Bachelorarbeit

Titel der Bachelorarbeit

"Cesium uptake of *Arabidopsis halleri* from heavy metal polluted soil"

Verfasser

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Contents

Abstract

By comparing leaf and root cross sections the heavy metal uptake from *Arabidopsis halleri*, usually an accumulator plant for many heavy metals becomes examined for the toxic chemical element Cesium. Therefore electron dispersive X-ray microanalysis (EDX) was used to determine eventually appearing aggregations of the target element whether in epidermal or mesophyll cells of leaves or in the cortex or the central cylinder of roots. Additionally a dot mapping of leaf hairs was used to show the composition of these structures and may find some Cesium deposits in the trichomes. By looking at the results *A. halleri* belongs to the group of accumulators concerning Cesium, by definition of Baker (1981) and Baker & Brooks (1989). Furthermore Cesium is also absent in the leaf hairs which differentiates them from Zinc and Cadmium polluted samples.

Keywords

Arabidopsis halleri, Cesium pollution, EDX, heavy metal stress

Introduction

Arabidopsis halleri (L.) is a perennial plant, which occurs in disjunctive areas of Europe and eastern Asia. This species is a member of a family called Brassicaceae and can be separated into different subspecies. The most common subspecies in Europe is *A. halleri* ssp. *halleri*, whereas *A. halleri* ssp. *ovirensis*, *A. halleri* ssp. *dacica* and *A. halleri* ssp. *tatrica* are typically encountered in more restricted areas like the Alps, the Tatra or the Carpathian mountains. In Japan or Taiwan *A. halleri* ssp. *gemmifera* is the most widespread subspecies (Clauss & Koch 2006).

Populations where often found at grassy meadows, forest margins but also at rocky slopes at altitudes above 600 m in Europe and in a range of sea level until 2600 m in Asia. These stoloniferous plants can grow up to 20-65 cm and show a basal rosette of orbicular to broadly ovate leafs. They are featured with a pinnatifid to lyrate pinnatifid margin and 1- or 2-forked trichomes. Different shaped stem leafs which are ovate to oblong, decrease in length from the bottom to the top of the stem. Flowers with white, lilac or purplish petals as well as linear and 1-2 cm long siliques were formed from June till August (Al-Shehbaz & O'Kane Jr 2002).

This species prefers to grow on fresh, sandy and oligotophic soils which can be contaminated with a very high amount of heavy metals (Bert et al. 2000).

To handle this rather lethal environment plants developed different strategies. At the one hand they can react with exclusion of the heavy metals through restriction of their root to stem transport. This leads to a stable concentration of the heavy metal elements in the shoot over a wide range of contamination concentrations in the soil. At the other hand some plants are able to accumulate heavy metals, this leads to a high concentration of them in the upper parts of the plant even in soils which contain only a small amount of these elements. (Baker 1981)

A third pathway to cope with these environmental conditions is called hyperaccumulation and is practiced only by a small number of plants. They take up an extremely high amount of heavy metals and prefer to store them in their shoots (Baker & Brooks 1989). Baker & Brooks (1989) defined plats as hyperaccumulating when they accumulate more than 1% Zinc or Manganese, 0.1% Nickel, Copper, Cobalt and Lead or 0.01% Cadmium in their

overground parts, in relation to their dry mass (when growing on their native soils).
In this study the dealing of *A. halleri* with Cesium contaminated soils gets obtained. Therefore they were cultivated in soils with different concentrations of Cesium but at the same climatic conditions.
It is already known that *A. halleri* is a hyperaccumulator for Zinc (Macnair et al. 1999) and Cadmium (Huguet et al. 2012) while it excludes Copper and Lead (Dahmani-Muller et al. 2001).
To investigate, if these plants accumulate or where they store the Cesium, taken up from polluted soil, Energy-dispersive X-ray spectroscopy (EDX) as well as Dot-mapping were used.

Materials and Methods

Cultivation and first preparation of the samples

As samples for this experiment seeds of *Arabidopsis halleri* were collected in Arnoldstein, Austria.
After imbibition of gibberellic acid (10%) and ethanol (70%) in distilled water for three days, the seeds were germinated in Petri dishes filled with vermiculite in the dark for four days. To prevent them from desiccation, the substrate was moistened by deionized water. After three more weeks in a growth chamber, three seedlings at a time were put into plastic pots filled with 500g vermiculite. Afterwards they were transferred into 1 M Nitsch's nutrient solution that consists of 0.95 M KNO_3, 0.72 M NH_4NO_3, 0.22M $CaCl_2*2H_2O$, 0.18 M $MgSO_4*7H_2O$, 0.027 M $FeSO_4*7H_2O$, 0.025 M $MnSO_4*4H_2O$, 0.01 M $ZnSO_4*7H_2O$, 0.01 M H_3BO_3, $2.5*10^{-5}$ M $CuSO_4*5H_2O$, $2.5*10^{-4}$ M $Na_2MoO_4*2H_2O$ and 0.037 M Na_2EDTA. Furthermore stable Cesium was added as Cs_2SO_4 in concentrations of 2 mM and 20 mM.
For the following two months they were then cultivated hydroponically under controlled conditions. The exchange of the Cs solution was done once per ten days, already transpired water gets replaced by distilled water.
After that time, the separated plant organs were air dried for further investigations.

Preparation for Energy Dispersive X-ray microanalysis (EDX) and Dot-mapping

The previously dried leaf and root samples were cut into fine sections with razorblades and afterwards fixed on an Aluminum-stub with Carbon-foil. Also precisely removed leaf hairs

were put on stubs for following measurements. For being able to differentiate the samples it is important to mark the stubs with a number and note which sample is mounted on it. Furthermore it is important to place the slices in a way that the electron beam is able to scan the cross section of the sample.
To be sure, that there are enough sections in the right position it is necessary to prepare two stubs per sample with three to five slices mounted on each.
To avoid charging effects, all stubs were put into a Carbon Thread Evaporation Device (Leica EM MED020. Leica Microsystems GmbH, Germany). Three short pulses of high voltage lead to the evaporation of pure Carbon from the two times coiled threads to a 20 nm thin Carbon layer above the sample surface. Because this device produces a high vacuum before the coating process starts it is important to use dried samples. To be sure that there are no liquids left in the sample they were put in an oven at low temperature of about 40°C for at least one hour. After coating, the samples were ready for observation. Root and leaf cross sections of the control group and from Cesium polluted soils (2 mM and 20 mM) get prepared in this way as well as leaf hairs from the 20mM Cs treatment.

