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URANIUM UPTAKE OF *VETIVERIA ZIZANIOIDES* (L.) NASH

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Лыу Вьет Хынг и др. E18-2010-71
Урановое обогащение ветиверовой травы

Произведена оценка извлечения урана ветиверовой травой из аллювиальных каштановых, серых, солевых и красно-желтых почв Северного Вьетнама. Ветиверовая трава высевалась в почвы, в которые добавляли уран в количестве 0, 50, 100, 250 мг/кг в виде водного раствора уранилнитрата. Эффективность извлечения урана определяли по передаточному фактору (ПФ_U, $\text{кг} \cdot \text{кг}^{-1}$), равному отношению содержания урана в растении и в почве при равновесии. Передаточный фактор ПФ_U зависит от свойств почв, таких как pH, количество органического материала, железистых и калиевых веществ. Показано, что ветиверовая трава может быть использована для рекультивации почв, зараженных ураном.

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Luu Viet Hung et al. E18-2010-71
Uranium Uptake of *Vetiveria zizanioides* (L.) Nash

Uranium uptake of vetiver grass (*Vetiveria zizanioides* (L.) Nash) from Eutric Fluvisols (AK), Albic Acrisols (BG), Dystric Fluvisols (HP) and Ferralic Acrisols (TC) in northern Vietnam is assessed. The soils were mixed with aqueous solution of uranyl nitrate to make soils contaminated with uranium at 0, 50, 100, 250 mg/kg before planting the grass. The efficiency of uranium uptake by the grass was assessed based on the soil-to-plant transfer factor (TF_U, $\text{kg} \cdot \text{kg}^{-1}$). It was found that the TF_U values are dependent upon the soils properties. CEC facilitates the uptake and the increased soil pH could reduce the uptake and translocation of uranium in the plant. Organic matter content, as well as iron and potassium, inhibits the uranium uptake of the grass. It was revealed that the lower fertile soil, the higher uranium uptake. The translocation of uranium in root for all the soil types studied is almost higher than that in its shoot. It seems that vetiver grass could potentially be used for the purpose of phytoremediation of soils contaminated with uranium.

The investigation has been performed at the Flerov Laboratory of Nuclear Reactions, JINR.

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1. INTRODUCTION

Uranium (U) exists in the nature in a mixture of the three isotopes, namely ^{238}U, ^{235}U, and ^{234}U, with a relative abundance of 99.27, 0.720, and 0.0055%, respectively. Uranium, as a natural radioactive heavy metal, widely occurs throughout the Earth's crust, and the average U concentration in soil ranges from 0.1 to 11 mg $\cdot$ kg^{-1} [4, 6]. Uranium contamination of surface soils originates from such sources as weapon research, nuclear fuel production, waste reprocessing, mining, military operations employing ammunition with depleted uranium (DU) and the use of phosphate (P) fertilizers in agriculture. Environmental behavior of uranium is similar to that of other heavy metals, and its physiological toxicity, other than damage from ionizing radiation, mimics that of lead. Uranium is chemically toxic to kidneys, and insoluble U compounds are carcinogenic [4–6, 15].

In situ remediation techniques can sometimes be more suitable for radioactive contaminants due to the lower health risks of workers resulting from construction or transportation processes. The remediation techniques currently used are soil excavation, which removes the soil with radionuclides in its present state or after stabilization in concrete or glass matrices [7], and soil washing, which also requires soil removal plus chemical manipulations [5]. However, the remediation of radionuclide-contaminated soils represents a significant expense to many industries and governmental agencies.

One alternative to traditional radionuclide treatments is the method of phytoremediation. Phytoremediation of radionuclides has many advantages over the traditional treatments such as potentially lower cost. Phytoremediation, a novel plant-based remediation technology, is being applied to a variety of radionuclide-contaminated sites. Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless. Phytoextraction (phytoaccumulation), a type of phytoremediation, is defined as the use of metal-accumulating plants (hyperaccumulators) that can transport metals from the soil to the roots and then translocate them in the above-ground shoots [2, 7].

