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THE UPTAKE OF URANIUM FROM SOIL TO VETIVER GRASS (*VETIVER ZIZANIOIDES* (L.) NASH)

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Abstract

Uranium uptake of vetiver grass (*Vetiveria zizanioides* (L.) Nash) from Eutric Fluvisols (AK), Albic Acrisols (LP), Dystric Fluvisols (TT) and Ferralic Acrisols (TC) in northern Vietnam is assessed. The soils were mixed with aqueous solution of uranyl nitrate to make soils be contaminated with uranium at 0, 50, 100, 250 mg per 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 , kg kg^{-1}). It was found that the TF_U values are dependent upon the soils properties. CEC facilitates the uptake and the increase soil pH could reduce the uptake and translocation of uranium in the plant. Organic matter content as well as ferrous and potassium inhibit the uranium uptake of the grass. It was revealed that the lower fertile soil the higher uranium uptake. The grass could tolerate to the high extent (up to 77%) of uranium in soils and could survive and grow well without fertilization. The translocation of uranium in root for all the soil types studies 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.

Keywords: Uranium uptake, transfer factor (TF), Vetiver grass, Vietnam soils

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 disperses throughout the Earth's crust and the average U concentration in soil ranges from 0.1 to 11 mg 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, 5, 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, soil excavation- removes the soil with radionuclides in its present state or after stabilization in concrete or glass matrices [7] and soil washing- also requires soil removal plus chemical manipulations [5]. However, the remediation of radionuclides contaminated in 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 are 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 aboveground 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 Southeast 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 has been introduced into Vietnam for 10 years ago and it 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 clean-up U 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 (LP), Dytric Fluvisols (TT) 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 color soil from Luong Phong, Hiep Hoa, Bac Giang province. The Dytric Fluvisols is an alluvial but saline soil from a coastal area of Toan Thang, Tien Lang, Hai Phong province (HP), and the Ferralic Acrisols is a red-yellow with high Fe and Al content soil from the midland of the Thu Cuc, Tan Son, Phu Tho province (TC). All the places are from the northern Vietnam. The soils were taken from its surface layer, i.e. from 0 to 20cm 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 grain soils obtained were mixed with different amount of uranyl nitrate [$\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] dissolved in distilled water to make soils be contaminated at levels of 50, 100 and 250 mg U per kg soil. The mixing process was performed by thoroughly shaking the fine grain soils with aqueous solution of the uranium salt in PE bags of appropriate thickness. After that each soil type was split in 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 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 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 outdoor under the natural condition of temperature and sunlight in the Hanoi area, usually 30-35°C and relative humidity of 80-90%. The grass was daily watered with tap U-free water. The duration of the experiment was three months.

2.3. Sample processing for chemical analysis

Grass samples were harvested after 3 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 75°C overnight. Biomass of plant was determined by weighing all together 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 performed.

The chemical composition of the grass samples both shoot and root was analyzed using 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 HNO₃ concentrated and 3ml 30% H₂O₂. After the digestion completed and the liquid became clear all the content was transferred

into metric bottle of 50 ml capacity. The content in the bottle was diluted with 2 M HNO₃ just to the marker level [16].

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

Parameter	Soil-AK	Soil-LP	Soil-TT	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
Cation exchange capacity (CEC) (meq/100g)	21.00	25.30	24.48	19.80
Organic matter (OM) (%)	3.50	2.62	4.45	4.18
Al (mg kg ⁻¹)	19281	1833.2	15522.4	51147.7
Cu (mg kg ⁻¹)	32.93	4.12	20.42	27.20
Fe (mg kg ⁻¹)	14731.03	989.02	14602.02	26447.10
K (mg kg ⁻¹)	6209.20	739.30	5074.60	3765.50
Mn (mg kg ⁻¹)	326.80	41.01	69.90	151.03
P (mg kg ⁻¹)	457	405	278	306
Pb (mg kg ⁻¹)	31.93	6.57	32.09	67.86
U background (mg kg ⁻¹)	2.16	0.42	2.54	27.69

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 grain soil samples were digested in a microwave followed by 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 HNO₃ 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) and Single-Element Standards manufactured by Inorganic Ventures (USA). 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

$$TF_U = \frac{[U_{plant}]}{[U_{soil}]}, \text{ kg kg}^{-1} \quad (1)$$

where [U_{plant}] and [U_{soil}] denotes as the concentration of uranium in root and shoot (stem and leaf) of the

grass and soil samples (mg kg⁻¹ dry weight, d.w), respectively.

