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MASTER THESIS

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Organic acid-induced solubilization of nickel and other metals in serpentine soils

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Abstract

On serpentine soil, which are characterized by naturally high nickel concentrations, a very specific plant community has developed. Some species, e.g. *Alyssum murale* or *Noccaea goesingensis*, are able to accumulate very high concentrations of nickel in their above-ground biomass. Whereas the physiological and molecular mechanism of these plants, the so-called metal hyperaccumulators, is already partially characterized, nickel mobilization processes in the rhizosphere are still unknown. It is hypothesized that a mix of organic acids is exuded into the rhizosphere to mobilize nutrients, metals and trace elements. However, the efficiency of single or combined organic acid compounds is insufficiently known.

To investigate the efficiency of two key organic acids – citric acid, oxalic acid and the combination of those compounds – on the solubilization of nickel and other trace elements in serpentine soils, two different extraction experiments with three different substrates (serpentine rock, serpentine soil and arable soil with serpentine properties) were conducted.

This study gave indications of biogeochemical rhizosphere processes that are induced by an increased release of organic acids originating from root exudates. The results of this thesis suggest that ligand-induced mineral weathering of serpentine rock may play a crucial role in nickel solubilization and that this effect is ligand concentration-dependent.

Kurzfassung

Serpentinböden zeichnen sich durch natürlich hohe Nickelkonzentrationen aus, auf denen sich spezielle Pflanzengemeinschaften entwickelt haben. Einige Pflanzenarten, wie beispielsweise *Alyssum murale* oder *Noccaea goesingensis*, können sehr hohe Nickelkonzentrationen in ihren oberirdischen Pflanzenteilen akkumulieren. Obwohl die Physiologie und die molekularen Mechanismen dieser sogenannten Hyperakkumulatoren bereits teilweise charakterisiert wurde, sind die Nickelmobilisierungsprozesse in der Rhizosphäre noch weitgehend unbekannt. Es wird vermutet, dass ein Mix aus organischen Säuren in die Rhizosphäre ausgeschieden wird, um Nährstoffe, Metalle und Spurenelemente zu mobilisieren. Die Effektivität von einzelnen oder der Kombination von mehreren organischen Säureverbindungen ist noch nicht hinlänglich bekannt.

Um die Effektivität von zwei entscheidenden, organischen Säuren – Zitronen-, Oxalsäure, sowie die Kombination der beiden – auf die Fähigkeit Nickel und andere Spurenelemente aus Serpentinböden zu mobilisieren, wurden zwei unterschiedliche Extraktionsexperimente mit drei unterschiedlichen Substraten (Serpentingestein, Serpentinboden und Ackerboden mit serpentinen Eigenschaften) durchgeführt.

Diese Versuche gaben Hinweise auf biogeochemische Rhizosphären-Prozesse, welche durch verstärkte Abgabe von organischen Säuren über Wurzelexsudate hervorgerufen werden. Die Ergebnisse dieser Arbeit deuten darauf hin, dass die Mineralverwitterung von Serpentingestein, welche von Liganden hervorgerufen wird, eine entscheidende Rolle in der Nickelmobilisierung spielt und dass dieser Effekt von der Liganden-Konzentration abhängig ist.

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1 Introduction

1.1 Serpentine Soil

Ultramafites are strongly enriched in elements such as iron, magnesium, nickel, cobalt and chromium in comparison to other rock types. The relatively high nickel and cobalt concentrations in serpentines depend on the ionic radii which are very close to that of Mg^{2+}. Therefore, an ionic substitution readily takes places into magnesium-rich minerals, which are dominant in ophiolitic rocks. (Kothe and Varma 2012).

As Nickel has a relatively high availability in the range of pH values of serpentine soils the values of bioavailability of Nickel is often significantly higher than the toxicity threshold in serpentine soils. A second possible and selective factor is the high Mg concentration/or the deficiency of Ca (Kothe and Varma 2012).

The characteristic flora of serpentine soils results from the extreme chemical nature of such soils with extremely high concentrations of Ni, Cr and Cd and low levels of macronutrients such as Ca, K and P (Bani et al. 2009).

Serpentine soils often offer hostile physical conditions to many plants. Serpentine outcrops are often relatively rocky, which makes them vulnerable to erosion (Kothe and Varma 2012).

Typical nickel concentrations in most natural soils vary from 1 to 450 mg kg^{-1}. In polluted soils the concentrations could be 20 to 30-fold higher than the overall range (varying from 10-1000 mg kg^{-1}). The Ni concentration in serpentine soils is relatively high, i.e. usually in the range of 0.1-3% Ni. The area under serpentine terrain capture less than 1% of the total earth surface, but it is abundant in ophiolite belts along tectonic plat margins but widely scattered throughout the world and usually supports a unique flora (He et al. 2012).

Physical weathering is defined as the decay of rocks and minerals into smaller particles without changing the minerals chemically. “It is mainly initiated by pressure release of overburden, by temperature, ice and salt bursts, through root pressure, as well as through mutual mechanical strain in the rocks” (Blume et al. 2016).

Physical weathering is a forerunner of chemical weathering. Chemical weathering occurs when rocks undergo chemical reaction to form new minerals. One important agent is water, which e.g. dissolves minerals or disintegrates them (Blume et al. 2016).

Through the weathering of rocks and ores heavy metals can naturally enter soils. Other sources of heavy metal contamination are continental dust immissions. Heavy metal contents in rocks are usually in the mg kg^{-1} range. Higher concentrations are found in ultrabasic extrusive igneous rocks and in the serpentine rocks. The natural occurrence of heavy metals is rarely at toxic levels (Sherameti and Varma 2010; Blume et al. 2016).

1.2 Soil and rhizosphere processes

The rhizosphere is a narrow region of soil, which is directly influenced by root exudation and is associated with soil microorganisms. Various soil animals and soil microorganisms prefer the direct surrounding of plant roots as microhabitat (Blume et al. 2016).

The main exudate compounds are amino acids, sugars, enzymes, aromatic acids, phenolic acids as well as organic acids. These released substances support the growth and metabolic-activity of soil microorganisms. (Oburger et al. 2013; Sessitsch et al. 2013; Blume et al. 2016). The replenishment of organic acid pools depends almost upon the steady release from plant or animal cells, therefore it can be hypothesized to be more temporally variable (Jones et al. 2003).

In Figure 1 the main biochemical processes in the rhizosphere are displayed.

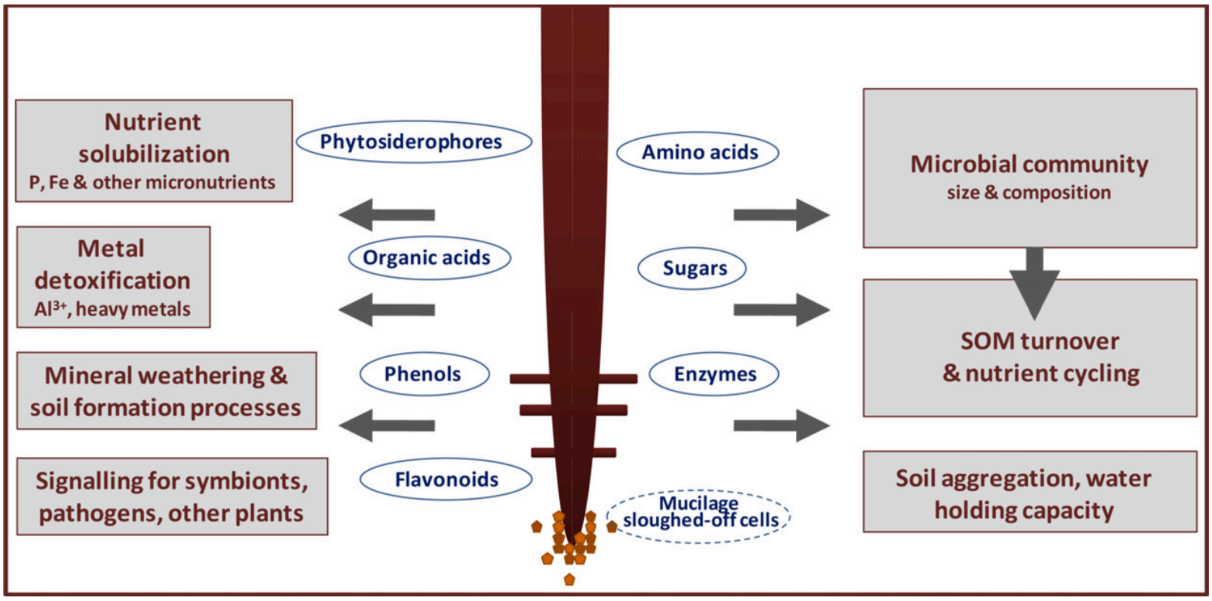

Figure 1: Biochemical processes in the rhizosphere driven by root exudates (Source: Oburger et al. 2013)

It is hypothesized that low-molecular organic acids are involved in many short-term soil processes e.g. nutrient mobilization and have the potential to significantly enhance the rate of primary mineral weathering. (van Hees et al. 2002; Van Hees et al. 2003)

The behavior of organic acids is difficult to predict because the reactions of organic acids with metals in soil is dependent on the complexation ability, the solid phase sorption/desorption reactions, slower diffusion rates, microbial degradation and also the hydrolysation of organic acids by metal oxides. (Jones and Darrah 1994; Jones et al. 2003)

Plant roots and microorganisms often respond to nutrient deficiency with the exudation of organic acids, which enables them to mobilize nutrients in the soil. White lupines are well known for high citrate exudation rates, e.g. under P deficiency. Likewise, the deficiency of micronutrients can similarly induce the exudation of organic acids in many plant species (Blume et al. 2016).

Organic acids are able to complex metal cations and therefore they are key factors in nutrient mobilization processes. Therefore, the total concentration of metals in solution is typically greater than the concentration of free ions because of complexation with organic ligands. Most of the organic acids in soils originate from root exudation and cell lysis, but also by the release from soil microorganisms. Examples of the main organic acids found in the rhizosphere include citric acid, malic acid and oxalic acid typically in the concentration range 1 to 100 µM. One main function of organic acids in soil is the mobilization and solubilization of nutrients by plants and microbes. The nutrient solubilization by organic acids (e.g. oxalate and citrate) is highly soil-dependent. However, it is well known that low-molecular weight organic acids also play a role in detoxification of metal (e.g. Zn and Al) and in the breakdown of wood (Blume et al. 2016; Jones et al. 2003; Molina Millán et al. 2006; van Hees, et al. 2002).

Enhanced rock weathering caused by microbial metabolites through chemical interaction or reactions may lead to mineral dissolution and metal solubilization. LMW organic acids seem to be the main agent in biogeochemical weathering of silicate minerals, in particular oxalic acid was found to be involved in these process (Becerra-Castro et al. 2013).

To buildup organic substances plants require carbon from the atmosphere as well as hydrogen from the soil water. All other nutrients are taken up from soil solution in their dissolved form. The majority of nutrients in Middle-European soils is native, which means they originate from the bedrock (Blume et al. 2016).

The main nutrient input in arable soils derives from mineral fertilization and manuring. Besides inputs more important are nutrient losses. There are four main factors influencing nutrient losses according to Blume et al. (2016):

- Uptake of nutrients by plants when the harvest products are removed, including wood out of forest
- Washing away with surface runoff
- Leaching with interflow and groundwater runoff as well as drainage water
- The erosion of topsoil material containing nutrients”

Many factors are influencing the removal of nutrients, e.g. plant species, variety and yield, nutrient supply in the soil and climatic conditions (Blume et al. 2016).

Only a small fraction of the total amount of nutrients contained in soils is present in direct plant-available forms. Plants can only take up nutrients that are present as ions in the soil solution, or in some cases as low-molecular organic or inorganic complexes. Therefore, the concentration and the respective chemical form of nutrient elements in the soil solution are critical factors for the absorption by roots (Blume et al. 2016).

Usually most nutrients are bound to soils in readily, moderately or poorly mobilized form. The availability of nutrients depends after Blume et al. (2016) on the following factors:

- The effective concentration of the chemical forms of nutrient elements in the soil solution. This is also called intensity.
- The total available quantity or the amount of solids that can be mobilized by roots during a vegetation period.
- The rate of replenishment from the available reserves of the solids into the soil solution or the other way around. These transitions between solution and solid phases involve adsorption, desorption, precipitation, dissolution and diffusion processes.
- The ability of plants to mobilize elements, this process is determined by rooting density, type and quantity of mobilizing root exudates and by diverse microbial processes in the surrounding rooting zone.

Besides macronutrients like N or P, plants also need other elements, i.e. micronutrients, in very small quantities (Cl, Fe, Mn, Ni), which are essential, e.g. for biosynthesis, growth, , chlorophyll formation and function, as well as for stress resistance.

Although these elements are essential they can cause toxic effects at high concentrations. The dose-response relationship for essential elements and heavy metals shows deficiency at suboptimal concentration, tolerance at optimal concentration and toxicity at high concentrations. This is also shown in Figure 2 (Blume et al. 2016; Sherameti and Varma 2010).

