Radiocesium uptake through leaf surfaces of tea plants (Camellia sinensis L.)

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Abstract

To clarify the source of radiocesium detected in newly emerged tea leaves contaminated just before the time of bud opening by fallout of radionuclides from Fukushima Dai-ichi Nuclear Power Plant, ^{137}CsCl solution (0.185 M Bq mL^{-1}) was applied to the front or the backside surfaces of mature leaves of tea plant (*Camellia sinensis* L. cv. Yabukita) at the time of bud opening. A 21 days after foliar application, the buds had grown and developed to the three- or four-leaf stage. In the front treatment, almost all (95%) of the applied ^{137}Cs was present in the mature leaves (hot mother leaves). In the backside treatment, 68% of applied ^{137}Cs also remained in hot mother leaves, but 22% and 10% was found in the new shoots attached to hot mother leaves and the other parts (non-applied mature leaves, stems and roots), respectively. The images of a hot leaf and its attached new shoots by imaging plate analysis revealed that the results coincided with those of the ^{137}Cs distribution above. These suggested that radiocesium was primarily absorbed from the backside surface of tea leaves through the stoma, and then the greater part was transported to newly emerged tea organs during the new shoot growth period.

Keywords

Tea plants (*Camellia sinensis* L.); Fukushima Dai-ichi Nuclear Power Plant; Radiocaesium; Transport

1. Introduction

On the 11th of March 2011, Fukushima Dai-ichi Nuclear Power Plant (FDNPP) was severely impacted by the Great East Japan Earthquake and tsunami, and was damaged by hydrogen explosions with the meltdown of the nuclear fuel. Consequently, a large amount of radionuclides was emitted and contaminated a wide range of land and many crops, mainly in the Tohoku and Kanto regions of Japan.

Tea plants were one of the crops contaminated by radionuclides. In the report of the Ministry of Health, Labour and Welfare (2011), the first crops of tea harvested in Ibaraki, Chiba, Gunma, Kanagawa and Shizuoka Prefectures after the accident were contaminated with radiocesium at a higher level than the guidance level in raw food materials set by the Food Safety Commission of Japan, 500 Bq kg^{-1} (sum of ^{134}Cs and ^{137}Cs activities). Because of the contamination, the sale of these tea products was suspended, resulting in huge damage to the Japanese tea industry. In these tea fields, new tea leaves of the first crop after the contamination event emerged around early-to-mid April and were harvested around early-to-mid May. According to the report of the Ministry of Education, Culture, Sports, Science and Technology (2011), the amount of radionuclide release from FDNPP was the highest from 15th to 21st March, but a lesser amount of radionuclide release was continuously observed during March. In early April, radionuclide release had almost decreased to the level normally observed in this area before the accident. That is, radionuclides were deposited on the canopy of tea plants covered with overwinter leaves (mature leaves) before the emergence of new leaves. Therefore, new tea shoots of the first crop season after the accident in Japan were not directly contaminated by radiocesium fallout from the FDNPP accident.

At the beginning of March 1986, fallout from the Chernobyl Nuclear Power Plant (CNPP) accident considerably contaminated the Black Sea coast, where tea plants had been densely cultivated in Turkey (Gökmen et al. 1995). In this case, heavy rains carried and precipitated radionuclides onto the tea leaves during the picking period of the first tea crop. Therefore, the first tea crop of Turkey in 1986 was contaminated directly with the radionuclides of the CNPP accident fallout.

