



## Original Article

## Evaluation of vetiver grass for radiocesium absorption ability

Nualchavee Roongtanakiat<sup>a,\*</sup> Thunyaras Akharawutchananon<sup>b</sup><sup>a</sup> Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand<sup>b</sup> Radioactive Waste Management Center, Thailand Institute of Nuclear Technology (Public Organization), Bangkok 10900, Thailand

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## ABSTRACT

Plantlets of the Surat Thani and Ratchaburi ecotypes of vetiver grass (*Chrysopogon zizanioides* L. (Roberty)) were hydroponically cultured in <sup>134</sup>Cs solutions to investigate their <sup>134</sup>Cs uptake ability. After 5 d of culture in <sup>134</sup>Cs solutions, the Surat Thani plantlets were still fresh and healthy without any evidence of toxicity symptoms, while the Ratchaburi plantlets were rather dry with some brown leaves. The information from the radiographic images and photo-stimulated luminescence (PSL) signals as well as the specific activity of cesium indicated that the Surat Thani plantlets cultured in <sup>134</sup>Cs solutions were significantly superior to the Ratchaburi vetiver plantlets with regard to <sup>134</sup>Cs absorption ability. The increase in the amount of <sup>134</sup>Cs in the vetiver plantlets with the level of <sup>134</sup>Cs in the culture solution was clearly demonstrated from the PSL signal, as the relationship between the PSL per square millimeter values and <sup>134</sup>Cs solution concentrations was linear. The data also indicated that both studied vetiver ecotypes accumulated more <sup>134</sup>Cs in the roots than in shoots; therefore, vetiver might be suitable for phytostabilization of radiocesium-polluted soil.

The Surat Thani vetiver plantlets were cultured in <sup>134</sup>Cs solution with a concentration of 5 MBq/L for different periods. The results of the radiographic image, PSL signals and the radioactivity levels in the vetiver samples strongly indicated that vetiver could absorb a greater amount of <sup>134</sup>Cs when the period of culture was longer. After vetiver culture periods of 3 d, 6 d, 9 d, 12 d, 15 d and 18 d, the activity of <sup>134</sup>Cs in the cultured solutions declined to 98.0%, 93.2%, 88.6%, 78.1%, 70.7% and 65.5%, respectively. These values indicated that vetiver could remediate <sup>134</sup>Cs in the cultured solution by 2.0%, 6.8%, 11.4%, 21.9%, 29.3% and 34.5% for the respective durations.

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## Introduction

Environmental contamination with radionuclides has resulted mainly from nuclear power accidents and nuclear weapon testing. For example, the Chernobyl accident in April 1986 resulted in a large area of Europe and the former Soviet Union being contaminated with radionuclides; in the Ukraine; over 260,000 km<sup>2</sup> were contaminated with Cs-137 levels exceeding 1 Ci/km<sup>2</sup> (Dushenkov, 2003). In March 2011, the Fukushima Daiichi nuclear power plant accident in Japan caused a month-long discharge of radioactive materials into the atmosphere (Chino et al., 2011). More than 90% of the radionuclides were distributed in the upper 6 cm of the soil column in a wheat field and within 4 cm of the surface in a rice paddy, orchard and cedar forest (Ohno et al., 2012). Radiocesium is one of the most important artificial radionuclides produced by

nuclear fission due to its long physical half-life—30 yr for <sup>137</sup>Cs and 2 yr for <sup>134</sup>Cs—and its high biological availability (Zhu and Smolders, 2000). Radionuclides in the soil are taken up by plants and become available for further entry into food chains.

Vetiver grass, a versatile plant in agriculture, is widely known for its effectiveness in soil and water conservation and furthermore, many researchers have reported on the use of vetiver for remediation of heavy metals (Roongtanakiat et al., 2007 2008; 2009; Roongtanakiat and Sanoh, 2011.), petroleum (Brandt et al., 2006), phenol (Singh et al., 2008b) and 2,4,6-trinitrotoluene (Markis et al., 2007a, 2007b; Das et al., 2010). Singh et al. (2008a) also indicated that vetiver might be a potential candidate plant for the phytoremediation of <sup>90</sup>Sr and <sup>137</sup>Cs. There are two species of vetiver in Thailand—*Chrysopogon nemoralis* (Balansa) Holttum or upland vetiver and *Chrysopogon zizanioides* L. (Roberty) or lowland vetiver. Both species have distinct ecological characteristics that allow them to adapt to different habitats and there are many ecotypes (Roongtanakiat, 2009). *C. nemoralis* and *C. zizanioides* grown in zinc

\* Corresponding author.

E-mail address: [nualchavee@gmail.com](mailto:nualchavee@gmail.com) (N. Roongtanakiat).

mine soil showed differences in growth performance, heavy metal transfer factors and heavy metal uptake (Roongtanakiat and Sanoh, 2011). Former research by the current authors also revealed that radiosensitivity varied between vetiver species and among ecotypes (Roongtanakiat et al., 2012).

Therefore, in this study, the objectives were to examine the possibility of using vetiver for radio cesium decontamination regarding  $^{134}\text{Cs}$  absorption ability and its distribution in two vetiver ecotypes cultured in  $^{134}\text{Cs}$  solutions. The influence of culture period on  $^{134}\text{Cs}$  uptake by vetiver grass was also investigated.

