CHAPTER 1

INTRODUCTION

Sulfur (S) occurs widely in soils in sulfide minerals and organic components which are potentially available to plants. It is also present in coal and crude oil and as hydrogen sulfide in natural gas. Sulfur, being one of the essential plant nutrients, has always been important for plant and crop production, however, its importance has been overshadowed by the nitrogen, phosphorus and potassium. Sulfur is required by plants in amounts similar to phosphorus (Morris, 1987) and has long been supplied incidentally by nitrogenous and phosphate fertilisers, crop residues, manures, precipitation, air, irrigation water, and pesticides.

Sulfur is a component of three amino acids, cystine, cysteine and methionine, and the various compounds in plants. Hence it is important in plants for protein formation, enzyme synthesis and other important functions (Anderson, 1975; Thompson *et al.,* 1986). Plants differ markedly in their sulfur demands with crops such as crucifers having a higher sulfur requirement than the others (Randall, 1988). Jordan and Ensminger (1958) stated that crops (such as vegetables e.g. radish) have a relatively short growth period and absorb sulfur at a rapid rate, hence, a supply of readily available sulfate is especially important. The legumes, cotton, tobacco and corn are grouped as intermediate in their growth period and sulfur requirement, whereas, crops having a prolonged growth period such as grasses and forage have a low sulfur demand rate.

The need for S in plant nutrition is well recognised and crop responses to S fertilisation have been reported worldwide (Morris; 1987; Messick *et al.,* 1992). Several researchers have summarised crop responses to S in some regions (Blair, 1979; Blair and Till, 1983; Tandon, 1991). A deficiency of sulfur can reduce crop yield and also adversely affect crop quality.

With the increasing use of high-analysis fertilisers which contain little or no S, increasing S removal with new varieties (high crop yields), reduced use of S containing fungicides and pesticides, and reduced emissions of S from industry, crops in many parts of the world receive less than adequate S for their nutrition. As a result, deficiencies of S have been reported with increasing frequency throughout the world in the last two decades (Blair, 1971; Blair, 1987; Messick, *et al.,* 1992). Because of this there is a need for a soil S test to be able to evaluate the S status of soils and crops, for use in the diagnosis of S deficiency and for the assessment of fertiliser requirements (Rayment *et al.,* 1993; Freney, 1986b; Jones, 1986).

Soil and plant analysis are useful to assess the S status of soils and crops. However, soil S tests and/or tissue testing have met with variable results (Jones, 1986; Blair *et al.,* 1992). Tissue testing studies have shown that the indices such as total S, sulfate-S and N/S ratio, vary widely, depend on crop type and age, portion of plant sampled and N status of the plant (Jones, 1986). However, an attempt to overcome some problems with plant S analysis had been investigated by Blair and Crofts (1970) , Anderson and Henderson (1986), and Littlefield *et al.* (1990).

Soil S testing was first attempted by Purri and Asghar (1938). Many procedure have been used to determine the S status of soils, including chemical extractants, sulfate released on incubation, microbial growth, and plant uptake. However most have met with poor success (Jones, 1986). Although research on soil S testing has concentrated on developing methods that extract soil S in proportion to its availability to plants, there is no general agreement on which estimate best defines a soil's S supply (Probert, 1976; Tsuji and Goh, 1979). The nature of the S cycle, which includes four main soil pools, is believed to be the reason for the poor performance of soil S testing (Blair *et al.,* 1992). These four main pools are soil solution sulfate, adsorbed sulfate, and ester sulfate and carbon bonded S. The organic S pool is believed to be a contributor to the S nutrition of crops and pastures (Freney *et al,* 1971; Till and May, 1971, McLaren and Swift, 1977, Goh and Tsuji, 1979; Tsuji and Goh, 1979; McLaren *et al.,* 1985; Blair *et al.,* 1992). Sulfur from the ester sulfate pool can undergo rapid mineralisation and/or splitting to plant available sulfate. The poor performance of soil S testing is believed to be related to the inability of the extractants to estimate the organic S pool that can mineralised.

Plants take up sulfate from the soil solution pool which receive S from both the adsorbed and organic S pools. Monocalcium phosphate is the most widely used extractant for S (Fox *et al.,* 1964; Ensminger and Freney, 1966; Barrow, 1967). Spencer *et al.* (1969) and Spencer and Glendinning (1980) were unable to establish satisfactory relationships with plant response with this extractant. Till (1975) also stated that current extractants and soil test procedures do not measure the replenishment rate of the water soluble sulfate pool over a season. The inability of the extractants to measure the organic S pool that can be mineralised will tend to underestimate the soil sulfate supplying capacity. In contrast, extractants which extract more organic S than that which may become available to the plant will tend to overestimate the size of available S pool. The soil S test should take into account both the availability of the sulfate-S pool and organic S turnover. A reliable soil S test must include a measure of the sulfate in solution, an estimate of adsorbed sulfate which is available for plant uptake and an estimate of potentially mineralisable organic S.

This study consisted of three separate experiments, investigating a range of soil S tests using various extractants to predict S status. The broad objectives of this study were; to develop a

reliable soil S test which takes into account the important role of organic S turnover in the soil for crops and pastures; to examine the sources of sulfur taken up by plants and extracted by a range of chemical extractants; to analyse the forms of the sulfur in the extracted solution, and to examine the ability of soil sulfate and organic S pools to supply S for a range of crops with differing growth periods. In many of these investigations, ^{35}S was used so the turnover rate of the S pools and the source of S incorporated into the plant could be calculated.

The literature review in Chapter 2 describes the role and requirements of S in plant nutrition, as well as S pools and transformations in the soil. It also describes soil S testing which includes the evolution of soil S tests, the problems encountered in soil S testing and reviews the relationships between soil S extracted by the various extractants and plant growth or S uptake. The results of experimental work undertaken are presented in Chapters 3, 4, and 5. Chapter 3 evaluates a range of soil S tests and Chapter 4 evaluates the sources of S taken up by pasture and measured by four chemical extractants. Chapter 5 examines the effect of growth period on the S supply for the range of crops and evaluates the ability of extractants to accommodate differing plant demand rates.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

Sulfur (S) is essential for plants, being accepted as the fourth primary essential nutrient element in crop production following N, P, and K (Olson and Rehm, 1986). Sulfur was described by Jordan and Ensminger (1958), as a neglected plant nutrient because crop responses to applied S were restricted to a few areas and to only a few crops (Stevenson, 1986). Sulfur responses are now being reported in many crops. An adequate sulfur supply to the plants is important because sulfur deficiency can reduce yields and can also affect product quality. Blair (1971) stated that reports of sulfur deficiencies have been increasing since 1966. He also listed the important reasons for the increase in S deficiency as follows;

i) in the use of the high-analysis, low-sulfur containing fertilisers
ii) increases in crop yield that make greater demands on soil nutrients
iii) the decrease in the use of sulfur containing pesticides and fungicides
iv) decreased release of SO_2 by industrial and domestic fuel burning.

Sulfur is also important in animal nutrition, particularly ruminant animal nutrition. Adequate sulfur levels in forage can increase palatability and digestibility of dry matter and result in increased meat, milk and wool production (Downes *et al.,* 1975; Moir, 1975; Tisdale and Bixby, 1983).

Sulfur, in general, occur in soils in a number of different sized pools with different turnover rates. These pools include sulfate in the soil solution, adsorbed sulfate and organic S pools (Till, 1979). Plants take up S from the sulfate pool which both receives S from and contributes S to the organic S pools. A better understanding of soil S pools is therefore required in order to develop a more reliable soil S test procedure.

In this review, role and requirements of sulfur in plant nutrition, as well as S pools and transformation in the soil, and soil S testing are discussed.

2.2 Role and requirement of S in plant nutrition

Sulfur is one of the sixteen essential plant nutrients (Tisdale *et al.,* 1985), and ranks third or fourth in the amount needed for optimum plant growth behind nitrogen and potassium, and sometimes, phosphorus (Tisdale and Bixby, 1983; Tandon, 1991; Messick, *et al.,* 1992). The essentiality of sulfur as a plant nutrient has been known for over 200 years, with the first report of the importance of S in plants coming from Switzerland in 1768 (Duke and Reisenauer, 1986).

2.2.1 Role of S in plant nutrition

Sulfur is a biologically important element which occurs in the compounds either in valency state -2 or +6, such as cysteine and methionine (S = -2) and some sulfate esters (S = +6) (Anderson, 1975). Sulfur is involved in numerous important compounds in plants (Blair, 1979; Tisdale *et al.,* 1985)), directly or indirectly in many plant processes (Blair, 1979), and has many essential functions in plants (Smith and Siregar, 1983). To understand the role of S in plant nutrition, a knowledge of the functions of S in plant growth and metabolism is necessary. The functions of sulfur in plants are as follows:

(a) Synthesis of the three sulfur-containing amino acids cystine, cysteine, and methionine, which are essential components of protein. These three amino acids are the primary products of S metabolism in plants, which account for approximately 90% of the S found in the plants (Allaway and Thompson, 1966). Dijkshoorn and Wijk (1967) found that organic S occurs mainly as cysteine and methionine and is directly related to protein metabolism in most species investigated.

These amino acids are constituents of cellular proteins, which accomplish two functions, catalytic (enzymes) and structural properties of the protein. Cysteine has a special role in determining the 3-dimensional shape of proteins. Whilst methionine, usually as S-adenosyl methionine, serves as a methyl group donor in biosynthesis of lignin, pectin, chlorophyll and flavonoids. Methionine also serves as the substrate for the synthesis of ethylene and the vitamin thiamine (Anderson 1975). Torchinsky, (1981 cited in Duke and Reisenauer 1986) reported that methionine and cysteine are complementary to the structure and function of many enzymes.

Tisdale *et al.,* (1950) reported that increasing levels of S in the culture medium increased the methionine, cystine, and total S content in two experimental strains of alfalfa. Sulfur addition have also been reported to increase the methionine and cystine content in clover, soybeans, and Sudan grass (Sheldon *et al.,* 1951; Bardsley and Jordan, 1957). Ismunadji and Miyake

(1978; cited in Blair *et al.,* 1978) found that rice grown in a sulfur-deficient area had a lower methionine content in grain than that grown with sufficient S.

(b) Formation of disulfide linkages, which are associated with the structural characteristics of protoplasm (Blair, 1979) and are important in stabilising and determining the configuration of proteins (Tisdale *et al.,* 1985). Of the sulfur-containing amino acids, cysteine has a structural role. (Anderson, 1975). The sulfhydryl group (-S-H) of cysteine is concerned with the catalytic function of enzymes (Tisdale *et al.,* 1985) through the provision of sites for attachment of metallic cations, which appear to act as the site of binding the substrate to the enzyme. This function of the sulfhydryl group is important in carbohydrate metabolism (Boyer, 1959 cited in Kurmarohita, 1973). The concentration or quantity of sulfhydryl groups in plant tissue has been associated with increased cold and drought resistance in some species (Blair, 1979).

(c) Synthesis of other metabolites, including coenzyme A, biotin, thiamine or vitamin B1, and glutathione, which are essential in small amount for plants (Thompson *et al.,* 1986).

Coenzyme A is involved in many basic processes, for instance the synthesis of amino acids, the oxidation and synthesis of fatty acids, and the oxidation of certain intermediates of the tricarboxylic acid or citric acid (Tisdale *et al.,* 1985). Coenzyme A also provides the acetyl group in many acetylation reactions (Goldman and Vagelos, 1964 cited in Kurmarohita, 1973).

Biotin is involved in the metabolism of the products of keto acid oxidation and acts as a carbon dioxide carrier in carboxylation reactions (Thompson, 1967 cited in Kurmarohita, 1973).

Thiamine performs the transfer of two carbon fragments of keto sugars to sugar phosphates in the process known as transketolation (Metzler, 1960 cited in Kurmarohita, 1973). Glutathione is an activator of the enzyme glyoxylase, which converts the methyl glyoxal to lactic acid, and functions in the maintenance of appropriate oxidation reduction in cells (Knox, 1960 and Loughman, 1964 cited in Kurmarohita, 1973). Glutathione is also a reducing agent which has a very important role in the detoxification of certain metabolites injurious to cells (Tisdale *et al.,* 1985).

Needham and Hauge (1952; cited in Blair *et al.,* 1978) reported that sulfur-deficient plants of *Medicago sativa* had a lower content of vitamins, biotin, folic acid, pantothenic acid, pyridoxine, riboflavin and thiamine.

(d) A component of other sulfur-containing substances, including S-adenosylmethionine, formylmethionine, lipoic acid, and sulfolipid acid. S-adenosylmethionine, a methyl group donor, is involved in methylation reactions. Formylmethionine is the amino acid at the N-terminal end of protein during synthesis. Lipoic acid, a cofactor, is necessary for the oxidation

of oxoacids in the tricarboxylic acic cycle. Sulfolipid is an important structural material of the chloroplast membrane (Tisdale *et al.*, 1985).

(e) S is required for the synthesis of chlorophyll. Thomas *et al.* (1950; cited in Blair *et al.*, 1978) found that sulfur-deficient plants of *Medicago sativa* has 40% less chlorophyll in the leaves than in non-deficient plants. Similarly Nanawati *et al.* (1973; cited in Blair *et al.*, 1978) showed that the content of chlorophyll in fresh leaves of rice seedlings was significantly reduced under S deficiency conditions Rendig *et al.* (1968; cited in Tisdale *et al.*, 1985) showed that a high level of sulfur nutrition increased the chlorophyll content of Kenland red clover.

(f) An essential part of ferredoxin which is important to plants (Anderson, 1975). Ferredoxin, a type of nonheme iron sulfur protein, occurs in the chloroplast. It functions as an electron carrier in the light and dark reactions of the photosynthetic process. Reduced ferredoxin is possibly the source of reducing power for the reduction of carbon dioxide in dark reaction. The oxidised form of ferredoxin is the receiver of the electrons exported from chlorophyll during photooxidation of chlorophyll in the light reaction. (Tisdale *et al.*, 1985).

(g) Formation of a ferredoxin-like compounds which is involved in the fixation of nitrogen by root nodule bacteria and free living nitrogen-fixation bacteria (Blair, 1979). Ferredoxin has a important role in nitrite reduction, sulfate reduction and assimilation of nitrogen by these bacteria, and in mitochondrial electron transport (Tisdale *et al.*, 1985). Tandon, (1991) reported that sulfur promoted nodulation in legumes thereby promoting N-fixation.

(h) Required for activation of certain proteolytic enzymes such as papainases (Blair, 1979).

(i) Required for activity of ATP sulfurylase, an enzyme that functions in the metabolism of sulfur in plants (Blair, 1979). Adams and Rinne, (1969 cited in Thompson *et al.*, 1986) found that the leaves of soybean (*Glycine max* L. Merrill) had the highest concentration of ATP sulfurylase activity and the younger leaves had more activity than the older ones. On the other hand, the roots had almost as much ATP sulfurylase enzyme activity as the leaves in maize, *Zea may* L., (Ohajobi *et al.*, 1973 cited in Thompson *et al.*, 1986) and in tomato, *Lycopersicon esculentum* L (Ellis, 1969 cited in Thompson *et al.*, 1986).

(j) Occurs in volatile compounds responsible for the characteristic taste and smell of plants such as mustard, onions, garlic and other cruciferous plants

(k) Enhances oil formation in crops such as soybeans flax, peanuts, and rapeseed. Biswas and Tewatia (1992) reported that the oil content of oilseed crops increased by 3-9% due to S application on a sulfur-deficient soil in India. A similar result was found by Walker and Booth (1992) who found that the application of S increased glucosinolate levels in oilseed rape. Nuttall and Ukrainetz (1983; cited in Beaton and Soper, 1986) also found that the

percentage of oil in canola seed was increased by S application in combination with N, P, and K.

The effects of S nutrition on oil content has received more attention in Europe than in the United States, where the emphasis has been on breeding and selection to increase the oil content of crops, rather than on the nutrition of the crop (Tisdale *et al.,* 1985).

(l) A nutritional value of plants. Sulfur is known to influence plant quality (Spencer *et al.,* 1977). Protein concentration in kernels of cereal crops and nutritive value of grain was increased by the presence of S fertilised legumes in crop rotations in the reports of Beaton and Soper (1986). Bettany *et al.* (1983) reported that both protein quality and quantity of wheat were increased significantly where wheat followed sulfur-fertilised alfalfa in rotation, and that S deficiencies can substantially affect crop quality of both forages and oilseed. Large amount of S containing amino acids and protein were formed in rapeseed when S was supplied (Beaton and Soper, 1986).

