METHODS OF ANALYSIS
FOR SULFUR IN SOILS

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ABSTRACT

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By

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A bibliographic review (including more than 70 references) of available methods for soil and plant analysis for sulfur (S) was made.

Based on a criterion of sensitivity, accuracy, and simplicity, three methods for total and extractable S, and extractable sulfate (SO_4) were selected.

The method used for total S consisted of an alkaline oxidation of the soil samples with sodium hypobromite which transformed all S form to SO_4. The determination procedure was based on SO_4 reduction to H_2S using a reducing mixture composed of HI, HOAc, and NaH_2PO_2. The H_2S generated was then measured colorimetrically by the methylene blue method.

Based on results of preliminary tests, it was possible to modify this procedure, thus reducing significantly the time of analysis and, in one soil sample, increasing the amount of S measured.

Three Michigan soils -- Morley, Hoytville, and Miami series -- were used in this study. Total S was measured in natural soil samples and in samples with 100 ppm of added S which were subsequently incubated for two weeks at 20 C. Original S contents were 222, 424, and 201 ppm of S, and the percent recoveries of added S were 100, 103.5, and

and 106.2, respectively.

Extraction with water and with a 500 ppm P solution was used to evaluate extractable S; this was measured with the methylene blue method. These extractions were made on soil samples which had 0 and 6 weeks of incubation time and with 0 and 100 ppm of added S. Significant increases in the amounts of extractable S due to incubation were found with both extractants in the samples without added S. Only one sample, with 100 ppm of added S, showed this same effect when extracted with water.

These same extracts, after being clarified with $FeCl_3$ or activated charcoal depending on their degree of turbidity, were used for SO_4 analysis.

The method initially selected consisted of a precipitation of the SO_4 with $Ba(IO_3)_2$. Colorimetry of the free IO_3 after its reduction to I_2, gave an indirect measurement of the SO_4 concentration.

As this method offered several problems, a new one was developed using the same precipitation technique. Although not standardized in a definite way, as little as 0.15 ppm of SO_4-S was measured by the colorimetric measurement of a iodine/acetonitrile system.

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This work is dedicated
to the memory of my father
and to my beloved families who,
by my side and from abroad,
helped me so much.

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INTRODUCTION

Sulfur, although an essential nutrient for plant growth, has not had the attention of researchers in the same proportion as other nutrients. This situation is presently changing due to several reasons, namely: large increases in crop yields with a consequent increased demand for this element; an increase in the use of S-free fertilizers; decrease in the use of pesticides containing S; and the use of energy sources with a relatively low S content, resulting in lower S additions from the air.

This same situation holds true for Argentina, where some reports of S deficiencies have appeared. These isolated reports will probably begin to extend to greater areas as more studies are performed, and as water, nitrogen, and phosphorus nutritional aspects are improved; thus, S and perhaps certain micronutrients may become limiting factors for plant growth. This problem could be particularly serious in farming areas far away from industrial centers in which atmospheric-S inputs would not be enough to compensate for soil-S deficiencies.

The main objectives of this study were: 1) to select and modify methods for S analysis applicable to research on S needs in Argentina; 2) to compare the quantity of S extracted with water to that extracted in a P solution; and 3) to study the effect of sample incubation upon extractable S.

LITERATURE REVIEW

A large number of different methods to evaluate the sulfur status in plants and soils are available from the literature of the last 20 years. These may be arbitrarily divided into methods of extraction and determination as follows:

A. Total soil S extraction
B. Total plant S extraction
C. Extraction of available S
D. Quantitative determination of extracted S

The word arbitrarily was used because most research work embodies both extraction and determination procedures. Nevertheless, in order to obtain a clearer picture of this subject, the above division will be kept through this literature review.

A. Total Soil S Extraction

Two general methods for extracting total soil S exist:

1. Reduction methods,
2. Oxidation methods.

1. Reduction Methods

Within this group, the technique most oftenly used is that of Johnson and Nishita (1952). It is basically a digestion of the soil sample at 115 C with a reducing mixture composed of hydriodic acid, formic acid and red phosphorus. Most of the S present in the sample is reduced to H_2S. This gas is then passed through a pyrogallol and NaH_2PO_4 gas washing solution by a constant N_2 flow, to finally react with a solution of ZnOAc-NaOAc. Both, the gas washing and the ZnOAc-NaOAc solutions, must be free of traces of heavy metals, particularly Cu, due to the precipitation of CuS (Johnson and Arkley, 1954).

A detailed study of the reduction and color reaction steps has been done by Gustafsson (1960a, 1960b). This researcher has modified, among other things, the composition of the reducing mixture, by substituting hypophosphorus acid for red phosphorus.

Several researchers, Spencer and Freney (1960), Dean (1966), Searle (1968), Roberts and Koehler (1968), Walker (1972), and Walker and Doornenbal (1972), have used this technique.

Johnson and Nishita (1952), proposed this same technique for the analysis of water samples, soil extracts, and plant samples.

Since this method does not distinguish between organic and inorganic S forms, Freney (1957) adapted it for SO_4 analysis of water samples and soil extracts. He proposed the use of bacteriological filters to clean the sample of organic and inorganic colloids. Finally the SO_4-S was separated by precipitation, as $BaSO_4$. This researcher also showed that the drier the soil samples were, prior to the extraction, the more was the SO_4-S extracted by water.

The reduction of soil elemental S to H_2S has been employed by Barrow (1968), who extracted this element with chloroform and reduced it afterwards with Fe powder in acid solution.

Magnesium metal in acid solution has also been used as a reducing agent for S by Steinbergs et al. (1962). They stated that total soil S values obtained by this technique were smaller than those obtained by oxidation methods.

Carson et al. (1972), based on Dean's (1966) modification of Johnson and Nishita's (1952) method, used a reducing mixture composed of hydriodic, formic, and hypophosphorus acid, avoided the use of the gas washing solution, and trapped the evolved H_2S in NaOH, thus simplifying the reduction-distillation apparatus.

2. Oxidation Methods

Different researchers have reported trying many different oxidation mixtures to oxidize all forms of S to SO_2, SO_3, and SO_4^{-2}.

Sodium peroxide has been used by Steinbergs (1955). Butters and Chenery (1959) tried HNO_3 and $Mg(NO_3)_2$ at 300 C for 10 hours. Bardsley and Lancaster (1960) and Spencer and Freney (1960), used $NaHCO_3$ at 500 C for 3 hours, and Steinbergs et al. (1962) used $NaHCO_3$ and Ag_2O at 550 C. Saalbach, Kessen, and Judel (1962) used $KClO_3$, and Bloomfield (1962) proposed the use of V_2O_5 in a N_2 flow; the complete oxidation of the different volatile compounds was achieved by passing the vapors through hot CuO. Chaudry and Cornfield (1966) used a HNO_3-KNO_3 mixture at 550 C for 3 hours. Jenkinson (1968) used a three steps method to evaluate total soil S. All S forms were first oxidized with $K_2Cr_2O_7$ and dehydrated H_3PO_4. The SO_4 produced was then reduced to SO_2 by heating with activated charcoal in H_3PO_4. This gas was trapped in H_2O_2 where it formed H_2SO_4. The quantification of the acid was done by a titrimetric technique. According to the author the overall sensitivity of the method could be increased by means of a colorimetric instead of the titrimetric determination. One of the drawbacks of this method was that the hot H_3PO_4 attacked the glass of the digestion flask, making it necessary to replace the flask each 20 to 50 digestions.

The often used high-frequency induction furnace technique is also an oxidation method. Searle (1971), Cadahia (1971), and Tiederman and Anderson (1971), among others, have used this technique. Basically, the soil sample is mixed with a catalytic oxidant mixture composed of powdered Fe, MoO_3 and CrO_3, and heated by a high-frequency electrical flux induced in the sample itself, in which Fe acts as the conductor. An O_2 stream carries the S oxides produced, which react with a solution of NaOH and are then measured by the Johnson and Nishita (1952) technique.