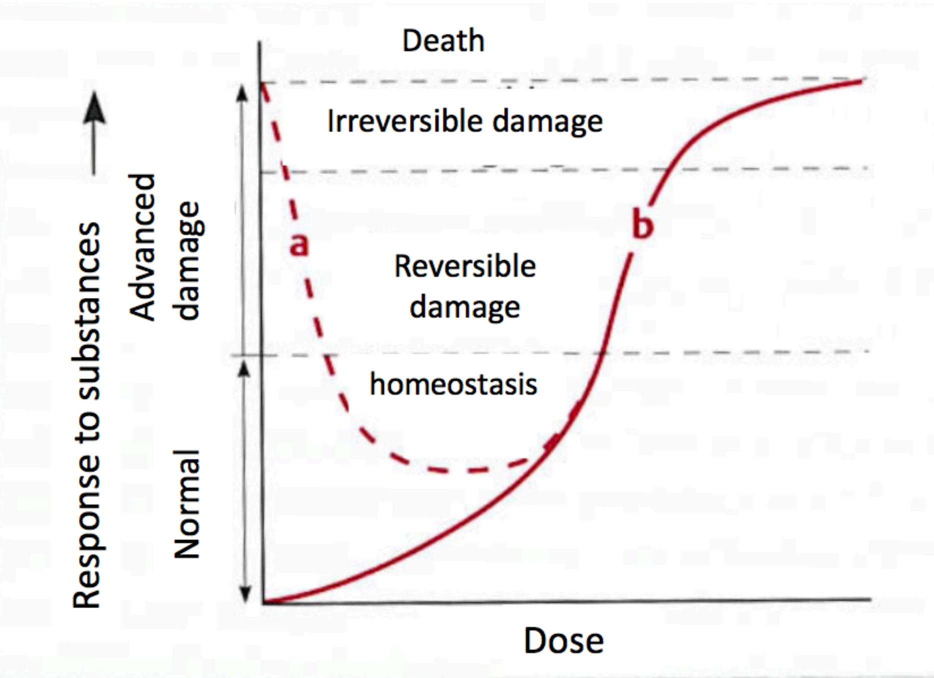

Figure 2: Dose-response relationship (modified after Fent (2013))
a = essential substances which have negative consequences at too low and too high levels
b = typical environmental substances where increasing the dose the effects change from reversible to irreversible damages ending up with death.

Ni is ubiquitously distributed in nature and constitutes a trace element in most living cells. In high concentrations, it is toxic to most cells and is also listed as a possible human carcinogen and associated with reproductive problems and birth defects. Nickel is an essential metal, physiologically required for several enzymatic reactions (e.g. urease activity) and other key cellular actives, but high Ni concentrations could impact soil organisms and limit plant growth (Davari, et al., 2015; Liu et al. 2015). Directly bioavailable for plants are Ni^{2+}, ion pairs, and simple complexes in soil solution. Organic substances such as organic acids and other dissolved organic matter form complexes with nickel readily. These processes enhance Ni desorption or dissolution in soils (He et al. 2012).

Another example of essential nutrients for all life forms is Manganese. For example, Manganese is liable for the activation of enzymes for photosynthesis. Plants take Mn mainly as Mn^{2+} ions up from the soil solution. In soil Manganese is found in organic complexes but also in exchangeable and dissolved forms. Heavy metals (e.g. Co, Ni and Zn) often accumulate with Mn oxides (Blume et al. 2016).

The most important processes taking place at interfaces between solid, gas and solution phases in soil are adsorption and desorption. The enrichment and release of dissolved or gaseous ions at the surfaces of solids is called adsorption and desorption. Besides dissolved ions also uncharged inorganic species and molecules can be absorbed on surfaces (Blume et al. 2016).

Jones et al. (2003) define sorption as a reversible binding of organic acids to the soil solid phase. One important factor influencing the adsorption to the soil solid phase is the ion charge. Monovalent organic acids as acetate and lactate are less strong adsorbed compared to divalent organic acids such as malate and fumarate, which are adsorbed more strongly (Jones et al. 2003).

The kinetics of chemical and physical reactions in soil are dependent on environmental conditions (e.g. temperature, pH value, ligands) and mineral type. As soils are open systems, chemical equilibria are frequently disturbed by fluxes of energy and matter. An example from Blume et al. (2016) is the under-saturated soil solution in humid climate conditions where the chemical weathering and soil formation are driven by frequent rainfall and leaching of dissolution products.

At water-solid interfaces (e.g. soil solution is in contact with the surfaces of soil minerals) dissolution and precipitation reactions take place. Such reactions play an important role according to the bioavailability of nutrients and toxicity of trace elements in soil (Blume et al. 2016). Also the chemical forms and binding types have impacts on the mobility, bioavailability and also the toxicity of heavy metals and other trace elements (Schreiber et al. 2005).

One important aspect of sorption processes is the reduction of the transport of heavy metals in the environment. It is difficult and critical to understand and predict the future mobility of sorbed metals (Bradl 2005).

Heavy metals occur in specific binding forms in soils depending on their origins. Most heavy metals are found as hydrated ions, inorganic pairs, or dissolved organic matter in solutions. The main reactions of heavy metals in soil are adsorption and desorption of defined metal compounds as well as precipitation and dissolution in case of strong soil contamination (Blume et al. 2016).

The delivery rate of metal ions to an absorbing root depends on the transport rate (diffusion and mass flow) through the soil and the desorption from the soil solution (Molina Millán et al. 2006).

Metal mobilization from the soil to the roots is according to He et al. (2012) influenced by:

- Plant exudates including organic acids (citric and oxalic acids)
- Metal-chelating compounds (e.g. phytosiderophores)
- Enzymes (reductase)
- Acidification by protons

Heavy metals can accumulate in plant tissue through root uptake from the soil solution or through the deposition from the air. Some plants are able to accumulate extremely high heavy metal concentrations – called hyperaccumulator plants. These pants are found in soils with higher heavy metal concentrations as usual. Prominent examples are the genera *Noccaea* and *Alyssum*, which can hyperaccumulate Zn, Cd and Ni (Blume et al. 2016).

1.3 Hyperaccumulator plants

“Metal hyperaccumulators are plants that are capable of extracting metals from soil and accumulating them to extraordinary concentrations in aboveground tissue (greater than 0.1% dry biomass Ni)” (Kramer et al. 1997, 1641).

“A number of taxa, most of which are endemic to serpentine soils, have been found to accumulate nickel to extraordinarily high levels, and have been designated hyperaccumulators of this element” (Reeves, Brooks, and Dudley 1983, 184).

Since hyperaccumulating plants were discovered over 500 plant species have been reported in the last decades (Sarma 2011).

Plant species which are growing on serpentine soil from the genus *Alyssum* are able to hyperaccumulate up to 30 g kg^{-1} Ni in their dry leaves (Centofanti et al. 2012).

Hyperaccumulating plant species represent perhaps the ultimate in plant tolerance to extremely hostile edaphic environments (Brooks 2008).

Accumulated metal concentrations in plant tissue may function as defense mechanism against enemies e.g. herbivores, pathogens (He et al. 2012).

As the threshold of hyperaccumulation in plants will vary for different trace elements it is necessary to define hyperaccumulators according to the elements. Whereas for Ni a threshold of 1000 µg g^{-1} seems appropriate, this is not the case for Zn because the natural abundance in plants is quite high (Brooks 2008).

Toxicity induced by Ni occurs usually at concentration levels higher than 10-50 mg kg^{-1} dry weight (DW). Therefore, it is remarkable that Ni hyperaccumulators contain >1000 mg Ni kg^{-1} DW (He et al. 2012).

There are different strategies developed by plants to manage potentially toxic metals: hyperaccumulation and exclusion. Hyperaccumulation enables plants to take up large amount of metals in their tissue without coming to harm. Metal-tolerant excluders growing on metallifours soils devoid of bioaccumulating metals (Puschenreiter et al. 2003; Wenzel et al. 2003; Massoura et al. 2004).

He et al. (2012) distinguish 2 types of metallophytes: strict metallophytes, which can exclusively live on metalliferous soils and facultative metallophytes. These plants can also live on non-metalloferous soils. However, they are more prevalent on metal-enriched habitats.

A hypothesis for the high metal concentrations in the tissue of hyperaccumulating species is that the plants take up metals from soil solution in elevated rates. Another point is the increased ability to translocate metals into the shoots (Kramer et al. 1997).

Some studies showed that hyperaccumulating plants absorb metals form the same labile pools in soil as normal plant species but hyperaccumulators are able to accumulate 100 times more nickel in their tissue. The Ni concentration in the above ground biomass in *Alyssum* species is in orders of magnitudes higher than the labile fraction of Ni in soil. Therefore there must be another mechanism that is responsible for the high Ni uptake in hyperaccumulating plants (Centofanti et al. 2012).

Root proliferation into soil patches containing elevated concentrations of heavy metals are suspected to be a possible mechanism that causes the high metal acquisition by hyperaccumulators (Whiting et al. 2000). But Moradi et al. (2009) showed in their study that the roots of *Berkheya coddii* does not forage toward the Ni rich patches.

The release of root exudates by hyperaccumulating species containing chelator agents may have the potential to enhance the metal uptake, translocation and resistance. To investigate the excessive metal uptake by hyperaccumulators one approach is to understand the rhizosphere processes driven by root exudation. Exudation rates and chemical composition of exudates of hyperaccumulating plants are relatively unknown and difficult to measure and collect from soil-grown plants (Wenzel et al. 2003).

Hyperaccumulating plants are able to deplete bioavailable pools of metals to extents where the chemical equilibria of the metal in soils in changed. An indirect mechanism of depleting the non-labile pools of metals in soil is the renewal of the labile pool from the non-labile pool during the equilibration over time (Centofanti et al. 2012). These results support the convection model of Ni uptake, which includes both the transpiration rate and the concentrations of the soluble Ni in solution. “Hyperaccumulators play a role in altering the concentration of soluble Ni in solutions through depletion of bioavailable pools of metals to extents where they change the chemical equilibria of that metal in the soil.” (Centofanti et al. 2012, 81).

An important factor for the bioavailability of metals to hyperaccumulating plants is the re-supply of depleted metals into the labile pools. Notably is that plants release approximately 25% of their assimilated carbon as root products into the rhizosphere which is suspected to have direct impact to the bioavailability of metals or indirect through enhanced microbial activity (Puschenreiter et al. 2003).

In *N. goesingensis* rhizosphere an elevated labile Ni was observed independent of the extent to which Ni was accumulated in the plant. This effect suggests that *N. goesingensis* mobilizes Ni generally when it is present in low concentrations in soil. Similar observations where reported previously for Zn in the rhizosphere of *N. caerulenscens* growing on non-contaminated soils. Therefore, it is hypothesized that hyperaccumulating species mobilize heavy metals actively in non-contaminated soils, reflecting an increase of the labile fraction (Puschenreiter et al. 2003).

Puschenreiter et al. (2003) investigated in their study that the hyperaccumulation of Zn was related to a decrease both the labile (NH_4NO_3-extractable) and EDTA-extractable pool.

Rhizosphere acidification and the release of organic acids that act as chelating agents by plant roots have been supposed as mechanisms to increase the Ni solubility in soils. The pH value decrease only slightly in the rhizosphere of the hyperaccumulator *Noccaea ochroleucum* and *N. caerulescens* which indicates that the increased metal uptake is not related to rhizosphere acidification (Wenzel et al. 2003; Robinson et al. 1999; Centofanti et al. 2012). There is also little evidence that hyperaccumulator plants secrete organic or amino acids to increase Ni solubilization or uptake (Centofanti et al. 2012).

The cultivation of metal accumulating higher plants to remove, transfer or stabilize soil contaminations is called phytoremediation. For a successful plant-based remediation of moderately polluted soils crops that are able to accumulate metals in a range of 1-2% would be needed. Thus, hyperaccumulating plants are considered as suitable plants for such purposes, given that the harvestable biomass is sufficient.

1.4 Hypotheses and Research Questions

As the phenomenon of nickel hyperaccumulation is still not fully understood we conducted two experiments on three different substrates.

The hypothesis of this master thesis are as follows:

Organic acids are responsible for mobilization of Ni and other trace elements. The co-solubilization of other trace elements (e.g. Mn, Cr, Fe, …) is an indicator for the mobilization processes and involved mineral fractions.

Derived from the hypothesis the following research questions were addressed:

- RQ1: How long does the mobilization take – mobilization kinetics?
- RQ2: Which concentrations of organic acids are necessary for the element mobilization?
- RQ3: Which substance (citrate, oxalate or the combination of these two) is more efficient and are there synergistic effects?
- RQ4: Besides Ni, which elements are co-solubilized?
- RQ5: To which extent is the primary serpentine mineral a Ni source in the rhizosphere?

With these experiments, we tested potential rhizosphere processes and the influence on serpentine primary mineral as well as on soils developed out of serpentine rock with different soil types (subsoil from a soil profile in a forest vs. an arable soil).

The results of these experiments should enable to estimate potential Ni solubilization processes that may enhance the Ni bioavailability in the rhizosphere of hyperaccumulating plants.

It is assumed that organic acids at the root tip could reach concentrations up to 250 µM (Jones et al. 2003). Therefore, these high concentrations of citrate, oxalate and the combination of these two were used in the concentration-dependent extraction experiment. The other concentrations used in this experiment were 10 and 50 µM which are shown as common in the rhizosphere (Van Hees et al., 2003).

2 Materials and Methods

Soil samples were collected at two different serpentine sites near Redlschlag in eastern Austria. Additional to soil samples, serpentine rock was ground to fine powder and analyzed.

The properties of the soil types are depicted in Table 1.

The first soil type was an arable soil with serpentine properties form Bernstein (following called B), the second soil was a serpentine soil (following called as S). (Wenzel et al. 2003).

The serpentine rock (following called as R) was analyzed using x-ray diffraction (XRD). The semi-quantitative constitution of R is described in m/m% as follows: serpentine 85%, chlorite 13%, magnetite 1-2% and traces of magnesite.

Table 1: Soil characteristics and total metal concentrations of soil types B, S and R[1] Metal concentrations are depicted in mg kg^{-1} n.d.= not determined.

Soil type	**pH**	**Ni**	**Cr**	**Mn**	**Pb**
B	6.2	1500	389	3384	6
S	6.6	2580	383	3622	5
R	n.d.	1758	2428	n.d.	1

Inductive Coupled Plasma Mass Spectrometry

The chemical analysis was conducted using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) unit type PerkinElmer ELAN 900DRCe. As internal standard Indium in concentration of 10 ppb was used.