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

Results presented in tables and figures are the mean value of three repeated analysis. The standard deviation of the analysis is always not exceeded 10%.

3. RESULTS AND DISCUSSION

3.1. Biomass and potential accumulation U 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 kg⁻¹ of uranium concentrations added the grass still survives and grows moderately well, non to show toxicity symptoms, e.g. chlorosis, burning of leaf margins, leaf abscission and shoot die black.

The biomass of vetiver grass harvested in the experiments is presented in Figure 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-LP with 35.95±1.79 g.pot⁻¹ and the lowest one from the control soil-AK of 29.27±1.46 g pot⁻¹. The biomass

of the grass planted on the soil-AK 28.11 ± 1.40 g.pot⁻¹, soil-LP 27.57 ± 1.37 g.pot⁻¹, soil-TT 32.58 ± 1.62 g.pot⁻¹, and soil-TC 29.90 ± 1.49 g.pot⁻¹ contaminated with uranium at 250 mg U kg⁻¹, reduced down, respectively, by 3.97%, by 22.84%, by 5.76%, and by 9.26% compared to the biomass of the grass planted on the control soils, i.e. no uranium added (Fig. 1). It was obvious the fact that the reduction of the grass biomass was not so significant even the concentration of the contaminant was increased 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 96.03%, 77.16%, 94.24% and 90.74%, respectively, for the soil-AK, soil-LP, soil-TT and soil-TC contaminated with 250 mg U kg⁻¹.

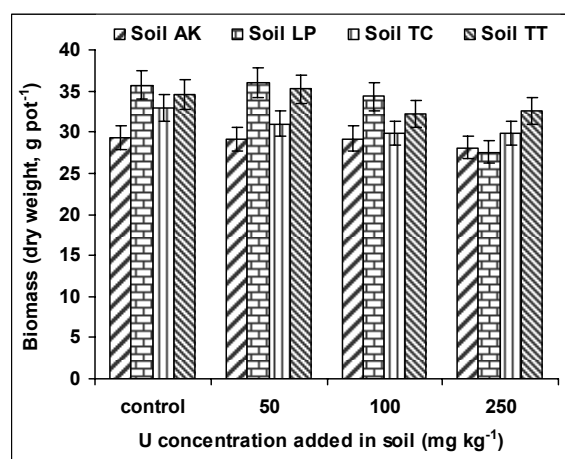


Figure 1: Effect of uranium concentration in soils on biomass of vetiver grass

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 in soils contaminated with different rates of the U is shown in Table 2. Data in Table 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 mg U kg⁻¹, the uptake is ranging within 2.11 ± 0.10 and 27.57 ± 1.37 mg U kg⁻¹ in shoot and between 16.29 ± 0.81 and 164.37 ± 8.21 mg U kg⁻¹ in

root (Tab.2). The extent of U accumulation in vetiver grass grown in farming soils such as soil-AK, soil-LP, and soil-TT was higher than that grown in bare soil-TC. Uranium concentration was the highest in the root of the grass grown in the low fertile soil-LP and the lowest in the bare soil-TC. On the other hand, the U concentration in the root of the grass in soil-LP was found to be about 6 times higher than that in its shoot in case of the soil was contaminated with 250 mg U kg⁻¹.

Uranium soil-to-plant transfer factors (TF_U) is the indices used to for screening plants and evaluating heavy metal uptake and accumulation in the phytoremediation [1, 13, 15].

