The influence of citric acid on mobility of radium and metals accompanying uranium phytoextraction

J. Mihalík[1,2], P. Tlustoš[1], J. Száková[1]

[1]*Faculty of Agrobiology, Food, and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*National Radiation Protection Institute, Prague, Czech Republic*

ABSTRACT

We have investigated the plant uptake of radium, iron and other elements linked with phytoextraction of uranium induced by citric acid. Experiments were carried out on soil from the surroundings of a uranium mine. Enhancement of U bioavailability was successful. Now we focused our attention on the uptake of the most important elements accompanying uranium in phytoextraction. Radium was analyzed in leaves of willows and sunflowers. Mainly in willows, Ra was accumulated 3–4 times more than in the control group. Analyses of sunflower leaves showed an increasing tendency of Ra activity; however, not as significant as in the willow. During the experiment, we recorded irregular increases of metal contents in leaves week by week. Whereas U did not increase significantly, iron reached a value 9.3 times higher than in the control group. This is the evidence of more effective iron mobilization than of uranium when citric acid was added into the soil. Manganese also competes with uranium in plant uptake. Its content in leaves increases after citric acid treatment and is always higher than uranium uptake. The uptake of ubiquitous metal elements is one of the limiting factors for phytoextraction.

Keywords: phytoextraction; radium; uranium; willow; citric acid

The former uranium mines can become an environmental risk due to the occurrence of natural radionuclides in a level overdrawing ambient values. The contamination of mines or mills in the surroundings is often caused by resuspension from abandoned tailing heaps or sludge, eventually by uncontrolled leaks of radioactive solution (e.g. Carvalho et al. 2007, Rood et al. 2008). Moreover, the source of U and Ra incorporated into the soil can be fertilizers (for example phosphor gypsum) made of apatite with their high natural contents (Mortvedt 1994).

Our previous experience pointed out the ability of the willow (*Salix smithiana*) to accumulate U from soil after citric acid treatment (Mihalík et al. 2010). This species was chosen due to its tolerance to toxicity of heavy metals. Pulford and Watson (2003) brought knowledge about uptake and translocation of heavy metals in trees with a stress on willow in their review.

In addition to high biomass productivity, willows also have an effective nutrient uptake and high evapotranspiration rate (Pulford and Watson 2003). Thanks to it, willow is well-known as a 'water pump'. These attributes make the willow an advantageous tool for phytoextraction.

Mahon and Mathewes (1983) examined some plant species growing in soil rich in radium and uranium. Willows (*Salix scouleriana*) contained relatively a small quantity of U (0.016 mg/kg) and ^{226}Ra (3.8 Bq/kg). They sampled above-ground biomass of the previous year. Nevertheless, they stated a positive correlation between soil and plant ^{226}Ra contents.

Radium tends to accumulate in leaves similarly to calcium (Mortvedt 1994, Rodríguez et al. 2006). It is feasible to assume that radium is also phloem-immobile and cannot be reutilised from leaves to other parts (Von Firck et al. 2002).

In the context of phytoextraction, Tomé et al. (2009) carried out experiments showing that the

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sunflower is well-adapted for the elimination of U. Ra in their experiment was concentrated in plant tissues mainly in deficiency of P in the nutrient solution and that increase was higher with the pH decrease at 5. Adding citric acid only caused a slight increase of Ra content in roots and shoots against the control variant. Rodríguez et al. (2006) were more successful in Ra uptake and translocation into sunflowers. Moreover, they presented a linear relation between Ra concentration in nutrient solution and tissues. Besides this, the sunflower was successfully employed in U phytoextraction (Huang et al. 1998, Mihalík et al. 2010).

Vandenhove and Van Hees (2007) dealt with soil parameters convenient for the increase of Ra uptake into plant. They believed that a difference in soil parameters can lead to a difference in the transfer factor in one order of magnitude. The highest accumulation was reached in soil with low organic matter, CEC and pH. The low content of P also has a positive impact on the increase of Ra uptake. They presented a linear relation between Ra concentration in tissues and in soil solution.

Citrate complexes. The release of organic acids from the root was suggested as a general mechanism for metal solubilisation from the soil insoluble mineral phase (Jones and Darrah 1994). Citric acid influences the mobility of elements present in the soil. Soil elements compete with uranium while citrate complexes form.

The stability constants of metal complexes with citric acid are often higher than for a uranium-citrate (for Fe^{3+} log $\beta_1 = 11.5$, Fe^{2+} log $\beta_1 = 4.4$, $(UO_2)^{2+}$ log $\beta_1 = 7.4$) (Kotrly and Sucha 1985). On the other hand, the stability constant of the radium-citrate complex (log $\beta_1 = 2.36$) (Clifford 1990) is markedly lower than of uranium-citrate and is also lowest among the complex of alkaline earth metals and citric acid. The efficiency of complex appearance varies as far as stability constants are considered. Finally, citric acid is consumed by the appearance of complexes with other elements detrimental to uranium. As Huang et al. (1998) stated that the complexation of iron can lead to the release of uranium bonded to iron oxides.

