doi:10.1246/cl.2011.1398

Published on the web December 3, 2011

SR-XRF Imaging of Cs Highly Accumulated in Vegetables

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Accumulation of Cs in vegetables was studied with regard to the remediation of radioactive Cs from a nuclear plant accident in Fukushima. It was found that *Brassica oleracea var. capitata, Brassica campestris var. perviridis*, and *Lactuca sativa* accumulated Cs to a level of more than 10000 ppm (dry weight) when they were cultivated in 1 mM Cs solution. Two-dimensional distributions of Cs were revealed by SR-XRF imaging showing a homogeneous distribution of Cs in the plant bodies.

After the disaster in Fukushima, radioactive Cs was defused across a wide area in the Tohoku–Kanto regions. It is now, therefore, crucial to effectively remove Cs from soils. With regard to this goal, it will be of interest to identify plants, known as hyperaccumulator plants, with an ability to absorb high levels of specific elements. More specifically, it will be desirable to find plants that are hyperaccumulators of Cs. In addition, it is clearly a serious problem that people unintentionally take in radioactive Cs as a result of eating vegetables, and movement of Cs up the food chain increases the complexity of this problem. Eating foods contaminated with Cs will cause internal radiation exposure. From this perspective, it will be interesting to determine which vegetables absorb high level of Cs and where Cs accumulates.

Based on the above, we have begun to cultivate several kinds of vegetables using liquid cultivation media containing Cs. The distribution of Cs in a plant can be revealed by synchrotron-radiation-excited X-ray fluorescence (SR-XRF) imaging. The application of this technique to the analysis of Cs in plants has in the past shown the distribution of Cs in *Arabidopsis thaliana*.[1,2] They used the Cs Lα line (4.287 keV) for the analysis, which required analysis to carry out in a vacuum. In contrast, we used the Cs Kα line (30.857 keV), which allowed us to carry out the analysis in vivo, a technique that has not previously been reported for Cs uptake. Use of the Kα line is also advantageous over L line analysis because of the small absorption effect due to the high X-ray energy, and one can obtain a bulk chemical composition. Cs Kα imaging can be realized only by using SR. It is a unique approach of the present study that our analysis was focused on vegetables considering the problem of the human uptake of hazardous radioactive Cs.

Seeds of three kinds of vegetables: *Brassica oleracea var. capitata* (cabbage), *Brassica campestris var. perviridis* (KOMATSUNA), and *Lactuca sativa* (garden lettuce) were placed on cotton in Petri dishes soaked with a mixture of 1/2MS medium[3] and 0 (control), 1, and 5 mM Cs solution prepared with CsCl. The seeds were germinated after placing the dish in a dark place and then kept in an incubator at 25 °C under a 16 h light/day. The collection of the plants for analyses was performed at 8 days after germination. Leaves and roots were rinsed in deionized water. The plants were then placed on a mylar film, and roots were kept in wet cotton and covered with another mylar film to prevent the sample from drying out. The sample was then subject to the in vivo measurement.

XRF imaging was carried out on BL37XU at SPring-8.[4] Monochromatic X-rays of 36 keV were obtained by a Si(511) double crystal monochromator. The X-ray beam size was adjusted by slits to 200 µm × 200 µm. The sample on a plastic holder was placed on an automatic XY stage. Elemental maps were obtained by scanning the sample with a 36 keV beam under the following conditions: step size 200 µm × 200 µm with a measurement time of 0.5 s/point. To evaluate actual Cs levels from the XRF intensity, a calibration curve of the Cs Kα intensity vs. Cs levels was prepared by using standard disk samples. One hundred milligrams of cellulose powder was mixed with a known amount of CsCl in agate mortar. The mixture was pressed into a solid disk with diameter of 1 cm. The intensity of the Cs Kα line was measured for 100 s with the same beam size as the one used in the XRF imaging. The calibration curve is shown in Figure 1. The dotted line is the net intensity (corrected for background), and the solid line is total intensity. Since XRF imaging is not corrected for background intensity, we should use the latter calibration curve to estimate the actual amount of Cs from the XRF intensity shown in the XRF image.

