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ISOTOPE DETERMINATION OF SULFUR BY MASS SPECTROMETRY IN SOIL SAMPLES(1)

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SUMMARY

Sulphur plays an essential role in plants and is one of the main nutrients in several metabolic processes. It has four stable isotopes (^{32}S, ^{33}S, ^{34}S, and ^{36}S) with a natural abundance of 95.00, 0.76, 4.22, and 0.014 in atom %, respectively. A method for isotopic determination of S by isotope-ratio mass spectrometry (IRMS) in soil samples is proposed. The procedure involves the oxidation of organic S to sulphate ($S\text{-}SO_4^{2-}$), which was determined by dry combustion with alkaline oxidizing agents. The total $S\text{-}SO_4^{2-}$ concentration was determined by turbidimetry and the results showed that the conversion process was adequate. To produce gaseous SO_2 gas, $BaSO_4$ was thermally decomposed in a vacuum system at 900 ºC in the presence of $NaPO_3$. The isotope determination of S (atom % ^{34}S atoms) was carried out by isotope ratio mass spectrometry (IRMS). In this work, the labeled material ($K_2{}^{34}SO_4$) was used to validate the method of isotopic determination of S; the results were precise and accurate, showing the viability of the proposed method.

Index terms: sample preparation, soil samples, isotopic dilution, labeled material, stable isotope, ^{34}S.

RESUMO: *DETERMINAÇÃO ISOTÓPICA DE ENXOFRE POR ESPECTROMETRIA DE MASSAS (IRMS) EM AMOSTRAS DE SOLO*

O enxofre tem papel essencial em plantas, sendo um dos principais nutrientes em diversos processos metabólicos; ele apresenta quatro isótopos estáveis (^{32}S, ^{33}S, ^{34}S e ^{36}S), com abundância natural de 95,00, 0,76, 4,22 e 0,014 % em átomos, respectivamente. Desenvolver

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um método para determinação isotópica do S por espectrometria de massas de razão isotópica (IRMS) em amostras de solo foi o objetivo deste trabalho. A oxidação de S orgânico a sulfato (S-SO_4^{2-}) foi avaliada utilizando a oxidação via seca com agentes oxidantes alcalinos. A concentração S-SO_4^{2-} foi determinada pelo método turbidimétrico, e os resultados mostraram que o processo de conversão foi adequado. A obtenção do gás SO_2 foi por decomposição térmica do $BaSO_4$ em uma linha de vácuo a 900 ºC, em presença de $NaPO_3$. A determinação isotópica do S (% em átomos de ^{34}S) foi realizada em um espectrômetro de massa (IRMS). Neste trabalho, a utilização de material marcado ($K_2{}^{34}SO_4$) teve como propósito validar o método para determinação isotópica do S; os resultados obtidos foram exatos e precisos, mostrando a viabilidade do método proposto.

Termos de indexação: preparo de amostras, amostras de solo, diluição isotópica, isótopos estáveis, ^{34}S.

INTRODUCTION

Sulphur is present in the soil in two basic forms; inorganic and organic S. In the inorganic form (sulphate S), S is available for plant uptake, but organic S accounts for 95 % of the total sulphur in most soils. This is due to the close relation of organic S with organic C and total N. Organic sulfur has two forms: organic S that is not bonded directly to C, consisting largely of S in the form of ester sulfates (organic sulfates containing C-O-S linkages) and organic S that is directly bonded to C (C-S), consisting largely of S in the form of S-containing amino acids, such as methionine and cysteine (Tabatabai, 1982; Freney, 1986; Havlin et al., 2005).

The method for S isotopic determination in soil samples involves several steps such as: converting all S-organic to sulfate ($BaSO_4$); preparation and purification of SO_2 (g) in a high-vacuum system and isotopic analysis of S (atom % or δ ^{34}S ‰) by isotope-ratio mass spectrometry (IRMS).

