THE SULPHUR STATUS OF QUEBEC SOILS WITH PARTICULAR REFERENCE TO THE AMOUNT OF ORGANICALLY COMBINED SULPHUR AND THE NATURE OF ITS COMBINATION

By

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INTRODUCTION

Interest in the sulphur status of soils stems from the fact that sulphur is an element essential for the growth of all plants. Although sulphur deficiencies have not so far been reported in the Province of Quebec, they are of considerable importance in some areas of North America, and the prevailing trend towards use of more concentrated fertilizers containing little or no sulphur in incidental impurities, has caused some workers to wonder whether such deficiencies should in the future be expected to occur in the province.

It was against this background that the present project was initiated in 1957, with the aim of ascertaining the sulphur status of some typical Quebec soils and of gaining information on the nature and behaviour of the sulphur in these soils. Such an investigation was considered a necessary prelude to any attempt to assess the present or future adequacy of the sulphur supply of Quebec soils.

Some of the results of the project have already been reported (51,52), and the present phase of the investigation, reported here, is concerned primarily with the distribution and chemical nature of the organic sulphur in soils.

REVIEW OF LITERATURE

The sulphur status of a soil results from the interplay of three major factors; first the initial sulphur content of the parent material from which the soil is formed, secondly the rate at which sulphur is added to the soil either from the atmosphere, by intentional additions of sulphur fertilizers, by additions of plant or animal residues, or by incidental additions in fertilizers added primarily to supply other nutrients, e.g., in superphosphate fertilizers, and thirdly the rate at which sulphur is lost from the soil, which in turn is affected by management of the soil, climate, nature of vegetation and drainage.

The importance of several of these factors has been clearly established. Mann (58) in 1955 after studying samples taken from the Woburn plots over a fifty-year period, concluded that atmospheric sources were sufficient to maintain adequate sulphur status for the crop grown (barley), whether the plots were fertilized or not. Tortensson (74) found a concentration gradient for sulphur in air at increasing distances from an industrial centre. In 1937 Alway et.al. (2) in Minnesota reported that the acquisition of 10 lb/acre/year of sulphur in precipitation was sufficient to maintain adequate sulphur status. He reported additions of from 5 to 100 lb/acre/year for different parts of the state. Bertramson et.al. (13) in Indiana reported additions of 20-127 lb/acre/year of sulphur in precipitation.

He concluded that such amounts would be sufficient to meet the needs of crops, if it were all retained in the soil in available form. Comparable figures have been reported for other regions, values of over 90 lb/acre/year being generally associated with areas of relatively heavy industrialization, whereas values below 30 lb/acre/year were generally reported for regions remote from any industrial areas. These values can be compared with those reported by Jordan and Ensminger (43) for the amounts of sulphur removed annually by average crops of alfalfa (20-24 lb/acre), turnips (25-30 lb/acre), wheat (9-12 lb/acre) and corn and grasses (8-10 lb/acre), and with the amounts removed by leaching, which can run as high as 60 lb/acre /year on well drained soils of relatively high pH (53). This comparison suggests that in regions remote from industry, on soils subject to substantial leaching losses, the atmospheric sulphur alone might be expected to be inadequate to meet the requirements of a sulphur demanding crop like alfalfa. The climate affects the importance of the atmospheric source of sulphur, since Leland (47) has shown that 75% of the sulphur reaching the soil in precipitation at Ithaca, N.Y., is brought down during the winter months when it could not be immediately utilized by plants and was therefore susceptible to leaching losses. The significance of the loss of sulphates in drainage waters has been demonstrated in lysimeter studies by Lyon and Bizzell (53), who reported that 31-62 lb/acre of sulphur were lost per year.

On the other hand, Martin (59) in California found that incidence of sulphur deficiency was not related to soil type or parent material, and MacLachlan (56) in Australia, found no correlation between incidence and intensity of sulphur deficiency and origin of parent material, climate, organic matter content or texture.

Various workers have reported values for the total sulphur content of soils, but considerable differences in the methods employed makes comparison difficult. Some of these values are reported in Table 1. In general it has been found that mineral soils have a total sulphur content ranging from 100 to 1200 p.p.m. of sulphur, whereas organic soils have a sulphur content in the range 2000-8000p.p.m.

Until comparatively recently, it was thought that most soils were well able to supply the needs of crops for sulphur, although this may have been due in part to failure to recognize sulphur deficiencies when they occurred. However during the last fifteen years, more and more cases of sulphur deficiency have been reported in many parts of the world. Widespread deficiencies have been reported in Australia (4,5,39,56,71) and in New Zealand (50). Deficiencies have also been reported in Brazil (54). In the U.S.A. Younge (87) has reported sulphur deficiencies on Coastal Plain soils and Martin (59) observed widespread deficiencies in California. Jordan and Bardsley (42) reported the existence of sulphur responsive soils in the

TABLE 1

Total sulphur content of soils as reported by various workers.

Worker	Location	Soils	Sulphur content p.p.m. S
Wyatt & Doughty (86)	Alberta	non-alkali	320-710
		organic	7300
Bentley *et.al.*(10)	Alberta	S responsive	130-190
		non S responsive	140-670
Lowe & DeLong (52)	Quebec	brown forest	1380
		podsol	440-525
Brown & Kellogg (17)	Iowa	mineral soils	800-1200
Evans & Rost (24)	Minnesota	chernozem	412-552
		black prairie	277-699
		podsol	48-131
Harpur (36)	Oklahoma	virgin mineral soils	50-1040
		cultivated mineral soils	10-750
Swanson & Latshaw (74)	Kansas	humid zone	260-350
		sub-humid zone	420-450
Little (49)	Great Britain	general agricultural soils	355-598
		hill farms	345-2430
Buchner (18)	Germany	mineral soils	200-800
		moor soils	up to 5000
Shkonde (67)	Russia	various	200-8000
Williams & Steinbergs (82)	Australia	various	40-1900

south-eastern states. In Canada sulphur deficiencies have been shown to occur on grey wooded soils in Alberta, British Columbia and Saskatchewan (10, 43, 61, 62). No sulphur deficiencies have yet been reported in Quebec.

The literature on microbiological transformations of sulphur is quite extensive, and has been reviewed recently by Pochon and de Barjac (64). However most of the work has been carried out _in vitro_, under conditions which may bear no relationship to those occurring in soils in the field.

Freney (31) has reported a study on the conversion of cysteine to sulphate in a whole soil, using a perfusion technique. Freney, on the basis of his results, postulated a mechanism for this conversion involving the following sequence:-
Cysteine → cystine → cystine disulphoxide → cysteine sulphinic acid → cysteic acid → sulphate.
These results may have relevance to the breakdown of plant residues in the soil, but may have little significance in relation to the liberation of sulphate from soil organic matter.

Comparatively little work has been done on the mineralization of sulphur in soil organic matter. Barrow (8) showed that when plant materials rich in nitrogen decomposed in the soil, accumulation of ammonium ions occurred, leading to a rise in pH. This increased pH was accompanied by increased mineralization of sulphur from the original soil organic matter. White (81) found that the relative rates of mineralization of nitrogen and sulphur from a group of soils were fairly constant, the two elements being mineralized in the ratio of approximately 10:1 in neutral soils and 20:1 to 40:1 in acid soils. Barrow (9) also showed that the mineralization of sulphur from added organic materials depended on the sulphur content of the materials in the same way as the nitrogen mineralization depended on the nitrogen content. Incubation of soils to which such materials had been added, led to mineralization or immobilization of sulphur depending on the composition of the materials added. Hesse (37) reported that soils can mineralize considerable amounts of carbon and nitrogen from added materials but yet lead to no net mineralization of sulphur. Freney and Spencer (33) reported that one out of five soils incubated for twelve months showed a decrease in extractable sulphate as a result of incubation. The mineralization of small amounts of sulphate from soil organic matter has also been reported by other workers (19, 33, 81).

The availability of soil sulphur to plants can be determined directly by measuring the uptake of the element by growing plants, and indeed in 1957 Walker (78) claimed that only field trials were useful in revealing deficient areas and that soil analysis was useless for this purpose. However several workers have attempted to find a satisfactory method of assessing available sulphur by means of soil analysis.

Aiyar (1) used water soluble sulphate values to predict rice crops in India, but other workers who studied the easily soluble sulphate content of soils in Australia, Great Britain and North America (7,49, 51, 70, 83) were unable to demonstrate that determination of this fraction was a satisfactory measure of available sulphur. In 1959 McClung et.al. (54) claimed that sulphate extractable with ammonium acetate solution correlated well with plant response on several Brazilian soils, using millett as the test plant. Williams and Steinbergs (83) in Australia studied various soil sulphur fractions as possible indices of available sulphur. These workers found that the sulphur uptake and yield of oats correlated well with 'heat soluble sulphur', a fraction obtained by heating the soil with water at 100°C and oven drying at 102°C, followed by extraction of sulphate. Bardsley and Lancaster (7) measured reserve sulphur as the difference between total soil sulphur and soluble sulphate sulphur, the latter being extracted with ammonium acetate-acetic acid reagent. They found that reserve sulphur correlated highly with yield of sulphur values for white clover, but that soluble sulphate sulphur did not reflect the levels of available sulphur. Kilmer and Nearpass (45) found a good correlation between sulphur 'A' values on thirty U.S. soils and the available sulphur as measured by extraction with 0.5 Molar sodium bicarbonate, followed by determination of sulphur in the extract by Johnson and Nishita's methylene blue procedure (41). This determination recovers organic as well as inorganic sulphate. Spencer and Freney (70) in 1960 compared nine methods of assessing sulphur availability in 24 Australian soils. The methods used were cold water extractable sulphate (30), extraction with potassium dihydrogen phosphate solution containing 500 p.p.m. of phosphorus (23), acetate extractable sulphate (54), heat soluble sulphur (83), hot water extractable sulphate, obtained by refluxing the soil with water for 30 minutes, reducible sulphur, obtained by digestion of the soil with a reducing mixture containing hydriodic acid (41) and determining the sulphide released, total sulphur by the method of Bardsley (6), reserve sulphur (7) and finally microbiological assay with Aspergillus niger. Aspergillus sulphur and phosphate extractable sulphur showed the greatest promise for predicting the adequacy of sulphur supply in these soils, although good correlation was also obtained between sulphur uptake or percentage yield and hot water extractable sulphate, cold water extractable sulphate and heat soluble sulphur for a range of deficient and non-deficient soils. Total sulphur and its derivative reserve sulphur showed poor correlation with uptake, a finding in agreement with earlier reports (10).

It is now well established that in temperate regions a substantial proportion of the soil sulphur is present in organic form (32). In 1945 Evans and Rost (24) reported that 65-70% of the total sulphur in Minnesota chernozems and black prairie soils was organic. The corresponding figure for podsols was 30-50%. Madanov reported mean values of 50% for chestnut soils and 75% for chernozems in Russia (55). Values of 60-70% for two podsols and 33% for a brown forest soil have been reported from Quebec (52).

Other workers have recently emphasized the importance of organic sulphur in soils (7, 37, 45, 70, 83).

The easily soluble sulphur fraction, extractable with water, dilute acid or salt solutions, has been shown to be very variable but in general to account for only a small fraction of the total sulphur of soils of temperate regions (7,24,49,51,52,58,70,83). Easily soluble sulphur values have normally been shown to be less than 10% of the total sulphur values in such regions, and often as low as 1-2%. It has been shown that extracts for easily soluble sulphur contain both organic and inorganic sulphur (51), and that the amounts of sulphate extracted vary considerably for a given soil with extractant and conditions of extraction (45, 51, 70).

