Heavy metal

PP Ecology of organisms on heavy metal sites: mechanisms of stress management

Abstract

Heavy metal contamination is a present problem in our modern society. In our study we chose one natural heavy metal contaminated site (Redlschlag, serpentine motherrock) and one former mining spot in Hirschwang. Special interest was taken in two known hyperaccumulators *Noccaea goesingensis* (Ni) and *Noccaea caerulescens* (Ni, Zn, Cd), but also *Rumex acetosella* and *Silena vulgaris*, which are not known to hyperaccumulate. The plants and the soils they grow on were studied in their physiology as well as their element composition using light microscope, the EDX analysis and AAS/ICP-MS analysis. Additionally comparative germination test on *Triticum aestivum* were performed to observe the effects of heavy metals on non-adapted plants, which showed strong effects on its growth and germination rates.

We found that N. goesingensis can accumulate Zn additionally to its Ni hyperaccumulation and the similarity of the accumulation elements and concentrations of these populations to their *N. caerulescens* neighbours. In Hirschwang *Rumex acetosella* showed signs of a lead accumulation, which has to be further studied.

1 Introduction

1.1 Heavy metals in plants

Per definition heavy metals (HMs) are elements with a density > 5 g cm^{-3}. HMs like Zn, Ni, Cu or Mo, are essential micronutrients for all Organisms. In addition animals need also Co, which is mostly bound and has small environmental relevance, and Cr, which has a low mobility in ecosystems. Although all HMs like Zn, Ni, Cu, Pb, Cd, As, Cr, Mo or Hg are, if elevated, toxic and endanger the normal life cycle of the plant (Hall 2002).

1.1.1 Nickel

Nickel (Ni) belongs to the transition metals and has the atomic number 28. It is biologically important as a cofactor of Urease (an enzyme that assists in the hydrolysis of urea).Ni can behave as an analog (functional or nonfunctional) of essential nutrients in plants (Cataldo, Garland and Wildung 1978).

1.1.2 Copper

Zinc belongs to the transition metals and has the atomic number 29. It is essential for organisms, but only required in small amounts of 5-20 mg/kg (Amberger 1988). It is necessary as a cofactor electron transporting protein in photosynthesis and respiratory pathways. Copper toxicity leads to chlorosis as it can replace iron ions in protein complexes (Schulze, Beck and Müller-Hohenstein 2002).

1.1.3 Zinc

Zinc (Zn) belongs also to the transition metals and has the atomic number 30. It is often a cofactor of dehydrogenases, carboanhydrase and nucleic acid binding proteins. It is adequately supplied at 20-150 mg/kg (Amberger 1988) and if overrepresented it can

replace manganese in the photosynthetic water oxidase (Schulze, Beck and Müller-Hohenstein 2002).

1.1.4 Manganese

Manganese (Mn) belongs to the transition metals as well and has the atomic number 25. It is essential for the water splitting complex.

1.1.5 Chromium

Chromium (Cr) has the atomic number 24 and belongs to the transition metals. Cr is toxic and nonessential to plants so they do not possess specific mechanisms for its uptake. The presence of Cr leads to changes in the growth and development pattern of the plant, even in small amounts (Shanker et al., 2005).

1.1.6 Lead

Lead (Pb) belongs to the poor metals and has the atomic number 82. It is nonessential and toxic for plants.

1.2 Availability and handling

Most of the HM uptake to the plant occurs through the roots, which can mobilize actively ions through acidic exudation. The lateral roots and the cortex act as an absorption zone for the soils solutes. Water and minerals can easily diffuse and saturate the apoplast, where the positive charged HM ions can bind to the negative charged pectin – molecules and are restrained in the donnan free space. The endodermis incorporates the casparian strip and forms an impermeable zone for big molecules and unfavourable ions. Carrier proteins in the plasmalemma regulate the active transport into the symplast. As ATPase-, antiporter-, CDF- (cation diffusion facilitator) and ZIP- (zinc and iron transporter) proteins have affinity to positive charged ions HMs can be translocate to the vascular bundles. Once imported, plants form complexes to reduce reactivity of the HMs.

Phytochelatins (PCs) are $(\gamma\text{-Glu-Cys})_n$–Gly (n=2-11) peptides formed by reduction of glutathione (GSH) and are no translation product. Metallothioneins (MTs) are associated to cysteine rich proteins of low molecular weight and are direct genetic products. Other complexes are ligated to organic acids such as oxalate, malate and citrate or amino acids, e.g. histidine. Once bound in molecular complexes HMs get inactive for physiological pathways and can be transported and compartmented into the vacuoles of the leaves or the generative organs. A secondary pathway for HMs is interception of resuspended ions through the stomata.
Whereas plants handle HM stress in different ways we can categorize them into hyper accumulator, accumulator, indicator or excluder. To evaluate the eco-types the BCF (bio concentration factor) is used which is the ratio of the soils and the plants HM concentration. Accumulators have a shoot : root ratio >1, excluders have it <1.
(Schulze, Beck & Müller-Hohenstein, 2002; Freeman et al., 2004; Taiz & Zeiger, 2006; Frey & Lösch, 2010)

1.3 Sites of research

1.3.1 Hirschwang (Lower Austria / Austria)

Knappenberg situated next to the location Hirschwang and the Rax Mountain in the area of Semmering and was an iron-mining site until the 1890s. Greywacke and sulphur or carbonate containing rocks such as Siderite, Chalcopyrite, Malachite, Cinnabar, Cuprite and Pyrite mostly forms the bedrock. Mining activity on Knappenberg is known before the 16th century and nearly the whole mountain was used for mining (Mohr H., 1956). By implication a very large amount of spoil were deposited. The main part of the gravel on the site consists of copper ore, so the spoil heap results to be toxic to most of the plants. The surrounding coniferous forest contributes to the soil acidity, due to acidic exudates of the mycorrhizal communities. The pH is between 2.5 and 4, which elevates the bioavailability of copper and other HMs. The spoil tips soil structure is composed mostly of rough gravel with a thin humus layer, which results in a low water retention capacity. Since no trees are growing on the heap an additive stress by high irradiation is induced to the organisms. The mining site we focused on owns three very large pits and

a destroyed shaft. In table 11(appendix) we listed the vegetation and the collected specimens. Some plants, e.g. *Larix decidua* or *Rumex sp.,* displayed nanism.

1.3.2 Redlschlag (Burgenland / Austria)

The bedrock of Redlschlag is composed of Serpentine, which contains high levels of Ni and Cr and some Zn and Co. Since the soil is alkaline (approx. pH 6), the main problems for plants are low concentrations and availability of micronutrients, whereby Mg is abundant (Reeves R. D. et al., 1984; Baker A. J. M., 1987; Baker A. J. M. et al., 1994; Frey & Lösch, 2010). We visited two sites, Ochsenriegel and Steinstückl, which differ from each other mostly in exposition and sparsely in soil composition. The Ochsenriegel is composed of dwarf shrubs and in all owns a low vegetation density and diversity. The stress factors are HMs, drought and eluviation. Steinstückl is surrounded by a pine forest, which results in a higher density and diversity of vegetation. Stress by irradiation and drought are reduced and mycorrhizal exudates may raise the nutrient availability. The observed and collected species are listed in table 11 (appendix).

1.4 Phytoremediation

Phytoremediation uses plants that are able to extract large amounts of heavy metals from the ground. There is either the continuous way of using hyper accumulating plants or the usage of fast growing plants in combination with the applying of artificial chelators to bind heavy metals.

2 Material and methods

2.1 Anatomic analysis

Before doing physiological and chemical analyses of the Specimens, we assayed if there are some anatomic features which support the plants to exclude, accumulate or hyper-accumulate heavy metals. We also noted the positions of the stomata (epi-, hypo- or amphi-), where interception with HMs occur. Cross-sections of leaves, roots and shoots were prepared for the evaluation under the microscope. The samples in question were *Arabidopsis halleri*, *Rumex acetosella*, *Vaccinium myrtillus*, *Noccaea goesingensis*, *Noccaea caerulescens* and *Silene nutans*. For the examination of the samples we used the *"Olympus CX-41"* light microscope with bright field, dark field, phase contrast and polarized light techniques.

2.2 Energy-dispersive X-ray spectroscopy

2.2.1 Introduction of the EDX technique

The Energy dispersive X-ray spectroscopy (EDX) is a technique to perform semi-quantitative measurements on specimen in the electron microscope.
The electron beam of the EM hits the atoms of the sample, interacting with them in a special way. A highly energized electron beam collides with the electrons of the different electron shells of the elements contained in the sample. The incident beam excites an electron in one of the shells, displacing it from the shell. An electron from an outer, higher-energy shell then fills the hole left by the electron and the energy difference between the higher-energy shell and the lower energy shell is released as an X-ray. These X-rays hit the detector in the EDX- unit, which then creates a signal and passes it on to the pulse processor. There the signals are measured and given to the analyser.

The data is displayed and further analysed via specialised software. The amount of energy released by the transferring electron depends on the shell it transferred from and to which shell it is transferred. Each element releases X-rays with distinct able amounts of energy during the transferring process. Therefore the identity of specific

elements can be derived. The output of an EDX analysis is an EDX spectrum, which is a plot of how frequently an X-ray is received for each energy level. An EDX spectrum normally displays peaks corresponding to the energy levels for which the most X-rays had been received. Each of these peaks is unique to a single element. The higher a peak in a spectrum, the more concentrated the element is in the specimen.

2.2.2 Preparation of the EDX samples

Different organ samples like leafs, stems and roots were dried at 105°C. The samples were manually cut, put on carbon foiled stubs and carbon coated before EDX analysis. They were measured for 100 seconds per measuring spot and 5- 10 spots per tissue was analysed.

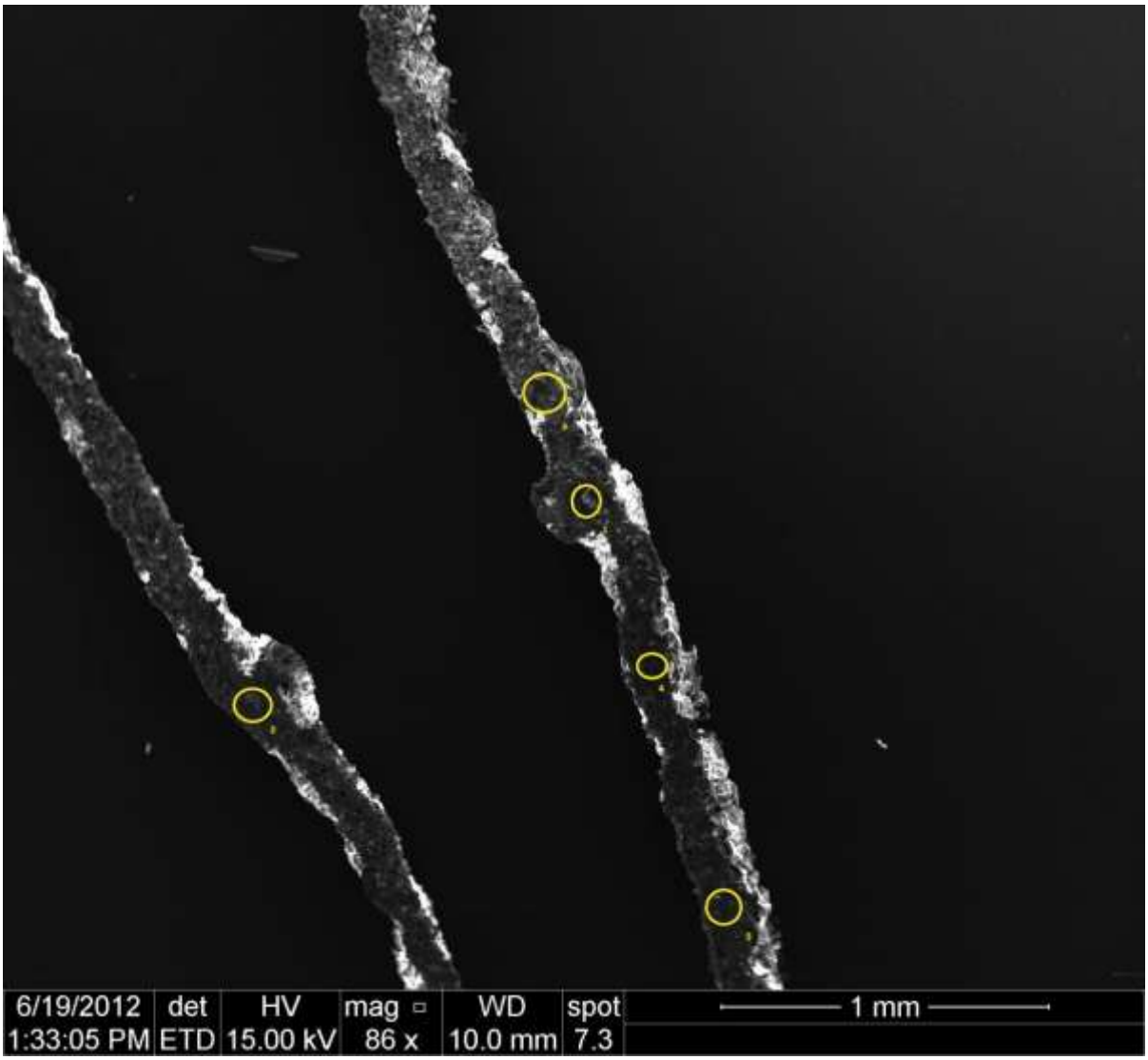


Figure 1: Overview of *N. caerulescens* in the scanning electron microscope. EDX measuring points are marked with yellow circles.

