AN INVESTIGATION OF SOME FACTORS INFLUENCING THE RATE OF OXIDATION OF ELEMENTAL SULPHUR FERTILIZERS

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ABSTRACT

Methodologies for measuring the particle size of S^o in pure and compound fertilizers (sulphurized superphosphates (SSP), reactive phosphate rocks (RPR) and partially acidulated phosphate rock (PAPR)) and for determining the rate at which S^o in these materials oxidises in soils were evaluated and improved. Sample dispersion in 10% HCl followed by wet sieving was the most successful method for sizing S^o in SSP, RPR and PAPR based fertilizers. S^o/bentonite fertilizers, however, were more easily dispersed in water than in acid.

Acetone extraction (40 g:200 ml acetone, using a 16 h shaking period) and determination of S^o in the extract proved to be a suitable method for measuring amounts of S^o in finely ground fertilizers and soils at concentrations above 5 $\mu g\ S\ g^{-1}$ soil and below 200 $\mu g\ S\ ml^{-1}$ acetone.

The rate of S^o oxidation in soil was determined by regularly measuring residual amounts of S^o. The influence of soil type and fertilizer history on the potential of soils to oxidise S^o was examined in incubation studies. On average, soils that had previously received S^o applications had higher initial rhodanese enzyme activities (RA) and higher S^o oxidation rates but there was no simple relationship between fertilizer history or RA and initial S^o oxidation rate.

Different sources of S^o, namely Rotokawa S^o (geothermal S^o), dark S^o, Damman S^o, and agricultural grade S^o had similar oxidation rates per unit surface area. Granules or prills oxidised slowly in incubated soil because they did not disintegrate when placed in soil and had small specific surface area.

On average, the oxidation rate of S^o was increased when mixed or granulated with reactive phosphate rocks and incorporated in soil but this effect was not consistently reproducible. Further incubations of S^o in the presence of various combinations, $CaHPO_4$, $CaCl_2$ and $CaCO_3$, demonstrated that the presence of $CaHPO_4$ and $CaCO_3$ could elevate S^o oxidation rates.

Granulation of RPR and PAPR with S^o did not significantly increase ($p > 0.05$) the oxidation rate of S^o surface applied to undisturbed pasture soils (glasshouse and field

trials). Under surface application conditions granulated S^o had similar oxidation rates to finely divided S^o forms.

An iterative computer program was developed to calculate specific oxidation rates (K, $\mu g\ S^o\ cm^{-2}\ day^{-1}$) from the amounts of acetone extractable S^o remaining in soils at different times. On average, K for <150 S^o μm was significantly lower ($p < 0.05$) when surface applied to undisturbed soil cores than when incorporated into incubated soils.

Specific oxidation rates of different particle sizes (<150, 150-250 and 250-500 μm) of surface applied S^o were similar (ranging form 11-19 $\mu g\ S^o\ cm^{-2}\ day^{-1}$) but were different ($P < 0.05$) for the two soil types used in glasshouse trials (means of 17 and 13 $\mu g\ S^o\ cm^{-2}\ day^{-1}$ for Ramiha and Tokomaru soil, respectively). Corrections for the effects of soil moisture on oxidation rates provided evidence that all S^o could have similar maximum potential K values (Kmax = 18 $\mu g\ S\ cm^{-2}\ day^{-1}$) in both soils. This suggested, with other evidence from the literature, that S^o oxidation in soil could be effectively modelled by knowing S^o particle size and the effects of soil moisture and temperature on S^o oxidation.

A S^o oxidation simulation model was constructed using a value for Kmax determined in the glasshouse trials. Within experimental error, the simulation model predicted S^o oxidation in field soil well (explaining between 76 and 97% of data variance at 3 field sites) and provides a useful basis for designing future research projects.

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CHAPTER 1

INTRODUCTION

Widespread use of single superphosphate (SSP) on New Zealand pastures to overcome phosphate (P) deficiency concurrently overcame the natural sulphur (S) deficiency that existed in many regions (Walker 1963; Ludecke 1966, 1969; During 1984), especially in inland areas of the South Island (Boswell, 1987; Boswell and Swanney, 1988). In the mid 1980's, however, high analysis P fertilizers such as reactive phosphate rocks (RPRs) and partially acidulated phosphate rocks (PAPRs) were introduced as P fertilizers that could reduce the cost of P application to many remote hill country areas (FMRA, 1985). Some high analysis P fertilizers were virtually S free. To provide S with these P fertilizers and not compromise their high P analysis, it was necessary to combine with them elemental sulphur (S^0), the ultimate in high analysis fertilizer.

Elemental S had previously been used to fortify single superphosphate for use in regions where S applied as sulphate was easily leached or regions where S deficiency but not P deficiency existed (During, 1984). Series of field trials conducted by Ministry of Agriculture and Fisheries (MAF) (Sinclair *et al.*, 1985) had shown that, to be effective, the particle size range of S^0 needed to be finely divided (<150 μm for cool temperate and <250 μm for warm temperate zones) in order to oxidise and to supply sulphate-S (SO_4-S) at a rate which satisfied plant (pasture) requirements in the year of application.

To reduce the explosive hazard of fine S^0 particles, S^0 products have been developed which incorporate S^0 into combined P and S granules (e.g. S^0/PAPR) or into prills and irregularly shaped 'chips' which are mixtures of molten S^0 and Na-bentonite (Rothbaum *et al.*, 1983; Boswell *et al.*, 1988a; 1988b).

This recent changing of the S fertilizer form from SO_4-S in SSP to high analysis S^0 forms has created a general need to reintroduce long term field trials to evaluate the agronomic value of such new materials (e.g. such trials reported by Boswell, 1987; Swanny et al., 1988 and Smith and McDougall, 1988). The number of such trials, however, may be reduced if the factors influencing S^0 oxidation rates in a range of New Zealand soils can be established and adequately modelled.

Recently MAF trials conducted throughout the country have identified the important effects of S^0 particle size on the performance of pure S^0 fertilizers in different climatic zones (Sinclair *et al.*, 1985; Boswell and Swanney, 1986, 1988) However, except for some regional evaluations conducted by Boswell and Swanney (1986) and Smith and McDougall (1988), there is still little information on the newly available S^0 fertilizer forms and the influence of fertilizer form on the rate of oxidation.

Some research has been done on the effect of PR on S^0 in incubated soils (Attoe and Olson, 1966; Lee *et al.*, 1987) but not under field application conditions. The effect of S^0 oxidation increasing PR dissolution has been measured in glasshouse and field soils (Rajan, 1983, 1987; Rajan and Gillingham, 1986; Friesen *et al.*, 1987) but S^0 oxidation rates were not measured.

The evaluation of experimental mixtures of S^0 and Triple superphosphate (TSP) and also S^0/PAPR has also highlighted the importance of the disintegration characteristics of fertilizer granules and the method of application of these types of fertilizers on S^0 oxidation rates (Boswell and Swanney, 1986; Boswell, 1987).

It appears therefore, that methods are required firstly, to routinely determine the particle size of S^0 in a range of S^0 containing fertilizer materials and secondly, to determine if other characteristics of the fertilizers influence the rate of S^0 oxidation. Such information would be valuable for use in assessing the agronomic value of current and new S^0 fertilizer forms.

Experiments described in this thesis attempt to provide some of these methods and information.

CHAPTER 2

REVIEW OF LITERATURE

2.1 THE CHEMICAL NATURE OF SULPHUR IN SOILS

Sulphur can occur in various states of oxidation ranging from -2, to 0, +2 and +6 and because of this undergoes a great diversity of chemical reactions in the soil environment. Most often more than 90% of the total S content of soils of humid and semi-humid regions are in organic forms (Freney, 1967; Syers *et al.*, 1987). However, the chemical nature of soil organic S forms is largely unknown. They are arbitrarily divided into carbon bonded and non carbon bonded S. Most of the non carbon bonded S (HI reducible fraction; Freney, 1961) is considered to be ester sulphates e.g. choline sulphate, phenolic sulphates, sulphated polysaccharides much of which occurs in the high molecular weight (humic acid) fraction of the soil organic matter (Freney, 1961; Williams, 1974).

The C bonded S is believed to be bonded directly to C as in amino acids and is not reduced by hydriodic acid. This fraction can be subdivided into two fractions depending on its reducibility with Raney nickel. The Raney Ni-reducible fraction (Freney *et al.*, 1962; 1975) is thought to contain S bound in the amino acids cysteine and methionine.

The non carbon bonded S (HI reducible S) generally accounts for 30-70% of the organic sulphur, while the Raney reducible S may account for up to 60% of the total organic sulphur (Freney and Williams, 1983).

Inorganic soil sulphur includes soluble sulphate, adsorbed sulphate, insoluble (precipitated sulphide), occluded (co-precipitated sulphate) sulphate, primary mineral S (sulphate or sulfide), elemental S (S^0) and sulfide-S. Most of the inorganic S in well-drained and well aerated soils occurs as sulphate, the most highly oxidized of the 6 valency states of sulphur (Starkey, 1966). Other inorganic compounds are less stable in aerated soil conditions and are generally less than 1% of the soil inorganic S (Williams, 1974). Sulfides can occur in poorly drained or water logged soils and soils of tidal swamps (Williams, 1975; Metson, 1979). Primary mineral sulphate and sulfides are not present in most well developed soils.

H_2S can be released by anaerobic microbial degradation of S containing compounds from soil organic matter, plant residues, animal manures and organic wastes, e.g. sewage sludges. The amounts of H_2S present are relatively small in comparison to S^o and other reduced S forms (polysulphides) that are normally present in soils or added as fertilizers or soil amendments.

The natural cycle in the biosphere involves transforming S between the forms described above and is summarized in Figure 2.1. Plants generally utilize S in the +6 oxidation state of SO_4^{2-}, and as a result the reduced forms of the element (e.g. R-SH and S^o) must be oxidized before they can become available to crops. The primary reduced S compounds found in soils are sulfides (S^{2-}), such as metal sulfides or H_2S, elemental sulfur (S^o). Thiosulphate ($S_2O_3^{2-}$), tetrathionate ($S_4O_6^{2-}$), and sulphite (SO_3^{2}) also occur. All these forms are not stable and eventually convert to sulphate by the processes of oxidation.

2.2 PROCESSES OF S^o OXIDATION

In soil, oxidation of S^o and other lower oxidation states of S to SO_4^{2-} can be both chemical (abiotic), microbiological (biotic), or a combinations of both (Konopka, *et al.*, 1986).

2.2.1 Abiotic Oxidation

Evidence for abiotic oxidation of S^o comes from studies in which S^o oxidation occurred in previously autoclaved soils (Nor and Tabatabai, 1977). According to Wainwright (1984) The abiotic oxidation of elemental sulphur to SO_4^{2-} comprises of two distinctly separate steps.

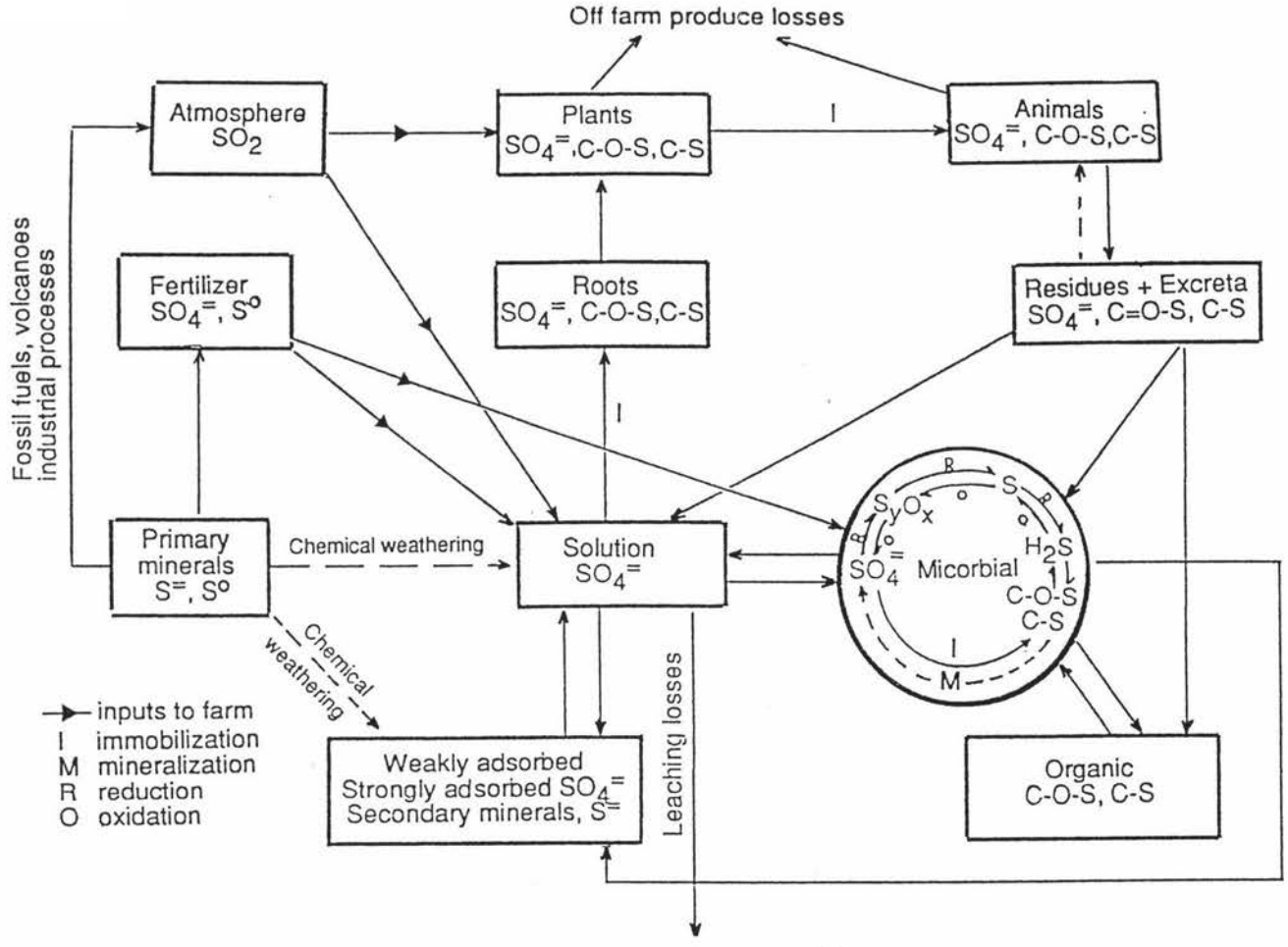


Figure 2.1 The S cycle (Hedley, M.J., 1990. Lecture notes of soil fertility and the environment, 89.412 Soil Science, Massey University).

1. Oxidation of sulphide to free sulphur

$$S^{2-} = S^{o} + 2e^{-} \qquad (2.1)$$

2. Oxidation of S^{o} to SO_4^{2-}

$$S^{o} = S^{6+} + 6e^{-} \qquad (2.2)$$

The abiotic oxidation of S^{o} to SO_4^{2-} can therefore be written as

$$2S + 2H_2O + 3O_2 \longrightarrow H_2SO_4^{2-} \qquad (2.3)$$

The following hypothetical course of abiotic oxidation may involve intermediates which are usually regarded as being indicative of microbial S oxidation (Wainwright, 1984):

$$S^{o} \longrightarrow S_2O_3^{2-} \longrightarrow S_4O_6^{2-} \longrightarrow SO_3^{2-} \longrightarrow SO_4^{2-} \qquad (2.4)$$

The oxidation of S^{2-} to S^{o} is rapid in aerobic soils, whereas the oxidation of S^{o} to SO_4^{2-} is slow (Wiklander *et al.*, 1950 as cited by Wainwright, 1984).

Abiotic oxidation of S^{o} to SO_4^{2-} is extremely slow at ambient temperature and pressures. Fast oxidation can be accomplished only at a temperature of 1200-1600°C where SO_2 forms and a catalyst of complex vanadium or platinum is required to oxidize SO_2 to SO_3, which eventually dissolves in water to form sulfuric acid. This process is commonly used to manufacture H_2SO_4 in the chemical industry.

Microbial oxidation in soil, however, takes place at ambient temperature and pressures in aerobic soils and these conditions contrast strongly with the severe conditions required for abiotic oxidations. Therefore, under normal soil conditions microbially mediated S^{o} oxidation is generally considered to be primarily responsible for oxidising most forms of S in lower oxidation states to SO_4^{2-}.

2.2.2 Microbial Oxidation

A wide spectrum of microorganisms are capable of oxidising S^o in the environment, including members of the genus *Thiobacillus*, a number of heterotrophs, photosynthetic sulphur bacteria, and colourless, filamentous sulphur bacteria. However, chemoautotrophic bacteria and heterotrophs (fungi and actinomycetes) play the most important role in S oxidation in most agricultural soils (Wainwright, 1984).

2.2.2.1 *Chemolithotrophs*

The obligate chemolithotrophs (autotrophs), of genus the *Thiobacillus* which gain energy from the oxidation of S and use CO_2 as their carbon source, are usually regarded as the main agents of S oxidation in agricultural soils (Starkey 1966; Vishniac and Santer, 1957; Wainwright, 1978).

Several species of the genus *Thiobacillus* are able to carry out S oxidation at normal soil pH (4-7) e.g. *T. thioparus* (is predominant) *T. neapolitanus* and *T. denitrificans*. However, *T. thiooxidans*, the most rapid S-oxidiser, oxidises S at pH 5 and below and continue to operate under conditions of extreme acidity (pH 2.5) (Vitolins and Swaby, 1969).

Most studies of *thiobacilli* populations in relation to S oxidation have used soil incubation techniques. The general results obtained have been as follows:

i) *Thiobacilli*, the most effective S oxidiser, are relatively rare compared to the heterotrophic oxidisers (Moser and Olson, 1953; Swaby and Fedel, 1973; Vitolins and Swaby, 1969; Germida *et al.*, 1985; Lee *et al.*, 1988a; Lawrence and Germida, 1988).

ii) *Thiobacilli* numbers increase substantially on the addition of S^o to soil (Adamczyk-Winiarska *et al.*, 1975; Lee *at al.*, 1987, 1988a, 1988b).

iii) There is a rapid decline in the number of sulphur oxidising bacteria present after a period (4-6 weeks) of incubation with elemental sulphur (Hart, 1959; Barrow, 1971; Lee *et al.*, 1987). This decline was not caused by an exhaustion of oxidisable sulphur (Hart, 1959).

iv) Substrates oxidised by these bacteria include sulphides, thiosulphate, tetrathionates, thiocyanates, sulphites and elemental-S which are products of organic-S mineralisation (Miller, 1949).

2.2.2.2 *Heterotrophs and their relative importance*

It has long been recognized that heterotrophic micro-organisms can also participate in sulphur oxidation. Soil fungi and some heterotrophic bacteria including actinomycetes can oxidize elemental sulphur and reduced forms of sulphur in vitro (Starkey 1934; Yagi *et al.*, 1971; Wainwright and Killham, 1980; Killham *et al.*, 1981). The details of S oxidation mechanisms of heterotrophic organisms have been studied intermittently for many decades and have been recently reviewed by Wainwright (1984).

In contrast to autotrophic oxidisers, the population of heterotrophic S oxidisers are abundant in soil. Agricultural soils 'polluted' with S^o, contain higher levels of heterotrophic S oxidisers than 'un-polluted' soils (Germida, 1985; Lawrence and Germida, 1988). Fungi have been the most readily isolated S oxidisers from agricultural soils that have not been treated with S^o, bacteria and actinomycetes able to oxidise sulphur have also been isolated (Germida, 1985). In vitro oxidation studies (Germida, 1985) also indicate that S oxidising heterotrophs from two physiological functional groups: those that are rapid, complete S oxidisers (i.e. fungi) and those that are slow, incomplete S oxidisers (i.e. bacteria and actinomycetes). Germida (1985) also observed that whereas fungi produced substantial quantities of sulphate, thiosulphate and tetrathionate, the bacteria and actinomycetes examined produced mostly thiosulphate during the oxidation of S^o.

A number of studies have suggested that heterotrophic organisms play a more important role in S oxidation in soils than autotrophic organisms. (Pepper and Miller, 1978; Germinda *et al.*, 1985; Wainwright *et al.*, 1986b; Lawrence and Germida, 1988). The positive correlations of S oxidation with the frequent isolation of heterotrophic S oxidisers, led to the general hypothesis that heterotrophic organisms may be important and the acidophilic microorganisms such as *T. thiooxidans* are of little importance in neutral to alkaline agricultural soil (Lawrence and Germida, 1988; Germida *et al.*, 1985; Vitolins and Swaby, 1969). However, no method is currently available for selectively inhibiting the activity of the heterotrophic sulphur oxidizers in soils; as a result it is impossible to determine which is the most active group in the process (Wainwright, 1984; Lawrence and Germida, 1988).

There are certain criteria which can be used to identify the relative importance of the heterotrophic microorganisms responsible for the S oxidation. If hetrotrophs are primarily responsible for the S oxidation in agriculture soil, then heterotrophically mediated processes should be stimulated by the addition of appropriate organic nutrients and thereby S^0 oxidation should increase (Wainwright 1984). Glucose addition into heterotroph inoculants in autoclaved soils have increased S oxidation (Pepper and Miller, 1978; Lawrence *et al.*, 1988). The amendment of autoclaved soil with 0.25% sugar beet extract or 1% straw also has increased the extent of oxidation (Wainwright *et al.*, 1986a).

The rate of S oxidation was found to be strongly correlated with microbial biomass C (Lawrence and Germida, 1988). Changes in the size and activity of the heterotrophic biomass were reflected in S oxidation rates. The microbial biomass, in turn, was a function of the organic C and clay content, pH and nutrient levels of the soils.

Regardless of the fact that heterotrophic microorganisms oxidise S^0, their oxidative capacity was significantly lower than that of the autotrophic *thiobacilli* (Germida, 1985).

It has been established that S^0 oxidation can be carried out by both chemolithotrophic and heterotrophic soil microorganisms. However, there is not sufficient information at present to identify which type of organism plays the greatest role in near neutral aerobic soils, which are the target for S^0 fertilizers in New Zealand.

2.2.3 Mechanisms of S^0 Oxidation

Two possible mechanisms may be envisaged for sulphur oxidation by micro-organisms. These include i) extracellular and ii) cell interface based mechanisms.

i) Extracellular: Sulphur may react with chemical or enzymic agents excreted by micro-organisms, with the formation of soluble compounds which are assimilated by the micro-organism prior to oxidation. However, there is no evidence to support this mechanism. Direct contact of the *thiobacilli* cells with solid sulphur particles has been considered to be necessary and the extent of contact is important in

controlling the microbial oxidation rate of sulphur (Vogler and Umbreit, 1941; Shaeffer *et al.*, 1963; Takakuwa *et al.*, 1979).

ii) **Interface**: A reaction between sulphur and a cellular component may take place at the surface of microbial cells. The reaction between S^o and cellular components involves two processes i). initial attachment of the bacteria to S^o particles and ii). subsequent oxidation (Takakuwa *et al.*, 1979).

Initial attachment of bacterial cells to S^o particles is achieved by chemical interaction with a cell envelope through either sulphydryl groups (*T. thiooxidans*) or by glycocalyx groups (*T. albertis*). Where the attachment through the former group is considered to be dependent upon physiological conditions such as pH and cellular energy (Takakuwa *et al.*, 1979), the attachment through the latter groups is independent of all these processes (Bryant *et al.*, 1984).

The attachment of bacterial cells to sulphur granules was critical for their proliferation and accounted for an apparent early lag phase in growth (Takakuwa *et al.*, 1979). Laishley *et al.*, (1986) found that at these cell-sulphur adhesion sites, microcolony development may occur where the initial adhering cell divides into two daughter cells after obtaining enough energy from the oxidation of S to fix CO_2 into cell constituents. The growth process repeats many times over 19 days allowing bio-films to grow on the sulphur surface.

Improved understanding of the effects of such mechanisms on the rate of microbial oxidation is essential for successful modelling of the rate of oxidation of S^o fertilizers in soil. However, the biochemical mechanisms and enzymes by which heterotrophs oxidize reduced S compounds remain to be elucidated.

2.3 FACTORS AFFECTING S^o OXIDATION

Being a biological process, S^o oxidation is affected by environmental factors. From an intuitive approach such a mechanism requires (McCaskill, 1984),

i, an exposed surface of the insoluble elemental sulphur,

ii, a film of water on the elemental sulphur surface in which the organisms can live,

iii, the presence of *thiobacilli* or other organisms capable of oxidising S^o,

iv, diffusion of oxygen from the soil atmosphere through the water film,

v, diffusion of CO_2 to supply a carbon source for the growth of *thiobacillus* cell bodies,

vi, diffusion of H^+ and SO_4^{2-} ions through the water film into the surrounding soil to remove waste products, and

vii, diffusion of nutrient ions such as phosphate to the reaction site to sustain microbial growth.

Suitable physical and chemical conditions, the size and composition of sulphur oxidising microorganism populations and the amount and form (particle size, shape and molecular structure) of S^o, factors that may affect the rate of oxidation, are discussed in the following review section.

2.3.1 S^o Allotropes

S^o exists in an unusually large number of different forms or allotropes. These allotropes can co-exist in a variety of multi-component systems; however, all but one of these allotropes (or combination thereof) are considered to be metastable. The most abundant and stable class of S^o under normal ambient conditions is $S_8\alpha$, which consists of a crown shaped eight membered sulphur atom ring arranged in an orthorhombic crystal lattice (Meyer, 1976). Small amounts of polymeric sulphur, μ-S, (helical chains up to 300,000 sulphur atoms long) and S_x (where **x** is number of atoms of sulphur in the molecule) composed of a mixture of non-S_8 membered rings (S_6 to S_{26}) and noncrystalline amorphous S_8 make up the other two major molecular classes found in S^o (Laishley *et al.*, 1986). The percent composition of these molecular classes found in S^o are dependent on the thermal history and mode of solidification (Kobryn and Hyne, 1981)

The composition of commercial S^o sources which consist of mixtures of various molecular arrangements of sulphur atoms can influence S oxidation rate (Higgins, 1983), however, relatively few studies have been undertaken.

Laishley *et al.* (1986) studied the effect of the molecular composition of S^o on the rate of S oxidation. Three different types of molecular sulphur with variable percentages of S species, namely high-purity orthorhombic (HPO) sulphur (91.4% $S_8\alpha$; 8.3% S_x; 0.3% μ-S), mixed molecular (MMS) sulphur (71.6% $S_8\alpha$; 17.9% S_x; 10.5% μ-S) and high-purity polymeric (HPP) sulphur (100% μ-S) were added into *T. albertis* solution culture at 28°C over a 3 weeks period. The initial oxidation of these powdered forms of sulphur were exponential up to 3 days at essentially identical rates. After which sulphate production MMS decreased substantially with time as compared with similar sulphate production rate generated for HPO and HPP oxidation. After 21 days, *T. albertis* utilized approximately 70% of the HPO and HPP sulphur forms, while only 35% of the MMS was used.

They concluded that the increased S_x content in MMS was responsible for the slower oxidation rate because the sulphur crystal lattice formation was altered reducing the number of sterically favourable oxidation binding sites for *T. albertis* growth.