Sulphur in other more or less arbitrary fractions has been determined. Evans and Rost (24) reported that 23% of the total sulphur in Minnesota podsols and 13% in black prairie soils occurred in the 'humus sulphur' fraction, extractable with 4% ammonium hydroxide solution. Bardsley and Lancaster (7) reported values for reserve sulphur obtained by difference between total sulphur and soluble sulphate. This fraction was claimed to represent the sum of the organic and reduced inorganic sulphur. For 23 soils, reserve sulphur accounted for a mean of 91% of the total soil sulphur. Spencer and Freney (70) measured adsorbed plus soluble sulphate in Australian soils by extraction with potassium dihydrogen phosphate solutions containing 500 p.p.m. of P. This fraction accounted for 4-13% of the total sulphur, although it was not established whether all adsorbed sulphate was recovered, or whether any organic sulphates were recovered by the procedure. The heat soluble sulphur fraction of Williams and Steinbergs (83) and the hot water soluble fraction of Spencer and Freney (70) probably included soluble sulphate plus various proportions of sulphate derived from organic combinations. Values between 3% and 34% of the total sulphur have been reported for these fractions in Australian soils (70). Freney (32) determined the soil sulphur reducible with Johnson and Nishita's reducing mixture (41) and found that this 'reducible sulphur' contained an average of 59% of the total sulphur of a group of Australian soils. Fifty-two percent of the total sulphur (i.e. the bulk of this fraction) was thought to be present in the form of organic sulphates. Freney also determined the amounts of inorganic sulphur in these soils in a lower state of oxidation than sulphate, and showed that it accounted for only 1% of the total sulphur. Williams <u>et.al.</u> (84) produced evidence that suggested the existence of small amounts of barium and strontium sulphates in Scottish soils.

Very little specific information has appeared in the literature concerning the chemical forms in which organic sulphur occurs in soil. A few sulphur containing compounds have been isolated from soils. Shorey (68) in 1913 isolated trithiobenzaldehyde from an unusual infertile soil, and Putman and Schmidt (65) identified cystine in small amounts among the free amino acids extracted from soils. Other reports have shown that cysteic acid, methionine, methionine sulphone, methionine sulphoxide and cystine can be isolated from soil hydrolysates (15, 69, 72), but are presumably present in soil in the combined state.

In 1946 Madanov (57) took the total nitrogen to total sulphur ratios of soils as evidence that the bulk of the sulphur like the nitrogen was in protein form. This idea has persisted in more recent publications, but has never been substantiated. The amounts of sulphur containing amino acids detected in soil hydrolysates have been far too small to account for more than a small proportion of the organic sulphur in soils. Indeed the evidence for the existence of significant amounts of protein in soil organic matter is far from conclusive. In 1961 Freney (32) reported evidence for the existence of substantial amounts of soil sulphur in the form of organic sulphates. He found a close correlation between the reducible sulphur fraction and the nitrogen and organic matter contents of the soils studied, and that substantial amounts of inorganic sulphate could be detected after hydrolysis of certain soil extracts, but not before hydrolysis. He also showed that when a soil was treated with methanolic HCl, a reagent which will react with covalent sulphate groups with the formation of methyl sulphate, extracts of the soil contained reducible sulphur but gave no precipitate of $BaSO_4$ when treated with barium chloride solution. However the extract did give a precipitate with barium chloride after acid hydrolysis. It did not prove possible to isolate methyl sulphate from the extracts, but after taking to dryness, the extract showed an infrared absorption band characteristic of the covalent sulphate group. This and other reports (51, 70, 83) suggest that some of the organic sulphates can be converted to inorganic sulphate by hydrolysis, heating or grinding of the soil. Freney suggested that the remaining organic sulphur, not present as sulphate, might occur in proteins, since the ratio of soil nitrogen to the remaining organic sulphur approached the value characteristic of protein, and that the organic sulphate might occur as sulphated polysaccharides or as choline sulphate.

Coffin (21) working on the B21 horizon of a podsol, observed three distinct types of organic sulphur compound in his fractions of the organic matter. The sulphur of the first type was reducible to hydrogen sulphide by Johnson and Nishita's hydriodic acid reagent (41) both before and after oxidation of the fraction with magnesium nitrate, and failed to yield hydrogen sulphide on fusion with potassium hydroxide. This fraction might be expected to contain organic sulphates. The second type was reducible with the HI reagent only after prior oxidation of the fraction with magnesium nitrate, but did yield hydrogen sulphide on fusion with KOH. The third type occurred in fractions which yielded sulphide on KOH fusion, and which released hydrogen sulphide on treatment with the HI reagent before but not after oxidation of the fraction. Coffin suggested that the failure to recover sulphide after oxidation with magnesium nitrate might have been due to volatilization of sulphur containing materials. Only one of the fractions yielding hydrogen sulphide with the HI treatment before oxidation, and therefore possibly containing organic sulphate, contained any appreciable amount of carbohydrate material (16.6%). The fractions isolated contained from 2.6% to 11.9% of sulphur and showed N/S ratios ranging from 0.06-1.22. All of the fractions studied contained substantial amounts of aromatic materials.

A number of workers have calculated C:S and N:S ratios of soils and have used them to infer the possible chemical nature of sulphur containing compounds in soil. In 1945 Madanov (57) reported that total nitrogen:organic sulphur ratios in chernozems and chestnut soils were fairly constant at 10.4-11.4, and took this as evidence that the sulphur like nitrogen was present in protein form. He also reported N:S values of 2-3 for bog soils. Evans and Rost (24) reported similar values for N:organic S for Minnesota soils, finding a similar constancy for soil types with values around 17 for podsols and 8.7 for chernozems and black prairie soils. They also reported fairly constant C:S values. In 1958, Williams and Steinbergs (82) reported values of 120 and 8 for C:organic S and N:organic S respectively for a group of Australian soils. In the same year Walker and Adams (79) recorded values of 92 and 7.7 for the C:total S and N:total S ratios of grassland soils. Walker <u>et.al</u>. (80) subsequently reported that as a result of 25 years of improvement practices, the N:S ratios of some grassland soils dropped from 33 to 11, while the C:S ratios fell from 270 to 33, indicating a greater relative rate of mineralization for carbon and nitrogen than for sulphur, as a result of the treatment. The improvement practices involved seeding with a grass-legume mixture, and annual dressings of superphosphate which added about 20 lb/acre of sulphur in the form of sulphate. White (81) in New Zealand found N:S values of 7-8 for the A horizon of neutral soils and a range of 9-14 for the corresponding layer of acid soils. He also found that mineralization of nitrogen and sulphur occurred in the fairly constant ratios of 10:1. Harpur (36) recorded a mean N:S ratio of 7.5 for 170 virgin Oklahoma soils and 7.6 for corresponding cultivated soils. He also reported that N:S ratios either were constant or else decreased with increasing depth in the profile. In 1960 Bardsley and Lancaster (7) reported a mean N:total S value of 8.2 for 23 surface soils. Williams <u>et.al.</u> (84) in Scotland studied fifty Scottish soils, 10 calcareous and the rest various non-calcareous soils. The non-calcareous soils had a mean N:total S ratio of 7.2 and a C:total S value of 104. The calcareous soils, which contained relatively large amounts of free inorganic sulphate, showed a N:non-sulphate S ratio of 7.9 and a C:non-sulphate S value of 88. For the soils of the same type the ranges of C:S and N:S values were quite narrow.

The relative constancy of these C:S and N:S ratios for soils of the same soil group, as reported by various workers, has in general been taken as strong evidence that most of the soil sulphur is present in organic combination. However, the use of N:S values by some workers as evidence for the protein nature of soil sulphur appears unjustified, since the existence of such protein has yet to be established and furthermore since it is now known that much of the organic sulphur in many soils is present in the form of covalent sulphate (32) which is not a normal constituent of protein.

MATERIALS

The soil samples used in the investigation were all from the Province of Quebec. The two podsol samples belonged to the Greensboro and Sherbrooke series, which have been described by Cann and Lajoie (20). One sample was taken from a brown forest soil of the Grenville series (46). These first three samples were taken from under permanent pasture, which as far as was known had never received applications of mineral fertilizers. The dark grey gleisolic sample belonged to the St.Rosalie series (46). The peat sample was collected near Sherrington, Quebec. Three other samples were collected on the Macdonald College property, namely a muck soil, a sample of the A_0 layer from under pine cover in the Morgan Arboretum, and a B_{21} horizon of a podsol belonging to the St.Amable series (46). With the exception of the A_0 layer and the B_{21} horizon sample, all the above samples were taken from the 0-6 inch layer. Further samples for the study of a podsol profile were taken from a well developed profile of the Ascot series (20) located under red maple near Beauvoir, Quebec. It should be noted that the Grenville, Muck, St.Amable, St.Rosalie and pine A_0 samples were collected from sites relatively close to the Montreal industrial area and to trunk railway and highway routes, and might therefore be more influenced by atmospheric sulphur sources than the other samples.

The activated charcoal used in fractionation studies was obtained from the Eastman Kodak Company under the trade name of Norit.

EXPERIMENTAL METHODS

Determination of total sulphur in soil

Total sulphur was determined by the method of Lowe and DeLong (52), employing the Parr oxygen bomb for oxidation of the sample, followed by reduction of the sulphate formed, by the action of Johnson and Nishita's reducing mixture (41) composed of hydriodic acid (HI), formic acid and hypophosphorous acid in the proportions of 4:2:1. The sulphide released in the reduction was determined colorimetrically in the form of methylene blue.

Determination of HI reducible sulphur

HI reducible sulphur was determined by the application of Johnson and Nishita's reduction method (41) directly to 0.05-0.50 gram samples of soil, ground to pass a 60 mesh sieve. The work of Johnson and Nishita and of Freney (32) indicates that this procedure should recover organic sulphates in addition to inorganic sulphate and sulphide.

Determination of carbon bonded sulphur

Carbon bonded sulphur was determined by a new procedure, previously outlined (21), based on the ability of Raney nickel catalyst to desulphurize organic compounds in which the sulphur is bonded directly to carbon (26, 27, 34). The procedure employed is described below.

Apparatus:- Digestion-distillation apparatus as described by Johnson and Nishita (41), but with the digestion flask replaced by one of 150 ml capacity.

Reagents:- The same reagents as described by Johnson and Nishita (41) are used for the wash solution, nitrogen purification, colour development and absorbing solution. The catalyst is prepared in the reduction flask from Raney nickel alloy obtained from the British Drug Houses Limited. (B.D.H.).

Procedure:- Place sample of fine soil (60 mesh) in digestion flask, add c.0.1 gm nickel alloy, 5 ml 5% NaOH and 25 ml distilled water. Connect nitrogen stream to the side arm of the flask and clamp the latter to the condenser. Digest over a low flame for 30 minutes. Remove the flame and allow the flask to cool somewhat, then add excess HCl (e.g. 5 ml 1:1 HCl) rapidly to the flask. Distil for 30 minutes and finally develop colour in the receiving flask as described by Johnson and Nishita (41).

Heating at the beginning of the digestion should be cautious to avoid excessive frothing.

Samples of 0.1-0.5 gm. of 60 mesh soil were used according to the nature of the sample, smaller amounts being used for organic samples.

The procedure was also applied to soil extracts by placing an aliquot of the sample directly in the digestion flask and proceeding as for solid samples except that no additional water need be added to the sample in the flask.