2.3 Element determining techniques

2.3.1 Atomic absorption spectroscopy

Atomic absorption spectroscopy (AAS) is a technique to analyse plant material quantitatively and qualitatively. An extract of the plant or soil sample is inserted ant atomized (Fig. 2). The air-acetylene flame with a temperature of about 2600 °K is infused with the atomized sample and the elements radiate element specific wavelengths due to their electron shell constellation. This image colour spectrum is detected and amplified and analysed by a high-end computer.

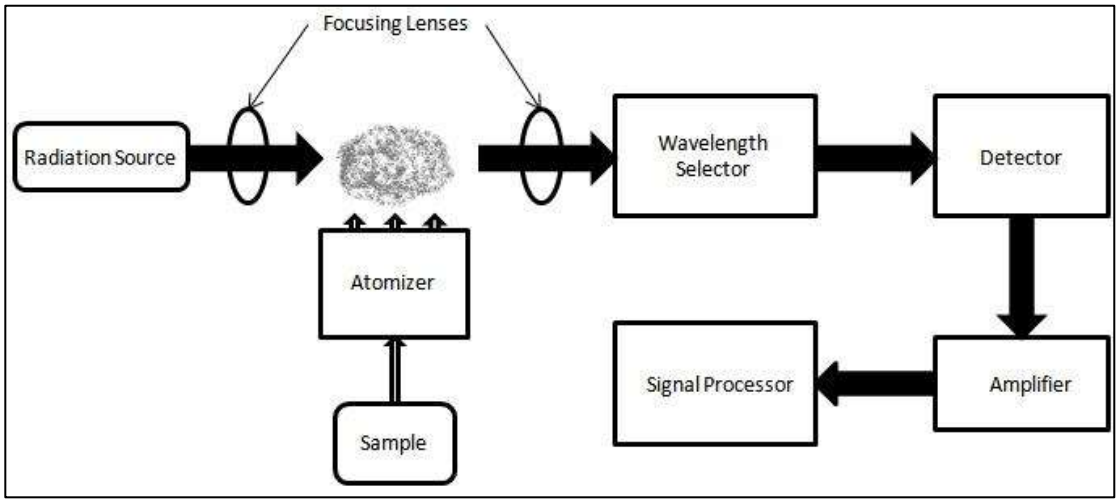


Figure 2: Schematic of AAS workflow(http://upload.wikimedia.org/wikipedia/commons/0/08/AASBLOCK.JPG 30.08.2012)

2.3.2 Inductively Coupled Plasma Mass Spectrometry

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an analytical technique used for elemental determinations. The technique has accurate detection capabilities, particularly for the rare-earth elements. ICP-MS has advantages over atomic absorption

spectrometry, as the detection limits for most elements are better than those when using AAS.
A high- temperature inductively coupled plasma source (Argon plasma: 6000- 10000°K) converts the atoms of elements in the sample into ions. Those are separated and detected by a mass spectrometer (http://minerals.cr.usgs.gov/icpms/intro.html 4.9.2012). This signal is then processed and can be read out at a computer.

2.3.3 Soil extraction

The soil samples were taken at the heaps in Redlschlag and Hirschwang and air dried for a weak. Soils were filtered for a grain size lower than 2mm.

2.3.4 Aqua regia extraction

For extraction an extracting agent of 3 parts HCl: 1 part HNO3 (Volume) was used. 2 g of soil were infused with 30 ml Aqua regia. After 3 hours of backflow distillation the sample was filled up to 100 ml.

2.3.5 Ammonia nitrate extraction

For the determination of plant- available heavy metal content the soil samples were infused with 1M ammonia nitrate(NH_4NO_3), where the ratio of fluid: solid matter is 2,5:1. The soil particles were filtered and the filtrate is stabilized with HNO_3.

2.3.6 Plant extraction

The harvested plants were divided into root, rosette leafs, stem and stem leafs. These plant organs were cleaned using distilled water and dried at 105°C for at least 48 hours. Afterwards their weight was determined and they were grinded.
It was planned to infuse 2 g dry matter with 24 ml acid mixture (5 parts nitric acid and 1 part perchloric acid), which was cooked until only little perchloric acid is left.
Due to the small sample mass, samples larger than 1,5 g were diluted to 100 ml and thosesmaller than 1,5 g were diluted to 45 ml.

2.4 Plasmolytic tolerance test

This test is determining the toxicity threshold of cells in heavy metal solutions. Cross-sections must have at least a thickness of two cell layers and are immersed into salt-solutes. After incubation the sections are treated with a sucrose solute to check if plasmolysis occurs and the cells are still alive. We prepared 0,1 M stock solutions of $CuSO_4$, $ZnSO_4$, $NiSO_4$ and $Cr_2(SO_4)_3$and diluted each to produce serial solutes from 10^{-7} to 10^{-1}mol/l. We used sulphate salts because of their low deleterious effect. After the cross- and length-sections of the specimens leaves, their state of vitality (if already plasmolysed) was checked under an *"Olympus CX-41"* light microscope with consequent immersion into the solutes. Incubation occurred for 45 minutes. The sections were then treated with a 1 M sucrose solute. We applied this test on ten different species (see Table 1) in order to evaluate the vitality and various resistances to each heavy metal and their concentrations by observing the quantity of plasmolysed cells and discoloured chloroplasts. In previous researches the phenomenon of "dead zone" was observed which means plasmatic resistance at high and low and plasmolysis at mean concentrations (Url, 1956; Sissolak, 1984; Hörmann 2001;), and was another fact of issue in our assay.

Table 1: Species of the plasmolytic tolerance test

Polygonaceae	*Rumex acetosella, Rumex acetosa;*
Brassicaceae	*Noccea goesingensis, Noccea caerulescens, Thlaspi minimum, Arabidopsis halleri;*
Plumbaginaceae	*Armeria obir, Armeria walles;*
Alliaceae	*Allium cepa*
Dicranaceae (moss)	*Cynodontium sp.*
Poaceae	*Triticum aestivum*

2.5 Wheat Germination test – tolerances of a crop plant

Since *Triticum aestivum* is one of the world's most important crop plants we used its seeds to observe and check heavy metal tolerances, viabilities and germination abilities for Cu, Zn, Ni and Cr. Recent studies showed that wheat is able to accumulate HMs in contaminated soils (Bose & Bhattacharyya, 2007; Shumaker & Begonia, 2005). This results in a risk for humans and animals as HMs can enter the food chain and disturb physiological pathways.
We prepared 0,2 M stock solutions of $CuSO_4$, $ZnSO_4$, $NiSO_4$ and $Cr_2(SO_4)_3$. The Stock solutions were diluted to form serial concentrations from 10^{-8} to 10^{-1}mol/l. Controls were treated only with distilled water. Seeds were applied on blotting paper and rolled. We produced eight germination-rolls for each concentration. Incubation occurred for two weeks in the departments `greenhouse. Since water evaporates and the solutes concentration elevates we marked the solutes quantity on the glasses and refilled them three times a week with distilled water. Due to microclimatic changes, positions of the solute-glasses were changed regularly. We wanted to understand whether different inhibitions by HMs can occur in germination and evaluated the ratios of the *root / shoot* lengths. Additionally we measured the photosynthetic activity via chlorophyll-fluorescence and weighed dry – and fresh – weight of the plants.

2.6 Soil analysis - photometric determination of humus content

The content of organic substances in soil can be detected trough wet oxidation with potassium dichromate ($K_2Cr_2O_7$) and sulphuric acid (H_2SO_4).
Organic substances in the sample get oxidised, while potassium dichromate gets reduced from Cr^{6+} to Cr^{3+}. With a photometer it is possible to measure the intensity of the change of colour from Cr^{6+} to Cr^{3+}. The more organic substances get oxidised, the darker the sample gets.

The soil samples were prepared the same way as for the aqua regia extraction. Dependent on the amount of humus 0,5 g to 2 g soil were mixed with 20 ml $K_2Cr_2O_7$ and 15 ml of concentrated H_2SO_4. The solution was then left under the extractor hood for to hours and was then filled up with distilled water to 100ml. After one night of

decantation 1 ml of the sample was mixed with 24 ml of distilled water and shaken. It was important to evade soil particles in the solution, as they would have falsified the measurements.
Calibration solutions was prepared containing 0, 116, 232 and 348 mg myo- inosit infused with 100 ml of distilled water and additionally one solution with 20 ml $K_2Cr_2O_7$ and 15 ml H_2SO_4. Those solutions correlated with 0, 4, 8 and 12% humus in our samples.

The results the photometer produces can be translated with following formula:

$$\frac{(VP - BW) \times 2}{EW}$$

BW… blank value
EW… net weight
VP … humus content (%)

3 Results

3.1 Anatomic analysis

On the roots of *Vaccinum myrtillus* we found ericoid mycorrhiza (endomycorrhiza). We estimate that hyphae protect the plant from heavy metals (see figure 3 A & B). We observed amphistomatous leaves.

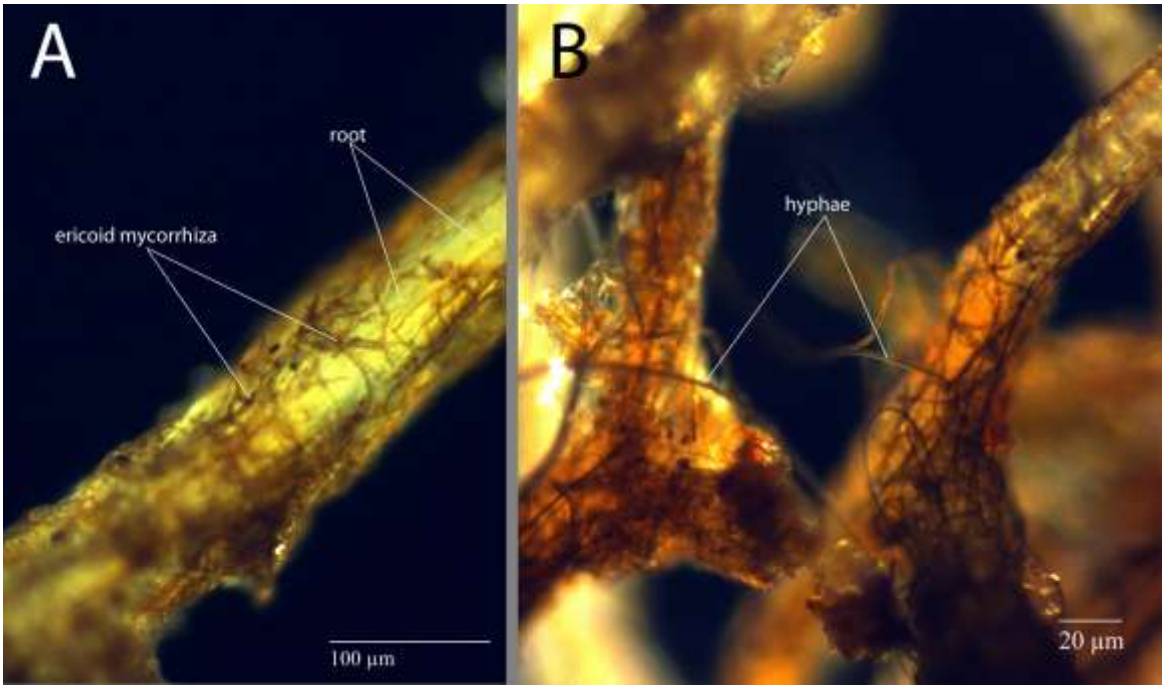


Figure 3:*Vaccinum myrtillus;* **A & B,** root*;*

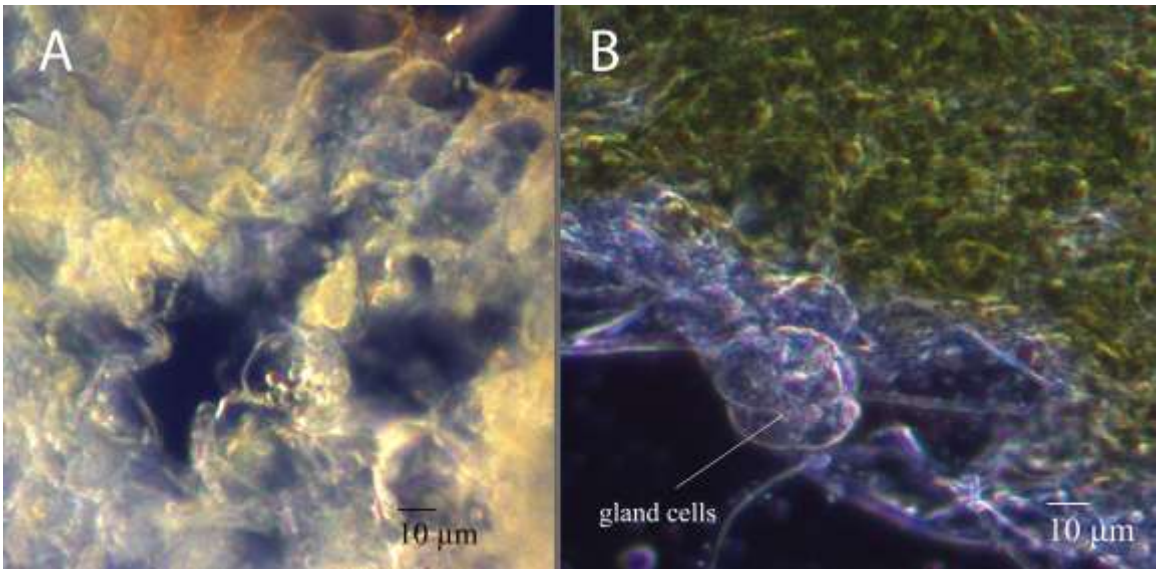


Figure 4: *Rumex acetosella;* **A,** root cortex; **B,** gland cell of a leaf;

