Quantitative mapping of elements in basil leaves (Ocimum basilicum) based on cesium concentration and growth period using laser ablation ICP-MS

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HIGHLIGHTS

- Quantitative elemental mapping of pollutants in sweet basil was studied by laser ablation ICP-MS.
- The concentration of Cs increased with growth period and pollutant concentration.
- The accumulation of Cs followed the order of leaf margin, petiole, midrib, and veins.
- Significant suppression of the growth rate was observed due to the presence of high-concentration Cs.
- The experimental model showed potential for studying the influence of radioactive pollutants on plants.

ABSTRACT

Quantitative elemental mapping of metallic pollutants in sweet basil was studied by laser ablation (LA)-ICP-MS. For this, the sweet basil was cultivated in Hoagland nutrient solution spiked with 100 and 1000 ng mL⁻¹ of Cs for 10–60 days. Then, the Cs distribution in collected leaves was determined by LA-ICP-MS using lab-synthesized standard pellets based on NIST 1573a tomato leaves. For comparison, S, Ca, and K were also simultaneously determined in this measurement with a¹³C⁺ signal from the leaves as an internal standard. The obtained calibration curves showed linear coefficient of determination (R²) of 0.991 for K and 0.999 for Cs. The concentration of Cs measured in the basil leaves increased with growth period and pollutant concentration, and accumulation followed the order of leaf margin, petiole, midrib, and veins. Although no visible symptom was detected, significant suppression of the growth rate was observed due to the presence of high-concentration Cs. The experimental model demonstrated herein showed potential for studying the influence of radioactive pollutants on plants and other organisms in the food chain.

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1. Introduction

The natural environment has been exposed to various metallic pollutants (e.g., heavy metals, engineered nanomaterials, and radioactive elements) due to the rapid expansion of their use in industry (Batley et al., 2013; Hough et al., 2004). Because such pollutants can interrupt biological and physiological systems of organisms directly or indirectly, many researchers have focused on the distribution and behavior of metallic pollutants (Rico et al., 2013; Zhang et al., 2007; Zheljazkov et al., 2008). In particular, plants are one of the important targets for research because of their...
intermediate role in the human food chain. That is, the investigation of how metallic pollutants contaminate and accumulate in leaves gives clues to pollution pathways and prospects for environmental protection (Conway et al., 2015; Dinh et al., 2015). For these kinds of studies, various analytical methods have been employed. For example, atomic spectroscopy, such as inductively coupled plasma mass spectrometry (ICP-MS) (Dan et al., 2015), inductively coupled plasma optical emission spectrometry (ICP-OES) (Barros et al., 2016), and atomic absorption spectrometry (AAS) (Paz-Rodríguez et al., 2015) were used for quantitative analysis of accumulated target elements. Proton induced x-ray analysis (PIXE) (Isaure et al., 2006), energy-dispersive x-ray spectroscopy (EDS) (Hong et al., 2014), and micro x-ray fluorescence spectroscopy (μ-XRF) (Hernandez-Viezcas et al., 2013; Wu et al., 2016) were applied for studying elemental distribution. Among them, μ-XRF has been well discussed for monitoring pollutants and imaging soft tissue samples, owing to its noteworthy feature of high resolution. However, although it showed lower limits of detection than did other non-destructive methods (Wu and Becker, 2012), the analysis of uncontaminated samples for comparison with the pollutant-exposed samples was unsatisfactory (Wang et al., 2011). For this reason, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been extensively used for various biological targets, such as rice (Choi et al., 2014), single eukaryotic cells (Drescher et al., 2012), thin mouse brain samples (Pozebon Smolders, 2000), and sunflower leaves (da Silva et al., 2013), as well as for samples of other plant tissues (Moradi et al., 2010; Narewski et al., 2000) and leaves (Kötscau et al., 2013). It is noteworthy that LA-ICP-MS provides information about time-resolved pollutant behavior in the targets, and provides the convenience of sampling through direct analysis of solid samples (i.e., no sample treatment is required because the target is directly ablated from the sample surface by laser and delivered into ICP-MS for detection (Pozebon et al., 2014)). Therefore, the probability of sample loss and contamination during sample preparation can be significantly lowered. Furthermore, fast analysis with simultaneous detection makes it possible to accomplish multi-elemental mapping with small sample consumption, which is a very big advantage for biological targets. So far, a few reports of radioactive elemental mapping for plant leaves has been published although environmental pollution by radioactive elements has been a major issue since the Chernobyl and Fukushima disasters in 1986 and 2011 (Müller and Mousseau, 2006; Steinhauser et al., 2014). Because plant leaves are at the root of the human food chain, the quantitative distribution data of the radioactive materials could give information important for human health from pollution. Among the radioactive elements, Cs is a well-studied alkali metal related to disasters at nuclear power plants (Yasunari et al., 2011). Because it is physicochemically similar to K (Kanter et al., 2010) (i.e., same surface charge and similar diameter), Cs passes through the K⁺ channels of plants and/or uses K⁺ transporters at the cell membranes (Zhu and Smolders, 2000).