The main components of an IPC-MS instrument are: sample introduction, ion generation in the ICP, plasma/vacuum interface, ion focusing, ion separation and measurement (Aligent Technologies 2005).

A schematic overview of the processes is presented in Figure 3.

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[1] The total metal concentration of B and S was analyzed in *aqua regina* whilst the metal concentration of R was analyzed using XRD.

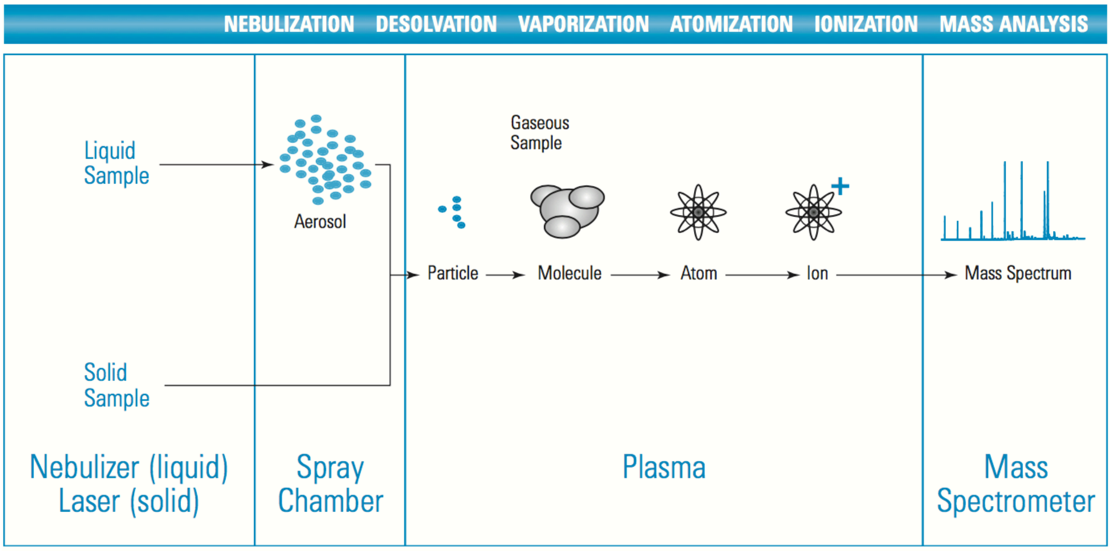

Figure 3: Schematic representation of processes in ICP-MS from sample introduction to mass analysis (Source: Aligent Technologies 2005)

2.1 Materials

- Soil samples
- Sieve (2 mm)
- shaking bottles (100 mL)
- centrifuge tubes (15 mL, 11 mL)
- funnels
- filter paper
- 10 mL syringes
- syringes filter (pore size 0.45 µm)
- overhead shaker
- centrifuge
- dispenser
- micropipettes (1000 µL, 5000 µL, 10000 µL)
- tips

Solutions:

- 2.5 mM $Mg(NO_3)_2$
- 0.2 g L^{-1} Bronopol
- 10 µM Citric acid
- 10 µM Oxalic acid
- 10 µM Citric acid + Oxalic acid
- 4% HNO_3
- 110 ppb Indium Standard
- 50 µM Citric acid
- 50 µM Oxalic acid
- 50 µM Citric acid + Oxalic acid
- 250 µM Citric acid
- 250 µM Oxalic acid
- 250 µM Citric acid + Oxalic acid

2.2 Methods

2.2.1 Soil preparation

Soil samples (S and B) were passed through a sieve with a pore size of 2 mm and afterwards spread on tables and left for one week to enable air-drying.

Before the experiments started the sieved soil was equilibrated with 2.5 mM $Mg(NO_3)_2$ for the soil types S and B 150 mL kg^{-1} and for R 75 mL kg^{-1} solution were used. The soil was incubated for at least 48 hours.

Molina Millán et al. (2006) showed in their study that the Ni desorption in water is higher than in $MgCl_2$ solution. The ionic strength of the liquid is influencing the Ni desorption. To investigate the nickel desorption in natural systems it is essential that the ionic strength of the extraction solution is near to the ionic strength of the soil solution (B and S), or the saturation extract of the serpentine rock. Therefore, we equilibrated the experimental substrates with 2.5 mM $Mg(NO_3)_2$. The results in our study were comparable to those with $MgCl_2$ solution from Molina Millán et al. (2006).

2.2.2 Soil Water Content

The soil water content was determined by gravimetric method with oven drying. Therefore, the air-dried soil samples were weighed in ceramic dishes and dried in the oven for 24 hours at 105°C. The samples were cooled down and reweighed.

The soil water content was afterwards calculated with formula (1). The weights are displayed in Table 2.

$$\%soil = \frac{weight\ of\ wet\ soil\ [g] - weight\ of\ dry\ soil\ [g]}{weight\ of\ dry\ soil\ [g]} * 100 \qquad (1)$$

Table 2: Weighted soil for gravimetric soil water content determination Weight wet soil represents the weight of the air-dried soil. Weight dry soil represents the soil after 24 hours in the oven at 105°C.

Soil type	Weight wet soil [g]	Weight dry soil [g]
S	32.16	31.60
B	23.53	23.10

The soil water content for the serpentine soil (S) was determined as 1.77% and for the arable soil with serpentine properties (B) 1.86%.

2.2.3 Determination of nutrient and trace element concentration

To determine the concentrations of nutrients and trace elements of the soils (S and B) an acid digestion with *aqua regia* according to ÖNORM L1085 was conducted. Therefore 0.5 g of the ground and homogenized soil samples were weighed into the digestion tubes. Afterwards the tubes were transferred under the fume hood and the acids were added with a dispenser. First 4.5 mL of HCl and then 1.5 mL HNO_3 was filled into the tubes. It is important to add the acids in the right order. To inhibit foaming one drop of octanol was added into each tube. Another important point is to ensure the whole samples is in contact with the acids. The next step is to put cooler on the tubes and let them react over night.

After the final temperature of 150°C is reached the tubes were left for another three hours. Then the cooler has to be rinsed with distilled water. After filling the tubes with distilled water up to the mark (50 mL) the samples were mixed with the vortex-shaker. The total weight of the tube (tube + sample + acid + water) was taken and noted down. Afterwards the samples were filtrated into appropriate vials.

2.2.4 Time-dependent mobilization of Ni

To determine the time-dependent mobilization of Ni and other trace elements a 1:10 solid:solution extraction was conducted. Therefore, 5 g of the equilibrated soil samples were put into 100 mL PE-bottles. 50 mL extraction solution (10 µM oxalic acid, 10 µM citric acid, 10 µM oxalic + citric acid and 0.2 g L^{-1} Bronopol) were added into the shaking bottles.

For the serpentine rock 0.5 g of grinded rock was mixed with 5 mL extraction solution.

The bottles were then put on the overhead shaker at 20 rpm for a defined extraction time. The extraction times were: 0.2, 0.5, 1, 6, 24, 72 and 168 hours. The experiment was conducted with 3 replicates for each soil type, each treatment and each time (252 samples plus 3 blanks for each treatment).

Then the soil samples were filtrated with funnels and filter paper (brand) and afterwards stored in the freezer until the sample preparation for the analysis was done.

The serpentine rock was put afterwards into the centrifuge at 5000 rpm for 5 minutes. Afterwards the supernatant was filled into a syringe and filtrated with syringe filters (pore size 0.45 µm).

2.2.4.1 Sample Preparation

The samples were put out of the freezer and left until they reached room temperature.

Afterwards the soil samples (S and B) were diluted (1:2) with 3 mL probe and 3 mL 4% HNO_3 and 0.6 mL 110 ppb Indium was added.

For the serpentine rock (R) 2.5 mL probe and 2.5 mL 4% HNO_3 were mixed with 0.5 mL Indium.

2.2.4.2 Analytics

The elemental concentrations were analyzed using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (PerkinElmer ELAN 9000 DRCe).

To investigate if the different treatments have statistically significant influences a covariance analyzes (ANCOVA) was conducted with the statistic program SPSS (19).

2.2.4.3 Preparation of standard solutions

To ensure an accurate and precise measurement standard solutions must be prepared for the calibration of the instrument. Therefore, seven solutions in different concentrations (1, 10, 25, 50, 75, 100 and 125 ppb – for Zn and Fe the concentrations were 10-fold higher) were prepared as followed: 5 mL standard were mixed with 0.5 mL Indium (final indium concentration: 10 ppb).

2.2.5 Preliminary Test

Before starting the concentration-dependent mobilization extraction a preliminary test was conducted. This was to investigate the extraction time for concentration-dependent Ni extraction efficiency.

For this test a 1:10 solid:solution extraction was conducted. 5 g soil were put into 100 mL PE-bottles and filled with 50 mL 250 µM citric acid or 0.2 g L^{-1} Bronopol. The extraction times were 0.5, 24 and 144 hours.

For analyzing the element concentrations, the samples were prepared as described in 2.2.4.1 and analyzed using ICP-MS.

2.2.6 Concentration-dependent mobilization of Ni

According to the results of the preliminary extraction the extraction time was set at 0.5 hours.

To determine the concentration-dependent mobilization of Ni and other trace elements a 1:10 solid:solution extraction was conducted. Therefore, 5 g of the equilibrated soil samples were put into 100 mL PE-bottles. 50 mL extraction solution (10, 50 and 250 µM oxalic acid, 10, 50 and 250 µM citric acid, 10, 50 and 250 µM oxalic + citric acid and 0.2 g L^{-1} Bronopol in each treatment) were added into the shaking bottles.

For the serpentine rock 0.5 g of ground rock was mixed with 5 mL extraction solution.

The bottles were then put on the overhead shaker at 20 rpm for 0.5 hours. The experiment was conducted with 3 replicates for each soil type, each treatment (90 samples).

Then the soil samples were filtrated with funnels and filter paper (brand) and afterwards stored in the freezer until the sample preparation for the analysis was done.

The serpentine rock was put afterwards into the centrifuge at 5000 rpm for 5 minutes. Afterwards the supernatant was filled into a syringe and filtrated with syringe filters (pore size 0.45 µm).

2.2.6.1 Sample preparation

The samples were prepared as described in 2.2.4.1. The element concentration was analyzed using ICP-MS.

2.2.6.2 Analytics

The elemental concentrations were analyzed using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (PerkinElmer ELAN 9000 DRCe).

To investigate if the different treatments have statistically significant influences an one way ANOVA was conducted with the statistic program SPSS (19).

2.2.6.3 Preparation of standard solutions

To ensure an accurate and precise measurement standard solutions must be prepared for the calibration of the instrument. Therefore, seven solutions in different concentrations (1, 10, 25, 50, 75, 100 and 125 ppb – for Zn and Fe the concentrations were 10-fold higher) were prepared as followed: 5 mL standard were mixed with 0.5 mL Indium (final In concentration: 10 ppb).

3 Results

In the following section the results of the conducted experiments are presented. The statistical analyzes were done separately for each soil type to ensure only the treatment is influencing the results.

3.1 Time-dependent mobilization of Ni and other trace elements

This chapter is structured as follows. The time-dependent mobilization of Nickel, Chromium and Manganese are described separated for each substrate (B, S and R).

3.1.1 Time-dependent mobilization of Ni

In the following Figure 4 the mobilization of Nickel over time is represented. There was no apparent difference in Ni mobilization between the different treatments (control, citrate, oxalate and combination of oxalate and citrate) for the investigated substrates.

The concentrations of R with values varying from 10 to 75 µg kg^{-1} were higher than the concentrations in the soils. The values from B were varying from 1 to 15 µg kg^{-1} and in S from 0.2 to 1 µg kg^{-1}.

The Ni concentrations of R rose monotone with the extraction time. The concentrations of the soils (arable and serpentine) were also rising with a decline after 72 hours and rising again to the highest values after 168 hours.

The analysis of the mobilization rates after 168 hours of the substrates showed that most Ni was mobilized in R of 79.05% (from 15.89 to 75.84 µg kg^{-1}) Ni, followed by S with a concentration increase of 73.3% (from 0.28 to 1.05 µg kg^{-1} Ni). In B the nickel concentration increased of 66.48% (from 4.84 to 14.44 µg kg^{-1} Ni).

The amount of mobilized nickel in respect to the total amount of B were 9.6 ppm. In R 31 ppm and in S 0.41 ppm of total nickel amount were solubilized.

