Transfer characterization of sulfur from coal-burning emission to plant leaves by PIXE and XANES *

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Abstract   The impact of coal-burning emission on sulfur in camphor leaves was investigated using Proton Induced X-ray Emission (PIXE) and synchrotron radiation technique X-ray Absorption Near-Edge Structure (XANES) spectroscopy. The PIXE results show that the sulfur concentrations in the leaves collected at the polluted site are significantly higher than those in controls. The Sulfur XANES spectra show the presence of organic (disulfides, thiols, thioethers, sulfonates and sulfoxides) and inorganic sulfur (sulfates) in the leaves. The inorganic sulfur in the leaves of camphor tree polluted by coal combustion is 15% more than that of the control site. The results suggest that the long-term coal-burning pollution resulted in an enhanced content of the total sulfur and sulfate in the leaves, and the uptake of sulfur by leaves had exceeded the metabolic requirement of plants and the excess of sulfur was stored as SO_4^{2-}. It can monitor the sulfur pollution in atmosphere.

Key words   sulfur, PIXE, XANES, atmospheric particulate patter, SO_2, leaf

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1   Introduction

Emission from coal combustion is the major source of SO_2 and airborne particulate matter (PM) in China due to huge amount of coal consumption. PM and SO_2 are always the top pollutants in many cities of China[1]. Studies show that SO_2 and PM have profound influence on acid deposition, climate change, aquatic and terrestrial ecosystems[2, 3]. Trees can improve the quality of urban air and reduce the effects of particulate matter pollution[4]. Trees also can act as biological filters because of large leaf areas and the physical properties of their surfaces, trapping and absorbing air pollutants. Research has showed that some plant leaves have the capability of absorbing and accumulating sulfur dioxide, and the amount of sulfur contained in plant is closely related to the concentration of SO_2 in the air. Sulfur content in leaves of plants from SO_2 polluted areas is higher than that from clear air areas[5—7].

Sulfur is an element of particular environmental and biochemical importance. It is essential for plants with vital functions in proteins and enzymes. It is ubiquitous with a large variety of species and valence states, from -II in sulfides to +VI in sulfates[8]. Although many studies focused on the effects of SO_2 on plant, such as Cicek studied the accumulation of sulfur in soil and tree leaves sampled from the surrounding of a thermal power plant[9], in which the total sulfur concentration in environmental samples by using wet chemical techniques; however, research on the speciation of sulfur was scarce as analytical methods were complicated. Sensitive analytical methods are needed to analyze and monitor the functions and

transformations of sulfur species in biochemical reactions and environment. Among analytical techniques, synchrotron radiation X-ray Absorption Near-Edge Structure (XANES) spectroscopy appears particularly suitable for the characterization of the chemical speciation of sulfur in plants. As XANES analysis does not require digestion and separation of sulfur species from samples before analysis, it has little risk of converting the forms of sulfur through redox reactions. In the past ten years, sulfur K-edge XANES spectroscopy has been successfully used to identify the characteristic sulfur forms in environmental samples[8]. The Proton Induced X-ray Emission (PIXE) is a multi-elemental, nondestructive, rapid, and sensitive method, and has been widely used for the analysis of environmental samples. In this study, we combined PIXE and sulfur K-edge XANES to determine the sulfur form and content in the leaves of camphor trees polluted by a coal-burning boiler and control site to monitor the sulfur metabolism in urban trees and evaluate the impacts of coal combustion pollution on plants.

2   Materials and methods

2.1   Sampling and sample preparation

The evergreen *Cinnamomum camphora* (L.) Presl (commonly known as camphor tree) is a native tree in China. These are very popular on the roadsides，in the parks and gardens in Shanghai. The leaves have a glossy, waxy appearance and camphor smell. Three sites of different type were selected: one is about 3 m away from a 600 kg/h coal-burning boiler (Site1). The coal consumption of this boiler is about 5 tons of anthracite coal monthly. Emission of SO_2 and PM from the boiler is serious due to the lack of effective pollution control process. It is estimated that about 60 kg SO_2 and 10—20 kg dust are released to the air when one ton of coal is burned based on the effluent concentration of flue dust and sulfur dioxide of this kind of boiler and sulfur content in feed coal. The contents and speciation of sulfur in coal, fly ash and $PM_{2.5}$ are determined by SRXRF and XANES as described elsewhere[10]. In order to know that the camphor trees are subjected to SO_2 and particulate matter, the other two sampling sites are chosen. The second site (Site 2) is about 20 m away from the boiler and the last one is on the campus of the Shanghai Institute of Applied Physics, the Chinese Academy of Sciences (SINAP, CAS) as a control site (Site 3).