In this work, we studied the influence of Cs pollution on plants through quantitative elemental mapping using LA-ICP-MS. Similarly, Kowata et al. used stable Cs for the discussion of uptake mechanism and qualitative distribution on Egeria densa using SR-μ-XRF (Kowata et al., 2014). Sweet basil (classified as an annual dicot herb) was herein selected as a model plant due to its high consumption as a cooking ingredient. After being nourished in Cs-spiked Hoagland nutrient solution, samples of basil leaves were collected based on their growth period and Cs concentration. Five elements (13C, 34S, 39K, 43Ca, and 133Cs) were simultaneously monitored using LA-ICP-MS and the mapped results were compared and discussed to understand the influence of pollutant uptake. Although a stable isotope of Cs (i.e., 133Cs) was used in this study, the influence of radioactivity can be estimated from the specific activity of 137Cs because they have similar transfer efficiencies to plants (Kamei-Ishikawa et al., 2008; Tsukada et al., 1998).

Therefore, this experimental model and result, including a time-resolved image of Cs distribution, will have potential for studying the influence of various radioactive materials on plants.

2. Experimental

2.1. Reagents and equipment

Hoagland nutrient solution was prepared using KNO₃ (99.0%), Ca(NO₃)₂·4H₂O (98.5%), MgSO₄·7H₂O (99.5%), KH₂PO₄ (99.0%), MnCl₂·4H₂O (99.0%), CuSO₄·5H₂O (99.5%), H₂MoO₄·(87.0%), and ZnSO₄·7H₂O (99.5%), purchased from Kanto Chemical Co., Inc. (Tokyo, Japan); and H₂BO₃ (99.5%) was obtained from Yoneyama Yakuhinn Kogyo Co. (Osaka, Japan). The detailed components and their concentrations in the nutrient solution are described in Supplementary Table–I. The sweet basil (Ocimum basilicum) seeds were purchased from Nikkoseed (0801, Utsunomiya, Japan).

The certified reference materials (CRMs) of NIST 1573a tomato leaves and NIST 1547 peach leaves were purchased from the National Institute of Standards and Technology (NIST) for standard pellets and method validation. A quadrupole-based ICP-MS (Agilent 7500ce, Tokyo, Japan) and laser ablation system (New Wave UP 213, Electro Scientific Industries, Inc., Portland, USA) were used for quantitative analysis. The optimized operation conditions are listed in Table 1.

