

Study of uranium bioaccumulation capacity of *Salvia officinalis* L. and *Ocimum basilicum* L. enhanced by citric acid

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Abstract: Plants possess various intrinsic mechanisms necessary to accumulate and either sequester or detoxify soil contaminants, including radionuclides. The aim of this study was to determine the bioconcentration factor of two fast growing plants *Salvia officinalis* L. (sage) and *Ocimum basilicum* L. (basil) that were cultivated in pH neutral soils artificially contaminated with three different concentrations of uranium (211, 352 and 470 mg/kg). The efficiency of citric acid was evaluated with respect to the enhancement of the phytoextraction process. The results showed that the bioconcentration factor did not differ significantly between the selected species (0.01 - 0.03). Citric acid was added in doses (50 mL, 30 mM) until the first effects of uranium phytotoxicity appeared. After four doses of citric acid, the bioconcentration factor reached 0.05 for both plants. The increase of uranium content taken up by the respective plants was more pronounced. Thus, the uranium content of sage grown in contaminated soil (470 mg/kg) increased from 6.03 to 21.28 mg/kg in citric acid-treated soil. The data obtained confirmed the efficiency of citric acid in enhancing phytoextraction of uranium and further suggest that even plants of a rather small biomass can be useful in phytoremediation given the appropriate treatment through induced phytoextraction with appropriate chemical agents.

INTRODUCTION

Having the ability to absorb and accumulate metals, plants have become valuable tools in controlling or remediating environmental contamination. Plant-assisted remediation of soil containing radionuclides generally occurs through one of the following mechanisms: phytoextraction, rhizofiltration, phytovolatilization or phytostabilization (Duschenkov, 2003). Apart from being a low-cost alternative to engineering-based remediation methods such as excavations, phytoremediation allows *in situ* treatment of soil and it is a technique that can be used as a long-term treatment (Gavrilescu *et al.*, 2009).

Numerous, and predominantly terrestrial plant species have been investigated for their capacity to accumulate uranium such as *Brassica* sp., *Trifolium* sp., *Helianthus annuus* L., *Zea mays* L., (Malaviya and Singh, 2013). Among 34 species that have been screened by Shahanadeh and Hossner (2002a), *Brassica juncea* (L.) Czern. and *Helianthus annuus* L., proved to be particularly suitable for phytoremediation of uranium.

The bioaccumulation of uranium depends not only on the plant species and its cultivation conditions, but also on the soil composition, soil pH, coexisting ions, contamination exposure, amendments and the very uranium speciation (Claus *et al.*, 2007; Schindler *et al.*, 2015; Černe *et al.*, 2018; Meng *et al.*, 2018; Khan, 2020). Various types of amendments and many methods, including agricultural strategies, are currently being used to improve phytoremediation processes (Rostami *et al.*, 2019; Li *et al.*, 2018). Low molecular organic acids, in particular citric acid (CA), have been used in many studies to enhance plant uptake of uranium during phytoextraction (Shahandeh and Hossner, 2002a). The aim of this work was to investigate and compare the bioaccumulation capacity of two fast growing plant species sage and basil and to evaluate the plants' responses to the chosen amendment - citric acid.

EXPERIMENTAL

Soil contamination

The used soil was commercially obtained. According to the declaration it contained 64% black peat, 34% white peat and 2% clay. The heavy metal content (mg/kg) was as follows: Cd<0.1, Cr<8.0, Cu<9.0, Hg<0.1, Pb<10.0, Zn<15.0. The soil was thoroughly homogenized and air dried for 10 days at room temperature.

The soil was portioned and irrigated using aqueous solutions of uranyl nitrate hexahydrate. Thus, three sets of soil samples were prepared containing 450 mg/kg, 700 mg/kg and 1000 mg/kg uranyl nitrate hexahydrate which corresponds to 211.5 mg/kg, 352.5 mg/kg and 470.0 mg/kg uranium. The soil was homogenized every two days for two weeks, after which it was used in pot experiments to cultivate sage from seeds and seedlings and basil from seeds. Non-contaminated soil was used as a control.

Determination of soil pH

The pH was determined in non-contaminated soil in triplicates. Soil samples (5 g) were suspended in 50 mL water (pH in H₂O) and in 50 mL 1 M KCl (pH in KCl), periodically mixed for 1 hour and the pH was measured using a glass electrode.