EDX with the Scanning Electron Microscope (SEM) Philips XL20

After venting the sample chamber, the already prepared stub gets mounted on the sample holder with a special formed forceps and liquid nitrogen gets filled into the Dewar of the EDX-detector. This step is needed to cool down the detector and the Field Effect Transistor (FET) for enhancing the resolution capacity of the device by reducing the mobility of Lithium. These two units were located in a vacuum which is separated from the vacuum in the sample chamber by a Beryllium window (Morgan 1985).
This part of the detection unit is available in different qualities: On the one hand as an 8 µm thick window, that filters out the signals of elements which were lighter then Sodium (With less than 2 keV), from atomic number 15 (Phosphorous) and above the transmission reaches 100%. Or on the other hand the Super Ultra-Thin Window that allows the measurement of all elements which are at least as heavy as Boron with the element number 5, unfortunatly they are more sensitive. (Warley 1997) To produce a stable high vacuum ten to fifteen minutes have to pass. If the device shows that the vacuum is ready, the operator is allowed to turn on the high tension. After localization of a definite tissue the working distance gets adjusted to 12 mm by using the focus function of the microscope. To ensure consistence during all measurements, this parameter is not allowed to be changed. To enhance the acuity of the images from now on, only the z-Axis is used for focusing

(because here the working distance stays at 12 mm). While looking at the software EDAX Genesis (EDAX Inc., USA) the dead time gets regulated to a value of around 30% by changing the spot size of the SEM.
When all these parameters were checked the 100 sec lasting measurement can be started by clicking collect in the EDX software. After finishing the data collection, all peaks were assigned to a specific element by using the EDX software. This step was followed by adjusting the background of the measurement. This is important to produce reliable results for the semi-quantitative EDX-analysis.

Dot mapping with the SEM Phillips XL20

For this method already prepared leaf hairs of *A. halleri* (by Anna Burger) were used. As well as the other samples they became carbon coated before the measurement. After putting the stub into the sample chamber up to 16 chemical elements were able to get measured at the same time to receive an element distribution pattern. For this specific sample Calcium, Cesium, Oxygen, Phosphorous and Sulfur were chosen. When the measurement gets started, the device produces an element distribution for each of the elements and marks their appearance with a colored dot. Because of the high number of measurements that are needed for this analysis one run takes about two to three hours or longer when more chemical elements were chosen. After the measurement is finished, it is possible to combine the dot map of every element with the prior taken SEM picture to determinate where the elements of interest are located exactly in the leaf hair. This measurement takes so much time, because the apparatus produces a whole EDX measurement for each dot that later on appears on the screen.

Results

The following table (Table 1) is showing the raw data which were received from the EDX measurements on SEM Phillips XL20 and processed by the software EDAX Genesis (EDAX Inc., USA). Beside the values of this table, also the percentage of Carbon, Oxygen, Sodium, Magnesium, Aluminum, Silicon, Phosphorous, Sulfur, Chlorine, Potassium, Calcium, Iron, Copper and Zinc were obtained but they are not necessary in this context. After exporting the data in Microsoft Office Excel 2007 (Microsoft Corporation, USA) they were standardized.

Table 1: Percentage of Cesium (Cs) in various plant tissues of *A. halleri* in weight and atomic percent

	Epidermis		**Mesophyll**		**Cortex**		**Central cylinder**	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
20 mM Cs	0.42	0.04	0.41	0.04	2.03	0.22	0.63	0.06
	0.15	0.01	0.37	0.04	3.39	0.39	0.30	0.03
	1.41	0.16	2.08	0.25	3.15	0.35	0.58	0.06
	0.65	0.07	2.08	0.24	0.78	0.08	0.28	0.03
	0.3	0.03	0.89	0.1	0.61	0.06	0.43	0.04
2 mM Cs	0.34	0.03	0.28	0.03	0.22	0.02	0.28	0.03
	0.38	0.04	0.18	0.02	0.20	0.02	0.18	0.02
	0.35	0.04	0.22	0.02	0.82	0.12	0.24	0.02
	0.40	0.04	0.28	0.03	0.19	0.02	0.26	0.03
	0.43	0.05	0.38	0.04	0.24	0.02	0.30	0.03
Control	0.00	0.00	0.07	0.01	0.04	0.00	0.10	0.01
	0.03	0.00	0.03	0.00	0.05	0.00	0.09	0.01
	0.09	0.01	0.33	0.05	0.00	0.00	0.12	0.01
	0.06	0.01	0.04	0.00	0.04	0.00	0.09	0.01
	0.03	0.00	0.13	0.01	0.02	0.00	0.00	0.00

For further calculations square root of the proportions ($x_{sqrt} = \sqrt{x/100}$) were transformed in angular degrees$x_{ang} = \sin^{-1}(x_{sqrt}) * \frac{180}{\pi}$. To define upper and lower limits of the confidence region (Table 2) following formula were used: $x_{UL} = x_{ang} + t * SE$ for the upper limit and $x_{LL} = x_{ang} - t * SE$ for the lower limit of the confidence interval ($SE = Standard\ Error = SD/\sqrt{n}$ where SD stands for standard deviation and n for the sample size). For the following figures the calculated parameters were again retransformed using at first the formula $x_{sqrt} = \sin x_{ang} * \frac{\pi}{180}$ to receive radian values

again, followed by a squaring of x_{sqrt} and a multiplication with 100 the values are back in their starting position with additional confidence limits which cover 95% of the measured values.