2.2. Preparation of lab-made standard pellets for calibration

Standard leaf pellets were prepared from spiking of standard aqueous solutions in the range of 2000–53,000 μg g⁻¹ for K and 100–50,000 ng g⁻¹ for Cs. For details, 1 mL of standard solution and 1 mL of 50 μg mL⁻¹ in as an internal standard were added to 0.5 g of NIST 1573a tomato leaves. After drying at 70 ˚C, the leaves were finely ground using a mortar and pestle, and then 0.1 g of the powder was pressed under 40 MPa for 10 min using a pellet maker.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Operating Condition</th>
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<tbody>
<tr>
<td>(a) ICP-MS</td>
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</tr>
<tr>
<td>Company, Model</td>
<td>Agilent, 7500ce</td>
</tr>
<tr>
<td>RF power (W)</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Dwell time (ms)</td>
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<tr>
<td>13C</td>
<td>3</td>
</tr>
<tr>
<td>34S</td>
<td>10</td>
</tr>
<tr>
<td>39K</td>
<td>1</td>
</tr>
<tr>
<td>43Ca</td>
<td>10</td>
</tr>
<tr>
<td>133Cs</td>
<td>100</td>
</tr>
<tr>
<td>(b) Laser Ablation</td>
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</tr>
<tr>
<td>Company, Model</td>
<td>ESI, New Wave UP 213</td>
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<tr>
<td>Wavelength of Laser (nm)</td>
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<td>Carrier gas flow rate (L min⁻¹)</td>
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</tr>
<tr>
<td>Repetition frequency (Hz)</td>
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</tr>
<tr>
<td>Average energy output (mJ)</td>
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<tr>
<td>Average fluence (J cm⁻²)</td>
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<tr>
<td>Energy output (%)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Spot diameter (μm)</td>
<td>110</td>
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<tr>
<td>Distance between lines (μm)</td>
<td>500</td>
</tr>
</tbody>
</table>
(Evacuable pellet press for FT-IR instrument, JASCO Corporation, Hachioji, Japan) to make a pellet with 1 mm of thickness.

2.3. Plant growth and sample preparation for uptake study

After germination for 24 h with de-ionized water (DIW, 18.2 MQ, Millipore-Q), the sweet basil seeds were planted into soil and cultivated in a growth chamber at constant temperature (25 °C), light for 12 h, and DIW supply twice a day. Two months later, they were transplanted into Hoagland nutrient solutions of pH 4.8 containing 100 or 1000 ng mL\(^{-1}\) of Cs and grown with an oxygen supply. After growth periods of 10, 20, 30, 45, and 60 days, the sample leaves were collected and attached to a Si-wafer with double-sided adhesive carbon tape for LA-ICP-MS measurement. The resolution of images can be improved by controlling the beam diameter of laser and the distance between lines, while samples have more chance of damages if analysis time is too long. Therefore, the operating parameters were optimized as listed in Table 1. It took 3–4 h to ablate a half of a leaf, of which the raw data was converted to images using a software (Origin 8, OriginLab, USA).

3. Results and discussion

3.1. Removal of polyatomic interference for \(^{39}\)K in ICP-MS

For study of the influence of Cs pollution on plants, quantitative mapping of \(^{133}\)Cs was carried out using LA-ICP-MS. Images related to growth period and Cs concentration were compared with that of the K distribution because both elements were expected to have similar behaviors in plants. Because multiplex detection is possible, C, S, and Ca were also monitored among various macro and micro elements for specific purpose. For example, \(^{13}\)C\(^{+}\) signal was monitored as an internal standard to reduce measurement error from fluctuations in the LA-ICP-MS measurements (Wu et al., 2009), and \(^{34}\)S\(^{+}\) and \(^{43}\)Ca\(^{+}\) were used as markers for proteins related to detoxification reactions and a representative element of macro nutrients, respectively. Since element analytes were exclusively transported as particulate phase from the carbon containing-gaseous species and carbon containing particles during laser ablation, the control leaf with the same matrix was used for the comparison of images in this work (Frick and Günter, 2012). Because the ions \(^{34}\)S\(^{+}\), \(^{43}\)Ca\(^{+}\), and \(^{39}\)K\(^{+}\) were subjected to severe interference from molecular ions in ICP-MS, such as Ar\(^{+}\), ArH\(^{+}\), O\(_2\)\(^{+}\), and CO\(_2\)\(^{+}\), the use of H\(_2\) gas through the gas flow reactors (CID) using H\(_2\) gas was employed in this study. For the removal of the interfering molecular ions, collisional-induced dissociation (CID) using H\(_2\) gas was employed in this work. For example, the interference of \(^{40}\)Ar\(^{+}\)H\(^{+}\) on the measurement of \(^{39}\)K\(^{+}\) was eliminated by the use of H\(_2\) gas through the proton transfer reaction, of which the optimization result for \(^{39}\)K\(^{+}\) is shown in Fig. 1. The best signal-to-noise ratio (S/N) was observed at the flow rate of 4 mL min\(^{-1}\) for both concentrations (10 and 100) ng mL\(^{-1}\). If the flow rate was too high, S/N was decreased. Under this condition, S/N was maximized at a level about 2-times higher than that of the baseline and similar optimization profiles were observed for \(^{34}\)S\(^{+}\), \(^{43}\)Ca\(^{+}\), and \(^{133}\)Cs\(^{+}\). Therefore, the flow rate of 4 min mL\(^{-1}\) for CID was used throughout this experiment.

