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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.

	Experiment title: Three dimensional structure of mineral pathway by analyzing frozen-hydrated Japanese cedar tree trunk using cryo-micro-XRF and cryo-micro-XANES	Experiment number: LS-2782
Beamline: ID21	Date of experiment: from: 06 July 2018 to:10 July 2018	Date of report: 02 Nov 2018
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 the 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 from leaves, and water and nutrients from roots are transported by phloem and xylem, respectively. An important mineral-transport pathway from the roots to the tree trunk is believed to be via conduit cells such as tracheids and vessels as sap solution. In addition to this pathway, minerals move radially in tree trunks through the symplasmic system. Wood rays in the tree trunk are believed to be responsible for this transportation [1, 2]. Although this function has been generally accepted, there are few experimental data showing that parenchyma cells are the exact place of chemical transportation, because analyzing the phenomena occurring inside the standing tree trunk is difficult.

The purpose of this study was to reveal the site of mineral transport from phloem to xylem of trees for understanding the mineral transportation mechanism in the tree trunk. In the normal procedure for analyzing the chemicals of trees, the samples are subjected to dry conditions, which may result in alteration of ionic distribution and/or chemical speciation from the original *in planta*. Therefore, we used a unique sampling method to analyze the distributions of water and chemicals, in which the samples were freeze-fixed in living standing trees; thus, the analyzed samples were hydrated[3, 4]. In our previous experiment on ID 21, we succeeded in obtaining clear elemental mapping of frozen-hydrated Japanese cedar tree by micro-X-ray fluorescence (μ-XRF) [LS-2565 report]. However, additional experiments of elemental mapping are needed to reach the conclusion. Besides determining the mineral transport pathway, determining the chemical form is also important to understand whether the minerals are moving or accumulating in xylem, especially in the heartwood, the inner part of the tree trunk. Therefore, in this experiment, we attempted to perform micro-X-ray absorption near-edge structure (μ-XANES) analysis to deermine the chemical form of minerals; which would help in differentiating whether the minerals are labile or bound to the cell structure.

(Experimental design)

We used the stable isotope cesium (Cs) as a tracer to reveal the mineral transport pathway in the tree trunk. Cs is an element that is homologous with potassium (K), which is an essential mineral element in plants. Hence, we expected that Cs movement was similar to that of potassium. Cesium chloride (CsCl) solution was injected into the trunks (ca. 15 cm in diameter) of standing Japanese cedar (*Cryptomeria japonica*) trees for 1 day and 60 days (Fig. 1A, B) [4]. For revealing the elemental distribution in a standing tree, we used the freeze-fixed-standing tree trunk parts (Fig. 1C, D). The trunk part of a standing tree was frozen by liquid nitrogen for more than 20 min and then cut down. The frozen samples were transferred to the laboratory and stored in the deep freezer (below −70°C).

For the cryo-μ-XRF and the cryo-XANES analyses, we used the sample parts where Cs detection was confirmed in our preliminary experiment by the cryo-SEM/EDX analysis. Block samples were cut into small pieces in a liquid nitrogen pool (Fig. 1E). We then made transverse, tangential, and radial thin sections from a frozen block of the sample using a cryomicrotome and subjected them to analyses (Fig. 1E–G). Thus, the frozen-hydrated samples were kept frozen until the end of the analysis. The details of the parameter setting of the apparatus were decided based on correspondence with a beam line scientist (Dr. Castillo-Michel at ID21), such as a sub-micrometric beam (~0.3 μm ver. × 0.7 μm hor.). We conducted low-resolution analysis for 1 h and then high-resolution analysis (100 × 100 μm, at dwell times of 100–200 ms with a step size of about 1 μm) multiplied by 30 scan (final scanned area was 300 μm × 1 mm).

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 the tree was felled (D). A part of the trunk was cut into small pieces (E, F) and subjected to cryomicrotome to prepare thin sections (G). Samples were under frozen state during the entire procedure from the cutting of the standing tree to the end of the analysis.

(Results and discussion)

We used thinner sections (about 10 μm thickness) in this experiment than those in the previous experiment, LS-2565 (15-μm thickness), to obtain the detailed structure to compare the elemental distribution between the cell wall and the lumen (inside a cell). We obtained clear elemental mapping of cryo-μ-XRF on the frozen-hydrated sections in three dimensions, i.e., transverse, tangential, and radial.

