 2) Do these differences vary according the characteristics of culture media, of metallic salts and of the composition of their mixtures?
- 3) Is there evidence of interactions among metals, particularly between Zn and Cd or between Zn and Ni?

Material and methods

Plant and soil material

N. caerulescens seeds were collected from 7 metalliferous sites, from 2 serpentine soils and from 9 non-metalliferous soils in Belgium, the Grand Duchy of Luxembourg and France (Table 1). In each population, seeds from 20–30 flowering plants were collected. To analyse the extractable Zn, Cd, Pb and Ni concentrations of soil, bulk samples were air-dried, ground and the <2 mm fractions were separated from coarse rock

Table 1 Characteristics of metallicolous, non-metallicolous and serpentine sites of *Nocca caerulescens* populations. Soil concentrations are expressed as mg kg⁻¹ ammonium acetate-EDTA extractable element. ABBR: Abbreviations of localities. Abbreviations of treatments: AS (Les Avinières soil), BM (Binary metallic salt mixtures), MS (Monometallic salts), HY (Hydroponics)

Localities	ABBR.	Treatments	Altitude (m)	Latitude (N)	Longitude (E)	Soil Zn	Soil Ni	Soil Cd	Environment
Metallicolous	Avinières mine	AS-BM-MS-HY	168	43°55'56"	003°39'58"	29279 ^a	22	360 ^a	Calcareous waste grassland
	Durfort mine	AS-BM	203	43°59'59"	003°57'08"	19348	<10	69	calcareous waste grassland
	Prayon smelter	AS-BM	160	50°35'03"	005°40'24"	16510 ⁽²⁾	<10 ^b	429 ^b	limestone and shale grassland
	St Felix mine	AS-BM-MS-HY	337	44°01'57"	003°56'21"	8051	<10	36	calcareous waste grassland
	Treves mine	AS-BM-MS	648	44°04'17"	003°23'50"	6887	<10	41	calcareous waste grassland
	Vermisserie mine	AS-BM	266	43°59'53"	003°56'24"	5716	<10	28	calcareous waste shrubland
	Vivez smelter	AS-BM	262	44°33'07"	002°13'21"	3098–99500 ^c	–	28–578 ^c	micaschist grassland
	Baraquette	AS-BM-MS-HY	745	43°55'06"	003°36'57"	60	<10	<10	calcareous shrubland
	Buege	AS-BM	120	43°50'27"	003°40'18"	–	–	–	calcareous shrubland
	Navacelles	AS-BM	610	43°53'15"	003°30'31"	18	<10	<10	calcareous shrubland
Non metallicolous	Ranquas	AS-BM	669	43°50'14"	003°31'58"	–	–	–	calcareous shrubland
	St Baudille	AS-BM	805	43°44'45"	003°29'12"	59	<10	<10	calcareous shrubland
	St Come	AS-BM-MS	982	44°18'14"	003°23'27"	114	<10	<10	calcareous shrubland
	St Michel	AS-BM	691	43°52'00"	003°25'41"	50	<10	<10	calcareous shrubland
	Seranne	AS-BM-MS-HY	902	43°52'11"	003°38'30"	<10	<10	<10	calcareous shrubland
	Wilwerwitz	AS-BM	350	49°59'04"	005°59'54"	15 ⁽²⁾	<10 ^b	<10 ^b	shale grassland
	Bergенbach	AS-BM-MS	829	47°54'39	006°57'15"	25	116 ^d	<10	serpentine grassland
	Puy wolf ^e	AS-BM-MS	479	44°32'56"	002°18'34"	69	371 ^e	<10	serpentine shrubland

^a(Frérot et al. 2006) Total Zn 126126±17620 mg/kg; total Cd 899±200 n=3 (Escarré et al. 2011)

^b(Dechamps et al. 2008a,b; Molitor et al. 2005)

^cTotal Zn concentration (Reeves et al. 2001)

^dTotal Ni concentration: 146 to 2500 mg/kg (Chardot et al. 2007)

^eTotal Ni concentration: 784–1984 mg/kg (Reeves et al. 2001)

fragments by sieving. All chemical analyses were performed on the <2 mm soil fractions. Mineral elements were extracted with ammonium acetate-EDTA 1 N (pH4.65) for 30 min (10 g dry soil in 50 ml of extractant) (Cottenie et al. 1982). The supernatant was filtered and analysed by inductively-coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX). This method is known to extract the 'labile' and 'less labile' pools of trace elements (Fangueiro et al. 2005; Labanowski et al. 2008), i.e. the mineral fraction potentially available to plants via root absorption. In previous experiments, this fraction showed a good correlation with the concentrations of Zn and Cd in the aerial tissues of *Noccaea caerulea* (Robinson et al. 1998). Extractable Ni concentrations were analysed only for the ultramafic soils from Bergenbach and Puy de Wolf. A sampling of the other soils from Southern France was also analysed but the Ni EDTA concentrations were all lower than 10 mg/kg.

Plant culture in soils

Germinated seedlings were transferred into 0.5 L containers and assigned to different treatments for 3 months (from May to July) in a glasshouse with natural day: night regime and watered with distilled water at the CEFÉ-CNRS experimental field Station in Montpellier (France). Containers were completely randomised in the glasshouse.

The following treatments were used:

1) Contaminated soil.

This was collected in a tailing pond at Les Avinières mine. The soil had high metal content (Table 1), low organic matter and a shortage of major plant nutrients. It was mixed with commercial compost (90 % soil-10 % compost) to facilitate plant growth. Initially, 10 plants per population were used, but the mortality in some populations reduced the number of surviving plants at the end of the experiment. Only plant Zn and Cd concentrations and mass were analysed because the Ni concentration values in this soil are very low. We used Les Avinières soil because in previous experiments (Escarré et al. 2000) it allowed to discriminate MET and NMET origins with low mortality. In addition it is a "true soil" used as a control to compare with the other treatments.

2) Garden soil with metallic (Zn, Cd, Ni) salts added.

- a) BINARY MIXTURES. The soil was a mixture of commercial garden compost and soil from the CEFÉ-CNRS experimental station with a very low organic C content (3.8 %) and a low C:N ratio (16) (see Escarré et al. 2000 for other characteristics of this soil). Zn was supplied to soil as $ZnSO_4 \cdot 7H_2O$ and mixed with two salts ($CdSO_4$ or $NiCl_2 \cdot 6H_2O$) in six treatments: 1) 250 mg/kg Zn – 100 mg/kg Cd (hereafter 250Zn100Cd; id for the following treatments); 2) 100 mg/kg Zn – 250 mg/kg Cd; 3) 250 mg/kg Zn – 250 mg/kg Cd; 4) 100 mg/kg Zn – 250 mg/kg Ni; 5) 250 mg/kg Zn – 250 mg/kg Ni; 6) 250 mg/kg Zn – 100 mg/kg Ni.
- b) MONOMETALLIC ZN, CD AND NI SALTS. Different Zn, Cd and Ni salts with contrasted solubility in water (20 °C) were used: 250 and 1500 mg/kg $ZnSO_4 \cdot 7H_2O$ (solubility 96.5 g/100 ml); 1500 mg/kg ZnO (solubility 0.00016 g/100 ml); 250 and 500 mg/kg $CdSO_4$ (solubility 76.4 g/100 ml); 500 mg/kg $CdCl_2$ (solubility 135 g/100 ml); 250 and 1000 mg/kg $NiCl_2 \cdot 6H_2O$ (solubility 254 g/100 ml); 1000 mg/kg $NiSO_4 \cdot 6H_2O$ (solubility 65 g/100 ml).

Five plants per population and per treatment were used in the experiments with metallic salts.

Soil metal concentrations of the mixtures were relatively low compared to those of soils from Les Avinières mine (Table 1) and in the range of ultramafic soils for Ni (Table 1). Our goal was to allow survival and optimal growth for populations from the three origins. Nevertheless, six of nine treatments with monometallic salts (Zn, Cd and Ni) had very high concentrations of metals with the purpose to test the tolerance of populations and the aerial metal concentration of the plants from three origins in extreme soil conditions.

Plant culture in hydroponics

Individuals of four populations (2 MET: AV, SF; 2 NMET: BA, SE) were randomly placed into 3.9 L containers with a capacity for 16 plants, in a growth room with artificial light, as detailed in Garnier (1992), and then filled in with a nutritive solution

described by Koch et al. (1987). The photoperiod was set at 16:8 h (day:night), the air temperature was 22:18 °C and the relative humidity was maintained above 60 %. The Photosynthetically Active Radiation flux (PAR) at seedling height was $515 \pm 8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($n=100$). The solution was renewed twice a week, the pH was adjusted to 5.5 with HCl every 2 days, and the containers were periodically moved to homogenize light distribution. Nine containers received 1.5 μM of zinc, and nine received 2000 μM ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ form). Plants were harvested at 7, 20, 40, 60, 80, 100 and 125 days after transplanting. The number of plants at each harvest was variable depending on the mortality. Two or three plants were collected per population and treatment at the first harvest, and five for the other harvests, while for the last harvest all survivors were collected (4–7 individuals) for a total of 265 plants. At each harvest, only one plant per population and per container was taken at random and the containers were also randomly selected.

Plant analysis

Aerial biomass of all plants grown in soil was harvested after 3 months, rinsed in deionised water and dried at 60 °C for 3d. Due to the very large number of elemental analyses, only a subset of MET and NMET populations were analysed for Cd and Ni in the experiments with salt mixtures and monometallic salts, whereas the two SERP were consistently analysed (Table 1). Some plants with too low biomass were excluded from Cd/Ni analyses. Dried samples were ground and a subset was mineralised in a mixture of nitric and perchloric acid with a Tecator digester, and their individual cadmium and nickel contents determined by ICP-OES (Varian Vista MPX). The rest of the ground material was used to measure the individual Zn concentration by the zincon method (see hereafter).

The totality of individuals grown in soil and in hydroponics was analysed for Zn concentrations with the Zincon method developed for *Arabidopsis halleri* (Macnair and Smirnov 1999). This method is based on UV-visible spectrophotometry using zincon as a coloured Zn-chelating agent and is less expensive than ICP. The Zincon method allows the analysis of individuals with low biomass. This method has been previously validated with *Noccaea caerulea* (Frérot et al. 2005).

Metal mass was calculated as the product of metal concentration, times the above-ground biomass.

Despite the fact that metal mass is concentration-dependent, unlike biomass, this measure was also selected because it provides a good idea of the potential of each *Noccaea caerulea* population to extract metals from soil.

Statistical methods

First, dry aerial metal concentrations and metal masses were analysed for all soil treatments by 6 three-way partially nested ANOVAs (SAS 2004) with the following factors: origins (MET, NMET and SERP), treatments (10 for Zn, 7 for Cd and 6 for Ni) and populations nested within origins and treatments. The population factor was considered as random. Type III sums of squares and the Satterthwaite approximation were used. Means were compared using least square means tests. These analyses were made to compare both the mean values in the different treatments and the performances of the three origins.

Secondly, because of the complex interactions between populations and treatments (see results), each treatment was also analysed independently by a nested ANOVA with the three origins and the populations nested within the origins. Population was considered as random. The ANOVAs for metal concentrations and masses were as follows: i) one for the experiment with the mine substrate, ii) 6 for each of the treatments with Zn binary salt mixtures (3 with Cd and 3 with Ni), and iii) 9 for the monometallic salt treatments (3 for each metal).

Lastly, differences in metal concentrations or metal masses among salt mixtures and monometallic salts for a population were performed by one-way ANOVAs followed by *a posteriori* contrasts.

Data from hydroponics were analysed independently because the experimental conditions were not the same as for the other experiments. Zn concentration and mass were analysed by partially-nested ANOVAs with the following factors and their interactions: dates (7), origins (3), treatments (2) and populations (4) nested within origins. Values were log-transformed prior to statistical analyses. Therefore, the results of the ANOVAs are given for log-transformed values, but means (\pm SE) are in arithmetical values for the sake of clarity.

Survival differences between origins and treatments were tested using Fisher's exact test (Statistix 2003).

Results

Comparisons among soil treatments and origins (populations nested within origin and treatment)

- a) *Biomass and survival.* Plant cultivated only with Cd or Ni monometallic salts had lower biomass (Fig. 1) than those grown with mixtures (ANOVA's Treatment effect significant). In most treatments MET had higher biomass than NMET and SERP (ANOVA's Origin effect significant). The latter had on average the lowest biomass (see the three histograms at the right end of the graph). The only exception was in the 250ZnSO₄ treatment and in the treatments with Ni monometallic salts where SERP biomass was equivalent to that of MET individuals.