Table 2: Mean, upper and lower confidence interval (CI) in the three Cs treatments of *A. halleri*

	Epidermis		**Mesophyll**		**Cortex**		**Central cylinder**	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
20 mM Cs								
Mean	0.51	0.05	1.03	0.12	1.80	0.20	0.43	0.04
CI high	1.20	0.14	2.33	0.28	3.80	0.43	0.65	0.06
CI low	0.11	0.01	0.25	0.02	0.53	0.05	0.26	0.03
2 mM Cs								
Mean	0.38	0.04	0.26	0.03	0.30	0.03	0.25	0.03
CI high	0.43	0.05	0.36	0.04	0.64	0.09	0.31	0.03
CI low	0.34	0.03	0.18	0.02	0.09	0.01	0.20	0.02
Control								
Mean	0.03	0.00	0.10	0.01	0.02	0.00	0.06	0.01
CI high	0.10	0.01	0.27	0.04	0.07	0.00	0.18	0.02
CI low	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00

The retransformed data was then used for designing different figures with the software IBM SPSS Statistics 20 (International Business Machines Corporation, USA) to visualize the discrepancies between the three approaches. In Figure 1, 2, 3 and 4 the weight percent (mass of the atoms of interest in relation to the total mass) of Cs in the four observed tissues were shown for each treatment, in Figure 5, 6, 7, 8 the atomic percent were shown (percentage of atoms of interest in relation to the total amount of atoms).

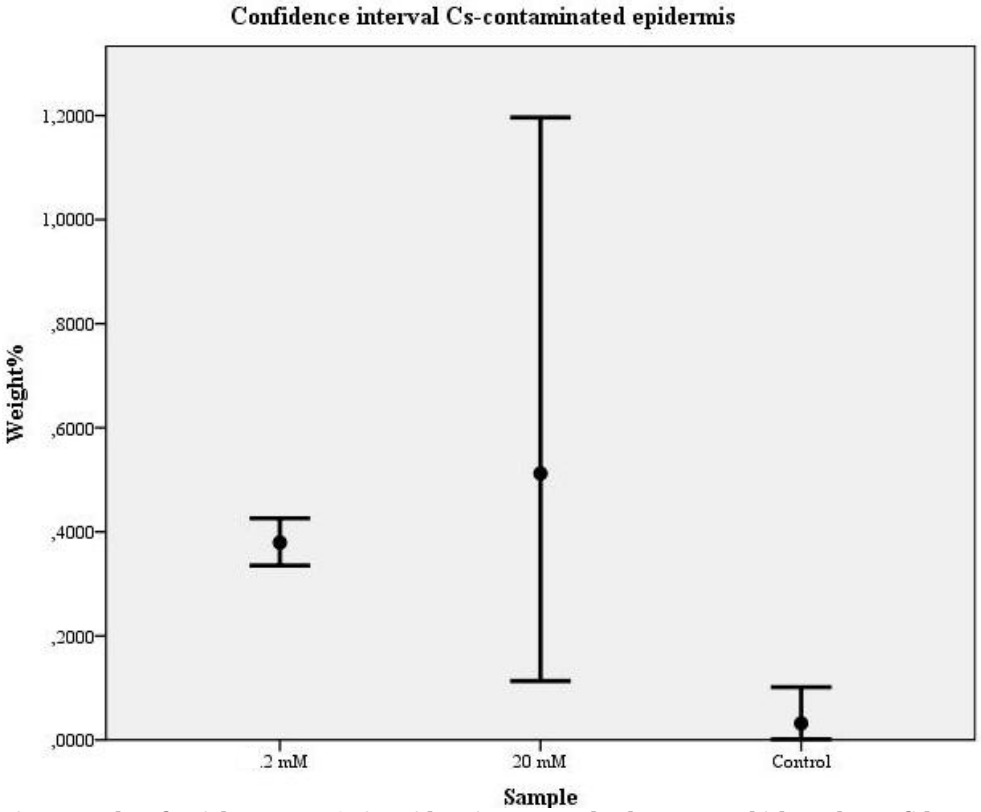

Figure 1: Plot of weight percent Cs in epidermis; Dot marks the mean, Whiskers the confidence interval