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) is a fast-growing, perennial, tussock grass belonging to the family Poaceae and a native of south-east Asia with a particular cultivar in the Indian subcontinent. The World Bank has initiated

several projects in India for systematic development of vetiver grass technology (VGT), now popularly known as vetiver system (VS). Vetiver grass is well known for its high tolerance for metals such as Al, Mn and heavy metals like Cd, Cr, Ni, Pb, Hg, Se, Zn, and metalloids such as arsenic (As) present in the soils [10–12]. Vetiver grass can also remove ^{90}Sr up to 90% and ^{137}Cs up to 60% from the water within 7 h [14].

The use of vetiver grass was introduced in Vietnam 10 years ago, and it is currently being used for the purpose of disaster mitigation, erosion control in Vietnam. Recently, several trials have been made with the use of vetiver grass for wastewater treatment, pollution control and reducing the toxins in soils, etc., and got promising results [9].

In this study, the uptake of U by vetiver grass from several typical soils in the northern Vietnam has been tested and evaluated. The purpose of the study is to evaluate the possibility of the use of the grass for U clean-up from soils contaminated with the metal.

2. MATERIALS AND METHODS

2.1. Soil Type and Uranium Contamination of the Soils for the Experiment. Four types of soil of the FAO classification, namely Eutric Fluvisols (AK), Albic Acrisols (BG), Dystric Fluvisols (HP) and Ferralic Acrisol (TC), were chosen for the study. The Eutric Fluvisols is the alluvium deposition of the Red River and it was taken from the Hanoi area. The Albic Acrisols is a low fertile and gray-silver color soil from Hiep Hoa, Bac Giang province. The Dystric Fluvisols is an alluvial but saline soil from a coastal area of Tien Lang, Hai Phong province (HP), and the Ferralic Acrisols is a red-yellow soil with high Fe and Al content from the midland of the Thu Cuc, Phu Tho province (TC). All the places are in the northern Vietnam. The soils were taken from its surface layer, i.e., from 0 to 20 cm depth. Some of the physical and chemical characteristics of the soils used in this study are presented in Table 1.

After the soils were taken, they were allowed to dry in the air, then were crashed by hand and sieved on a sieve of 2 mm mesh. The fine-grained soils obtained were mixed with different amount of uranyl nitrate $[UO_2(NO_3)_2 6H_2O]$ dissolved in distillated water to make soils contaminated with U at levels of 50, 100 and 250 mg per kg of soil. The mixing process was performed by thoroughly shaking the fine-grained soils with aqueous solution of the uranium salt in PE bags of appropriate thickness. After that each soil type was split into portions of 3 kg each and transferred to ceramic pots of D200 and H180 (mm). The soils contaminated with uranium in pots were left for a week in order that uranium could equally distribute within the soil mass. The uranium salt was PA grade and from the Merck (Germany) supplier. The experiment was conducted with

Table 1. Physical and chemical characteristics of the soils used in this study

Parameter	Soil-AK	Soil-BG	Soil-HP	Soil-TC
pH_{KCl}	6.22	5.75	5.88	6.13
Sand (%)	33.28	30.96	14.35	35.72
Silt (%)	31.15	56.30	36.64	37.13
Clay (%)	35.57	12.74	49.01	27.15
CEC* (meq/100 g)	21.00	25.30	24.48	19.80
OM** (%)	3.50	2.62	4.45	4.18
Al ($mg \cdot kg^{-1}$)	19281	1833.2	15522.4	51147.7
Cu ($mg \cdot kg^{-1}$)	32.93	4.12	20.42	27.20
Fe ($mg \cdot kg^{-1}$)	14731.03	989.02	14602.02	26447.10
K ($mg \cdot kg^{-1}$)	6209.20	739.30	5074.60	3765.50
Mn ($mg \cdot kg^{-1}$)	326.80	41.01	69.90	151.03
P ($mg \cdot kg^{-1}$)	457	405	278	306
Pb ($mg \cdot kg^{-1}$)	31.93	6.57	32.09	67.86
U background ($mg \cdot kg^{-1}$)	2.16	0.42	2.54	27.69

*Cation exchange capacity.
**Organic matter.

triplicate including 12 pots as controls (no uranium added) for 4 soil types, so totally there were 48 pots.

2.2. Grass Planting. Mature tillers of vetiver grass (*vetiveria zizanioides* (L.) Nash) with at least three or four well-developed leaves and bases, together with some roots, were separated, cut off the body above the leaves, leaving only a section of about 15 cm length [12], then planted into the pots. The pots were left outdoors under the natural condition of temperature and sunlight in the Hanoi area, usually $30-35\,^{\circ}C$ and relative humidity of 80–90%. The grass was daily watered with tap U-free water. The duration of the experiment was six months, from March to August.

