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Ramie (*Boehmeria nivea*)'s uranium bioconcentration and tolerance attributes



Wei-hong Wang^a, Xue-gang Luo^{b,*}, Lai Liu^a, Yan Zhang^a, Hao-zhou Zhao^a

- ^a College of Environment and Resources, Southwest University of Science and Technology, Mianyang, 621010, China
- ^b Engineering Research Center of Biomass Materials, Ministry of Education, Mianyang, 621010, China

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ABSTRACT

The authors sampled and analyzed 15 species of dominant wild plants in Huanan uranium tailings pond in China, whose tailings' uranium contents were 3.21–120.52 μ g/g. Among the 15 species of wild plants, ramie (Boehmeria nivea) had the strongest uranium bioconcentration and transfer capacities. In order to study the uranium bioconcentration and tolerance attributes of ramie in detail, and provide a reference for the screening remediation plants to phytoremedy on a large scale in uranium tailings pond, a ramie cultivar Xiangzhu No. 7 pot experiment was carried out. We found that both wild ramie and Xiangzhu No. 7 could bioconcentrate uranium, but there were two differences. One was wild ramie's shoots bioconcentrated uranium up to $20\,\mu$ g/g (which can be regarded as the critical content value of the shoot of uranium hyperaccumulator) even the soil uranium content was as low as $5.874\,\mu$ g/g while Xiangzhu No. 7's shoots could reach $20\,\mu$ g/g only when the uranium treatment concentrations were $275\,\mu$ g/g or more; the other was that all the transfer factors of 3 wild samples were > 1, and the transfer factors of 27 out of 28 pot experiment samples were < 1. Probably wild ramie was a uranium hyperaccumulator. Xiangzhu No. 7 satisfied the needs of uranium hyperaccumulator on accumulation capability, tolerance capability, bioconcentration factor, but not transfer capability, so Xiangzhu No. 7 was not a uranium hyperaccumulator.

We analyzed the possible reasons why there were differences in the uranium bioconcentration and transfer attributes between wild ramie and Xiangzhu No. 7., and proposed the direction for further research.

In our opinion, both the plants which bioconcentrate contaminants in the shoots and roots can act as phytoextractors. Although Xiangzhu No. 7's biomass and accumulation of uranium were concentrated on the roots, the roots were small in volume and easy to harvest. And Xiangzhu No. 7's cultivating skills and protection measures had been developed very well. Xiangzhu No. 7's whole bioconcentration factors and the roots' bioconcentration factors, which were 1.200-1.834 and 1.460-2.341, respectively, increased with the increases of uranium contents of pot soil when the soil's uranium contents are $25-175\,\mu\text{g/g}$, so it can act as a potential phytoextractor when Huanan uranium tailings pond is phytoremediated.

1. Introduction

Ramie (*Boehmeria nivea*), a fiber economic crop native to China, belongs to nettle family and ramie genus, and has been planted in middle and lower reaches of the Yangtze River since the Neolithic Age, known as "Chinese grass" in the international community. The world's ramie producing areas are between 50° S and 38° N latitude, among which China has the largest output, accounting for about 90% of the world's total output, and Brazil and the Philippines ranks the second and third respectively. In addition, Indonesia, North Korea, Vietnam and other Southeast Asian countries have a small amount of cultivation, and it was reported that in the Mediterranean region ramie was also

successfully artificially cultivated (Angelini and Tavarini, 2013).

There are very rich wild ramie germplasm resources in China. From 1995 to 1998, Lai Zhanjun et al. collected 130 copies of wild ramie species from 34 counties of 14 provinces and autonomous regions, which are situated in the Yangtze River basin and its south; covering 20 species and 6 variants (Lai et al., 1999). A number of studies found that wild ramie was often a pioneer or dominant plant in the mining area or smelter tailings, with excellent quality to adapt to environments lacking of water and fertilizer and endure heavy metal pollution, which had a bioconcentration effect on arsenic, cadmium, lead, antimony, copper, zinc, etc. And wild ramie performed some hyperaccumulative characteristics on above mentioned heavy metals. (She et al., 2010, 2011;

E-mail address: lxg@swust.edu.cn (X.-g. Luo).

^{*} Corresponding author.

Table 1
The properties of soil where wild ramie samples were collected and the pot experimental soil.

Soil	PH	Organic matter (g.kg ⁻¹)	Total N (g.kg ⁻¹)	Total P (g.kg ⁻¹)	Total K (g.kg ⁻¹)	Alkaline N (mg.kg ⁻¹)	Available P (mg.kg ⁻¹)	Available K (mg.kg ⁻¹)
Wild ramie sampling	5.8	21.0	1.43	0.48	18.5	82.8	9.6	65.7
Pot experiment	7.4	22.8	1.50	0.56	25.4	109.0	9.0	131.0

Wei and Chen, 2002; Yue, 2004).

At the end of July 2015, the authors of this paper sampled and analyzed 15 species of dominant plants in Huanan uranium tailings, whose tailings' uranium contents were $3.21-120.52\,\mu\text{g/g}$. Among the 15 species of wild plants, ramie had the strongest uranium bioconcentration and transfer capacities. It was the first time finding that ramie had good uranium bioconcentration potential. In order to further study the uranium bioconcentration and tolerance attributes of ramie, and provide a reference for the screening work of remediation plants in uranium tailings, a ramie pot experiment was carried out.

