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UPTAKE EVALUATION OF CAESIUM BY GLASSHOUSE GROWN GRASSES FOR RADIOPHYTOREMEDIATION OF CONTAMINATED SOIL

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ABSTRACT

A glasshouse experiment was performed to evaluate the uptake of grasses viz. Napier and Vetiver in radiophytoremediation of caesium-contaminated soil. The radiophytoremediation experiment was designed according to the Randomized Complete Block Design (RCBD). The grasses were grown in troughs filled with soil mixed with a known specific activity of ^{134}*Cs. Initial Cs activity and activity after different cultivation time intervals of 1, 3, 6 and 9 months were analyzed using gamma spectrometer direct measurement. The results showed the uptake of caesium by Napier and Vetiver after 9 months with the transfer factors (TF) were 4.70 and 6.25, respectively. The remediation of caesium from the contaminated soil during the study period was 98.08% for Napier and 98.21% for Vetiver. Both grasses have been found to accumulate caesium, with Vetiver accumulating higher than Napier. Thus, the present study suggests that Vetiver could be used as a potential plant for radiophytoremediation of caesium.*

Keywords: Bioaccumulation, caesium, grasses, Napier, radiophytoremediation, soil, Vetiver

INTRODUCTION

Various physical and chemical methods have been employed for remediation of radionuclides. Unfortunately, these methods are not practically effective when concentrations of radionuclides are low and areas/volumes involved are large. However, the use of plants for remediation of contaminants, namely as phytoremediation agents, has been recognized as an aesthetically pleasing, low cost *in situ* successful method, which can be used for remediation of low levels of toxic pollutants from the environment (Arthur et al., 2005; McGrath et al., 2002). Phytoremediation is an emerging green technology that uses plants to remediate soil, sediment, surface water, and groundwater environments contaminated with toxic metals, organics and radionuclides (Alkorta and Garbisu, 2001). Singh et al. (2009) suggested that phytoremediation could be promoted as a promising technique useful in cleaning up of radionuclides such as caesium (^{137}Cs, ^{134}Cs). This method has the benefits of contributing to site restoration when remedial action is ongoing. In phytoremediation, plants play important role in *in situ* removing of contaminants from soil and solutions (Kang et al., 2012). The action of plants can include the degradation, adsorption, accumulation and volatilization of compounds or the enhancement of soil rhizosphere activity

(Janngam et al., 2010). The plants take in deposited radionuclides from soil, commonly expressed as soil-to-plant transfer factor (TF), which is widely used for calculating radiological human dose via the ingestion pathway. The soil-to-plant TF is regarded as one of the most important parameters in environmental safety assessment for nuclear facilities (IAEA, 1994). This parameter is necessary for environmental transfer models, which are useful in the prediction of radionuclide concentration in agricultural crops for estimating dose impact to human being (Chakraborty et al., 2013).

Cs is a trace element in soils (White and Broadley, 2000), occurring in the form of radioactive isotopes and circulates in the environment (Krajewski and Rosiak, 2001). Nuclear technologies, however, have produced several long-lived Cs isotopes, most notably ^{134}Cs and ^{137}Cs, that are radioactive and can make their way into the environment through nuclear facility accidents and operational activities or through detonation of nuclear weapons (White and Broadley, 2000). These isotopes of Cs emit beta and gamma radiation that is harmful to organisms, including humans, especially if the isotopes are ingested (White and Broadley, 2000). Moreover, these radioisotopes are of environmental concern due to their relatively long half-life, emission of gamma radiation during decay and rapid incorporation into living organisms (Pipíška et al., 2004). Removal of these isotopes from contaminated soils is a priority if radiation exposure is to be minimized (Zhu and Smolders, 2000). Thus, there is considerably interest in remediation using extraction by plants (phytoextraction) that do not enter the human food chain (Pipíška et al., 2004). Cs is an alkali metal and analogue to potassium (K) that a macronutrient for plants and both has similar chemical properties (Pipíška et al., 2004). It has known role whereas K concentration in soil increases lead to Cs uptake by plant decreases (Zhu and Smolders, 2000).