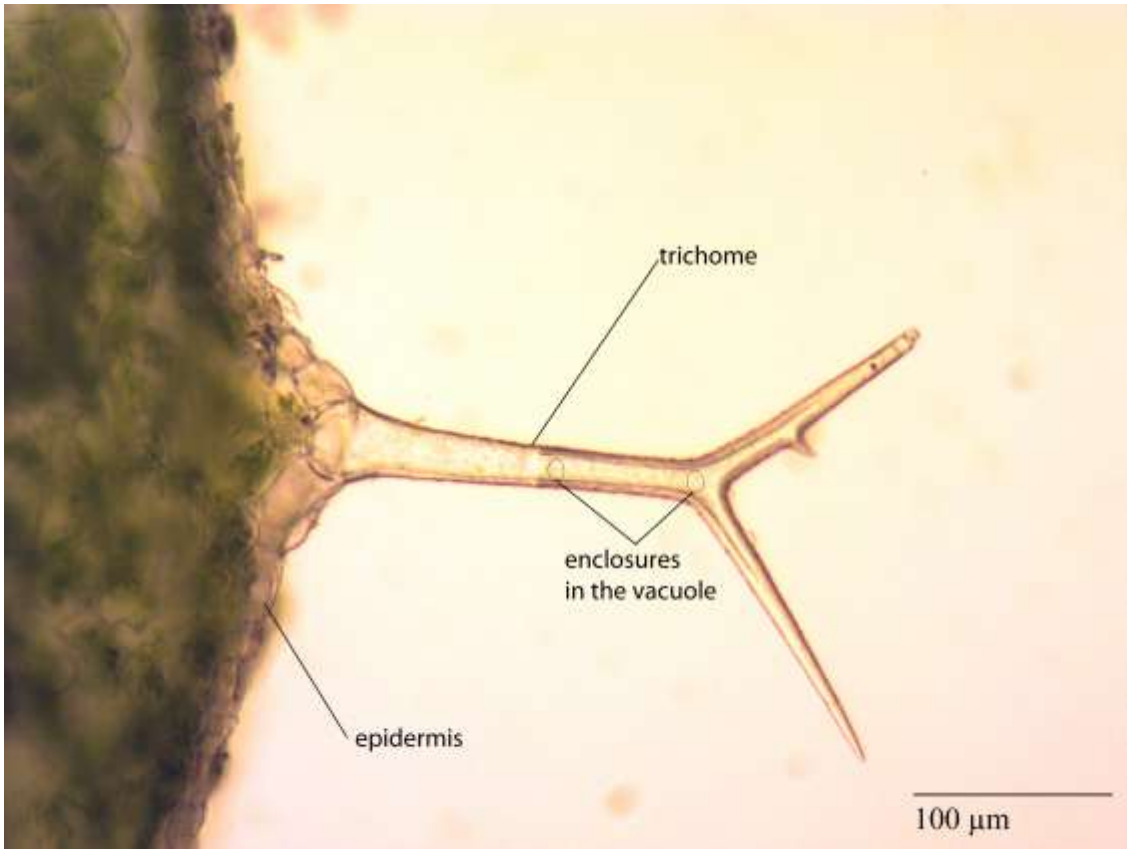


Figure 5: *Arabidopsis halleri;* enclosures in the trichome of a leaf;

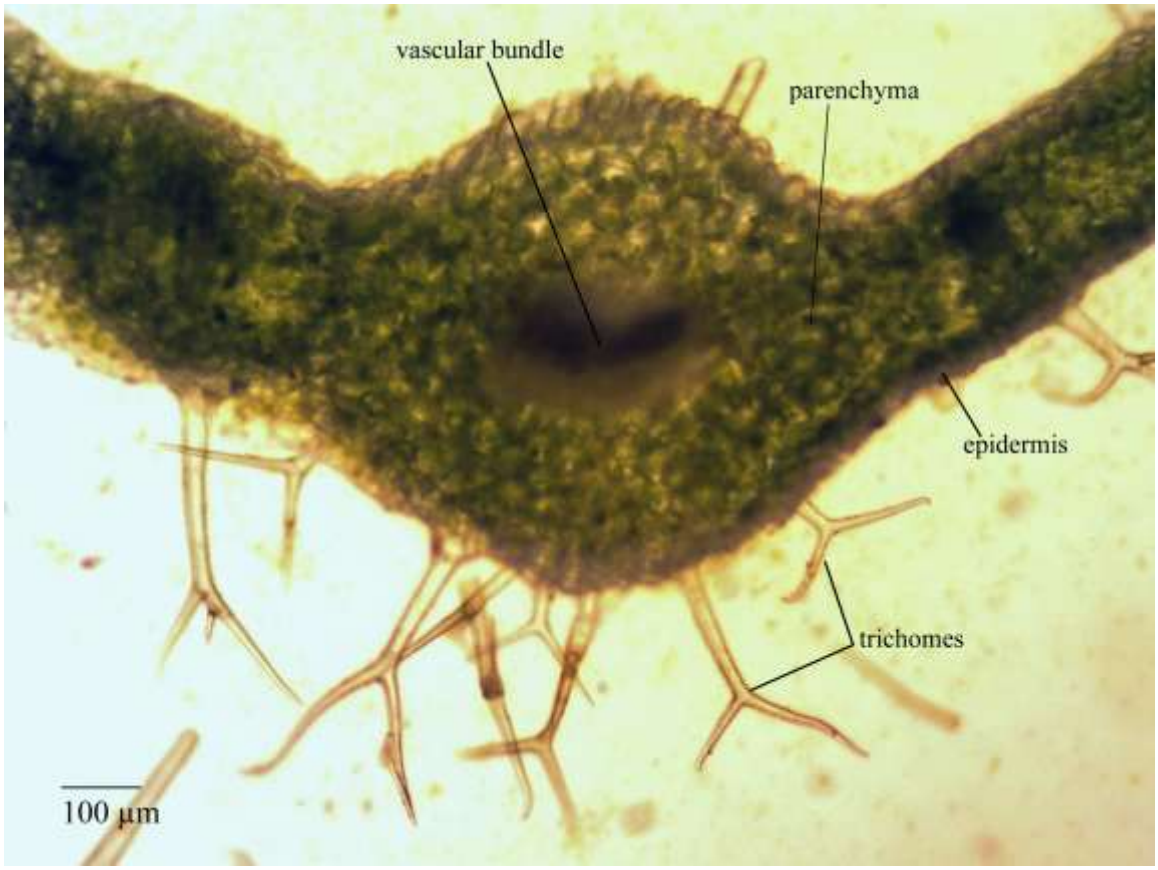


Figure 6: *Arabidopsis halleri;* cross -section of a leaf;

Figure 7: *Silene nutans;* cross-section of a leaf and large trichomes;

Leaves of *Rumex acetosella* possess large gland cells and are amphistomatic. In the root cortex we observed a large number of metalloid shining droplets or enclosures with dark field technique Leaves and shoots of the Brassicaceae *Arabidopsis halleri*, *Noccaea goesingensis* and *Noccaea caerulescens* and the Caryophyllaceae *Silene nutans* all feature emergences such as papillae or trichomes. mes. Especially *Arabidopsis halleri* and *Silene nutans* possess very large trichome cells. In their vacuoles we observed a high density of grains. All plants possess amphistomatous leaves.

3.2 EDX analysis

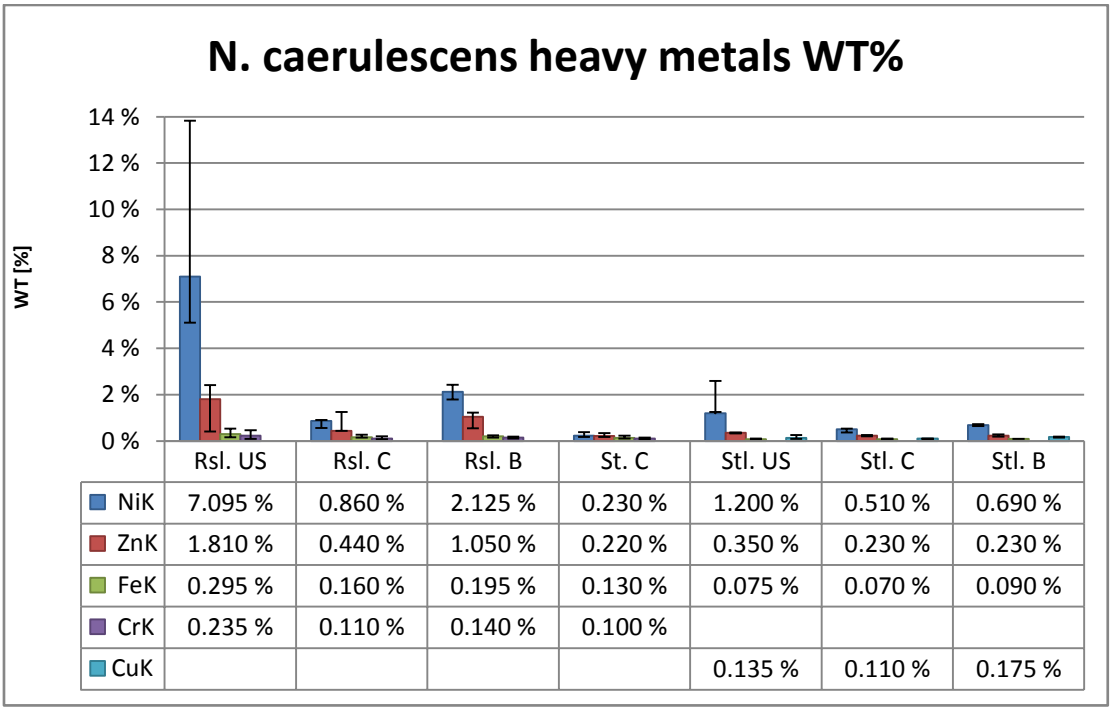
Noccaea caerulescens and Noccaea goesingensis were analysed for nickel (Ni), zinc (Zn), copper (Cu), Chromium (Cr) and iron (Fe).

3.2.1 Noccaea caerulescens

Table 2: Abbreviations used in the following figures concerning N*occaea caerulescens*

Organ	Abbreviation	Location	Abbreviation
Rosette leaf	Rsl	Upside	US
Stem	St	Bottom	B
Stem leaf	Stl	Cross-section	C

The heavy metals most abundant in N. caerulescens leafs were nickel and zinc (Fig. 8).



	Rsl. US	Rsl. C	Rsl. B	St. C	Stl. US	Stl. C	Stl. B
NiK	7.095 %	0.860 %	2.125 %	0.230 %	1.200 %	0.510 %	0.690 %
ZnK	1.810 %	0.440 %	1.050 %	0.220 %	0.350 %	0.230 %	0.230 %
FeK	0.295 %	0.160 %	0.195 %	0.130 %	0.075 %	0.070 %	0.090 %
CrK	0.235 %	0.110 %	0.140 %	0.100 %			
CuK					0.135 %	0.110 %	0.175 %

Figure 8: Median values of the EDX measurements in WT%. Error bars show the first and third quartile

Especially in the rosette leafs higher concentrations could be found whereas nickel was always present in the highest percentage followed by zinc. Stem leafs showed lower levels of heavy metal content than the rosette leafs. Furthermore the stem showed altogether lower heavy metal levels and the heavy metals were evenly distributed (Fig. 9).

