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Assessment of atmospheric sulfur with the epilithic moss *Haplocladium microphyllum*: Evidences from tissue sulfur and $\delta^{34}S$ analysis

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Mosses on open rocky surfaces are reliable bioindicators of atmospheric sulfur deposition.

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ABSTRACT

The application of geochemical signals in mosses is more and more popular to investigate the deposition of atmospheric pollutants, but it is unclear whether records of atmospheric sulfur in mosses differ between their diverse habitats. This study aimed to investigate the influence of growing condition on tissue sulfur and $\delta^{34}S$ of *Haplocladium microphyllum*. Epilithic and terricolous mosses in open fields, mosses under different canopy conditions were considered. We found that tissue sulfur and $\delta^{34}S$ of mosses under different habitats were not consistent and could not be compared for atmospheric sulfur research with each other even collected at the same site, moss sulfur and $\delta^{34}S$ records would be distorted by subsoil and upper canopies in different degrees, which possibly mislead the interpretation of atmospheric sulfur level and sources. Consequently, mosses on open rocks can be used reliably to assess atmospheric-derived sulfur in view of their identical sulfur and $\delta^{34}S$ evidences.

1. Introduction

It is well known that sulfur derived from human activities has exceeded the average natural release into the atmospheric system, which has long been seen as a pollutant central to the acid deposition since the early 20th century (Brimblecombe et al., 1989; Lefohn et al., 1999). Together, natural and anthropogenic global SO_2 sources emit an estimated 194 million tones annually, of which about 83% is anthropogenic from fossil fuel combustion (Botkin and Keller, 2005). Even in the Rocky mountain region, atmospheric sulfur was found being chiefly of anthropogenic sources at several high-elevation sites (Heuer et al., 2000).

Currently, the sulfur biogeochemical cycle has been vastly affected and altered by atmospheric inputs, thus it is of both environmental and ecological importance to understand the level and source of atmospheric sulfur deposited onto vegetation and soil (Prietzel et al., 2004; Puig et al., 2008). In many forested ecosystems, the increase of sulfur deposition has been found to cause various adverse impacts on vegetation (Novák et al., 2000), such as chlorophyll degradation in leaves, damage to biological membranes, chloroplasts and photosynthesis reduction (Legge and Krupa, 2002). Recently, Swanepoel et al. (2007) reported that increased uptake of SO_2 caused toxicity and reduced growth as well as productivity in plants due to accumulation of sulfite and sulfate in tissues. Latestly, Barrelet et al. (2008) assessed the suitability of sulfur in Norway spruce wood as an environmental archive of SO_2 pollution in Switzerland, and suggested the need of a multidisciplinary approach as well as several analytical methods for biomonitoring atmospheric sulfur.

The stable sulfur isotope ($\delta^{34}S$) has been known as an important tool holding source-specific information that can serve as a fingerprint to identify sulfur sources at scales ranging from highly localized to regional or even global (Krouse, 1977; Nriagu et al., 1991; Alewell and Novák, 2001). In the past two decades, great interest in measuring sulfur content and $\delta^{34}S$ in plants has produced a considerable literature on the potential of plants as bioindicators of isotopically different sulfur pollutants (e.g. Novák et al., 2000, 2001). However, the utility of plant $\delta^{34}S$ as tracer in sulfur biogeochemical processes is only beginning to be realized and more works in this field are still needed to identify various coping mechanisms employed by different plants in response to sulfur loading (Trust and Fry, 1992).

Among terrestrial plants, the applicability of mosses as monitoring organisms of atmospheric pollutants is today a world-wide accepted technique due to their special biological and morphologic characteristics as nonvascular plants (Thompson and Bottrell, 1998; Vingiani et al., 2004; Rühling and Tyler, 2004; Adamo et al., 2008).

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Therefore, the marriage of moss biomonitoring with sulfur isotope was considered as a sensitive, easy and low-cost approach to specifically shed light on the long-term and integrative assessment of atmospheric sulfur (e.g. Nrlagu and Glooschenko, 1992; Novák et al., 2001). For instances, an earlier comparison by Winner et al. (1978) found that moss δ^{34}S (+16‰ to +32‰, +24‰) around natural gas smelts closely reflected the influence of SO_2 from smelts ($\delta^{34}S = +25‰$), while δ^{34}S of *Picea glauca* and *Abies balsamea* (+18‰) was deviated from the atmospheric source because of the buffering effect of soil sulfur ($\delta^{34}S = +7‰$ to +12‰). Zhao et al. (2003) also reported that δ^{34}S of most higher plants could only grossly reflect the shift of atmospheric sulfur sources, δ^{34}S of wheat (*Triticum aestivum* L.) influenced by coal combustion ($\delta^{34}S = -6‰$ to −10‰) decreased from +6‰ to +7‰ in 1845 to −2‰ to −5‰ in the early 1970s, while in the late 1990s, it returned to +0.5‰ to +2‰ due to the banning of coal combustion. Therefore, moss δ^{34}S method plays a critical role in the biomonitoring of atmospheric sulfur sources, which represents an important supplement to instrumental monitoring methods.

