Heavy metal stress in plants; a closer look

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Introduction

Plants often have to deal with different stress factors like drought, radiation, availability of water, etc. But if all this happens on a heavy metal rich soil, it would be much difficult conditions for plants to survive. Most of the elements which are presented on such sites are essential in small amounts. To those micronutrients count B, Cl, Cu, Fe, Mn, Mo, Ni, Zn and for some plants also Co, Na and Si. For adequate growth the average concentration in the plants ranges from 0.1mg/kg (Mo, Ni) to 100mg/kg (Fe, Cl) in the shoot (Marschner, 1995). In addition to them, there are also non-essential elements like Ar, Cd, Cr or Pb. However, in high concentrations they become all toxic.
Areas with high heavy metal contents can have its seeds in natural geological aggregations like serpentine (ultramafic rocks). The soil in those sites have low Ca-content relative to Mg, low amount of macronutrients and a high concentration of Ni, Cr, and Co (Kazakou et al., 2010). But more often result heavy metal contaminated land from anthropogenic deposit like mining waste heaps or agricultural treatment like in vineyards. For example, a normal untreated soil contains 20-50mg/kg Cu but in a vineyard it could be over 300mg/kg (Schulze et al., 2005).
To deal with the high concentrations of heavy metals, the plants which grow on the contaminated soil developed two main strategies. On the one hand the plant excludes all heavy metals by stopping them from entering the plant. This occur either through stopping the uptake of the metals into the root or with the immobilization in the rhizodermis. It is also possible that the plant produces root-exudates to reduce the availability in the soil.
On the other hand the plant accumulates the metals and stores them in parts of the plant where they cannot interact with the metabolism. This happens mainly in the apoplast of the cells or in the vacuoles. The metals additionally get bound to special complexes by plant-chelators like phytochelatins or metallothionins (Frey & Lösch, 2010, Assunção et al. 2003).
Some plants accumulate the heavy metals more active. In so called hyperaccumulators the concentration of the metal is 100 times higher than in normal plants. Many studies reported Ni and Zn contents of *Noccaea* species >1000mg/kg each (Reeves and Baker, 1984; Reeves, 1988). Metal hyperaccumulation occurs approximately in 500 plant taxa worldwide (Krämer 2010).
In this work I want to show data from soil and plant samples collected from two different sites to evaluate: (a) if there are specific anatomic features for the heavy metal stress; (b) if the cells of the metalophytes tolerate higher metal concentrations than cells of non-metalophytes; (c) what influence has heavy metal stress on germination and growth on plants, (d) which plants accumulate heavy metals and which exclude it; and (e) in which plants and plant tissues respectively is the metal stored and how high is the concentration.

Material/Method

Study sites

Two sample sites were chosen; an old Cu heap on the Knappenberg in Hirschwang (Styria) and a natural serpentine (ultramafic) site on the Ochsenriegel in Redlschlag (Burgenland).

Hirschwang (7.5.2012)

In this area Cu ore was mined until the 1890s. On one of the old mining waste tip the samples were collected. The tip was surrounded by a fir forest. On the heap there is much rough gravel and less fine material. The vegetation is also very marginal and small in size (petticoat formation). Because of absence of vegetation the sun radiation is higher and therefore the

microclimate is warmer and drier than in the surrounding forest. For the plants on the heap it means beside the toxic stress of the Cu an additional drought stress.

Redlschlag (9.5.2012)
On the Ochsenriegel there is a natural deposit of serpentine, a Ni-rich rock. The samples where taken on two different sites; the Steinstückl and the Ochsenriegel. The main difference between those two sites is the vegetation. On the Steinstückl the vegetation has a high cover and diversity dominated by *Noccaea*. It is surrounded by a thin pine forest. The Ochsenriegel-site is more opposite. There is less vegetation and less diversity and is surrounded by heather dwarf shrubs. The main reason for the difference is maybe the additional drought stress on the second site.

Collected plants:

Hirschwang	Redlschlag
Avenella flexuosa	*Noccaea caerulescens*
Betula pendula	*Noccaea goesingensis*
Rumex acetosella	*Silene vulgaris*
Vaccinium myrtillus	*Myosotis sp.*
Dryopteris filix-mas	*Hieratium sp.*
Silene nutans	*Biscutella laevigata*
Arabidopsis halleri	*Asplenium cuneifolium*

Anatomic features

Six plants (respectively three plants from the two sites) were selected for the anatomic analysis; *A. halleri*, *R. acetosella* and *V. myrtillus* from Redlschlag and *N. caerulescens*, *N. goesingense* and *S. nutans* from Ochsenriegel. For the analysis a light-microscope was used (Olympus CX-41) with its different contrast techniques: bright and dark field, phase contrast and polarized light.
First different sections were made of the shoot and the root (cross and longitudinal sections). From all remarkable structures of the sections pictures were made and discussed afterward.