The representative and homogeneous samples of 40 mature leaves (about 11 months old) were collected at the height of 3—6 m from the canopy of at least 20-year old trees at each site and taken to the laboratory in plastic bags. Soil samples were collected from each site at 0—5 cm depth (top), 5—20 cm depth (bottom). The leaves were washed repeatedly to clean off the surface contaminants such as dust and fly ash by using tap water, and then rinsed with de-ionized water several times and placed in ultrasonic wash for 20 minutes. The leaf and soil samples were dried at 65℃ in an oven until constant weight was achieved, and then ground into fine powder by an agate mortar and passed through an 80-mesh sieve. About 1g of powdered sample was weighed out and pressed at 14-ton oil press machine for 120 seconds to obtain a cylindrical pellet in a diameter of 10 mm. For each sample three powdered pellets were pressed and sulfur analysis were determined by PIXE and XANES.

2.2   PIXE analysis and S K-edge XANES Spectroscopy

The analysis of sulfur content was carried out using PIXE at SINAP, CAS, installed in the $-45°$ line at the 4 MU pelletron accelerator. The 3.0 MeV proton beam of 6 mm diameter was used for PIXE measurements. The typical beam currents ranged between 10 and 20 nA and the average measuring time per sample was 30 minutes. A Si(Li) detector with an energy resolution of 180 eV at 5.9 keV and an active area of 32 mm^2 was positioned at 135° relative to the beam incidence to detect the characteristic X-rays from elements, and the sample surface was perpendicular to the beam direction. In this work the relative method was used for the determination of the contents of sulfur. Three standard reference materials of bush branches and leaves (GBW07603), poplar leaves (GBW07604), and soil (GBW07405) from the National Research Centre for Certified Reference Materials, Beijing, China, were used as external reference samples for quantitative analysis. The reference standards were in the form of fine powder and also pressed into pellets for PIXE analysis. The experimental PIXE spectra were fitted by using iterative least square (AXIL) code[11].

XANES experiments were conducted on Beamline 4B7A of the Beijing Synchronization Radiation Facility (BSRF). The electron energy was 2.5 GeV and the beam current was 80—180 mA. A Si (111) double-crystal monochromator was used to vary the X-ray energy from 2420 eV to 2570 eV. The energy resolution of the spectrometer was better than 0.3 eV. The X-ray energy was calibrated by elemental sulfur, the white-line peak positions in XANES spec-

tra of elemental sulfur were set at 2472 eV to define the zero points of energy. The X-ray beam was maintained in vacuum from the synchrotron ring to the experimental hutch in order to minimize the absorption of soft X-rays by air. The sulfur K-edge XANES spectra were collected as fluorescence mode using a Si(Li) detector. Samples of standard compounds in various oxidation states such as pyrite (inorganic sulfide), elemental sulfur, glutathione oxided (disulfide: R-S-S-R'), glutathione reduced (thiol:R-SH), dibenzothiophen (DBT), sulfoxide (R-S(O)-R'), sulfone, $Fe_2(SO4)_3$, were prepared respectively by mixing them thoroughly with boron nitride (1 wt% sulfur) and then pressing the mixtures into pellets. The purpose of mixing the standard compounds with boron nitride was to reduce the self-absorption effect that commonly happens in a solid sample with higher concentration. It was reported that self-absorption is not a problem for a thin sample (30 μm) if the sulfur weight concentration is less than 5%[12, 13]. The self-absorption effect was corrected using the self-absorption routine of IFEFFIT package[14]. In this work, the impacts of self-absorption in thick samples on sulfur quantitative analysis can be neglected after self-absorption correction.
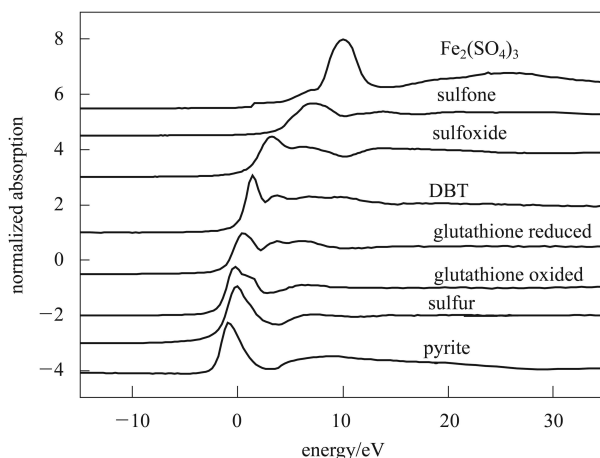

Fig. 1.   The normalized sulfur K-edge XANES spectra for sulfur standard compounds.