The conversion of organic S to sulphate by various methods was described elsewhere (Krouse et al., 1996; Menegário et al., 1998; Rossete et al., 2008a), but the complexity of the organic matrix of the soil hampers the procedures of sample preparation for isotope analysis of S. Most methods employed for sulfur oxidation in soil analysis involve wet or dry-ash oxidation (Rossete et al., 2008a). In comparison with wet oxidation, digestion by nitric and perchloric acids is more frequently used, but requires safety measures against explosion and fire, aside from possible material losses, that can make the method unserviceable (Tabatabai, 1982).

The conventional method for oxidation of organic S to sulphate uses nitric acid and liquid bromine (Krouse & Tabatabai, 1986; Krouse et al., 1996). This method is seldom recommended due to the risks of Br_2 and toxic waste generation. The biological action of bromine in its liquid and gaseous states is highly noxious. Bromine causes strong irritation in eyes and skin, necroses, and inflammation of the breathing system.

In the preparation of SO_2, a gas suited for studies on isotope determination by isotope-ratio mass spectrometry (Hamilton et al., 1991; Bendassolli et al., 1997), procedures of combustion, purification and preparation of the gas in vacuum system were used, avoiding isotopic fractionation of the sample (Fritz et al., 1974; Schoenau & Bettany, 1988; Krouse et al., 1996).

Of the known isotopes of Sulfur, four are stable: ^{32}S, ^{33}S, ^{34}S, and ^{36}S, with natural abundances of 95.02; 0.75; 4.21 and 0.02 atom %, respectively (Krouse et al., 1996). Some researchers used the radioisotope ^{35}S as tracer, especially in studies involving the dynamics of this nutrient in the soil-plant system (Lal & Dravid, 1990; Arora et al., 1990; Sharma & Kamath, 1991; Bansal & Mortiramani, 1993; Patnaik & Santhe, 1993; Fitzgerald et al., 1999). However, compounds labeled with the stable isotope ^{34}S have some advantages over the radioisotope, because the experiments are not related to the decay rate and if there is no exposure to radiation, no safety measures against radiation are required. The first studies using the ^{34}S isotopic tracer were developed by Hamilton et al. (1991) and Awonaike et al. (1993) and in Brazil by Trivelin et al. (2002), who used sulphate-^{34}S in studies on sulphur in soil under rice and sunn hemp (*Crotalaria juncea*).

In a study addressing the isolation of the ^{34}S isotope, Bendassolli et al. (1997) were able to produce a series of different labeled compounds, e.g., ammonium sulfate, gypsum, potassium sulfate, and single superphosphate (Maximo et al., 2005; Rossete et al., 2006, 2008b). Recently, the stable isotope ^{34}S has been used to study sulphur uptake and distribution in wheat plants (Zhao et al., 2001). These compounds motivate the use of the isotopic technique (^{34}S) in many research studies, and shed light on a number of aspects related to isotope determination (sample preparation) (Carneiro, et al., 2008).

In this context, the objective of this work was to develop a method for isotopic determination of S (atom % ^{34}S) in different soils by Isotope ratio mass spectrometry (IRMS). The proposed method was validated by the use of labeled material ($K_2{}^{34}SO_4$).

MATERIAL AND METHODS

Equipment

The Isotope Ratio Mass Spectrometer (IRMS), model CH4 ATLAS-MAT, is equipped with a molecular-flow admission system and Faraday-cup ion collector. The analytical system comprises a vacuum system with a mechanic vacuum pump, a diffuser vacuum pump, an active vacuum measurer and a vacuum filament sensor.

Sample preparation and total sulfur determination in soil

Different soil types with sandy and clayey texture, were used to evaluate the total S determination method (Rossete et al., 2008a). Soil samples were collected from the surface horizon (0-20 cm), at different locations in the State of São Paulo. The soils were classified according to the Brazilian Soils Classification System (Embrapa, 2006).