Plasmolytic tolerance

To determinate the toxic thresholds of heavy metal concentration on the cells itself the plasmolytic tolerance test is used. This test shows the vitality of the cells by testing the function of the plasmolysis. For this test plants from the heaps and from the laboratory were used:

R. acetosella	*A. halleri*
R. acetosa	*Armeria sp. from Carinthia (O)and Wales (W)*
N. caerulescens	*Allium cepa*
N. goesingense	*Cynodontium strumiferum (Bryophyte)*
N. minima	

The leaves of the plants were prepared by making cross-sections which are around two to three cells thin, so that at least one row of cells is intact for the plasmolysis. These cross-sections were incubated 48h in a heavy metal solution of different concentrations. For that a 0.1M solution of Cu-, Ni-, Zn- or Cr-sulfate was gradually diluted from 10^{-1} to 10^{-7}.
After the incubation time the cross-sections were transferred into a 1M sucrose solution and observed with the microscope. If the cells became plasmolysed by the sucrose solution the

cells were alive. By using the different concentrations of heavy metal solution it is possible to detect the toxicity thresholds of the cells.

Germination-rolls

To observe the heavy metal stress under the other stresses like heat, radiation or microbial activity, we had to exclude the external influences in a standard treatment. For this we used seeds of *Triticum aestivum* which have no specific adaptations to heavy metal stress. Ten seeds each were put on a filter paper which was rolled up and put into different metal (Ni, Zn, Cu, and Cr) concentrations from 10^{-1} to 10^{-8}. After 12 days it was observed the account of germinated seeds and its shoot and root length. Additionally, the chlorophyll fluorescence was measured to correlate it with the heavy metal stress in the wheat.

Humus content

0.5- 2g of soil was diluted with 20ml potassium dichromate and afterward with 15ml sulfur acid. After 3h the solution was filled up to 100ml with distilled water. After 24h 1ml of the solution is mixed with 24ml distilled water and measured with the photometer against a standard solution on 570nm. The standard was made with definite amounts of myo-inosit which present definite humus contents.

AAS/ICP-MS

In order to be able to compare absolute and plant-available heavy metal it was necessary to use quantitative methods on soil and plant samples. Therefore the samples from the two sites have been examined using atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS).
The soil samples were air dried and then filtered for a grain size lower than 2mm. To determinate the absolute heavy metal content, 2g of soil samples were digested with 30ml Aqua Regia ($HCl:HNO_3$ = 3:1). After 3 hours of backflow distillation the extract was filled up to 100ml with distilled water. For the plant-available heavy metal content the soil was mixed with 1M Ammonium nitrate (NH_4NO_3) in the ratio 2.5 to the solid content. Then the extraction was filtrated and stabilized with HNO_3.
The plant samples were divided into rosette leaves, stem leaves, stem, seeds and roots. Then the samples were cleaned from soil and other sediments and dried at 105°C. For the analyzing 1-3g dry matter was mixed with 24ml acid (Nitrite acid:perchloric acid = 5:1) and cooked until only little perchloric acid was left. Afterward the solution was diluted to 100ml with distilled water. If the dry matter was less than 1.5g the solution was diluted only to 45ml otherwise the AAS could not detect the heavy metal content.
With the AAS the soil extracts were measured for Cu and Ni and the plant extracts for Cu, Ni and Zn. The ICP-MS measured parallel the elements Cd, Cu, Mn, Ni, Pb and Zn in the soil and plant samples.

EDX

With the EDX-analysis (energy dispersive X-ray) it is possible to locate and semi quantify the heavy metal content in different plant organs and tissue layers. For the measurement the plant organs of *N. caerulescens* and *N. goesingense* were divided into the different plant parts (rosette leaves, stem leaves, stems, seeds, roots).The different plant organs were air dried and then the cross-sections or the epidermal layers were carbon-coated and fixed for the REM (XL

20) coupled with the EDX. Ten measurements of each layer/cross-section were made to get the relative atomic weight of the heavy metal.

Results

Anatomic features

The most interesting structures of *A. halleri* were the leaves which showed a high amount of branching trichomes which were up to 400µm long (Fig.1A & B).
R. acetosella showed two interesting features; vesicle-rich cells around a large intercellular space in the root (Fig.1C) and gland cells on the upper and lower epidermis of the leaves (Fig.1C).
A special anatomic feature of *V. myrtillus* was the root-associated mycorrhiza. The hyphae of the fungi were observable all along the roots (Fig.2E & F).
S. nutans had also trichomes on the leaves but in contrast to *A. halleri*, were those multi cellular and unbranched (Fig.2D). The size was up to 500µm.