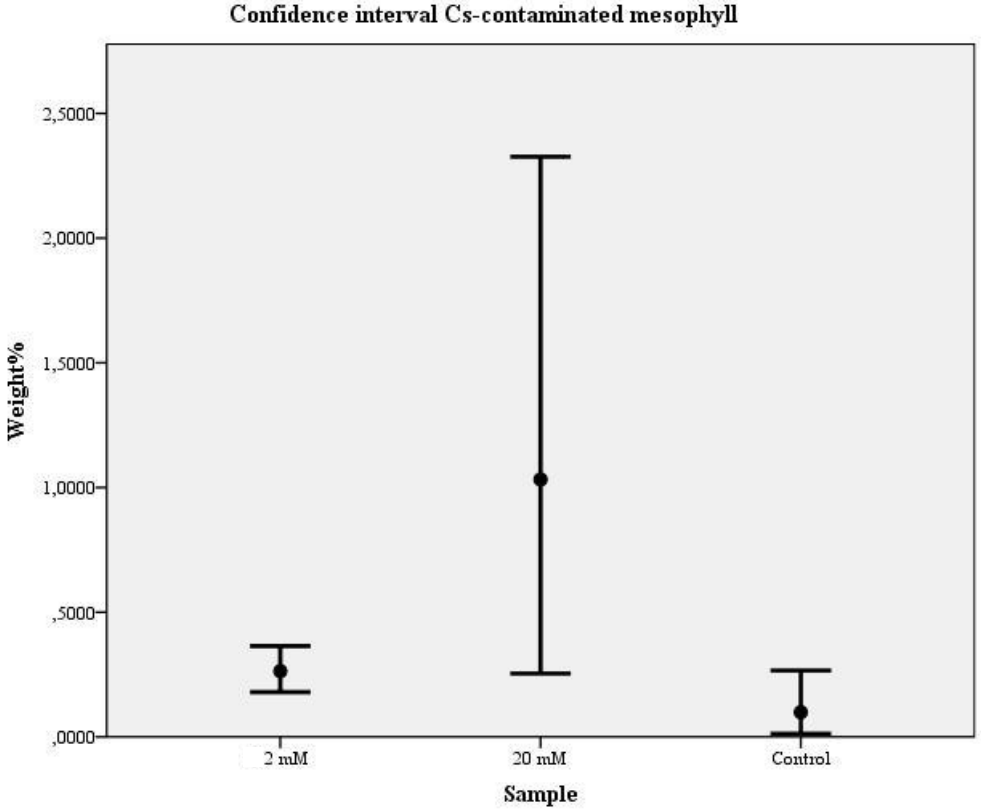

Figure 2: Plot of weight percent Cs in mesophyll; Dot marks the mean, Whiskers the confidence interval

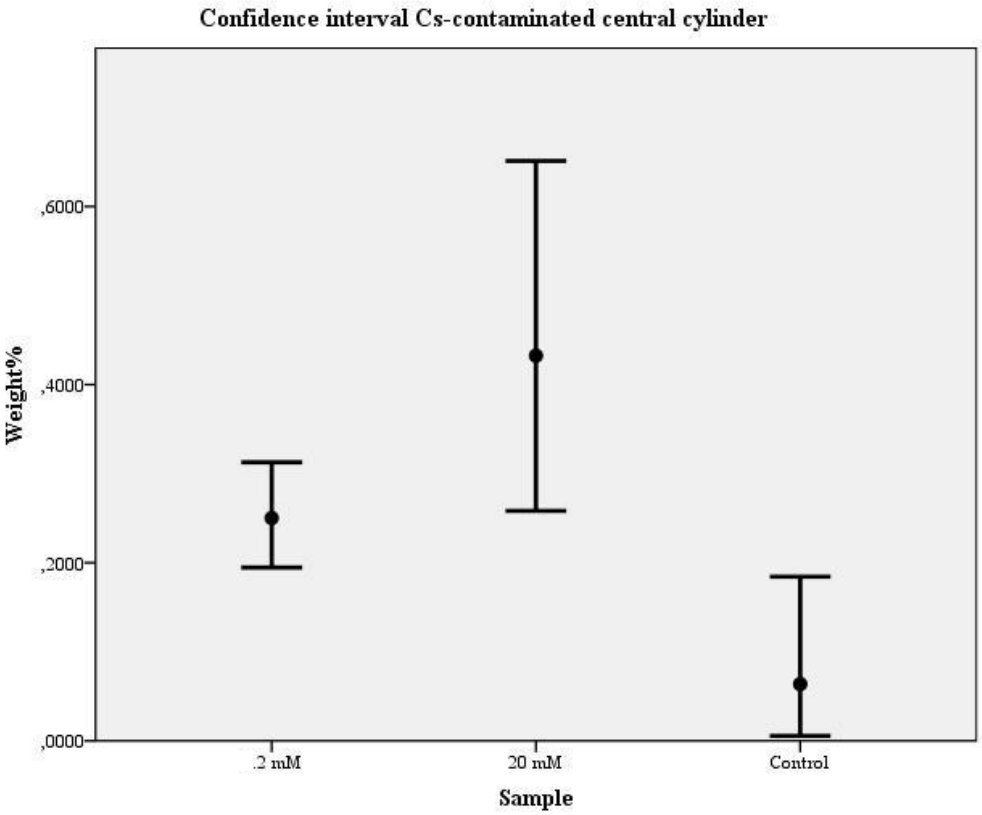

Figure 3: Plot of weight percent Cs in cortex; Dot marks the mean, Whiskers the confidence

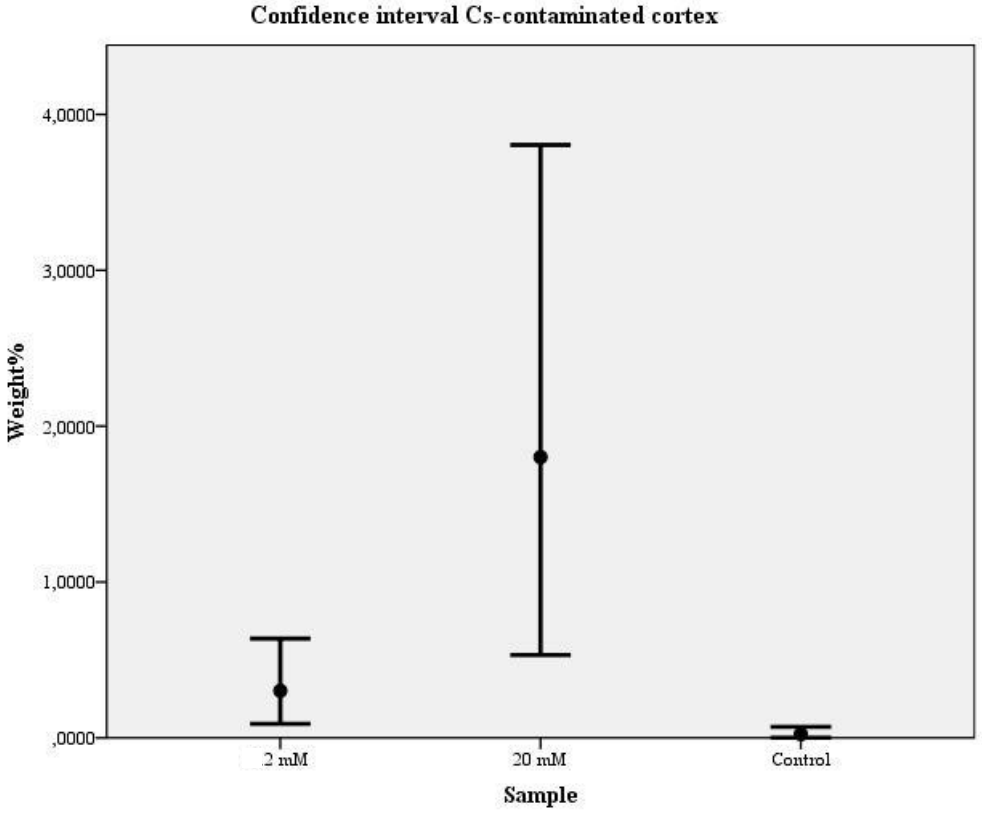

Figure 4: Plot of weight percent Cs in central cylinder; Dot marks the mean, Whiskers the confidence interval