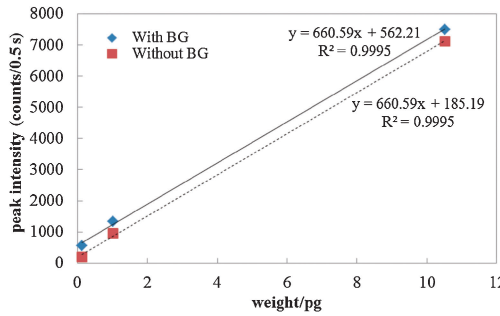

Figure 1. Calibration curve for Cs Kα X-ray intensity vs. Cs level.

The bulk concentrations of Cs in the plants were determined using an inductively coupled plasma mass spectrometer (ICP-MS), Agilent 7500c. The plants were harvested, washed with distilled water three times, and then freeze-dried for 24 h. The dried samples were ground into powder. Approximately 10 mg of sample was weighed into a teflon vessel, digested with concd HNO_3 in a microwave oven and then subjected to ICP-MS analysis.

The plants died when exposed to 5 mM Cs in a few days. After 8 days exposure to 1 mM Cs, growth of the vegetables was reduced compared with the control (0 mM Cs). Figure 2 compares the biomass of three plants after 30 days of exposure to 1 mM Cs and their control. After the exposure, biomass was the largest for *Brassica campestris var. perviridis* and the smallest for *Lactuca sativa*, which showed a whitish color on some of the leaves. The results indicate the toxicity of the Cs, but the results suggest that the former plant may be suitable for phytoremediation.

The Cs concentrations of the plants after Cs exposure were determined by ICP-MS and are compared in Figure 3a. It is remarkable that after only 8 days exposure, *Brassica oleracea var. capitata* accumulated Cs up to 10000 ppm (expressed in dry weight) in the body. After 30 days of exposure, Cs accumulated in *Lactuca sativa* up to 18000 ppm, and the other two plants showed

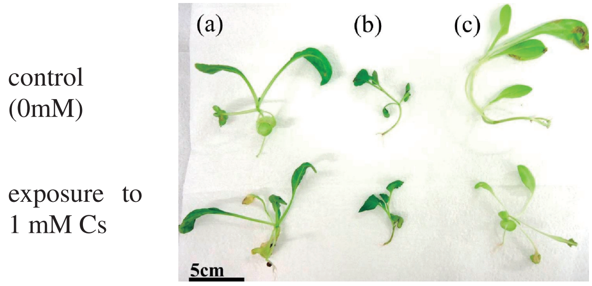

Figure 2. Influence of Cs expossure for 30 days on biomass of (a) *Brassica campestris var. perviridis*, (b) *Brassica oleracea var. capitata*, and (c) *Lactuca sativa*.

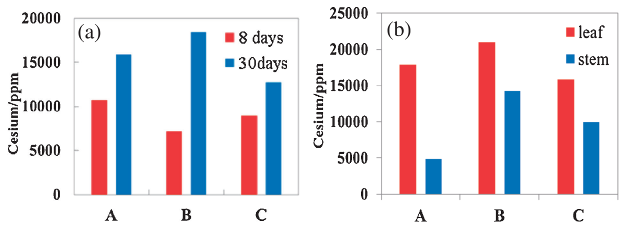

Figure 3. Comparison of the Cs concentration levels determined by ICP-MS: (a) after 8 and 30 days exposure (b) in leaf and stem. A: *Brassica oleracea var. capitata*, B: *Lactuca sativa*, C: *Brassica campestris var. perviridis* (these abrevitations are used in Figures 4 and 5).

accumulation higher than 10000 ppm. From the perspective of concentration only, these plants may be considered hyperaccumulators of Cs. We also compared the parts of the plants shown in Figure 3b with regard to accumulation. It was found that, generally, the leaves accumulated higher levels of Cs than the stems. This tendency is remarkable for *Brassica oleracea var. capitata*.

In order to clarify the accumulation behavior of Cs and Zn in the plants, SR-XRF imaging of Cs and Zn was carried out. Elemental maps of the three plants after 8 days of Cs exposure are shown in Figure 4 for the whole plant and in Figure 5 for the leaves. The Cs Kα intensity was expressed by color scale from lowest (blue) to highest (red). The top figure shows a photograph of each analyzed sample, the middle figure an elemental map of Cs, and the bottom a similar map of Zn. To evaluate actual Cs levels, the calibration curve in Figure 1 can convert the Cs Kα intensity into the Cs level (weight/pg).

