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Fundamental Study on an Arsenic Hyper-Accumulating Plant Using Submilli-PIXE Camera

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Abstract. We have developed an in-air PIXE analysis system which provides elemental distribution images in a region of 3×3 cm^2 with a spatial resolution of ~0.5 mm. We call this system an in-air submilli-PIXE camera. This system consists of a submilli-beam line, beam scanners and a data acquisition system for elemental mapping. We applied the in-air submilli-PIXE camera to phytoremediation research. Phytoremediation is a technology for cleaning metal-contaminated soils using plant physiology. *Pteris vittata*, which is known as a hyper-accumulator of As, was analyzed by the PIXE camera. Elemental images of leaves were obtained *in-vivo* without sample preparation. The elemental map of the leaves in several centimeters region showed that arsenic was accumulated in the edges of *Pteris vittata* leaves and redistributed in the fronds at different growth stages. The submilli-PIXE camera is an effective tool for undertaking phytoremediation research.

Keywords: Submilli-PIXE Camera, PIXE Analysis, Imaging of Heavy Metals, Phytoremediation, Plant, Soil.

INTRODUCTION

A number of technologies for remediation of soils contaminated by heavy metals have been developed. Most of these technologies, however, are expensive, and they occasionally produce secondary waste [1]. Recently, environmentally friendly, low-input approaches such as phytoremediation have been proposed to cleanup soils contaminated with heavy metals and metalloids [2]. Phytoremediation is a technology for cleaning environments using the metabolism of plants, and interaction between plants and microorganisms in the rhizosphere. To develop practical applications of this technology, it is necessary to explicate effective accumulation mechanisms for heavy metals. In general, contaminated soil and plant samples are chemically analyzed using atomic absorption spectrometry (AAS) and inductively coupled plasma-mass spectrometry (ICP-MS), after oxidizing pre-treatment. However, these methods are time-consuming and provide only average metal concentrations in each plant organ. For this reason, Particle-Induced X-ray Emission (PIXE) analysis is an attractive analytical tool. PIXE analysis has high sensitivity and multi-elemental capability, and this non-destructive technique can be applied quickly and simply to analyze living plant samples [3,4].

The purpose of this work is to obtain a fundamental information on a mechanism of arsenic accumulation in *Pteris vittata,* expected to be an arsenic hyper-acccumulator plant, in an *in-vivo* investigation by using a submilli-PIXE camera.

SUBMILLI-PIXE CAMERA

The submilli-PIXE camera has been settled at Dynamitron laboratory of Tohoku University [5]. A single-ended type 4.5 MV Dynamitron accelerator with a high-current duo-plasmatron ion source can provide the maximum current of 3 mA for accelerate hydrogen, deuteron or helium ions and the brightness of 3.3 pA mrad^{-2} mm^{-2} MeV^{-1}.As shown schematically in Fig.1, submilli-beams are formed with two slits of 1.5m spacing; the first slit is four-pole type with copper jaws of 2mm thick and the second slit at downstream side consists of two wedge-shape jaws made by tantalum plates of 0.1mm thick. Both slits are remotely controlled in an operating room and beam currents are monitored at each slit. To scan a wide area, we use two magnets which are an air-core coil and a

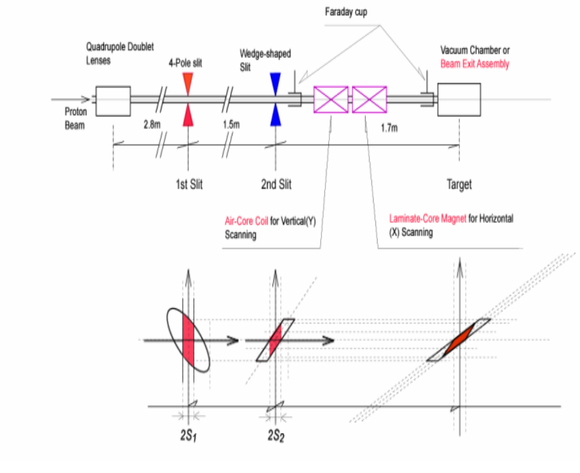

FIGURE 1. Schematic view of submilli-beam line.

laminate-core magnet for vertical (Y) and horizontal (X) scanning in high-speed, respectively. These magnets are controlled by a function generator, and scanning patterns can be freely made by a computer program. The maximum area of scanning region is 3 × 3 cm^2.

The beam exit assembly is shown in Fig.2. A beam exit window is a Kapton foil of 12.5 μm thickness and its area is 20 cm^2. The lifetime of the Kapton foil window until breakdown by beam irradiation was longer than 2000 seconds under beam current density of 300 nA/mm^2 (100 nA for beam spot size of 0.7mm in diameter) [6]. Since the lifetime is made to elongate by beam scanning, the endurance of the Kapton foil window is sufficient for the submilli-PIXE analysis. A specimen is set just after the exit window. X-rays from a specimen are detected with two Si(Li) detectors; No.1 detector (7.5 μm thick Be window, 10 mm^2 active area) with a low geometric efficiency is well suited for detection of an element of low atomic number, and No.2 detector (12.5 μm thick Be window, 50 mm^2 active area) with a 100-μm Mylar absorber allows detection of X rays > 4 keV and the removal of recoil protons.

