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REGULAR ARTICLE

Nickel bioavailability in an ultramafic toposequence in the Vosges Mountains (France)

**V. Chardot · G. Echevarria · M. Gury ·
S. Massoura · J. L. Morel**

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Abstract A serpentinised harzburgite outcrop located in the Vosges Mountains hosts a population of the Ni-hyperaccumulator *Thlaspi caerulescens* J. & C. Presl. A complete study was undertaken to relate the variability of Ni availability along the ultramafic toposequence to pedogenesis, soil mineralogy and functioning with X-Ray Diffraction, Transmission Electron Microscope observations coupled with Isotopic Exchange Kinetics and diethylenetriamine pentaacetic acid extraction of Ni. The soil profiles ranging from Dystric Cambisol to Hypermagnesic Hypereutric Cambisol were distributed unevenly along the toposequence probably due to geochemical variability of the bedrock and also complex quaternary erosion features. The richest soils were characterised by slight mineral weathering leading to Ni, Cr and Fe accumulation in the B horizons whereas the lowest saturated soils had very low-metal contents. Most soil minerals were inherited from the parent materials and there were only few traces of formation of secondary minerals. Primary minerals (e.g. serpentine, chlorite) contained low Ni concentrations (0.2%) whereas neoformed goethite, mainly in the B horizons of the richest soils, contained up to 4.3% Ni. Ni was probably sorbed onto amorphous Fe oxy−hydroxide particles (oxalate extraction) rather than incorporated within the crystal lattice of goethite. Ni availability in the B horizon of Hypereutric Cambisols was extremely high and so was the oxalate extractable Fe. At the toposequence level, there was a high level of Ni availability in the least weathered soils and a very low-availability level in the more intensively weathered soils (strongly acidic pH). Ni availability was unexpectedly positively correlated to pH and was controlled by soil mineralogy and Ni-bearing mineral phases. Ni hyperaccumulation (above 1,000 mg kg^{-1}) by native *T. caerulescens* was only reached in the Ni-rich soils as a consequence of the local edaphic factors. Ni uptake by *T. caerulescens* is strongly regulated by Ni availability in soils and therefore related to pedogenesis.

Keywords Ultramafics · Bioavailability · Soil mineralogy · Isotopic exchange kinetics · Thlaspi caerulescens · Metal hyperaccumulators

Introduction

The worldwide distribution of ultramafic rocks is of great interest because they cover only a small portion

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V. Chardot · G. Echevarria (✉) · M. Gury ·
S. Massoura · J. L. Morel
Laboratoire Sols et Environnement, UMR INPL-INRA
1120, Nancy-Université, ENSAIA, BP 172, 54505
Vandoeuvre-les-Nancy Cedex, France
e-mail: guillaume.echevarria@ensaia.inpl-nancy.fr

S. Massoura
Andra (Agence nationale pour la gestion des déchets
radioactifs), Parc de la Croix Blanche, 1-7, rue Jean
Monnet, 92298 Chotenay-Malabry Cedex, France

of the earth's land surface (<1%) and yet are host to distinctive flora (Brooks, 1987). Serpentinite is a metamorphic rock formed from a low temperature hydrothermal alteration of igneous ultramafic rocks, classified as peridotites (O'Hanley, 1996). Ultramafic rocks and especially serpentinites contain very high magnesium (18–24%) and high iron (6–9%) but very low Ca (1–4%) and aluminium (1–2%) (Alexander, 2004). In a region with a cold or a humid temperate climate, three important specific pedogenic processes can develop on such ultramafic outcrops, provided that there is no interference with other pedogenic processes: (a) fast Ca leaching, (b) formation on the surface of a black humus of the *mull* type, (c) formation, under the surface, of a clay texture horizon, generally poorly structured following Ca leaching (Duvigneaud, 1966). These soils, generally termed serpentine soils, are also strongly influenced by the geochemistry and mineralogy of the parent material (Alexander, 2004). They share a number of chemical particularities, including a high content of specific heavy metals (nickel, chromium and cobalt), a low-Ca:Mg concentration ratio and low concentrations of macronutrients (Brooks, 1987). Serpentinites and serpentinised peridotites generally contain the following primary minerals according to their genesis: Serpentine (chrysotile, antigorite and lizardite), pyroxenes, magnetite, chlorite, talcs and other trace minerals. Serpentine is, of course, the most abundant one. However, the minerals found in serpentine soils will differ considerably according to soil genesis and age (Bonifacio, Zanini, Boero, & Franchini-Angela, 1997; Caillaud, Proust, Righi, & Martin, 2004; Gasser & Dahlgren, 1994; Lee, Sears, Graham, Amrhein, & Vali, 2003). According to drainage properties, lixiviation of Mg and Si, secondary clay minerals may appear, such as Mg-rich or Fe-rich smectites, high- and low-charge vermiculites (Bonifacio et al., 1997; Bulmer, Lavkulich, & Schreier, 1992; Lee et al., 2003; Rabenhorst, Foss, & Fanning, 1982). Secondary Fe oxides are also frequent in serpentine soils and often accumulate (Alexander, 2004; Bonifacio et al., 1997). Hematite, and more importantly, goethite form after release of Fe and other insoluble metals during weathering of clay minerals. Neo-formed goethite may be a very important sink for Ni and Cr in serpentine soils (Becquer et al., 2001; Schwertmann & Latham, 1986). The serpentine soils are often of ecological, agronomic or environmental interest due to their specific functioning and to the nutrition stress they impose on plants (Alexander, 1988; Brooks, 1987; Gasser & Dahlgren, 1994). However, very few studies have been published on the relationships between those processes and the availability of associated trace metals (Quantin, Becquer, Rouiller, & Berthelin, 2001) since the works of Gasser et al. in the mid-nineties.

Among the metals widely presents in ultramafic soils, Ni is probably the one that causes most significant toxicity to non-adapted plants. Ni bioavailability is, in general, sufficient to create a physiological stress on most of the plant species growing on such soils. Accordingly, the flora and vegetation of serpentine areas often differ markedly from that of adjacent, normal soils (Brooks, 1987). The vegetation growth is limited by a number of factors, including soil shallowness and permeability, low-nutrient levels including Ca deficiency, Mg toxicity and high concentrations of potentially toxic elements, such as Cr, Ni and Co (Brooks, 1987). Ni-hyperaccumulators plants, which can exceed 1,000 μg g^{-1} of Ni (Jaffré, Brooks, Lee, & Reeves, 1976), are very often endemic to serpentine soils. These unique plants are potentially important tools for the phytoremediation of soils contaminated with metals from anthropogenic sources (Baker, McGrath, Sidoli, & Reeves, 1994).