As for biomass, survival values (Table 2) were lowest for plants grown without Zn but with Cd salts (including 500 CdCl₂: 43 % survival) and to a lesser extent with Ni salts of (1000 NiSO₄: 63 % survival). Overall survival of plants growing with Zn salts (in mixtures or with monometallic salts) was significantly higher (97.6 % 645/660) than

plants without Zn salts (69.6 % 167/240). There was no difference in survival between origins for mixtures. On the other hand NM survival (66.6 % 90/135) was significantly lower than that of MET (79.2 % 90/135) and that of the SERP (82.2 % 74/90) when plants were cultivated in Zn, Cd and Ni monometallic salts. Survivals of individuals grown in Les Avinières soil was 76 % (137/180) similar to that of monometallic salts but significantly lower than that of mixtures. There were no significant survival differences among origins in this soil.

- b) *Zinc.* Plants cultivated in monometallic salts or in Les Avinières soil had the highest Zn concentration values per plant (significant treatment effect, capital letters above bars in Fig. 2a) and plants from Zn-Cd mixtures had the lowest Zn concentration values (Fig. 2a). Overall, plants from MET origin had the lowest mean Zn concentration (MEAN, last three bars at the right of each graph) but a high Zn mean mass (with NMET) because the high biomass of MET individuals compensated for the low Zn concentration. SERP individuals had overall the highest Zn concentration and the

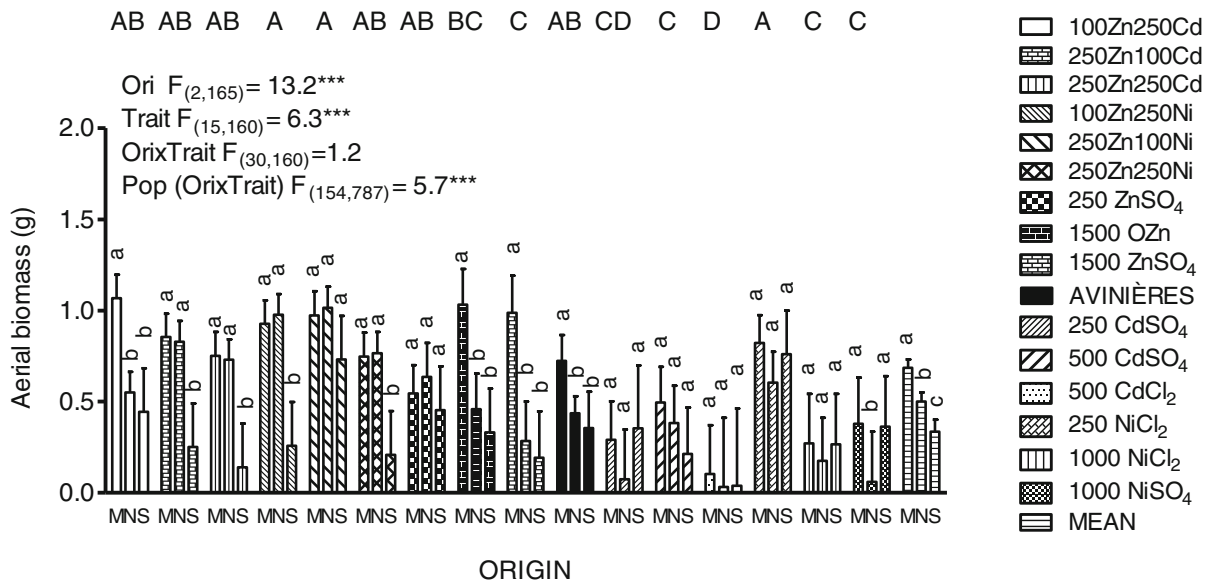


Fig. 1 Mean (\pm SE) aerial biomass value per individual of metallicolous (M), non-metallicolous (N) and serpentine (S) *Noccaea caerulea* plants cultivated in a garden soil contaminated with different metal concentrations and in Les Avinières mine soil. In binary mixtures, Zn was provided as ZnSO₄, Cd as CdSO₄ and Ni as NiCl₂. In monometallic salts, Zn was provided as ZnSO₄ and ZnO, Cd as CdSO₄ and CdCl₂ and Ni

as NiCl₂ and NiSO₄. For each treatment, bars topped with the same lower letter are not significantly different with a least squares means test (SAS). Results of the mixed nested ANOVA including all treatments are shown. Treatments with the same capital letter are not significantly different with a Tukey test (SAS). Means for origins across treatments are shown as the last three bars

Table 2 Survival (percentage number of living individuals/total number) of metallicolous, non-metallicolous and serpentine populations of *Noccaea caeruleascens* cultivated in 3 Zn/Cd and 3 Zn/Ni salt mixtures, in Zn, Cd and Ni monometallic salts and in a contaminated soil from the tailing basins of Les Avinières mine. For salt mixtures and monometallic salt treatments, survival values with the same letter are not significantly different with a Fisher's exact test

Survival (%)	100Zn250Cd	250Zn100Cd	250Zn250Cd	100Zn250Ni	250Zn100Ni	250Zn250Ni	AVINIÈRES SOIL
Salt mixtures							
Metallicolous	97 (34/35)	100 (35/35)	97 (34/35)	100 (35/35)	100 (35/35)	97 (34/35)	73 (51/70)
Non-metallicolous	97 (44/45)	97 (44/45)	100 (45/45)	100 (45/45)	97 (44/45)	95 (43/45)	80 (71/90)
Serpentine	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	75 (15/20)
Total	98 (88/90) a	99 (89/90) a	99 (89/90) a	100 (90/90) a	99 (89/90) a	97 (87/90) a	76 (137/180) b
Monometallic salts							
Metallicolous	250ZnSO ₄ 93 (14/15)	1500ZnSO ₄ 93 (14/15)	1500ZnO 100 (15/15)	250CdSO ₄ 60 (9/15)	500CdSO ₄ 100 (15/15)	500CdCl ₂ 53 (8/15)	1000NiCl ₂ 53 (8/15)
Non-metallicolous	86 (13/15)	86 (13/15)	100 (15/15)	40 (6/15)	93 (14/15)	26 (4/15)	73 (11/15)
Serpentine	100 (10/10)	90 (9/10)	100 (10/10)	50 (5/10)	90 (9/10)	50 (5/10)	80 (8/10)
Total	93 (37/40) a	90 (36/40) a	100 (40/40) a	50 (20/40) bc	95 (38/40) a	43 (17/40) c	68 (27/40) b
							1000NiSO ₄ 60 (9/15)
							53 (8/15)
							80 (8/10)
							63 (25/40) bc

lowest Zn mass due to their small biomass, particularly in some Zn-Cd treatments and Cd monometallic salts, probably due to the low Cd tolerance of these populations. NMET individuals had intermediate Zn mean concentration. In the 1500 ZnSO₄ treatment (the most soluble salt) plants from SERP had values above 1,0000 mg Zn/kg (the admitted threshold for Zn hyperaccumulation).

Zn concentrations were significantly lower in metallic salt mixtures (Zn-Cd or Zn-Ni) than in monometallic salts (250ZnSO₄) at equivalent Zn concentration (250 mg/kg) (Fig. 2a). Zn mass was significantly lower for Zn-Cd (but not for Zn-Ni) mixtures (250Zn100Cd and 250Zn250Cd), than in monometallic salts (Fig. 2b).

- c) *Cadmium*. The highest Cd concentrations were measured in plants grown with monometallic Cd salts (Fig. 2c), whereas plants grown in Les Avinières soil had the lowest Cd concentrations and Cd mass (Fig. 2c, d). No differences among the origins were found for Cd concentration, but MET populations had a significantly higher Cd mass overall than the others. In all treatments and origins Cd concentrations were above the Cd hyperaccumulation threshold.

Cd concentrations (but not Cd mass) were significantly lower in salt mixtures (100Zn250Cd and 250Zn250Cd) than in monometallic salt (250 CdSO₄) at equivalent Cd concentrations (250 mg/kg) (Fig. 2c, d). Even at high Cd concentrations (especially in 500 mg/kg CdCl₂) Cd mass was very low because Cd (particularly in the most soluble salt CdCl₂) decreased biomass and therefore Cd mass.

- d) *Nickel*. Plants cultivated with the highest concentration of Ni monometallic salts (1,000 NiCl₂ or 1000 NiSO₄) had the highest Ni concentrations, and those with Zn-Ni binary mixtures had the lowest (Fig. 2e). Overall, SERP populations had significantly higher mean Ni concentrations and mass; while MET had the lowest concentrations but similar masses as NMET (Fig. 2e, f). In 1000 mg Ni/kg treatments, SERP populations had mean Ni concentration values above the Ni hyperaccumulation threshold (1000 mg Ni/kg plant dry mass).

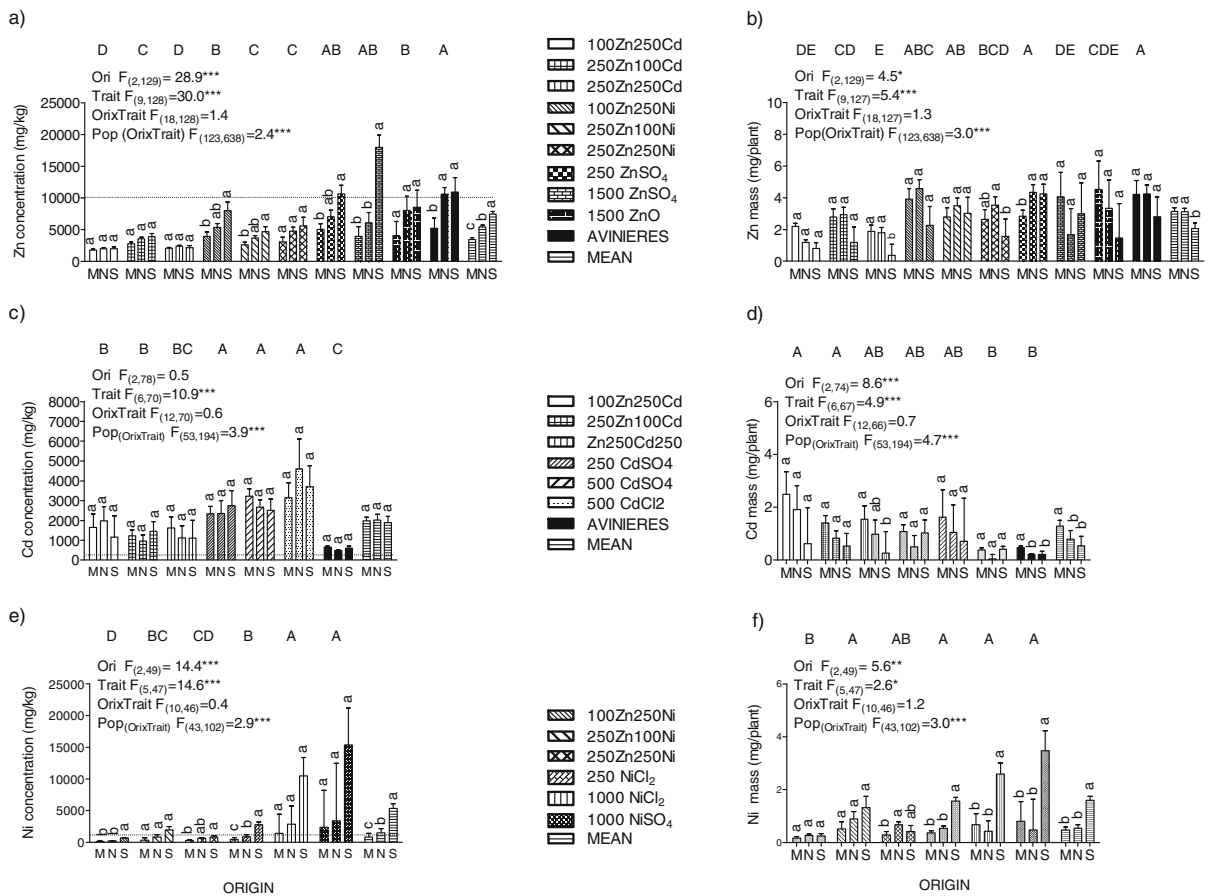


Fig. 2 Mean (\pm SE) Zn (a), Cd (c) and Ni (e) concentrations (mg/kg) and mean (\pm SE) Zn (b), Cd (d) and Ni (f) masses (mg/plant) per individual in *Noccaea caerulescens* aerial parts of metallicolous (M), non-metallicolous (N) and serpentine (S) *Noccaea caerulescens* plants cultivated in a garden soil contaminated with different metal concentrations and in Les Avinières mine soil. In binary mixtures, Zn was provided as ZnSO₄, Cd as CdSO₄ and Ni as NiCl₂. In monometallic salts, Zn was provided as ZnSO₄ and ZnO, Cd as CdSO₄ and CdCl₂ and Ni as NiCl₂ and NiSO₄. For

each treatment, bars topped with the same lower letter are not significantly different with a least squares means test (SAS). Treatments with the same capital letter are not significantly different with a least squares means test (SAS). Means for origins across treatments are shown as the last three bars in each graph. Hyperaccumulation thresholds for Zn (a), Cd (c) and Ni (e) are indicated with a dotted horizontal line. Results of the mixed nested ANOVA including all treatments are shown

The comparison of Ni concentration and mass between Zn-Ni mixtures and monometallic salts (250NiCl₂) at the same soil Ni concentration (250 mg/kg) did show a significant decrease in Ni concentration but not in Ni mass (Fig. 2e, f).