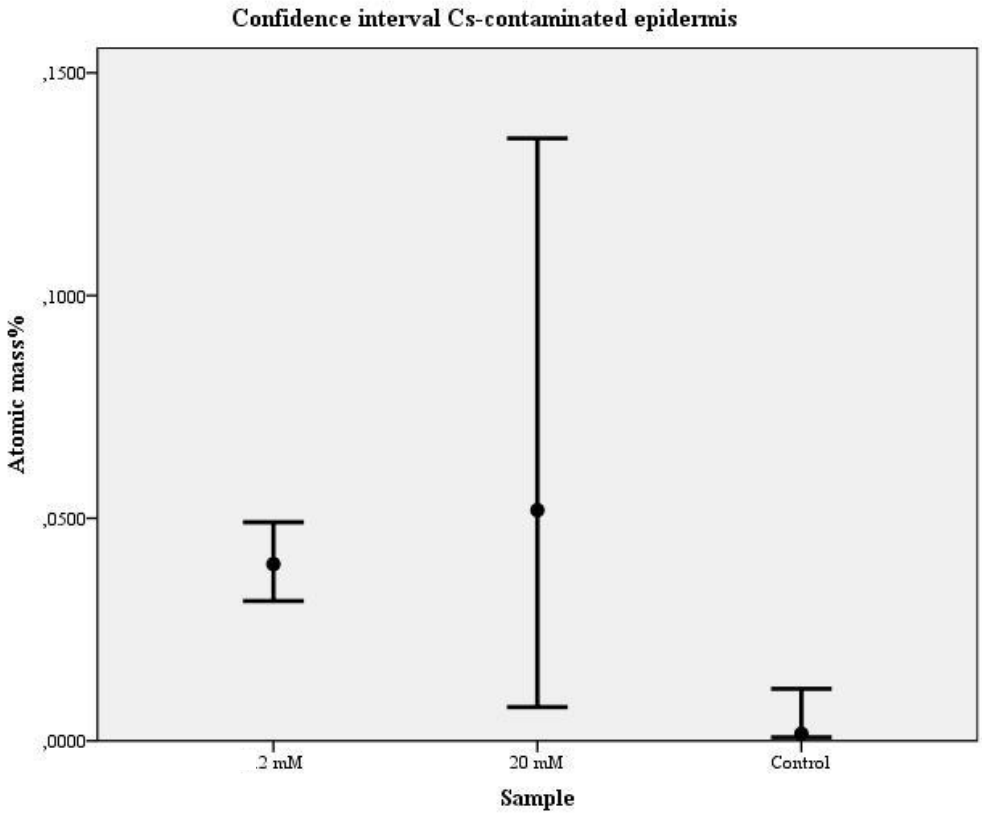

Figure 5: Plot of atomic mass percent Cs in epidermis; Dot marks the mean, Whiskers the confidence interval

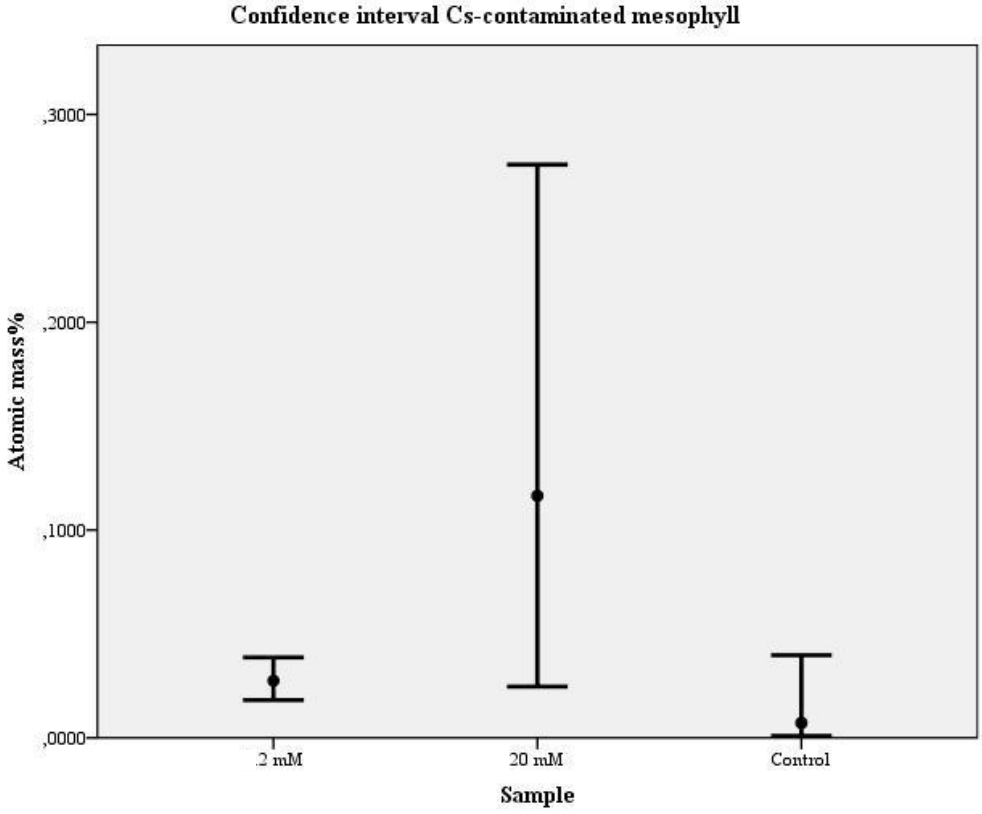

Figure 6: Plot of atomic mass percent Cs in mesophyll; Dot marks the mean, Whiskers the confidence interval

