

# Potential of *Brassica juncea* and *Helianthus annuus* in phytoremediation for uranium

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**Abstract.** To investigate the efficiency of the phytoremediation of uranium-contaminated soil by *Brassica juncea* and *Helianthus annuus*, greenhouse experiments with both plant species and soil from the “Fuhrberger Feld” (near Hanover, Germany) with defined enhancement of the uranium concentrations up to approximately 6 mg kg<sup>-1</sup> have been carried out. Part of the test plants was treated with citric acid buffer (pH =4.8). Plants of both species were grown in soil from the “Fuhrberger Feld” without any treatment (control plants). The plants were harvested 13 weeks after settling and the uranium concentration in shoots and roots and the remaining soil was measured. The uptake of uranium into the shoot was clearly lower than into the roots. Uranium concentration in the shoots of the control plants was below the detection limit. The addition of the citric acid buffer caused an enhancement of the uranium concentration in the soil water by factors of up to approximately 80. A visible effect of this enhanced concentration on the uptake into the plant and on the distribution on shoot and root was only shown for *Brassica juncea*, where the concentration in the shoot doubled. The reverse effect was shown for *Helianthus annuus*, where the citric acid buffer treatment caused a lower concentration in roots and shoots compared to the untreated soil with the same uranium concentration.

Generally, the uranium uptake into the plants was very low ( $<1 \mu\text{g}$  per plant) for all experiments.

The results of these plant experiments point out that the effectiveness of uranium uptake into plants cannot be increased by citric acid buffer treatment in every case, even if the uranium concentration in soil solution is increased considerably. No decrease of uranium concentration in soil due to the uptake into plants could be shown. Thus a special suitability of *Brassica juncea* and *Helianthus annuus* for phytoremediation could not be proven.

## Introduction

Mankind affects the cycles of many elements and compounds. These changes become important, if they cause an endangerment for humans or the basis of human existence. In the last decades the investigation of uranium in soils and in ground waters gained importance due to its potential hazard. Thereby especially the chemotoxic effect of uranium is relevant, since uranium compounds cause damages of liver and kidneys among others (Merkel and Dudel 1998).

Since uranium, like all metals, is not degradable, its entries in soils or ground waters should be reduced or prevented and already entered uranium must be removed from the environmental compartments.

An in situ method to remove metals from soils is phytoremediation. Thereby plants take up metals from the soil and accumulate them in their harvestable aboveground organs, mainly in their shoots. The aboveground plant biomass is then harvested and burned, in order to remove the contaminants permanently from the soil (Cherian and Oliveira 2005)

These practices are suitable for treatments of large areas with minor concentrations of impurities (Negri and Hinchman 2000). The success of phytoremediation depends on different factors such as a sufficient production of biomass by the plants in which they have to enrich high quantities of the metals. Indeed finding a suitable plant for removing metals is not that easy, because most of the known hyperaccumulator plants have small biomasses (Baker and Brooks. 1989). In addition these plants need to be plantable and harvestable with agricultural methods. Also they should mainly accumulate the hazardous metals in large quantities (Blaylock et al. 1997).

Uranium which is sorbed at clay minerals and humus particles, as well as uranium in connection with the Fe-Mn-oxides is immobile and therefore not available for plants. However, roots can mobilize metals by acidifying with citric acid or other organic acids, changes of redox conditions or formation of organic complexes (Alloway 1999). These so called chelates increase the fraction of dissolved metals in the soil solution. Chelates can make insoluble cations plant available,

they increase there transport to the plant roots and supply more of the already absorbed cations (Shahandeh and Hossner 2002b).

The uptake of actinides from soils by terrestrial plants is considered as low. Thereby the enrichment of uranium in plants occurs particularly in their roots (Hossner et al. 1998).

*Helianthus annuus* (Annual sunflower) and *Brassica juncea* (Indian mustard) show a high ability for the accumulation of uranium in comparison with other plants (Ebbs et al. 1998, Huang et al. 1998, Shahandeh and Hossner 2002, Chang et al. 2005). However, prior examinations were accomplished mostly with high soil uranium contents ( $>100 \text{ mg U kg}^{-1}$  soil) or non soil typical substrates (Schönbuchner 2002). No publications were available about soils with lower uranium contents, wherefore Phytoremediation is mainly applicable in practice. Therefore, a controlled greenhouse experiment was set up to investigate how much uranium can be taken up by the plants with different soil uranium content and how the addition of citric acid buffer affects the uptake of uranium.