In the determination of total sulphur, HI reducible sulphur and carbon bonded sulphur, the intensity of the methylene blue colour was determined with an Evelyn colorimeter having a 1 inch light path.

Discussion of the methods for determining sulphur fractions

Total sulphur:- The oxygen bomb method employed has been shown to give higher recoveries of sulphur than some other methods, to be relatively rapid and to involve no difficult separations such as are involved in some other methods. It is sufficiently sensitive to give satisfactory results on soils with very low sulphur content (51).

HI reducible sulphur:- The procedure will recover both inorganic and organic sulphates and also thiosulphates and sulphides. It achieves partial recovery of other inorganic forms of sulphur such as dithionite, dithionate, bisulphite and elemental sulphur. It does not however recover any significant amounts of organic sulphur compounds in which the sulphur is bonded directly to carbon (30, 41). The presence of high concentrations of nitrates or selenium has been shown to interfere with the determination, preventing or reducing the formation of methylene blue (41, 60).

Carbon bonded sulphur:- Raney nickel catalyst has been used for desulphurizing a wide variety of organic sulphur compounds, including sulphinic and sulphonic acids, sulphides, disulphides and aryl sulphones (26, 27, 34). However alkyl sulphones are unaffected by the reagent (34). Thus the procedure described can be expected to recover all forms of organic sulphur in which the sulphur is bonded directly to carbon, with the exception of alkyl sulphones, and it has been demonstrated by the author that the procedure will not recover inorganic sulphate or the covalent sulphate groups in such materials as the sulphated polysaccharides carrageenin and agar. Of the inorganic forms of sulphur, the procedure will only recover elemental sulphur and sulphides, although recovery of the more refractory mineral sulphides may not be complete. Thus if the method is applied to soils containing significant amounts of elemental sulphur or mineral sulphides, a correction must be applied to obtain a true estimate of the carbon bonded sulphur. Due to the uncertainty of the forms of carbon bonded sulphur present in soil, and hence the inability to test the efficiency of their recovery, there inevitably must remain some doubt as to the completeness of recovery of carbon bonded sulphur by this procedure.

Testing for the presence of inorganic sulphides in soil

A soil sample was digested with 6N HCl in the digestion apparatus used for other sulphur determinations for 30 minutes, the gases evolved being passed through the zinc acetate absorbing solution, and the colour being developed in the same manner as that employed in the determination of HI reducible sulphur.

Testing for the presence of inorganic sulphur in forms other than sulphide or sulphate

Soil samples were digested in the same manner as in the test for sulphides but with the addition of about 0.1 gm of powdered zinc. Colour development was the same as in the previous test. Any increase in the amount of hydrogen sulphide recovered in this test as compared to that recovered in the test for sulphide was taken as a measure of the amounts of inorganic sulphur in forms other than sulphide or sulphate. Elemental sulphur, thionates and thiosulphates would be recovered in this fraction, if present.

Extraction procedures used in the study of soil sulphate

One hundred gram samples of the soils were extracted by gravity flow leaching in columns of 5 cm diameter. The columns were filled with a glass wool plug, a layer of acid washed sand, the sample and finally a second layer of sand. The columns were leached until 400 ml of leachate had been collected. Additional leaching was found to remove negligible amounts of sulphate. This arrangement permitted efficient extraction while keeping the volume of extract reasonably low. The extracting solutions used were 1N ammonium acetate adjusted to appropriate pH levels by additions of ammonium hydroxide or acetic acid, and 0.1 M and 0.5 M phosphate buffers prepared by mixing solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate in suitable proportions.

The adsorbed plus soluble sulphate values recorded in Table 10 were obtained by leaching the sample as described above, with 0.5 M phosphate buffer at pH 7, and determining the HI reducible sulphur content of the extract.

Extraction procedure used in the study of extractability of carbon bonded sulphur

Samples of 0.5-2.0 grams of 60 mesh soil (depending on the sulphur content) were placed in 15 ml centrifuge tubes. Each sample was then extracted with 10 ml of extractant for 30 minutes at 90°C and then centrifuged. The extraction was repeated twice and the residue washed with distilled water and centrifuged again, the extracts and washings from each sample being combined. The extractions were carried out first with 1 N HCl and then with 1% NaOH solution on each sample. In each case an aliquot was taken for the determination of carbon bonded sulphur, the acid extracts being neutralized prior to the determination.

Preparation of humic acid and fulvic acid fractions

These fractions were prepared by leaching the soil with 0.1 N HCl and then extracting with 1% NaOH solution at room temperature. The humic acid fraction was precipitated by acidifying to pH 2 and separated from the fulvic acid by filtration. The humic acid was then washed with 0.1 N HCl and redissolved in dilute NaOH, followed by removal of salts and alkali by prolonged dialysis against distilled water.

Chromatographic methods

Whatman No. 1 paper was used in all cases, employing the descending technique. A development time of approximately 16 hours was used for all solvents. The solvents employed were n-butanol, acetic acid, water in the proportions 4:1:2.2 (14) and 40% n-propanol (21). Sulphated materials of high molecular weight were detected by staining with 0.125% toluidine blue in 80% acetone (14). Other stains employed were 0.25% ninhydrin in acetone (75) for amino compounds, ammoniacal silver nitrate as described by Partridge (63) for reducing compounds, diazotized sulphanilic acid (14) and ferric chloride-ferricyanide reagent (14) for phenolic materials and p-anisidine phosphate (14) and naphthoresorcinol (14) for sugars.

Forsyth fractionation of acid soluble materials (28)

The materials to be fractionated were passed in acid solution through a bed of charcoal in a Buchner funnel. The filtrate was designated fraction A. The charcoal was then eluted successively with 500 ml of 90% acetone, 500 ml distilled water and 500 ml 1% NaOH solution, yielding fractions B, C, and D respectively.

Other methods

Nitrogen determinations on soils and soil extracts were made by the microkjeldahl procedure (16). The Walkley-Black dichromate oxidation procedure (40) and ignition for 3 hours at 450°C were employed for the determination of soil organic matter. All pH measurements were carried out with a Beckman Model G pH meter, those on soil samples being made in a 1:2 aqueous suspension. Alkali fusions with KOH were carried out according to the procedure of Coffin (21). Hydrolysis of polysaccharide fractions was effected with 6N HCl at 100°C for 6 hours in a sealed tube. Hydrolysates were desalted by passing successively through short columns of Dowex 50 X 8 in hydrogen form and then Dowex 1 X 8 in carbonate form. Sugars were determined by means of an anthrone reagent (25), and hexosamines by a colorimetric method based on the use of p-dimethylamino benzaldehyde (3).

RESULTS AND DISCUSSIONS

Studies on soil sulphate

Various reagents have been employed for the extraction of sulphate from soils, including 0.1 N HCl (23, 24), 0.001 N HCl (48), ammonium acetate (54), potassium phosphate (23) and water (83). In all cases interest was confined to the inorganic sulphate extracted; indeed the possibility of extracting organic sulphates was not even considered. It has been shown that the conditions of extraction and reagent used lead to considerable differences in the amounts of sulphate extracted (51). The results of several workers have indicated that pH especially has an important effect on sulphate release from soils. Lyon and Bizzell (53) showed that increasing the pH of a soil by liming led to increased losses of sulphate in drainage waters, which suggested that sulphate was less strongly held by soils at higher pH. However this effect may have been due in part to increased mineralization of organic sulphur as a result of increased microbiological activity. A similar effect was demonstrated more recently by Ensminger (23), who showed that liming reduced the ability of soils to retain sulphate from solutions of calcium sulphate leached through the soil. He claimed that hydrated alumina was active in holding sulphate in the soil. Kamprath et.al. (44) showed that the adsorption of sulphate by soils increased with increasing pH and decreased with increasing phosphate ion concentration. Volk and Bell (77) showed that 90% more sulphate was leached from a sandy soil in lysimeter studies at pH 6.9 than at pH 6.1. The importance of the anion used for extraction is probably considerable, since Berg and Thomas (11) have shown that the chloride ion is less strongly held by acid soils and soil clays than the divalent sulphate ion, whereas several workers have demonstrated the efficiency of the phosphate ion in displacing sulphate (23).

In the present investigation an attempt was first made to find the amounts of sulphate extracted by ammonium acetate buffers over the pH range 5-9. The extracted sulphur was determined by the methylene blue procedure of Johnson and Nishita (41) in order to recover both organic and inorganic sulphates extracted. Samples of the Grenville (brown forest soil) and Sherbrooke (podsol) soils were leached with 1 N ammonium acetate buffered at the following pH levels: 5,6,7,8 and 9. With the acid Sherbrooke soil more sulphate sulphur was extracted at pH 5 (19.2 p.p.m.) than at pH 7 (13.6 p.p.m.) or at pH 9 (8.5 p.p.m.), whereas the nearly neutral Grenville soil released significantly more sulphate sulphur at pH 9 (39.5 p.p.m.) than at pH 5 (26.6 p.p.m.) or pH 7 (20.0 p.p.m.). However erratic colour development was experienced when applying the methylene blue method to these ammonium acetate extracts, which was in accord with the findings of Kilmer and Nearpass (45). Hence the use of ammonium acetate as extractant was rejected as unsatisfactory for the present purpose.

The next extractants employed were 0.1 M phosphate buffers over the same pH range, 5-9. Phosphate solutions have been used for extracting soil sulphate (23, 70) due to their ability to displace adsorbed as well as freely soluble inorganic sulphate, and phosphate buffers have been employed for the extraction of soil polysaccharides (12), which are materials which could possibly contain ester sulphate groups. The amounts of sulphate extracted are shown in Table 2. In this case the erratic colour development found with the acetate extracts was not encountered.

Table 2

Amounts of sulphate extracted from two soils
with 0.1 M phosphate buffers.
(Expressed in p.p.m. S, air dry soil basis)

Soil	pH 5	6	7	8	9
Grenville	25.4	17.7	24.4	21.6	19.3
Sherbrooke	2.2	8.1	10.8	11.9	21.0

It was found that after collection of 400 ml of leachate, no further sulphate was removed from the soil by further leaching. The extracts obtained at higher pH levels were much darker in colour than those at low pH, indicating the removal of substantial amounts or organic matter at pH levels of 7 or above. The main disadvantage of this extractant proved to be its inadequate buffering capacity, especially when used on the Grenville soil, leading to failure of the pH to remain constant during the course of the extraction. It was therefore decided to employ 0.5 M buffers instead, and because of the apparent effect of pH on the amounts of sulphate extracted in the case of the Sherbrooke soil, to extend the range of pH of the buffers down to pH 1.

The same two soils were next leached with 0.5 M phosphate buffers at the pH levels, 1, 3, 5, 7 and 8, using 50 gm of soil and collecting 200 ml of leachate. Determinations were made of the sulphate extracted and also of the sulphate remaining in the extract after shaking for 1 minute with activated charcoal and filtering. It was hoped that this procedure would indicate how much organic sulphate was present in each extract, since activated charcoal was known to remove many organic materials from solution, and was observed to completely decolorize the extracts. However it has subsequently been shown that some organic sulphates are not held on charcoal under these conditions, although the highly coloured humic materials which can contain covalent sulphate groups, are removed from solution by this treatment. This aspect will be discussed in a later section. The results of these extractions are presented in Tables 3 and 4, and are expressed in p.p.m. S, air dry soil basis. It was found that using the 0.5 M phosphate buffers, in no case did the pH of the extract vary from that of the extractant by more than 0.2 pH units.