Radiocesium is transferred to plants by foliar and root uptake pathways (Russell, 1966). The radiocesium uptake by plant roots was expected to be useful for phytoremediation as a potential environmental technology (Zhu and Smolders, 2000). Different plant species have different abilities to take up radiocesium from soil. It was reported in Turkey that ^{137}Cs from the CNPP accident was stored in stems from old leaves of tea plants and was then translocated to new leaves (Topcuoğlu et al., 1997). Additionally, Calmon et al. (2009) reported that the primary source of tree contamination in European countries was the direct dry or wet interception of airborne radionuclides by the canopy, and then by translocation from the foliar surface to structural components. Research in our group has also demonstrated that the solution/plant ^{137}Cs transfer factor of tea plant roots (4–7 g mL^{-1}) was lower than those of wheat and spinach (10–15 g mL^{-1}; data not shown). These results suggested the possibility that the major source of radiocesium in newly emerged tea leaves after the FDNPP accident in Japan might be the translocation from overwinter leaves. However, there is still no evidence whether the main source of radiocesium contamination in Japanese green tea leaves was the foliar absorption, root uptake from soil or both. Additionally, the front surface of tea leaves is covered by a thick cuticular layer that limits the penetration of solutes.

In this study, to clarify the mechanisms of radiocesium translocation to newly emerged tea leaves, ^{137}Cs was applied to the front or the backside surface of tea plant leaves, and the distribution of ^{137}Cs in tea plants during the development of new leaves was examined.

2. Materials and methods

2.1 ^{137}Cs uptake by tea leaves

On 14th May 2012, 1-year-old rooted tea cuttings (cv. Yabukita) were transplanted to 1 a/10,000

Wagner pots containing tea field soil collected in Makinohara City, Shizuoka, Japan. At transplanting, 0.12 g N, 0.06 g P_2O_5 and 0.08 g K_2O per pot were applied as a basal fertilizer. Tea plants were then cultivated according to conventional methods in the greenhouse in Shizuoka University, Shizuoka, Japan. To synchronize the growth stage of each tea plant, one bud and three leaves of every new shoot were cut off on 20th July 2012.

At the time of bud opening (on 17th August 2012), ^{137}CsCl solution was applied on the front surface or the backside surface of mother leaves that are cultivated in different pots for each of the treatments with a paintbrush in the draft chamber of the Radiochemistry Research Institute, Shizuoka University (Fig. 1). Mother leaves were the most upper mature leaves with a lateral bud developing a new shoot. To diminish contamination by ^{137}Cs, mother leaves overlapping with other mature leaves were excluded. Consequently, 4 to 6 mother leaves per tea plant (per pot) were treated with ^{137}CsCl solution. To apply radiocesium at the similar level to that of the fall out in tea fields resulting from the FDNPP accident (several hundred Bq per g dry weight of tea leaves, Shiraki et al., et al. 2013), we pretested how to apply radiocesium with a paintbrush by using other tea plants. Then we gave mature tea leaves two coats of 1.85 KBq mL^{-1} of ^{137}Cs solution by a paintbrush. After ^{137}Cs treatment, the tea plants were cultivated in the draft chamber under a controlled condition to avoid radiation exposure to the environment; 25 °C, 70-80 RH (%), 12/12 hr day/night cycle and light intensity was maintained at around photosynthetic photon flux density of 200 μmol m^{-2} s^{-1} with two white LED lamps (Fig. 2). Each treatment was replicated three times.

After 3 weeks (on 7th September 2012), at the opening time of the third to fourth leaves, tea plants were harvested and separated into nine parts; mother leaves with applied ^{137}Cs (hot mother leaves), new shoots attached to hot mother leaves, mother leaves without applied ^{137}Cs (cold mother leaves), new shoots attached to cold mother leaves, other mature leaves, upper stems, lower stems, stem bases and roots, as shown in Fig. 3. Stem bases and roots were washed with tap water to remove the soil. The fresh weight of each part was measured, and then one set of a hot mother leaf and a new shoot in each of the front and backside surface treatments was used for the imaging plate analysis. The dry weights of the other fresh samples were measured after drying in the draft chamber. Samples for imaging plate analysis were also dried and weighted after imaging plate analysis. All of dry samples were ground with a pestle and mortal, and used for the measurement of radiocesium activity.