Similar results were also obtained by Headford and McSweeney (1984), in which allotropic forms of sulphur were incubated with moist soil for two months. Although all forms were oxidised appreciably to sulphate, the results suggested that species other than orthorhombic α-sulphur had reduced oxidation rates. These observations suggests that the microbial oxidation is more sensitive to physical factors, such as the crystal microstructure of the sulphur, rather than to its specific molecular composition.

2.3.2 Particle Size

As described in Section 2.2.3, sulphur oxidation is exclusively a surficial reaction. If all the surface area of the sulphur particle is exposed to attack by S oxidising microorganisms and if the population sizes of these organisms and other factors were not limiting, the rate of oxidation of a given mass should be a function of its surface area (Janzen and Bettany, 1987b; Watkinson, 1989). Linear relationships between the amount of S oxidised and the surface area (inverse relation to radius) of S applied to soil have been established by several researchers (Fox *et al.*, 1964; Koehler and Roberts, 1983; Laishley *et al.*, 1983; Janzen and Bettany, 1987b).

The effect of S^o surface areas on the rate of S oxidation is covered in more detail in Section 2.3.8 and Chapter 8

2.3.3 Effect of Previous S^o Application on the S^o Oxidation Potential of Soil

Many workers (Vitolins and Swaby, 1969; Fawzi, 1976; Li and Caldwell, 1966; Pepper and Miller, 1978; Germida *et al.*, 1985; Kittams and Attoe, 1965; Janzen and Bettany, 1987c) have found that inoculation of soils with *thiobacilli* can increase the number of these organisms in the soils and hence the rate of S^o oxidation. These results suggest that if S^o fertilizers stimulate S^o oxidising populations (Germida *et al.*, 1985) then the effectiveness of subsequently applied S^o products may be enhanced on previously S^o fertilized areas.

For example, Lawrence *et al.* (1988) studied the effect of fertilizer history on S^o oxidation rate in two Canadian soils. In one soil, application of S^o fertilizer caused an increase in the populations of autotrophic thiosulfate-oxidising microorganisms while in the other soil S^o fertilization did not stimulate their growth. Previous S^o fertilization resulted in increased S oxidation rates in both soils. However, the extent of the increasing oxidation rate was found to depend on the rate of previously applied fertilizer. For instance there was a threefold increase at the lower rate of cumulative addition of 110 kg S over 5 years (i.e. 22 kg S^o ha^{-1} yr^{-1}) in one soil, but the oxidation rate only increased by 17% following a single application of 100 kg S^o to the other soil.

In a field study conducted in New Zealand, Lee *et al.* (1988b) found that the second application of S^o was oxidised more rapidly than the first application, because of the larger populations of *thiobacilli* and the warmer and more moist soil conditions.

Although there was an increase in the numbers of sulphur oxidizing microorganisms due to S^o fertilizer pretreatment, some workers (Solberg and Nyborg, 1983; Germida *et al.*, 1985) have failed to observe increases in the oxidative capacity of the soils. This is mainly due to the significant role of heterotrophic sulphur oxidizers in these soils.

It is also possible that a non-biological soil parameter (e.g. level of available organic substrate or soil pH) may have restricted the proliferation of oxidizing organisms. In some studies, the stimulatory effect of S^o depended on the type of microorganisms responsible for the S oxidation process. It would appear that further research is required to determine the reasons for the different effects observed in different studies.

2.3.4 Temperature

Soil temperature would be expected to affect S oxidation because of the normal temperature influence on microbial activity. S oxidation is much more responsive to the change in soil temperature than most other biological mediated reactions as indicated by the Q_{10} values ranging from 3.2-4.3 reported by Janzen and Bettany (1987a). The New Zealand Ministry of Agriculture and Fisheries (Sinclair *et al.*, 1985) classify the effectiveness of different S^o particle sizes according to climatic zone. This implies that sensitivity to temperature probably accounts for the limited effectiveness of most S^o fertilizer in the cooler regions.

S^o oxidation rate is very slow at temperatures below 4°C, but increases rapidly with increasing temperature to a maximum at approximately 40°C (Burns, 1967; Weir, 1975; Li and Caldwell, 1966; Nor and Tabatabai, 1977). The optimum temperature range for *thiobacilli* was 28-30°C (Waksman and Joffe, 1922). However, this information may not accurately reflect the relationship between the oxidation of surface applied S^o and temperature under field conditions, because the surface applied S^o would be exposed to large temperature fluctuations, both seasonal and diurnal.

Shedley (1982) studied the effect of temperature range on S oxidation, using undisturbed soil cores in controlled temperature cabinets, at four diurnally fluctuating temperatures; 14/8, 20/14, 24/18 and 30/24°C day/night, respectively. The results showed oxidation rates increase linearly between 14/8°C to 20/14°C day/night temperature but there was no further increase to 30/24°C.

Janzen and Bettany (1987a) found the oxidation rate was exponentially related to temperature, each subsequent increase in temperature from 3°C had a progressively greater stimulatory influence on oxidation rate as shown in Figure 2.2.

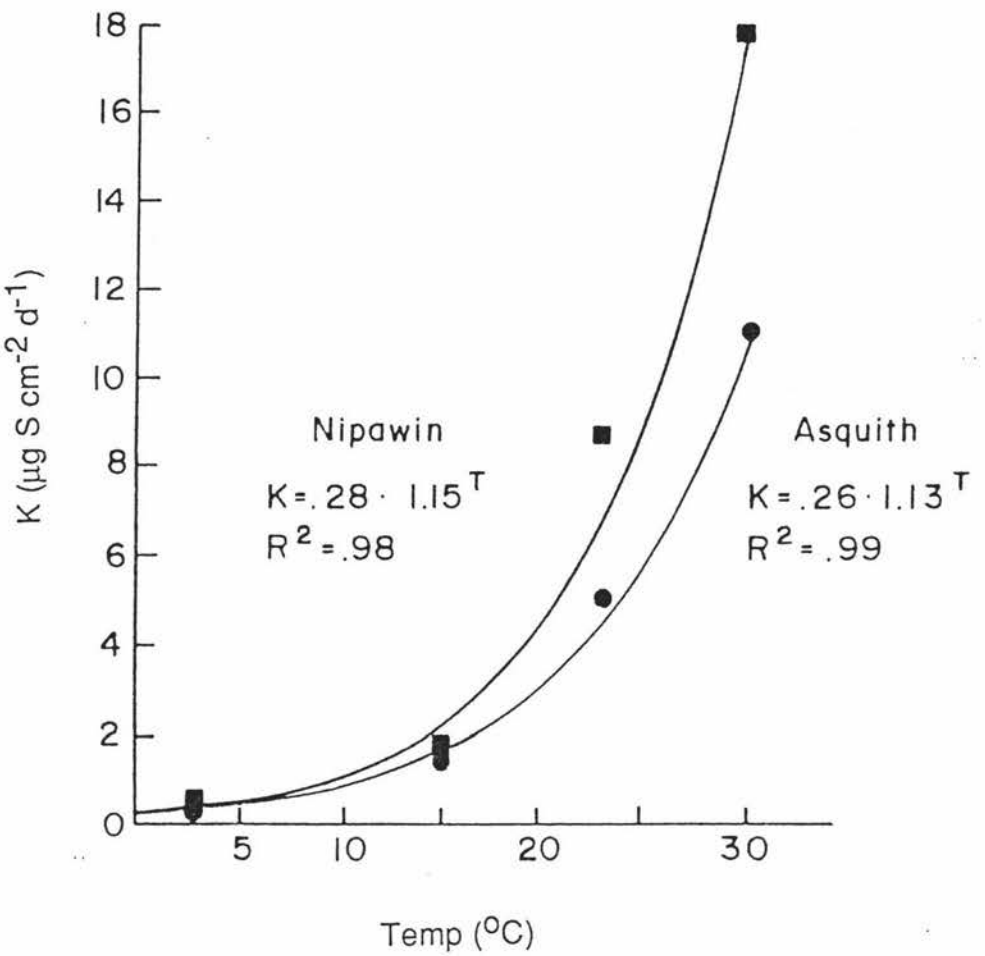


Figure 2.2 Effect of temperature and on S^0 oxidation in two Canadian soils (Janzen and Bettany, 1987a).

For these results, the relationship between oxidation rate and temperature was accurately described by a van't Hoff-type equation (Janzen and Bettany, 1987a):

$$K = a.b^T \qquad (2.5)$$

where k = oxidation rate ($\mu g\ S\ cm^{-2}\ day^{-1}$); a, b = constants and T = temperature °C. This relationship was established in a relatively short incubation period (6 days). Under these conditions the population of S^o oxidisers may not have reached a steady state. The exponential increase in S^o oxidation rate with temperature may reflect the exponential increase in the micro-organism population. However, in a longer period of incubation (Li and Caldwell, 1966) and a pot experiment (Shedley, 1982) the oxidation rate was found to increase linearly over most of the temperature range 14/8°C day/night temperature and 20/14°C but there was no further increase to 30/24°C.

Obviously more information is required before the effects of temperature on the growth of S^o oxidisers and S^o oxidation rates are fully understood.

2.3.5 Soil Moisture

Sulphur oxidation has been shown to proceed most rapidly at a soil water potential close to field capacity with much reduced rates at low moisture contents and at higher soil moisture contents as shown in Figure 2.3 (Moser and Olson, 1953; Kittams and Attoe 1965). Generally S oxidation was minimal at moisture tensions ranging from 0.1 to -1 MPa (Attoe and Olson, 1966). Moser and Olson (1953) found the moisture tension of 0.003 to 0.006 MPa were optimum for oxidation in four soils with textures ranging from loamy sand to silty clay.

The optimum water potential varied with soil type, the different optima reflect variations in the degree of aeration, which is positively related to air-filled porosity and negatively related to volumetric moisture content (in Chapter 8, this data is re-evaluated in the light of a recent publication by McCaskill and Blair, 1989).

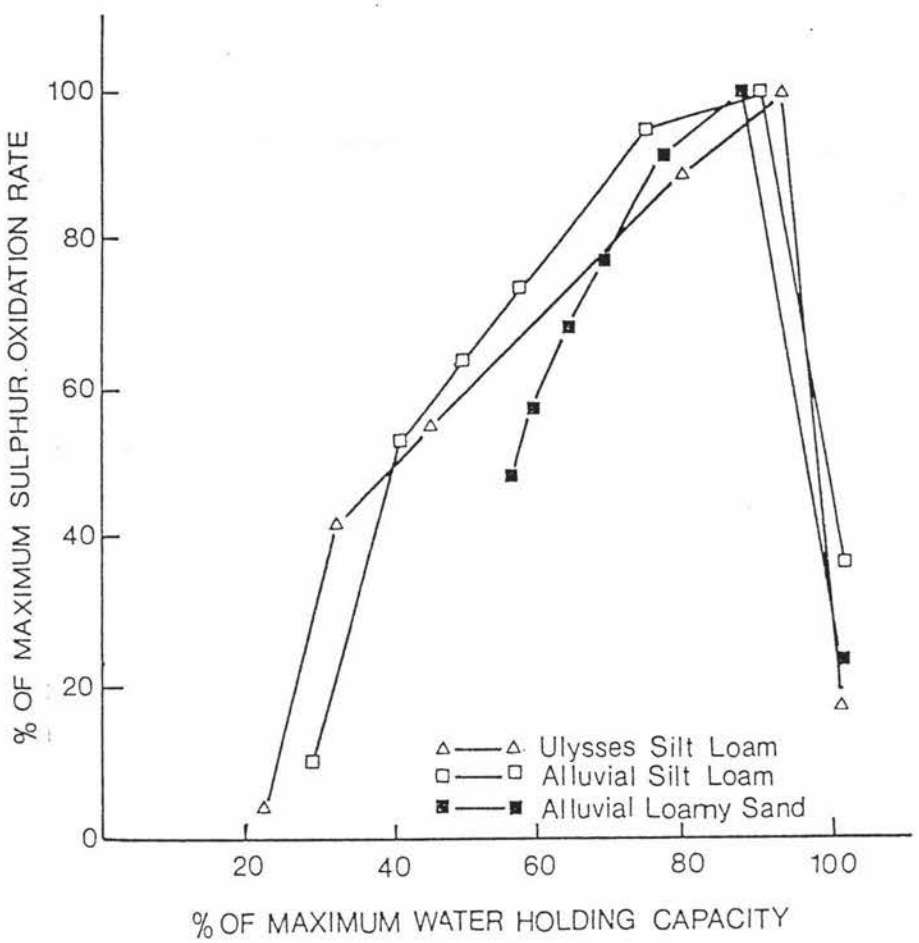


Figure 2.3 Percentage of maximum S^0 oxidaiton as a function of percent maximum water holding capacity (re-expressed from Moser and Olson, 1953 by Shedly, 1982).

At high water potential the rate of oxidation was lower through insufficient aeration (Janzen and Bettany, 1987a), especially in fine textured soils because the fraction of the pore space occupied by water at any potential is much higher in a fine-textured than in a coarse-textured soil. At lower water potentials the lower rate of oxidation is more likely to be the result of slower aqueous transport of nutrients to the microbes or reduced accessibility of the S^o particles, which are highly hydrophobic, rather than lowered water availability as concluded by Moser and Olson (1953). Only a very thin water film is needed for oxidation to occur (Watkinson, 1988).

Low water potentials appear to be less limiting to S oxidation than to plant growth (Janzen and Bettany 1987a). Under these conditions, SO_4^{2-} tends to build up in soils because of limited diffusion of the oxidised product. This in turn will reduce the rate of succeeding S^o oxidation.

Thiobacilli are believed to be very susceptible to long periods of drought, hence their absence in many Australian soils (Vitolins and Swaby, 1969). Moser and Olson (1953) and Kittams and Attoe (1965) also found negligible sulphur oxidation in incubated soils inoculated with *thiobacilli* when soil water contents were below 30% of maximum water holding capacity. However, other groups of S^o oxidising microorganisms seem to withstand long periods of drought and can become active when adequate soil moisture becomes available. Bollen (1977), found that soil samples in dry storage for 54 years were able to oxidised S^o after rewetting. Janzen and Bettany (1987a) found that during the incubation of soil with S^o of known particle size, the rate of oxidation was parabolically related to soil water potentials in the range 0.007 to -10MPa. The results probably reflect the interactive effects of aeration and water availability (Burns, 1967).

2.3.6 Interactive Effects of Temperature and Moisture

Increasing the temperature was found to enhance the restrictive effect of high water potential on oxidation rate as shown in Figure.2.4 (Janzen and Bettany, 1987a). At higher temperatures oxidation rates declined by large percentages compared to low

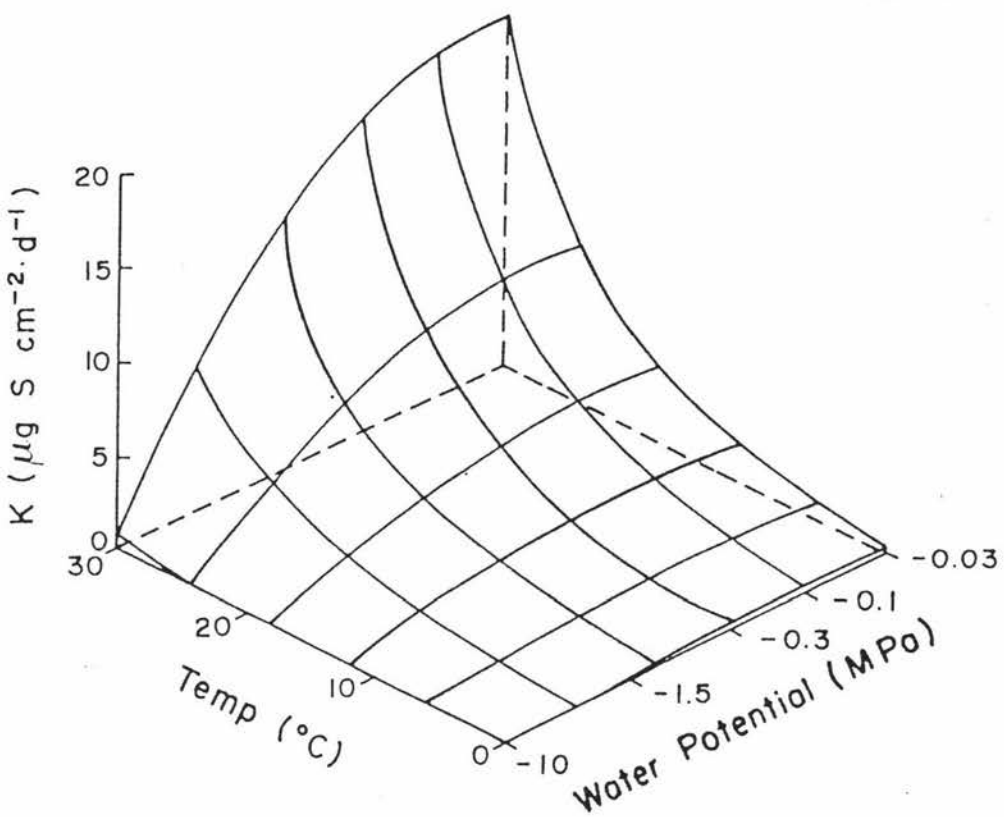


Figure 2.4 Effect of soil temperature and water potential on S^0 oxidation in a Canadian soil (Janzen and Bettany, 1987a).

temperatures. This interactive effect probably reflects the higher demand for oxygen, the lower solubility of O_2 in water at high temperatures and hence a greater restriction is imposed by a low air-filled porosity at high temperatures.

Janzen and Bettany (1987a) successfully defined the oxidation rate as a function of both temperature and water potential by defining **b** (a constant for a given soil) in terms of a function describing the response to temperature using the exponential equation above and defining **a** as a function of water potential (using best fit quadratic relationship). However, the response of oxidation rate to temperature was also significantly affected by soil type. A generalized mathematical expression describing the effect of temperature and water potential on the oxidation rate, therefore, must include the effect of soil type on the temperature dependent constant **a** (Janzen and Bettany, 1987a).

Despite the large amount of information gained by Janzen and Bettany (1987a) the applicability of their relationships, which describe the effect of temperature and moisture on the rate of oxidation of S^o freshly added to soil, to long term S^o oxidation is questionable, for the reasons described earlier (Section 2.3.4)

2.3.7 Soil Type or Texture

Variation in soil type and texture do not have any consistent effect in the rate of S oxidation. Kittams and Attoe (1965) in a study of S oxidation in 54 Wisconsin soils, reported no consistent relationship between the amount of S oxidized and soil type or soil pH. Vitolins and Swaby (1969), incubated 273 Australian soils at 25°C for 10 weeks and also found no obvious correlation between the extent of oxidation and a wide range of soil parameters e.g. great soil group, geology, geography, storage time before analysis, moisture content at time of sampling, season when sampled, field pH, final pH, and texture. McCaskill and Blair (1987) also observed no relationship between soil texture and S^o oxidation rates, measured over 238 days of a pot experiment in soils with a range of clay contents from 9-52%. In all studies there was, however, a tendency for oxidation to be lower in alkaline soils. Both the Australian

and Wisconsin studies (Vitolims and Swaby, 1969; Kittams and Attoe, 1965) attributed variability in S^o oxidation rates to differences in the number and kinds of sulphur oxidising microorganisms present in the soils studied.

Conversely, Moser and Olson (1953) studied four soils which were incubated at different moisture tension ranging from 30 to 60 cm of water which is considered to be the optimum for oxidation in each soil. These results indicate that the differences in maximum oxidation rates between soils was related to soil texture. A clay soil had a higher oxidation rate than a sandy soil. These results may not be solely related to moisture and nutrient availability. Soil organic C content, which has been found to be highly correlated with the number and activity of heterotrophs oxidisers, was also likely to be higher in the clay soil. Other effects of O_2 availability were discussed in Sections 2.3.5 and 2.3.6.

The stimulatory effects of clays (Montmorillonite proved more stimulatory to the growth and S-oxidizing ability of *T. thiooxidans* and *T. denitrificans* than did kaolinite) on S^o oxidation by *thiobacilli* in vitro was shown by Mouraret and Baldensperger (1977) and in soils by Fawzi (1976). Stotzky and Rem (1966) suggest that microbes obtain nutrient cations by ionic exchange with clay surfaces, probably with H^+ as the exchanging ion. This may be important for *thiobacillus* which produces H_2SO_4 as a product of sulphur oxidation.

A number of workers have attempted to investigate the effect of soil pH on sulphur oxidation rates (Kittams and Attoes 1965, Vitolins and Swaby, 1969) and the effects of liming (Fox *et al.*, 1964; Arif, 1967; Bloomfield, 1967). They failed to find any relationship between S oxidising ability and pH of the soils, except at pHs greater than 8. The effects of liming on sulphur oxidation have been confounded by the stimulatory effect of liming on soil organic matter mineralization (Williams, 1967).

Lawrence and Germida (1988), in the studies of S oxidation of 28 Canadian agricultural soils, found that soil microbial biomass C, clay content and other soil properties such as pH, organic C, P and S constituents were correlated with the level of S^o oxidation.

Contradictory results on the effect of soil type or texture on the rate of oxidation may be related to the different types of S^o oxidiser operating in different soils and to stimulatory effects which are peculiar to the dominant oxidising population. Other parameters like moisture, soil pH, buffering and organic matter content may have a major influence because these factors were not adequately controlled in many of the experiments conducted. In soils where heterotrophs are primarily responsible for S oxidation, the heterotrophic microbial biomass and activity are positively correlated with soil pH, clay content, organic C, and soil P levels (Germida *et al.*, 1985; Lawrence and Germida, 1988) or the addition of appropriate organic nutrients (Wainwright 1984).

2.3.8 Application Rate

Published literature is full of conflicting results relating to the effect of S^o application rate on the S^o oxidation. The independence of oxidation rate from the rate of S^o application rates has been observed by Li and Caldwell (1966), Weir (1975), Lettl *et al.* (1981) and Janzen and Bettany (1987b).

There was no effect of a range of application rates on oxidation rates measured in the incubation studies of Li and Caldwell (1966), when they used S^o particles of 60-80 mesh (180- 240 μm) size mixed with soil at 100 to 1,000 μg S^o g^{-1} soil for 60 days. The only trend was a slight increase in the recovery of sulphate at the higher application rates after 60 days. Rates of 100 and 500 μg S^o g $soil^{-1}$ of a range of particle sizes were used in another of their experiments, but again no effect of sulphur application rate was evident.

Janzen and Bettany (1987b) used S^o of 106 to 150 μm (mean diameter of 119 μm) particle size and applied a range of rates from 0 to 4,000 μg S^o g^{-1} soils to two soils of different pH (pH 6.1 and 7.2 in 0.01 M $CaCl_2$, solution:soil, 5:1) and pH buffering capacities at -0.03 MPa water potential for a relatively short (6 days) period of time. The oxidation rates were measured in terms of sulphate produced per unit surface area per unit time. The results showed that rate of S oxidation (μg S^o oxidised per cm^{-2} day^{-1}) was not affected by rate of S application, but a linear relationship existed between application rate and the amount of sulphate produced over the range of 0-4,000 μg S^o g^{-1} soil (Figure. 2.5).

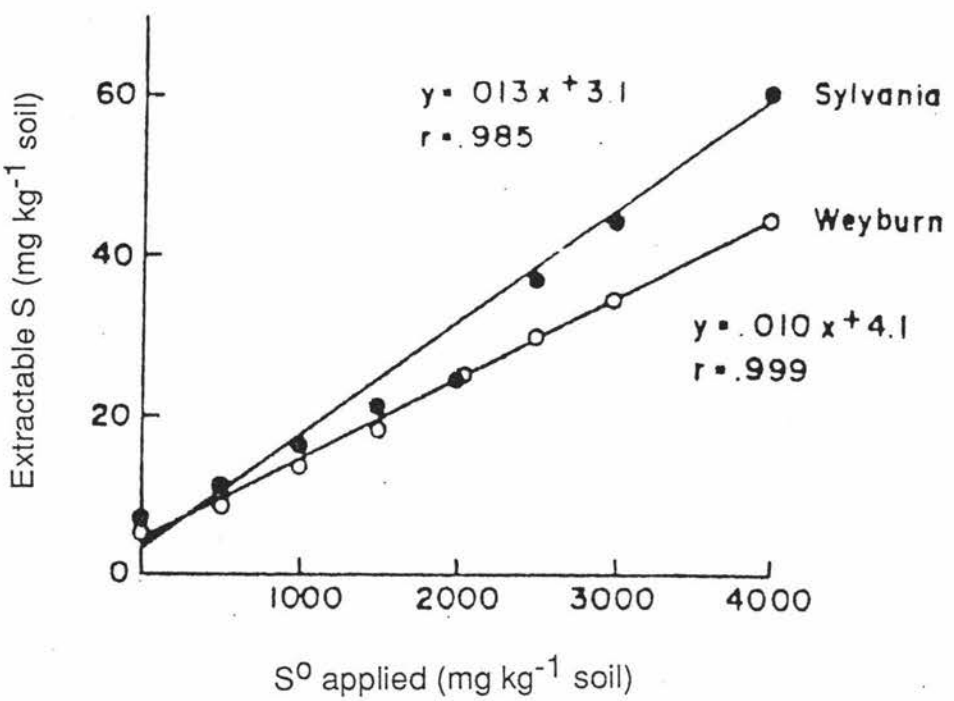


Figure 2.5 Effect of rate of S^0 application on extractable sulphate-S produced during a 6-day incubation (Janzen and Bettany, 1987b).

They concluded that S oxidation is not limited by the number of S oxidising organisms in the soil, because every additional increment of applied sulphur resulted in a proportional increase in sulphate production. However, the linear relationship was maintained only up to a certain threshold application rate (total applied surface area of 0.2188 $cm^2\ g^{-1}$ soil; Table 2.1) as indicated by another incubation study, using finely divided sulphur (<0.053 mm), in which a decline in oxidation rate occurred when application rates exceeded 400 $\mu g\ S^o\ g^{-1}$ soil. This decline will occur at progressively lower application rates as specific surface area is increased (Janzen and Bettany, 1987b). They suggested that oxidation was limited by the amount of available substrate, rather than by the oxidative capacity of the microbial population. Janzen and Bettany (1987b) considered sulphur oxidation is likely to be limited by an initial chemical solubilization process that renders the sulphur accessible for oxidation, not by the biological capacity of the microbial population to oxidise sulphur.

Depressions in apparent oxidation rates of sulphur were found at high rates of application (Barrow, 1971) when very fine S^o was used (< 200 mesh or 53 μm) and applied at a ranges of rate from 50 to 1,000 $\mu g\ g^{-1}$ soil in two soil types. Barrow (1971) found decreasing percentage recoveries of sulphate with increasing application rates. The percentage recovery, of applied sulphur as sulphate, at the 1,000 $\mu g\ g^{-1}$ application rate was in all cases less than 20% of the recovery at lower rates of application. The usual reason for the apparent differences in the effect of application rate on S^o oxidation rates is the difference in S^o particle size used in each experiment.

In order to compare the effect of application rate on S^o oxidation using the results of various researchers, the total S^o surface area applied rate was calculated as shown in Table 2.1. The threshold application rate (of Janzen and Bettany (1987b) of <53 μm particle size at 400 $\mu g\ g^{-1}$) had only 0.219 $cm^2\ g^{-1}$ soil of surface area compared to 0.974 $cm^2\ g^{-1}$ soil for the 119 μm particle size applied at the rate of 4000 $\mu g\ g^{-1}$ soil. However, the oxidation rate of the latter was still represented by a linear relationship, indicating that the threshold application rate may depend on the size of the S^o particle, and an interaction between the application rate and the particle sizes may exist.

