## Acute Lung injury of mouse Caused by PM<sub>2.5</sub> Aerosols studied by

## Synchrotron Microradiograph

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#### Abstract

Six solutions contained  $PM_{2.5}$  aerosol particles,  $FeSO_4$ ,  $ZnSO_4$  and their mixtures were used to be instilled into mouse lungs. By two days after instillation, the live mice were checked in vivo by synchrotron refractive-index microradiography at Pohang Light Source. In addition after extracted and examined by dissection, the right lobes of lung were fixed by formalin, then imaged by synchrotron microradiography again. Corresponding parts of those lung tissues were embedded in paraffin for histopathologic study. The synchrotron x-ray microradiographs of live mouse lung showed different lung structure after instilled different toxic solutions. Hemorrhage points and texture changes in lung were observed for the mice instilled by toxin solutions. The synchrotron x-ray microradiographs and the histopathological study of fixed tissues showed consistent results. By compared with the conventional x-ray radiography of mouse lung the synchrotron x-ray microradiography showed much high resolution. It was found that the acute lung injury of mice caused by solution of  $PM_{2.5}+FeSO_4+ZnSO_4$  was more serious than other toxin solutions and the composition of bioavailable metals played a major role in the toxicity of aerosol particles The synchrotron refractive-index microradiography may be further developed to observe the pneumonia, lung cancer and other disease at early stage in future.

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# Characterization of pH-responsive vinyl polymer/silica colloidal nanocomposite particles in the wet state by soft x-ray spectromicroscopy

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Polymer-silica colloidal nanocomposites were prepared by free-radical copolymerization of 4vinylpyridine (4VP) monomer and ethylene glycol dimethacrylate (EGDMA) in the presence of 20 nm silica sols in aqueous media. These nanocomposite particles swell and acquire microgel character at low pH due to protonation of the 4VP residues, as confirmed by dynamic light scattering (DLS). For example, the intensity-average particle diameter was 230 nm at pH 8.8 but increased to 550 nm at pH 2.5. This pH-responsive behavior is critical for the use of these nanocomposite particles as 'Pickering' emulsifiers [1].

In this work scanning transmission X-ray microscopy (STXM) studies at energies near the C-1s, N-1s, and O-1s absorption edges using the beamline 5.3.2 at Advanced Light Source provide direct experimental evidence that the nanocomposite particles swell at low pH. We conducted the STXM measurement in a wet cell between two silicon nitride membranes (the cell thickness is less than 1 micron). Nanocomposite particles were dispersed in water at between pH 2 and pH 10 adjusted by adding HCl and NaOH. The observed particle sizes at both low pH and high pH correspond well to the values determined by DLS. The acid-base interaction between the silica sol and the pyridine was studied using nitrogen 1s NEXAFS spectroscopy [2].



**Figure 1**: Left and right images show the STXM optical density images at N1s absorption edge energy, at pH 2.3 and pH 8.2, respectively. Image size:  $(5 \mu m)^2$ 

#### References

- [1] Fujii, S., Read, E. S., Binks, B. P., Armes, S. P., Adv. Mater., in the press 2005 (and refs therein).
- [2] Gyan K. Agarwal et al., J. Phys. CHem. B, 107, 12497 (2003)

## Cadmium Distribution in a Cadmium Hyperaccumulator Plant by micro-XRF imaging

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Cadmium is well-known as one of the most toxic heavy elements for animals. Some specific kinds of plants can grow in the contaminated soils and absorb a large amount of Cd in their bodies. Such hyperaccumulator plants are expected to be used for remediation of environments. However, the accumulation mechanism has not been revealed yet. One of the solutions to be solved is nondestructive two dimensional analysis of trace cadmium in the plant tissues. In the present study, we applied high-energy micro-XRF system to investigate the accumulation mechanism of Cd in cellular level.