As one of the most abundant and widespread plants, mosses are characterized by diverse habitats and often form the dominant vegetation in montane, boreal and arctic ecosystems (Ayres et al., 2006). They could reside and survive healthily in multiple growing conditions such as under canopies, on soils, rocky surfaces, rotten woods, barks and leaves. However, it is an important but unrevealed question whether mosses under different growing conditions could still consistently provide reliable information for indicating atmospheric sulfur, and it is unclear mosses in which kinds of habitats could be confidently employed for atmospheric biomonitoring purposes. The lack of understanding of habitats' differences will make us confront with one potential trouble in selecting suitable moss samples for δ^{34}S analysis and possibly mislead the interpretation of atmospheric sulfur sources.

In the light of the above context, we undertook a survey of tissue sulfur and δ^{34}S of a pleurocarpous moss *Haplocladium microphyllum* under five groups of growing conditions commonly existing in natural ecosystems (epilithic species on open rocky surfaces, terricolous species in open fields and under three kinds of canopies). It was designed to specifically elucidate whether and how sulfur content and isotopic signal in mosses could be affected by different habitats, and finally attempt to determine a reliable bioindicator of atmospheric-derived sulfur and optimize the method of moss δ^{34}S for monitoring atmospheric sulfur.

2. Materials and methods

2.1. Study area

This study was carried out in a small woodland with an area of about 0.2 km^2 in the southeast of Guiyang downtown (26°34′N, 106°43′E), SW China. Guiyang has a subtropical monsoon climate with an annual average temperature of 15.3 °C, an average rainfall of 1170 mm (900–1500 mm) and an average relative humidity (RH) of 86%. With the mild climate and higher humidity, there are abundant moss resources, so it is an ideal place to carry out moss biomonitoring studies. Difference of original atmospheric deposition could be avoided in such a small area. Elevation within the studying area is 990 ± 3 m and there is no high building or covering around. The dominant taller plants within the woodland were *Cryptomeria japonica* (evergreen), *Osmanthus fragrans* (evergreen) and *Platanus acerifolia* (deciduous) (Table 1).

The major air pollution in Guiyang is from coal combustion (high sulfur coal of 1.86%; Ni, 1997), which accounts for over 90% of energy consumption of the whole city. With pillar industries including electric power, ferric, steel, phosphorus and rubber, it was once one of the most serious acid deposition cities in SW China (Galloway et al., 1987). In 1997, most small-sized mills and mines as well as workshops with heavy pollution were forced to shut down and some heavy polluting industries were relocated from downtown to suburbs, and now over 49% of the city residents have replaced coal by natural gas. Thus, air quality (e.g. TSP, SO_2, NO_x) in Guiyang improved from 1996 to 2003 (Guiyang Environmental Protection Bureau, 2006; Xiao and Liu, 2002, 2004).

2.2. Moss collection and treatment

In August 2005, moss materials (epilithic and terricolous) were collected in open fields and under different canopy conditions (Tables 1 and 2). Characterized by high presence in disturbed environment, *H. microphyllum* has been considered as an efficient accumulator of air pollutants in existing studies (e.g. Liu et al., 2008). However, our sampling design required the same species in different conditions and the occurrence of *H. microphyllum* tended to be patchy, thus the amount of eligible samples was limited within such a small area and the number of samples widely varied especially under different canopy thicknesses (Table 2). At each sampling location, 2–5 sub-samples were combined into one sample, a subjective judgement of sample age (the upper green leafy shoot) was made at the time of collection to keep every sample as uniform as possible. Mosses at open sites must not be influenced by any canopy or overhanging vegetation, epilithic mosses must be on the bare rocky surfaces without soil. Besides, sample collection was restricted at slightly higher places (>10 cm) above ground to avoid surface water, samples possibly disturbed by bird roosts and human trampling were also excluded.

Canopy thickness was measured by pole, the thicknesses of *C. japonica*, *O. Fragrans* and *P. acerifolia* canopies were about 10 m, 4 m and 10 m respectively. Fresh mosses were stored in cleaned plastic bags before transported to laboratory. As this study focused on tissue sulfur (not adsorbed), all moss samples were gently rinsed in 1.5 mol L^{-1} HCl solution, then sonicated and washed with deionized water (Milli-Q). The main purpose of this washing procedure was to remove pollutants adsorbed on moss tissues thoroughly. Samples were finally dried in a vacuum oven at 70 °C and re-dried after being ground separately in liquid N into fine powders.

2.3. Element analysis and isotopic determination

Tissue sulfur of mosses (%, dry weight) was determined with an elemental analyzer (PE2400II, USA) with an analytical precision of ±0.1%. For the δ^{34}S analysis of moss, all forms of sulfur in moss tissues were converted to sulfate based on Eschka method, and subsequently sulfate was recovered from washings by precipitating as $BaSO_4$ with enough 2 mol L^{-1} $BaCl_2$ solution. After precipitating for 24 h, the mixture was filtered through a dense ashless quantitative filter paper. The precipitate ($BaSO_4$) on the filters was carefully rinsed with enough Milli-Q water to remove Cl (silver nitrate was used to check), and then transferred into crucibles with the filters and combusted at 850 °C for 1 h in air. The results of X-ray diffractometry showed >99% $BaSO_4$ in the white powder in the crucible. The $BaSO_4$ was weighed into tin capsule and δ^{34}S determination was conducted with an elemental analyzer combustion continuous flow isotope ratio mass spectroscopy (EA-C-CF-IRMS, EA-IsoPrime, Euro3000, GV instrumnents, United Kingdom). The standard deviation for the δ^{34}S analysis of NBS127 (barium sulfate, $\delta^{34}S = +20.3‰$) was better than ±0.2‰ ($n = 5$).