2.3. Sample Processing for Chemical Analysis. Grass samples were harvested after six months of the experiments. The samples were separated into two parts, above-ground one, i.e., shoot part, consisting of the stem just above the soil surface and root part. The plant roots were harvested by soaking the pots and their contents in a water bath and gently washing off the soil adhered to take all of the roots. The roots and shoots were rinsed with deionized water before drying at $85^{\circ}C$ overnight. Biomass of plant was determined by weighing alltogether the shoot and root part. The dried plant samples were crushed into small size and

then ground to < 1 mm powder and stored in glass bottles until the analysis is performed.

The chemical composition of the grass samples, both shoot and root, was analyzed using the Inductive Couple Plasma Mass Spectrometry (ICP-MS) method at a Perkin Elmer, Elan 9000, USA. To do so, around 200 mg of dry plant sample was digested in a microwave with 7 ml HNO_3 concentrated and 3 ml 30% H_2O_2. After the digestion completed and the liquid became clear, all the content was transferred into a metric bottle of 50 ml capacity. The content in the bottle was diluted with 2 M HNO_3 just to the marker level [16].

The soil samples were dried at $105°C$ overnight, then finely ground, sieved through a sieve of mesh size < 1 mm. Around 200 mg of the fine-grained soil samples were digested in a microwave oven following EPA Method 3052 [16]. After the solution became clear, it was filtered to remove sand retained. All the clear solution was then transferred into metric bottle and diluted with 2 M HNO_3 solution for further analysis by the same ICP-MS technique.

For each sample the analysis was repeated three times to derive the mean value and standard deviation. The QA/QC procedure for the analysis was performed using a certified reference material (CRM) soil-7 supplied by the International Atomic Energy Agency (IAEA) in Vienna. It was revealed that the accuracy of the analysis was less than 10% for all the elements, including uranium.

2.4. Data Processing. In this study the uranium uptake of the grass was assessed based on the soil-to-plant transfer factor of uranium (TF_U) that is defined as

$$TF_U = \frac{[U_{plant}]}{[U_{soil}]}, \quad kg \cdot kg^{-1}, \tag{1}$$

where $[U_{plant}]$ and $[U_{soil}]$ denote the concentration of uranium in root and shoot (stem and leaf) of the grass and soil samples ($mg \cdot kg^{-1}$, dry weight), respectively.

The quantity of TF_U will tell us about the ability of the grass in phytoextraction of the uranium contamination from uranium-contaminated soil.

The results presented in tables and figures are the mean value of the three repeated analyses. The standard deviation of the analysis is always no more than 10%.

3. RESULTS AND DISCUSSION

3.1. Biomass and Potential U Accumulation of Vetiver Grass. During the experiment no signs showing the addition of uranium to soil affect the growth of vetiver grass. At a level of 250 $mg \cdot kg^{-1}$ of uranium concentrations added, the grass still survives and grows moderately well, showing no toxicity symptoms, e.g., chlorosis, burning of leaf margins, leaf abscission and shoot die black.