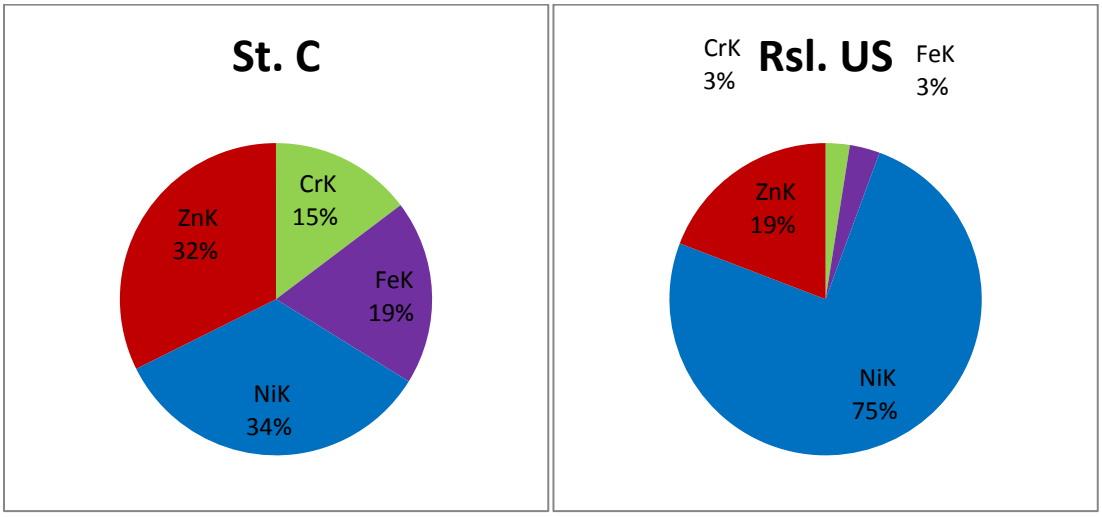


Figure 9: Cross- section of a stem leaf compared to the bottom of the rosette leaf

Comparativ analysis of the rosette leaf epidermis of the upside, bottom and cross-section have shown that the metal content is in general higher in the leaf epidermis (Fig. 10). Within the parenchymatic cells of the leaf the heavy metal content seems to be much lower.

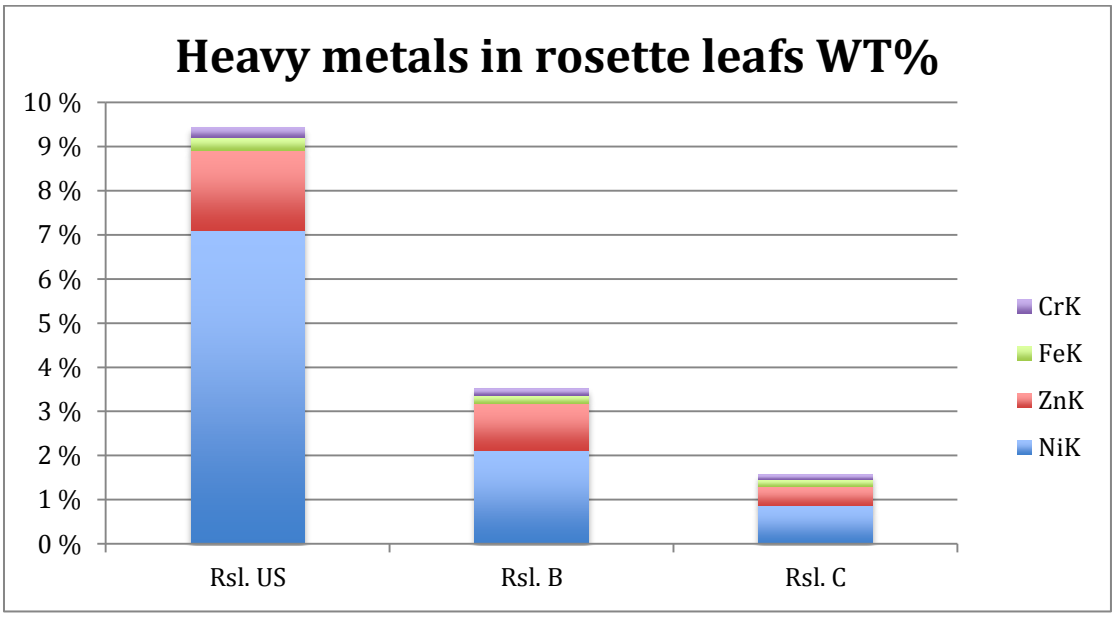


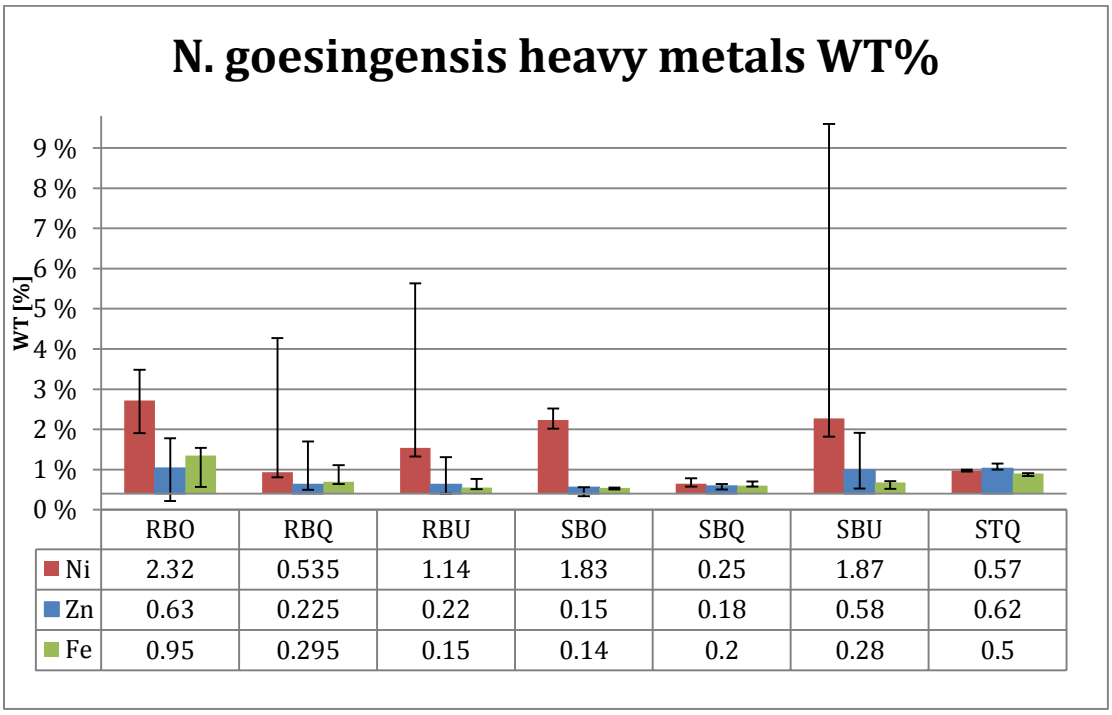
Figure 10: Heavy metal percentage in the rosette leaf epidermis und the upside and bottom compared to the cross-section

3.2.2 Noccaea goesingensis

Table 3: Abbreviations used in the following figures concerning *Noccaea goesingensis*

Organ	Abbreviation	Location	Abbreviation
Rosette leaf	RB	Upside	O
Stem	ST	Bottom	U
Stem leaf	SB	Cross-section	Q

The heavy metals most abundant in N. goesingensis leafs was nickel (Fig. 11), whereas zinc and iron abundance changed between the different tissues.



	RBO	RBQ	RBU	SBO	SBQ	SBU	STQ
Ni	2.32	0.535	1.14	1.83	0.25	1.87	0.57
Zn	0.63	0.225	0.22	0.15	0.18	0.58	0.62
Fe	0.95	0.295	0.15	0.14	0.2	0.28	0.5

Figure 11: Median values of the EDX measurements in WT%. Error bars show the first and third quartile

The concentration of the heavy metals was quite even in the parenchymatic cells of the leafs and the stem. The epidermis cells of the rosette and stem leafs show higher heavy metal percentages.

There is a correlation between the nickel and zinc uptake in the tissues of Noccaea goesingensis. Figure 12 suggests a strong correlation in the rosette leaf cross-section and not quite as strong correlation in the bottom of the rosette and stem leaf.

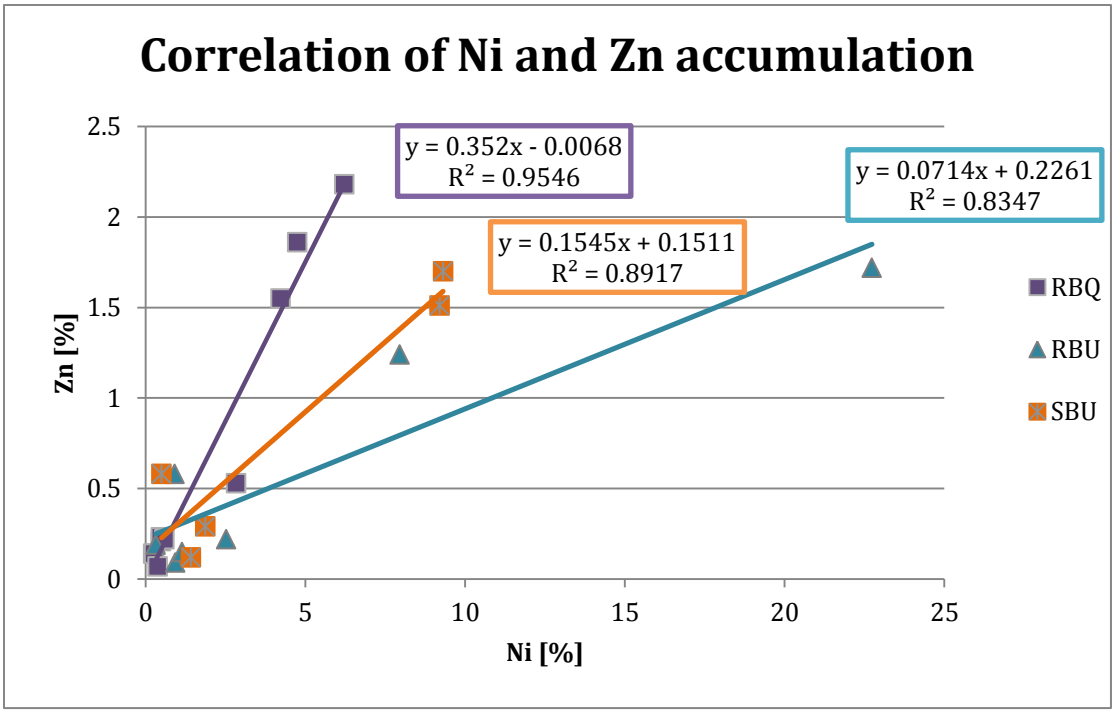


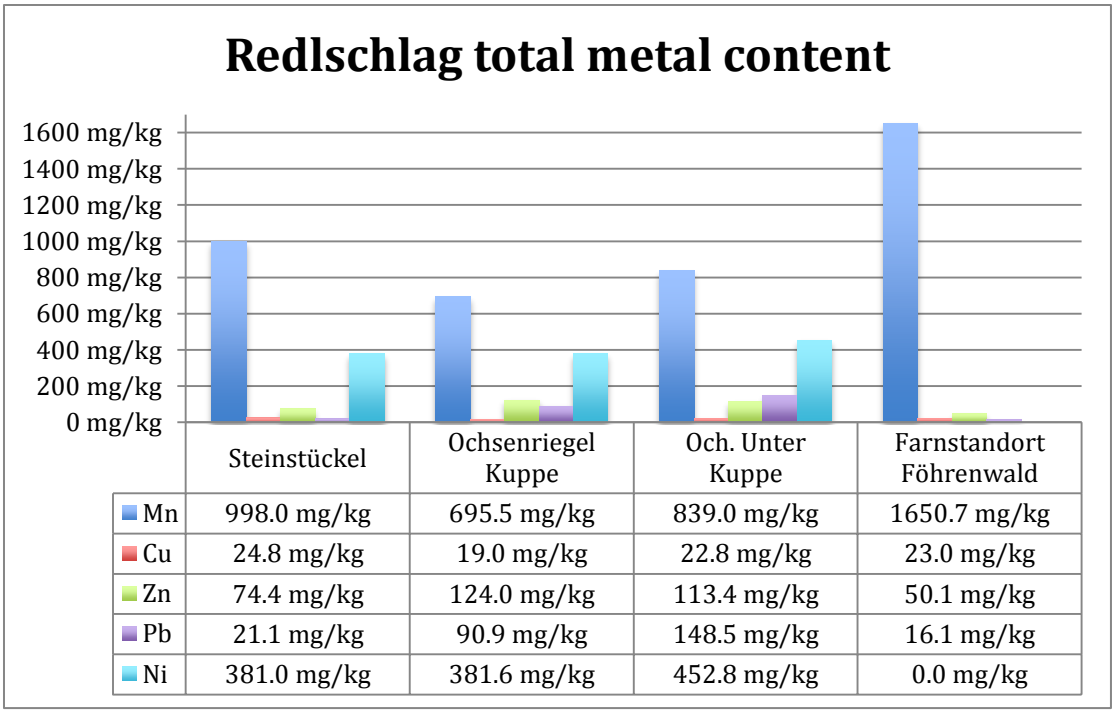
Figure 12: Correlation of nickel and zinc accumulation in the rosette leaf cross-section (violet), rosette leaf bottom (turquois) and stem leaf bottom (orange)

3.3 AAS and ICP-MS analysis

Using the AAS and ICP technique soil and plant samples of both spoil heaps have been analysed.

3.3.1 Analysis: Redlschlag

The most abundant heavy metal in Redlschlag was manganese, followed by nickel (Fig. 13). There were also quite large amounts of zinc and lead present.