## Methods

The goal of this work was to determine whether soils with uranium contents of slightly over  $5 \text{ mg U kg}^{-1}$  can be regenerated by phytoremediation. The amount of  $5 \text{ mg U kg}^{-1}$  is the tolerance value for a gardening-agricultural use from soils after Eikman and Kloke (1993).

**Table 1.** Overview over the most important treatment steps for investigation of the potential for Phytoremediation of *Brassica juncea* and *Helianthus annuus*

time [d]	
0	sowing of the seeds into a planting bowl
11	Sorting of the plants at the two leaves state into a planting pallet <sup>1</sup>
31	Repot into planting containers with a diameter of 11 cm - volume about $0.8 \text{ l}^1$
51	Repot into planting containers with a diameter of 20 cm - volume about 5 l arrange plants over control treatments and prepared soils
53	Installation of the suction cups in the planting pots and beginning of the soil water sampling
70	1. citric acid buffer treatment
73	2. citric acid buffer treatment
77	Harvest

<sup>1</sup>soil was mixed with Basacote Plus 9M- fertilizer (fertilizer contents: 16% N, 8%  $\text{P}_2\text{O}_5$ , 12%  $\text{K}_2\text{O}$ , 2% MgO, 5% S, 0.02% B, 0.05% Cu, 0.4% Fe, 0.06% Mn, 0.015% Mo, 0.02% Zn) to avoid any kind of nutrient limitation

Therefore two plant species *Brassica juncea* and *Helianthus annuus* were planted in sandy farmland soil from northern Germany (Fuhrberger field), which has an uranium content of  $0.96 \text{ mg kg}^{-1}$  soil due to the application of uranium containing P fertilizers. A natural soil should be used, because artificial substrates have clearly different sorption characteristics.

The plants were grown in a greenhouse under natural light conditions (temperature range  $7\text{-}39 \text{ }^\circ\text{C}$ ). The most important vertices of the procedure are held in table 1

After 51 days 12 plants of each species were set in 5L pots, whereby 8 of 12 plants were set in soils to which  $5 \text{ mg}$  uranium per kg soil was added. Uranium was added as uranyl acetate dihydrate solution ( $(\text{CH}_3\text{COO})_2\text{UO}_2 \cdot 2 \text{ H}_2\text{O}$ ). Additionally  $3.7 \text{ mg P}$  per kg soil was added as dipotassium phosphate ( $\text{K}_2\text{HPO}_4$ ) to simulate the input of uranium via fertilizing. In each case 4 plants were grown with the same treatment: soil with natural uranium content, soil with additional uranium, soil with additional uranium and citric acid treatment (Table 2). Plants were grown for 20 days in the different soils.

Citric acid buffer was chosen because it is environmentally harmless and completely naturally degradable as well as citric acid has shown a relatively constant effectiveness in removing metals (Francis and Dogde 1998). In previous studies the addition of citric acid to the soils has led to a strong increase of the uranium uptake into the plants (Huang et al. 1998, Chang et al. 2005).

After 70 days of growing 250 ml of citric acid buffer solution was added to 4 of the pots of *Brassica juncea* and *Helianthus annuus* respectively. The amount of solution added should not correspond to more than 5-10 Vol% of the soil in order to prevent a leakage of solution. Since citric acid has a pH value below 3 and such a pH value causes damages to plant roots a citric acid buffer solution with  $4.8 \text{ mmol l}^{-1}$  citric acid ( $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ ) and  $5 \text{ mmol l}^{-1}$  trisodium citrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2 \text{ H}_2\text{O}$ ) with a pH value of 4.8 was used.

It is assumed that plant metabolism changes after the bloom time to a smaller transpiration. Therefore blooms of both plant species were cut, those of *Brassica juncea* twice, and those of *Helianthus annuus* were only removed once.