Organic S in soil samples was oxidized to sulphate to determine S by dry-ash oxidation, using $NaHCO_3$ (alkaline medium) and Ag_2O as oxidizing agents (Rossete et al., 2008a). The soil sample (5.0 g) and $NaHCO_3/Ag_2O$ mixture (2.0/0.2 g) were mixed in a porcelain mortar. Thereafter, the material was combusted at 550 ºC in a muffle for 8 h, inducing the oxidation of organic S to sulphate. To establish the analytical calibration curve for 2.5; 5; 10; 15; and 20 mg L^{-1} $S\text{-}SO_4^{-2}$, the proportional quantity of $NaHCO_3/Ag_2O$ mixture was burned under the same conditions as the soil. Then, the samples were cooled to room temperature and the resulting sulphate was solubilized in 30 mL of 0.15 % (w/v) $CaCl_2$ solution and 1.0 g of activated charcoal. All soil extraction solutions were shaken on a horizontal circular shaker at 200 rpm for 15 min. Finally, 20 mL of 0.15 % (w/v) $CaCl_2$ solution were added and the extract was filtered through cellulose ester filter (diameter 45 mm, mesh 0.45 μm). The $S\text{-}SO_4^{2-}$ concentration was then determined by turbidimetry (Tabatabai & Bremner, 1970; Andrade et al., 1990; Cantarella & Prochnow, 2001).

Soil labeling procedure

The labeled material ($K_2{}^{34}SO_4$) was used in this study to validate the proposed method. In experiments analyzing isotopic dilution, the diferente isotope values of the labeled material ($K_2{}^{34}SO_4$) were 6.15 ± 0.07; 8.97 ± 0.01; 12.51 ± 0.01 and 15.03 ± 0.04 atom % ^{34}S. The labeled potassium sulphate ($K_2{}^{34}SO_4$) was produced at USP/CENA Stable Isotope Laboratory.

For this purpose, 5.0 g of Nitossolo Vermelho eutroférrico (NVef) and 5.0 g of Argissolo Vermelho-Amarelo distrófico soil (AVAd), were mixed with approximately 700.0 and 400.0 μg of S contained in the labeled material ($K_2{}^{34}SO_4$), respectively.

The theoretical isotopic abundance (Ab_t) should be calculated by equation 1. The value obtained for total S should represent the sum of total initial S (ST_o) and $^{34}S_l$. Based on the results obtained by the isotopic analysis (Ab_{exp}) after adding the labeled material; it was possible to evaluate the method for organic S oxidation (dry-ash) by applying equation 1. Using the values for experimental isotopic abundance (Ab_{exp}) and theoretical isotopic abundance (Ab_t), the analytical error can be calculated by:

$$(m_0soil * ST_0) * Ab_0 + {}^{34}S_l * Ab_l = [(m_0soil * ST_0) + {}^{34}S_l] * Ab_t \qquad (1)$$

m_0soil = total S in the sample (μg S); ST_0 = total initial S (μg g^{-1}); Ab_0 = natural isotopic abundance of S (atom % ^{34}S); $^{34}S_l$ = spiked amounts of $K_2{}^{34}SO_4$-labeled material (μg ^{34}S); Ab_l = isotopic abundance of labeled material ($K_2{}^{34}SO_4$ (atom % ^{34}S); Ab_t = theoretical isotopic abundance (atom % ^{34}S).

Sample preparation for isotopic determination of S (atom% ^{34}S)

Soil-extractable SO_4^{2-} was acidified and subsequently precipitated as $BaSO_4$ by adding 10 mL of 10.0 % (w/v) $BaCl_2.2H_2O$ solution under agitation. After this procedure, the precipitated $BaSO_4$ was washed with deionized water and centrifuged at 2500 rpm for 5 min. This procedure was repeated three times to eliminate impurities of $BaCl_2.2H_2O$ excess. Then the precipitate was dried to constant weight at 60 ºC and 5.0 mg $BaSO_4$ precipitate was mixed with 15 mg $NaPO_3$ (Halas & Wolacewicz, 1981). $NaPO_3$ was obtained by burning $NaH_2PO_4.H_2O$ in a muffle at 200 ºC for 2 h. This step is necessary to remove the structural water from the compound and to eliminate possible organic impurities.