The stable isotopic signatures were reported in standard delta notation (units permill, ‰) as:

$$\delta^{34}S[‰ versus\ CDT] = \left(R_{sample}/R_{standard} - 1\right) \times 1000$$

where $R = {}^{34}S/{}^{32}S$. The international standard for δ^{34}S is the Canyon Diablo Troilite (CDT), a meteorite of FeS used as a standard zero point for expression of sulfur isotopes. All experimental analyses were performed in the State Key Laboratory of Environmental Geochemistry at the Institute of Geochemistry, Chinese Academy of Sciences.

2.4. Statistical analysis

Statistical analysis was conducted by using SPSS11.5 and graphs were created with SigmaPlot2000 software (both SPSS Science, Chicago, USA). A multiple comparison test (Tukey HSD, LSD) was used to determine significant differences of mean sulfur contents and δ^{34}S values between different growing conditions ($P < 0.05$).

3. Results

3.1. Moss sulfur content

Sulfur content of mosses under different growing conditions ranged between 0.25% and 0.56%. In open fields, terricolous mosses presented significantly higher tissue sulfur (0.47 ± 0.06%) than epilithic mosses (0.39 ± 0.04%), and mosses in open fields had generally lower sulfur contents than those under canopies (Table 1). The lowest sulfur contents (0.33 ± 0.04%) occurred in mosses under the canopy of *O. fragrans*, possibly suggesting the highest retention capacity of sulfur deposition among the three canopies, but there was lack of a linear correlation between moss sulfur (0.25–0.41%) and canopy thickness (1–4 m) ($R^2 = 0.14$, $P > 0.05$).

Table 1
Sulfur contents and $\delta^{34}S$ values of mosses under different growing conditions. Values (means ± SD) not sharing the same superscript letters (a, b, c) in the same column are significant different ($P < 0.05$).

Substratum	Upper canopy	Sample (n)	Tissue sulfur (%, DW)	$\delta^{34}S$ (‰, CDT)
Terricolous	*Cryptomeria japonica* (Linn. f.) D. Don. (evergreen)	5	0.45 ± 0.03 (0.40–0.47)[a]	−2.25 ± 0.99 (−3.33 to −1.65)[bc]
Terricolous	*Osmanthus fragrans* Lour (evergreen)	13	0.33 ± 0.04 (0.25–0.41)[b]	−1.49 ± 0.46 (−2.26 to −0.61)[c]
Terricolous	*Platanus acerifolia* (Ait.) Willd (deciduous)	3	0.41 ± 0.01 (0.40–0.42)[ab]	−2.42 ± 0.53 (−2.76 to −1.18)[b]
Terricolous	Open field	5	0.47 ± 0.06 (0.39–0.56)[a]	−1.80 ± 0.36 (−2.36 to −1.45)[bc]
Epilithic	Open field	3	0.39 ± 0.04 (0.35–0.44)[ab]	−4.22 ± 0.90 (−3.20 to −4.88)[a]

3.2. Moss sulfur isotope

$\delta^{34}S$ values of mosses under different growing conditions varied from −4.88‰ to −0.61‰ (Table 1). In open fields, mosses on rocky surfaces expressed significantly more negative $\delta^{34}S$ signatures (−4.22 ± 0.90‰) than those growing on soil (−1.80 ± 0.36‰). Some differences of $\delta^{34}S$ could be seen between mosses under different canopies, the most positive value (−1.49 ± 0.46‰) occurred in mosses under *O. fragrans* (Fig. 1), in which a significant enrichment of ^{34}S (−2.26‰ to 0.61‰) was observed along the canopy thickness transect (1–4 m) (Fig. 2).

4. Discussion

4.1. Tissue sulfur in epilithic mosses for assessing the level of atmospheric SO_2

Mosses might show multiple biological responses to changes of atmospheric sulfur, among which sulfur content was an important parameter to reflect the level of atmospheric sulfur deposition (especially gaseous SO_2) because higher atmospheric sulfur directly caused higher tissue sulfur in mosses (Novák et al., 2001; Vingiani et al., 2004). In this study, all sampling sites were within a small area of about 0.2 km^2 to keep the same sulfur context (level and sources) in soil and atmosphere. Therefore, the variation of moss sulfur (0.25–0.56%, Table 1) mainly reflected the difference of atmospheric sulfur flux regulated by different growing conditions. In open fields, sulfur in terricolous mosses (0.47 ± 0.06%) was significantly higher than that of epilithic mosses (0.39 ± 0.04%), indicating that soil-growing mosses were confronted with higher sulfur supply owing to the contribution from subsoil. Terricolous *H. microphyllum* weft-built on soil was likely to uptake soluble sulfur (e.g. SO_4^{2-}) directly under the help of rainwater or dew on their pinnate leaves and shoots, while it is unlikely for epilithic mosses to take up sulfur from carbonate rocks in the Karst region. A similar study by Ayres et al. (2006) also demonstrated unequivocally that carpet-formed mosses could absorb nutrients from the soil and transport them to feed growing tissues, and uptake and transport of soil-derived N to shoots could be up to 9% of wet deposition-derived N. These findings of substratum effects may place some terricolous mosses at greater uncertainty in bioindicating atmospheric deposition (Van Tooren et al., 1990).