**Table 2.** Different plant treatments after 50 days of growing in farmland soil for the investigation of the potential for phytoremediation

<i>Brassica juncea</i> (Indian mustard)	<i>Helianthus annuus</i> (Annual sunflower)
● 4 x control (farmland soil with approx. $1 \text{ mg/kg}$ )	● 4 x control (farmland soil with approx. $1 \text{ mg/kg}$ )
● 4 x farmland soil + approx. $5 \text{ g U/ kg}$ and $3.7 \text{ mg phosphate/ kg}$	● 4 x farmland soil + approx. $5 \text{ g U/ kg}$ and $3.7 \text{ mg phosphate/ kg}$
● 4 x farmland soil + approx. $5 \text{ g U/ kg}$ and $3.7 \text{ mg phosphate/ kg}$ + two times citric acid buffer treatment	● 4 x farmland soil + approx. $5 \text{ g U/ kg}$ and $3.7 \text{ mg phosphate/ kg}$ + two times citric acid buffer treatment

Plants were harvested after 77 days, 4 days after the second citric acid treatment, since the highest uranium uptake should take place up to then, according to literature (Huang et al. 1998). Plants were cut off 1 cm above soil surface, weighed (leaves, stem and roots separately) and dried afterwards at 70°C to 80°C. Roots were washed carefully before drying.

Plants samples were milled with a ultra centrifugal mill (Retsch ZM 1000, Speed 10000 rpm) and sieved to <0.63 µm. To quantify the amount of uranium absorbed by the plants uranium contents per g plant (dry weight) were examined after digestion according to DIN EN 13805. To control the completeness of the digestion the reference material Virginia Tobacco Leaves (CTA-VTL-2) from Poland was used.

To investigate the mobility of uranium in the soil solution, soil water was sampled with suction cups (Al<sub>2</sub>O<sub>3</sub>-cups, Haldenwanger, Waldkraiburg, Germany), which were conditioned before with 8.44 µg l<sup>-1</sup> uranyl nitrate solution. The soil solutions of the 4 pots with the same treatment were sampled in one bottle. When a sufficient amount of sample was present, uranium content and pH values of the soil solution were measured.

To control the distribution of uranium in the soils with added uranium 3 soil samples were examined for their uranium content after digestion. Also the used fertilizer was investigated for its uranium content. The samples were dried at 70 to 80°C, milled, sieved to <63 µm and digested by aqua regia dissolution according to DIN EN 13346 (2001-04).

The concentration of trace elements was measured in the soil solutions as well as in the solutions from digestion of plants, soil and fertilizer. Trace elements concentrations were determined with the ICP-MS (VG Elemental, Winsford, England). The detection limits for uranium were between 0.006 and 0.009 µg l<sup>-1</sup>.

## Results

### Uranium concentrations and pH in soils

Uranium concentrations of three sub samples of the soils with added uranium differ strongly (4.7-18.6 mg kg<sup>-1</sup>). The average value was 10.4 mg U kg<sup>-1</sup> soil. The different uranium concentrations seem to have no influence on the uranium concentration in the soil solution (see Uranium contents in soil solution). The fertilizer used contained 4.0 mg U kg<sup>-1</sup>.

The amount of the sampled soil solutions was prone to strong fluctuations. Therefore it was not possible to get constantly enough soil solution for pH measurement. Generally the pH values of the soil solutions in the pots without treatment with citric acid buffer were within the range of 5.2-7.1 for *Helianthus annuus*, 5.8-6.8 for *Brassica juncea*. There was no apparent pH difference between the solutions of the soils with different uranium concentrations.

A growth of algae was determined in some of the soil water sample bottles 14 days after beginning of the sampling. Since the algae could affect the water characteristics due to metabolic procedures, they were rinsed out immediately and slight remaining remnants in the bottles were removed mechanically.

After the first treatment with citric acid buffer the pH value of the soil solutions of both plant species increased to 7.4 for *Helianthus annuus* and 7.3 for *Brassica juncea*. Three days after the second citric acid buffer addition, the pH values in the soil of both plant species amounted 6.6 and was higher than for the parallel treatment without citric acid buffer. In addition to the increasing pH value the citric acid buffer led to a staining of the soil water by dissolved humic acids.

Because of defects of the suction cups soil solutions could not be sampled regularly, three times there was not enough sample material for the measurement of the uranium concentrations. The soil solution of the *B. juncea* control treatment had uranium concentrations in the range of 0.2-1.5  $\mu\text{g l}^{-1}$ , those of *H. annuus* in the range 0.2-2.9  $\mu\text{g l}^{-1}$ . Soil solutions of soils treated with uranyl acetate dihydrate and dipotassium phosphate had uranium concentrations of 0.4-2.5  $\mu\text{g l}^{-1}$  for both species. The uranium concentrations in these solutions were mostly higher than those of the control soils (Table 3).