TABLE 3

Sulphate extracted from two soils with
0.5 M phosphate buffers.

Soil	pH 1	3	5	7	8
Grenville	51	34	51	63	84
Sherbrooke	90	67	42	52	50

TABLE 4

Percentage of sulphate extracted with
0.5 M phosphate buffers not held on
activated charcoal

Soil	pH 1	3	5	7	8
Grenville	96	102	98	96	92
Sherbrooke	79	78	102	80	93

The figures in Table 3 show that the Grenville soil released the largest amounts of sulphate at the higher pH levels, having a minimum release at pH 3, whereas the Sherbrooke soil released more sulphate at pH 1 and 3 than at higher pH levels. The results shown in Table 4 supply no evidence for the retention of significant amounts of organic sulphates by the charcoal in the case of the Grenville soil, although some organic sulphates are apparently present in the extracts obtained from the Sherbrooke soil at low pH. In this connection it is of interest to record that later work reported elsewhere in this thesis provided evidence for the presence of sulphated polysaccharide complexes in extracts obtained from the Sherbrooke soil with phosphate buffers. With reference to Table 4 it can not be assumed that all the sulphate not retained by charcoal was in fact in inorganic form. The fact that the sulphate extracted from the Grenville soil at pH 1 was no greater than that at pH 5 or 7 may indicate that no significant hydrolysis of organic sulphate had occurred as a result of the low pH, although such a hydrolytic effect may have contributed to the high value for sulphate extracted from the Sherbrooke soil at pH 1.

Although extraction with phosphate buffers was expected to remove freely soluble sulphate and some or all of the adsorbed sulphate from soils, it was not known whether less soluble forms like calcium sulphate or barium sulphate would be recovered. Accordingly this point was studied by repeating the leachings on columns of soil to which the equivalent of 100 p.p.m. of sulphur (soil basis) had been added in the form of either solid calcium sulphate or barium sulphate, the salt being mixed with the top half inch of the soil column. Table 5 shows the percentage of the added sulphate recovered in the extracts at the same pH levels as those previously used.

TABLE 5

Percentage of added calcium sulphate or barium sulphate recovered by leaching with 0.5 M phosphate buffers.

Soil	Salt added	pH 1	3	5	7	8
Grenville	$CaSO_4$	103	9	3	91	98
Sherbrooke	$CaSO_4$	93	0	44	72	103
Sherbrooke	$BaSO_4$	-52	-60	15	2	4

It was found that the recovery of added calcium sulphate at the extremes of the pH range used was almost complete for both soils, whereas at intermediate pH levels, especially at pH 3 and pH 5, the recovery of sulphate from calcium sulphate was low, only 0% and 9% at pH 3 for the Sherbrooke and Grenville soils respectively. This latter finding was of considerable interest, both because many workers have attempted to extract soil sulphate in this pH range, and because sulphate in the form of calcium sulphate can be expected to be available to plants over a growing season, judging by crop responses obtained by additions of this material to sulphur deficient soils. Hence such methods of extraction must be a poor basis on which to form an assessment of available soil sulphate. The results for the recovery of sulphate in the form of barium sulphate from the Sherbrooke soil were unusual. There appeared to be negligible recovery at higher pH levels and a net immobilization of sulphate at pH 1 and pH 3. No explanations can be offered for this phenomenon. In any case barium sulphate, when present, would clearly not be recovered by this procedure.

It was assumed initially that the efficiency of the phosphate buffer in extracting sulphate was due primarily to the high concentration of phosphate ions and the consequent ability to displace adsorbed sulphate. However the possibility existed that, in the case of the buffers at low pH, some of the sulphate might have been released by hydrolysis of organic sulphates. Hence a comparison was made of the amounts of sulphate leached from four different types of soil by phosphate buffers and by 0.1 N HCl. The results are presented in Table 6, the extracted sulphate being expressed in p.p.m. of sulphur.

TABLE 6

Sulphate extracted from four soils with
0.1 N HCl and with phosphate buffers.

Soil	0.1 N HCl	Phosphate buffers pH 1	 pH 5	 pH 8
Grenville (brown forest)	2.4	51	51	84
Sherbrooke (podsol)	2.5	90	42	50
St.Rosalie (dark grey gleisolic)	1.0	48	50	48
A_0 layer under pine	5.0	10	10	43

The amounts of sulphate extracted by the phosphate buffers from all soils were markedly higher throughout the pH range studied than those obtained with 0.1 N HCl. Hence at pH 1, the low pH itself is clearly not responsible for the higher release of sulphate from the Sherbrooke soil. In the case of the A_o layer, the difference between the sulphate extracted by 0.1 N HCl and by phosphate buffers at pH 1 and 5 was less than for the other soils, but the sulphate extracted at pH 8 was much greater than that extracted by the acid. These results suggested that in this material, the level of adsorbed sulphate was low, while the sulphate released at pH 8 might well have been in organic forms more readily dispersed at high pH than at low.

Ensminger (23) employed phosphate solutions containing 500 p.p.m. of phosphorus in extracting adsorbed plus soluble sulphate from soils. The concentration of phosphorus in Ensminger's extractants was much lower than that employed in this work, and it was decided to investigate the effects of phosphate concentration on the extraction of sulphate from one of these soils. The same leaching procedure was employed as in the preceding experiments, using phosphate buffers at pH 5 as extractants and the Grenville soil as test sample. The results are shown in Table 7.

The results demonstrate that there is a marked effect of concentration on the amount of sulphate extracted by this phosphate buffer, and that there is no reason to select 500 p.p.m.

TABLE 7

Effect of phosphorus concentration on extraction of sulphate from the Grenville soil with phosphate buffer at pH 5.

P concentration p.p.m.	Sulphate extracted p.p.m. S
100	3.8
500	6.0
2500	24.4
7500	37.4
15000	51.0

P as a suitable concentration for extracting adsorbed plus soluble sulphate.

While several important questions remain to be answered concerning sulphate in soils, some conclusions can be drawn from the results reported here. First, the amounts of sulphate extracted by 0.5 M phosphate buffers were substantially higher than those extracted by other reagents commonly employed for the extraction of soil sulphate, namely dilute HCl, neutral ammonium acetate and more dilute phosphate solutions. Some of the sulphate extracted from the Sherbrooke soil was apparently organic in nature, although it was not established how much was in this form. However since organic sulphates might be expected to become available to plants over a growing season, use of this extractant might give a better indication of available sulphur than the other extractants mentioned above. The variation with pH of the amounts of sulphate extracted by phosphate buffers, and the apparent inability of the extractant to recover calcium sulphate at pH 3, emphasize the importance of the pH of the extractant in attempting to measure extractable sulphate. However the results obtained indicate that the selection of a single pH level for extraction of a group of soils would give an incomplete picture of their sulphate status, since intermediate pH levels would not give full recovery of calcium sulphate, and extreme pH levels (pH 1 or 8) probably recover variable but substantial amounts of the organic sulphate. If a single pH level is to be preferred, then perhaps pH 7 should be selected, since this should effect good recovery of calcium sulphate and of adsorbed sulphate.

Carbon bonded sulphur in soils

The organic sulphur fraction has been known for some time to make up the bulk of the sulphur in many soils. However little has been done in attempting to break down the organic sulphur into fractions having some chemical significance in terms of the forms in which the sulphur is present. The first significant step in this direction was made by Freney (32), who pointed out that the sulphur recovered from a soil by the hydriodic acid reduction method of Johnson and Nishita (41), termed 'reducible sulphur' by Freney, probably represented the reducible inorganic sulphur plus the organic sulphates. Thus by substracting the value for the inorganic sulphur present from the value for reducible sulphur, he obtained an estimate for the organic sulphur present in the form of covalent sulphate. In spite of the difficulties in distinguishing between the organic and inorganic components of this reducible sulphur fraction, this approach appears to give a reasonable estimate of the organic sulphate in soil. However no record could be found in the literature of attempts to measure directly other forms of organic sulphur, in which the sulphur was bonded directly to a carbon atom. It was decided to refer to the sulphur bonded in this manner as the 'carbon bonded sulphur' fraction. A method was then developed for the direct determination of this fraction, based on the desulphurizing powers of Raney nickel catalyst. The procedure has been described and discussed in the section on methods. The procedure was tested satisfactorily on a variety of soils and soil extracts, and significant amounts of carbon bonded sulphur were detected in all soils examined, and in soil extracts obtained with acid and alkaline reagents and with phosphate buffers.

Levels of carbon bonded sulphur in soils

Seven soil samples were selected to include a wide variety of soil types and soil organic matter. The carbon bonded sulphur was determined on each, the results being presented in Table 8, together with values for the total sulphur of these samples. To obtain a measure of the precision of the determination of carbon bonded sulphur in soils by the procedure employed, the coefficient of variation (V) was calculated from the replicate values obtained on each soil, using the expression

$$V = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Values for V ranged from 2.35% to 4.61% for the organic soils, with a mean of 3.66%, and from 4.16% to 6.23%, with a mean of 4.96% for the mineral soils.

TABLE 8

Carbon bonded sulphur and total sulphur in various soils and soil horizons.

Soil	Total sulphur p.p.m.S	Carbon bonded sulphur p.p.m.S	Carbon bonded sulphur as % of total S
Muck	6450	3710	58
Peat	4580	2160	47
A_0 layer	1620	940	58
Podsol 0-6" (Greensboro)	440	119	27
Podsol B_{21} (St.Amable)	236	84	35
Brown forest soil (Grenville)	1385	172	12
Dark grey gleisolic (St.Rosalie)	410	130	32

In all seven soils significant amounts of carbon bonded sulphur were found, and as might be expected from the higher contents of organic matter, the levels of carbon bonded sulphur were considerably higher in the organic soils and in the A_0 sample than in the mineral soils. Furthermore the percentage of the total sulphur recovered as carbon bonded sulphur was significantly higher in the organic materials (47-58%) than in the mineral samples (12-35%). The amounts of carbon bonded sulphur in the mineral surface soils were of the same order for each of the soil groups represented, although the brown forest soil contained more than three times as much total sulphur as the podsol or dark grey gleisolic samples. The procedure employed will recover elemental sulphur or mineral sulphides if present, but no significant amounts of these materials could be detected in any of the samples studied. One type of sulphur containing material which could not be expected to be recovered by the procedure is the alkyl sulphone. It is not known whether such materials occur in soils in significant amounts, but their presence would mean that the estimates of carbon bonded sulphur would be low.

Extractability of carbon bonded sulphur

The distribution of carbon bonded sulphur in the same seven samples between acid extractable, alkali extractable and non-extractable fractions was studied by making successive extractions with 1 N HCl and 1% NaOH solutions, followed by determination of the carbon bonded sulphur in each of the fractions and in the residue. The proportion of the total carbon bonded sulphur recovered in each of the fractions is shown in Table 9, together with the total percentage recovery of the carbon bonded sulphur for each soil.