Many recent laboratory scale experiments and field-site-scale studies have shown that caesium can be taken up by plants such as grasses, ferns, mosses, lichens as well as vascular plants. To fulfil our goal, we used grasses which can easily grow in a caesium contaminated site and has efficiency to take up this radionuclide from soil; thus, we selected Napier (*Pennisetum purpureum*) and Vetiver (*Vetiveria zizanioides*) grasses. Napier grass occurs naturally throughout tropical Africa and particularly in East Africa. It is a tall, stout and deep-rooted perennial bunch grass well known for its high yielding capability and usage as forage for livestock (Woodard and Prine, 1991). Napier grass which is also known as “elephant grass” was first introduced in Malaysia in the 1920’s from East Africa and is currently the most popular fodder grass in dairy and feedlot production systems (Halim et al., 2013). Vetiver grass is a perennial grass belonging to the Poaceae family. It has short rhizomes and a massive, finely structured root system. The deep root system makes the Vetiver grass extremely drought tolerant and very difficult to dislodge when exposed to a strong water flow. Likewise, the Vetiver grass is also highly resistant to pests, diseases, fire (Dudai and Putievsky, 2006). It is also known to be tolerant to heavy metals (Andra et al., 2009). There are reports on the use of these plants for phytoremediation of soils contaminated with heavy metals (Chen et al., 2004; Wilde et al., 2005), radionuclides and nuclear waste (Singh et al., 2008).

Realising the lack of phytoremediation studies in Malaysia and limited data available in the open literature, this study was performed with the immediate goal to evaluate radiocaesium bioaccumulation by grasses *viz.* Napier and Vetiver from Cs-spiked soils.

MATERIALS AND METHODS

Plant Materials

Napier and Vetiver grasses (Figure 1) were used for the glasshouse radiophytoremediation experiments involving caesium-contaminated soil. The stem of Napier grasses were cut at 15 cm length before planted in soil spiked with ^{134}Cs. The age of the Vetiver plantlets used was 3 weeks after sowing.

Figure 1: Type of grasses, Napier (left) and Vetiver (right) used in the glasshouse radiophytoremediation experiments on Cs-contaminated soil

Glasshouse Experiment

About 150 kg of soil at field capacity was loaded and mixed thoroughly into square fibreglass trough measuring 0.5 m height × 1 m length x 1 m width. A known specific activity of ^{134}Cs was added to tap water and stirred with Teflon stirrer until well mixed. Then, 15 L of these spiked solutions with an approximately 150 Bq of total activity of ^{134}Cs were spread into each trough (except for control) containing soiland mixed thoroughly. The Napier and Vetiver grasses were planted in the troughs according to the randomized complete-block design (RCBD). Stem cuttings of Napier grass at 15 cm length and Vetiver planting material were then planted to a depth of 10 – 15 cm. The grasses were planted in rows with five rows per trough spaced at 15 cm between rows and 15 cm between points in each row. The grasses were grown in a glasshouse having an ambient temperature of around 30^{o}C. During the study period, the grasses were exposed to sunlight which had a photosynthetically active radiation and a 10 – 12 h naturally photoperiod. Each trough was watered three times in a week. The grasses were allowed to establish for a period of one month before the first sampling was taken. There were a total of four harvests taken over a period of 9 months (11 June 2012 – 13 March 2013) with harvesting interval of 1, 3, 6, 9 months depending on the growth rate of the grasses.

Grass shoots were harvested after harvest interval period by cutting the leaves, and soil samples were taken for gamma measurement of ^{134}Cs. The leaf and soil samples were weighed fresh, then dried, reweighed, ground and filled in counting container. Ground leaf and soil samples were counted using HpGe Gamma spectrometry system for 15 hours to analyze the activity concentrations of ^{134}Cs. Lastly, the values of transfer factor (TF) for ^{134}Cs in both grasses were calculated. Some parameters of soil classification such as moisture content (%), specific gravity and particle size distribution were also measured.

Statistical Analysis

Each treatment for grasses, soil and uptake consisted of three replicates. The data presented for each treatment in this study were represented as means of samples with their uncertainty ($x \pm 2\sigma$). The data obtained in the experiments were subjected to paired sample t-test analysis to determine the mean values for selecting a suitable species of grass in remediation of contaminated soil.

RESULTS AND DISCUSSION

Soil Analysis

The soil used in this experiment was topsoil. Its particle size distributions showed higher content of clay (42%) compared to silt (32%) and sand (26%). The percentage of moisture content of the soil was 21% and the specific gravity was 2.63. The values have profound effect upon the properties of soil including its water supplying power, rate of water intake, aeration, fertility, ease of tillage, elemental and radionuclides binding; and efficient uptake of nutrients and compounds for proper growth of plants (Chakraborty et al., 2013).