Prior to this study, Qu et al. (1994) has adopted moss bags to monitor atmospheric sulfur pollution at 13 sites of Guiyang city, and a strong correlation was observed between atmospheric SO_2 and moss sulfur ($R^2 = 0.9723$, $P < 0.05$). As can be seen in Fig. 3, moss sulfur in 1989 (1.65 ± 0.63%) was much higher than that measured in this study (0.25–0.56%), suggesting there was serious atmospheric sulfur pollution in the early 1990s. According to Wang (1993), the concentration of ground SO_2 at Guiyang reached 400–500 μg m^{-3} during 1981–1990, which is three times higher than the average level of 10 major cities in northern America (Huang et al., 1995). Even the average atmospheric SO_2 at Guiyang is still as high as 300 μg m^{-3} in 1996, about six times above the WHO annual guideline of 50 μg m^{-3}. However, due to the banning of coal combustion, only 87 μg m^{-3} of SO_2 concentration was determined at Guiyang in 2003 and lower value of 70 μg m^{-3} was found in 2005 (Xiao and Liu, 2002) (Fig. 3). The SO_4^{2-} concentration of rainwater (20 mg L^{-1}) in 2001 was 50% lower than that in 1987 (43 mg L^{-1}) (Galloway et al., 1987; Xiao and Liu, 2002). Based on the SO_2 data and the regression equation obtained by Qu et al. (1994), the corresponding moss sulfur content in 2005 could be calculated as 0.41%, which is very close to the sulfur content of mosses on open rocky surfaces (0.39 ± 0.04%, Table 1). This clearly suggested that it is more accurate to use sulfur content of epilithic mosses in open fields for assessing the level of atmospheric sulfur.

4.2. Effects of substratum on moss $\delta^{34}S$ for indicating atmospheric-derived sulfur

Plants' $\delta^{34}S$ could be used to identify their long-term sulfur sources because most sulfur in different ecosystem pools had distinct $\delta^{34}S$ signals (Mektiyeva et al., 1976; Trust and Fry, 1992). $\delta^{34}S$ of epilithic mosses (−4.22 ± 0.90‰) was significantly more negative than that of terricolous mosses (−1.80 ± 0.36‰) in this study (Table 1), demonstrating that the allochthonous sulfur sources were different between terricolous and epilithic species. Compared with atmospheric $\delta^{34}S$ data, the $\delta^{34}S$ of epilithic mosses (−4.22 ± 0.90‰) was similar to the $\delta^{34}SO_4^{2-}$ of rainwater (−4.9 ± 2.8‰) collected in the same study area in 2001 (Xiao and Liu, 2002), even much closer to the average atmospheric $\delta^{34}S$ value (−4.0 ± 3.6‰) and $\delta^{34}SO_2$ value (−4.3‰, −7.8‰ to 2.7‰) reported by Mukai et al. (2001) for Guiyang city (Fig. 1).

Similarly, our recent studies in some Chinese mountains and cities showed the consistency of $\delta^{15}N$ and $\delta^{34}S$ signatures in

Table 2
Sulfur contents and $\delta^{34}S$ values of mosses under a canopy of *Osmanthus fragrans* with different canopy thicknesses (values represent means ± SD of n samples range).

Canopy thickness (m)	Sample (n)	Tissue sulfur (%, DW)	$\delta^{34}S$ (‰, CDT)
1.0	1	0.27	−2.26
1.5	2	0.31 ± 0.01 (0.30–0.31)	−1.67 ± 0.18 (−1.79 to −1.54)
2.0	2	0.38 ± 0.05 (0.35–0.41)	−1.69 ± 0.13 (−1.78 to −1.60)
2.5	1	0.25	−1.69
3.0	1	0.36	−2.06
3.5	3	0.33 ± 0.01 (0.32–0.34)	−1.00 ± 0.28 (−1.27 to −0.61)
4.0	3	0.34 ± 0.04 (0.29–0.37)	−1.26 ± 0.35 (−1.50 to −1.01)

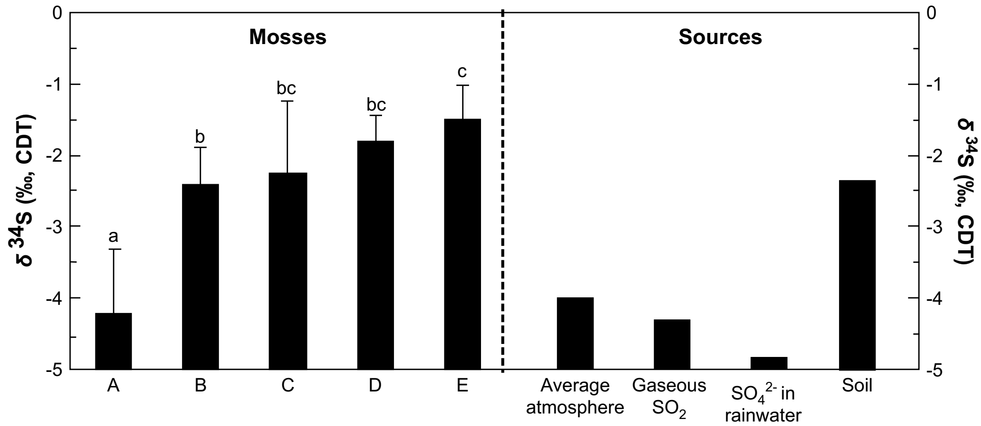

Fig. 1. Comparison of moss $\delta^{34}S$ between different growing conditions and $\delta^{34}S$ of soil and atmosphere, where A represents epilithic mosses, B represents mosses under *P. acerifolia*, C represents mosses under *C. japonica*, D represents mosses at open sites and E represents mosses under *O. fragrans*. Data of $\delta^{34}S$ in soil and atmosphere were cited from Liu and Hong (1996), Mukai et al. (2001), and Xiao and Liu (2002).