Another method for total S determination is the one proposed by Tabatabai and Bremner (1970a). The soil sample undergoes a wet oxidation with NaBrO and the oxidized S is quantified afterwards by the Johnson and Nishita (1952) method.

Tabatabai and Bremner (1970b) also published a critical comparison of several of these oxidation methods.

B. Total Plant S Extraction

Almost all the methods mentioned above can be used for S analysis in plants, although some minor modifications may be needed.

Among those not mentioned for soils, Lysyj and Zarembo (1958), developed a technique to measure the S content of organic compounds, adapted afterwards by Iismaa (1959) for plant analysis. This method is based on the combustion of the sample in an Erlenmeyer flask with NaOH and H_2O_2; the S is then measured by Johnson and Nishita's (1952) method.

The wet digestion with HNO_3 and $HClO_4$ has been proposed by Shaw (1959), the SO_4-S being measured by gravimetry or any other available technique.

C. Extraction of Available S

Several studies to correlate extractable S with plant yields or S content in plants have been done. Ensminger and Freney (1966) published a bibliographic review (73 references) related to the diagnosis of S deficiencies by plant and soil analysis.

The expression "extractable S" includes many S fractions. Williams and Steinbergs (1958) correlated eight of these fractions (SO_4-S, total water-soluble S, heat-soluble S, SO_4 soluble after ignition, alkali soluble S, reducible S, and SO_4 released by H_2O_2 oxidation), with S uptake and yield of dry matter of oats.

Spencer and Freney (1960) correlated S uptake of Phalaris tuberosa with the following S fractions: acetate-extractable S, cold-water extractable S, PO_4-extractable S, Aspergillus S, heat-soluble S, hot-water-extractable S, reducible S, reserve S, and total S.

Good correlations were found between S uptake by plants and SO_4-S, total water-soluble S, and heat-soluble S in the research by Williams and Steinbergs (1958); while Spencer and Freney (1960) found good correlations with Aspergillus S and PO_4-extractable S.

Fox, Olson and Rhoades (1964) found that water-extractable S and a 100 ppm P solution as $Ca(H_2PO_4)_2$ gave the best agreement with sulfur A values and total S uptake by alfalfa. However, they pointed out that while both extractants behaved the same in unweathered soils, this did not hold true for weathered soils (with sorbed SO_4) in which the P extraction correlated better with S uptake than did water-extractable S.

Magnesium chloride and LiCl were used by Roberts and Koehler (1968) and $CaCl_2$ by Walker and Doornenbal (1972) -- all found good correlations between extractable S with these solutions and plant yield.

In a recent work, Hoeft, Walsh, and Keeney (1973) used four different P solutions, $CaCl_2$ and Bray P_1 for S extraction. The best correlation with alfalfa yield was given by the S extracted by a 500 ppm P-2N HOAc solution, with the P as $Ca(H_2PO_4)_2$. They also used a regression equation to predict the yield response of alfalfa with good results.

From this review it can be concluded that there is a tendency to use salt solutions, particularly of P, to study correlations between extractable S and plant yields. Total S and other artificial S fractions have been set aside, while in some cases microbiological assays and water-extractable S could still be used with some advantage.

D. Quantitative Determination of Extracted S

A great variety of methods are actually available, even though none of them satisfy all of the requirements of a good analytic technique, namely: accuracy, precision, sensitivity, quickness and economy.

Although much has been done in the last 20 years in terms of methods and instrumentation, S still offers some difficulties in its quantification, especially with minute quantities.

The methods to be presented will be grouped according to the physical, physical-chemical or chemical principle upon which they are based.

1. Turbidimetry

One of the first applications of turbidimetric methods to soils was that of Chesnin and Yien (1950). These authors briefly reviewed an early application of it, particularly in the field of medicine. To extract the soil S, they used the "Morgan solution" (1941) formed by NaOAc and HOAc. Then, they precipitated the extracted SO_4 with 30-60 mesh $BaCl_2$ crystals. The $BaSO_4$ precipitate was stabilized by a gum acacia suspension and the readings made in a colorimeter with a blue filter. Reported results with this technique were compared with those obtained by gravimetry.

Steinbergs (1953), found that the sensibility and reproducibility of the method was somewhat affected by insoluble impurities present in the $BaCl_2$ used as the precipitating agent. These impurities acted as crystallization nuclei causing a more homogeneous $BaSO_4$ precipitate.

Due to the high purity of commercial $BaCl_2$, Steinbergs (1955) prepared standardized solutions seeded with $BaSO_4$ crystals and with excess Ba. where these crystals acted as crystallization nuclei. He added the unknowns or the standards to these solutions and read the transmission 60 to 90 minutes after mixing. This same technique was used to study different fractions of S as indexes of available S (Williams and Steinbergs, 1959). According to the indicated procedure no gum acacia solution was needed with this technique.

Since Morgan's solutions extracted colloidal organic matter which interferred with the analytical method, Hesse (1957) proposed a modification of Chesnin and Yien's (1950) method. The organic matter was removed by precipitation with $Fe(OH)_3$ and separated afterwards by filtration. According to Bartlett and Meller (1960), when soil extracts from Morgan's solution contained less than 20 ppm SO_4 (6.7 ppm S), more than 6 g of $BaCl_2$ per 50 ml of extract should be added to obtain a quantitative precipitation of the $BaSO_4$.

A very detailed study of the best conditions to use turbidimetry for total S in plants and soils has been done by Butters and Chenery (1959). They studied the effect of such variables as size of the $BaCl_2$ crystals, buffered medium to obtain a good dissolution rate of these crystals, the use of H_3PO_4, time of precipitation, and interferences from other ions present. They also used the gum acacia solution as a stabilizer, prepared according to Chesnin and Yien's (1950) procedure.

Massoumi and Cornfield (1963), used this method to analyze soil extracts by introducing some modifications to increase its sensitivity. With these modifications they were able to detect 2 ppm of S, (also the method could be used for either total soil or plant S).

Tabatabai and Bremner (1970a) compared turbidimetry with the Johnson and Nishita (1952) procedure with good correlation.

Due to the need of a very careful standardization of this technique in each of its steps plus the problem of the size of the $BaCl_2$ crystals, Basson and Bohmer (1972) proposed using the Technicon autoanalizer for the SO_4 turbidimetry. With this type of analyzer each standard and each sample are treated in the same way, maintaining these conditions through all the analysis. The authors used this method to analyze SO_4 content in plant samples digested with HNO_3 and $HClO_4$.

Several researcher, for example, Bardsley and Lancaster (1960), Dodgson (1961), Chaudry and Cornfield (1966), and Souty and Guennelon (1967), have used turbidimetry as a mean for SO_4 determination. Only a portion of those who have improved this technique have been mentioned here.

2. Nephelometry

An important contribution to the standarization of this technique for SO_4 determination as $BaSO_4$ was done by Toennies and Balsay (1953). These researchers studied the effect of organic solvents, pH and interferences of other ions on the sensitivity and reproducibility with this method. According to the authors, the main reasons for erratic results were: deficient washing of the tubes, SO_4 contamination of the distilled water, and aging of the $BaCl_2$ reagent.

Another modification of this method was by the use of organic precipitants to precipitate the SO_4. Based on early works by Belcher, Nutten, and Stephen (1953), Belcher et al. (1956), and Belcher and Stephen (1959) -- who found that the 4-amine 4'-chlorodiphenyl chloride (CAD) gave a highly insoluble salt with SO_4 -- Martin and Stephen (1967a) applied this reaction to the nephelometry of this anion. They described in detail a technique to measure between 2.5 and 25 ppm of SO_4 (0.83 to 8.3 ppm of S) using CAD and a stabilizing solution of gum ghatti. They also studied some possible anionic interferences. In a short communication, Martin and Stephen (1967b) reported increased sensitivity to 1 ppm detectable SO_4 through improvement of the stabilizing solution.