For SO_2 production, the $BaSO_4/NaPO_3$ mixture was placed in a quartz tube (QT) (length about 30 cm). A copper metal ring (about 2 g) and quartz wool was always placed in the tube approximately 2 cm above the mixture (sample/reagents). Copper is used to retain oxides formed during combustion and to convert possible SO_3 into SO_2, avoiding isotopic fractionation (Rafter, 1957; Yanagisawa & Sakal, 1983). Next, the QT was connected to a vacuum system by mechanic (MP) and diffuser (DP) pumps. A cylinder with dry ice/ethanol (-70 ºC) and another with liquid N (-196 ºC) were introduced around the traps Tr_1 and Tr_2, respectively, which were used to purify and separate the gases (SO_2, N_2, CO) in the vacuum system. The traps Tr_1 and Tr_2 were designed to trap vapors of water and SO_2, respectively, formed during combustion. The vacuum system configuration was based on the method developed by Bailey & Smith (1972) (Figure 1).

In the next step, the MF heated to 900 ºC was shifted upwards to the QT, where it was maintained for 10 min for the combustion of $BaSO_4$ and further SO_2 gas formation (Equation 2).

$$BaSO_4 + NaPO_3 \rightarrow NaBaPO_4 + SO_{2(g)} + 1/2O_{2(g)} \quad (2)$$

Depending on the conditions (O_2 partial pressure), SO_3 gas could be produced, which is undesirable, since it could cause isotopic fractionation in analysis. Equations 3 and 4 show the conversion of SO_3 to SO_2 by reduced copper introduction.

$$SO_{2(g)} + 1/2O_{2(g)} \rightleftharpoons SO_{3(g)} \quad (3)$$

$$SO_{3(g)} + Cu^o \rightarrow SO_{2(g)} + CuO \quad (4)$$

The SO_2 formed after combustion was transferred into the stock flask (B) by replacing the bottle containing liquid N_2 from trap Tr_2 by the stock sample tube (ST). Next, the ST containing the SO_2 was transferred to the mass spectrometer admission system and the isotopic abundance of S (atom % ^{34}S) was determined.

Isotopic determination of (atom % ^{34}S)

The ^{34}S isotope was determined (in atom %) in the mass spectrometer (Bendassolli et al., 1997). The analysis based on isotopic ratios or atom % (abundance) of light elements is frequently carried out using properly purified gaseous samples. The proposed method involved procedures for chemical transformation in order to eliminate gas interferences, and the combustion process for SO_2 production was carried out in a vacuum system to avoid interferences of atmospheric gases.

The intensities of the ion peaks of the species SO_2^+ and SO^+ were high and both could be used for the determination of isotopic S. Therefore, it was decided to work with SO^+ species for having a lower number of combinations between S and O elements. For SO molecular species, ion currents appear at m/z 48 ($^{32}S^{16}O$); 49 (^{32}S, ^{17}O and $^{33}S^{16}O$); 50 ($^{32}S^{18}O$; $^{33}S^{17}O$ and $^{34}S^{16}O$); 51 ($^{33}S^{18}O$ and $^{34}S^{17}O$); 52 ($^{34}S^{18}O$ and $^{36}S^{16}0$); 53 ($^{36}S^{17}O$) and 54 ($^{36}S^{18}O$). For analysis, the mass spectrometer worked with an admission system heated to 70 °C due to the polar nature of the SO_2 and SO molecules, thus avoiding a memory effect. A cryogenic trap containing dry ice and ethanol (-70 °C) was placed below the mass spectrometer admission system to retain undesirable residual water from samples. The tests were carried out with five replications, in a completely randomized design, in factorial combination. The means were subjected to statistical analysis (Tukey 5 %) (SAS, 2008).