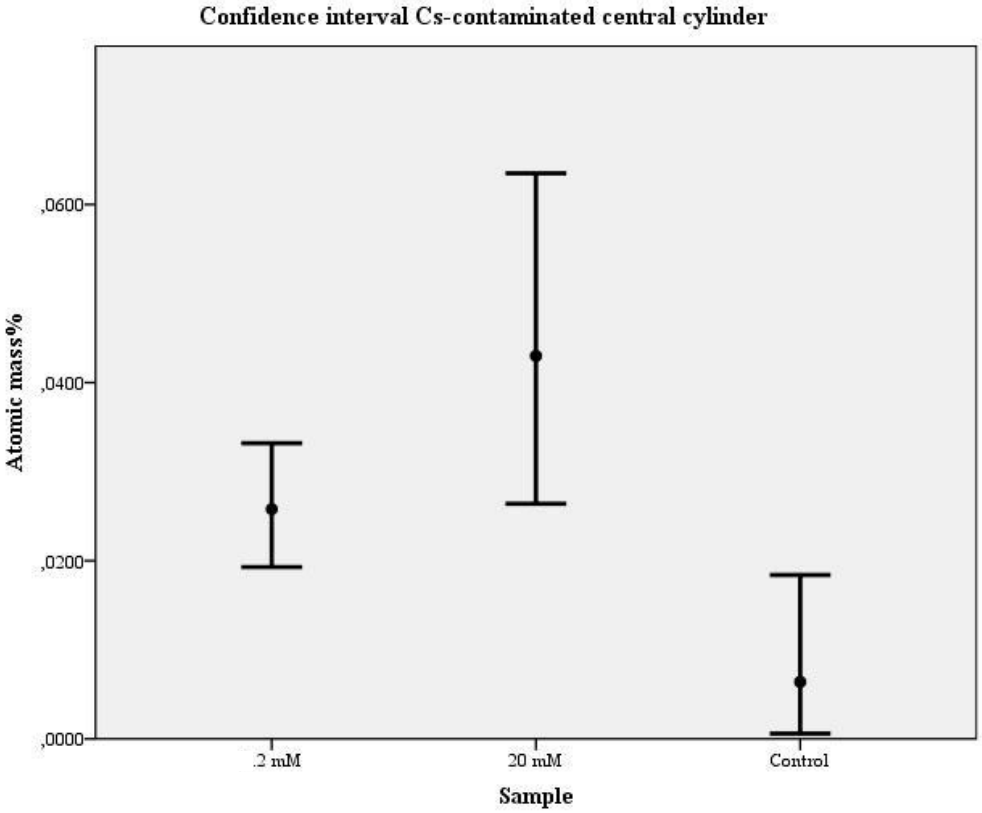

Figure 7: Plot of atomic mass percent Cs in cortex; Dot marks the mean, Whiskers the confidence

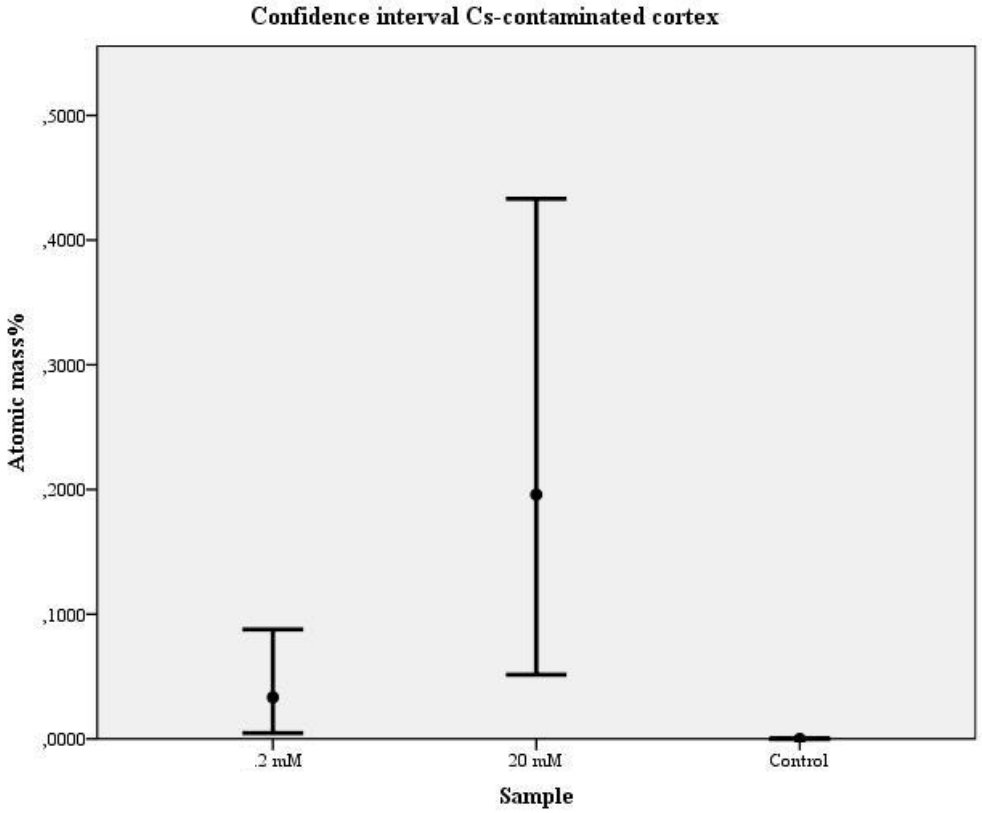

Figure 8: Plot of atomic mass percent Cs in central cylinder; Dot marks the mean, Whiskers the confidence interval

For a better ability of comparing the calculated means, they were plotted on two extra diagrams which were shown as Figure 9 and 10.