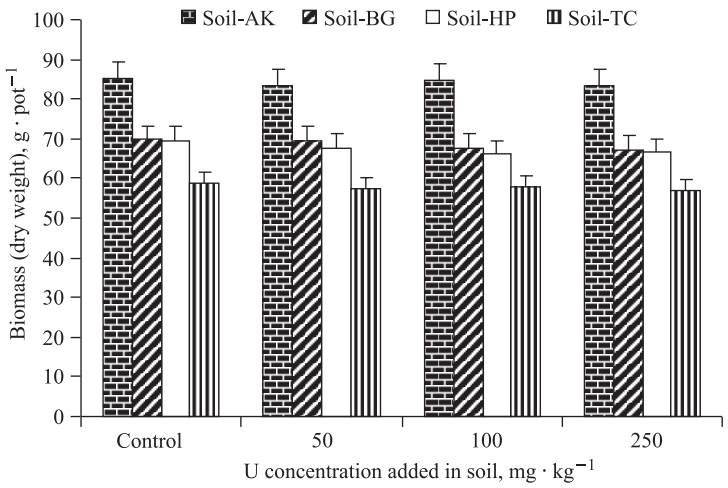

Fig. 1. Effect of uranium concentration in soils on biomass of vetiver grass

The biomass of vetiver grass (dry weight, d.w.) harvested in the experiments is presented in Fig. 1. The results showed that the biomass of the grass grown in soil contaminated with high content of uranium does not significantly differ from those planted on the control soils. It was found that the highest grass biomass was harvested from the control soil-AK with (85.25 ± 4.26) g $\cdot$ pot^{-1} and the lowest one from the control soil-TC of (58.75 ± 2.94) g $\cdot$ pot^{-1}. The biomass of the grass planted on the soil-AK (83.25 ± 4.16) g $\cdot$ pot^{-1}, soil-BG (67.25 ± 3.36) g $\cdot$ pot^{-1}, soil-HP (66.75 ± 3.34) g $\cdot$ pot^{-1}, and soil-TC (56.75 ± 2.84) g $\cdot$ pot^{-1} contaminated with uranium at 250 mg $\cdot$ kg^{-1}, reduced down, respectively, by 2.35%, 3.58%, 3.96%, and 3.40% compared to the biomass of the grass planted on the control soils, i.e., no uranium added (Fig. 1). It was obvious that the reduction of the grass biomass was not so significant even though the concentration of the contaminant was up to hundred times higher than the control.

Tolerance of plant to U contamination level in soils is expressed as the ratio percent between the biomass of plant species grown in the soil treated with uranium and that of plant species grown in control soil. This index is used for screening plants and evaluating heavy metal uptake, accumulation and tolerance in the phytoremediation [1, 13]. In this experiment the uranium tolerance index of vetiver grass was found to be 97.7%, 96.4%, 96.0% and 96.6%, respectively, for the soil-AK, soil-BG, soil-HP and soil-TC contaminated with U at 250 mg $\cdot$ kg^{-1}.

3.2. Uranium Uptake and Accumulation in Plant. The extent of U uptake and translocation in shoots (stems and leaves) and roots of vetiver grass grown

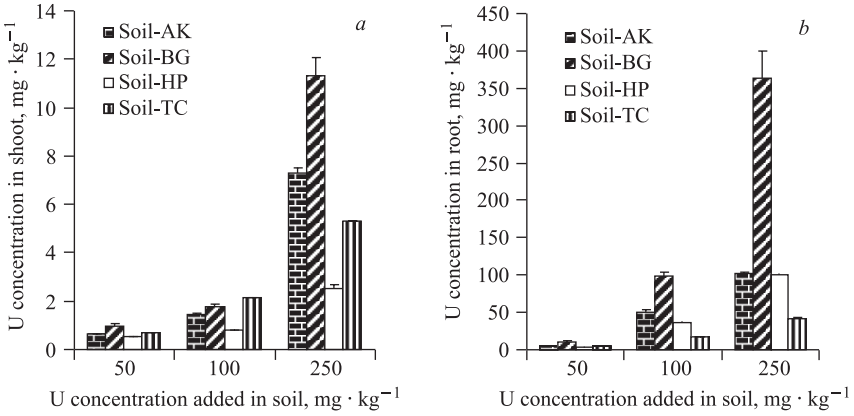

Fig. 2. Uranium uptake and translocation in shoots and roots of vetiver grass

in soils contaminated with different rates of the U is shown in Fig. 2. Data in Fig. 2 show that U concentration in grass shoot and root increase with increasing the content of uranium in the soils. Uranium translocated more in roots than in shoots, regardless of U content and soil types. At the level of 250 $\text{mg} \cdot \text{kg}^{-1}$, the U uptake is ranging within (2.51 ± 0.15) and (11.33 ± 0.75) $\text{mg} \cdot \text{kg}^{-1}$ in shoot and between (42.32 ± 0.59) and (363.72 ± 35.68) $\text{mg} \cdot \text{kg}^{-1}$ in root (Fig. 2). The extent of U accumulation in vetiver grass grown in farming soils such as soil-AK, soil-BG, and soil-HP was higher than that in vetiver grass grown in bare soil-TC. Uranium concentration was the highest in the root of the grass grown in the low fertile soil-BG and the lowest in the bare soil-TC. On the other hand, the U concentration in the root of the grass in soil-BG was found to be 32 times higher than that in its shoot in case of the soil was contaminated with U to 250 $\text{mg} \cdot \text{kg}^{-1}$.