	Steinstückel	Ochsenriegel Kuppe	Och. Unter Kuppe	Farnstandort Föhrenwald
Mn	998.0 mg/kg	695.5 mg/kg	839.0 mg/kg	1650.7 mg/kg
Cu	24.8 mg/kg	19.0 mg/kg	22.8 mg/kg	23.0 mg/kg
Zn	74.4 mg/kg	124.0 mg/kg	113.4 mg/kg	50.1 mg/kg
Pb	21.1 mg/kg	90.9 mg/kg	148.5 mg/kg	16.1 mg/kg
Ni	381.0 mg/kg	381.6 mg/kg	452.8 mg/kg	0.0 mg/kg

Figure 13: Total heavy metal content (ICP-MS) in the soil in Redlschlag using aqua regia extraction and ICP-MS for analysis

In plants of the genus Noccaea especially high concentrations of nickel could be found within the roots and shoots (Figure 14). The shoot uptake is exceptionally stronger.

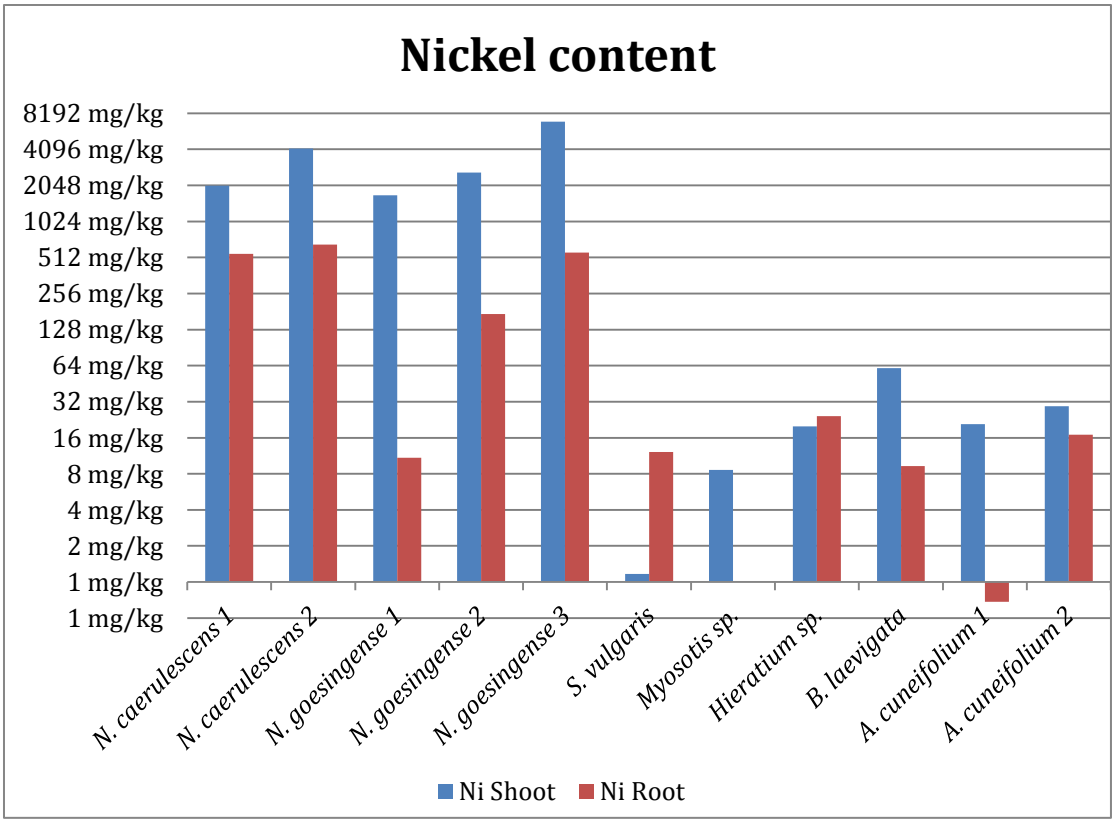


Figure 14: Ni concentration (ICP-MS) in roots and shoots of plants growing on serpentine

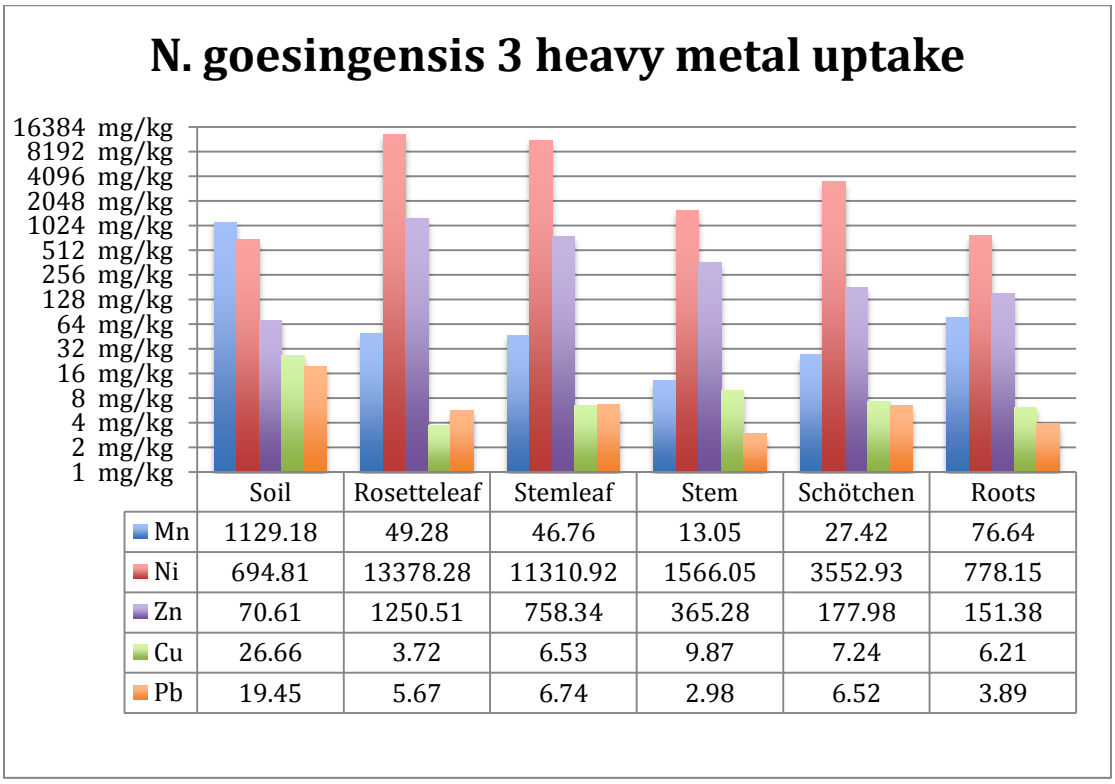
SIlene vulgaris shows nearly no nickel uptake with a very low TF and BCF value (Table 4). *Noccaea goesingensis* shows the highest TF and BCF values, followed by *Noccaea caerulescens.*

Table 4: Nickel concentration (ICP-MS) in the roots and the shoot as well as the BCF (bio concentration factor (shoot/soil) →<1 Exclusion, >1 Accumulation) and TF (translocation factor (shoot/root) →<1 more metal in the root, >1 more metal in the shoot) values

Species	Shoot	Root	TF	BCF
N. caerulescens 1	2036,6 mg/kg	549,1 mg/kg	3,71	3,86
N. caerulescens 2	4149,0 mg/kg	656,7 mg/kg	6,32	7,85
N. goesingense 1	1687,6 mg/kg	10,9 mg/kg	154,58	4,37
N. goesingense 2	2620,1 mg/kg	172,8 mg/kg	15,16	8,20
N. goesingense 3	6951,1 mg/kg	562,9 mg/kg	12,35	10,00
S. vulgaris	1,2 mg/kg	12,2 mg/kg	0,10	0,00
Myosotis sp.	8,6 mg/kg			0,01

Hieratium sp.	19,9 mg/kg	24,3 mg/kg	0,82	0,06
B. laevigata	61,1 mg/kg	9,3 mg/kg	6,57	0,19
A. cuneifolium 1	20,8 mg/kg	0,7 mg/kg	30,41	0,05
A. cuneifolium 2	29,4 mg/kg	17,0 mg/kg	1,73	0,04

Considering the total uptake of heavy metals, *Noccaea goesingensis 3*showed the largest heavy metal concentrations. They were not evenly distributed throughout the plant but rather focused onto the rosette and stem leafs (Fig. 15).



	Soil	Rosetteleaf	Stemleaf	Stem	Schötchen	Roots
Mn	1129.18	49.28	46.76	13.05	27.42	76.64
Ni	694.81	13378.28	11310.92	1566.05	3552.93	778.15
Zn	70.61	1250.51	758.34	365.28	177.98	151.38
Cu	26.66	3.72	6.53	9.87	7.24	6.21
Pb	19.45	5.67	6.74	2.98	6.52	3.89

Figure 15: Heavy metal uptake in *N. goesingensis* 3 compared to the soil content (ICP-MS, except for the nickel soil value which is from the AAS data)

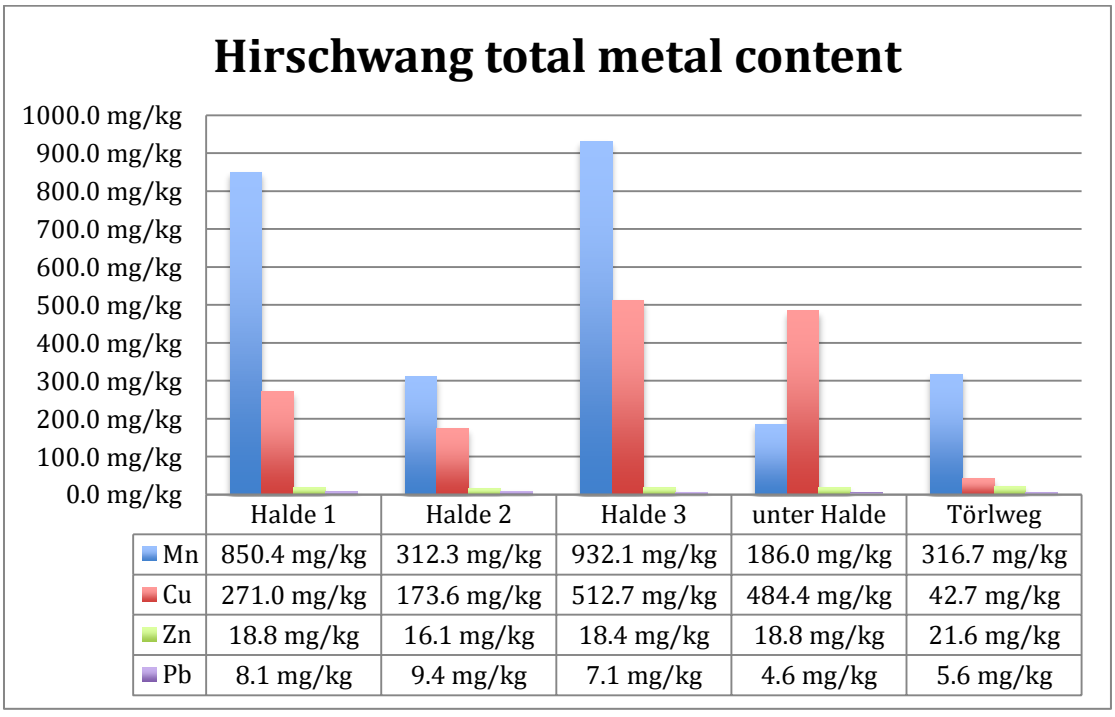
The largest transfer rates from the soil to the shoot occurred in rosette leafs and stem leafs for nickel, followed by zinc (Table 5). For Mn, Cu and Pb there was no considerable transfer into the plant tissues.

Table 5: BCF of every tissue compared to the soil of *N. goesingensis* 3. High values are highlighted brighter

Plant tissue	Mn	Ni	Cu	Zn	Pb
Rosetteleaf	0,04	19,25	0,14	17,71	0,29
Stemleaf	0,04	16,28	0,24	10,74	0,35
Stem	0,01	2,25	0,37	5,17	0,15
Schötchen	0,02	5,11	0,27	2,52	0,34
Roots	0,07	1,12	0,23	2,14	0,20

3.3.2 Analysis: Hirschwang

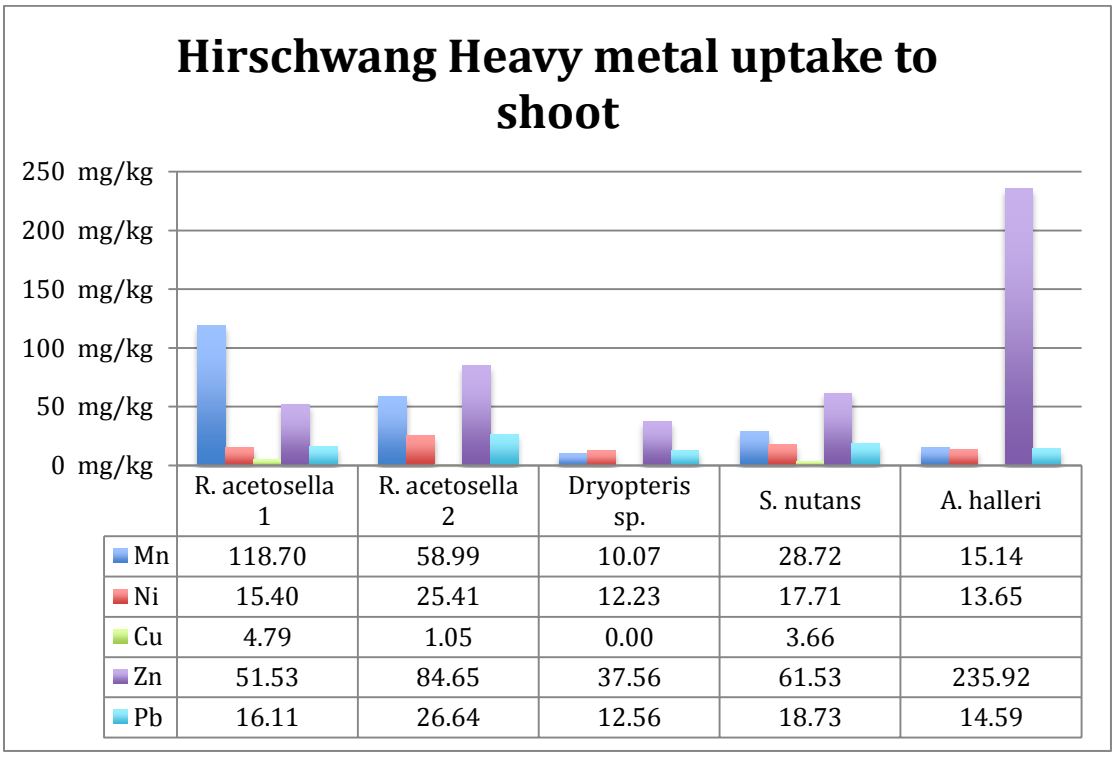
In the spoil heap in Hirschwang manganese is the dominating heavy metal followed by copper (Figure 16). Only at a point below the spoil heap (see "unterHalde") copper showed a larger concentration.