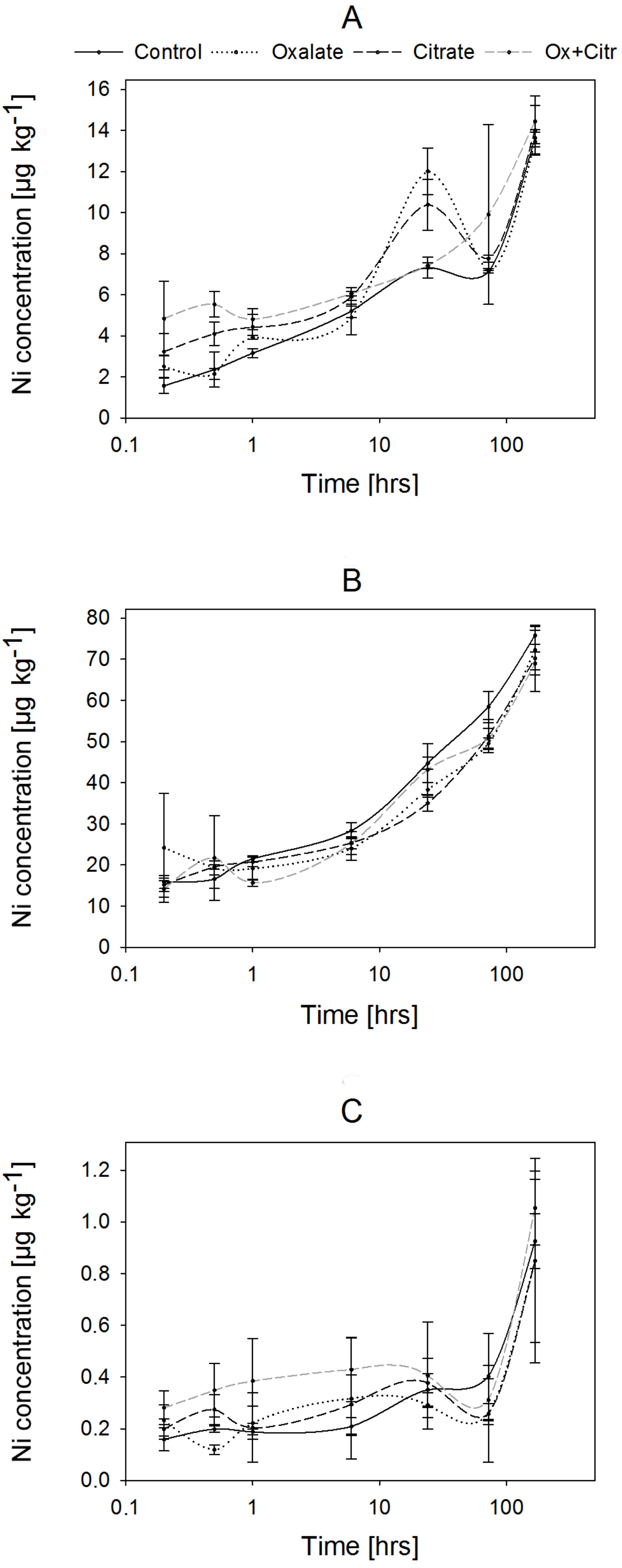

Figure 4: Ni concentration over time in the different soil types when exposed to 10 µM citrate, 10 µM oxalate, or 10 µM citrate+oxalate. A represents the arable soil in Bernstein, B represents serpentine rock and C displays the serpentine soil. The extraction time was between 12 minutes and 168 hours. Error bars show standard deviation of the mean (n=3).

In Table 3 the changes of the nickel concentration from 0.2 to 24 hours and from 0.2 to 168 hours are displayed.

Within 24 hours the Ni concentration increased in Bernstein the most in the oxalate treated samples with a factor of 4.78 followed by the control group with an increasing factor of 4.66. In R citrate had the most influence increasing the concentration (factor = 3.05) followed by the control group with an increasing factor of 2.82. In S the control group showed the most concentration increase with a factor of 2.19 followed by the combination of oxalate citrate with a factor 1.90.

Within 168 hours the largest increase over time in Bernstein soil was in the control group with a factor of 8.69 followed by the oxalate treatment with an increase of a factor 5.36. In R the treatment with citrate had the most influence in concentration increase with factor 4.87 followed by the control group with an increase factor of 4.77. In S the control group induced the most concentration increase with factor 5.81 followed by the combination of oxalate and citrate with an increasing factor of 4.25.

Table 3: Changes of Ni concentration from 0.2 to 24 hours and from 0.2 to 168 hours. The changes of concentration are displayed as factors.

	Bernstein	**Serpentine Rock**	**Serpentine Soil**
0.2 to 24 hours			
Control	4.66	2.82	2.19
Oxalate	4.78	1.58	1.26
Citrate	3.38	3.05	1.46
Oxalate and Citrate	1.53	2.31	1.90
0.2 to 168 hours			
Control	8.69	4.77	5.81
Oxalate	5.36	2.99	3.70
Citrate	4.34	4.87	3.75
Oxalate and Citrate	4.84	4.63	4.25

In the following table (Table 4) the statistical output of the ANVOCA for the time-dependent Ni mobilization in Bernstein, Serpentine Rock and Serpentine Soil is listed.

The treatment has no significant influence on the results. The p-value was for all substrates >0.05 (0.330 for B, 0.861 for R and 0.514 for S).

This result is also depicted in Figure 4.

Detailed statistical information is listed in the appendix.

Table 4: Statistical output of ANCOVA for time-dependent Ni mobilization There is no statistical difference between the treatments. p-value > 0.05.

Soil type	**p-value Treatment**
Bernstein	0.330
Serpentine Rock	0.861
Serpentine Soil	0.514

3.1.2 Time-dependent mobilization of Cr and Mn

Besides Nickel other trace elements were also mobilized by organic acids.

In the following Figure 5 the mobilization of Mn and Cr over time is represented.

For Mn the course of the curves of the different treatments (control, citrate, oxalate and combination of oxalate and citrate) appears very similar for each substrate.

The concentrations of R with values varying from 40 to 50 µg kg^{-1} were higher than the concentrations in the soils. The values from B were varying from 1 to 5 µg kg^{-1} and in the serpentine soil from 0.9 to 1.5 µg kg^{-1}.

The Mn concentrations of R rose slightly with the extraction time. The concentrations of B were also rising but with a decline after 72 hours and rising again to the highest values after 168 hours. The concentration of S was almost stable, there was no concentration increase over time visible.

For Cr the course of the curves for of the different treatments (control, citrate, oxalate and combination of oxalate and citrate) was very similar.

The concentrations of R with values varying from 56 to 148 µg kg^{-1} were higher than the concentrations in the soils. The values from B were varying from 0.3 to 15 µg kg^{-1} and in S from 0.8 to 2.1 µg kg^{-1}.

The Cr concentrations of R were rising with the extraction time with a plateau after 72 hours. The concentrations of B were also rising with a decline after 72 hours and rising again to the highest values after 168 hours. The extractable Cr concentrations of S were initially stagnating at a low level, but after 72 hours the concentration clearly increased.

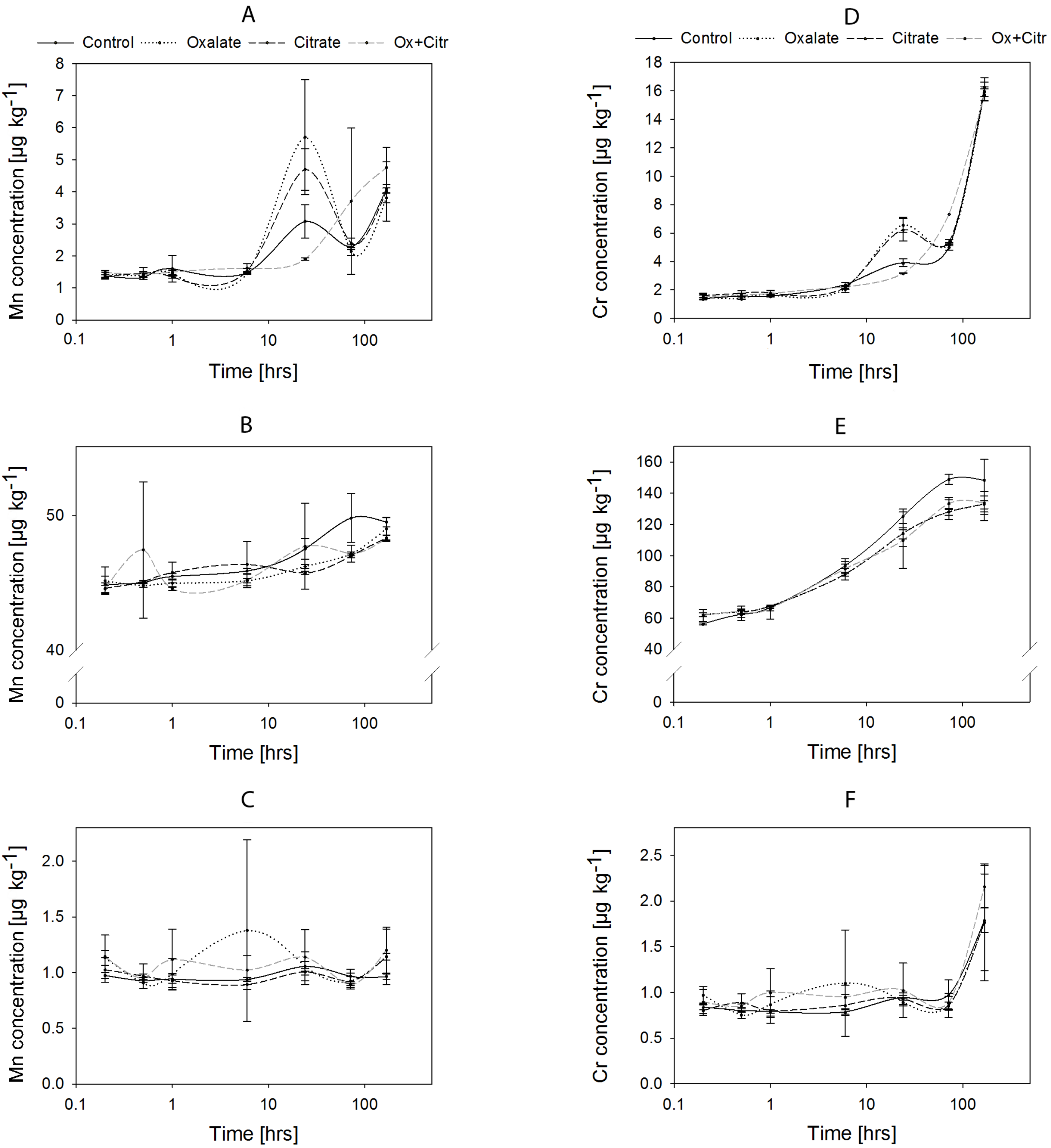

Figure 5: Mn and Cr concentrations over time in different soil types when exposed to 10 µM citrate, 10 µM oxalate or 10 µM citrate+oxalate. A and D represents the arable soil in Bernstein, B and E represents serpentine rock and C and F displays the serpentine soil. The extraction time was between 12 minutes and 168 hours. Error bars show standard deviation of the mean (n=3)

3.1.2.1 Time-dependent mobilization of Mn

The changes of manganese concentration over time is depicted in Table 5.

Within 24 hours the Mn concentration increased in B the most in the oxalate treated samples with a factor of 3.83, followed by the citrate group with the increasing factor of 2.92. In R citrate had no effect and there was no difference compared to the control. In S, there was no effect detectable for the control group and citrate treated samples (Factors 1.09 and 1). For the combination of oxalate and citrate and the oxalate treated samples a decrease in Mn concentration was recognizable.

For Mn the largest concentration increase over time (0.2 to 168 hours) in B was with the combination of oxalate and citrate with a factor of 3.21 followed by the control group with an increase of a factor 2.96. In R the control group had the most influence in concentration increase with factor 1.11, the oxalate treatment showed no effect (Factor = 1.09). In S the combination of oxalate and citrate induced the most concentration increase with factor 1.22, the citrate treated samples showed no effect (Factor = 1.05) In the control group, there was a slightly decrease in Mn concentration (factor = 0.99).

Table 5: Changes of Mn concentrations from 0.2 to 24 hours and from 0.2 to 168 hours. The changes of concentration are displayed as factors.

	Bernstein	**Serpentine Rock**	**Serpentine Soil**
0.2 to 24 hours			
Control	0.70	1.06	1.09
Oxalate	3.83	1.02	0.94
Citrate	2.92	1.07	1
Oxalate and Citrate	1.52	1.03	0.99
0.2 to 168 hours			
Control	2.96	1.11	0.99
Oxalate	2.66	1.09	1.01
Citrate	2.95	1.08	1.05
Oxalate and Citrate	3.21	1.08	1.22

In the following table (Table 6) the statistical output of the ANVOCA for the time-dependent Ni mobilization in Bernstein, Serpentine Rock and Serpentine Soil is listed.

The treatment had no significant influence on the results. The p-value was for all soil types >0.05 (0.941 for B, 0.355 for R and 0.185 for S).

This result is also illustrated in Figure 5.

Detailed statistical information is listed in the appendix.

Table 6: Statistical output of ANCOVA for time-dependent Mn mobilization There is no statistical difference between the treatments. p-value > 0.05.

Substrate	**p-value Treatment**
Bernstein	0.941
Serpentine Rock	0.355
Serpentine Soil	0.185

3.1.2.2 Time-dependent Cr mobilization

The concentration changes of chromium over time are displayed in Table 7.

Within 24 hours the Cr concentration increased in B the most in the citrate treated samples with a factor of 4.66 followed by the oxalate group with an increasing factor of 4.60. In R the control group had the most influence increasing the concentration (factor = 2.22) followed by the combination of oxalate and citrate with an increasing factor of 1.87. In S the combination of oxalate and citrate had the most increase in concentration with a factor of 1.16 followed by citrate with a factor 1.13. For the oxalate treated samples a decrease in Cr concentration was recognizable.

The largest concentration increase of Cr over time (from 0.2 to 168 hours) in B was in the control group with a factor of 11.55 followed by oxalate treated samples with an increase of a factor 10.72. In R the control group had the most influence in concentration increase with factor 2.63 followed by the citrate treatment with an increase factor of 2.17. In S the citrate induced the most concentration increase with factor 2.4 followed by the combination of oxalate and citrate treated samples with an increasing factor of 2.21.

Table 7: Changes in Cr concentrations from 0.2 to 24 hours and from 0.2 to 168 hours. The changes of concentration are displayed as factors.

	Bernstein	**Serpentine Rock**	**Serpentine Soil**
0.2 to 24 hours			
Control	2.83	2.22	1.12
Oxalate	4.60	1.84	0.92
Citrate	4.66	1.78	1.13
Oxalate and Citrate	2.16	1.87	1.16
0.2 to 168 hours			
Control	11.55	2.63	2.13
Oxalate	10.72	2.14	1.82
Citrate	10.11	2.17	2.4
Oxalate and Citrate	10.34	2.13	2.21

In the following table (Table 8) the statistical output of the ANVOCA for the time-dependent Ni mobilization in Bernstein, Serpentine Rock and Serpentine Soil is listed.

