University of Natural Resources and Life Sciences,
Vienna

**MASTER THESIS**

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# Characterisation of serpentine soil and Ni accumulation by *Odontarrhena chalcidica* along a gradient of total Ni concentration and soil development

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Author: Simon Obenaus
Supervisor: Priv.-Doz. Dr. Markus Puschenreiter
Co-Supervisor: prof. Ing. Pavel Tlustoš, CSc.

*Thesis submitted in partial fulfilment of the requirements*
*for the degree of "Master of Science" in the study program Natural Resources Management and Ecological Engineering*
*at the*
Institute of Soil Research

Vienna, November 2019

# Abstract

Serpentine soils are characterized by strong chemical peculiarities like low nutrient content, a low Ca/Mg ratio and high metal contents such as Ni, Co or Cr. This barren habitat constitutes a niche for a highly adapted and robust vegetation, including both non-hyperaccumulators and hyperaccumulators. Hyperaccumulators have received increased attention in the last decades, due to the potential of using their ability to accumulate heavy metals for practical applications like phytomining or phytoremediation.

However, dependency of Ni-hyperaccumulation on soil parameters has not yet been fully understood. In this context, this study aims at examining biogeochemistry of pedogenic Ni along a toposequence of serpentine soil and its relation to Ni accumulation by the hyperaccumulator *Odontarrhena chalcidica* (syn. *Alyssum murale*).

Six soil samples were taken along a toposequence near Redschlag in Burgenland and a pot experiment has been set up. *O. chalcidica* was planted into soils obtained from the six sites and harvested after 66 days.

Standard soil parameters (C, N, pH, CEC, etc.) revealed the typical infertility of serpentine soils. Ni fractions of different operational defined extractability (*aqua regia*, DTPA , $Sr(NO_3)_2$, $H_2O$) were determined and the ratio between amorphous Fe-oxides and well crystallized Fe-oxides was determined, as it is a relative indicator of the degree of soil development and weathering intensity. Pearson correlation matrix was calculated for all soil physico-chemical parameters and elemental concentrations in the shoots of *O. chalcidica*, in order to relate Ni accumulation to soil properties and identify the Ni fraction mainly supplying Ni to *O. chalcidica.*

$Ni_{Total}$ and Ni associated with amorphous Fe-oxides showed to be the best indicators for Ni uptake by *O. chalcidica* as these two fractions showed strong correlations with $Ni_{Shoot}$ values. In contrast to the single Ni extractions (DTPA , $Sr(NO_3)_2$, $H_2O$), which were no sufficient indicator for Ni uptake as they showed no significant correlation with $Ni_{shoot}$. Ni associated with amorphous Fe-oxides seems to be the main contributor to Ni taken up by *O. chalcidica.* Therefore, pedogenesis, weathering intensity and Fe-geochemistry is of great influence on Ni availability. Furthermore, antagonistic relationships between $Ni_{shoot}$ and $Fe_{shoot}$ were revealed and allowed assumptions related to the Ni uptake mechanisms of *O. chalcidica.* It is assumed that Ni is taken up via Fe transporters, for which they compete. No mobilisation of Ni by *O. chalcidica* could be detected since there was a depletion of $Sr(NO_3)_2$ and DTPA extractable Ni after growth of *O. chalcidica,* rather than an increase. Furthermore, species distribution among dissolved Ni species in soil solution showed no significant change during growth of O. chalcidica, which again suggests no mobilisation of Ni. On the other hand, Ni uptake showed significant correlations with $Ni_{Total}$ and Ni associated with amorphous Fe-oxides, both fractions that are not directly plant available. Therefore, mobilisation of Ni by O. chalcidica may take place, but could not be proven by the collected data.

# Acknowledgment

Before the I dive into this master thesis, I would like to address some special thanks to the people who supported me in the course of completing this work. I would like to thank Markus Puschenreiter for providing the topic and all his support whenever it was needed. Thank you for answering the big comprehensive questions as well as handling trivial struggles in the lab! Many thanks to Theresa Rosenkranz, Christina Hipfinger and Christina Roschitz for helping me out with any arising question during the lab work. Special thanks to Veronika Slavik for her support and infinite patience handling the ICP-OES and ICP-MS measurements. I felt genuinely integrated into the rhizo-research group which offered a familiar working environment.

# Affidavit

I hereby declare that I am the sole author of this work; no assistance other than that permitted has been used and all quotes and concepts taken from unpublished sources, published literature or

the internet in wording or in basic content have been identified by footnotes or with precise source citations.

Simon Obenaus

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# Introduction

## Serpentine Soils

Serpentine soils originate on bedrocks that are constituted of ultramafic rocks or serpentinites (Coleman, 1977). Ultramafic rocks are defined as igneous rocks that contain more than 90% mafic minerals. Ultramafic rocks (e.g. peridotites) are generally dominated by pyroxenes and olivine (Le Bas and Streckeisen, 1991; Alexander *et al.*, 2007). Serpentinites are formed of peridotites, (mostly dunite or harzburgite) due to the process of serpentinization (Vinx, 2015). In the course of this metamorphosis , due to the supply of $H_2O$ under pressure and relatively low temperatures (<500°), the OH-free olivine contained in the peridotites is being converted into OH-containing secondary serpentine minerals (Vinx, 2015). Thus, a rock is classified as serpentinite, if most of the olivine and pyroxene of the prior peridotite has been transformed to serpentine minerals. Serpentinite is a term used for rocks that are containing one or more serpentine minerals (Morris, Scheckel and McNear, 2019). Serpentines are 1:1 clay minerals and the most recognized and abundant of the serpentine subgroup minerals are chrysotile, lizardite and antigorite (Echevarria, 2018). Serpentine-rich rocks have an olive greenish colour, streaked with stripes of various shades that remind of a snakeskin, which explains the origin of the name serpentine (Brooks, 1987).

### Serpentine soil development

The major factors influencing the development of soils on ultramafic rocks is the parent ultramafic rock itself, climatic conditions, topographic situation, and vegetation cover (Echevarria, 2018). Echevarria (2018) reviewed 39 soils developed on ultramafic bedrock and described the main paths of pedogenesis. He partitioned the sites into soils derived from serpentinite bedrock and soils originated from non-serpentinized peridotite. Soils developed on serpentinite tend to be similar in pedogenesis and functioning regardless of latitude or elevation. Soils on serpentinite in temperate and Mediterranean climate mostly develop to cambisols, while in humid climates of subtropical to tropical areas, serpentinite soils are mostly dominated by cambic leptosols and cambisols. On the other hand, soils developed on non-serpentinized periodite are dominated by camibsols in termperate climate, cambisols and luvisols in Mediterranean climate and gerric ferrasols in humid climate (Echevarria,

2018) . According to Echevarria (2018) Mg is extremely depleted during pedogenesis of serpentine soils . Additionally, Silicon is depleted especially in Mediterranean climate. As a consequence, the relative concentrations of Fe and Cr increase in the soil, whereas Ni is slightly depleted in temperate soils and is stable in Mediterranean soils. The presence of serpentine will considerably reduce the loss of Si, and most mineral phases will be primary and secondary clay minerals (Echevarria, 2018).

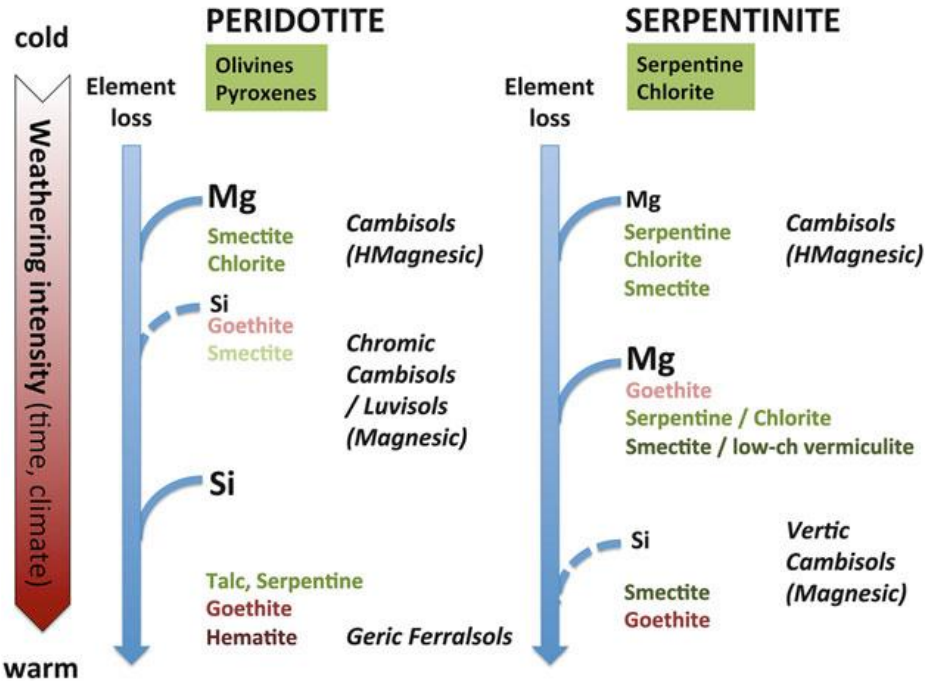


*Figure 1: A simplified description of soil genesis and evolution on ultramafic bedrock: non-serpentinized peridotite and serpentinite. In warmer climates, the difference between the two types of ultramafic bedrocks becomes more pronounced* (Echevarria, 2018) .

## Characteristics of serpentine soils

Serpentine soils have substantial fertility constraints. They generally have low Ca/Mg ratios, with lower Ca contents than soils in the surroundings and high Mg concentrations (Echevarria, 2018). These soils are also poor in essential macronutrients like N,P,K, but show high levels of geologically derived metals like Ni, Cr and Co which could be toxic for most plants (Brooks, 1987). Ni concentrations in serpentine soils range widely around the world but can exceed 1000 mg/kg. In comparison, the content of Ni in non-serpentine soils around the world ranges from 0.2-450 mg/kg (Gasparatos and Barbayiannis, 2018). The native vegetation on serpentine is able to handle these special conditions and is well adapted to them. Plants growing on serpentine soils can cope Ca, K and P deficiencies (Brooks, 1987). But also factors directly concerning the soil, like soil structure, organic matter content,

temperature or water holding capacity determine these harsh conditions which were named the “serpentine syndrome” (Jenny, 1980). As a consequence, demanding species are not able to survive on these poor soils, whereas some species, which are poor in competition are thriving. In the course of evolution these species developed physiological adaptions, which enable them to survive on these sites (Michalek *et al.*, 2015).

## Hyperaccumulators

Exceptional high heavy metal concentrations in soils lead to severe toxicity effects in most plants. Phytotoxic effects caused by heavy metals may result in changes of several physiological processes at a cellular and molecular level. Heavy metals may inactivate enzymes, block functional groups of metabolically important molecules, they may replace essential elements or disrupt membrane integrity (Rascio and Navari-Izzo, 2011). Most plants manage high metal concentrations with several defence mechanisms that regulate uptake and translocation of these elements. A prevalent strategy is to hinder the entrance of toxic elements into the root cells through trapping them in the apoplastic environment by binding them to organic acids (Watanabe and Osaki, 2002) or to anionic groups of cell walls (Rascio *et al.*, 2008). This hinders the transport to above-ground biomass thus protecting the leaves and in particular the metabolically active photosynthetic cells from damage (Rascio and Navari-Izzo, 2011). So called „excluders“ comprise plants that tolerate exceptional high heavy metal concentrations relying on strategies that restrict metal entrance . By retaining and detoxifying most of the heavy metals in the root tissues, they minimise the transport to the leave tissues which are still sensitive to heavy metal phytotoxicity (Hall, 2002). However, a number of species are behaving just the opposite way in terms of heavy metal uptake and distribution in the plant. Plants which are actively taking up large amounts of one or more heavy metal from the soil are defined as „hyperaccumulators“ (Brooks *et al.*, 1977). Furthermore, metals are not held back in the roots but are transported to the leaf tissues, where they show 100-1000 fold higher concentrations than non-hyperaccumulators, without showing symptoms of phytotoxicity (Reeves, 2006). About 450 angiosperm species have been identified so far as heavy metal hyperaccumulators (Rascio and Navari-Izzo, 2011). Metals that have been shown to be taken up by hyperaccumulators include As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn (Rascio and Navari-Izzo, 2011). Threshold values define the hyperaccumulation of each heavy metal. For example, a plant is defined as nickel-hyperaccumulator if the nickel concentration in its aerial organs is >1000 µg/g (>0.1%)

(Brooks *et al.*, 1977). The extent of hyperaccumulation can vary vastly in different species but also in different populations and habitats of the same species (Roosens *et al.*, 2003). However, there are three important indicators that distinguish hyperaccumulators from related non-hyperaccumulator taxa. These features are: a much greater ability to take up heavy metals from the soil, a very fast and efficient translocation of heavy metals from root to shoot, and a high capability to sequester vast amounts of heavy metals in the leaf tissues (Rascio and Navari-Izzo, 2011). An important role in driving this excessive behaviour is played by the overexpression of genes regulating metal homoeostasis.

## Physiological features of hyperaccumulators

Studies comparing hyperaccumulators and non-hyperaccumulators have shown that an enhanced Zn uptake can be ascribed to an overexpression of genes of the ZIP (Zinc-regulated transporter & iron-regulated transporter protein) family (Assunção *et al.*, 2001). The expression of these genes occurs in non-hyperaccumulating plants only at Zn deficiency, whereas in hyperaccumulators the expression of this gene is still present at high Zn availability (Assunção *et al.*, 2010). However, transporters responsible for Ni uptake have not been recognised, but it is assumed that transport systems for Zn (Assunção and Bleeker, 2008), Ca or Fe (Mohseni, Ghaderian and Schat, 2019) are possibly utilised for Ni entrance into roots.

In terms of root-to-shoot translocation, free amino acids, which form stable complexes with bivalent cations, seem to play a major role. According to Callahan and Baker (2006) free histidine is regarded as the most important ligand involved in Ni hyperaccumulation. For *Odontarrhena lesbiaca* (syn. *Alyssum lesbiacum*), again an overexpression of a gene (TP-PRT1) has been shown, which leads to a larger stock of histidine, which furthermore favours the loading of the xylem as a nickel-histidine complex (Kerkeb and Krämer, 2003). In contrast, Centofanti *et al.*, (2013) suggested that most of the nickel in the xylem is transported as hydrated cation. Moreover a class of proteins, also called HMAs (heavy metal transporting ATPases) seem to be important in operating heavy metal transport, while also playing a role in metal homeostasis and tolerance (Axelsen and Palmgren, 2001).

High effectiveness in sequestration and detoxification allow hyperaccumulators to build up high concentrations of heavy metals without damaging the photosynthetic machinery. This detoxification/sequestration happens in locations such as epidermis (Asemaneh *et al.*, 2006),

trichomes (Küpper *et al.*, 2000) or cuticle (Robinson *et al.*, 2003). Detoxifying/sequestering consist mainly in heavy metal complexation with ligands and removing them from metabolically active cytoplasm to inactive parts, like vacuoles and cell walls (Rascio and Navari-Izzo, 2011). Again, in leaves of Zn and Ni hyperaccumulators an overexpression of a gene (MTP1) could be shown, which encodes proteins which mediate bivalent cation efflux from the cytosol (Kim *et al.*, 2004). When it comes to detoxification, small ligands, such as organic acids play an important role in preventing heavy metals to persist as free ions and enabling their storage in vacuoles (Krämer *et al.*, 2000). For example, in the leaves of the hyperaccumulator *Noccaea goesingensis* (syn. *Thlaspi goesingense*), the main ligand for Ni is citrate (Krämer *et al.*, 2000).

