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ORIGINAL PAPER

Efficient phytoremediation of uranium mine tailings by tobacco

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Abstract This investigation shows that tobacco plant roots and leaves accumulate 60 times more uranium than previously reported. Phytoremediation is a convenient technique to clean up polluted soils using herbaceous plants and trees. Increasing research aims to identify novel plant species that accumulate toxic metals. Tobacco plant (*Nicotiana tabacum* L.) is a promising cultivar for phytoremediation because tobacco is fast growing and easily propagated. Here, we study phytoremediation of uranium by two tobacco varieties Virginia and Burley, bred in natural conditions. Plants were grown on uranium mine tailings with an average uranium content of 15.3 mg kg^{-1}. Each shoot sample was cross-sectioned into five uniform groups of leaves and stem segments. Results show a substantial variance in uranium uptake according to the section elderliness and origin of the plant parts. The highest concentrations of uranium values recorded in leaves of Burleys and Virginias nearest root shoot sections were 4.18 and 3.50 mg kg^{-1}, respectively. These values are 60 times higher rates than those previously published for leaves of cultivars grown under similar conditions. Taking into account the level of soil contamination, the content of accumulated uranium demonstrates uranium hyperaccumulatory properties of tobacco plant and its potential utilization in phytoremediation of uranium-contaminated mediums.

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Introduction

Uranium is one of the most frequent pollutants of groundwater and surface soils (Riley et al. 1992). Considerable amounts of its inputs have occurred over the last 50 years, mainly through the processes of soil fertilization, mining, nuclear industry and military activities (Meca et al. 2011; Stojanovic and Milojkovic 2011). Since uranium is chemically toxic to kidneys and its insoluble compounds are highly carcinogenic (Hossner et al. 1998), inappropriate counseling of uranium-contaminated soils may represent significant risks to human health, primarily via food chain (Duquene et al. 2006).

Typical concentration range of uranium in non-contaminated soils ranges from 0.40 to 6.00 mg kg^{-1} (Shacklette and Boerngen 1984; United Nations 2010). Even though uranium has not been shown to be essential to either plants or animals, plants will absorb uranium and incorporate it into their biomass, mainly in roots along with other heavy metals (Hossner et al. 1998). This observation suggests the possibility for remediation of uranium-contaminated soils through plant uptake (Salt et al. 1995).

The uranium uptake has commonly been studied in plant roots indigenous to mine sites rather than the above-ground parts of cultivars, which are normally consumed by humans (Chang et al. 2005; Chen et al. 2005; Shtangeeva 2010). Contrary to this can be seen in Sarić et al.'s study from 1995, which reported the levels of adopted uranium concentrations in older leaves of different cultivars grown in real conditions at medium-polluted uranium tailings ranged from 0.15 and

0.76 mg kg^{-1}, respectively. Later, Ebbs et al. (1998) in their pot study reported levels of uranium adopted in beet and vetch shoots to be 2.80 and 3.50 mg kg^{-1}, respectively. Similarly, Shahandeh and Hossner (2002), studying uranium uptake at uranium mine tailing and soils contaminated with 100 mg U kg^{-1}, found the highest uranium accumulation in sunflowers and Indian mustards the above-ground parts of 24.6 and 21.8 mg kg^{-1}, respectively. Sand culture method was also used and cultivated plants accumulated from 4.00 to 416 mg U kg^{-1} dry tissue weight, as demonstrated in Hashimoto et al.'s research (2005). Recently, Straczek et al. (2010) reported the results of a hydroponic experiment where cultivars have been exposed for 7 days to 100 mmol l^{-1} U nutrient solution. In relation to the other plant species, Indian mustard exhibited the highest shoot uranium uptake (122 ± 46.0 mg kg^{-1}), which could be expected for hyperaccumulators.

Since it has been noted that plants vary in their uranium uptake capacities, assortment of plant species plays a key role in the development of phytoremediation method. Therefore, an increasing attention has been paid to the identification of the novel plant species with a high heavy metal–accumulating potential.

To better define the extent of carcinogenic pollutants, such as cadmium and lead, which people are directly exposed to, heavy metal mass abilities of tobacco plant (*Nicotiana tabacum* L.) have been studied extensively (Tsotsolis et al. 2002; Lugon-Moulin et al. 2006).