The treatment had no significant influence on the results. The p-value was for all soil types >0.05 (0.992 for B, 0.468 for R and 0.853 for S).

This result is also illustrated in Figure 5

Detailed statistical information is listed in the appendix.

Table 8: Statistical output of ANCOVA for time-dependent Cr mobilization There is no statistical difference between the treatments. p-value > 0.05.

Substrate	**p-value Treatment**
Bernstein	0.992
Serpentine Rock	0.468
Serpentine Soil	0.853

3.2 Concentration-dependent mobilization of Ni and other trace elements

This chapter is structured as follows. The concentration-dependent mobilization of Nickel, Chromium and Manganese are described separated for each substrate (B, R and S).

3.2.1 Concentration-dependent mobilization of Ni

In the following section the concentration-dependent Ni mobilization was analyzed. The statistical output of the ANOVA for the different soil types/substrates is listed in the following table (Table 9). Find the detailed statistical information in the appendix. As the p-value was below 0.05 there was a significant difference between the treatments. To identify which groups were different a post hoc test (Turkey test) was conducted. The results of this test are displayed in the following sections separated for each substrate.

Table 9: Statistical output of ANOVA for concentration-dependent Ni mobilization There is a statistical difference between the treatments. p-value < 0.05.

Substrate	p-value Treatment
Bernstein	0.00
Serpentine Rock	0.00
Serpentine Soil	0.00

3.2.1.1 Bernstein

Figure 6 illustrates the Nickel mobilization dependent on the concentration.

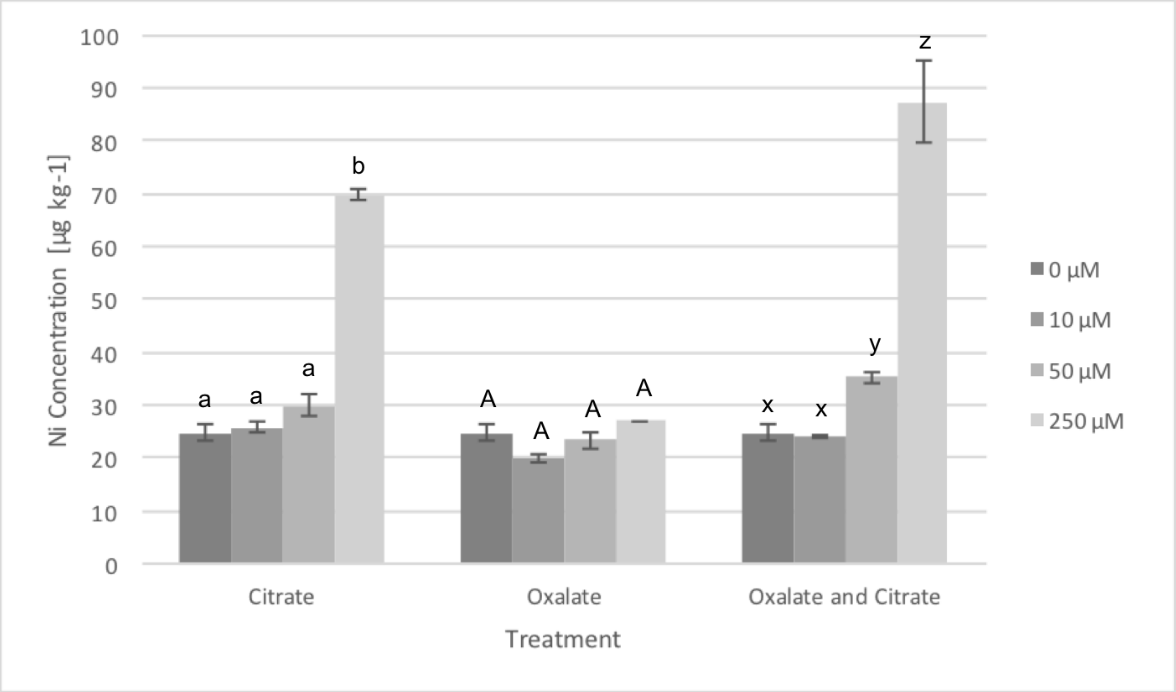

Figure 6: Concentration-dependent mobilization of Nickel in Bernstein Mobilization of Nickel with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test, p >0.05).

As shown in Table 9 there were statistical significant differences between the treatments (p <0.05). This is also illustrated in Figure 6. Within group 1 (citrate) the treatment 250 was statistically different from 50, 10 and the control group. In the samples treated with oxalate no statistical significant differences were detected. The combination of citrate and oxalate the samples treated with 250 µM differed statistically form 50, 10 and the control group. The group treated with 50 µM was different from 10 and the control. There were no statistical significant differences between 10 µM and the control group.

3.2.1.2 Serpentine Rock

Figure 7 illustrates the Nickel mobilization dependent on the concentration.

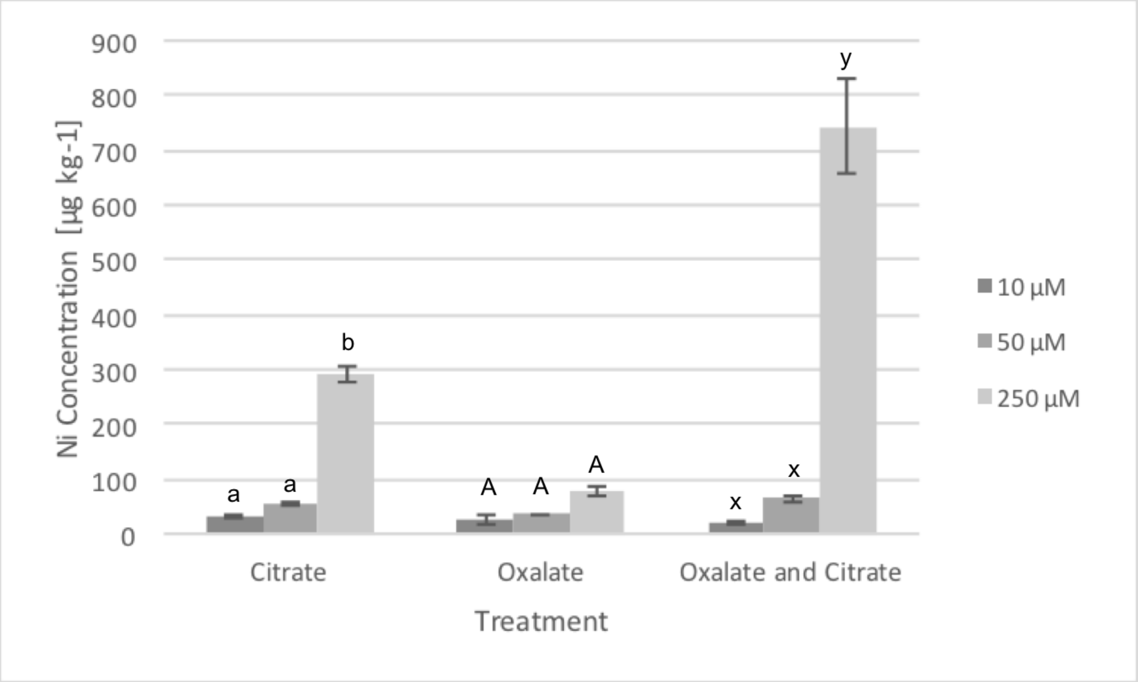

Figure 7: Concentration-dependent mobilization of Nickel in Serpentine Rock Mobilization of Nickel with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test, p >0.05).

As shown in Table 9 there were statistical significant differences between the treatments (p <0.05). This is also illustrated in Figure 7. Within group 1 (citrate) the treatment 250 was statistically different from 50 and 10 µM. In the samples treated with oxalate no statistical significant differences were detected. The combination of citrate and oxalate the samples treated with 250 µM differed statistically from 50, 10 and the control group. The group treated with 50 µM was statistically not different from 10 µM.

3.2.1.3 Serpentine Soil

Figure 8 illustrates the Nickel mobilization dependent on the concentration.

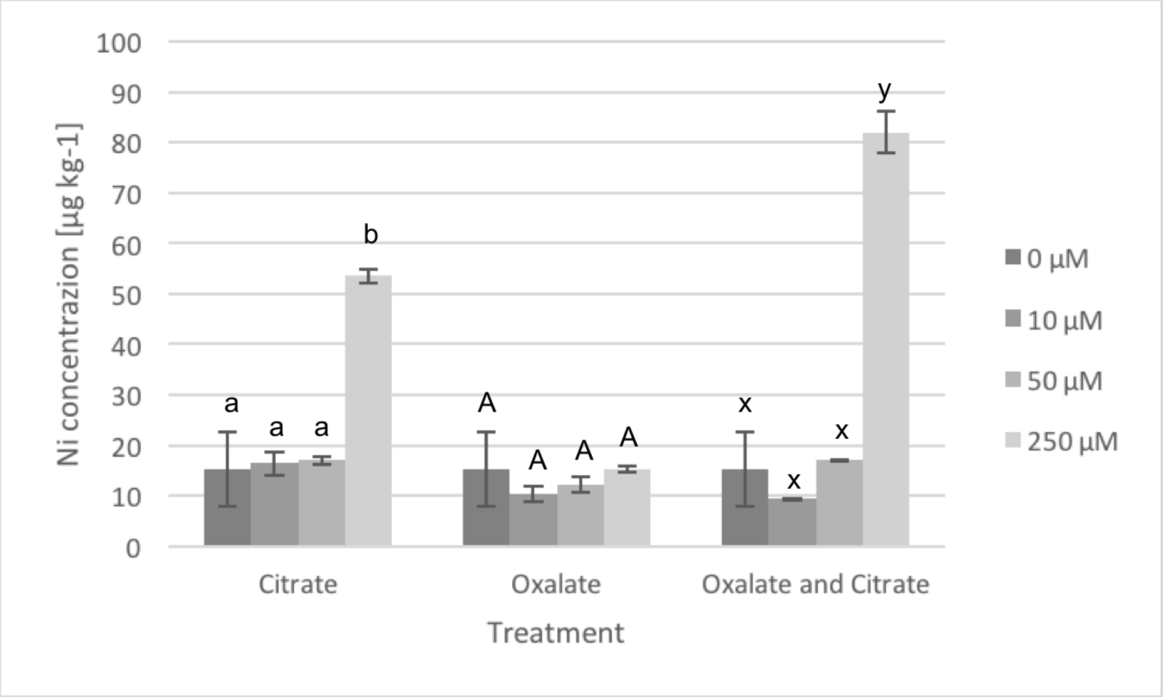

Figure 8: Concentration-dependent mobilization of Nickel in Serpentine Soil Mobilization of Nickel with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test, $p > 0.05$).

As shown in Table 9 there were statistical significant differences between the treatments ($p < 0.05$). This is also illustrated in Figure 8. Within group 1 (citrate) the treatment 250 µM was statistically different from 50, 10 µM and the control group. In the samples treated with oxalate no statistical significant differences could be detected. The combination of citrate and oxalate the samples treated with 250 µM differed statistically from 50, 10 µM and the control group.

3.2.2 Concentration-dependent mobilization of trace elements

3.2.2.1 Mobilization of Cr

In the following section the concentration-dependent Cr mobilization is analyzed. The statistical output of the ANOVA for the different substrates is listed in the following table (Table 10). Find the detailed statistical information in the appendix. As the p-value was below 0.05 there were significant differences between the treatments. To identify which groups were different a post hoc test (Turkey test) was conducted. The results of this test are displayed in the following sections separated for each substrate.

Table 10: Statistical output of ANOVA for concentration-dependent Cr mobilization There is a statistical difference between the treatments Bernstein and Serpentine Rock (p-value < 0.05) and no differences within Serpentine Soil (p-value >0.05).

Substrate	p-value Treatment
Bernstein	0.00
Serpentine Rock	0.00
Serpentine Soil	0.255

3.2.2.1.1 Bernstein

Figure 9 illustrates the mean Cr mobilization dependent on the concentration.

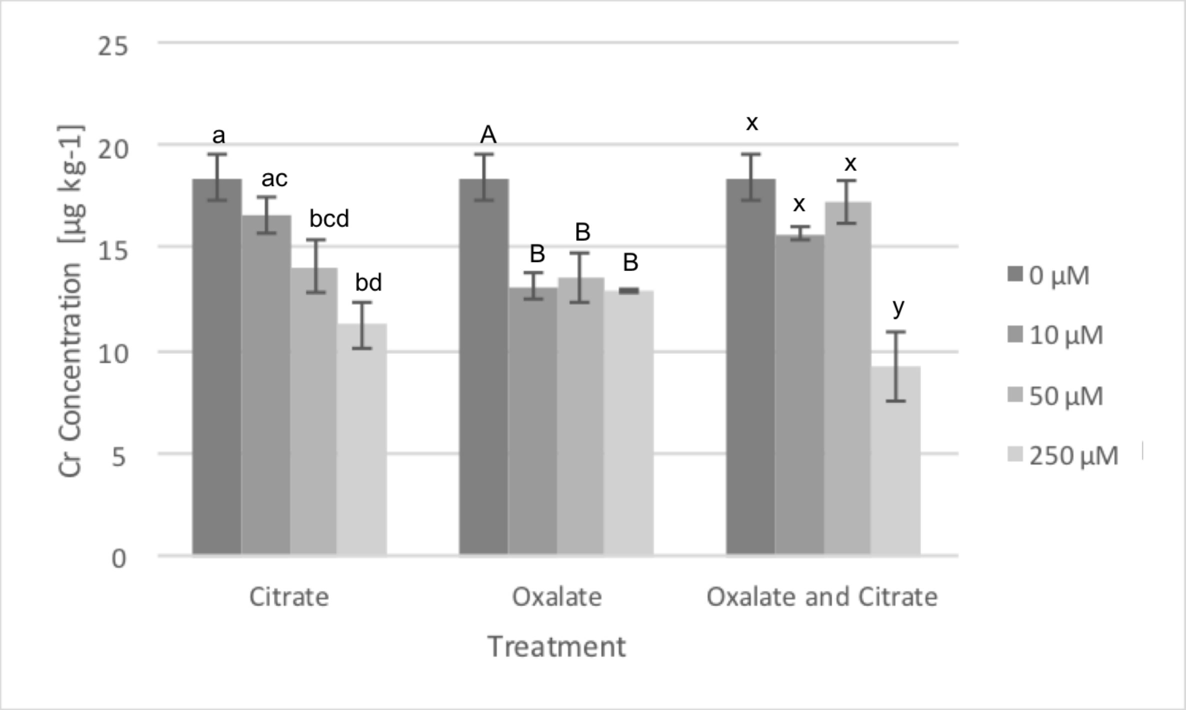

Figure 9: Concentration-dependent mobilization of Chromium in Bernstein Mobilization of Cr with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test p >0.05).