2.2 Measurement of ^{137}Cs radiation dose

Aliquots of dried samples were weighed and put into polystyrene tubes (10 mL) for the radiation measurement. The ^{137}Cs radiation activity in each tube was measured by the auto well gamma system ARC-380CL (Aloka, Tokyo, Japan) for 10 min and calibrated with a standard ^{137}Cs source (CS404, Japan Radioisotope Association). Radioactivities of tea plant samples were obtained after subtraction of the values from samples without applied ^{137}Cs calculated from each dry weight of samples. Total activity of ^{137}Cs of plant was determined by summing the activities of the measured sample components.

2.3 Imaging plate analysis

One set of a hot mother leaf and its attached new shoot in each of the front and backside surface treatments was put into a polyethylene film bag, closely set on the imaging plate (BAS-MS2040, FUJIFILM, Tokyo, Japan), and exposed in a dark place for 90 days. After that, the radioactivity distribution images were obtained by scanning the imaging plates with the Molecular Imager FX Pro Plus (BioRad Laboratories Inc., Hercules, CA, USA).

3. Results and Discussion

The dry weight of each part of the tea plants at the end of the treatments were shown in Table 1. Although the dry weight of roots was around 2 fold higher in the front surface application than the backside one, there were no significant differences in the dry weights of other parts or of the total dry weight between these two treatments.

In terms of the total ^{137}Cs activity per plant, the front application (338 Bq plant^{-1}) was 1.5 times higher than the backside application (221 Bq plant^{-1}) (Table 2). In the backside treatment, a small amount of ^{137}CsCl solution was spread smoothly onto the leaf surface, because there were many trichomes and a thin cuticular layer on the backside surface of tea leaves. On the front surface of tea leaves, the thick cuticular layer repelled the solution, meaning that it was necessary to apply a larger amount of ^{137}CsCl solution for it to spread uniformly. Therefore, the quantity of ^{137}Cs solution applied was lower in the backside surface treatment than in the front surface treatment. During the treatment period, no fallen leaves were observed in either treatment, suggesting that there was no loss of ^{137}Cs from the tea plants after foliar application.

The ratio of ^{137}Cs contents of each part to the total applied (Table 3) was used for the estimation of ^{137}Cs distribution after the foliar application, because there was a difference in the total amount of ^{137}Cs application between the two treatments. In the front treatment, almost all (95 %) of the ^{137}Cs applied was present in the hot mother leaves. The highest ^{137}Cs values were found in the hot mother leaves in the backside treatment, but their percentage of the total was 68 %, lower than that of the front treatment. ^{137}Cs activities of new shoots attached to hot mother leaves and the other parts were only 3% and 2%, respectively, in the front treatment, but as high as 22% and 10%, respectively, in the backside treatment. The images of hot leaves and their attached new shoots by imaging plate analysis were shown in Fig. 4. The color in the image was changed from blue to white, yellow and red with the increments in radioactivity. In the front treatment, the hot mother leaf was entirely reddened, but its new shoots showed blue color, despite showing several whitish small spots in their stems and in the midribs of new leaves. In the backside surface treatments, the mother leaf with applied ^{137}Cs was also very red, but part of the leaf tip was yellow. Moreover, the color of the new stems and the midrib of new leaves was from yellow to red. These images agreed with the results of the ^{137}Cs distribution (Table 3). In radish, more than 40% of the radiocesium applied on the surface of the leaves was absorbed within a few days (Oestiing et al. 1989), and a rather similar uptake was found for beans and potatoes when harvested a month after foliar application of radiocesium. It was estimated that the foliar uptake rate of ^{137}Cs applied on the backside surface of tea plants was comparable with these crops. Also it was suggested that ^{137}Cs applied on the backside surface of mother leaves was easily absorbed through stomas and translocated into their attached new shoots, but ^{137}Cs uptake from the front surface of leaves was completely inhibited by the thick cuticle layer. However, the rate of ^{137}Cs absorbed by tea plant leaves was generally lower than those of radish, bean and potato, because uptake of foliar applied ^{137}Cs of tea plants was restricted to the front surface of leaves.