	Halde 1	Halde 2	Halde 3	unter Halde	Törlweg
Mn	850.4 mg/kg	312.3 mg/kg	932.1 mg/kg	186.0 mg/kg	316.7 mg/kg
Cu	271.0 mg/kg	173.6 mg/kg	512.7 mg/kg	484.4 mg/kg	42.7 mg/kg
Zn	18.8 mg/kg	16.1 mg/kg	18.4 mg/kg	18.8 mg/kg	21.6 mg/kg
Pb	8.1 mg/kg	9.4 mg/kg	7.1 mg/kg	4.6 mg/kg	5.6 mg/kg

Figure 16: Total heavy metal content in the soil in Hirschwang (ICP-MS)

Only *Rumex acetosella* took up larger amounts of manganese than the other plant samples (Fig. 17). *Arabidopsis halleri* showed a high zinc uptake to the shoot with over 200 mg/kg.



	R. acetosella 1	R. acetosella 2	Dryopteris sp.	S. nutans	A. halleri
Mn	118.70	58.99	10.07	28.72	15.14
Ni	15.40	25.41	12.23	17.71	13.65
Cu	4.79	1.05	0.00	3.66	
Zn	51.53	84.65	37.56	61.53	235.92
Pb	16.11	26.64	12.56	18.73	14.59

Figure 17: Heavy metal uptake of plants to the shoot in Hirschwang (ICP-MS)

None of the plants showed considerable uptake of manganese and copper, but the BCF for zinc and lead was always in favour of the shoot (Table 6).

Table 6: BCF values of the plants on the spoil heap in Hirschwang. High values are highlighted brighter

Species	Mn	Cu	Zn	Pb
R. acetosella 1	0,14	0,02	2,89	2,07
R. acetosella 2	0,07	0,00	4,74	3,42
Dryopteris sp.	0,05	<det. Limit	2,00	2,75
S. nutans	0,15	0,01	3,28	4,10
A. halleri	0,05	<det. Limit	10,94	2,60

Rumex acetosella 1 and *2* showed translocation to the shoot for nickel, zinc and lead (Table 7). *Silene vulgaris* shows low translocation rates from the roots to the shoot for all heavy metals.

Table 7: TF values of *Rumex acetosella* and *Silene nutans*. High values are highlighted brighter

Species	Mn	Ni	Cu	Zn	Pb
R. acetosella 1	1,38	2,57	0,15	2,25	2,57
R. acetosella 2	1,27	4,45	0,02	4,02	4,60
S. nutans	1,13	0,66	0,12	0,64	0,66

3.4 Plasmolytic tolerance test

Evaluation occurred according the key in table 8. For complete results see table 9.

Table 8: Evaluation key

+	+/-	-	-/+	P
> 80 % alive	> 50 % alive	> 80% dead	> 50% dead	Plasmolysis before incubation

3.4.1 *Armeria sp.* (Obir / Slovenia)

Most of the sections survived the copper treatment. *Armeria sp.* from Obir seemed to be very resistant and tolerated concentrations up to 10^{-2} mol/l $CuSO_4$. Surprisingly the cells of *Armeria sp.* resisted all concentrations of the serial $NiSO_4$ solutes up to 10^{-1} mol/l. Although tissues incubated to the high concentrated solutes were pre-plasmolysed. Zinc was at least tolerated by the plant cells. More than 50% of the cells died already in the 10^{-5} mol/l solute. The toxicity threshold for chrome resulted at 10^{-3}mol/l $Cr_2(SO_4)_3$ whereby plasmolysis started already at 10^{-4}mol/l.

3.4.2 *Armeria sp.* (Wales / GB)

The highest tolerance was observed in copper were the threshold was the 10^{-3} $CuSO_4$ solute. In nickel and zinc cells lived until 10^{-5}mol/l and in chrome the plasmolytic tolerance was the lowest.

3.4.3 *Rumex acetosella* (Hirschwang / Austria)

In the copper solutes *R. acetosella* had the highest tolerance of all plants. Furthermore we found a "dead zone" from 10^{-2}mol/l to 10^{-4} mol/l $CuSO_4$. Most of our sections for nickel were pre-plasmolysed and we observed tolerances only up to 10^{-4}mol/l. Since the big leaf section in 10^{-5}mol/l $NiSO_4$ was pre-plasmolysed we consider this square not as a

"dead zone". In the section of a big leaf we found a high plasmolytic tolerance at 10^{-1} mol/l $ZnSO_4$. Small leafs section resisted up to 10^{-4} mol/l the cells of a small leafs section resisted the zinc treatment only until 10^{-4} mol/l. The $Cr_2 (SO_4)_3$ treatment resulted in a low tolerance, nevertheless we observed a "dead zone" for a big leafs section of *Rumex acetosella.*

3.4.4 *Noccaea goesingensis* (Redlschlag / Austria)

We observed a low plasmolytic resistance. The section for copper survived up to 10^{-5}mol/l. The test resulted in a low zinc tolerance at 10^{-6} mol/l $ZnSO_4$ and the cells in the serial $Cr_2 (SO_4)_3$ solutes were all dead.

3.4.5 *Noccaea caerulescens* (Redlschlag / Austria)

Copper was tolerated up to 10^{-5}mol/l, however sections for concentrations from 10^{-1} mol/l to 10^{-4}mol/l were pre-plasmolysed. In the nickel solutes all sections were injured before incubation. The tissue in 10^{-7} mol/l recovered. Plasmolytic tolerance at 10^{-7}mol/l was extreme low for $ZnSO_4$.In the chrome solutes we measured no tolerance.

3.4.6 *Thlaspi minimum*

The plants sections showed up high tolerances in copper, nickel and zinc. In $NiSO_4$ we observed cell survival until 10^{-1}mol/l. The least tolerated metal was chrome at 10^{-5}mol/l.

3.4.7 *Allium cepa*

The cells resisted copper treatment up to 10^{-2}mol/l and in the $ZnSO_4$ solutes until 10^{-3}mol/l. Due to our pre-plasmolysed sections we observed only one value in the serial $NiSO_4$ solutes. Chrome was tolerated until 10^{-5}mol/l.

3.4.8 *Cynodontium sp.*

The moss cells resisted to high nickel, 10^{-2}mol/l, and high zinc concentrations, 10^{-1}mol/l. For chrome and nickel we observed intermediate values.

3.4.9 *Triticum aestivum*

The plant demonstrated high cytoplasmic tolerances in our nickel, zinc and chrome solutes and we observed concentrations up to 10^{-4}mol/l for each heavy metal. The cells resisted copper treatment until 10^{-5}mol/l.

3.4.10 *Arabidopsis halleri*

In nickel and chrome the leaves sections resisted the 10^{-5}mol/l solutes. $ZnSO_4$ was the most tolerated metal at a concentration of 10^{-3}mol/l. In the copper solutes the cells resisted at least, however some sections were pre-plasmolysed.

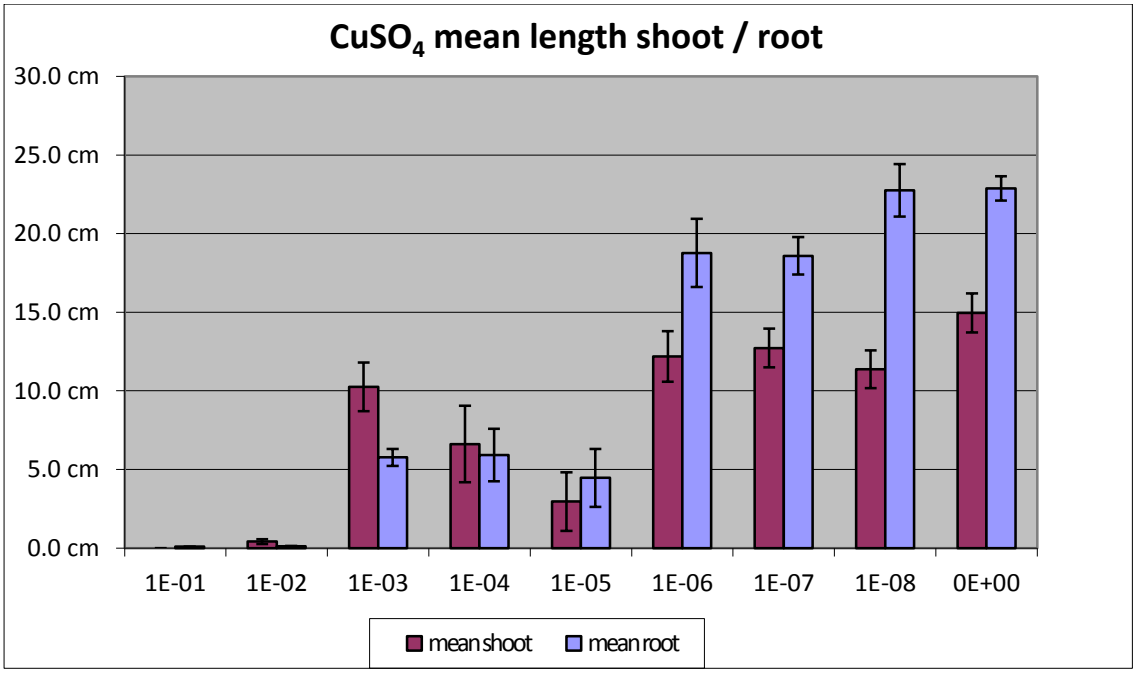
Table 9: Results of the plasmolytic tolerance test; green: „dead-zone“, yellow: non-„dead-zone“

	Cu							**Ni**							**Zn**							**Cr**						
10^x [mol/l]	-1	-2	-3	-4	-5	-6	-7	-1	-2	-3	-4	-5	-6	-7	-1	-2	-3	-4	-5	-6	-7	-1	-2	-3	-4	-5	-6	-7
Armeria sp. (Obir)	-/+	+	+	+	+	+	+	P+/-	P+/-	P+/-	+	+/-	+	+	-	p-	-/+	-/+	+/-	+	+	-	-	+/-	+/-	+	fehlt	+
Armeria sp. (Wales)	-	-	P-/+	P+	+	+	+	-	-	-	-	+/-	+	+	-	-	-	-	+/-	+	+	-	-	-	-	-	+/-	+
Rumex acetosella	-	-	-	-	+	+	+	-	-	-	-	-/+	+	+	-	-	-/+	+/-	+/-	+	+	-	-	-	-	-/+	+	+
Rumex grosse Blätter	+	-/+	-/+	-/+	+	+	+	p-/+	p-/+	p-/+	+	p-/+	P+/-	+	+/-	+/-	+/-	+/-	+/-	+/-	+	-	-	+/-	-	-	fehlt	+/-
T. goesingense	-	-	-	-	+/-	+	+	P -	P -	P -	P -	P-/+	P-/+	-/+	-	-	-	-/+	-/+	+	+	-	-	-	-	-	-/+	-/+
T. caerulescens	P-	P-	P-	P-	+	+	+	P-	P-	p-		p-	p-	P+	-	-	-	-	-	-/+	+	-	-	-	-	-	-	-
T. minimum	-	+/-	+	+	+	+	+	+/-	+	+	+	+	+	+	+/-	+	+	+	+	+	+	-	-	-	-	+/-	+	+
Allium cepa	-/+	+	+	+	+	+	+	fehlt	p-	p-	+/-	p-	p-	p-	-	-	+/-	+/-	+/-	+	+	-	-	-/+	-/+	+/-	fehlt	+
Cynodontium sp.	-	-	-	-	+	+	+	-	+/-	+/-	+	+	+	+	+/-	+/-	+	+	+	+	+	-	-	-	+/-	+/-	+/-	+
Triticum aestivum	-	-	-	-/+	+	+/-	+	P-	-	-/+	+	+	+	+	-	-	-/+	+/-	+	+	+	-	-	-/+	+	+	+	+
Arabidopsis halleri	-	P-/+	P-/+	-/+	-/+	+/-	+	-	-	-	-/+	+/-	+	+	-/+	-/+	+/-	+/-	+	+	+	-	-	-	-	+/-	+	+