The presence of other metals like Zn and Fe calls for an answer what roles these metals play when competing with U complexed by citric acid when they are taken up and translocated into plants.

Our experiments were carried out on soil with anthropogenic contamination. They were close to real conditions, which can occur during application of the phytoextraction method. We can observe competition among elements mobilized by citric acid.

The next part of the experiment was arranged in order to observe the evolution of U uptake during multiple treatments by citric acid. The creation of new leaves demands an increase of nutrients and water uptake and, in consequence, it should also lead to a higher uptake of uranium. We decided to harvest young leaves after separate treatments and to compare each and also compare to older leaves in treated and control groups.

The objectives of our study were:

- to analyse the U, Ra, Fe, Cu and Mn concentration in leaves in order to show the competition between them in the phytoextraction induced by citric acid;
- to investigate the effect of biomass growth on U accumulation.

MATERIAL AND METHODS

Uptake of ^{226}Ra. The plant experiment took place in an open-air greenhouse in 2008. The willows and sunflowers were planted for 1 and 2 months, respectively, in pots with a 30 cm diameter and 5 kg content of soil in order to study induced U phytoextraction. Three groups differing in the number of citric acid application were designed in the experiment with willows. In the case of the sunflower, four groups were designed. Unfortunately, we lost the control group of sunflowers.

Four sunflower seeds were inserted into the soil of each pot. One willow cutting was planted into each pot. In the control, no citric acid was applied. After one month of growth, treatment with the 0.08 mol/L solution of citric acid began. A dose of 300 mL of the solution was poured over the top of the pots once per week.

The total amount of citric acid in one dose was 5 mmol per 1 kg of soil. Five doses of agent were applied during a 5-week period: the total amount of citric acid was 25 mmol per 1 kg of soil. Each group contains three replicates.

Ra was determined in leaves that were dried and pulverized before analyses.

^{226}Ra was determined with the aid of gamma spectrometry. For the assessment we used a 186 keV photopeak that also contains contribution from ^{235}U. Natural uranium was determined by ICP-OES (Vista Pro, Varian, Australia) (Mihalík et al. 2010). With the help of relation between activity and number of impulses, we calculated its contribution to photopeak 186 keV.

For gamma spectrometry, the High Purity Germanium (HPGe) detector (Canberra, USA)

Table 1. Content of some metals in soil (dry mass) used for phytoextraction of Ra

U	Fe	Mn	Cr	Cu	^{226}Ra	^{40}K
(mg/kg)					(Bq/kg)	
520 ± 40	4550 ± 390	31.4 ± 0.77	12.5 ± 0.38	5.2 ± 0.87	2490 ± 250	58 ± 6.6

Content relates to the start of the experiment and was determined by ICP – OES and HPGe gamma spectrometry

– with 30% efficiency – was employed. Pulverized samples were placed on top of the detector in 50 or 100 mL vessels.

Experiment of willow adaptability. To observe the adaptation of a willow on the application of citric acid and parallel increase of uranium uptake by plants, we realized the following experiment in 2009. Once every two weeks, the pots occupied by willow cuts were watered by 300 mL of 0.03 mol/L solution of citric acid. All experiment took ten weeks.

Plants were divided into three groups:

- Control group – The plants grew without citric acid treatment. The composition of soil was identical to the other two groups. The samples were prepared from biomass collected at the end of the entire experiment.
- The second group was treated every second week by the identical dose of citric acid. Before each application of complexing agent, we collected the green biomass of the plants and analysed it.
- The plants in the third group were also watered by the solution of citric acid every two weeks. The samples were prepared from biomass collected at the end of the entire experiment.

Soil cultivation and analyses of biomass were described in Mihalík et al. (2010).

Experimental soil. Soil coming from Ralsko is typically sandy and contains low organic matter (< 0.5%). Low pH (4.5) and low P (40 mg/kg) predestined this soil for a successful phytoextraction experiment. It was self-planted by unspecific plants in the original place.

The contents of objected elements are listed in Tables 1 and 2. An experiment with Ra uptake was realised on soil with higher contents of Ra and U than in the experiment with willow adaptability.

Table 2. Content of some metals in soil (dry mass) used for an adaptability experiment

U	Fe	Mn	Cr	Cu
(mg/kg)				
240 ± 30	4360 ± 300	53.6 ± 3.1	13.5 ± 1.2	4.4 ± 0.50

Content relates to the start of the experiment and was determined by ICP – OES

Statistical analysis. All statistical analyses were performed with R software (R Development Core Team, 2009). Results were subjected to the analysis of variance (one-way ANOVA) and the Tukey's test. Metals concentration in plant tissues and transfer factor were log-normally transformed before statistical analyses. Data presented are mean values ± standard deviation. Uncertainty of transfer factor (TF) was calculated in accordance with propagation of uncertainty.