Table 2.1 S^o oxidation rates per unit area (μg SA^{-1} d^{-1} soil) taken from a range of published studies in which the effects of application rate (μg g^{-1}) and applied S^o surface area (cm^{-2} g^{-1} soil) were examined.

average diameter μm	Application rate μg g^{-1}	surface area appl.rate cm^2 g^{-1}soil	Oxidation rate μg $SA^{-1}d^{-1}$		Remark
Incubation studies.					
200	100	0.0145	11.49		Li and Caldwell (1966)
	300	0.0435	11.49		60 days
	500	0.0725	14.48		sandy loam soil
	700	0.1014	13.64		
	1000	0.1449	13.92		
			soil A	soil B	
<75	500	0.1932	19.59	23.66	Barrow (1971)
	1000	0.3865	13.49	20.88	70 days
	2000	0.7729	7.02	13.49	Soil A, sandy soil
	6000	2.3188	2.19	6.59	Soil B, well buffered
	10000	3.8647	1.40	3.88	lateritic red earth.
119	500	0.1218	12.31	15.05	Janzen and Bettany (1987)
	1000	0.2436	8.89	10.95	6 days
	1500	0.3654	8.21	9.58	pH 7.2, and 6.1
	2000	0.4872	8.21	8.21	
	2500	0.6089	8.21	9.85	
	3000	0.7307	7.45	9.42	
	4000	0.9743	7.53	10.26	
<53	400	0.2188			threshold appl.rate

average diameter μm	Applicatn. rate mg pot^{-1}	surface area application cm^{-2} pot^{-1}	Oxidation rate μg $pot^{-1}d^{-1}$	Remark
Pot experiment				
50	4	12.32*	2.32	Shedly (1982)
	8	12.32*	4.64	140 days
	32	12.32*	18.55	
100	8	24.64*	2.32	
	16	24.64*	4.64	
	64	24.64*	18.55	
200	32	46.21	4.64	
	64	34.65	9.28	
	256	21.56	37.10	
400	60	57.50	4.35	
	120	50.93	8.70	
	480	27.52	34.78	
1000	400	66.54	11.59	
	800	54.21	23.19	
	3200	22.18	92.75	

* presumably all S^o oxidised less than 140 days.
Vol.of a particle ($V = 4/3 \text{ pi } r^3$); wt.of a particle ($M = 2.07 \times 4/3 \times \text{pi} \times r^3$)
No.of particle g^{-1} soil ($n = \text{Rate} / M$); Surface area ($SA = 4 \text{ pi } r^2$).
Surfacr area application rate = n x A;
Oxidation rate = Appl.rate/(surface area* days) (μg S g^{-1} day^{-1})

In the intact soil core pot experiment of Shedley (1982), similar S^o surface areas were applied at each rates of the various particle sizes (50, 100, 200, 400 and 1000 μm) used. The rate of sulphur oxidation was shown to be inversely related to both the particle diameter of the sulphur used (surface area effect) and the rate at which it was applied. Proportional oxidation rates were depressed at the higher application rates. The maximum size of the depression was a 3.4 fold difference between the low and the high application rates of the 1000 μm treatment, which was less than that reported by Barrow (1971) who showed a fourteen fold difference in oxidation rates between the low (50 $\mu g\ g^{-1}$ soil) and high (1,000 $\mu g\ g^{-1}$ soil) application rates of <75 μm particle size of S^o. However, the relative range of application rates used by Shedley (1982) (a factor of eight within each particle size) was less than that half the range (twenty fold) range used by Barrow (1971).

The depression of oxidation rate at the very high surface application rate (Barrow, 1971) may be due to reduced numbers of sulphur oxidizers per unit surface area, the limitation of the organic C substrate (especially when heterotrophs are the major microbes responsible in the process), the adverse effects of acidity or oxidation intermediates that may accumulate at high application rates, and/or saturation of chemical solubilising agents (these effects may also explain the lower oxidation rates of sulphur applied in bands compared with the oxidation rates of sulphur mixed into soil (Fox *et al.*, 1964). Hart (1959) reported a decline in the size of the *T. thiooxidans* population with time in an S^o enriched soil, despite the presence of large amounts of oxidisable sulphur. This implied the presence of some mechanism regulating the size of stable populations of these organisms. Alternatively, the effect may be microbial, with some self imposed or environmental limitation on the population density of sulphur oxidising organisms, e.g. the suitable sites of micro-environments may have been filled, a decline in oxidation rates occurs as the less favourable sites are occupied with increasing application rates. Again insufficient information is available in the literature to develop relationships which accurately explain the rate of S^o oxidation in soils for all S^o particle sizes and rates of application.

2.3.9 Rate of S^o Oxidation under Incubation vs Field

In order to compare the rate of oxidation under laboratory incubation and field conditions, oxidation rates from published data were expressed as the proportional oxidation rates (percent of applied S day^{-1}). It was generally found that the proportional oxidation rates of the pot experiments were much greater than the incubation studies. For example, the mean oxidation rate of 200 μm diameter S^o at four temperature regimes (Shedley, 1982) was 0.49% of applied S^o day^{-1} which is much higher than the 0.13% S^o day^{-1} of the same particle size at 40°C reported by Li and Caldwell (1966). This may be due to the method of estimating oxidation rate by measuring the changes in soil sulphate concentrations which is likely to underestimate S^o oxidation, because the sulphate produced can be rapidly immobilised into organic forms (Till and May, 1971). Whereas in the pot experiment the estimated oxidation rate was achieved by directly measuring the residual S^o by solvent extraction. However, under field conditions in a perennial pasture (of Australia, Shedley, 1982) the mean oxidation rates of the three particle sizes, 0.15, 0.39 and 0.93 mm applied at 25 kg ha^{-1} were 0.18% day^{-1} over the 308 days of the experiment. These rates are only 40-50% of those measured in the pot experiment of Shedley (1982), presumably due to the less optimum climatic conditions of the field experiment compared to the pot experiment.

It appears that faster oxidation rates can be measured under laboratory and glasshouse conditions than under field conditions. Explanations for this are that sub optimal climatic conditions are likely to occur in the field.

2.3.10 Oxidative Potential of Soil

Information on the oxidative potential of the soil is necessary to determine whether some soils have sufficient S^o oxidising capacity to benefit from the addition of S^o fertilizers, and to determine whether the level of variability in oxidation capacity among soils warrants compensatory adjustment of application rate or particle size of the S^o fertilizers. The literature (Nor and Tabatabai, 1977; Wainwright 1978; Skiba and Wainwright, 1983; Ray *et al.*, 1985; Lawrence *et al.*, 1988) suggests that the

rhodanese enzyme may play an important role in the oxidation of S^o. Rhodanese activity can be easily measured, therefore it may provide a rapid method suitable for assessing the S^o oxidising potential of soils.

Relatively few enzymes of *thiobacilli* have been demonstrated to be positively involved in S^o oxidation. Kelly (1988) summarised several complementary or conflicting schemes for thiosulphate oxidation, it was found that the enzyme converting thiosulphate to tetrathionate is present in most *thiobacilli*, and the enzyme rhodanese appears ubiquitous.

The enzyme rhodanese (thiosulphate-cyanide sulphurtransferase, EC 2.8.1.1) will catalyse the formation of thiocyanate from thiosulphate and cyanide:

$$S_2O_3^{2-} + CN^- \longrightarrow SCN^- + SO_3^{2-} \qquad (2.6)$$

Its significance lies in the fact that thiosulphate is a frequent intermediate in S oxidation, and sulphite is rapidly converted in the soil to plant available sulphate. Thus it is assumed to play an important role in the oxidation of S^o to sulphate in the soil (Tabatabai and Singh, 1979).

Rhodanese is widely distributed in nature, and has been reported occurring in sand dune and saltmarsh soils (Wainwright, 1981; Skiba and Wainwright, 1983). Lettl (1981) showed that 63% of the heterotrophic bacteria isolated from a spruce humus soil were able to produce rhodanese enzyme. This is further evidence for the involvement of heterotrophs in S^o oxidation.

Rhodanese is the only S oxidising enzyme that can be measured in soil, thus it may provide an index of the oxidation potential of a soil and the oxidative response to S^o addition. Its activity is also found to increase in soils in which S oxidation is occurring (Wainwright 1978). Wainwright (1978) attempted to use rhodanese activity as an indicator of microbial S oxidation in soils heavily polluted with high concentrations of S products. He attempted to show that a portion of the decrease in pH in heavily polluted soils was due to oxidation of S compounds, rather than solely a result of sulphuric acid input. The polluted soils had a higher rate of rhodanese activity than non-polluted soils of a similar soil type. It was concluded that this resulted in a higher rate of S-oxidation, although this was not in fact measured at the time. Ray *et al.*

(1985) found that rhodanese activity in aerobic soil was related to microbial oxidation of S^o. Enzyme activity was shown to increase when the soil was incubated with S^o. Activity was always higher in rhizosphere soil compared to non-rhizosphere soil of rice. This was probably a result of the increased microorganism activity in the nutrient rich rhizosphere soil or through increased O_2 supply (Ray *et al.*, 1985).

Lawrence *et al.* (1988) found that the stimulation of S^o oxidation in a Waitville soil parallelled increases in the autotrophic thiosulphate oxidizing population and rhodanese activity. The increase in rhodanese activity was also correlated with the increase in autotrophic thiosulphate-oxidising microorganisms. Rhodanese activity decreased markedly with depth down the soil profile. This decrease was associated with a reduction in the soil's organic carbon content.

Preincubation of soils with glucose increased the microbial population and increased the level of rhodanese activity (Singh and Tabatabai, 1978)

The rhodanese activity in soil is sensitive to various soil preparation and storage procedures. Air drying causes a marked decrease in rhodanese activity, however, storage of fresh soil at -20°C for one month, and 5°C for two months had no effect on the level of rhodanese activity (Singh and Tabatabai, 1978). Singh and Tabatabai (1978) determined the effect of various inorganic anions on rhodanese activity. It was found that at the concentration of 1mM NO_3^-, SO_3^-, $H_2PO_4^-$ and HCO_3^- inhibited activity. This was explained in part by the increase in the ionic strength of the medium, but no explanation was found for the activation of rhodanese activity in soils by NO_2^-, SO_4^{2-} F^- and Cl^-. However, the activation and inhibition of rhodanese activity by the inorganic anions tested varied considerably among the soils. Most trace elements, at a concentration of 50 μmoles g^{-1} soil, inhibited rhodanese activity by at least 50% due to their reaction with the enzyme protein. Zinc alone, enhanced activity.

Rhodanese activity in soil is affected by a number of factors. It is important that these factors be identified and kept constant if rhodanese activity is to provide a reliable measure of a soils S^o oxidation potential. Firstly, however, research is required to further clarify the role of rhodanese in S^o oxidation.

2.4 THE STUDY OF S^o OXIDATION IN NEW ZEALAND SOIL

A major study of the activities of sulphur oxidising microorganisms in New Zealand soil was recently completed by Lee *et al.*, (1987, 1988a, 1988b). These authors concluded that *thiobacilli* are important S^o oxidisers but heterotrophs probably have a role in oxidising S^o under field conditions in New Zealand soils. This aspect is discussed in this section.

2.4.1 Chemolithotrophs in New Zealand Soils

i) Incubation studies:

Lee *et al.* (1987, 1988a), used 48 topsoils from grazed pastures in the Waikato region of the North Island. Six major New Zealand soil groups were represented. In an incubation study they found that these soils had very low native *thiobacilli* population numbers which comprised more neutrophiles than acidophiles. This can probably be attributed to the pH range of the soils which was between 5-6 and are more suited to neutrophiles. However, the *thiobacilli* population increased very rapidly from <10 to 10^7-10^8 *thiobacilli* g^{-1} soil two weeks after S^o was added. In detailed studies of 11 soils from three major soil groups, namely gley, organic and yellow-brown earth (YBE), the oxidation of S^o was most rapid between 0 and 6 weeks, after which the oxidation rate decreased. *Thiobacilli* numbers on the gley and organic soils were similar although the amount of S oxidised in the gley soil was less than most of the other soils. This may indicate a higher population of hetertrophic oxidisers in the organic soil than the gley.

ii) Field studies:

In field studies (Lee *et al.*, 1987, 1988b) on a S deficient yellow brown pumice soil (YBPS) under a ryegrass dominant sward (North Island) and, on another site, on the ryegrass-clover pasture of a yellow-grey earth/yellow-brown earth intergrade (YGE/YBE; South Island of cool temperature environment), the average *thiobacilli* numbers were <10 *thiobacilli* g^{-1} soil which is consistent with the population observed in the incubation study using the 48 North Island soils. At most of the sampling times the *thiobacilli* numbers were higher in S^o treated than untreated plots. The *thiobacilli* population fluctuated with season. There was no consistent relationship between

reapplication of S^0 or the amount of residual S^0 in the soil and the *thiobacilli* population.

It was concluded that *thiobacilli* numbers in the soil were affected by increases in the availability of S^0, the particle size (surface area) of S^0 exposed and possibly the soil moisture and temperature conditions. However, the initial numbers of *thiobacilli* in a soil were a poor indicator of the rate of S oxidation (Lee *et al.*, 1987).

The small *thiobacilli* numbers found in most of the studies may result from the small soil samples taken, may not have provided a representative soil sample and may underestimate the importance of the autotrophic sulphur bacteria. Possibly the Most Probable Number (MPN) method used to determine *thiobacilli* numbers did not detect low populations of *thiobacilli* since only one g of soil was used for each determination (Shedley, 1982). However, there was no information available on the spatial distribution of the microbial population in the soil. There was a large variation between soils in the size of the inoculation effect, depending on the type of oxidising organisms initially present in the soil (Kittams and Attoe, 1965). The time span over which each soil's S^0 oxidising ability was examined was, as in most other incubation experiments, very short in agronomic terms (Shedley, 1982). However, the ability of *T. thiooxidans* populations to respond rapidly to changes in substrate concentration, indicates that, where present, would be very important S^0 oxidisers.

Although the *thiobacilli* population increases rapidly on the addition of S^0 to the soil and is generally considered responsible for the oxidation occurring. It can be argued that even after the dramatic increase in number of *thiobacilli* their numbers may only reach those normally found for herterotrophic bacteria and these also often increase in number in response to S addition (Wainwright, 1984)

There have been relatively few studies of *thiobacilli* numbers in field soils. Most field studies of *thiobacilli* have involved surveys of polluted soils from mine spoils or near industrialised cities (Wainwright 1979; Olson *et at.*, 1981). Germida, (1985) found that soils that had been subjected over a period of time to excessive applications of S^0, and that had a pH ranging from 1.5-3.5, contained a considerable number of autotrophic *thiobacilli* and he suggested that these *thiobacilli* only became dominant after considerable S oxidation had occurred and the soil pH had decreased to a low level (i.e. pH = 3.4).

2.4.2 Heterotroph Numbers in New Zealand Soil

Lee *et al.* (1987), in the same incubation study mentioned above, found that at all sampling dates, there were no changes in numbers of S oxidising heterotrophic bacteria between control, S^0 and S^0/PR treatments, presumably due to a limitation imposed by a lack of oxidisable organic C since only S^0 was added. This result was consistent with the results of Lawrence *et al.* (1988); Gupta *et al.* (1988). Lee *et al.* (1987) observed a small decrease in the numbers of S oxidising heterotrophic bacteria in the S^0 treatment but the number did not significantly change in the S^0/PR treatment. Possibly the PR/S^0 treatment allowed more S-oxidising heterotrophic bacteria to survive because neutralisation of the product H^+ by PR may have reduced acidic soil conditions.

It was found that the initial heterotrophic population before S addition was the best estimator of S^0 oxidation in these soils. However, Lee *et al.* (1987) concluded that the role of heterophic S oxidisers in New Zealand soil is unclear as their number did not change when S was added. On the other hand, increases in the numbers of heterotrophic S oxidisers were found in the repeated S^0 application in the field studies of Germida, (1985) but this did not increase the oxidative capacity of the soil. In contrast the results of Kittams and Attoe (1969) showed increase in the amounts of S oxidised from 23 to 54% and were related to an increase in the population of unspecified S oxidisers. However, results from Germida *et al.* (1988) and Vitolins and Swaby (1969) would suggest that such an increase in oxidative capacity is unlikely unless a significant population of *thiobacilli* were present in those soils.

It appears that both heterotrophs and chemolithotrophs may be equally important in S oxidiser in soils. Evidence for the greater in importance of either chemolithotrophs and heterotrophs in New Zealand pasture soils is inconclusive.

2.5 AGRONOMIC EFFECTIVENESS OF S^o IN NEW ZEALAND PASTURE

Elemental S has proven to be an effective fertilizer for legume based pastures provided that the correct particle size range is chosen with respect to a particular climatic zone. The recommendations for S^o use (Table 2.2.A) are mainly based on research field trials (MAFtech) summarised by Sinclair *et al.*, 1985; Boswell and Swanney, 1986, 1988; Lee and Boswell (1988) and Swanney *et al.*, (1988). Boswell and Swanney (1988) have extended these recommendations to three climatic zones (Appendix 2.1) and to an application strategy every 4 years (Table 2.2.B).

Table 2.1 Recommended S^o particle size ranges (μm) for maximum agronomic effectiveness for New Zealand pasture (A) by Sinclair *et al.* (1985) and the extended recommendation (B) by Boswell and Swanney (1988).

A.

Application	Climatic zone (Temperature) Cool	Warm
		mm
Annual	<0.15	<0.25 (50% < 0.15)
Biannual	<0.25	<0.5 (50% < 0.25)

B.

Application	Climatic zone 1	2	3
Annual	<0.15	<0.25	<0.25
Biannual	<0.25 (50%<0.15)	<0.50 (50%<0.25)	<0.50 (50%<0.25)
4 years	<0.50 (25%<0.15, 50%<0.25)	<1.0 (25%<0.25, 50%<0.5)	<1.0 (25%<0.25, 50%<0.5)

In the past, sulphur deficiencies have been alleviated by the extensive use of single superphosphate to correct P deficiencies (During, 1984). Sulphurized superphosphate has been used in areas where extensive leaching of soil sulphate occurs or in areas where P deficiency does not exist and the main nutrient limiting legume growth is sulphur (During, 1984). Since 1985, in an effort to reduce fertilizer transport and application costs in New Zealand, there has been a trend towards increased usage of high analysis P and S fertilizers. With S, interest has centred on S^0, the ultimate high analysis S fertilizer. Further, when added to high analysis P fertilizers at the 1:1, S:P ratio, S^0 does not significantly reduced the P content of the fertilizer.

To be effective S^0 fertilizers must be applied in finely divided particles in order to be readily oxidised to supply sulphate-S at a rate which satisfies plant requirements (Sinclair *et al.*, 1985). However, these particle sizes create difficulty in spreading due to their explosive nature (Rothbaum *et al.*, 1983).

To overcome the above limitation of powdered S^0, technology has been developed to combine molten S^0 with high analysis P fertilizer e.g. sulphurized reactive phosphate rock (S^0/RPR) and sulphurized partially acidulated phosphate rock (S^0/PAPR). This method of S^0 addition can produce finely divided S^0 in these compound fertilizers, part of which approximately meets the particle size range recommendation by MAFTech, New Zealand. Often, however, a large percentage of the S^0 remains in a coarse form not suitable for short term agronomic use (Boswell, 1987; Bolan *et al.*, 1988).

The evaluation of experimental mixtures of S^0 and TSP and also S^0/PAPR (NCPR) has also highlighted the importance of the disintegration characteristics of the granules and the method of application of these fertilizers (Boswell, 1987).

Already considerable amounts of S^0 are added to New Zealand pasture in these fertilizer forms, when really little is known about how these fertilizer forms influence the rate of S^0 oxidation and SO_4^{2-} release.

Some work has been done on the effect of RPR on S^0 oxidation in incubated soils (Attoe and Olsen, 1966; Lee *et al.*, 1987). The effect of S^0 oxidation increasing PR dissolution has been measured in glasshouse and field soils (Rajan, 1983, 1987; Rajan and Gillingham, 1986; Friesen, *et al.*, 1987) but S^0 oxidation rates were not measured.

It appears therefore, that methods are required firstly to determine the particle size of S^o in a range of S^o containing fertilizer materials and secondly to determine if other characteristics of the fertilizers influence the rate of S^o oxidation. Such information would be valuable for use in assessing the agronomic value of current and new S^o fertilizer forms.

2.6 REVIEW OF METHODS FOR MEASURING S^o OXIDATION

Various methods have been used to measure the rate and extent of S^o oxidation. These include: (i) a decrease in soil pH; (ii) increases in soil SO_4-S levels and (iii) decreases in residual S^o.

2.6.1 Measurement of pH Change

Sulphuric acid is produced during the oxidation of S^o and as a result, soil will become more acidic:

$$2S^o + 3O_2 + 2H_2O \longrightarrow 2H_2SO_4 \qquad (2.7)$$

For 1 mole of S^o oxidised 1 mole of H_2SO_4 (or 2 mole of H^+) will be produced. The decreased pH in a sandy loam soil was found to be highly correlated with the amount of S^o oxidised (Li and Caldwell, 1966). Changes in soil pH, however, provide a useful index of S^o oxidation only in incubation studies where soils with low pH buffering capacities are used and when the product SO_4 ions are not reimmobilised (i.e. being reduced into organic sulphur compounds by soil microorganisms). The reduction of sulphate ions will consume H^+ ions as shown by De Vries and Breeuwsma (1987).

$$ROH + SO_4^{2-} + 2H^+ + 2CH_2O \longrightarrow RSH + 2CO_2 + 3H_2O \qquad (2.8)$$

Normal field application rates of 30 kg S^o ha^{-1}, equivalent to 66 μg S^o g^{-1} soil, (this assumes the top 5 cm of soil has a bulk density of 900 kg m^{-3}) would produce only 4 μmole H^+ g^{-1} if fully oxidised and the changes in bulk soil pH caused by one application would not be detectable for most New Zealand soils which may have soil

pH buffering powers ranging from 30-100 μmoles H^+ per unit pH change (Bolan *et al.*, 1990). Furthermore, in long term field studies, H^+ can be lost or gained by mechanisms other than S^o oxidation such as the microbial oxidation of soil organic N and C, and plant induced processes (De Vries and Breeuwsma, 1987), denitrification (Pierre *et al.*, 1971), rainfall and drainage.

In incubation studies where research workers have added between 60 to 300 times this quantity (1000 to 1600 $\mu g\ g^{-1}$) pH changes have been detectable (Li and Caldwell, 1966; Rajan and Edge, 1980; Lee *et al.*, 1987). The very high application rates of S^o may reduce soil pH and the rate of S^o oxidation may be reduced also due to the depression of heterotrophic activity at low pH levels (Bryant *et al.*, 1979; Bewley and Parkinson 1984; Lawrence and Germida 1988).

It must be concluded that measuring change in soil pH is not a reliable estimate of the extent of S^o oxidation because other H^+ generating and buffering mechanisms operate in soil. Furthermore at normal field application rates pH changes in bulk soil would not be detectable.

2.6.2 Measurement of Sulphate Production

The amount of sulphate produced has widely been used to measure the rate and extent of S^o oxidation (Li and Caldwell, 1966; Bloomfield, 1967; Keller, 1969; Vitolins and Swaby, 1969; Wainwright, 1978). Janzen and Bettany (1987b) appear to have used this technique successfully in short term (6 days) laboratory incubations in the absence of plants. In pot or field studies, however, plant uptake of the SO_4 oxidised, and SO_4 immobilisation in response to root carbon released into the rhizosphere would make quantitative measurement of the total amount of SO_4 release impossible (Till, 1975; Goh and Gregg, 1982). Sulphate produced by S^o oxidation can be rapidly immobilised when organic matter is present (Wainwright *et al.*, 1986a) because of the rapid growth and a consequent increase in assimilation of sulphate by microorganisms.

The removal of SO_4-S by plant uptake, leaching or immobilization makes the quantitative measurement of the total amount of SO_4-S produced through S^o oxidation difficult.

2.6.3 Measure of Residual S^o

The actual amount and rate of oxidation at any one time following S^o application can be determined most reliably by direct measurement of residual S^o in soils. In general, the method of S^o determination is a two-stage process, involving firstly extraction of elemental sulphur and secondly analytical assay of elemental sulphur in the extract.

2.6.3.1 *Extraction of S^o*

Various solvents have been used to extract S^o including benzene (Chopra, 1963), carbon disulphide (Flierman and Brock, 1973), chloroform (Barrow, 1968; Watkinson *et al.*, 1987) and acetone (Hart, 1961; Shedley, 1982; Nguyen, 1988). Benzene and carbon disulphide are too hazardous for routine use and will not be considered in this review.

The efficiency with which chloroform and acetone extract S^o from soils is affected by the soil moisture content. Chloroform was recommended as a suitable extractant for S^o from soils which have a moisture content equal or less than field capacity (Barrow, 1968; 1970). However, extraction was incomplete for soils with moisture contents within the plastic range, due to the water-immiscible nature of chloroform, which makes it unable to penetrate the plastic soil aggregates. Watkinson *et al.* (1987), however, demonstrated that for New Zealand soils with moisture levels above and below the plastic range, the soil dispersed well in the chloroform extract, permitting dissolution of S^o.

Although chloroform is suitable for extracting commercial grade S^o which consists of up to 99% S_8 (Lauren and Watkinson 1985). Barrow (1968) found that sublimed S^o and polymeric amorphous S^o had low solubility in chloroform (7 mg l^{-1}). The latter form which is produced through rapid cooling can be present in S^o produced at natural gas plants by the Stretford process (Maynard and Addison, 1985). It can also form 5% of fertilizer grade S^o (Ludwig and Dale, 1973).

Acetone was originally used by Hart (1961) to extract S^o from tidal mangrove soils. Since S^o is much less soluble in water/acetone mixtures than acetone alone, it is less efficient in extracting S^o from soil which has a moisture content over 75% of field capacity (Barrow, 1970; Nguyen, 1988).

The advantage of acetone is that different forms of S^o including sublimed, polymeric amorphous S^o and S^o produced at natural gas plant are soluble in acetone (Maynard and Addison, 1985). Acetone may also dissolve some forms of organic sulphur (e.g. some sulpholipids) but only small quantities are present in soil (0.5-4.8 $\mu g\ g^{-1}$ soil) and it is not necessary to pre-extract organic S from the soil prior to S^o extraction with acetone (Nguyen, 1988).

Acetone has been found to be an effective solvent for extracting S^o from soil both in pot and field experiments (Shedley, 1982; Nguyen, 1988).

2.6.3.2 *Analytical assay*

S^o extracted from soil by various solvents can be measured by several methods some of which have been reviewed recently by Watkinson *et al.* (1987) and Nguyen (1988).