After the citric acid buffer addition the uranium concentrations of the soil solutions increased. The uranium concentrations in soil solution of *B. juncea* reached a concentration of 19  $\mu\text{g l}^{-1}$  three days after the first citric acid treatment, three days after the second citric acid buffer treatment 76  $\mu\text{g l}^{-1}$ . This is an 8 and 32 time increase of the uranium concentration in the soil solution compared to soil without citric acid buffer treatment.

The uranium concentrations of the soil solutions of *H. annuus* were even higher. The maximum concentrations appeared after the first citric acid buffer treatment (241  $\mu\text{g l}^{-1}$ ), three days after the second treatment the concentration reaches 93  $\mu\text{g l}^{-1}$ , which correspond to an 83- and 32-fold increase, respectively, compared to soil without citric acid buffer treatment.

**Table 3.** Ranges of the uranium concentrations in the soil solution of the different plant treatments and number of measured samples (U = uranyl acetate dihydrate and dipotassium phosphate, C= citric acid buffer)

Treatment	<i>Helianthus annuus</i>		<i>Brassica juncea</i>	
	number of measurements	U in soil solution [ $\mu\text{g l}^{-1}$ ]	number of measurements	U in soil solution [ $\mu\text{g l}^{-1}$ ]
control	3	0.2-2.9	4	0.2-1.5
with U	6	0.4 -2.4	4	0.4-2.5
with U and C	2	93.1-240.9	2	19.3-76.1

## Uranium concentrations of the plants

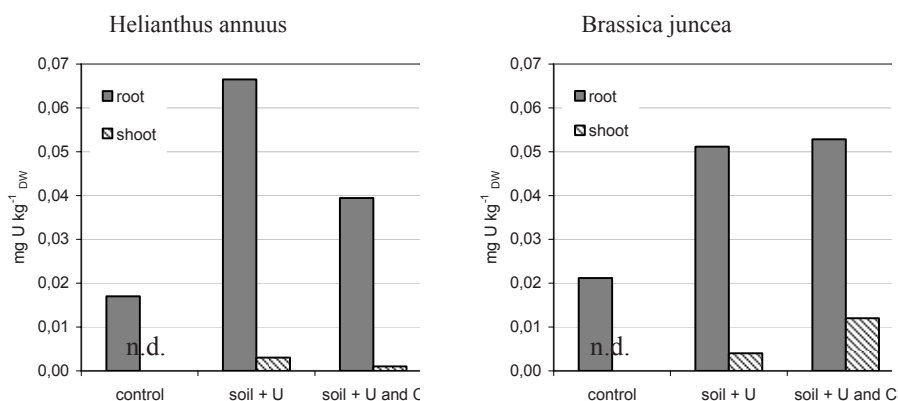
For all plants an uptake of uranium in their roots could be proven. Also the uranium concentrations of the roots were substantially higher than in the shoots (Fig.1).

The uptake of uranium from soils of the control treatments ( $0.96 \text{ mg U kg}^{-1}$  soil) in the roots accounted for both plant species  $0.02 \text{ mg U kg}_{\text{DW}}^{-1}$  on average. Since the average dry weight of the roots of both species was not different, the average uranium content in the roots was the same. An uptake of uranium into the shoots could not be detected for the control plants of both species ( $<7 \cdot 10^{-4} \text{ mg U kg}_{\text{DW}}^{-1}$ ).

Plants which have been grown in soils with added uranium showed a higher uptake of uranium than control plants. Uranium concentrations in the roots of *H. annuus* were on average  $0.07 \text{ mg kg}_{\text{DW}}^{-1}$  and higher than those of *B. juncea* ( $0.05 \text{ mg kg}_{\text{DW}}^{-1}$ ).

For both plant species the addition of citric acid buffer to the soil did not yield any increase of the uranium concentration in the roots. *H. annuus* even had lower uranium concentrations in roots and shoots. However *B. juncea* showed an increased uranium concentration in the shoots.

Even if the uranium concentrations of *H. annuus* were lower than that of *B. juncea* after citric acid treatment, the total amount of uranium in the plants was higher for *H. annuus* ( $0.49 \mu\text{g}$ ) than that of *B. juncea* ( $0.45 \mu\text{g}$ ) due to its larger biomass (Table 4).