In all analyses there was a significant interaction effect for Pop (Ori x Trait), indicating that metal concentration and metal mass among populations within origins and treatments were heterogeneous. For this reason, data were reanalysed treatment by treatment.

Comparisons for each soil treatment (populations nested within origin)

Biomass, survival, Zn and Cd concentration and mass in Les Avinières soil (Fig. 3)

Overall, MET populations (but Prayon) had high mean biomass values (Fig. 3e). NM and SERP had lower but similar values. The two SERP populations had very different biomass with BE having high and PW having low values (similar to PR and WI). Survival was very

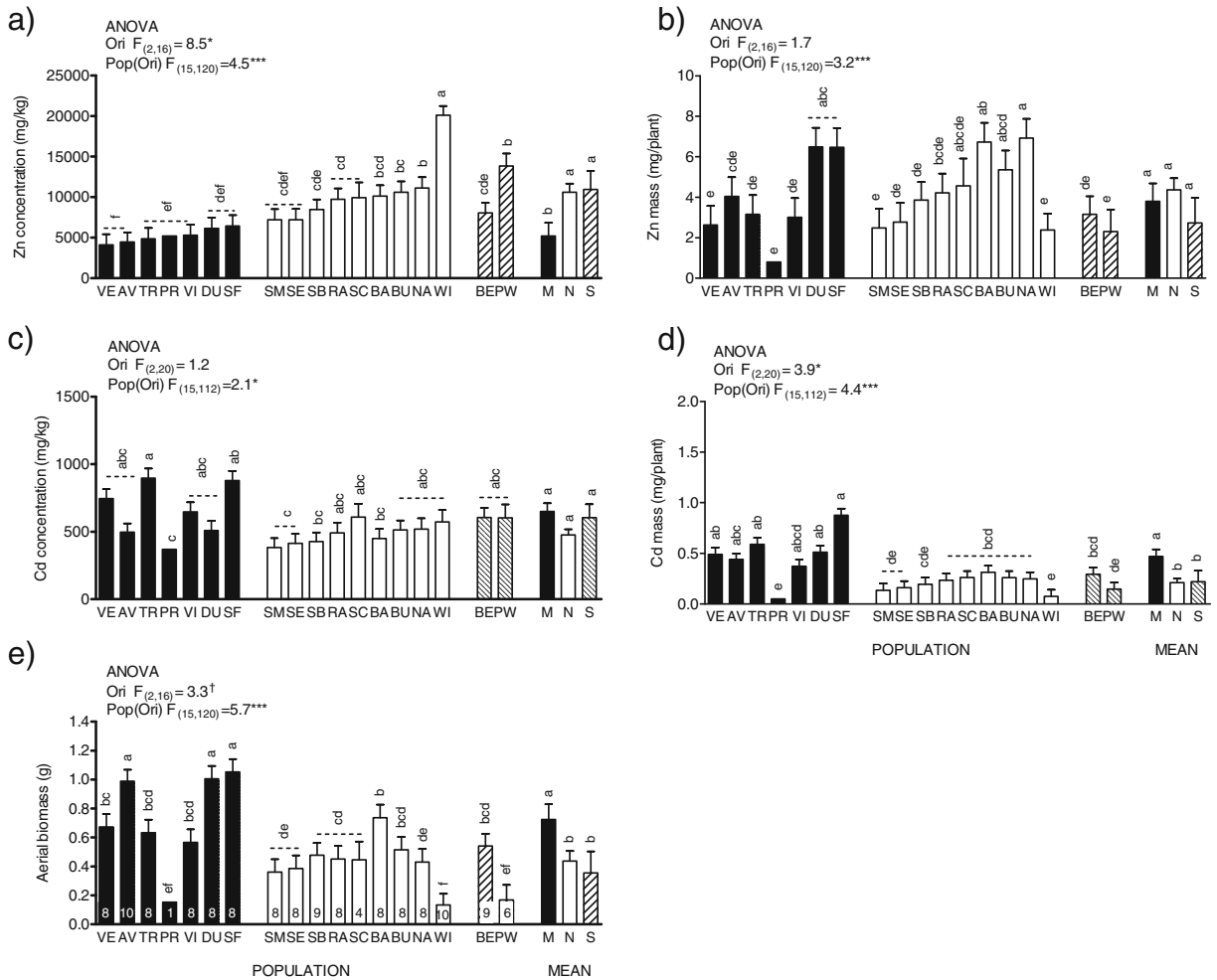


Fig. 3 Mean (\pm SE) Zn (a) and Cd (b) concentration (mg/kg), mean (\pm SE) Zn (c) and Cd (d) mass (mg/plant) and mean (\pm SE) biomass (g) (e) per individual of metalicolous (black bars), non-metallicolous (white bars) and serpentine (hatched bars) populations of *Noccaea caerulea* cultivated in Les

Avinières soil. For population names please see Table 1. Population means with the same letter are not significantly different with a least squares means test (SAS). *M* metallicolous, *N* non-metallicolous, *S* serpentine. Results of the nested ANOVA are shown

low for PR (1/10), SC (4/10) and PW (6/10). Surprisingly, all WI individuals were alive despite their low biomass.

MET populations had Zn concentrations lower than NMET and SERP (significant differences among origins). There was a high heterogeneity within origins: individuals from WI had two times higher zinc concentrations than the other NMET populations and the PW (SERP) accumulated 80 % more Zn than BE (SERP). There were no differences among the origins for Zn mass (Fig. 3c), but the northern populations PR (MET), WI (NMET) and PW (SERP) had the

lowest zinc mass (due to low biomass), and the southern populations DU and SF (MET), NA and BA (NMET) had the highest [significant population (origin)].

There were no significant differences among origins for Cd concentration (Fig. 3b), but a great heterogeneity occurred, particularly among MET populations [significant population (origin)]. Overall, MET populations had higher Cd masses than NMET or SERP (Fig. 3d). However, there was a high heterogeneity within MET, with SF showing the highest values and the northern PR the lowest.

Biomass, survival, Zn concentration and Zn mass in binary mixtures and in Zn monometallic salts (Table 3)

In 4 of 9 treatments there were significant differences among origins for plant biomass with MET having higher values than NMET and SERP. In 5 of 6 mixture treatments, NM populations had the highest biomass (exception: MET PR in the 100Zn250Cd). This highlights the high tolerance of NM to moderate levels of metals. In 6 of 9 treatments, there were significant differences among populations. This shows that within origins population respond differently to metals. Survival of the different populations was very high (see also Table 2) in mixtures as in treatments with monometallic salts (two of which had very high concentrations of Zn). Despite that SERP had low biomass there was no mortality in any treatment.

Zn salt mixtures (Zn-Cd and Zn-Ni) generated contrasting responses among populations for Zn concentration and mass (Table 3a). Thus, for the three Zn-Cd treatments there was only one significant difference ($p < 0.10$) in the Zn concentration among origins and among populations (250Zn100Cd). The mixture with 250Zn250Cd showed significant differences for zinc mass among origins (SERP had the lowest values, see also Fig. 2b) and among populations, with the northern populations WI (NMET) and PW (SERP) having the lowest Zn mass values (Table 3b).

Out of the three Zn-Ni treatments, two (100Zn250Ni and 250Zn100Ni) showed significant Zn concentration differences among origins (Table 3a) with SERP showing high values (Table 3b & Fig. 2a). However, all of the Zn-Ni treatments had a significant population (origin) effect for Zn concentration and Zn mass. Unexpectedly, some populations had contrasted differences in Zn mass for the same soil Zn concentration (250 mg/kg). For instance, WI (NMET) had the highest Zn mass values in the 250Zn250Ni treatment (7.3 mg/plant) and the lowest in the 250Zn100Ni treatment (0.7 mg/plant).

The two zinc sulphate treatments (monometallic salts) showed significant differences for Zn concentration among the origins (Table 3a) with the two SERP populations having the highest values (Table 3b). There were large variations for some populations among the three treatments with monometallic salts (e.g. Zn concentration values among BE (SERP) individuals did vary from 4,000 to nearly 20000 mg/kg),

and the treatment with the lowest Zn concentration in the soil (250 mg/kg) in other populations (BA, TR, SF) gave the highest values of Zn concentration in plants. Both 1500 Zn treatments showed significant differences among populations, particularly for the two SERP populations. Thus, PW had a higher Zn concentration than BE for zinc oxide, but had the lowest zinc mass. However, for zinc sulphate at 1500 mg/kg, BE had the highest Zn concentration.

Previously (see Fig. 2a), it was shown that Zn concentrations were significantly lower in metallic salt mixtures (Zn-Cd or Zn-Ni) than in monometallic salts (250ZnSO₄) at equivalent Zn concentrations (250 mg/kg). We checked whether this occurred in the 8 populations tested or if only some populations showed such a decrease. The one-way ANOVAs followed by *a posteriori* contrasts showed that the three MET populations (AV, SF and TR), two NMET (BA and SC) and the two SERP (BE and PW) populations had significantly ($p < 0.05$) lower Zn concentrations values in the Zn-Cd mixtures compared to monometallic salts. Only one NMET (SE) showed non-significant Zn concentration differences ($p > 0.05$). For the Zn-Ni TR (MET), BA and SC (NMET) and PW (SERP) showed a significant ($p < 0.05$) decrease of Zn concentration values in mixtures, whereas the other populations AV, SF (MET), SE (NMET) and BE (SERP) did not show any significant differences.

The results of the treatments with Zn salts clearly showed that the highest Zn concentrations and masses were achieved by different populations in different treatments (Table 3b). Thus, the maximum Zn concentration in each treatment was achieved by 6 different populations (4 NMET and the 2 SERP), and the maximum Zn mass in each treatment was achieved by 8 different populations (2 MET, 4 NMET, 1 SERP) (Table 3).

Biomass, survival, Cd concentration and Cd mass in binary mixtures and monometallic salts (Table 4)

There were no significant biomass differences among origins for any of the treatments, but for all treatments, there were significant differences among populations within origin. Some populations had an important mortality at high Cd concentrations. This was the case of SF (MET), BA and SC (NMET) and PW (SERP). The latter population had only one survivor in the 500 CdCl₂ treatment.