TABLE 9

Percentage of total carbon bonded sulphur recovered in three fractions

Soil	Extracted by N HCl %	Extracted by 1% NaOH, %	Total % extracted	Residue %	Total Recovery %
Muck	24	70	94	1	95
Peat	23	76	99	2	101
Pine A_0	22	80	102	1	103
Grenville	38	42	80	4	84
Greensboro	30	38	68	5	73
St.Rosalie	19	27	46	6	52
St.Amable B_{21}	54	35	89	4	93

It was found that a very high proportion of the carbon bonded sulphur in the three organic materials was extracted (94% or more), whereas the proportion recovered in the extracts from the mineral soils ranged from 46% to 89%. Very little carbon bonded sulphur was detected in the residue of any of the samples after extraction. Hence the total recovery of carbon bonded sulphur followed the same pattern, being virtually complete with the three organic soils and significantly lower for the three mineral surface soils. The recovery from the St.Rosalie sample (dark grey gleisolic) was particularly low, only 52%. With the exception of the podsol B_{21}, all samples released more carbon bonded sulphur to the alkaline than to the acid extractant, the proportions in the alkaline extracts being substantially higher for the organic soils than for the mineral surface soils. In contrast to the high recovery of carbon bonded sulphur from the organic soils, it may be noted that 16-48% of the carbon bonded sulphur of the mineral surface soils could not be accounted for in any of the three fractions. This low recovery from the mineral soils, especially marked in the dark grey gleisolic sample, may have been due to any of a number of reasons. Loss of hydrogen sulphide from mineral sulphides may have occurred during the acid extractions, although this factor was presumably not significant in this investigation, since no significant amounts of sulphides could be detected in any of the samples used. Other volatile sulphur containing materials may have been lost during the extractions and hence not recovered. However, perhaps the most likely cause of the low recovery of carbon bonded sulphur is the conversion of carbon bonded sulphur during the extractions to other forms of sulphur not recovered by the procedure used. It is known that heating sulphonic acids in acid solution can in some cases give rise to free inorganic sulphate (73), and that the conditions required to split off sulphate from sulphonates vary widely depending on the nature of the compound to which the sulphonate is attached (38), but that relatively mild hydrolysis is effective in some cases. The oxidative effects of hot alkaline solutions may also have contributed to the destruction of groups containing carbon bonded sulphur. An example of such a reaction is the conversion of alkyl sulphides or sulphoxides to alkyl sulphones which would not be recoverable.

The relatively high proportion of the carbon bonded sulphur not extractable by acid treatment, but extractable by alkali suggests that much of the carbon bonded sulphur may well be associated with the humic acid materials, and its resistance to the conditions employed for the extraction may indicate somewhat greater stability than that of the other major fraction of organic soil sulphur, the organic sulphate. The latter has been shown by Freney (32) to release inorganic sulphate when treated with hot acid or alkali.

The sulphur status of seven Quebec soils

The availability of a method for determining the carbon bonded sulphur made possible a more meaningful study of the overall sulphur status of soils, by determining the carbon bonded sulphur as well as the HI reducible sulphur, adsorbed plus soluble sulphate and the total sulphur content. These fractions were determined on a variety of soils, and the results are presented in Table 10. Some other chemical properties of the soils are shown in Table 11. If the procedure for carbon bonded sulphur recovers all of this form but no inorganic forms of sulphur, and if the determination of HI reducible sulphur recovers all sulphate but no carbon bonded sulphur, then the sum of these two fractions should approximate to the value obtained for the total sulphur, provided that only negligible amounts of inorganic forms other than sulphate are present. Thus a determination of these three fractions can be used as a test of the validity of these assumptions.

It was found that for the three organic soils and for two of the mineral soils, the sum of the carbon bonded sulphur and the HI reducible sulphur fractions accounted for 98-109% of the values obtained for the total sulphur. However for the other two samples, the Grenville and the B_{21} of a podsol, these two fractions only accounted for 57% of the total sulphur. The low recovery for these two samples may have been due either to the presence in these samples of carbon bonded sulphur in a form not recovered or incompletely recovered by the procedure employed, or to chemical interference with the methylene blue method in the determination of HI reducible sulphur. The latter

TABLE 10

The sulphur status of seven Quebec soils.

Soil	Total sulphur p.p.m. S	Carbon bonded sulphur p.p.m. S	HI reducible sulphur p.p.m. S	Adsorbed plus soluble sulphate p.p.m. S
Muck	6450	3710	3240	164
Peat	4580	2160	2312	92
Pine A_0	1620	940	656	36
Grenville	1385	172	616	63
St.Rosalie	410	130	318	50
Greensboro	440	119	322	52
St.Amable B_{21}	236	84	51	1

TABLE 11

pH, nitrogen content and organic matter content of seven Quebec soils.

Soil	pH	Total nitrogen %	Organic matter	
			by ignition %	by dichromate oxidation %
Muck	6.1	1.75	65	68
Peat	5.0	1.86	79	90
Pine A_0	3.8	1.48	79	88
Grenville	6.6	0.34	9.2	8.5
St.Rosalie	6.4	0.27	8.6	6.0
Greensboro	5.5	0.25	5.7	4.7
St.Amable B_{21}	4.7	0.14	6.9	4.9

seems the more likely explanation, since relatively large amounts of mineral material are present during the determination and since nitrates and selenium are known to interfere if present in high enough concentration. However other possible explanations can not be ruled out. The Grenville soil sample, in previous studies by the author (51), had shown somewhat unusual behaviour. When this sample had been subjected successively to prolonged extractions with hot alkali and then hot concentrated HCl, 32.5% of the total sulphur remained in the residue after removal of the extracts, as determined by the oxygen bomb procedure for total sulphur, although none of this sulphur could be recovered directly by the HI reduction method. Now since such treatment should remove virtually all carbon bonded sulphur, in view of the results shown in Table 9, any sulphur remaining in the residue must be in a form chemically distinct from those forms recovered in the carbon bonded or HI reducible sulphur fractions. If this material is considered as a separate fraction, then almost 90% of the total sulphur of the Grenville soil can be accounted for. The nature of this fraction has not been established, but it does not appear to be of significance in any of the other surface soils studied. The adsorbed plus soluble sulphate fraction recorded in Table 10 is one component of the HI reducible sulphur fraction, and probably represents a maximal estimate of this inorganic sulphur fraction due to the possible recovery of organic sulphates in the extracts. For the organic soils this fraction represented only 4.0-5.5% of the HI reducible sulphur, or 2.0-2.5% of the total sulphur. However for the mineral surface soils it accounted for 10.2-16.6% of the HI reducible sulphur or 4.6-12.2% of the total sulphur. Thus about 95% of the HI reducible sulphur in the organic samples and at least 85% of that in the mineral soils is probably present as organic sulphates.

With respect to the relative proportions of sulphate sulphur (as measured by HI reducible sulphur) and carbon bonded sulphur, it was observed that the organic soils contained as much or more carbon bonded sulphur than sulphate sulphur, whereas the sulphate form showed a marked predominance in the mineral soils, which are the ones of most general agricultural interest. In the absence of information on the relative rates at which the organic sulphates and the carbon bonded sulphur can be mineralized to sulphate in a soil in the field, and indeed of more precise information on the chemical nature of these forms, it is not possible to compare the importance of these two fractions in terms of their ability to supply the sulphur needs of crops. However, quantitatively speaking, it appears that the sulphate fraction, including both inorganic and organic forms, is probably the more important in many of the agricultural soils of Quebec.

Variation of sulphur status down a podsol profile

The podsol is one of the most important soil types in Quebec. It was therefore of interest to study the variation in the sulphur status down through the various horizons of a well developed podsol profile. For this purpose a profile from the Ascot series was selected. In Table 12 are presented the values obtained for the major sulphur fractions and for other chemical properties of the horizons.

The levels of total sulphur, HI reducible sulphur and carbon bonded sulphur all varied with depth in the profile roughly in parallel with variations in the organic matter content, being relatively high in the surface layer and in the illuvial horizons, and very low in the eluvial horizons and in the C horizons. There was no evidence of accumulation of sulphate in the C horizon. As with the other mineral soils previously studied (Table 10), the HI reducible sulphur fraction was

TABLE 12

The sulphur status and other properties of the horizons of a podsol.

Horizon	pH	Total N %	Organic* matter %	Total sulphur p.p.m.	Carbon bonded sulphur p.p.m.	HI reducible sulphur p.p.m.
A_0	4.9	0.70	26	2070	214	378
A_2	4.8	0.04	0.6	147	3	10
AB	4.9	0.08	1.7	210	8	13
B_{21}	4.9	0.24	8.8	596	43	148
B_{22}	5.4	0.20	6.1	505	41	122
B_{23}	5.7	0.13	3.0	318	26	84
C_1	6.1	0.0	0.02	0	0	0
C_2	6.3	0.0	0.01	0	0	0

* On these samples, organic matter was determined by the dichromate method.

substantially higher in all horizons than the carbon bonded sulphur, although it is of interest to note that the B_{21} horizon of the St.Amable podsol (Table 10) differs from the corresponding horizon of the Ascot podsol in this respect. On the other hand the Ascot B_{21}, and in fact all the horizons of this soil, resemble the B_{21} of the St.Amable soil in that the sum of the HI reducible sulphur and the carbon bonded sulphur by no means accounts for all of the total sulphur. In the case of the Ascot A_0 and B horizons the sum of these two fractions only accounts for 28-35% of the total sulphur. No adequate explanation of this was found, although the arguments used in connection with the Grenville soil may also be relevant in this case.

Carbon:sulphur and nitrogen:sulphur ratios of some Quebec soils

Workers in several countries have reported values for C:S and N:S ratios of soils. In some cases ratios were calculated in terms of total soil sulphur, and in others in terms of organic sulphur. In general it has been found that both ratios vary between fairly narrow limits and show some degree of constancy for a given soil type. The C:S ratio was found to be of the order of 100:1 and the N:S ratio of the order of 10:1.

Similar ratios were calculated from the data presented in Tables 10 and 11 for two organic and three inorganic Quebec soils to establish whether the C:S and N:S ratios of these soils were similar to those reported for other regions. In this instance it was also decided to calculate the ratios in terms of total sulphur, carbon bonded sulphur and of HI reducible sulphur. The results of these calculations are given in Table 13. The figures are based on carbon values as determined by dichromate oxidation for the mineral soils and by ignition for the organic soils, since these were expected to give a more reliable estimate of the carbon contents.

TABLE 13

Carbon:nitrogen, carbon:sulphur and nitrogen: sulphur ratios for five Quebec soils.

Soil	C/N	C/Total S	C/Carbon bonded S	C/HI reducible S	N/Total S	N/Carbon bonded S	N/HI reducible S
Muck	21	58	102	120	2.7	4.7	5.4
Peat	25	100	212	200	4.1	8.6	8.1
Grenville	14	36	290	80	2.4	20	5.5
Greensboro	11	62	230	85	5.7	21	7.7
St.Rosalie	13	85	270	110	6.6	21	8.5

The C:total S and N:total S values for the two organic soils were found to be in the range of values that can be calculated from the data of Wilson and Staker (85) for five New York muck soils. These New York soils showed a range of 30-100 for the C:total S ratio, and 1.5-5.2 for the N:total S ratio. The corresponding ratios for the three Quebec mineral soils were of the same order, but somewhat lower than most of those reported by workers in other regions, as can be seen on comparison with the data in Table 14 quoted from various sources in the literature.

Divergent results by different workers for similar soils, such as the N:S ratio of 17 for Minnesota podsols as compared to values of 5.4 and 5.7 obtained for two Quebec podsols by the author, may be due in part to differences in methods employed for the determination of total sulphur, some of which achieve more efficient recovery of soil sulphur than others.

TABLE 14

Values for carbon:sulphur and nitrogen:sulphur ratios quoted from the literature.