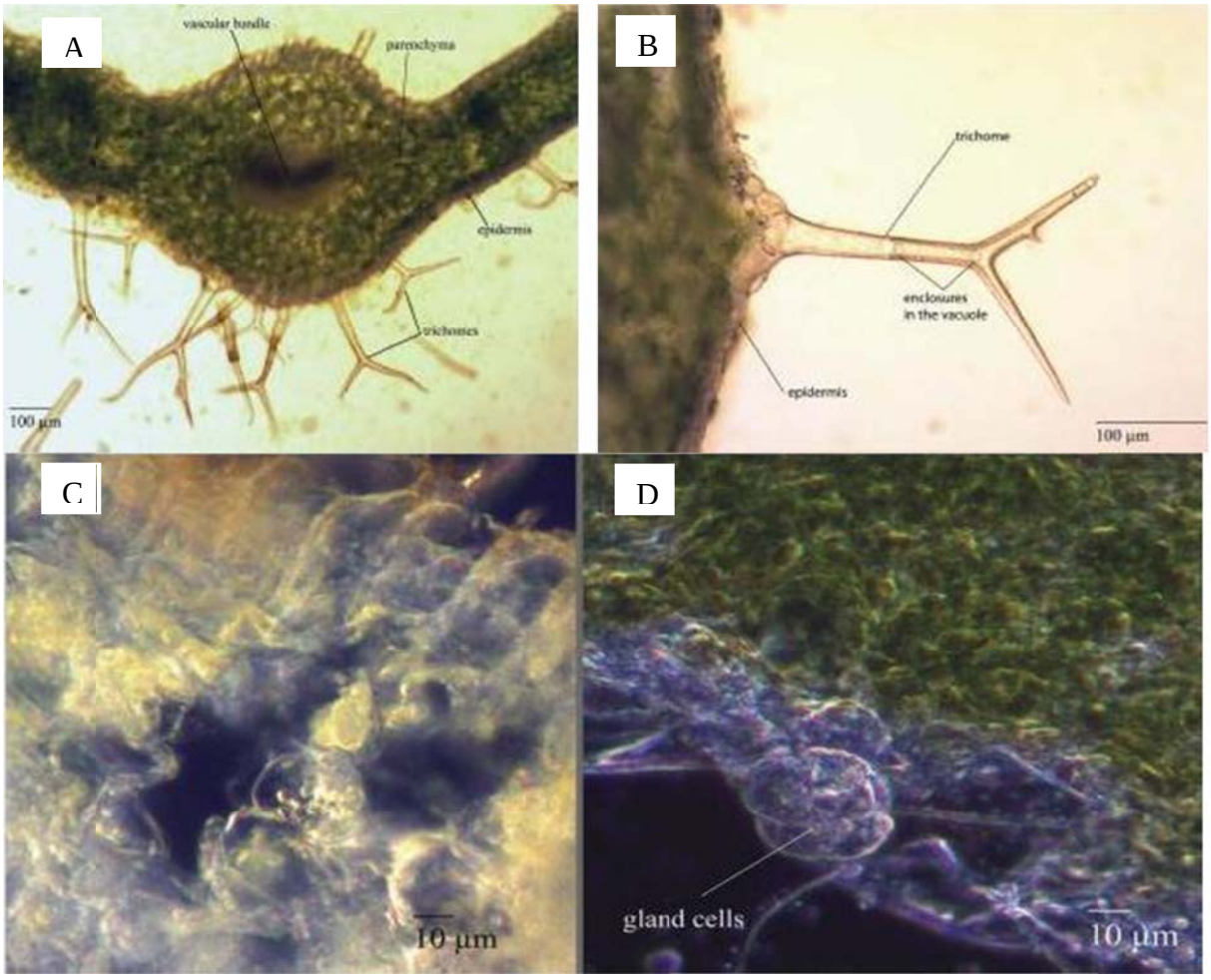

Fig.1: (A) Cross-section of an *A. halleri* leaf in the bright field with trichomes; (B) magnification of one trichome in the bright field; (C) Intercellular space with surrounding vesicle-rich cells in polarized light and (D) gland cells on the leaf-epidermis in the phase contrast of *R. acetosella*

