

## SULFUR SPECIATION IN INTACT PLANT LEAVES BY XANES SPECTROSCOPY

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Synchrotron-based X-ray absorption near edge structure (XANES) spectroscopy is a technique which can be used to identify characteristic sulfur groups in natural samples (Rompel 1998; Frank *et al.* 1999; Yu *et al.* 2001). The K-edge absorption energy of a sulfur atom (exciting a 1s core electron), shows a strong correlation with the oxidation state, with a large chemical shift ( $\sim 14$  eV) from the oxidation state -2 to +6 (Pickering *et al.* 1998; Vairavamurthy 1998). Pre-edge excitations to unoccupied valence states are sensitive to the arrangement and type of atoms surrounding the sulfur atom, and provide characteristic shapes of the X-ray absorption features in the spectrum. Therefore, sulfur XANES spectroscopy offers a unique non-destructive tool to identify, and with appropriate data analysis, to determine *in situ* the relative amounts of functional sulfur species down to low concentrations, about 0.05 mass % S (500 ppm). This method of analysis is in many ways superior to classic wet laboratory methods, which require digestion/separation of each sulfur species from a large amount of sample before quantitative analysis, with clear risk of converting the functional groups through redox reactions. Recently, we used sulfur XANES spectroscopy to discover an unexpected accumulation of reduced sulfur compounds causing severe problems with acidic precipitates on the marine archaeological wood of the 17<sup>th</sup> century Swedish warship *Vasa* (Sandström *et al.* 2002).

To identify the functional sulfur groups of the major metabolites in intact plant leaves from trees in urban areas, leaves were collected during fall and winter from six common species: (a) Southern Magnolia (*Magnolia grandiflora*), (b) Monterey Pine (*Pinus radiata*), (c) Giant Sequoia (*Sequoiadendron giganteum*), (d) California Juniper (*Juniperus Californica*), (e) California Redwood (*Sequoia sempervirens*) and (f) Bluegum Eucalyptus (*Eucalyptus globulus*) near the Stanford campus (a, b, d and f) and the Golden Gate Park (c, e) in California, US. Sulfur K-edge XANES spectra were measured of intact leaves without any pre-treatment in atmospheric pressure of helium, in fluorescence mode at beam line 6-2 of the Stanford Synchrotron Radiation Laboratory (SSRL) (Hedman *et al.* 1986). For each sample 8-10 scans were collected during about 2 hours of exposure to X-rays. The scans overlapped, indicating that no significant chemical transformations were induced by the radiation during the measurements. The EXAFSPAK software package was used for energy calibration of the scans by setting the maximum of the first XANES peak of sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) to 2472.02 eV. The averaged and normalized XANES spectra are shown in Fig. 1.

The spectral features show similar distributions of the major sulfur species, only with slightly different ratios. The peaks at 2473, 2476 and 2482 eV are characteristic for thiols (R-SH), sulfoxides (R-S(O)-R') and sulfates, respectively. To obtain the relative ratios (S in

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atom%), a theoretical model, comprising a sum of standard XANES spectra of sulfur compounds with different functional groups, was fitted by least-squares methods to the spectrum of the sample. The standards used were aqueous solutions (pH 7.0) of cysteine, cystine, methionine, methionine sulfoxide, methyl sulfonate, sulfate and *myo*-inositol hexasulfate (a sulfate ester,  $R-O-SO_3^-$ ).