As shown in Table 10 there were statistical significant differences between the treatments. This is also illustrated in Figure 9. Within the treatment citrate, samples treated with 50 µM were statistically different from the control. Samples treated with 250 µM differed statistically from the control and 10 µM treated ones.

The samples treated with oxalate (10, 50 and 250 µM) were statistically different from the control.

The combination of citrate and oxalate the samples treated with 250 µM differed statistically from the control group, 10 and 50 µM treatments.

3.2.2.1.2 Serpentine Rock

In Figure 10 the mean Cr mobilization concentration-depended of Serpentine Rock is illustrated.

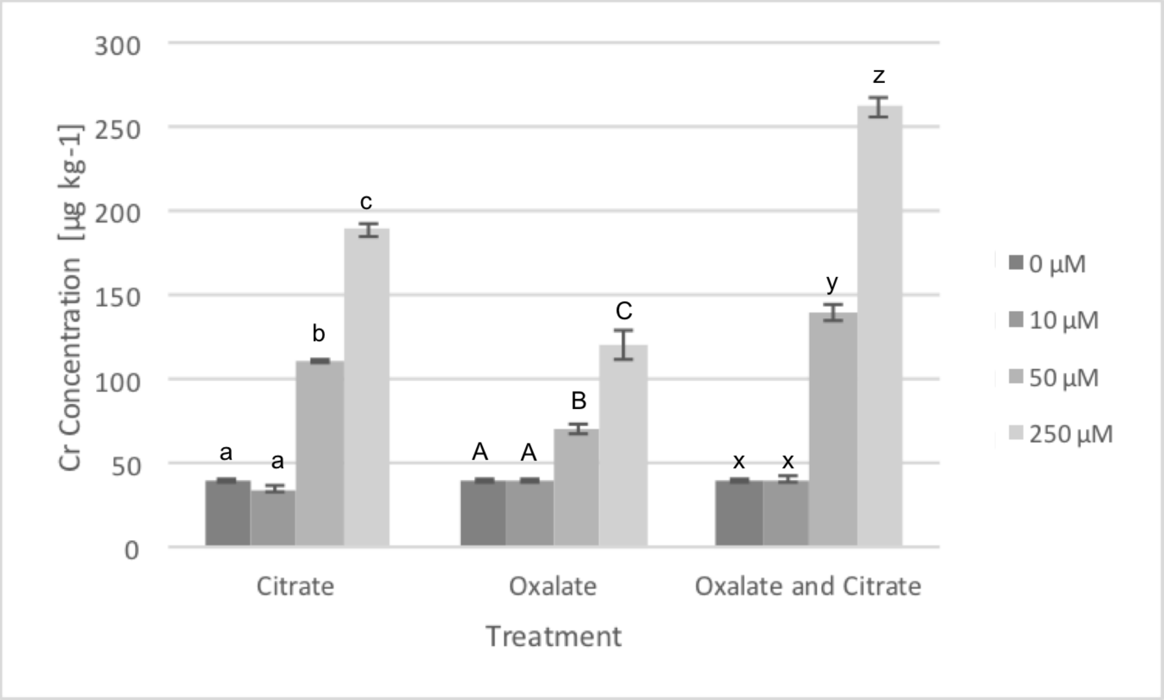

Figure 10: Concentration-dependent mobilization of Chromium in Serpentine Rock Mobilization of Cr with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test p >0.05).

As shown in Table 10 there were statistical significant differences between the treatments. This is also illustrated in Figure 10. Within group 1 (citrate) the treatment 250 µM was statistically different from 50, 10 µM and the control. The samples treated with 50 µM were different from 10 µM and the control group. In the samples treated with 250 µM oxalate were statistically different from the control, 10 and 50 µM treated samples. Samples treated with 50 µM oxalate were statistically different from the control group and samples treated with 10 µM.

The combination of citrate and oxalate the samples treated with 250 µM differed statistically from 50, 10 and the control group. The 50 µM treatment was statistically different from 10 µM treated samples and the control.

No differences could be detected between the control group and samples treated with 10 µM. This was regarded for all treatments.

3.2.2.1.3 Serpentine Soil

In Figure 11 is the concentration-dependent Cr mobilization depicted.

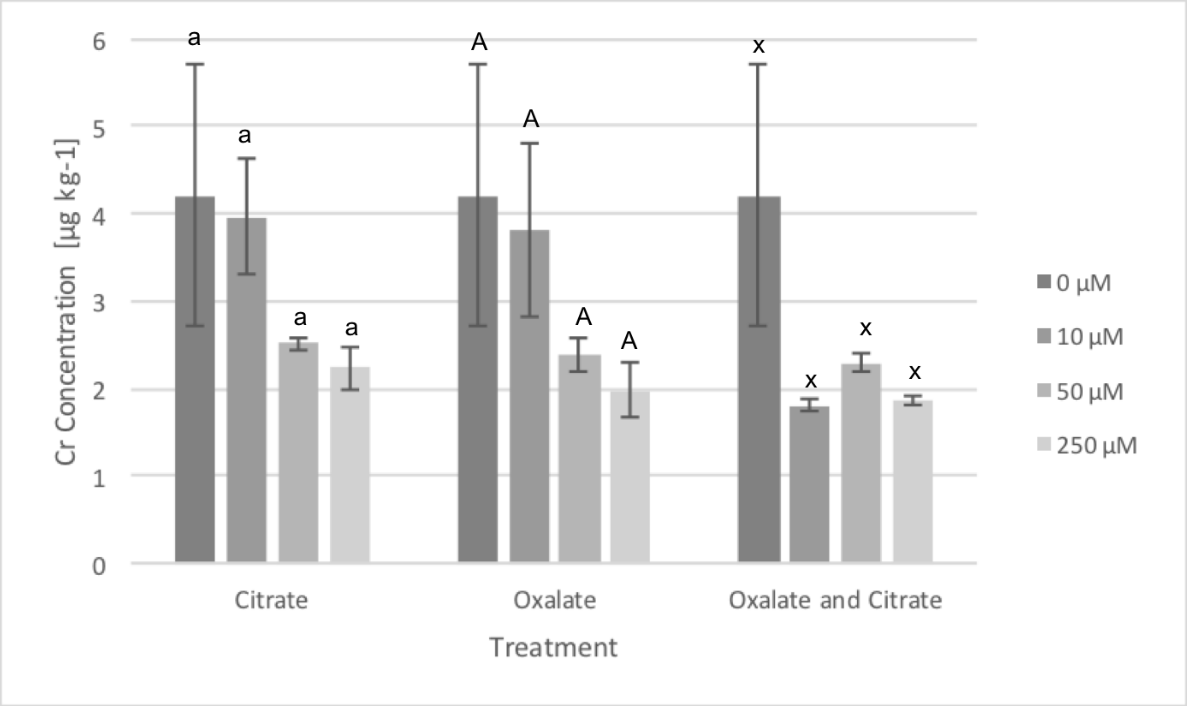

Figure 11: Concentration-dependent mobilization of Cr in Serpentine Soil Mobilization of Cr with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test p >0.05).

As shown in Table 10 there were no statistical significant differences between the treatments. The p-value was 0.255.

3.2.2.2 Mobilization of Mn

In the following section the concentration-dependent Mn mobilization was analyzed. The statistical output of the ANOVA for the different soil types is listed in the following table (Table 11). Find the detailed statistical information in the appendix. As the p-value was below 0.05 there were significant differences between the treatments. To identify which groups were different a post hoc test (Turkey test) was conducted. The results of this test are displayed in the following sections separated for each soil type.

Table 11: Statistical output of ANOVA for concentration-dependent Mn mobilization There is a statistical difference between the treatments in Bernstein (p-value < 0.05) and no differences within Serpentine Soil (p-value >0.05).

Soil type	p-value Treatment
Bernstein	0.00
Serpentine Soil	0.384

3.2.2.2.1 Bernstein

In Figure 12 the concentration-dependent Mn mobilization is displayed.

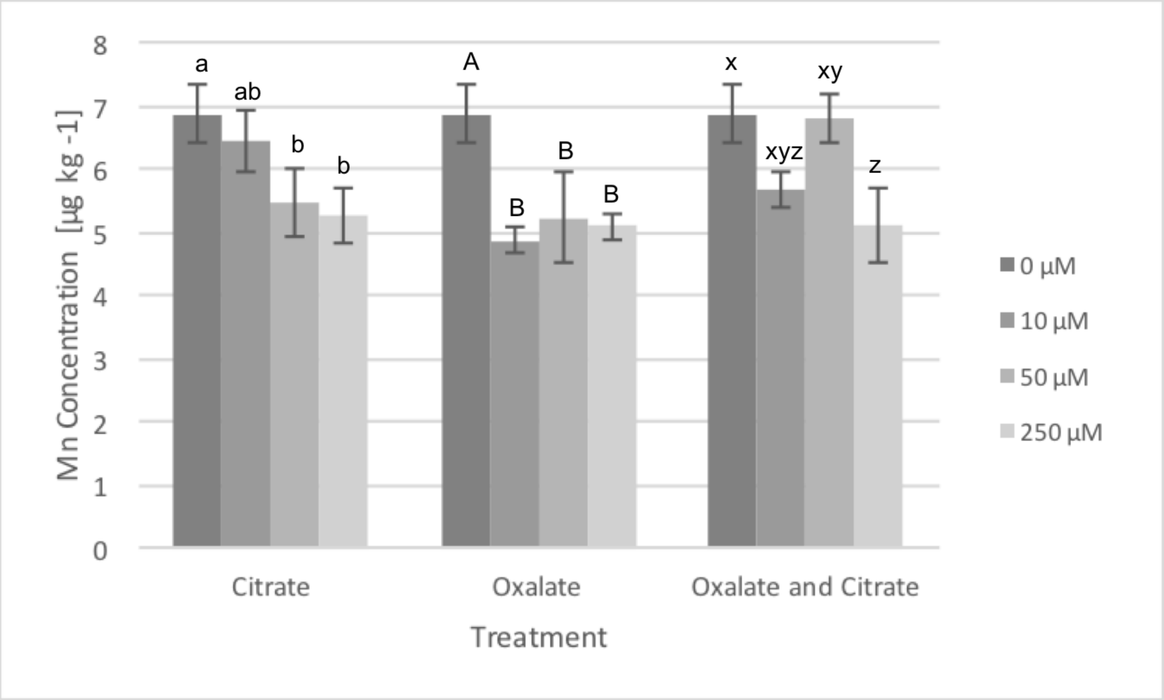

Figure 12: Concentration-dependent mobilization of Manganese in Bernstein Mobilization of Mn with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test p >0.05).

As shown in Table 11 there were statistical significant differences between the treatments. This is also illustrated in Figure 12. Within group 1 (citrate) the treatment 250 µM was statistically different from the control. Besides that, no further statistical differences could be detected.

The control group was statistically different from the samples treated with 250, 50 and 10 µM oxalate. Within the treatments no differences could be detected.

The combination of citrate and oxalate the samples treated with 250 µM differed statistically from 50 µM and the control group. No differences could be identified between the control, 10 and 50 µM treated samples.

3.2.2.2.2 Serpentine Rock

All data of Mn analyzes were below the detection limit of the ICP-MS. Therefore, no data is shown in this section.

3.2.2.2.3 Serpentine Soil

The concentration-dependent Mn mobilization is illustrated in Figure 13.

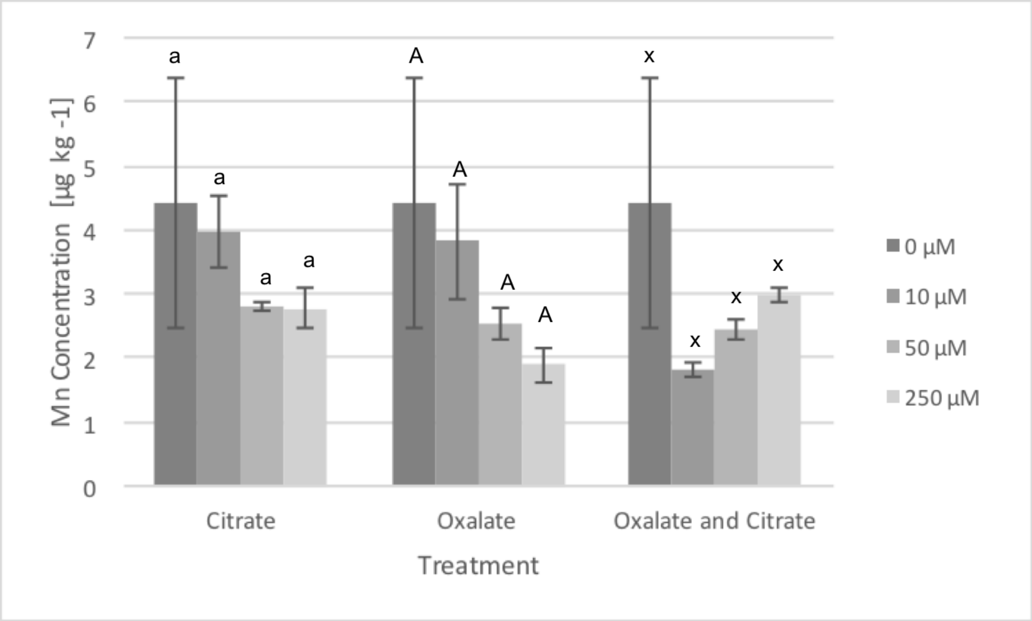

Figure 13: Concentration-dependent mobilization of Manganese in Serpentine Soil Mobilization of Mn with three different treatments and concentrations. Error bars show standard deviation of the mean (n=3). Bars showing the same letter code are not significant different (Turkey test $p > 0.05$).