The effect of sulfur on baking quality of grain has been reported by Beaton *et al.* (1971; cited in Beaton and Soper, 1986) who found that the largest loaves of the best quality bread were obtained from wheat receiving S fertiliser. Moss *et al.* (1981) also found that a restricted supply of S seriously affected the baking quality of wheat by producing dough that was excessively tough and unsuitable for normal use. These changes in dough quality were associated with decreases in the proportion of albumins and of high mobility gliadins in the total protein in the low S grain. Haneklaus *et al.,* (1992) stated that increasing S concentrations in grain were directly related to improved baking quality of the flour.

The concentration of S-containing compounds and the baking quality of wheat grain is dependent upon both genetically fixed and environmental factors (Byers *et al.*, 1987; Byers *et al.,* 1987 cited in Haneklaus *et al.,* 1992). Recently, Haneklaus and Schnug (1992) found that the environmental influences on S concentration in grain are of more importance than varietal effects.

Blair (1979) stated that sulfur deficiencies affect not only plant yield also protein quality through their effects on the synthesis of the amino acids. Samosir (1989) also reported that S deficiency could result in delayed maturity of from nil to 25 days in rice.

Sulfur is absorbed by plant roots almost exclusively as the sulfate (SO_4^{2-}) ion (Bouma, 1975; Blair *et al.,* 1978; Clarkson *et al.,* 1983; Tisdale *et al.,* 1985; Thompson *et al,* 1986), and transported to leaves. However plants can take up sulfur gases (SO_2) directly through the leaves (Thomas and Hendricks, 1943; Fried 1948; Tisdale *et al.,* 1985), while high concentrations of SO_2 are toxic to the plants (Tisdale *et al.,* 1985). Plants utilise inorganic sulfate as the source of S for the synthesis of cysteine and methionine or other S containing compounds (Anderson, 1975; Tisdale *et al.,* 1985; Thompson *et al.,* 1986). Sulfate may also accumulate in leaf tissue if it is in

excess of the requirement for protein synthesis (Dijkshoorn *et al.*, 1960; Huang *et al.*, 1992). The metabolism of S in plants has been summarised by Anderson, (1975) and Thompson *et al.*, (1986). Anderson (1975) summarised the sulfur metabolism in plants as follows (Figure 2.1) and he stated the two essential functions of proteins are:

"1) Catalytic proteins (enzyme) catalyse the biosynthesis and catabolic reactions required for maintenance, growth and development of cells and organisms.

2) Structural proteins are constituents of the various cell membranes which act as a boundary between the organisms and the internal environment".

Sulfur containing amino acids are involved in both of these functions.

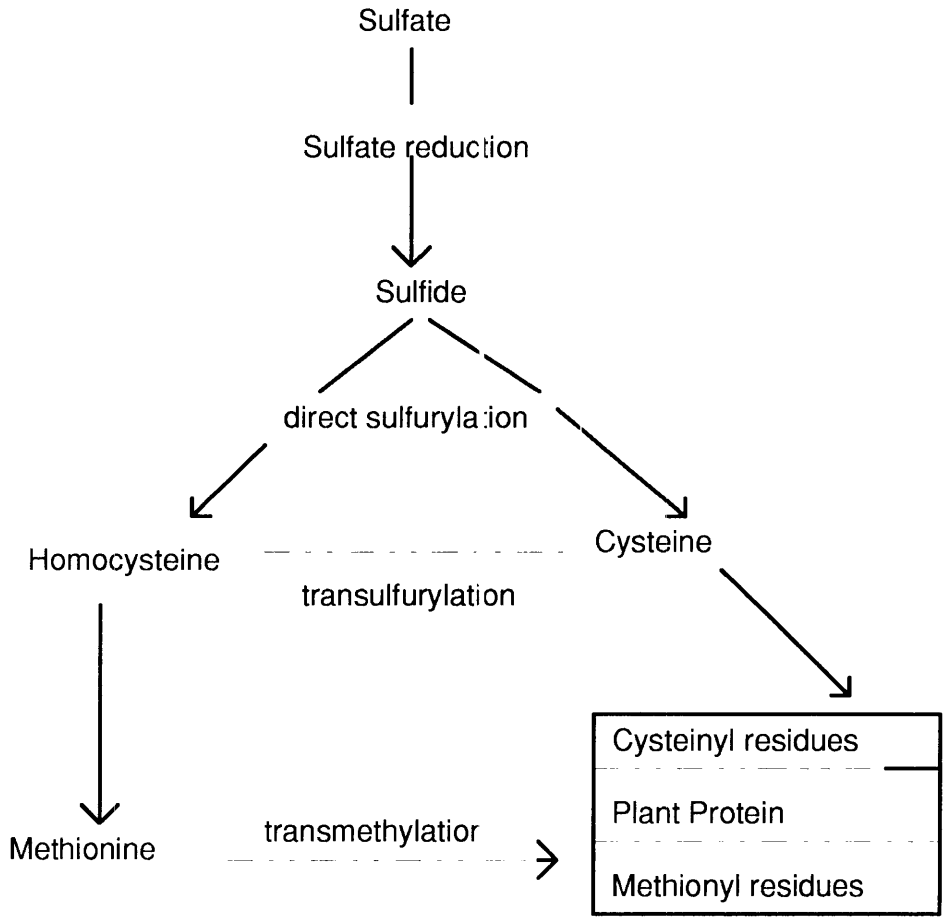
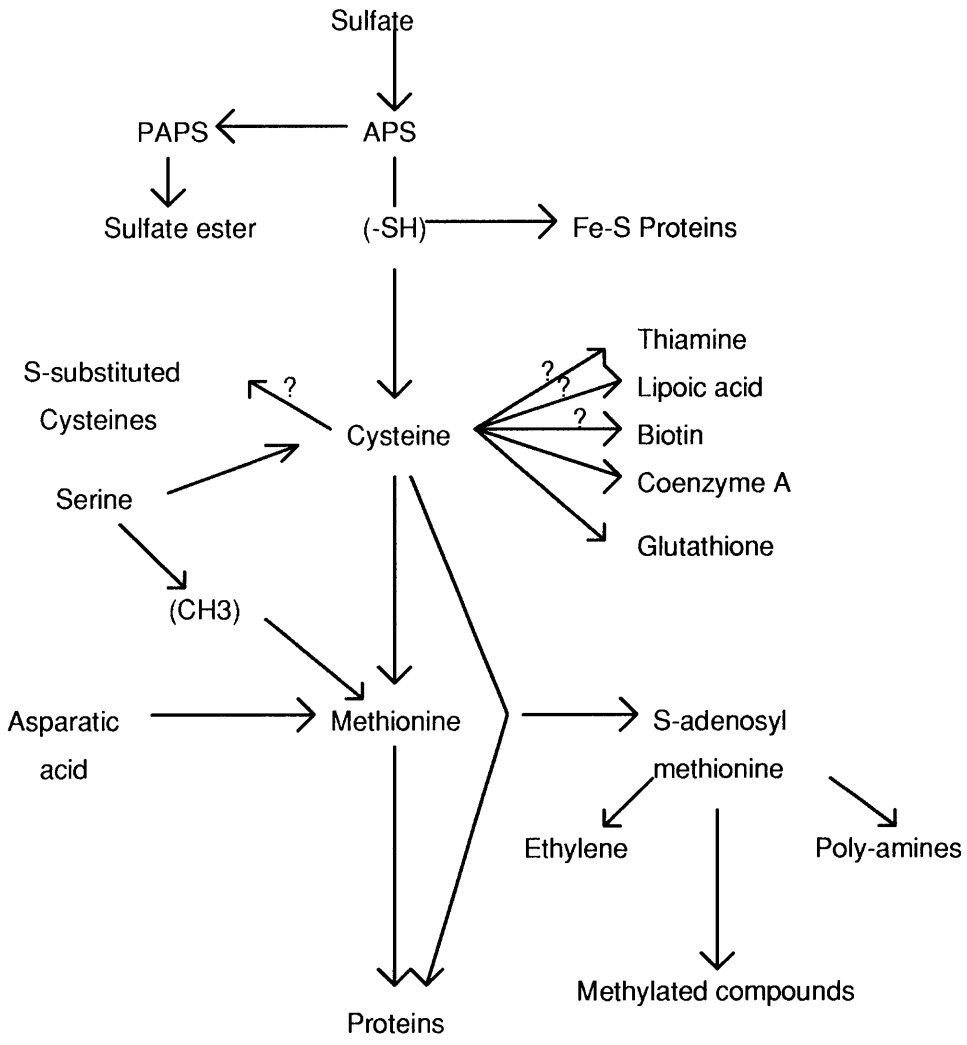


Figure 2.1 *Summary of the metabolism of sulfur in plants (Anderson, 1975).*

Thompson *et al.* (1986) presented the pathways for the synthesis of essential S compounds (Figure 2.2)



APS = adenosyl 5' -phosphosulfate

PAPS = 3' -phospho-APS

Figure 2.2 *Pathways for the synthesis of the essential sulfur compounds (Thompson et al., 1986).*

They categorised the flow of sulfur from SO_4^{2-} to various sulfur containing compound into six steps:

(1) Sulfate is activated. Sulfate (SO_4^{2-}) is absorbed by plant roots, transported to the endodermis and loaded into the xylem. Sulfate is transported to the leaves in the transpiration stream, and unloaded into the mesophyll cells. In the chloroplast, SO_4 is activated

(2) Activated sulfate is reduced (to a -2 valence state).

(3) Reduced S is incorporated into cysteine.

(4) The sulfur of cysteine is transferred into methionine and other essential compounds.

(5) Methionine is transformed into S-adenosylmethionine, which is a methyl donor and a precursor of important non sulfur compounds.

(6) Cysteine and methionine are incorporated into proteins.

Finally, he stated that S is also found in a host of other compounds whose function is unknown, and that the present knowledge of S metabolism in plants is incomplete.

It can be concluded that sulfur is necessary for plants because it is involved directly or indirectly in many plant processes. Sulfur compounds are essential for the proper functioning of the plant.

2.2.2 Sulfur requirement of plants

Plants require S for numerous functions as described in section 2.2.1. The S requirement of plants has been defined by Stanford and Jordan (1966), as the minimum uptake of S related to the maximum dry matter yield. Similarly, Loneragan (1968; cited in Duke and Reisenauer, 1986) stated that "a crop's requirement is commonly defined as the minimum content of that nutrient associated with the maximum yield or the minimum rate of the nutrient intake associated with the maximum growth rate". The first of these is the relation between growth and the element's internal concentration within the plant or plant part (critical concentration). The second is the relation between growth and the external concentration of the element in the soil or culture solution. These two relations are the bases of plant analysis and soil testing (Spencer, 1975).

Spencer (1975) stated that "the sulfur requirement has been considered as the amount of sulfur necessary for the plant to absorb over some given period of time. This infers that when the requirement is just being met, sulfur compounds will occur inside the plant at a particular concentration. In a given situation, can also be considered in terms of how much sulfur must be provided so that the plant grows optimally."

According to Spencer (1975) the internal S requirement of plants, can be described in term of the critical percentage of one or more forms of S in a plant for particular growing conditions, stage of growth and plant part, which is an indirect measure of just-adequate nutrition. On the other hand, the external S requirement can be elucidated in terms of a just-adequate concentration of S in the solution bathing the root.

The internal and external S requirements of tropical crops has been described by Fox and Blair (1986). Internal S requirements of crops can be expressed as the quantity of total S at near maximum yield or gross S uptake or concentration of total S (sometimes as SO_4-S) in the particular plant part. Whilst the external sulfur requirements are those concentrations of SO_4^{2-} in the solution bathing roots that produce near maximum yields.

Spencer (1975) classified crops into three groups on the basis of their S fertiliser requirement:

(1) crops with high S fertiliser requirement such as cruciferous forage, lucerne, rapeseed and canola.

(2) crop with the moderate S fertiliser requirement such as coconuts, sugar cane, clovers and grasses, coffee, and cotton.

(3) crop with the low S fertiliser requirement such as sugar beet, cereal forages, cereal grains and peanut.

Randall (1988) stated that plants differ markedly in their S demands with crops such as crucifers having a higher S requirement than cereals.

The S concentrations found in plants varies among species, among cultivars within a species, and with stage of development of the crop (Duke and Reisenauer, 1986), and different parts of the same plant (Blair, 1979). Blair *et al.,* (1978) reported that there is a general decline in the sulfur concentration of the tops of cereals with age, with the sulfur concentration of IR8 rice declining from 0.26% two weeks after transplanting to 0.11% at flowering. Duke and Reisenauer (1986) compiled a list of the S content of various crops (Table 2.1) and stated that the tissue S content within the cultivar can vary widely depending on the amounts supplied. He also stated that the halophytes and most species of the *Cruciferae* and *Liliacae* families contain the largest amount of S, with cotton, the legumes and tobacco being intermediate, and the small grains and maize (*Zea mays*) containing the lowest S content. Jones (1975) reported that the plant part or age of the plant often markedly affects the concentration of S found.

Table 2.1 *The S content of selected crops at harvest (Duke and Reisenauer, 1986).*

Crop	Component	S content (kg ha^{-1})
Alfalfa	Hay	45
Barley	Grain	12
Barley	Straw	12
Cabbage	Head	73
Maize	Grain	17
Maize	Stover	20
Maize	Silage	36
Cotton	Lint	7.5
Cotton	Stalks	23
Mixed grasses	Hay	52
Onion	Bulbs	44
Peanut	Nuts	11
Rice	Grain	6
Rice	Straw	8
Sorghum	Grain	22
Sorghum	Stover	16
Soybean	Grain	14
Soybean	Straw	15
Sugarbeet	Roots	12
Sugarbeet	Tops	41
Sugarcane	Tops	40
Tomato	Fruit	24
Tomato	Vines	23
Turnip	Roots	17
Turnip	Tops	6
Wheat	Grain	9
Tobacco	Leaves	14
Tobacco	Stalks	8

Tandon (1991) also reported that the S concentration in plants varies in different parts of the same plant and that the S concentration in the grains is higher than in harvested residues with the widest differences in cruciferous crops and the smallest in legumes (Table 2.2).

Table 2.2 *The average S concentration in some plant parts (Tandon, 1991).*

	In seed (%S)	In harvested residue (%S)
Cruciferous oilseed	1.19	0.13
Sunflower/Sesame	0.34	0.22
Legumes	0.24	0.20

2.3 Sulfur pools and transformation in the soil

The original source of most soil S is the metal sulfides (Fe, Ni, Cu) of plutonic rocks, with most unweathered igneous rock containing 0.05-0.30% S (Kurmarohita, 1973). Tisdale and Nelson (1975) estimated that the earth's crust contains about 0.06% S. Generally, basic igneous rocks have a higher S content than acidic rocks (Kurmarohita, 1973). During the weathering process much of the S in pyrites and other metallic sulfides are transformed to sulfates (Jordan and Ensminger, 1958). These sulfates are then precipitated as soluble and insoluble salts in arid or semiarid climates, absorbed by living organisms, or reduced by other organisms to sulfides or elemental sulfur under anaerobic conditions (Tisdale *et al.*, 1985). In many soils a major portion of sulfur is combined in the soil organic matter (Barber, 1984).

The main sulfur-bearing minerals in rocks and soils are gypsum ($CaSO_4.2H_2O$), anhydrite ($CaSO_4$), epsomite ($MgSO_4.7H_2O$), mirabilite ($NaSO_4.10H_2O$), pyrite and marcasite (FeS_2), sphalerite (ZnS), chalcopyrite ($CuFeS_2$), and cobaltite (CoAsS), and other important sulfides, including pyrrhotite ($Fe_{11}S_{12}$), galena (PbS), arsenopyrite ($FeS_2.FeAS_2$) and pentlandite $(FeNi)_9S_8$ (Tisdale *et al.*, 1985).

Another source of soil sulfur is the atmosphere. Fox *et al.* (1983) reported that atmospheric S derives from numerous sources: emissions of sulfur dioxide from industry, agriculture, and the burning of fossil fuels and plant materials, smelting, volatilisation of hydrogen sulfide and dimethyl sulfide during biological decay, reduction of sulfates in waterlogged soils and swamps, volcanic emissions, and sea spray. In industrial areas where coal and other sulfur containing products are burned, sulfur dioxide is released into the air, and much of this gas is returned to the soils by rain (Tisdale *et al.*, 1985).

2.3.1 Total soil sulfur

Sulfur (S) occurs in soils in both organic and inorganic forms but the proportion of organic to inorganic S may vary widely according to the soil type (pH, drainage status, organic matter content, mineralogical composition) and depth in the profile (Williams, 1975; Stevenson, 1986; Freney, 1986a). Bohn *et al.*, (1986) stated that the S content of soil is complex and depends on the organic S content, charge on the soil colloids, S input and sulfate leaching. The total sulfur content of soils ranges from approximately 20 mg kg^{-1} to 3 or 4%, and 100 to 1000 mg kg^{-1} in the humid and semi-humid regions (Syers *et al.*, 1988), or 22 to 35 000 mg kg^{-1} for a number of soils and in excess of 50 000 mg kg^{-1} in the severely industrially polluted areas (Bettany and Stewart, 1983).