It was found that Cs was present throughout the entire plant body, more or less homogeneously. The apparent accumulation points in Figure 4 are due to the steric effect: the analysis of the plant was carried out under living conditions, and the sample was not flat. In contrast, the leaf samples shown in Figure 5 were cut from the plant and were pressed onto a plastic film. It can be seen in Figure 5 that the vascular systems tended to contain high levels of Cs in all the samples. The distributions of Zn seem to be similar to those of Cs. A similar distribution of Cs has been reported for *A. thaliana*.[1] The highest concentrations of Cs were found in the vascular system of stem and leaves in the plant. Cs and K belong to the same periodic group, the alkali metal group. It has been reported that Cs behaves similarly to K in *A. thaliana*.[1] The leaves make up a high percentage of the total plant body in our vegetable samples, and the high Cs levels in the leaves may make these plants suitable for phytoremediation application. The SR experiments were carried

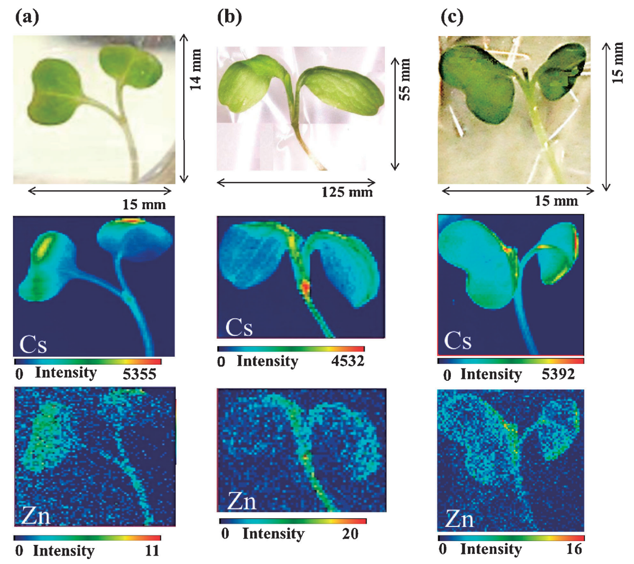

Figure 4. SR-XRF imaging of whole plant at 8 days after Cs exposure. Photographs of (a) A, (b) B, and (c) C. Beam size: 200 µm (V) × 200 µm (H), Step size: 200 µm × 200 µm, Measurement time: 0.5 s/point.

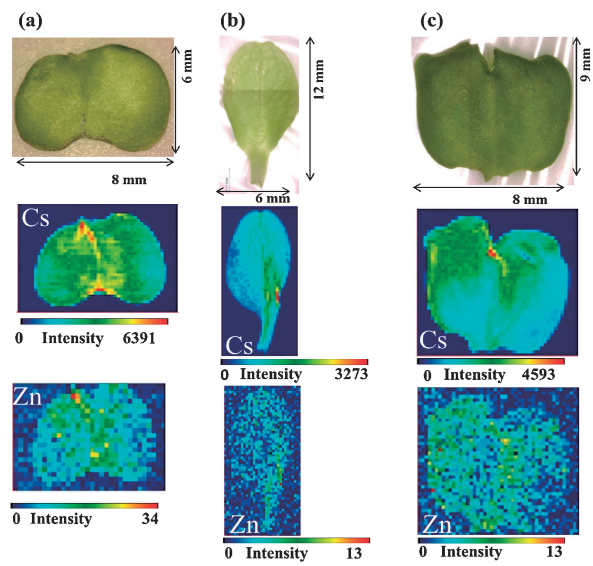

Figure 5. SR-XRF imaging of leaf at 8 days after Cs exposure. Photographs of (a) A, (b) B, and (c) C. Beam size: 200 µm (V) × 200 µm (H), Step size: 200 µm × 200 µm, Measurement time: 0.5 s/point.

out with the approval of the SPring-8 program Advisory Committees (Proposal No. 2011A1620).

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