As shown in Table 11 there were no statistical significant differences between the treatments. The p-value is 0.384.

4 Discussion

Nickel occurs in exchangeable forms, bound to carbonate, bound to Fe-Me oxides, associated with organic matter and residual in soil. In soil solution, this metal occurs as free ion (Ni^{2+}) as well as in complexes with organic and inorganic ligands. The bioavailability of Ni in ultramafic soils depends on soil characteristics (e.g. pH, organic matter and clay content), on possible rhizosphere effects, the efficiency of plant-root uptake and processes such as dissolution, precipitation of Fe/Mn oxides, adsorption on soil particles (Molas 2002; Massoura et al. 2004; Becerra-Castro et al. 2013).

Nickel minerals are poorly stable and undergo rapid weathering. Therefore, nickel is in developed soils typically associated with clays as vermuculites, saponite in serpentine soils, and Fe or Mn (hydro)oxides. The concentration range depends on the soil properties. Clay soils are richer in Ni than coarser ones.

The concentrations of Ni and Cr in serpentine soils are often similar but Cr is less soluble than Ni. About 1% of the total Ni in soil is available to plants, this exceeds the percentage of available Cr (Gonnelli and Renella 2013).

Nickel has a relatively high affinity for soil organic matter. The nickel (II) organic complexes in soil can be formed with particulate organic matter, humic substances or low molecular weight organic ligands (LMWOL) (Gonnelli and Renella 2013).

“Microbial influence on trace element speciation and mobility is an important component of biogeochemical cycle of trace elements. Sorbed, precipitated and occluded trace elements can be solubilized by acidification, chelation and ligand-induced dissolution.”(Sessitsch et al. 2013, 185)

The interaction of organic acids which were released by the roots of the hyperaccumulator *N. goesingesis* and the soil solid phase appear to be a key process. In ecosystems, the concentrations of organic acids in soil solution has been shown to be low, varying from 1 to 100 µM. (Jones et al. 2003; Puschenreiter et al. 2005).

LMWOLs that are released by plant roots may affect the solubility of Ni in the rhizosphere. Several examples showing that particular compounds in the substrate can either decrease or increase the Ni uptake. These effects depend on their quantity, quality and the characteristics of the plant. The presence of organic acids or inorganic ligands in soil solution results in the formation of Ni complexes with different characteristics that may inhibit or enhance root uptake (Gonnelli and Renella 2013).

Wenzel et al. (2003) found a significant correlation between water extractable Ni and Mg. This aspect supported that ligand-induced co-dissolution of Ni-bearing minerals may be involved in Ni mobilization in the rhizosphere. Comparable results were reported by Puschenreiter et al. (2005) where it is suspected that the enhanced Ni solubility in the rhizosphere of *N. goesingensis* was recognizable because of the adsorption of organic ligands onto surfaces on Ni-bearing primary minerals and ligand promoted dissolution of Ni (Puschenreiter et al. 2005).

It was investigated that plants which suffer nutrient deficiency (e.g. P-deficiency) show enhanced organic compounds exudation for example citric and malic acid (Fitz et al. 2003).

To enhance the nutrient uptake plants and especially hyperaccumulators modify the rhizosphere through e.g. acidification, release of root exudates and decrease of redox potential. However, rhizosphere acidification was found by several authors not to be involved in the metal solubilization processes of hyperaccumulators. Suggested mechanisms that increase Ni solubility in soil were e.g. the release of organic acids that act as chelating agents (Fitz et al. 2003; Puschenreiter et al. 2003; Centofanti et al. 2012).

Furthermore, Li et al (2003) found in their study that more nickel was accumulated in two *Alyssum* species with increasing pH. Therefore, another effect has to be responsible for the extreme metal uptake of hyperaccumulating plants.

Another important point in the study of Puschenreiter et al (2003) was the decrease of the labile Zn fraction. Only 10% of the total Zn uptake resulted from the labile Zn pool. They concluded that hyperaccumulators are able to mobilize and sequester Zn from less soluble fractions. In a well buffered soil solution the uptake of free Zn^{2+} induces further dissolution of Zn^{2+} into the soil solution. This is because of the maintenance of initial free Zn^{2+} concentration (Knight et al. 1997; Puschenreiter et al. 2003).

A depletion of labile metal pools in the rhizosphere often has been found to be associated with sustained or enhanced solubility but no direct evidence for metal mobilization induced by root exudation was reported so far (Puschenreiter et al. 2005).

Hyperaccumulating plants deplete bioavailable pools of metals and therefore change the chemical equilibria of the metal in soil. No mechanism is known by now where plants replenish Ni from the non-labile Ni pools. An indirect mechanism by which plants can deplete non-labile pools is the renewal of the labile pool. (Centofanti et al. 2012).

However, Centofanti et al. (2012) reported also that the labile pool of Ni is orders of magnitudes lower than the total Ni amount in above-ground biomass in *Alyssum* species. Therefore, other mechanisms have to be responsible for the high Ni uptake in hyperaccumulating plants.

Puschenreiter et al. (2005) found in their study that the decrease of Ni is clearly related to excessive Ni uptake by hyperaccumulators which is associated on the one hand with depletion of labile (easy bioavailable) metal pools and on the other hand with an active root proliferation towards contaminated soil areas. This is consistent with previous field observations of *N. goesingensis*. The metal depletion in the rhizosphere was also reported for other hyperaccumulator species such as Zn hyperaccumulator *N. caerlescens*, As hyperaccumulator *Pteris Vittata* and Tl hyperaccumulator *Iberis intervedia* (Puschenreiter et al. 2005; Sessitsch et al. 2013).

The replenishment of soluble Ni from sources other than the adsorbed fraction through interactions with the soil solid phase is triggered by root activities such as exudation of organic acids. One example is the ligand-promoted dissolution of Ni from fersetite-type minerals. The enhanced dissolution of Ni-bearing silicates could explain the concomitant increase of Ni, Ca and Mg in the soil solution (Puschenreiter et al. 2005).

Massoura et al. (2004) investigated in their study whether plants take up Ni from the same available pool or if some induced changes in the rhizosphere result in a larger reserve of soil Ni. Their experiment was based on a set of plants that exhibit different strategies for Ni uptake and transport. The three *A. murale* populations, which were investigated, accumulated very different amounts of Ni in their tissue. Interestingly none of them modified the pool of bioavailable soil Ni and all plants foraged the same Ni pool.

To recall the research questions asked in the introduction, they are presented here:

- RQ1: How long does the mobilization take – mobilization kinetics?
- RQ2: Which concentrations of organic acids are necessary for the element mobilization?
- RQ3: Which substance (citrate, oxalate or the combination of these two) is more efficient and are there synergistic effects?
- RQ4: Besides Ni, which elements are co-solubilized?
- RQ5: To which extent is primary serpentine mineral contributing to Ni solubilization processes?

4.1 Time-dependent mobilization of elements

In respect to the mobilization kinetics of Ni, different approaches were used in previous studies, varying from 30 minutes to 24 hours equilibration time. (Molina Millán et al. 2006; Robinson et al. 1999). Hence, to determine the mobilization kinetics for nickel and thus the extraction time of the second experiment, a preliminary test was conducted. The results of this preliminary test corresponded with the ones from Molina Millán et al. (2006). Therefore, the extraction time of 30 minutes was selected for the concentration-dependent extraction.

The time-dependent mobilization showed no differences ($p > 0.05$) between the treatments. As there were no statistically significant changes between the treatments there it is assumed that the concentrations used (10 µM citrate, 10 µM oxalate and 10 µM citrate and oxalate) were too low to mobilize elements (Ni, Mn and Cr) more efficient compared to the control. This is also discussed in the following section (concentration-dependent mobilization of elements). One possible explanation of this effect could be that one part of the organic acids were absorbed rapidly onto the soil matrix, which resulted in a lower effective concentration of organic acids than the concentrations used.

Molina Millan et al. (2006) investigated in their study that most of the Ni was desorbed within less than 15 minutes. Within 6 hours no significant changes in the nickel concentration was observed. In the present study, the general trend of the Ni mobilization was an increase of the concentration with the extraction time. The concentration increase was depending on the substrate.

An interesting point is the extreme drop of Ni concentrations at 72 h. A possible explanation could be an increased sorption of Ni on soil particles.

One possible reason for the concentration rise from 72h to 168 h could be abrasion on the soil particles. This effect of artificially high Ni concentrations was also described by Robinson et al. (1999).

The mobilization of the trace elements Cr and Mn was different from the mobilization of nickel. Only a slight increase of the Mn concentration over time could be detected. However, the increase of chromium was more pronounced, depending on the substrate. A possible explanation could be the different binding forms and chemical properties, such as speciation and complex formation (Blume et al. 2016)

In soils Mn is usually fixed in Mn oxides as well as in silicates and carbonates. Besides that, manganese can also be found in organic complexes as well as in exchangeable and dissolved forms as Mn^{2+} (Blume et al. 2016). Because no manganese was mobilized in the soils (arable soil with serpentine properties B and serpentine soil S) it could be assumed that Mn oxides were not involved in nickel mobilization.

4.2 Concentration-dependent mobilization of elements

As Molina Millán et al. (2006) showed in their study the nickel concentrations in the solutions increased with increasing concentration of oxalate or citrate. This effect was confirmed by the present study.

Another point which is similar to the study of Molina Millán et al. (2006) was that citrate and the combination of citrate and oxalate was generally more effective than oxalate in desorbing Ni from the soil. For oxalate, no significant differences could be detected in any substrate. In each substrate – the arable soil with serpentine properties (B), serpentine soil (S) and serpentine rock (R) - only the highest concentration (250 µM of citrate and the combination of citrate and oxalate) was statistically different from the other treatments. One implication therefore could be that very high concentrations of organic acids are needed to mobilize Ni substantially in soils. However, this study also showed that very high concentrations of organic acids and therefore high exudation rates are necessary to mobilize nickel substantially. Both potentially occurs at the root tip (Jones et al. 2003).

Chromium is only sufficiently stabile in the environment in the oxidations states Cr(III) and Cr(VI). The reactivity of Cr in soil is dependent on the soil pH, redox potential, presence of potential electron donors and Cr adsorption on colloids. In (sub-)neutral soil pH and natural Cr concentrations, both oxidation states may form various hydrolysis and deprotonated products. The hydrolysis products are commonly adsorbed onto clay minerals. (Gonnelli and Renella 2013).

The results of R showed a Cr mobilization as the chromium concentration increased with an increase of organic acid concentration. Hence, the organic acids (oxalate and citrate) induced a mineral solubilization. With each mol nickel 0.4 mol chromium were mobilized. This effect is a clear indication of an induced mineral weathering.

As in the serpentine soils (B and S) nickel was mobilized but no release of chromium was recognizable two different assumptions are suggested: first, an enhanced re-adsorption of chromium into the soil matrix (as CrO_4-ions) proceeded and/or second, the Ni was mobilized out of other fractions (e.g. organic matter) as the Ni was mobilized in the serpentine rock.

Due to the ionic properties, Cr is strongly retained by the solid phases, and generally poorly soluble and not mobile. Soil acidification may drastically increase the chromium solubility (Gonnelli and Renella 2013). "Cr(III) can form complexes with organic and inorganic ligands. Relatively small ligands (e.g. LMWOL) increase the Cr solubility, whereas relatively large or particulate organic ligands such as humic substances immobilize Cr(III)" (Gonnelli and Renella 2013, 321).

In another survey of six French soils it was reported that Ni was more soluble than Cr, with soil pH and dissolved organic matter having the most significant effect on Ni and Cr partitioning. (Gonnelli and Renella 2013).

Jones (1997) investigated in his study that more than 99% of the minerals were associated with the exchangeable phase. Al^{3+} release at $pH < 4.5$ and organic matter dissolution at $pH > 5.0$ were the main factors controlling the Cr solubility. Another result of this study was the competitive binding of carboxylic acid. This may have implications of Cr phytoavailability in contaminated soils, due to the relatively high concentrations of carboxylic acid in the rhizosphere.

S has a low content of organic matter with high pH values. Therefore, it was concluded that the pH value had more influence in the mobilization processes. For B the parameters were vice versa and therefore it is assumed that the effects are exactly the other way around.

The mobilization processes of Mn in the arable soil in B were comparable to the mobilization of chromium as the manganese concentrations decreased with increasing organic acid concentrations. *This is an interesting aspect as the concentration of Mn (cation) as well as the concentrations of chromium (anion) decreased.*

As Blume et al. (2016) showed that nickel often is found in organic complexes and accumulates with Mn oxides this can be excluded in this study because no Mn was mobilized in the soils.

The concentration-dependent extraction showed no statistically significant differences in S.

A different progress was recognizable in the samples treated with the combination of citrate and oxalate. It was remarkable that the Mn mobilization increased with an increase of organic acid concentration. This effect could occur because of synergistic effects of the combination of the substances.