The large range has been reported in total S concentration of soils both within and between different regions of the world.

Table 2.3 *The total S concentration (mg kg^{-1}) in soils from different regions of the world.*

Country	Region/Area	Total S	References
Australia	-	30-545	Spencer and Freney (1960)
	-	38-371	Freney (1961)
	north Queensland	11-725	Probert (1977; cited in Rayment *et al.*, 1983)
Canada	-	440-820	Lowe and DeLong (1961)
	eastern	800-2000	MacKenzie *et al.* (1967)
China	southern	190-580	Liu *et al.* (cited in Blair 1983)
India	-	19-9780	Tandon (1991)
Indonesia	-	49-149	Ismunadji *et al.* (1983)
New Zealand	grasslands	280-910	Walker and Adams (1958)
Philippines	-	167-626	Islam and Ponnamperuma (1982 cited in Mamaril *et al.*, 1983)
Scotland	northern	200-690	Williams *et al.* (1960)
Thailand	-	57-302	Chaiwanakupt *et al.* (1987)
USA	Iowa	57-618	Tabatabai and Bremner (1972a)
	Oregon	82-784	Harward *et al.* (1962b)
	Mississippi	120-565	Nelson (1964)
	Minnesota	131-940	Rehm and Caldwell (1968)

In general, temperate and tropical soils vary greatly in their total S content (Blair and Lefroy, 1987), the total S content of tropical soils is generally lower than that of temperate soils due to low

S containing parent material, higher temperature and high intensity rainfall, and resultant high weathering, leaching, and low organic matter content (Blair, 1979).

The C:N:S ratio of soils has been reviewed by Kurmarohita (1973) and found to vary widely from 147:10:1.4 for soils from Northern Scotland, 121:10:1.17 and 155:10:1.4 for pasture and podzolic soils of Australia, 130:10:1.3 for grassland soils of New Zealand, 114:10:1.5 and 145:10:1.01 for Minnesota and Oregon soils. Tabatabai and Bremner (1972b) showed an average ratio of 109:10:1.54 for Iowa soils. Freney (1986a) reported that agricultural soils, native grass and woodland soils, and peats and organic soils have C:N:S ratios of 130:10:1.3, 200:10:1 and 160:10:1.22 respectively.

Table 2.4 *Effect of parent material, weathering, and pasture improvement on C:N:S, C:S, and N:S ratios of soils.*

Factor	Ratios		
	C:N:S	C:S	N:S
Parent material (Williams *et al.*, 1960)			
Granite	169:10:1.45	117	6.9
Slate	148:10:1.42	104	7.0
Old Red Sandstone	130:10:1.37	95	7.3
Basic Igneous	140:10:1.37	102	7.3
Calcareous	113:10:1.27	89	7.9
Weathering stage (Walker and Adams, 1959)			
Weakly weathered	172:10:1.1	156	9.1
Moderately weathered	180:10:1.2	150	8.3
Strongly weathered	206:10:1.3	158	7.7
Years under pasture (Walker *et al.*, 1959)			
0	300:10:1.2	250	8.3
1.5	210:10:1.9	110	5.3
3	210:10:1.8	117	5.6
5	160:10:1.6	100	6.3
8	130:10:1.4	93	7.1
15	140:10:1.4	100	7.1
25	110:10:1.2	92	8.3

Considerable variations can occur in the C:N:S ratio between soils, but the mean ratio for various groups of soils from different parts of the world are generally similar (Williams, 1975;

Biederbeck, 1978; Stevenson, 1986; Freney, 1986a). Differences in the C:N:S ratios of soil groups (Table 2.4) have been attributed to such factors as parent material, climate, type of vegetation and pasture improvement (Walker and Adam, 1958, 1959; Williams and Steinbergs, 1958; Williams *et al.*, 1960; Bettany *et al.*, 1973 Neptune *et al.*, 1975).

Generally, virgin soils have a wider C:N:S ratio than those of the cultivated soils in different parts of the world (Cowling and Jones, 1970; Bettany *et al.*, 1973; McLaren and Swift, 1977). This suggests that sulfur is relatively more resistant to mineralisation than carbon and nitrogen (Freney, 1986a). It is generally reported that there is a closer correlation between total N and organic S in soils than between organic C and organic S (Tabatabai and Bremner, 1972b; Bettany *et al.*, 1973; Neptune *et al.*, 1975). This close N:S ratio is probably due to the similarity in the cycling of N and S within the soil-plant system (Biederbeck, 1978).

Organic sulfur accounts for >95% of the total S in the most soils from humid and semi-humid regions (Tabatabai, 1982; Freney, 1986a). Kurmarohita (1973) found that approximately 80% of the total S was present in organic forms in Thai soils. Factors that affect the organic matter content of soil also influences the amount of both total and organic S in these soils (Stevenson, 1986). This has been demonstrated by many researchers from different regions of the world (e.g., Williams and Steinbergs, 1959; Freney, 1961; Cooper, 1972; Jones *et al.*, 1972; Tabatabai and Bremner, 1972a,b). Freney (1986a) has collated data on the organic and inorganic S content of soils from different locations and this is presented in Table 2.5.

Table 2.5 *Concentration of organic and inorganic S (mg kg^{-1}) in soils from various locations (Freney, 1986a).*

	Location	Soil type	Total S	Inorganic S	Organic S
Australia	Queensland	Agricultural	11-725	2-18	82-98
	New South Wales	Agricultural	38-545	4-13	87-96
	New Zealand	Agricultural	240-1360	2-9	91-98
Europe	Scotland	Calcareous	460-1790	21-89	11-79
	Scotland	Acid	300-800	2-10	90-98
	England	Organic	405	5	95
North America	Albert	Agricultural	80-700	8-15	85-92
	Hawaii	Volcanic ash	180-2200	6-50	50-94
	Iowa	Agricultural	78-452	1-3	97-99
South America	Brazil	Agricultural	43-398	5-23	77-95
	West Indies	Agricultural	110-510	2-10	90-98

2.3.2 The inorganic sulfur pool

a) Introduction

In most surface soils, inorganic sulfur accounts for only a small fraction of the total S (Bohn *et al.*, 1986). Freney (1961) found that the amounts of inorganic S in surface soils of Australia was about 7% of total S. Tabatabai and Bremner (1972b) and Rehm and Caldwell (1968) found that inorganic S accounted for only 3% and 3.2% for Iowa and Minnesota soils respectively. Similarly, Jones *et al.* (1972) found that sulfate S constituted only 5.8% in English soils, and Acquaye and Beringer (1989) reported values of 2.3 to 14.8% for Ghanaian soils. Nguyen and Goh (1992a) recently also found that the inorganic sulfur (readily soluble and adsorbed S) accounted for less than 5% of the total S in grazed pasture soils in New Zealand.

Inorganic S in soils may occur as sulfate and compounds of lower oxidation state such as sulfide polysulfide, sulfite, thiosulfate, and elemental sulfur ((Williams, 1975; Tabatabai, 1982). Most inorganic sulfur occurs as sulfate, and the amounts of reduced-S compound are generally less than 1% of the inorganic S pool in well drained, well aerated soils (Freney, 1961). Fitzgerald (1976) reported that only 5.2% of the total S was present as inorganic sulfate and little was found as elemental S or sulfide. Stanko-Golden *et al.* (1992) found large amounts of soluble and adsorbed sulfate in forest soils of the Southern Appalachians, U.S.A., and the thiosulfate content was <1% of total S.

b) Sulfate

Sulfate is the primary source of sulfur taken up by plants (Blair, 1988). In general, tropical soils have lower levels of sulfate in the soil solution compared to temperate soils (Blair, 1979) and the subsurface layers usually contain more sulfate and are capable of adsorbing more than the surface layers (Ensminger, 1954). The availability of sulfate (adsorbed sulfate) in the subsoil for plants depends on the plant rooting habits and the release of sulfate for plant uptake (Bohn *et al.*, 1986). Harward and Reisenauer (1966) stated that the amount of sulfate in the subsoil may represent a significant proportion of plant available S pool in soil. Sulfate may occur in soils as water soluble salts, adsorbed to soil colloids or as insoluble forms (*e.g.* $CaSO_4$; Fe and Al-sulfate) (Williams, 1975). Bohn *et al.* (1986) stated that the major form of inorganic S in the soil is inorganic sulfate in both the soil solution and on the adsorption sites, and these two fractions are the S sources readily available to plants. The concentration of sulfate in the soil has been observed to vary throughout the year (Barrow, 1966; Williams; 1968; Vaughn, *et al.*, 1986; Ghani *et al.*, 1990).

(i) Water soluble sulfate

The amount of water-soluble sulfate in soils varies widely both between soil type and within the soil profile. Small but appreciable amounts of sulfate are usually found in surface horizons of most well drained soils and aridity or poor drainage conditions can result in appreciable accumulations of this fraction in subsoil horizons (Williams, 1975, Tabatabai, 1982). Kurmarohita (1973) reported that the water soluble S is the dominant form of the inorganic S in the surface horizons in Thai soils. Considerable seasonal fluctuation in the concentration of water-soluble sulfate in surface soils is observed due to the effect of seasonal conditions on mineralisation of organic S, leaching of soluble sulfate, and sulfate uptake by plants (Tabatabai, 1982). Tabatabai (1982) also reported that the application of fertilisers and the sulfate content of rain and irrigation waters may affect the concentration of water-soluble sulfate in soils. The amount of S accessible to plants is determined by the soil soluble sulfate concentration, and the supply of S to this pool from either organic matter via the microbial pool or directly from fertiliser, animal residues, or atmospheric inputs (Blair, 1988).

Soluble sulfate in soils is extractable with water (Spencer and Freney, 1960; Fox *et al.*, 1964; Walker and Doornenbal, 1972; Anderson, 1992), and with salt solutions such as $CaCl_2$, LiCl, $MgCl_2$ and NH_4Cl (Williams and Steinbergs, 1959; Tabatabai and Bremner, 1972a; Roberts and Koehler, 1968; Maynard *et al.*, 1987). A salt solution is frequently preferred because of the dispersion difficulty associated with aqueous extractants (Tabatabai, 1982). Williams and Lipsett (1961) found that calcium chloride is generally preferred due to dispersion difficulties with aqueous extractants and a lower solubility of organic matter. Maynard *et al.* (1987) also observed that salt solutions, particularly NH_4Cl were more effective in extracting sulfate-S than water. Freney *et al.* (1969) stated that these extracts usually contain some organic sulfur which is reducible by hydriodic acid and precautions must be taken to avoid errors from this source.

(ii) Adsorbed sulfate

Soils vary widely in their capacity to adsorb sulfate. In some soils, sulfate adsorption plays an important part in the retention of sulfate against leaching, but others possess little or no sulfate adsorption capacity.

Sulfate is adsorbed by hydrous oxides of Fe and Al and by the edges of aluminosilicate clay particles (Chao *et al.*, 1962c; Harward *et al.*, 1962a). The hydrous oxide may be as coatings on clay minerals (Fox *et al.*, 1971; Parfitt, 1980) or as free oxides (Sander and Tinker, 1975). The amount of sulfate adsorbed on the Fe and Al oxide depends on their specific surface area and

the density of the surface OH^- groups. Generally, Al oxides have greater sulfate adsorption capacities than Fe oxides (Ensminger, 1954; Aylmore *et al.,* 1967).

Chang and Thomas (1963) reported that adsorption of sulfate involves the replacement of OH^- groups. Rajan (1979) also reported that in the process of sulfate adsorption by allophanic clay, OH^- is released and surface charge changed, and that sulfate is adsorbed to clay by displacing H_2O and OH^- bonded to Al and Fe ions. This change in the surface charge is influenced by the concentration of sulfate with sulfate adsorption at low concentrations ($<10^{-4}$ M) neutralising the positive charge of the clay and at high concentrations increasing the negative charge. Adsorption of sulfate on pure hydrous oxides and clay is rapid (Rajan, 1978, 1979). In soils, the retention rate is slower (Barrow, 1967), possibly because of the incomplete exposure of the adsorption sites. Bloom (1981) stated that sulfate was also adsorbed onto organic matter, probably onto (Al, Fe) - humus complexes. This was confirmed by Harrison *et al.* (1989) who reported that organic matter may increase sulfate adsorption. On the other hand Singh (1984) reported that organic matter had a negative effect on sulfate adsorption.

Sulfate is retained on the adsorption complex in soils by three mechanisms: (1) coulombic attraction on positively charged sites, (2) formation of the inner sphere complexes with Fe or Al through replacement of surface water or hydroxyl groups and (3) precipitation of basic aluminium sulfate (Adams and Rawajfih, 1977; Parfitt, 1978).

Sulfate adsorption is influenced by different soil properties such as type of clay, soil pH and the presence of cations and anion in soils.

Clay content and type of clay mineral

Adsorption of sulfate usually increases with the clay content of soils. Harward and Reisenauer (1966) reported that sulfate retention on different clay mineral types is in the order kaolinite (1:1) > illite (2:1) > montmorillonite (2:1). The greater retention of sulfate by kaolinite is due to the higher proportion of anion exchange sites on 1:1 type clays and higher negative charge with associated anion repulsion on 2:1 type clays. The capacity for sulfate adsorption by hydrogen saturated clays has been reported by Chao *et al.* (1964) who found that S absorption by clay mineral types increases in the order bentonite, illite, and kaolinite, with Al clays adsorbing more S than H clays. When saturated with aluminium, adsorption is about the same for kaolinite and illite but much lower than bentonite. Fox *et al.* (1971) found that highly weathered soils, which normally contain higher amounts of 1:1 clays and Fe and Al hydrous oxides, can absorbed large quantities of sulfate. Fox (1974) stated that the amount of sulfate adsorbed in tropical soils varies due to their dominant mineralogy, with amorphous hydrated oxides > crystalline oxides > kaolin clay > 2:1 clay.

The lower soil horizons may adsorb more sulfate than the surface layers (Stevenson, 1986). In the subsoil of highly weathered soils of warm and humid climates adsorbed sulfate can be the dominant form of S present (Harward and Reisenauer, 1966; Kamprath, 1968). Hasan *et al.* (1970) showed that a subsoil of a well drained latosol from Hawaii contained as much as 7000 mg kg^{-1} of adsorbed S. Similar high amounts of adsorbed S has been found in subsoils by many investigators (Ensminger, 1954; Jordan and Bardsley, 1958; McClung *et al.*, 1959; Chao *et al.*, 1962b; Kamprath, 1968; Roberts and Koehler, 1968).

Soil pH

Sulfate adsorption is strongly related to soil pH; the amount of sulfate adsorption decreases with increasing pH from 4 to 7 due to a decline in the electrostatic potential of the adsorption plane (Kamprath *et al.*, 1956; Chao *et al.*, 1962c; Barrow, 1970; Fox *et al.*, 1971; Gebhardt and Coleman, 1974; Couto *et al.*, 1979). Chao *et al.* (1963) found that no sulfate adsorption occurred at a pH value above 6.5, due to the reduction in positive charge on the hydroxyl complex and increased OH^- concentration. However, Hingston *et al.* (1972) reported that sulfate adsorption by geothite and gibbsite declined with increasing pH up to 8, beyond which no adsorption occurred. In the study of Nodvin *et al.* (1986) the highest sulfate retention occurred at pH about 4.0. Marsh *et al.* (1992) also found that increased pH results in decreased sulfate retention which has implications for sulfate mobility in field soils.

There is a close direct relationship between sulfate adsorbed by soils and surface positive charge (Hingston *et al.*, 1972; Parfitt and Smart, 1978; Marsh *et al.*, 1987). Haynes (1983) stated that limed soil had little or no sulfate adsorption because liming increases soil pH which decreases surface positive charge and increases surface negative charge which decreases sulfate sorption. Recently, Marsh *et al.* (1992) reported that the effect of lime were more significant for sulfate than for phosphate.

The presence of cations and anions in soils

Sulfate adsorption is directly influence by the presence of cations and anions. Chao *et al.* (1963) observed that the relative effect of different cations on the adsorption of sulfate is Al^{3+} > Ca^{2+} > K^+. Tisdale *et al.* (1985) summarised the effect on sulfate adsorption of the associated cation of salts or the exchangeable cation as follows: H^+ > Sr^+ > Ba^{2+} > Ca^{2+} > Mg^{2+} > Rb^+ > K^+ > NH^{4+} > Na^+ > Li^+. Sulfate adsorption is also affected by the concentration of cations in solution. Barrow (1972) found that sulfate adsorption increased as concentration of calcium increased.