The K-edge absorption energy of a sulfur atom shows a strong correlation with the oxidation state; the energy shift of XANES is about 13.0 eV from S^{-2} to S^{+6} as shown in Fig. 1. The existence of the correlation between oxidation state, intensity and peak position of K-edge energy makes possible to analyze the sulfur speciation. In this paper we used the least square fitting method developed by Huffman and Xia to quantitatively determine the composition of sulfur species[12, 15, 16]. The method is based on the assumption that the experimental spectrum can be modeled

as the sum of one or two arctangent functions and a number of Gaussian functions. The relative concentration of each sulfur species was determined from the area under the respective Gaussian peak relative to the total area under the several Gaussian peaks using the Peak Fitting Analysis routine of IFEFFIT package[14]. Several assumptions were made in the fitting process: (1) The arctangent step functions represent the transition of ejected photoelectrons to the continuum; (2) The Gaussian functions represent 1s to $3p$ transitions; (3) The full width at half-maximum (FWHM) of each Gaussian function is constrained depending on their intensity and resolution. For low-valent sulfur (less than or equal to 4) FWHM is less than 0.7 eV, for high-valent (+5,+6) is between 0.7—1.0 eV; and (4) The speciation of sulfur was identified by the energy position of the Gaussian peak and the relative abundance of each sulfur was determined from the peak area. The areas were corrected for the change in absorption cross section with increasing oxidation state[13]. The error for the estimation of the percentage of each sulfur group is 5%[16].

3   Results and discussion

3.1   The total concentration of sulfur determined by PIXE

Figure 2 shows the typical PIXE spectrum of the camphor leaves from the coal-burning boiler site illustrating characteristic features. The S, K, Ca, Fe, Zn, and Sr peaks can be clearly observed. The concentrations of each element of samples were obtained by comparing with the standard reference materials as external reference samples. The results of sulfur in the leaves, soil, coal, fly ash and PM are summarized in Table 1. As revealed by the mean and standard deviation values, sulfur concentration in the same deep
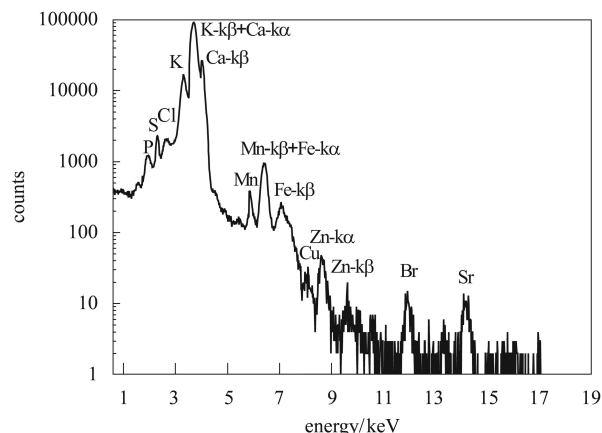

Fig. 2.   PIXE spectrum for sample of leaves collected from Site1.

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soil stays in the same level of order for three sites, except in the upper layer at Site 1, which sulfur concentration is about one time higher than others due to coal-burning boiler. The leaf concentration of sulfur at Site 1 is about two times that of the control site. The sulfur contents of coal, fly ash and $PM_{2.5}$ were significantly higher than the sulfur content of soil.

Table 1. Sulfur concentrations in leaves, soil, coal, fly ash and $PM_{2.5}$. (mg/kg in dry weight, mean value ±SD).

Sulfur in Plants		Sulfur in Soil	
		Surface	deep
Site 1	4870±429	405±241	238±65
Site 2	3191±382	165±50	222±84
Site 3	2487±320	151±41	276±115
Fly ash		2205±990	
$PM_{2.5}$		34858±5023	
Coal		19000±2000	

SD: standard deviations. Mean value from three determinations. Site 1: 3-m away from the boiler; Site 2: 20-m away from the boiler; Site 3: the control site.