A. *Colorimetric method*

A number of colorimetric methods have been used for S^o assay. Ory *et al.* (1957) used a method involving production of a blue colour through the reaction between S^o and Schoenberg's reagent (N-(4,4-dimethoxybenzohydrilidine) benzylamine). The method appears unnecessarily complicated, and is not sensitive to soil samples containing low content of S^o (soil extract should contain >40 $\mu g\ S^o$) and gives erroneous results if soil are rich in organic matter (Chopra 1963)

Bartlett and Skoog (1954) and Fliermans and Brocks (1973) used sodium cyanide to form thiocyanate with the S^o in the acetone or C_2S extracts which is estimated colorimetrically using ferric chloride, the method was claimed to be sensitive at concentrations as low as 8 $\mu g\ g^{-1}$ soil. However, this method is not suitable for routine use because of the toxic chemical involved.

Chopra (1963) reduced S^o in the extract to hydrogen sulphide with Raney nickel under alkaline conditions, hydrogen sulphide is then released from the nickel sulphide with H_2SO_4 and determined as methylene blue. The method can be sensitive at S^o concentrations as low as 5 $\mu g\ g^{-1}$ soil, however in this range, it is necessary to remove organic matter before soil is extracted, and the use of H_2O_2 to remove organic matter from the soil is an undesirable step because of the possible oxidation of organic soil S.

Barrow (1968) investigated different reducing procedures and suggested that S^o can be reduced to H_2S by stannous chloride, by iron powder and HCl and by tin and HCl. The reduction techniques were not specific to S^o and a few compounds which were soluble in chloroform were also reduced. The hydrogen sulphide was then absorbed in sodium hydroxide solution and assayed by titration against mercuric chloride, using dithizone as an indicator. The titration step is considerably time consuming and the method is not routinely used. If the titration method is replaced by Dean's (1966) colorimetric finish, then the tin/HCl reduction using a modified Johnson and Nishita apparatus appears to be the most suitable of the colorimetric methods and can be automated relatively easily (CSIRO Division of Forest Research, Method No. PS17).

B. *Chromatography*

Gas chromatography of S^o in solvent extracts of marine sediments is unsatisfactory because S^o does not chromatograph homogeneously (Struble, 1972; Chen *et al.*, 1973) and the linear range of detectors is very small. Heim *et al.* (1984) reduced the S^o to hydrogen sulphide before chromatography but method is lengthy.

C. *High-performance liquid chromatography*

Elemental S^o can be extracted into chloroform and measured by high-performance liquid chromatography (HPLC) (Watkinson *et al.*, 1987), the method is accurate and precise down to a detection limit of 0.1 mg S kg^{-1} soil.

2.7 SUMMARY OF LITERATURE REVIEW AND RESEARCH OBJECTIVE

All soils, unless present in an extremely arid or cold environment, appear to possess microorganisms with ability to oxidise S^o. Studies to determine the relationship between soil organism numbers and the extent of long term S^o oxidation appear inconclusive but it is clear that S^o oxidation is carried out by both chemolithotrophs and heterotrophs in most soils. The oxidation process is a surficial reaction and can be limited by the ability of an organism to attach to a S^o surface. Considerable information is available showing that the pure S^o oxidation rates are primarily a

function of S^0 particle size (surface area of S^0 exposed) and are also influenced to some extent, by soil characteristics, mainly soil moisture and temperature, with a few researchers indicating various effects of different soil types.

Pasture field trials conducted in New Zealand have provided recommendations for specific particle sizes of the S^0 to be used in different climatic zones. Little information is available on the effects of fertilizer form on the rate of S^0 oxidation. As S^0 is now applied to New Zealand soils in various forms such information would be valuable for assessing the agronomic suitability of a S^0 fertilizer.

Measurement of residual S^0 in soils appears to be the only method suitable for obtaining accurate estimates of S^0 oxidation rates in both field and laboratory studies. Provided care is taken preparing representative samples, extraction with acetone is simple and the determination of S^0 concentration in the acetone or chloroform extracts by colorimetric assay or HPLC, respectively is readily achieved.

The above review indicates that future research is required in the following areas in order to refine agronomic recommendations for S^0 use.

i, Routine methods are required for determining the S^0 particle size analysis in a range of S^0 containing fertilizers.

ii, An index of a soils potential to oxidise S^0 is required so that the S^0 oxidation potential in particular climatic zones can be defined. This could be achieved by examining more closely the physical, chemical and biological factors influencing the oxidation rate of S^0 in soil.

iii, Information is required on the influence of fertilizer form and placement in soils on S^0 oxidation.

This thesis reports a series of studies which embrace all three aspects listed above.

CHAPTER 3

EVALUATION OF METHODS FOR MEASURING THE RATE OF ELEMENTAL SULPHUR OXIDATION IN SOILS

3.1 INTRODUCTION

The use of elemental sulphur (S^0) fertilizers on New Zealand pastures is likely to increase (Sinclair *et al.*, 1985). With suitable choice of particle size and the amount and timing of application, the oxidation rate of applied S^0 to sulphate can be made to match closely the plant requirements. Investigations of the effectiveness of S^0 fertilizers requires that their rate of oxidation in soil be determined. Various methods have been used to measure the rate and extent of S^0 oxidation and these were briefly reviewed in Section 2.6. There has been no published study, however, in which these methods of measuring the extent of S^0 oxidation have been compared.

No soils in New Zealand have been found to be devoid of S^0 oxidising ability (Sinclair *et al.*, 1985), but rates of oxidation vary considerably (Watkinson, 1989). S^0 oxidation in soil is microbially mediated, and therefore the rate is affected by climate and the characteristics of both soil and fertilizers. A reliable indicator of the S^0 oxidising potential of a soil would assist in making decisions as to the suitability of S^0 as an effective S source for pasture grown under different soil and climatic conditions. There appears to be a paucity of information available concerning methods for rapidly ranking the S^0 oxidation potential of soils. It has been shown that rhodanese enzyme activity (RA) is involved in S^0 oxidation (review of RA is covered more fully in Section 2.3.10). Its activity can be easily measured and it may provide a rapid method suitable for assessing the S^0 oxidation potential of soils.

3.2 OBJECTIVES

The objectives of the experiments described in this chapter are to evaluate:

i) a technique suitable for the direct measurement of residual S^0 in the soil and to investigate some factors affecting the accuracy of the technique.

ii) the possibility of using RA as an index of soil S^0 oxidation potential and

iii) to compare methods suitable for measuring the rate and extent of S^o oxidation in a range of soils.

3.3 MATERIALS AND METHODS

3.3.1 Soils

3.3.1.1 *Incubation study*

Surface (0-5 cm) soil samples, were collected from 6 pasture sites, representing different soil types and different fertilizer histories as shown in Table 3.1. Field moist soils were sieved to < 2 mm and stored at -4°C.

Table 3.1 Fertilizer history of soil samples used in the incubation study.

Soil type	Code	Previous fertilizer history	Initial RA nmole SCN^{-1} g^{-1} soil h^{-1}
Tokomaru silt loam (Typic Fragiaqualf)	1.	control, no fertilizer over past 5 years	167
	2.	Sechura[a] + S^o at 20,20,10,20,20 kg S ha^{-1} yr^{-1} for previous 5 years.	819
	3.	Elemental S at 30 kg ha^{-1} for past 2 years.	387
Makotuku fine sandy loam (Aquic Dystrochrept)	4.	control, no fertilizer application.	1328
	5.	Elemental S at 30 kg ha^{-1} for past 2 years.	1536
Egmont black loam (Entic Dystrandept)	6.	1983, 350 kg ha^{-1} 30% potassic-superphosphate (28 kg S ha^{-1}); 1984/85, 300 kg ha^{-1} 30% potassic-superphosphate (33 kg S ha^{-1}).	586

a Chemical characteristics of these soil are described in Table 6.1. Tokomaru soil are described more fully in Chapter 7. The site and chemical characteristics of
b Egmont black loam are described in William, 1988.
Peruvian reactive phosphate rock, 13% P

3.3.1.2 *Pot experiment*

Makotuku and Tokomaru soils which contrasted in their initial RA, measured in the preliminary incubation (Section 3.3.3.1), were chosen to represent soils with potentially high and low S^o oxidising capacity. The field sites were revisited and fresh samples of Makotuku fine sandy loam (0-3 cm; no.4 Table 3.1) with a high level of initial RA (1326 nmole SCN g^{-1} h^{-1}) and samples of Tokomaru silt loam (3-5 cm; no.1 Table 3.1) with a low level of initial RA (168 nmole SCN g^{-1} h^{-1}) were sampled, sieved to <2 mm and used in the pot experiment.

3.3.2 S^o Fertilizer

Agricultural grade, Canadian bright sulphur with the particle size distribution shown in Table 3.2 was used in all incubation and pot experiments discussed in this chapter.

Table 3.2 The particle size distribution of the S^o fertilizer used.

	sieve size (μm)		
	250-150	150-75	<75
	(% by weight)		
Agr.grade S^o	13	40	47

3.3.3 Experimental Procedure

3.3.3.1 *Incubation study*

For measuring the rate of S^o oxidation, agricultural grade S^o was added in the form of fine particles (Table 3.2) at the rate of 190 μg S^o g^{-1} soil. Duplicate samples of each soil were also prepared without the addition of S^o. The soils were moistened to 'field capacity' in a plastic bag and incubated at room temperatures (20°C) for 60 days.

Subsamples of the soil were removed at fortnightly intervals to measure soil pH, rhodanese enzyme activity, 0.04 M $Ca(H_2PO_4)_2$ extractable SO_4-S and the amount of residual S^o remaining in the soil.

3.3.3.2 *Glasshouse trial, pot experiment and incubation*

In a separate glasshouse trial, the use of RA as an index of soil S^o oxidation potential and the effect of plants on RA and the rate of S^o oxidation were examined. Each pot contained 480 g of field moist, sieved (<2 mm particle size) soil. The soil was separated by nylon bolting (1 mm mesh size) into 3 layers. For the middle layer which contained the fertilizer treatment, 40 g of soil was spread over the nylon bolting, then 40 g of soil, premixed with the fertilizer S^o at 120 μg S g^{-1} dry soil, was added followed by a third lot of 40 g soil (Figure 3.1). All fertilizers were added to the soil in a granulated form (granule size 0.2-0.5 mm) and where necessary the Tokomaru soil was used as a filler to obtain the same concentration of S^o per granule and the same number of granules per treatment (see Section 6.2.3 for the granulation method and Table 6.4 for a list of fertilizers). The top and bottom layers contained 120 g and 240 g soil, respectively. The nylon bolting mesh served to identify the fertilized soil zone for easy recovery and analysis of the S^o remaining in the soil. Additional fertilizer treatments were added and results from these treatments are discussed in Chapter 6. In one set of pots, white clover (*Trifolium repens* cv Huia) seeds were sown with a rhizobia inoculum. After germination of the seeds, the plants were thinned to approximately 30 plants per pot. The remaining set of the pots remained unseeded.

The pots were kept under glasshouse conditions, at a maximum temperature of 24°C, and a minimum of 17°C. Artificial lighting was provided until the plants were 10 days old. The pots were watered regularly to 'pot capacity' by applying water to the saucer below the pot to meet a previously determined goal weight. The goal weight was determined by recording the weight of a pot plus wet soil which had been saturated with water for 2 days and then allowed to drain for 48 hours. A nutrient solution (Middleton and Toxopeus, 1973) was added to provide K, Mg and Ca fortnightly. The frequency of watering and nutrient solution addition was kept the same for pots with and without plants.

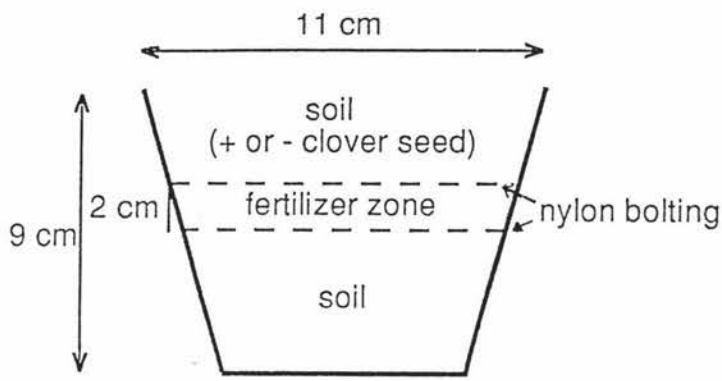


Figure 3.1 The layering of soil and fertilized soil in the glasshouse trial.

The pots were completely randomised and re-positioned every week to minimize any effects of uneven environmental factors such as light and temperature. At fortnightly intervals one pot per treatment was destructively sampled until a final harvest which was at 98 days after seeding. A total of 128 pots were used for each soil. At each harvest all plants were also cut to a level of 2 cm above the soil surface for the measurement of dry matter yield. The three layers of soil were separated and the bottom layer discarded. The top layer of soil, which contained the major portion of plant roots, was used for RA analysis. The soil from the fertilized middle layer was air dried and analysed for S^0 and SO_4-S.

3.3.4 Soil Analysis

3.3.4.1 *Elemental S*

The soil samples were ground using a coffee grinder to give an uniform subsample and analysed for S^0 as described in Section 3.4.1.9 (see below).

3.3.4.2 *Sulphate analysis*

Sulphate produced was extracted from the soil as described by Blakemore *et al.*, (1981). Soil samples (5 g), air dried and ground (<2 mm), were weighed into a 250 ml centrifuge tube and 25 ml of the extracting solution (0.04 M $Ca(H_2PO_4)_2$, pH 4.0) added. The mixture was shaken for 16 hours on an end-over-end mechanical shaker, before being centrifuged at 8000 rpm for 10 minutes in a Sorvall RC5C centrifuge using an SS-34 head. An appropriate aliquot of extract was transferred into a digestion tube and evaporated to dryness in an oven at 60°C. The sulphate content was determined by reducing it to hydrogen sulphide at 120°C with a strong reducing mixture (containing hydroiodic acid, hypophosphoric acid and formic acid, ratio 9:1:3) using a modified Johnson and Nishita (1952) apparatus. The hydrogen sulphide evolved was trapped in the Na/Zn-acetate solution and analysed as described in Section 3.4.1.9. Later SO_4 concentrations in extracts were measured by the autoanalyser method (CSIRO Division of Forest Research, Method No PS17).

In this thesis S extracted by Ca-P and measured by Johnson and Nishita analysis is called 'extractable SO_4-S and will include a small amount of organically bound SO_4.

3.3.4.3 *pH*

Soil pH was measured in distilled water at soil:solution ratio of 1:2.5, after a 24 h equilibrium period using a combination glass electrode and a Radiometer PHM 82 pH meter.

3.3.4.4 *Rhodanese enzyme (RA) assay*

Soil rhodanese enzyme activity was determined according to the method of Tabatabai and Singh (1976) which utilizes the reaction described in Chapter 2 section 2.3.10 and summarized in Equation 3.1. Four grams of soil were weighed into a 50 ml flask. Toluene (0.5 ml), 8 ml of Tham buffer at pH 6.0 and 1 ml each of $Na_2S_2O_3$ and KCN (in Tham buffer) were added and the flask was incubated for 1 hour at 37°C in a water bath. After 1 hour 10 ml of $CaSO_4$-formaldehyde solution was added. Formaldehyde inhibits further rhodanese activity, and prevents formation of a blue Fe-S_2O_3 complex. After standing the mixture for 20-30 minutes, the solution was filtered and 5 ml of the filtrate was pipetted into a test tube and one ml of ferric nitrate reagent added. This forms a yellow/brown ferrithiocyanate complex which was measured colorimetrically at 420 nm on a Pye Uricam SP8-300 spectrophotometer using 1 cm glass cuvettes.

$$\begin{array}{l} \qquad\qquad\quad CN \qquad\qquad\quad SCN^- + Fe \longrightarrow FeSCN \\ \qquad\qquad\quad \overset{\curvearrowright}{\qquad\quad Rhodanese \qquad\quad} \\ S^o \longrightarrow S_2O_3^{2-} \longrightarrow SO_3^{2-} \longrightarrow SO_4^{2-} \end{array} \qquad (3.1)$$

3.3.4.5 *Root length and rhizosphere soil*

The length of a subsample of roots (l) was determined by the method of Marsh (1971). The roots occupying the top layer of the soil were removed and washed. A subsample was floated on a grid of squares 1.5 cm wide. The number of times the roots intercepted the grid lines was counted. The subsamples were dried and weighed. The The subsample root length (l) and total root length (L) was calculated from the following relationships:

$$l = 11/14 \text{ N x A}$$

where l is the total length of root, N is the number of intersections between the root and counting grid, A is the width (cm) of a single grid square.

$$L = l \text{ x total dry wt of roots / dry wt of root subsample}$$

The rhizosphere soil or the effective zone of soil exploitation by the root is defined to be a cylinder along the length of the root of a certain radius. The volume of rhizosphere soil of this experiment was estimated from the average root length and the assumption that the rhizosphere extended to 1 mm either side of the root (Russell, 1976). However, in practice the rhizosphere soil was sampled from soil that remained adhering to roots after a gentle shaking.

3.4 RESULTS AND DISCUSSION

3.4.1 Evaluation of a Technique for Measuring the S^o Content of Soil Samples

3.4.1.1 *The proposed method*

Various organic solvents such as acetone, chloroform and carbon tetrachloride (CCl_4) have been used to extract S^o from soil samples (see Chapter 2 section 2.6.3.1). In the present study acetone was evaluated as the solvent suitable for the extraction of S^o from soil samples because of its relatively low cost and lack of poisonous qualities. Factors influencing the suitability of acetone as a extractant of S^o from soils were discussed in Chapter 2 section 2.6.3.1.

The S^o concentration in the acetone extract is determined by using a combination of reduction and colorimetric finish procedures described in part by Johnson and Nishita (1952) and Chopra (1963) and modified by Nguyen (1988). The following preliminary procedure was adopted for the determination of S^o in soil. Air-dried or moist soil of <2 mm particle size is shaken with acetone at a soil:solution ratio of 1:5 for 1 hour.

Duplicate aliquots (1-5 ml) of the acetone extracts were placed in distillation tubes and evaporated to near dryness at room temperature. Acetone (0.2 ml), tin powder (0.2 g) and hydrochloric acid (4 ml, 1:1 HCl:H_2O) are then added to each digestion tube and the tubes were quickly connected to a modified Johnson-Nishita apparatus. After reduction/distillation for 30 minutes at (40-60°C), the evolved hydrogen sulphide was collected in Na/Zn-acetate and its concentration determined using the colorimetric method of Johnson and Nishita (1952). The absorbance of the resulting solution was measured at a wavelength of 670 nm using a Pye Unicam SP8-300 spectrophotometer and 1 cm glass cuvettes. The proposed method is based on methods described by Barrow (1968) and Nguyen (1988). The suitability of the above method for measuring S^o remaining in soil was evaluated and is discussed below.

3.4.1.2 *Solubility of S^o fertilizers in acetone*

Solubility of S^o in pure acetone on a weight per volume basis is less (20.84 mg S^o ml^{-1}) than that in toluene (36.32 mg S^o ml^{-1}) at 25°C (Linke, 1958). Toluene, however, is the more expensive solvent and is less easily evaporated prior to the assay of S^o in a Johnson and Nishita apparatus (Johnson and Nishita, 1952). There is some advantage then, if acetone can be used as the extractant. It has been reported, however, that the solubility of S^o in acetone varies depending on S^o fertilizer form (Shedley, 1982). For this reason the solubilities of a range of commonly available S^o fertilizers in New Zealand were tested in acetone and toluene. The S^o:solvent ratio was maintained at 200 μg S^o ml^{-1} solvent, well below the published solubility limits. After extraction the solvents were analysed for S^o content and the results are presented in Table 3.3. There was no significant difference between the two extractants in the recovery of S^o from the fertilizer samples. The different S^o forms were completely dissolved at the concentration of 200 μg S^o ml^{-1}.

Similarly, Shedley (1982) obtained maximum dissolution of different forms of S^o namely, flowers, crushed, and sulphur recrystallized in acetone at concentrations ranging from 110-182 μg S^o ml^{-1}. However, with pure S^o of particle size <104 μm up to 1000 μg S^o ml^{-1} acetone can be achieved (Nguyen, 1988).

Table 3.3 S^o content (% w/w) of different S^o forms measured by extracting with either toluene or acetone.

S^o form[a]	Code	S^o content (% w/w) toluene		acetone	
Dark prill	K	98.1	±1.76[b]	102.9	±1.01
Prill S^o rolled	L	100.0	±1.62	101.9	±1.36
S^o mini prill	-	88.1	±1.29	91.0	±1.20
Tiger 90	O	91.3	±1.34	92.2	±1.64
Sulphur 85 prill	-	81.4	±1.53	82.2	±1.36
Ag.Grade S^o	B	100.0	±1.52	101.3	±1.67
Rotokawa ore	P	65.7	±1.32	64.5	±1.94

a see Table 5.1 in Chapter 5 for fertilizer characteristics.
b standard deviation.

The solubility of S^o in acetone varies considerably depending on the purity of acetone. The purity of acetone used in this thesis was 95% by wt (Shell chemicals, specific gravity of 0.791 g cm^{-3}). In the presence of 10% water only half of the S^o (10.58 mg ml^{-1}) is dissolved compared to 20.84 mg ml^{-1} at 100% acetone, Table 3.4. If moist soil containing S^o is extracted, sufficient acetone should be added to give a final concentration of S^o in the acetone extract of less than 200 µg S^o ml^{-1}. At this acetone:S^o ratio the possible dilution of acetone by soil moisture should not effect the efficiency of extraction.

Table 3.4 Solubility of elemental sulphur in aqueous acetone at 25°C (Linke, 1958).

% acetone in solvent(wt/wt)	Sp.Gr of solution	Solubility (mg ml^{-1})
100.00	0.7854	20.84
95.36	0.7911	14.42
90.62	0.8165	10.58
85.38	0.8295	8.11

3.4.1.3 *Selectivity of acetone to S^o*

Extraction of moist soil with acetone may remove S forms other than S^o, especially organic ester S or SO_4-S. Also moist acetone may extract other ions such as NO_3^- which may interfere with the colour development during S^o measurement. To examine these possibilities the following experiment was conducted.

Replicated 20 g samples of air dried Tokomaru soil, which had received radioactive $^{35}SO_4$-S labelled gypsum and superphosphate fertilizers (specific activity of 340 KBq mg^{-1} S) at the rate of 30 kg S ha^{-1} and had been incubated under field conditions for 60 days (S. Phimsarn, Ph.D studies. Massey University), were extracted with 100 ml of acetone for 16 hours. Then 1 ml of the extract was counted in liquid scintillation system (For details of the liquid scintillation system see Chapter 7 section 7.3.7. S^o in the extract was measured using a mild reducing agent which reduces only S^o (tin/HCl) and a strong reducing agent (HI) which reduces both S^o and SO_4-S.

Although considerable ^{35}S (approximately 0.8 KBq ml^{-1}) activity was measured in 0.04 M CaH_2PO_4 extracts (S. Phimsarn, pers. comm.) no ^{35}S activity could be detected in acetone extracts indicating that sulphate was not recovered in acetone. Further only negligible amounts of S were measured by both the reducing agents (The selectivity of tin/HCl reduction to S^o is discussed later in Section 3.4.1.5). Of the six different surface soil samples (Section 3.3.1.1) incubated with S^o, the background interference (i.e. the contribution to H_2S recovered from the tin/HCl reduction of the acetone extract from soil with no S^o added) ranged from 0-3 μg S^o g^{-1} soil. The contribution of acetone extractable S from plant root material in these soil samples therefore appears to be small and does not confound the S^o measurement.

It would appear that acetone extraction of soil samples containing plant material can be used to selectively recover S^o from soil containing quantities greater than 3 μg S^o g^{-1} soil.

3.4.1.4 *Effect of particle size and time of extraction on S^o solubility*

The amount of S^o dissolved in acetone also depends on the particle size of the S^o, the S^o:solution ratio and the time of shaking. The complete dissolution of S^o, when shaking with acetone, can take from five minutes to several hours, probably due to difference in S^o particle size (Hart, 1961; Shedley, 1982). Nguyen (1988) was unable to fully dissolve S^o particles of 1-2 mm in diameter in acetone even after 7 hours of shaking which maintained a S^o concentration of up to 1 mg ml^{-1}. The extraction of coarse particle size S^o (>2 mm diameter e.g. Dark S prill (K) and Tiger 90 (O)) in section 3.4.1.2 was complete but the concentration of the commercially available S^o fertilizers did not exceed about 0.2 mg ml^{-1} of acetone.

The effect of time on the dissolution of S^o of different particle size in acetone was investigated as follows. Replicate samples of about 30 mg Agr.grade S^o, in five particle size ranges (1-2, 1-0.5, 0.5-0.25, 0.25-0.150 and 0.150-0.075 mm) were shaken with 200 ml of acetone for 8, 16 and 24 hours. Aliquots (0.2 ml, 150 $\mu g\ ml^{-1}$) were taken and analysed for S^o by the method described above. The results (Table 3.5) indicate that there was no effect of particle size at any shaking time, This indicated that most of the S^o was completely dissolved in a shaking period of 8 hours. which is consistent with previous results (Section 3.4.1.2). However, there was a slight but significant increase ($P < 0.05$) in the recovery of S^o at the longest shaking time (24 hours) for the coarser particle size ranges. This suggests that to dissolve S^o of particle size >0.25 mm diameter a shaking period of 16 hours is required.

Table 3.5 Recovery of S^o in acetone extracts from S^o of different particle size at three different shaking times.

Particle size(mm)	Shaking time (% recovery of S^o) 8 h		16 h		24 h		Mean
1-2	98.50	(1.54)[1]	93.86	(4.85)	109.44	(1.44)	100.60
0.5-1	91.49	(1.63)	94.89	(4.45)	107.80	(2.24)	98.06
0.25-0.5	86.02	(0.28)	93.09	(1.15)	106.65	(1.65)	95.26
0.15-0.25	96.65	(2.66)	101.13	(1.87)	104.42	(1.24)	100.74
0.075-0.15	96.01	(0.98)	101.41	(1.72)	98.50	(1.54)	98.08
Means	93.74		95.96		105.95		

1 Standard error.
Treatment mean LSD 5% = 3.24.

3.4.1.5 *Reduction of So by tin/HCl*

Stronger reducing agents such as HI are used for reducing SO_4-S to H_2S. Several weaker reducing agents such as tin/HCl, stannous chloride, Fe/HCl and Raney nickel are used for reducing S^o to H_2S and most of them are subject to certain limitations. For example, stannous chloride was found to give variable recovery of hydrogen sulphide (Barrow, 1968). Raney nickel reduction also suffered from the interference of organic matter (Chopra, 1963). Although Fe/HCl was claimed to be an effective reducing agent, it does not give a complete reduction of the S^o added (Barrow, 1968). Also the reduction step was not specific to S^o, but inorganic salts containing sulphur in a lower valency state, sulphydryl groups of cysteine, thio groups of dithiocarbamate and of dithizone were also partly reduced (Barrow, 1968).

Tin/HCl was found suitable to reduce S^o in the modified Johnson and Nishita procedure proposed by Nguyen (1988). Tin/HCl was chosen and tested for its selectivity in reducing S^o.

Known volumes of a solution containing SO_4-S (20 µg) were placed in a series of distillation tubes and spiked with different levels of S^o (dissolved in acetone). The S forms in these tubes were reduced either with HI or tin/HCl and analysed for S.

The results in Table 3.6 show clearly that the reduction step of tin/HCl was specific to the S^o form; SO_4 was not reduced which is consistent with Barrow's (1968) results.

Table 3.6 S^o recovery by HI and tin/HCl reduction.