Author and reference	Region	Soil type	C/S	N/S
Madanov (57)	Russia	chernozem and chestnut soils		10.4-11.4
Evans and Rost (24)	Minnesota	podsols	230	17
		chernozems and black prairie soils	100	8.7
Walker and Adams (79)	Australia	grassland soils	92	7.7
Williams <u>et.al</u> (84)	Scotland	calcareous soils	62-132 (mean 88)	6.1-11 (mean 7.9)
		non-calcareous	79-154 (mean 104)	5.9- 9.2 (mean 7.2)
Harpur (36)	Oklahoma	various		3.9-11 (mean 7.5)
Freney (32)	Australia	various	37-148 (mean 100)	3.5-12 (mean 8.5)

It is difficult and perhaps unwise to draw any definite conclusions from such limited data as was obtained in this investigation, but a few observations can be made. The muck sample was much richer in all forms of sulphur, relative to carbon and nitrogen, than the more acid peat sample. The peat was less well decomposed than the muck, hence this observation is consistent with the report of Hesse (37) that carbon and nitrogen can in some cases be relatively more rapidly mineralized than sulphur during the decomposition of plant remains. For the three mineral soils, representing three different soil groups, the relative constancy of the C:carbon bonded S and N:carbon bonded S ratios, as compared to the corresponding ratios for total sulphur and HI reducible sulphur, suggests that the carbon bonded sulphur is more closely associated with the carbon and nitrogen levels than is the total sulphur or HI reducible sulphur. No C:S or N:S ratios could be found in the literature in terms of carbon bonded sulphur, but Freney (32) has determined HI reducible sulphur (termed reducible sulphur by Freney) on 24 Australian soils, and N:HI reducible S ratios calculated from his data range from 4.4 to 33 with a mean of 18. These figures compare with values of 5.4-8.4, with a mean of 7.0, for the five Quebec soils studied in this investigation.

In general it was found that the C:S and N:S ratios for the Quebec soils were of the same order as those for soils of other regions, although somewhat lower than most. The difference was due primarily to higher sulphur values rather than lower carbon or nitrogen values. The carbon bonded sulphur levels appeared to be more closely related than the total sulphur levels to the carbon and nitrogen contents of the soils.

C:S and N:S ratios were also calculated for the horizons of the Ascot profile, omitting those fractions containing very low levels of sulphur, since the possible percentage error on such figures was considerable. The results are shown in Table 15.

TABLE 15

Variation in carbon:nitrogen, carbon:sulphur and nitrogen:sulphur ratios between the horizons of a podsol profile

Horizon	C/N	C/total S	N/total S	N/carbon bonded S	N/HI reducible S
A_0	21	72	3.4	33	19
A_2	7.7	23	3.0		
AB	12	67	3.7		
B_{21}	21	86	4.0	56	16
B_{22}	18	70	4.0	49	16
B_{23}	13	55	4.1	50	16

The Ascot samples contained negligible amounts of soluble or adsorbed inorganic sulphates, and the essentially organic nature of the soil sulphur is perhaps reflected in the relatively constant N:S ratios down the profile. However there appeared to be a slight increase in the HI reducible sulphur and decrease in the carbon bonded sulphur levels relative to the nitrogen content, on passing from the A_0 layer to the illuvial B horizons. The N:total S and C:total S values were of the same order as those of the other mineral soils studied (Table 13), although the other N:S ratios were somewhat wider owing to the failure to recover all the soil sulphur in the carbon bonded and HI reducible sulphur fractions.

Qualitative investigations on the organic soil sulphur

The results obtained from the quantitative studies of the HI reducible sulphur and carbon bonded sulphur yielded no information on the precise nature of the sulphur compounds involved in these fractions. They did not indicate the nature of the organic materials to which covalent sulphate might be bonded, or the chemical groupings in which the carbon bonded sulphur was present. While it was realised that little real progress in the characterization of organic sulphur compounds in soil could be expected until more was known of the structure of fulvic acid and humic acid complexes, it was hoped that preliminary exploratory studies might yield some information on both these questions and indicate lines of research that might be developed in the future.

1. Qualitative studies on the organic components of the HI reducible sulphur fraction.

Earlier work by Freney (32) and by the author (51) and results reported earlier in this thesis indicated that substantial amounts of organic sulphate occur in the extracts obtained from soils with alkaline reagents, much of it probably being associated with the humic materials of dark colour and high molecular weight. It has also been shown in this investigation that considerable amounts of sulphate can be extracted from soils by leaching with 0.5 M phosphate solutions at pH 7. A comparatively small proportion of the latter was freely soluble sulphate, but it was not established how much of the remainder was adsorbed sulphate and how much was in organic combination.

Several procedures were tried for the fractionation of these neutral and alkaline extracts, with a view to isolating fractions rich in organic sulphate. Humic fractions were obtained by the classical procedure (see methods) by extraction of the muck, Grenville and Sherbrooke soils, and also by milder extraction with 0.5 M sodium bicarbonate at pH 10. After dialysis, the extracts still contained a relatively high concentration of HI reducible sulphur, presumably organic in nature since the soluble inorganic forms should have been removed by dialysis. Attempts were made to fractionate these humic acid fractions on cellulose columns and also on charcoal columns according to the procedure of Forsyth (28). The former was unsatisfactory since the HI reducible sulphur was not retained on the column, and the latter because the high molecular weight material was adsorbed too strongly and could not be recovered from the column with any of the solvents employed. With the charcoal columns up to 80% of the HI reducible sulphur could not be recovered in the fractions obtained from the column. Fractionation of a sodium hydroxide extract of the Grenville soil on a column of the anion resin Permutite S, in hydroxyl form, was also unsatisfactory, since most of the HI reducible sulphur was not held on the column and only about 4% was recovered by elution of the column with 2% NaCl solution. Similar results were obtained for both the Grenville and muck soils with Dowex 1, a strongly basic resin. In the case of the muck soil, a fraction was obtained by passing an alkaline extract through a column of Dowex 1, and after washing the column with water, leaching it with 2% NaCl. The material precipitated from the eluate by addition of three volumes of acetone was separated, washed with 70% acetone and dried in vacuo at room temperature. It was found to contain 0.6% nitrogen and 11% HI reducible sulphur. The fraction was a light brown powder, soluble in water to give a yellow solution. However the fraction accounted for less than 2% of the total soil sulphur, and was not characterized further. Fractionation of humic acid fractions of the Grenville and muck soils on columns of the cation resins Dowex 50 and Amberlite IRC-50 were also of little value since there was negligible retention on the column of materials containing HI reducible sulphur. Attempts to obtain fractions rich in HI reducible sulphur by fractional precipitation with the cations of calcium, barium, mercury and copper also met with little success.

Extracts obtained from the Greensboro, Grenville and muck soils by leaching with neutral phosphate buffers were also studied. Similar extractions have been employed in the isolation of polysaccharides from soils (12). It was considered a possibility therefore that some of the organic sulphate extracted in this manner might be associated with polysaccharide material in ester linkage. Accordingly fractions were isolated from these extracts by precipitation with three volumes of acetone made 1% in acetic acid. After redissolving in water, the fractions were reprecipitated as before. The yield of organic material represented 8%, 2% and 1% respectively of the organic matter of these soils. The Greensboro fraction was further analysed and found to contain 0.11% HI reducible sulphur, 0.026% carbon bonded sulphur, 0.18% nitrogen and 37% ash. All three fractions were studied by paper chromatography in 40% n-propanol. All three samples contained a stationary component which was not mobile in this solvent, and a mobile component having an R_f value between 0.7 and 0.8. In each case, both the stationary and the mobile components stained metachromatically with toluidine blue, a stain specific for sulphated materials of high molecular weight (55), whereas only the mobile component stained with ninhydrin reagent (purple-brown) and with thymol blue indicator dye (blue). The Greensboro and muck fractions were investigated further to find out whether they were of polysaccharide nature. After hydrolysis with 6N HCl in a sealed tube, they were analysed for sugars and hexosamines. For the Greensboro and muck fractions respectively, 3% and 14% of the fraction could be accounted for as sugars and no hexosamines could be detected.

Since the presence of polysaccharide materials in the phosphate extracts was established, it was decided to find an alternative extractant for these polysaccharides which would remove less humic materials, and preferably not displace adsorbed sulphate from the soil. For this purpose 2% NaCl was selected. Accordingly an extract was obtained from the muck by leaching with 2% NaCl, and a fraction prepared from it by precipitation with two volumes of acetone, which on reprecipitation from aqueous solution with acetone, was pale brown in colour and relatively rich in HI reducible sulphur. This fraction, which will be referred to as the muck polysaccharide fraction, was studied by chromatography both before and after hydrolysis, and by comparison of its behaviour with that of a known naturally occurring sulphated polysaccharide, carrageenin. The latter resembles the muck fraction in its solubility in water, insolubility in 70% acetone and possession of covalent sulphate groups. Solutions of the muck polysaccharide and of carrageenin were prepared to contain approximately 30 p.p.m. of HI reducible sulphur. Chromatograms of both samples were run in 40% n-propanol. No staining materials were observed in either sample when the chromatograms were treated with ferric chloride-ferricyanide reagent or diazotized sulphanilic acid, stains for phenolic materials, or with ammoniacal silver nitrate. On the other hand, when treated with toluidine blue, both chromatograms revealed spots showing pink metachromatic staining, indicative of sulphated materials. In the case of the carrageenin, spots were observed with R_f values of 0.0, 0.74 and 0.95, the latter being the most intense. On the chromatogram of the muck fraction, distinct spots were observed with R_f values of 0.0 and 0.80, and a third staining region was seen running in a streak from R_f 0.1 to R_f 0.65. This behaviour was similar to that observed by Bernier (12) who observed both stationary and mobile components on chromatograms of soil polysaccharides.

In earlier work by the author (51), activated charcoal had been considered as a possible means of separating the inorganic sulphate in soil extracts from organic forms of sulphur.

This technique would prove useful if the organic forms were held on the charcoal, to be removed by subsequent elution, while the inorganic sulphate would not be held. It was necessary for this, to establish what proportion of the sulphate sulphur of the fraction was adsorbed on passage through the charcoal, and whether the sulphate not held on the charcoal contained any organic sulphate. When the carrageenin solution was treated with charcoal at pH 4, only 34% of the sulphate sulphur was retained on the charcoal, and chromatograms of the fraction showed the same spots with toluidine blue both before and after the charcoal treatment. This indicated that much of the sulphated polysaccharide was not retained on the charcoal. When the muck fraction was treated in the same way, 49% of the sulphate sulphur was adsorbed on the charcoal, and although the same spots were obtained on chromatographing the treated extract as were observed on the untreated extract, the colour intensity of the spots was markedly decreased. Since the muck polysaccharide was found to resemble in its behaviour both the carrageenin sample and similar fractions extracted from various soils by Bernier (12), it was decided to make a cursory examination of its composition, to ascertain whether the components of the polysaccharide were similar to those of the materials examined by Bernier.

A sample of the muck polysaccharide was hydrolysed with 6N HCl. After the hydrolysis, a considerable amount of dark, humic acid like materials remained, confirming the experience of Bernier that soil polysaccharides are closely associated with other complex materials of the humic acid type. The hydrolysate was then subjected to chromatography, using a butanol:acetic acid:water system as solvent. A number of ninhydrin reactive spots were detected but at first no sugars were detected by staining with ammoniacal silver nitrate or with p-anisidine phosphate reagents. However, after desalting and concentrating the hydrolysates and rechromatographing, several characteristic sugar spots were obtained both with silver nitrate and with p-anisidine phosphate. From staining characteristics, R_f values and behaviour when co-chromatographed with standard sugars, the following compounds were tentatively identified as being present in the hydrolysate:- glucose, galactose, xylose and glucuronic acid. All these compounds had previously been identified in soil polysaccharides (12).