There are marked differences in the retention of different anions and on the effects of anion competition. The retention of anions by soil is hydroxyl > phosphate > sulfate = acetate > nitrate > chloride (Chang and Thomas, 1963). In general, sulfate is considered to be weakly held in

comparison to phosphate. The stronger adsorption of phosphate than sulfate is the basis for extraction of adsorbed sulfate (Ensminger, 1954; Fox *et al.*, 1964; Barrow 1969a). Increasing the phosphate concentration in solution leads to complete desorption of adsorbed sulfate (Rajan and Fox, 1975) and decreases sulfate adsorption from a solution containing both phosphate and sulfate (Parfitt, 1982). Ensminger (1954) found that the application of superphosphate to a sandy loam from Alabama decreased the capacity of soil to adsorb sulfate. Chao *et al.* (1962a) also found that the application of phosphorus increased sulfur leaching. Metson and Blakemore (1978) observed that phosphate application decreased sulfate adsorption more in weakly weathered than in highly weathered soils.

Barrow (1971) stated that sulfate adsorption capacity is affected by pedological factors such as parent material, rainfall, and drainage.

(iii) Insoluble sulfate

Various forms of insoluble sulfates can occur in soils, including barium and strontium sulfates, sulfate associated with calcium carbonate and basic iron and aluminium sulfate (Williams, 1975).

In arid regions, sulfates can account for more than 90% of the total S in surface soils by forming insoluble salts like gypsum ($CaSO_4.2H_20$), barytes ($BaSO_4$), epsomite ($MgSO_4.7H_2O$) and strontium sulfate (Kurmarohita, 1973). Sulfate can occur as a co-crystallized impurity in calcium carbonate and this has been found to account for almost 95% of total S in certain calcareous soils of Australia. (Williams and Steinbergs, 1962, 1964; Williams *et al.,* 1960). In subsoil horizons of calcareous soils Williams (1975) found that it accounted for 40 to 50% of the total S. Beattie and Haldane (1958) identified that barytes occurred as small concretions in certain soils. Freney (1967; cited in Kurmarohita, 1973) reported that inorganic S can also occur as insoluble minerals, such as sphalerite (ZnS), chalcopyrite ($CuFeS_2$) or pyrite (FeS_2) associated with sedimentary rocks, shale and limestone. Basic iron sulfate, jarosite [$KFe_3(OH)_6(SO_4)_2$] and coquinbite, [$Fe_2(SO_4)_3.5H_2O$] have been identified in separates from tidal marsh soils (Clark *et al,* 1961; Fleming and Alexander, 1961).

(iv) Compounds of lower oxidation state than sulfate

Under anaerobic conditions, particularly in tidal swamps and poorly drained or waterlogged soils, the main form of inorganic sulfur in soils is sulfide and often elemental sulfur (Harmsen *et al.,* 1954; Hart, 1959; Fleming and Alexander, 1961). Elemental sulfur and pyrite (FeS_2) occur predominantly in acid sulfate soils (Kurmarohita, 1973), with sulfide dominating in the subsoil at

depths below the watertable (Brummer *et al.*, 1971 cited in Williams, 1975). The rapid oxidation of sulfide to sulfate in such soils often leads to acidity problems (Hart, 1959; Fleming and Alexander, 1961; Walker, 1972). Under waterlogged or submerged conditions, sulfide, if not precipitated by iron, causes toxicity problems in rice crops (Greer, 1957; Vamos, 1964; Blair *et al.*, 1978) resulting in a physiological disease in rice known as "Akiochi" (Aomine, 1962). Wainwright (1984) stated that in well drained soil, where reduced S is found, its presence could be indicative of atmospheric pollution of industrial origin.

2.3.3 The organic sulfur pool

Stevenson (1986) stated that organic forms of sulfur dominate in soils of humid and semi-humid regions. In general, organic sulfur compounds occur in plants, animals and microorganisms growing in or on soils and these sulfur compounds can be formed in soils during the life or death of the organisms. Most of the sulfur (approximately 90%) in plants is found in the amino acids cysteine, cystine and methionine (Blair, 1979). Freney (1986a) reported that the majority of the sulfur in microorganisms occurs in the sulfur containing amino acids, cysteine, cystine, and methionine with some sulfur in compounds such as choline sulfate, sulfolipid, sulfonic acid, and sulfate polysaccharides. Freney (1986a) also reported that the bulk of the amino acid sulfur in soil is bound in some manner to the mineral and humus fractions. Recently, Autry and Fitzgerald (1990) reported that sulfonate sulfur is a major form of organic sulfur in forest soils.

The presence of other organic sulfur compounds in soil such as trithiobenzaldehyde has been shown in early work and other reports have shown that small amounts of cysteine and methionine can be present as constituents of polypeptides (Tabatabai, 1982). Lowe (1968) extracted sulfated polysaccharides from the surface horizons of chernozemic and podzolic soils and found that polysaccharide sulfur accounted for less than 2% of total sulfur in these soils. Freney *et al.* (1972) reported that amino acid sulfur accounted for 21 and 30% of the total organic sulfur in two podzolic soils, and approximately 60% of the amino acid sulfate was cystine sulfur. Scott *et al.* (1981) found that the S containing amino acids accounted for 11 to 15% of the total S in several Scottish soils. Chae and Tabatabai (1981) indicated that sulfolipids are found in photosynthetic organisms associated with soils such as plants, algae, bacteria, and protozoa, and they also found little sulfolipid sulfur in Iowa soils (0.29-0.45% of the total). Bettany and Stewart (1983) stated that sulfolipids have been found in bacteria, and that small amounts of choline sulfate have been identified in bacterial cell walls, whilst fungi have a larger portion of choline sulfate and significant amounts of various aryl sulfates. Autry and Fitzgerald (1993) recently found that prokaryotes (gram-negative bacteria) were the microorganisms primarily responsible for organic sulfur formation at all depths within the soil profile.

Characterisation of the soil organic sulfur pools has been achieved by the use of chemical extraction techniques. Freney (1986a) used a sequential process to characterise the organic sulfur complexes in soils (Figure 2.3). A grouping of organic sulfur compounds based on the reactivity of organic sulfur with certain reducing agents has been made (Williams, 1975; Tabatabai, 1982, Freney, 1986b), These are:

a) Organic sulfur that is reduced to hydrogen sulfide (H_2S) by hydriodic acid. This sulfur is not bonded directly to carbon and is believed to be largely in the form of ester sulfate (e.g. phenolic sulfate).

b) Organic sulfur that is reduced to inorganic sulfide by Raney nickel and that seems to consist almost entirely of sulfur in the form of amino acid (e.g. cysteine and methionine).

c) Organic sulfur that is not reduced by either hydriodic acid or Raney nickel. This fraction is assumed to consist of sulfur bonded directly to carbon, but not recoverable by current methods used for estimation of carbon bonded sulfur.

a) Hydriodic acid-reducible sulfur (HI reducible S)

Freney (1986a) stated that the hydriodic acid-reducible sulfur is believed to be comprised largely of ester sulfates, and that organic sulfur compounds which are not directly bonded to carbon will be reduced to H_2S by hydriodic acid. That is sulfur in ester sulfate (-C-O-S-), sulfamic acid (-C-N-S), and the second S^* in S-sulfocysteine (-C-S-S^*-), where the S and C atoms are separated by O, N or S atoms, respectively. The fraction of ester sulfate in soils is sometime described as organic SO_4^{2-}-S. Ester sulfates have been found to be the most labile fraction of the soil organic S (Freney *et al.,* 1971; Cooper, 1972; McLaren and Swift, 1977; Goh and Tsuji, 1979; McLaren *et al.*, 1985; Lou and Warman 1992a), and can be split from the organic fraction during drying of the soils (Barrow, 1961; Williams and Steinbergs, 1964). The labile ester sulfate fraction in soil may serve as a temporary storage of soil sulfate (Lou and Warman, 1992b), thus potentially available to plants (Freney *et al.,* 1971, 1975; Schnitzer, 1991).

Freney *et al.* (1971, 1975) found that when fallow soil or soil awaiting planting was incubated in the presence of ^{35}S labelled SO_4^{2-}, ^{35}S was incorporated into both the HI-reducible S and the C bonded S fractions with the HI-reducible S exhibiting a greater specific activity, with 75% of ^{35}S present in the fulvic acid.

There is evidence that the HI-reducible S may also be adsorbed to soil particle. Thus Houghton and Rose (1976) found that a wide variety of ^{35}S labelled sulfate esters were adsorbed to Welsh soils to the extent of 67% of the total concentration of the ester that was added to these soils.

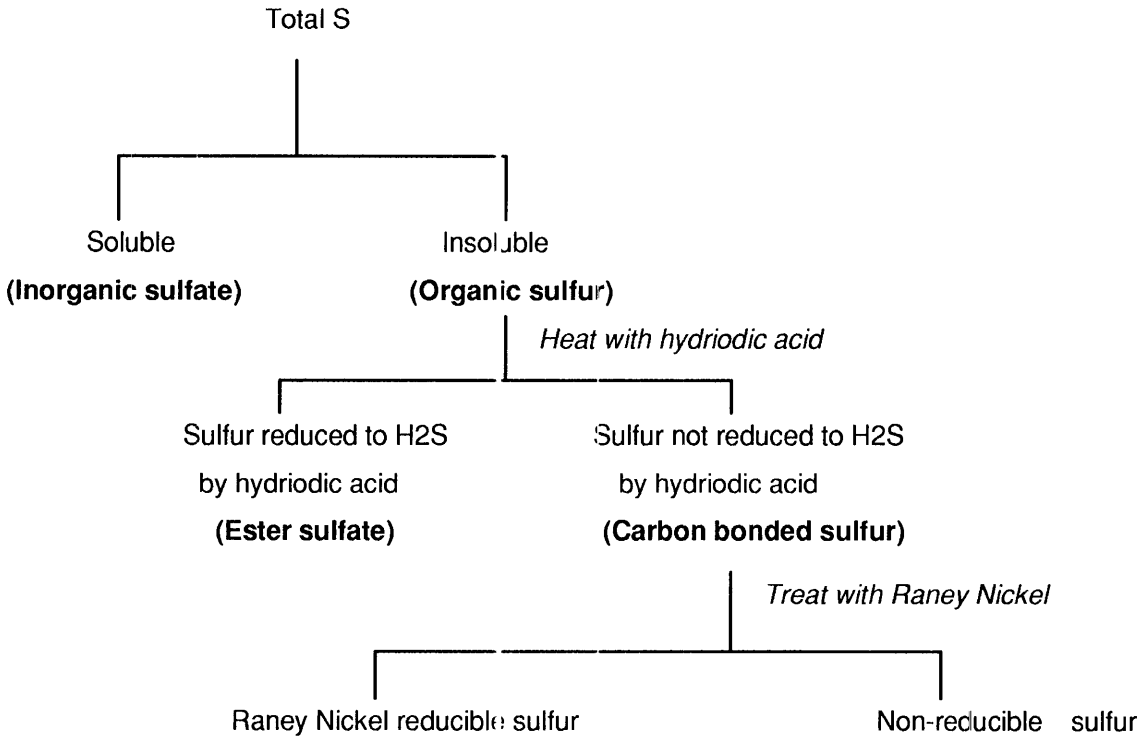


Figure 2.3 *Flow sheet of the fractionation of soil sulfur (Freney, 1986).*

Freney *et al.* (1969) extracted soils with sodium bicarbonate ($NaHCO_3$) at pH 10 and with chelating resin and they found that much of HI-reducible sulfur occurs in the high molecular weight (humic acid) fraction of the soil organic matter. Bettany *et al.* (1973) indicated that the HI reducible sulfur is believed to be largely associated with certain side chain components of fulvic and humic materials. Recently Lou and Warman (1992a,b) reported that a portion of ester sulfate in soil organic matter is biochemically reactive, i.e. "labile", and ester sulfate groups bound to external surfaces of soil humic polymers may be easily accessible to sulphatase enzyme and thus readily mineralisable during incubation or extraction of soil organic matter.

The possible origin of ester sulfates in soil has been discussed by Fitzgerald (1976) who indicated that ester sulfates are derived mainly from microorganisms and that the wide range of microorganisms within the soil having a variety of enzyme systems results in a range of ester-S forms. He also reported that the ester sulfates derived from animals include heparin, arylsulfates, ester sulfate of steroids, amino acid O-sulfate and ascorbic acid 2-O-sulfate. Ester sulfate compounds isolated from soil microorganisms include arylsulfotransferase enzyme from the fungus *Aspergillas oryzae* (Burns and Wynn, 1975), choline O-sulfate from algae (Ikawa and Taylor, 1973) and fungi (Catalfomo *et al.*, 1973). Plants have been found to contain ester sulfate with sulfate thioglyioxides (Virtanen, 1965) and sulfur bearing lipids linked as ester sulfate (Kate, 1970).

Freney (1967 cited in Tabatabai, 1982) stated that the major portion of HI-reducible sulfur in soils is present as sulfated polysaccharides, phenolic sulfates, choline sulfate, or sulfated lipid.

Ester sulfates are reduced to H_2S by hydriodic acid and the pool extracted is referred to as HI reducible S (Freney, 1961). In general, HI-reducible S accounts for 30 to 80% of the organic sulfur in soils, with the variation attributed to cultivation, organic matter, and climatic factors (Williams and Steinbergs, 1959; Freney, 1961; Lowe and DeLong, 1963; Cooper, 1972; Tabatabai and Bremner, 1972b; Bettany *et al.*, 1973; Neptune *et al.*, 1975, Biederbeck, 1978). Tabatabai and Bremner (1972b) found that the level of ester sulfate was higher under grassland than forest. McLaren and Swift (1977) also found that the ester sulfate level was higher under pastures than under crops. In some soils, the percentage of the organic S present as HI reducible sulfur increases with depth in the profile (Tabatabai and Bremner, 1972b; Nguyen and Goh, 1990), but in others the percentage remains constant with depth (Williams, 1975). Bettany and Stewart, (1983) also stated that "the % HI reducible S showed considerable variation across environmental gradients and cultivation chronosequences, generally increases with depth in the soil profile and is concentrated in the clay and fulvic acid fractions of soils".

b) Carbon bonded sulfur (C bonded S)

Carbon bonded sulfur is not converted to inorganic sulfate by hot acids or alkalis. This fraction includes the sulfur containing amino acids, mercaptans, disulfides, and sulfonic acids, etc. (Freney, 1986a). Bettany *et al.* (1973) suggested that C bonded S is primarily associated with the aromatic core of humic acids on the basis of its correlations with certain humus properties and resistance to several extractants. Williams (1975) reported that C bonded S is closely related to N suggesting that there was little change in the chemical nature of this fraction throughout the profile.

c) Raney-nickel reducible sulfur

The Raney-nickel reducible sulfur is a sub-fraction of carbon bonded sulfur (Williams, 1975). The first attempt to measure the C bonded S directly by reaction of the soil with Raney Ni was proposed by DeLong and Lowe (1961) and made by Lowe and DeLong (1963). The method involves desulfurization by Raney Ni in the presence of alkali in the Johnson and Nishita apparatus, liberation of hydrogen sulfide by acidification, and estimation of the sulfide released colorimetrically as methylene blue. Since then, it has been used by many workers to estimate C bonded S in soils (Freney *et al.*, 1970; Tabatabai and Bremner, 1972b; Scott and Anderson, 1976). However Freney *et al.* (1970) stated that Fe and Mn in soil interfere with this reduction procedure, and that the result obtained depends on the amount of Raney Ni, alkali concentration, reduction time, and the amount of sulfur dissolved in the alkali. Although the Lowe-DeLong procedure has been criticised for failing to reduce all C bonded S compound and for the possibility of producing artefacts it still is the best method available for estimating this component (Biederbeck, 1978). This reagent reacts with all

reduced S bonded to C (such as occurs in mercaptans) and oxidised S in the form of sulfoxides, sulfinic acids, or sulfonic acids attached to an aromatic nucleus, (e.g., sulfanilic acid) but Raney Ni does not react with sulfur in the form of aliphatic sulfones (such as methionine sulfone) or aliphatic sulfonic acids (such as cysteic acid) (Freney, 1986a).

Lowe and DeLong (1963) found the C bonded S accounted for 46 to 58% of the total S in organic soils. However, Lowe (1965) later reported much lower value in mineral soils from Canada, ranging from 12 to 32 % of the total S. Freney *et al.* (1970), who investigated the procedure of Lowe and DeLong, found that only 12% of the total S in Australian soils could be accounted for as C bonded S. Similarly, Tabatabai and Bremner (1972b) also reported a range of 5 to 20% (mean 11%) for Iowa surface soil.

d) Non-reducible sulfur

This sulfur may be in form of aliphatic sulfones or aliphatic sulfonic acids that are not reduced to sulfide by either hydriodic acid or Raney Ni (Freney, 1986a).