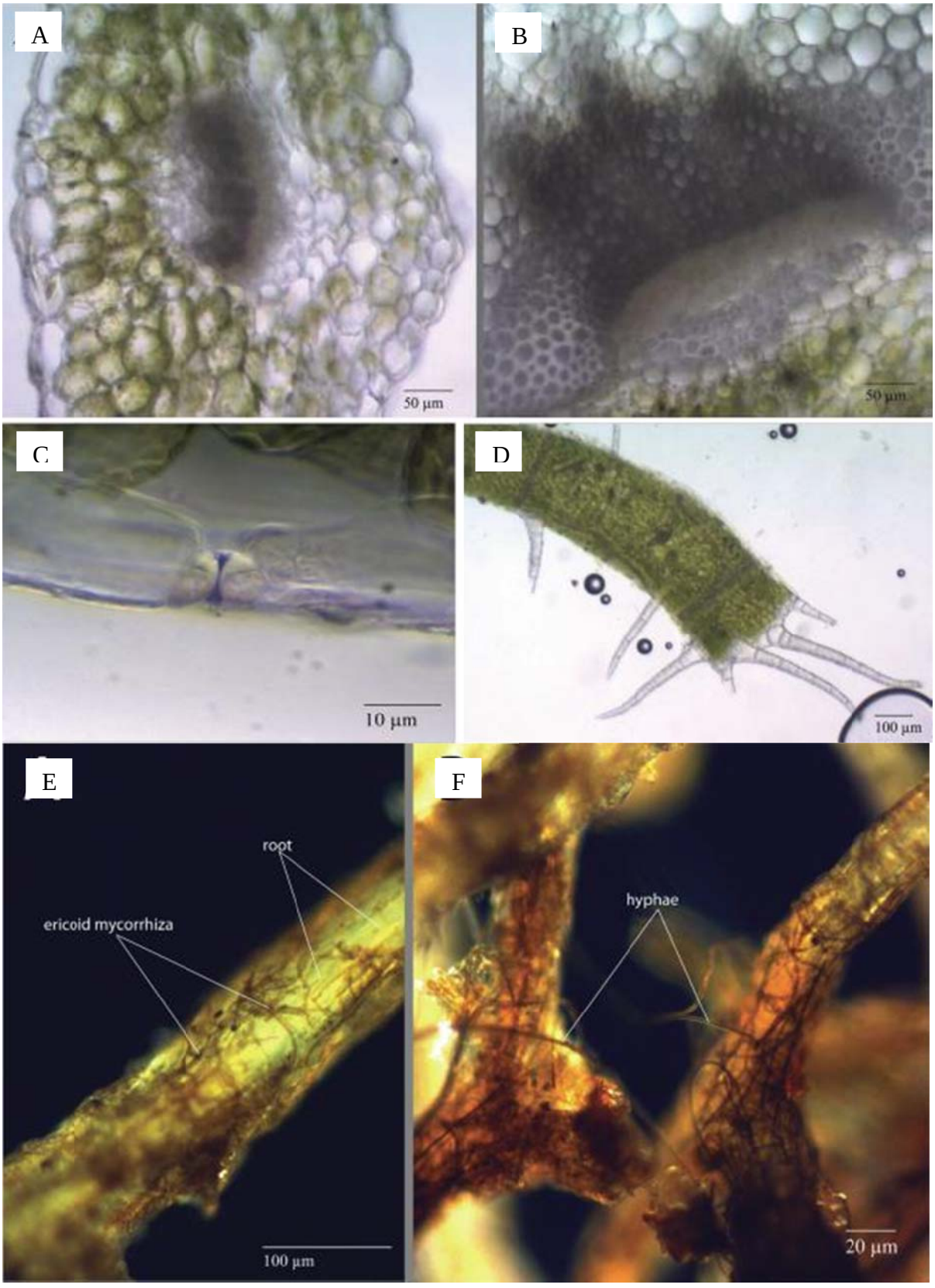

Fig.2 (A & B) Cross-sections of the stem of *N. caerulescens* in the bright field; (C) Cross-section of a stomata of N. *goesingense* in the bright field; (D) Epidermal layer of *S. nutans* with trichomes in the bright field; (E & F) Hyphae of the mycorrhiza fungi on *V. myrtillus* roots in the dark field

Tab.1: Tolerance of the cells from chosen species against different heavy metal concentrations (from 10^{-1} to 10^{-7}): (+) all cells, /+/-) over 50%, (-/+) under 50%, (-) no cells made plasmolysis, (P) means that the cells were plasmolysed cause of the high concentration of the heavy metal solution

	Cu							Ni							Zn							Cr						
10^	-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. (O)	-/+	+	+	+	+	+	+	P+/-	P+/-	P+/-	+	+/-	+	+	-	P-	-/+	-/+	+/-	+	+	-	-	+/-	+/-	+	n.d.	+
Armeria sp. (W)	-	-	P-/+	P+	+	+	+	-	-	-	-	+/-	+	+	-	-	-	-	+/-	+	+	-	-	-	-	-	+/-	+
R. acetosella	-	-	-	-	+	+	+	-	-	-	-	-/+	+	+	-	-	-/+	+/-	+/-	+	+	-	-	-	-	-/+	+	+
R. acetosa	+	-/+	-/+	-/+	+	+	+	P-/+	P-/+	P-/+	+	P-/+	P+/-	+	+/-	+/-	+/-	+/-	+/-	+/-	+	-	-	+/-	-	-	n.d.	+/-
N. goesingense	-	-	-	-	+/-	+	+	P-	P-	P-	P-	P-/+	P-/+	-/+	-	-	-	-/+	-/+	+	+	-	-	-	-	-	-/+	-/+
N. caerulescens	P-	P-	P-	P-	+	+	+	P-	P-	P-	n.d.	P-	P-	P+	-	-	-	-	-	-/+	+	-	-	-	-	-	-	-
N. minimum	-	+/-	+	+	+	+	+	+/-	+	+	+	+	+	+	+/-	+	+	+	+	+	+	-	-	-	-	+/-	+	+
A. cepa	-/+	+	+	+	+	+	+	n.d.	P-	P-	+/-	P-	P-	P-	-	-	+/-	+/-	+/-	+	+	-	-	-/+	-/+	+/-	n.d.	+
C. strumiferum	-	-	-	-	+	+	+	-	+/-	+/-	+	+	+	+	+/-	+/-	+	+	+	+	+	-	-	-	+/-	+/-	+/-	+

Plasmolytic tolerance

Tab.1 gives an overview of the results of the plasmolytic tolerance test. The treatment of the cells with Cu showed that all the plants were living until the 10^{-5} dilution. Up to the 10^{-2} dilution only one *Armeria*, *T. minimum* and *A. cepa* were alive. The cells of *R. acetosa* showed again plasmolysis on the highest concentration.
With Ni, *Armeria sp.*(O) and *T. minimum* showed plasmolysis at all concentration and *C strumiferum* on the 10^{-2} dilution. In contrast *N. caerulescens* and *N. goesingense* were not alive at all respectively only on the lowest concentration. A special case was observable at *A. cepa*. There was only plasmolysis at the 10^{-4} dilution.
In the Zn-treatment the cells of *R. acetosa*, *T. minimum* and *C strumiferum* made plasmolysis in all the concentrations. *N. goesingense* and *N. caerulescens* were again only at the lowest concentrations alive.
The treatments with higher Cr concentrations were toxic for all the cells. *R. acetosa* and the two *Noccaea* showed less respectively no plasmolysis on the lowest concentrations.
Altogether were the cells of *T. minimum* and *Armeria sp* (O). more resistant against the heavy metal than *N. goesingense* and *N. caerulescens* which showed the lowest resistant.