Taking the above-mentioned findings into consideration, the paper presented here provides the results of a study aimed at monitoring uranium adoption levels and distribution trends in two tobacco plant varieties across shoot sections from near mine, medium-polluted soils. Using multivariate analysis, it has been investigated whether it is possible, based on adopted uranium levels, to distinguish the tobacco types and its above-ground parts regarding their origin and the position and to establish tobacco plant as a bioindicator and potential uranium hyperaccumulator.

Experimental

An experiment was conducted on the barren soil of closed Kalna-Gabrovnica uranium mine in southeast Serbia. Following a period of exploitation, which lasted from 1953 to 1962, the place was covered in tailings. Nowadays, deposit tailings cover an area of approximately 0.1 km^2, which on average contains 15.3 mg U kg^{-1} at 8.24 pH values (Stojanović et al. 2009).

Two types of tobacco plant (*N. tabacum* L.), Virginian and Burley, were planted on deposit during the last week of May and picked in September, during the phase of full maturation. The experiment was carried out in five repetitions on the elementary plots of one square meter in size. During the vegetative period of plants, nitrogen, phosphorous and potassium mineral fertilizers were applied. After the vegetation period, plant shoots were cross-sectioned into five equal segments of leaves and stems, rinsed with distilled water and dried at 105 °C.

Dried ground samples (20 g) of above-ground plant parts, leaves and stalk sections, were ashed at 450 °C in a muffle furnace for 2 h, after which the ash was dissolved in 5.0 ml 10.3 M HNO_3 + 5.0 ml of 24 M HF and then dried on a hot plate. This residue was redissolved in 5.0 ml 10.3 M HNO_3 and dried again, to be followed by dissolution in 5.0 ml 10.3 M HNO_3 and dried again in order to remove free fluoride. The final ash was dissolved in 25.0 ml 12.7 M HNO_3 for the determination of U. Aliquot samples (5.0–10.0 ml) of the dissolved ash were transferred to 125-ml separate funnels containing 10.0 ml saturated $Al(NO_3)_3$ and 10.0 ml 0.1 M TOPO (trioctylphosphinoxide, $[CH_3(CH_2)_7]_3PO$] in ethyl acetate. Funnels were shaken vigorously (5 min), and the organic (upper) and aqueous (lower) phases were allowed to separate. The uranium complex separated into the organic phase. Small volumes (0.1 ml) of the organic phase were transferred to platinum fusion dishes (10 mm in diameter) containing 0.75 mg 9 % NaF + 91 % $NaKCO_3$ pellets, dried under high intensity lamps and fused at 700 °C for 5 min., and then cooled. The intensity of fluorescence was determined in a fluorimeter Thermo-Jarrell Ash Corp., Franklin. The concentration of U was determined from standard U calibration curves (detection limit 0.005 mg kg^{-1}, range 0.05–5 mg kg^{-1}, correlation coefficient $R > 0.997$).

Statistical analysis

Statistical analyses were performed using software package Minitab 16 (Minitab Inc.). Normality was assessed with the Anderson–Darling test. Even when no normal distribution of the data transformation (logarithmic, exponential, power) was obtained, data treatment was done with the original data, unless indicated differently. Significant differences were considered at $p = 0.05$, and mean values were ranked by Tukey's multiple range tests when more than two groups were compared with ANOVA. Single-parameter regression analysis was performed with Minitab 16. Marked correlations are significant at $p < 0.05$ level, unless otherwise mentioned.

Results and discussion

The uranium concentrations in different shoot sections and their standard deviations for two tobacco types are given in Table 1. The uranium content in both of the types varies according to the age of their leaves and corresponding stem sections.

The mean values of uranium concentrations in the uppermost sections were 0.29 mg kg^{-1} for Virginia and 0.36 mg kg^{-1} for Burley type, whereas the means of uranium for the nearest root parts were 2.36 and 2.80 mg kg^{-1}, respectively. Latter concentrations are four times higher than the uranium levels accumulated in oldest leaves of maize, potato, cabbage, sunflower and bean, grown under the same conditions previously reported by Sarić et al. (1995).

The highest concentrations and their standard deviations of mean uranium values recorded in Burleys oldest leaves and stems were 4.18 ± 0.15 mg kg^{-1} and 1.42 ± 0.05 mg kg^{-1}, respectively. Corresponding uranium values for Virginia type were 3.54 ± 0.36 mg kg^{-1} for leaves and 1.17 ± 0.06 mg kg^{-1} for stems. These are almost 60 times higher uranium concentrations registered in leaves than in the leaves of crops cultivated on soil of a similar pollution level (Sarić et al. 1995). The lowest uptake of uranium was observed in the uppermost sections of both tobacco types, for Virginia type of 0.38 ± 0.07 mg kg^{-1} in leaves and 0.20 ± 0.04 mg kg^{-1} in stems, and for Burley type leaves and stem of 0.45 ± 0.06 mg kg^{-1} and 0.22 ± 0.04 mg kg^{-1}, respectively. From the results, it can be observed that with the growth period uranium content in various sections of the plant increases gradually. Lower, nearer root shoot sections generally accumulated more uranium than the younger, upper ones. Also, leaves accumulated more of uranium than their corresponding stems.