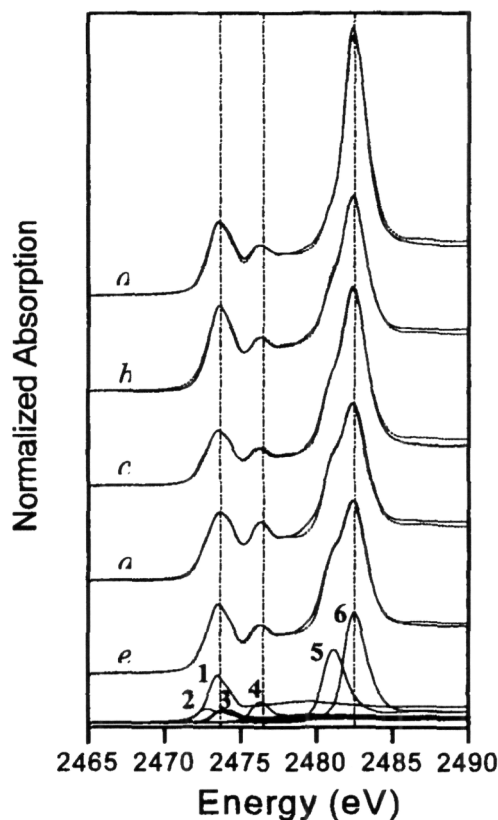


Fig. 1. Sulfur K-edge XANES spectra of intact plant leaves (—) from trees in the urban Stanford area (California, US): (a) Southern Magnolia, (b) Monterey Pine, (c) Giant Sequoia, (d) California Juniper and (e) California Redwood. The standard XANES spectral components (bottom) in the least squares model fitting (....) are: cysteine (1), cystine (2), methionine (3), methionine sulfoxide (4), methyl sulfonate (5) and sulfate (6) in aqueous solution (see Table 1).

The fitting indicated that the major chemical forms of sulfur in the leaves were thiols ( $-SH$ ) and thioethers ( $R-S-R'$ ), disulfides ( $R-S-S-R'$ ), sulfoxides ( $R-S(O)-R'$ ), sulfonates ( $R-SO_3^-$ ) and sulfate (Fig. 1, bottom). The total amount of the reduced sulfur forms (thiol + disulfide + thioether) was obtained as about 44-55 atom % S, and the oxidized forms (sulfonate + sulfate) between 38-50 atom% S (Table 1). About 6-10 atom % S was found to be present as sulfoxide. The peak shapes and energies in the standard sulfur K-edge XANES spectra of cysteine and methionine are very similar and thus strongly correlated in the fitting. Therefore, thiols and thioethers have been combined in Table 1. However, the

fitting improved in most cases by including the standard methionine in the model. Only for Redwood and Sequoia, could this component (~5-7 S atom %) be excluded without significant effect, by a corresponding increase in the amount of thiols.

*Table 1.* The relative amount (atom% S) of each characteristic sulfur group by least squares fitting of sulfur XANES spectra for samples of intact leaves from: (a) Southern Magnolia, (b) Monterey Pine, (c) Giant Sequoia, (d) California Juniper, (e) California Redwood and (f) Bluegum Eucalyptus (wiped) collected from Stanford area and Golden Gate Park (California, US). Total sulfur was measured using the ICP method.

	a	b	c	d	e	f
R-SH + R-S-R'	24 + 17	26 + 18	28 + 5	15 + 17	33 + 7	32 + 6
R-S-S-R'	7	11	11	17	11	21
R-S(O)-R'	6	6	6	10	7	12
R-SO <sub>3</sub> <sup>-</sup>	9	15	20	20	20	12
SO <sub>4</sub> <sup>2-</sup>	37	23	30	21	22	5
R-O-SO <sub>3</sub> <sup>-</sup>						12
Total S (mass %)	0.21	0.22	0.11	0.12	0.19	0.13

The accuracy in determining the relative amounts of the overlapping reduced sulfur forms is usually lower than for the oxidized sulfur forms. One reason is that the peak intensity for sulfate (SO<sub>4</sub><sup>2-</sup>) is almost three times that of elemental sulfur S<sub>8</sub> for similar concentration, because of the larger number of available unoccupied molecular orbitals of 3p character. When comparing different models in the least-square fitting, e.g. adding/removing one component or replacing cysteine with another similar standard such as glutathione, variations of 1-3 atom% S occurred in the oxidized forms (sulfoxide, sulfonate and sulfate) and 5-7 S atom% in the reduced forms (thiol + thioether and disulfide). Similar variations also occur between samples taken from the same tree.

An interesting observation was that wiping the surface of Bluegum Eucalyptus leaves with a moist paper tissue caused a significant decrease of the oxidized forms in the 2480-2485 eV region of the XANES spectrum (Fig. 2), indicating removal of sulfate. Similar effects for pine and spruce needles were observed using XANES and scanning X-ray microscopy (unpublished results). Moreover, to obtain a satisfactory fit for the split peak in this region, *myo*-inositol hexasulfate (a sulfate ester) had to be introduced as one of the standards in the model. As shown in Fig. 2, the XANES spectrum of *myo*-inositol hexasulfate (7) resembles a combination of the sulfonate (5) and sulfate (6) standard spectra. Hence, it is possible that part of the sulfonate and sulfate of the samples a – e (Table 1) is in fact a sulfate ester. However, for the wiped Eucalyptus leaves, the sulfate ester *myo*-inositol hexasulfate was necessary for a satisfactory model.