Table 3 Results of nested ANOVAs for aerial biomass, Zn concentration and Zn mass (a) and means (\pm SE) for aerial biomass (g) (bold), Zn concentration (mg/kg) (normal) and Zn shoot mass (mg/plant) (*italics*) per individual (b) of metallicolous, non-metallicolous (**bold**) and serpentine (*italics*) populations of *Nocca caerulea* cultivated in six Zn salt mixtures (ZnSO₄) and in three Zn monometallic salts (ZnSO₄, ZnO). For each salt treatment, population means with the same letter (**bold for biomass**, normal for Zn concentration and *italics for Zn mass*) are not significantly different with a least squares means test (SAS). Maximal biomass, Zn concentration and mass values for each treatment are underlined. Sampling number is enclosed in parentheses after the mean aerial biomass

		Zinc salt mixtures										Zinc monometallic salts									
		100Zn 250Cd	250Zn 100Cd	250Zn 250Cd	100Zn 250Ni	250Zn 100Ni	250Zn 250Ni	250Zn 250Ni	250Zn 250Ni	1500ZnSO ₄	1500ZnO										
a ANOVAS	Shoot biomass	Ori F_{2,15}=3.1†	Ori F_{2,15}=1.6 ns	Ori F_{2,15}=4.0*	Ori F_{2,15}=4.2*	Ori F_{2,15}=0.1 ns	Ori F_{2,15}=5.7*	Ori F_{2,15}=0.8 ns	Ori F_{2,15}=1.0 ns	Ori F_{2,15}=1.2 ns	Ori F_{2,15}=1.2 ns										
	Pop(Ori) F_{15,70}=1.6 ns	Pop(Ori) F_{15,70}=4.6***	Pop(Ori) F_{15,70}=1.0 ns	Pop(Ori) F_{15,72}=3.2**	Pop(Ori) F_{15,68}=6.6**	Pop(Ori) F_{15,67}=2.4**	Pop(Ori) F_{15,28}=1.7 ns	Pop(Ori) F_{15,28}=8.9 ***	Pop(Ori) F_{15,32}=16.9***												
	<i>Ori F_{2,15}=1.2 ns</i>	<i>Ori F_{2,15}=2.9†</i>	<i>Ori F_{2,15}=1.6 ns</i>	<i>Ori F_{2,15}=3.7*</i>	<i>Ori F_{2,15}=3.1†</i>	<i>Ori F_{2,15}=2.0 ns</i>	<i>Ori F_{2,15}=7.4*</i>	<i>Ori F_{2,15}=1.1 ns</i>													
concentration	Pop(Ori) F _{15,70} =1.3 ns	Pop(Ori) F _{15,70} =1.7†	Pop(Ori) F _{15,70} =1.0 ns	Pop(Ori) F _{15,72} =3.5**	Pop(Ori) F _{15,68} =5.2**	Pop(Ori) F _{15,67} =2.0*	Pop(Ori) F _{15,28} =0.7 ns	Pop(Ori) F _{15,28} =2.2†	Pop(Ori) F _{15,32} =6.5***												
Shoot zinc mass	<i>Ori F_{2,15}=0.9 ns</i>	<i>Ori F_{2,15}=1.3 ns</i>	<i>Ori F_{2,15}=3.8*</i>	<i>Ori F_{2,15}=1.7 ns</i>	<i>Ori F_{2,15}=0.7 ns</i>	<i>Ori F_{2,15}=2.6†</i>	<i>Ori F_{2,15}=3.2†</i>	<i>Ori F_{2,15}=0.5 ns</i>	<i>Ori F_{2,15}=0.8 ns</i>												
	Pop(Ori) F _{15,70} =1.4 ns	Pop(Ori) F _{15,70} =3.1***	Pop(Ori) F _{15,70} =2.5**	Pop(Ori) F _{15,72} =1.9*	Pop(Ori) F _{15,68} =5.1***	Pop(Ori) F _{15,67} =1.6†	Pop(Ori) F _{15,28} =0.7 ns	Pop(Ori) F _{15,28} =8.3***	Pop(Ori) F _{15,32} =9.2***												
b Aerial biomass (g)/Zn Concentration (mg/kg)/Zn MASS (mg/plant)																					
Avimères (AV)	0.90±0.34b (5)	1.34±0.14 a (5)	0.61±0.23 abc (4)	1.08±0.24 a (5)	0.78±0.11 ab (5)	0.38±0.21 abcd (4)	0.76±0.09 a (5)	1.58±0.23 a (5)	1.29±0.26 a (5)												
	1289±384 a	2804±300 ab	1763±673 a	4934±926 defg	3428±122 cdef	3987±224 bcd	4429±966 b	4615±299 cd	4701±756 bc												
	<i>1.6±0.7 a</i>	<i>3.7±0.4 abc</i>	<i>1.6±1 abc</i>	<i>5.3±1.4 ab</i>	<i>3.0±0.2 abc</i>	<i>2.1±1.7 b</i>	<i>3.6±0.7 a</i>	<i>7.2±0.9 a</i>	<i>6.3±1.6 a</i>												
Durfort (DU)	1.04±0.42 ab (5)	1.19±0.26 abc (5)	0.71±0.15 abc (5)	0.81±0.17 ab (5)	1.10±0.16 abc (5)	0.71±0.22 abcd (5)	–	–	–												
	1455±306 a	2813±575 ab	2178±249 a	6165±659 cd	3725±289 bcde	3052±851 cde	–	–	–												
	<i>1.6±0.8 a</i>	<i>3.8±0.9 abc</i>	<i>1.7±0.5 abc</i>	<i>5.3±1.4 a</i>	<i>4.2±0.9 ab</i>	<i>2.9±1.2 ab</i>	–	–	–												
Prayon (PR)	1.37±0.24 a (5)	0.37±0.22 abcd (5)	0.97±0.32 abc (5)	0.71±0.17 ab (5)	0.28±0.07 bc (5)	0.63±0.22 abcd (5)	–	–	–												
	2456±319 a	1585±235 b	2572±415 a	3045±591 e fgh	2310±447 efg	2810±992 cde	–	–	–												
	<i>3.3±0.6 a</i>	<i>0.7±0.5 de</i>	<i>3.3±0.7 a</i>	<i>2.6±0.8 b</i>	<i>0.8±0.3 cd†</i>	<i>1.80±0.7b</i>	–	–	–												
St Felix (SF)	1.16±0.36 ab (5)	0.99±0.22 abc (5)	0.99±0.38 abc (5)	1.14±0.29 a (5)	1.44±0.22 a (5)	1.05±0.26 abc (5)	0.35±0.13 a (4)	1.04±0.33 ab (5)	1.33±0.24 a (5)												
	1902±240 a	4356±384 a	2034±485 a	4812±834 defg	3718±590 bcde	4443±453 bcd	6209±2010 b	3026±1875 cd	3938±558 c												
	<i>2.4±0.8 a</i>	<i>4.6±1.2 ab</i>	<i>2.6±1.2 ab</i>	<i>6.2±2.3 a</i>	<i>5.7±1.6 a</i>	<i>4.8±1.2 ab</i>	<i>2.9±1.1 a</i>	<i>3.3±1.1 ab</i>	<i>5.4±1.5 a</i>												
Treves (TR)	1.08±0.57 ab (4)	0.62±0.28 abcd (5)	0.21±0.08 bcde (5)	1.34±0.34 a (5)	1.07±0.43 ab (5)	0.62±0.29 abcd (5)	0.58±0.17 a (5)	0.34±0.3 bc (4)	0.48±0.20 b (5)												
	2222±482 a	2763±723 ab	2284±511 a	3990±759 defg	1594±232 g	2121±383 e	5129±623 b	4195±2096 d	3575±444 c												
	<i>2.5±1.2 a</i>	<i>1.9±0.8 cd</i>	<i>0.5±0.2 abc</i>	<i>4.6±0.7 ab</i>	<i>1.9±0.8 bcd</i>	<i>1.6±0.8 ab</i>	<i>2.9±1.0 a</i>	<i>1.7±1.1 bc</i>	<i>1.9±0.9 bc</i>												
Vernissière (VE)	0.87±0.3 ab (5)	0.34±0.14 cd (5)	1.18±0.3ab (5)	0.62±0.21 ab (5)	0.95±0.35 abc (5)	1.12±0.14 ab (5)	–	–	–												
	1343±417 a	2258±594 ab	1793±188 a	1832±344 h	1475±138 g	3113±796 cde	–	–	–												
	<i>1.6±0.7 a</i>	<i>1.2±0.5 de</i>	<i>2.3±0.6 abc</i>	<i>1.3±0.5 b</i>	<i>1.4±0.6 abcd</i>	<i>3.8±1.5 ab</i>	–	–	–												
Viviez (VI)	1.03±0.22 ab (5)	1.12±0.16 abc (5)	0.60±0.18 abc (5)	0.79±0.12 ab (5)	1.18±0.08 a (5)	0.70±0.04 abcd (5)	–	–	–												
	1912±317 a	3103±424 ab	1794±306 a	2792±450 e fgh	2128±202 fg	2083±782 e	–	–	–												
	<i>2.1±0.6 a</i>	<i>3.6±0.8 abc</i>	<i>1.2±0.5 abc</i>	<i>2.2±1.2 b</i>	<i>2.5±0.2 abc</i>	<i>1.5±0.6 b</i>	–	–	–												
Baraquette (BA)	0.84±0.18 ab (5)	1.54±0.13 a (5)	1.81±0.16 a (5)	1.67±0.20 a (5)	1.18±0.17 a (5)	1.60±0.21 a (5)	0.52±0.14 a (5)	0.05±0.01 c (5)	0.13±0.07 c (5)												
	2541±656 a	2611±222 ab	2078±555 a	2876±612 gh	3179±266 defg	3676±606 cde	7539±774 b	4288±2563 cd	4510±1328 ab												

Table 3 (continued)

	Zinc salt mixtures								Zinc monometallic salts			
	100Zn 250Cd	250Zn 100Cd	250Zn 250Cd	100Zn 250Ni	250Zn 100Ni	250Zn 250Ni	250ZnSO ₄	1500ZnSO ₄	1500ZnO			
Buege (BU)	2.0±0.6 a	4.0±0.5 abc	3.6±0.8 a	4.5±0.9 ab	3.6±0.4 ab	5.6±0.6 ab	4.3±1.6 a	0.1±0.1 c	0.6±0.4 bc			
	0.65±0.16 ab (5)	1.01±0.19 abc (5)	0.85±0.13 abc (5)	0.93±0.23 a (5)	1.13±0.11 ab (5)	0.87±0.14 abcd (5)	—	—	—			
	2270±225 a	4561±495 a	2714±213 a	5412±756 cde	4195±537 bcd	3458±260 cde	—	—	—			
	1.6±0.6 a	4.6±0.9 ab	2.4±0.5 ab	5.0±1.4 ab	4.7±0.6 a	3.0±0.6 ab	—	—	—			
Navacelles (NA)	0.61±0.21 ab (5)	0.72±0.21 abc (5)	0.48±0.17 abcd (5)	0.92±0.10 a (5)	1.17±0.17 ab (5)	0.53±0.13 abcd (5)	—	—	—			
	2338±332 a	3670±616 ab	2795±257 a	4847±748 defg	4606±492 bc	4961±833 bc	—	—	—			
	1.2±0.3 a	2.8±0.7 abcd	1.4±0.6 abc	4.7±1 ab	5.3±0.8 a	2.9±0.7 ab	—	—	—			
Ranquas (RA)	0.15±0.03 b (4)	0.47±0.13 abc (5)	0.48±0.13 abc (5)	0.56±0.10 ab (5)	1.01±0.05 ab (5)	0.26±0.07 bcd (5)	—	—	—			
	1482±178 a	2740±523 ab	1415±298 a	5997±409 cd	2911±287 defg	2649±780 de	—	—	—			
	0.2±0.1 a	1.5±0.5 de	0.7±0.7 abc	3.5±0.8 ab	3.0±0.3 abc	0.8±0.3 b	—	—	—			
Seranne (SE)	0.74±0.27 ab (5)	0.66±0.23 abc (5)	0.84±0.15 abc (5)	1.78±0.12 a (5)	1.1±0.13 ab (5)	1.15±0.27 ab (5)	0.87±0.08 a (3)	0.74±0.17 ab (5)	1.13±0.09 a (5)			
	1862±356 a	3327±555 ab	2525±325 a	4501±544 defg	2849±407 defg	2821±667 de	4116±445 b	4472±1875 cd	7392±742 c			
	1.4±0.7 a	2.5±1 bcde	2.2±0.5 ab	8.0±0.9 a	3.1±0.4 abc	3.5±1.1 ab	3.6±0.6 a	3.7±1.1 ab	8.1±0.3 a			
St Baudille (SB)	0.61±0.12 ab (5)	1.3±0.31 ab (5)	0.99±0.15 ab (5)	0.94±0.2 a (5)	0.92±0.06 ab (4)	0.91±0.17 abcd (5)	—	—	—			
	1787±137 a	3607±333 ab	2750±337 a	4577±958 defg	3478±401 cde	3947±863 cde	—	—	—			
	1.1±0.2 a	4.7±1.3 a	2.9±0.7 a	5.0±1.6 ab	3.4±0.4 abc	3.9±0.9 ab	—	—	—			
St Come (SC)	0.70±0.29 ab (5)	1.11±0.37 abc (5)	0.57±0.17 abc (5)	1.12±0.19 a (5)	1.57±0.20 a (5)	0.69±0.20 abcd (5)	0.48±0.03 a (5)	0.063±0.04 c (3)	0.12±0.09 cd (5)			
	1980±349 a	3530±552 ab	2885±397 a	4745±516 defg	2941±336 defg	4871±770 bc	6477±537 b	11695±4639 abc	12286±2716 a			
	1.5±0.7 a	3.7±1 abc	1.6±0.7 abc	5.0±0.7 ab	4.4±0.2 a	3.7±1.3 ab	3.1±0.3 a	1.1±1.0 bc	1.3±0.9 bc			
St Michel (SM)	0.41±0.10 ab (5)	0.55±0.09 abc (5)	0.49±0.06 abc (5)	0.69±0.20 ab (5)	0.92±0.23 ab (5)	0.29±0.03 abcd (5)	—	—	—			
	1921±388 a	4365±372 a	2599±313 a	4283±1245 defg	4307±600 bcd	3631±1259 cde	—	—	—			
	0.8±0.3 a	2.3±0.4 cde	1.2±0.1 abc	3.2±1.2 ab	3.5±0.6 abc	1.2±0.3 b	—	—	—			
Wilwerwiltz (WI)	0.23±0.16 b (5)	0.08±0.03 d (4)	0.06±0.01 e (5)	0.17±0.06 b (5)	0.12±0.04 c (5)	0.58±0.14 abcd (3)	—	—	—			
	2351±600 a	3723±897 ab	1930±329 a	10836±2317 a	4953±945 b	12660±568 a	—	—	—			
	0.9±0.7 a	0.3±0.2 e	0.1±0.04 c	2.1±0.9 b	0.7±0.3 d	7.3±1.9 a	—	—	—			
Bergenbach (BE)	0.43±0.16 ab (5)	0.28±0.15 abcd (5)	0.18±0.06 cde (5)	0.35±0.15 ab (5)	0.91±0.05 ab (5)	0.17±0.06 d (5)	0.56±0.10 a (5)	0.36±0.14 abc (5)	0.65±0.19 ab (5)			
	2 349±429 a	3992±1091 ab	2337±845 a	8737±663 ab	2722±653 efg	6579±1487 b	8301±2345 ab	19748±3852 a	4010±605 c			
	0.8±0.2 a	1.4±0.8 cde	0.5±0.3 abc	3.2±1.3 ab	2.6±0.7 abcd	1.4±0.5 b	4.4±1.1 a	5.9±1.9 ab	2.7±0.8 ab			
Pip de Wolf (PW)	0.45±0.05 ab (5)	0.22±0.06 abcd (5)	0.10±0.04 de (5)	0.16±0.05 b (5)	0.55±0.08 ab (5)	0.24±0.12 cd (5)	0.35±0.08 a (5)	0.03±0.005 c (4)	0.01±0.003 d (5)			
	1647±225 a	3832±533 ab	2093±212 a	7281±893 bc	6696±1074 a	4629±1589 bcd	13068±3633 a	15801±3744 ab	13076±2428 a			
	0.8±0.2 a	1.0±0.4 de	0.2±0.1 bc	1.3±0.4 b	3.5±0.6 abc	1.8±1.2 b	4.1±1.5 a	0.4±0.1 bc	0.2±0.1 c			