Stephen (1970) described the precipitation of SO_4 with 2-amino pyrimidine. He found this salt to be even more insoluble than CAD-SO_4

forming regularly shaped and sized crystals with a very low tendency to coagulate even after standing for several hours. The author reported that SO_4 concentrations as low as 0.05 ppm yielded a visible precipitate.

3. Absorption Spectrometry

As mentioned early, one of the most widely used methods for soil and plant S analysis, is that of Johnson and Nishita (1952). The H_2S produced during the reduction step reacts with the ZnOAc-NaOAc, yielding ZnS. Due to the acidification with a sulfuric solution of p-amine dimetylaniline, the H_2S is liberated reacting with the base (the resulting solution is colorless in the reduced form, Bardsley and Lancaster, 1965). By adding Fe^{+3} ions, the base changes to the oxidized form, generating a blue color (Johnson and Urlich, 1959), which is measured with a spectrophotometer.

A detailed study of the experimental conditions that affect this reaction has been done by Gustafsson (1960b).

In a recent work, Kirsten and Patel (1971) proposed the addition of pyridine or sodium dodecyl benzene sulfonate to reduce or eliminate the association among methylene blue molecules caused by S concentrations exceeding 50 micrograms/100 ml (Johnson and Nishita, 1952).

To increase the sensitivity of the method, they also proposed the extraction of the methylene blue with a small volume of organic solvent, namely 1,1,2,2,-tetrachloroethane and n-octanol-2. In this way they reported detecting as little as 0.2 micrograms of S.

Dean (1966) and Carson et al. (1972), collected the H_2S in NaOH. Following this, a reagent composed by $Bi(NO_3)_3$., HOAc and gelatine was added, and the absorbancy of the resulting solution measured with a spectrophotometer.

Bloomfield (1962), utilized p-rosaniline and a formaldehyde solution to measure total S in soils colorimetrically.

Another technique is based on the reaction of S^{-2} with organic disulfides. Several of these disulfides were studied by Humphrey et al. (1971) and according to the authors, between 0.05 and 21 ppm of S can be detected with this technique.

4. Atomic Absorption Spectrometry

This method is generally based on the precipitation of the SO_4 ions with $BaCl_2$, filtering the precipitate and measuring the excess Ba left in solution. Thus, the amount of SO_4 in the sample is determined by difference, (Warley and Poou Yien Chin, 1970; Borden and McCormick, 1970; and Burge, 1970).

The major difference between the two first studies is that, in the first one they did the conductimetric measurement of the extract to approximate the concentration of SO_4. In this way they were able to save time by taking aliquots that would fall within the range of the standard curve; thus they did not need to make any dilution after beginning with a group of samples.

5. Emission Spectrometry

This technique has been recently applied by Forbes (1973) to measure SO_4 in an indirect way (by the same technique as in atomic absorption), adding excess $BaCl_2$ to the sample containing SO_4 with 50 % propan-2-ol. Following precipitation and centrifugation, the unreacted Ba content of the solution is measured by its emission in a nitrous oxide-acetylene flame. According to the author, SO_4 contents ranging from 0.5 to 5.0 and 1.0 to 10 ppm have been measured with this procedure.

6. Potentiometric Titrations

This technique is based on the titration of the sample containing SO_4 with a Pb salt solution, where a change in potential indicates the end point of the titration.

Cortellessa and Napoli (1969) titrated the SO_4 solution with a $Pb(NO_3)_2$ solution, after addition of potassium hexacyanoferrate II and potassium hexacyanoferrate III, with a Pt wire as the measuring electrode and a Ag reference electrode. At the equivalence point, the precipitation of hexacyanoferrate II ocurred with a resulting sharp change in the potential of the Pt electrode. Ross and Frant (1969) and Goertzen and Oster (1972) used a Pb selective electrode and titrated the SO_4 solution with a standard $PbClO_4$ solution. According to the latter, 0.5 meq/l of SO_4 could be measured with this technique.

7. Polarography

Khan and Webster (1968) based their technique on the precipitation of SO_4 in soil extracts as $PbSO_4$. The excess of Pb was measured by a dropping Hg electrode polarized at a potential corresponding to the diffusion current of Pb ions. According to the authors they were able to measure a minimum SO_4 concentration of about 3 ppm, with a non-commercial polarograph.

Garber and Wilson (1972) applied differential polarography to measure SO_2 by bubbling the gas in a dimethyl sulfoxide solution.

8. X-ray Emission Spectrography

Robert and Koehler (1968a) used this technique to analyze for S extractable with $MgCl_2$ from soils of Washington. They also did a very detailed study to determine its application to soil S and compared it with the methylene blue method (Roberts and Koehler, 1968b). According to the authors, this technique makes possible the analysis of 40 soil extracts per day with a S content varying between 0.5 and 14 ppm and a precision of ±2 to ±10 % in the determinations.

Souty and Guennelon (1967), used the same technique to analyze the S content in plants, avoiding in this way the calcination of the samples. They compared it with the oxygen bomb method followed by the turbidimetric determination of the SO_4 ions. Results by both methods were comparable in samples containing 0.3 % S or more. In samples with less S content, the oxygen bomb-turbidimetric determination was found to be more reliable than the X-ray emission spectrography.

Another comparative study with four other methods has been done by Tabatabai and Bremner (1970b) who used it for total soil S. They reported that results with this technique were comparable with other methods, but it had the disadvantage of a very time consuming preparation of the sample if only S was to be analyzed.

9. Coulometry

Coulometric methods of analysis are based on the exact measurement of the quantity of electricity that passes through a solution during the occurrence of an electrochemical reaction. The substance of interest may be oxidized or reduced at one of the electrodes (primary coulometric analysis) or may react quantitavely in solution with a single product of electrolysis (secondary coulometric analysis), (Willard, Merrit, and Dean, 1965).

In a recent work, Bailey and Bishop (1972) applied the secondary coulometric analysis to measure SO_2 concentration in water. They used a potentiometric method to find the end point of the redox reaction that took place in solution, obtaining a 900 mV potential change at this point, independent of the SO_2 concentration. The sensitivity of the method lay within 10^{-4} to 10^{-5} molar, with a precision better than 1 %.

10. Volumetry

There are several published works that use this principle to measure S in water extracts.

Belcher et al. (1953) studied the possibility of using substituted benzidines as precipitating agents for SO_4. Later, Belcher and Stephen (1959) developed a turbidimetric method using 4-amino4-chlorodiphenyl (CAD) to precipitate SO_4 which proved to be more insoluble than $BaSO_4$. In another work, Belcher et al. (1956) described a technique by which this anion was precipitated with excess CAD, and the excess reagent titrated with NaOH using a mixed indicator to determine visually the end point of the titration.

Fritz and Freeland (1954) also used volumetry for SO_4 analysis in aqueous extracts by a direct titration of the anion with a Ba standard solution using alizarin red as the indicator. The titration with Ba has been used by Armenau and Dragusin (1969) and by Ignatova and Abaturova (1970), employing carboxiazo III or carboxisulfonazo III and nitrocromazo respectively.

Sulfonazo III has been used as an indicator by Jenkinson (1968) in the titration of the excess of added Ba with a standard K_2SO_4 solution.

When measuring elemental S in soils by reduction with Fe powder in acid medium (Barrow, 1969), the H_2S produced was titrated with a standard $HgCl_2$ solution, using ditizone as an indicator.

Searle (1971), Cadahia (1971), Tiedeman and Anderson (1971), and Tabatabai and Bremner (1970b), who worked with the oxidation of soil and plant samples in a high-frequency induction furnace, also used volumetry. The SO_2 produced during the oxidation was trapped in HCl and titrated automatically with a KI standard solution.