RESULTS AND DISCUSSION

Effect of citric acid on the accumulation of ^{226}Ra. The transfer factor of Ra determined for sunflower spans a broad interval from 0.085 to 1.10 with geometric mean: 0.42 (Vandenhove et al. 2009). Soudek et al. (2004) listed a number of Ra TF determined for various plant species growing in natural conditions or in greenhouses without special treatments. For sunflowers they mentioned TF 0.121 (Soudek et al. 2004) and 0.018–0.037 (Soudek et al. 2010), depending on specific activity of radium in the soil. Their experiments employed soil with much higher specific activities of ^{226}Ra than in our present experiment. These specific activities were from 7.1 to 25.6 kBq/kg or 15.5 kBq/kg (Soudek et al. 2004) and 4.5–13 kBq/kg (Soudek et al. 2010).

The most similar species to the willow is the poplar. According to Soudek et al. (2004), TF of Ra for the poplar is posed at 0.0434.

The increase of ^{226}Ra is evident mainly after repeated doses of citric acid in both cases (Tables 3 and 4). In comparison with Soudek et al. (2004

Table 3. Specific activity (in dry mass) of ^{40}K and ^{226}Ra in willow leaves after citric acid treatment

	^{40}K	^{226}Ra	TF (^{226}Ra)
	(Bq/kg)		
Control	480 ± 200	840 ± 420	0.34 ± 0.17
1 dose	610 ± 250	970 ± 290	0.39 ± 0.12
5 doses	630 ± 130	3130 ± 560	1.26 ± 0.26

Table 4. Specific activity (in dry mass) of ^{40}K and ^{226}Ra in sunflower leaves after citric acid treatment

Doses	^{40}K	^{226}Ra	TF (^{226}Ra)
	(Bq/kg)		
1[a]	1000 ± 120	2300 ± 620	0.92 ± 0.27
1[b]	890 ± 40	1280 ± 670	0.51 ± 0.27
3	1500 ± 880	3120 ± 1300	1.25 ± 0.54
5	1300 ± 460	3730 ± 1100	1.50 ± 0.47

Group 1[a] was treated once and let to grow to the end of the experiment. Group 1[b] was also treated once, but one week after treatment it was harvested. However, there was not a control group in this trial

and 2010), the TF for the sunflower leaves determined for the 1[b] and 1[a] groups in our experiment is significantly higher, nevertheless within the range referred by Vandenhove et al. (2009). The repeated treatment caused the increase of TF over the higher limit mentioned in Vandenhove et al. (2009).

Comprehensive analysis of the effect of various parameters on Ra uptake was listed in Vandenhove and Van Hees (2007). Alkali earth metals most likely compete with Ra for a sorption place on roots and their excess inhibit radium uptake. They did not observe correlation between Ca or Mg uptake and Ra and only slight correlation with phosphorus. According to them, the pH of the soil solution does not influence Ra uptake. The transfer factor of Ra is controlled by concentration of Ra in the soil solution. The content of organic matter in soil makes a serious impact on the inhibition of Ra uptake.

The significant increase of ^{226}Ra in the willow leaves was determined only after the fifth dose of agent (Table 3). No significant increase was observed in ^{40}K content in willow leaves.

Citric acid is markedly more effective as far as Ra is concerned. Ra bioavailability could slightly increase by a pH change as well as by complexation. The stability constant of the Ra-citrate complex is lower than K_d of the uranium-citrate complex; therefore, we can consider the uptake of Ra after the treatment by citric acid as a bias effect.

We did not observe a significant difference in ^{226}Ra contents between sunflower leaves and willow leaves in the plants treated by one or five doses of citric acid.

Five doses of complexing agent caused distinguishable increases of radium in leaves of willow as well as of sunflower. Tomé et al. (2009), similarly to us, observed only a small effect of citric acid on Ra accumulation in sunflowers.

However, the amount of ^{226}Ra calculated of its activity is negligible in comparison with U and, therefore, without crucial influence on U uptake.

Willow adaptability

Uranium. We did not observe a significant increase in U content in willow leaves. The increase of U concentration in leaves in Mihalík et al. (2010) was 3.3-times higher after one dose and 21-times after 5 doses.

The control group and group '0' were not the same in Table 5. The leaves from group '0' were collected at the start of the experiment before the first treatment with citric acid, but the leaves from the control group were collected only at the end of the whole experiment. In the range of standard deviation, we can consider the content of uranium in leaves as equal. As opposed to previous results (Mihalík et al. 2010), we reached a markedly lower content of uranium in leaves when we added five doses of citric acid into the soil. The only difference between the experiment in 2008 and the last, from this point of view, was the time period between individual doses. In the last experiment, one week was replaced by a two-week period. A weaker effect of citric acid on the accumulation of U could be caused by a shift of this U into a pool less available than an original (Ebbs et al. 1998).