epilithic mosses with atmospheric isotopic ratios (Liu et al., 2008; Xiao et al., in press). Moss isotopic ratios at Emei Mountain (SW China) averaged +4.63%, comparable to the reported mean value of rainwater sulfate (+4.68%, Yanagisawa et al., 2003). The conclusion was also drawn by Nrlagu and Glooschenko (1992) that *Sphagnum fuscum* on low hummocks in ombrotrophic bogs acquired $\delta^{34}S$ values similar to those found for atmospheric SO_2, and Thompson and Bottrell (1998) observed that in the polluted sites more HSO_3^- is available for uptake and the isotopic shift between *Sphagnum* and aqueous sulfur species is smaller. In this study, $\delta^{34}S$ of soil-growing mosses ($-1.80 \pm 0.36‰$, Table 1) was obviously less negative than epilithic mosses, suggesting the influence of soil-derived sulfur ($\delta^{34}S = -2.17‰$, Liu and Hong, 1996).

Accordingly, epilithic mosses preserved the average $\delta^{34}S$ signal of sulfate in atmosphere because they acquired sulfur dominantly from the atmosphere and could be used to discriminate atmospheric-derived sulfur more reliably. Secondly, there was possibly little sulfur isotopic legacy left during moss sulfur assimilation, at least for *H. microphyllum*. Before this study, some researchers also assumed that there is possibly no significant isotopic fractionation occurring during the direct influx and absorption of nutrients or pollutants to the living moss cells (e.g. *Sphagnum* plants) as they have no cuticles to block atmospheric inputs (Mektiyeva et al., 1976; Trust and Fry, 1992; Bragazza et al., 2005).

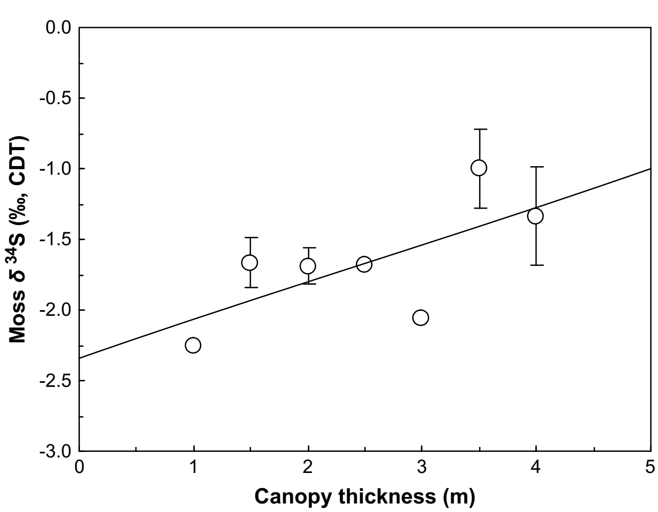

Fig. 2. Moss $\delta^{34}S$ plotted against canopy thickness of *O. fragrans* ($Y = 0.283X - 2.367$, $R^2 = 0.502$, $P = 0.0163$).

4.3. Effects of upper canopies on mosses' sulfur and $\delta^{34}S$ for indicating sulfur deposition

Canopies represent a distinct natural barrier to light and atmospheric deposition for under-growing vegetation. Canopy retention of atmospheric deposition broadly refers to uptake via adsorption, absorption, assimilation and other processes, in which the chemical composition and isotopic signals of original atmospheric deposition might be markedly distorted (Lindberg, 1992; Hietz et al., 2002; Wania et al., 2002). Mosses under canopies generally had lower tissue sulfur than those in open fields (Table 1), showing that *H. microphyllum* could respond to the reduction of atmospheric sulfur input into ground flora due to the canopy retention. Besides, moss sulfur content under different canopies was related to the retention capacity of upper canopy. Compared with *O. fragrans*, canopy of *P. acerifolia* has shorter annual retention time due to its deciduous habitus, and *C. japonica* has lower retention capacity of both particulate and wet deposition due to its smooth needle-like leaves (lower wettability and shorter residence time) and smaller canopy. Thus, tissue sulfur of mosses under *C. japonica* ($0.45 \pm 0.03\%$) and *P. acerifolia* ($0.41 \pm 0.01\%$) did not differ significantly from those in open fields ($0.47 \pm 0.06\%$), but was much higher than those ($0.33 \pm 0.04\%$) under evergreen *O. fragrans* with dense and broad leafage (Table 1).