Despite recent advancements in understanding the physiological mechanisms of Ni uptake and translocation to shoots several key hyperaccumulation mechanisms have still not been described. It has been shown that hyperaccumulators absorb metals from the same labile pools in soils as normal plants (Massoura *et al.*, 2004). However, Ni hyperaccumulators are able to accumulate 100 times more Ni in their shoots than normal crops. Moreover Bani *et al.*, (2007)) showed that *Odontarrhena* species accumulate Ni concentrations by an order of magnitude higher than the labile Ni pool in the soil. This suggests that there is apparently another process in the rhizosphere supporting the high Ni uptake by hyperaccumulators.

## Possible utilities of Hyperaccumulators

Hyperaccumulator plants have received increased attention, because of the potential of using their ability to accumulate heavy metals for practical implications. The usage of plants to remediate polluted soils, generally known as phytoremediation, has seen a lot of development and research in the last decades. This approach on remediation is seen as promising, as it could be an eco-friendly and cheap alternative to conventional, civil-engineering methods. However, the potential for remediation is limited due to the low speed of metal removal and the metal selectiveness of most plants. (Cunningham, Berti and Huang, 1995). Nevertheless, phytoremediation could be an option for the remediation of low to moderate contaminated soils (Rascio and Navari-Izzo, 2011).

Another way of making use of hyperaccumulator's potential is phytomining. Phytomining aims to generate profit by recovering valuable metals by growing hyperaccumulators on contaminated or mineralized soils, incinerating plant biomass and recovering valuable metals from the "bio-ore"(Brooks et al. 1998). Phytomining allows for sustainable metal recovery without causing the environmental impact associated with conventional mining activities (Kidd, 2018). Many field trials have been successfully conducted and particularly high biomass Ni hyperaccumulators like *Odontarrhena chalcidica (*syn. *Alyssum murale)* have been shown to be economically viable, especially on infertile serpentine soil (Chaney, 2019).

## Rhizosphere effect

The fate of Ni soils and its availability is not only influenced by major soil variables like pH, organic matter, soil development or parent material, but is also expected to deoend on the activities of roots and their associated microbial communities in the rhizosphere (Wenzel *et al.*, 2003). Rhizosphere is defined as the area influenced by root activities which is characterized by a physical, chemical and biological distinction from the bulk soil (Liepec and Glinski, 2011).The uptake of elements as well as the release of root products like amino acids, fatty acids, sugars, cellular debris and $CO_2$ can modify the soil chemistry near the roots substantially. Root products affect the behaviour and availability of nutrients as well as trace metals directly by affecting pH, redox potential and by providing organic ligands and complexing agents. (Hinsinger, Plassard and Jaillard, 2006). Root products may also affect the availability of nutrients and trace elements indirectly by changing the microbial activity. Root deposits are substrate for the growth of microbial communities. Roots are embedded in mucilaginous layers formed through the release of cellular debris and mucigel, which are habitat for root microbiome (Lynch and de Leij, 2012). This rhizosphere biota may affect further variation in the behaviour of chemical elements in the rhizosphere.

These rhizosphere processes are known to induce transport processes like mass flow and diffusion, and they cause changes of chemical speciation and solubility. Furthermore metal release during weathering, bioavailability and leachability of trace elements, both in terms of mobilization and immobilization may be modified by rhizosphere processes (Hinsinger and Courchesne, 2007). Changes of biogeochemical parameters of the soil in the vicinity of the roots affect a lot of reactions at the interface of solid soil and soil solution. As a consequence higher plants may have an influence on the weathering of soil minerals, soil formation

processes and the biogeochemistry of elements that are either beneficial or potentially toxic to themselves (Hinsinger and Courchesne, 2007). Root-induced changes such as rhizosphere acidification and the release of organic acids acting as chelating agents have been suggested to be responsible for increasing Ni solubility in soil. However, several authors (Li *et al.*, 2003; Kukier *et al.*, 2004) have shown that metal solubilization processes in *Odontarrhena chalcidica* (syn. *Alyssum murale*) rhizosphere does not involve rhizosphere acidification. Hyperaccumulators deplete bioavailable pools of metals to extents where they change the chemical equilibria of that metal in the soil. Replenishment of the labile pool from the non-labile pool during equilibration over time is an indirect mechanism by which hyperaccumulators increase the desorption of labile metals to soil solution (Centofanti et al., 2012). However, no mechanism is yet known where plants directly attack the non-labile pool of Ni, e.g. via root exudates.

## Nickel in soils

The characterization of Ni in the soil solid phase as well as Ni speciation in soil solution is essential for the understanding of Ni behaviour in soils. Fractionation and speciation of Ni determine Ni bioavailability in soil and therefore play a major role in the development of management strategies for Ni rich soils (Tsadilas, 2019).

The main inorganic species of Ni in soil solutions include $Ni^{2+}$, $NiSO_4^0$ and $NiHCO_3^+$ in acidic soils, and $NiCO_3^0$, $NiHCO_3^+$ and $Ni^{2+}$ in alkaline soils (Kirk, 2004). But also organic Ni complexes can add to speciation, especially in non-alkaline soils (Kirk, 2004). Free $Ni^{2+}$ in soil solution is relatively stable but can also be associated with organic and inorganic ligands (Uren, 1984; Ma and Hooda, 2010). For example, in rhizosphere soil solutions organic complexes are influencing Ni speciation as plants roots typically increase the quantity and change the composition of dissolved organic matter in soil solution. As DOM in rhizosphere increases, more Ni becomes complexed. As a consequence there is a shift from free $Ni^{2+}$ to Ni complexed with DOM which can cause solubilization of Ni from the soil solid phase (Wenzel et al., 2003).

In general, the basic mechanisms that govern the distribution of Ni between liquid and solid phases and therefore its bioavailability are dissolution/sorption, hydrolysis, oxidation/reduction and carbonization (Tsadilas, 2019).

Ni in solid soil phase can be bound to exchange sites, specifically adsorbed, or occluded into sesquioxides, bound to the clay lattice or bound to organic matter (Adriano, 2001). Ni can be bound to the surface of the soil solid phase as in inner-sphere complexes or outer sphere complexes. Where the former is characterized by low availability and the latter is easily soluble, existing as hydrated Ni species bound to the surface of those minerals (Uren, 1984; Ma and Hooda, 2010). pH, competing metal species, organic compounds or complex formation are having an effect on the sorption of Ni to the soil mineral phase (Tsadilas, 2019).

Although pH appears to be the most influencing factor on Ni solubility in soils (Anderson and Christensen, 1988), availability of Ni in serpentine soils seems to be determined mainly by the mineralogy and the nature of Ni- bearing phases rather than soil solution chemistry (Becquer *et al.*, 2001; Chardot *et al.*, 2007)

## Ni in serpentine soils

The formations and distribution of Ni bearing minerals are highly variable and are controlled by the geochemistry and mineralogy of the parent material as well as the other soil forming factors including climatic conditions , topography, biota and time. (Kierczak *et al.*, 2016)

The primary sources of geogenic nickel in serpentine soils are primary minerals like pyroxene, olivine, spinel, amphibole as well as serpentine minerals like chrysotile, antigorite and lizardite. (Massoura *et al.*, 2006; Quantin *et al.*, 2008; Ratié *et al.*, 2015). Ni substitutes for Fe or Mg in those minerals, because of the similarity of the ionic radii of these elements (Becquer *et al.*, 2006). However, these Ni-rich minerals are not stable in the soil environment, thus chemical weathering of the Ni-bearing minerals leads to the release of Ni (Massoura *et al.*, 2006). The intensity of chemical weathering, which varies greatly during pedogenesis according to climate and moisture regimes, defines the formation of newly formed minerals(Echevarria *et al.*, 2006). Subsequently the released Ni is associated with these newly formed, secondary minerals that are clay minerals (e.g smectite or vermiculite) and Fe-Mn oxides (Echevarria, 2018). In the early stages of soil development Ni may be adsorbed on the surfaces of clay minerals or incorporated into their lattices, as it is again

able to substitute Mg (Hseu *et al.*, 2007). Also Fe oxides of different crystallization stages are considered an important host for Ni, especially in surface horizons (Ratié *et al.*, 2015). Then, Ni-bearing secondary minerals are quite different from the original bedrock, but Ni concentration in the soil stays almost the same (Massoura *et al.*, 2006). As soil development progresses, the incorporation of Ni into lattices of secondary minerals is promoted, as a consequence Ni is found in mineral structures rather than on the surface charge of particles (Hseu and Iizuka, 2013). Consequently the Ni -bearing phase that are formed during soil development determine the mobility and availability of Ni in the soil profile (Gasparatos and Barbayiannis, 2018) .The availability of Ni in primary minerals and primary clay minerals (i.e. serpentine minerals) is very low due to the presence of Ni in the crystal lattices. However some high solubility might be observed due to slight weathering of these minerals and absence of secondary high-charge minerals for further resorption. (Echevarria *et al.*, 2006). Ni availability tends to be high in moderately weathered soils where Ni is associated with poorly crystallized phyllosilicates, clay minerals and amorphous Fe oxides (Echevarria *et al.*, 2006). Soils exposed to more intense chemical weathering and soils being in an advanced stage of soil development, show a low Ni availability due to the high contents of well-crystallized Fe-oxides and high contents of goethite, where Ni is incorporated in the crystal structure and hardly available (Massoura *et al.*, 2006).

Generally, the most important factor determining Ni availability in serpentine soils are the bearing phases, respectively the minerals Ni is associated with. As a consequence soil development and factors influencing it, like climate and topography play a key role in the fate of Ni (Echevarria *et al.*, 2006; Massoura *et al.*, 2006; Cheng *et al.*, 2012; Bani *et al.*, 2014; Kierczak *et al.*, 2016)

## Estimation of Bioavailability

It is well established that information about the total concentrations of mineral elements in soil is not sufficient to draw conclusions about the element's mobility or availability to plants. Therefore, a range of chemical soil extractions have been developed in order to assess element availability for plants. A review of the methods used to extract different Ni fractions shows a great variety of extractions commonly used (Nikoli and Matsi, 2019). The most common single extraction methods to estimate bioavailability of Ni can be arranged into three groups, that are methods using extracting solutions containing complexing substances, (diluted)acids or salts. (Nikoli and Matsi, 2019). The former group uses

complexing substances like DTPA or EDTA which are complexing water soluble Ni and exchangeable Ni (Loeppert and Inskeep, 1996). They also access Ni ions weakly bound to organic and inorganic complexes that are eventually available and accessible to plants (Echevarria *et al.*, 1998). By complexing water soluble and exchangeable Ni, the extractant forces different pools of the soil solid phase to release further Ni into soil solution. (Nikoli and Matsi, 2019). Chemical extractions using salts like $CH_3COONH_4$, $NH_4NO_3$, $CaCl_2$ or $Sr(NO_3)_2$ target on extracting only the most mobile forms of Ni like the readily exchangeable Ni and water soluble Ni by replacing exchangeable cations present in soil. Also water can be used as a Ni extractant, however it is faulted to dilute the soil solution and changing the ionic strength, which is why it is more sensible to use salt solutions having a similar ionic strength as the soil solution (Markus Puschenreiter, personal communication, October 13 ,2019) . In numerous studies the above mentioned single extraction methods showed significant correlations with Ni uptake by accumulating as well as non-accumulating plants, therefore they are commonly used as indicator for available Ni (Nikoli and Matsi, 2019). Furthermore sequential extractions are also used to characterize metal fractionation in soil (Krishnamruti and Naidu, 2007). Different Ni fractions are consecutively targeted by different extraction solutions, from the most soluble to the more persistent fractions. These fractions are operationally defined and include soluble and exchangeable Ni, Ni associated with Mn-oxides or carbonate, Ni bound to soil organic matter or to amorphous Fe-Oxid, Ni occluded into crystalline Fe-oxides and the residual fraction associated with silicates (Tessier, Campbell and Bisson, 1979). However, the modification of the sample and reallocation of Ni from one fraction to the other cannot be excluded during extraction steps (Tessier, Campbell and Bisson, 1979). Nevertheless, these fractions are only operationally defined, since they are establishing an equilibrium between the soil solid phase and the extractant. But it does not mimic solute uptake by plants or gradual depletion of bioavailable Ni (Kreuzeder *et al.*, 2013). Therefore, methods which are including the factor time into their consideration about bioavailability have been used. For example isotopic exchange kinetics are used to determine the amount of Ni retained on soil particles through sorption and complexation as well as the weakly bound fraction (Echevarria *et al.*, 1998). IEK enables the distinction of these different fractions and gives information about the chemical state of the element (Echevarria *et al.*, 2006). Another example of a time dependent extraction technique is the diffusive gradient in thin film technique (DGT). It is an infinite sink extraction method and

thus mimics the root and it's diffusive uptake of metals and includes the additional metals being resupplied from the labile fraction during the application time (Zhang *et al.*, 2001).

Mitchell, Bingham and Page (1978) proposed basic qualifications of a soil extraction method used as bioavailability indicator. Namely the effectiveness over a wide soil pH range on the one hand, and a significant correlation with plant concentration and uptake on the other hand. As an additional qualification of the soil test, the correlation of the extracted element with other soil parameters that influence the element's bioavailability can be named (Nikoli and Matsi, 2019).

It can be noted that none of the above described methods accounts for the true bioavailability, but can only be an indicator giving, in combination with other soil parameters, a deeper insight into the fractions of Ni taken up by plants.

## Goals of this study

Despite the increasing research in the field of Ni-hyperaccumulation, the driving factors of the nickel uptake by hyperaccumulators are not yet fully understood. It is still debated which nickel fraction mainly supplies Ni uptake by the hyperaccumulator *O. chalcidica* and whether or not *O. chalcidica* is able to mobilise Ni and accesses non-labile pools of Ni.
In this context a detailed study was conducted to relate the hyperaccumulation of Ni by *O. chalcidica* along an ultramafic toposequence to physio-chemical soil properties, different Ni fractions of defined extractability, pedogenesis and soil mineralogy.
It is hypothesized that a particular Ni fraction mainly provides Ni for uptake by *O. chalcidica.* Further it is assumed that *O. chalcidica* is able to mobilise Ni from a distinct pool and increases the labile Ni pool in the surrounding soil, depending on Ni concentration. Additionally, it is hypothesized that Ni accumulation by *O. chalcidica* is different along stages of soil development.
Several research questions arising from these hypotheses are formulated as follows:

- Does Ni accumulation by *O. chalcidica* correlate with a Ni fraction of defined extractability or physio-chemical properties of the soil?
- Is Ni-availability and accumulation by *O. chalcidica* associated with the degree of soil weathering?
- Is *O. chalcidica* able to mobilise Ni and increase the labile Ni pool as a consequence of limited Ni supply form soil?

# Material and Methods

## Study site

This study was performed using soil sampled on a toposequence on serpentine soil near Redschlag, in the state of Burgenland, eastern Austria.

The climate is characterised by an annual rainfall of 718 mm precipitation over the year, and a mean temperature of 8°C (ZAMG, 2002). This area is part of the geological region "Rechnitzer unit", with the area around Redschlag being one of its few serpentinite outcrops. The Rechnitzer unit belongs to the Peninikum, which is very old rock pressed deeply under the alps. Only at some sites it is exposed due to tectonic folding of the alps (Michalek *et al.*, 2015). The studied soils are developed on a serpentinite bedrock (Figure 2).