A detailed fractionation of soil organic sulfur was undertaken by Bettany *et al.* (1979), who separated the organic sulfur in four soils by reaction with 0.1M NaOH, 0.1M $Na_4P_2O_7$ at pH 13 under N_2, and dispersion, acidification, and centrifugation. This method extracted 63 to 72% of the total S, and the S was categorised as follows: fulvic acid A (36%); fulvic acid B (3%); humic acid A (13%); humic acid B (21%); humin >2 µm (15%), and humin <2 µm (11%). Bettany *et al.* (1979) also stated that fulvic acid A is presumed to contain microbial metabolites and relative young materials which are not associated with the soil colloids, fulvic acid B contains part of the extracellular enzymes, lytic products and soluble cytoplasmic materials released during sonication, humic acid A is considered as the more recalcitrant fraction with a structure of highly condensed aromatic units surrounded by adsorbed amino rich, aliphatic side chains, which are chemically stable and the most resistant to microbial degradation, humic acid B is a high molecular weight, weakly aromatic structure, less humified, physically stabilised fraction, and closely associated with soil clays and humin <2 µm fraction and is regarded as a physically protected, less humified, potentially labile fraction.

Nguyen and Goh (1992a) found that soil organic sulfur, hydriodic reducible sulfur and carbon bonded sulfur were significantly correlated with organic carbon and total N indicating that all these soil sulfur fractions are integral components of soil organic matter, and they indicated that similar relationships have been reported for soils in other countries (Freney, 1986a; Acquaye and Kang, 1987). Distinct differences in the forms and distribution of organic N and S among particle size fractions have been reported by Bettany and Stewart (1983) who stated that S follows different

pathways in humus formation and transformation than carbon and nitrogen, S was found to be more concentrated in the fine clay fractions and the majority of this S is HI-reducible.

In conclusion, total sulfur pools contain both organic and inorganic forms in which the proportion of organic to inorganic S may vary widely according to soil type and depth in the profile. Sulfur is found in soils as a variable mixture of the following; primary minerals, soil soluble sulfate or sulfate ion in solution, adsorbed sulfate, organically bound ester sulfates, and organic sulfur compounds. The organically bound ester sulfates, and organic sulfur compounds act as a reserve of S.

2.3.4 Sulfur transformations in soils

The transformation of sulfur in soils is regarded as resulting both from microbial activity (Freney *et al.*, 1971; Stevenson, 1986) and chemical processes (Bloomfield and Coulter, 1973). Freney and Swaby (1975) stated that "sulfur transformation in soil are many and varied and often the changes are cyclic; the element changes from inorganic to organic forms and back again due to the presence of living organisms." Bettany and Stewart (1983) stated that microbial sulfur transformations can be grouped into four distinct processes:

a) *Mineralisation*: a process in which large organic molecules containing sulfur are broken down to smaller units and ultimately to inorganic sulfate

b) *Immobilisation*: simple inorganic sulfur molecules (mainly sulfate) are converted to organic compounds.

c) *Oxidation*: inorganic sulfur of lower oxidation state (elemental sulfur, thiosulfates, polythionates, sulfides, etc.) are generally converted to sulfate as a stable end product.

d) *Reduction*: sulfate and other partially reduced sulfur-containing anions are converted to sulfide.

Mineralisation and immobilisation occur simultaneously in soils wherever organic debris is undergoing microbiological decomposition (Maynard *et al.*, 1983, 1985).

a) Mineralisation

Mineralisation is the conversion o organic forms of S into inorganic forms as a result of chemical and/or biological action. Biological mineralisation occurs as a result of carbon oxidation to provide energy for the microorganisms when S is released from carbon bonded compounds. Whilst biochemical mineralisation occur as a result of the stimulation of soil microorganisms to produce enzymes which hydrolyse sulfate from ester compound because of the low level of inorganic S for microbial S requirements (McGill and Cole, 1981). During the breakdown of organic substrates, some of S is used for synthesis of new cell material (immobilisation) and only excess amounts are released to soil as inorganic sulfate (Bettany and Stewart, 1983; Freney, 1986a).

Originally it was thought that N and S were closely associated in soil organic matter and that these two elements would mineralise at similar rates, however, many experiments have shown that this is not so (Biederbeck, 1978, Bettany and Stewart, 1983). A possible explanation for the behaviour of S in mineralisation process was proposed by McGill and Cole (1981) who presented a conceptual model which explained changes in the C:N:P:S ratio over geological and biological time scales.

Freney (1986a) suggested that four different patterns of inorganic sulfate release could occur; i) initial immobilisation of sulfur followed by sulfate release (net mineralisation) at a later stage; ii) a steady, linear release of sulfate with time; iii) an initial rapid release of sulfate during the first few days followed by a slower linear release and iv) a rate of release which decreases with time.

Two incubation techniques have been used to study the sulfur mineralisation process, namely, a closed system where the sulfur release is allowed to accumulate (Barrow, 1961; Williams, 1967; Kowalenko and Lowe, 1975; Maynard *et al.*, 1983, 1985), and an open system where mineralised sulfur is removed by eluviation at regular intervals (Tabatabai and Al-Khafaji, 1980; Maynard *et al.*, 1983; Pirela and Tabatabai, 1988; Ghani *et al.*, 1991; Nguyen and Goh, 1992b). Differences in net sulfur mineralised between closed and open soil incubation systems have been observed by Maynard *et al.* (1983) who found that the open system incubation resulted in a higher rate of net mineralisation than the closed system due to the removal of S by plant or leaching under field condition.

Freney (1986a) suggested that the sulfatase (sulfohydrolase) enzyme may play an important role in the mineralisation of organic sulfur in soil. Ester sulfates are considered to be the substrate for the sulfatase enzyme in soil. Arylsulfatase was first reported in Iowa soils by Tabatabai and Bremner (1972a). Soils from many different region of the world have been found to contain

enzymes that hydrolyse aryl sulfates and a variety of other sulfatase (Tabatabai and Bremner, 1972a,b; Cooper, 1972; Speir and Ross, 1975; Speir, 1976, 1977a,b; Al-Khafaji and Tabatabai, 1979; Lee and Speir, 1979; Stott and Hagedorn, 1980; Sarathchandra and Perrott, 1981; Neal and Herbein, 1983). The hydrolysis of ester sulfate to release inorganic sulfate can occur either intercellularly or in the periplasmic space, depending on the organism and substrate (Fitzgerald, 1978, cited in Strickland and Fitzgerald, 1986). The level of arylsulfatase activity in soils has been found to vary with soil type, depth, season and climate (Biederbeck, 1978; Stevenson, 1986). Tabatabai and Bremner (1972b) showed that arylsulfatase activity in six Iowa soils was correlated with organic carbon and that activity decreased markedly with depth as organic matter decreased. The relationship between arylsulfatase activity and organic matter has been studied by many investigators (Cooper, 1972; Speir, 1977a,b; Lee and Speir, 1979; Stott and Hagedorn, 1980; Sarathchandra and Perrott, 1981). Speir (1977b) suggested that there is a relationship between sulfatase activity and S mineralisation in the presence of the plant. Freney (1986a) suggested that a strong relationship between sulfate release and arylsulfatase activity may be unreasonable because arylsulfatase is only one of the enzymes involved in organic matter decomposition.

Numerous factors affect the rate of S mineralisation. Microorganisms are involved in the mineralisation of S and addition of microbial inhibitors will suppress mineralisation (Alexander, 1961; Williams, 1967). Any variable which influences microbial growth and the production and activity of enzyme will affect S mineralisation, thus temperature, moisture, pH, mineral content of organic matter, presence and absence of plants, and availability of food supply affect microbial activity related to mineralisation (Barrow, 1960b; Chaudhry and Cornfield, 1967a,b; Williams, 1967; Biederbeck, 1978; Tabatabai and Al-Khafaji, 1980; Tisdale *et al.*, 1985).

Mineralisation of S has been found to increase with increasing temperature from 20 to 40°C but was less at 50°C than at 40°C (Chaudhry and Cornfield, 1967b; Williams, 1967). Williams (1967) found that mineralisation of S was markedly suppressed at 10°C and that no mineralisation occurred in some soils at this temperature over a 64 day incubation period, and that increasing temperature from 10 to 30°C resulted in a 6.3 fold increase in S mineralisation. Pirela and Tabatabai (1988) observed that the rate of S mineralisation in Iowa and Chilean soils were increased 1.91 and 1.73 times greater when incubated at 30°C compared to 20°C.

The effect of soil moisture on the mineralisation of S has been investigated by Williams (1967) who found that at low (<15%) and high (>40%) moisture levels mineralisation of sulfur was considerably retarded, and maximum rates of sulfur mineralisation occured with a soil moisture content ranging between 20 to 30%. Williams (1967) also stated that the effect of moisture on sulfur mineralisation was less than the effect of temperature. Chaudhry and Cornfield (1967a) also

found that the optimum moisture content for mineralisation was 60% of maximum water holding capacity.

In a number of soils studied by Williams (1967) the amount of S mineralised was directly proportional to pH up to a value 7.5. Above pH 7.5 mineralisation increased more rapidly suggesting that chemical hydrolysis was possibly affecting the process.

The addition of calcium carbonate ($CaCO_3$) to soil has been found to increase soluble sulfate on incubation (White, 1959; Nelson, 1964; Williams, 1967). This increase has been explained by many workers. Barrow (1960a) suggested that sulfate maybe released from organic matter by chemical hydrolysis under alkaline conditions. Freney (1986a) suggested that sulfate maybe released from organic sulfur because of better growth of microorganisms under a more favourable pH environment. Chao *et al.* (1964) and Williams and Steinbergs (1962) have suggested that sulfate desorption at high pH could be responsible for the observed increase in sulfate concentration. Williams and Steinbergs (1962, 1964) have indicated that sulfate may be added in the $CaCO_3$.

Cycles of wetting and drying accelerate the breakdown of soil organic matter (Birch, 1960a,b). Barrow (1961) stated that these factors affect sulfur availability under field conditions. Drying results in mineralisation of sulfate (Freney, 1958; Barrow, 1961; Williams, 1967; David *et al.*, 1982). Williams (1967) found that more sulfate is released when soils are dried and re-moistened prior to incubation than when they are incubated without prior drying. They also found a cumulative effect of cycles of wetting and drying on sulfur mineralisation in fallow soils in the laboratory. The flush of mineralisation immediately following the addition of moisture to dry soils has been ascribed to an enhanced accessibility of organic matter to microbial attack and to decomposition of cellular material of the dead organisms (Sorensen, 1974; Jarger and Bruins, 1975).

Mineralisation of S depends on the S content of decomposing material in much the same way that the mineralisation of N depends on the N content. Barrow (1960b) observed that smaller amounts of sulfate were liberated from the materials containing a smaller percentages of S and that an analogous situation exists for the mineralisation of N. The C:S ratio of organic materials provides a rough guide to their ability to release mineral sulfur to soil (Freney, 1986a; Stevenson, 1986). Barrow (1960b) found that the initial C:S ratios of 200 and 420 were the minimum and maximum value at which no mineralisation of S occurred. At or below a C:S ratio of approximately 200:1, only mineralisation of S occurs, and above this ratio immobilisation is favoured, particularly where the ratio is greater than 400:1. Biederbeck (1978) illustrated that sulfur mineralisation increased as the C:S ratio decreased. Stevenson (1986) explained that there is a net gain of

sulfate when the C:S ratio of added plant residues is below 200, and that there is a net loss when C:S ratio exceeds 400 while there is neither a gain nor a loss of sulfate for the C:S ratios between 200 and 400. The C:S ratios of 200 and 400 correspond to a sulfur concentration of about 0.25 and 0.5%, respectively (Stevenson, 1986). The N:S ratio of organic materials is an indication of the ability to mineralise S in soil. Stewart *et al.* (1966) found that the addition of straw with a low S content to the soil tied up the soil available S due to immobilisation by soil microorganisms during decomposition of the straw, and they also found that when the N:S ratio of wheat straw was 14:1 or less, S was no longer limiting.

Generally, S mineralisation in the presence of growing plants is greater than in fallow soil (Freney and Spencer, 1960; Barrow, 1967; Cowling and Jones, 1970; Nicolson, 1970; Jones *et al.*, 1972; Freney *et al.*, 1975; Tsuji and Goh, 1979; Maynard *et al.*, 1985). This may be due to the rhizosphere effect brought about by the excretion by plant roots of amino acids and sugars and the resultant stimulation of microbial activity (Freney and Swaby, 1975, Stevenson, 1986). Seasonal fluctuations in mineralised S have been observed by many workers (Barrow, 1966, 1969b; Williams, 1967, 1968; Tisdale *et al.*, 1985; Ghani *et al.,* 1990). Williams (1968) found that sulfate accumulated in surface soil under subterranean clover pasture during summer and decreased to low levels in winter and spring. He concluded that the high levels in summer probably resulted from mineralisation of soil organic sulfur under favourable moisture and temperature conditions and lack of plant uptake while low levels in winter were due to leaching, plant uptake and low rates of mineralisation at low temperature. In other studies (Freney, 1958; Barrow, 1961, 1966; Williams, 1967) the high levels of mineral sulfur in soil during summer were found to be due to S release from organic matter by desiccation. However, Williams (1968) argued that only small amounts of sulfate were released in his study.

Bettany *et al.,* (1980) reported that the contributions of six soil organic matter fractions to S mineralisation during 65 years of cultivation were, in decreasing order, clay-associated humic acid (36%), conventional humic acid (26%), humin <2 μm (18%), conventional fulvic acid (14%), clay-associated fulvic acid (4%), and humin >2 μm (3%).

There is little information on S mineralisation in flooded soils. Some sulfide is produced as a result of protein decomposition, and many heterotrophic microorganisms can convert organic S to sulfide under anaerobic conditions (Blair *et al.*, 1992).

b) Immobilisation

This process is the transformation of inorganic sulfate to organic compounds in cellular material, termed "assimilatory sulfate reduction" and has been reviewed by Bettany and Stewart (1983). Briefly, the process depends upon the activation of the sulfate ion by a two step process leading to the production of energy rich sulfate nucleotides (ester) APS (adenosine 5'-phosphosulfate) and PAPS (3'-phosphoadenosine-5'-phosphosulfate). The nucleotides are then used in the synthesis of the sulfur containing amino acids (e.g. cysteine, methionine). The pathway is still uncertain, but it is thought that PAPS is reduced to sulfite and then sulfide. The amino acid serine is then combined with sulfide to produce cysteine. Thiosulfate is readily used by microbes in place of sulfate and is thought to be on the direct reduction pathway. Cysteine is then used as the building block for the other S-amino acids, which are then combined into proteins.

The mechanism of the breakdown of plant, animal and microbial compounds and their subsequent incorporation into soil organic matter has been studied by many investigators (Scharpenseel and Krause, 1963 cited in Bettany and Stewart, 1983; Freney *et al.*, 1971: Sachdev and Chhabra, 1974; Saggar *et al.*, 1981a,b etc.). An early study by Scharpenseel and Krause (1963 cited in Bettany and Stewart, 1983) with rye plants grown with ^{35}S labelled sulfate and residues incorporated into soils showed that appreciable amounts of ^{35}S label were found in the soil humic acids . Later, Panak (1966 cited in Freney and Swaby, 1975) found that only 16% of the ^{35}S from the incubated $Na_2{}^{35}SO_4$ cow manure was incorporated into organic compounds in the first month, mainly into the humic acid fraction, and approximately 44% of ^{35}S occurred in the organic substances with 20% in the humic acid fraction and significant amounts in the volatile and insoluble fractions at the end of two months. Freney *et al.* (1971) observed that labelled S was incorporated into the soil organic fraction as both hydriodic acid reducible and carbon bonded S after 168 days of incubation, with a greater specific activity in the HI reducible S fraction. Over 90% was present in the HI reducible form and about 75% in the fulvic acid fraction. Fitzgerald (1978, cited in Strickland and Fitzgerald 1986) also indicated that the organic S in humic acid occurs as ester sulfate and carbon bonded S.

A study of the transformation of added ^{35}S labelled sulfate-S in alluvial soil has been made by Sachdev and Chhabra (1974) who found that 28.1 % of the added ^{35}S was incorporated into organic forms. Later, studies have shown that substantial amounts of ^{35}S added sulfate are incorporated into organic sulfur fractions over time, ranging from 2 to 24 weeks (Freney *et al.*, 1975; Tsuji and Goh, 1979; Goh and Gregg, 1982). Saggar *et al.* (1981b) found that inorganic sulfate was mostly immobilised into the fulvic acid fraction after 64 day incubation, and that the transformation of immobilised sulfur into the more resistant humic acids and humin

fractions occurred in long term incubation studies. Goh and Gregg (1982) observed that immobilisation of applied inorganic S in soil is rapid with 17 to 40% of the applied S appearing in organic soil S forms within 34 to 75 days after application. They also reported that the rates of immobilisation are expected to vary throughout the year due to variations in microbial activity. Strickland and Fitzgerald (1985) found that ^{35}S labelled sulfate was incorporated into amino acid-S, sulfonate-S and ester sulfate linkages and that this process was stimulated by adenosine 5'-triphosphate (ATP), cellulose, cellobiose, glucose, succinate and pyruvate. ^{35}S labelled adenosine 5'phosphosulfate (APS) or 3'-phosphoadenosine-5'-phosphosulfate (PAPS) also served as S donors for organic S formation.