Reduction method	SO_4-S added / S^o added	20 µg			
		10	20	40	60 µg
HI		30.5	41.3	59.0	71.4[1]
Tin/HCl		10.2	19.6	40.8	59.8

1 At levels above 60 µg S sample^{-1} the standard volume of 10 ml zinc acetate becomes less efficient at trapping H_2S evolved from the Johnson and Nishita apparatus (Saggar, S.K., pers.comm).

3.4.1.6 *Temperature of the reduction*

The effects of the temperature of the tin/HCl reduction on length of reaction time for complete reduction of S^o and on the possibility of reduction of SO_4-S at higher temperatures were examined.

Known volumes of a solution containing SO_4-S (20 µg S) were placed in a series of distillation tubes and evaporated to dryness and were spiked with S^o (in acetone) at the rate of 30 µg S. The samples were reduced by tin/HCl using varying times and temperatures (Figure 3.2). The results indicated that the reduction of S^o at the three temperatures was completed after 20 minutes and at higher temperatures the SO_4-S was not reduced by the tin/HCl reduction. However, reduction at the higher temperatures (100-110°C) caused a slight decrease in the recovery of S^o after 20 minutes. The reason for this decrease in S^o recovery is unclear.

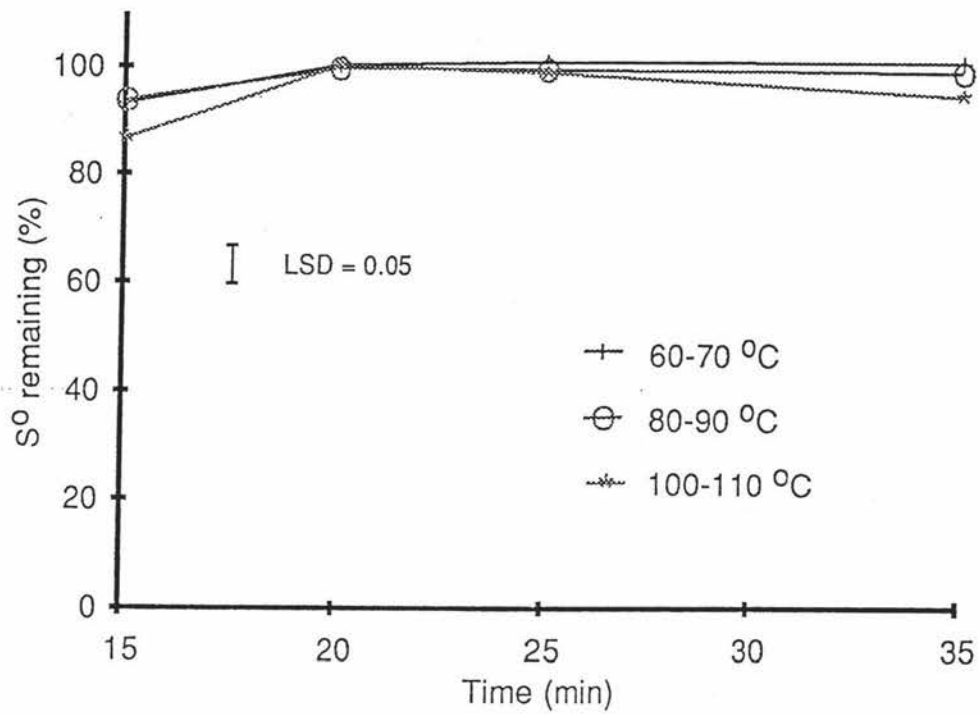


Figure 3.2 The effect of temperature and time on the recovery of S^o by Tin/HCl reduction,.

It is clear from the research discussed above that selective measurement of S^o can be achieved by using tin/HCl as the reducing agent. Literature indicated (Barrow, 1968; Shedley, 1982 and Maynard and Addison, 1985) that the pure acetone extractant may also be a selective extractant for S^o in the absence of significant levels of compound such as sulpholipids. The level of these organic S compounds in the soils being studied in this thesis are not high enough to confound the measurement of S^o residues in soils that contain more than 3 $\mu g\ S^o\ g^{-1}$ soil.

3.4.1.7 *Effect of different soil drying methods*

Barrow (1970) showed that varying amounts of S^o were lost from soil samples depending on the drying conditions. The mild conditions of air-drying at 30°C or freeze-drying and microwave drying of soils containing fertilizer S^o (<0.15 mm fraction) lead to losses of no more than 5% (Barrow 1970; Watkison *et al.*, 1987). But where finer crystals of S^o are present (i.e. after evaporation of chloroform solutions containing S^o) losses can be from 20% to 100% (Watkinson *et al.*, 1987).

Standard S^o dissolved in acetone was added to 5 g of Tokomaru silt loam (moisture content just less than 80% field capacity) at a rate of 40 $\mu g\ S^o\ g^{-1}$. This would result in S^o of <10 μm particle size on the soil surface (S. Phimsarn, pers comm). The soil samples were dried by three different methods (air-dried, oven dried at 30°C and freeze-dried) and moist and dried soil samples were extracted with acetone using two different shaking times (1 and 16 hours). Forced air-drying at 30°C and freeze-drying resulted in 35% and 10% losses of S^o, respectively. There was, however, no significant difference between the recovery of S^o from air-dried and moist samples at both extraction times (Table 3.7). The results were in good agreement with the findings of Watkinson *et al.* (1987).

Table 3.7 Percent of S^o extracted from soil by acetone as influenced by different soil drying methods (40 µg S^o added per g of moist soil).

	Air dry	Dry at 30°C	Freezed-dry	Moist extraction 1 h	16 h
	97.5	62.1	95.8	95.0	98.5
	96.5	70.1	89.3	111.5	110.2
	101.7	63.8	81.8	114.0	103.7
	97.0	64.8	88.8	100.7	104.7
mean	98.2	65.2	88.9	105.3	104.3
Stdev	2.4	3.4	5.7	8.9	4.8

3.4.1.8 *Size of soil sample required for extraction*

It is considered that in the determination of S^o and total S in fertilized soil samples, a major source of variability is introduced from the non-uniform mixing of particles of S^o through a large bulk of soil. Thus it is difficult to obtain representative subsamples with uniform S^o content. The size of soil sample taken for analysis should depend on the rate and particle size of S^o applied (Barrow 1968, Watkinson *et al.*, 1987).

At the rate of 30 kg S^o ha^{-1} the mean number of particles of two S^o samples of particle size ranges, 150-250 µm and 250-500 µm in a 1 g sample of soil were estimated by counting several sub-samples using a microscope. The probable numbers of particles per g of the topsoil (0-3 cm) were calculated. It was found that only 13.7 and 1.8 particles, for the small and large particle size respectively would be contained in a 1 g soil sample. This clearly shows that the smallest soil sample size taken, when soils are fertilized with these two particle size ranges at 30 kg S^o ha^{-1}, should be at least more than 2-3 g and more than 10 g soil per subsample, respectively. This especially applies to field experiments, where it is advisable to take a much larger soil sample (several hundred grams) which is ground finely prior to subsampling for analysis.

In order to determine the percentage recovery of S^o applied to the surface of a field soil, and the appropriate size of soil samples for analysis, two particle size ranges of S^o (<150 μm and 250-500 μm in diameter) were applied to the surface of undisturbed soil cores (isolated by a 15 cm diameter galvanised steel cylinder) at three different rates of application (50, 25 and 12.5 mg $core^{-1}$, equivalent to 30, 15 and 7.5 kg S^o ha^{-1}). Each treatment was prepared in triplicate. The top 3 cm soil layer was immediately sampled and frozen to prevent S oxidation occurring during storage. The samples were freeze dried and ground using a hammer mill. Duplicate subsamples of soil (40 g) were shaken overnight in 200 ml acetone and the concentration of S^o in the acetone was measured as described in section 3.4.1.9.

Overall 90% of the applied S^o was recovered regardless of the particle size and rate of application. There was a slight decrease in the recovery of S^o at the lower rate of S application for both particle sizes (Table 3.8). However, the effects of particle size and rate of application on recovery were not significant. The results indicate that the sample size of 40 g (out of 400 g of dried soil from the top 3 cm of the intact soil core) was sufficient to reduce the variability to an acceptable level when recovering S^o from a field experiment using the galvanised cylinder technique.

Table 3.8 Elemental S recovery of surface application on the undisturbed soil cores as influenced by S^o particle size and rate of application

Particle size (μm)	50 kg ha^{-1}	25 kg ha^{-1}	12.5 kg ha^{-1}	Mean
		(S^o recovery %)		
250-150	90.68 (3.67)*	91.43 (2.69)	83.72 (1.51)	88.61[a]
<150	96.94 (0.86)	91.79 (1.44)	87.24 (1.77)	91.99[a]
Mean	93.81[a]	91.61[a]	85.48[a]	

* Standard error of the means.
[a] Lsd at 0.05.

Several other authors who have sampled field soils fertilized with S^o have found large spatial variations in S^o content. Barrow (1968) measured S^o in samples taken from field soils. This involved collecting the samples, bulking them to give a composite sample and sub-sampling to give a sample for analyses. The standard deviations associated with field sampling, subsampling and analysis were 34.4, 4.9 and 1.1 kg S ha^{-1} (mean value 43.7 kg S ha^{-1}). This standard deviation was higher than that usually found for soil analysis even though Barrow used up to 80 g of soil sample for analysis. In more recent studies, Watkinson *et al.* (1987) also found large field sampling errors, but did not find mixing and sub-sampling to be a serious problem when the S^o size was <0.25 mm in diameter. They suggested that a 10 g soil sub-sample be used and one or two determinations are adequate for S^o analysis. The lower variation found in the study of Watkinson *et al.* (1988) may be due to the use of small plot size (9 m^2) rather than the fertilized paddocks used by Barrow (1968). An optimum number of 9-15 core samples (2.5 cm in diameter) per plot of 9 m^2 was recommended and the plot size of the field experiment involving S^o needs to be large enough to supply the total number of cores required at all subsequent sampling times without increasing the block to blocks differences in soil characteristics (Watkinson *et al.*, 1987).

When the fertilizer S^o is 'held captive' in a galvanised steel cylinder, standard deviations of mean S^o contents of soils (Table 3.8) lower than those values reported by Barrow (1968) and Watkinson *et at.* (1987) can be achieved with a smaller number of replicated soil samples. The cylinder technique was used for future field observations on S^o oxidation.

3.4.1.9 *Technique adopted*

Based on the results obtained from testing the proposed technique and from the literature, the method adopted for extraction and analysis of S^o in soil samples was as follows:

Air-dried (or freezed dried) ground, surface soil was extracted with acetone in screw capped glass bottles. The soil to solution ratio used varied with the estimated sulphur content of the soil. Generally for incubation studies the whole soil sample (100 g soil) and for pot or field studies up to 40 g of surface soil were taken for extraction.

However, in each case the final sulphur concentration in the acetone extract was kept below 200 S^o $\mu g\ ml^{-1}$ to ensure the complete extraction of S^o. After overnight (end over end) shaking of the sample the samples were allowed to stand for 24 hours. Duplicate aliquots (<2 ml) of the clear acetone extracts were placed in distillation tubes and evaporated to near dryness at room temperature. Acetone (0.2 ml), tin powder (0.2 g) and hydrochloric acid (5 ml 1:1, HCl:water, volume basis) were then added to each digestion tube and the tubes were quickly connected to a modified Johnson-Nishita (1952) apparatus. After reduction/distillation for 30 minutes at (60-70°C), the evolved hydrogen sulphide was collected in Na/Zn-acetate and its concentration determined using the colorimetric method of Johnson and Nishita (1952). The absorbance of the resulting solution was measured at a wavelength of 670 nm on a Pye Unicam SP600 spectrophotometer using a 1 cm glass cuvette.

3.4.2 Evaluation of Rhodanese Activity as An Index of Oxidation Potential

In the previous section a method suitable for extracting and analysing S^o remaining in soil has been proposed. To examine the rate and extent of S^o oxidation in soil, it must first be established that soils used for such studies have significant S^o oxidising power. (i.e. the potential ability of the soil to oxidise S^o needs to be known.) Rhodanese enzyme activity (Wainwright, 1978) and Most Probable Number (MPN) of S^o oxidising microorganisms (Lee *et al.*, 1987) have been proposed as indices of the oxidation potential of soils. RA has the advantage of easier measurement than MPN. The RA activity in soil is affected by a number of factors which were discussed in Chapter 2, Section 2.3.10. It is important that the effect of these factors on the RA are fully understood if RA is to provide a useful index of the potential of soils to oxidise S^o.

3.4.2.1 *Effect of fertilizer history*

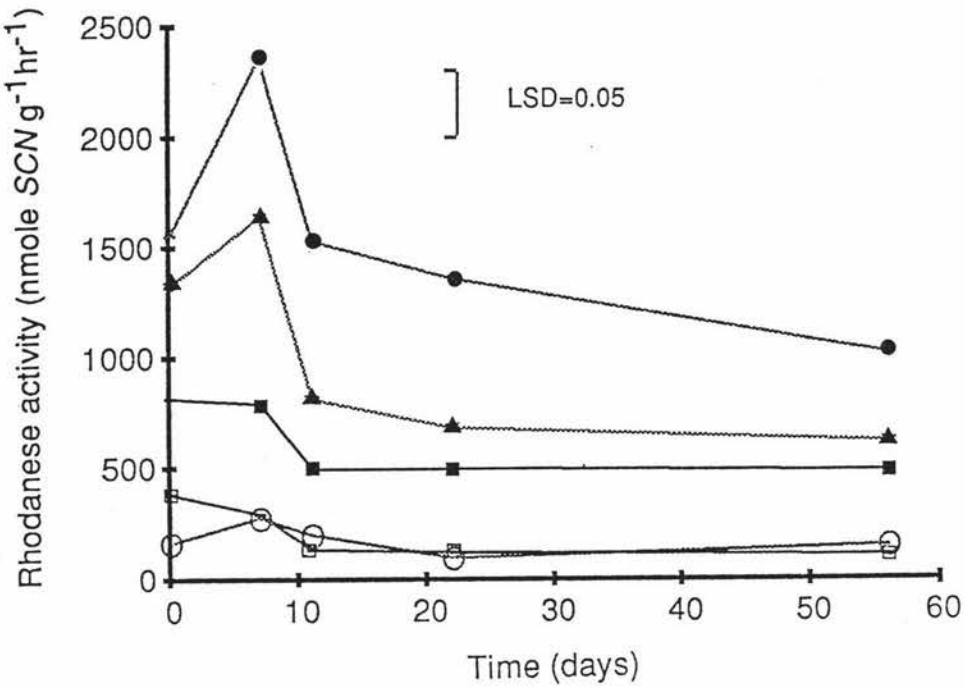
The initial RA in samples of fresh soil varied with soil type (Makotuku >Egmont >Tokomaru) and the history of fertilizer use (Table 3.1, Figure 3.3). Initial RA was higher in the field soils that had been fertilized with S^0. This is probably due to the establishment of a stable S oxidising microbial population in the soils receiving previous applications of S^0 (Janzen and Bettany, 1987c; Lawrence *et al.*, 1988; Lee *et al.*, 1988b).

Although initial RA appeared to be higher in soils that had previously received S^0 fertilizer (Table 3.1), S^0 fertilizer history did not significantly influence the amounts of sulphate produced (Figure 3.8), or S^0 remaining (Figure 3.10), at any sampling date in the incubation study.

3.4.2.2 *Effect of incubation and addition of S^0 to incubating soil*

Throughout the incubation period (Figure 3.4) there was no difference in RA between soils incubated with and without S^0. This was consistent with data published by Ray *et al.* (1985).

The level of RA in both S^0 amended and control soils decreased with time of incubation which may be due to the increase in the concentration of oxidation products such as SO_4^{2-} and H^+. However, others have observed that RA level increases with an increase in soil sulphate concentration (Wainwright, 1978). Singh and Tabatabai (1978) also demonstrated that sulphate activates RA and the level of activation and inhibition of RA by inorganic anions varied considerably among different soils. The decrease in RA in the present experiment could possibly be due to inhibition of the enzyme by the reaction product SO_3^{2-} (Singh and Tabatabai, 1978).



Soil and previous fertilizer

- ■ Tokomaru, Sechura + S^o (18 kg ha^{-1} for 5 yrs)
- □ Tokomaru, Sechura + S^o (30 kg ha^{-1} for 2 yrs)
- ○ Tokomaru, Control (0 kg ha^{-1})
- ● Makotuku, S^o (30 kg ha^{-1} for 2 yrs)
- ▲ Makotuku, S^o (0 kg ha^{-1})

Figure 3.3 The effect of soil type, S fertilizer history and inoculation time on the rhodanese enzyme activity in soil (laboratory incubation).

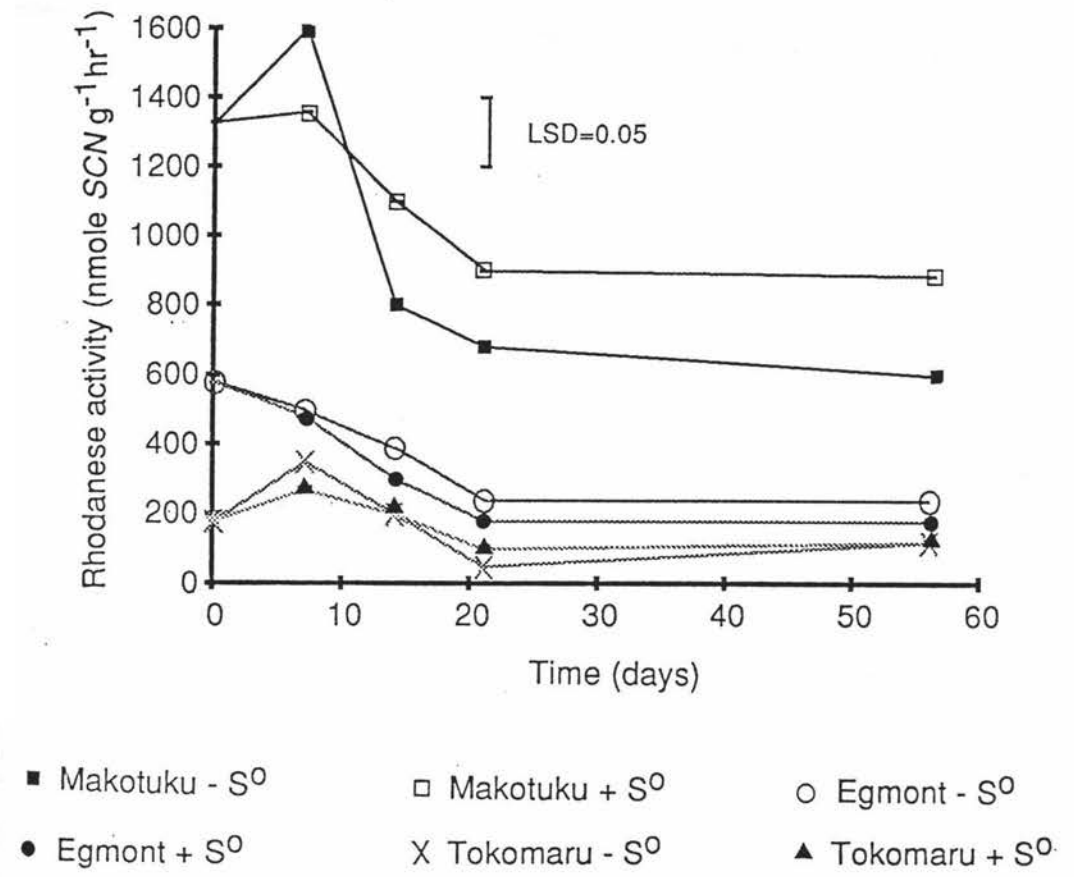


Figure 3.4 Effect of S^0 addition on rhodanese enzyme activity in incubated soil samples that had no recent history of S^0 fertilizer use (laboratory incubation).

3.4.2.3 *Effect of plants on RA*

The effect of root activity on soil RA was analysed using soil samples taken from the pot trial. Fertilizers, other than straight S^o, used in this pot trial are described in detail in Chapter 6 section 6.3.

There was no effect of clover plants on the RA of the whole soil. The RA in the rhizosphere soil at the end of the experiment, however, was significantly higher than the non-rhizosphere soil (mean of 436 compared to 356 nmoles g^{-1} soil h^{-1}; Figure 3.5). It has been observed that the presence of plants enhance the RA in soils (Ray *et al.*, 1985). The volume of rhizosphere soil in this experiment was estimated from the average total root length and by assuming that the rhizosphere extended to 1 mm radius around the root (Russell, 1976). The volume of the rhizosphere soil increased with time and ranged from 13% at the first sampling (20 days after seeding) to 39% of the whole soil volume at the last sampling (98 days after seeding). However, the contribution of RA of the rhizosphere soil to the weighted mean RA of the whole soil was too small to cause any significant increase in RA of the whole soil, especially at the early stage of the experiment.

3.4.2.4 *The use of RA as an index of the S^o oxidation potential of a soil*

The initial rates of S^o oxidation in the laboratory incubation experiment calculated from Figure 3.10 were not highly correlated (Figure 3.6) with initial RA from Figure 3.3. A positive trend of increasing S^o oxidation with increasing RA does, however, exist.

Two soils with contrasting RA levels, namely Tokomaru and Makotuku which had not received previous applications of S^o fertilizer, were used to investigate further the relationship between RA and the rate of S^o oxidation, in soils incubated with S^o (13% <250, 40% <150 and 47% <75 μm in diameter, Section 3.3.3.2).

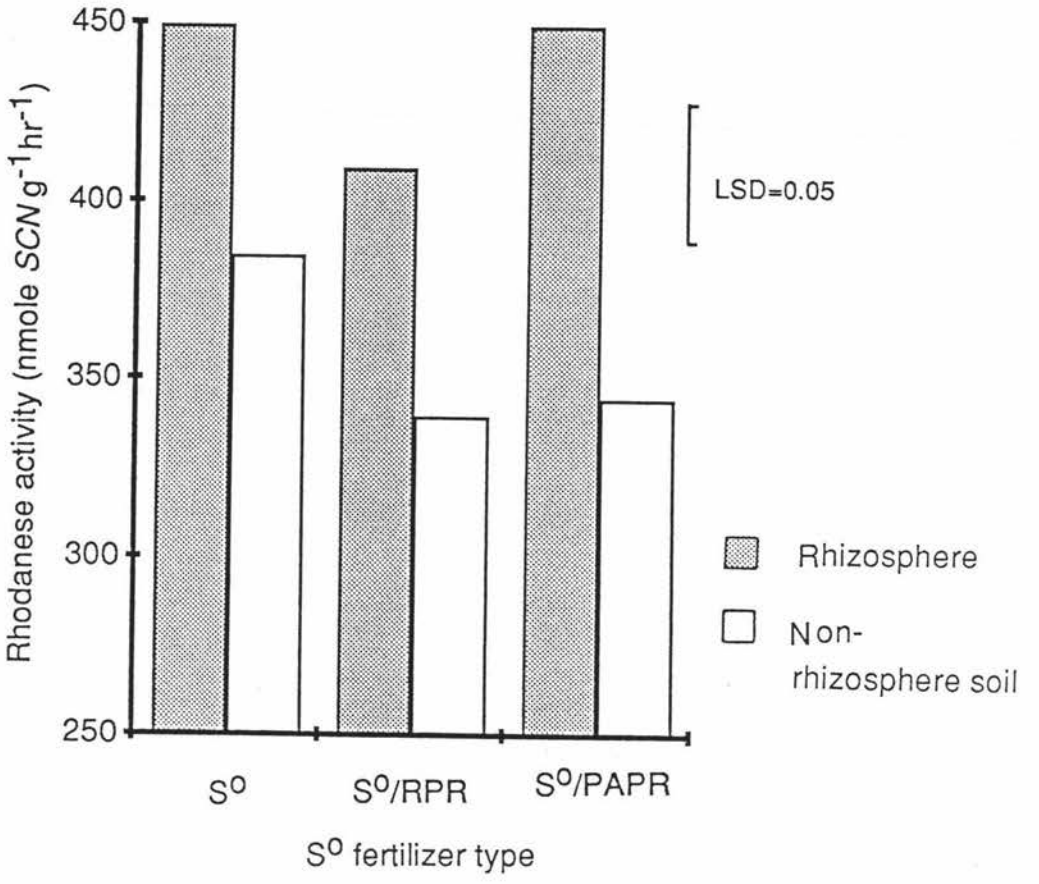


Figure 3.5 Rhodanese activities of clover rhizosphere and non-rhizosphere soil in pots fertilized with different S^0 fertilizers.

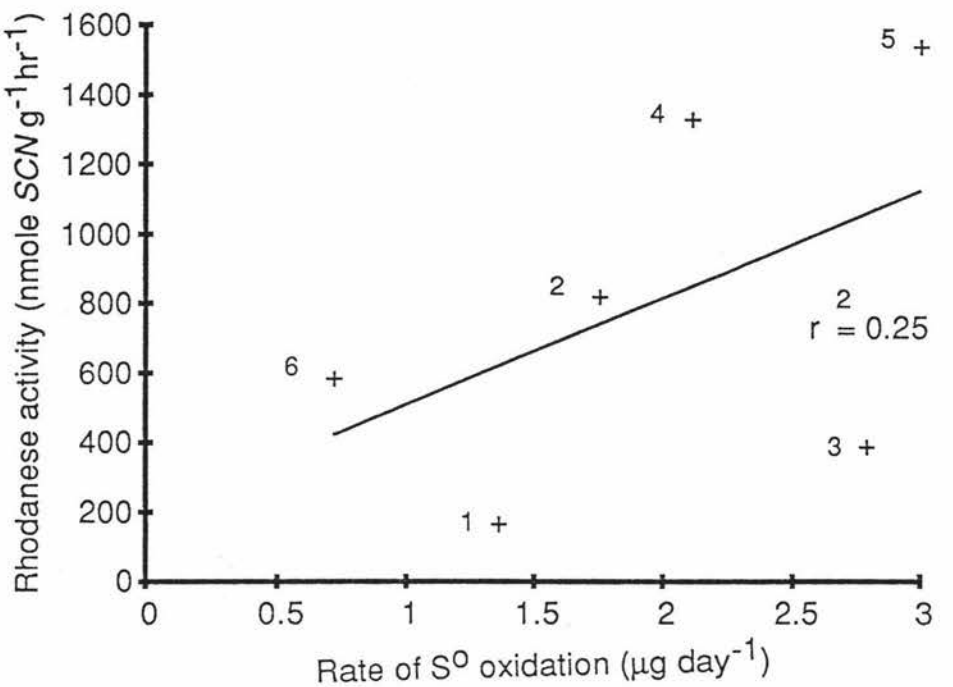


Figure 3.6 Relationship between initial rhodanese enzyme activities and the amounts of S^0 oxidised during the first 30 days of the incubation (laboratory incubation). Numbers refer to soil codes in Table 3.1.

The S^0 oxidation rates at each sampling were calculated using the differential of the exponential decay functions which were fitted to observed data (S^0 treatments only Figure 6.1b and 6.2b.) collected in Chapter 6 section 6.3.1. Again there was a poor relationship between the rate of S^0 oxidation calculated from the amount of residual S^0 and RA (Figure 3.7). The average rate of S^0 oxidation was higher for the Tokomaru soil than for the Makotuku soil (Figure 3.7), despite the fact that the Makotuku fine sandy loam had higher initial RA (Table 3.1, Figure 3.7), which may have been expected to indicate a higher S^0 oxidation potential than the Tokomaru soil.