## Materials and methods

### Plant material and culture

*C. zizanioides*, (the Surat Thani ecotype) and *C. nemoralis* (the Ratchaburi ecotype) were used in this study. Vetiver plantlets were prepared using tissue culture techniques before being transferred to potting soil for further growth in the greenhouse. Uniform plantlets with 15 cm height and well developed roots were selected for use in two sets of experiments. The objective of the first experiment was to compare the  $^{134}\text{Cs}$  uptake ability of the Surat Thani and Ratchaburi vetiver ecotypes. The influence of culturing duration on the  $^{134}\text{Cs}$  uptake was investigated in the second experiment. The  $^{134}\text{Cs}$  uptake was measured using photo-stimulated luminescence (PSL) signals via autoradiography involving  $^{134}\text{Cs}$  activity via gamma spectrometry.

The culture solutions were prepared from the  $^{134}\text{CsCl}$  standard solution obtained from the CERCA LEA radioactive standard laboratory, Pierrelatte, France. In the first experiment, three concentrations of  $^{134}\text{Cs}$  culture solution (2.5 MBq/L, 5.0 MBq/L and 7.5 MBq/L) were used and 20 mL of each solution concentration was pipetted into a 50 mL tube. One vetiver plantlet was cultured in each  $^{134}\text{Cs}$  solution concentration. There were three replicates of each vetiver ecotype. The plantlets were harvested 5 d after culturing. In the second experiment, the Surat Thani vetiver plantlets were cultured in  $^{134}\text{Cs}$  culture solution with a concentration of 5.0 MBq/L for 3 d, 6 d, 9 d, 12 d, 15 d and 18 d with three replications for each culture period.

### Autoradiography of plant samples

At the end of each experiment, plantlets were thoroughly washed with distilled water. Each plantlet was then well flattened by pressing with papers followed by air drying until the plant samples were completely dried. The individual flat-pressed samples were wrapped with polyethylene (PE) film to protect the imaging plate from contamination by radio cesium. The wrapped plantlets were placed against the imaging plate (BAS-MS; Fujifilm Co.; New York, USA) for 10 min before reading with an imaging analyzer (Bas-2500; Fujifilm; New York, USA) and the photo-stimulated luminescence (PSL) signals per square millimeter from each plantlet were recorded.

### $^{134}\text{Cs}$ activity determination

For  $^{134}\text{Cs}$  activity determination, 0.1 mL of  $^{134}\text{Cs}$  solution sample was pipetted onto a 25-mm diameter planchet then dried under ultra violet light. Three replications of each studied solution concentration were performed both before and after the experiment. The gamma spectrometric analyses were carried out using an HPGe detector, (GMX60-P4-83; ORTEC; Atlanta, USA) with a 6.1% efficiency for gamma energy of 604.7 keV. Each sample was measured for 600 s. The  $^{134}\text{Cs}$  activity in each sample was calculated to the harvest date for an activity comparison at different times.

After autoradiography, the dried vetiver plantlet samples from the first experiment were separated into shoot and root parts and then cut into small pieces. Each sample was weighed and placed on a 25 mm diameter planchet, covered with a thin PE film, and then the  $^{134}\text{Cs}$  activity was measured using the same procedure as for the  $^{134}\text{Cs}$  solution mentioned above. The specific activity of  $^{134}\text{Cs}$  in each plant sample was determined in kilobecquerels per gram of dry plant.

## Results and discussion

### $^{134}\text{Cs}$ and radiation toxicity

At 5 d after culturing, the vetiver plantlets in the solution with three different concentrations of  $^{134}\text{Cs}$  of the two vetiver ecotypes were visually compared (Fig. 1). It was clear that the Surat Thani plantlets were still fresh and healthy in the radio cesium solution without any toxicity symptoms even at a concentration of Cs as high as 7.5 MBq/L while the plantlets of the Ratchaburi ecotype were rather dry with some brown leaves. White and Broadley (2000) indicated that cesium toxicity in plants was manifested by necrosis or chlorosis because it interfered with the K uptake and biochemistry or both (Hampton et al., 2004).  $^{134}\text{Cs}$  emits both beta and gamma radiation and may be radiotoxic as Sahr et al. (2005) reported that a low level of radio cesium exposure altered gene expression in the root of *Arabidopsis* sp. However, the study by Roongtanakiat et al. (2012) showed that vetiver was quite resistant to radiation compared to other plants. Therefore, in this study it was expected that radio cesium and radiation would not be toxic to vetiver growth. The unhealthy Ratchaburi plantlets might have been due to growth in the acidic radio cesium solution (pH 4.1) as the Ratchaburi ecotype is an upland vetiver which prefers dry areas and well-drained soil (Roongtanakiat, 2006).

The radiography images of Ratchaburi and Surat Thani vetiver grown in  $^{134}\text{Cs}$  solutions with three different concentrations are shown in Figs. 2 and 3 which indicate the localization of  $^{134}\text{Cs}$  in the vetiver plantlets. This distribution in plantlets is explained by the intensity of blackening on the image. It was clear that both vetiver ecotypes could absorb  $^{134}\text{Cs}$  which was transported from the root to the shoot. The vetiver plantlets cultured in solutions with higher cesium concentrations gave darker images as they absorbed the higher amount of  $^{134}\text{Cs}$  that was transported to leaves.