Changes in the specific activity of the soil sulfate pool during mineralisation and immobilisation experiments indicates that, although one process may be dominant at any given time, both are always taking place concurrently (Swift, 1985).

c) Oxidation

The oxidation process is of considerable importance, because the ultimate source of S in soil is sulfate, and many S fertilisers are based on the reduced form of S. In general, elemental sulfur, sulfide and several other inorganic S compounds can be oxidised slowly in soils by a purely chemical process (Weir, 1975), whilst microbiological oxidation by various groups of photosynthetic, autotrophic and heterotrophic bacteria, fungi and actinomycete is much more rapid under favourable conditions (Bettany and Stewart, 1983). Reduced inorganic S compounds (e.g. elemental sulfur, H_2S, FeS_2, etc.) are readily oxidised in soils under appropriate conditions by a group of bacteria or other microorganisms that use the energy thus released to carry out their life processes (Stevenson, 1986).

The most important group of S oxidising microorganisms are the autotrophic bacteria belonging to the family *Thiobacteriaceae*, genus *Thiobacillus*. These autotrophic organisms seem to be ubiquitous, operate over a wide variation of temperature and pH, and are capable of very rapid oxidation rates *in vitro* (Weir, 1975; Bettany and Stewart, 1983). Other microorganisms that are capable of oxidising reduced-sulfur compounds, include heterotrophic yeasts, the facultative autotrophic bacteria e.g. *Beggiatoa*, the aerobic and facultative aerobic heterotrophic bacteria e.g. *Bacillus* and *Aerobacter*, the aerobic heterotrophic fungi e.g. *Saccharomyces* and *Penicillium*, the anaerobic photoautotrophs e.g. the purple sulfur bacteria *Chromatium* and *Thiocystis* and the green sulfur bacteria *Chlorobium* and *Chlorobacterium*, (Vitolins and Swaby, 1969; Wainwright, 1984).

The most common form of autotrophic bacteria belong to the genus *Thiobacillus*. Vitolins and Swaby (1969) found that *T. thiooxidans*, *T. thioparus*, *T. denitrificans*, *T. ferrooxidans*, and *T intermedius* were more numerous than all the other species of *Thiobacilli* together.

Autotrophic sulfur bacteria use the energy released from the oxidation of the inorganic S for the fixation of CO_2 into organic matter. In the case of the photosynthetic bacteria such as *Chromatium* (purple sulfur bacteria) and *Chlorobium* (green sulfur bacteria), sunlight is used as a source of energy and electrons derived from S oxidation are used to reduce CO_2, and this oxidation can occur in flooded soil, muds, the photic zone of lake and lagoons, and in reducing sediments. (Alexander, 1961; Vitolins and Swaby, 1969).

The various members of the *Thiobacilli* can oxidise hydrogen sulfide, elemental sulfur, thiosulfate, and polythionate (Suzuki, 1974 cited Bettany and Stewart, 1983), with the end product being sulfate (Goldhaber and Kaplan, 1975 cited in Bettany and Stewart, 1983). The mechanism of oxidation of S by autotrophic bacteria is unclear, although several schemes or pathways have been suggested (Weir, 1975).

Sulfur oxidation is affected by a large number of environmental and microbiological factors. Numerous studies have shown that the rate of oxidation is affected by the particle size of elemental sulfur (Fox *et al.*, 1964; Li and Caldwell, 1966; Barrow, 1971; Shedley, 1982; Blair, 1987), soil temperature (Li and Caldwell, 1966; Nor and Tabatabai, 1977; Shedley, 1982; Janzen and Bettany, 1987b), soil moisture and aeration (Jones *et al.*, 1971; Kittams and Attoe, 1965; Janzen and Bettany, 1987b), soil texture (Rehm and Caldwell, 1969; McCaskill and Blair, 1987), soil pH (Vitolins and Swaby, 1969; Nor and Tabatabai, 1977; Janzen and Bettany, 1987a), microbial population and inoculation (Moser and Olsen, 1953; Kittams and Attoe, 1965; Li and Caldwell, 1966; Vitolins and Swaby, 1969; Barrow 1971), and nutrient supply (Parr and Papendick, 1978; Bloomfield, 1967; Keller, 1969; Weir, 1975), and organic matter (Li and Caldwell, 1966; Vitolins and Swaby, 1969; Jones *et al.*, 1971).

d) Reduction

This process is known as dissimilatory or respiratory sulfate reduction (Bettany and Stewart, 1983). Dissimilatory sulfate reduction is brought about by a special group of bacteria, which use sulfate as the terminal electron acceptor for their respiratory process, and leads to the formation of sulfide (H_2S) as the end product. Konopka *et al.* (1986) reported that sulfate reduction will occur if the environment is anaerobic, and there are adequate concentrations of electron donors (organic matter) and electron acceptors (sulfate). The predominant microorganisms involved in this reaction are obligate anaerobes belonging to two groups of bacteria, *Desulphomaculum* consisting

of straight or somewhat curved, sporulating rods, and *Desulfovibrio*, which are curved, rod shaped cells. The typical habitats for reducing bacteria include soils and waters, sewage, polluted waters, deep-sea sediments, muds, and estuarine sands (Stevenson, 1986). The reduction of sulfate occurs when the environment is favourable for the growth of anaerobic bacteria. Flooding or waterlogged soils where quantities of readily decomposable plant residue are present, often contain the large amounts of hydrogen sulfide. The pathway of dissimilatory sulfate reduction in *Delsulfovibrio* has been shown by Roy and Trudinger (1970; cited in Bettany and Stewart, 1983). The sulfate reducing bacteria all contain cytochrome respiratory pigments, which act as electron donors in the reduction process. *Desulfuricans* can reduce sulphite, thiosulfate and tetrathionate but the pathways have yet to be defined (Bettany and Stewart, 1983). The hydrogen sulfide formed commonly reacts with iron compounds to form ferrous sulfide in soil, and under some conditions, such as coastal areas the slow formation of pyrite can be observed (Bettany and Stewart, 1983).

Freney (1986a) stated that the two main reactions of organic sulfur in soils which are important for agriculture are mineralisation and release of volatiles to atmosphere. Volatile sulfur compounds are produced through microbial transformations of soil S compounds under both aerobic and waterlogged conditions. The volatile compounds have so far been isolated from soils or during decomposition of pure organic compounds, plant materials, animal manures and sewage sludge. These are carbon disulfide, carbonyl sulfide, methyl mercaptan, dimethyl sulfide and dimethyl disulfide (Greenwood and Lees, 1956; Frederick *et al.,* 1957; Lewis and Papavizas, 1970; Elliott and Travis, 1973; Banwart and Bremner, 1975; Banwart and Bremner, 1976a,b;). The various volatile compounds may have important side effects. Those substances released from soil during the decomposition of cruciferous crop residues have been reported to control root rot in peas, beans, and sesame. Carbon disulfide is a strong inhibitor of nitrification while methyl mercaptan, dimethyl sulfide, and dimethyl disulfide are also capable of retarding nitrification. The annoying and sometimes toxic odors from decomposing animal manures are caused by volatile sulfur compounds such as hydrogen sulfide, dimethyl sulfide, carbonyl sulfide, carbon disulfide, and methyl mercaptan (Tisdale *et al.,* 1985). Soils have the capacity to sorb these volatile organic sulfur compound from the atmosphere (Bremner and Banwart, 1976).

2.4 Soil testing for S status

Tisdale *et al.* (1985) stated that a soil test is a chemical method for estimating the nutrient supplying power of a soil and the objective of soil testing is to obtain a value that will help to predict the amount of nutrients needed to supplement the supply in the soil. Doyle and Bacon (1990) described that a soil test will show the availability of a particular nutrient which limits growth of a

crop and the amount of fertiliser required for optimal plant growth. Blair (1993) indicated that a soil test can only provide information on the amount of a particular nutrient present in the soil at the time of sampling and in some cases the amount of nutrient that may become available during the life of the crop or throughout the year (e g. pasture). Rayment (1993) argued that a soil test has little utility unless they are accurate, timely and capable of meaningful interpretation.

In the case of sulfur, the S cycle in soil includes different sized pools, with different flow rates between the pools (Till, 1979) as discussed earlier. Determination of the S needs by soil testing is complicated by the various forms by which S is held in the soil. Blair (1979) stated that the major problem encountered in studies of soil S in temperate areas is the variability in the size of soil S pools over short periods of time, which is brought about by the nature of S sources present.

2.4.1 The evolution of soil S testing

There has been interest in measuring soil S since 1938 (Purri and Asghar, 1938) when ammonium carbonate solution was used to estimate total sulfate in soils. Evans and Rost (1945) attempted to determine the total organic S in soils by oxidation of acid-washed soil with hydrogen peroxide. Chesnin and Yien (1950) used a solution of acetic acid buffered with sodium acetate to extract soil S, and developed a turbidimetric methods for sulfate determination. The sulfate extracted was measured by precipitation as $BaSO_4$.

Later, Smittenberg *et al.* (1951) reported a rapid method for determining different types of S compounds in soils. These were the total sulfur, oxidisable S, monosulfidic S, and extractable S (free and organically bound). Total S was determined via hydrogen sulfide, obtained by ignition of the sample with reduced iron in a nitrogen current and liberation of H_2S with HCl. Oxidisable S was measured by exposing the sample to tin and concentrated hydrochloric acid. Monosulfidic S was determined by boiling the soil sample with HCl and distilling the liberated H_2S with a nitrogen current into a cadmium acetate solution, after which the cadmium sulfide precipitate was determined iodometrically. Acetone was recommended for determining solution + adsorbed S.

Johnson and Nishita (1952) published their reduction/distillation method for sulfur measurement. This method involves the reduction of S compound to H_2S with a mixture of hydriodic acid, formic acid and hyphophosphorus acid, distillation and collection in Na-Zn acetate solution. The hydrogen sulfide concentration is measured colorimetrically by reaction of hydrogen sulfide with p-amino dimethyaniline.

Ensminger (1954) determined the sulfate concentration in soil by extracting with six different solutions and sulfate extracted was measured turbidimetrically. The solutions used were neutral

sodium acetate solution, sodium acetate buffered at pH 4.8, KH_2PO_4 solution containing 100 ppm P, KH_2PO_4 solution containing 500 ppm P, 0.1N HCl solution and water. He found that the first four extracting solutions extracted similar amounts of sulfate from adsorbing soils while the 0.1N HCl solution and water extracted little or no sulfate.

Steinbergs (1955) described a turbidimetric method of S measurement which used a "seeded" barium chloride suspension which gave reproducible results with the $BaSO_4$ precipitation method of Chesnin and Yien (1950). Hesse (1957) later stated that the method for determination of soluble sulfate in soil by Chesnin and Yien (1950) was possibly the most convenient and gave the most reproducible result, however, they argued that the sodium acetate solution extracted considerable amounts of colloidal organic matter, which interfered with the formation of the precipitate. At low S concentration these organic colloids suppress the precipitation of sulfate resulting in a lower S value and at high S concentration organic colloids are co-precipitated with the barium sulfate resulting in an inflated S value. Removing organic matter from the solution was achieved by treated the sample with ferric hydroxide. In the same year Freney (1957 cited in Williams and Steinbergs, 1959) proposed the use of the reducible S fraction to estimate S supply to plants. Soil was digested with a reducing mixture and sulfate determined by the reduction procedure (Johnson and Nishita, 1952). This reducible S appeared to be inorganic sulfate plus sulfate associated with the organic matter.

Freney (1958) used water as an extractant and the reduction method of Johnson and Nishita (1952), to determine the soil soluble sulfate. He suggested that to measure the true water soluble sulfate content of a soil it was necessary to remove the soil colloids and isolate the sulfate from other inorganic sulfur compounds by precipitation as barium sulfate.

Williams and Steinbergs (1959) developed the "heat soluble sulfur method" by using a 1% sodium chloride solution to extract sulfur from soil following a heat treatment of the soil sample. They measured the sulfur fractions namely, total sulfur, sulfate sulfur, total water-soluble sulfate, heat soluble sulfate (sulfur released by heating), sulfate soluble after ignition, alkali soluble S, reducible sulfur, sulfate released by hydrogen peroxide oxidation. They found that most of the S was present in organic forms in all of soils examined, and approximately half of this was extracted by 0.1N sodium hydroxide. Similar amounts were obtained by the ignition and reduction methods. These three fractions (sulfate soluble after ignition, alkali soluble sulfur and reducible sulfur) were correlated well with total sulfur and with one another, but not with either yield or S uptake of oats in a pot experiment. In addition, they found that the amount of water soluble sulfate was small and an unsatisfactory indicator of yield. On the other hand, heating the soil prior to extraction substantially increased the amount of water soluble S. Heat soluble S correlated well with both yield and S uptake by oats so it provided a satisfactory index of available S. Later, Bardsley (1959

cited in Spencer and Freney, 1960) suggested reserve S as an indicator of S status because this fraction was basically organic S.

McClung *et al.* (1959) used neutral 1.0N NH_4OAc to extract S from several Brazilian soils. They found that the neutral ammonium acetate solution extracted S fractions which correlated well with plant response.

Anderson and Webster (1959) used Morgan's extractant (1N sodium acetate solution adjusted to pH 4.8) for the extraction of sulfate. They found that this extractant did not cause problems of deflocculation and that the extractant removed S that was related to the S nutrition of plants.

Spencer and Freney (1960) compared several procedures for estimating the S status of soils by using chemical and microbiological assays. They used eight chemical assays to measure various soil S fractions. These were cold-water extractable sulfate (Freney, 1958), phosphate-extractable sulfate (500 ppm. P solution) Ensminger, 1954), acetate-extractable S, heat-soluble S, hot-water-extractable sulfate, reducible S (Johnson and Nishita, 1952), total S, and reserve S. They found that extraction of the soil with a KH_2PO_4 solution containing 500 ppm. P was superior than the other extraction procedures, due to its ability to displace adsorbed sulfate. This extractant also showed the best correlation with sulfur supplying ability of the soils. The microbiological assay using *Aspergillus niger* was also reliable for assessing the S status of soil.

Bardsley and Lancaster (1960) determined the reserve S (organic and reduced inorganic S) fraction and soluble sulfates in soils. For reserve S, soils were ignited with sodium bicarbonate at 500°C, then extracted with 2N acetic acid and 0.1N $NaH_2PO_4.H_2O$ and determined turbidimetrically with $BaSO_4$ after acidifying a portion of the filtrate with 1:1 HCl. Soluble sulfates were extracted with 0.5N NH_4OAc + 0.25N HAc. They found that the use of Norit "A" activated charcoal to decolorize the extracts was useful. To measure sulfate they increased the sensitivity of the turbidimetric method by adding an acidic "seed" solution. Kilmer and Nearpass (1960) later compared sodium oxalate pH 5.0, sodium acetate pH 4.8, neutral ammonium acetate, and sodium bicarbonate at pH 8.5 or 10.0 as extractants of plant available S. They found that sodium bicarbonate at pH 8.5 was the best extractant. In the same year, Bartlett and Neller (1960) used the Bray-1 extractant and turbidimetric method for the determination of sulfate S in soils.

Barrow (1961) extracted the soil with 0.15% calcium chloride solution. He reported that this solution seemed to give a good indication of available S. Freney (1961) used six procedures for the fractionation of sulfur in soils into : (i) total S (ii) adsorbed plus soluble sulfate (iii) reducible S

(iv) inorganic S compounds of lower oxidation state than sulfate (v) organic S and (vi) sulfate attached covalently to soil organic matter. He found that the reducible S and inorganic S compounds accounted for 59% and 7% of the total S respectively. The remaining portion appeared to be organic based with a strong correlation with soil organic matter. Most of this reducible S occurred in the fulvic acid fraction and could be hydrolysed to inorganic sulfate. He also suggested that a considerable amount of sulfate in soils was covalently bound to certain compounds of the organic matter. In the U.S.A., Arkley (1961; cited in Kurmarohita, 1973) used 0.1M LiCl and 0.005M $MgCl_2$ to characterise soil available S.

Lowe and DeLong (1961) determined the S status of three Quebec soils by measuring total S following oxidation in the Parr oxygen bomb by the colorimetric methylene blue method, easily soluble S by using 0.001N HCl and humus S by 4% ammonium hydroxide according to the Evans and Rost (1945) procedure. They found that the total S determined by the Parr oxygen bomb was markedly higher than that obtained by the gravimetric method following bomb oxidation or oxidative fusion with sodium peroxide and sodium carbonate. They suggested that sulfate-S existed in association with the organic fraction.