Nickel availability in soils depends on many environmental and pedological factors. Although pH appears to be the most influencing factor on Ni solubility in soils (Anderson & Christensen, 1988), available compartments of Ni seem to depend mainly on the mineral bearing phases and the speciation of Ni within these species (Becquer et al., 2001; Quantin et al., 2001). Either high charge clay minerals (e.g. smectites) or poorly crystallised Fe oxides are the main contributing phases to Ni availability depending on pedological processes occurring in ultramafic areas (Massoura et al., 2006). However, Ni availability is extremely low in strongly weathered ultramafic soils when well-crystallized Fe oxides are the main Ni-bearing mineral phase, such as in ultramafic laterites (Massoura et al., 2006).

Isotopic exchange kinetics (IEK) have been used to assess plant available Ni (Echevarria, Morel, Fardeau, Leclerc-Cessac, 1998). Labile Ni was also shown to be the available pool of Ni-hyperaccumulator plants despite their incredibly high Ni uptake from soils (Shallari, Echevarria, Schwartz, & Morel, 2001; Massoura, Echevarria, Leclerc-Cessac, & Morel, 2004). The IEK method is therefore suitable to assess the potential risk of transfer of Ni from soils to plants, through the characterisation of intensity, buffer capacity and quantity. The isotopically exchangeable pool refers mostly to the element retained through sorption and surface-complexation onto soil particles or accessible through solid-diffusion processes and may provide information as to whether the element is included or not in crystal lattices (Massoura, 2004; Massoura et al., 2006; Sarret et al., 2004).

One of the smallest and most isolated serpentine outcrops hosting a Ni-hyperaccumulator plant- namely *Thlaspi caerulescens*- is located in the Vosges Mountains (Reeves, Schwartz, Morel, & Edmondson, 2001). The aim of this study was to relate the variability of Ni availability along the ultramafic toposequence to soil genesis, mineralogy and functioning. Also, the distribution pattern and Ni uptake by native *T. caerulescens* was studied according to pedological properties and Ni availability as a first step to understand the behaviour of this plant in situ. Therefore, fine soil characterisation and mineralogy (XRD), identification of Ni-bearing mineral phases with TEM–EDX, full description of Ni availability with the IEK method and complementary diethylenetriamine pentaacetic acid (DTPA) extractions were carried out on nine pedons along a transect on the ultramafic outcrop. Distribution of plant species and especially *T. caerulescens* was also studied along the toposequence and their Ni content was determined.

Materials and methods

Study area and soil characteristics

The study was carried out in the Vosges Mountains on the ultramafic outcrop of Bergenbach in the municipalities of Oderen and Fellering (47°54.5′N – 06°57.5′E) located in the Haut-Rhin district (north-east of France). The climate is characterised by an annual rainfall of ~1,500 mm with evenly distributed precipitation over the year, and a mean temperature of ~7°C (closest meteorological station). The studied soil is developed on serpentinite bedrock (highly serpentinised peridotite: harzburgite). The outcrop (a few tens of ha) is however surrounded by gneiss, which is present above at the hilltop down to the highest point of the outcrop (with a 20 m elevation difference between the hilltop and the upper part of ultramafics). Therefore, some non-ultramafic material from glacial ages may have contaminated the site surface at some points. Soil occupation is permanent pasture.

A toposequence was studied across the whole outcrop from 840 m downhill to 764 m (Fig. 1). Soil profiles were observed throughout the toposequence. Nine soil observations (from I down to IX) were made and each pedon was carefully described. Soil horizons were each fully characterised (e.g. colour and structure) and sampled whenever it was possible. Samples of each horizon were taken, air-dried and sieved at a 2-mm mesh size, prior to analyses.

On 21 April 2006, a survey of the distribution of the Ni hyperaccumulator *T. caerulescens* J. & C. Presl over the whole site was realised. At each location, its presence and abundance was recorded as follows: absent (−), rare (+), common (++) or abundant (+++). Shoot samples of *T. caerulescens* were collected for analysis whenever it was present (samples consisted of mixed plant material of at least three individuals). Samples were rinsed with deionised water and dried for 48 h at 70°C. They were then ashed in a microwave oven in concentrated acids 0.5 g DM in 2 ml H_2SO_4 (98%), 6 ml HNO_3 (98%) and 6 ml H_2O_2 (30%). Solutions were filtered and adjusted to 25 ml with 0.1 M HNO_3. Acid 0.2 ml HNO_3 (98%) was added to soil solution sample extracts to prevent Ni from sorbing onto the vial surface before analysis.

Identification of soil minerals and location of Ni

To achieve the identification of Ni-bearing phases, two contrasted pedons from the toposequence were chosen. The first one (V) was a typical moderately desaturated Cambisol whereas, the second one (VIII) was a saturated Mg-rich, Fe-rich and Ni-rich Cambisol (See soil description in the Results section). X-Ray diffraction (XRD) analysis was performed on the surface horizon samples of soils V and VIII on the < 50-μm fraction using a D8 Bruker diffractometer with Co Kα1 radiation ($\lambda = 1.7902$ Å). The diffractometer is

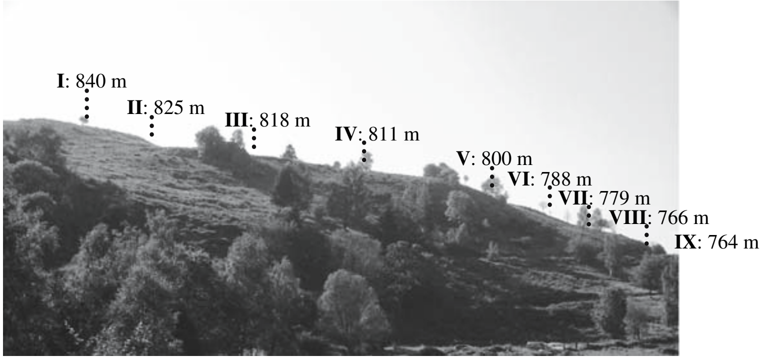


Fig. 1 Profile of the ultramafic toposequence sampled in Bergenbach (Oderen, Vosges Mountains, Haut-Rhin) on a harzburgite outcrop. Locations of the nine different observation sites are reported with their number (in roman) and altitude

equipped with a (θ, 2θ) goniometer and a position sensitive detector (PSD). X-ray diffractograms were collected on powder samples at room atmosphere and temperature, within the 2θ range [3, 65°], with 0.035° step and 2 s collecting time. Organic matter (OM) was removed and samples were decarbonated prior to analysis.

The surface horizon samples were also observed with a transmission electron microscope (TEM) (Phillips CM 20). The finely powdered soil samples were suspended in ethanol under ultrasonication. A drop of suspension was then deposited on a carbon-coated film supported by a copper grid. EDX analysis of selected particles was carried out using an EDAX spectrometer mounted on a Phillips CM 20 TEM operating at 200 kV.