RESULTS AND DISCUSSION

In experiments related to the use of metallic copper in the quartz tube, it was observed that 2 g of material was necessary to avoid undesired oxide formation (BaO and CaO) in the vacuum system. In the assessment of the time required for SO_2 formation in the high-vacuum system, the oven-burning time of $BaSO_4$ varied from 4 to 10 min. Results indicated that combustion was complete after 10 min, and no alteration of the signal was observed after longer periods. Therefore, 10 min was determined for this step, for improving parameters related to volatile compound formation. For the isotopic determination of S, the peaks in the mass spectrum were related to the species SO^+ (Figure 2).

Results for isotopic determination of ^{34}S in SO_2 samples obtained applying the $BaSO_4$ p.a. (natural abundance) was 4.23 ± 0.02 atom % ^{34}S. The corresponding values of samples with natural variation are in agreement with the expected values, demonstrating that the process for SO_2 production in presence of $NaPO_3$ and the method for isotopic determination of S in the mass spectrometer were suitable. Validation of the proposed method was

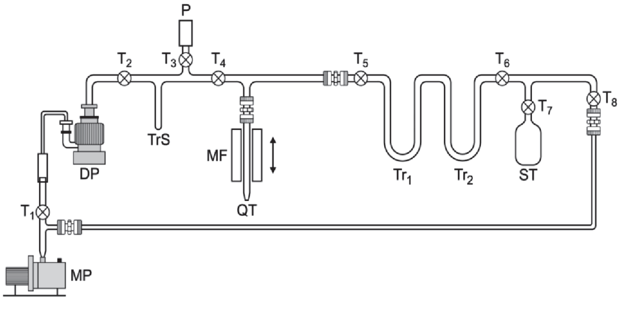

Figure 1. Vacuum system for SO_2 production and purification. QT = quartz tube; MF = movable furnace (900 °C); Tr_1 = trap with dry ice / ethanol (-70 °C); Tr_2 = trap with liquid nitrogen (-196 °C); ST: stock sample tube; P = Pirani vacuum gauge filament sensor (Edwards); TrS = security trap with liquid nitrogen (-196 °C); DP = diffusion pump (high-vacuum); MP = mechanical pump; T_1 to T_8 = Taps

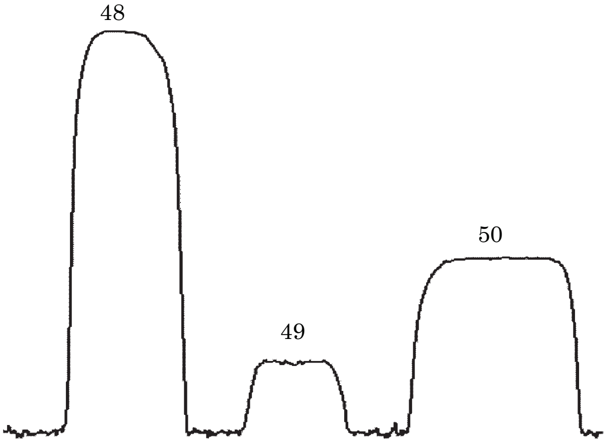

Figure 2. Mass spectrum related to SO^+ species by IRMS. Peaks were measured at m/e 48 ($^{32}S^{16}O$), 49 ($^{32}S^{17}O$ and $^{33}S^{16}O$), 50 ($^{32}S^{18}O$, $^{33}S^{17}O$ and $^{34}S^{16}O$).

accomplished by analysis of different soil types classified according to the Brazilian System of Soils Classification (Embrapa, 2006). The initial amount of S (µg g^{-1} of S) and isotopic abundance (atom% of ^{34}S) was evaluated (Table 1).