The influence of the  $^{134}\text{Cs}$  concentration level in the culture solution on  $^{134}\text{Cs}$  absorption and accumulation in vetiver plantlets appeared similar between the Ratchaburi and Surat Thani ecotypes.

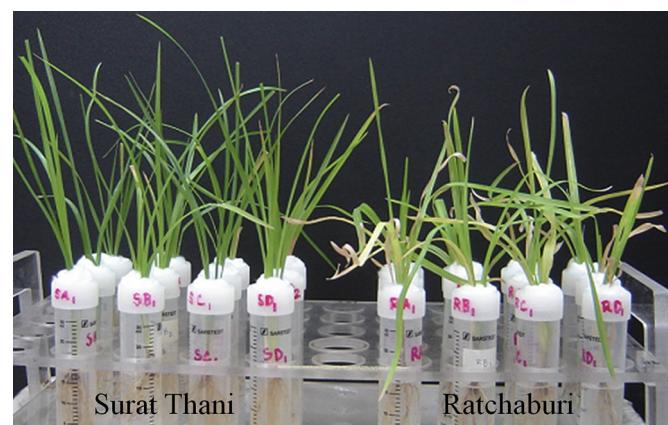
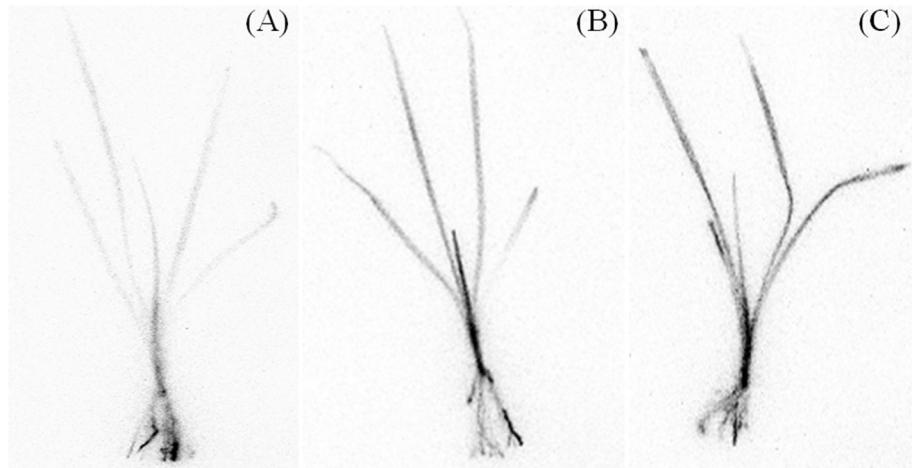
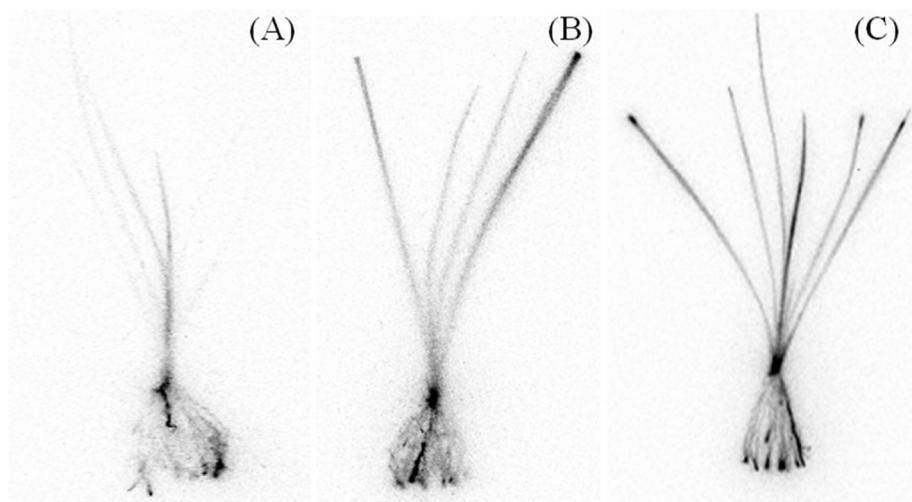


Fig. 1. Surat Thani and Ratchaburi vetiver plantlets after 5 d culture in  $^{134}\text{Cs}$  solution,  $^{134}\text{Cs}$  absorption: effect of  $^{134}\text{Cs}$  concentration.



**Fig. 2.** Radiographic images of Ratchaburi vetiver plantlets cultured in solutions with  $^{134}\text{Cs}$ , concentrations of: (A) 2.5 MBq/L; (B) 5.0 MBq/L and (C) 7.5 MBq/L.



**Fig. 3.** Radiographic images of Surat Thani vetiver plantlets cultured in solutions with  $^{134}\text{Cs}$ , concentrations of: (A) 2.5 MBq/L; (B) 5.0 MBq/L and (C) 7.5 MBq/L.