Table 1 Average uranium concentrations and their standard deviations in tobacco plant types across five shoot sections ($n = 5$)

Section	U (mg kg^{-1}) in			
	Virginia type		Burley type	
	Leaves	Stem	Leaves	Stem
1[a]	3.54 ± 0.36	1.17 ± 0.06	4.18 ± 0.15	1.42 ± 0.05
2	1.24 ± 0.04	0.29 ± 0.07	1.65 ± 0.20	0.40 ± 0.02
3	0.64 ± 0.04	0.27 ± 0.04	0.74 ± 0.06	0.37 ± 0.02
4	0.44 ± 0.03	0.27 ± 0.05	0.58 ± 0.06	0.38 ± 0.02
5[b]	0.38 ± 0.07	0.20 ± 0.04	0.42 ± 0.06	0.22 ± 0.04

[a] Nearest root shoot section

[b] Uppermost shoot section

Regression analysis of uranium uptake for both tobacco types is given in Fig. 1. The positive correlation between uranium concentrations in Virginia leaves and stems via different sections was statistically significant, with $p = 0.004$ ($p < 0.05$) and $R^2 = 0.94$ of variation in Virginia leaves accounted by the regression model (Fig. 1a). The relationship between uranium content in Virginia leaves and Burley leaves and Virginia stems and Burley stems was equal, highly statistically significant with $p = 0.000$ ($p < 0.05$) in both cases and with $R^2 = 0.99$. The relationship between uranium content in Virginia stems and Burley leaves was notable statistically significant as well with $p = 0.007$ ($p < 0.05$) and with $R^2 = 0.91$. The correlation between uranium concentrations in leaves and in stem sections in Burley tobacco was positive and statistically significant with $p = 0.006$ ($p < 0.05$) and $R^2 = 0.92$ (Fig. 1b). Also, positive correlation was found between Virginia leaves and Burley stems with p-value of 0.004 and $R^2 = 0.95$. These six strong positive correlations, and results suggest that the trend of the uranium uptake is very similar within the types of *N. tabacum* genus, as well as within different shoot sections across both of the types.

When considering each tobacco type separately, the allocation of the uranium via sections deviated from a normal distribution (Anderson–Darling test, $p < 0.05$, for each tobacco type). The p-value of 0.05 indicates that the null hypothesis of normality should be rejected at a confidence level of 95 %. Despite this, variations within the type did not significantly affect uranium concentration

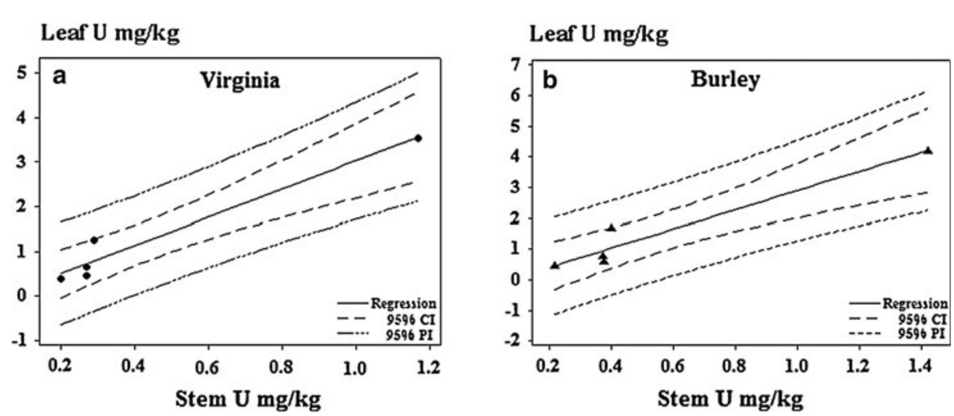

Fig. 1 The relationship between uranium concentrations in Virginia (**a**) and Burley (**b**) leaves and stems is statistically significant ($p < 0.05$), with $p = 0.004$ ($R^2 = 0.94$) and $p = 0.006$ ($R^2 = 0.92$), respectively. These strong positive correlations suggest a very similar trend of the uranium uptake within the tobacco types and within different shoot sections