To obtain a comparison between RA and S^0 oxidation rate it is assumed that the RA catalysed thiosulphate oxidation and this is a limiting step in S^0 oxidation. The RA levels, in the two soils, which were equivalent to theoretical S^0 oxidation rates ranging from 175 to 1053 μg S^0 oxidation g^{-1} soil day^{-1}, were far in excess of actual rates of S^0 oxidation in the glasshouse incubation soil which ranged from 0 to 3.3 μg S^0 oxidised g^{-1} soil day^{-1}. The RA levels in the soils would only reflect the S oxidising capacity of the soil if the enzyme is both essential for, and limiting the rate of, oxidation. Thus level of RA does not appear to be a useful index of a soils' S^0 oxidation potential, other than indicating that if a soil does have RA activity it is likely to have an ability to oxidise S^0.

3.4.3 Evaluation of Methods for Measuring the Rate of S^0 Oxidation

Measuring residual S^0 appears to be a suitable method for monitoring the rate of S^0 oxidation. However, other methods have been used. These include measuring the amounts of SO_4-S produced and the change in soil pH. This section examines the relationship between the extent of S^0 oxidation determined from measuring amounts of residual S^0 and changes in soil pH and the amounts of SO_4 produced.

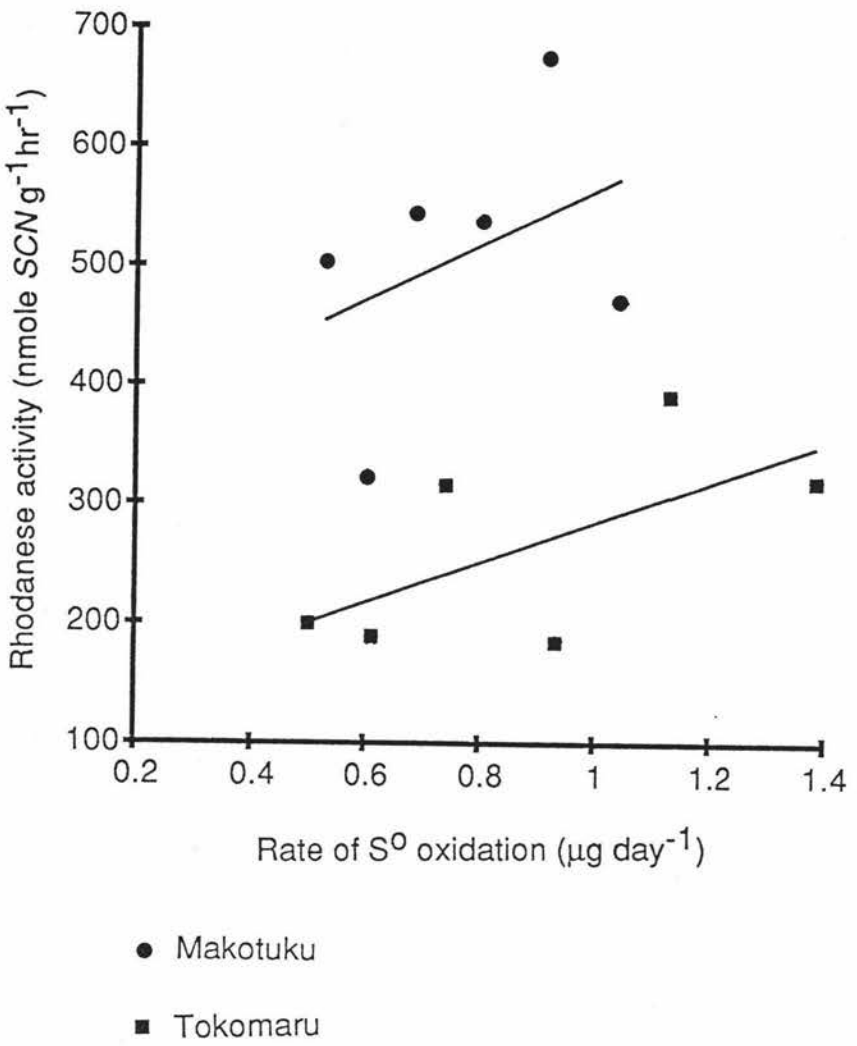


Figure 3.7 Relationship between the rate of S^0 oxidation on the RA measured at every harvest date for previously unfertilized samples of Makotuku and Tokomaru soil (glasshouse experiment - no plants).

3.4.3.1 *Soil pH*

Decreases in soil pH have been found to be highly correlated with amounts of S^o oxidised and have been used as indices for estimating the extent of S^o oxidation (Li and Caldwell, 1966; Fox *et al.*, 1964; Kittams and Attoe, 1965; Skiba and Wainwright, 1984).

The initial pH of the soils used in this experiment varied from 5.4 to 5.8 but no significant difference was found between the pH of the control and S^o amended soils measured at intervals during the pot incubation (data not presented). Even if all the S^o applied (120 μg S^o g $soil^{-1}$) is oxidized, only 7.5 μmoles of H^+ g^{-1} soil are produced. From the pH buffer curve of Tokomaru soil (Appendix 3.1) it was calculated that approximately 16-20 μmoles of H^+ g^{-1} soil are required to cause a drop in pH of 0.1 unit. The changes in soil pH caused by complete S^o oxidation would be too small to be determined accurately. Changes in soil pH may be used as an index of S^o oxidation only in soil with low pH buffering capacities and with very high application rates of S^o (Skiba and Wainwright, 1984) and when the product SO_4 ions are not reimmobilised and reduced into organic sulphur compounds by soil microorganisms i.e., when a biocide is used or when there is no net immobilization of inorganic S. Furthermore, in long term studies, H^+ can be lost or gained by mechanisms other than S^o oxidation as described earlier in Section 2.6.1.

Lee *et al.* (1985) observed a large drop in soil pH when soils were incubated with large amounts of S^o (10,000 μg S^o g^{-1}). However, in their experiment the extent of S^o oxidation, calculated from the alkali titration of soil acidity, was much less than that determined from the amount of S^o oxidised (added - residual). This suggests that protons released during the oxidation of S^o were involved in reactions other than reversible pH buffering.

3.4.3.2 *Extractable sulphate*

Measurement of the amount of sulphate produced is the most commonly used index of S^o oxidation in incubation studies where amounts of S^o converted to SO_4-S are small and low levels of native soil SO_4 exist, e.g. the short term studies of Wainwright (1978) and Janzen and Bettany (1987a, 1987b).

The amount of sulphate sulphur (SO_4-S) extracted by a solution of 0.04 M $Ca(H_2PO_4)_2$ from the laboratory incubated soil is presented in Figure 3.8. The extract has been shown to remove mostly the inorganic SO_4-S released during the mineralization of organic S and oxidation of S^o (Searle, 1979). In the case of control (no S^o added) soils, there was no change in the amounts of extractable S during the incubation indicating that there was no net mineralization or immobilisation of organic S. In the case of soils incubated with S^o, however, the amount of extractable SO_4 increased with the incubation time (Figure 3.8). It was assumed that addition of S^o had no effect on the rate of mineralization or immobilization of organic S and the amount of S^o oxidized was calculated from the difference in the amounts of SO_4-S extracted from the control and S^o amended soils. The increase in SO_4-S produced by S^o oxidation during the incubation studies showed a good agreement with the decreasing amounts of S^o residue ($r = 0.96$), but the extent of S^o oxidation as measured by extractable SO_4-S slightly over estimates (Figure 3.9) those calculated from measuring the residual S^o (Figure 3.10). This is probably due to a slight net mineralisation of an organic sulphur fraction caused by the increased microbial activities in the S^o amended treatments.

In the presence of plants, it is expected that some of the sulphate produced by S oxidation will be rapidly immobilised by assimilation of sulphate by roots and by microorganisms stimulated by root carbon released into the rhizosphere (Till and May, 1971; Freney *et al.*, 1971; Boswell, 1984). Thus it is unlikely that measurement of the SO_4-S increase in field soils will be a useful measure of the extent of S^o oxidation, especially in soils that have high SO_4-S leaching indices (Sinclair, 1982). These expectations were confirmed in the glasshouse pot experiment (Figure 3.11) in which after 6 weeks sulphate levels dropped when plants were present in S^o amended soil. During the experiment the relationships between the amounts of sulphate produced and the amount of S^o oxidised (calculated from residual S^o measurements) were poor (Figure 3.12).

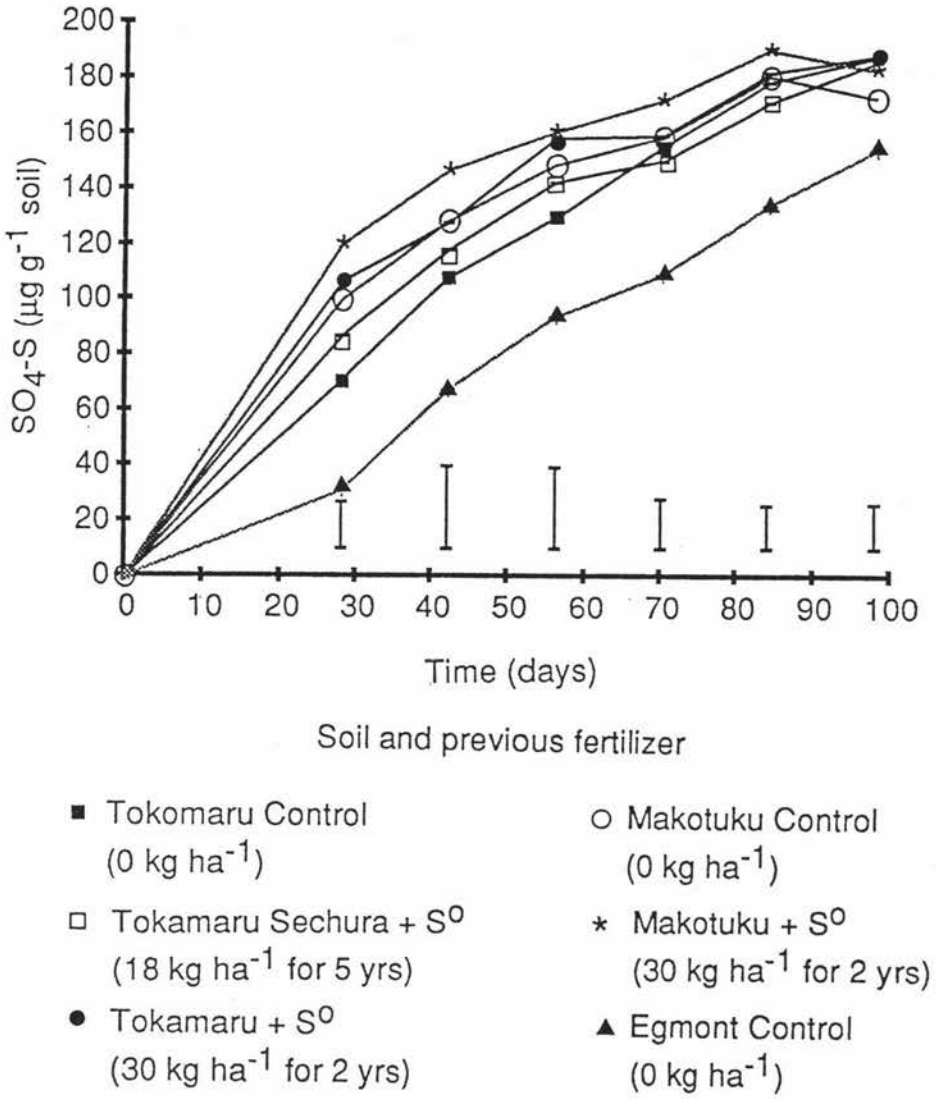


Figure 3.8 Differences in the amounts of 0.04M $Ca(H_2PO_4)_2$ extractable sulphur between S^o amended soil and control soil in the laboratory incubation.

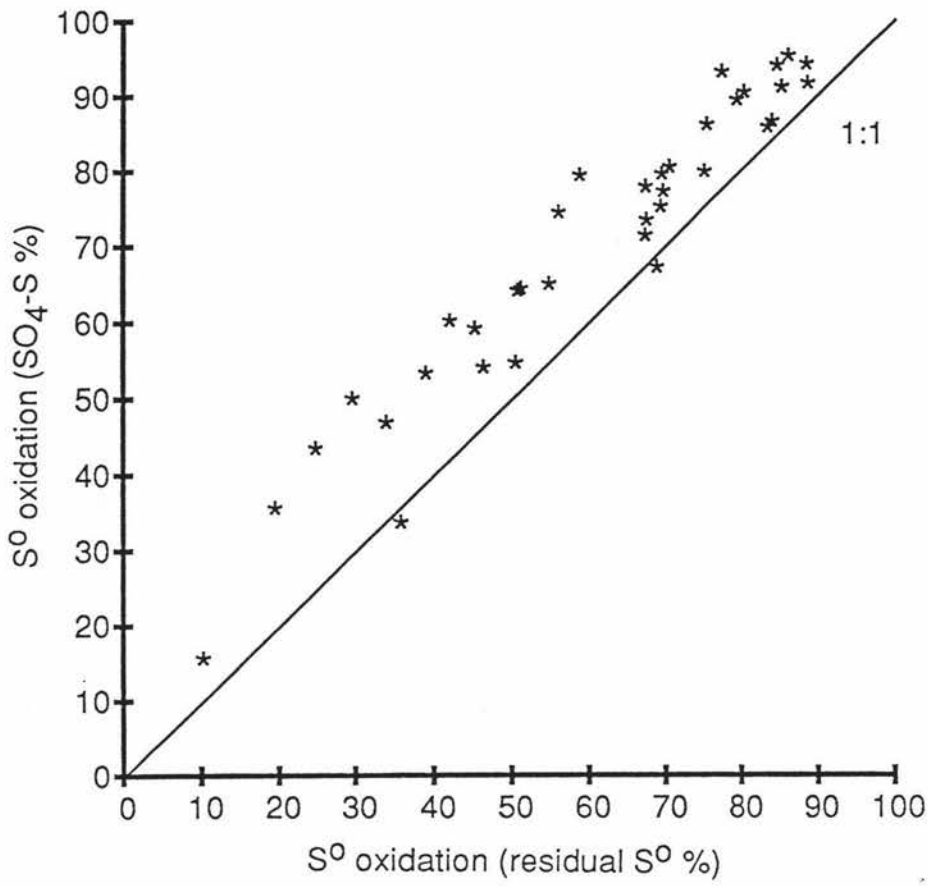


Figure 3.9 Relationship between percentage S^0 oxidation, measured by acetone extractable residual S^0 and 0.04M $Ca(H_2PO_4)_2$ extractable SO_4-S in the absence of plants (laboratory incubation).

3.4.3.3 *Residual S^o*

The amounts of S^o extracted by acetone from various soils incubated with S^o is presented in Figure 3.10. Only small amounts (0-3 μg S g^{-1} soil) of S were extracted by acetone from the control soil which indicates that negligible amounts of native soil S are are extracted by acetone and reduced by tin/HCl.

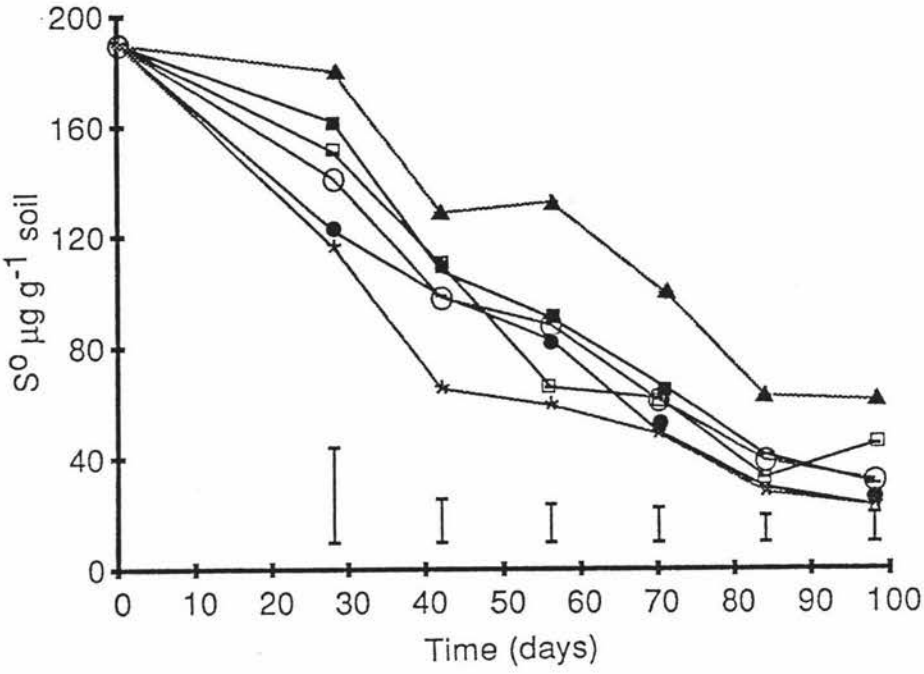
The difference in the amounts of acetone extractable S between control and the S^o amended soils gives the amount of residual S^o remaining in the soils. From this the amounts of S^o oxidised were calculated using the following relationship:

Amount of S^o oxidised = $S^o(t_0)$ - acetone extractable $S(t_n)$ (3.2)

where $S^o(t_0)$ = amount of S^o added at time 0
$S(t_n)$ = harvest at time n
= (S^o amended soil - control soil)

There is a strong relationship between the amounts of S^o oxidized measured by SO_4-S and residual S^o in the absence of plants for soils incubated in the laboratory Figure 3.9. In the glasshouse trial, the amounts of residual S^o in S^o amended soils decreased with time at a rate similar to the incubation study both in the presence and absence of plants (Figure. 3.10 and Figure 3.13). These results are discussed in more detail in Chapter 6, section 6.3.2.

In the presence of plants, the measurement of SO_4-S greatly underestimated the extent of S^o oxidation (Figure 3.12). As discussed earlier (section 3.4.3.2) there was no consistent relationship between the amounts of SO_4 extracted and the amount of S^o oxidised (calculated from residual S^o). Inclusion of S taken up by plants in SO_4-S, only slightly improved the relationship between the amount of S^o oxidation measured by these two methods (data not shown). This indicated that in the presence of growing plants a large portion of the S^o oxidized is immobilized in root or soil organic S and measurement of SO_4-S plus increments in above ground plant S may result in an underestimation of the extent of S^o oxidation.



Soil and previous fertilizer

- ■ Tokomaru Control (0 kg ha^{-1})
- □ Tokamaru Sechura + S^o (18 kg ha^{-1} for 5 yrs)
- ● Tokamaru + S^o (30 kg ha^{-1} for 2 yrs)
- ⁎ Makotuku Control (0 kg ha^{-1})
- ○ Makotuku + S^o (30 kg ha^{-1} for 2 yrs)
- ▲ Egmont Control (0 kg ha^{-1})

Figure 3.10 The amounts of acetone extractable sulphur in S^o sulphur amended soil (laboratory incubation).

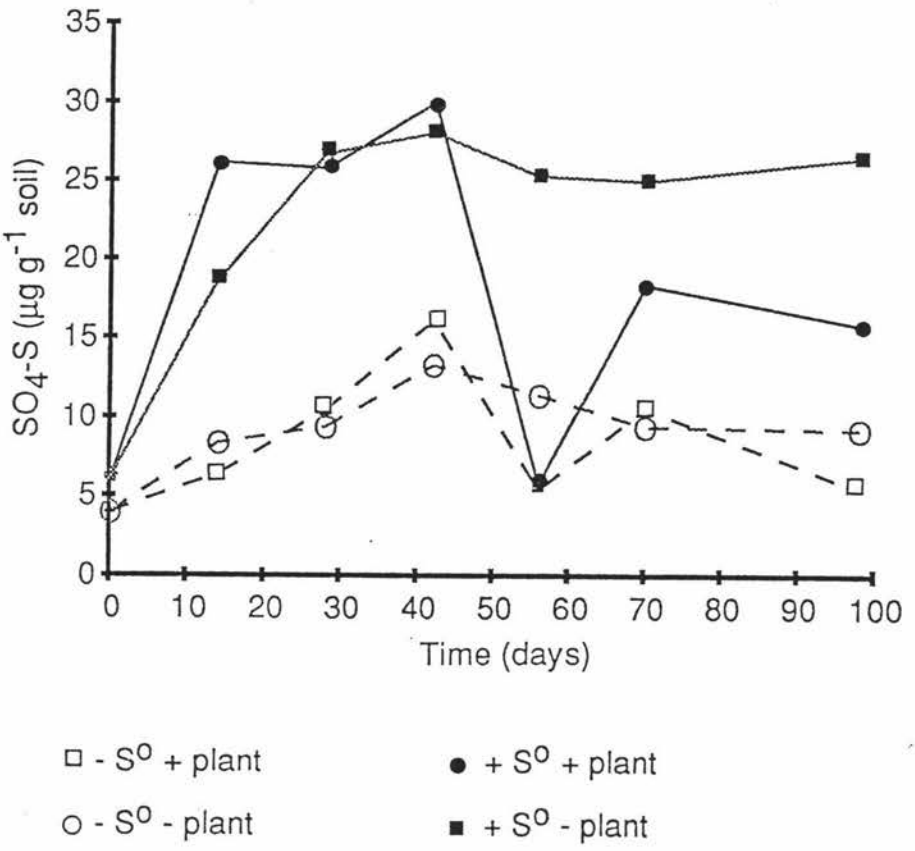


Figure 3.11 Soil sulphate-S levels in Tokomaru soil in the presence and absence of plants during the glasshouse incubation and plant growth experiment.

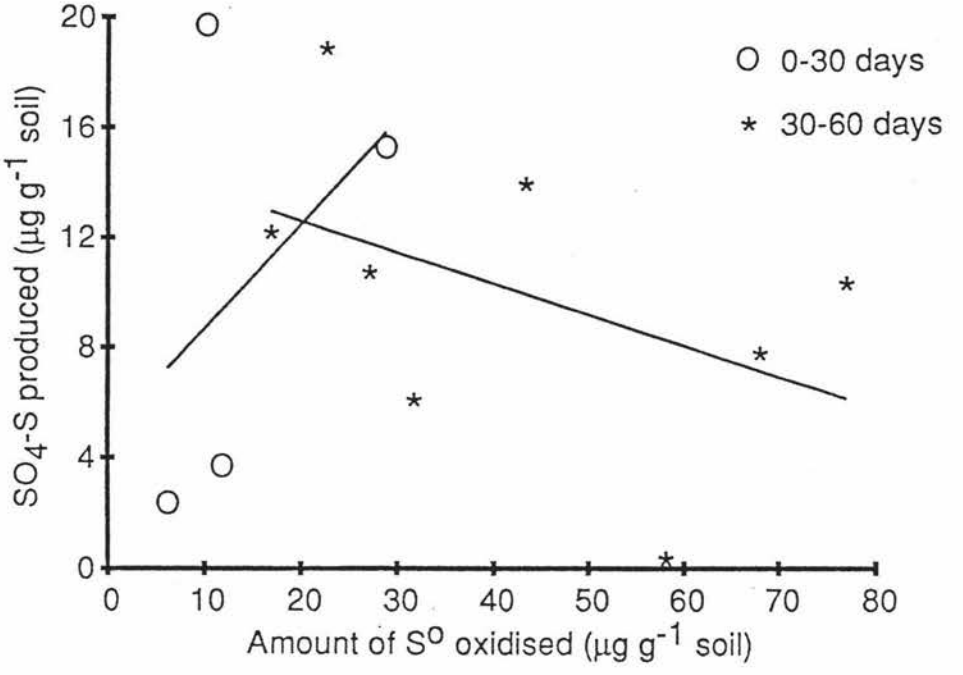


Figure 3.12 A comparison of the amounts of S^o oxidised calculated from measurements of residual S^o or from extractable sulphate (above control soil) during the growth of clover plants in the Tokomaru soil (Glasshouse Trial).

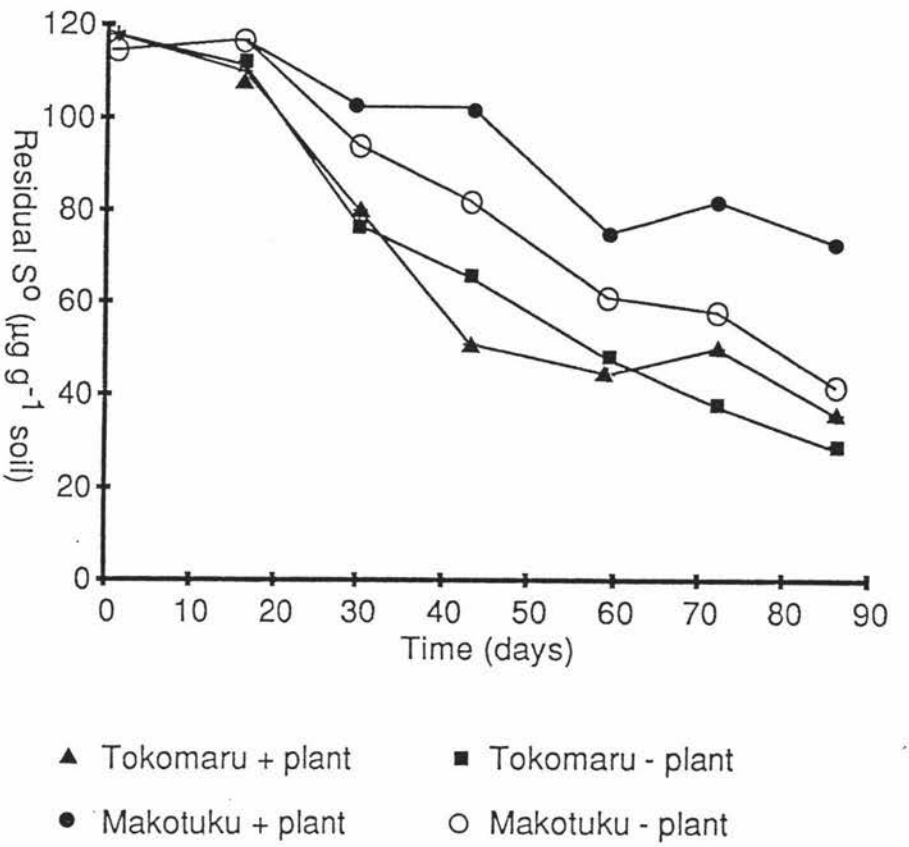


Figure 3.13 Residual amounts of S^o in Tokomaru and Makotuku soils in the presence and absence of plants during the glasshouse experiment.

Although measuring the amount of residual S^o appears to be the only accurate measure of S^o oxidation in field soils, there are some limitations of the method. It was generally found that there was a larger error involved in determining the amount of residual S^o rather than soil sulphate content. This is due to the non-uniform mixing of S^o particles through a large bulk of soil (Barrow, 1968). In this study, in order to use residual S^o as a measure of the oxidation rate, the actual amounts of S oxidised (Equation 3.2) at a certain time need to be at least 6 $\mu g\ S\ g^{-1}$ soil which is the value of twice the standard deviation of the S^o determination using 10 g soil sample.