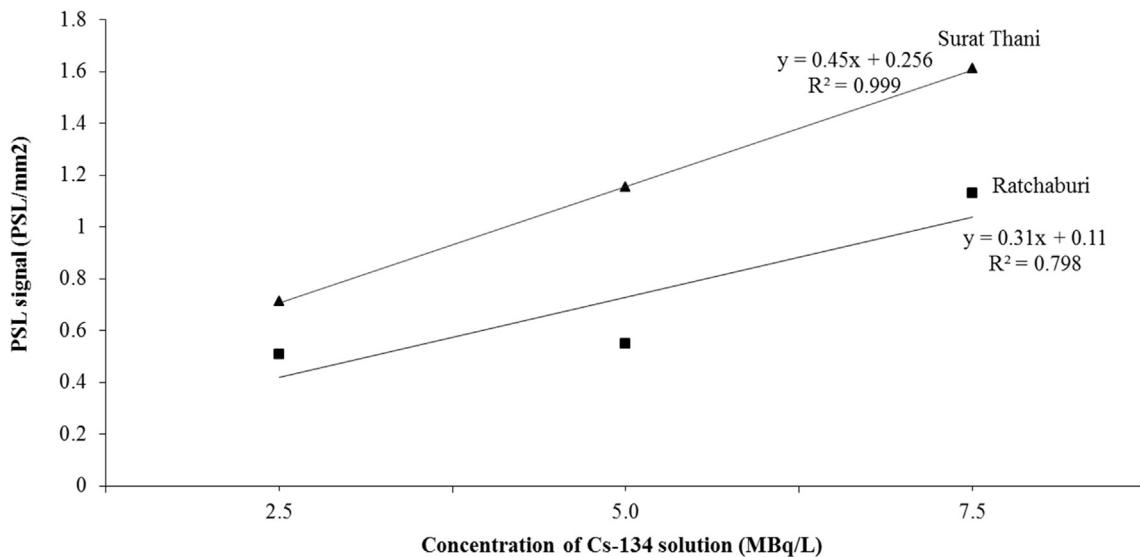
The images obtained from both ecotypes could not be used for distinguishing their absorption ability of  $^{134}\text{Cs}$ . However, the data of the PSL signal indicated that the specific activity in the treatments for all three  $^{134}\text{Cs}$  concentrations with the Surat Thani plantlet were significantly higher than those of the Ratchaburi plantlet (Fig. 4) which clearly demonstrated an increase in the  $^{134}\text{Cs}$  amount in the vetiver plantlets with increasing concentration of  $^{134}\text{Cs}$  in the culture solution. The relationship between the specific activity and the  $^{134}\text{Cs}$  solution concentration was linear with  $R^2$  (coefficient of determination) values of 0.99 and 0.80 for the Surat Thani and Ratchaburi ecotypes, respectively.

The concentrations of  $^{134}\text{Cs}$  in the shoots and roots of vetiver were compared using gamma radiation detection and expressed as specific activity. Fig. 5 shows that both vetiver ecotypes accumulated more  $^{134}\text{Cs}$  in the roots than in the shoots. These results support the findings of Singh et al. (2008a) that  $^{137}\text{Cs}$  accumulation was greater in vetiver roots than shoots. Cesium has high mobility within a plant (Zhu and Smolders, 2000) and many authors—Cline and Hungate (1960), Clint and Dighton (1992), Smolders and Shaw (1995), Adler (1996), Eapen et al. (2006) and Singh et al. (2009)—reported that plants preferentially accumulated radiocesium in the roots. However, Tang et al. (2004) showed that *Amaranthaceae* accumulated more radiocesium in leaf tips and young leaves than

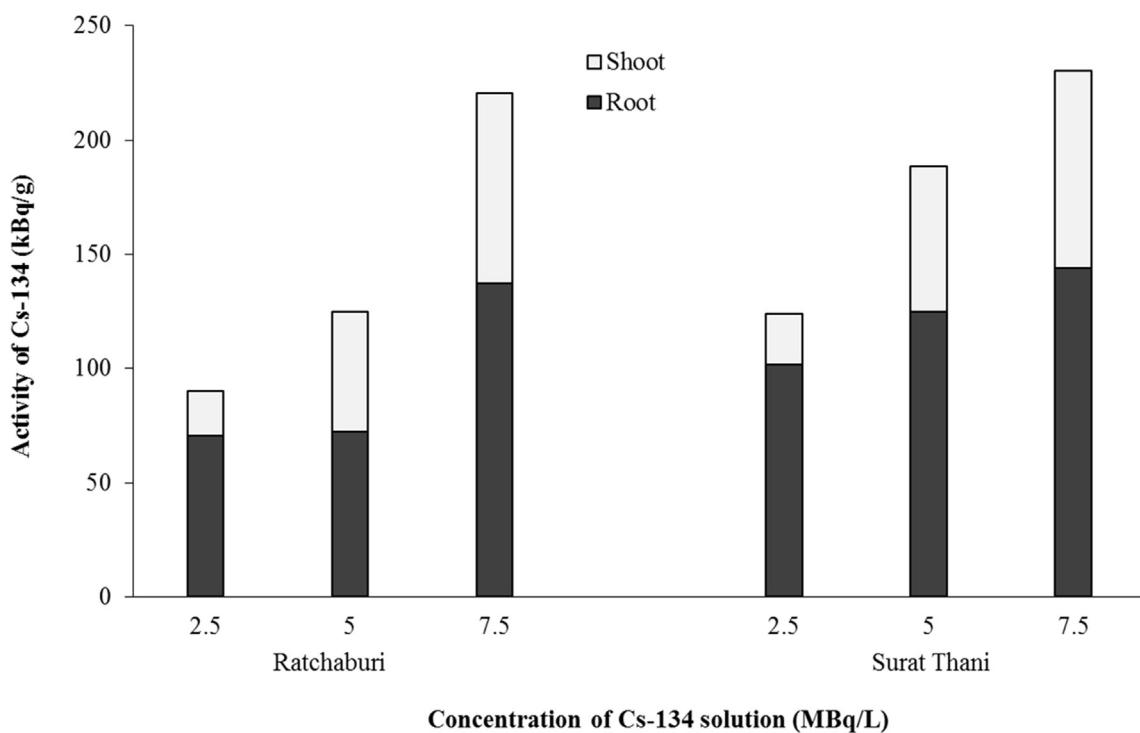
in other tissues. Bineva and Stoeva (2002) and Bineva et al. (2005) concluded that radiocesium accumulated unevenly in different plants organs, as its highest concentration was found in the plants leaves.