Germination-rolls

Up to dilution factor of 10^{-6} all plants of the four treatments showed similar growth like in the control (Fig. 1, A & B). In the Ni and Zn treatment the growth length was constant until 10^{-5} in the Cr treatment until 10^{-4}. In the two highest concentrations the growth of the roots was repressed and the shoot showed very low growth rates fewer than 5cm.
The chlorophyll fluorescence was relative constant over the whole treatment (Fig 1, C). Cu showed a reduction of the fluorescence at 10^{-4} down to 0.770. In the highest concentrations the analyses were not possible realizable because of the small size or the absence of the leaves.

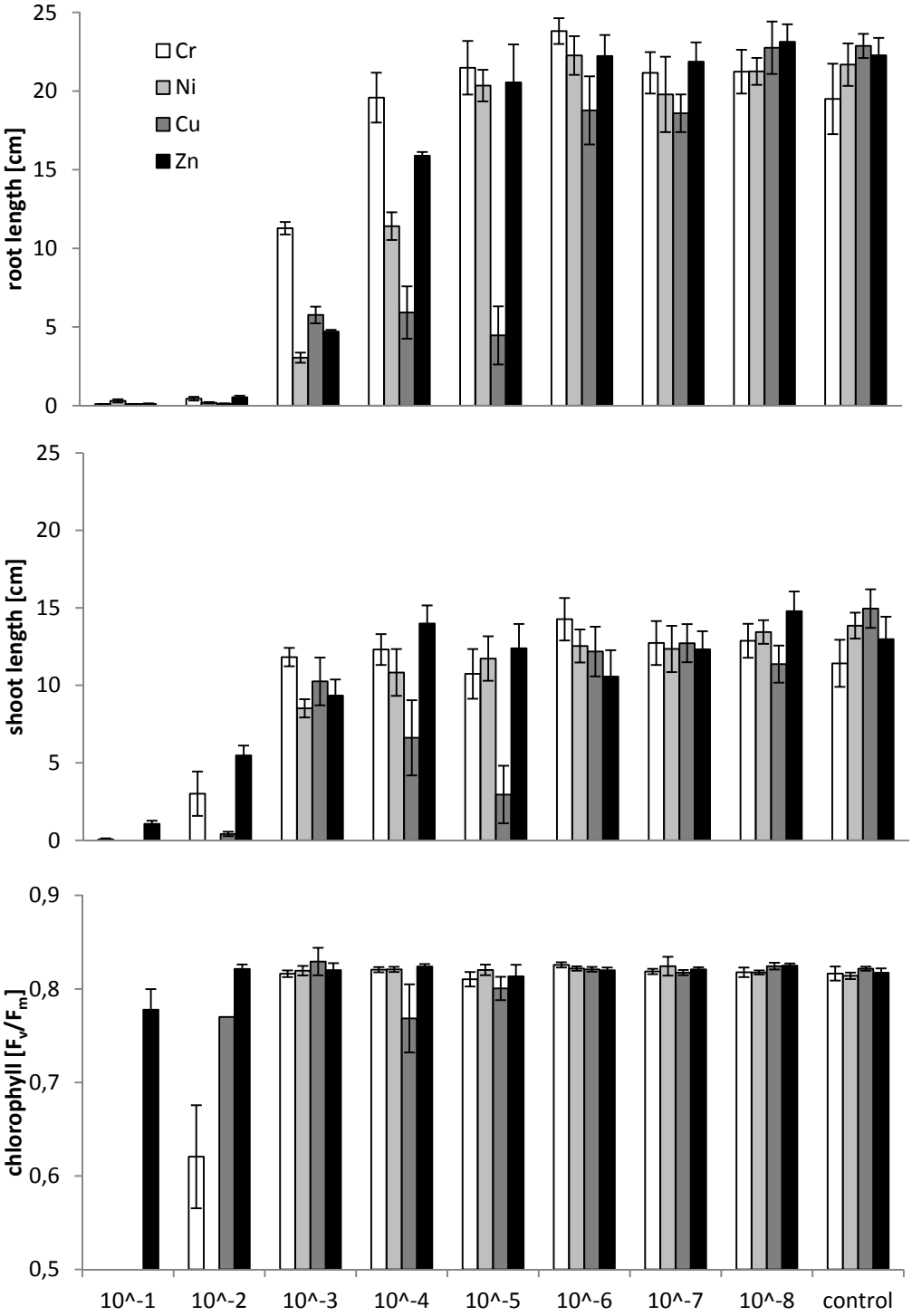

Fig.3: (A) root and (B) shoot length of the *T. aestivum* after 48h in different heavy metal solutions of Cr, Ni, Cu and Zn, (C) Chlorophyll F_v/F_m of the shoots after the treatment

Soil/Humus

The humus content of the heap in Hirschwang lay between 10 and 25% of the soil. On the serpentine the humus content ranged due to the two different sites between 1 and 60%. The content of available Cu in Hirschwang was between 0.7 to 1.1%. In Redlschlag was the available Ni content more heterogenic between 0.05 to 0.85%.

Comparing the humus content with the availability of Cu or Ni then a correlation is only observable in Hirschwang. With higher humus content the availability of cupper gets reduced.