Selective dissolution of Fe oxides was carried out using two chemical reactants to characterise the degree of crystallisation and therefore the degree of evolution of the soils. The two extraction procedures were carried out independently on different aliquots of each soil sample, thus it was not a sequential extraction procedure. An oxalate extraction was performed (McKeague & Day, 1966) for amorphous ferrous oxy–hydroxides. The dithionite–citrate–bicarbonate (DCB) extraction was run (Mehra & Jackson, 1960) for well-crystallised Fe oxy–hydroxides. Ni concentrations in each of the Fe extraction solutions was measured (Massoura et al., 2006). Ni and Fe extracted by oxalate are denoted, respectively, by Ni_o and Fe_o. Ni and Fe extracted by DCB are denoted, respectively, by Ni_d and Fe_d. Fe_o quantifies Fe in amorphous Fe oxy–hydroxides. Well-crystallised Fe oxy–hydroxides are calculated as Fe_d–Fe_o. Metal surface-complexed to OM may also be extracted by oxalate.

Nickel availability

The IEK in soil suspensions allow the description of Ni availability in a given soil: It determines the contribution of the various pools available in the solid phase to buffer the concentration of the element in the soil solution. The method consists in introducing tracer $^{63}Ni^{2+}$ ions in a soil:solution suspension to monitor with time the pools in the solid phase that exchange Ni with the soil solution. The kinetics of disappearance of the $^{63}Ni^{2+}$ ions in solution and their dilution within solid phase exchangeable pools allow calculation of the various time-dependent labile pools (i.e. E_t). Full details for the calculation of the different pools E_t are given in earlier work (Echevarria et al., 1998). The method quantifies: the intensity -C_{Ni}- (i.e. the concentration of Ni in the soil solution, in μg l^{-1}), the quantity or labile pool—E_t—(i.e. the Ni ions from the soil solids that are able to supply the soil solution according to their average time of exchange, t, in mg kg^{-1}) and the buffer capacity –C–, i.e. the capacity of the soil solid phase compartments to thermodynamically maintain Ni ions in the soil solution, in l kg^{-1}). Weakly sorbed Ni, or instantaneously exchangeable Ni, along with soluble Ni, is quantified by the labile metal after 1 min—$E_{0-1\ \mathrm{min}}$—(Echevarria et al., 1998). The exchangeable metal over a period of 3 months—$E_{1\ \mathrm{min-3\ months}}$—corresponds to more strongly bound sorbed pools or diffusion-limited available Ni and generally represents the biggest reservoir of available Ni in soils (Echevarria et al., 2006). Unavailable metal, either located in the microporosity of soil constituents or occluded in the crystal lattices of minerals, is assessed by the unexchangeable pool (Sarret et al., 2004).

Isotopic exchange kinetics were performed on the dry material from each of the nine surface horizons of the toposequence. IEK were replicated three times on each soil sample. Suspensions with 10 g of soil and 100 ml of deionized water [1:10 soil : solution ratio (w : v)] were mixed in an end-over-end shaker for 17 h until the concentration of Ni in the solution was constant (three replicates). About 1 ml of a $^{63}Ni^{2+}$ solution was then injected into the suspension, which was shaken continuously. At this addition rate, there was no effect on stable Ni solubility. Five aliquots of the suspension (3 ml) were sampled with a syringe after 1, 4, 10, 40 and 100 min immediately filtered through cellulose nitrate filters (porosity 0.2 μm, SARTORIUS, Edgewood, NY, USA). Radioactivity in the solution was measured in the five filtered aliquots. C_{Ni} in the solution was measured in the filtered solutions from the suspension in the 100 min aliquot (a greater volume was filtered).

The DTPA–TEA extraction solution consisted of 0.005 M DTPA with 0.01 M $CaCl_2$ and 0.1 M triethanolamine (TEA) at pH 7.3 (Lindsay & Norvell, 1978). Soil samples from each horizon of the nine pedons were extracted with the DTPA–TEA solution. Extractions were performed in triplicate. A ratio of 1:10 soil : DTPA-TEA solution was shaken for 2 h; then the suspension was centrifuged at 4,000*g* for 20 min, filtered through a 0.2 μm porosity cellulose nitrate filter (SARTORIUS). The quantity extracted per kg of soil is noted Ni_{DTPA}.

Radioactive Ni in solution samples (IEK) was determined by β^- counting using a liquid scintillation spectrometer (liquid scintillation cocktail: Ready Safe, BECKMAN, Fullerton, CA, USA; spectrometer PACKARD 460 CD). The concentration of Ni in solutions (IEK and DTPA extractions) was determined by ICP-AES (VARIAN, Palo Alto, CA, USA).

Statistical analyses

Pearson correlation matrix was calculated for all soil physico-chemical parameters with STATBOX-PRO™ (GRIMMER LOGICIELS, Clignancourt, France) and Newman–Keuls tests ($P = 0.05$). Linear regressions were calculated with the statistical tool of Excel software (MICROSOFT®).

Results

Soil variability and classification along the toposequence

The richest soils found in the toposequence were classified as Magnesic Hypereutric Cambisols (WRBSR-FAO classification). The term ‘Magnesic’ refers to an exchangeable Mg : Ca ratio >1 (Table 1) but we proposed the term Hypermagnesic to specifically designate soils with exchangeable Mg:Ca ratio >3 that are more typical from ultramafic origin. Therefore, those soils could be named Hypermagnesic Hypereutric Cambisols (I, II, III, VII, VIII). Two of the less saturated soils were classified as Magnesic Eutric Cambisols (V & VI), one was classified as an Eutric Cambisol (IX), and the more weathered soil was classified as Dystric Cambisol (IV). The Mg:Ca ratio in the toposequence was inversely correlated to base saturation, so no Magnesic Dystric Cambisol was found. The distribution of the soils in the toposequence did not follow a simple pattern. The native vegetation found on most of the site (e.g. *Calluna vulgaris*, *Vaccinium myrtillus*, *Pteridium aquilinum*) is usually found on humus of the mor/moder type in the Vosges Mountains, however, the humus in this soil is an organic carbon-rich mull, except on the pedon IV (Dystric Cambisol). In general, all soils from the area have surprinsingly highly saturated cation exchange capacity (CEC) with regard to usual soils developed on Gneiss or Granite at this same altitude in the Vosges. Their pH may also vary throughout the year but it is also moderately acidic compared to similar conditions elsewhere in the Vosges. The soils are also extremely infertile with regard to K and P availability (The bedrock shows very low-K contents). There is an obvious lack of fertilisation in this area despite its use for pasture.