Radionuclide Activities in Grasses and Soils; Transfer Factor and % Remediation

The activities of anthropogenic radionuclide ^{134}Cs in grasses and soil samples collected at different harvest time from the glasshouse radiophytoremediation experiment are shown in Table 1. The uncertainty values are presented in 2σ variation due to counting errors. The average ^{134}Cs activities in different grass samples for 1.5 – 9 months cultivation were found to vary from 3.37 ± 0.54 to 33.69 ± 5.32 Bq/kg dw. for Napier and 3.03 ± 0.49 to 41.71 ± 6.67 Bq/kg dw. for Vetiver. These differences are due to in-homogeneity of ^{134}Cs distribution in the soil, change of soil parameters/properties during the grasses growth phase, different ^{134}Cs uptake by these different type of grasses and other factors (Prorok et al., 2006). This finding was strongly supported in a previous study where the accumulation of ^{134}Cs in plants or grasses is a complex process that is determined by an interaction of numerous factors. Soil type and physicochemical properties, timing from the ^{134}Cs deposition, type of radionuclide deposition, and plant species physiology are among the major factors affecting radiocaesium accumulation in plants (Dushenkov et al., 1999). In this study, activities of ^{134}Cs were found to be relatively higher in Vetiver grass than in Napier grass. In alignment with that we can conclude that the absorption and accumulation of ^{134}Cs in Vetiver grass was higher than in Napier grass. It also revealed the activities of ^{134}Cs in both grasses increased with length of cultivation time. Meanwhile, the activity of ^{134}Cs in the soil for both cultivations of Napier and Vetiver grasses were observed to be constant throughout the study.

Soil-to-grass transfer factor (TF) of ^{134}Cs was calculated by using ^{134}Cs activity concentration in part of grass (e.g. leaf, stalk, root etc.) and the mean value of ^{134}Cs activity in soil. The value of TF for ^{134}Cs in leaves of different grass species are shown in Table 1 and Figure 1. In this experiment, significant variation of TF was observed among the grass species of Napier and Vetiver. This is due to the bioaccummulation of a particular radionuclide depends on plant species and physiology of the plant (Sadhasivam et al., 2010). On the other hand, different TF can also result from differences in rooting pattern, rooting depth, absorption characteristics of the root surface, root turn over, root growth rate etc. Since crops differ with regard to their physiological and metabolic characteristics they vary in their ability with regard to uptake, accumulation and translocation of ^{134}Cs (Sadhasivam et al., 2010).

Table 1: Activity of ^{134}Cs in grasses and soils obtained during 9 months of glasshouse radiophytoremediation experiment

Harvest Time (months)	Napier Grass				Vetiver Grass			
	^{134}Cs Activity (Bq/kg dw.)		Transfer Factor, TF (Unitless)	% Remediation	^{134}Cs Activity (Bq/kg dw.)		Transfer Factor, TF (Unitless)	% Remediation
	Leaves	Soil			Leaves	Soil		
1.5	3.37 ± 0.54	5.87 ± 0.94	0.56	98.41	3.03 ± 0.49	6.01 ± 0.94	0.47	98.38
3	2.15 ± 0.34	6.50 ± 1.04	0.33	98.24	1.96 ± 0.31	6.24 ± 0.99	0.31	98.31
6	4.86 ± 0.76	6.34 ± 1.01	0.79	98.29	9.37 ± 1.50	5.71 ± 0.92	1.64	98.46
9	33.69 ± 5.32	7.12 ± 1.13	4.70	98.08	41.71 ± 6.67	6.63 ± 1.05	6.25	98.21