MATERIALS AND METHODS

A. Materials

1. Soils

During the present research three surface soils from Michigan belonging to the series Morley, Hoytville and Miami were used. Some of their characteristics are given in Table 1. These soils developed under different drainage conditions. They present differences in texture and organic matter content, but the pH among them is very similar.

B. Methods

1. Preparation of the Samples

The soil samples were initially air dried and then ground to pass a 10 mesh sieve (2 mm).

Two hundred grams of each soil, replicated six times, were weighed and placed in plastic bags with hermetic sealing. Three replications were brought to field capacity with S-free water and used as controls. The rest were brought to field capacity with a K_2SO_4 solution, adding 0.100 g of S per 1000 g of soil (100 ppm) to each. Both, the controls and the treated samples, were incubated for 0, 2, and 6 weeks at 20 C. After incubation the samples were dried at 80 C and ground to pass a 35 mesh sieve (0.5 mm).

Table 1. Soil series, chemical and physical properties of the 3 Michigan soils used. (1)

Soil Series	Natural Drainage	Texture	Horizon	pH	Eh	Organic Matter
					mV	%
Morley	well drained	clay loam	A	6.38	569	3.09
Hoytville	poorly drained	clay	A	6.50	599	4.66
Miami	well drained	loam	A	6.48	544	2.84

(1) Data from Manoowetaya Srisen, M.S. Thesis, 1971. Michigan State University.

2. Extraction Procedures

a. Water extractable S

Reagents

Sulfur-free water: pass distilled water through different resins to extract organic matter, cations, and anions, (organic removal, D8904; cation removal, D8905; and mixed bed, D8907, from the Barnsted Sybron Corp., Boston, Mass.), and finally through an anion exchange resin in the Cl form, (Amberlite IRA-400 Ar).

Procedure

Weigh 20 g of sample and place in 100 ml centrifuge tube; add 50 ml of S-free water (soil:water ratio 1:2.5) and shake for 45 minutes on a wrist action shaker. Afterwards, centrifuge for 30 minutes at 2200 rpm, (an International centrifuge, Model 5V was used) and then filtrate through Whatman No. 42 filter paper. Each soil sample was extracted in triplicate.

b. Extractable S with a 500 ppm P solution

Reagents

Extractant solution: prepare a 500 ppm P solution in 2 N HOAc by dissolving 2.034 g of $Ca(H_2PO_4)_2.H_2O$ in 114.38 ml of HOAc (glacial) in a 1 liter volumetric flask. Dilute to 1 Liter with S-free water.

Procedure

The same procedure outlined for water extractable S was used for these extracts.

c. Water extractable SO_4

Reagents

1 N sodium hydroxide: dissolve 40 g of NaOH in S-free water and make to 1 liter.

5 % ferric chloride solution: dissolve 5 g of $FeCl_3.6H_2O$ in 100 ml of S-free water.

Procedure

While shaking add 2 ml of $FeCl_3$ solution to 25 ml aliquot of the extracts obtained according to 2.a. Then add 0.5 ml of the 1 N NaOH, shake and let the suspension stand for 2 or 3 minutes. If the supernatant liquid is still turbid and/or colored, add one drop of the base, shake and let stand for another 2 or 3 minutes. Repeat this procedure until the supernatant liquid is perfectly clear and colorless. Then filter the solution through Whatman No. 41 filter paper.

The pH of 54 extracts clarified according to the indicated procedure in every case was between 6 and 6.5.

The present technique is based on Hesse's (1957) work, and was adapted to use with aqueous extracts.

d. Extractable SO_4 with a 500 ppm P solution

Reagents

Washed activated charcoal: wash approximately 20 g of activated charcoal with 500 ml of 0.1 N HCl. Filter under vacuum with a Buchner funnel.

Procedure

Measure 25 ml of the extracts obtained according to 2.b. and place in a small Erlenmeyer. Add a small amount of washed activated charcoal, shake and filter through Whatman No. 42 filter paper. Controls using S-free water following this procedure did not show detectable SO_4 levels.

3. Total S in Soils

Tabatabai and Bremner's (1970a) procedure was followed for the alkaline oxidation step, and Gustafsson's (1960a, 1960b) for the reduction and color reaction steps. As these two last steps are common to several types of extraction procedures for both, total and extractable S, they are described as an independent method in Section 4.

Modifications introduced to these procedures are justified in the next chapter.

Alkaline oxidation (Tabatabai and Bremner, 1970a)

Apparatus: the digestion flask corresponds to Tabatabai and Bremner's (1970a) distillation unit.

Reagents

2 N sodium hydroxide: dissolve 80 g of NaOH in S-free water and make to 1 liter.

Sodium hypobromite solution: add slowly with continuous stirring (approximately 0.5 ml/min) 3 ml of Br_2 to 100 ml of 2 N NaOH. This reagent should be prepared fresh before using it.

Formic acid: concentrated, analytical reagent grade.

Procedure

Place sufficient sample to have between 10 and 60 micrograms of S (approximately 0.1 to 0.3 g of soil) in a digestion flask. Add 3 ml of the NaBrO solution, mix the contents gently and let stand for 5 minutes. Mix the contents again and place the flask in a sand bath at 250-260 C. Once the residue is dry (15 to 20 min), leave it in the bath for 30 additional minutes at the same temperature. Then remove the flask from the bath and allow it to cool at room temperature; add 1 ml of formic acid and continue with the reduction step (see 4.a.). Perform a blank for each set of samples.

4. Sulfur Determination by the Methylene Blue Method (Gustafsson, 1960a, 1960b).

a. Total S determination

Apparatus: A diagram of the reduction-distillation unit is shown in Tabatabai and Bremner's (1970a) work.

Reagents: For a complete list of reagents see Gustafsson (1960a, 1960b).

Procedure

Place 15-20 ml of S-free water in the gas washing column, wet both ends of the U tube with a drop of H_3PO_4 (Tabatabai and Bremner, 1970a) and attach it to the unit. Immerse the gas outlet tube in a 100 ml volumetric flask -- containing 65 ml of water and 10 ml of the ZnOAc-NaOAc solution -- with the end of the tube at 1 or 2 mm from the bottom of the flask. Begin the water circulation in the condenser and the N_2 flow, regulating it to obtain 150-200 ml/min with 0.75 atm of pressure.

Wet the ground glass connection of the unit with one drop of H_3PO_4. Add 4 ml of the reducing mixture to the flask that contains the digested sample with formic acid, and connect it to the unit by means of the two springs.

To prevent overheating, place an asbestos board fitted with a 3 cm diameter hole in the middle in contact with the flask, then light the burner regulating the tip of the blue flame to finish a few millimiters from the bottom of the flask. The sample should boil within 60 seconds and the distillation should be continued for 10 to 15 minutes.

Once this period is finished, disconnect the volumetric flask, leaving the gas outlet tube in the flask with its latex connection. Then add 10 ml of the methylene blue solution through the tube with a quick delivery pipette forming a layer in the bottom of the flask; raise the tube and rinse it with one or two jets of S-free water. Rotate the flask gently and immediately add 2 ml of the $Fe(NH_4)(SO_4)_2$ solution with a quick delivery pipette. Close the flask rapidly and shake it vigorously for 30 seconds. Then bring to volume with S-free water and leave it 15 minutes or more in subdued light for color development.

It is important to note that the time between the addition of the first solution and the shaking of the flask should be as short as possible to prevent gas losses.

All the readings were done with a Bausch and Lomb spectrophotometer (Model Spectronic 20) at 667 nm. Results were compared with those of a standard curve with 20, 40, 50, and 60 micrograms of S.

The unit was cleaned by attaching the gas outlet to the distillation unit and applying vacuum in the N_2 inlet, immersing the gas outlet in a beaker with S-free water 3 or 4 times. Once finished, the gas washing column was drained of the remaining water and refilled to the 15-20 ml mark by carefully applying vacuum. Once the digestion flask was disconnected, the unit was ready for another reduction.

