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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

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The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

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All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Injected cesium analysis of frozen-hydrated Japanese cedar tree trunk by cryo-μ-XRF and cryo-μ-XANES.	**Experiment number**: LS-2565
Beamline: ID21	**Date of experiment**: from: 27 Jan 2017 to:31 Jan 2017	**Date of report**: 05 Mar 2017
Shifts: 12	**Local contact(s)**: Dr. Hiram Castillo-Michel	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

(Background)

In tree trunk, phloem (bark) and xylem (wood) lie side by side along the vertical axis of a tree, and are joined by cambium. Assimilation products such as sugars and starch, and minerals from leaves, and water and nutrients from roots are transported by phloem and xylem, respectively. These are thought to be transported in radially by living cell such as parenchyma cells [1, 2]. This aspects are generally accepted and proposed in many text books, although there are no experimental data that parenchyma cells are the exact place of chemical transportation. The experimentation on standing trees is difficult, because the phenomena occur inside the tree trunk.

The purpose of this study was to reveal the site of mneral transport from phloem to xylem of trees for understanding the nineral transportation mechanism in tree trunk. We have analyzed the mineral distribution using frozen-hydrated tree samples which were injected Cs as a tracer into the trees (Fig. 1). In this experiment, we analyzed the sub-micron level element mapping of Cs (μ-XRF) to reveal the mineral pathway from phloem to xylem via cambium using frozen-hydrated tree samples.

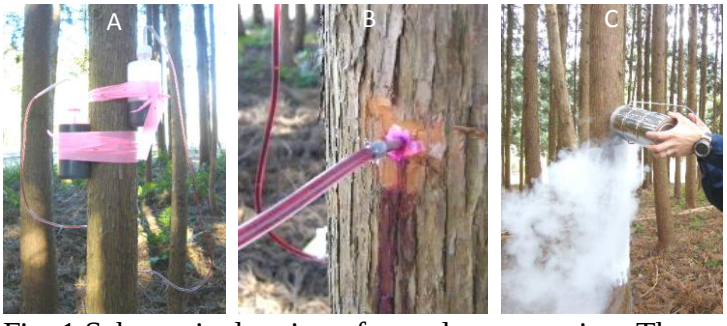

Fig. 1 Schematic drawing of sample preparation. The cesium chloride solution was injected into the trunk of a standing Japanese cedar tree using a stainless tube (A, B). After the injection, the trunk part was freeze-fixed with liquid nitrogen (C), and then tree was felling.

(Experimental design)

We use stable isotope cesium (Cs) as a tracer. Cs is a homologous element with potassium that is an essential mineral element in plants. Thus, we expect that Cs moves similarly to potassium. Cs is injected into trunks (ca. 15 cm in diameter) of standing Japanese cedar (*Cryptomeria japonica*) trees for 1day. Since

chemicals in standing trees are of in wet condition, we use a unique sampling procedure to reveal the element distribution in a standing tree (Fig. 1). Trunk part of a standing tree is frozen by liquid nitrogen for 20 minutes, and then cut down. Frozen samples are cut into small pieces in a liquid nitrogen pool and kept frozen until the end of the analysis [3].

We used 4 areas of different distance from injection point (Fig. 2A), since our preliminary results from cryo-SEM/EDX and ICP-MS analyses showed that Cs concentration was different from the distance from the injection point. Frozen block of the tree sample was cut into small blocks (Fig. 2B), and transverse, tangential, radial thin-sections were prepared by using of cryomicrotme (Fig. 2C), and subjected to the cryo-μ-XRF analysis. The details of the setting was decided based on the correspondence with beam line scientist (Dr. Castillo-Michel at ID21), such as a sub-micrometric beam (~0.3μm ver. x 0.7μm hor.). We conducted low resolution analysis for 1 h, and then high relosution analysis (100 x 100μm, at dwell times of 100-200 ms with step size about 1 μm) multiplied by 30 scan (final scanned area was 300μm x 1 mm).