During new shoot growth, around 20% and 10% of the ^{137}Cs applied to the backside was transported into new shoots and lower parts (mature leaves, stems and roots), respectively. This was suggested that Cs absorbed from tea plant leaves was easily mobile through the phloem, similar to other crops (Coughtrey and Thorne, 1983).

In conclusion, radiocesium was primarily absorbed from the backside surface of tea leaves through the stoma, and then the greater part was transported to newly emerged tea organs during the new shoot growth period.

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References

Calmon, P., Thiry, Y., Zibold, G., Rantavaara, A., Fesenko, S., 2009. Transfer parameter values in temperate forest ecosystems; a review. J. Environ. Radioact. 100, 757-766

Coughtrey P.J., Thorne M.C., 1983. Radionuclide distribution and transport in terrestrial and aquatic ecosystems. A critical review of data, vol. 1. Balkema, Rotterdam, pp.1-496

Gökmen, I.G., Birgül, O., Kence, A., Gökmen, A., 1995. Chernobyl radioacitivity in Turkish tea and its possible health consequences. J. Raioanal. Nucl. Chem., 198, 487-4197

Ministry of Health, Labor and Welfare, 2011. Test Results o Radionuclide in Foods since 19 March 2011 Available at. http://www.mhlw.go.jp/english/topics/2011 eq/index.html

http://www.mhlw.go.jp/stf/houdou/2r98520000029prx-att/2r98520000029rvk.pdf

Ministry of Education, Culture, Sports and Technology, 2011. Reading of Radioactivity Level in Fallout by Prefecture. http://radioacitivity.mexto.go.jp/en/monitoring_by _fallout/.

Osestiing O., Kopp P., Burkart W., 1989. Foliar uptake of cesium, iodine and strontium and their transfer to the edible parts of beans, potatoes and radishes. Int. J. Radiation Appl. Inst. Part C. Radiation Physics and Chemistry, 33, 551-554

Russell, R.S., 1966. Raioacitivity and human diet. 1st edn. Pergamon Press, Oxford.

Shiraki Y., Takeda H., Okamoto T. Kita N., 2013. The Correlation of the Radiocesium Concentration of New Shoots Harvested in 2012 and 2013 and Old Leaves, and New Shoot in 2011 Grown in

Kanagwa Prefecture. RADIOISOTPES, 62, 183-190 (in Japanese with English abstract)
Topcuoğlu. S., Güngör, N., Köse, A., Varinlioglu, A., 1997. Translocation and depuration of ^{137}Cs in tea plants. J. Radioanal. Nucl. Chem. 218, 263-266
Zhu, Y-G., Smolders E., 2000. Plant uptake of radiocesium: a review of mechanisms, regulation and application. J. Exp. Bot., 51, 1635-1645

Figure

Fig. 1. Foliar application of $^{137}CsCl$ solution (1.85 KBq mL^{-1}) with a paintbrush.

Fig. 2. Tea plants cultured in the draft chamber after foliar application.

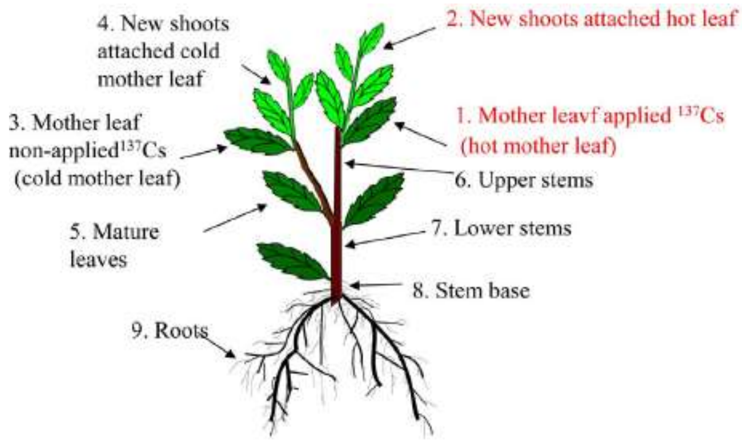

Fig. 3. Separation into nine parts of tea plants at harvest after foliar treatment.