Soil mineralogy and identification of Ni-bearing phases

The XRD analysis on the different horizons of two representative pedons of the toposequence (Hyperamagnesic Hypereutric Cambisol ‘VIII’ and Magnesic Eutric Cambisol ‘V’) revealed the presence of various mineral phases varying from primary to secondary minerals (Table 2). In general, the

Table 1 Physico-chemical characteristics of different horizons of the nine soil profiles along the toposequence in Bergenbach

Location	Horizon	Sampling depth	Colour	pH		Particle size distribution			Organic C	C : N ratio
			Munsell's code	Water	KCl 1 M	Clay	Silt g kg^{-1}	Sand		
		Cm								
Bedrock			–	7.9						
I	A_1	0–15	10 YR 2/3	5.3	4.8	329	507	164	87.8	11.5
II	A_1	0–15	7,5 YR 2/2	5.8	5.3	343	446	211	67.3	10.9
III	A_1	0–20	10 YR 2/3	5.6	4.7	209	426	365	33.8	11.4
	B	20–40	10 YR 2/3	6.0	5.3	187	450	363	14.4	12.3
	B/C	40–60	10 YR 3/3	6.3	5.4	159	411	430	7.7	12.5
IV	A_0	0–5	10 YR 2/1	4.8	4.1	197	387	416	73.6	14.0
	A_1 (A_2)	10–15	10 YR 2/3	4.8	4.4	140	383	477	37.2	16.8
	$B_{(hs)}$	25–35	10 YR 3/3	5.0	4.7	115	461	424	40.6	19.7
V	A_1	0–15	10 YR 2/3	4.8	4.2	221	361	418	59.5	14.0
	B	20–35	10 YR 3/4	4.9	4.2	175	341	484	35.8	16.3
	B/C	40–55	10 YR 4/4	5.2	4.4	130	331	539	16.2	19.0
VI	A_1	0–15	10 YR 2/3	4.7	4.2	212	336	452	70.5	13.6
	B	15–30	10 YR 3/4	5.2	4.4	151	339	510	33.3	16.0
	B/C	35–55	10 YR 4/4	5.1	4.5	103	329	568	16.4	16.9
VII	A_1	0–15	10 YR 2/3	5.4	4.8	214	367	419	43.5	14.5
	B	15–30	10 YR 3/3	5.5	4.8	192	390	418	21.6	15.1
	C	35–45	10 YR 3/2	6.1	5.5	151	374	475	10.8	16.0
VIII	A_1	0–20	7,5 YR 2/1	6.0	5.6	161	517	322	79.5	12.2
	B_W	40–60	7,5 YR 3/3	6.8	6.2	220	394	384	20.4	13.7
	C	40–60	–	7.5	ND	137	254	609	10.4	14.9
IX	A_1	0–15	10 YR 2/2	5.1	4.5	182	502	502	66.0	12.9
	B	20–30	10 YR 2/3	5.1	4.4	174	473	473	42.3	15.1

Table 1 continued

Location	Horizon	CEC (Cobaltihexamine)	Exchangeable cations (Cobaltihexamine)							Base saturation	Total major elements				Total trace elements		
			Ca^{2+}	Mg^{2+}	K^+	Na^+	Al^{3+}	Fe^{3+}	Mn^{2+}		Ca	Mg	Fe	K	Co	Cr	Ni
						$cmol^+ kg^{-1}$				%			$g\ kg^{-1}$			$mg\ kg^{-1}$	
Bedrock											17.0	218	63	2.3	ND	1,760	2,059
I	A_1	13.8	3.4	9.0	0.46	0.04	0.76	0.008	0.375	96	24.5	83	61	5.4	116	2,230	505
II	A_1	19.5	3.0	15.3	0.23	0.06	<0.02	<0.001	0.224	97	7.4	70	103	10.2	202	4,810	842
III	A_1	8.0	1.2	6.3	0.10	0.04	0.11	0.005	0.093	97	8.4	51	55	22.1	104	2,240	372
	B	7.1	0.8	6.1	0.03	0.02	<0.02	<0.001	0.065	98	8.2	51	59	23.9	112	2,950	385
	B/C	6.9	0.7	6.0	0.02	0.02	<0.02	<0.001	0.036	98	10.4	56	60	22.9	116	3,160	474
IV	A_0	6.1	1.2	1.1	0.29	0.08	3.48	0.053	0.052	46	8.7	22	32	23.2	18	296	146
	A_1 (A_2)	2.3	0.1	0.1	0.08	0.08	1.50	0.011	0.027	18	9.8	26	33	26.2	21	283	164
	$B_{(hs)}$	1.8	0.2	0.1	0.07	0.09	0.86	0.012	0.013	28	10.6	25	36	21.7	20	244	176
V	A_1	5.3	1.2	2.7	0.17	0.05	1.59	0.037	0.057	79	12.0	45	31	7.7	24	677	269
	B	3.6	0.4	1.4	0.07	0.03	1.49	0.025	0.018	54	12.7	50	34	7.4	29	806	311
	B/C	2.4	0.2	0.8	0.06	0.03	0.88	0.019	0.009	49	11.3	63	38	6.0	43	722	502
VI	A_1	9.0	3.2	3.9	0.25	0.03	1.41	0.023	0.078	83	8.6	32	31	10.5	29	611	279
	B	3.5	0.5	1.3	0.09	0.01	1.25	0.014	0.014	55	9.0	36	32	11.8	31	518	322
	B/C	2.1	0.2	0.6	0.03	<0.01	0.79	0.001	0.006	42	7.9	33	38	14.7	38	414	388
VII	A_1	7.0	1.3	5.0	0.10	0.03	0.29	0.082	0.034	94	10.0	40	39	15.0	43	1,010	312
	B	5.5	0.8	3.7	0.08	0.02	0.25	0.006	0.022	83	10.2	45	42	15.7	54	844	354
	C	13.1	0.9	11.3	0.04	0.05	<0.02	<0.001	0.019	94	4.1	130	81	5.5	165	2,400	1,550
VIII	A_1	23.7	5.9	16.2	0.24	0.08	<0.02	<0.001	0.159	95	23.4	52	86	11.7	173	2,350	682
	B_W	22.9	4.4	17.1	0.09	0.04	<0.02	0.006	0.039	95	5.2	142	101	3.6	147	2,380	2,270
	C	ND									25.0	159	92	ND	ND	2,490	2,507
IX	A_1	6.8	2.5	2.3	0.53	0.06	1.11	0.021	0.077	81	6.0	17	27	28.2	17	273	125
	B	3.8	0.6	0.8	0.20	0.04	1.85	0.023	0.019	45	5.9	18	29	28.7	18	308	132

ND: not determined

Table 2 Abundance of minerals in different horizons of a Hypermagnesic Hypereutric Cambisol (VIII) and a Magnesic Eutric Cambisol (V) determined by X-ray diffraction of fine powder (<50 μm)