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; † $p < 0.10$; ns $p > 0.10$.

Table 4 Results of nested ANOVAs for aerial biomass, Cd concentration and mass (a) and mean (\pm SE) aerial biomass (g) (**bold**), Cd concentration (mg/kg) (normal) and mean (\pm SE) Cd shoot mass (mg/plant) (*italics*) per individual (b) from metallic salts (normal), non-metallicolous (**bold**) and serpentine (*italics*) populations of *Nocccaea caeruleascens* cultivated in three Cd salt mixtures (CdSO₄ and in three Cd monometallic salts (CdSO₄, CdCl₂). For each salt treatment, population means with the same letter (**bold for biomass**, normal for Cd concentration and *italics for Cd mass*) are not significantly different with a least squares means test (SAS). Maximal Cd concentration and mass values for each treatment are underlined. Sampling number is enclosed in parentheses after the mean aerial biomass. Degrees of freedom differ among biomass and Cd analyses because sampling number was not the same

	Cadmium salt mixtures			Cadmium monometallic salts		
	100Zn 250Cd	250Zn 100Cd	250Zn 250Cd	250CdSO ₄	500CdSO ₄	500CdCl ₂
a ANOVAS						
Shoot biomass	Ori F_{2,9}=1.9 ns Pop(Ori) F_{9,47}=2.0†	Ori F_{2,9}=1.5 ns Pop(Ori) F_{9,47}=7.0***	Ori F_{2,9}=2.3 ns Pop(Ori) F_{9,47}=6.3***	Ori F_{2,5}=0.1 ns Pop(Ori) F_{5,12}=5.6**	Ori F_{2,5}=0.14 ns Pop(Ori) F_{5,30}=28.5***	Ori F_{2,2}=1.0 ns Pop(Ori) F_{2,12}=15.8***
Shoot cadmium concentration	Ori F _{2,9} =0.2 ns Pop(Ori) F _{9,23} =3.6**	Ori F _{2,9} =0.3 ns Pop(Ori) F _{9,21} =6.6***	Ori F _{2,9} =1.4 ns Pop(Ori) F _{9,19} =4.2**	Ori F _{2,3} =0.2 ns Pop(Ori) F _{3,4} =15.8**	Ori F _{2,4} =0.9 ns Pop(Ori) F _{4,7} =0.7 ns	Ori F _{2,3} =3.2 ns Pop(Ori) F _{1,3} =0.1 ns
Shoot cadmium mass	Ori F _{2,9} =0.7 ns Pop(Ori) F _{9,23} =2.2†	Ori F _{2,9} =0.8 ns Pop(Ori) F _{9,21} =3.6**	Ori F _{2,9} =3.2 ns Pop(Ori) F _{9,19} =4.8**	Ori F _{2,3} =0.4 ns Pop(Ori) F _{3,4} =13.6**	Ori F _{2,4} =0.2 ns Pop(Ori) F _{4,7} =7.5*	Ori F _{2,3} =8.7 ns Pop(Ori) F _{1,3} =1.4 ns
b Aerial biomass (g)/Cd Concentration (mg/kg)/Cd MASS (mg/plant)						
Avinières (AV)	0.90±0.34 ab (5) 575±94 cd <i>0.8±0.1 b</i>	1.34±0.14 a (5) 1131±353 bcd <i>1.5±0.6 ab</i>	0.61±0.23 abc (4) 803±92 cd <i>0.8±0.3 cd</i>	0.34±0.24 ab (4) 1991±199 bc <i>1.4±0.9 a</i>	0.78±0.30 a (5) 3547±387 a <i>1.3±0.1 ab</i>	0.13±0.01 a (4) 3199±1105 a <u><i>0.5±0.2 a</i></u>
Prayon (PR)	1.37±0.24 a (5) 635±312 cd <i>1.0±0.5 b</i>	0.37±0.22 abcd (5) 300±56 d <i>0.2±0.2 d</i>	0.97±0.32 abc (5) 673±92 cd <i>0.9±0.2 cd</i>	— — —	— — —	— — —
St Felix (SF)	1.16±0.36 ab (5) 3152±621 ab <i>5.2±0.6 a</i>	0.99±0.22 abc (5) 1644±91 ab <u><i>2.1±0.5 a</i></u>	0.99±0.38 abc (5) 2369±64 b <u><i>3.6±0.8 a</i></u>	0.012 b (1) — —	0.67±0.22 a (5) 3497±674 a <u><i>3.5±0.7 a</i></u>	dead — —
Treves (TR)	1.08±0.57 ab (4) 3417±1594 ab <i>4.7±2.2 a</i>	0.62±0.28 abcd (5) 1570±478 ab <i>1.3±0.4 ab</i>	0.21±0.08 bcde (5) 2092±941 b <i>0.8±0.6 cd</i>	0.18±0.04 ab (4) 3406±383 ab <i>0.7±0.1 ab</i>	0.03±0.01 b (5) 2660 a ^a <i>0.1 c</i>	0.07±0.02 ab (4) 3097±456 a <i>0.3±0.1 a</i>
Viviez (VI)	1.03±0.22 ab (5) 432±37 d <i>0.6±0.1 b</i>	1.12±0.16 ab (5) 1447±142 b <i>1.9±0.3 a</i>	0.60±0.18 abc (5) 2181±32 b <i>1.7±0.4 bc</i>	— — —	— — —	— — —
Baraque (BA)	0.84±0.18 ab (5) 890±410 bcd <i>0.9±0.4 b</i>	1.54±0.13 a (5) 566±160 cd <i>0.9±0.3 abc</i>	1.81±0.16 a (5) 479±100 cd <i>0.8±0.1 cd</i>	0.02 ab (1) — —	0.07±0.04 b (5) 1844±316 a <i>0.3±0.2 c</i>	0.03±0.01 b (4) <u>4603 a^a</u> <i>0.1 a</i>
Buege (BU)	0.65±0.16 ab (5) 629±116 cd <i>0.6±0.2 b</i>	1.01±0.19 abc (5) 402±49 d <i>0.5±0.1 bcd</i>	0.85±0.13 abc (5) 550±95 cd <i>0.6±0.2 cd</i>	— — —	— — —	— — —

Table 4 (continued)

	Cadmium salt mixtures			Cadmium monometallic salts			
	100Zn 250Cd	250Zn 100Cd	250Zn 250Cd	250CdSO ₄	500CdSO ₄	500CdCl ₂	
Seranne (SE)	0.74±0.27 ab (5) 1.567±.514 abc <i>1.5±0.3 b</i>	0.66±0.23 abc (5) 581±91 cd <i>0.6±0.2 bcd</i>	0.84±0.15 abc (5) 543±7 cd <i>0.6±0.1 cd</i>	0.05±0.01 ab (3) <i>915 c^a</i> <i>0.1 ab</i>	1.1±0.2 a (5) 2602±681 a <i>2.8±1.1 ab</i>		dead
St Come (SC)	0.70±0.29 ab (5) 4759±1210 a <i>4.9±2.4 a</i>	1.11±0.37 abc (5) 1334±475 bc <i>1.9±0.8 a</i>	0.57±0.17 abc (5) 3921±236 a <i>2.9±0.7 ab</i>	0.16±0.13 ab (2) <i>3871 a^a</i> <i>1.1 a</i>	0.02±0.004 b (4) <i>3565 a^a</i> <i>0.1 c</i>		dead
Wilwerwiltz (WI)	0.23±0.16 b (5) 2050±1796 abcd <i>1.6±1.6 b</i>	0.08±0.03 d (4) 1969±113 ab <i>0.2±0.1 cd</i>	0.06±0.01 e (5) 148 d ^a <i>0.1 d</i>	–	–		–
Beygenbach (BE)	0.43±0.16 ab (5) 461±29 cd <i>0.3±0.1 b</i>	0.28±0.15 abcd (5) 576±122 cd <i>0.3±0.1 cd</i>	0.18±0.06 cde (5) 1510±633 bc <i>0.4±0.2 cd</i>	0.70±0.18 a (2) 2823±251 ab <i>2.0±0.6 a</i>	0.40±0.16 a (4) 2503±632 a <i>0.7±0.2 bc</i>		0.08±0.02 ab (4) 3700±441 a <i>0.4±0.1 a</i>
Puy de Wolf (PW)	0.45±0.05 ab (5) 1847±231 abc <i>0.9±0.2 b</i>	0.22±0.06 abcd (5) 2312±352 a <i>0.8±0.3 abc</i>	0.10±0.04 de (5) 687±659 cd <i>0.2±0.1 d</i>	0.01±0.002 b (3) 2682 ab ^a <i>0.2 b</i>	0.025±0.008 b (5) – –		0.002 c (1) – –

^a n = 1

*** p<0.001, ** p<0.01; * p<0.05; † p<0.10; ns p>0.10

We did not find statistical differences in Cd concentrations among origins when plants were cultivated in mixtures of Zn-Cd, but large significant differences were found among populations of the same origin (Table 4a). For instance, SC individuals (NMET) had high Cd concentration values (3921 mg/kg) in the treatment 250Zn250Cd, whereas the other NMET populations had values below 550 mg/kg (Table 4b). Similar contrasting results were obtained between this SC population and the other NMET in the treatment 100Zn250Cd.

Likewise, the MET populations SF and TR had values above 3000 mg/kg in the 100Zn250Cd treatment, whereas the other MET populations had values lower than 650 mg/kg. For Cd mass in the same treatment, SC (NMET), SF and TR (MET) showed values of around 5 mg/plant whereas SERP individuals had Cd values lower than 1 mg/plant (Table 4b). In monometallic salts, there was a high mortality (80-100 %) for SF (MET), BA, SE and SC (NMET), and PW (SERP). Plants in 500CdCl₂ soil showed low Cd mass despite high concentration values, probably because of the high solubility of this salt decreased biomass (4 of 8 populations died in this treatment).