3.5 Wheat Germination test – tolerances of a crop plant

3.5.1 CuSO4

Figure 18: $CuSO_4$ mean length shoot / root



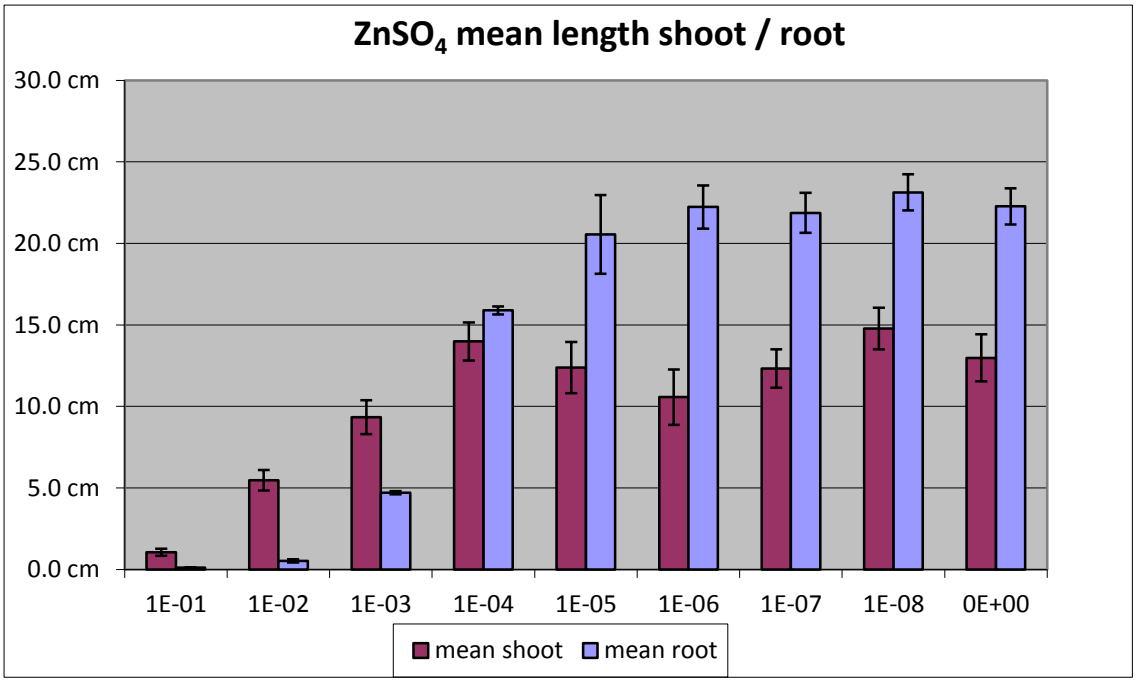
In the highest copper concentrations most of the wheat seeds failed to germinate. The 10^{-3}mol/l and 10^{-4}mol/l $CuSO_4$ solutions showed us that the shoot growth was promoted whereas in the control groupsroots were longer than shoots (see Fig. 18). High concentrations from 10^{-5}mol/l $CuSO_4$ up let us observe extreme issues in germination and viability and tendencies to nanism. The specimens weight measurements let us observe an increased dry weight and a decreased fresh weight in the highest concentrations. The chlorophyll-fluorescence varies a lot in high and low concentrations and showed us no obvious trend for the photosynthetic activity.

3.5.2 ZnSO4

For zinc we observed less germination issues. First symptoms of inhibition were observed at 10^{-4}mol/l zinc sulphate. Suppressed root elongation occurred from

10^{-3}mol/l up (see Fig. 19). The weighs showed us increased dry weights in the two highest concentrations. The specimens showed few variations in the photosynthetic activity. A downward drift was noticed only in the highest concentration of 10^{-1}mol/l $ZnSO_4$.

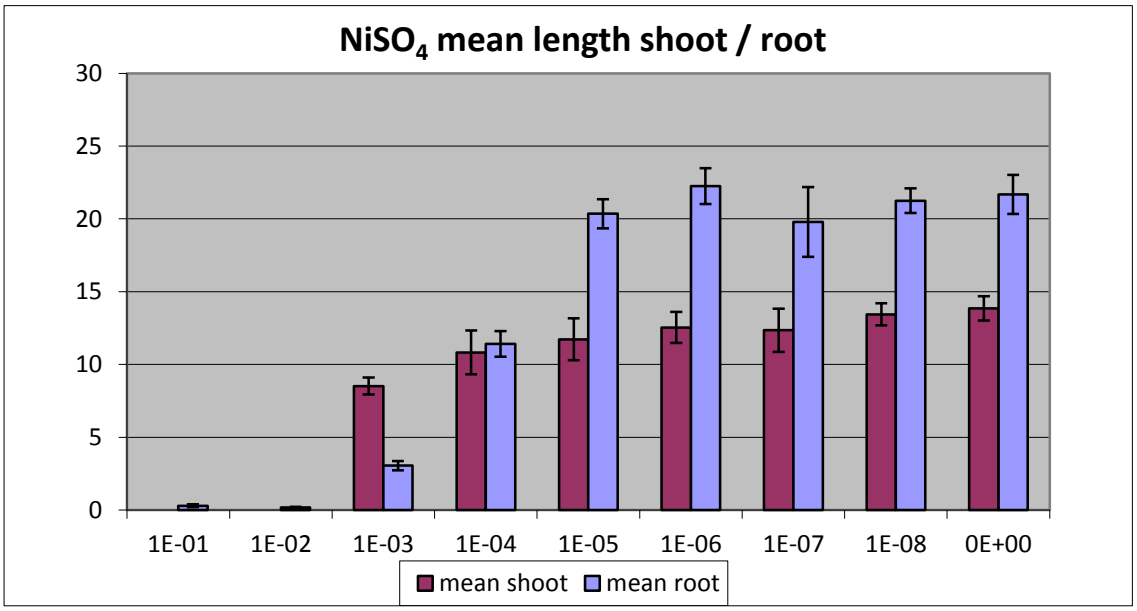
Figure 19: $ZnSO_4$ mean length shoot / root



3.5.3 NiSO4

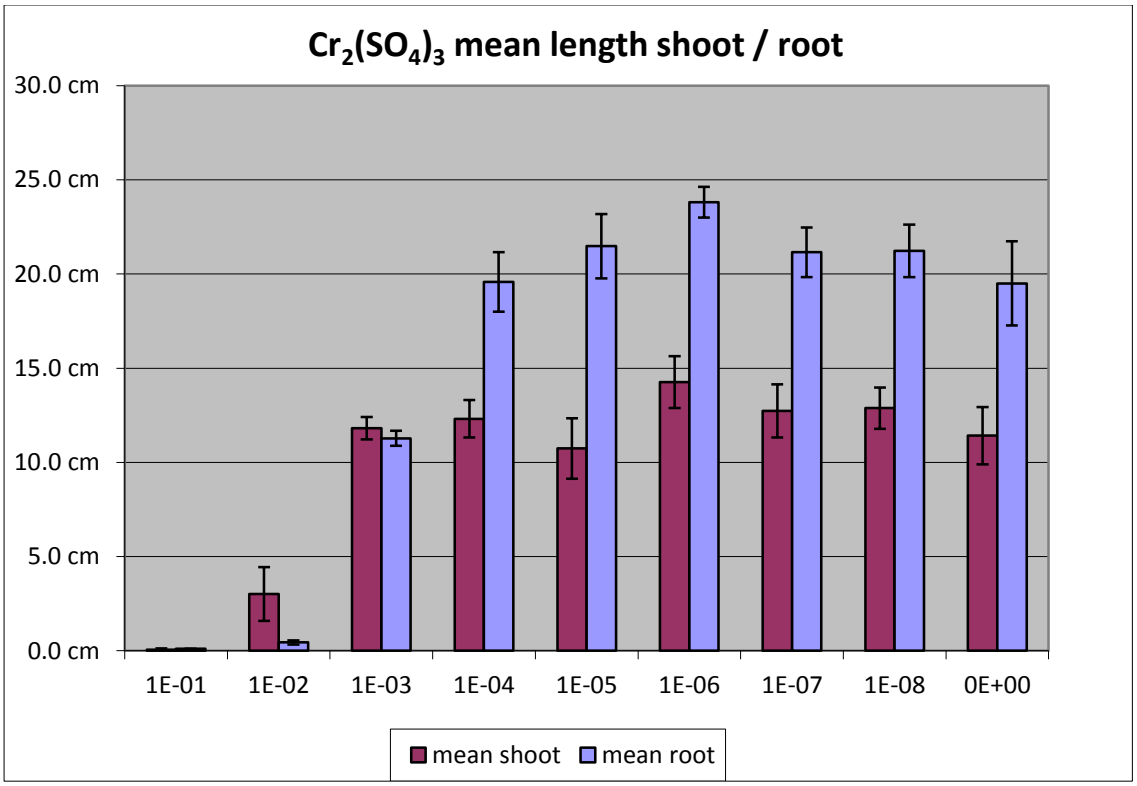
In the highest concentrations of the nickel solutes almost no germination occurred. 10^{-4}mol/l $NiSO_4$ started the inhibition on the root elongation, which got more obvious in the 10^{-3}mol/l solute (see Fig. 20). The elevation in concentration showed a decrease in fresh weight and an elevation in dry weight. The highest photosynthetic activity was measured at 10^{-7}mol/l, nevertheless we observed no clear trend. No activity was measured for the two highest concentrations.

Figure 20: $NiSO_4$ mean length shoot / root



3.5.4 Cr2(SO4)3

Figure 21: $Cr_2(SO_4)_3$ mean length shoot / root



In the chrome solutes we observed a promoted root elongation, especially at values from 10^{-8}mol/l up to 10^{-5}mol/l, which exceeds the length of the control group. At 10^{-2}mol/l $Cr_2(SO_4)_3$ we found shoot elongation with consequent absence of germination at 10^{-1}mol/l (see Fig. 21). The peaks for dry weight were noticed at the two highest concentrations, whereas fresh weight peaks lay at 10^{-3} and 10^{-4}mol/l $Cr_2(SO_4)_3$. Highest photosynthetic activity was measured with 0,825 (F_v/F_m) in the 10^{-6}mol/l, followed by the 10^{-4}mol/l$Cr_2(SO_4)_3$ solute whereby the control group had a lower fluorescence value.

3.6 Soil analysis - photometric determination of humus content

Table 10: Humus content and pH of the heavy metal sites

Site	Spot	pH	Humus percentage
Hirschwang	Halde 1	3,44	23,70%
Hirschwang	Halde 1	3,45	12,10%
Hirschwang	Halde 2	3,02	11,10%
Hirschwang	Halde 3	3,78	15,80%
Hirschwang	unter Halde	4,37	12,20%
Hirschwang	Törlweg	6,95	12,20%
Redlschlag	Steinstückel	5,11	16,00%
Redlschlag	Steinstückel	5,45	10,10%
Redlschlag	Steinstückel	5,14	19,20%
Redlschlag	Steinstückel		21,60%
Redlschlag	Steinstückel	5,45	3,40%
Redlschlag	Steinstückel	5,47	7,10%
Redlschlag	Steinstückel	5,16	18,00%
Redlschlag	Ochsenriegel Kuppe	5,52	61,70%
Redlschlag	Och. Unter Kuppe	4,87	59,60%
Redlschlag	Farnstandort Föhrenwald	5,08	4,00%

The soil samples of Hirschwang (Table 10) are always greater than 11,1 % Humus, with a peak at Halde 1 (23,7 %). Their pH varies between 3,02 and 4,37 an can be called sour, the sample from the Törlweg is quite an exception as it does not belong directly to the heap.

For Redlschlag, even though all the samples are quite distributed within a larger area, the average pH lays at 5,25 with a standard deviation of 0,23. Within the Steinstückel the humus content varies greatly from 3,4- 21,6 %, whereas Ochsenriegel shows by far greater humus content of about 60 %.

4 Discussion

4.1 Anatomic analysis

As soil acidity elevates cation availability for the plants by implication HM availability increases too (Taiz & Zeiger, 2006; Schulze, Beck & Müller-Hohenstein, 2002). Previous studies by Bradley R. et al. (1982) showed that plants of the Ericaceae family not only benefit in the nutrient uptake by the presence of ericoid mycorrhiza. They also can colonize HM contaminated sites such as spoil heaps, as the fungus reduces HM accumulation potential into the shoot. For *Vaccinium sp.* the ecto-mycorrhiza may exclude HMs via direct adsorption at hyphae or by incorporation into the cell wall, by chelation binding through exsudations of organic acids and HM immobilisation in the fungal apoplast (Frey & Lösch, 2010; Jentschke & Goldbold, 2000).