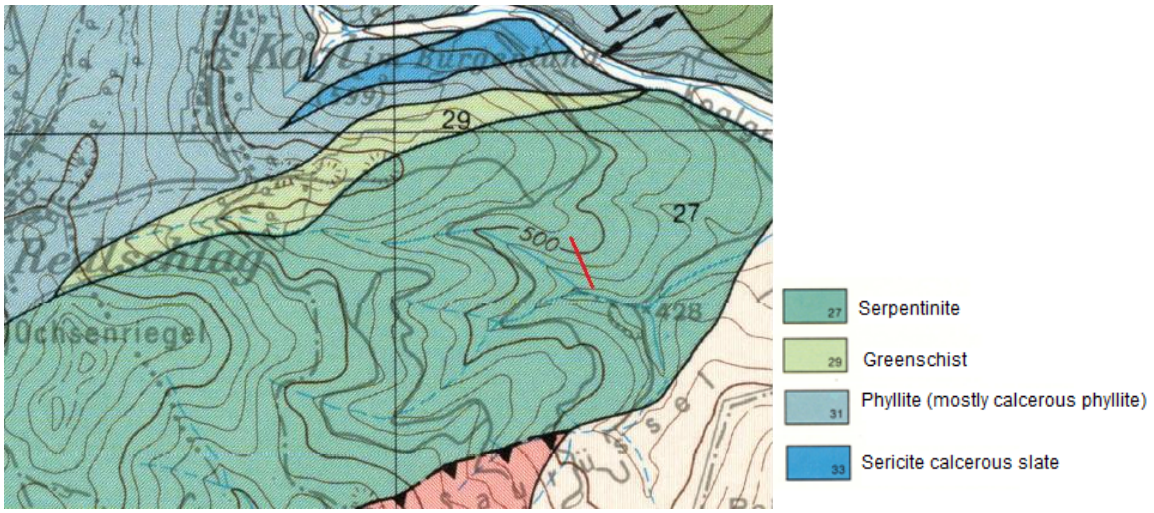


*Figure 2: The sampled toposequence is marked by a red line. The bedrock consists of serpentinite.* (Erich, Hermann and Pahr, 1982)

Six samples were collected along a SSE facing hillslope, which exhibits a gradient of Ni concentration. The first sample was collected at the bottom of the slope (450m.a.s.l) which was characterized by an erosion edge or rather a small landslide. Soil 1 had hardly any humus layer, had shallow soil depth and was rich in coarse fragment. All further 5 samples were collected in similar distances to each other throughout the toposequence, with the last

sample located at the shoulder of the slope (518 m.a.s.l). The study site is in very close proximity to an active quarry. The hillslope is facing to the quarry with sample point 1 being the closest to the quarry.

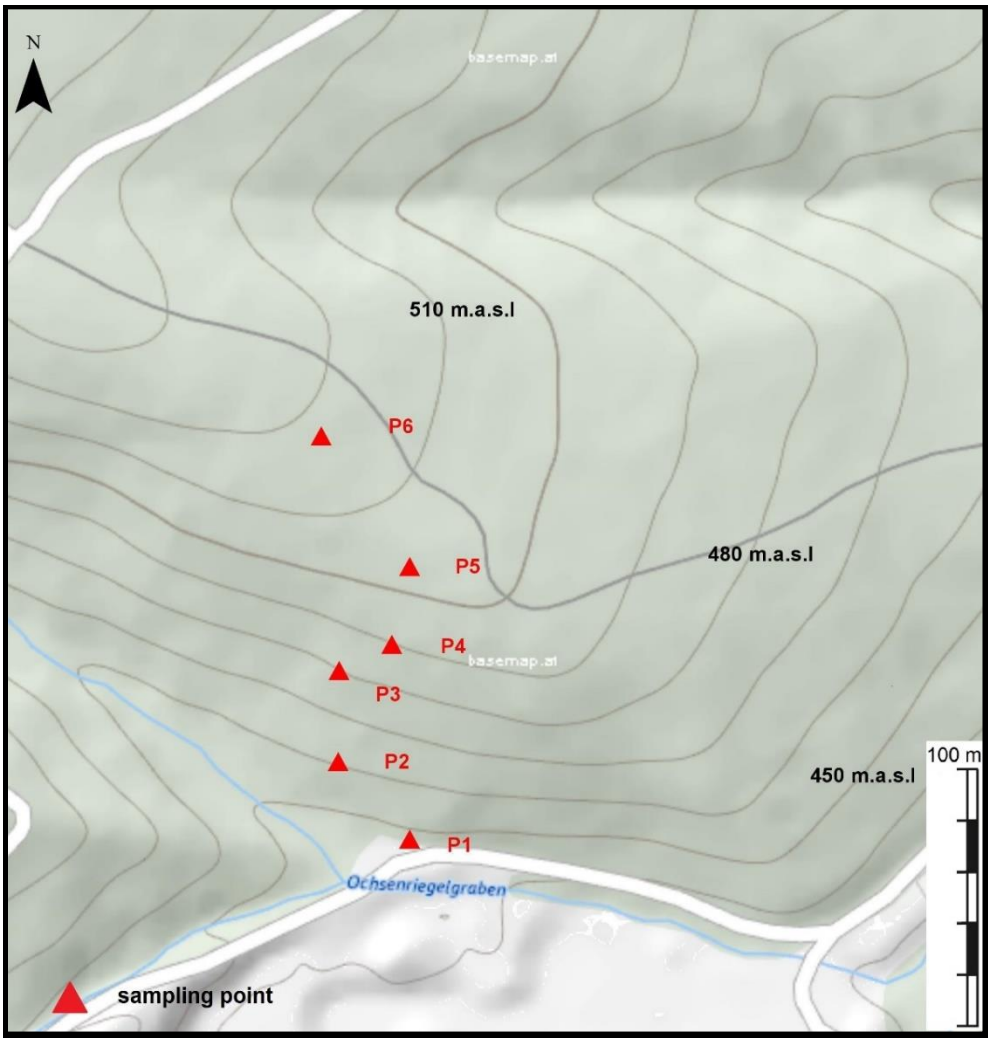


*Figure 3: Six soil samples were collected to similar distance to each other along the toposequence.*

Wenzel and Jockwer( 1999) and Millan *et al.*(2006) analysed soil samples from this site in earlier studies and classified the soil as an eutric leptosol, showing a sandy loam soil texture (in[%]:Sand(46.8), Silt (35.9), Clay (17.3)). The soils obtained from the six sample sites were air-dried and passed through a 2mm mesh screen before using it in the pot experiment and before analysing.

## Pot-experiment

A pot experiment was set up using soil from the six soil samples taken at the site described above. First, seedlings of *Odontarrhena chalcidica* (syn. *Alyssum murale*) were germinated in

a tray filled with water-saturated vermiculite. Pots were filled with 500 g of soil and a soil solution sampler was added in each pot (Rhizosphere Research Products B.V., Rhizon MOM, pore size 0,15 µm). Four replicates were set up for each soil. After 2 weeks of germination plants developed 3-4 leaves and they were transplanted into the pots. Pots were set up in a greenhouse at a set temperature of 21 °C and average air humidity of 40%. Since the pot experiment went over into middle of June temperatures rose and differed from the set temperature and could reach over 30°C on some days. Soil moisture was maintained at 80% of water holding capacity. Soil solution was sampled using a soil solution sampler every 2 weeks, 4 times in total. After 66 days, plants were harvested. Shoots were cut at 0.5 cm above soil surface, washed with deionized water and dried in paper bags at 80°C for 48 h. After determining the weight, plant shoots were ground for homogenization.

After the pot experiment the soil from each pot was air-dried and passed through a 2mm sieve before further analysis.

## Plant digestion

The digestion of the dried plant material was performed following Zhao, McGrath and Crosland (1994). Therefore 0.2 g of the ground and homogenized sample was weighed into a digestion tube. 5 ml of $HNO_3$, 1 ml of $H_2O_2$ and subsequently, one drop of iso-octanol (using a Pasteur-pipette) was added to prevent strong foaming. The tubes plus cooling unit were left overnight under a fume hood at room temperature. The following day, the digestion heating-program was run (3 h at 150 °C). After the digestion, the tubes were filled with purified water up to approximately 50 ml and weighed again. After homogenizing, the solution was filtered using folded paper filters (Munktell 14/N, pore size 2-3 µm). The elements Ca, Fe, Mg, K, Ni, P were measured on ICP-OES (Optima 8300, Perkin Elmer) while Cr, Co, Cu and Zn were measured using ICP-MS (Elan DRCe 9000, Perkin Elmer). For the ICP-OES measurement a 1:4 dilution of the samples was prepared using 2% $HNO_3$. For the ICP-MS measurement a 1:10 dilution using 2% $HNO_3$ was prepared.

Using the data from the soil and plant digestion, the bioaccumulation factor (BAF) was derived. It expresses the uptake of Ni into the plant by relating the Ni accumulated in the aerial tissue of *O. chalcidica* to the total Ni concentration in the soil:

$$BAF = \frac{Ni_{shoot}[\frac{mg}{kg}]}{Ni_{total}\,[\frac{mg}{kg}]} \quad (1)$$

## Soil physicochemical analysis

### Gravimetric water content

Soil water content was determined by the gravimetric method with oven drying. 50 g of soil were oven dried at 105° for 24 hours. The exact weight was noted to three decimal places. After 24 hours, the samples were cooled down in an exsiccator and afterwards reweighed. The measured dry weight was used to calculate the mass of water lost using formula 2:

$$soil\ water\ content = \frac{mass\ of\ wet\ soil\ [g] - \ mass\ of\ dry\ soil\ [g]}{mass\ of\ dry\ soil\ [g]}\ x\ 100 \quad (2)$$

### Soil pH

Soil pH was measured following OENORM L 1083-89 using bi-distilled water in one measurement and 0.01M $CaCl_2$ in another measurement. 25 ml of $CaCl_2$ solution, respectively 25ml of bi-distilled water were added to 10 g of air-dried soil (< 2 mm) in a 50 ml bottle. The suspension was shaken by hand until all soil material was in contact with the solution and left 2 hours for equilibration. Subsequently, the ProLab 4000 pH meter (SCHOTT Instruments GmbH, Weilheim, Germany) was used for pH measurement, three-point calibrated at pH 4, 7 and 10.

### Water holding capacity

To identify water holding capacity 10-15 g of air-dried soil (< 2 mm) were put into folded paper filters (Munktell 14/N, pore size 2-3 µm) seated into funnels. The soil was wetted to saturation and afterwards it was drained for one hour. This process was repeated twice. Subsequently the moist soil sample was transferred to a porcelain cup and the exact weight was noted to three decimal places. The soil sample was dried in an oven at 105° for 24 hours and reweighted. The measured dry weight was used to calculate the water content of the saturated samples using formula 1 which equals the water holding capacity.

## Total C and N

Total Carbon ($C_{Total}$) and Nitrogen ($N_{Total}$) was determined by dry combustion (ÖNORM L1080). The soil samples were oven-dried and finely ground using a ball mill at 80 rpm for 5 minutes. 100 mg of the homogenized, fine grained soil samples were weighed and packed into tin foil. The samples were analysed using combustion analysis (vario MACRO cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). This analytical method is based on the oxidation of the sample by "flush combustion". In the course of oxidation all organic and inorganic substances are converted into combustion products. The combustion gases $CO_2$ and $N_2$ are detected by the thermal conductivity detector.
Previous measurements conducted by (Noller, 2017) at the same site revealed, that the content of inorganic carbon, measured as $CaCO_3$, can be neglected. Therefore, in this study it is assumed that $C_{Total}$ originates entirely from organic sources.

## Cation-exchange-capacity

In order to determine the cation exchange capacity a Barium chloride extraction was performed following ÖMORM L 1086-89. Therefore 100 ml of a 0.1 mol/L $BaCl_2$ solution was added to 5 g of soil. The sample was mixed carefully to make sure the whole sample is in contact with the solution. After letting the samples to equilibrate overnight, they were agitated for 2 hours at 20 revolutions per minute. After shaking, the samples were filtered using folded paper filters (Munktell 14/N, pore size 2-3 µm). The samples were diluted 1:2 using 4% $HNO_3$ before measuring them on ICP-OES. The concentrations of Al, Ca, K, Mg, Ni and Na were measured in order to calculate the exchange capacity in the solution considering their specific charge using the following formula:

$$\sum_{i=1}^{k} \frac{n_i * z_i}{m} \quad (3)$$

Where $n_i$ = amount of substance

$z_i$ = valence

$k$ = total number of absorbed species

$m$ = reference mass

## Determination of OLSEN-P

To identify the plant available P content of the soils the method of OLSEN-P was applied. Therefore 2 g of air-dry soil were weighed into a 100 ml PE shaking bottle. 40 ml of 0.5 mol/L $NaHCO_3$- solution was added. The 0.5 mol $L^{-1}$ $NaHCO_3$- solution was priorly adjusted to a pH of 8.5 using a 1mol/L NaOH solution. The samples were put into an overhead shaker for 30 minutes and then filtered using folded paper filters (Munktell 14/N, pore size 2-3 µm). The samples were measured on a UV/VIS spectrophotometer. Therefore, the samples were coloured using the molybdate blue colorimetry. Before staining the samples were diluted by a factor of two. 5 ml of a sample were diluted with 4.5ml of bi-distilled water and 0.5 ml of $H_2SO_4$. $H_2SO_4$ was added to neutralise the buffering capacity of the carbon in the samples. After diluting, 1 ml of the sample mixed with 0.2 ml of the staining solution, which consisted of 10ml 2.5 mol/L $H_2SO_4$, 3ml 35 mmol/L $(NH_4)_6Mo_7O_{24}$ , 1ml 4.5 mmol/L $C_8H_4K_2O_{12}Sb_2$ and 6 ml and 0.1 ml/L $C_6H_8O_6$. After letting the mixture react for 20 minutes the samples were measured at the UV/VIS spectrophotometer at a wavelength of 881 nm.

## Total metal concentrations in *aqua regia*

The Ni concentration in the soil samples was analysed by four different extraction methods, providing an fractionation of Ni into pools with different operationally defined extractability. The quantification of these could help to identify the Ni fractions available to *O. chalcidica* for uptake.

The (pseudo)-total metal concentration in soil ($Ni_{Total}$) was measured using *aqua regia* according to OENORM L 1085. 0.5 g of ground and oven dry soil was weighed into acid washed glass tubes of known weight. 4.5 ml of 37% HCl and 1.5 ml of 65 % $HNO_3$ was added to the tubes under the fume hood. Subsequently, one drop of iso-octanol (using a Pasteur-pipette) was added to prevent strong foaming. The tubes plus cooling unit were left overnight under a fume hood at room temperature. The following day, the digestion heating-program was started (3 h at 150 °C). After the digestion program, the tubes were filled with purified water up to approximately 50 ml and weighed again. After homogenizing, the solution was filtered using folded paper filters (Munktell 14/N, pore size 2-3 µm). The elements Al, Ca, Fe, K, Mg, Mn, P, Ni, Zn were measured on an ICP-OES while Co, Cu, Zn were measured on an ICP-MS. For the

ICP-OES measurement a 1:10 dilution of the samples was prepared using 2% $HNO_3$. For the ICP-MS measurement a 1:20 dilution using 2% $HNO_3$ was prepared.

### Sr $(NO_3)_2$-extractable metals

The $Sr(NO_3)_2$ extraction is a single chemical extraction that uses the strontium salt of nitric acid to replace exchangeable cations present in soil. Cation concentrations determined by this extraction method are referred to as exchangeable fraction (Santelli and Freire, 2015). A 10mmol/L $Sr(NO_3)_2$ solution was added to 10 g of air-dry soil. The sample was mixed to make sure the whole sample is in contact with the solution. The sample was agitated for 2 hours at 20 rpm and filtered using folded paper filters (Munktell 14/N, pore size 2-3 µm). The samples were diluted 1:5 using 2.5% $HNO_3$. Metal concentrations were determined using ICP-MS.