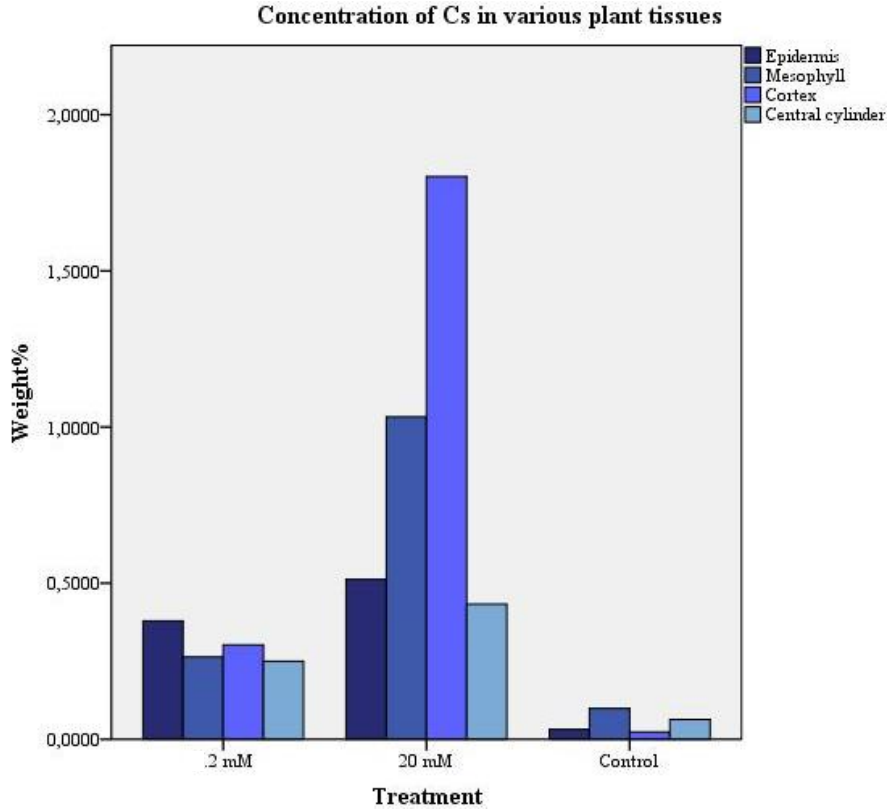

Figure 9: Comparison of the mean Cs-concentration in weight percent for each

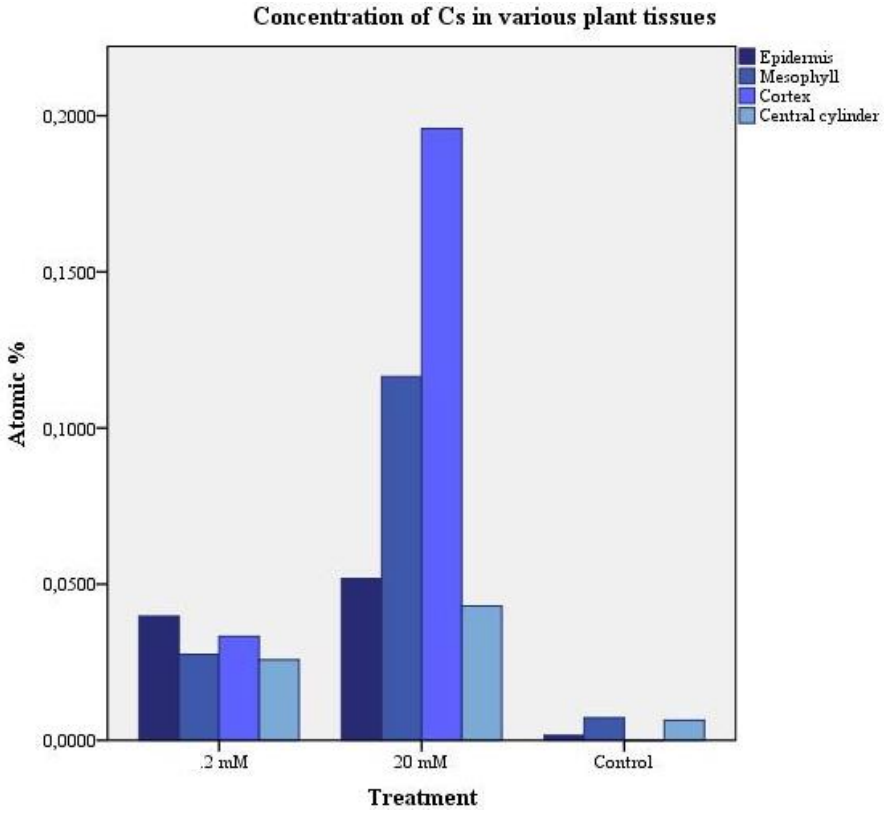

Figure 10: Comparison of the mean Cs-concentration in atomic mass percent for each treatment

The following Figure 11 shows the results, received from the dot mapping of an *A. halleri* leaf hair (20 mM Cs polluted). The visible element distributions of the other elements (which are not Cesium) are not in focus of this experiment. For producing these images also EDAX Genesis (EDAX Inc., USA) was used.