The TF_U determined as the ratio of the uranium concentration in plant to those in the soils at the equilibrium. Figure 2 presents the value of TF_U of vetiver grass. The values of TF_U of the grass presented in Fig 2 were determined for the case of U added to the soils at a concentration of 250 mg U kg⁻¹. The TF_U was found to be the highest with the soil-LP representing the lowest nutrition one among all the soil types studied, and it was 0.269 ± 0.013 kg.kg⁻¹, followed by soil-AK of 0.059 ± 0.002 kg.kg⁻¹, soil-TT of 0.077 ± 0.003 kg.kg⁻¹ and lowest soil-TC of 0.022 ± 0.001 kg.kg⁻¹. These finding is in good agreement with those reported by Vandenhove, H [15].

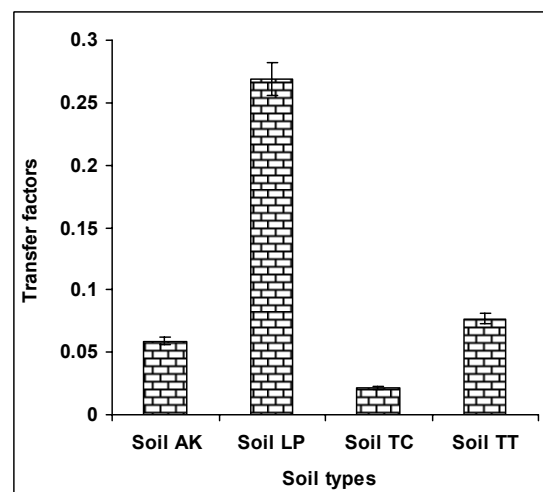


Figure 2: Uranium soil-to-plant transfer factors (TF_U) of soils mixed with 250 mg U kg⁻¹

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 present primarily (80-90%) in the +VI oxidation state as the uranyl (UO₂²⁺) cation.

Free UO_2^{2+} species of U in soil was proven to be easiest for plant to uptake and translocation in 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 $R^2 = 0.60$ (figure 3). 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 uptake. The availability of uranium cations for plant

to uptake seems to depends 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 0.73 (Figure 4). Note that the TF_U values presented in Figures 4 and 5 are the mean one that derived from four levels (0, 50, 100, and 250 $mg\ kg^{-1}$ soil) of uranium added to each of the soils studied and its deviation is within 30% as maximum.

Table 2: The uptake of uranium in vetiver grass

Soil types	U added in soil ($mg\ kg^{-1}$)	U contents in stems and leaves ($mg\ kg^{-1}$)	U contents in roots ($mg\ kg^{-1}$)
Soil AK	0	0.1±0.00	0.25±0.01
	50	1.3±0.06	0.35±0.01
	100	2.3±0.12	13.92±0.69
	250	2.43±0.12	44.02±2.20
Soil LP	0	0.05±0.00	0.19±0.00
	50	0.3±0.01	4.79±0.23
	100	2.8±0.14	42.88±2.14
	250	27.57±1.37	164.37±8.21
Soil TC	0	0.05±0.00	0.24±0.01
	50	0.23±0.01	2.13±0.10
	100	0.59±0.02	6.85±0.34
	250	2.11±0.10	16.29±0.81
Soil TT	0	0.18±0.00	0.66±0.03
	50	0.14±0.00	2.32±0.11
	100	1.2±0.06	28.33±1.41
	250	5.41±0.27	65.55±3.27