Soil profile	VIII			V		
Horizon	A_1	B	C	A_1	B	C
Pyroxene			+			
Amphibole				+	+	+
Serpentine	++	+++	+++	(+)	(+)	+
Talc	+		+	+	(+)	(+)
Chlorite	+++	++	++	+	++	+++
Smectite		(+)	(+)			
Kaolinite	(+)	(+)	(+)	(+)		
Illite		+				
Goethite		+		(+)		
Quartz	(+)			+++	++	+
Feldspar		(+)		++	++	+

+++: most abundant; ++: common; +: minor; (+) traces

detection of minor mineral phases corresponds roughly to a content of 5%. There was a notable difference in the mineralogy of the two soils. Serpentine was present as a major mineral phase in the three horizons of soil VIII and so was chlorite. Chlorite, talc, serpentine and quartz were present in this order of abundance in the A_1 horizon. In the B horizon, we detected serpentine and chlorite but also secondary minerals such as illite, goethite and traces of smectite. Primary minerals were predominant in the C horizon: serpentine, chlorite, talc and pyroxenes were the main mineral phases with still traces of smectite. On soil V, we detected less contribution of ultramafic primary minerals; amphibole, another mineral inherited probably from the bedrock was present throughout the pedon. Chlorite and talc were also present in the whole pedon but less than in soil VIII. Chlorite also tended to disappear from the surface. No secondary minerals could be detected in any of the horizons but some important contamination by non-ultramafic minerals (i.e. quartz and feldspars and probably kaolinite) from the surface was obvious. Ultramafic minerals seemed to be diluted in the surface horizons.

On the surface horizons of the Hypermagnesic Hypereutric Cambisol (VIII) and the Magnesic Eutric Cambisol (V), we tried to spot all the minerals previously identified with XRD analysis with the TEM and to obtain an EDX spectrum for each of them. In the Hypermagnesic Hypereutric Cambisol, the identified bearing phases showing more than 0.01% of atomic composition of Ni were goethite (0.8%), chlorite (0.5%), serpentine (0.2%) and talc (0.2%) particles (Table 3). The original Ni content of the bedrock was 0.2% in weight. In the Magnesic Eutric Cambisol, the Ni bearing phases were goethite (4.3%), chlorite (0.05%) and talc (0.01%). The goethite was not a predominant mineral phase in the second soil but its Ni content was always extremely high when spotted under the microscope.

In all the soils from the toposequence, total Ni content was correlated with total Fe content ($R^2 = 0.96$, $P < 0.01$). Therefore, it was crucial to know under which chemical forms Fe was present in these soils. The results from oxalate and DCB extractions of, respectively, amorphous and well-crystallised Fe oxides were rather contrasted between Hypermagnesic Hypereutric Cambisols and Dystric Cambisols (Table 4). The fraction of free Fe in the surface horizons of soils V and VIII represented more than half of total Fe content (i.e. 56 and 58%, respectively) (Fig. 2). In both soils, free Fe was mainly present as well crystallised oxy–hydroxides. The fraction of amorphous Fe oxy–hydroxides in the surface horizons of soils V and VIII represented 25 and 20% of total Fe content, respectively. According to the chemical analyses on the soil samples (Table 1), the exchangeable Fe fraction was very low; it represented less than 1% of the CEC.

Table 3 Identification of Ni-bearing phases and associated Ni content (% atomic) in the surface horizon of a Magnesic Eutric Cambisol (V) and a Hypermagnesic Hypereutric Cambisol (VIII) determined by TEM-EDX technique

	Ni-bearing phases	Non-Ni-bearing phases
V (A_1)	Goethite (4.3%) [8]	Quartz [1]
	Chlorite (0.04%) [3]	Feldspar [1]
	Talc (0.01%) [8]	Kaolinite [1]
VIII (A_1 & B)	Goethite (0.8%) [7]	Quartz [5]
	Chlorite (0.5%) [7]	Kaolinite [5]
	Serpentine (0.2%) [7]	
	Talc (0.2%) [7]	

[*n*]: number of analyses (EDX)

Table 4 Selective extractions of Fe and Ni in soil samples with water, oxalate (McKeague & Day, 1966) and dithionite–citrate–bicarbonate (Mehra & Jackson, 1960)

Sites	Horizon	Water Ni (C_{Ni}) (mg l^{-1})	Oxalate extraction		DCB extraction		Oxalate/DCB	
			Fe_o (%)	Ni_o (mg kg^{-1})	Fe_d (%)	Ni_d (Mg kg^{-1})	Fe_o/Fe_d	Ni_o/Ni_d
I	A_1	0.050	2.02	32.4	3.86	46.9	0.52	0.69
II	A_1	0.201	3.00	151.0	5.62	191.4	0.53	0.79
III	A_1	0.080	1.34	41.6	3.04	62.2	0.44	0.67
	B	ND	1.13	53.4	2.99	72.0	0.38	0.74
	C	ND	1.03	61.4	2.94	83.6	0.35	0.73
IV	A_0	0.011	0.83	6.9	1.69	16.1	0.49	0.43
	A_1	ND	0.77	8.0	1.59	18.0	0.49	0.45
	B	ND	0.93	8.2	1.75	19.9	0.53	0.41
V	A_1	0.035	0.76	12.3	1.73	19.8	0.44	0.62
	B	ND	0.84	14.9	1.69	24.2	0.50	0.62
	C	ND	0.70	16.9	1.82	33.8	0.38	0.50
VI	A_1	0.047	0.67	18.8	1.63	34.3	0.41	0.55
	B	ND	0.66	16.2	1.55	35.8	0.42	0.45
	C	ND	0.50	18.6	1.33	42.2	0.38	0.44
VII	A_1	0.065	0.84	21.5	2.18	33.9	0.38	0.63
	B	ND	0.74	24.6	2.27	38.0	0.33	0.65
	C	ND	1.72	272.0	3.73	209.0	0.46	1.30
VIII	A_1	0.122	1.72	114.7	4.98	203.8	0.58	0.56
	B	ND	2.40	475.1	5.32	493.0	0.53	0.96
	C	ND	1.30	425.0	4.75	658.0	0.27	0.64
IX	A_1	0.036	0.75	13.6	1.81	21.5	0.42	0.63
	B	ND	0.87	11.8	1.89	23.3	0.46	0.51

ND: not determined

In soil IV, most of the Ni associated with Fe oxides was found in resistant well-crystallised structures (Ni_o:Ni_d < 0.5) whereas in the saturated soils (e.g. VIII), it was found essentially with the amorphous phase (Ni_o:Ni_d > 0.56). In some cases, in the C horizons, the presence of large quantities of unweathered materials showed Ni_o:Ni_d > 1. It should not be possible in theory but it is probably due to the fact