2. Experimental materials and methods

2.1. Experimental materials

Both the soil where wild ramie samples were collected and the pot experimental soil were loam, with their properties being shown in Table 1. The pot experimental soil was taken from the farmland (104° 42′ E, 31° 32′ N) next to the greenhouse in the Engineering Research Center of Biomass Material, Ministry of Education. It passed through 1.4 cm sieve, and was loaded into the pots (2 kg DW per pot), whose depths were 15.5 cm, and the upper and bottom diameters 18.5 and 13.0 cm, respectively.

Many experiment reports showed that in low-dose radionuclide contaminated soil, the chemical toxicity of radionuclides was much stronger than its radiotoxicity, so radioactive toxicity can be negligible, and the stable isotope can replace radioactive isotope to carry out related researches (Wang et al., 2016). Since the uranium contents of the mineral waste residue and soil stored in the uranium tailings pond were between 3.21 and $120.52\,\mu\text{g/g}$, U^{238} derived from analytically pure uranyl acetate was adopted in this pot experiment.

Taking account into that it is difficult for wild ramie to reproduce by seeding, while cultivating skills and protection measures of cultivars had been developed very well and cultivars are more suitable to phytoremedy on a large scale, we decided to carry out the pot experiment with cultivar Xiangzhu No. 7, being successfully co-cultivated in 2013 by Hunan Agricultural University and Yuanjiang Bureau of Agriculture, whose breeding spot and suggested planted areas were less than 100 km apart from the uranium tailings pond, ensuring the similarity between the wild variety and cultivar as much as possible. And Xiangzhu No. 7 had been proven to have high yield and satisfactory tolerance on heavy metals Cd and As (Zhao, 2015).

2.2. Evaluation indices

2.2.1. Evaluation indices of uranium bioconcentration attributes

(1) Bioconcentration factor (BCF)

Uranium bioconcentration factor = plant uranium content $(\mu g/g)$ / soil uranium content $(\mu g/g)$, among which: plant uranium content = (U content in stem \times stem DW + U content in leaf \times leaf DW + U content of root¹ of plant \times DW of root)/(stem DW + leaf DW + DW of root).

(2) Transfer factor (TF)

Uranium transfer factor = U content of the shoot of plant $(\mu g/g)/U$ content of the root of plant $(\mu g/g)$, among which: U content of the shoot of plant = $(U \text{ content in stem } \times \text{ stem DW} + U \text{ content in leaf } \times \text{ leaf DW})/(\text{stem DW} + \text{ leaf DW})$.

(3) Plant uranium accumulation quantity

Plant uranium accumulation quantity = uranium accumulation quantity of shoot of plant + uranium accumulation quantity of root of plant.

Uranium accumulation quantity of shoot of plant = U content in stem \times stem DW + U content in leaf \times leaf DW.

Uranium accumulation quantity of root of plant = U content in root of plant \times DW of root.

2.2.2. Evaluation indices of uranium tolerance attributes

Tolerance Indices (TI) of plant response to uranium stress were used as the evaluation indices to tolerance attributes of uranium. Tolerance index = (biological character value of U-treatment/biological character value of CK) \times 100% (Baker, 1987; Bona and Kang, 2003).

The influence of the external environmental factors on the photosynthetic state can be reflected by the change of the chlorophyll fluorescence parameters. Therefore, it is often necessary to measure the chlorophyll fluorescence parameters in the studies of stress physiology of higher plants. The maximum quantum yield reflects the quantum yield when the photosystem II complex is in a fully open state. It is the most widely and frequently used parameter among all chlorophyll fluorescence parameters, reflecting the potential maximum photosynthetic capacity of the plant.

If the plant cannot tolerate heavy metal pollution stress or tolerance is low, the plant's growth will be inhibited because the transport of essential nutrients in the plant is cut off, and its vegetative characters such as leaf color and plant height will change to various degrees, eventually the biomass of the shoot will reduce significantly (Wei, 2004; Sun, 2001; Zhou et al., 2003).

The root system is the first part to be affected by the heavy metal pollution of the entire plant. When the plant is less resistant to heavy metal pollution, its root growth will be inhibited. Root morphological indices are often used to evaluate the metal tolerance attributes of a plant. Wilkins proposed to use the "root elongation method" to quantify the inhibitory effect of plant root on the metal ions in the heavy metal solution (Wilkins, 1957), and the simplest and most convenient index of root morphological indices is the longest root extension before and after the plant's migration into the culture medium of pollutants. It is best to carry out observation and measurement of the root morphology in the solution. However, the hydroponics cultivation environment is different from the soil environment, and a terrestrial plant has different root growth attributes and heavy metal absorption attributes in the solution and soil, so it is not accurate to evaluate the remediation ability of a terrestrial plant by hydroponics (Tang et al., 2013). Therefore, the authors did not measure the response of the root length of Xiangzhu No. 7 on the uranium stress by hydroponics. Since seeding, rather than transplantation, is used in this soil culture experiment, the longest root length can be used as one of the indices for evaluation of ramie tolerance to uranium. In order to avoid the influence of the errors of root

 $^{^{1}}$ In this paper, root refers to the underground part of ramie including underground stem.

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