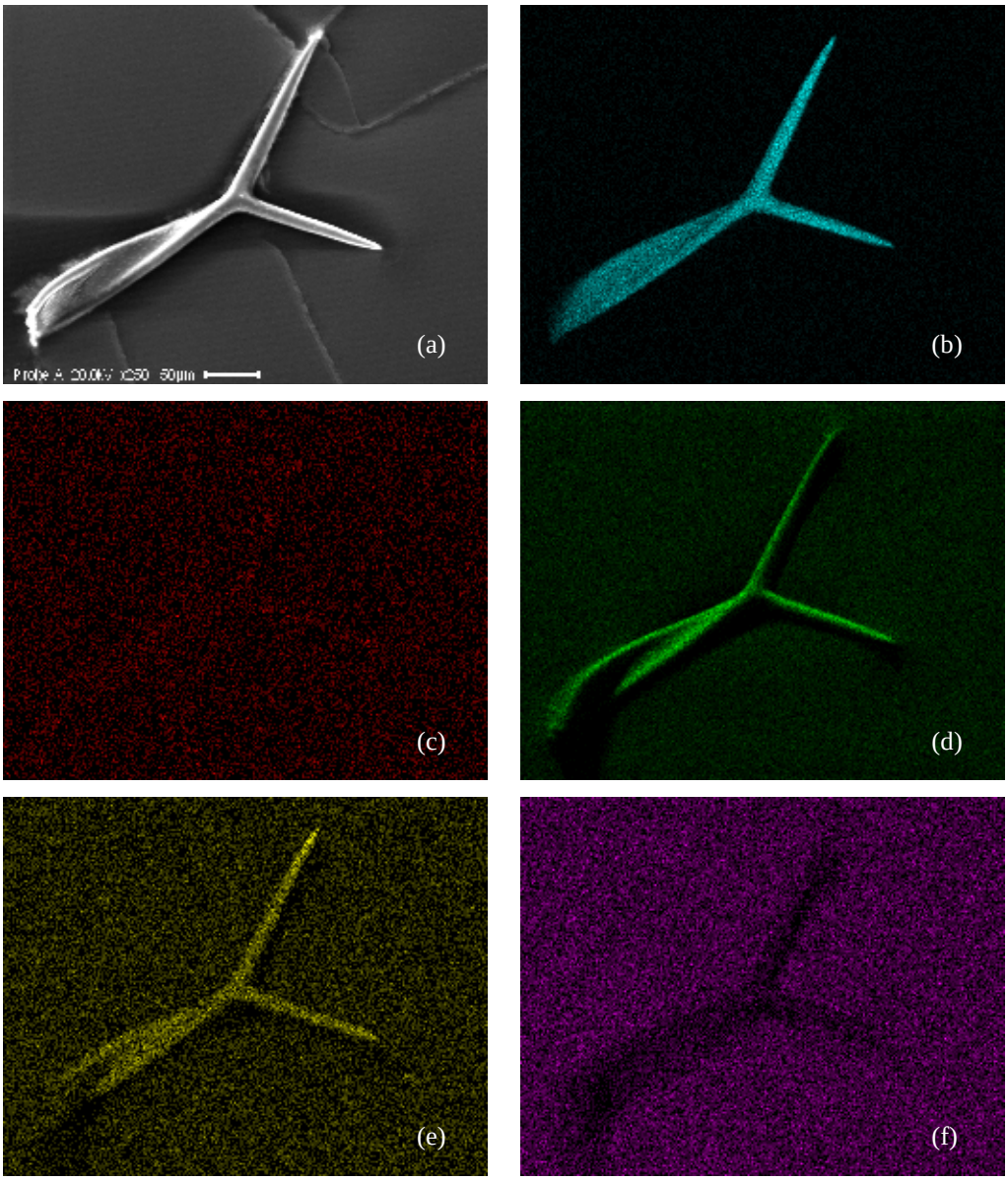

Figure 11: Dot mapping of A. halleri leaf hair (20 mM Cs); (a) standard image of Leaf hair, 20 kV, 250x; (b)Ca-, (c) Cs-, (d) O-, (e) P- and (f) S- distribution

Discussion

By looking at the figures 1 to 8 it is shown, that the main differences between the three approaches are visible by comparing the cesium treated plants and the control. But there are no significant differences between the different cesium concentrations. To obtain closer confidence intervals it would be necessary to make more measurements with every tissue to get more informative data.

But there are some different tendencies between the treatments concerning the storage of this heavy metal. While the plants of the 2 mM treatment are storing them more often in the epidermis of their leafs, the individuals from the 20 mM treatment prefer to store them in the cortex of their roots followed by the deposition in the mesophyll (Figure 9 and 10).

By comparing the mean values of the cesium polluted treatments in figure 9 and 10 there are visible differences in the concentration of this element in some plant tissues but these differences are not necessarily proportional to the concentration variation between the two approaches. At cortex and central cylinder the percentage of cesium is at least ten times higher in the 20 mM treatment than in the 2 mM treatment. At the epidermis and the mesophyll this is not the case so it can be assumed, that the plant tolerates cesium in the cells of the above ground tissue in a minor concentration than in their root tissues.

If they would hyperaccumulate this heavy metal the concentration in the shoot tissue would be much higher than in the root tissue like in individuals from Zinc polluted soils (Macnair *et al.* 1999) and if they would exclude this element like Copper and Lead (Dahmani-Muller *et al.* 2001) there would not be that high concentration of Cesium in the root tissues.

Concerning the definitions of Baker (1981) as well as Baker and Brooks (1989) this species corresponds most likely to the normal Cesium accumulators. But to verify these results it is also necessary to take more measurements.

The second part of the investigations, the dot mapping of an *A. halleri* leaf hair, results in the pictures seen in figure 11.

Whereas the trichome contains a lot of Calcium, Oxygen and Phosphorous, they do not contain Sulfur or Cesium. While Oxygen and Calcium were distributed all over the leaf hair, Phosphorous is absent at its base. This fits to the experiments of Küpper, et al. (2000) but in contrast to Cesium, Zinc and Cadmium are associated with the basal structure of the trichome because of their atomic similarities.

The high content of Calcium and Oxygen is maybe an evidence for Calcium carbonate

incorporations in the cell wall, which could lead to a protective function against tiny herbivores.

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