Based on the specific activity, the  $^{134}\text{Cs}$  concentration in the vetiver shoots and roots increased when the vetiver was grown in solutions with higher Cs concentrations, which was similar to the results of the autoradiography and the study by Singh et al. (2009) regarding the  $^{137}\text{Cs}$  concentration in the shoots and roots of *Chromolaena odorata* which increased as the  $^{137}\text{Cs}$  concentration in the culture solution increased. Fuhrmann et al. (2002) also reported that the  $^{137}\text{Cs}$  concentration in redwood pigweed and tepary bean increased as its concentration in the soil increased. However, the level of  $^{134}\text{Cs}$  in the shoots of Ratchaburi vetiver grown in the 5.0 MBq/L solution was not significantly higher than that in the 2.5 MBq/L solution due to the unhealthy plantlet condition through having many dry, brown leaves.

The ratio between the specific activity in the shoots and roots of vetiver increased from 0.28 to 0.60 for the 2.5 MBq/L and 5.0 MBq/L treatments, respectively, in the Surat Thani vetiver and from 0.22 to 0.60 in the Ratchaburi vetiver for the same respective treatments. This indicated that when vetiver was grown in the high  $^{134}\text{Cs}$  concentration solution, vetiver not only could absorb more  $^{134}\text{Cs}$



**Fig. 4.** Photo-stimulated luminescence (PSL) signals and linear relationship plots of Surat Thani and Ratchaburi vetiver plantlets cultured in solutions with different  $^{134}\text{Cs}$  concentrations.



**Fig. 5.** Specific activity of  $^{134}\text{Cs}$  in shoots and roots of Ratchaburi and Surat Thani vetiver plantlets cultured in  $^{134}\text{Cs}$  solutions with different concentrations.

from solution but also stimulated translocation of Cs from the roots to the shoots. This result agreed with the study of Cook et al. (2009) on Cs absorption by crested wheatgrass, cheatgrass, bluebunch wheatgrass and Great Basin wild rye.

A comparison of the Cs absorption ability of the two vetiver ecotypes showed that the Surat Thani plantlets grown in  $^{134}\text{Cs}$  solution concentrations of 2.5 MBq/L, 5.0 MBq/L and 7.5 MBq/L for 5 d had higher specific activities in the shoots and roots than those of the Ratchaburi ecotype (Fig. 5). This confirmed the results of the PSL signal values in Fig. 4. Many researchers—Willey and Martin (1997), Lasat et al. (1998), Fuhrmann et al. (2002), Massas et al.

(2002), Soudek et al. (2004), Cook et al. (2009) and Massas et al. (2010)—reported that plants differed in their ability to absorb and translocate radionuclides. Moreover, the plant variety affected the radionuclide absorption as shown by the current study results which were in agreement with the previous studies of Bineva and Stoeva (2002) who reported that there were cultivar differences in radiocaesium absorption by bean, pea and soybean.

The activities of  $^{134}\text{Cs}$  in the culture solutions were measured after harvesting the vetiver plantlets and were compared with the initial values. The results showed that the  $^{134}\text{Cs}$  activity in the culture solutions of Surat Thani was reduced more than in the