Furthermore, sulfur assimilation for most plants is associated with an isotopic effect of discriminating against the heavier isotope ^{34}S (Heaton et al., 1997; Novák et al., 2001). The mechanism of ^{34}S discrimination (^{32}S preference) during canopy retention would cause $\delta^{34}S$ of atmospheric sulfur available for ground mosses more ^{34}S-enriched than that of original deposition in different degrees. Thus mosses under canopies had generally higher $\delta^{34}S$ ratios than those in open fields (Table 1). Similarly, Heaton et al. (1997) found that $\delta^{34}S$ of throughfall (4.7–9.8‰, $6.1 \pm 1.9‰$) was more ^{32}S-depleted than that of rainwater collected in open fields (3.5–6.4‰, $5.1 \pm 1.1‰$). A comparison of ^{34}S ratios of atmospheric input (canopy throughfall) and ecosystem sulfur pools (forest floor) in Norway spruce (*Picea abies*) showed a systematic shift toward lower ^{34}S, on average by 2‰ (Novák et al., 2001).

In addition, the isotopic fractionation during canopy retention of sulfur deposition might be rather selectively associated with canopy type and leaf morphology. Among the three kinds of canopies, mosses under *O. fragrans* showed the most positive $\delta^{34}S$ ($-1.49 \pm 0.46‰$, Table 1), which might be ascribed to the strong and prolonged interception of *O. fragrans* canopies, while lower retention capacities of *P. acerifolia* and *C. japonica* canopy should

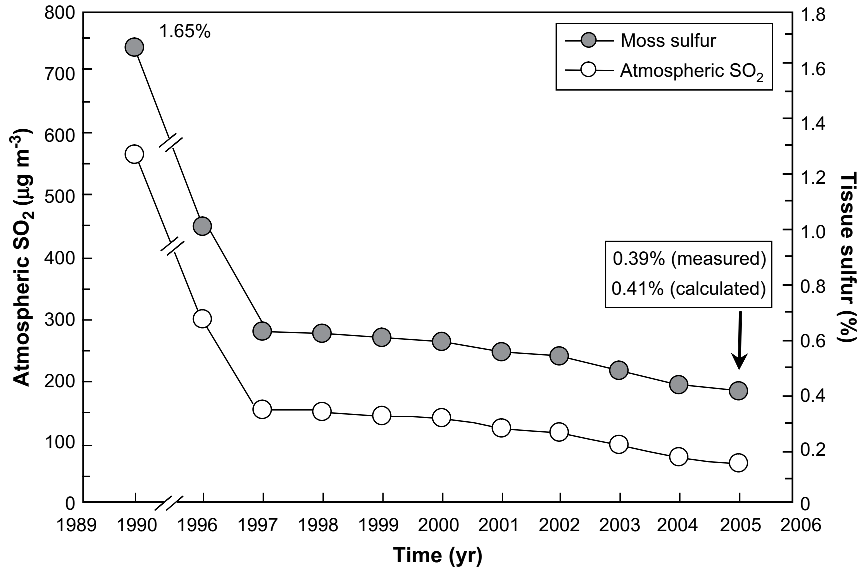

Fig. 3. Annual variation of atmospheric SO_2 concentration in Guiyang and comparison of moss sulfur contents calculated through atmospheric SO_2 with that measured in this study. Data were cited from Qu et al. (1994) and Guiyang Environmental Protection Bureau (2006).

partly be responsible for more negative $\delta^{34}S$ of mosses (-2.42 ± 0.53‰ under *P. acerifolia* and -2.25 ± 0.99‰ under *C. japonica*) (Fig. 1).

Besides, canopy thickness was an important factor to directly regulate the capacity of retention of atmospheric deposition: thicker canopy means higher retention and ^{34}S discrimination (Heaton et al., 1997; Filoso et al., 1999). A study by Krouse et al. (1984) indicated that trees may show a height trend, with the uppermost needles/leaves having a higher proportion of atmospheric sulfur than lower ones, which was thought to be the result of upper foliage exerting a canopy effect on the lower branches. As shown in Fig. 2, mosses $\delta^{34}S$ (-2.26‰ to -1.00‰) under *O. fragrans* were linearly correlated with the upper canopy thickness ($R^2 = 0.502$, $P = 0.0163$), showing that the thickness of canopy played a significant role in long-term influencing the isotopic composition of original sulfur deposition; the isotopic effect during canopy retention would increase with canopy thickness. Wania et al. (2002) also found a similar isotopic mechanism that $\delta^{15}N$ of epiphytes got less negative (^{15}N-enriched) with increasing canopy thickness from upper to lower parts within canopy.

Altogether, canopies with different features would perform differently when influencing $\delta^{34}S$ of atmospheric sulfur deposited onto ground soil and plants, sulfur isotope in mosses can potentially be used to understand the effect of canopy retention and simultaneously strengthen the utility of the moss isotope method in monitoring atmospheric sulfur.

5. Conclusions

Tissue sulfur of epilithic mosses in open fields was consistent with atmospheric SO_2, while terricolous mosses presented higher sulfur due to the additional soil contribution. Evidences from $\delta^{34}S$ strongly corroborated the evidence that mosses on open rocky surfaces could escape the influences of substratum, exhibiting much closer $\delta^{34}S$ to that of atmospheric sulfur; on the other hand, mosses growing on soil showed a source signal approaching soil.

Mosses under canopies showed generally lower sulfur contents but higher $\delta^{34}S$ than those in open fields, showing the effects of canopy retention and accompanying isotopic mechanism. Mosses under *O. fragrans* exhibited the lowest sulfur content but the highest $\delta^{34}S$, and a positive correlation was found between canopy thickness and mosses' $\delta^{34}S$, indicating that the influence of canopy on records of atmospheric sulfur in mosses was related to factors regulating the retention capacities of different canopies.