### DTPA- extractable metals

To determine the diethylenetriaminepentaacetic acid (DTPA) extractable metal fraction the extraction was performed following Loeppert and Inskeep (1996). DTPA is a chelating agent that forms complexes with soluble and exchangeable metals. (Loeppert and Inskeep, 1996). The DTPA extraction accesses exchangeable Ni and Ni ions weakly bound to organic and inorganic complexes that are eventually available and accessible to plants (Echevarria *et al.*, 1998). According to Echevarria *et al.* (2006) the DTPA extraction can serve as a fairly good indicator of available Ni in soils.

To prepare 1 L of DTPA extraction solution 14.91 g of TEA, 1.957 g of DTPA and 1.47 g of $CaCl_2$ was dissolved in 1L deionized water. The solution was adjusted to a pH of 7.3 by adding 6mol/L HCL. 20 ml of the DTPA solution was mixed with 10 g of soil. The mixture was shaken for 2 hours on a reciprocating shaker. After 2 hours the samples were centrifuged at 3000 * G, decanted and filtered through a folded paper filter (Whatman, Nr 42). Before measuring metal concentration on ICP-MS the samples were diluted 1:250 using 2% $HNO_3$.

### Citrate-Bicarbonate-Dithionite-extractable Iron

Selective dissolution of Fe oxides was achieved using two extractants to characterise the degree of crystallisation of Fe oxides and therefore be able to draw conclusions about the

degree of evolution of the soils. The Citrate-Bicarbonate-Dithionite extraction and Ammonium-Oxalate extraction were carried out independently on different aliquots of each soil sample, therefore it was not a sequential extraction procedure.
The oxalate extraction affects Fe oxides under amorphous forms ($Fe_o$)
whereas the dithionite extraction dissolves both, amorphous and well-crystallized Fe oxides ($Fe_d$). According to Blume and Schwertmann (1969) the crystallization of iron oxides in soils is a major process of soil genesis and the ratio $Fe_0/Fe_d$ ("activity ratio") is used as a relative measure of the degree of aging of free iron oxides. Ni associated with the two fractions was also measured and is supposed to originate from the same bearing Fe oxide particles (Massoura *et al.*, 2006).

The Citrate-Bicarbonate-Dithionite extraction enables the solubilization of pedogenic crystalline iron oxides (e.g hematite, goethite) and non-crystalline iron oxides. This method is based on a reduction process. Reducible elements like iron are reduced and kept in solution by complexing with citric acid (Santelli and Freire, 2015).

In order to assess the content of free iron oxides ($Fe_d$) a modification of the Citrate-Bicarbonate-Dithionite extraction by Mehra and Jackson (1960) was performed.
0.4 g of air-dried soil was weighed into a 50mL plastic centrifuge tube. 18 ml of 0.3 mol/L sodium citrate and 2.8 ml of 1mol/L sodium bicarbonate was added to the soil. The samples were placed into an 80° warm water bath until they reached that very temperature. Next, 0.4 g of sodium dithionite powder was added and the sample was stirred constantly for 1 minute and then intermittently every 5 minutes for 15 minutes. After 15 minutes a second 0.4 g portion of sodium dithionite was added and the sample was stirred occasionally for another 10 minutes. After the digestion, 1 ml of saturated sodium chloride solution was added, the samples were taken out of the water bath and were allowed to cool. Samples were centrifuged at 1200 rpm for 15 minutes. After centrifugation no flocculation could be observed, so samples were brought to temperature again and 1 ml of acetone was added. After cooling the samples were centrifuged again and the supernatant was decanted and filtered using folded paper filters (Munktell 14/N, pore size 2-3 µm). A 1:50 dilution of the samples was prepared using 2% $HNO_3$ before analysing them on ICP-OES.

Fe and Ni extracted using the Citrate-Bicarbonate-Dithionite method will be referred to as $Fe_d$ respectively $Ni_d$.

## Ammonium-Oxalate extractable Iron

To determine the share of amorphous Iron oxides ($Fe_o$)in the soil samples an acid ammonium Oxalate in Darkness extraction was performed following (Loeppert and Inskeep, 1996).

An acidified ammonium oxalate solution was prepared by dissolving 12.435g of ammonium oxalate and 6.305g of oxalic acid in 1L of deionized water. The solution was adjusted to a pH of 3 by adding HCL.

The acidified ammonium oxalate solution was added to 500mg of air-dried soil. The samples were placed in a light proof container. The container was placed on a reciprocating shaker and the samples were agitated for 2 hours. After 2 hours the samples were centrifuged, decanted and filtered through a folded paper filter. The samples were diluted 1:50 with 2% $HNO_3$ and stored in a dark container before analysing them on ICP-OES.

Fe and Ni extracted using the Ammonium-Oxalate method will be referred to as $Fe_o$ respectively $Ni_o$.

## Soluble Cations

To determine soluble salts and complexed compounds which are readily available to plants a 1:10 water extraction according to ÖNORM; L 1092-93 war conducted. 50 ml of bi-distilled water was added to 5 g of air-dried soil. The samples were shaken by hand in order to get the whole sample in contact with the water. The samples were let overnight to react. The next day the samples were shaken for 1 hour at 20 revolutions per minute. After shaking the samples were decanted and filtered using folded filter papers (Whatman, Nr 42). Before determining solved cations on ICP-MS the samples were diluted 1:2 with 4% $HNO_3$.

## Soluble Anions

In order to identify the concentrations of soluble anions, the undiluted 1:10 water extraction samples were analysed using Ion chromatography (881 Compact IC pro, Metrohm). For this analysis a “Metrosep A Supp 5 150/4.0” column was used.

### DOC

For the determination of the dissolved organic carbon, 10 ml of the 1:10 water extracts were mixed with 80 µl of 10% HCL. The samples were analysed using a TOC Variocube (by Elementar Analysensysteme GmbH, Langenselbold, Germany)

### Mineral Analysis

Mineral phases were recorded qualitatively and semi- quantitatively by using x-ray diffraction. Soil samples were dried and ground using a disc vibrating mill. The measurement was made using a Panalytical XPert Pro MPD Diffractometer with automatic divergence slit, Cu LFF tube, 45 kV, 40 mA and a X'celerator detector. The measuring time was 25 seconds. In addition, the samples were analyzed by simultaneous thermonanalysis in order to estimate the amorphous fraction of soils.

### Speciation

Finally, to identify the different chemical species of Ni present in soil solution geochemical modelling was performed. Therefore, following data was entered the chemical equilibrium software Visual MINTEQ vers. 3.1.:

- concentrations of $Ni^{2+}$, $Mn^{2+}$, $Fe^{3+}$, $Zn^{2+}$, $Mg^{2+}$, $K^{+}$, $Ca^{2+}$, $Al^{3+}$, $Na^{+}$ in soil solution sampled at four sampling dates
- pH measured in the soil solution at four sampling dates
- DOC measured in 1:10 water extract, due to lack of sufficient amount of soil solution.
- Concentrations of $NO_2^{-}$, $NO_3^{-}$, $PO_4^{3-}$, $SO_4^{3-}$, $F^{-}$, $CL^{-}$, measured in 1:10 water extract due to lack of sufficient amount of soil solution.

Geochemical speciation was calculated using Visual MINTEQ vers. 3.1. employing the Nica-Donnan model.

## Statistical Analysis

To determine if the variation of the soil physico-chemical parameters along the toposequence are of statistical significance, an Analysis of Variance was conducted. If the requirements for an ANOVA (variance homogeneity and normal distribution) were not fulfilled a Kruskal Wallis test or a Welch test was performed. Furthermore, an Analysis of

Variance with repeated measures was performed to ascertain if the $Ni_{DTPA}$, $Ni_{SR(NO3)2}$ and the Ni concentrations in soil solution showed a significant change over time.

Finally, a pearson correlation matrix was calculated for all soil physico-chemical parameters and elemental concentration in the shoots of *O. chalcidica.*

# Results

## Physicochemical soil properties

*Table 1: Physio-chemical characteristics of the six soils along the toposequence*

| Sample Point | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| pH | | | | | | | |
| H20 | | 7.9 | 7.2 | 7.4 | 7.3 | 6.2 | 6.0 |
| CaCl2 | | 7.1 | 6.7 | 6.8 | 6.7 | 5.5 | 5.4 |
| Exchangable Cations | [mmol$^c$/kg] | | | | | | |
| $Al^{3+}$ | | 0.05 | 0.04 | 0.03 | 0.03 | 0.06 | 0.11 |
| $Mg^{2+}$ | | 114 | 179 | 140 | 219 | 162 | 237 |
| $Ca^{2+}$ | | 15.3 | 51.1 | 53.0 | 96.7 | 46.8 | 90.6 |
| $K^+$ | | 1.62 | 1.21 | 1.24 | 1.43 | 0.94 | 2.33 |
| $Na^+$ | | 0.36 | 0.44 | 0.53 | 0.64 | 0.39 | 0.70 |
| $Ni^{2+}$ | | 0.22 | 0.33 | 0.34 | 0.52 | 1.07 | 0.70 |
| CEC | | 132 | 232 | 195 | 318 | 212 | 331 |
| Olsen-P | [mg/kg] | 1.93 | 6.47 | 6.10 | 6.37 | 2.98 | 9.74 |
| Total N | [mg/kg] | 0,31 | 2.74 | 2.24 | 4.36 | 3.62 | 5.98 |
| Total C | [mg/kg] | 6,49 | 50.4 | 40.3 | 76.6 | 68.3 | 122 |
| DOC | [mg/L] | 8,06 | 21.0 | 17.0 | 17.0 | 11.5 | 82.6 |
| Mg/Ca ratio | | 40,9 | 39.2 | 37.9 | 33.9 | 87.3 | 23.3 |
| Total major elements | | | | | | | |
| Ca | [g/kg] | 3.14 | 3.84 | 4.12 | 4.18 | 1.61 | 4.23 |
| K | [g/kg] | 2.51 | 0.67 | 0.57 | 0.45 | 0.45 | 0.85 |
| Al | [g/kg] | 22.1 | 13.8 | 12.6 | 11.9 | 13.2 | 18.1 |
| Mg | [g/kg] | 128 | 151 | 156 | 141 | 140 | 98 |
| Fe | [g/kg] | 54.0 | 63.7 | 65.3 | 55.7 | 65.3 | 45.0 |
| P | [mg/kg] | 104 | 245 | 222 | 268 | 350 | 360 |
| Total trace elements | | | | | | | |
| Co | [mg/kg] | 126 | 145 | 193 | 189 | 197 | 148 |
| Cu | [mg/kg] | 47.7 | 21.9 | 21.4 | 30.9 | 13.7 | 20.0 |
| Zn | [mg/kg] | 64.5 | 64.3 | 63.2 | 76.5 | 79.0 | 90.7 |
| Ni | [mg/kg] | 1713 | 914 | 926 | 1185 | 720 | 526 |
| Mn | [mg/kg] | 1111 | 1298 | 1424 | 1256 | 1448 | 1378 |

The pH-values of the soil samples followed a clear trend along the toposequence. With a $pH_{H2O}$ of 6.0 measured at the top of the slope, the pH-value gradually increased to 7.9 at the

bottom of the slope. CEC values showed a more random pattern without a clear trend along the slope to be recognized. Sample point 1 showed the lowest CEC value of 132 $mmol^c$/kg and sample 6 the highest value of 330 $mmol^c$/kg with the other soils varying in between these two soils. $Mg^{+2}$ clearly dominated the exchange complex of all soils followed by $Ca^{+2}$, $K^+$, $Na^+$ and $Al^{+3}$. $Mg^{+2}$ proportion of the exchange complex was rather high with values ranging between 87% (site 1) and 69% (site 4). Exchangeable Mg/Ca ratio showed the highest value at site 1 with 7.41, which shows a clear differentiation to the other soils. Exchangeable Mg/Ca at the sites 2,3,4,5,6 showed values of 3.51, 2.65, 2.26, 3.47, 2.61 respectively.

## Ni extractability along toposequence

*Table 2: : Ni pools of operationally defined extractability.*

**Soil Ni fractions**

| Sample point | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Ni total | [mg/kg] | 1713 | 914 | 926 | 1185 | 720 | 526 |
| Ni DTPA | [mg/kg] | 16,0 | 44,8 | 43,2 | 69,8 | 32,5 | 21,1 |
| | % | 0,94% | 4,90% | 4,66% | 5,89% | 4,51% | 4,01% |
| Ni $Sr(NO_3)_2$ | [mg/kg] | 0,57 | 0,81 | 0,79 | 0,96 | 1,21 | 0,95 |
| | % | 0,03% | 0,09% | 0,08% | 0,08% | 0,17% | 0,18% |
| Ni $H_2O$ | [mg/kg] | 0,55 | 1,11 | 0,78 | 1,48 | 0,80 | 1,20 |
| | % | 0,03% | 0,12% | 0,08% | 0,12% | 0,11% | 0,23% |

To be able to split the total Ni concentration into pools of different extractability, four different extractions were conducted. Total Ni concentration showed a significant ($p<0.05$) increase downhill along the slope. With the highest concentration at the lower most sample point 1 (1712 mg/kg), $Ni_{total}$ concentration gradually decreased along the toposequence, reaching its lowest value at the upper most sample point 6 (526 mg/kg). On the other hand $Ni_{Sr}$ showed a reversed trend to $Ni_{total}$. $Ni_{Sr}$ showed a significant ($p<0.05$) decrease downhill along the toposequence. The data obtained from the DTPA and $H_2O$ extraction revealed no trend; however, sample point 1 showed clearly the lowest Ni values in both of these two Ni fractions. The exchangeable and soluble Ni, detected by $Ni_{Sr}$ and $Ni_{H2O}$ respectively, represented a relatively small fraction of the total Ni content, with a proportion of below 1%. The $Ni_{DTPA}$ fraction took up a higher percentage of total Ni, which varies between 0.94% and 5.89%.