**Fig 1.** Uranium concentrations (mean value of 4 plants) of *Helianthus annuus* and *Brassica juncea* in the roots and in the shoots for the different treatments, U = uranyl acetate and dipotassium phosphate, C =citric acid buffer, DW = dry weight, n.d. = not detectable

For the calculation of the soil/plant transfer factor a soil uranium concentration of  $6 \text{ mg kg}^{-1}$  was assumed. This value was calculated as mean value for the soil uranium concentration after the treatment with uranyl acetate dihydrate.

**Table 4.** Uranium fraction transported from the roots into the shoots (amount (branch) [ $\mu\text{g}$ ] /amount (plant) [ $\mu\text{g}$ ]), total uranium uptake (mean value) and Transfer factor plant/soil for the different treatments of *Brassica juncea* and *Helianthus annuus*, C =citric acid buffer

	From roots into shoots shifted fraction [%]	Total uptake into the plant [ $\mu\text{g}$ ]	Transfer factor plant/soil
<i>Helianthus annuus</i>			
control	-	0.17	$1.6 \cdot 10^{-3}$
with U	0.14	0.76	$6.2 \cdot 10^{-3}$
with U + C	0.11	0.49	$3.7 \cdot 10^{-3}$
<i>Brassica juncea</i>			
control	-	0.16	$1.8 \cdot 10^{-3}$
with U	0.10	0.42	$4.9 \cdot 10^{-3}$
with U + C	0.18	0.45	$5.6 \cdot 10^{-3}$

The soil/ plant transfer factor was in the order of  $10^{-3}$  for all plant treatments (Table 4). The higher soil uranium concentration led to an increase of 2-4 times for *H. annuus* and 3 times for *B. juncea*. The reaction to the addition of citric acid buffer to the soil differs depending on the plant specie.

## Discussion

### Uranium concentration and pH in soils

The differences of the uranium concentrations in the prepared farmland soil are caused by inhomogeneous distribution of the uranyl acetate dihydrate in the soil. These differences of the soil uranium concentrations are assumed to be at a small scale compared to the pot and the root volume, and therefore did not affect the total uranium content in one pot and in the soil solutions.

The uranium concentration in the used fertilizer ( $4.0 \text{ mg U kg}^{-1}$ ) is small compared to literature data ( $6\text{-}39 \text{ mg U kg}^{-1}$ , Kratz and Schnug 2005, Uyanik et al. 1999). Also only small amounts of this fertilizer were used for fertilization. Therefore they should not have any important effect on the measured uranium concentrations of the soils and plants of this investigation.

The determined effective soil pH of the farmland soil from the Fuhrberger Feld amounts 5.8. As far as it was recognizable from the measured pH values the addition of uranyl acetate dihydrate solution and dipotassium phosphate had no effect on the pH value of the soil solution.

The first citric acid buffer treatment of the soils led to two clearly visible responses: First the pH value of the soil solution increased 8-83 times. This increase



can possibly be explained by the dissolution of alkaline acting cations. The second reaction the yellow colouring of the solution is probably due to the protonation and simultaneous dissolving of humic substances.

The increase of the pH value of the soil solution to greater 6.5 after the citric acid buffer treatment could be one reason for the higher solubility of uranium and thus a higher concentration of uranium in the soil solution. However the chelate forming agency of the citrate is more likely the reason for the increased uranium concentration. Anyway the mobilization of uranium by citric acid as described in the literature (Huang et al. 1998) is confirmed by these results. The addition of the citric acid buffers to the soil and the following increased concentration of the uranium in the pore water should be a good precondition for the uptake of uranium into the plants.

### **Uranium uptake by *Helianthus annuus* and *Brassica juncea***

The uptake of uranium into the plants was with a maximum concentration of  $<1\mu\text{g}$  per plant and  $0.07\text{ mg kg}_{\text{DW}}^{-1}$  in the roots very low, compared to the available uranium per pot (approx. 30 mg). In comparison to the literature values of Kabata Pendias (2001) who stated concentrations of plant ashes with  $0.6\text{ mg kg}^{-1}$  no notable uptake has been shown for *H. annuus* and *B. juncea* in this investigation.

The analysis of a reference material (Virginia Tobacco Leaves) indicted that the digestion was not complete (systematic error up to 30.5%), however, this error has only small influence because of the low amount of uranium in the plants.