Uranium soil-to-plant transfer factors (TF_U) are the indices used for screening plants and evaluating heavy metal uptake, and accumulation in the phytoremediation [1, 13, 15]. The TF_U is determined as the ratio of the uranium concentration in plant to those in the soils at the equilibrium. Figure 3 presents the value of TF_U of vetiver grass. The values of TF_U of the grass presented in Fig. 3 were determined for the case of U added to the soils at a concentration of 250 $\text{mg} \cdot \text{kg}^{-1}$. The TF_U was found to be the highest with the soil-BG representing the lowest nutrition one among all the soil types studied, and it was (0.510 ± 0.137) $\text{kg} \cdot \text{kg}^{-1}$, followed by soil-AK of (0.148 ± 0.003) $\text{kg} \cdot \text{kg}^{-1}$, soil-HP of (0.126 ± 0.015) $\text{kg} \cdot \text{kg}^{-1}$ and lowest soil-TC of (0.069 ± 0.012) $\text{kg} \cdot \text{kg}^{-1}$. These findings are in good agreement with those reported by Vandenhove H. [15].

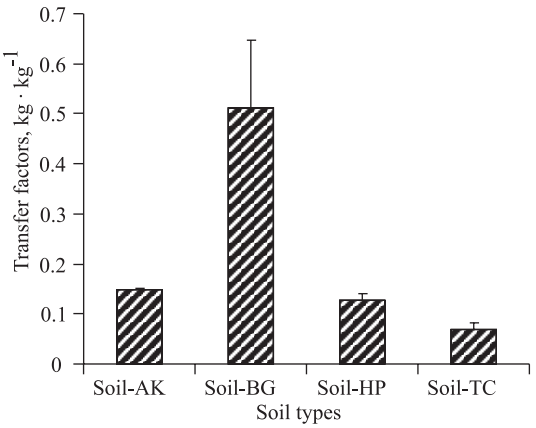

Fig. 3. Uranium soil-to-plant transfer factors (TF_U) of soils mixed with 250 mg $\cdot$ kg^{-1}

3.3. Effect of Soil Properties on the Uranium Uptake of Vetiver Grass. The data presented in Table 1 show that the soils used in this study are acidic with pH ranging from 5.75 to 6.22 (Table 1). Under the acidic condition, U in soil is present primarily (80–90%) in the +VI oxidation state as the uranyl (UO_2^{2+}) cation. Free UO_2^{2+} species of U in soil was proven to be easiest for plant to take up and translocate different parts [3, 15]. In this study, it was found that the correlation between the TF_U values and soil pH was good enough that the correlation coefficient was $R^2 = 0.54$ (Fig. 4). The TF_U values decrease with the increase of pH, meaning the higher soil pH the less bioavailability of uranium in soil for plant to take up. The availability of uranium cations for plant to take up seems to depend upon the CEC of soils, so it was found that the TF_U of the plant is positively correlated with the soils CEC with a correlation coefficient R^2 close to 0.60 (Fig. 5). Note that the TF_U values presented in Figs. 4 and 5 are the mean one derived from four levels (0, 50, 100, and 250 mg $\cdot$ kg^{-1} soil) of uranium added to each of the soil types studied and its deviation is within 30% as a maximum.

It was revealed also that the TF_U values are negatively correlated with the content of organic matter (OM), of clay, of ferrous and potassium concentration in the soils studied. The correlation of TF_U and [SOM], [clay], [Fe] and [K] is described by respective equations presented in Table 2.

A. Piccolo [8] studied the structure of soil organic matter (SOM) by different techniques like permeable chromatography, nuclear magnetic resonance (^{13}C-NMR) and proved that SOM has supermolecular and micelle-like structure. This structure could entrap constituents presented in soil inside, making the contami-