3.2 Speciation of sulfur by XANES

Figure 3 is the normalized sulfur K-edge XANES spectra of leaf samples. Three major absorption peaks at 2472.7, 2475.7, and 2481.4 eV are observed in the spectra for leaves. By comparing the relative energy shift to the standard samples and published experimental results[11, 15, 16], the species of sulfur could be determined. The first strong peak at 2472.7 (whiteline) is the typical energy position for C-S and S-H bonds of the sulfur containing amino acids cysteine and methionine, which are characteristic for thiols (R-SH) and thioethers (R-S-R'). The peak at 2475.7 eV is the characteristic of sulfoxides (R-S(O)-R') and the peak at 2481.4 eV of sulfate, which are present within the majority of plants[17]. The normalized XANES spectra show similar distributions of the major sulfur species in the leaves, only with slightly different ratios.

The sulfur XANES spectra were fitted as discussed above to derive quantitative information. The deconvolution of sulfur spectra is shown in Fig. 4. Different sulfur species produce multiple peaks indicative of different oxidation states of the component sulfur atoms. The energy positions of these peaks are used to assign sulfur forms and the areas of the peaks represent the abundance of the different species of sulfur compounds. The fitting results indicating the major chemical forms of sulfur in the leaves are disulfides (R-S-S-R'), thiols (-SH)/thioethers (R-S-R'), sulfoxides (R-S(O)-R'), sulfonates (R-SO_3^-) and sulfates (SO_4^{2-}). The results correspond to the literature[8],

in which the sulfur XANES spectra for intact plant leaves were fitted to linear combination of standard reference XANES spectra of sulfur compounds by the least square method. Table 2 summarizes the relative percentage of each sulfur species in all samples.
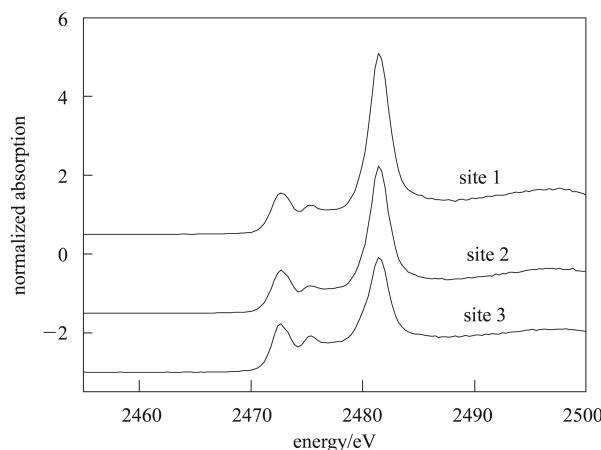

Fig. 3. The normalized K-edge XANES spectra for leaves collected from three sites.
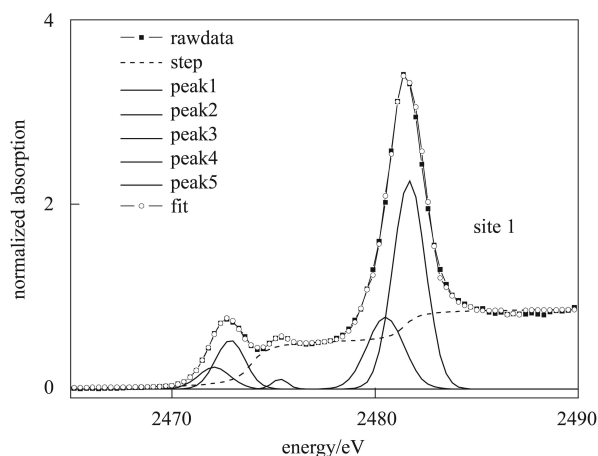

Fig. 4. The least square fits sulfur K-edge XANES spectrum for the leaf sample collected from Site 1.

The contents of major sulfur oxidation groups are grouped into organic sulfur (sum of disulfides, thiols/thioethers, sulfoxides, sulfonates), and inorganic sulfur (sulfates) as shown by the bar diagram in Fig. 5. The total amount of the organic sulfur forms in the three kinds of leaves are 54, 59, 69 atom% sulfur, respectively, and the inorganic forms are 46, 41 and 31%, respectively. The inorganic sulfur form in the leaves of camphor pollution by coal combustion is 15% more than that of the control site.