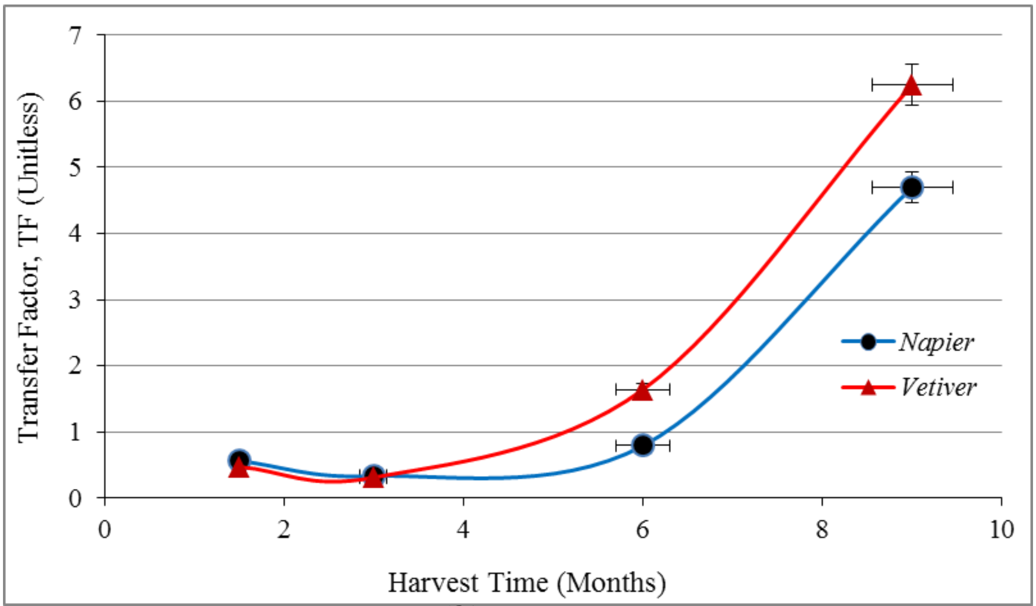

Figure 1: Glasshouse experiment for ^{134}Cs uptake by Napier and Vetiver grasses from Cs-contaminated soil

It can be observed from the results of ^{134}Cs activity that both grasses could accumulate caesium with the TF value in the leaves of Vetiver grass was higher than the leaves of Napier grass. In other word, Vetiver is an effective accumulator of ^{134}Cs, which is characterized by high degree of uptake of ^{134}Cs. In view of the fact, it was proven by paired sample t-test analysis that there has mean value of TF for Vetiver grass (2.1675) more than *Napier* grass (1.5950), thus this indicated Vetiver grass has characteristic to accumulate more ^{134}Cs. Uptake of radionuclide does not only depend on the grass or plant species but also depend on quantity/density of root growth and shoot biomass (Sadhasivam et al., 2010). Moreover, the actual TF of ^{134}Cs in the part of the grass species also depend on caesium contents in soil, including its availability to the plant and the metabolic characteristics of the grass species. Chemical factors such as the amount of exchangeable potassium in the soil probably might affect the uptake of caesium by grass (Chakraborty et al., 2013). This revealed the continuous accumulation of potassium through root uptake over a period of time as indicated by high concentration of potassium (K) in soil which was cultivated with Napier grass (Table 2). It is well known that potassium is an essential element of metabolism; plants take up potassium from soil in varied amounts depending upon their metabolism and potassium concentration, respectively. Thus, this suggested higher levels of potassium uptake by Napier grass compared to Vetiver grass. Other than K or ^{40}K, some ions such as K^+, NH_4^+, Rb^+ and Cs^+ form a homologous series with considerable physicochemical similarity with ^{134}Cs probably affected its uptake by grasses or plants (Dushenkov et al., 1999). It is interesting to note that although all the grass species are grown in soils of similar characteristics, the TFs are different for different species (Chakraborty et al., 2013).

Table 2: Concentration of potassium (K) in soil grown with each type of grass

Harvest Time (months)	Potassium (K) Concentration in Soil Grown with Different Grasses (ppm)	
	Napier Grass	**Vetiver Grass**
1.5	3393 ± 167	2193 ± 170
3	-	-
6	3019 ± 218	2118 ± 124

Generally, the TF of ^{134}Cs in both grasses species of Napier and Vetiver gradually increased but inhibited with length of exposure time (6 and 9 months) and grasses height (Figure 1). This finding may be closely related to root growth rate and quantity/density of root which affect the activities of root uptake, xylem loading, and vacuoles transport. McGrath and Zhao (2003) suggested a possible contribution of the root uptake mechanism to the hyper-accumulation of elemental such as heavy metal and radionuclide; and xylem loading (root-to-shoot). In addition, vacuoles sequestration may also be a key component affecting this mechanism (Kang et al., 2012). These increments of TF between 6 to 9 months might be due to the development stage of the plant that plays an important role in radionuclide accumulation. Whereas in this stage the grass shoots being uptake more and concentrated radiocaesium; and changed in K concentrations (Dushenkov et al., 1999). The result showed that ^{134}Cs remediation/removal from soil was very high (Table 1). About 98% of ^{134}Cs in soil was lost after growing Napier (98.08%) and Vetiver (98.21%) grass for 9 months. Thus, this is an advantage for these grasses in particular Vetiver grass for radiophytoremediation.

CONCLUSIONS

The results showed the uptake of caesium by Napier and Vetiver after 9 months cultivation with the transfer factors (TF) was 4.70 and 6.25, respectively. Meanwhile, the remediation of caesium from contaminated soil at the same time was 98.08% for Napier and 98.21% for Vetiver. Both grasses have been found to accumulate caesium, with Vetiver accumulating higher than Napier. Thus, the present study suggests that Vetiver could be used as a potential candidate grass for radiophytoremediation of caesium (^{134}Cs) from contaminated soil which needs remediation and containment. The data from this study will help to develop a reference database on this important issue so that any change in this respect in future due to nuclear phenomenon can be ascertained and radiation safety measurements may be taken accordingly.

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