Generally some water remained in the N_2 inlet tube and between this tube and the ground glass connection. This would cause dilution of the reducing mixture, therefore, the N_2 inlet tube was washed with one or two jets of acetone and the inner side of the ground glass connection dried with absorbing paper.

b. Extractable S determination

Place an aliquot containing between 10 and 60 micrograms of S in the reduction flask. Dry the content in an oven at 120-130 C. Cool the flask to room temperature and add 4 ml of the reducing mixture. Then follow the same procedure as described above.

5. Sulfate Determination by I_2 Colorimetry

Reagents

Potassium iodide: KI crystals of reagent quality.

Acetonitrile: glass-distilled quality.

Barium iodate: dissolve 10 g of KIO_3 in 500 ml of S-free water and 5 g of $Ba(NO_3)_2$ in another 500 ml of S-free water. Mix both solutions and stir for 4 or 5 minutes. Let it stand and eliminate excess water. Repeat these washings with water 3 or 4 times. Do the last one with ethyl alcohol, filter under vacuum and dry the residue in an oven at 90 C.

Sulfur standard solution: dissolve 5.43 g of K_2SO_4 (dried at 105 C for 2 hours) in S-free water and dilute to 1 liter. This solution contains 1000 ppm S as SO_4.

Procedure

Place 16 ml of acetonitrile in a 50 ml Erlenmeyer, add an aliquot containing between 3 and 15 micrograms of SO_4-S and dilute to 20 ml with S-free water. Add about 30 to 40 mg of $Ba(IO_3)_2$ to the solution and let it stand for 1 hour, shaking occasionally.

Filter afterwards through Whatman No. 42 filter paper, receiving the filtrate in a 25 ml volumetric flask. Add some KI crystals, shake until dissolved, and then add 2 ml of glacial HOAc.

Dilute to the mark with S-free water and measure after 10 minutes at 400 nm. Perform a blank for each set of samples.

Compare the results with those of a standard curve with 3, 5, 10, and 15 micrograms of SO_4-S using the same procedure.

RESULTS AND DISCUSSION

1. Total S Determination

In the present investigation, Tabatabai and Bremner's (1970a) method was used.

Two modifications were introduced to their original procedure in an attempt to reduce the time of analysis per sample and to increase, in some cases, the amount of S measured.

Tabatabai and Bremner's (1970a) original contribution was in the procedure to oxidize the organic and inorganic S present in the sample and also in the modification of the distillation unit. In the second part of the method -- SO_4 reduction and color reaction steps -- they used the method of Johnson and Nishita (1952), in which these authors proposed a one hour reduction-distillation period. Nevertheless, Gustafsson (1960a) in a very detailed study of the reduction step, suggested that a 10 minute reduction-distillation period was enough for the S reduction and transfer of the H_2S formed.

Since this would mean a great time saving per sample, some preliminary studies were performed to determine if accuracy was still mantained. Results can be seen in Table 2.

The analysis of variance (Table 3) shows that the recovery of S between 10 minutes and 1 hour of reduction-distillation did not vary significantly, at the 1 % level, for any of the soils. Therefore, a 10 minute reduction-distillation was adopted for all the analysis performed by this method.

Table 2. **Influence of the time** of reduction-distillation and of **residue** dilution upon total soil S **determination.**

Soil Series	Reduction-Distillation Time											
	10 minutes						1 hour					
	No dilution			Dilution			No dilution			Dilution		
	ppm*	SD	CV(%)	ppm*	SD	CV(%)	ppm*	SD	CV(%)	ppm*	SD	CV(%)
Morley	221	3.7	1.6	157	7.3	4.6	214	15.2	7.1	158	8.5	5.4
Hoytville	422	18.8	4.4	465	9.7	2.1	445	23.5	5.3	406	17.5	4.3
Miami	233	17.5	7.5	213	4.3	2.0	218	7.5	3.4	189	17.6	9.3

(*) Micrograms of S per gram of soil, average of two replicates.

Table 3. Analysis of variance of experiment of the influence of the reduction-distillation time and of residue dilution.

Source of Variation	Degrees of Freedom		Sum of Squares	Mean Squares	F
MORLEY SERIES					
Treatments	3		7350.0		
Time		1	13.9	13.9	0.15
Dilution		1	7306.2	7306.2	82.16 *
Interaction (TxD)		1	29.9	29.9	0.33
Experimental error	4		355.7	88.9	
Total	7		7706.3		
HOYTVILLE SERIES					
Treatments	3		4098.0		
Time		1	680.2	680.2	2.10
Dilution		1	7.3	7.3	0.02
Interaction (TxD)		1	3410.5	3410.5	10.52
Experimental error	4		1259.0	323.9	
Total	7		5393.0		
MIAMI SERIES					
Treatments	3		1971.0		
Time		1	749.1	749.1	4.67
Dilution		1	1174.4	1174.4	7.47
Interaction (TxD)		1	47.5	47.5	0.30
Experimental error	4		628.7	157.2	
Total	7		2599.7		

(*) Significant at the 1 % level.

The second modification is related to the dilution of the sample as proposed by Tabatabai and Bremner (1970a).

According to their procedure, once the alkaline oxidation is finished, and with the flask at room temperature, 1 ml of S-free water should be added to the residue heating for a few seconds in order to resuspend it. After this, 1 ml of formic acid and 4 ml of the reducing mixture are added and the reduction-distillation begins immediately.

In the work by Gustafsson (1970a), already mentioned, the author stresses the effect of diluting the reducing mixture. She found that the addition of 0.5 ml of water to an evaporated SO_4 sample decreased the recovery of SO_4 by 6 %. Two mililiters of water diminished the yield by 35 %.

Results from preliminary tests conducted during this investigation were not so conclussive. It can be seen in Table 3 that only one soil (Morley series) showed a significant difference at a 1 % level not favoring the dilution of the sample.

Nevertheless, from Table 2 it is clear that the sample of the Miami series shows the same tendency, although at a different level of significance, (6 %).

The most difficult results to explain are those of the Hoytville series which showed a significant interaction between the two variables at the 5 % level, thus not allowing a separate analysis of the behavior of both variables.

Therefore, although the results of the analysis of variance at the 1 % level of significance would allow the modification of the original procedure with respect to the dilution of the oxidation residue, they are not sufficiently conclusive. It might be suggested, thus, that more soils should be analyzed with respect to this behavior before adopting a definite procedure.

A side effect of this latter modification was seen during the washing of the digestion flasks. The formic acid did not suspend the residue nor did the reducing mixture, therefore leading to difficulties in washing the glassware.

This problem, although not sufficiently tested, was overcome by reversing the order of addition of the reagents, namely adding first the reducing mixture, then the formic acid, and beginning the distillation immediately. No variation in the results was observed when this procedure was followed.

The values of total S for the three soils analyzed can be seen in Table 4. The percentage of recovery for the samples which received 100 ppm of SO_4-S are also given in this table.

These values are sufficiently accurate and comparable with those given by Tabatabai and Bremner (1970a), if the differences in the S application techniques are considered. These authors added the organic and inorganic S to the subsample to be analyzed (0.1 to 0.2 gr of soil) with 1 ml of water, while in the present investigation the S was added to 200 gr of soil, of which the subsamples for analysis were withdrawn after a 2 weeks incubation period.

Considering the sources of error -- sampling, subsampling, weighing error, intrinsical error of the method, heterogeneity due to an uneven mixing of the added S, instrumental error and reading error-- the variation coefficients are within acceptable limits, (except for a 6.6 % value, all the rest are below 4 %). With a better standarization

Table 4. Total soil S and percent recovery of added S.