3.5 CONCLUSIONS

The level of RA in a soil sample may indicate a S^o oxidising ability but, over the range of RA's measured in this thesis (200 - 1600 nmole SCN $g^{-1}\ h^{-1}$), level of RA does not provide an index of the extent to which the soil will oxidise S^o. RA varied with soil type, but within any soil type was higher in soils that had a recent history of S^o use. The rate of sulphur oxidation in soil, however, was influenced significantly by soil type but not by fertilizer history. RA of rhizosphere soil was found to be higher than non-rhizosphere soil.

The methods for estimating sulphur oxidation by measuring changes in soil pH or extractable sulphate are useful only in soil of low buffering capacity and in short incubation studies. The direct measurement of residual elemental sulphur is the only appropriate method, especially for use in pot trials with plants or under field conditions. Some general precautions need to be taken in the preparation of the soil samples. Larger soil samples may be needed for the extraction of S^o especially when coarse particles of S^o have been applied. In S^o extraction, the precision of the subsampling can be lower than is usually obtained with other soil analyses, This is due to the non-uniform mixing of S^o particles through the soil.

For measuring residual S^o in field moist or dry soils, acetone is a suitable extractant provided the extractant S^o concentration is not allowed to exceed 200 $\mu g\ S\ ml^{-1}$. Extraction of dry soil is recommended however. If soil samples are dried the drying temperature should not exceed 30°C. S^o concentration in the extract can be readily

measured using tin/HCl reduction in a modified Johnson and Nishita apparatus. This combination of acetone and tin/HCl reductant measures negligible S from soils other than S^{o} residues, even when they include root material. An extraction time of 16 hours is recommended to ensure the dissolution of larger particle sizes of S^{o}.

CHAPTER 4

EVALUATION OF METHODS FOR DETERMINING THE PARTICLE SIZE OF ELEMENTAL SULPHUR IN FERTILIZER MATERIALS

4.1 INTRODUCTION

In a particular climatic zone, S^0 particle size, which determines the specific surface area, is the most important factor controlling the rate of S^0 oxidation and release of plant available sulphate. This aspect has been reviewed in detail in Section 2.5. In summary, at normal application rates (10-30 kg S^0 ha^{-1}) particle sizes >250 μm will oxidise too slowly to maintain an adequate supply of S to pasture plants even in the temperate climate which covers most pastures in New Zealand's North Island.

To judge the suitability of a S^0 source as a S fertilizer, there is a need to develop methods that can be used to determine routinely the S^0 particle size distribution in the wide range of P and S compound fertilizers that are available for application to pasture. These materials include processed fertilizer such as S^0 fortified superphosphate (S^0-SSP), sulphurised partially acidulated phosphate rock (S^0-PAPR), S^0-reactive phosphate rock blends (S^0-RPR), S^0 prills (Boswell and Swanney, 1986) and raw ores such as Rotokawa pumice sulphur (Bell-Booth *et al.*, 1988). Other than the unpublished work of Rogers and Braithwaite (1984) no methods suitable for measuring S^0 particle size in fertilizer compounds have been published.

4.2 OBJECTIVES

The objective of this chapter is to examine the suitability of different methods for determining the S^0 particle size in a range of fertilizer materials. These methods generally involve three steps, particle dispersion, particle size determination and measurement of S^0 content of each size fraction. Therefore, within the main objective, the influence of dispersant type on the measured S^0 particle size and two techniques for measuring the S^0 content of specific particle size fractions were also examined.

4.3 MATERIALS AND METHODS

4.3.1 Materials

A wide range of S fertilizers was used including sulphurized superphosphate, sulphurized partially-acidulated phosphate rock, prilled S^0 and Rotokawa pumice sulphur (Table 4.1). All materials except Rotokawa S^0 were obtained from Ravensdown Fertilizer Co-operative Ltd. All are commercially available for application to pasture soils.

Table 4.1 Percent moisture content, total P and S^0 content of the range of fertilizers used for S^0 particle size analysis.

Fertilizer	Moisture	Total P	S^0
		(% w/w)	
Ground S^0			
Ag.Grade S^0	0.25	-	100.0
Rotokawa S^0(50 grade)	0.94	-	53.9
Granulated/prills			
Sul85 prill	0.55	-	82.2
Tiger 90	0.33	-	92.2
Sulphurised SSP			
40% S Super	5.72	6.0	34.8
33% S Super	4.98	7.0	24.1
S-super extra	2.32	7.0	18.9
Sulphurised PAPR			
Hyphos Supreme	2.90	13.8	11.0
Hyphos S	3.68	14.5	6.0
Rockphos	3.53	10.7	4.0

4.3.2 Methods

The method for the particle size analysis of S^o in compound fertilizers involves three steps: i) particle dispersion, ii) sieving to different particle size fractions and iii) analysis of S^o in each fraction.

4.3.2.1 *Sample preparation*

The bulk fertilizer samples (20 kg) were successively riffled to produce subsamples for the determination of moisture content, S^o content and particle size analysis.

4.3.2.2 *Moisture content*

The moisture contents of fertilizer samples were determined by drying in an oven overnight at temperatures below 30°C. This avoids a major loss of microfine S^o which may occur at higher temperatures, as described in (Section 3.4.1.7).

4.3.2.3 *Dispersion techniques*

Dispersion of S^o particles can be achieved in alcohol (Janzen and Bettany, 1987b), water or mineral acids (Bolan *et al.*, 1988; Rogers and Braithwaite, 1984).

A. Dispersion in water and wet sieving

Replicate sub-samples (10 g) of each fertilizer were shaken (end over end) overnight with 1000 ml of distilled water. The fertilizer suspension was then washed with water through a nest of stainless steel sieves (mesh openings 0.5 mm, 0.25 mm, 0.15 mm, 0.075 mm). Separation of a particular size fraction was considered complete when the effluent leaving a sieve was completely clear. The fraction passing 0.075 mm was collected on a milipore filter (0.45 μm). A total water volume of approximately 3 litres was used. All fractions were washed with alcohol prior to drying at 30°C for 20 minutes and weighed.

B. Acid wash and dry sieving

Replicate sub-samples (35 g) of fertilizer were shaken (end over end) in 1000 ml of HCl (1:3, acid volume:water volume, approximately 10% HCl w/w) overnight, filtered and extensively washed with distilled water. The dispersed samples were dried at 30°C and sieved using a Rotap shaker for 15 minutes. This method is modified from that of Rogers and Braithwaite (1984) who used a stirring technique which lasted 2 hours rather than overnight shaking.

C. Acid wash and wet sieving

Replicated sub-samples (7 g) of fertilizer were shaken (end over end) in 200 ml of dilute HCl (1:3, acid:water) overnight. After centrifuging at 3000 rpm for 15 minutes on a Sorvall RC25 centrifuge using a SS34 head, the acid was decanted and the samples were washed through a nest of sieves, using approximately 1 litre of water (as described in section 4.3.2.3. A). This modification to the acid dispersion technique was adopted because during the filtration (Section 4.3.2.3.B) some residues formed cakes on the filter paper which did not disintegrate upon dry sieving, giving rise to an overestimate of S^o particle size >500 μm.

D. Double acid wash and wet sieving

Two hundred mls of dilute HCl (1:3, acid:water) were added to each replicated sub-sample (7 g) of fertilizer in 250 centrifuge bottle. After some gentle shaking and standing for two hours the bottle was centrifuged at 3000 rpm for 15 minutes and the acid was decanted. This was repeated with another lot of 200 ml of dilute HCl. The samples were then washed through a nest of sieves as described above.

4.3.2.4 *Elemental S determination*

A. Loss on ignition

Replicated known weights (about 3 to 5 g) of ground fertilizer samples were pre-dried at 30°C overnight before being weighed and heated in a muffle furnace at 550°C for 3 hours. The percent of S^o in each sample was calculated from the percent weight loss during ignition.

B. Solvent extraction

Ground (<150 μm particle size) whole fertilizer samples and sieve fractions containing 10-30 mg S^o were shaken with 200 ml of acetone in a glass bottle for 16 hours. The acetone suspension was allowed to settle and clear. An aliquot (0.2 to 1 ml) of the extract, containing 5 to 50 μg S^o, was analysed for S^o by the method described in Section 3.4.1.9.

4.3.2.5 *Solubility of gypsum and P fertilizer components in dilute HCl*

Gypsum at various concentration levels (10, 15, 20 and 25 mg ml^{-1}) in combination with different levels of MCP (0, 5, 10, and 15 g ml^{-1}), as shown in Table 4.4, were shaken overnight with 40 ml of 10% HCl. After centrifuging at 10,000 rpm for 10 minutes using Sorvall RC5C centrifuge with a SS-34 head, the supernatants were subsampled for the analysis of P and SO_4. The undissolved residue in the centrifuge tubes were dried overnight at 60°C and weighed.

Various concentrations of gypsum, MCP and North Carolina Phosphate Rock (NCPR) (Table 4.5) were also extracted in 10% HCl to determine the effect of PR residues on the efficiency of acid dispersion.

4.4 RESULTS AND DISCUSSION

4.4.1 Selection of Dispersants for the Determination of S^o Particle Size

Information on the chemical nature of the different components of S^o fertilizers is required in order to choose solvents, which will completely dissolve the agents binding these fertilizer materials into aggregates or granules. The current forms of fertilizer containing S^o which are used on New Zealand pastures can be categorised in terms of their components as follows:

A. *Pure S^o*

Screened agricultural grade S^o contains 100% sulphur, and varies considerably in particle size depending on supplier. Within a sample there is also a wide particle size distribution. For example, Boswell (1987) reported the following particle size distribution, 19-30%, <250 μm; 4-18%, <150 μm and 10%, <75 μm in diameter for a sample of screened S^o.

B. *Geothermal S^o*

This S^o occurs naturally in Taupo-Rotorua geothermal area. The estimated subsurface deposit of 2.6 million tones of S^o and considerable quantities of potentially useful S are also found in the surface (e.g. Sulphix S with 12, 20, 50% by fresh weight S^o, Table 4.1).

C. *Mixtures of S^o and bentonite clays*

These prilled or granular fertilizers are prepared by mixing molten or screened S^o with sodium bentonite clay and prilling in oil. The product contains between 60 to 95% S^o (e.g. Sul85 prill and Tiger 90, Table 4.1).

D. *S^o/anhydrite*

This fertilizer is prepared by heating a mixture of S^o and gypsum to 450°C and then granulating the product (experimental fertilizers) (Rothbaum *et al.*, 1980).

E. *Mixtures of P and S^o fertilizers*

These products can be subdivided into:

a). S^o/fortified superphosphate (10 to 50% S^o) This is prepared either by dry-mixing screened agricultural S^o with superphosphate (dry mix, sulphurised superphosphate), or by adding molten S^o during superphosphate production (wet mix, sulphurised superphosphate e.g. sulphurised SSP, Table 4.1).

b). Sulphurised partially acidulated reactive phosphate rock. This is produced by adding molten S^o to the mixer during partial acidulation of a reactive phosphate rock (e.g. Hyphos Supreme, Hyphos-S and Rock phos made by Ravensdown Fertilizer Co-op. Ltd., Table 4.1).

c). Dry mixtures of S^o and partially acidulated reactive phosphate rock (10 % S^o; Charleston and Laing, 1985) or reactive phosphate rocks (9.5% S^o; Rogers, 1985).

The major cementing agents or materials other than S^o in these fertilizers (Section E only) are gypsum ($CaSO_4.2H_2O$), andhydrite ($CaSO_4.1/2H_2O$), monocalcium phosphate (MCP), reactive phosphate rocks and phosphate rock residues. Removal of these components prior to particle size analysis of S^o is necessary for the following reasons. Firstly, gypsum and MCP can act as binding agents and unless they are removed, complete dispersion of S^o particles may not be achieved. Secondly, during the measurement of S^o by loss on ignition the loss of water by crystallization from gypsum or other hydrated components can result in an overestimation of S^o content.

Gypsum can be removed by many ways. The technique of overnight heating at 105°C followed by washing in water (Rivers *et al.*, 1982), is normally used in the removal of gypsum prior to the particle size analysis of soil samples, but this procedure could not be applied due to the accelerated loss of S^o at elevated temperatures (Section 3.4.1.7). The standard solubility data published by Linke (1958) in Table 4.2 show that gypsum has its highest solubility in dilute HCl (1.85 g in 9.15% w/w HCl). This concentration of dilute HCl (10%) is similar to that used by Rogers and Braithwaite (1984) for removal of gypsum and MCP in the particle size analysis of S^o enriched TSP and SSP fertilizer samples.

Table 4.2 Solubility of calcium sulphate in hydrochloric acid solutions (Linke, 1958).

g per 100 g saturated solution at 25°C	
HCl	$CaSO_4$
3.56	1.50
6.48	1.80
9.15	1.85
12.08	1.73
15.09	1.56
18.33	1.27
26.73	0.75

In preliminary laboratory tests, the solubility of gypsum in several dispersants such as citric acid, EDTA, NH_4Cl, was compared with that in 10% HCl. Combinations of gypsum with either MCP or NCPR were shaken overnight with the dispersants mentioned above and the amounts of residue weight were measured (Table 4.3). The results confirmed that 10% HCl dissolved the maximum amount of each mixture.

Therefore 10% HCl was selected as a dispersant for the particle size analysis of S^o enriched P fertilizers. The estimation of the amount of acid to be used for complete dispersion of different S^o enriched P fertilizers and the evaluation of other dispersants are discussed in the following sections.

Table 4.3 The amount of residue remaining after mixtures of MCP or NCPR with Gypsum were shaken for 16 h in different dispersants (40 ml).

Dispersants	Concentration	MCP added (mg)	Gypsum	Residue weight mg in 40 ml
HCl	10%	600	1000	458
HCl/Citric	10%/sat.	600	1000	455
HCl/EDTA	10%/sat.	600	1000	1060
HCl/NH4Cl	10%/0.37 M	600	1000	468
NH4Cl	0.37 M	600	1000	901
		NCPR	Gypsum	
HCl/NH4Cl	10%/0.37 M	480	704	322
NH4Cl	0.37 M	480	704	901
HCl	10%	480	704	233

NB: Results are means of 3 replicates and in all cases standard deviation was less than 5% of mean unless stated.

4.4.1.1 *Applying solubility criteria to the dissolution of gypsum in HCl*

Determination of solubility products for gypsum and MCP in HCl is difficult because the equilibrium solution is complex. The solubility product of gypsum can be expressed as (Drever, 1982):

$$CaSO_4(s) \text{ ==== } CaSO_4^{o}(aq) \text{ ==== } Ca^{2+} + SO_4^{2-} \qquad (4.1)$$

$$Ksp\ CaSO_4 = [Ca^{2+}][SO_4^{2-}] / [CaSO4]^{o} \qquad (4.2)$$

$$= 1.95 \times 10^{-4}$$

Following equations can be written to account for the ions present in the system of gypsum/MCP/HCl.

$$Ca(H_2PO_4)_2^{o} \text{ ==== } Ca^{2+} + 2H_2PO_4^{-} \qquad (4.3)$$

$$Ca^{2+} + 2H_2PO_4^{-} \text{ ==== } CaHPO_4^{o} + H_3PO_4^{o} \qquad (4.4)$$

$$H_3PO_4^{o} \text{ ==== } H^{+} + H_2PO_4^{-} \qquad (4.5)$$

$$HCl^{o} \text{ ==== } H^{+} + Cl^{-} \qquad (4.6)$$

$$SO_4^{2-} + H^{+} \text{ ==== } HSO_4^{-} \qquad (4.7)$$

$$HSO_4^{2-} + H^{+} \text{ ==== } H_2SO_4^{o} \qquad (4.8)$$

The presence of acid (10% HCl) keeps the SO_4^{2-} concentration low by forming HSO_4^{-} and $H_2SO_4^{o}$ ion pairs (equation 4.7 and 4.8), thus causing the solubility of $CaSO_4$ to increase (equation 4.1). Also in the presence of Ca^{2+} the excess H^{+} ensures that the phosphates remain soluble as $H_2PO_4^{-}$ and H_3PO_4 (equation 4.4 and 4.5) forms without allowing the formation of less soluble HPO_4^{2-} and PO_4^{3-}. Addition of HCl also keeps the ionic strength of the system high which reduces the activity coefficients of Ca^{2+} and SO_4^{2-} ions, thus causing higher solubility of gypsum.

An accurate prediction of $CaSO_4$ solubility can be achieved by knowing the concentration of all the ion species in equilibrium with $CaSO_4$ in the system which may be computed using a computer program such as 'Geochem' (Sposito, 1988).

A successful dispersant for all fertilizer materials containing S^o needs to dissolve the cementing agents, gypsum and MCP, and in the case of partially acidulated fertilizers and RPR/S^o blends it must dissolve the unacidulated phosphate rock residue. In dissolving the residue the HCl concentration must not be significantly reduced through reaction with the residue, otherwise the dissolution of gypsum may be incomplete.

The solubility of gypsum, MCP and rock residues in dilute HCl is affected by the concentration of Ca^{2+} and SO_4^{2-} in solution (Kemper *et al.*, 1975) and by the extent to which phosphate rock residues neutralize the HCl. A preliminary experiment was undertaken to examine the effect of different concentrations of Ca^{2+}, SO_4^{2-} from fertilizer materials (gypsum, MCP and phosphate rock) on the dissolution of gypsum in 10% HCl used for the dispersion of S^o (Section 4.3.2.5).

The range of MCP and gypsum mixtures were chosen to simulate different fertilizer materials used for S^o particle size analysis in this chapter. The residue weight was recorded and from the concentration of SO_4 in the solvent the amount of gypsum dissolved was calculated (Table 4.4).

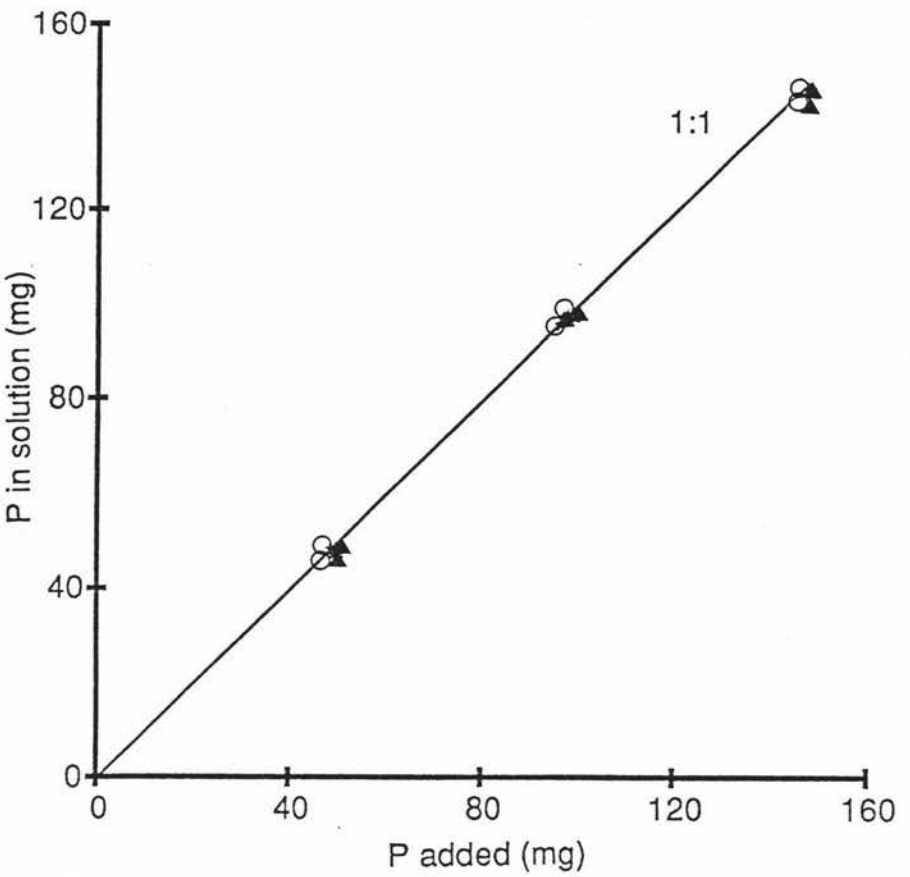
The amounts of solution P obtained when a range of MCP and gypsum mixtures were shaken in HCl (10%) are shown in Figure 4.1. The maximum amounts of gypsum dissolved, as calculated by the solution SO_4-S concentrations are shown in Figure 4.2. The maximum amount of gypsum dissolved in 10% HCl was 1.95 g per 100 ml of HCl which was consistent with the solubility value (1.85 g per 100 ml of 10% HCl) published by Linke (1958). There was an interaction between the amount of gypsum dissolved and the level of MCP and gypsum added. The solubility of gypsum decreased when the level of MCP added increased and solubility decreased markedly at the higher level of gypsum addition (25 mg ml^{-1}) than at the lower level (20 mg ml^{-1}) (Figure 4.2). This result was expected because the Ca^{+2} concentration in solution increased (Table 4.4) with the addition of MCP. This increase in the concentration of the 'common ion' Ca^{+2} caused the precipitation of gypsum at lower SO_4^{2-} concentrations.

Table 4.4 The amount of added and recovered P and S after HCl extraction of various MCP and gypsum mixtures.

MCP Added	Gypsum (mg)	P added	P recovery	S added	S recovery
		(mg in 40 ml 10% HCl)			
600	1000	148	140.3	186	148
400	1000	98	94.7	186	157
200	1000	49	48.2	186	167
0	1000	0	na	186	182
600	800	148	142.0	149	140
400	800	98	95.1	149	140
200	800	49	46.7	149	142
0	800	0	na	149	144
600	600	148	144.6	112	112
400	600	98	96.6	112	106
200	600	49	47.5	112	108
0	600	0	na	112	112
600	400	148	141.6	74	76
400	400	98	97.7	74	76
200	400	49	47.9	74	76
0	400	0	na	74	74

NB: Results are means of 2 replicates and in all cases standard deviation was less than 5% of mean unless stated.
na = non applicable.

The solubility of MCP in water is 7.5 - fold higher than that for gypsum (18 g and 2.4 g per litre respectively, Weast 1972) indicating that little MCP will be precipitated if these mixtures were dissolved in water. In these MCP/gypsum/10% HCl systems, although the ionic compositions are not known, the MCP is expected to be completely soluble even in the presence of solution Ca concentration sustained by insoluble $CaSO_4.2H_2O$.



Source of P added

▲ MCP/Gypsum (Table 4.4)

○ MCP/RPR/Gypsum (Table 4.5)

igure 4.1 The relationship between the amount of P recovered in 10% HCl extracts and the amount added.

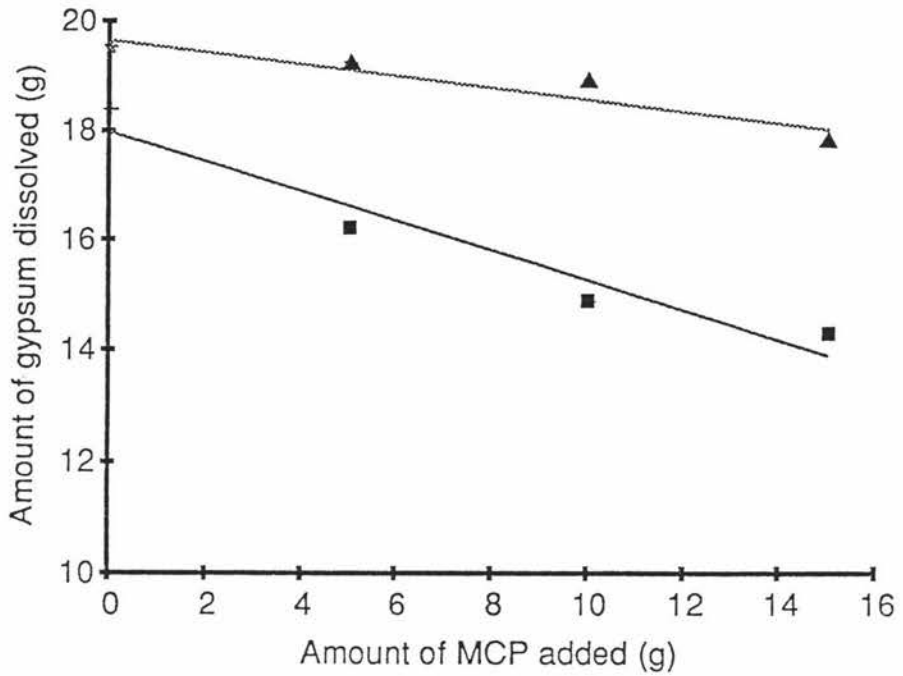


Figure 4.2 The effect of increasing amounts of MCP on the amount of gypsum dissolved in 10% HCl extracts.

About 95-99% of P added as MCP was recovered in the 10% HCl solution at the highest level of addition of the MCP and gypsum mixture (0.6 g MCP added with 1 g gypsum in 40 ml HCl; Table 4.4). Within an experimental error (cv 5%) this indicated that all MCP was dissolved. However, the recovery of SO_4-S in the 10% HCl was decreased when higher rates of gypsum were added. This indicates that for fully acidulated P fertilizers that contain essentially only MCP and/or gypsum, the amount of gypsum in the fertilizer is the prime factor controlling the minimum amount of acid needed for complete dispersion of the fertilizer for particle size analysis.

4.4.1.2 *Estimation of the amount of acid used for complete dispersion*

For S^0/PAPR fertilizers, however, it would be expected that some acid would be involved in the acidulation of RPR-residue and the concentration of HCl will fall below 10% and maximum gypsum dissolution may not occur (Linke, 1958). Thus less gypsum would be expected to be dissolved. The influence of the consumption of HCl for RPR dissolution on the dissolution of gypsum was examined. Mixtures of NCPR, MCP and gypsum, in a proportion similar to those found in a range of acidulated P and S fertilizers, were dispersed in 10% HCl. The amounts of P and S added and recovered in HCl as well as the residue weights are presented in the Table 4.5.

The amount of rock phosphate residue in PAPR and the corresponding amount of HCl to acidulate it can be calculated from the following equation (Braithwaite, 1986).

$$2Ca_5(PO_4)_3F + 12yH_3PO_4 \xrightarrow{H_2O} 9yCa(H_2PO_4)_2.H_2O + 2(1-y)Ca_5(PO_4)_3F + yCaF_2 \qquad (4.9)$$

where y = acidulation factor, for PAPR 0.75> y >0.

$$2Ca_5(PO_4)_3F + 12\,HCl \xrightarrow{H_2O} 3Ca(H_2PO_4)_2.H_2O + 6\,CaCl_2 + CaF_2 \qquad (4.10)$$

For example, 7 g of 30% partially acidulated phosphate rock (y = 0.3) would contain 3.5 g of NCPR residue which requires 0.042 mole or 1.52 g of HCl for complete dissolution. This suggests that it would neutralize 7.3% of total acid (20.9 g) in 200 ml of the 10% HCl used for the dispersion. If the fertilizer also has a high gypsum content then the decrease in the acid strength may reduce $CaSO_4$ solubility. The gypsum content in these PAPR fertilizers is considerably smaller than that in sulphurized superphosphate especially when phosphoric acid is used for partial acidulation. Normally in commercial grade H_3PO_4 one can expect up to 5.8% by weight of H_2SO_4 (Braithwaite, 1986).