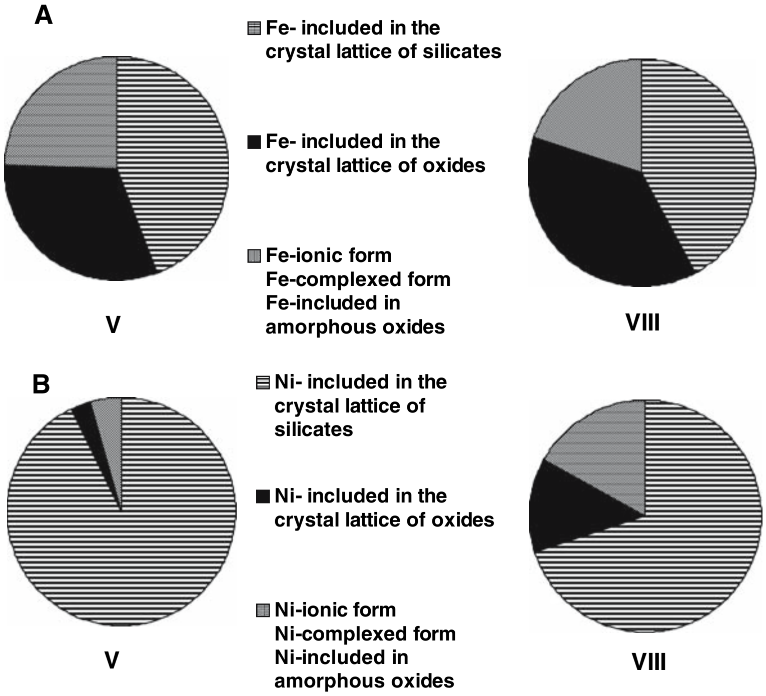


Fig. 2 Distribution of Fe (**a**) and Ni (**b**) within the different mineral compartments in the surface horizons of a Magnesic Eutric Cambisol (soil V) and a Hypermagnesic Hypereutric Cambisol (soil VIII). Total Fe and Ni contents in soil V are, respectively, 31 g kg^{-1} and 269 mg kg^{-1}, and in soil VIII, 86 g kg^{-1} and 682 mg kg^{-1}

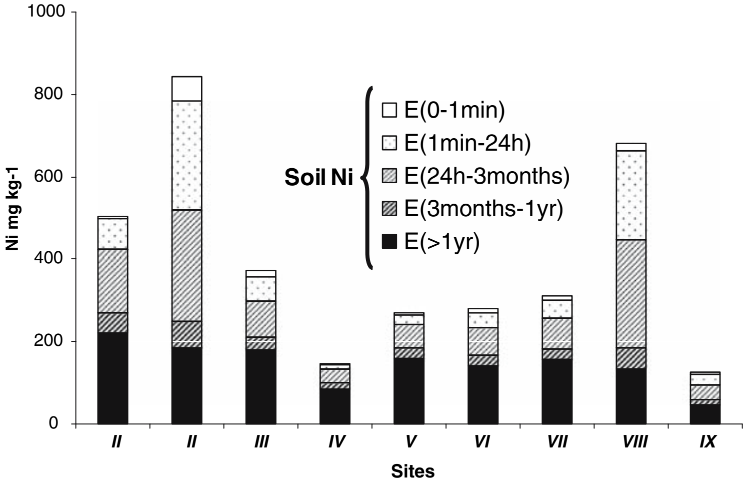
that oxalate is probably enhancing clay mineral dissolution and releasing more Ni than contained in the free Fe oxides. When compared to the Fe distribution between amorphous and well-crystallised oxides, Ni was always more bound to amorphous Fe oxides:Ni_o:Ni_d was always higher than Fe_o:Fe_d except for the Dystric Cambisol (IV). In the surface horizons of soils V and VIII, Ni was majoritarily contained in the crystal lattice of silicates (Fig. 2). In both soils, a small part of total Ni was trapped in the crystal lattice of neoformed Fe oxy–hydroxides. Ni_o was quite similar in quantity with Ni_{DTPA}: it should then correspond to the bioavailable fraction estimated by IEK because Ni_{DTPA} and E-values are strongly correlated.

Variability of Ni availability and correlation to soil properties along the toposequence

Ni in solution (i.e. C_{Ni}-values) varied from 0.01 in Dystric Cambisol (IV) to 0.20 mg l^{-1} in the Hypermagnesic Hypereutric Cambisol (II) (Table 4). Ni availability expressed by the distribution of Ni in the labile pools with increasing average time of exchange showed clear variations according to the landscape position (Fig. 3). These relationships were clearly due to the type of soils. Instantaneously labile Ni (i.e. $E_{0-1\ min}$) was lowest for the Dystric Cambisol (1.0 mg kg^{-1}) and highest (57.5 and 19.1 mg kg^{-1}, respectively) for the two Hypermagnesic Hypereutric Cambisols (II and VIII). The same order applied for medium term labile Ni (e.g. $E_{1\ min\text{–}3\ months}$): it varied from 45.4 mg kg^{-1} (IV) to 477 mg kg^{-1} (VIII) and 534 mg kg^{-1} (II). It was obvious from the results that the saturated soils showed much higher Ni availability than the more intensely weathered soils. Labile Ni represented more than 50% of total soil Ni in all soils—except IV and V- and the buffer capacity in most of the soils was also extremely high.

DTPA extractable Ni was correlated at a highly significant level with isotopically exchangeable Ni as assessed by IEK (Fig. 4) although the range of values was incomplete for appropriate linear regression. However, DTPA extractable Ni (Ni_{DTPA}) could be used as a suitable estimate of Ni bioavailability. We thereafter used this variable as the indicator of Ni bioavailability of the different soil horizons at each site. According to Pearson's correlation coefficients calculated for the whole matrix of physico-chemical parameters measured for each soil horizon, Ni_{DTPA} was significantly and positively correlated to pH, clay content, Mg and Fe total contents, CEC,

Fig. 3 Ni availability assessed by isotopic exchange kinetics in the surface horizons of the nine soils sampled in the toposequence



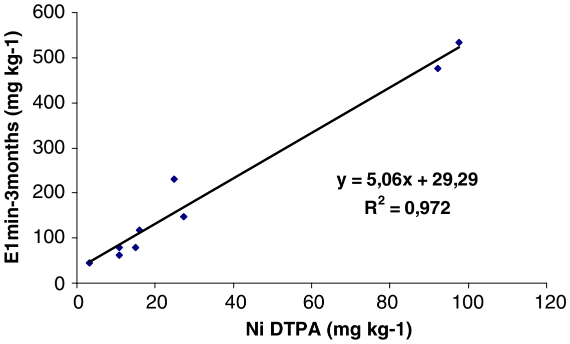


Fig. 4 Relationship between Ni extracted by DTPA (Lindsay & Norvell, 1978) and isotopically exchangeable Ni (Echevarria et al., 1998) in the nine surface horizons