Thus it appeared that some of the HI reducible sulphur in the muck and Greensboro soils, and probably in most soils, was present in the form of covalent sulphate groups attached to polysaccharides, the latter being associated with other humic type materials. However the sulphur determined in these fractions only accounted for between 1% and 2% of the HI reducible sulphur in these two soils.

Chromatography of humic acid fractions of the muck and Greensboro soils in 40% n-propanol also revealed spots staining metachromatically with toluidine blue, indicating the presence of covalent sulphate groups, but in view of the complexity of such materials and lack of knowledge of their structure, no attempt was made to characterize the materials carrying the sulphate groups.

2. Qualitative studies on carbon bonded sulphur in soil.

It had already been shown that for a variety of surface soils much of the carbon bonded sulphur was extractable with hot alkali, but not with acid, suggesting that much of this fraction was associated with the humic rather than the fulvic acid fraction. Isolation and analysis of the classical humic and fulvic acid fractions from several of these soils confirmed this suggestion. However no information was obtained on the type of chemical group involved, except that much of the carbon bonded sulphur was stable to both hot acid and alkaline treatment, in so far as it remained in carbon bonded form after the treatment.

Fractionation of humic acid preparations has proved to be a stumbling block for soil workers for many years, and no significant progress was made in this direction in the present investigation. However attempts were made to gain information on the type of sulphur containing groups that might be present, by observing the effects of various hydrolytic and oxidative treatments on the fractions. For this purpose a humic acid fraction obtained from the muck soil with NaOH solution was employed. It was first subjected to hydrolysis with 6N HCl in a sealed tube at 100°C. After this treatment, 74% of the initial carbon bonded sulphur content was recovered in the acid insoluble residue, 8% in the acid soluble hydrolysate and 18% could not be recovered, presumably as a result of conversion to forms of sulphur other than carbon bonded forms. Some naturally occurring sulphonates are known to give rise to inorganic sulphate on hydrolysis under similar or even milder conditions (35), and it is perhaps reasonable to suggest that similar sulphonic or even sulphinic acid groups might account for some of the carbon bonded sulphur destroyed by the acid hydrolysis of the humic acid fraction. However the bulk of the carbon bonded sulphur in the fraction must be in some relatively stable combination resistant to hydrolysis.

Treatment of a solution of the humic acid fraction with hydrogen peroxide, at 70°C for 30 minutes, apparently caused complete oxidation of the carbon bonded sulphur in the material, since no carbon bonded sulphur could be recovered after the treatment. Similarly after treatment with potassium permanganate solution under the same conditions, no carbon bonded sulphur could be detected. The disappearance of carbon bonded sulphur on oxidation can be accounted for either by conversion to other nonrecoverable forms of sulphur, or by loss of volatile materials during the treatment. However since it was subsequently shown that no carbon bonded sulphur could be detected in the distillate after normal or steam distillation of aqueous solutions of the fraction in either basic or acidic solution, it seems that conversion of the carbon bonded sulphur to other forms is the most probable explanation. Now in general sulphone groups are resistant to such oxidising agents as hydrogen peroxide and permanganate, and organic sulphides and sulphoxides are normally converted to sulphones by permanganate oxidation, and since aryl sulphones are recoverable by the procedure used for the determination of carbon bonded sulphur, such sulphur groups are probably not present in this material attached to aromatic nuclei. However alkyl sulphones are not recovered in the procedure, hence alkyl sulphides if not volatile, and alkyl sulphoxides are among those groups which might be expected to exhibit behaviour similar to that observed in the carbon bonded sulphur of the muck humic acid fraction. On the other hand, sulphonate groups are normally unaffected by permanganate treatment as far as oxidative effects are concerned, although if hydrolysing conditions exist, there may be some release of inorganic sulphate depending on the substance to which the sulphonate is attached. Hence this group could perhaps account for some of the carbon bonded sulphur in the fraction.

Attention was next turned to the acid soluble materials obtained from the muck soil, either in the fulvic fraction or obtained directly by treating the soil with hydrochloric acid. In general these materials have been found to be somewhat more amenable to fractionation and are probably a little less complex than the humic materials. Various attempts were made to fractionate such extracts and to isolate materials containing carbon bonded sulphur. A fulvic acid fraction from the muck soil was fractionated on a short column of charcoal, following the procedure of Forsyth (28). Only one fraction, that eluted from the charcoal with sodium hydroxide, contained any significant amount of carbon bonded sulphur. However the fractionation was considered unsatisfactory since over 70% of the carbon bonded sulphur placed on the column was not recovered from it. This observation was in agreement with the report of Schlichting (66) who concluded that Forsyth's fractionation of fulvic acids was not suitable for some soils, since their high molecular weight, acid soluble materials were strongly adsorbed on the charcoal. Other studies were carried out on extracts made by direct extraction of the soil with concentrated HCl at 70°C for 10 minutes. These extracts were expected to contain similar materials to those occurring in the fulvic acid fraction, and were simpler to prepare. The classical fulvic acid fraction is an arbitrary preparation and itself may well contain artefacts of the preparation. Hence it was decided that results obtained on the HCl extracts would be as valuable as those obtained on the fulvic acid fractions. HCl extracts from the muck soil were subjected to the Forsyth fractionation on charcoal. The results were the same as those obtained on the fulvic acid fraction of this soil. Similar extracts from three other soils, namely the pine A_0, the Grenville and the St.Amable B_{21}, were also fractionated on charcoal. In general the results were the same as for the muck extract, in that 65-80% of the carbon bonded sulphur in the fraction could not be recovered from the charcoal. However there were marked differences between the four soils in the distribution of the carbon bonded sulphur that was recovered between the four fractions, A, B, C and D, obtained from the fractionation. In the muck soil most carbon bonded sulphur was recovered in the D fraction (eluted from charcoal with NaOH), with traces in the B fraction (eluted with acetone), and none in the A and C fractions. In the case of the pine A_0 extract, most carbon bonded sulphur was recovered in the B and D fractions, whereas for the Grenville soil the C fraction (eluted with water) and D fraction accounted for nearly all of the carbon bonded sulphur recovered. The St.Amable extract yielded some carbon bonded sulphur in the A fraction (not retained on charcoal), traces in the B and C fractions and the largest amount in the D fraction. The HCl extract of the muck was next fractionated on a column of cellulose powder. The extract was concentrated to dryness <u>in vacuo</u> at room temperature over NaOH, and placed at the top of the column, which was then successively eluted with acetone (A), alcohol (B), water (C), 0.1 N HCl and 1% NaOH solution. All five fractions were coloured, the last one quite highly, but only the first three contained any carbon bonded sulphur. Addition of 1 volume of ether to the first two fractions produced precipitates which contained no carbon bonded sulphur and were discarded. The three fractions A, B and C were then chromatographed using butanol:acetic acid:water as solvent. Fraction A contained one material showing fluorescence under ultraviolet illumination, but no spots could be detected on the chromatograms when stained with ninhydrin reagent, ammoniacal silver nitrate or diazotized sulphanilic acid. Chromatograms of fraction B revealed eight distinct spots on staining with ninhydrin, and probably contained a number of amino acids. Two additional spots were obtained with silver nitrate, indicating the presence of reducing groups, possibly sugars. Fraction C contained seven ninhydrin reactive materials and four materials detectable with silver nitrate. Chromatograms of all three fractions were treated with a reagent containing N-ethylmaleimide, designed to reveal the presence of compounds containing the -SH group. No spots were obtained with this reagent.

Thus although a number of fractions containing carbon bonded sulphur were obtained, little definite information was obtained on their chemical nature. Fractionation of this acid soluble material by using differences in the solubilities of the acidic materials and their barium salts in organic and inorganic solvents was also investigated. It appeared to be a promising approach, free from the hazards of losing part of the material on columns, but it was not worked out in detail.

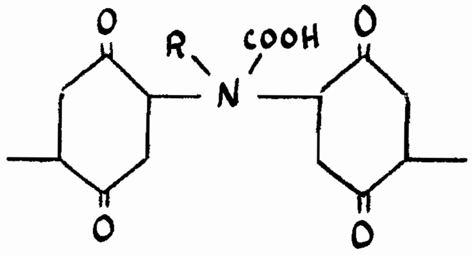
A fulvic acid fraction of the muck soil was subjected to the same hydrolytic and oxidative treatments as described earlier for the humic acid fraction. After hydrolysis with 6N HCl, only 73% of the carbon bonded sulphur in the fraction could be recovered. This represented a greater destruction of carbon bonded sulphur than was observed with humic acid. This indicates that there is a higher proportion of carbon bonded sulphur materials converted to non-carbon bonded forms in the fulvic than in the humic acids. As was suggested above, this hydrolysable fraction may contain sulphonated materials. When oxidised with potassium permanganate only 66% of the carbon bonded sulphur was destroyed, the remainder being recoverable in carbon bonded form after oxidation. This result indicated the presence in this fraction of materials resistant to oxidation. Aryl sulphones and some sulphonic acids might be expected to show such behaviour, although it must be emphasized that no conclusive evidence has yet been obtained for the existence of such materials in soil organic matter.

Discussion of the nature of carbon bonded sulphur in soil

Although it will probably prove impossible to establish the precise nature and structure of the carbon bonded sulphur compounds in soils, until progress has been made in elucidating the structure of the complex humic materials in soils, the possibility of establishing the nature of the sulphur containing groupings and their relative abundance appears to be somewhat more promising. For this reason it has been considered worthwhile to discuss the various forms in which sulphur might occur in carbon bonding, in the light of the known chemical behaviour of these forms and of the limited observations thus far available concerning the behaviour of carbon bonded sulphur in soils.

Thiols, sulphides and disulphides

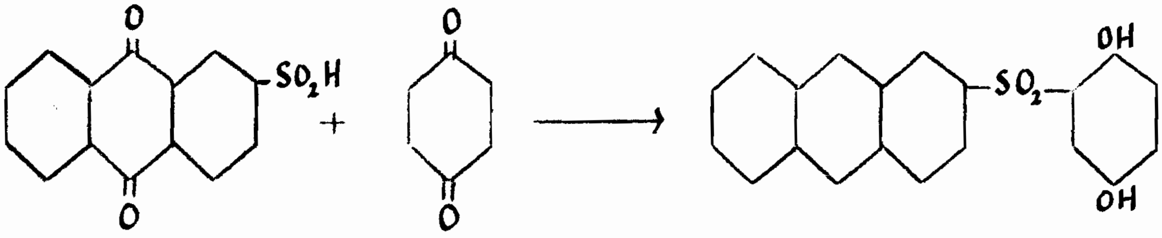
Traces of free amino acids including cystine, a disulphide, have been detected in soils, but they would not be expected to persist in soils in the presence of an active microbial population. Quite substantial amounts of amino acids have been isolated from the hydrolysates of soil organic matter. Among these several sulphur containing materials have been detected, namely methionine and its sulphone and sulphoxide, cystine and cysteic acid. However the amounts of sulphur containing compounds obtained were far too small to account for more than a very small proportion of the carbon bonded sulphur in soils. The occurrence of a sulphone and a sulphoxide was of considerable interest, although some doubt existed as to whether these materials were artefacts of the procedure employed (72). The possibility of sulphur containing amino acids present in other forms than proteins and peptides must be considered. Swaby has postulated the existence of amino acids in soil organic matter as bridges in quinonoid or phenolic chains of the general type



In this form the amino acids might prove more resistant to hydrolysis than when present in normal peptide linkage, and would therefore not be recovered by the hydrolytic procedures employed. Aliphatic thiols and sulphides are known to be produced by some soil microorganisms, but except in relatively anaerobic conditions such as occur in swamps, they would not be expected to persist in soil in any significant amounts since they are relatively easily oxidised, and also since many are volatile.