Soil Series	Sample Weight	Total S	Added S	S.D.	Recovery	C.V.
	mg	ppm*	ppm		%	%
Morley	200	222	---	8.2	---	3.7
Hoytville	200	424	---	7.7	---	1.8
Miami	200	201	---	13.3	---	6.6
Morley	100	322	100	5.9	100	1.8
Hoytville	100	557	100	13.1	106.2	2.3
Miami	100	304	100	10.6	103.5	3.5

(*) Average of three replicates.

of the technique these values could have been kept below 3 %. All results were reproducible.

Based on the accquired experience, three points could be subject of further standarization:

a. Nitrogen flow: Although disagreement exists among researchers as to which is its optimum value, the important factor, within limits, is to have the same flow for each unit. Thus, a good calibration at each outlet is required, particularly since reducing the reduction-distillation time makes this variable more critical.

b. Heating: According to Johnson and Nishita's (1952) technique, an asbestos board is interposed between the flame and the distillation flask. Gustafsson (1960a) fitted the board with a 3 cm diameter hole in its middle part. With this modification she obtained a faster boiling of the solution while preventing overheating of the flask walls. This researcher also reported an incomplete S reduction the to distillations carried without boiling the solution.

Nevertheless, care should be taken not to allow direct contact between the flame and the flask, thus, overheating the sample. During the first distillations of standard S solutions, working with 4 units simultaneously, bad flame regulations allowing overheating of the flasks yielded losses in the S recovery.

Therefore, use of good quality burners is recommended, in which an accurate regulation of the flame is permitted.

c. Temperature regulation during the color development reaction: Gustafsson (1960b) studied the influence of this variable on the color reaction step. Her conclusions are summarized as follows: A compromise relation exists between the temperature of the solution before the addition of the reagents and the intensity of the blue color formed. With increasing temperatures, the H_2S tends to escape to the gaseous phase, therefore diminishing the color intensity. With lower temperatures this problem is minimized but the speed of the color formation reaction is much slower, thus allowing secondary reactions to occur which reduce the yield of the methylene blue color.

According to the author, some researchers have found an optimum temperature for the reaction at 24 C, although she does not confirm those same results.

The second compromise relation is established between the temperature at the moment of measurement and the absorbance obtained. To measure this effect, Gustafsson (1960b) worked with a thermo-regulated cell-compartment in the spectrophotometer. Her conclusions were that in this case the temperature effect was positive, that is, the absorbance increased with the temperature. She proposed 20 C as the optimum temperature to satisfy both relations.

2. Extractable S Determination

Sulfur-free water and a 500 ppm P solution were used as extractant solutions. Gustafsson's (1960a, 1960b) procedure was followed for the analysis of the extracted S. The comments regarding this technique were already made and they will not be discussed further; the three points mentioned -- N_2 flow, heating, and temperature regulation in the color reaction step, apply equally here.

The results obtained from the analysis of the aqueous extracts and of the P solution extracts can be seen in Tables 5 and 6, respectively.

The extracts were done in triplicate in all cases, and the reported results are the average of each triplicate. This factor must be taken into account, particularly when analyzing the standard deviations. These are, in some cases, somewhat high due to the fact that they include both variation "among extracts" and "within extracts". Therefore confounded effects exist that could have been avoided by analyzing each extract two or three times. In this way, variability would have been divided into "within extracts" (errors of the method, instrumental, and reading error), and "among extracts" (sampling, subsampling and weighing error). This procedure was not followed due to a lack of time.

The variable studied was the change in extractable S with time of incubation. The results obtained were analyzed in the following way:

a) Test of hypothesis using the F distribution of Snedecor that relates two sample variances, in order to compare two population variances, (Cappelletti, 1972). None of the variances compared in pairs showed significant differences at a 5 % level and were considered homogeneous from a statistical point of view.

b) Comparison of treatment means using the Student distribution with the pooled estimate of the common variance for the treatments under consideration. The comparisons were made between the means of samples with and without incubation, and for both extractants.

Asterisks in Tables 5 and 6 show the soils in which there was a significant increase, at the 5 % level, in the S content due to incubation.

Table 5. Water-extractable S.

Soil Series	Added S ppm	Incubation weeks	Extractable S ppm (1)	S.D.	C.V. %
Morley	0	0	10.5	1.0	9.6
"	0	6	* 16.0	1.2	7.4
"	100	0	96.5	8.4	8.7
"	100	6	112.6	6.5	5.7
Hoytville	0	0	13.5 (2)	1.3	9.8
"	0	6	* 22.8	0.8	3.4
"	100	0	101.4	7.4	7.4
"	100	6	113.4	3.7	3.2
Miami	0	0	7.4	0.4	6.1
"	0	6	* 13.5	0.8	6.3
"	100	0	103.0	4.1	4.0
"	100	6	* 119.5	7.3	6.1

(1) Average of three replicates.

(2) Average of two replicates.

(*) Significant difference at the 5 % level between treatments.

Table 6. Extractable S with a 500 ppm P Solution.

Soil Series	Added S ppm	Incubation weeks	Extractable S ppm (1)	S.D.	C.V. %
Morley	0	0	12.6	0.6	5.2
"	0	6	21.3 *	0.9	4.1
"	100	0	114.0	7.0	6.2
"	100	6	112.5	6.8	6.1
Hoytville	0	0	14.4	0.7	4.9
"	0	6	28.3 *	1.3	4.6
"	100	0	117.1	3.8	3.2
"	100	6	110.9	7.9	7.2
Miami	0	0	8.6	0.3	3.6
"	0	6	18.0 *	0.5	2.8
"	100	0	115.9	3.1	2.6
"	100	6	119.1	6.0	5.0

(1) Average of three replicates.

(*) Significant difference at the 5 % level between treatments.

All the samples without added S showed significant differences with both extractants. Of those samples with 100 ppm S added, instead, only one belonging to the Miami series showed a significant increase when using water as an extractant.

Considering that the method used to measure S embodies both, the organic and inorganic forms of this element, its increase with both extractants after incubation could be explained by the decomposition of the organic matter originally present in the soils.

Williams (1967) studied the effect of several factors upon the organic S fraction during incubation. Two bibliographic reviews related to this matter were done by Starkey (1966) and by Freney and Stevenson (1966). From these studies, it can be concluded that the soil organic S fractions suffer biodegradation processes, as happens with N, P and other nutrients. These processes would lead to the formation of simpler S compounds and of inorganic S, that would be extracted more readily by several extractants.

With the method employed to measure S it is not possible to determine which of these two forms is most important in explaining the increase of this element after incubation.

Nevertheless, if the amounts of S extracted with water and P in the control samples are compared, one may conclude that the differences of 1 to 2 ppm of extracted S between both solutions is due to the greater exchangeable power of the P solution as compared to the water. This solution develops an acid pH at equilibrium, therefore intensifying anionic exchange, in which the $H_2PO_4^-$ tends to replace the sorbed SO_4 (Reisenauer et al. 1973).

On the other hand, the differences in water extractable S between

0 and 6 weeks of incubation were 5.5, 9.3, and 6.1 for the Morley, Hoytville, and Miami series, respectively. In this same order, the differences using the P solution were 8.7, 13.9 and 9.4 ppm.

Based on the prior discussion it is suggested that the differences in water-extractable S are due to an increase in the amount of simpler S compounds plus a fraction of the completely mineralized S. The increase in P-extractable S could be explained by the fact that the P solution extracted the same fraction as the water, plus that amount of mineralized S subsequently sorbed.

The analysis of the results in those samples with 100 ppm of added S becomes more difficult because it is not possible to differentiate between the S originally present in the soil from that added.

Nevertheless, it is worthwhile to compare the results of both extractants with the unincubated samples: A sorption phenomena can be noted, particularly in the Morley and Hoytville series, when water was used as the extractant, which may be explained by their higher clay content. This process was not as evident in the Miami series, especially when the standard deviations are considered for this set of values. The samples extracted with the P solution did notshow the sorption process due to the greater exchange power of this extractant. Therefore the S originally present plus the added, was recovered in the unincubated samples.