## Iron Oxides and associated Nickel

*Table 3: Selective extractions of Fe and Ni in soil samples with ammonium-oxalate ($Fe_O$, $Ni_O$) and citrate-bicarbonate-dithionite( $Fe_d$, $Ni_d$)*

| Sample point | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| $Fe_d$ | [mg/kg] | 10209 | 10534 | 15274 | 13644 | 21235 | 17285 |
| $Fe_d$ | % | 18.8% | 16.4% | 23.3% | 24.4% | 32.4% | 38.2% |
| $Ni_d$ | [mg/kg] | 507 | 292 | 328 | 471 | 198 | 167 |
| $Ni_d$ | % | 29.6% | 32.0% | 35.5% | 39.7% | 27.5% | 31.7% |
| $Fe_o$ | [mg/kg] | 2470 | 2598 | 2267 | 2219 | 2903 | 3111 |
| $Fe_o$ | % | 4.54% | 4.05% | 3.46% | 3.97% | 4.43% | 6.87% |
| $Ni_o$ | [mg/kg] | 261 | 158 | 171 | 236 | 96.6 | 76.6 |
| $Ni_o$ | % | 15.2% | 17.3% | 18.5% | 20.0% | 13.4% | 14.6% |
| $Fe_c$ | [mg/kg] | 7739 | 7936 | 13008 | 11424 | 18332 | 14174 |
| | % | 14.2% | 12.4% | 19.8% | 20.4% | 28.0% | 31.3% |
| Activity Index | | | | | | | |
| $Fe_o$/ $Fe_d$ | | 0.24 | 0.25 | 0.15 | 0.16 | 0.14 | 0.18 |

The contents of free iron oxides ($Fe_d$), amorphous iron oxides ($Fe_o$) and well crystallized iron oxides ($Fe_d$ -$Fe_o$) are shown in table 3. The measurements revealed that the content of amorphous iron oxides was relatively low, ranging from 3.46% in soil 3 to 6.87% in soil 6 of the total Fe. Also the concentrations of $Fe_o$ were relatively stable among the six samples, as there was no significant difference of the $Fe_o$ between the samples revealed. On the other hand, the free iron oxides and well crystallized iron oxides showed significant differences ($p<0.05$) between the soils. Also $Fe_c$ comprised a high percentage total Fe in soils. The content of $Fe_c$ showed an increase uphill along the toposequence from 12.4 % (soil 2) to 31.3% (soil 6) of total Fe. The activity index, which is used as a relative measure of the degree of aging of iron oxides also showed significant differences ($p<0.05$) and decreased uphill along the toposequence. The Activity indices ranged from 0.24 at soil 1 to 0.18 at soil 5. Considering these values one can derive that there is a gradient of progress in soil development and weathering along the toposequence, with soil 1 being the least weathered and least developed soil. When proceeding uphill, the soils show a progress in soil development and weathering intensity, with soil 5 being the most developed having the highest concentration of well crystallized iron oxides (16469 mg/kg) and the lowest activity index (0.14).

Although free Fe was mainly under well-crystallised forms, Ni was associated with both, well crystallized and amorphous Fe-oxides. In fact, Ni associated with Fe oxides was evenly distributed between the well crystallized and amorphous phase as $Ni_o$:$Ni_d$ ratios were approximately 50. In downhill and mid slope soils 1,2,3,4 the $Ni_o$:$Ni_d$ ratios were 0.51, 0.53,

0.52, 0.50 respectively, which means that more than half of the Ni associated with Fe oxides was bound to or occluded in amorphous Fe oxides. Whereas in uphill soils 5 and 6 the $Ni_o$:$Ni_d$ ratios are 0.48 and 0.45 respectively, which implies that more than half of the Ni was associated with well crystallized iron oxides. In contrast to the Fe allocation between amorphous and well-crystallised oxides, Ni was always more bound to amorphous Fe oxides as $Ni_o$:$Ni_d$ was always higher than $Fe_o$:$Fe_d$.

Furthermore, relatively large portion of the total Ni in soils was associated with free Fe-oxides. The fraction of $Ni_o$ represented between 13.4% (soil 5) and 20% (Soil4) of total Ni in soils. $Ni_d$ represented between 27.5% (Soil 5) and 39.7% (Soil 4) of total Ni in soils. Compared to other Ni pools extracted from the soils these were by far the biggest pools.

## Ni uptake by *O. chalcidica* along the toposequence

*Table 4:Concentrations of trace elements and nutrients in leaves of O. chalcidica. Results are given as mean values of four replicates ±standard deviations*

| Sample point | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Biomass | [g] | 0.15 ± 0.04 | 0.57 ± 0.27 | 0.46 ± 0.09 | 0.52 ± 0.17 | 1.22 ± 0.19 | 0.52 ± 0.27 |
| Ni | [g/kg] | 15.2 ± 2.8 | 8.36 ±0.5 | 12.4 ± 0.1 | 11.3 ± 3.2 | 9.36 ± 1.1 | 2.16 ± 0.6 |
| BAF Ni | | 8.92 | 9.16 | 13.4 | 9.57 | 13.0 | 4.11 |
| Fe | [mg/kg] | 177 ± 97.8 | 138 ± 35.4 | 88.0 ± 22.4 | 109 ± 5.7 | 114± 12.1 | 146 ± 84.0 |
| Ca | [g/kg] | 24.4 ± 3.4 | 23.1 ± 2.8 | 29.3 ± 1.8 | 28.4 ± 1.7 | 27.1 ± 2.5 | 21.3 ± 4.7 |
| K | [g/kg] | 26.9 ± 2.7 | 26.4 ± 3.3 | 24.7 ± 1.7 | 24.7 ± 3.1 | 24.3 ± 2.6 | 19.3 ± 3.1 |
| Mg | [g/kg] | 8.88 ± 2.1 | 13.8 ± 2.3 | 10.7 ± 1.1 | 15.1 ± 3.9 | 11.7 ± 2.7 | 24.2 ± 3.8 |
| P | [g/kg] | 2.16 ± 0.4 | 3.10 ± 0.4 | 2.70 ± 0.3 | 2.72 ± 0.3 | 1.75 ± 0.3 | 2.64 ± 0.5 |
| Cr | [mg/kg] | 11.2 ± 13.4 | 7.96 ± 4.4 | 4.10 ± 3 | 6.32 ± 2.4 | 6.78 ± 0.8 | 14.6 ± 13.3 |
| Mn | [mg/kg] | 132 ± 52.2 | 108 ± 50.5 | 103 ± 12.9 | 227 ± 63.8 | 268 ± 43.7 | 149 ± 37.8 |
| Co | [mg/kg] | 70.9 ± 11.1 | 132 ± 43.8 | 204 ± 12.6 | 201 ± 71.1 | 313 ± 39.8 | 83.1 ± 38.1 |
| Cu | [mg/kg] | 11.0 ± 3.1 | 7.25 ± 0.5 | 5.94 ± 0.5 | 6.47± 0.6 | 7.53 ± 0.5 | 6.48 ± 1.1 |
| Zn | [mg/kg] | 50.2 ± 4.2 | 42.7 ± 9.9 | 44.9 ± 5.3 | 69.6 ± 18.7 | 177 ± 31.7 | 127 ± 53.3 |

The amount of Ni accumulated by *O. chalcidica* varied greatly throughout the plants. The Ni concentrations in the aerial tissues of the plants grown on the different soils were significantly different ($p<0.05$) . However, table 4 also displays a great variety of Ni hyperaccumulation by plants grown on same soils, especially on soil 1 and 4. The highest Ni concentrations were shown by the plants growing on soil 1 ranging from 17295 mg/kg and

11334 mg/kg. By far the lowest Ni accumulation could be shown for plants growing on soil 6. The uptake efficiency was calculated as bioaccumulation factor. The BAF values showed that Ni in the plant tissue of *O. chalcidica* was 4 to 13 times higher than in soil. These values demonstrate once again the astonishing ability of *O. chalcidica* to accumulate Ni in its shoots. The plants were also able to take up high amounts of P and Ca despite the low supply in soils.

## Mineral analysis

*Table 5:Abundance of minerals in soil samples determined by X-ray diffraction of fine powder. Amorphous materals were detected by simultaneous thermoanalysis.*

| Sample point | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Serpentine | % | 40 | 70 | 70 | 74 | 71 | 58 |
| Chlorite | % | 29 | 23 | 24 | 20 | 22 | 30 |
| Quartz | % | 19 | 5 | 3 | 4 | 5 | 11 |
| Magnetite | % | 1 | 2 | 3 | 2 | 2 | 1 |
| Amphibole | % | 3 | tr | nd | nd | nd | tr |
| Muscovite | % | 8 | nd | nd | nd | nd | nd |
| Amorphous material | | | * | * | ** | ** | *** |

tr=traces, nd=not detectable
*=rare, **=common, ***=abundant

In all studied soil samples the main mineral component was lizardite, a silicate mineral from the group of serpentine minerals. The serpentine contents ins soils were between 40% (soil 1) and 74% (Soil 4). The second most abundant mineral group was chlorite with contents between 20% and 30%. Quartz was detectable in all six soil samples, with highest contents measured in soil 1 (19%) and soil 6 (11%). Low contents of amphiboles could be detected only in soil 1 and 6. Muscovite could be detected in soil 1 (8%) only. Nickel minerals could not be detected as they were below the detection limit of the x-ray diffraction. The simultaneous thermo analysis revealed the content of amorphous material in the samples. That can be organic material or weakly crystallised or amorphous Fe-(hydro)oxides. Since the result of the thermo analysis correlated with the distribution of $C_{total}$ over the toposequence, it is assumed that the amorphous material measured in the thermo analysis is mainly organic material.

## Speciation

The total dissolved Nickel in soil solution showed a change during the period *O. chalcidica* grew in the soils six soils. While soils 1,2,3 show a gradual and statistically significant ($p<0.05$) decrease of dissolved nickel over time, this effect is not as distinct and not significant ($p>0.05$) in soils 4,5,6.

Figure 4 shows the outcome of the geochemical modelling using the speciation software Visual MINTEQ. The percentual distribution of nickel species over time in the modelled solution showed hardly any change. No significant alteration of percentual contents of $Ni^{2+}$, organically complexed Ni, $NiOH^{+}$, $NiSO_4$ or $Ni^{2+}$ weakly bound to dissolved fulvic acid could be observed. According to the geochemical modelling the percentual distribution among dissolved species in solution stayed almost the same.

However, the modelled solution also displayed a charge difference of around 90% for each soil and each date, which displays a drastic disequilibrium, caused by a dominance of cations in solution.

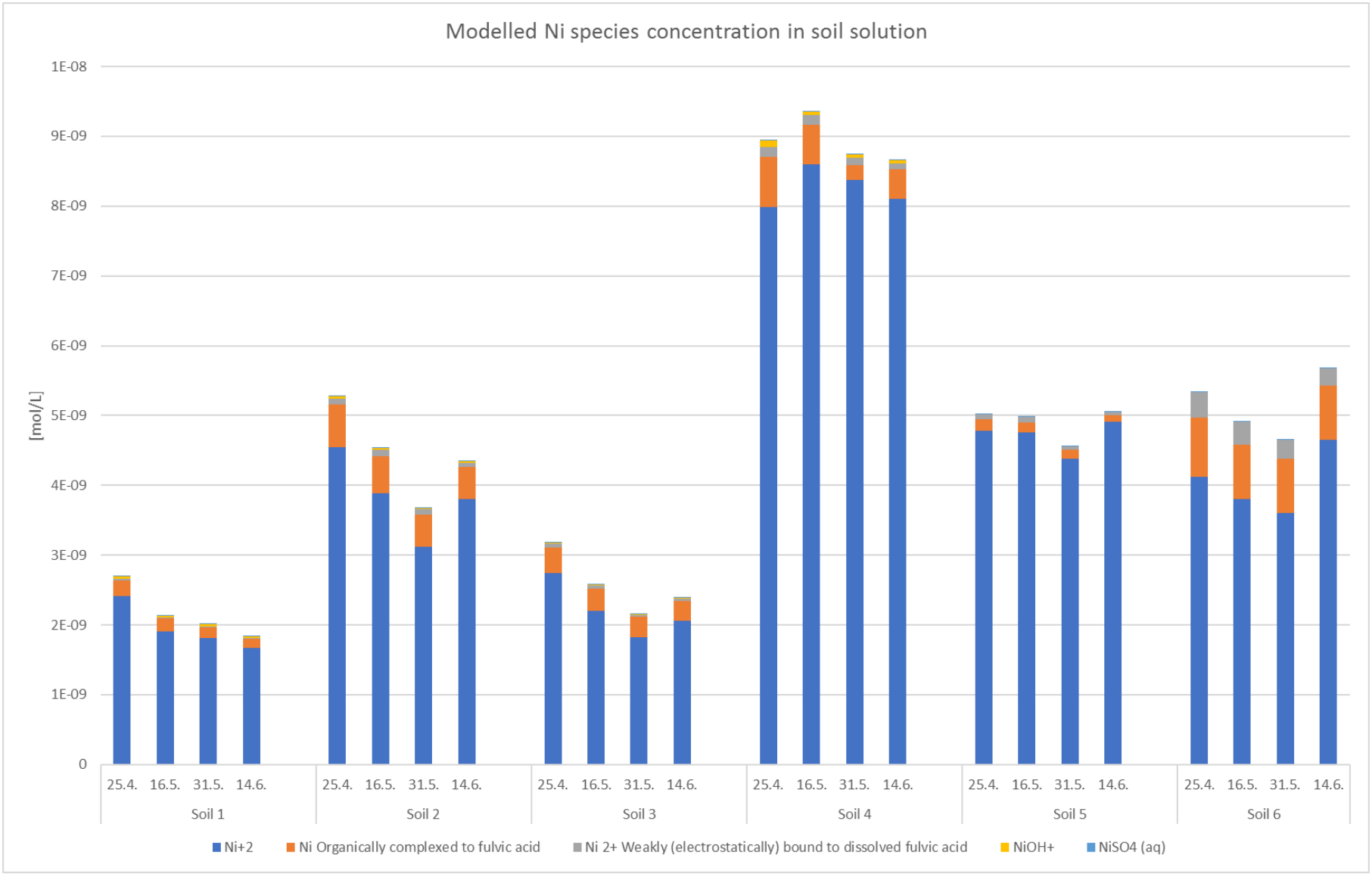


*Figure 4:Species distribution among dissolved Ni species in soil solution. Results of each sampling date are given as mean values of four replicates.*

## Correlation between Ni uptake by *O. chalcidica* and fractions of Ni and soil properties along the toposequence

*Table 6: Pearson's correlation matrix between Ni uptake by O. chalcidica and fractions of Ni and physico-chemical soil properties (n=6).*

| Pearson correlation coefficient | Shoot Ni | pH H20 | CEC | Mg/Ca | Olsen P | C/N | DOC | Activity Index | AquReg Ni | DTPA Ni | Ni $H_2O$ | $Sr(NO_3)_2$ Ni | Fe o | Fe d | Fe cr | Ni o | Ni d | Ni cr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shoot Ni | 1 | | | | | | | | | | | | | | | | | |
| pH H20 | **,847*** | 1 | | | | | | | | | | | | | | | | |
| CEC | -0,742 | -0,585 | 1 | | | | | | | | | | | | | | | |
| Mg/Ca | 0,196 | -0,278 | -0,389 | 1 | | | | | | | | | | | | | | |
| Olsen P | -0,790 | -0,465 | **,828*** | -0,653 | 1 | | | | | | | | | | | | | |
| C/N | -0,191 | -0,135 | -0,225 | -0,124 | -0,111 | 1 | | | | | | | | | | | | |
| DOC | **-,886*** | -0,657 | 0,681 | -0,506 | **,823*** | 0,432 | 1 | | | | | | | | | | | |
| Activity Index | 0,145 | 0,526 | -0,320 | -0,381 | -0,128 | 0,405 | -0,058 | 1 | | | | | | | | | | |
| Ni $_{Total}$ | **,850*** | **,884*** | -0,595 | -0,120 | -0,658 | 0,157 | -0,622 | 0,507 | 1 | | | | | | | | | |
| Ni $_{DTPA}$ | 0,147 | 0,178 | 0,433 | -0,095 | 0,214 | **-,921**** | -0,313 | -0,300 | -0,030 | 1 | | | | | | | | |
| Ni $_{H2O}$ | -0,515 | -0,300 | **,924**** | -0,415 | 0,713 | -0,453 | 0,410 | -0,157 | -0,377 | 0,686 | 1 | | | | | | | |
| Ni $_{Sr(NO3)2}$ | -0,496 | **-,817*** | 0,501 | 0,588 | 0,164 | -0,319 | 0,169 | -0,723 | -0,714 | 0,255 | 0,371 | 1 | | | | | | |
| $Fe_o$ | -0,785 | **-,833*** | 0,275 | 0,224 | 0,270 | 0,589 | 0,672 | -0,047 | -0,635 | -0,620 | -0,020 | 0,466 | 1 | | | | | |
| $Fe_d$ | -0,441 | **-,838*** | 0,284 | 0,578 | 0,100 | -0,043 | 0,267 | **-,842*** | -0,705 | -0,098 | 0,016 | **,865*** | 0,551 | 1 | | | | |
| $Fe_{cr}$ | -0,392 | -0,803 | 0,273 | 0,585 | 0,080 | -0,097 | 0,220 | **-,876*** | -0,681 | -0,048 | 0,018 | **,863*** | 0,489 | **,997**** | 1 | | | |
| Ni o | **,835*** | **,924**** | -0,397 | -0,264 | -0,475 | -0,125 | -0,623 | 0,399 | **,941**** | 0,283 | -0,120 | -0,672 | **-,829*** | -0,745 | -0,706 | 1 | | |
| Ni d | **,819*** | **,892*** | -0,357 | -0,266 | -0,466 | -0,104 | -0,594 | 0,361 | **,937**** | 0,282 | -0,094 | -0,641 | -0,811 | -0,709 | -0,670 | **,997**** | 1 | |
| Ni cr | 0,762 | **,829*** | -0,488 | -0,203 | -0,579 | 0,239 | -0,505 | 0,532 | **,987**** | -0,057 | -0,292 | -0,708 | -0,566 | -0,708 | -0,690 | **,926**** | **,931**** | 1 |

*The correlation is significant at the level of 0.05 (2-sided).