Because the synthesized standard pellets and/or basil leaves contain high-concentration K (i.e., NIST 1571a contains 27,000 ± 500 ng g\(^{-1}\)), further study was carried out on the interference with the K matrix by other elements. Fig. 2 (a) shows the S/N change of \(^{34}\)S, \(^{39}\)K, and \(^{43}\)Ca with the change in concentration of K, while \(^{13}\)C\(^{+}\) peak was used as an internal standard. Although a significant matrix effect of K on S and Ca was expected, no change in of the S/N of \(^{34}\)S\(^{+}\) and \(^{43}\)Ca\(^{+}\) was observed as the concentration of K was increased (i.e., 0.071 ± 0.002 for \(^{34}\)S\(^{+}\) and 0.287 ± 0.004 for \(^{43}\)Ca\(^{+}\)) indicating that there was almost no matrix interference. For further study of interference, time-resolved profiles of \(^{34}\)S\(^{+}\), \(^{39}\)K\(^{+}\), and \(^{43}\)Ca\(^{+}\) were obtained as well. As shown in Fig. 2 (b), the peaks of \(^{34}\)S\(^{+}\) and \(^{43}\)Ca\(^{+}\) did not exhibit interference from K because relatively low background peaks were not increased even at the concentration of 39,000 µg g\(^{-1}\) of K. However, some transient background peaks for \(^{39}\)K\(^{+}\) were often detected because it is generally distributed throughout a whole leaf. In addition, relative standard deviations (rsd) of the background level were low (i.e., 1.45% for S and 2.36% for Ca), indicating that the weakly observed transient background peaks showed no regularity and no proportion to the concentration of K. Therefore, the effect of the presence of the K-matrix on the background of \(^{34}\)S\(^{+}\) and \(^{43}\)Ca\(^{+}\) was negligible for plant samples studied via LA-ICP-MS.

3.2. The calibration curve for quantitative analysis

Because a standard with composition identical to sweet basil leaves was not available, known concentrations of Cs and K solutions added to NIST 1573a tomato leaves, which were then pelletized for calibration. Prior to the analysis, 100 ng mL\(^{-1}\) of In was also added to test homogeneity. Typically obtained \(^{115}\)In/\(^{13}\)C was 0.169 ± 0.006 with rSD of 3.5% for the measurement of 19 samples. This indicated that the prepared pellets were sufficiently homogeneous to be used as a standard for calibration. Calibration curves using the synthesized standard pellets revealed good linearity coefficient of determination (R\(^{2}\)) of 0.999 in the range of (100–50,000) ng g\(^{-1}\) for K and 0.991 in the range of (2000–53,000) µg g\(^{-1}\) for Cs (Supplementary Fig. S1). Using this calibration curve, the concentration of K and Cs in NIST 1573a was estimated to be (25,454 ± 616) µg g\(^{-1}\) and (49.87 ± 2.27) ng g\(^{-1}\), respectively, of which the recovery was near 94% for both elements with respect to the certified values. For further confirmation, K in the NIST 1547 peach leaves was also determined to be (21,403 ± 414) µg g\(^{-1}\) using the synthesized pellets, which was close to the certified value of (24,300 ± 300) µg g\(^{-1}\). Therefore, the LA-ICP-MS method using the synthesized standard pellets had enough accuracy and precision for the quantification of real leaf samples.