In the transverse sections of the 1-day-injection sample, K was distributed on the cell wall of all cell types (tracheid, ray, and axial parenchyma cells and axial parenchyma cells) and the cell lumina of ray and axial parenchyma cells, whereas calcium (Ca) was distributed on the cell wall. The injected Cs appeared to distribute on the cell wall of all cell types (tracheid, ray, and axial parenchyma cells) and the cell lumina of ray and axial parenchyma cells, which was highly similar to the distribution of K. This result was extremely similar to the previous result of LS-2565 and our cryo-SEM/EDX result [4]. On the other hand,

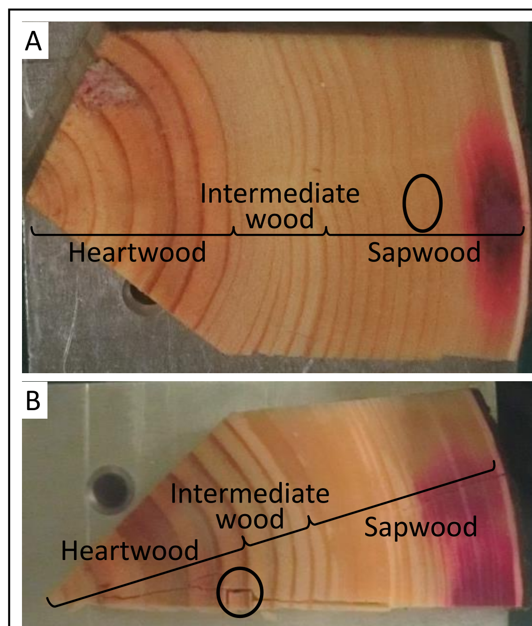

Fig. 2. Samples used in this experiment. The middle part of sapwood of 1-day-injection sample (A) and the outer part of heartwood of 60-day-injection sample (B) were used (circles).

in the tangential and radial sections, the distributions of Cs and K appeared to be different from those in the transverse section; Cs and K were primarily distributed in the cell lumina of ray and axial parenchyma cells and weakly distributed on the cell wall of all cell types. We believe that the different patterns on the mapping between transverse and tangential or radial sections might be caused due to the anatomical feature of ray parenchyma cells of Japanese cedar. Since Japanese cedar has uniseriate rays, the tangential and radial sections include a part of one parenchyma cell in the 10-μm thickness sections. Thus, the cell lumen and the cell wall of a parenchyma cell could be separately analyzed. On the other hand, a transverse section often contains a pair of cell wall and lumen of parenchyma cells. The X-ray goes down several micrometers from the surface, which implies that the X-ray fluorescence signal can always be detected from both the cell lumen and the cell wall of the parenchyma cell, and it is difficult to distinguish between the cell wall and the lumina of parenchyma cells in the transverse section of Japanese cedar. Thus, analysis using tangential and radial sections was found to be effective for determining the mineral distribution, with differentiation between the cell wall and the cell lumen.

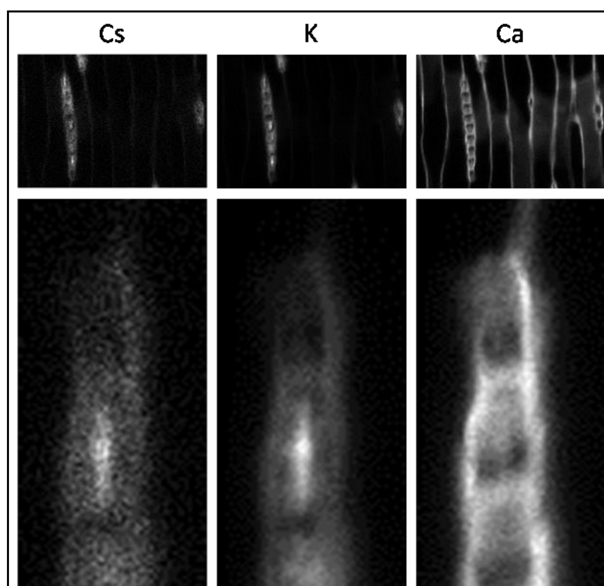

Fig. 3. Representative elemental mappings of the tangential section of the middle part of sapwood of 1-day-injection Japanese cedar sample by cryo-μ-XRF. Lower (upper row) and higher magnification (lower row).

In this experiment, we made sections from the middle part of sapwood of 1-day-injection samples (Fig. 2). We believe that this part was almost tip of the Cs transportation from outside (injection point) to innerward based on our preliminary experiment by the cryo-SEM/EDX point analysis. Here, a higher distribution of Cs was detected in the lumina of parenchyma cells than in the cell wall of all cell types, whereas Cs distribution was even in the cell lumina and the cell wall in phloem and current-year xylem in the LS-2565 experiment. These results indicated that Cs moved via the lumina of the ray parenchyma cells. In our previous experiment, we were not able to distinguish between the cell lumen and the cell wall of a parenchyma cell and concluded that Cs moved in the parenchyma-related parts (i.e., the parenchyma–tracheid cell wall, the parenchyma–parenchyma cell wall, and the parenchyma lumen) [4]. These results indicate that Cs first moves radially in xylem from the outer to inner parts via the cell lumina of the ray parenchyma cells and then moves through the cell wall by diffusion.