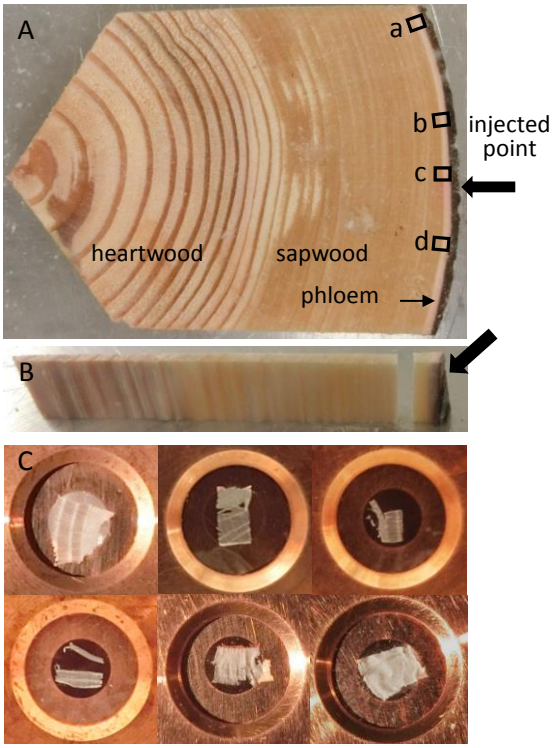

Fig. 2 Samples used for cryo-μ-XRF analysis in this experiment. Four areas (a, b, c, d) were selected according to the distance from the injection point (A), and the pholem-xylem parts were used (B). The sections of 15μm or 20μm thickness were mounted on the sample holder of μ-XRF aparatus (C) and subjected to the analysis.

(Results and discussion)

The results are presently being analyzed.

Our experimts mainly conducted using transverse sections. We obtained elemental mappings of cryo-μ-XRF in this experiment (Figs. 3 and 4). We compared the images among the difference of section thickness, and decided to use 15μm thickness section for the analysis.

We used the area a as a control, where Cs had low concentration as same as no-injected tree sample detected by ICP-MS analysis (our unpublished data). Cs was not detected in the area a, although potassium (K) and calsium (Ca) represented cell structure and phosphate (P) represented living cells (Fig. 3). On the other hand, Cs was clearly detected in the areas b, c, and d (Fig. 4). The results indicated that Cs distributed in the cell walls of all types of cells such as tracheids and parenchyma cells. The results of this experiment showed some similarity and some differences between Cs and K distribution. To unravel these properties, we need to conduct further analysis. In addition to the transverse sections, we obtained mapping of tangential and radial surface. Unfortunately, because of the limit of the beam time, we analyzed only one scan in each of tangential and radial section. These results also indicate that Cs distributed in the cell walls of all types of cells, although one mapping was not enough to arouse conclusion. However, we conclude that these 3 dimensional data seem crucial to understand the spatial distribution of minerals.

In the submitted proposal of this experiment, we proposed the XANES analysis to investigate Cs speciation (identify movable vs bound to the cell structure). In this experiment, however, the XANES analysis was not performed because our first experiment preference was given to XRF mapping. The chemical form of Cs analyzed by μ-XANES in frozen-hydrated tree samples will be needed to unravel the mechanism of mineral distribution in standing trees.

In our ongoing study, we have analyzed the mineral distribution using frozen-hydrated tree samples by cryo-SEM/EDX. We obtained the cesium distribution by point analysis, but not obtained subcellular elemental images because of technical problems. In this experiment at ESRF ID21, we succeeded to obtain Cs mapping in the cryo-μ-XRF analysis, which helps us to understand the details of the cesium transportation mechanism in the trees. These data are also important to reveal how the trees were contaminated by radiocesium after Fukushima nuclear power plant accident in Japan [4], and to predict the future distribution of radiocesium in trees.