3.3. Monitoring of Cs in the sweet basil leaves

Fig. 3 shows the experimental scheme to monitor Cs distribution...
and accumulation in sweet basil according to growth period and pollutant concentration. For the pollutant nutrient solution, Hoagland solution was spiked with Cs standard solution and diluted twice (i.e., 50%). Hoagland solutions containing (100 and 1000) ng mL\(^{-1}\) of Cs was prepared. If the concentration was >10,000 ng mL\(^{-1}\), the sweet basil plants died within a week. As shown in the figure, after the basil seeds were cultivated in the soil for two months, the young plants were transplanted into the prepared 50% Hoagland solution spiked with Cs. For monitoring the influences and results of Cs on the plant growth, leaves of the young plants were collected after 10, 20, 30, 45, and 60 days and analyzed by LA-ICP-MS. For laser ablation, the collected leaves were attached to a Si-wafer using carbon tape. The detailed measurement parameters of the ICP-MS and laser ablation systems are described in Table 1. For elemental mapping, the line-scanning mode of the laser ablation process was employed with a beam diameter of 110 \(\mu\)m. The distance between lines was optimized to be 500 \(\mu\)m. If the distance was too narrow for higher resolution, sample damages were observed during ablation.

The mapping results of K and Cs are shown in Fig. 4. The basil leaves shown were exposed to a blank, 100 ng mL\(^{-1}\), and 1000 ng mL\(^{-1}\) of Cs within the growth interval of 10–60 days. In order to keep the consistency of sampling, young basil leaves were collected from the highest place on the sweet basil plants with similar growth rate, so that the sample leaves collected were of sizes as similar as possible in order to eliminate the size effect. The averaged half size of a leaf (shown in the figure) was (1.61 ± 0.22) cm (height) × (0.54 ± 0.17) cm (width). Because of transient particle generation by laser ablation, the dwell time of all the ions measured in this work was optimized for simultaneous determination, as listed in Table 1. As expected, a high concentration (red) of K was found all over the basil leaves, even in the blank sample, due to its significant roles in maintaining the physical activities in the plants (e.g., photosynthesis, enzyme activation, water regulation) (Leigh and Wyn Jones, 1984; Wang et al., 2013; Hopkins and Hüner, 2008). In contrast, almost no Cs signal was observed without Cs spiking during the growth period, except for 60 days. When exposed for 60 days, the averaged Cs concentration was

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**Fig. 2.** Study on the spectral overlap between \(^{34}\)S, \(^{43}\)Ca, and \(^{39}\)K ions: (a) Signal to noise ratio (S/N) with the concentration of K in ICP-MS and (b) Time-resolved profile of ions in LA-ICP-MS.

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**Fig. 3.** Experimental scheme for the monitoring of Cs contamination in sweet basil leaves.
(64.10 ± 61.26) ng g⁻¹ while the background level was (18.97 ± 5.77) ng g⁻¹ due to the carbon tape used for leaf adhesion. When exposed to 100 ng mL⁻¹ of Cs, a weak ¹³³Cs⁺ signal was appeared after 10 days, and the signal intensity gradually increased with the length of the growth period, as shown in Fig. 4 (b). Interestingly, K and Cs showed similar transport and distribution patterns (Fig. 4c for Cs and Fig. 4a for K). The presence of both elements was observed at the petiole and/or midrib near the leaf base after 10 days exposure, and then expanded to the whole leaf blade (including the leaf tip and margin) after 20 days. This result is explained by a report on the relationship between them. The physical similarities of surface charge and hydrated ionic size result in very similar plant uptake and accumulation processes, even though Cs has no functions in plants (White and Broadley, 2000).

The concentration of Cs in the sample leaves increased with the length of the growth period and the Cs concentration of the nutrient solution. From Fig. 4, it was also found that the accumulation of Cs in parts of the plants, increased in the order of leaf margin, petiole, midrib, and veins. The highest concentration of Cs was detected at the vein because most elements are transported through it.