The ^{34}S (atom %) in soil samples was determined in three replications and in the isotopic results evaluation the natural variation from -30 to +30 ‰ was taken into account (Krouse & Tabatabai, 1986). These natural variation values correspond to a variation of 4.15 - 4.38 atom % ^{34}S. The analytical precision of the mass spectrometer is approximately 1 %. After addition of 700.0 and 400.0 µg of S in labeled $K_2{}^{34}SO_4$ to 5 g of Nitossolo Vermelho eutroférrico (NVef) and Argissolo Vermelho-Amarelo (AVAd), respectively, the values for experimental isotopic abundance (Ab_{exp}) and theoretical isotopic abundance (Ab_t) were determined (Table 2). The values of Ab_t and Ab_{exp} were obtained by equation 1 and by IRMS, respectively.

Results indicated no significant difference between experimental isotopic abundance (Ab_{exp}) and theoretical isotopic abundance (Ab_t) for the Argissolo Vermelho-Amarelo (Table 2). However, for the Nitossolo Vermelho eutroférrico, the statistical analysis indicated significant differences ($p < 0.05$) when $K_2{}^{34}SO_4$, labeled on 6.15 atom % ^{34}S, was used.

Based on results obtained for S isotopic determination (atom % ^{34}S) by using ^{34}S-labeled K_2SO_4, the analytical error was calculated as about 1.1 %. This result was considered low, considering the number of steps in the proposed method, and the complexity of the analytical process.

CONCLUSIONS

1. The use of a high-vacuum system for conversion of $Ba^{34}SO_4$ to SO_2 using $NaPO_3$ and combustion is suitable for the proposed method. Based on the results obtained in the validation experiments (isotopic dilution), it was verified that dry oxidation is feasible for the conversion of organic S to sulphate.

2. The results obtained in isotopic determination of S by isotope-ratio mass spectrometry (IRMS) were precise and accurate, showing the viability of the proposed method.

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Table 1. Total S and isotopic determination (atom % ^{34}S) in soil samples

Soil (1)	ST_0 (2)	Ab_0 (3)
	µg g^{-1}	atom % ^{34}S
Latossolo Vermelho eutroférrico (LVef)	250.1±1.2	4.43±0.01
Neossolo Quartzarênico órtico (NQo)	61.0±5.4	4.41±0.02
Nitossolo Vermelho eutroférrico (NVef)	222.5±4.8	4.41±0.01
Latossolo Vermelho distrófico (LVd)	167.7±3.5	4.43±0.03
Argissolo Vermelho-Amarelo distrófico (AVAd)	135.6±2.2	4.43±0.03
Argissolo Vermelho distrófico (AVd)	103.9±4.8	4.45±0.02

(1)Brazilian Soils Classification System; (2)Results of total initial S determined by turbidimetry; (3)Results of isotopic S initial (natural abundance) determined by IRMS; ± Relative standard deviations (n=5).

Table 2. Isotopic determination of S (atom % ^{34}S) in soil samples with application of labeled $K_2{}^{34}SO_4$

Labeled $K_2{}^{34}SO_4$	Nitossolo Vermelho eutroférrico (NVef)		Argissolo Vermelho-Amarelo distrófico (AVAd)	
	Ab_{exp}	Ab_t	Ab_{exp}	Ab_t
6.15	5.21±0.03a	5.08±0.01b	5.15±0.01a	5.07±0.01a
8.97	6.25±0.02a	6.17±0.02a	6.06±0.03a	6.11±0.02a
12.51	7.64±0.08a	7.54±0.03a	7.49±0.06a	7.43±0.03a
15.03	8.55±0.07a	8.51±0.04a	8.29±0.08a	8.36±0.03a

Results represent the S isotopic abundance in the soil samples, determined by mass spectrometry. Means followed by the same letter in the rows did not differ statistically (Tukey test, $p<0.05$, n=5); ± Relative standard deviations.

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