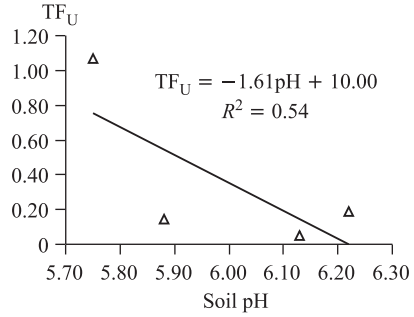

Fig. 4. The correlation of TF_U with soil pH

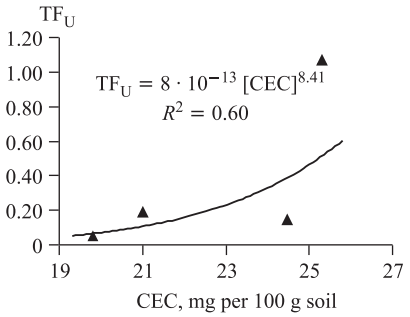

Fig. 5. The correlation of TF_U with soil CEC

nant less available for plant to take up or translocate. This might be the reason why SOM reduces the uptake of uranium of the grass. On the other hand, clay possesses a lot of alumina hydroxide acting as active centers to adsorb metallic cations like uranyl. By this adsorption mechanism the clay component in soils seems to be a good adsorbent to fix uranium not allowing the constituent to be available to the plant. Iron and potassium seem to compete with uranyl cation for translocation and uptake by the plant. The effect of decrease in the plant uptake of other metallic cations with the increase of ferrous content in soils was also observed by the authors of [13].

Figure 6 depicts the accumulation of uranium in shoot of the grass planted in four soil types mixed with different rate of uranium.

As seen from Fig. 6, the lower fertile soil the higher uranium uptake and translocation by the grass. In the case of the soil-BG (poor fertile soil) totally the grass could accumulate as high as up to (126.45 ± 12.04) mg · kg^{-1} of plant biomass (Fig. 2) if soil was contaminated with uranium at a concentration of 250 mg · kg^{-1}. Additionally, the grass was grown well under the natural conditions without fertilization with any nutrients during the six-month experiment. The grass has high biomass productivity and its ability to tolerate high concentra-

Table 2. Equation describing the relationship between TF_U and content of soil organic matter, clay, iron and potassium (the correlations were not shown graphically here)

Correlation pair	Correlation equation	Correlation coefficient, R^2
TF_U – SOM	$TF_U = -0.52[OM] + 2.29$	0.81
TF_U – [clay]	$TF_U = -0.02[clay] + 1.10$	0.58
TF_U – [Fe]	$TF_U = -4 \cdot 10^{-5}[Fe] + 0.95$	0.81
TF_U – [K]	$TF_U = -2 \cdot 10^{-4}[K] + 1.03$	0.72

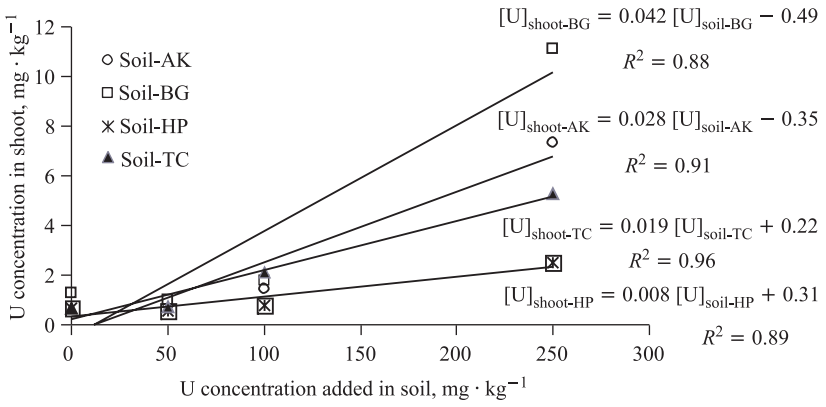

Fig. 6. The accumulation of uranium in the grass shoot in relation to the soil types

tions of pollutants, over 95% at 250 mg · kg^{-1} of U concentration in soil. This result shows that vetiver grass could effectively be used to treat soils contaminated with high uranium content.

4. CONCLUSIONS

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) appears to be a good plant to take up uranium from soil contaminated with high concentration of the metal. More uranium is taken up and accumulated mainly in the root than in the shoot of vetiver grass. The uptake is dependent upon the soil properties. The soil CEC facilitates but pH, OM, ferrous, potassium, and clay content of soils, reversely, reduces the uranium uptake of the grass. The poorer fertile soils, the higher uranium uptake by the plant. The tolerance of the grass to uranium contamination is as high as up to 95%, at the same time the plant could survive and grow well under the natural conditions without any fertilization. All this makes the grass a potential plant for effective phytoremediation of soils contaminated with uranium and other heavy metals as well as radionuclides.

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