Sulphenic, sulphinic and sulphonic acids and their salts

The sulphenic, sulphinic and sulphonic acid groups, either in free acid or salt form may well account for some of the carbon bonded sulphur in soils. The sulphenic acid group is unstable and is probably of little importance, but Freney (31) has shown that sulphinic acids are probable intermediates in the breakdown of cysteine or cystine in soil. Sulphinic acids are readily oxidised to sulphonic acids, and might be expected to have but a transitory existence in soil, but it has been shown that they can condense with α/β unsaturated acids to form sulphones, and also that they can condense with quinones to form sulphones (38), e.g. -



Now sulphones in general are very stable, and if formed in such a manner from sulphinic acids could be expected to persist in soils. Sulphonic acids vary considerably in their stability, both in the ease with which the acid group can be split off as sulphate by hydrolysis (73), and in their resistance to attack by micro-organisms. For example taurine was completely decomposed to sulphate in a six-week experiment by Frederick <u>et.al</u>. (29), whereas sulphanilic acid persisted in the soil unchanged for

21 weeks. In general the stability depends on the nature of the compound to which the sulphonate group is attached, and in particular whether it is aromatic or aliphatic. The presence of aromatic materials in soil organic matter is well established, and the existence of relatively stable aromatic sulphonates in soils is a distinct possibility. The observation in this investigation that some of the carbon bonded sulphur in both humic and fulvic acid fractions of a muck soil was destroyed by acid hydrolysis suggests that some alkyl sulphonates may be present in these fractions.

Sulphoxides and sulphones

In general the sulphoxides, like the sulphinic acids, are relatively unstable, being readily reduced to sulphides or oxidised to sulphones. Hence they are unlikely to make up any significant proportion of the carbon bonded sulphur in soils, although they may perhaps be intermediates in the formation of other materials. In contrast, sulphones exhibit remarkable stability, being resistant to the usual oxidising and reducing agents, and to aqueous acids and alkalis (38). Although there is no experimental evidence for the existence of sulphones in soils (except traces of methionine sulphone isolated from soil hydrolysates), there are theoretical grounds for suggesting that they may occur in soil organic matter. There are several ways by which they might be formed in soil from other materials by oxidation or condensation, and their exceptional resistance to modification by chemical means suggests that they might also be more resistant to microbiological attack than other forms of organic sulphur.

Other sulphur groups

Among other sulphur containing groups, there are some, for example the thioamides and thioaldehydes, which like the thiols and sulphides are unlikely to persist in soil in aerobic conditions, while others show considerable stability as in the case of some heterocyclic sulphur compounds. Except in the case of thioaldehydes, of which one example has been isolated from an American soil by Shorey (68), there is little to indicate whether these types of materials occur in soils or not.

In conclusion it may be said that at the present time the limited information available suggests that the most likely groups to account for the carbon bonded sulphur in soil are the sulphonate, the sulphone and the thiol in some combined form. However much more work must be undertaken before this question can be answered satisfactorily.

SUMMARY AND CONCLUSIONS

Studies on extractable sulphate in Quebec soils showed that 0.5 M phosphate buffers extracted more sulphate than 0.1 N HCl, 1 N ammonium acetate or more dilute phosphate buffers. The increased extraction was attributed to more efficient displacement of adsorbed sulphate as a result of the high phosphate concentration and to the extraction of some organic sulphate. The pH of the extractant had a marked effect on the amounts of sulphate extracted from the Grenville and Sherbrooke soils by phosphate buffer. Both soils were extracted over the pH range 1-8, and the nearly neutral Grenville soil showed maximum sulphate release at pH 8, whereas the more acid Sherbrooke soil showed greatest sulphate release at pH 1. The concentration of phosphate in the buffers had a marked effect on the amounts of sulphate extracted at pH 5, the latter increasing more than tenfold over the concentration range 100-15,000 p.p.m. P in the phosphate buffer. 0.5 M phosphate buffers failed to recover added barium sulphate from the Sherbrooke soil over the range pH 1-8. They also achieved negligible recovery of added calcium sulphate from the Grenville and Sherbrooke soils at pH 3, only partial recovery at pH 5 and 7, and virtually complete recovery at pH 1 or 8. These results indicated that some of the reagents commonly employed to measure available sulphates by extractions in the pH range 3-5 would not only fail to recover all adsorbed sulphate but also would not recover calcium sulphate if present.

The concept of the 'carbon bonded sulphur fraction' in soils was established, and a method for its determination developed, based on the desulphurizing ability of Raney nickel catalyst. Significant amounts of carbon bonded sulphur were found in all soils studied, ranging from 84-172 p.p.m. in mineral soils and from 940-3710 p.p.m. in organic soils. These levels represented 12-35% of the total sulphur of the mineral soils and 47-58% of the total sulphur of the organic soils. Studies on the extractability of the carbon bonded sulphur fraction by successive treatments with dilute acid and alkali, showed that more than 94% of the fraction could be extracted from the organic soils whereas only 46-89% could be extracted from the mineral soils. Little carbon bonded sulphur could be detected in the residue of any soil after extraction, and although recovery of the carbon bonded sulphur was virtually complete for the organic soils, recovery from the mineral soils ranged from 52% to 93%. With the dark grey gleisolic sample 48% of the carbon bonded sulphur could not be accounted for. This loss of carbon bonded sulphur was attributed primarily to conversion to non-carbon bonded forms during the extractions.

The HI reducible sulphur, representing basically the sum of the organic and inorganic sulphates, comprised 40-50% of the total sulphur of the organic soils and 44-77% of that of the mineral surface soils. The adsorbed plus soluble sulphate, which gives a rough estimate of the inorganic sulphate content and is therefore a component of the HI reducible sulphur fraction, accounted for 2.0-2.5% of the total sulphur of the organic soils and 4.5-12% of the total sulphur of the mineral surface soils.

It was observed that for three organic and two mineral soils, the sum of the carbon bonded sulphur and the HI reducible sulphur accounted for virtually all the total soil sulphur, but in one sample, the Grenville soil, the sum of these fractions only amounted to 56% of the total sulphur content.

An examination of the sulphur fractions of the horizons of a well developed podsol profile, showed that the total sulphur, HI reducible sulphur and carbon bonded sulphur levels all paralleled the organic matter content, being greatest at the surface, very low in the eluviated horizons, higher in the B_{21} horizon and decreasing with depth from the B_{21} downwards through the profile.

Calculated values for the C:total S and N:total S values were similar to values reported for other regions in the case of the organic soils, but somewhat lower than those recorded elsewhere in the case of the mineral soils. C:carbon bonded S and N:carbon bonded S ratios showed less variation between soil types than did C:total S and N:total S ratios.

Materials containing organic sulphate groups were present in soil extracts obtained with alkali, neutral sodium chloride and phosphate buffer solutions. Attempts to fractionate the sulphate containing materials in the humic and fulvic acid fractions were not successful, but sulphated fractions were isolated from the muck and Sherbrooke soils with NaCl and phosphate buffers, containing polysaccharide material in association with other complex humic type materials. Sulphur recovered from these soils in the form of sulphated polysaccharides only accounted for 1-2% of the HI reducible sulphur of the soil. In the case of the muck polysaccharide, glucose, galactose, xylose and glucuronic acid were tentatively identified among the products of acid hydrolysis.

Fractionation of the carbon bonded sulphur in the humic and fulvic acid fractions by the Forsyth procedure, or on exchange resins were unsatisfactory. However 74% of the carbon bonded sulphur in the muck humic acid proved resistant to hydrolysis, with 18% being converted to non-carbon bonded forms, whereas all the carbon bonded sulphur was converted to other forms by permanganate oxidation. In contrast only 66% of the carbon bonded sulphur in the fulvic acid was oxidised by permanganate and 27% destroyed by acid hydrolysis. A consideration of some of the possible forms of combination of organic sulphur suggested that sulphones, sulphonates and free or combined thiol groups might perhaps account for most of the carbon bonded sulphur in soils.

In general it was concluded that the bulk of the sulphur in the Quebec soils studied was organic in nature, and that the levels of sulphur found in these soils gave no indication of any likelihood of sulphur deficiencies occurring in the near future.

CONTRIBUTIONS TO KNOWLEDGE

1. 0.5 M phosphate buffers were found to extract significantly more sulphate from two soils than other solutions currently employed for the extraction of soil sulphate, namely 0.1 N HCl, ammonium acetate and more dilute phosphate solutions. The increased extraction was attributed to more efficient extraction of adsorbed sulphate by the higher phosphate concentration and to the extraction of organic sulphates.

2. Variation in the pH of 0.5 M phosphate buffers over the pH range 1-8, caused considerable variation in the amounts of sulphate extracted from soils. Soils differed with respect to the pH level at which maximum extraction occurred.

3. Phosphate buffers failed to recover added $BaSO_4$ at pH levels from 1-8, achieved negligible recovery of added $CaSO_4$ at pH 3, partial recovery at pH 5 and 7 and virtually complete recovery at pH 1 or 8.

4. The concept of a 'Carbon bonded sulphur fraction' in soils was established and a method for its determination developed.

5. Levels of carbon bonded sulphur ranged from 84-172 p.p.m. in mineral soils and 940-3710 p.p.m. in organic soils. These levels represented 12-35% of the total sulphur of the mineral soils and 47-58% of the total sulphur of the organic soils.

6. More than 94% of the carbon bonded sulphur of the organic soils and 46-89% of that of the mineral soils was extractable by successive treatments with acid and alkali. In all cases except that of the B_{21} horizon of a podsol, more carbon bonded sulphur was extractable with alkali than with acid.

7. For three organic and two mineral soils the sum of the carbon bonded sulphur and the HI reducible sulphur accounted for 98-109% of the total soil sulphur. For the Grenville soil these fractions only accounted for 56% of the total sulphur.

8. The total sulphur, HI reducible sulphur and carbon bonded sulphur all varied with depth in a podsol profile, being greatest at the surface, very low in the eluviated horizons, higher in the B_{21} horizon and decreasing from this point down through the lower horizons.

9. C:carbon bonded S and N:carbon bonded S ratios for the Quebec soils showed less variation between soil types than the C:total S and N:total S ratios. For the organic soils C:total S and N:total S ratios were similar to values reported for other regions, but for the mineral soils these ratios were lower than those reported for other areas.

10. Sulphur in the form of sulphated polysaccharides accounted for 1-2% of the sulphate sulphur in a muck soil and in a podsol.

11. 74% of the carbon bonded sulphur in a muck humic acid fraction proved resistant to hydrolysis, with 18% being converted to non-carbon bonded forms, whereas all the carbon bonded sulphur was converted to other forms by permanganate oxidation. In contrast only 66% of the carbon bonded sulphur in the fulvic acid was oxidised by permanganate and 27% was destroyed by hydrolysis.

12. The levels of sulphur in the Quebec soils studied indicated that there is no likelihood of sulphur deficiencies on these soils in the near future.

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