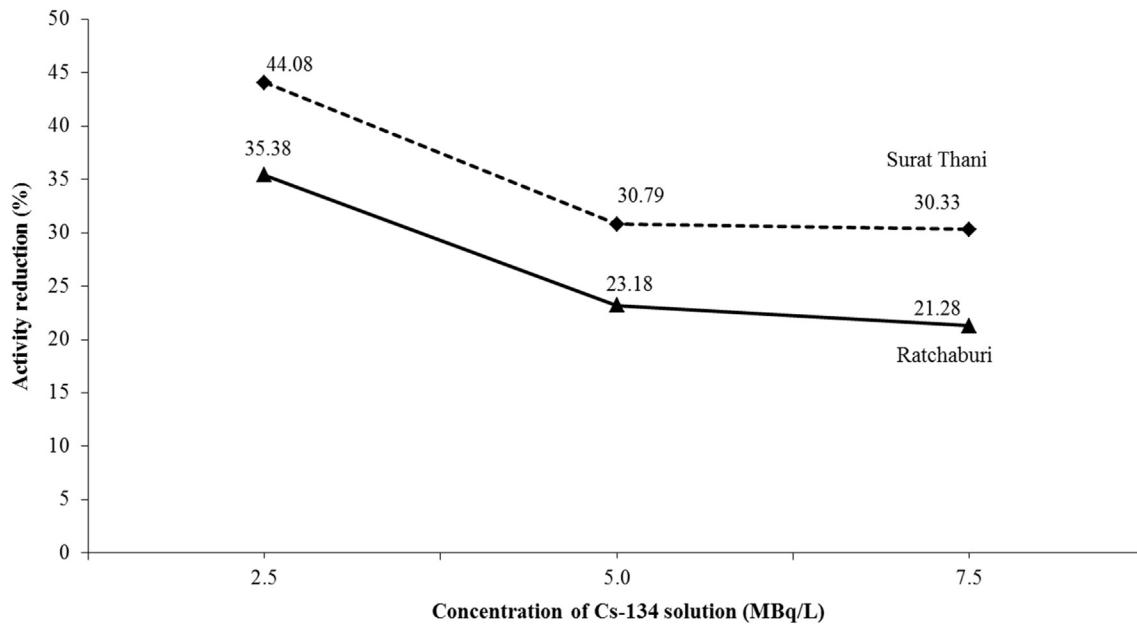


Fig. 6.  $^{134}\text{Cs}$  activity reduction of  $^{134}\text{Cs}$  solution with different concentrations after vetiver culture for 5 d.

Ratchaburi treatments (Fig. 6). This information was supported by the specific activity data as the Surat Thani vetiver absorbed more Cs than the Ratchaburi vetiver. In the solution concentrations of 2.5 MBq/L, 5.0 MBq/L and 7.5 MBq/L, the Surat Thani vetiver could reduce  $^{134}\text{Cs}$  by 44.08%, 30.37% and 30.33%, respectively, while the Ratchaburi vetiver produced reductions of 35.38%, 23.18% and 21.28%, respectively, which indicated that the Surat Thani ecotype had better ability than the Ratchaburi ecotype in the removal of  $^{134}\text{Cs}$  from the solution and the  $^{134}\text{Cs}$  removal efficiency for both ecotypes decreased as the  $^{134}\text{Cs}$  concentration in the solution increased. For the solution concentration of 5.0 MBq/L, the Surat Thani vetiver could remove 30.37% of  $^{134}\text{Cs}$  from the solution after 5 d of culture while Singh et al. (2008a) reported that vetiver could remove 59% of  $^{137}\text{Cs}$  at the end of 7 d. These differences might have been due to differences in the vetiver variety and culture period.

#### $^{134}\text{Cs}$ absorption: effect of culture period

From the radiographic images of the Surat Thani plantlets after 3 d, 6 d, 9 d, 12 d, 15 d and 18 d of culture (Fig. 7), it was evident that a small amount of  $^{134}\text{Cs}$  was transported from the roots to the shoots of vetiver plants after 3 d of culture. An increased intensity in the blackening on images was found when the culture time was

longer which indicated that vetiver plants could absorb more Cs as the culture time increased. The PSL signal data showed that the specific activity values of the vetiver increased as the culture time increased. A rather strong linear relationship between the PSL signal value and culture period was found with an  $R^2$  value of 0.94 (Fig. 8).

The results of radioactivity measurement in the vetiver shoots and roots revealed that  $^{134}\text{Cs}$  accumulated in vetiver roots rather than in shoots, which was similar to the results in the first experiment, except in the plants that were cultured for 6 d (Fig. 9). The ratio of the specific activity in the shoots to that in the roots decreased from 0.53 to 0.32 when the length of culture period increased from 3 d to 18 d, showing that Cs transportation from the roots to the shoots in vetiver was limited, similar to the case of more accumulation of uranium in vetiver roots than shoots as reported by Roongtanakiat et al. (2010). Therefore, it could be concluded that vetiver is suitable for the phytostabilization of radionuclides. Similar results were shown by Yang et al. (2003) and Roongtanakiat et al. (2008, 2009) on vetiver in studies on the remediation of heavy metals.

Information from the radiographic image (Fig. 7), PSL signal values (Fig. 8) and radioactivity in vetiver (Fig. 9) strongly indicated that vetiver could absorb a greater amount of  $^{134}\text{Cs}$  when the period

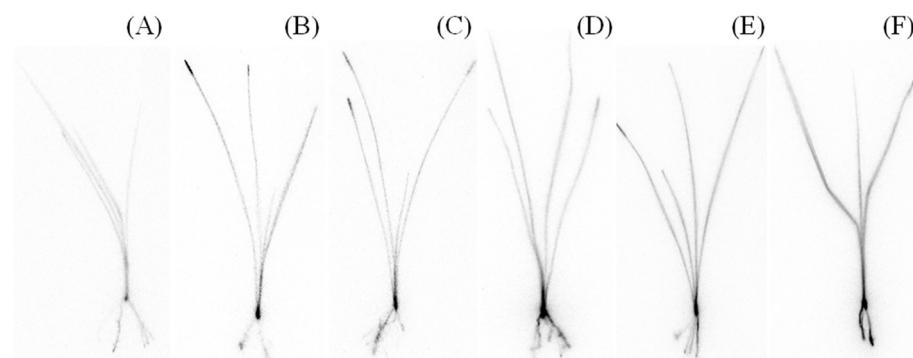


Fig. 7. Radiographic images of Surat Thani vetiver plantlets after culture in  $^{134}\text{Cs}$  solutions for time periods of: (A) 3 d; (B) 6 d; (C) 9 d; (D) 12 d; (E) 15 d; (F) 18 d.