Plant culture

Sage and basil seeds were commercially obtained (producer: Royal seeds) and used to grow seedlings in non-contaminated soil for 8 weeks. After that period, the sage seedlings were transferred into pots containing cca. 300 g soil and watered with aqueous solutions of uranium every other day. At the same time, sage and basil seeds were planted into the already contaminated soil. Thus, the seedlings were allowed to grow for 8 weeks. To evaluate the effects of additive on bioaccumulation, plants grown in the highest uranium concentration were watered with 50 mL of 30 mM citric acid every other day, 4 times in total.

Soil analysis

Soil samples were subjected to acid digestion according to EPA method (3050B). Each air-dried soil sample (3g) was transferred into a vessel and mixed with 21 mL conc. HCl, 7 mL conc. HNO₃ and 1 mL distilled water. The suspension was left overnight (16 hours) at room temperature before another 5 mL HNO₃ were added, and heated. After cooling down, 2 mL of distilled water and 3 mL 30% H₂O₂ were added to the suspension. To achieve complete oxidation, the suspensions were additionally heated and small volumes of 30% H₂O₂ were added gradually. The suspensions were then filtrated, transferred into measuring flasks (50 mL) and filled up to the mark with distilled water.

Plant analysis

The harvested plant samples (roots and areal parts) were washed with distilled water and soil particles were removed from the roots by immersing them into a 0.5 M CaCO₃ solution for 3 hours. Then, the roots were washed out with distilled water again, excess of water on the surface was wiped off with absorbent paper and the plants were dried in an oven at 70°C to constant weight.

After weighing the biomasses, the plant material was pulverized into fine powder. The plant samples were analyzed by weighing 0.45 g plant powder and leaving it overnight suspended in 3 mL conc. HNO₃. Then, the suspension was heated for 1 h at 125°C, 2 mL 30% H₂O₂ and 0.5 mL distilled water were added, while continuing heating and adding more H₂O₂ until the solution became clear. The sample was filtrated and filled with distilled water to 10 mL.

ICP-MS Analysis

Soil and plant sample solutions were analyzed in triplicate using mass spectrometry with inductive coupled plasma (ICP-MS Agilent 7700x) and the obtained data processed with Agilent Mass Hunter. Operating parameters for the ICP-MS in He mode were as follows: voltage power (RF) 1550 W; sample depth 7-10 mm, points per peak 3; resolution at 10% peak's height 0.65 - 0.8; carrier gas 1.01 - 1.11 L/min; He gas 2.5-6 mL/min; S/C temperature 2°C; nebulizer pump 0.1 rps.

RESULTS AND DISCUSSION

The soil pH, determined before the contamination process, was in the neutral range. The pH measured in water was 6.98±0.05 and 6.70±0.03 measured in water and KCl, respectively.

Table 1: Uranium concentration in analysed soil and plant samples (mg/kg) determined by ICP-MS

Contaminated soil	Sage (whole plant)		Basil (whole plant)
U (mg/kg)	Seeds	Seedlings	Seeds
211.5	7.1 ± 0.5 (6.6 – 7.6)	3.6 ± 0.1 (3.5 – 3.7)	7.8 ± 0.5 (7.3 – 8.3)
352.5	6.6 ± 0.5 (6.1 – 7.1)	11.4 ± 1 (10.5 – 12.4)	3.5 ± 0.5 (3.2 – 4.1)
470.0	6.0 ± 0.5 (5.6 – 6.5)	9.8 ± 1 (8.8 – 10.8)	8.0 ± 0.5 (7.5 – 8.5)
470.0 + CA	21.3 ± 2 (23.2 – 25.0)	21.6 ± 2 (19.6 – 23.6)	23.1 ± 2 (19.2 – 22.9)

* The results are expressed as Mean value ± SD

Due to the relatively short growth period of nine weeks and the low biomass of the selected plants that did not exceed 0.5 g dry weight, the uranium concentration was determined as a total present in root and areal parts (stem and leaves). As can be seen from Table 1, the uranium concentration in sage and basil grown from seeds does not differ much, except for samples grown in 352.5 mg/kg U contaminated soil. Similarly, a significant difference between uranium concentrations in plants grown from seeds and those from transplanted seedlings was not established.

The bioconcentration factor (BCF), also known as plant transfer factor (TF), measures the bioaccumulation or bioconcentration capacity of the whole plant to remove contaminants and is calculated according to the equation (Dushenkov, 2003):

$$BCF = \frac{\text{plant uranium content (mg/kg)}}{\text{soil uranium content (mg/kg)}}$$

Most of the studies (Wang *et al.*, 2018; Mihalik *et al.* 2010), represent the BCF of the shoot and root system separately, however the BCF values of this study refer to the uranium content in the whole plant / uranium content in soil upon contamination, on dry weight basis (presented in Fig. 1).