Physiological studies investigating Cd and Zn influx in the root apoplast suggested that the Cd transport from root to shoot was not Zn suppressible and mediated by the same transporter with a higher affinity for Cd than for Zn in the MET AV (called “Ganges ecotype”; Lombi et al. 2001b; Zhao et al. 2002). Roosens et al. (2003) further showed that the addition of Zn (10 or 100 µM) in hydroponic solutions containing Cd (30 µM) did not decrease leaf Cd concentration in SF population (also called “Ganges ecotype”), confirming the idea that Cd is accumulated independently of Zn in these populations. We tested this hypothesis for the AV population and two others by comparing the Cd concentration between the two salt mixtures (100Zn250Cd and 250Zn250Cd) and the monometallic salt 250 CdSO₄ at the same Cd concentration (250 mg Cd/kg). Cd concentrations and mass differences were tested with one-way ANOVAs followed by *a posteriori* contrasts for each population (it was not possible to test SF, BA, SE, SC and PW because of high mortality (≥80 %) in the 250 CdSO₄ treatment. In contrast to precedent studies we showed a significant decrease of leaf Cd concentrations in MET AV ($p < 0.05$) and in SERP BE ($p < 0.10$) in the two salt mixtures compared with monometallic salts.

For TR (MET), the mean Cd concentrations and mass values were not significantly different between monometallic salts and salt mixtures (see Table 4b for mean values).

Compared to Zn, only a reduced number of populations achieved the highest Cd concentrations and mass values in the different treatments (Table 4). Thus, SC (NMET) had the highest concentration values in 4 treatments and BA (NMET) and PW (SERP) in the other two treatments. The SF (MET) population showed the highest Cd mass values for 4 treatments and AV (MET) and BE (SERP) for the other two treatments.

Biomass, survival, Ni concentration and Ni mass in binary mixtures and Ni monometallic salts (Table 5)

Only 2 of the 6 treatments showed significant differences among origins. None of them involved monometallic salts. On the other hand, 5 of 6 treatments showed significant differences among populations within an origin.

Population survival was higher in mixtures since all plants had survived until the end of the experiment. Again plants cultivated in monometallic salts with high concentrations of Ni (1000 NiCl₂ and 1000 NiSO₄), had a high mortality e.g. MET (SF, TR), NM (SE, SC) and to a lesser extent in the SERP (PW). BE was the only population which showed no mortality.

There were significant differences among origins for shoot Ni concentrations in treatments with 250 mg Ni/kg only (binary mixtures and monometallic salts) (Table 5a). In addition, significant differences among populations were found in all treatments for Ni concentrations and masses, with the northern PR (MET) having less Ni than the others (Table 5b). Unexpectedly, the highest Ni concentration in mixtures was obtained for PW (SERP) at the lowest soil Ni concentration (250Zn100Ni). Similar results were obtained with TR (MET), SE and WI (NMET). The PW population had the highest Ni concentration with monometallic salts (25451 mg Ni/kg with 1000 NiSO₄).

Previous results (see Fig. 2e) showed that the Ni concentrations were significantly lower in metallic salt mixtures (Zn-Ni) than in monometallic salts (250NiCl₂) at equivalent Ni concentrations (250 mg/kg). As for Zn and Cd, we checked whether the 8 populations tested all showed such a decrease. One of the three MET

Table 5 Results of nested ANOVAs for aerial biomass, Ni concentration and mass (a) and mean (\pm SE) aerial biomass (g) (**bold**), Ni concentration (mg/kg) (normal) and mean (\pm SE) Ni shoot mass (*italics*) (mg/plant) per individual (b) from metallicous (normal), non-metallicous (**bold**) and serpentine (*italics*) populations of *Noccaea caerulea* cultivated in three Ni salt mixtures (NiCl₂) and in three Ni monometallic salts (NiCl₂, NiSO₄). For each salt treatment, population means with the same letter (**bold for biomass**, normal for Ni concentration and *italics for Ni mass*) are not significantly different with a least squares means test (SAS). Maximal biomass, Ni concentration and mass values for each treatment are underlined. Sampling number is enclosed in parentheses after the mean aerial biomass. Degrees of freedom differ among biomass and Ni analyses because sampling number was not the same

	Nickel salt mixtures			Nickel monometallic salts		
	100Zn 250Ni	250Zn 100Ni	250Zn 250Ni	250NiCl ₂	1000NiCl ₂	1000NiSO ₄
a ANOVAS						
Shoot biomass	Ori F_{2,9}=3.4† Pop(Ori) F_{9,48}=5.2***	Ori F_{2,9}=0.1 ns Pop(Ori) F_{9,48}=8.0***	Ori F_{2,9}=12.5*** Pop(Ori) F_{9,45}=1.3 ns	Ori F_{2,5}=0.4 ns Pop(Ori) F_{5,32}=6.4***	Ori F_{2,5}=0.3 ns Pop(Ori) F_{5,8}=3.8*	Ori F_{2,3}=1.4 ns Pop(Ori) F_{3,19}=8.7***
Shoot nickel concentration	Ori F _{2,9} =3.6† Pop(Ori) F _{9,22} =7.4***	Ori F _{2,9} =1.2 ns Pop(Ori) F _{9,23} =2.3*	Ori F _{2,9} =3.2† Pop(Ori) F _{9,21} =3.2*	Ori F _{2,8} =10.6*** Pop(Ori) F _{8,22} =2.2†	Ori F _{2,5} =2.9 ns Pop(Ori) F _{5,8} =10.4**	Ori F _{2,3} =2.8 ns Pop(Ori) F _{3,6} =8.1*
Shoot nickel mass	Ori F _{2,9} =0.4 ns Pop(Ori) F _{9,22} =7.3***	Ori F _{2,9} =0.6 ns Pop(Ori) F _{9,23} =2.7*	Ori F _{2,9} =3.5† Pop(Ori) F _{9,21} =3.2*	Ori F _{2,8} =6.0* Pop(Ori) F _{8,22} =1.3 ns	Ori F _{2,5} =3.0 ns Pop(Ori) F _{5,8} =3.1†	Ori F _{2,3} =2.4 ns Pop(Ori) F _{3,6} =2.3 ns
b Aerial biomass (g)/Ni Concentration (mg/g)/Ni MASS (mg/plant)						
Avinières (AV)	1.08±0.24 a (5) 330±78 b <i>0.3±0.01 ab</i>	0.78±0.11 ab (5) 417±158 bc <i>0.3±0.01 cd</i>	0.38±0.21 abcd (4) 345±28 bc <i>0.2±0.01 cd</i>	0.99±0.14 a (5) 432±119 b <i>0.4±0.1 a</i>	0.09±0.04 bc (3) 857±369 d <i>0.1±0.01 d</i>	0.64±0.09 a (5) 1994±501 c <i>1.1±0.04 ab</i>
Prayon (PR)	0.71±0.17 ab (5) 25±9 d <i>0.02±0.005 c</i>	0.28±0.07 bc (5) 76±18 c <i>0.03±0.004 d</i>	0.63±0.22 abcd (5) 124±28 c <i>0.1±0.007 d</i>	0.91±0.09 a (5) 428±140 b <i>0.4±0.2 a</i>	0.65±0.33 a (3) 1793±379 bcd <i>1.8±0.9 ab</i>	dead
St Felix (SF)	1.14±0.29 a (5) 124±37 c <i>0.2±0.01 bc</i>	1.44±0.22 a (5) 638±122 bc <i>1.1±0.2 bc</i>	1.05±0.26 abc (5) 621±89 abc <i>0.8±0.1 a</i>	0.66±0.11 ab (5) 573±34 b <i>0.4±0.1 a</i>	0.07±0.005 b (2) 1663 cd ^a <i>0.1 cd</i>	0.12±0.07 c (4) 2738±177 bc <i>0.5±0.04 ab</i>
Treves (TR)	1.34±0.34 a (5) 110±19 c <i>0.2±0.01 bc</i>	1.07±0.43 ab (5) 433±30 bc <i>0.8±0.1 bcd</i>	0.62±0.29 abcd (5) 157±24 c <i>0.1±0.03 d</i>	0.66±0.11 ab (5) 573±34 b <i>0.4±0.1 a</i>	0.07±0.005 b (2) 1663 cd ^a <i>0.1 cd</i>	0.12±0.07 c (4) 2738±177 bc <i>0.5±0.04 ab</i>
Viviez (VI)	0.79±0.12 ab (5) 132±28 c <i>0.1±0.03 bc</i>	1.18±0.08 a (5) 255±23 bc <i>0.3±0.05 cd</i>	0.70±0.04 abcd (5) 161±15 c <i>0.1±0.01 d</i>	0.66±0.11 ab (5) 573±34 b <i>0.4±0.1 a</i>	0.07±0.005 b (2) 1663 cd ^a <i>0.1 cd</i>	0.12±0.07 c (4) 2738±177 bc <i>0.5±0.04 ab</i>
Baraquette (BA)	1.67±0.20 a (5) 157±40 c <i>0.3±0.1 ab</i>	1.18±0.17 a (5) 612±74 bc <i>0.9±0.2 bc</i>	1.60±0.21 a (5) 524±83 abc <i>0.9±0.2 a</i>	0.33±0.09 c (5) 963±208 b <i>0.3±0.04 a</i>	0.05±0.04 c (3) 3553 bc ^a <i>0.07 cd</i>	0.04±0.007 c (5) 2035 bc ^a 0.02 b
Buege (BU)	0.93±0.23 a (5) 364±76 ab	1.13±0.11 ab (5) 521±90 bc	0.87±0.14 abcd (5) 563±80 abc	0.33±0.09 c (5) 963±208 b <i>0.3±0.04 a</i>	0.05±0.04 c (3) 3553 bc ^a <i>0.07 cd</i>	0.04±0.007 c (5) 2035 bc ^a 0.02 b

Table 5 (continued)

	Nickel salt mixtures			Nickel monometallic salts		
	100Zn 250Ni	250Zn 100Ni	250Zn 250Ni	250NiCl ₂	1000NiCl ₂	1000NiSO ₄
Seranne (SE)	0.5±0.1 a 1.78±0.12 a (5) 105±10 cd 0.2±0.01 bc	0.7±0.1 bcd 1.1±0.13 ab (5) 1097±87 bc 1.4±0.1 b	0.5±0.1 abc 1.15±0.27 ab (5) 449±116 bc 0.7±0.1 ab	0.74±0.06 ab (5) 748±185 b 0.6±0.2 a	0.31±0.06 ab (5) 744±227 d 0.3±0.1 bcd	dead
St Come (SC)	1.15±0.19 a (5) 222±23 bc 0.3±0.05 ab	1.57±0.20 a (5) 619±394 bc 1.2±0.7 b	0.69±0.20 abcd (5) 866±70 ab 0.8±0.2 a	0.87±0.06 a (5) 1020±110 ab 0.8±0.1 a	0.16±0.11 abc (3) 4495±828 b 0.9±0.6 abc	0.08±0.06 c (3) 4751 bc ^a 0.9 ab
Wilwerwiltz (WI)	0.17±0.06 b (5) 115±3 c 0.03±0.01 c	0.12±0.04 c (5) 1333±121 b 0.3±0.05 cd	0.58±0.14 abcd (3) 716±34 abc 0.4±0.1 bcd	–	–	–
Bergenbach (BE)	0.35±0.15 ab (5) 522±263 ab 0.2±0.1 b	0.91±0.05 ab (5) 323±81 bc 0.3±0.1 cd	0.17±0.06 d (5) 474±446 bc 0.2±0.1 cd	1.1±0.12 a (5) 1486±637 ab 1.4±0.7 a	0.38±0.13 ab (5) 4360±273 b 2.5±0.7 a	0.63±0.22 ab (5) 5231±1107 b 4.7±2 a
Puy de Wolf (PW)	0.16±0.05 b (5) 789±146 a 0.2±0.02 b	0.55±0.08 ab (5) 3549±495 a 2.3±0.3 a	0.24±0.12 cd (5) 1201±422 a 0.7±0.2 ab	0.46±0.12 bc (5) 4025±823 a 1.7±0.5 a	0.15±0.07 abc (3) 16632±2611 a 2.7±1.3 a	0.09±0.03 bc (3) 25451±1797 a 2.2±1 a

^a n=1

***p<0.001; **p<0.01; *p<0.05; † p<0.10; ns p>0.10

populations (TR), the three NMET (BA, SE and SC) and the two SERP populations showed significantly ($p < 0.05$) lower Ni concentrations values in the Zn-Ni mixtures compared to the monometallic salts (one-way ANOVAs followed by *a posteriori* contrasts). These populations were the same (except SE) as those that showed a decrease of Zn mass in Zn-Ni mixtures. PW (SERP) was the only population that showed a decrease of Ni mass between monometallic Ni salts and salt mixtures.

As for Cd, only a reduced number of populations achieved the highest Ni concentrations and mass values in the different treatments (Table 5). Thus, PW (SERP) had the highest concentration values for all treatments. The SERP populations also showed the highest Ni mass values in 4 treatments and BA and BU (NMET) in the other 2 treatments.