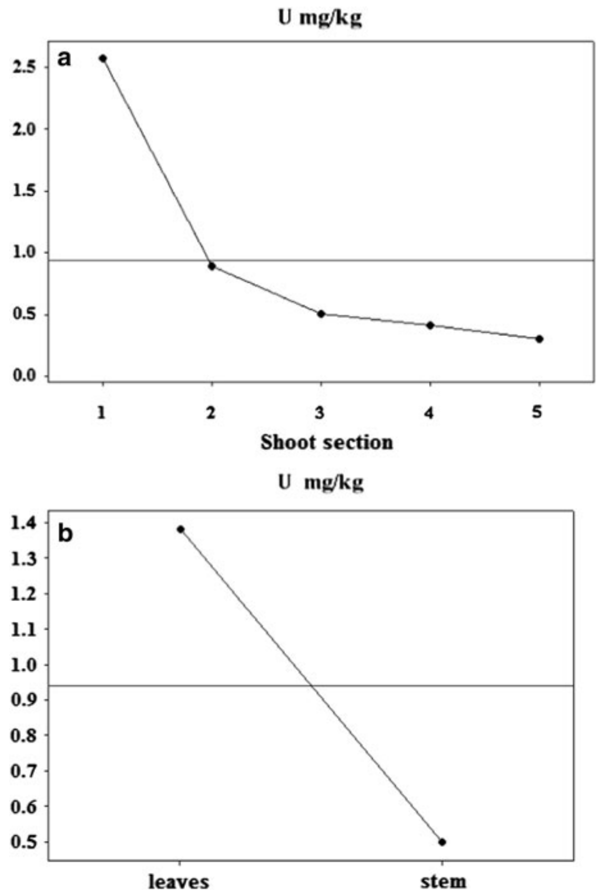

Fig. 2 The main effect plots for uranium concentrations regarding the shoot section (**a**) and plant part (**b**), Minitab Inc. Trend of data means indicate that the position of the shoot section (ANOVA, $F = 101.04$, $p = 0.000$), and the origin of plant parts (ANOVA, $F = 110.68$, $p = 0.000$) significantly affect uranium mass

(one-way ANOVA, $F = 0.15$, $p = 0.704$). Although not significantly different, stem samples showed a generally lower mean uranium concentration than leaves from the same section in the both of the types (one-way ANOVA, $F = 3.75$, $p = 0.069$). The mean values of uranium significantly differed over the sections within the types (one-way ANOVA, $F = 6. 38$, $p = 0.003$).

A wide variation in uranium concentration was found in both the types regarding the position of the stalk section and part of the plant. Analysis of variance using general linear model ANOVA indicated that the interaction regarding part and type of the plant did not significantly affect uranium concentration ($F = 0.02$, $p = 0.880$), as well as interaction regarding the type and section ($F = 0.03$, $p = 0.998$). In contrast to this, the position of the section affected uranium concentration ($F = 101.04$, $p = 0.000$) (Fig. 2a), in relation to origin of plant parts ($F = 110.68$, $p = 0.000$) (Fig. 2b). The interaction term (i.e., part $\times$ section) was also highly significant ($F = 29.23$, $p = 0.000$), which confirms that there were a substantial variance of uranium uptake in relation to section elderliness and plant parts genre.

Conclusion

The values of uranium accumulated in oldest leaves of both tobacco varieties are almost 60 times higher than the uranium levels in oldest leaves previously issued in cultivars grown under the similar conditions. Lower parts of the tobacco plant accumulated more uranium than the younger, upper ones, and leaves accumulated more uranium than the corresponding stems. Stems generally showed a lower adoption capability of uranium than leaves from the same section in the both of the tested types. Considering the level of soil contamination, values of accumulated uranium in nearest root sections of tobacco plant could speak in favor of the potential hyperaccumulatory properties of tobacco plant.

The trend of uranium uptake is very similar within the types of the genus, as well as within different shoot sections across the types. Substantial variance of uranium uptake was found in relation to section elderliness and the plant parts origin.

In summary, presented results point to two major implications of this study: firstly, the accumulated uranium levels evidence that tobacco plants (in particular, Virginia and Burley types) exhibit potential uranium hyperaccumulatory abilities convenient to phytoremediation, and secondly, a wider ramification of the results concerns the industrial production of tobacco and cigarettes from both theoretical and practical standpoint.

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