*Brassicae Arabis gemmifera* [1] cultivated with culture medium containing Cd was subjected to the analysis. The micro-SR-XRF imaging was carried out at BL37XU of SPring-8, JASRI. Monochromatic X-ray microbeam of 37 keV was produced by Fresnel zone plate [2] or Kirkpatrick-Baez mirror. The beam size was ca. 1.5x1.5 or  $3x3 \ \mu\text{m}^2$ . The Cd imaging of the plant tissue was obtained by detection of Cd K $\alpha$ .

The concentration of Cd in the leaves was ca. 500  $\mu$ g g<sup>-1</sup> dry weights when they were cultivated on 5  $\mu$ g ml<sup>-1</sup> of culture medium for several days. The distribution of Cd in the leaves was revealed very clearly. It was found that Cd highly accumulated in trichomes, which are epidermal outgrowth cells protruding from the surface of leaves. The high-energy micro-XRF imaging has given useful information as to the distribution of Cd in sub-cellular levels for understanding of the accumulation mechanism.

[1] H. Kubota, C. Takenaka, Int. J. Phytoremediation, 5, 197 (2003).
[2] M. Awaji et al., Rev. Sci. Inst., 74, 4948 (2003).

## Characterization of individual aerosol particles using an x-ray microprobe

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The X-ray microprobe installed to the BL37XU in the SPring-8 has the capability to analyze trace elements less than 1 fg [1]. The microprobe system has been utilized for characterizing inorganic components of individual aerosol particles, and the results are being utilized to categorize particles according to their origin and the chemical transformation during the transportation [2]. Though the characterization of the particles is successfully carried out, the possibilities of the aggregation between the particles of different phases make the identification of their origin difficult. One of the practical ways to overcome the difficulty is to measure the x-ray diffraction (XRD) from the same region, and we have developed an experimental setup for micro XRD measurements.

In this presentation the setup of the microanalysis, principles of elemental quantification using x-ray fluorescence analysis and the results of applications will be discussed.

1) S. Hayakawa et al., Anal. Sci. 17s, i115 (2001).

2) C.-J. Ma et al., Atmospheric Environment 38, 1133(2004).

## Arsenic Distribution and Speciation in the Arsenic Hyperaccumulator Fern by micro-XRF imaging and micro-XANES analysis

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Some specific kinds of plants are known as the hyperaccumulator for heavy metals. It was reported that, for example, Chinese brake fern (*Pteris vittata* L.) contained large amounts of arsenic (As: *ca.* 22,000  $\mu$ g g<sup>-1</sup> dry weight) when they grew in the contaminated soils [1]. It is quite interesting to know how they efficiently extract such a toxic heavy element from the contaminated soil into its fronds. Studies on the distribution and chemical form of As are increasing recently, however only limited information is available as to the chemical behavior of arsenic in the fern in relation to the function of the plant tissue and organs. In the present study, microbeam synchrotron-radiation X-ray fluorescence ( $\mu$ -SR-XRF) analysis was applied to the hyperaccumulator for As in order to reveal the distribution of toxic heavy elements in their tissues and cells and to investigate their physiology and accumulation mechanism.

The Chinese brake fern cultivated in a culture medium containing As was subjected to the analysis. The samples were prepared by microtome as a slice of tissues. The  $\mu$ -SR-XRF imaging was carried out at BL37XU of SPring-8, JASRI and at BL4A of PF. Monochromatic X-ray microbeam (beam size  $< 2 \times 5 \ \mu m^2$ ) was produced by Fresnel zone plates[2] or K-B mirror. The chemical form of As in plant tissue was investigated by micro-XANES analysis of As K-edge.

The two-dimensional distributions of As and some trace elements in the plant tissue and cells were clarified. It was demonstrated that As accumulated in the vascular tissue and the neighboring tissue of spore. The results from micro-XANES of fern tissue showed that As(V) in the culture medium was reduced to As(III) in the tissue after absorption.

[2] Y. Suzuki et al., Jpn. J. Appl. Phys., 40, 1508 (2001).

<sup>[1]</sup> L.Q. Ma, K.M. Komar, C. Tu, W. Zhang, and Y Cai, *Nature*, **409**, 579 (2001)