Figure 3 shows the schematic diagram of beam scanning system and a data acquisition system for elemental imaging. The concept of the scanning system and data taking system are almost the same as that reported in a previous paper [5], but the components of the system are updated with new models. In the PIXE camera, the X-ray energy and the beam position are simultaneously measured in order to obtain a spatial distribution of element. The system consists of a multi-parameter data acquisition unit, two ADCs for X-ray detector signals and two ADCs for position signals. The position signals are derived from the control signals for X-and Y-magnets. Analog signals from the detectors trigger the ADCs for position signals. After conversion, the digital data are saved in a list file. It takes 20 μsec for one data acquisition cycle. The list mode data acquisition is useful to observe changes in elemental imaging during irradiation, because the system can sort the data for a selected element / energy region and generate an elemental image even while accumulating data.

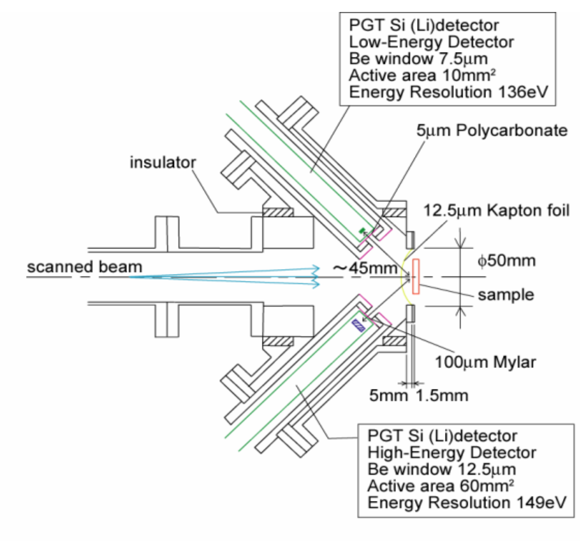

FIGURE 2. Cross section view of beam exit assembly.

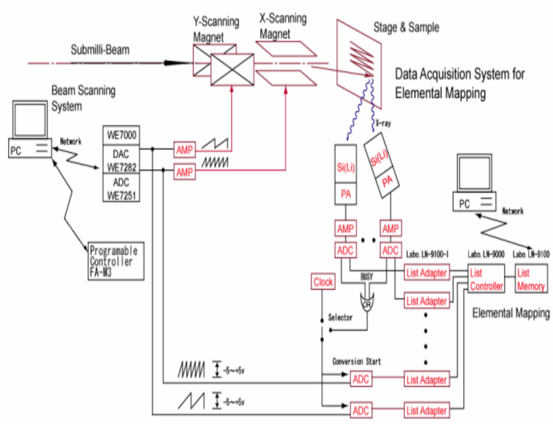

FIGURE 3. Schematic diagram of data acquisition system for elemental imaging.

PERFORMANCE OF PIXE CAMERA

The proton beams were first focused at the end of the submilli-beam line using two quadrupole double lenses with opening the slits maximally, and then the four-pole slit at upstream side was closed to 0.5mm by monitoring the beam spot size at both X and Y axes. Finally, the wedge-shape slit at downstream side was adjusted. A beam spot size was measured in air by scanning beams across a 1.5mm wide tungsten mesh. The distance from the beam exit window and the mesh was 5mm. The FWHM of beam spot was obtained by differentiating the spectra derived from the cross section of the spatial distribution of characteristic X-ray yields form the tungsten mesh, as shown in Fig. 3. Beam spot size was 0.65mm (X) × 0.54mm (Y) and the intensity of the beam halo was ~1/200 fraction of the beam center for average beam currents of 1 nA.

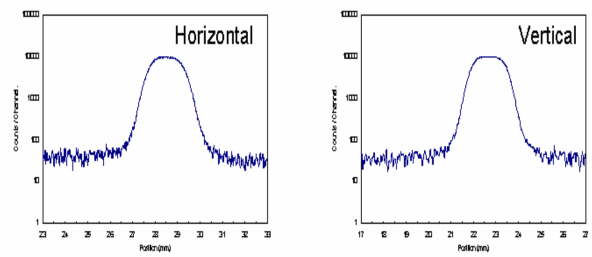

FIGURE 4. X-ray yield-curve of the 1.5mm W-mesh.

ELEMENTAL MAPPING IN ARSENIC HYPERACCUMULATOR PLANT

Arsenic contamination of soils and groundwater from various sources such as mine and urban wastes, wood preservatives and pesticides is of great environmental problem. Phytoremediation using an arsenic hyperaccumu-lator, *Pteris vittata* L., has generated increasing interest worldwide, for its both environmentally sound and cost effectiveness [7]. Recently, Poynton *et al.* showed that arsenate influx into roots of hyper-accumulating ferns is greater than in non-hyper-accumulating ferns and speculated that the phosphate transporting protein has a responsibility for the influx [8]. However, the mechanisms of arsenic uptake and accumulation by this plant are not clear at this time.