**The correlation is significant at the level of 0.01 (2-sided).

Pearson's correlation coefficients were calculated for the whole matrix of physico-chemical parameters, different Ni pools, and plant uptake (table 5). As shown in Figure 5 and 6, Ni uptake by *O. chalcidica* showed a significantly ($p<0.05$) positive correlation with total Ni and amorphous Fe-oxides. Surprisingly Ni uptake also showed a significantly ($p<0.05$) positive correlation with pH ($H_2O$). However, accumulation of Ni was not reflected by equivalent changes of DTPA extractable Ni or $Sr(NO_3)_2$ extractable Ni. $Sr(NO_3)_2$ extractable Ni showed no significant correlation with plant uptake but with pH ($H_2O$). Water soluble Ni showed no correlation with plant uptake either, but with CEC. DTPA extractable Ni was neither correlated to plant uptake nor to any physiochemical soil property but C/N ratio. The assumption that DTPA extractable Ni is associated with organic matter was discarded due to non-significant correlation of $Ni_{DTPA}$ with DOC or total C.

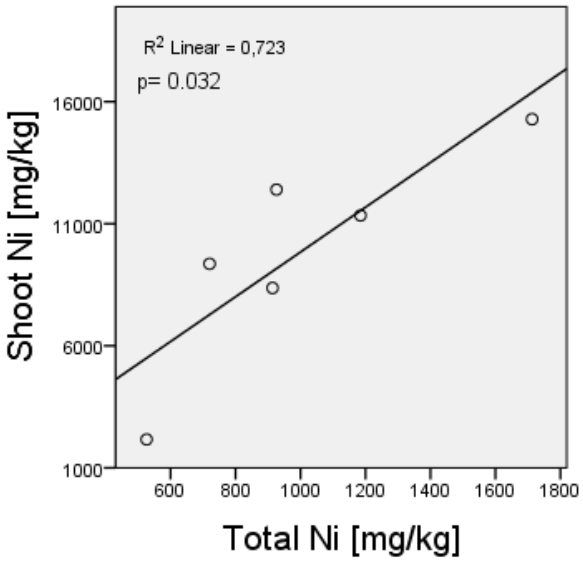


*Figure 5: Significant positive regression between Ni in shoots of O. chalcidica and total Ni in soils.*

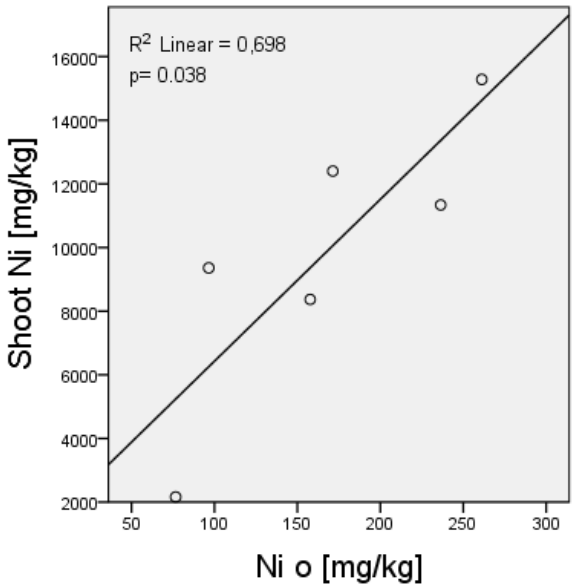


*Figure 6: Significant positive regression between Ni in shoots of O. chalcidica and selective extractions of Ni with ammonium-oxalate.*

## Plant induced changes in soils

By comparing values measured in the soils with values measured in the same soils after growth of *O. chalcidica*, it appears that *O. chalcidica* had a significant effect on some soil properties. After the pot experiment the soils showed a significant ($p< 0.05$) depletion of

$Sr(NO_3)_2$extractable Ni and DTPA extractable Ni. The reduction of $Sr(NO_3)_2$ and DTPA extractable Ni shows that these pools were depleted due to root uptake of Ni. However, no increase of SrNO3 extractable Ni and DTPA extractable Ni took place in any of the samples, therefore no mobilisation or solubilisation of Ni from non-labile to labile pools could be verified. Furthermore, there was a significant ($p< 0.05$) increase in pH values, measured in $H_2O$ as well as $CaCl_2$.

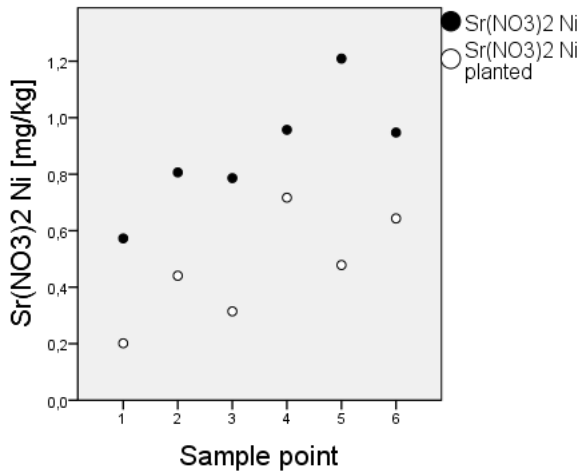


*Figure 7: Average of $Sr(NO_3)_2$ extractable Ni in planted and unplanted soils (N=4)*

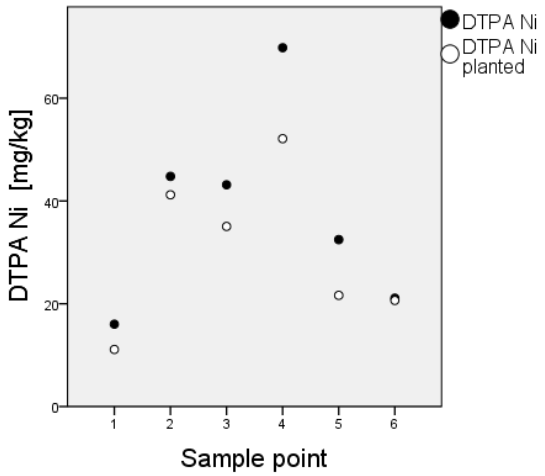


*Figure 8: Average of DTPA extractable Ni in planted and unplanted soils (N=4)*

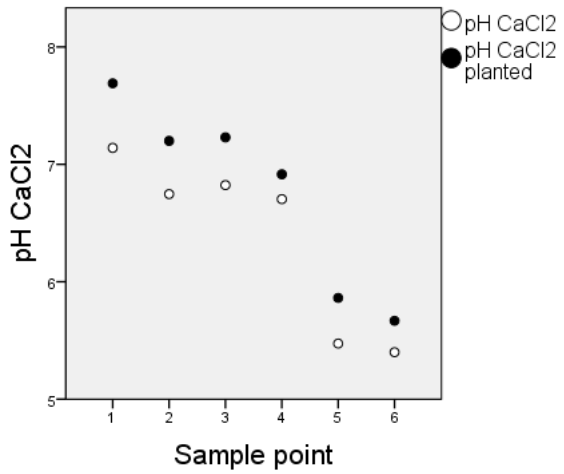


*Figure 9: Average pH-values in planted and unplanted soils (N=4)*

# Discussion

The data obtained from the soil sample analysis generally illustrated typical characteristics of serpentine soils (Alexander, 2004). The very low values of Olsen-P, K, Ca and the very high C/N ratio confirmed once again the infertility of serpentine soils.

The term 'Magnesic' refers to an exchangeable Mg/Ca ratio >1, therefore all of these soils can be considered as magnesic soils. Furthermore, soils exceeding an exchangeable Mg/Ca ratio of 3 can be considered "hypermagnesic" (Chardot *et al.*, 2007). Therefore, soils 1, 2 and 5 could be called hypermagnesic. The investigation of total element concentration also revealed extremely high $Mg/Ca_{total}$ ratios. With a $Mg/Ca_{total}$ of 87.3 site 5 showed the highest value, while the other soils showed values between 40.9 (at site 1) and 23.3 (at site 6). High $Mg/Ca_{total}$ ratios are a typical feature for serpentine soils and could also be found in the processed samples.

## Distribution of Ni fractions along the toposequence

Distributions of Ni in the serpentine soils depend on the parent material and on soil developing processes that are influenced by the landscape position (Lee et al., 2004). In the present study, the toposequence revealed an increase of $Ni_{Total}$ downhill along the slope, with soil 1 showing the highest and soil 6 the lowest $Ni_{Total}$ concentrations. Since the $Ni_{Total}$ levels along the slopes are not reflected by equivalent distribution of the Ni-bearing serpentine minerals, a redistribution of Ni might have taken place.

Cheng *et al.* (2012) attributed a similar distribution of total Ni and Cr along a Taiwanese toposequence to erosion and transport of solutes by lateral flow. Analogous the difference in total Ni at this current studied site may be attributed to erosion or surface runoff containing suspended particles. The deposition of sediments at the footslope and the transport of suspended particles by runoff might have led to a redistribution of Ni along the toposequence.

However, differences in total Ni levels between the soils may also be influenced by the activity of the quarry located next to the sampling sites. Due to the mining activity large amounts of dust from the serpentinite bedrock are released and deposited again in the area

next to the quarry. With sample site 1 being the closest and site 6 being the furthest from the quarry, the gradient of total Ni along the toposequence may be affected by the dust deposition.

Strontium nitrate extractable Ni ($Ni_{Sr}$) showed a reversed trend to $Ni_{total}$. $Ni_{Sr}$ showed a decrease downhill along the toposequence. The sample site 1 at the foot of the slope was the least weathered soil which had a very shallow soil depth and it contained a low amount of compounds able to retain Ni on their exchange sites, such as organic matter or Fe oxides. The other sample sites along the toposequence showed a higher amount of these compounds with soil 6 showing the highest C values and soil 5 the highest $Fe_d$ values. These compounds that are able to retain Ni on their exchange sites may explain the opposed trend of $Ni_{Sr}$ to $Ni_{total}$. This coherence might be confirmed by the positive significant ($p<0.05$) correlation between $Ni_{Sr}$ and $Fe_d$. Furthermore the labile and readily exchangeable Ni fraction ($Ni_{Sr}$) is connected to pH by a significant ($p<0.05$) negative correlation, as with higher pH the retention of Ni to the solid phase increases (Blume *et al.*, 2016).

Similarly, soluble $Ni_{H2O}$ is connected with the abundance of exchange sites and their ability to hold Ni, as $Ni_{H2O}$ showed a significant positive correlation with the cation-exchange-capacity. $Ni_{DTPA}$ distribution along the toposequence is hard to comprehend as it was barely correlated with any physico-chemical soil parameter, apart from a negative significant correlation with the high C/N ratios. In previous studies $Ni_{DTPA}$ showed correlations with isotopically exchangeable Ni (Echevarria *et al.*, 2006) or TOC (Bani *et al.*, 2014). In a very similar study conducted by Chardot *et al.* (2007) at a serpentine toposequence in the French Vosges mountains, $Ni_{DTPA}$ also showed a significant negative correlation with C/N ratio, and it was reasoned with C/N being an indicator for high weathering intensity and soil evolution.

## Soil genesis and its influence on Ni availability

The nature of soil mineralogy strongly affects Ni availability. As soil mineralogy depends on pedogenic properties, one can eventually deduct that Ni availability depends on soil genesis (Bani *et al.*, 2014). The main minerals found in the six topsoils are serpentine group and chlorite group minerals, both of them are not stable in soil environment(Kierczak *et al.*, 2007). Serpentine tends to weather to smectite during pedogenesis while chlorite is weathered to vermiculite before transforming to smectite(Lee *et al.*, 2004). Since both

groups are still the main component of the mineral composition, soil development is estimated not to be very advanced. Rather the present soils are rated to be in the early stage of soil development and only moderately weathered.

Blume and Schwertmann (1969) stated the crystallization of iron oxides in soils is a major process of soil genesis and the ratio $Fe_o/Fe_d$ ("activity ratio") is used as a relative measure of the degree of aging of free Fe-oxides. At the studied site the activity ratio decreased uphill along the toposequence from 0.24 at soil 1 to 0.18 at soil 5. Considering these ratios, one can derive that there is a gradient of progress in soil development and weathering intensity along the toposequence, with soil 1 being the least weathered and least developed soil. The more uphill soils show a progress in soil development and weathering intensity, with soil 5 being the most developed having the highest concentration of well crystallized Fe-oxides (16469 mg/kg) and the lowest activity index (0.18). Decreased pH values and higher levels of total C originating from organic sources, affirm the assumption of a gradient in weathering intensity. Alexander *et al.* (1989) and Lee *et al.* (2004) recorded that higher developed serpentinic soils showed higher Ca/Mg respectively lower Mg/Ca ratios, due to the leaching of Mg and deposition of plant detritus containing Ca. A similar trend could be observed at the studied toposequence. The least developed soil at site one showed the highest Mg/Ca ratio followed by a decrease of Mg/Ca ratios at the more developed soils uphill. However, soil 5 constitutes an outlier, having the highest Mg/Ca ratio, despite its high soil development stage according to the activity index. Neither the decreasing pH, nor the increasing carbon content or the decreasing Mg/Ca ratios showed statistically significant correlations with $Fe_d$ content or the activity ratio.

Nevertheless, these indicators and the selectively enrichment of Ni in Fe-oxides illustrate moderately weathered soils and the soils along the toposequence showed differences in weathering intensity.