(Extra)

During our experiment, it was honour to be introduced our experiment at “weekendusers”. Please see the ESRF web page, http://www.esrf.eu/home/news/general/content-news/general/tree.html.

(References)

[1] Spicer R. (2014) Symplasmic networks in secondary vascular tissues: parenchyma distribution and activity supporting long-distance transport. *Journal of Experimental Botany* **65**: 1829-1848.

[2] van der Schoot C, van Bel AJE. (1990) Mapping membrane-potential differences and dye-coupling in internodal tissues of tomato (*Solanum lycopersicon L.*). *Planta* **182**: 9–21.

[3] **Kuroda K**, et al. (2014) The accumulation pattern of ferruginol in the heartwood-forming *Cryptomeria japonica* xylem as determined by time-of-flight secondary ion mass spectrometry and quantity analysis. *Annals of Botany* **113**:1029-1036.

[4] **Kuroda K**, et al. (2013) Radiocesium concentrations in the bark, sapwood and heartwood of three tree species collected at Fukushima forests half a year after the Fukushima Dai-ichi nuclear accident. *Journal of Environmental Radioactivity* **122**: 37-42.

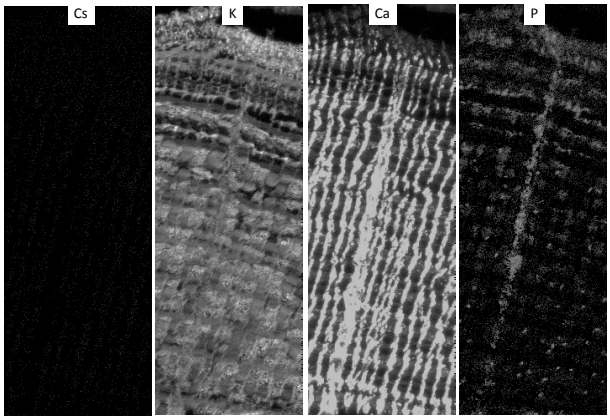

Fig. 3 Representative elemental mappings of Cs-injected Japanese cedar tree by cryo-μ-XRF. Cs was not detected in the area a (as a control).

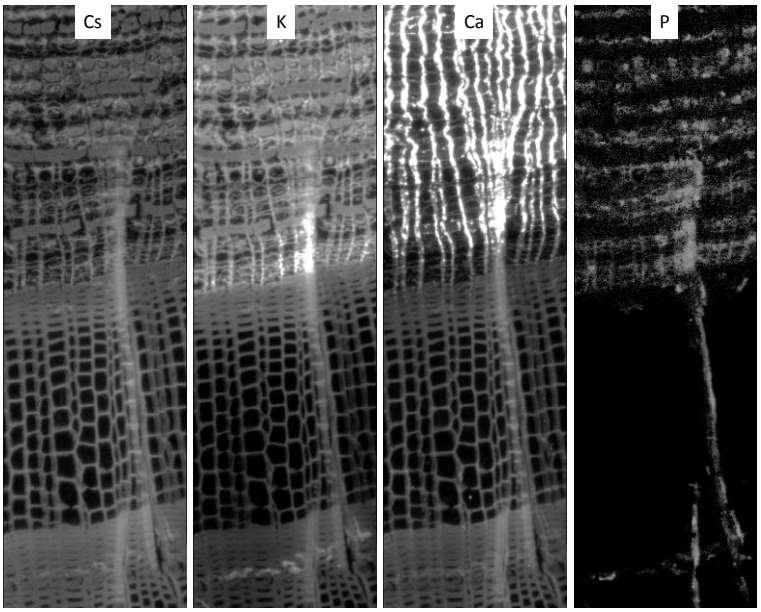

Fig. 4 Representative elemental mappings of Cs-injected Japanese cedar tree section by cryo-μ-XRF. Cs was detected in the cell walls of all types of cells both in phloem and xylem in the areas b, c, and d.