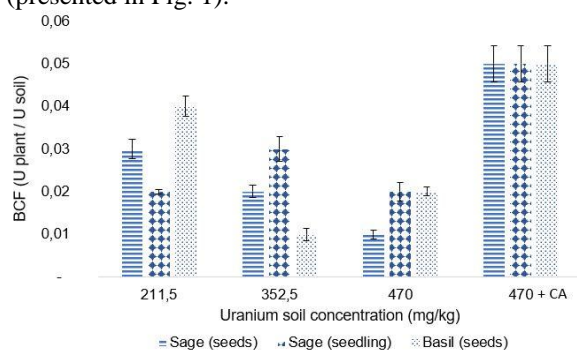


Figure 1: Bioaccumulation of uranium in roots and areal parts of sage and basil grown from seeds and seedlings.

The aim and purpose of phytoextraction is maximum extraction of contaminants - a process determined by many factors. The efficiency of that process is often evaluated by the BCF. In the case of uranium, the BCF is small and typically in a range between 0.0002 - 0.38 for shoots and between 0.007 - 8.1 for roots (Malaviya and Singh 2013). Leafy vegetables generally show higher BCFs, followed by root, fruit and grain crops (Vandenhove *et al.*, 2001). The current contribution aimed to explore the capacity of sage and basil, two fast growing medicinal plant species from the Lamiaceae family, to accumulate uranium with and without any amendment addition. The plants grown from seeds and the transplanted seedlings were exposed equally long to the different uranium concentrations. Changes observed in the uranium content in the whole plants grown from sage seeds (7.05 to 6.03 mg/kg) imply a gradual decline with the increase of uranium concentrations in the soil. In the case of sage grown from seedlings, the U content in the plant increased more than 3-fold in the soil contaminated with 352.5 mg/kg U compared to the soil contaminated with 211.5 mg/kg uranium. The same pattern is reflected in the BCF values, as well. A nonlinear correlation between the uranium soil concentration and BCF has been reported by Alsabbagh and Abuqudaira (2017) who concluded that while the uranium removal percentage values were close, they did not directly depend on the three different soil concentrations to which the sunflower plants were exposed during the 10 weeks of the pot experiment. Wang *et al.*, (2018), on the other hand, demonstrated an inversely proportional relationship. They conducted a pot experiment sowing *Boehmeria nivea* seeds in contaminated soil, and for the three uranium concentrations of 175.275 and 485 mg/kg the BCF was 1.834, 1.084 and 0.295, respectively. *Helianthus annuus* L. is one of the most extensively analysed plant species for uranium bioaccumulation and there are reports of uranium content in whole plants such as 44 mg/kg where C uranium in soil = 480 mg/kg (Mihalik *et al.* 2010) or cca. 16 mg/kg where C uranium in soil = 253 mg/kg (Alsabbagh and Abuqudaira, 2017). In this study, the uranium content in sage and basil at the initial soil

concentration (211.5 mg/kg) was 7.05 mg/kg and 7.79 mg/kg, respectively which is remarkable considering the differences in biomass and morphology of the selected plants compared to sunflower.

The results of this study (Fig. 1.) confirmed the effectiveness of CA on the enhancement of uranium phytoextraction and consequently on the BCF. The newly created conditions after CA treatment caused a 3-fold increase in the uranium concentration in sage and basil grown from seeds. This effect was even more pronounced in sage grown from seedlings where the initial uranium concentration rose from 3.6 to 21.56 mg/kg. Interestingly, the BCF reached the value of 0.05 for both sage and basil, regardless of the cultivation type. Most probably the uranium in the complex with CA improved the metal uptake by both plants. Organic acids, can directly complex cations, change the soil pH, and increase the uranium mobility in the soil (Vandenhove *et al.*, 2001; Shahnadeh and Hossner, 2002a; Duquène *et al.*, 2008). Ideally, phytoextraction amendments should improve plant accumulation of the targeted metal but also stay environmentally friendly (Parra *et al.*, 2008). Citric acid as a natural alternative to synthetic chelators has a shorter environmental half-life, it is biodegradable, less toxic and can be obtained at relatively low-cost (Malaviya and Singh 2013). The results of this study are in line with literature findings (Li Chen *et al.* 2019; Hu *et al.* 2019.). One of the early studies (Huang *et al.* 1998) showed that shoot U concentrations of *Brassica juncea* (L.) Czern. and *Brassica chinensis* L. grown in a U-contaminated soil (750 mg/kg) increased from less than 5 mg/kg to more than 5000 mg/kg in citric acid-treated soils. Chang *et al.* (2005) expanded their research evaluating the CA effects on uranium accumulation in aboveground biomass of *Brassica juncea* (L.) Czern, *Brassica chinensis* var. *oleifera*, *Brassica napus* L. var. *napus* and *H. annuus* L. considering two soil types. For all investigated plants, CA was efficient in boosting the bioaccumulation of uranium. Mihalik *et al.*, (2010) compared the effectiveness of a single and repeated dose of CA using *H. annuus* and *Salix* spp. for phytoremediation. A single treatment with 300 mL 80 mM CA was only effective for *Salix* spp., however after 5 doses the uptake of uranium increased in both plants. In our experiment, CA was used in four doses (50 mL 30 mM CA, every other day) after which the treatment was stopped because plant withering became apparent. The different uranium soil concentrations visibly reduced the growth rate of the selected plants; however, the toxic threshold of soil uranium became evident after the repeated CA additions. Thus, it is most probably the synergistic effects of uranium and CA that caused the negative effects, which is not surprising as several other authors reported inhibited plant growth upon CA addition (Vandenhove *et al.*, 2001; Shahandeh and Hossner, 2002a, b; Lesage *et al.*, 2005).