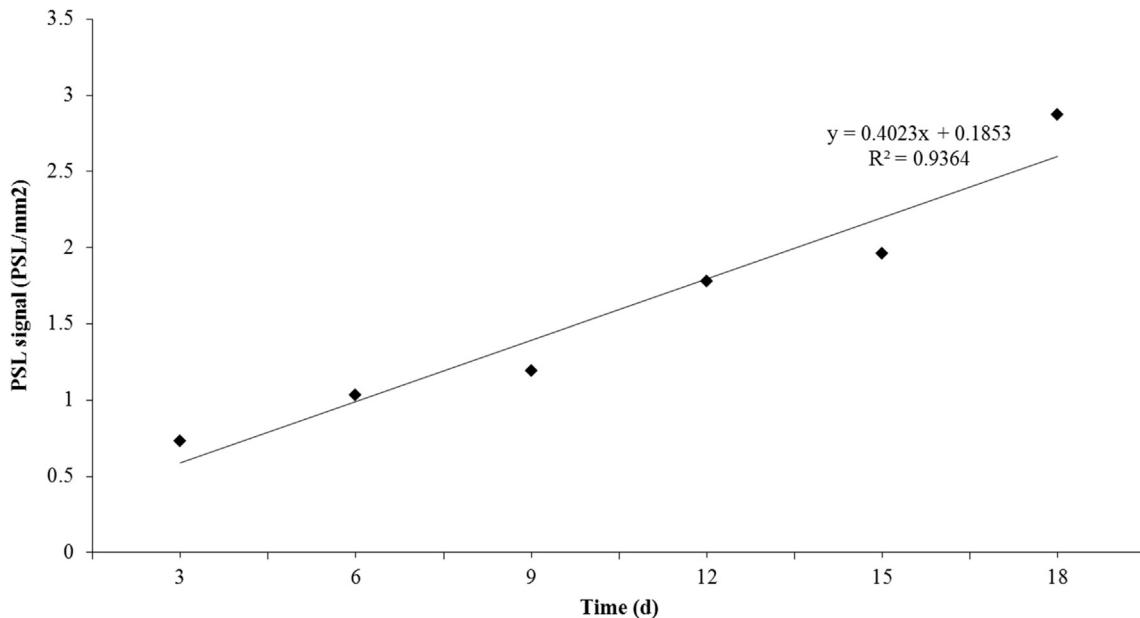


Fig. 8. PSL signal of Surat Thani vetiver plantlets cultured in  $^{134}\text{Cs}$  solutions for different time periods.

of culture was longer. This finding was in agreement with previous studies reporting that plants absorbed more radionuclides when they were grown longer in a radioactive solution (Dushenkov et al., 1997; Singh et al., 2008b; Roongtanakiat et al., 2010).

After the Surat Thani plantlets had been cultured in  $^{134}\text{Cs}$  solution with activity of 5 MBq/L for 3 d, 6 d, 9 d, 12 d, 15 d and 18 d, the activity of  $^{134}\text{Cs}$  in the solutions declined to 98.0%, 93.2%, 88.6%, 78.1%, 70.7% and 65.5% (Fig. 10), respectively, indicating that vetiver could remediate  $^{134}\text{Cs}$  in the cultured solution by 2.0%, 6.8%, 11.4%, 21.9%, 29.3% and 34.5% of the respective durations. These figures were lower than those reported by Singh et al.

(2008a), where at the end of 96 h, 38% and after 168 h, 61% of Cs was removed from the solution when vetiver was incubated in solution. These deviations might have been due to the different vetiver varieties and culture conditions. Soudek et al. (2004) reported that poplar, reed and sunflower could remove 68%, 15% and 8%, respectively, of  $^{137}\text{Cs}$  from 14 MBq/L solution. Ikeura et al. (2014) also proved that when sunflower, rapeseed and cosmos were exposed to three different concentrations of  $^{133}\text{Cs}$  (0.5 mg/L CsCl, 2 mg/L CsCl and 5 mg/L CsCl), more than 48% of the  $^{133}\text{Cs}$  was remediated after 7 d of culture, and the highest remediation rate was 67%, by sunflower.