Biomass, Zinc concentration and Zn mass in hydroponics (Fig. 4)

Differences in aerial biomass (Fig. 4a, b) were logically significant for all of the dates of harvest for the two treatments. For the treatment at 2,000 μM (but not for the other treatment at 1.5 μM) there were significant differences between origins with the MET having higher biomass than NMET in the last three harvests. In the 1.5 μM treatment MET had higher biomass than NMET only in the last harvest.

The two treatments did not show significant differences in Zn concentration between origins (Fig. 4c, d). There were significant differences between populations (within origin) in the two last harvests in 2,000 μM with SER populations having the highest Zn values. In the low Zn treatment there were also significant differences between populations (within origin) but in this treatment the two metallicolous populations had the highest values in the last harvest. By way of comparison, when the 4 populations used in hydroponics were cultivated in Les Avinières soil, the two MET populations (AV and SF) had significantly lower Zn concentrations (mean \pm SE: 5,337 \pm 654, $n = 18$) than NMET (BA and SE; 8,674 \pm 694, $n = 16$).

There were significant differences in Zn mass between origins (Fig. 4e, f) in the two treatments with the MET populations having values largely higher than those of NMET because of their greater aerial biomass in high Zn solution and higher Zn concentration in low Zn solution. However, in low Zn solution the differences were significant in the last harvest only. Zn mass values of NMET in high Zn solution were close to

Fig. 4 Mean (\pm SE) biomass (g) (a, b), mean (\pm SE) Zn concentration (mg/kg) (c, d) and mean (\pm SE) Zn mass (mg/plant) per individual (e, f) in aerial parts of two metallicolous (Avinières, St Félix) and two non-metallicolous (Baraquette, Séranne) populations of *Noccaea caerulea* cultivated in hydroponic solutions with two Zn ($\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ form) concentrations: 1.5 μM of zinc (open symbols: b, d, f), and 2000 μM (black symbols: a, c, e). Results of mixed nested ANOVAs are shown

those of MET in the low Zn solution due to the low biomass produced by NMET in the treatment with high Zn concentration.

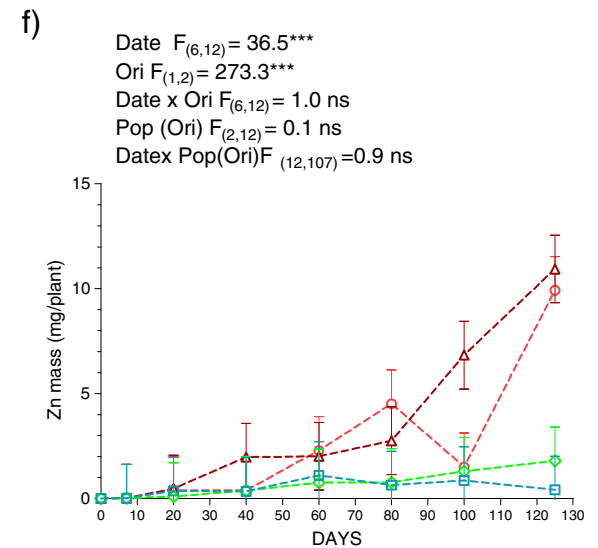
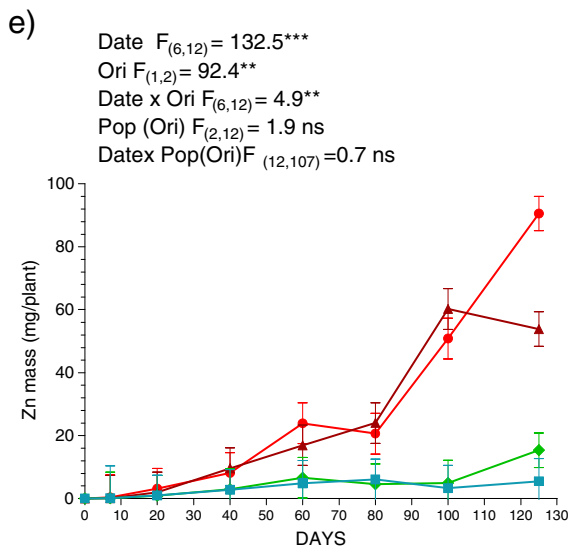
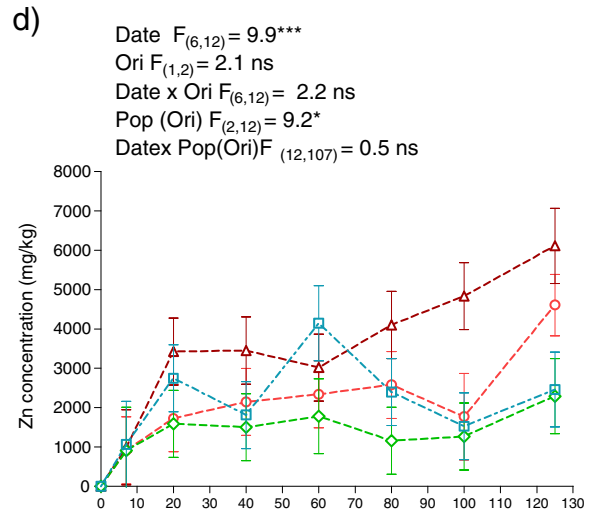
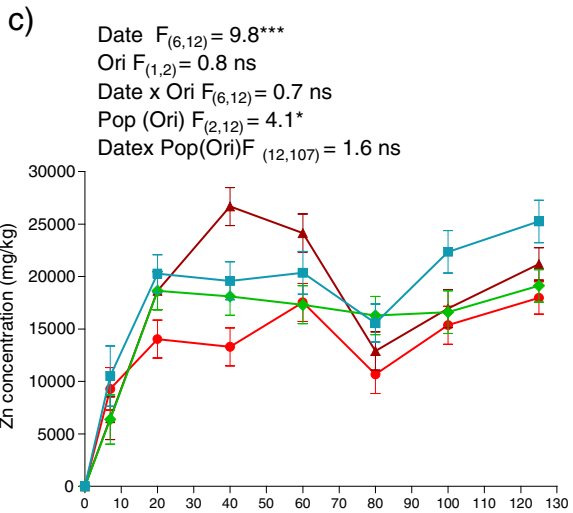
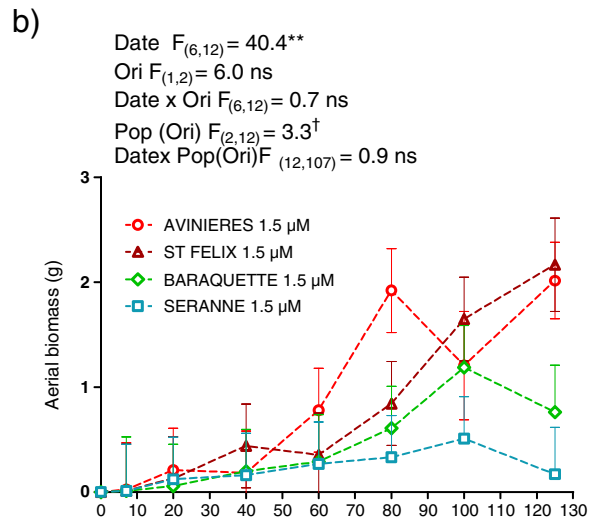
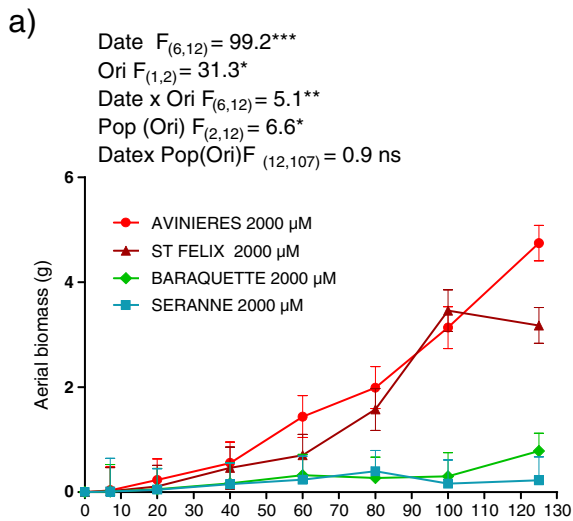
For comparison, in Les Avinières soil, Zn mass was not significantly different between the two origins (MET: 5.12 \pm 0.60, mean \pm SE, $n = 18$; NMET: 4.75 \pm 0.64, $n = 16$).

Discussion

For the first time, Zn, Cd and Ni concentrations and mass of 18 *Noccaea caerulea* populations from MET, NMET and SERP origins were compared. The analyses with the totality of treatments showed significant differences between origins for Zn and Ni concentration and mass and for Cd mass. However, there was a large heterogeneity of responses among populations depending on the substrate used in the experiments. For this reason, the origin factor was often non-significant when data were analysed treatment by treatment. Thus, even if it is possible to find *a posteriori* statistical differences in metal content or metal mass among origins (when the edaphic origin of each population is known) with many experiments, it is not possible to correctly assign a single population to its accurate origin on the basis of only one experiment.

Zinc concentration and Zinc mass

Variations among origins The present study is the first to assess metal concentration and mass of SERP populations in soil conditions. Overall, SERP populations (and particularly PW) had low biomass values but the highest Zn concentrations, followed by NMET and MET. Several studies comparing MET and NMET populations showed that NMET have higher Zn concentration capacities than MET (Meerts and Van Isacker 1997; Escarré et al. 2000; Meerts et al. 2003; Dechamps et al. 2007), which was accounted for by the need for *N. caerulea* growing on Zn-poor soil



to compensate for the low Zn availability in their natural environment (Meerts and Van Isacker 1997). The results of this study suggest that SERP populations behave like NMET with huge capacities of foliar Zn concentration. This is in agreement with Reeves et al. (2001), who showed that field-collected *N. caerulea* of PW accumulated up to 6000 mg/kg, despite the fact that the soil Zn concentration was below 70 mg/kg (Table 1). In hydroponic experiments, SERP populations had higher (Roosens et al. 2003) or similar (Assunção et al. 2003a; 2008) values of foliar Zn concentration compared to MET populations.

SERP populations had the lowest biomass and the lowest Zn mass, showing that a high Zn concentration was associated with a low biomass and therefore a low metal tolerance. Such an inverse relationship had already been suggested by comparisons between NMET and MET, with NMET populations accumulating more Zn but being less tolerant (i.e. lower biomass) to this metal (Escarré et al. 2000). Assunção et al. (2003c) also showed a negative correlation between metal tolerance and concentration among individuals from crosses between metalliculous and non-metalliculous populations of *Noccaea caerulea*. The high Zn concentration of SERP individuals did not result in a mortality increase because survival values were similar to M and higher than NM individuals.

Variations among populations In the present study, the use of several replicate populations within each origin allowed us to highlight the huge variability of foliar Zn concentration among populations of the same origin. For instance, when MET and NMET populations were cultivated in Les Avinières soil, a continuous distribution of Zn concentration values was observed, but there was still a significant mean difference between origins overall (with NMET showing higher values than MET, Fig. 2) which confirms previous results (Escarré et al. 2000; Frérot et al. 2005).

Mass values, which combine concentration and tolerance (assessed by biomass and survival), were also highly variable among populations, mainly for SERP and NMET. For instance, in 1500Zn, the SERP PW had Zn mass levels 10 times lower than the SERP BE, and the NMET BA had Zn mass levels 13–30 times lower than the NMET SER.

Interactions between elements The results of this study showed that 7 out of 8 populations had lower

Zn concentration values in Zn-Cd salt mixtures than in monometallic salts at the same Zn concentration (250 mg Zn/kg). Similarly, Roosens et al. (2003) showed that exposing two French MET populations (among which SF) to high Cd decreased their Zn concentrations. Zn and Cd are classed in the transition-elements subgroup of the Periodic Table. The addition of Cd to the soil resulted in a decrease in the leaf Zn concentration either by inhibiting the root Zn uptake or because they share a common transport, such as those of the ZIP family (Hart et al. 2005). For instance, ZIP2 is a Zn transporter but also has a good affinity for Cd and Cu (Grotz et al. 1998).

Overall, in Zn-Ni mixtures plants had Zn concentrations lower than in monometallic salts. However, only 4 of 8 populations had significantly lower Zn concentration values in Zn-Ni mixtures. There were non-significant differences for the 4 others. This suggests that Zn-Ni interaction is weaker than Zn-Cd interaction. Peer et al. (2003) analysed the Zn-Ni interference with many plant species from MET and SERP sites, including some *N. caerulea* populations from this study. They showed that PW individuals had higher Ni and Zn concentration in mixtures (Ni (NO₃)₂·6H₂O and Zn(NO₃)₂·6H₂O with 100 µg Ni g⁻¹ dry weight and 100 µg Zn g⁻¹ dry weight) than in a single metal soil, whereas individuals from SF did not show significant differences in Zn concentration in either treatments. The results from SF are in agreement with the current results, whereas those of PW are the opposite because PW individuals showed a significant decrease in Zn concentration in mixtures. These differences illustrate the difficulty of comparing experiments performed with different salts and concentrations.