Finally, the transfer factor of uranium in the control group (calculated for leaves) was 0.040 ±

Table 5. Content of chosen elements (in dry mass) in willow leaves after citric acid soil treatment

Group	Cu	Mn	Fe	U
	mg/kg			
Control	1.13 ± 0.34	41.6 ± 20	12.1 ± 1.2	9.67 ± 4.4
0	2.8 ± 0.14	11.3 ± 2.1	9.82 ± 3.2	9.72 ± 0.79
1[a]	21.1 ± 0.03	129 ± 9	153 ± 80	16.5 ± 10.2
1[b]	10.6 ± 8.4	129 ± 91	211 ± 149	29.5 ± 17.5
1[c]	2.71 ± 0.25	33.9 ± 3.6	34.3 ± 1.1	13.4 ± 1.3
1[d]	3.10 ± 0.34	45.7 ± 9.2	60.2 ±19	9.50 ± 5.0
1[e]	2.76 ± 0.55	57.9 ± 9.2	106 ± 8.6	13.5 ± 0.069
5 doses	1.86 ± 0.21	128 ± 6.6	63.7 ± 17	19.6 ± 4.3

[a–e]symbolize the number of citric acid doses i.e. a – 1 dose; b – 2 doses, etc.

Table 6. Tukey's contrast between individual samples listed in Table 5

Groups	Cu	Fe	Mn
Control – 0	–	–	*
Control – 1^a	***	***	–
Control – 1^b	+	*	–
Control – 1^c	–	*	*
Control – 1^d	–	***	–
Control – 1^e	–	*	–
Control – 5 doses	–	***	*
0 – 1^a	*	***	***
0 – 1^b	–	**	*
0 – 1^c	–	**	*
0 – 1^d	–	***	**
0 – 1^e	–	**	+
0 – 5 doses	**	***	***
1^a – 1^b	–	–	–
1^a – 1^c	*	**	–
1^a – 1^d	*	+	–
1^a – 1^e	–	–	–
1^a – 5 doses	*	–	–
1^d – 5 doses	–	–	+

The couples without significant contrast are not listed here. Significance between groups represented $+P < 0.1$, $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$

0.19 and in the group treated by five doses of CA was 0.082 ± 0.21.

Heavy metals. The effect of citric acid on Fe is well known. Tiffin (1966) described a role of citrate in the transport of iron in xylem. Moreover, citric acid is able to dissolve Fe oxides which leads to the release of U bonded to this soil fraction (Huang et al. 1998). Therefore, after adding citric acid, the total amount of U available for plants is enhanced by the portion of U bonded to iron oxide.

The contents of Fe (Table 5) were significantly higher in the leaves of plants treated by five doses of citric acid than in the control group (Table 6). Iron achieved the highest increase of the observed elements. Its content was nearly six times more in comparison with the control group. In this case we must take into consideration that with the increase of mobility and uptake of metals, the competition among them also increases. We can observe the priority of iron followed by manganese what is in accordance with the stability constant of their complexes.

Similarly to U, differences between the contents of Fe and Cu in the leaves of the control group and group '0' were not statistically different (Table 6).

Fe, Mn and Cu achieved their maximal contents in the leaves when the first or second dose of CA was applied (Table 5). We can expect similar trend for U, although its values did not have statistically significant contrasts.

Herein, it is desirable to attempt to elucidate the turning point in metal uptake:

- The regulation of metal uptake by plants seems to be the most feasible. The period between two doses could be employed by plants to regeneration of the root's membranes which were finally more resistant.
- Creation of less soluble compounds seems to be less likely – uranium is still taken up in a higher amount than before treatments.

The experiment to verify the phenomenon that young leaves take up more uranium than older as a consequence of higher nutrient demand was not successful. Moreover, the uranium uptake was markedly lower than Mn or Fe uptakes even after the second citric acid dose. In accordance with knowledge of CA effect on alkaline metals, K content did not increase significantly under influence of citric acid. More surprisingly, no significant increase was observed in U content in willow leaves. We observed significant contrasts in Fe content in willow leaves, between control group and each treated group (1^a – 1^e, 5 doses) as well as between group '0' and each treated group.

Addition of citric acid provokes an enhanced uptake of Fe, Mn and Cu that compete with uranium for complexing agent.

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Corresponding author:

Ing. Ján Mihalík, Státní ústav radiační ochrany, v.v.i., Bartoškova 28, 140 00 Praha 4, Česká republika
e-mail: jan.mihalik@suro.cz