This SO_4 sorption process has been related to the presence of organic matter, free Fe- and Al-oxides, (Chao, Harward and Fang, 1962); in the acid range it was essentially proportional to the content of hydrous Fe- or Al-oxides, although when both were present, Al-oxides were more effective in this process (Chao et al. 1964). The Fe system exhibited increasing adsorption with increased acidity whereas the Al system showed a maximum at pH 4.0. Dissolution of the hydrous Al-oxide phase decreased the SO_4 adsorption in Al-coated soils when going below this pH, (Chao et al. 1964). The nature of soil acidity was shown to be important in that the Al-clays retained more SO_4 than H-clays, (Chao et al. 1962), and according to these authors the possible mechanisms of SO_4 adsorption would be given by:

a. Anion exchange due to positive charges developed on hydrous Fe- or Al-oxides, or on the crystal edges of clays, especially kaolinite, at low pH's.

b. Retention of SO_4 ions by hydroxyl-Al complexes by coordination.

c. "Salt adsorption" resulting from attraction between the surface of soil colloids and the salt.

d. Amphoteric properties of soil organic matter.

Chang and Thomas (1963) proposed a mechanism that involved a cation and anion reaction; due to it, K^+ would be adsorbed by exchange and/or hydrolysis of Al^{3+} on the clay surfaces, giving H^+ into solution. At the same time, SO_4^{2-} would replace OH^- ions from R(OH) coatings on the clay and substitute for them. The replaced OH^- ions will react with the H^+ ions. Whether or not there is a change on the system's pH would depend on the relative rates of the two reactions. For the SO_4 retentive soils, it has been shown (Fang et al. 1962, Chao et al. 1962) that the SO_4 adsorption data can be fitted into either the Freundlich or the Langmuir isotherms, depending on the range of SO_4 concentrations of the equilibrium solutions.

After 6 weeks of incubation, this sorption process was apparently compensated by mineralization. The Hoytville series was the only one that did not recover the S level of the control with incubation. The increase in water extractable S after incubation in the Miami series samples was significant at the 5 % level, being the only case of all the samples with added S.

The behavior of the P extractant varied drastically as compared to water, in the samples with incubation. The original values were recovered only in the Miami series, and no indication of a mineralization process was shown by any of the samples. In fact, this is not possible because if mineralization occurred, as appears from the extraction with water, it should have also been shown by the other extractant solution.

The similarities in the values of S extracted with both extractants in the samples with 6 weeks of incubation should be noted.

Somewhat high standard deviations and the small number of soils tested did not allow a definitive conclusion with respect to this aspect.

3. Extractable SO_4 Determination

As was mentioned before, several techniques are available for the SO_4 analysis -- the turbidimetric one probably being the most widely used.

The indirect measurement of this anion using atomic absorption spectrophotometry is presently being developed.

The lower detection limit is the principal problem of both techniques as neither one will measure concentration of 1ppm or less with sufficient accuracy.

In a recent work, Hinze and Humphrey (1973) used a so called amplification reaction, which consists of precipitating the SO_4 with $Ba(IO_3)_2$ -- a relative insoluble reagent -- as $BaSO_4$ (even more insoluble). Two moles of IO_3^- are produced for each mole of SO_4 present. In an acid medium and with excess I^-, 3 moles of I_2 are formed per each IO_3^- present.

Other anions have been previously measured with this technique, and Bertolacini and Barney (1957, 1958) used Ba chloranilate for the precipitation of SO_4. They measured the liberated chloranilate ion with visible and ultraviolet spectrophotometry.

Hinze and Humphrey (1973), following Lamber and Zitomer's (1963) procedure, used a reagent composed of starch and CdI_2 to measure the I_2 formed in the reaction. The I_2 reacts with the starch, forming a blue compound. Volumetry with thiosulfate would be another way to measure the I_2 formed (Weisz and Fritsche, 1970).

Detection limits are 1.2 and 0.5 ppm of SO_4 (0.4 and 0.16 ppm S) for the Hinze and Humphrey (1973) and Weisz and Fritsche (1970) methods respectively.

The first method was tried during this investigation. It was a highly sensitive method but offered some problems for its standarization. These were, basically:

a. Somewhat high blank values, due to the solubility of the $Ba(IO_3)_2$ in the alcoholic solution.

b. Turbidity of the starch-iodide reagent that would not disappear upon dilution.

c. Difficulties in reproducing a standard curve.

It was thought, therefore, that the I_2 could be measured by direct spectrophotometry of its yellow color.

Several tests and experiments were conducted, and although no definitive conclusions were obtained, the possibility exists of having found a very simple and accurate method, of a high sensitivity for SO_4 and eventually also for IO_3. Nevertheless, it is important to point out that the procedure outlined in the preceding chapter is by no means a definitive one.

To improve the results, several variables were studied: Type of solvent, optimum solvent proportion to minimize the blank values, effect of evaporating the solvent in the absorbance values, optimum dilutions, amount and type of acid to drop the pH. The advantage of adding the KI as crystals or as solution and the stability of the color as a function of time were also studied.

A standard curve is shown in Figure 1, where each value is the mean of triplicate standards. The molar absortivity calculated as a function of S concentration was 21,950. In this curve, zero absorbance corresponded to the blank. Its absorbance value, when compared with an 80 % acetonitrile solution was always below 0.013 (greater than 98 % transmitance).

The minimum concentration used was 0.15 ppm SO_4-S. A tendency to lose linearity was observed when trying to go above 0.7 ppm SO_4-S, so other dilutions should be tried.

The continuous spectrum between 460 and 320 nm can be seen in Figure 2. It was made in a Beckman automatic spectrophotometer, Model DB-G. Routine measurements were done with a Bausch and Lomb spectrophotometer, Model Spectronic 20. The minimum transmission peak (350 nm) could not be used for them, because the blank would notread 100 % transmitance at this wavelength; therefore, the 400 nm band was used.