As observed in other studies, fate of Ni in serpentine soils is strongly connected with fate of free Fe and distribution of Ni associated with free Fe is mostly in favour of amorphous Fe-oxides (Massoura *et al.*, 2006; Chardot *et al.*, 2007; Cheng *et al.*, 2012; Bani *et al.*, 2014). This principle could be affirmed in this study. A high proportion of total Ni was connected to free Fe-oxides (29.6%-39.7%). Ni was always more bound to amorphous Fe oxides rather than incorporated into the lattices of well crystallized Fe-oxides as $Ni_o$:$Ni_d$ was always higher

than $Fe_o$:$Fe_d$. Furthermore studies by Massoura *et al.* (2006), Chardot *et al.* (2007), Bani *et al.* (2014), Kierczak *et al.* (2016) and Pędziwiatr *et al.* (2018) stated the Ni fraction sorbed to amorphous Fe-oxides as one of the main contributors to available Ni. Likewise, in in this study Ni availability to *O. chalcidica* was shown to depend strongly on Ni associated with Fe-oxides, specifically with amorphous Fe-oxides ($Ni_o$) as shown in figure 6. As crystallization of Fe oxides and partitioning of Ni amongst Fe oxides depends on soil age and weathering, it can be derived that Ni availability and Ni uptake by *O. chalcidica* is indeed related to weathering intensity and pedogenic processes. Due to the fact, that $Ni_{shoot}$ showed no correlation with the activity index, Ni availability to *O. chalcidica* does not strictly increase with increase of weathering intensity or soil development. Instead it seems that Ni availability is higher in moderately weathered soils, than in intensely weathered soils, due to its affinity to amorphous Fe-oxides. Just as *Echevarria et al. (2006 )* stated, Ni availability is observed to be higher in moderately weathered soils where Ni is associated with poorly crystallized phyllosilicates, clay minerals and amorphous Fe-oxides rather than occluded into the lattices of well crystallized Fe-oxides. Furthermore some high solubility might be observed due to slight weathering of primary minerals and absence of secondary high-charge minerals for further resorption (Echevarria *et al.*, 2006). This might be the case for sample point 1 which showed the highest Ni accumulation by *O. chalcidica,* while having the lowest values of total C, total N, cation-exchange-capacity and free Fe oxides.

Hence, the present data allow the conclusion of a coherence of Ni availability and pedogenic processes and weathering intensity.

## Ni fractions accessed by *O. chalcidica*

Ni uptake by *O. chalcidica* strongly depends on total Ni concentrations in soils. Ni uptake in this study can best be explained considering the total Ni levels in soils and Ni connected with amorphous Fe-oxides rather than any single Ni extraction.

The single Ni extractions ( DTPA, $Sr(NO_3)_2$, $H_2O$) were performed to evaluate Ni bioavailability and for detecting potential relationships between these fractions and shoot Ni concentrations.. By complexing soluble and exchangeable metals, the DTPA extraction accesses Ni-ions weakly bound to organic and inorganic complexes that are eventually

available and accessible to plants (Echevarria *et al.*, 1998). It includes the other two fractions, the exchangeable $Ni_{Sr}$ and the soluble $Ni_{H2O}$. All of them are considered to be potentially bioavailable. Echevarria et al. (2006) used an isotopic exchange and dilution technique to determine Ni plant availability for 100 ultramafic soils. The study showed that $Ni_{DTPA}$ generally equals the isotopic findings and can be used as a fairly good indicator of available Ni in soils. Nevertheless, in this study $Ni_{DTPA}$ was not significantly correlated to Ni uptake as shown in previous studies (Massoura *et al.*, 2004; Chardot *et al.*, 2006), which is why it is assumed that another fraction of Ni mainly provides Ni to *O. chalcidica.*
The residual Ni fraction generally accounts for the biggest part of $Ni_{Total}$ in serpentine soils and is integrated into silicates (Wenzel and Jockwer, 1999). Likewise, in the conducted study, the majority of $Ni_{Total}$ was not accessed by the single Ni extraction methods.
The results of this study showed that none of the conducted single element extractions assessed the Ni fraction predominantly accessed by *O. chalcidica.* The obtained data clearly suggest that Ni extracted with ammonium oxalate (therefore assumed to be associated with amorphous Fe-oxides), was the main contributor to plant available Ni. However, in theory oxalate extraction can also desorb Ni bound to organic matter (Chardot *et al.*, 2007), but since total carbon showed no correlation with Ni availability among soils, amorphous Fe-oxides can be expected to be the main contributor to $Ni_o$. Ammonium oxalate extraction revealed the high importance of amorphous Fe-oxides in scavenging Ni as potentially available forms. While well crystallised Fe-oxides occlude Ni into their lattices and can be considered as stable sink of metals (Pędziwiatr *et al.*, 2018), Ni is sorbed or complexed at the surface of amorphous Fe-oxides and therefore exchangeable and thus potentially plant available (Chardot *et al.*, 2007).

Since Fe geochemistry, especially Fe-oxides seem to play a key role in Ni availability and Ni uptake by *O. chalcidica,* a closer look into the interactions of Ni and Fe was implicated.

## Effects of interactions between nickel and other micronutrients on their accumulation in the hyperaccumulator *O. chalcidica*

Regarding the Fe concentrations in the shoots of *O. chalcidica,* they are rather low compared to other studies recently conducted using *O. chalcidica* on serpentine soils (Bani *et al.*, 2014; Broadhurst and Chaney, 2016; Xhaferri *et al.*, 2018). Furthermore, in a study conducted by

Noller (2017), local and at least one year old plants of the hyperaccumulator *Noccaea goesingensis* were harvested at some of the same sites investigated in this study. The shoots analysed by Noller also revealed much higher Fe levels than *O. chalcidica* took up in this study. Despite the relatively young age of the *O. chalcidica* plants in this study, the low Fe values are still surprising as the $Fe_{DTPA}$ values of the soils indicate a sufficient Fe supply in the studied soils. Despite the high Fe supply in soils, all studied plants showed relatively low Fe levels, apart from plants grown on soil 1, which oddly showed the highest Fe values despite the fact that soil 1 had the lowest $Fe_{DTPA}$ levels.

As the low Fe uptake of *O. chalcidica* cannot be explained by Fe deficient soils, low Fe values in the shoots might be connected to competition of Fe with other elements, like the excessively present Ni. Similar hypotheses were studied by Nishida *et al.* (2011), Deng *et al.*(2019) and by Mohseni, Ghaderian and Schat (2019).

By growing *Odontarrhena* species in Fe deficient nutrient solution, Mohseni, Ghaderian and Schat (2019) could show that Fe starvation lead to an increase of Ni uptake velocity in *Odontarrhena bracteata.* They concluded that "Ni is taken up via a Fe-deficiency induced Fe transporter, and that Ni and Fe compete for this transporter".

Nishida *et al.* (2011) described that the non-hyperaccumulator model plant *Arabidopsis thaliana* takes up Ni through the Fe uptake system. Using a hydroponic experimental system, they found that Fe-deficient treatments increased Ni accumulation in plants, implying that Ni is absorbed by the Fe uptake system. They were able to identify iron-regulated transporter 1 (IRT1) as transporter for Ni into the roots. Furthermore, (Nishida *et al.*, 2011) demonstrated that exposure to excess Ni induced an Fe-deficient response in *A. thaliana*, which would stimulate the expression level of the IRT1. Consequently, excess Ni further accelerated Ni and Fe accumulation via IRT1.

Deng *et al.* (2019) studied the interactions between Ni and other trace elements during the uptake and transport process of hyperaccumulator *Noccaea caerulescens.* They found that Ni exposure increased Fe accumulation, while Fe deficiency stimulated Ni uptake in *N. caerulescens.* Thereby they could affirm the results of Nishida *et al.* (2011) and adapt them to a Ni-hyperaccumulator species. However, Deng *et al.* (2019) concluded that *N. caerulescens* mainly takes up Ni by Zn transporter, while Fe transporters play a smaller role.

Reasoned by these recent studies and the low Fe levels in the shoots of *O. chalcidica* in this study, a concurrent relationship between Fe an Ni in the early growing stage of *O. chalcidica* can be assumed. When excluding sample site one, due to its special Fe uptake behaviour, this assumption can be affirmed by the significant negative regression between $Fe_{shoot}$ and $Ni_{shoot}$ demonstrated in figure 10.

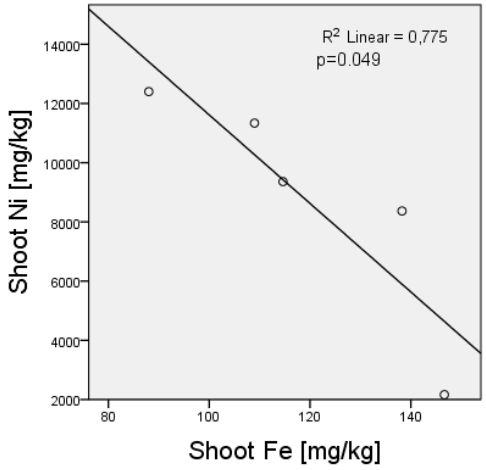


*Figure 10: Competitive relationship between Fe and Ni expressed as a negative correlation of $Ni_{shoot}$ and $Fe_{shoot}$. Samle site one was ignored in this regression due to its exceptional Fe uptake behaviour.*

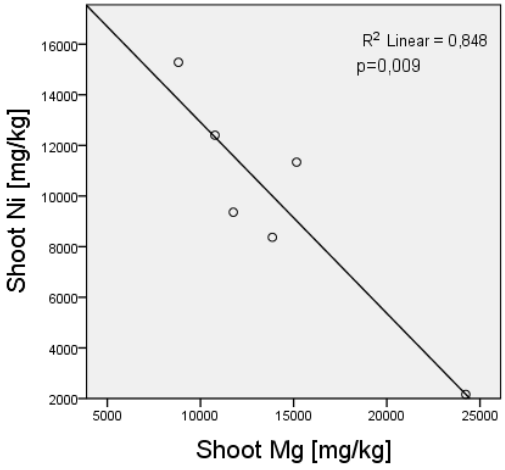


*Figure 11: Competitive relationship between Mg and Ni expressed as a negative correlation of $Ni_{shoot}$ and $Mg_{shoot}$.*

Consequently, there is reason to assume that excess Ni induces Fe deficiency in *O. chalcidica* in the early growing stage, because Ni can be taken up via Fe transporters due to their same valency and similar mass. Therefore, Ni and Fe compete for this transporter, as described by Mohseni, Ghaderian and Schat( 2019). Subsequently the plants may initiate a Fe-deficiency induced response in order to increase Fe uptake, similar as described by Nishida *et al.* (2011) for *A. thaliana.* As a result, plants manage to increase Fe uptake and overcome Fe deficiency, just as Noller's (2017) *Noccaea goesingensis* showed no noticeable low Fe levels. By increasing Fe uptake, Ni accumulation continues as Ni might be taken up via Fe uptake system.

A similar antagonistic relationship may be present between Ni and Mg, as there is an even stronger negative correlation between $Mg_{shoot}$ and $Fe_{shoot}$ considering all 6 sample sites. Exchangeable $Mg^{2+}$ levels in soils were extremely high as shown in table 1. It is possible that Mg is able to limit Ni accumulation, as shown by Robinson, Brooks and Clothier (1999). They showed by adding $MgCO_3$ to serpentine soil planted with hyperaccumulator *Berkheya coddii,* accumulation of Ni by *B. coddii* was significantly decreased. Therefore a similarity of the Ni-Mg relationship to the Ni-Fe relationship described above could be possible.

## Mobilisation of Ni by *O. chalcidica*

When comparing $Sr(NO_3)_2$ and DTPA extractions before plant growth with the extractions after growth of *O. chalcidica*, a significant depletion of $Sr(NO_3)_2$ extractable Ni and DTPA extractable Ni is evident (Figure 7 and 8). The depletion of $Ni_{DTPA}$ and $Ni_{Sr}$ would indeed suggest, that the plants access Ni from these fractions. But reductions observed in labile ($Ni_{DTPA}$) and exchangeable pools ($Ni_{Sr}$) of Ni generally accounted for a low percentage of the total metal uptake by the plants, indicating that Ni is mainly acquired from less available pools. The depletion of $Ni_{DTPA}$ might not solely be caused by plant uptake as explained by Saad *et al.* (2018). $Ni_{DTPA}$ depletion may also be reasoned by the alkalinisation of the soil after growth of *O. chalcidica,* similar to the findings of Cerdeira-Pérez *et al.*(2019). However, since a decrease and no increase of $Sr(NO_3)_2$ extractable Ni and DTPA extractable Ni took place in the samples, no mobilisation or solubilisation of Ni from non-labile to labile pools could be verified. Furthermore, geochemical modelling showed no change in species distribution among dissolved Ni species in soil solution during growth of *O. chalcidica.* There was no increase in Ni associated with organic compounds which would speak for a mobilisation. Again, the results of the geochemical modelling showed no evidence that could indicate a mobilisation of Ni caused by *O. chalcidica*.

On the other hand, Ni uptake ($Ni_{shoot}$) showed significant correlations with $Ni_{Total}$ and $Ni_o$, both fractions that are not immediately available for plants. Moreover, Ni uptake by *O. chalcidica* showed to be independent of pH and did not increase with decreasing pH values as one would expect. This is an indication that $Ni_{shoot}$ mainly originates from non-labile fractions which are independent of pH.

Since $Ni_{shoot}$ showed correlations with Ni fractions which are not directly available to plants, mobilisation of Ni by *O. chalcidica* may take place. However, mobilisation could not be proven by the collected data. Mobilized Ni may be taken up by the plants immediately and therefore could not be shown by the DTPA and $Sr(NO_3)_2$ extractions of the planted soil samples. Only assumptions can be made about the mechanism and process of mobilisation. But since $Ni_o$ seems to be the main contributor to Ni accessed by *O. chalcidica,* and Ni is assumed to be taken up via Fe transporters, Ni mobilisation might be related to a Fe-deficiency induced response as described above. But the precise mechanisms of mobilization remain unclear and could not be revealed in this study.

# Conclusion

This study aimed at examining biogeochemistry of pedogenic Ni along a toposequence of serpentine soil and its relation to Ni accumulation in the hyperaccumulator *Odontarrhena chalcidica* (syn. *Alyssum murale*). $Ni_{Total}$ and Ni associated with amorphous Fe-oxides showed to be the best indicators for Ni uptake by *O. chalcidica* as these two fractions showed strong correlations with $Ni_{Shoot}$ values. On the contrary, the DTPA, $Sr(NO_3)_2$ and $H_2O$ extractions were no sufficient indicator for Ni uptake as they showed no significant correlation with $Ni_{shoot}$. Ni associated with amorphous Fe-oxides seems to be the main contributor to Ni taken up by *O. chalcidica*. Therefore, pedogenesis and weathering intensity is of great influence on Ni availability. Furthermore, antagonistic relationships between $Ni_{shoot}$, $Mg_{shoot}$ and especially $Fe_{shoot}$ were revealed and allowed assumptions related to the Ni uptake mechanisms of *O. chalcidica.* It is assumed that Ni is taken up via Fe transporters, for which Ni and Fe compete. No mobilisation of Ni by *O. chalcidica* could be detected since there was a depletion of $Sr(NO_3)_2$ and DTPA extractable Ni after growth of *O. chalcidica,* rather than an increase. Furthermore, species distribution among dissolved Ni species in soil solution showed no significant change during growth of *O. chalcidica,* which again suggests no mobilisation of Ni. On the other hand, Ni uptake ($Ni_{shoot}$) showed significant correlations with $Ni_{Total}$ and Ni associated with amorphous Fe-oxides, both fractions that are not directly plant available. Therefore, mobilisation of Ni by *O. chalcidica* may take place, but could not be proven by the collected data.

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**Appendix**

# Statistical Analysis

### Variation of the soil physico-chemical parameters along the toposequence

**ANOVA**

$Ni_{total}$

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2572892,444 | 5 | 514578,489 | 458,059 | ,000 |
| Within Groups | 13480,667 | 12 | 1123,389 | | |
| Total | 2586373,111 | 17 | | | |

**ANOVA**

$pH_{H2O}$

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 8,806 | 5 | 1,761 | 9606,358 | ,000 |
| Within Groups | ,002 | 12 | ,000 | | |
| Total | 8,808 | 17 | | | |

## ANOVA

Biomass

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2,453 | 5 | ,491 | 12,703 | ,000 |
| Within Groups | ,695 | 18 | ,039 | | |
| Total | 3,149 | 23 | | | |

## Robust Tests of Equality of Means

$Ni_{H2O}$

| | Statistic[a] | df1 | df2 | Sig. |
|---|---|---|---|---|
| Welch | 2022,733 | 5 | 5,260 | ,000 |

a. Asymptotically F distributed.