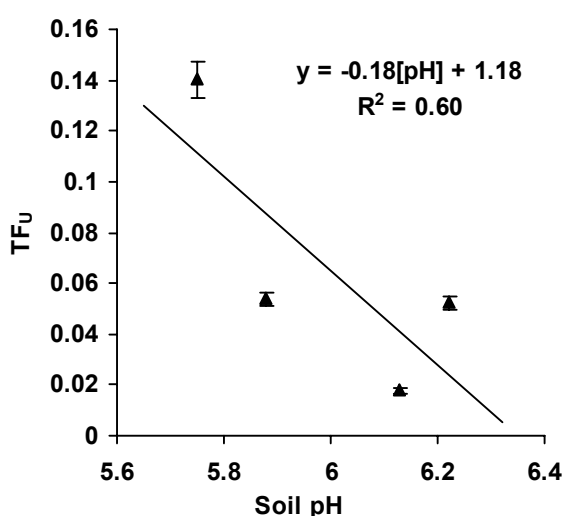


Figure 3: The correlation of TF_U with soil pH

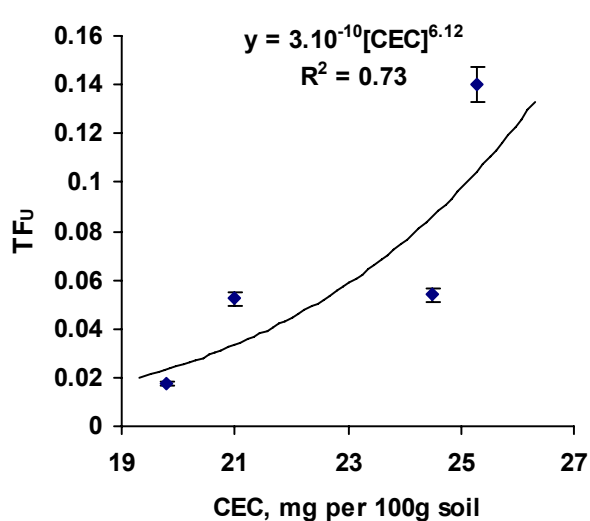


Figure 4: The correlation of TF_U with soil CEC

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.

Piccolo, A., [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 contaminant to be less available

for plant to uptake or translocation. 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 clay component in soils seems to be a good adsorbent to fix uranium not allow the constituent to be available to the plant. Ferrous and potassium seems to compete with uranyl cation for translocation and uptake by the plant. The effect of decrease the plant uptake of other metallic cations with the increase of ferrous content in soils was observed also the authors of [13].

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

Correlation pair	Correlation equation	Correlation coefficient, R^2
TF_U - SOM	$TF_U = -0.05[OM] + 0.26$	0.73
TF_U - [clay]	$TF_U = -0.06 \text{Ln}[\text{Clay}] + 0.29$	0.55
TF_U - [Fe]	$TF_U = -5.10^{-6}[\text{Fe}] + 0.13$	0.94
TF_U - [K]	$TF_U = -0.04 \text{Ln}[\text{K}] + 0.43$	0.74

4. CONCLUSION

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) appears to be a good plant to uptake uranium from soil contaminated with high concentration of the metal. Uranium uptake and accumulates mainly more in the root than in the shoot of vetiver grass. The uptake is dependent upon the soil properties. The soil CEC facilitates but 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 so high as up to 77%, at the same time the plant could survive and grow well under the natural conditions without any fertilization. All this makes the grass to be a potential plant for effective phytoremediation of soils contaminated with uranium and other heavy metals as well as radionuclides.