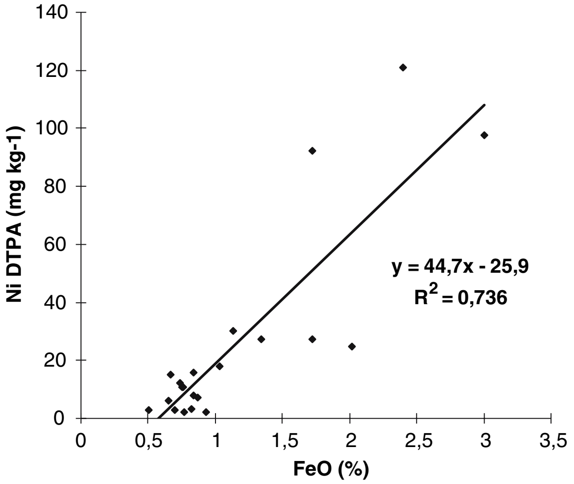


Fig. 5 Relationship between Ni extracted by DTPA and oxalate extractable Fe (Fe_0) in samples from the different horizons of the nine pedons

exchangeable Ca and Mg and to free Fe oxide contents (Fe_o and Fe_d), most of which are typical indicators of soil saturation and lack of weathering. In particular, we illustrate the correlation with oxalate extractable Fe (Fe_o) with a highly significant linear regression (Fig. 5). Conversely, Ni_{DTPA} was significantly but negatively correlated with C : N ratio and exchangeable Al, both indicators of high weathering intensity and soil evolution. There was no significant correlation of Ni_{DTPA} with total OM content.

Distribution of *T. caerulescens* and uptake of Ni along the toposequence

The presence and abundance of *T. caerulescens* along the toposequence was uneven (Table 5). Concentration of Ni in *T. caerulescens* shoots was surprisingly highly variable from 396 mg kg^{-1} on site VI to 3057 mg kg^{-1} on site VIII. *T. caerulescens* was therefore not reaching hyperaccumulation on sites I, V, VI and IX. Ni concentration in shoots was significantly correlated to labile Ni (Fig. 6) and was also influenced by the level of soluble Ni (C_{Ni}) in surface horizons, although labile Ni was better correlated than C_{Ni} with Ni concentrations in shoots ($R^2 = 0.95$ vs. $R^2 = 0.67$). It was therefore lower in the more weathered acidic soils (Eutric and Dystric Cambisols) and much higher in the Hypermagnesic Hypereutric Cambisols.

Table 5 Distribution, abundance and Ni concentration in shoots of *Thlaspi caerulescens* along the toposequence

Site	Abundance	Ni concentration in shoots (mg kg^{-1})
I	+++	833
II	+++	2,288
III	−	−
IV	−	−
V	+	467
VI	++	396
VII	+	−
VIII	++	3,057
IX	+++	531

Absent (−), rare (+), common (++) or abundant (+++)

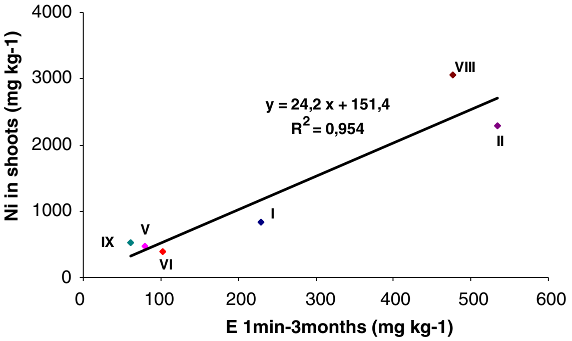


Fig. 6 Relationship between isotopically exchangeable Ni in surface horizons and averaged Ni concentrations in shoots of *Thlaspi caerulescens* in soils where it was naturally growing

Discussion

Soil type and pedogenesis along the toposequence

The soils along the toposequence show a strange pattern. All of them belong to the Cambisol class although soil IV shows the first steps of intense weathering with the premises of an elluvial horizon. Therefore, soil differenciation within the toposequence relies mostly on the ultramafic influence on soil genesis. Soils IV and IX seem to be of none or very limited ultramafic origin (e.g. higher Ca : Mg ratio, much higher total K content, lower total metal contents). These soils are therefore the more acidic and weathered soils (least saturation of CEC). The mineralogy of soil V also suggests contamination with non-ultramafic material and this pedon lies between the previous type and the richest type (e.g. soil VIII). All of the other soils are very slightly desaturated or saturated as a consequence of the geochemical properties of the ultramafic bedrock. Furthermore, soils II and VIII as well as the C horizon of soil VII show relative enrichment of less-soluble metals in lower horizons (Fe, Cr and Ni) and strong losses in Mg and Si as compared to bedrock composition. No losses of Ca seem to have taken place in these more eutric soils as opposed to previous statements on Ca losses in ultramafic soils elsewhere in France (Duvignaud, 1966). Such modifications could be due to more intense weathering under warmer climates in the past. Soil II seems to have evolved from the truncated lower part of a soil that was initially similar to soil VIII.

Some hypotheses can be formulated to explain such a strange distribution pattern of soil types in this toposequence. It could be explained by two scenarios. A heterogeneity in chemical composition of the bedrock with particularly low-ultramafic minerals underneath soils V and IX. The lesser abundance of serpentine in the C horizon of soil V and the relatively high content of amphibole could also be due to this local change in the bedrock. Alternatively, the observed pattern could be due to a complex evolution of soil cover. Total Cr, Ni and Fe total concentrations are strongly lower in soils IV to VI and especially higher in soils II and VIII. Moreover, the colours of the A and B horizons of soil VIII and the A horizon of soil II recall typical colour patterns found in tropical environments and seldom in temperate environments. Ancient pedogenesis on serpentine could have led to intensely weathered soils. Some remains of this material that was not or only partially eroded during glacial cycles could explain soil composition at several locations in the toposequence (II, VII and VIII). Glacial erosion could have removed part of this cover and also have brought new non-ultramafic material from upslope (gneiss) that accumulated preferentially on flat sections of the toposequence (e.g. soil IV). The intermediate soils could be a mixture of ultramafic and non-ultramafic materials (e.g. soil V). Therefore, some of the soils show total Ni-values that are not as high as those found in soils developed on serpentine elsewhere in Europe.