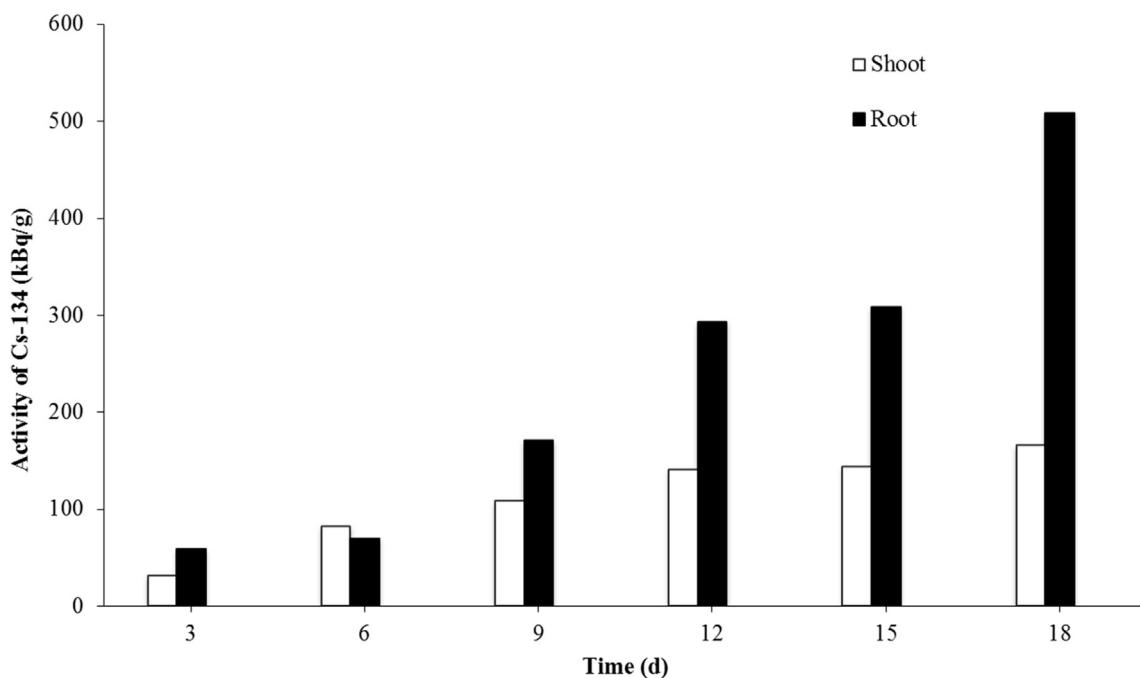


Fig. 9. Specific activity of  $^{134}\text{Cs}$  in shoots and roots of vetiver after culture in  $^{134}\text{Cs}$  solution for different time period.

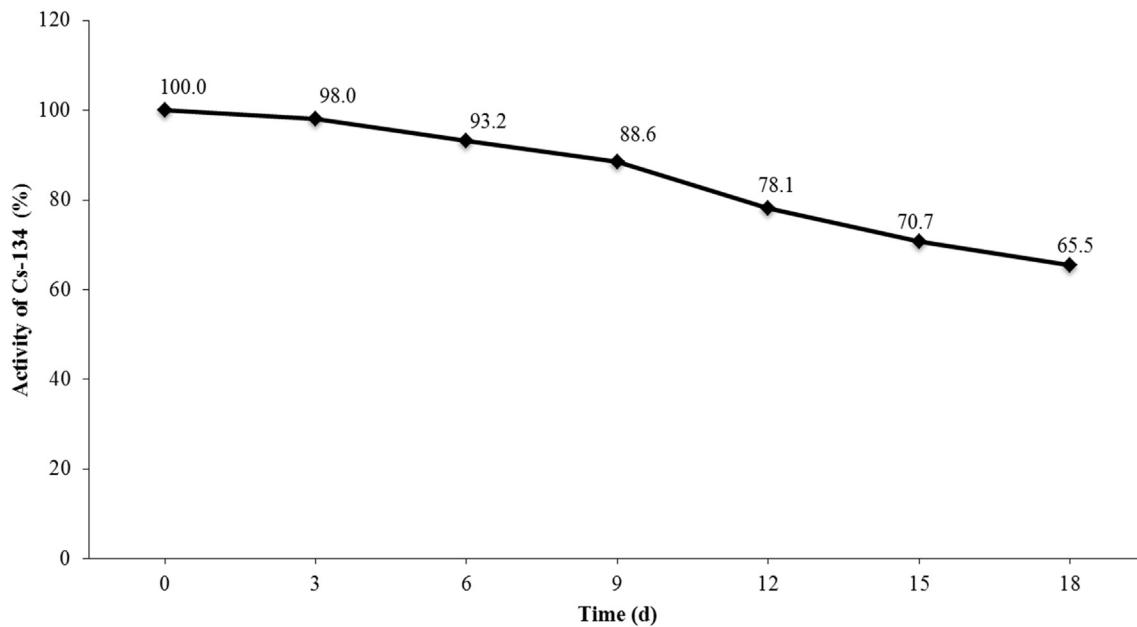


Fig. 10.  $^{134}\text{Cs}$  activity in solutions after vetiver culture for different time periods.

Worldwide, it is known that vetiver is a very beneficial plant for soil and water conservation and erosion control (Truong, 1999). For phytoremediation application, vetiver can be used for cleaning up soil and water contaminated with heavy metals (Roongtanakiat, 2009). This study showed that vetiver could absorb radiocesium and accumulated more in the root parts; therefore, vetiver might be suitable for the phytostabilization of radiocesium-polluted soil. As the vetiver variety, the cesium concentration in the growth medium and the culture period all affected the cesium absorption ability of vetiver, proper practices and management are necessary for effective radiocesium remediation using vetiver grass.

### Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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