## Robust Tests of Equality of Means

$Ni_{shoot}$

| | Statistic[a] | df1 | df2 | Sig. |
|---|---|---|---|---|
| Welch | 60,575 | 5 | 8,109 | ,000 |

a. Asymptotically F distributed.

## Robust Tests of Equality of Means

$Fe_{shoot}$

| | Statistic[a] | df1 | df2 | Sig. |
|---|---|---|---|---|
| Welch | 1,449 | 5 | 7,605 | ,309 |

a. Asymptotically F distributed.

## Test Statistics[a,b]

| | C/N | CEC | OlsenP | Fe d | Fe o | Activity Index |
|---|---|---|---|---|---|---|
| Chi-Square | 10,308 | 10,769 | 9,615 | 10,769 | 8,846 | 10,462 |
| df | 5 | 5 | 5 | 5 | 5 | 5 |
| Asymp. Sig. | ,067 | ,056 | ,087 | ,056 | ,115 | ,063 |
| Exact Sig. | ,002 | ,000 | ,014 | ,000 | ,050 | ,001 |
| Point Probability | ,001 | ,000 | ,003 | ,000 | ,006 | ,000 |

a. Kruskal Wallis Test

b. Grouping Variable: Samling point

**Test Statistics[a,b]**

| | Ni $Sr(NO_3)_2$ | Ni DTPA | Total_N | Total_C | Ni o | Ni d |
|---|---|---|---|---|---|---|
| Chi-Square | 10,462 | 10,462 | 10,769 | 10,769 | 10,538 | 10,538 |
| df | 5 | 5 | 5 | 5 | 5 | 5 |
| Asymp. Sig. | ,063 | ,063 | ,056 | ,056 | ,061 | ,061 |
| Exact Sig. | ,001 | ,001 | ,000 | ,000 | ,001 | ,001 |
| Point Probability | ,000 | ,000 | ,000 | ,000 | ,000 | ,000 |

a. Kruskal Wallis Test

b. Grouping Variable: Samling point

## Depletion of DTPA Extractable Ni after planting *O. chalcidica*

**Tests of Within-Subjects Effects**

Measure: Ni DTPA over time

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| NiDTPA | Sphericity Assumed | 173,242 | 1 | 173,242 | 9,232 | ,029 | ,649 |
| | Greenhouse-Geisser | 173,242 | 1,000 | 173,242 | 9,232 | ,029 | ,649 |
| | Huynh-Feldt | 173,242 | 1,000 | 173,242 | 9,232 | ,029 | ,649 |
| | Lower-bound | 173,242 | 1,000 | 173,242 | 9,232 | ,029 | ,649 |
| Error(NiDTPA) | Sphericity Assumed | 93,828 | 5 | 18,766 | | | |
| | Greenhouse-Geisser | 93,828 | 5,000 | 18,766 | | | |
| | Huynh-Feldt | 93,828 | 5,000 | 18,766 | | | |
| | Lower-bound | 93,828 | 5,000 | 18,766 | | | |

## Depletion of $Sr(NO_3)_2$ Extractable Ni after planting *O. chalcidica*

**Tests of Within-Subjects Effects**

Measure: N $iSr(NO_3)_2$ over time

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| $NiSr(NO_3)_2$ | Sphericity Assumed | ,514 | 1 | ,514 | 34,261 | ,002 | ,873 |
| | Greenhouse-Geisser | ,514 | 1,000 | ,514 | 34,261 | ,002 | ,873 |
| | Huynh-Feldt | ,514 | 1,000 | ,514 | 34,261 | ,002 | ,873 |
| | Lower-bound | ,514 | 1,000 | ,514 | 34,261 | ,002 | ,873 |
| Error(NiSrNO32) | Sphericity Assumed | ,075 | 5 | ,015 | | | |
| | Greenhouse-Geisser | ,075 | 5,000 | ,015 | | | |
| | Huynh-Feldt | ,075 | 5,000 | ,015 | | | |
| | Lower-bound | ,075 | 5,000 | ,015 | | | |

## Ni concentration measured in soil solution over time

### Tests of Within-Subjects Effects

Measure: Ni in soil solution over time soil 1

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni solution 1 | Sphericity Assumed | ,006 | 3 | ,002 | 7,539 | ,008 |
| | Greenhouse-Geisser | ,006 | 1,943 | ,003 | 7,539 | ,025 |
| | Huynh-Feldt | ,006 | 3,000 | ,002 | 7,539 | ,008 |
| | Lower-bound | ,006 | 1,000 | ,006 | 7,539 | ,071 |
| Error(Nisolution) | Sphericity Assumed | ,002 | 9 | ,000 | | |
| | Greenhouse-Geisser | ,002 | 5,829 | ,000 | | |
| | Huynh-Feldt | ,002 | 9,000 | ,000 | | |
| | Lower-bound | ,002 | 3,000 | ,001 | | |

### Tests of Within-Subjects Effects

Measure: Ni in soil solution over time soil 2

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni solution 2 | Sphericity Assumed | ,018 | 3 | ,006 | 23,624 | ,000 |
| | Greenhouse-Geisser | ,018 | 2,049 | ,009 | 23,624 | ,001 |
| | Huynh-Feldt | ,018 | 3,000 | ,006 | 23,624 | ,000 |
| | Lower-bound | ,018 | 1,000 | ,018 | 23,624 | ,017 |
| Error(Nisolution) | Sphericity Assumed | ,002 | 9 | ,000 | | |
| | Greenhouse-Geisser | ,002 | 6,148 | ,000 | | |
| | Huynh-Feldt | ,002 | 9,000 | ,000 | | |
| | Lower-bound | ,002 | 3,000 | ,001 | | |

### Tests of Within-Subjects Effects

Measure: Ni in soil solution over time soil 3

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni solution 3 | Sphericity Assumed | ,008 | 3 | ,003 | 28,924 | ,000 |
| | Greenhouse-Geisser | ,008 | 2,151 | ,004 | 28,924 | ,001 |
| | Huynh-Feldt | ,008 | 3,000 | ,003 | 28,924 | ,000 |
| | Lower-bound | ,008 | 1,000 | ,008 | 28,924 | ,013 |
| Error(Nisolution) | Sphericity Assumed | ,001 | 9 | 9,158E-5 | | |
| | Greenhouse-Geisser | ,001 | 6,453 | ,000 | | |
| | Huynh-Feldt | ,001 | 9,000 | 9,158E-5 | | |
| | Lower-bound | ,001 | 3,000 | ,000 | | |

## Tests of Within-Subjects Effects

Measure: Ni in soil solution over time soil 4

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni solution 4 | Sphericity Assumed | ,004 | 3 | ,001 | ,276 | ,841 |
| | Greenhouse-Geisser | ,004 | 1,250 | ,003 | ,276 | ,678 |
| | Huynh-Feldt | ,004 | 1,713 | ,002 | ,276 | ,739 |
| | Lower-bound | ,004 | 1,000 | ,004 | ,276 | ,636 |
| Error(Nisolution) | Sphericity Assumed | ,043 | 9 | ,005 | | |
| | Greenhouse-Geisser | ,043 | 3,749 | ,011 | | |
| | Huynh-Feldt | ,043 | 5,139 | ,008 | | |
| | Lower-bound | ,043 | 3,000 | ,014 | | |

## Tests of Within-Subjects Effects

Measure: Ni in soil solution over time soil 5

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni solution 5 | Sphericity Assumed | ,002 | 3 | ,001 | ,650 | ,603 |
| | Greenhouse-Geisser | ,002 | 1,280 | ,002 | ,650 | ,506 |
| | Huynh-Feldt | ,002 | 1,814 | ,001 | ,650 | ,544 |
| | Lower-bound | ,002 | 1,000 | ,002 | ,650 | ,479 |
| Error(Nisolution) | Sphericity Assumed | ,010 | 9 | ,001 | | |
| | Greenhouse-Geisser | ,010 | 3,840 | ,003 | | |
| | Huynh-Feldt | ,010 | 5,441 | ,002 | | |
| | Lower-bound | ,010 | 3,000 | ,003 | | |

## Tests of Within-Subjects Effects

Measure: Ni in soil solution over time soil 6

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni solution 6 | Sphericity Assumed | ,009 | 3 | ,003 | 1,048 | ,418 |
| | Greenhouse-Geisser | ,009 | 1,458 | ,006 | 1,048 | ,396 |
| | Huynh-Feldt | ,009 | 2,485 | ,003 | 1,048 | ,413 |
| | Lower-bound | ,009 | 1,000 | ,009 | 1,048 | ,381 |
| Error(Nisolution) | Sphericity Assumed | ,024 | 9 | ,003 | | |
| | Greenhouse-Geisser | ,024 | 4,374 | ,006 | | |
| | Huynh-Feldt | ,024 | 7,455 | ,003 | | |
| | Lower-bound | ,024 | 3,000 | ,008 | | |

## Percentage of Ni species in soil solution over time

### Tests of Within-Subjects Effects

Measure: $Ni^{2+}$ (%) over time

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| $Ni^{2+}$ | Sphericity Assumed | 17,501 | 3 | 5,834 | 3,233 | ,052 |
| | Greenhouse-Geisser | 17,501 | 1,495 | 11,704 | 3,233 | ,105 |
| | Huynh-Feldt | 17,501 | 1,989 | 8,798 | 3,233 | ,083 |
| | Lower-bound | 17,501 | 1,000 | 17,501 | 3,233 | ,132 |
| Error(Ni) | Sphericity Assumed | 27,063 | 15 | 1,804 | | |
| | Greenhouse-Geisser | 27,063 | 7,476 | 3,620 | | |
| | Huynh-Feldt | 27,063 | 9,946 | 2,721 | | |
| | Lower-bound | 27,063 | 5,000 | 5,413 | | |

### Tests of Within-Subjects Effects

Measure: NiOH (%) over time

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| NiOH | Sphericity Assumed | ,079 | 3 | ,026 | 2,422 | ,117 |
| | Greenhouse-Geisser | ,079 | 2,000 | ,039 | 2,422 | ,150 |
| | Huynh-Feldt | ,079 | 3,000 | ,026 | 2,422 | ,117 |
| | Lower-bound | ,079 | 1,000 | ,079 | 2,422 | ,195 |
| Error(NiOH) | Sphericity Assumed | ,130 | 12 | ,011 | | |
| | Greenhouse-Geisser | ,130 | 8,000 | ,016 | | |
| | Huynh-Feldt | ,130 | 12,000 | ,011 | | |
| | Lower-bound | ,130 | 4,000 | ,032 | | |

## Tests of Within-Subjects Effects

Measure: $NiSO_4$ (%) over time

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| NiSO4 | Sphericity Assumed | ,002 | 3 | ,001 | 5,606 | ,012 |
| | Greenhouse-Geisser | ,002 | 1,194 | ,001 | 5,606 | ,064 |
| | Huynh-Feldt | ,002 | 1,415 | ,001 | 5,606 | ,052 |
| | Lower-bound | ,002 | 1,000 | ,002 | 5,606 | ,077 |
| Error(NiSO4) | Sphericity Assumed | ,001 | 12 | ,000 | | |
| | Greenhouse-Geisser | ,001 | 4,776 | ,000 | | |
| | Huynh-Feldt | ,001 | 5,659 | ,000 | | |
| | Lower-bound | ,001 | 4,000 | ,000 | | |

## Tests of Within-Subjects Effects

Measure: Ni weakly bond to OM (%) over time

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni_weakly_bond_to_OM | Sphericity Assumed | 1,929 | 3 | ,643 | 4,135 | ,025 |
| | Greenhouse-Geisser | 1,929 | 1,194 | 1,616 | 4,135 | ,085 |
| | Huynh-Feldt | 1,929 | 1,356 | 1,422 | 4,135 | ,076 |
| | Lower-bound | 1,929 | 1,000 | 1,929 | 4,135 | ,098 |
| Error(Ni_weakly_bond_to_OM) | Sphericity Assumed | 2,332 | 15 | ,155 | | |
| | Greenhouse-Geisser | 2,332 | 5,968 | ,391 | | |
| | Huynh-Feldt | 2,332 | 6,781 | ,344 | | |
| | Lower-bound | 2,332 | 5,000 | ,466 | | |

## Tests of Within-Subjects Effects

Measure: Ni complexed to OM (%) over time

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Ni_complexed_toOM | Sphericity Assumed | 6,941 | 3 | 2,314 | 1,699 | ,210 |
| | Greenhouse-Geisser | 6,941 | 1,188 | 5,843 | 1,699 | ,247 |
| | Huynh-Feldt | 6,941 | 1,345 | 5,161 | 1,699 | ,245 |
| | Lower-bound | 6,941 | 1,000 | 6,941 | 1,699 | ,249 |
| Error(Ni_complexed_toOM) | Sphericity Assumed | 20,427 | 15 | 1,362 | | |
| | Greenhouse-Geisser | 20,427 | 5,939 | 3,439 | | |
| | Huynh-Feldt | 20,427 | 6,725 | 3,038 | | |
| | Lower-bound | 20,427 | 5,000 | 4,085 | | |

# Additional data

**DTPA Extract**

| Sample point | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Cr | [mg/kg] | 0.50 | 0.29 | 0.17 | 0.11 | 0.13 | 0.15 |
| Cr planted | [mg/kg] | 0.05 | 0.07 | 0.02 | 0.02 | 0.09 | 0.07 |
| Fe | [mg/kg] | 11.07 | 41.21 | 23.24 | 29.49 | 75.82 | 108.65 |
| Fe planted | [mg/kg] | 6.05 | 40.46 | 21.86 | 37.06 | 65.88 | 102.82 |
| Co | [mg/kg] | 0.23 | 0.60 | 0.79 | 0.29 | 0.81 | 1.94 |
| Co planted | [mg/kg] | 0.12 | 0.67 | 0.67 | 1.58 | 0.93 | 2.80 |
| Cu | [mg/kg] | 1.02 | 0.62 | 0.60 | 0.67 | 0.31 | 0.51 |
| Cu planted | [mg/kg] | 0.83 | 0.87 | 0.68 | 0.88 | 0.38 | 0.51 |
| Zn | [mg/kg] | 0.86 | 0.95 | 0.87 | 1.35 | 1.21 | 2.66 |
| planted | [mg/kg] | 0.73 | 0.91 | 0.38 | 0.68 | 0.71 | 1.91 |
| Ni planted | [mg/kg] | 11.11 | 41.21 | 35.04 | 52.13 | 21.63 | 20.66 |

**$Sr(NO_3)_2$ Extract**

| Sample point | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Mn | [mg/kg] | 0.31 | 0.46 | 0.63 | 0.05 | 0.38 | 5.64 |
| Mn planted | [mg/kg] | 0.03 | 0.10 | 0.05 | 0.21 | 0.58 | 5.64 |
| Fe | [mg/kg] | 0.34 | 0.70 | 0.79 | 0.95 | 0.71 | 1.76 |
| Fe planted | [mg/kg] | 0.64 | 0.79 | 0.91 | 1.08 | 0.88 | 1.42 |
| Ni planted | [mg/kg] | 0.20 | 0.44 | 0.31 | 0.72 | 0.48 | 0.64 |