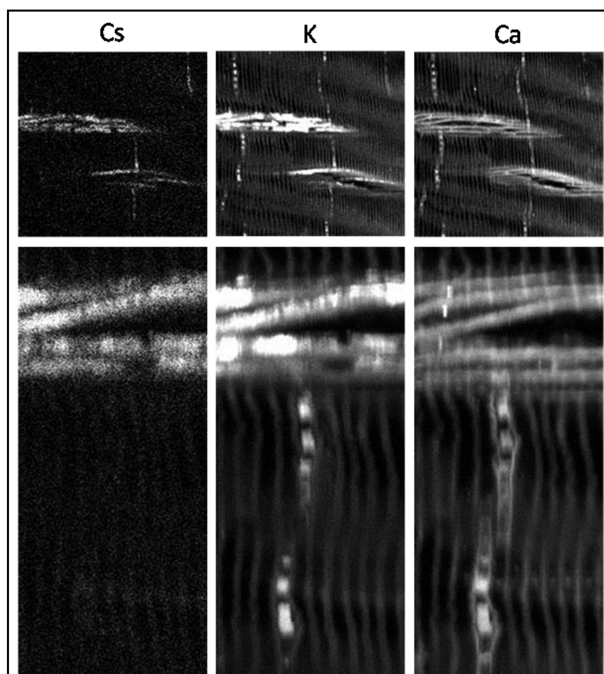

Fig. 4. Representative elemental mappings of the radial section of the outer part of heartwood of 60-day-injection Japanese cedar sample by cryo-μ-XRF. Cs distribution in the lumina of ray parenchyma cells was similar to K distribution. Some axial parenchyma cells lacked Cs even in the lumina that contained K. Lower (upper row) and higher magnification (lower row).

In addition to the middle part of sapwood, we used the heartwood of 60-day-injection sample (Fig. 2). The distribution pattern of Cs was different in the 60-day-injection sample from that in the 1-day-injection sample, although the distributions of K and Ca were highly similar among the two samples. Cs was distributed in the lumina of almost all ray parenchyma cells and some axial parenchyma cells but not in the lumina of some axial parenchyma cells (Fig. 4). Unlike sapwood, all ray and axial parenchyma cells were dead in the heartwood, suggesting that Cs movement in the heartwood was through diffusion. Since axial and ray parenchyma cells contact each other, Cs in the axial parenchyma cells might move from ray parenchyma cells by diffusion.

In the proposal of this experiment, we hypothesized that the cryo-μ-XANES analysis of the frozen-hydrated tree

sample could unravel the forms of Cs identified from the speciation, i.e., whether labile or bound to the cell structure. We used CsCl powder and frozen CsCl solution dissolved in water for the cryo-μ-XANES analysis to prepare standard profiles of crystalline and ionized Cs. We obtained clear differences in profile between the powdered and frozen Cs solution; the crystalline Cs had two peaks, and the frozen CsCl solution had one peak after a large peak (Fig. 5). We performed cryo-μ-XANES analysis of several sections of 1-day- and 60-day-injection samples. Several patterns of profiles were obtained, not only similar standard profiles but also several different patterns. The results are currently being analyzed to quantify the chemical form of Cs, which is required to understand the mechanism of mineral movement in the tree trunk.

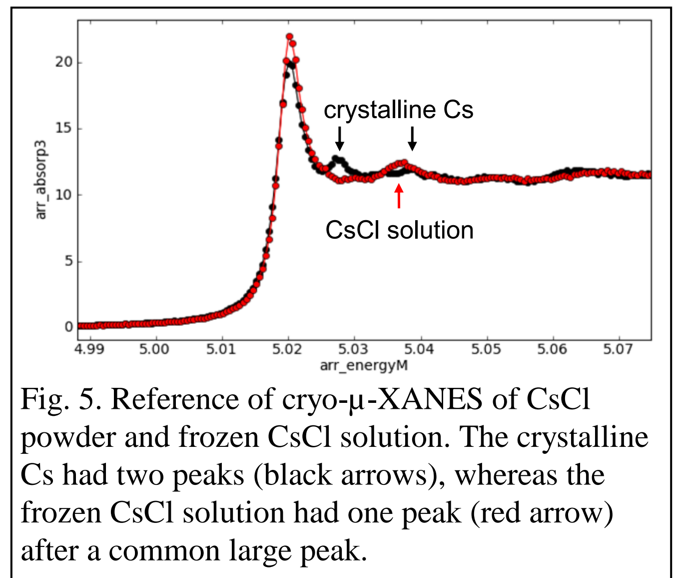

Fig. 5. Reference of cryo-μ-XANES of CsCl powder and frozen CsCl solution. The crystalline Cs had two peaks (black arrows), whereas the frozen CsCl solution had one peak (red arrow) after a common large peak.

(Conclusion)

The results of the LS-2782 experiments demonstrated that Cs was transported from the outer sapwood to the inner sapwood (including the intermediate wood between sapwood and heartwood) via the lumina of axial and ray parenchyma cells, and in the heartwood, Cs moved to ray parenchyma cells and then diffused to axial parenchyma cells. Japanese cedar is known to accumulate high content of K in the heartwood in both axial and ray parenchyma cells. Although our results of K distribution are consistent with this theory, the results of Cs distribution in the heartwood are different. To unravel this contrariety, we need further experiments on the transportation mechanism of minerals in the standing tree trunk.

In addition to the scientific understanding, these results help us understand how the trees were contaminated by radiocesium after the Fukushima nuclear power plant accident in Japan [5] and predict the future distribution of radiocesium in the trees.

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