AAS/ICP-MS

Hirschwang-

All concentrations of the heavy metals and the corresponding transfer factors are presented in Tab.2. The Cu content in the soil of the heap was 351.6-484.4mg kg^{-1} (mean 418mg kg^{-1}). The content in *R. acetosella* and *S. nutans* was 2.9 mg kg^{-1} respectively 3.7 mg kg^{-1}. The BCF (bio concentration factor (shoot/soil): <1 Exclusion, >1 Accumulation) is far less than 1. Zn was found in all of the plants in higher concentration than in the soil (mean 18.7mg kg^{-1}). In *R. acetosella* and *S. nutans* it was under 70mg kg^{-1} in *A. halleri* it was 235.9mg kg^{-1} (BCF = 10.94). The Mn content was higher in the soil than in the plants. The BCF of all three plants was between 0.1 and 0.15. Ni was not detected in the soil but in the plants in higher concentrations.

Redlschlag

The Ni content of the soil reached from 466.9-795.6mg kg^{-1} (mean 596.8mg kg^{-1}). The content in *N. caerulescens* and *N. goesingense* was 12981.6mg kg^{-1} respectively 18076.2mg kg^{-1}. The BCF was above 25 respectively 38. In comparison, *S. nutans* had in the shoot only 9.1mg kg^{-1} (Tab.3)

Zn was detected in the soil with 66.3-77.6mg kg^{-1} (mean 72.5mg kg^{-1}) but in *N. caerulescens* and *N. goesingense* with 4726.6mg kg^{-1} (BCF = 64) respectively 2536.6mg kg^{-1} (BCF = 33) and in *S. nutans* only with 58mg kg^{-1}

The Mn content in the soil lay at 995.2-1058.1mg kg^{-1} (mean 1008.5mg kg^{-1}). In spite of the high content was less Mn in the plant (BCF > 0.3)

Tab.2: The concentrations of different metals in the soil, shoots and roots in Hirschwang with the corresponding bio concentration factor (BCF)

	Ni	Cu	Zn	Mn	Pb
R. acetosella					
soil	< det. limit	351,6	18,6	877,6	7,8
shoot	20,4	2,9	68,1	88,8	21,4
root	5,9	48,6	22,0	66,2	6,0
BCF	--	0,01	3,65	0,10	2,76
S. nutans					
soil	< det. limit	484,4	18,8	186,0	4,6
shoot	17,7	3,7	61,5	28,7	18,7
root	27,0	31,6	96,1	25,5	28,5
BCF	--	0,01	3,28	0,15	4,10
A. halleri					
soil	< det. limit	42,7	21,6	112,6	5,6
shoot	13,6	< det. limit	235,9	15,1	14,6
root	--	--	--	--	--
BCF	--	--	10,94	0,13	2,60

Tab.3: The concentrations of different metals in the soil, shoot and root in Redlschlag with the corresponding bio concentration factor (BCF)

	Ni	Cu	Zn	Mn	Pb
N. caerulescens					
soil	527,9	27,7	73,7	972,2	19,4
shoot	12981,6	< det. limit.	4726,6	< det. limit.	< det. limit
root	602,9	--	810,6	--	--
BCF	24,59	--	64,1	--	--
N. goesingense					
soil	466,9	20,1	77,6	995,2	23,2
shoot	18076,2	31,3	2536,6	294,7	55,6
root	364,9	8,1	126,5	56,6	9,6
BCF	38,7	1,6	32,7	0,3	2,4
S. vulgaris					
soil	795,6	32,9	66,3	1058,1	17,8
shoot	9,1	13,1	58,0	40,4	4,3
root	18,96	6,8	42,1	37,85	4,9
BCF	0,01	0,4	0,8	0,04	0,2

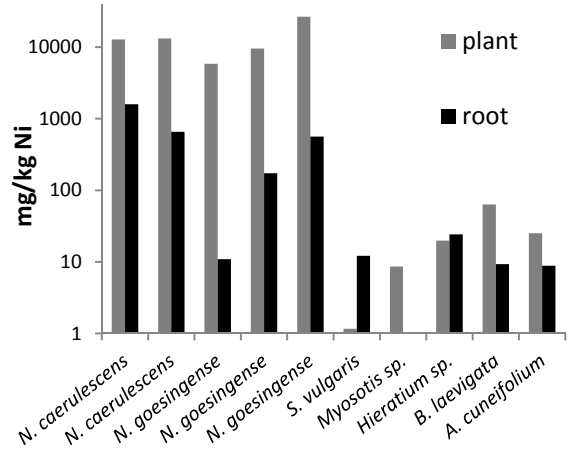

Fig. 4: Ni content in the shoot and root of the plants collected on the serpentine in Redlschlag, (log scale)

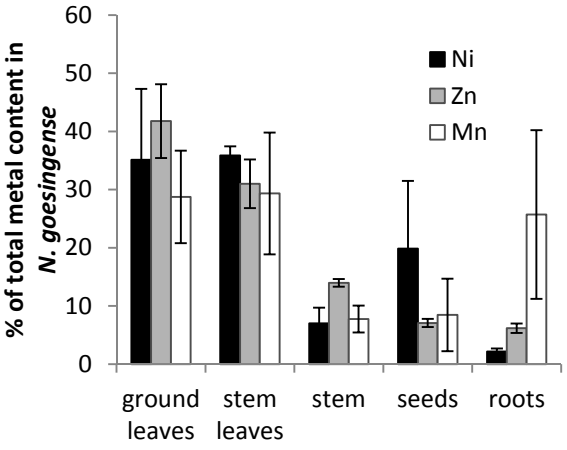

Fig. 5: Relative Ni, Zn and Mn content in the different plant parts of *N. goesingense*

The comparison of the different plant parts showed that around 70% of the total Ni and Zn content is stored in the leaves (Fig.5).