Soil mineralogy

The minerals identified can be classified as follows: (a) primary minerals derived from the serpentinised peridotite (i.e. serpentine, talc, chlorite, pyroxene, magnetite and amphibole), (b) secondary minerals from the weathering of the primary minerals (i.e. goethite and smectite) and (c) minerals that are non-specific to serpentinites (i.e. kaolinite, illite, feldspars and quartz). Mineral contamination from upslope soils developed on gneiss bedrock may explain the presence of illite, quartz and feldspars in the surface (e.g. quartz found only in the A_1 horizon). Moreover, none of these three minerals appeared to bear Ni, which, in this case, could be used as a tracer for ultramafic origin. Chlorite was a major component in the three horizons of soil VIII as well as in soil V. Serpentine is still highly present in the whole profile of soil VIII but is less present in soil V. In both soil profiles, the mineral composition of clays from A_1 to C horizons was roughly the same, indicating that pedogenic clay formation had not occurred to a very significant extent (Bulmer et al., 1992). However, the B horizon in soil VIII presented various neoformed minerals such as smectite that were not found above and which could be the product of primary chlorite transformation (Lee et al., 2003). There was also a significant increase in less-soluble metals (especially Fe) in this horizon. Amorphous Fe oxides and goethite probably derived from the weathering of primary Fe-rich chlorite but also from the release of Fe during the weathering of serpentines. During the alteration of serpentines, Mg was strongly leached which was not the case of Ca that was progressively enriched through preferential retention on the CEC although Ca was less abundant originally.

Iron and trace metal localisation in soils

The accumulation of Fe up to 10% in the A_1 horizon of soil II, in the B horizon of soil VIII, and to a lesser extent in the B/C horizons of soils VII and VIII, reaches uncommon levels for temperate soils but is typical from serpentine soils (Alexander, 2004). In draining conditions, Fe is the witness of mineral weathering and accumulation of less-soluble metals in the B horizon whilst soluble cations (Si and Mg) are leached during pedogenesis. The presence of quartz does not seem to result from secondary precipitation of Si within the profiles, but mainly from eroded materials from upslope gneiss soils. Ni seems to follow the same fate as Fe (Gasser, Juchler, Hobson, & Sticher, 1995) and tends to accumulate in the horizons in which Fe precipitates as oxy–hydroxides such as goethite. However, it is mostly accumulated in the amorphous fraction of Fe oxides and the partition coefficient $Ni_o : Ni_d$ is much higher than $Fe_o : Fe_d$ (Massoura et al., 2006). In the case of Cr, there was notable enrichment in soil II and slight enrichment in lower horizons of soils III, VII and VIII.

The Ni-enriched goethite found in both soils really results from weathering and is a sink mineral for released Ni. This mineral was the only one to reach such an accumulation level of Ni when compared with primary mineral concentrations (Becquer et al., 2001). It is not known whether Ni is sorbed onto the goethite surface or it is incorporated within the crystal lattice during goethite precipitation. The extremely high Ni availability in this horizon suggests that it is not due to Ni present in the crystal lattice of goethite, which is highly unavailable (Becquer T, Bourdon E, & Pétard, 1995; Massoura, 2004; Massoura et al., 2006; Quantin et al., 2001). Although oxalate extraction can desorb OM-bound Ni in theory, it revealed the high importance of amorphous Fe oxides (e.g. ferrihydrite) in Ni scavenging and especially under highly available forms (Massoura et al., 2006). The fact that total organic carbon fails to explain differences in Ni availability among soils tells that amorphous oxides are the main contributors to Ni_o. High $Ni_o : Ni_d$ ratios are usually the condition of high Ni availability in soils where high exchange capacity clays are scarce (Massoura et al., 2006). In our case, the highest values correspond to the highest Ni availability levels. Ni associated with goethite is therefore mostly incorporated in the crystal lattice whereas Ni associated with amorphous Fe oxides is mostly complexed at the surface. Ferrihydrite shows high-specific surface for metal sorption with pH-dependant capacity. The higher pH values in saturated soils favour Ni sorption onto amorphous ferrous hydroxides.

Nickel bioavailability in the soils

In some cases, availability levels were extremely high but similar to those observed on California Mollic Haploxeralfs (Gasser & Dahlgren, 1994). Except in the two most acidic soils, Ni available pools of topsoil horizons accounted for more than 50% of total Ni. Such values are seldom reached in soils, even in serpentine environments (Echevarria et al., 2006). However, Ni concentration in the soil solution was not critical to plants (generally lower than 0.2 mg l^{-1}). Soil mineralogy and the nature of Ni-bearing phases both control Ni availability rather than soil solution chemistry. The retention of Ni and other cations by a much higher CEC when soils are saturated protects the soils from leaching over time. This leads to higher exchangeable Ni than in soils with lower pH where Ni is leached more easily. This is evidenced by the highly significant positive correlation between Ni_{DTPA} and pH, which seems contradictory at first sight (Anderson & Christensen, 1988).

Due to the lack of formation of secondary smectites that could be significant sources of highly available Ni, the Fe geochemistry governs the fate of Ni in this toposequence (Becquer et al., 2006; Massoura et al., 2006). The moderate weathering intensity and the humid climate that favours accumulation of amorphous free Fe oxides (instead of well-crystallised goethite) is responsible for the high Ni availability in this site. However, when goethite is formed, it results in Ni trapping within the crystal lattice. Such neoformed goethite reached Ni concentrations 4–12 times higher than original bedrock. Pedogenesis is therefore the key to understanding Ni availability in the soils of this toposequence.

Interactions between Ni availability and behaviour of *T. caerulescens*

In this work we clearly showed that Ni uptake by *T. caerulescens* responds to Ni availability as assessed by IEK. Concentration of Ni in shoots responded specifically to labile Ni in soils but less clearly to Ni concentration in the soil solution. This can be explained by the large quantities of Ni taken up by this plant. *T. caerulescens* in the Vosges occurs indifferently in ultramafic and non-ultramafic sites (Reeves et al., 2001). The Bergenbach area is almost the only location where it accumulates Ni instead of Zn in the Vosges. It is therefore possible that Ni is secondary to the populations of the Vosges as some specimen from Bergenbach seemed to better accumulate Zn when both were present at similar concentrations (Peer et al., 2006). The field work carried out by Reeves et al. (2001) clearly showed some important features of *T. caerulescens* that may help to understand what was observed in this study. The tolerance of and hyperaccumulation of Zn is often reported in Zn-rich soils. Although Zn uptake seems to be reduced by massive Ni uptake in serpentine soils, Zn hyperaccumulation also occurs from non-metalliferous soils, which is not the case of Ni hyperaccumulation.

Considering these features, our results have two main issues. The first one, is that *T. caerulescens* does not seem to look specifically for Ni in soils since it is present in some of the Ni-poor soils and absent from some of the Ni-rich. In the Ni-poor soils, it is very likely, as in all other *Thlaspi* sites in the Vosges (Reeves et al., 2001), that *T. caerulescens* is accumulating Zn. The second one is that differences in Ni concentrations in site-collected individuals cannot be interpreted only as genetic variability but more certainly as an indication of metal bioavailability in soils. Therefore, clear information on soil properties and metal biovailability is essential when plant material, such as seeds, is collected. Genetical variations between populations and individuals should then be assessed in controlled conditions.

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