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TABLE OF CONTENTS

	<i>Page</i>
111 Preparation of a new cyclobutane building block for the synthesis of nucleoside analogues. <i>Tran Thi Thu Thuy, Ngo Ngoc Thang, Nguyen Quyet Chien, Stephanie Legoupy</i>	533
112 Diarylheptanoids and flavanones from <i>Alpinia napoensis</i> . <i>Phan Minh Giang, Dang Thi Tuyet Anh, Le Thu Huong, Phan Tong Son</i>	537
113 The suzuki reaction using palladium complex immobilized on magnetic nanoparticles (CoFe ₂ O ₄) as a catalyst. <i>Phan Thanh Son Nam, Nguyen Tan Tai</i>	542
114 QSSR models: prediction of physicochemical property and anticancer activity of structurally similar flavone and isoflavone derivatives. <i>Bui Thi Phuong Thuy, Pham Van Tat</i>	550
115 Multivariate QSAR study on antioxidant activity of tripeptides using 2D and 3D structural descriptors. <i>Nong Thi Hong Duyen, Pham Van Tat</i>	557
116 Influence of acetic anhydride treatment on hydrophilic properties of cotton fiber and tensile strength of polymer composite. <i>Nguyen Nhat Trinh</i>	562
117 Carbon nanotubes field-effect transistor for rapid detection of DNA. <i>Nguyen Thi Thuy, Nguyen Duc Chien and Mai Anh Tuan</i>	566
118 Effect of polyethylene glycol on morphology, properties and hydrolysis of poly(lactic acid)/chitosan composites. <i>Thai Hoang, Nguyen Thi Thu Trang, Nguyen Thuy Chinh</i>	570
119 Study on steel corrosion inhibition effect of caffeine extracted from green tea byproduct. <i>Luc Van Thu, Le Xuan Que</i>	575
120 Conversion reaction of 4-(tetra- <i>o</i> -acetyl- β -D-glucopyranosyl)thiosemicarbazones of substituted benzaldehydes with ethyl bromoacetate. <i>Nguyen Dinh Thanh, Le The Hoai, Le Khanh Toan</i>	579
121 Synthesis of 4-(tetra- <i>o</i> -acetyl- β -D-galactopyranosyl)thiosemicarbazones of <i>n</i> -alkylisatins. <i>Nguyen Dinh Thanh, Nguyen Thi Kim Giang, Nguyen Viet Hung, Vu Duc Trung</i>	585
122 Synthesis of some 1,3,5-triaryl-2-pyrazolines as fluorescence materials. <i>Nguyen Ngoc Linh, Nguyen Thi Hong Nhung, Chu Thi Huong, Pham Van Ben, Tran Minh Thi, Vu Quoc Trung</i>	591

- 123 Radiation polymerization and crosslinking of poly(n-isopropylacrylamide) hydrogels. 597
Hoang Dang Sang, Phạm Duy Duong, Tran Minh Quynh
- 124 Microwave-assisted suzuki reaction using palladium complex immobilized on SBA-15 as an efficient catalyst. 601
Phan Thanh Son Nam, Nguyen Thi Quynh Ngoc
- 125 Lupane triterpenoids from *Acanthopanax trifoliatum* - isolation and chemical transformation. 609
Dao Duc Thien, Lam Hoang Tu, Le Thi Hong Nhung, Tran Van Chien, Nguyen Thanh Tam, and Tran Van Sung
- 126 Preparation of phosphorescent organometallic complexes of iridium (III) and platinum (II) used for the fabrication of polymer light emitting diode device. 613
Nguyen Tien Thao, Vu Nhu Nang, Shigeyuki Yagi
- 127 Characterization of morphology and structure of Co and Fe doped manganese oxides for supercapacitive application. 619
Le Thi Thu Hang, Nguyen Thi Lan Anh, Mai Thanh Tung
- 128 Chemical constituents of *Adenosma caeruleum* R.Br. 624
Nguyen Thanh Tam, Nguyen Tuan Thanh, and Tran van Sung
- 129 Study on chemical constituents of rhizomes of *Polygonum cuspidatum* Siesb. et Zucc. (polygonaceae) growing in Vietnam. 627
Vu Dinh Hoang, Ba Thi Cham, Bui Kim Anh, Tran Viet Hung, Tran Dai Lam
- 130 Carbon nanotubes-based biosensor: a review. 630
Phuong Dinh Tam
- 131 Electrochemical preparation of magnetic layered structures materials with giant magnetoresistant (GMR) and giant magnetoimpedance (GMI). 647
Mai Thanh Tung, Le Thi Thu Hang, Dang Viet Anh Dung
- 132 The uptake of uranium from soil to vetiver grass (*Vetiver zizanioides* (L.) Nash). 656
Luu Viet Hung, Bui Duy Cam, Dang Duc Nhan, Tran Thi Van

Xinhe bìa 4

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