Consequently, epilithic mosses in open fields hold reliable information for assessing the level and source of atmospheric sulfur. This finding is important for sample selection when using mosses as bioindicators of atmospheric sulfur. Our results supported the fact that moss $\delta^{34}S$ variation did not simply elucidate the utilization of isotopically different sulfur sources, but also mirrored some processes during sulfur acquisition by ground flora, which would benefit for better understanding the isotopic mechanisms of sulfur biogeochemical cycle in forest ecosystems.

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References

Adamo, P., Bargagli, R., Giordano, S., Modenesi, P., Monaci, F., Pittao, E., Spagnuolo, V., Tretiach, M., 2008. Natural and pre-treatments induced variability in the chemical composition and morphology of lichens and mosses selected for active monitoring of airborne elements. Environmental Pollution 152, 11–19.

Alewell, C., Novák, M., 2001. Spotting zones of dissimilatory sulfate reduction in a forested catchment: the ^{34}S–^{35}S approach. Environmental Pollution 112, 369–377.

Ayres, E., van der Wal, R., Sommerkorn, M., Bardgett, R.D., 2006. Direct uptake of soil nitrogen by mosses. Biology Letters 2, 286–288.

Barrelet, T., Ulrich, A., Rennenberg, H., Zwicky, C.N., Krähenbühl, U., 2008. Assessing the suitability of Norway spruce wood as an environmental archive for sulphur. Environmental Pollution 156, 1007–1014.

Bragazza, L., Limpens, J., Gerdol, R., Grosvernier, P., Hájek, M., Hájek, T., Hajkova, P., Hansen, I., Iacumin, P., Kutnar, L., Rydin, H., Tahvanainen, T., 2005. Nitrogen concentration and δ^{15}N signature of ombrotrophic *Sphagnum* mosses at different N deposition levels in Europe. Global Change Biology 11, 106–114.

Botkin, D.B., Keller, E.A., 2005. Environmental Science: Earth as a Living Planet. Wiley, USA.

Brimblecombe, P., Hammer, C., Rodhe, H., Ryaboshapko, A., Boutron, C.F., 1989. Human influence on the sulfur cycle. In: Brimblecombe, P., Lein, A.Y. (Eds.), Evolution of the Global Biogeochemical Sulphur Cycle, vol. 39. Wiley, Chichester, pp. 77–121.

Filoso, S., Williams, M.R., Melack, J.M., 1999. Composition and deposition of throughfall in a flooded forest archipelago (Negro River, Brazil). Biogeochemistry 45, 169–195.

Galloway, J.N., Zhao, D.W., Xiong, J.L., Likens, G.E., 1987. Acid rain: China, United States, and a remote area. Science 236, 1559–1562.

Guiyang Environmental Protection Bureau, 2006. Bulletin of Environmental Conditions of 2005 in Guiyang (in Chinese).

Heaton, T.H.E., Spiro, B., Robertson, S.M.C., 1997. Potential canopy influences on the isotopic composition of nitrogen and sulphur in atmospheric deposition. Oecologia 109, 600–607.

Heuer, K., Tonnessen, K.A., Ingersoll, G.P., 2000. Comparison of precipitation chemistry in the central Rocky Mountains, Colorado, USA. Atmospheric Environment 34, 1713–1722.

Hietz, P., Wanek, W., Wania, R., Nadkarni, N.M., 2002. ^{15}N natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131, 350–355.

Huang, M.Y., Chen, Z.L., Liu, S.R., Wu, Y.X., Xiao, H., Lei, H.C., Bai, C.H., 1995. A study on the formation processes of acid rain in some areas of southwest China. Scientia Atmospheric Sinica 19, 359–366 (in Chinese).

Krouse, H.R., 1977. Sulphur isotope abundance elucidate uptake of atmospheric sulphur emissions by vegetation. Nature 265, 45–46.

Krouse, H.R., Legge, A.H., Brown, H.M., 1984. Sulphur gas emissions in the boreal forest: the west Whitecourt Case Study V. Stable sulphur isotopes. Water, Air and Soil Pollution 22, 321–347.

Lefohn, A.S., Husar, J.D., Husar, R.B., 1999. Estimating historical anthropogenic global sulfur emission patterns for the period 1850–1990. Atmospheric Environment 33, 3435–3444.

Legge, A.H., Krupa, S.V., 2002. Effects of sulphur dioxide. In: Bell, J.N.B., Treshow, M. (Eds.), Air Pollution and Plant Life. John Wiley and Sons, Ltd., England, pp. 135–162.

Lindberg, S.E., 1992. Atmospheric deposition and canopy interactions of sulphur. In: Johnson, D.W., Lindberg, S.E. (Eds.), Atmospheric Deposition and Forest Nutrient Cycling. Springer, New York, pp. 152–166.

Liu, G.S., Hong, Y.T., 1996. The sulfur isotopic composition characteristics of atmospheric particulates from near the ground in the urban and suburban areas of Guiyang city. Acta Mineralogica Sinica 16, 353–357 (in Chinese).

Liu, X.Y., Xiao, H.Y., Liu, C.Q., Li, Y.Y., Xiao, H.W., 2008. Atmospheric transport of urban-derived NH_x: evidences from nitrogen concentration and δ^{15}N in epilithic mosses at Guiyang, SW China. Environmental Pollution 156, 715–722.