In this study, *P. vittata* L. was grown on the soil contaminated with arsenic used as a raw material of agricultural chemicals for 3 months in a growth chamber with a 16 hours light period, 25 ^{0}C/20 ^{0}C day/night temperature and 70 % relative humidity. A part of lamia of *P. vittata* was gathered at three different growth stages; an active growth stage, a mature stage without arsenic harm and the eldest stage with discoloration by arsenic harm. Plant samples were fixed directly to a target frame with dipping the stalks into tap water. Irradiation was carried out with 3 MeV proton beams; beam currents of ~200 pA, total accumulated charges of 0.4~0.7 μC and scanning area of 10 × 13mm^2. X-rays from plant samples passed through the exit window of 12.5 μm thick Kapton and were measured with two Si(Li) detectors which viewed the targets in a geometry shown in Fig.2. Mylar absorber of 300 μm thickness was set in front of the detector for higher energy X-rays while no absorber was used for the detector for lower energy X-rays. This resulted in a decrease of the dead time of signal processing. Quantitative PIXE analysis was performed using the GeoPIXEII software [9].

Figure 5 shows the elemental distributions in fronds of *P. vittata* at three growth stages. The numerical

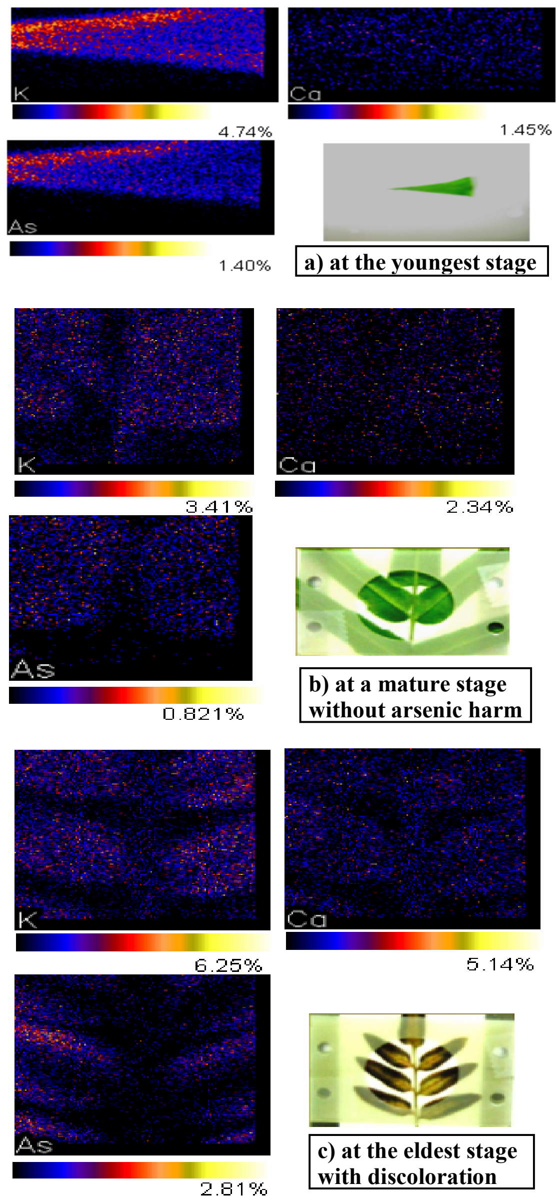

FIGURE 5. Photograph and elemental maps of lamias of *P. vittata* at three growth stages.

value in the color bar shows the maximum weight percent of a selected element in the plant of which composition is assumed to be 80 % H_2O + 20 % $C_4H_6O_3$. In the case of *P. vittata* at the eldest stage with arsenic harm, arsenic and essential elements of plants such as potassium and calcium are detected in a similar concentration level. However, arsenic concentrated especially in the near edge of fronds which corresponds to the discoloration part of the lamina, but arsenic occurs at a low level in the vein. Calcium is uniformly distributed in both the vein and the fronds, but potassium disappears to some extent from the withering part of the fronds with arsenic accumulation. In the case of younger *P. vittata* laminas, these elements are distributed rather uniformly in a whole part of the lamina including the vein, excluding that arsenic and potassium concentrate in some degree into the tip of the fronds at the youngest stage.

These results leads to the speculation that *P. vittata* acquires tolerance for arsenic by accumulating it in the edge of the lamina in stead of redistributing arsenic in the fronds without arsenic accumulation. Hence, the cm-scale area mapping of elements by the subumilli-PIXE camera reveals arsenic redistribution in the fronds of *P. vittata* at different growth stages.

For the eldest growth stage of *P. vittata*, the amount of arsenic accumulated in the fronds was chemically analyzed 12000 mg/kg. This concentration was much higher than the contaminated level of 600 mg/kg for the soil used in cultivating *P. vittata* plants. So, *P. vittata* can be potentially utilized to cleanup soil that is contaminated by arsenic originated from agricultural chemicals or drain of factories and smelting works.

The submilli-PIXE camera enables to map the distribution of a harmful element and to detail the location in a living plant without laborious pretreatments. Therefore, PIXE analysis by the subumilli-PIXE camera is an effective tool for undertaking phytoremediation research.

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