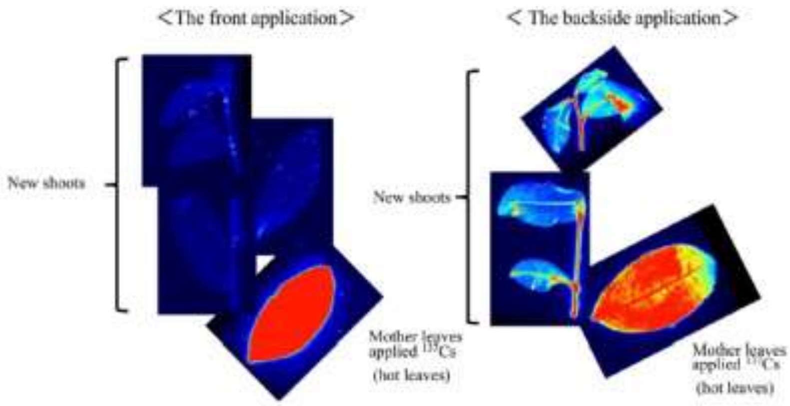

Fig. 4. Images of hot leaves and their attached new shoots by imaging plate analysis.

Table 1 Dry weights of tea plants (g plant^{-1}).

Treatments	Mother leaves applied ^{137}Cs	New shoots attached hot leaves	Mother leaves non-applied ^{137}Cs	New shoots attached cold leaves	Mature leaves	Stems			Roots	Sum
						Upper	Lower	Base		
Front	0.93±0.21	0.32±0.19	0.67±0.47	0.04±0.01	1.13±0.22	0.76±0.24	1.97±0.43	0.95±0.38	2.31±0.71	9.09±1.52
Backside	0.70±0.18	0.35±0.04	0.88±0.14	0.13±0.09	1.93±1.00	0.62±0.18	2.40±0.54	1.13±0.27	4.54±1.48	12.68±3.11

Values were average ± S.D. (n = 3).

Table 2 ^{137}Cs radioactivities of tea plants (Bq plant^{-1}).

Treatments	Mother leaves applied ^{137}Cs	New shoots attached hot leaves	Mother leaves non-applied ^{137}Cs	New shoots attached cold leaves	Mature leaves	Stems			Roots	Sum
						Upper	Lower	Base		
Front	322±55	10±7	0.3±0.2	0.0±0.0	0.8±0.4	2.1±1.8	1.3±0.4	0.3±0.3	0.6±0.5	338±49
Backside	144±33	47±7	0.2±0.2	0.1±0.1	3.4±2.9	3.3±1.2	6.8±2.0	1.8±0.9	4.1±3.4	211±33

Values were average ± S.D. (n = 3).

Table 3 Rates of ^{137}Cs radioactivities of tea plants (%).

Treatments	Mother leaves applied ^{137}Cs	New shoots attached hot leaves	Mother leaves non-applied ^{137}Cs	New shoots attached cold leaves	Mature leaves	Stems			Roots	Sum
						Upper	Lower	Base		
Front	95±3	3±2	0.1±0.1	0.0±0.0	0.2±0.1	0.7±0.5	0.4±0.1	0.1±0.1	0.2±0.1	100±0
Backside	68±5	22±0	0.1±0.1	0.1±0.0	1.9±1.7	1.6±0.8	3.2±0.9	0.9±0.5	2.0±1.6	100±0

Values were average ± S.D. (n = 3).