DeLong and Lowe (1961) first attempted to measure the carbon bonded S in soils. They proposed a method which involved desulfurization by Raney Ni in the presence of alkali in the Johnson and Nishita apparatus, liberation of H_2S by acidification, and estimation of the sulfide released colorimetrically as methylene blue.

Freney *et al.* (1962) in a review indicated that no one extraction method had been established as having general application, due to variable handling of the soil samples prior to analysis. As a result they made a plea for workers to standardise the procedure for collection and preparation of soils for S analysis.

Bardsley and Kilmer (1963) confirmed that the acetate extractant of Bardsley and Lancaster (1960) and sodium bicarbonate were both suitable extractants for predicting crop response to applied S. In the same year, Massoumi and Cornfield (1963) described a modification of the turbidimetric method which increased the sensitivity of measurement of sulfate in water extracts of soils. The main modification involved the use of (i) a dilute seed suspension of barium sulfate (ii) the stabiliser gum acacia reagent addition and (iii) Norit "NK" charcoal. This method was found to be suitable for determining sulfate in soils where the sulfate-S content was low compared with that of total S.

Lowe and DeLong (1963) estimated carbon bonded S in selected Quebec soils by developing the method of DeLong and Lowe (1961). This method recovered all forms of

organic sulfur except organic sulfate and alkyl sulfones. They also found that >95% of the total sulfur in four of five Quebec soils was present as carbon bonded S and HI reducible S.

In 1964, the calcium phosphate solution [$Ca(H_2PO_4)_2$] (MCP) was first introduced to estimate available S of Nebraska and Hawaiian soils by Fox *et al.* (1964) who compared the MCP solution with potassium phosphate solution (KH_2PO_4), water, heat soluble S and autoclave soluble S. They concluded that phosphate extraction had the widest application and that calcium phosphate solution was more convenient than potassium phosphate because it did not disperse the soils. Finally, they commented that $Ca(H_2PO_4)_2$ was viewed as suitable for the assessment of S availability especially for soils in the tropics which disperse easily.

In the same year, Williams and Steinbergs (1964) determined soluble sulfate by using 0.15% $CaCl_2$ solution, adsorbed sulfate by KH_2PO_4 solution containing 500 ppm P and $CaCO_3$, heat soluble S by the Williams and Steinbergs (1959) method and S by 0.5M $NaHCO_3$ at pH 8.5. Later, Lowe (1964) studied the S status of Quebec soils by using 0.5M NaH_2PO_4 at pH 7.0 and .001N HCl. Lowe (1965) used the same extractants for the determination of S in Alberta soils.

Ensminger and Freney (1966) grouped the methods for the determination of available S into: (i) extraction of sulfate-sulfur (ii) extraction of sulfate sulfur plus a fraction of the organic sulfur and (iii) use organic and total sulfur as an indicator of soil sulfur status and (v) microbial assays. The first group included extraction with water, neutral salts (e.g. $CaCl_2$), phosphate solution (e.g. $Ca(H_2PO_4)_2$ solution and KH_2PO_4 solution), acidic solutions (e.g. sodium acetate pH 4.8). The second group contained the heat soluble S method, neutral ammonium acetate, and the 0.5M $NaHCO_3$ solution at pH 8.5. They concluded that solutions containing phosphate extracted most of the sulfate S in soils and offered promise for determining available S. The acetate solutions were not as effective as phosphate solutions in extracting adsorbed sulfate, and low amount of sulfate were extracted by water. In the same year, Lowe (1966) extracted soils with 0.1N NaOH extractant and 0.5M NaH_2PO_4 at pH 7.0 to determine the organic and inorganic sulfate in soils.

Dean (1966) proposed an alternative method to the methylene blue colour finish for the Johnson and Nishita (1952). In this method the H_2S evolved was adsorbed in a NaOH solution and treated with bismuth reagent to develop colloidal bismuth sulfide.

Barrow (1967) chose 0.01M MCP at pH 4 to extract S from soils. He found that this solution contained sufficient phosphate to displace the greatest quantity of adsorbed sulfate at a soil:solution ratio of 1:5 for most soils, but that a wider soil:solution ratio was needed for some soils and that extraction should take place over 24 hours for maximum displacement of adsorbed sulfate. He also stated that the 0.01 M MCP extractant was the one most worthy of further study.

Freney and Spencer (1967) reviewed the diagnosis of S status by soil and plant analysis. They concluded that although plant analysis was superior to soil analysis in field trails, that limitations in plant analysis and further development of soil tests should continue. Cooper (1968; cited in Saunders and Cooper, 1975) found that the 0.03M $NaH_2PO_4.2H_2O$ in the 2M acetic acid extractant of Bardsley and Lancaster (1960) was the best of the five extractants they used, the other four being 0.01M $CaCl_2$, 500 ppm P as KH_2PO_4, 0.5M $NaHCO_3$ at pH 8.5 and heat soluble sulfur extracted with 1% NaCl.

Roberts and Koehler (1968) introduced x-ray fluorescence spectrometry for the determination of total S in soil extracts. In the same year, Rehm and Caldwell (1968) measured total S, $NaHCO_3$ extractable S, sulfate-S by $NH_4C_2H_3O_2$ and sulfate-S extracted by MCP. Their conclusion was that $NaHCO_3$, $NH_4C_2H_3O_2$, and MCP extractable S were satisfactory estimators of plant available S.

Spencer *et al.* (1969) confirmed the inferiority of soil S tests and plant analysis in the field. They used heat soluble S, KH_2PO_4 solution and the *Aspergillus niger* method and found that all the soil S tests performed poorly in correlating with pasture yield. They proposed that the problems in correlation could be improved by (i) sampling to greater depth and (ii) taking more account of sampling date.

Tabatabai and Bremner (1970a) proposed a method for the determination of total S in soils by heating soils with alkaline sodium hypobromite solution with the sulfate formed being reduced to H_2S by a modified Johnson and Nishita (1952) procedure, and determined colorimetrically as methylene blue. They found that this method gave quantitative results with pure organic and inorganic S compounds, including amino acids, sulfonic acids, organic sulfates, sulfoxides, thioureas, sulfates, sulfites, sulfides, and elemental S. Later, Tabatabai and Bremner (1970b) compared five methods for determining total sulfur. These were (i) the alkaline oxidation method of Tabatabai and Bremner (1970a), (ii) the acid oxidation method of Arkley (1961 cited in Tabatabai and Bremner, 1970b), (iii) the dry ashing method of Steinbergs *et al.* (1962 cited in Tabatabai and Bremner, 1970b), (iv) X-ray fluorescence spectrography as described by Norish and Hutton (1964; cited in Tabatabai and Bremner, 1970b) and (v) the use of a Leco Sulfur Analyzer. They showed that digestion with either a nitric and perchloric acid mixture or sodium hypobromide, gave similar values and was more precise than the other methods, while ashing with sodium bicarbonate/silver oxide gave slightly lower values. They also found that X-ray fluorescence spectrography could be used satisfactory for routine S analysis.

In Canada, Walker (1972) and Walker and Doornenbal (1972) studied soil S testing by using the $CaCl_2$ extractant and the Johnson and Nishita (1952) reduction/distillation procedure for

measuring extracted S. They suggested that the best prediction of soil S status was attained by soil sampling to 30 cm depth, sampling in early spring before active plant growth commenced, and air drying samples before analysis.

Tabatabai and Bremner (1972a) determined the S status of Iowa soils by (i) the total S by the method of Tabatabai and Bremner (1970a), (ii) the HI-reducible S by the method of Freney (1961), (iii) carbon bonded S by method of Lowe and DeLong (1963), (iv) inorganic non-sulfate S as described by Freney (1961), (v) sulfide-S by Smittenberg *et al.* (1951), and (vi) sulfate-S by using 0.1M LiCl and determining S by the Johnson and Nishita (1952) reduction procedure. They found that inorganic S occurred entirely as sulfate and that it accounted for only 1-5% of the total S in these soils. Ester sulfate and carbon bonded S accounted for 50% and 11% of the total S respectively.

In the same year, Kirkbright *et al.* (1972) introduced inductively coupled plasma-atomic emission spectrometry (ICP-AES) for the determination of S and P. They commented that this method was relatively free from chemical and physical interferences, though spectral interferences from metal ions were observed.

Sinclair (1973) described a turbidimetric autoanalyser method for the determination of sulfate in 0.01M MCP extracts. Also in 1973, Hoeft *et al.* (1973) evaluated the effectiveness of six soil S extractants; MCP-HOAc, MCP, $NaH_2PO_4.H_2O$-HOAc, $CaCl_2$, Bray-1, and H_3PO_4. They found that MCP-HOAc was the best extractants for predicting yield and S uptake of alfalfa. In the same year Kurmarohita (1973) determined the S status of Thai soils using the alkaline oxidation procedure of Tabatabai and Bremner (1970a), water soluble S and S determined by the Johnson and Nishita procedure (1952) and 0.01M MCP solution. They found that the inorganic S as extracted by MCP, which is the sum of water-soluble and adsorbed S, was the best estimator of plant available S in these soils.

Westermann (1974) extracted soils with KH_2PO_4 and LiCl extractants and derived very good correlation coefficient values with S response in alfalfa in a field study.

Reisenauer (1975) stated that the extractants of Bardsley and Kilmer (1963), Cooper (1968; cited in Saunders and Cooper, 1975) and Hoeft *et al.* (1973) were good predictors of S response in field trials because they removed soluble, adsorbed and portions of the organic sulfur. He commented that acidic solutions should not be used on calcareous soils. He also stated that the turbidimetric method had become the most widely used procedure for the determination of sulfate while biological evaluations using *Aspergillus* and plants were of limited utility for routine assay.

Small *et al.* (1975) first introduced the determination of sulfate by using ion exchange chromatography using low pressure liquid chromatography for separating anions using a combination of resins to eliminate background. In Australia, Andrew (1975) concluded that no one soil sulfur test had been accepted, but the MCP plus charcoal test was being developed, in Victoria which showed promise.

Probert (1976) studied available and isotopically exchangeable S in north Queensland soils by using 0.01M MCP (pH 4.0) and 0.5 M $NaHCO_3$ extractants. He found that the S extracted with 0.01M MCP was from a similar pool as that used by the test plants and obtained near full recovery of added $^{35}SO_4$. and that 0.5 M $NaHCO_3$ removed some soil sulfur that was not available to the plants.

Arora and Sekhon (1977) evaluated ten extractants and found that 0.5M $NaHCO_3$ at pH 8.5 was the best extractant for routine analysis. They argued that an additional advantage in using this extractant in their laboratory was that it was also used as a soil P test.

Spencer (1979) developed a new extractant by using a magnesium phosphate solution for extracting adsorbed sulfate in soils. He found that 0.04 M magnesium phosphate at pH 5 or 0.0075M at pH 6.8 were more efficient in extracting adsorbed sulfate than MCP. Later, Searle (1979) recommended a procedure for estimation the solution + adsorbed sulfate by extracting the soil with 0.04M MCP (pH 4) at a soil:solution ratio of 1:5, shaking on an end-over-end shaker for 16 h at 20°C, and determining sulfate by the reduction method as described by Johnson and Nishita (1952). In the same year, Dick and Tabatabai (1979) used the ion exchange chromatography (IC) method for the analysis of sulfate in soil extracts. They found that the results obtained for S by IC was essentially the same as those obtained from the reduction/distillation method.

Wall *et al.* (1980) developed a rapid and accurate method for the determination of sulfate in soils by an automated turbidimetric method. They reported that this method was accurate and precise and that sulfate in soil extracts could be determined in the range 0-15 ppm, with from 180 to 240 sample solutions analysed per day.

Scott (1981) compared five methods for extracting sulfate from soils. These were (i) Dowex AG21 resin Cl^- form, (ii) $CaCl_2$ (iii) potassium dihydrogen phosphate, (iv) sodium acetate-acetic acid, and (v) sodium bicarbonate. He found that sulfate extracted with calcium chloride, potassium di-hydrogen phosphate and sodium bicarbonate gave the best relationships with the crop yields. Lee and Pritchard (1981) determined S by inductively coupled plasma atomic emission spectrometry (ICP-AES) and observed that aluminium, calcium, iron, and manganese could

interfere in the measurement of S. He stated that the interference from calcium and manganese could be reduced by observing the S emission at a lower observation height in the plasma.

Sinclair and Enright (1982) automated the turbidimetric procedure of Sinclair (1973) to enable one user analyse over 200 soil sulfate tests per day. They compared the turbidimetric method with the reduction/distillation method of Johnson and Nishita (1952), and found standard deviations of 0.64 and 1.23 ppm sulfate S respectively. They emphasised the requirement for an effective charcoal grade to be used and recommended checking for interference from precipitation inhibitors in turbidimetric analysis by occasional comparison with the reduction/distillation method

Randall and Sakai (1983) stated that "the multiplicity of methods testifies to the difficulties experienced by analysts in their attempts to achieve simplicity and accuracy.

Till *et al.* (1984) established the automated turbidimetric procedure for the simultaneous determination of S and P. They also developed a scintillation mixture for simultaneous determination of sulfur-35 and phosphorus-32 in a portion of the digest which was used for chemical analysis. They also overcame three problems associated with the automated turbidimetric method; (i) deposition of barium sulfate especially in the flow cell by devising a wash technique to remove barium sulfate films during analysis, (ii) low sensitivity at sulfur concentrations less than 10 µg S mL^{-1} by maintaining a constant level of detectable sulfur in the system and (iii) the stabilisation of the barium sulfate precipitation by using polyvinyl alcohol as a suspending agent.

Freney (1986b) stated that the magnesium phosphate extractant developed by Spencer (1979) was the most suitable available as it is necessary to have a high pH in the suspension as well as a high concentration of phosphate to desorb all of the sulfate from strongly sorbing soils. Also in 1986, Jones (1986) concluded that "in general, chemical analysis as a means of evaluating the sulfur status of soils under field conditions have not been very helpful."

Vaughn *et al.* (1987) evaluated the effectiveness of four extractants for determining sulfur status. They found that the Bray-1 extractant (ammonium fluoride in hydrochloric acid) was more suitable in predicting pasture response to residual sulfur fertiliser than MCP-HOAc, MCP, and LiCl.

Maynard *et al.* (1987) studied the measurement of sulfate-S and total extractable S in surface organic horizons of five forest soils. They used eight extractants (water, 0.01M $CaCl_2$, 0.01M KCl, 0.01M LiCl, 0.003M NH_4OAc 0.003 and 0.01M NH_4Cl and 500 mg P L^{-1} as MCP for

extraction and determined sulfate-S by ion chromatography (IC) and total extractable S by ICP-AES. They found that the amount of SO_4-S removed by the weak salt extractants was similar, however water was a less desirable extractant due to the removal of more organic sulfur than the salt extractants, and was the most variable for IC analysis with inconsistent results. They also concluded that the 0.01M NH_4Cl was the most consistent extractant for IC or ICP-AES analyses with a solution:soil of 10:1. They also found that the storage of air dried material at room temperature (20 ± 4°C) for 1.5 yr resulted in increased total extractable S and SO_4-S concentrations.

In 1989, Appiah and Ahenkorah (1989) measured the sulfate-S in some Ghanian soils by using five extraction methods; 0.1N HCl (Barrow, 1967), sodium acetate pH 7.0 (Anderson and Webster, 1959), KH_2PO_4 solution containing either 3 or 16 mM P (Ensminger, 1954), and distilled water (Spencer and Freney, 1960), with two soil-extractant ratios; 1:5, 1:10. They found that the highest amount of sulfate-S was extracted at the 1:5 ratio and at the 1:10 ratio by water and KH_2PO_4 extractants, respectively. The lowest amount of S was extracted by 0.1 N HCl.

Vendrell *et al.* (1990) determined soil S status by ICP-AES, using MCP and neutral ammonium acetate extractants, and determined sulfate-S by IC. They found that soil sulfate-S analysed by IC averaged 21% lower than ICP-AES, and suggested that the higher values from ICP-AES was likely due to S in forms other than sulfate. ICP-AES analysis of ammonium acetate indicated that 13% more S was determined than in MCP. They suggested that this additional S might represent a pool of easily mineralisable S. They also commented that S could be determined in the same ammonium acetate extract used to determine exchangeable cations and the additional S determined in the ammonium acetate extract by ICP-AES analysis could improve correlations between soil S test and plant growth.

Watkinson and Perrott (1990) developed a soil sulfur test for sulfate and mineralisable organic S. This test measures the pools of sulfate and organic S (labile organic S) which will provide sulfate-S for plant uptake. This method involves extracting the soil with 10 mM MCP at pH 4.0 for 30 minutes and the single extract is then analysed by ICP-AES which measures the total S in the extract. Sulfur in the extract is also measured by HPLC which measures only the sulfate-S concentration in the extract. Labile organic sulfur in the extract is determined by the difference between total S and sulfate-S.