EDX

N. goesingense

The EDX showed that most of the heavy metals were stored in the leaves, especially in the epidermis layers (Fig.6A). The Ni content ranched in the leaves from 0.3% (cross section) up to 2.3% (epidermis) of the total plant mass. The highest concentrations of the heavy metals were in the upper epidermis layer (Ni 2.3%, Zn 0.6, Fe 0.9%) of the rosette leaves. In the stem leaves the highest content is in the lower epidermis layer (Ni 1.9%, Zn 0.6%, Fe 0.3%)

N. caerulescens

Like in *N. goesingense* the heavy metal content was highest in the upper epidermis layer of the rosette leaves (Fig.6B). Especially the Ni content is over 7% of the total atomic mass. Also the Zn content reached there their highest value (1.8%) In the lower epidermis the Ni content reached 2% and the Zn content 1%. In contrast to the leaves the stem had the lowest heavy metal content (under 0.23%)

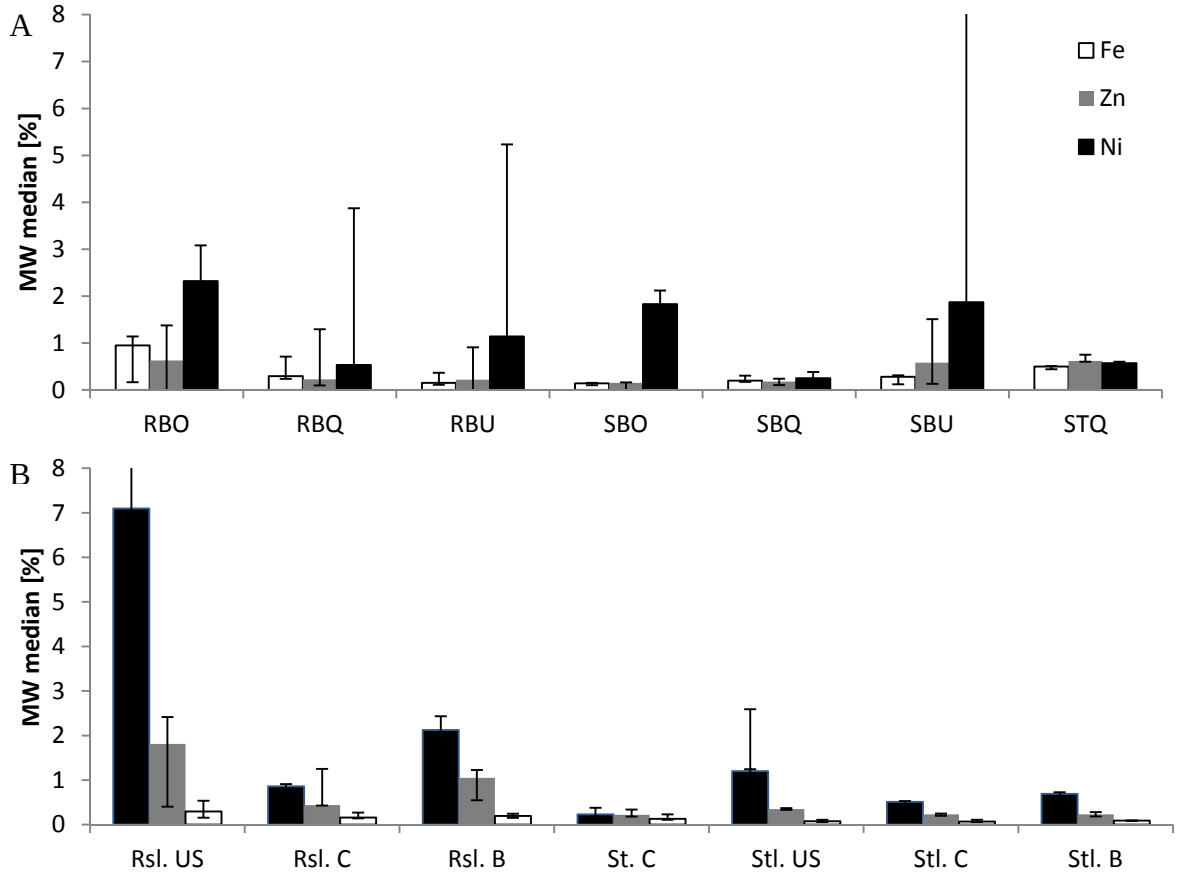

Fig. 6: Relative mass weight content of Ni, Zn, and Fe in the rosette leaves (RB), the stem leaves (SB) and the stem (ST) of (A) *N. goesingense* and (B) *N. caerulescens*. The plant tissues were divided in upper epidermis layer (O/US), lower epidermis layer (U/B) and cross-section (Q/C).

Discussion

Are there specific anatomic features?