Sulfur is essential for the plant growth with vital functions in proteins and enzyme as a major element. Generally, sulfate taken up by the root is the main source of sulfur for plant. But the leaves deposited atmospheric sulfur from SO_2 and particulate matter can also be used as the sulfur source if the sulfate supply

to the roots from the soil is limited, especially in the case of high SO_2 concentration due to air pollution. SO_2 and particulate matter in a size of 1 μm or less can be taken up via the stomates. Although particles with a diameter large than 1 micrometer can not pass into the stomates, particles can be absorbed on the surface of the leaves and infiltrate into the biological tissue if they are dissolved. Fly ash and fine particles were enriched sulfur and major sulfur was presented as sulfate, with the relative concentrations of 79% and 94%, respectively[10]. The sulfur concentration in the leaves and soil reflects sulfur pollution and deposition which is caused by the coal-burning boiler

emission. The total sulfur concentrations in leaves varied significantly among three sites, the sulfur contents increased from control to polluted leaf sample, and the maximum value was observed at Site1. The sulfur concentrations of the deep soil of the three sites were in close values, so there were no significant differences in sulfur uptakes from soil for the control site and the polluted site. As camphor roots are in deep soil, the sulfur absorption differences from the upper layer soil can be ignored. According to the above discussion, the increase of sulfur concentration of leaves can ascribe to SO_2 and PM pollution from coal-burning boiler

Table 2. Results from the least-square fitting of XANES spectra for the camphor leaf samples. (atom% S).

	disulfides	thiols/thioethers	sulfoxides	sulfonates	sulfates
Site 1	12	21	2	19	46
Site 2	14	26	2	17	41
Site 3	16	32	4	17	31

Sulfur is absorbed and accumulated in the leaves, after a series of biochemical reactions; its metabolism has impact on plant growth. The leaves of camphor close to the boiler had morphological deformations as color change and had brownish or black leaf spots. This shows that the harmfulness of SO_2 pollution causes leaf spot disease. The spots varied in size and were most brownish or black. The plants in the control site had little leaf spot. This morphological difference reflects the deleterious effect of the deposited sulfur on leaf surfaces.
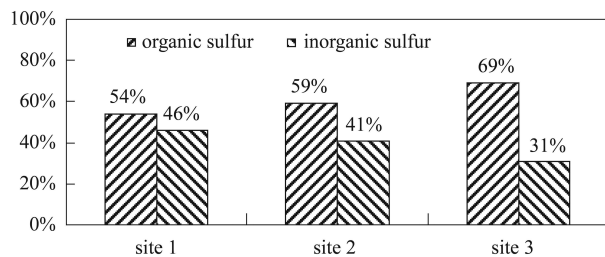

Fig. 5. Sulfur percentages derived from the XANES.

Sulfur in plants is present in both organic and inorganic forms[18]. Organic sulfur is a major component of plant protein composed of sulfur amino acids such as methionine, cystine, and cysteine. Inorganic sulfur is present in SO_4^{2-} form stored at vacuole, which contents depended on the level of sulfur supply. Sulfate can enter the sulfur assimilatory pathway

and is utilized for organic sulfur compound synthesis as sulfur nutrient, but if it is at levels exceeding the sulfur requirement for growth, the redundant sulfate will be stored into the vacuoles. Therefore, organic sulfur content represents the assimilation of sulfur, and the inorganic sulfur represents the accumulation of sulfur in the leaves. In the present study, an increase in the total sulfur and SO_4^{2-} content suggests that the uptake of SO_2 and PM by leaves had exceeded the metabolic demand of plants for organic sulfur compound synthesis and hence the excess of sulfur was stored as SO_4^{2-}.

4 Conclusion

In this study, the camphor leaves and soil samples in the vicinity of a coal-burning boiler and control site were monitored by PIXE and XANES to evaluate the influence of coal combustion emission on plants. It demonstrates that coal combustion causes SO_2 and particulate matter pollution, and the combined effects of SO_2 and particulate pollution resulted in the accumulation of the total sulfur and sulfate in the leaves of camphor trees. Therefore, the SO_2 and particulate matter pollution can be monitored by the sulfur contents in leaves. Moreover it may remind people to find a plant to be used to remove SO_2 and particulate matter to improve air quality.

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