As far as we know *Rumex acetosella* is not associated with any type of mycorrhiza (Varma A. (ed.), 2008). The observed droplets in the plants root cortex show a high exposure to the soils solutes and the unprotected state of the plants radix. We estimate that the enclosures are compartmented heavy metal complexes (PCs or MTs) or detached cation - pectin complexes in the pores of the donnan free space. The leaves of *Rumex acetosella* possess a large number of gland cells wherefore we estimated an active secretion of heavy metal exsudates. To prove our estimations we used EDX techniques, which showed us more detailed results. As the detection in the tissues of the root parenchyma and the leaf gland cells resulted in no altered copper, zinc or nickel concentrations another semi-quantitative detection method has to be found.

Arabidopsis halleri and *Silene nutans* possesses a vast number of large trichomes with a high density of enclosures in their vacuoles. We estimate that the plants get rid of their HM complexes (PCs & MTs) by compartmentation into the emergences.

The observed plants all possess amphistomatous leaves, which may double the possibility of HM contamination by interception of re-suspended ions through the upper and lower stomata.

4.2 EDX Analysis of the genus *Noccaea*

4.2.1 *Noccaea caerulescens*

Noccaea caerulescens (formerly *Thlaspi caerulescens*) is known to be a self-compatible, biannual Zn and Cd hyperaccumulator (Krämer 2010). It is mostly known to hyperaccumulate Zn and Cd but also Pb has been suggested (Baker, Reeves and Hajar 1994).
Hyperaccumulation properties towards nickel were already knownof(Peer, et al. 2006) and in the case of this study it became clear that the population in Redlschlag has the abilities to do so. The EDX analysis showed that especially in the rosette leafs nickel and zinc were stored. Due to the biannual nature of the rosette leafs it is possible that it can store high levels of heavy metals. The stems showed little heavy metal content, which is probably becausethe heavy metals are only transported through them in a complexed form but are not stored there.

4.2.2 *Noccaea goesingensis*

For Noccaea goesingensis (formerly Thlaspi goesingense) is usually seen as a Ni hyperaccumulator (Krämer, Smith, et al. 1997). In EDX it showed large amounts of Nickel stored especcially in the epidermis of the rosette leaf, which relates to the observations with *N. caerulescens*. But in *N. goesingensis* the showed also nickel levels of about 1,8 % WT in the epidermis of the stem leafs, which has to be further researched. The second quite abundant heavy metal to be found was zinc, which indicates that the plant can also accumulate it.

4.3 AAS and ICP-MS analysis

4.3.1 Redlschlag

The soil in Redlschlag is dominated by manganese, followed by nickel, then zinc and lead which is quite typical for serpentine soils. *Noccaea caerulescens*, *Noccaea goesingensis* and *Silene vulgaris* could be found in this landscape and all of them are known for their heavy metal tolerance.
For nickel the momentarily used value to qualify for hyperaccumulation lies at >1000 mg/kg (or µg * g^{-1}) dry mass in the plants(Krämer 2010), which is easily met by *N. caerulescens* as well as *N. goesingensis* (Table 4).

In *N goesingensis* the highest concentration of heavy metals in the rosette and stem leafs, which is backed up nicely by the observations in the EDX analysis.
An interesting addition to the nickel hyperaccumulating observation of *N. caerulescens* was the discovery of a specimen near the serpentine location, which shows the typical Zn hyperaccumulation, without an over exposition to nickel (Fig. 22).

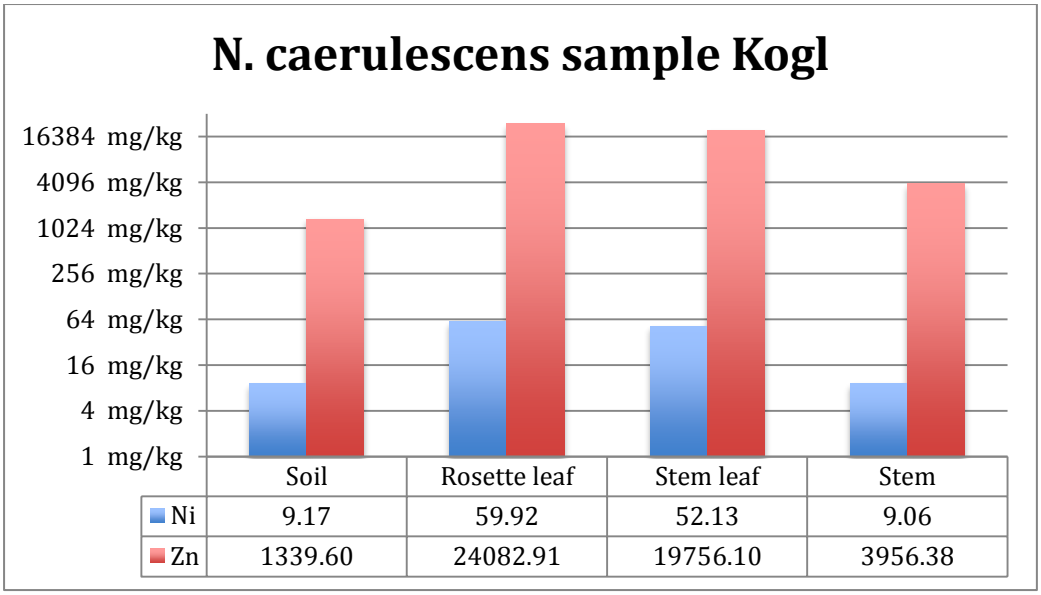


Figure 22: *N. caerulescens* specimen analysed by AAS

For the future it would be very interesting to test *N. caerulescens* samples with the ICP-MS for additional metals, especially cadmium to confirm a possible accumulation.

Silene vulgaris showed a different strategy of tolerance by excluding nickel from its tissues nearly completely, which is sometimes referred to as hypertolerance(Macnair 1993).

4.3.2 Hirschwang

In Hirschwang the soil was dominated by manganese followed by copper. Directly at the spoil heap no hyperaccumulators were found, solely *Arabidopsis halleri* grew on a spot nearby without elevated copper values. The plants growing on the heap showed excluding properties. Neither manganese nor copper was taken up to the shoot in large quantities.
Quite surprisingly *Rumex acetosella* as well as *Silene nutans* and *Dryopteris sp.* took up considerable amounts of zinc and lead (Table 5) with BCFs between two and four. *R. acetosella* transferred lead and

zinc from the roots to the shoot, whereas *S. nutans* kept most of it in the roots.

As a future aspect it would be interesting to determine if the elevated zinc an lead uptake is a, by the plant, unwanted process or a tolerance mechanism to cope with the high copper and manganese content. Furthermore all data has to be measured again, as it is highly probable that a dilution error has occurred, as the values are about ten times smaller than in previous works based on Hirschwang. Still BCF and TF values should still have comparable value.

4.4 Plasmolytic tolerance analysis

In the observations of our test we found an unexpected cytoplasmic reaction of some plants, which in literature are described as zinc, nickel, copper or other HM ecotypes (Frey & Lösch, 2010; Prasad & de Olivera Freitas, 2003).

Especially our main specimens *Noccaea goesingensis* and *Noccaea caerulescens* showed low plasmolytic tolerances in nearly all of our metal solutions used. As *N. goesingense* and *N. caerulescens* in literature are especially described as nickel and zinc hyper-accumulators but are also known to have constitutive properties of multiple tolerances (Reeves R. D. et al., 1984; Baker A. J. M., 1987; Baker A. J. M. et al., 1994; Krämer U. et al.; 1997; Krämer U., 2010), our results surprised us. The fact that a vast number of our sections in both specimens were pre-plasmolysed before incubation, let us conclude that the test has to be repeated due to the uncertainness of our operation. For the Brassicaceae we could observe only in *Thlaspi minimum,* which is defined as accumulator-type species (Aigner B., 2005), a multiple tolerance in mid to high concentrations.

Armeria spp. from Obir (Slovenia) and Wales (Great Britain) instead resulted to have a high protoplasmic resistance. Also in previous studies (Aigner B., 2005) high plasmolytic tolerances for *Armeria alpine* could be observed.

The tolerances observed for *Rumex acetosella* were surprisingly high. The plant showed the ability to tolerate almost each of the used metal solutions up to high concentrations and resulted as an all-rounder. Maybe this plant will be issue in future researches and in the phytoremediation for copper contaminated sites, such as vineyards, orchards or spoil tips of ancient mines (Tang et al., 1999; Schulze, Beck & Müller-Hohenstein, 2002; Babalonas et al., 1987).

Since a high number of specimens used in assays by Saukel J. (1980), Sissolak M. (1984), Hörmann D. (2001) and Sassmann S. et al. (2010) were mosses we merely found result - correlation for *Cynodontium sp.* but low to no result - correlation with our studies on higher plants. The presence of vascular bundles

clearly promotes another type of stress – management. According to past studies (Saukel J., 1980; Sissolak M., 1984; Hörmann D., 2001) our observations also indicate that probably there is only a low relation between the plasmolytic tolerances and the eco-typical adaptions of a plant.

4.5 Wheat germination analysis- tolerance of the crop plants

Former studies (Wong M. H. & Bradshaw A. D., 1981; Sharma D. C. et al., 1995; Peralta J. R. et al., 2001; Munzuroglu O. and Geckil H., 2002) on wheat and other plants showed that the presence of altered HM concentrations in the growth medium clearly induces stress, retards germination and altogether lowers the germination rate of the plants. We showed that at high concentrations in nearly all HMs, root and shoot elongation is inhibited almost completely.
As assayed by Munzuroglu O. and Geckil H. (2002), we also noticed a fast decrease of germination in the Cu solutes. Since some HMs also act as micronutrients we observed some shoot and root elongation in 10^{-8}mol/l to 10^{-5}mol/l of the Zn and Ni solutes. Observations in *Medicagosativa* resulted in an elongation in Zn (Peralta J. R. et al., 2001), but none in Ni. Nevertheless El-Ghamery A. A. et al. (2003) showed that Zn lowers the mitotic activity within incubation time. Surprisingly in the 10^{-8}mol/l to 10^{-4}mol/l Cr solutes we found promoted root and shoot elongations and increased DWs at 10^{-3}mol/l and 10^{-4}mol/l (see table 12). An altered photosynthetic activity was monitored in the 10^{-6} mol/l, followed by the 10^{-4} mol/l $Cr_2(SO_4)_3$ solute. As far as we know such metabolic effects were never observed for Cr till now (Huffman E. W. D. & Allaway W. H., 1973; Sharma D. C. et al., 1995; Shanker A. K. et al., 2005) and may be issue of further investigations. However we confirm that even low concentrations of Zn, Ni, Cu and Cr has a deleterious effect on *Triticum aestivum.*

4.6 Soil analysis- photometric determination of the humus content

In Hirschwang there was comparatively small variation within the soil pH and humus content, yet no significant correlation can be drawn. Sadly it was not possible to connect humus und pH to the available heavy metals, as ICP-MS for the ammonia nitrate extraction has not been measured yet.

In Redlschlag there is huge variation within pH and humus and again it was not possible to correlate the pH and the humus content. As mentioned above it is not yet possible to compare them to the available heavy metal, which would be very interesting.

For the future it would be important to measure the ammonia nitrate extraction samples, so that the available heavy metal should correlate to pH or humus content.

Appendix

Table 11: Species / site list

Hirschwang		
Halde	Untere Halde	Törlweg
Halde	Unter Halde	*Arabidopsis halleri*
Larix decidua	*Dryopteris carthusiana*	
Pinus sylvestris	*Silene nutans*	
Vaccinium myrtillus	*Dryopteris filix-mas*	
Betula pendula		
Picea abies		
Rumex acetosella		
Avenella flexuosa		
Redlschlag		
Steinstückl (way side)	Steinstückl (hill side east)	Ochsenriegel
Achillea millefolium	*Euphorbia amygdaloides*	*Dianthus carthusianorum cf. subsp. capillifrons*
Campanula persicifolia	*Fagus sylvaticus*	*Galium sp.*
Chamaecytisus ratisbonensis	*Fragaria sp.*	*Genista pillosa*
Euphorbia cyparissias	*Genista pillosa*	*Hieracium pilosella*
Festucca pallens	*Luzula luzuloides*	*Polygala amara*
Hieracium sylvatica	*Myosotis sp.*	*Polygala chaebuxus*
Knautia sylvestris	*Noccaea caerulescens*	*Polygonatum odoratum*
Myosotis sp.	*Noccaea gaesingensis*	*Pyrus communis*
Noccaea caerulescens	*Picea abie*	*Rumex acetosa*
Noccaea gaesingensis	*Quercus robur*	*Silene vulgaris*
Polygala amara	*Rubus sp.*	*Sorbus aria*
Potentilla alba	*Sambucus nigra*	*Sorbus aucuparia*
Potentilla crantzii	*Sorbus aucuparia*	*Verbascum sp.*
Pteridium aquifolium	*Stellaria holostea*	*Viola sp.*
Ranunculus acris	*Symphytum tuberosum*	*Biscutella laevigata*
Sedum telephium	*Taraxacum sp.*	*Sorbus aria*
Silene vulgaris		*Asplenium cuneifolium*
Stellaria holostea		*Biscutella laevigata*
Tanacetum corymbosum		*Hieracium pilosella*
Thymus sp.		*Noccaea goesingensis*
		Dicranum cf. scoparium
		Hypnum cupressiforme
		Leucobryum glaucum
		Polytrichum formosum
		Vaccinium myrtillus

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