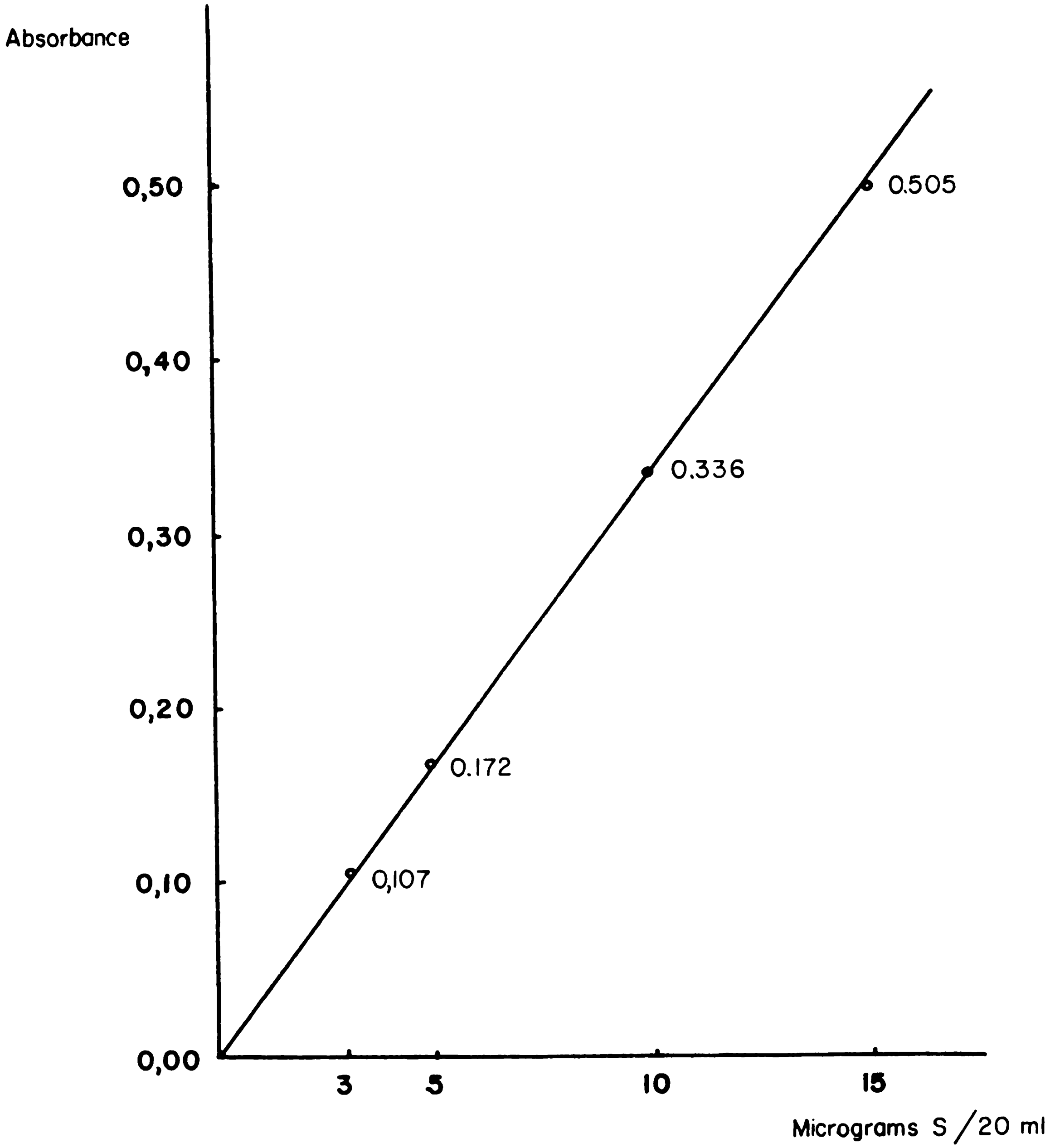

Figure I: Relationship between absorbance and S concentration. (λ = 400 nm, d = I cm).

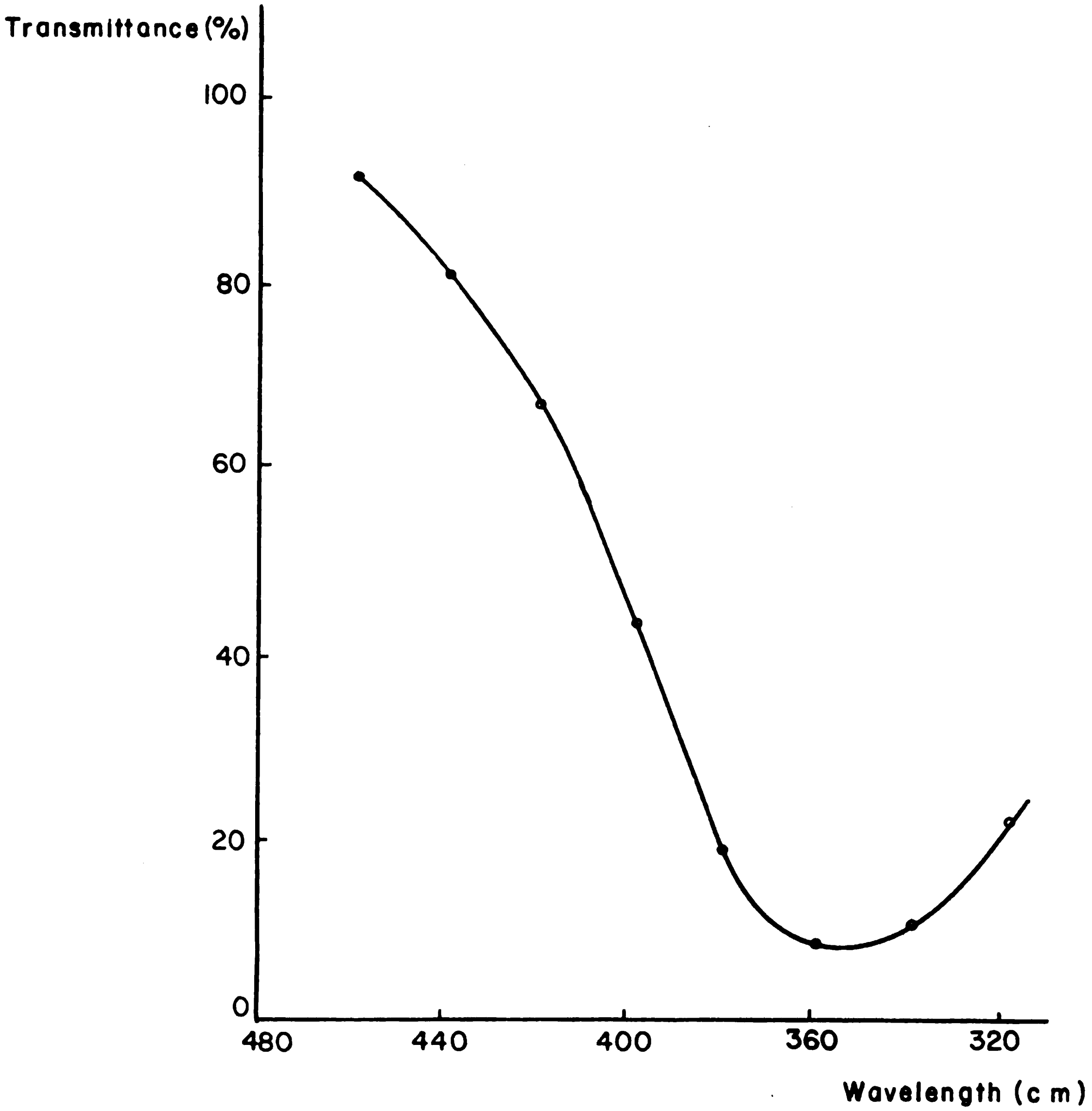

Figure 2: Absorption spectra of the iodine / acetonitrile system (80 % acetonitrile, 20 % water, 5 ug IO_3^- / 25 ml, d = 1cm).

Two aspects should be further studied, namely:

a. Optimization of the conditions for the SO_4 precipitation as $BaSO_4$.

b. Possible interferences from ions commonly present in soil extracts.

Although several extracts obtained according to the procedure outlined in the preceding chapter were analyzed, the results will not be presented until the points mentioned above have been studied in detail.

SUMMARY AND CONCLUSIONS

Samples of three Michigan surface soils -- Morley, Hoytville, and Miami series -- were incubated 0, 2, and 6 weeks at 20 C.

Half of the samples received 100 ppm of S (as K_2SO_4), and the other half were used as controls.

Total S content was measured in the samples after 2 weeks of incubation, with and without added S.

The samples with 0 and 6 weeks of incubation -- with and without added S -- were analyzed for their content of extractable S, with water and a 500 ppm P solution as extractants.

The following conclusions were drawn:

1. The total S content of the Morley, Hoytville and Miami series were 222, 424, and 201 ppm of S, respectively.

2. The percentage S recovery in the samples with 100 ppm of added S was 100, 103.5, and 106.2, respectively.

3. The results of preliminary tests with the alkaline oxidation method allowed for the reduction of the time of analysis per sample from 1 hour to 10 minutes.

4. The data suggested that the dilution of the residue with water should not be done for all type of samples. It was shown that in some cases this would cause a reduction in the recovery of total S.

5. A significant increase in the S content, at the 5 % level, due to 6 weeks of incubation was found in all the samples, using both extractants.

6. In the samples with 100 ppm of added S, these same differences were significant only in the Miami series samples where water was used as the extractant.

7. A new method for SO_4 analysis was developed. Although still in its standarization stage, SO_4 concentration of as little as 0.45 ppm was measured with good reproducibility.

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