While sulfur K-edge XANES spectroscopy allows non-destructive *in situ* analysis of the relative amount of major sulfur functional groups, such as thiols, disulfides, sulfoxides, sulfonates and sulfates in intact samples of leaves, the major limitations of this technique are: 1) Compounds with similar functional groups, such as cysteine and glutathione, cannot be distinguished; 2) Proper standard compounds must be chosen with functional groups in a

surrounding similar to that in the sample; there are still difficulties in distinguishing between e.g. thiols and thioethers, or sulfate esters and combinations of sulfonates and sulfates; 3) The limitations of beam time allocated at the synchrotron facilities make satisfactory statistics difficult to obtain.

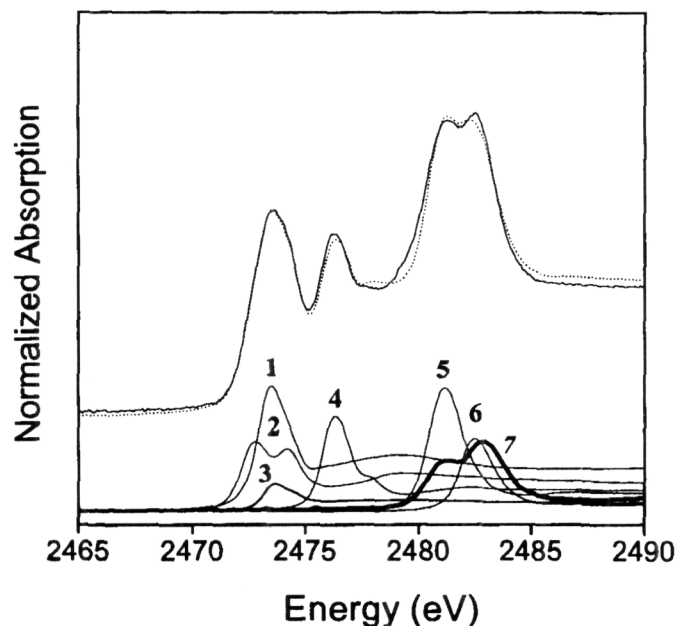


Fig. 2. Sulfur K-edge XANES spectrum of wiped Bluegum Eucalyptus leaves (upper trace, —) from Stanford University campus area (California, US); The standard XANES spectra (Table 1) from the least squares fitting (....): cysteine (1), cystine (2), methionine (3), methionine sulfoxide (4), methyl sulfonate (5), sulfate (6) and *myo*-inositol hexasulfate (7) in aqueous solution at pH 7.

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#### References

- Frank, P., Hedman, B. & Hodgson, K.O. 1999. *Inorg. Chem.* 38: 260-270.
- Hedman, B., Frank, P., Penner-Hahn, J.E., Roe, A.L., Hodgson, K.O., Carlson, R.M.K., Brown, G., Cerino, J., Hettle, R., Troxel, T., Winick, H. & Yang, J. 1986. *Nucl. Instrum. Methods Phys. Res. A.* 246: 797-800.
- Pickering, I.J., Prince, R.C., Divers, T. & George, G.N. 1998. *FEBS Lett.* 441: 11-14.

- Rompel, A., Cinco, R.M., Latimer, M.J., McDermott, A.E., Guiles, R.D., Quintanilha, A., Krauss, R.M., Sauer, K., Yachandra, V.K. & Klein, M.P. 1998. *Proc. Natl. Acad. Sci. USA* 95: 6122-6127.
- Sandström, M., Jalilehvand, F., Persson, I., Gelius, U., Frank, P. & Hall-Roth, I. 2002. *Nature* 415: 893-897.
- Vairavamurthy, A. 1998. *Spectrochim. Acta Part A* 54: 2009-2017.
- Yu, E.Y., Pickering, I.J., George, G.N. & Prince, R.C. 2001. *Biochim. Biophys. Acta* 1527: 156-160.