To the best of our knowledge, the uranium bioaccumulation capacity of sage and basil has not been previously investigated and a direct comparison of the BCF results is therefore not at hand. A comparison to other species would need to take into account a rich set of parameters such as plant species and its mechanisms to sequester unwanted contaminants, biomass, uranium

concentration and exposure time, uranium speciation, soil type, pH, amendment and its concentration and whether BCF is calculated for the whole plant, root system or shoot system. On these grounds, we restricted to the results obtained by this study and concluded that basil and sage are equally suitable for phytoremediation purposes and that their capacity for uranium bioconcentration can be significantly increased with the addition of an appropriate amount of CA, which will not induce the uranium phytotoxicity. It is known that the soil pH must decline below 5.0 to effectively transport uranium to the shoots due to the predominance of the soluble uranyl cation (Ebbs *et al.*, 1998). In our study, the soil pH was in the neutral range which may explain the low uranium concentrations determined in the plants prior to the addition of CA. The type of soil and its properties will play an additional role in the mobility, binding capacity and chemical reactivity of uranium and thus affect the overall bioaccumulation capacity of plants (Yan and Luo, 2015).

CONCLUSIONS

The search for plants that have the highest potential towards uranium bioconcentration as well as the optimal conditions are essential for successful soil remediation of this radionuclide. Although the obtained results are based on a pot experiment, it has been clearly demonstrated that sage and basil grown from seeds have similar BCF values that were not in a linear correlation with the uranium concentration in the soil. After the addition of four doses of citric acid, when the first signs of phytotoxicity became visible, the BCF has increased to 0.05 for both plant species. The low uranium content in whole plants prior to the addition of CA may be partially explained by the neutral pH of the soil. In summary, this study has provided first data on the potential of sage and basil for uranium bioaccumulation and demonstrated that even plants with a small biomass may be utilized for phytoremediation in combination with suitable amendment agents.

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Summary/Sažetak

Biljke posjeduju različite unutrašnje mehanizme koji su neophodni za akumulaciju i detoksikaciju kontaminanata iz tla a koji uključuju i radionuklide. Cilj ove studije bio je utvrditi biokoncentracijski faktor dvije brzo rastuće biljke, *Salvia officinalis* L. (kadulja) i *Ocimum basilicum* L. (bosiljak). Naime, biljke su kultivirane u pH neutralnim tlima i kontaminirane sa tri različite koncentracije urana (211-470 mg/ kg), a ispitana je i efikasnost limunske kiseline na unapređenje procesa fitoekstrakcije. Rezultati su pokazali da se biokoncentracijski faktor ne razlikuje značajno između dabranih biljnih vrsta (0.01-0.03). Naime, limunska kiselina je dodavana u dozama (50 mL, 30 mM) dok se nisu pojavili prvi efekti fitotoksičnosti urana, pri čemu je nakon četiri doze limunske kiseline biokoncentracijski faktor dostigao vrijednost od 0.05 za obje ispitivane biljke i izražen je povećani udio urana kod obje. Tako je pri istoj koncentraciji urana (470 mg/kg) u kontaminiranom tlu, udio urana u kadulji je porastao sa 6.03 na 21.28 mg/kg nakon dodatka limunske kiseline. Dobiveni rezultati potvrdili su efikasnost limunske kiseline u poboljšanju procesa fitoekstrakcije urana i nadalje sugeriraju da čak i biljke relativno male biomase mogu biti korisne u fitoremedijaciji ukoliko se primjeni odgovarajući tretman hemijskim sredstvima.