The plants have shown a higher accumulation of uranium in roots than in shoots, as expected. However a high uptake of uranium into the shoots is desirable, as the part of the plant which can be harvested easily.

Influences that could have affected the uranium uptake into the plants are the following:

*Phosphate:* Rufyikiri et al. (2006) determined that an increased P-content in the soil leads to a decreased U absorption into the plants. Hence, the phosphate which was added to the soil together with the uranium can increase the fixation of uranium in the soil and thus reduce the availability of uranium for plants.

*Soil pH value:* The soil pH of 5.8 is near the sorption maximum of clay minerals at pH 6.0 to 6.5 (Scheffer and Schachtschabel, 2002). Therefore due to adsorption processes less uranium can get into to the mobile phase than at lower pH values. Also this is the pH range in which stable uranium phosphate complexes are formed.

*Nutrient conditions of the soil:* The uptake of metals and probably also that of uranium into the roots is influenced by the nutrient conditions in the soil. Lower nutrient availability in the soil leads to higher metal accumulation in the roots (Schroetter et al. 2004).

In this study the plants were provided sufficiently with nutrients to exclude any limitation by lack of nutrition. Due to the addition of the Basacote plus 9M fertilizer (NPK fertilizer) it can be assumed that the soil contained sufficient amount of

plant-available potassium, whereby no KCl addition was necessary as compensation for the addition of dipotassium phosphate in the treated soils.

*Uranium concentration of the soil and the soil solution:* A higher uranium concentration in the soil and thereby soil solution led to an increased uranium uptake into the plants, for both plant species. However, the presence of larger quantities of uranium in the soil solution alone cannot be crucial for an improved uptake of uranium by plants, because the higher uranium concentration in the soil solution caused by the citric acid buffer only leads to a higher uptake of uranium for *Brassica juncea* (uranium concentration in the shoots doubled).

*Citric acid buffer:* The strongly increased availability of uranium in the soil solution of *H. annuus* (32-83 times higher than without treatment) did not yield a higher uranium content in the plant, whereas *B. juncea* showed a higher uptake. A reason for the different responses of the two plant species to the treatment with citric acid buffer could be a different absorption ability of uranium citrate complexes due to different metabolic characteristics of *H. annuus* and *B. juncea*.

Dushenkov et al. (1997) determined a high affinity for uranium for *Helianthus annuus* in a hydroponic. Probably *H. annuus* preferentially takes up water dissolved uranium species, but no citric complexes. Possibly the citric acid buffer has even led to a release of uranium which was adsorbed at the root surface of *H. annuus*.

A further reason for the small effect of the citric acid addition on the uranium uptake could be the late growth stage of the plants at that time. With a smaller growth rate the uptake of nutrients and metals into the plant decreases. Therefore the effect of the higher concentrations of uranium in the soil solution is weakened by a smaller uptake. The success of this method could possibly be increased by the addition of the citric acid buffers at an earlier time.

Two methodical facts have to be mentioned especially for further investigations in this field: the timing of the investigation and distribution of the artificially added uranium to the soil.

The time window of the investigations has to be smaller and seed should grow up already in the soil with higher uranium content, as it would be in a large scale application. Plants that are used for phytoremediation in the fields should not grow there for a long time, because after blooming the uptake of plants is lower than in the growing stage. Also it should be avoided that the contaminated plants are eaten by animals. This should be reflected in the experiments for phytoremediation.

Even if we act on the assumption that the irregular distribution of the artificially added uranium in the soils did not affect the uptake of uranium into the plants, a clear definition of the reference parameters (in this case soil uranium concentration) for the calculation of the uptake of uranium from the soil would be preferable. Thereby it must be noted that evenly addition of uranium to natural soil represents a certain challenge, since natural soils are less homogeneous than artificial substrates.

However, the determined soil/plant transfer factors at this investigation are in the same range as that of the control plants (approx.  $10^{-3}$ ) as well as determined transfer factors for field grown dump plants without any treatment  $10^{-4}$  to  $10^{-3}$  (Schönbuchner 2002, Whicker et al 1999).

In conclusion it can be said, that *Brassica juncea* and *Helianthus annuus* under the accomplished experimental condition and the determined soil removed only very small quantities of uranium from the soil. Thus for both plant species no suitability for phytoremediation could be proven.

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