Mektiyeva, V.L., Gavrilov, E.Y., Pankina, R.G., 1976. Sulphur isotopic composition in land plants. Geochemistry International 13, 85–88.

Mukai, H., Tanaka, A., Fujii, T., Zeng, Y.Q., Hong, Y.T., Tang, J., Guo, S., Xue, H.S., Sun, Z.L., Zhou, J.T., Xue, D.M., Zhao, J., Zhai, G.H., Gu, J.L., Zhai, P.Y., 2001. Regional characteristics of sulfur and lead isotope ratios in the atmosphere at several Chinese urban sites. Environmental Sciences and Technology 35, 1064–1071.

Ni, J.Y., 1997. Composition character of sulfur and trace elements in late Permian coal of Guizhou province, China. Ph.D. Dissertation. Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, China.

Novák, M., Bottrell, S.H., Přechova, E., 2001. Sulfur isotope inventories of atmospheric deposition, spruce forest floor and living *Sphagnum* along a NW–SE transect across Europe. Biogeochemistry 53, 23–50.

Novák, M., Kirchner, J.W., Groscheová, H., Havel, M., Černý, J., Krejčí, R., Buzek, F., 2000. Sulfur isotope dynamics in two Central European watersheds affected by high atmospheric deposition of SO_x. Geochimica et Cosmochimica Acta 64, 367–383.

Nriagu, J.O., Coker, R.D., Barrie, L.A., 1991. Origin of sulphur in Canadian arctic haze from isotope measurements. Nature 349, 142–145.

Nrlagu, J.O., Glooschenko, W.A., 1992. Isotopic composition of sulfur in mosses across Canada. Environmental Science and Technology 26, 85–89.

Prietzel, J., Mayer, B., Legge, A.H., 2004. Cumulative impact of 40 years of industrial sulfur emissions on a forest soil in west-central Alberta (Canada). Environmental Pollution 132, 129–144.

Puig, R., Àvila, A., Soler, A., 2008. Sulphur isotopes as tracers of the influence of a coal-fired power plant on a Scots pine forest in Catalonia (NE Spain). Atmospheric Environment 42, 733–745.

Qu, L.Y., Lin, Q.W., Li, M., Li, Q.X., 1994. Application and study of *Hypnum* sp. and *Thuidium* sp. plant about air impact assessment. Journal of Guizhou Normal University (Natural Sciences) 12, 45–48 (in Chinese).

Rühling, Å., Tyler, G., 2004. Changes in the atmospheric deposition of minor and rare elements between 1975 and 2000 in south Sweden, as measured by moss analysis. Environmental Pollution 131, 417–423.

Swanepoel, J.W., Krüger, G.H.J., Heerden, P.D.R., 2007. Effects of sulphur dioxide on photosynthesis in the succulent *Augea capensis* Thunb. Journal of Arid Environment 70, 208–221.

Thompson, A., Bottrell, S., 1998. Sulphur isotopic investigation of a polluted raised bog and the uptake of pollutant sulphur by *Sphagnum*. Environmental Pollution 101, 201–207.

Trust, B.A., Fry, B., 1992. Stable sulphur isotopes in plants: a review. Plant, Cell and Environment 15, 1105–1110.

Van Tooren, B.F., Van Dam, D., During, H.J., 1990. The relative importance of precipitation and soil as sources of nutrients for *Calliergonella cuspidata* (Hedw.) Loeske in chalk grassland. Functional Ecology 4, 101–107.

Vingiani, S., Adamo, P., Giordano, S., 2004. Sulphur, nitrogen and carbon content of *Sphagnum capillifolium* and *Pseudevernia furfuracea* exposed in bags in the Naples urban area. Environmental Pollution 129, 145–158.

Wang, Y.Q., 1993. Conditions and strategy of Chinese environmental pollution. China Environmental Science 13, 241–245 (in Chinese).

Wania, R., Hietz, P., Wanek, W., 2002. Natural ^{15}N abundance of epiphytes depends on the position within the forest canopy: source signals and isotope fractionation. Plant, Cell and Environment 25, 581–589.

Winner, W.E., Bewley, J.D., Krouse, H.R., Brown, H.M., 1978. Stable sulfur isotope analysis of SO_2 pollution impact on vegetation. Oecologia 36, 351–361.

Xiao, H.Y., Liu, C.Q., 2002. Sources of nitrogen and sulfur in wet deposition at Guiyang, Southwest China. Atmospheric Environment 36, 5121–5130.

Xiao, H.Y., Liu, C.Q., 2004. Chemical characteristics of water-soluble components in TSP over Guiyang, SW China, 2003. Atmospheric Environment 38, 6297–6306.

Xiao, H.Y., Xiao, H.W., Liu, X.Y., Liu, C.Q. Sulphur isotopic characteristics of mosses at southern Chinese cities. Geochimica (in Chinese), in press.

Yanagisawa, F., Jia, S., Masuda, H., Yang, H., Ueda, A., Kawabata, A., Akata, N., Motoyama, R., 2003. Sulphur isotopic ratio of sulphate in the rain collected in November and December. Journal of Chengdu University of Technology 30, 96–98 (in Chinese).

Zhao, F.J., Knights, J.S., Hu, Z.Y., McGrath, S.P., 2003. Stable sulfur isotope ratio indicates long-term changes in sulfur deposition in the broadbalk experiment since 1845. Journal of Environmental Quality 32, 33–39.