Perspectives from field data Four populations, two NMET (SC, WI) and the two SERP populations, exceeded the hyperaccumulation threshold for Zn in a few treatments. Even in Les Avinières soil, only WI (NMET) showed Zn values above 10000 mg/kg. According to these results, MET populations from southern France are not zinc hyperaccumulators on average according to the “classic” concentration threshold (Baker and Brooks 1989). AV individuals harvested in situ had Zn mean values of 7310 mg/kg (±646) (Escarré et al. 2011). However, *Noccaea caerulea* from PR (MET) (Zn in soil 18360 mg/kg) had mean leaf Zn concentrations up to 13400 mg/kg (Faucon 2004).

In 9 NMET soils in Switzerland (<120 mg/kg of Zn in soil), Zn leaf concentrations values averaged 4857 mg/kg (Basic et al. 2006), and in 15 NMET populations from Luxembourg (soil=8.6 mg/kg Zn) mean Zn leaf values were 7300 mg/kg (Molitor et al. 2005). Banášová et al. (2008) found mean Zn concentrations of 13650 mg/kg and of 10729 mg/kg in MET and NMET populations, respectively, in central Slovakia. However, the SE populations as well as other NM populations from the Larzac plateau had concentrations of up to 2510 mg/kg Zn (Noret et al. 2005; Lefèbvre and Escarré, unpublished results) for the same soil Zn levels as in Luxembourg.

The numerous Zn treatments used in this study showed that the highest Zn concentrations and masses were achieved by different populations. This suggests that any Zn phytoremediation program should first allow a preliminary experiment to select the best accessions in those particular conditions. Of particular note, a large within-population variation also exists in some accessions, showing that artificial selection of efficient tolerant and hyperaccumulating genotypes might be possible.

Cd concentration and mass

Variations among origins There were no differences in Cd concentration among origins, nor were there differences between MET and NMET from southern France as found previously by Escarré et al. (2000) in Les Avinières soil with few populations. Similarly, Dechamps et al. (2005) did not find any differences in Cd concentration between MET populations from Belgium (including PR) and NMET populations from Luxembourg (including WI). However, in the current study, MET had overall a higher mean Cd mass than NMET and SERP populations, which can be accounted for by a higher tolerance (estimated here by overall mean biomass and a slightly higher survival) of MET on high soil Cd concentration (MET:71 %; NMET:53 %; SERP: 63 %). Hydroponic experiments (Assunção et al. 2003a; Roosens et al. 2003) had shown that SERP populations are particularly intolerant to Cd.

Variations among populations The results of this study highlight important variations within origins, i.e. among populations of the same origin. The SF (MET) had the greatest Cd values (in particular for

the Cd mass), which was in agreement with the hydroponic results of Roosens et al. (2003). This high Cd mass capacity does not seem to be related to the soil Cd concentration of the SF site (36 mg Cd/kg; Table 1) as similar levels were measured in sites of populations that did not accumulate as much Cd (e.g. TR 41 mg Cd/kg). Some populations considered to be high Cd accumulators such as “Ganges ecotype” (here AV) can show extraordinary fluctuations in their shoot concentrations depending on treatments used whereas other populations such as SC (NMET) and BE (SERP) showed high Cd concentrations in many treatments. Therefore, the use of the term “ecotype”, which refers to “an intraspecific product of environmental selection arising as a result of genotypic response to a particular habitat” (Gregor and Watson 1961) should be avoided to refer to AV and SF populations for two reasons. First, the adaptive function of Cd accumulation has not been shown yet, and secondly, the high Cd foliar concentration is not only limited to these populations, but also to other NM and SERP populations.

This study also showed an important mortality in the 3 monometallic Cd treatments, even at 250 mg Cd/kg (e.g. 5 of 8 populations had at least 80 % mortality, among which was the SF population). As there was almost no mortality in binary mixtures with the same Cd concentration, this suggests that the lack of Zn in the presence of Cd decreases survival.

Interactions between elements The addition of Zn in the soil significantly decreased the shoot Cd concentration in the 2 salt mixtures compared with monometallic salts at the same Cd concentration, suggesting that they probably share a common transporter. However, some studies have shown that the influx of Cd in root apoplast was not Zn-suppressible in some MET populations, such as AV or SF (Lombi et al. 2001b; Roosens et al. 2003), suggesting that the accumulation of Cd was mediated by a transporter with a higher affinity for Cd than for Zn. Here, after 3 months of growth in soil a significant decrease in leaf Cd concentration in salt mixtures was found in AV, as well as in BE (SERP), compared with those obtained in monometallic salts at the same concentration (250 mg Cd/kg). This indicates that the leaf Cd concentration was lowered by the addition of Zn in soil. However, for TR (MET), the mean Cd concentration was not significantly different among monometallic salts and salt mixtures, and the Cd mass was even

higher in a salt mixture (100Zn250Cd) compared to monometallic salts. In the current study, the Cd mass difference is linked to differences in biomass because the Cd concentrations were very similar between a salt mixture (100Zn250Cd) and the monometallic salt (250CdSO₄).

Perspectives with field data All populations were found to hyperaccumulate Cd over the threshold (100 mg/kg), even in Les Avinières soil, where the Cd concentration of plants was the lowest. NMET plants collected in situ in southern France accumulate very small amounts of Cd (mean 29 mg/kg Cd, $n=5$; Lefèbvre and Escarré, unpublished results) compared with plants from mine sites around Saint-Laurent-le-Minier (mean 1280 mg/kg, $n=24$; Escarré et al. 2011). Molitor et al. (2005) obtained an average of 31 mg/kg ($n=15$) for the NMET from Luxembourg. Swiss and Slovak NMET populations had higher Cd concentrations (263 and 127 mg/kg, respectively; Basic et al. 2006; Banášová et al. 2008).

The Cd concentrations of plants cultivated in Les Avinières soil were largely lower than those obtained when using metal salts. With monometallic salts, most populations showed Cd values higher than those found in plants collected in situ and may provide inaccurate information on the phytoremediation performances of the species. However, the values obtained with salts were still largely below the level of 14000 mg/kg obtained in hydroponics by Lombi et al. (2001b).

Ni concentration and mass

Variations among origins SERP plants generally showed the highest values of Ni concentration and mass with Ni salts, while MET and NMET had rather similar lower values. A similar ranking among origins was obtained in hydroponics by Assunção et al. (2003a; 2008). In soil experiments, Dechamps et al. (2008a) showed that a NMET population accumulated more than 15 times more Ni than the MET PR. This suggests that SERP *Noccaea* plants are better adapted to Ni availability than MET and NMET. The adaptation of plant species to serpentine soil is frequent. For instance, in *Helianthus exilis* populations from serpentine and normal soils, growth was significantly higher in their respective soils, showing ecotypical differentiation (Sambatti and Rice 2006). The same result was found for *Collinsia sparsiflora* (Wright et al. 2006)

and *Cerastium alpinum* (Berglund et al. 2004). An exception is the case of *Thlaspi goesingense*, where plants from serpentine and normal soils had a similar growth on both soils (Reeves and Baker 1984) showing that tolerance to Ni is constitutive in this species.

Variations among populations There were significant variations for biomass, Ni concentrations and mass among populations for almost all Ni treatments. In particular, the two SERP populations often showed different responses, with PW having lower biomass values and higher Ni concentrations than BE, which was in agreement with field concentration results (BE 1,882–5945 mg Ni/kg; $n=4$; PW 3170–8550 mg Ni/kg $n=4$, Reeves et al. 2001). PW was the population which showed the highest Ni concentration values for all of the Ni treatments and the highest Ni mass values in 3 of 6 treatments. The 1000 NiSO₄ treatment decreased survival as 4 of 8 populations had a mortality of ≥ 80 %.

Interactions between elements Cataldo et al. (1978) showed that the transfer of Ni from root to shoot in soybean was inhibited by the presence of Zn²⁺. In the current study, the antagonism between Zn and Ni appeared at the concentration of 250mgNi/kg, particularly for serpentine populations. The latter had Ni concentration values that were four times lower, and Ni mass values two to seven times lower in binary mixtures than in monometallic salts at equivalent concentrations. Surprisingly, in salt mixtures, the lowest concentrations of Ni (100 mg/kg) produced the highest Ni concentration values for TR (MET), SE and WI (NMET) and PW (SERP). A similar phenomenon was reported by Assunção et al. (2008), where the highest concentration of a metal (in our case 250Zn) inhibited the concentration of another metal less than a lower concentration (100Zn250Ni). Taylor and Macnair (2006) showed an inhibition of Ni translocation from roots to shoots in presence of Zn in two serpentine endemic species, *Thlaspi pindicum* (*Noccaea tymphaea*) and *T. alpinum* var. *sylvium* (*Noccaea sylvia*), and concluded that Ni transport was achieved by acting on a pre-existing Zn transporters and therefore that Zn accumulation may have evolved first.

Perspectives with field data The SERP PW was the only one to almost always reach the Ni hyperaccumulation threshold of 1000 mg/kg, even in the 250Zn100Ni

treatment. Nickel can be highly accumulated in leaves of field-growing NMET plants (178 mg Ni/kg with a soil Ni concentration of 1 mg/kg, $n=15$; Molitor et al. 2005). It is therefore obvious that any phytoextraction program intending to improve Ni extraction should use serpentine populations.

Comparisons of Zn concentration and mass between soils and hydroponics

The results of Zn concentrations in hydroponics are close to those of Shen et al. (2000; Zn concentration in solution of 500 μ M), Zhao et al. (1998; 1000 μ M) or Brown et al. (1995; 3600 μ M Zn in solution) after approximately 1 month of culture when using the metallicolous PR population. Brown et al. (1995) showed values of Zn concentration of 26,000 mg/kg and Zn mass of 35 mg/plant with 3600 μ M Zn after 30 days of growth. In the present study, about 22000 mg/kg was obtained in 2 mM Zn concentrations for MET and NMET plants, and masses of around 70 and 10 mg/plant for MET and NMET, respectively after 3 months of growth. These values are considerably higher than those obtained with the soil from Les Avinières mine which, nevertheless, had a very high Zn concentration. When AV and SF (MET) populations, were cultivated in soils, they had a Zn concentration of about 1/4 and a Zn mass of 1/10 of those obtained in hydroponics. In addition, when MET and NMET populations were cultivated in Les Avinières soil, they showed significantly different mean Zn concentrations, which was not the case in high Zn hydroponic solution. In addition, the results between the two Zn treatments in hydroponics were very different. In the low Zn solution differences between origins for biomass, Zn concentration and Zn mass were significant only in the last harvest after 125 days of growth. If we had stopped the experiment after 90 days of growth (as for the experiments with soil) results obtained in the low Zn concentrations would not have shown a clear pattern between the populations of the two origins. In the high Zn solution, MET had higher biomass and Zn mass than NMET in the last three harvests.

In mine soil, there was no Zn mass differences between MET and NMET compared to the large differences in hydroponics. Thus, the results obtained in hydroponics and in Les Avinières mine soil are contradictory.

We suggest that these contradictory results may be due to the fact that in hydroponic culture, Zn is directly available because of the low pH of the nutrient solution and there are no complex molecules like organic matter or clay, which may interact with Zn absorption. Similarly, Assunção et al. (2003a) compared Zn concentrations in MET and NMET in hydroponics but did not find significant differences between the Ganges population (named here AV) and the non-metallicolous population from Lellingen (close to WI).

Conclusion

This study shows that differences in concentrations and masses occur between MET, NMET and SERP origins when taking into account all treatments. However, there was also a large heterogeneity of responses in terms of metal concentration among populations of the same origin. The numerous treatments used in this study clearly show that the highest Zn concentrations and Zn masses were achieved by different populations. MET populations have higher Cd masses, mainly due to higher Cd tolerance in Cd-contaminated soil. It was also shown that the addition of Cd or Ni to a Zn-contaminated soil significantly decreased the shoot Zn concentration. Ni experiments showed that serpentine populations were particularly adapted to Ni-rich soils as they had the highest values of Ni concentration, but they were also able to have high Zn concentrations in Les Avinières mine soil. Finally, the experiments in hydroponics give Zn concentrations and Zn mass far higher and contradictory with those found in a mine soil. Data on metal concentration obtained in culture soils are closer to those in field soils than those from hydroponics so that they could give a more accurate information on the accumulating capacity of *Noccaea caerulea* and its use in phytoextraction of metals in field conditions.

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