Searle (1991) proposed a simple manual method for measuring phosphate extractable sulfate. Sulfate was extracted by a phosphate anion-exchange membrane which, after elution, was measured turbidimetrically as barium sulfate. He concluded that this manual method compared well with a continuous flow analyser method.

Anderson (1992) developed the KCl method for extracting the soluble organic S by modifying the method of Gianello and Bremner (1986 a,b) for assessing the available organic N . This method involved determining soil S by heating the soil sample with 0.25M KCl in the oven at 98°C for 4 hours, filtering and analysing the filtrate for S by ICP-AES. He found that the KCl method extracted more S than MCP and H_2O and was better correlated to plant response than the other 2 extractants, when the level of inorganic S was low. However, the KCl method tended to overestimate the size of the available S pool with only 6 to 55% of the extracted S being taken up by plant.

Since soil S testing began in 1933, many procedure have been used to determine the S status of soils, including chemical extractants, sulfate released on incubation, microbial growth, and plant uptake. However, there is still no general agreement on which estimate best defines a soil's sulfur supply.

2.4.2 Problems encountered in soil S testing

Spencer *et al.* (1969) stated that soil S tests performed poorly in correlating with pasture yield. They proposed that the problems in correlation could be improved by (i) sampling to greater depth and (ii) taking more account of sampling date. Later, Blair (1979) indicated that the error in the stages of sample preparation and analytical methods were the major problems in S correlation studies. He suggested that precautions should be taken in the sampling, sample preparation, S extraction, analysis, and interpretation steps.

i) Sampling

Care should be taken to obtain a representative sample of the soil or area of the field to be sampled (Jones, 1986). Blair (1979) indicated three aspects of sampling that should be considered, and these are pattern and intensity, depth, and timing. Freney *et al.* (1962) made a plea for the standardization of collection and preparation of soils for soil S analysis. Reisenauer (1975) stated that guidelines commonly recommended 15-30 subsamples be taken in either a systematic or random manner throughout a field. Blair (1979) suggested "the use of several separate bulk samples (made of at leas 20 cores each) from the test area and comparing the results from each. Variation among samples should be less than 10%. If there are different soil types, drainage areas, etc., within the test area, separate samples should be taken from each area. Core samples, generally of 2.5 cm diameter, should be taken either from a zig zag of the whole area, or as a pattern sample from a random area within the test area. In both cases, a minimum of 20 cores should be used as a bulk sample for testing". Later, Friesen and Blair (1984)

found that the cluster or monitor plot sampling procedure to be more efficient in time, accuracy and cost than the more conventional zig zag sampling in low and high nutrient status pasture paddocks.

The most appropriate sampling depth for S determination in cropping soils has been found to vary from, 0-30 cm (Walker, 1972), 0-60 cm (McGill, 1984), 0-80 cm (White *et al.,* 1981), 0-90 cm (Rayment, 1983), or 0-100 cm (Probert and Jones, 1977). Blair (1979) argued that a 0-7.5 cm soil sampling depth, which is commonly used, especially for pastures, was probably not deep enough for a mobile nutrient such as S, which is subject to leaching and accumulation at variable depths in the profile, especially in tropical soils or weathered soil. Blair *et al.*, (1978) also indicated that the sulfate content of a sandy layer may be low but the plant may take up S in the clay layer that is below the sampling depth in soils with a sandy surface horizon and a clay layer at depth. Nguyen and Goh (1992c). also found sulfur leaching beyond a soil depth of 30 cm. This leaching losses involved the SO_4^{2-} and organic S from the soil S pools, plant litter and animal excreta as the soil has a low SO_4^{2-} retention capacity. Lipsett and Williams (1971) reported that S showed appreciable leaching and accumulated at depth in at least 6 out of 10 sites. Hue and Cope (1987) found that sorghum obtained 55% of its S requirement from 0-20 cm horizon and 45% from the subsoil. However, Brown (1993) revealed that little change in available S with soil depth in a study in Victoria whilst in the volcanic ash soils and sedimentary soils in New Zealand the available S concentration was twice as high in the 0-15 cm soil layer compared to the 0-7.5 cm layer. Blair (1979) recommended that experiments where S soil tests were evaluated should include a study of vertical distribution of S at the start of the experiment. Brown (1993) suggested that soil sampling from the surface only may over-estimate S need for deep rooting crops in some soils.

The timing of sampling of the field is also important. Blair (1979) stated that the time of the year when the soil sample is taken must also be carefully considered due to the dynamics of S in soils. Studies of seasonal changes in soil available S (Barrow, 1966 and 1969b; Williams, 1968; Nguyen *et al.,* 1989; Ghani *et al.,* 1990; Anderson, 1992) have shown consistent trends of accumulation over summer and autumn decline over winter and spring. Walker (1972) suggested that sampling should be done in early spring before active plant growth commences. Blair (1979) recommended the sampling be conducted as long as possible after the last fertiliser application and as close as practicable to the planting of the crop. Later McGill (1984) recommended that the field should be sampled on an annual basis as well. Brown (1993) reported that an autumn sampling in alpine areas of East Gippsland (where considerable mineralisation of organic matter can take place) may provide the best assessment of S status during the critical time of pasture re-establishment and early growth, whereas a spring sampling may grossly overestimate this requirement. Jones (1986) stated that soil samples, time and depth of sample may be important on some soils, but not the others.

ii) Sample preparation

Once the sample has been collected it must be carefully prepared before chemical extraction if meaningful estimates of S status are to be made (Blair *et al.*, 1978). Large fluctuations in the amount of sulfate S can occur because of the mineralisation of organic to inorganic sulfate which take place after sampling (Williams, 1967). The factors that have been found to affect mineralisation are soil moisture, soil temperature and time of sample storage (Barrow, 1961; Williams, 1967; Peverill *et al.*, 1975). Sample drying and storage are also important. Spencer (1974, cited in Rayment, 1993) confirmed that levels of phosphate extractable S in two air-dry soils increased with storage for about 13 weeks then decreased slightly over the following 20 weeks to level 2.5 to 3 times higher than those present immediately following air drying and that low temperature storage (3°C) did not prevent changes occurring in soil S status. Maynard *et al.* (1987) also found that storage of air dried material at room temperature (20 ± 4°C) for 1.5 yr resulted in increased total extractable S and SO_4-S concentrations. The major problem of sample preparation is one of drying the sample before analysis. Drying the soil hydrolyses the organic sulfate and increases the extractable S level (Barrow, 1961). Williams (1967) found a tenfold increase in extractable S in some soils dried at 100°C. However, Reisenauer (1975) recommended that samples be air dried because of the "relatively minor effects " as Williams (1968) found air drying to release only 0.7 to 1.1 ppm S. Air-drying at 20°C has been found to result in least change in the sulfate content and is the recommended procedure. Walker and Doornenbal (1972) and McGill (1984) recommended that air drying of the soil should be done immediately after sampling.

iii) S extraction

The methods used for S extraction have been described in Section 2.4.1.

iv) Analysis

Blair (1979) indicated that "lack of an accurate, rapid and uncomplicated method for sulfate analysis is a major factor contributing to the lack of interest in this nutrient". The methods used for the measuring of S in extracts has been described in Section 2.4.1.

v) Interpretation

The normal procedure for the interpretation of a soil S test is to correlate the S test with some parameter of plant response, e.g. S uptake % maximum yield. This has been described in the section 2.4.3.

2.4.3 Relationship between extracted soil S and plant growth or S uptake .

Many correlation studies of soil S status and plant response have been published . The two general methods of calibration are (i) a relationship between percent of maximum yield [(yield without sulfur/yield with sulfur) x 100] or S uptake and soil test, and (ii) the selection of a value above which little or no response is likely (critical level).

a) Relationship between % maximum yield or sulfur uptake and soil test.

In general, this calibration method involves the establishment of a coefficient of determination (r^2) between extractable sulfur and either dry matter yield or S uptake by plants.

Anderson (1992) summarised the published reports of the relationship between the level of S extracted by various extractants and plant growth in field and glasshouse or pot studies (Tables 2.6 and 2.7). He indicated that the poorest correlation of pasture yield with soil S test was found in the measurement of inorganic S after pre-treatment with charcoal. An exception was the results by Vaughn *et al.* (1987) who found a significant relationship between Bray-1 extractable S and yield of a legume/grass mixture. Anderson (1992) also observed that techniques that extract inorganic S and a fraction of organic S correlated better with yield response to applied S than when charcoal was used to remove the soluble organic S from the solution prior to analysis. These conclusions are similar to those of Reisenauer (1975) who reported an excellent prediction of response in field trials for extractants that removed soluble, adsorbed and a portion of the organic S.

Table 2.6 *The relationship between soil sulfur extraction technique and yield or sulfur content expressed by coefficient of determination (r^2) for field experiments (Anderson, 1992).*

References	Crop	Extractants	Method	Yield (r^2)	S content (r^2)
Hoeft *et al.* (1973)	Alfalfa	$CaCl_2$	TC	0.28	0.36
		Bray 1	TC	0.13	0.24
		$Ca(H_2PO_4)_2$	TC	0.32	0.34
Hoque *et al.* (1987)	Rye-grass	$CaCl_2$	TC	NS	-
		$Ca(H_2PO_4)_2$	TC	NS	-
		Incubation	TC	NS	-
		Incubation +S	TC	0.69	-
		Plant assay	-	0.71	-
Vaughn *et al.*, (1987)	Clover/Grass	LiCl	TC	0.06	-
		$Ca(H_2PO_4)_2$	TC	0.40	-
		$Ca(H_2PO_4)_2$ -HOAc	TC	0.52	-
		Bray 1	TC	0.76	-
		Total	TC	0.17	-
Bardsley&Kilmer (1963)	Clover/Grass	Acetate	TC	0.55	-
	Clover/Grass	$NaHCO_3$	JN	0.59	-
	Cotton	Reserve	TC	0.44	-
Westermann (1974)	Alfalfa	water	JN	0.62	0.76
		LiCl	JN	0.84	0.81
		KH_2PO_4	JN	0.70	0.88
Walker and Doornenbal (1972)	Legume/Grass	water	TO	0.77	-
		water	TT	0.76	-
		$CaCl_2$	TO	0.71	-
		$CaCl_2$	JN	0.76	-

TO Turbidimetric with no pre-treatment to the samples, NS non significant r^2

TC Turbidimetric after sample have been treated with charcoal - data not given.

TT Turbidimetric after sample have been evaporated and digested,

JN Reduction method

The coefficient of determination (r^2) is generally higher from glasshouse or pot experiments than in the field because rooting density is higher and there is minimal influence of other factors (i.e. climate, nutrient) on the plant growth (Anderson, 1992).

Table 2.7 *The relationship between soil S extraction technique and yield or S content expressed by coefficient of determination (r^2) for glasshouse experiments (Anderson, 1992).*

Reference	Crop	Extractant	Method	Yield (r^2)	S content (r^2)
Fox *et al.* (1964)	Alfalfa	H_20	TC	-	0.94
		KH_2PO_4	TC	-	0.94
		$Ca(H_2PO_4)_2$	TC	-	0.95
		Heat Soluble	TC	-	0.84
		auto-clave	TC	-	0.83
Scott (1981)	Oat	$CaCl_2$	TC	0.63	0.82
		KH_2PO_4	TC	0.63	0.91
		Resin	TC	0.60	0.82
		Na-Acetate	TC	0.38	0.81
		L-value	TC	0.60	0.90
		Heat Soluble	TC	0.60	0.90
		$NaHCO_3$	TC	0.65	0.90
		Total	TC	0.31	0.62
Kang *et al.* (1981)	Corn	$LiCl_2$	TC	0.66	0.77
		$CaCl_2$	TC	0.56	0.73
		KH_2PO_4	TC	0.71	0.79
		CaH_2PO_4	TC	0.75	0.76
		NH_4-Acetate	TC	0.64	0.73
		Heat Soluble	TC	0.79	0.75
		Total	TC	0.56	0.53
Spencer and Freney (1960)	Phalaris	H_2O	JN	-	0.62
		$Ca(H_2PO_4)_2$	JN	-	0.82
		NH_4-Acetate	JN	-	0.69
		Heat Soluble	JN	-	0.41
		Hot H_2O	JN	-	0.68
		HI	JN	-	0.75
		Total	JN	-	0.80
Lee and Speir (1979)	Ryegrass	$Ca(H_2PO_4)_2$	JN	0.84	0.94
		HI	JN	0.64	0.70
		C-Bonded	JN	NS	0.72
		Total	JN	0.59	0.74
		Sulfatase	-	NS	NS
Williams and Steinbergs (1959)	Oats	$CaCl_2$	TO	0.89	0.78
		$CaCl_2$	TT	0.89	0.80
		Heat soluble	TT	0.88	0.90
		Ignition	TT	-	0.30
		NaOH	TT	NS	0.41
		HI	JN	NS	0.46
		Total	TT	0.61	0.37
Williams and Steinbergs (1964)	Oats	$CaCl_2$	JN	-	NS
		KH_2PO_4	JN	-	0.24
		Heat Soluble	JN	-	0.53
		$NaHCO_3$	JN	-	0.53
		Total	JN	-	NS

TO = Turbidimetric with no pre-treatment to the samples, JN = Reduction method

TC = Turbidimetric after sample have been treated with charcoal, NS = non significant r^2

TT = Turbidimetric after sample have been evaporated and digested, - = data not given.

b) Selection of a value above which little or no response is likely (critical value)

Several workers have listed the critical value of soil S obtained by various extractants (Ensminger and Freney, 1966; Reisenauer, 1975; Blair, 1979; Jones, 1986). Examples of critical values are presented in Table 2.8.

Table 2.8 *The critical levels of extractable S of different extractants for pot and field study (Adapted from Blair 1979).*

Method of determination	crop	Critical level (ppm)	References
Readily soluble sulfate			
Water	Legume-grass	3	Walker and Doornenbal (1972)
0.1M $CaCl_2$	Legume-grass	3	Walker and Doornenbal (1972)
0.01M $CaCl_2$	Pastures	6	Beaton and Fox (1971)
LiCl	Pasture	3	Westermann (1974)
Readily soluble plus portions of adsorbed sulfate			
KH_2PO_4-500 ppm P	Pasture	4	Spencer *et al.* (1969)
	Pasture	4	Westermann (1974)
	Pasture	8-10	Scott (1981)
	Maize	4	Kang and Osiname (1976)
$Ca(H_2PO_4)_2$-500 ppm P	Alfalfa	10	Fox *et al.* (1964)
	Maize	8	Fox *et al.* (1964)
	Various crops	13	Andrew (1975)
	Maize	4	Kang and Osiname (1976)
	Pasture	4	Probert and Jones (1977)
	Pasture	3.5	White *et al.* (1981)
	Pasture	4	Rayment (1983)
NaOAc-HOAc	Pearl millet	6-7	McClung *et al.* (1959)
	Alfalfa	12	Harward *et al.* (1962b)
	Maize	4	Kang and Osiname (1976)
	Maize	6	Enwezor (1976)
NH_4OAc	Pasture	10.5	Arora and Sekhon (1977)
0.5M $NaHCO_3$	Cotton	10	Kilmer and Nearpass (1960)
	Pasture	22	Arora and Sekhon (1977)
	Pasture	8	Scott (1981)
Readily soluble, adsorbed, and portion of organic sulfate			
$Ca(H_2PO_4)_2$-2M HOAc	Alfalfa	10	Hoeft *et al.* (1973)
NaH_2PO_4-2M HAOc	Pasture	10	Hoeft *et al.* (1973)
NH_4F+HCl	Pasture	10	Vaughn *et al.* (1987)
0.01M HCl	Pasture	200	Andrew (1975)
Total S	Various crops	Variable	Andrew (1975)
"A" value	Pearl millet	70	McClung *et al.* (1959)
	Cotton	15	Nearpass *et al.* (1961)
	Alfalfa	10	Harward *et al.* (1962b)

Although there is variability between the various extractants Blair (1979) stated that this variability does not make the soil test procedure useless but instead it serves to emphasise the need for correlation studies to be conducted in defined areas with specific crops, soil extractants, and methods.

2.7 Conclusion

This review of literature has indicated that S is involved in numerous and important compounds in plants and plants require S for numerous functions Sulfur in soil is contained in different sized pools with different flow rates between the pools. Sulfur can be transferred from available to unavailable pools over short periods of time. Plants take up S from the sulfate-S pool. In general, the size of sulfate pool (soil solution) taken up by plant is very small relative to the adsorbed sulfate and organic S pools. Transfer rates between these pools can be large or small depending on the climatic conditions. Attempts to measure soil S status have been described however, there is no general agreement on which estimate best defines a soil S supply. The experiments reported in this thesis were undertaken with the objective of developing a soil S test which better estimates S supplying capacity of a soil and also to measure which components of the soil S pool are being extracted and to relate these to plant uptake.