Conclusively, several important points were cleared up by this experiment. The accumulation of Cs started from the base of leaf because the uptake proceeded up through the vascular bundle of the leaf stem, and the accumulation increased with the length of the growth period and the Cs concentration of the nutrient solution. In addition, although it was difficult to distinguish between xylem and phloem due to the lack of resolution, the mapping images showed that Cs ions were transported in the same uptake channels as K ions. For exposure to 1000 ng mL⁻¹ of Cs solution, similar results were obtained. With, the longest growth period (up to 60 days), the concentration of accumulated Cs increased continuously while K was saturated regardless of the growth period. The averaged Cs concentration after 60 days exposure was (5740 ± 592) ng g⁻¹ for 100 ng mL⁻¹ and (39,396 ± 942) ng g⁻¹ for 1000 ng mL⁻¹ Cs-spiked nutrient solutions (n = 3). This was approximately 97 and 665 times higher than without the Cs exposure. No evidence of element exchange between K and Cs was observed in this study. The distribution mapping of two other elements (S and Ca) are shown in Supplementary Fig. S2. Sulfur was monitored as an indicator of metalloproteins, phytochelatins, and/or other S-containing biomolecules related to protection reactions in plants (Cobbett, 2000; Dixon et al., 1998). The distribution of Ca was also determined for general knowledge. As shown in the figure, both elements accumulated at the midrib when plants were exposed to the Hoagland solution spiked with 1000 ng mL⁻¹ Cs, which was similar to the behavior of Cs.

### 3.4. Influence of Cs uptake on the elongation of the sweet basil leaves

The Cs uptake can affect not only elemental distribution, but also plant elongation (Hampton et al., 2004). In this work, the influence on the growth rates was estimated by measuring the elongation of the leaves after exposure for 0, 20, 30, 40, and 60 days.
As described in Table 2, at Day 0, the sweet basil plants showed similar elongation of (5.2 ± 1.3) cm, (5.8 ± 1.1) cm, and (5.4 ± 1.6) cm at the beginning of their exposure to 0, 100, and 1000 ng ml⁻¹ of Cs nutrient solution, respectively. The growth rates, however, changed with the Cs concentration and the growth period. The measured length from the ground to the leaf top (i.e., the root length was excluded) is listed in Table 2. The measured length after 60 days was 72.5% of the leaf raised without pollutant for 100 ng ml⁻¹ of Cs and 58.7% for 1000 ng ml⁻¹. That is, a higher concentration of Cs in the nutrient solution suppressed the growth rate of sweet basil plants without any visible symptoms of tissue necrosis. This was expected due to K deficiency induced by the uptake of Cs ions in the same transportation channel (Hopkins and Huner, 2008).

Similar suppression of elongation due to the uptake of Cs ion has been reported by others (Hampton et al., 2004; Adams et al., 2013), in which the stable Cs influenced the growth rate of the model plants through extraordinary gene expression. Although genetic mutation that affected the next generation was not reported in those papers, the distinct result of the accumulation of radioactive Cs was discussed. Using the specific radioactivity of 137Cs (i.e., 3.2 × 10¹³ Bq kg⁻¹), the degree of Cs radioactivity on the plants can be estimated (Bodansky, 2005) (i.e., 1.0 × 10⁻¹² g g⁻¹ of 137Cs in a plant can be converted to 3.2 × 10³ Bq kg⁻¹). Reportedly, 3.4 × 10³ Bq kg⁻¹ was determined in Houttuynia cordata in Koriyama City near the Fukushima Dai-ichi nuclear facility (Sugiura et al., 2016) after the accident. In addition, increased mortality and abnormality was reported as well for a pale grass blue butterly fed leaves contaminated with 134Cs and 137Cs (Hiyama et al., 2012).

Conclusively, the designed experimental procedure employing cultivation and elemental mapping using LA-ICP-MS was successfully applied to study the influence of Cs exposure on sweet basil. In particular, LA-ICP-MS using synthesized standard pellets showed excellent performance for elemental mapping and provided the information of Cs movement and accumulation in the leaves. Moreover, simultaneously monitoring of five elements (C, S, Ca, K, and Cs) using LA-ICP-MS resulted in reliable quantification of information about environmental pollution of plants with small sample consumption and rapid analysis. The measured Cs concentration in sample leaves increased with growth period and pollutant concentration in the nutrient solution, and that the uptake movement of Cs was in the order of petiole, midrib, veins, and leaf margin. Therefore, the experimental model of plant cultivation in various nutrient solutions, followed by quantitative elemental mapping using LA-ICP-MS, will be useful for studies on the influence of radioactive exposure to plants, intermediate organisms, and predators in the food chain.

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