The analyses of the different plant structures showed no apparent features for the heavy metal stress. The existing structures are more common to heat adapted plants. Features like trichomes by *A. halleri* and *S. nutans* create a less turbulent air layer around the leaf to reduce the transpiration. But Küpper et al. (2000) reported that *A. halleri* strongly accumulate Zn and Cd into the trichome base.

The symbiosis of V. *myrtillus* with the mycorrhiza fungi is a typical feature of the order Ericales. This ericoid mycorrhiza facilitates the uptake of nutrients in edaphically stressful environments where the plants usually appear (Read, 1992; Read, 1996). Maybe the mycorrhiza additionally absorbs the heavy metals or produces chelates to reduce the toxicity for the plant.

The gland cells of *R. acetosella* were suspected to excrete the heavy metals out of the plant. An investigation with the EDX showed no enrichment of heavy metals in those cells but higher concentration of Na which hypothesize that the gland cells are used for salt excretion. This feature is also found by Armeria (Frey & Lösch, 2010) and an adaption for water deficiency. Also in the vesicle-rich cells close to the intercellular space showed no higher concentrations of heavy metal than in the rest of the root. Further analysis must be made to clear the function of those cells.

Are the cells of metalophytes more tolerant to heavy metal?

Surprisingly are the cells of *N. goesingense* and *N. caerulescens* average less tolerant than the non-adapted species like *A. cepa* or *C. strumiferum*. In contrast shows *N. minimum* a very high tolerance to the different heavy metals though they are close related.

R. acetosella hade average tolerance threshold than expected too. A very good tolerance shows beside *N. minimum Armeria sp*. (O) which had similar thresholds except Zn. The plasmolysis of *R. acetosa* in the highest Cu concentration can be explained that in high concentrations the heavy metal atoms develop a shell on the surface of the plasma membrane and prevent them from entering the cell (Kaho, 1993). Url (1956) reported similar date about the Cu and Cr resistance of different moss species. Summarily the data of the plasmolytic tolerance test hypothesize that the exclusion of the heavy metals out of the symplast works as good that the cells itself do not need a special adaption to heavy metals. In opposite, plants which had no adaption on heavy metal stress could have a general higher tolerance to toxic elements. Because of the missing barriers the cells will be confronted more often with them.

What influences have heavy metals on the germination and growth of plants?

Our data shows that low concentrations up to 10^{-6} had no negative effect on the germination and the growth of the wheat seedlings. The most toxic element was Cu, because concentration above 10^{-6} caused a dramatic reduction in the growth length. The plants in the other elements had a more continued reduction in length size with higher concentrations. In the highest concentration the germination rate in Cu and Ni was reduced to the half. On interesting observation was that the root length reduces more than the shoot in higher concentrations of all four treatments. Because the root is responsible for the uptake of the toxic metals the plant tried to reduce the size and thereby the absorbing surface.

Are there any accumulators/excluders on the heaps?

The results from the AAS show that the *R. acetosella* and *S. nutans* exclude the Cu of the heap in Hirschwang. Despite the high concentration in the soil was less than 1% of it in the shoot. The rest is stored or attached at the roots. Brun et al. (1998) reported a similar concentration in roots of *R. acetosella* growing on Cu-enriched vineyards (soil 102mg kg^{-1}, root 32.7mg kg^{-1}). But both plants accumulated Zn in the plants. In *R. acetosella* the Zn concentration was also higher in the shoot than in the root what indicate that the Zn is actively stored there. Mn is a common metal in rocks which explains the high concentration in the soils. It was also excluded from the plants and lay far under the critical toxicity level of 200-3500mg kg^{-1} (Krämer, 2010). *A. halleri*, which is well known for hyperaccumulation (Krämer, 2010), accumulated strongly Zn into the shoot despite the low concentration in the soil.

The data from Redlschlag demonstrate the expected high Ni concentration in the ultramafic soil. The two collected *Noccaea* species accumulate high amounts of Ni and Zn which is in evidence with the high BCF. Their specific affinity to one metal is obviously on the respective metal. *N. caerulescens* is primer known as a Zn hyperaccumulator and has the higher Zn content than *N. goesingense* which is primer a Ni hyperaccumulator (Assunção, 2003). But the heterogeneous content of metals in the collected plants complicates a clearer conclusion (Fig.4). In contrast, *S. vulgaris* seems to be a strict excluder. The heavy metal content in the shoot is at all measured metals lower than in the soil.

Where exactly is in the plant the heavy metal stored?

The data from the AAS of *N. caerulescens* show that the more than 70% of Ni and Zn was stored in the leaves. This seems to be the main storage of the heavy metals. In contrast to that, the stem and the roots had very low concentrations although the metals will be transported through them to the leaves. This argues for the efficient root-to-shoot transport in the xylem

with the water stream and the enhanced uptake of the metals in the vacuoles of leaf cells (Krämer, 2010). Our data from the EDX support these results and also other studies (Frey & Lösch, 2010; Küpper et al., 1999) illustrate that the main storage occur in the epidermis layers of the leaves. The rosette leaves are more preferred than the stem leaves. In *N. goesingense* the average value of Ni and Zn was lower than in *N. caerulescens* despite the also high metal content in the shoot. The reason for this lies in the very high metal content of the upper epidermis layer of the rosette leaves. It could represent older leaves where the heavy metal content is much higher. Similar data was shown by Küpper et al. (1999) for Zn in *N. caerulescens* where the mature leaves have the double of the young leaves.

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