The presented data are approaches to field conditions. Additional factors, which might lead to increase or decrease of Ni availability in the soil such as climate, weathering or pH have not been considered. Some other factors have also be taken into account. Without sterilization of soil, a significant microbial degradation could take place through the shaking processes and therefore the sorption is overestimated. The degradation through microorganisms can be suppressed with the addition of chemical sterilants but many of these interact with the soil's solid phase, possibly reducing organic acid sorption. (Jones et al. 2003; Robinson et al. 1999). The Bronopol used in the present study has only low interactions with the serpentine soils respectively serpentine rock. A reduced adsorption of organic acids because of the interaction with the steriliant used could be excluded.

It is most likely that other substances contribute to nickel mobilization e.g. phenols or phytosiderophores in special plant communities. Another factor, which can conduce to the nickel mobilization are microorganisms. This is also shown in the study of Becerra-Castro et al. (2013).

Rhizospheric studies of hyperaccumulating plant species have shown contrary results. Some authors found a depletion of labile metal fraction because of the plant uptake, while others observed an increase in such fractions in the rhizosphere. Wenzel et al. (2003) concluded in their study that these controversial results could be because of an enhanced nickel complexation with organic ligands.

Fitz et al. (2003) observed in their study a drastic increase of Fe solubility and assumed this effect was related to the enhanced DOM concentration in the rhizosphere soil solution. The DOM causes Fe complexation and hence, higher total concentrations of Fe in solution. A similar result for Ni was detected by Wenzel et al. (2003).

The nature of DOM differs among plant species. Therefore, the DOM in the rhizosphere of hyperaccumulator forms stronger complexes with Ni than the DOM from the bulk soil or from the rhizosphere from the rhizosphere excluders (Wenzel et al. 2003).

Enhanced DOM concentrations in the rhizosphere affect the metal mobilization with several mechanisms e.g. shift of the equilibrium towards more total dissolved Ni as revealed by chemical speciation. Another mechanism is de sorption of DOM onto surfaces of Ni-bearing minerals, weakening metal-oxygen bonds. The rate of metal dissolution was proportional to the surface concentration of organic ligands (Wenzel et al. 2003).

Several studies reported an increase of soluble Ni in the rhizosphere of the hyperaccumulator *N. goesingesis*. They reported a highly significant correlation between Ni and DOM in soil solution. This provided evidence for enhanced Ni-DOM complexes in the rhizosphere of *N. goesingesis.* One implication for the detected enhanced root exudation could be the larger DOM concentration in the soil solution of *N. goesingesis* rhizosphere (Wenzel et al. 2003; Puschenreiter et al. 2005).

It is still unclear whether hyperaccumulating plants are able to access metal fractions which are not bioavailable to non-accumulating plants or if hyperaccumulators directly change the soluble pool via root exudation (Becerra-Castro et al. 2013).

For detailed and solid information about the solubilization of Ni and other elements in the soil, further research under field conditions need to be performed.

To get information about the interaction and releases of organic acids under natural conditions by plants into the soil, further investigations with respect to the soil-plant-relationship should be conducted.

5 Conclusion

This study gave indications of soil processes, which are induced by an increased release of organic acids originating from root exudates. The results of this study clearly show that induced mineral weathering of serpentine rock was recognizable, this effect was concentration-dependent.

The mobilization kinetics were determined in the preliminary test with 30 minutes. This corresponds with previous findings (Molina Millán et al. 2006).

The time-dependent experiment showed that nickel can be mobilized through low concentrations of organic acids or totally without them.

However, this study also showed that very high concentrations of organic acids and therefore high exudation rates are necessary to mobilize nickel substantially. Both potentially occurs at the root tip (Jones et al. 2003).

Citrate was in the majority of the cases the most efficient substance in solubilizing metals. The combination of citrate and oxalate was in one case more efficient in mobilizing manganese.

The results of this study indicate that the mobilization processes of chromium and manganese differ from the mobilization processes of nickel.

Because of the chromium mobilization in the serpentine rock it is assumed that nickel could be solubilized out of primary serpentine mineral. This effect was not obvious for the serpentine soils (B and S), as there was no release of chromium into the soil solution recognizable. Possible reasons therefore could be an enhanced re-adsorption into the soil matrix or the nickel in the serpentine soils was mobilized from other fractions (e.g. organic matter) compared to the serpentine rock, which is assumed to be more likely.

For detailed and solid information about the solubilization of Ni and other elements in the soil, further research under field conditions needs to be performed.

To get information about the interaction and releases of organic acids under natural conditions by plants into the soil, further investigations with respect to the soil-plant-relationship should be conducted.

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7 List of Tables

8 List of Figures

9 Appendix (List of Figures and Tables, Documentation)

9.1 Time-dependent mobilization of Ni

The following tables list the statistical output of the ANCOVA for the time-dependent Ni mobilization in Bernstein, Serpentine rock, and serpentine soil.

A.Table 1: Statistical output ANCOVA Bernstein
No significant differences between the treatments $p>0.05$

Tests of Between-Subjects Effects

Dependent Variable: Ni

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	323.464[a]	4	80.866	23.110	.000	.801
Intercept	.052	1	.052	.015	.904	.001
Treatment	12.646	3	4.215	1.205	.330	.136
Time	310.818	1	310.818	88.827	.000	.794
Error	80.481	23	3.499			
Total	1683.429	28				
Corrected Total	403.944	27				

A.Table 2: Statistical output ANCOVA Serpentine Rock No significant difference between the treatments p >0.05.

Tests of Between-Subjects Effects

Dependent Variable: Ni

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	9045.038[a]	4	2261.260	34.041	.000	.855
Intercept	2.411	1	2.411	.036	.851	.002
Treatment	49.655	3	16.552	.249	.861	.031
Time	8995.383	1	8995.383	135.417	.000	.855
Error	1527.831	23	66.427			
Total	45249.231	28				
Corrected Total	10572.869	27				

A.Table 3: Statistical output ANCOVA Serpentine Soil No significant difference between the treatments p >0.05.

Tests of Between-Subjects Effects

Dependent Variable: Ni

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	.869[a]	4	.217	6.841	.001	.543
Intercept	.007	1	.007	.208	.653	.009
Treatment	.075	3	.025	.787	.514	.093
Time	.794	1	.794	25.005	.000	.521
Error	.730	23	.032			
Total	5.455	28				
Corrected Total	1.599	27				

9.2 Time-dependent mobilization of Mn

The following tables list the statistical output of the ANCOVA for the time-dependent Mn mobilization in Bernstein, Serpentine rock, and serpentine soil.

A.Table 4: Static Statistical output ANCOVA Bernstein of Mn
There is no significant difference between the treatments p >0.05

Tests of Between-Subjects Effects

Dependent Variable: Mn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	24.877[a]	4	6.219	6.716	.001	.539
Intercept	1.283	1	1.283	1.385	.251	.057
Time	24.515	1	24.515	26.473	.000	.535
Treatment	.362	3	.121	.130	.941	.017
Error	21.299	23	.926			
Total	200.813	28				
Corrected Total	46.176	27				

A.Table 5: Static Statistical output ANCOVA Serpentine Rock of Mn
There is no significant difference between the treatments p >0.05

Tests of Between-Subjects Effects

Dependent Variable: Mn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	49.118[a]	4	12.279	14.673	.000	.718
Intercept	10745.319	1	10745.319	12839.577	.000	.998
Time	46.263	1	46.263	55.280	.000	.706
Treatment	2.854	3	.951	1.137	.355	.129
Error	19.248	23	.837			
Total	60286.303	28				
Corrected Total	68.366	27				

A.Table 6: Statistical output ANCOVA Serpentine Soil of Mn
There is no significant difference between the treatments p >0.05

Tests of Between-Subjects Effects

Dependent Variable: Mn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	.070[a]	4	.017	1.406	.264	.196
Intercept	5.569	1	5.569	449.016	.000	.951
Time	.005	1	.005	.371	.548	.016
Treatment	.065	3	.022	1.751	.185	.186
Error	.285	23	.012			
Total	29.649	28				
Corrected Total	.355	27				

9.3 Time-dependent Cr mobilization

The following tables list the statistical output of the ANCOVA for the time-dependent Cr mobilization in Bernstein, Serpentine rock, and serpentine soil.

A.Table 7: Statistical output ANCOVA Bernstein of Cr There is no significant difference between the treatments p >0.05

Tests of Between-Subjects Effects

Dependent Variable: Cr

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	433.406[a]	4	108.351	10.710	.000	.651
Intercept	51.996	1	51.996	5.140	.033	.183
Time	432.457	1	432.457	42.747	.000	.650
Treatment	.949	3	.316	.031	.992	.004
Error	232.685	23	10.117			
Total	1314.676	28				
Corrected Total	666.091	27				

A.Table 8: Statistical output ANCOVA Serpentine Rock of Cr There is no significant difference between the treatments p >0.05

Tests of Between-Subjects Effects

Dependent Variable: Cr

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	25315.597[a]	4	6328.899	63.596	.000	.917
Intercept	7009.763	1	7009.763	70.437	.000	.754
Time	25054.206	1	25054.206	251.756	.000	.916
Treatment	261.391	3	87.130	.876	.468	.102
Error	2288.906	23	99.518			
Total	281402.523	28				
Corrected Total	27604.503	27				

A.Table 9: Statistical output ANCOVA Serpentine Soil of Cr There is no significant difference between the treatments p >0.05.

Tests of Between-Subjects Effects

Dependent Variable: Cr

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1.523^a	4	.381	4.260	.010	.426
Intercept	1.847	1	1.847	20.668	.000	.473
Time	1.453	1	1.453	16.259	.001	.414
Treatment	.070	3	.023	.261	.853	.033
Error	2.056	23	.089			
Total	33.282	28				
Corrected Total	3.579	27				

9.4 Concentration-dependent mobilization of Ni

The following tables list the statistical output of the ANOVA for the concentration-dependent Ni mobilization in Bernstein, Serpentine rock, and serpentine soil.

A.Table 10: Statistical output ANOVA Bernstein There is a significant difference between the treatments p <0.05.

Tests of Between-Subjects Effects

Dependent Variable: Ni

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	13969.323[a]	9	1552.147	212.416	.000	.990
Intercept	40378.492	1	40378.492	5525.912	.000	.996
Treatment	13969.323	9	1552.147	212.416	.000	.990
Error	146.142	20	7.307			
Total	54493.957	30				
Corrected Total	14115.465	29				

A.Table 11: Statistical output ANOVA Serpentine Rock There is a significant difference between the treatments p <0.05.

Tests of Between-Subjects Effects

Dependent Variable: Ni

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1355305.678[a]	8	169413.210	199.411	.000	.989
Intercept	605117.016	1	605117.016	712.266	.000	.975
Treatment	1355305.678	8	169413.210	199.411	.000	.989
Error	15292.198	18	849.567			
Total	1975714.892	27				
Corrected Total	1370597.876	26				

A.Table 12: Statistical output ANOVA Serpentine Soil There is a significant difference between the treatments p <0.05.

Tests of Between-Subjects Effects

Dependent Variable: Ni

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	15022.087[a]	9	1669.121	27.544	.000	.929
Intercept	19520.970	1	19520.970	322.140	.000	.944
Treatment	15022.087	9	1669.121	27.544	.000	.929
Error	1151.358	19	60.598			
Total	36561.708	29				
Corrected Total	16173.444	28				

9.5 Concentration-dependent Cr mobilization

The following tables list the statistical output of the ANOVA for the concentration-dependent Cr mobilization in Bernstein, Serpentine rock, and serpentine soil.

A.Table 13: Statistical output ANOVA Bernstein of Cr There are significant differences between the treatments p<0.05

Tests of Between-Subjects Effects

Dependent Variable: Cr

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	215.912[a]	9	23.990	22.605	.000	.910
Intercept	6056.229	1	6056.229	5706.457	.000	.997
Treatment	215.912	9	23.990	22.605	.000	.910
Error	21.226	20	1.061			
Total	6293.367	30				
Corrected Total	237.138	29				

A.Table 14: Statistical output ANOVA Serpentine Rock of Cr There are significant difference between the treatments p<0.05

Tests of Between-Subjects Effects

Dependent Variable: Cr

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	156440.637[a]	9	17382.293	1062.437	.000	.998
Intercept	327479.180	1	327479.180	20016.123	.000	.999
Treatment	156440.637	9	17382.293	1062.437	.000	.998
Error	327.215	20	16.361			
Total	484247.032	30				
Corrected Total	156767.853	29				

A.Table 15: Statistical output ANOVA Serpentine Soil of Cr There is no significant difference between the treatments p>0.05

Tests of Between-Subjects Effects

Dependent Variable: Cr

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	249.668[a]	9	27.741	1.395	.255	.386
Intercept	423.870	1	423.870	21.316	.000	.516
Treatment	249.668	9	27.741	1.395	.255	.386
Error	397.700	20	19.885			
Total	1071.238	30				
Corrected Total	647.367	29				

9.6 Concentration-dependent Mn mobilization

The following tables list the statistical output of the ANOVA for the concentration-dependent Mn mobilization in Bernstein and serpentine soil.

A.Table 16: Statistical output ANOVA Serpentine Soil of Mn There are significant difference between the treatments $p<0.05$

Tests of Between-Subjects Effects

Dependent Variable: Mn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	14.860[a]	9	1.651	7.703	.000	.776
Intercept	965.654	1	965.654	4505.026	.000	.996
Treatment	14.860	9	1.651	7.703	.000	.776
Error	4.287	20	.214			
Total	984.802	30				
Corrected Total	19.147	29				

A.Table 17: Statistical output ANOVA Serpentine Soil of Mn
There is no significant difference between the treatments $p>0.05$

Tests of Between-Subjects Effects

Dependent Variable: Mn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1380.116[a]	9	153.346	1.137	.384	.338
Intercept	853.070	1	853.070	6.324	.021	.240
Treatment	1380.116	9	153.346	1.137	.384	.338
Error	2697.835	20	134.892			
Total	4931.020	30				
Corrected Total	4077.951	29				