Table 4.5 The amounts of added and recovered P and S after 10% HCl extraction of various MCP/NCPR gypsum mixtures and the calculated final acid concentration.

MCP added mg	NCPR added mg	Gypsum	MCP/PR P ratio	P added mg	P recov. in 40 ml	S added	S recov. 10% HCl	Acid depleted (%)	Acid straight
300	556	800	1	144	141	149	94	5.5	8.7
200	370	800	1	96	96	149	112	3.7	9.2
100	185	800	1	48	48	149	134	1.8	9.6
480	222	800	4	146	142	149	112	1.2	9.7
320	148	800	4	98	98	149	130	1.5	9.7
160	74	800	4	49	47	149	138	0.7	9.8

NB: Results are means of 3 replicates and in all cases standard deviation was less than 5% of mean unless stated.

The results in Table 4.5 show that as the ratio of soluble P to rock phosphate P increases the recovery of S increases indicating that less gypsum is dissolved. This is simply due to the consumption of HCl for the dissolution of RPR. From equation 4.10 it can be calculated that the strength of HCl is decreased from 10% to about 9% at the highest addition of NCPR (556 g). Thus to ensure that compound fertilizers containing S^o, gypsum and unacidulated phosphate rock are fully dispersed in 10% HCl, additional HCl should be added to account for the acid consumed by the phosphate

rock residue. The minimum amount of acid required for dispersion can be calculated by assuming that:

(i) 18 g of gypsum dissolves in 1000 g of 10% acid (Table 4.2) and
(ii) 1 mole of rock phosphate P requires 2 moles of H^+ for complete acidulation (Equation 4.10) (10% HCl contains 3.3 g HCl per 100 ml)

A) Amount (g) of 10% HCl required for the dissolution of gypsum
= wt. fertilizer x (% SO_4 x 172 x 1000)/ (100 x 32 x 18)
= wt. fertilizer x 2.98 x % SO_4-S

B) Amount (g) of 10% (w/w; or 1:3 v/v HCl:H_2O) HCl required for the dissolution of residual PR
= wt. fertilizer x (% P in residue x 36.5 x 2 x 100) / (100 x 31 x 10)
= wt. fertilizer x % P in residue x 0.24

C) Total minimum amount (g) of 10% HCl required (simplified)
= wt. fertilizer [(% SO_4-S x 2.98) + (%P residue x 0.24)]
= wt. fertilizer (% SO_4-S x 3 + % residue P x 0.25) (4.11)

Residue P is calculated as the difference between water soluble P and total P and expressed as a percent by weight of the original fertilizer.

4.4.2 Evaluation of Dispersants Using Various Fertilizer Materials

Different solvents namely, alcohol, water and HCl were evaluated as dispersants for a range of S^o fertilizer materials. The suitability of these dispersants were compared using groups of similar S^o containing fertilizers. Water was found to be an effective dispersant for the S^o granule or prilled fertilizers such as S^o-bentonite prill (Boswell *et al.*, 1988a). Pure S^o fertilizers screened agricultural grade S^o because of its hydrophobic nature, however, disperse better in ethyl alcohol than in water (Janzen and Bettany, 1987b).

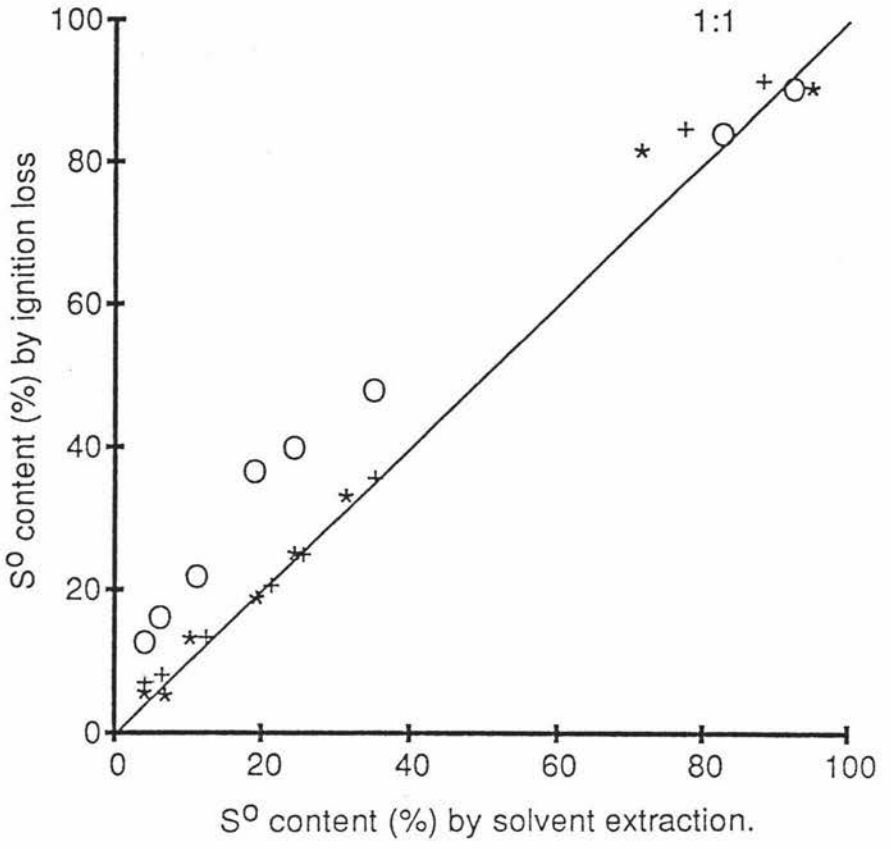
4.4.2.1 *Measurement of elemental S in fertilizers by loss on ignition*

Prior to examining the effects of dispersants, the effect of fertilizer form on the accuracy of S^o determination by loss on ignition was examined. The loss on ignition method is a simple technique requiring inexpensive equipment which leads itself to routine analyses for fertilizer quality control. Several components of the residues obtained after sieve analyses may also contribute to losses on ignition e.g., $CaSO_4.2H_2O$ and PR residues. The S^o of whole fertilizer samples (Table 4.6) and their residues (after water or acid washing) were determined by both the loss on ignition method and the standard acetone extraction technique developed in Chapter 3 Section 3.4.1.9.

There was a good agreement between the S^o content determined by solvent extraction and loss on ignition in fertilizers and their residues containing greater than 80% S^o, such as the prilled forms of S^o. For fertilizers with lower S^o contents the loss on ignition method greatly over-estimated the S^o contents, unless the whole sample is prewashed in water or 10% HCl. Even with prewashing the loss on ignition method was inaccurate for determining S^o contents below 10% (Table 4.6 and Figure 4.3). Inaccuracy of the ignition method at S^o contents <10% is presumably due to the very small absolute weight losses with the chosen sample size. It would be possible to overcome this inaccuracy with phosphate rock based fertilizer by increasing the weight of fertilizer dispersed, provided sufficient acid is used to dissolve the phosphate rock and gypsum in the fertilizer samples. With larger weights of sieved separates a significant weight loss will occur upon ignition for S^o determination.

4.4.2.2 *Sulphurized superphosphate*

The major binding agents, MCP and gypsum in these fertilizers can be dissolved by water and HCl. The efficiency of the two dispersants in the two wet sieving technique were compared and the particle size distributions after dispersion are shown in Table 4.7 and Figure. 4.4.



gure 4.3 Relationship between the amount of S^0 in a range of fertilizers measured by acetone extraction and by loss on ignition.

Figure 4.4 S particle size distribution in sulphurized superphosphates measured by three techniques. Water dispersion/wet sieving (WW), Acid dispersion/wet sieving (AW) and Acid dispersion/dry sieving (AD).

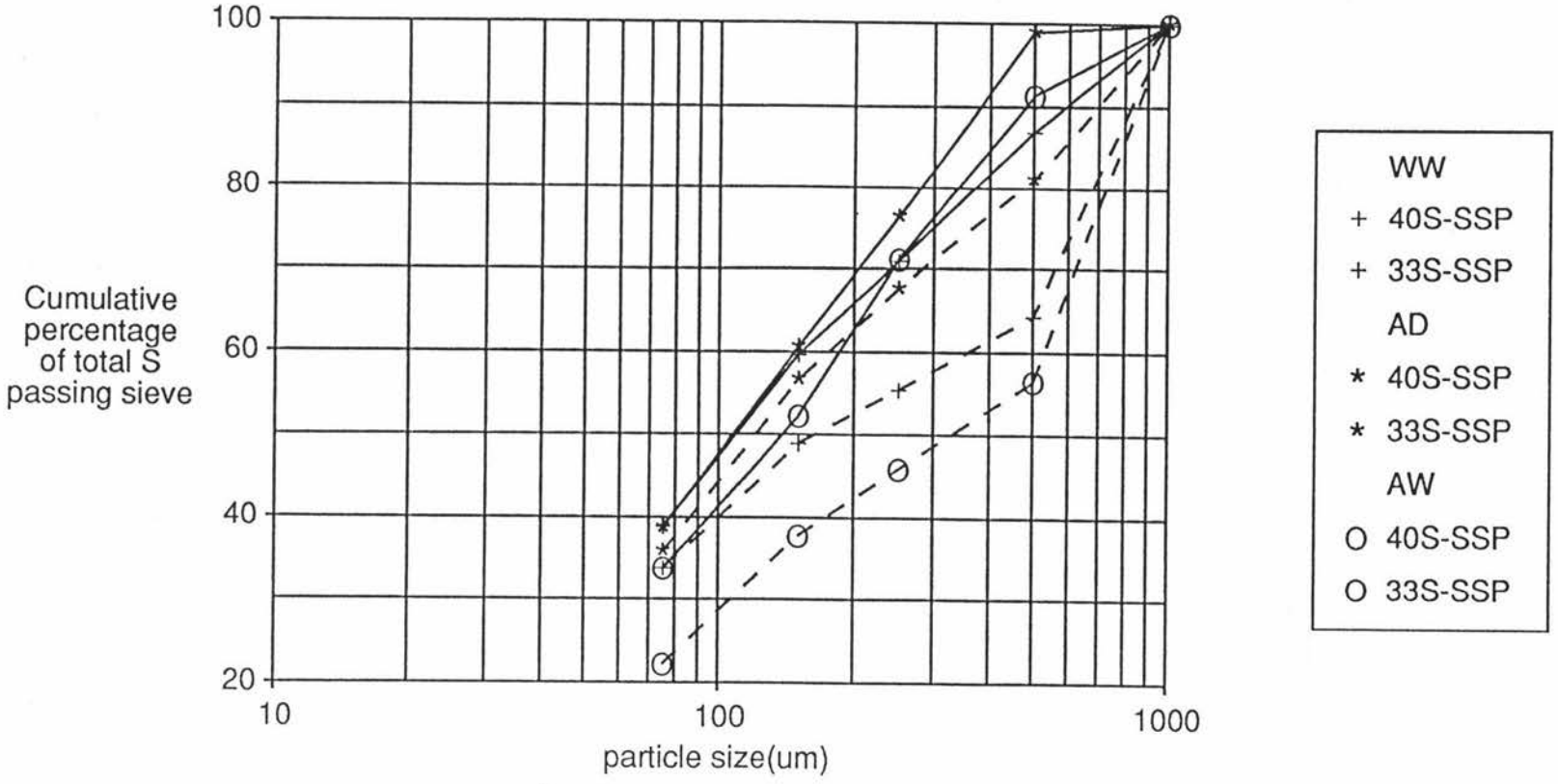


Table 4.6 Total S^o content as analysed by loss on ignition and solvent extraction on the fertilizers samples after water or acid washed compared to the whole sample analysis.

Fertilizer	moisture content %(wt/wt)	P%	S^o Content % (wt/dry wt) Solvent Extract. Whole sample	Loss on ignition Whole sample	Water washed	Acid washed
Tiger 90	0.33	-	92.2	90.6	90.3	91.6
Sul85 prill	0.55	-	82.2	84.2	81.5	84.8
40% S^o Super	5.72	6.0	34.8	48.2	33.0	35.9
33% S^o Super	4.98	7.0	24.1	40.1	25.0	25.0
S-Super extra	2.32	7.0	18.9	36.9	18.8	20.7
Hyphos Supreme	2.90	13.8	11.0	22.0	13.1	13.5
Hyphos S	3.68	14.5	6.0	16.4	5.2	8.2
rockphos	3.53	10.7	4.0	12.9	5.5	7.1
Ag.Grade S			100.0			

Dispersion in acid produced lower residue weight than dispersion in water. Even with fully acidulated superphosphate further acidulation of the residue was achieved when the fertilizer is dispersed in 10% HCl or some gypsum remains in the water washed residues. The finest fraction (<75 μm) obtained with water dispersion has the lowest S^o content and thus appears to contain most of the phosphate rock residue (for SSP manufacture in New Zealand approximately 75% of the PR is ground to <75 μm). The S^o content of most fractions increased when acid dispersants were used, indicating that PR components are dissolved. With water dispersion this would remain undissolved.

4.4.2.3 *Sulphurized PAPRs*

All these fertilizer forms produce lower residue weights upon dispersion in acid than in water because they contain between approximately 50 to 70% unacidulated reactive phosphate rock (see equation 4.8) which mostly dissolves in the 10% HCl wash (Table 4.8). The removal of residual PR and other components such as mono-calcium phosphate and gypsum by acid washing allows the loss on ignition method of S^o analysis to give a closer estimate of the actual (determined by acetone extraction) S^o content of each fraction (Table 4.6).

Table 4.7 Particle size analysis of non-water soluble and non-acid soluble residues of a range of sulphurized superphosphates

Fert. particle size (mm)	Residue weight as cumulative % of fertilizer weight			($\%$ S^o)[a]			Cumulative $\%$ S^o passing each sieve opening		
	WW[b]	AW[c]	AD[d]	WW	AW	AD	WW	AW	AD
40% S^o-SSP									
>0.5	45.9	36.9	41.1	67.4	98.8	73.5	100	100	100
<0.5	29.4	22.4	32.1	81.2	86.2	82.3	64	56	81
<0.25	26.0	18.3	26.4	79.6	96.2	92.3	55	46	68
<0.15	23.4	15.5	22.2	82.4	100.0	97.8	49	39	57
<0.075	17.7	10.4	14.7	59.3	70.9	85.5	34	22	36
33% S^o-SSP									
>0.5	42.3	25.2	37.2	60.9	76.2	41.6	100	100	100
<0.5	37.2	22.8	36.6	73.4	100.0	49.3	87	91	99
<0.25	31.9	18.6	25.0	78.6	86.3	63.2	71	71	76
<0.15	28.5	14.0	18.7	88.3	85.4	77.1	60	52	60
<0.075	22.8	9.5	11.6	41.2	74.9	84.9	39	33	39
S^o-SSP extra									
>0.5	38.1	20.4	33.8	71.0	60.7	61.8	100	100	100
<0.5	34.9	17.7	33.5	78.6	55.0	50.5	88	89	99
<0.25	31.5	15.1	23.2	84.4	48.9	58.5	74	79	74
<0.15	29.0	12.7	16.8	81.6	59.0	69.0	63	71	56
<0.075	22.8	8.3	9.1	31.1	95.0	72.6	37	53	31

[a] S^o content determined by acetone extraction on each sieve separation.
WW[b] water dispersion/wet sieving; AW[c] acid dispersion/wet sieving
AD[d] acid dispersion/dry sieving.
NB: Results are means of 3 replicates and in all cases mean deviation was less than 10% of mean unless stated.

Table 4.8 Particle size analysis of non-water soluble and non-acid soluble residues of a range of sulphurized partially acidulated phosphate rocks.

Fert. particle size (mm)	Residue weight as cumulative % of fertilizer weight			($\%$ S^o)[a]			Cumulative $\%$ S^o passing each sieve opening		
	WW[b]	AW[c]	AD[d]	WW	AW	AD	WW	AW	AD
Hyphos-supreme									
>0.5	81.7	15.1	16.0	56.2	92.2	86.9	100	100	100
<0.5	72.3	8.3	9.1	27.7	49.5	77.2	48	38	51
<0.25	65.1	5.6	5.2	7.4	76.6	66.4	28	25	26
<0.15	46.1	4.2	3.1	5.0	35.6	61.6	14	14	15
<0.075	30.2	2.9	1.3	2.0	34.8	57.6	6	10	6
Hyphos-S									
>0.5	37.5	21.6	10.3	97.0	65.0	89.1	100	100	100
<0.5	20.2	19.6	5.4	90.0	3.2	66.0	29	25	41
<0.25	16.8	18.3	3.3	60.0	1.3	50.8	16	14	22
<0.15	14.5	14.6	2.0	39.0	1.0	50.5	10	11	12
<0.075	11.6	9.3	0.8	10.0	1.6	40.8	5	8	4
Rockphos S									
>0.5	73.5	9.7	14.8	44.6	90.0	75.9	100	100	100
<0.5	71.4	8.6	14.6	40.8	92.0	35.4	76	73	97
<0.25	69.7	7.7	9.3	6.0	75.0	21.7	58	53	52
<0.15	66.2	7.3	5.7	1.7	32.0	22.4	52	45	34
<0.075	48.6	6.5	2.1	3.6	23.0	31.0	45	38	15

[a] S^o content determined by acetone extraction on each sieve separation.
WW[b] water dispersion/wet sieving; AW[c] acid dispersion/wet sieving
AD[d] acid dispersion/dry sieving.
NB: Results are means of 3 replicates and in all cases mean deviation was less than 10% of mean unless stated.

4.4.2.4 *Prilled forms of S^o*

A. Water vs acid dispersion

The prilled products which are cemented with bentonite dispersed to a greater extent in water than in acid. The poor dispersion of bentonite in 10% HCl is probably because bentonite clay tends to flocculate at lower pH. However, irrespective of the dispersion media, particle breakdown was poor and over 84% of the S^o remained >250 μm in diameter (Table 4.9. and Appendix 4.2).

Table 4.9 Particle size analysis of non-water soluble and non-acid soluble residues of prilled S^o fertilizers.

Fert. particle size (mm)	Residue weight as cumulative % of fertilizer weight			($\%$ S^o)[a]			Cumulative % S^o passing each sieve opening		
	WW[b]	AW[c]	AD[d]	WW	AW	AD	WW	AW	AD
Tiger 90									
>0.5	98.9	98.4	98.6	96.9	88.4	89.5	100	100.0	100
<0.5	16.5	4.6	7.4	100.0	45.9	87.2	16	3	7
<0.25	13.5	3.9	3.8	100.0	85.1	89.1	12	3	4
<0.15	11.0	3.5	2.4	97.9	77.8	83.0	10	2	2
<0.075	7.6	3.0	1.2	78.3	54.7	79.8	6	2	1
Sulphur 85									
>0.5	100.0	95.5	96.2	78.2	87.9	76.0	100	100	100
<0.5	23.6	9.4	68.3	78.6	54.4	87.5	14	2	73
<0.25	16.6	6.7	40.5	80.1	35.0	95.6	6	0	41
<0.15	15.4	6.5	21.9	72.7	39.3	57.5	5	0	18
<0.075	10.6	6.3	4.4	1.6	0.1	86.0	0	0	5

[a] S^o content determined by acetone extraction on each sieve separation.
WW[b] water dispersion/wet sieving; AW[c] acid dispersion/wet sieving
AD[d] acid dispersion/dry sieving.
NB: Results are means of 3 replicates and in all cases mean deviation was less than 10% of mean unless stated.

B. Alcohol vs water dispersion

Various S^o fertilizers were dispersed by shaking with either water or alcohol. The particle size distribution was determined by wet sieving. A better dispersion of ground S^o was obtained with alcohol than with water resulting in a greater proportion of S^o (85%) passing through the 500 μm sieve in alcohol than in water (75%) (Bolan *et al.* 1988). For prilled S^o fertilizers, however, there was less dispersion in alcohol presumably because water was not available for hydration and subsequent expansion of the Na-bentonite. Also for fertilizer samples containing other components such as phosphate rock (Hyphos S), there was absolutely no dispersion in alcohol. This clearly indicates the limitation of the use of alcohol for wet sieving of compound fertilizers containing S^o (Bolan *et al.* 1988).

4.4.3 Effect of Fertilizer to Solution Ratio on the Determination of S Particle Size

Earlier work using gypsum, MCP and RPR mixtures (Section 4.4.1.2) demonstrated that, for S^o particle size analysis in P and S compound fertilizers, the fertilizer to solution ratio must be controlled to ensure complete dissolution of the cementing agent and dispersion of the fertilizers granule. Water at laboratory temperature (about 16°C) will dissolve 1.8 g of MCP and 0.2 g of gypsum per 100 g (The solubility product of gypsum in water is 1.95×10^{-4}). In HCl, maximum gypsum solubility occurs in approximately 10% HCl (1.8 g 100 g^{-1}). Rockphos (Sulphurised PAPR with 4.5% SO_4-S and 4.0% S^o) was dispersed in either water at ratios of 1:100 and 3.5:100 (fertilizer:solution) or in 10% HCl at 3.5:100.

The results are presented in Table 4.10. Rockphos contained 4.5% S as sulphate, which would be equivalent to 24% gypsum. Thus at the 3.5:100 (Rockphos:water) ratio the solubility of gypsum in water was exceeded. Significantly more residue (40%) and S^o(46%) remained in the >500 μm particle size fraction (Table 4.10), when the narrower (3.5:100) fertilizer:water ratio was compared to the wider (1:100) solution ratio. When 10% HCl was used as the dispersant the gypsum solubility is not exceeded and the S^o particle size distribution was similar to that measured after dispersing the sample in water at the wider (1:100) fertilizer to solution ratio.

4.4.4 Comparison of Double and Single Acid Wash

The dispersion procedure using a double 10% HCl wash (Rogers and Braithwaite, 1984) was compared with the modified single 10% HCl wash and overnight shaking using a range of sulphurized fertilizers. For both methods the dispersed samples were wet sieved for particle size analysis. Residue weights and the S^o size distribution of the samples are presented in Table 4.11 (and Appendix 4.3)

Table 4.10 The effect of solvent and fertilizer to solvent ratio on the measurement of the S particle size distribution in Rockphos S^o.

Solvent; Fert:Solution ratio Particlsize (μm)	Cumulative residue weight of fertil.	residue	S^o (%)	Relative S in each residue fraction
Water (1:100)				
>500	73.5	100.0	45.3	100
<500	71.4	97.0	41.7	76
<250	69.7	95.0	6.1	58
<150	66.2	90.0	1.7	52
<75	48.6	66.0	3.6	45
Water (3.5:100)				
>500	79.8	100.0	3.9	100
<500	47.5	59.9	1.3	54
<250	45.9	56.6	3.2	44
<150	42.3	53.4	1.3	39
<75	30.1	37.7	3.9	34
10% HCl (3.5:100)				
>500	9.7	100.0	90.0	100
<500	8.6	88.2	92.0	73
<250	7.7	79.4	75.0	53
<150	7.3	74.8	32.0	45
<75	6.5	66.8	23.0	38

NB: Results are means of 3 replicates and in all cases mean deviation was less than 10% of mean unless stated. S^o content determined by acetone extraction on each sieve separation.

Table 4.11 The residue weight and S particle size distribution of fertilizer as dispersed by single and double 10% HCl.

Fertilizer particle (μm)	Single HCl			Double HCl		
	cumulat. resid wt.	cumulat. S %	% S of fraction	cumulat. resid wt.	cumulat. S %	S % of fraction
50%S^{o}-SSP						
<75	15.0	15.0	98.7	8.7	8.6	97.9
>75	30.1	30.1	99.1	21.0	20.8	99.1
>150	44.2	44.2	99.3	35.0	34.6	98.6
>250	66.0	66.2	99.8	56.3	56.1	99.9
>500	00.0	100.0	98.4	100.0	100.0	99.9
sum %S^{o} by wt.		44.0			42.8	
40%S-SSP						
<75	26.8	26.7	98.7	16.9	16.9	98.9
>75	40.6	40.4	98.8	29.9	29.8	98.8
>150	48.0	47.8	98.9	37.6	37.4	99.1
>250	58.6	58.4	99.8	46.8	46.5	98.0
>500	100.0	100.0	99.7	100.0	100.0	99.8
sum %S^{o} by wt.		38.7			38.4	
33%S-SSP						
<75	39.2	39.1	98.3	25.7	26.0	98.3
>75	60.6	60.6	99.0	45.3	46.0	98.5
>150	71.6	71.2	95.2	58.7	57.7	84.8
>250	85.6	85.4	99.7	76.0	75.4	99.2
>500	100.0	100.0	99.9	100.0	100.0	99.8
sum %S^{o} by wt.		26.6			25.1	
Rockphos						
<75	56.0	57.0	76.1	56.5	57.2	74.8
>75	68.0	63.1	38.3	67.5	62.6	35.5
>150	75.5	72.2	89.6	74.1	71.4	99.3
>250	87.5	87.4	95.1	86.2	85.9	88.6
>500	100.0	100.0	75.5	100.0	100.0	75.3
sum %S^{o} by wt.		5.8			5.9	
Hyphos-S						
<75	34.5	35.0	69.5	29.5	29.6	66.9
>75	44.5	41.6	45.4	39.3	36.3	45.7
>150	54.5	55.1	92.8	48.6	49.5	94.8
>250	67.2	71.8	90.1	59.3	64.0	89.9
>500	100.0	100.0	59.0	100.0	100.0	58.8
sum %S^{o} by wt.		6.1			6.4	

NB: Results are means of 3 replicates and in all cases mean deviation was less than 10% of mean unless stated. S^{o} content determined by acetone extraction on each sieve separation.

The total dispersed residue weights were similar for both dispersion methods. This confirmed that the single acid wash with overnight shaking was able to completely dissolve the gypsum present in each fertilizer. There was a slightly larger residue weight in the finer (<75 μm) particle size fraction but slightly less residue weight in the coarser fraction (>500 μm) after the single acid than after the double acid wash. This was possibly due to the breakdown of the coarser particles during the overnight shaking. However, as discussed in the next section these differences in particle size would not influence the agronomic classification of these fertilizers according to the recently published New Zealand MAF-Tech criteria for S^0 use (Sinclair *et al.*, 1985; Boswell and Swanney, 1988; see Chapter 2, Table 2.2).

4.4.5 Evaluation of Sieving Techniques

The techniques of water dispersion and wet sieving (WW) and wet (AW) and dry (AD) sieving after the acid dispersion were compared using a range of S^0 containing fertilizers.

4.4.5.1 *Sulphurized superphosphate*

The results for the S^0 particle size analysis of a range of sulphurized superphosphate (S^0-SSP) using the three techniques described above were presented in Table 4.7; Figure 4.4.

The 33% S^0-SSP and S^0-SSP extra contain S^0 of finer particle size than 40% S^0-SSP. All three techniques of S^0 particle size analysis gave similar results for the two supers containing the finer S^0. For the coarser particle sized 40% S^0-Super, however, acid washing followed by dry sieving (AD) produced a finer S^0particle size analysis than either of the two wet sieving methods (WW and AW). It is believed that the dry sieving technique (Rotap shaker) is moderately abrasive and serves to reduce the particle size of the coarse S^0 fraction which is greater than 0.5 mm diameter in sulphurised superphosphates.

4.4.5.2 *Sulphurized PAPRs*

Acid dispersion and dry sieving produced marginally less coarse particle size S^O (>500 μm) than the other two methods (Table 4.7), particularly with the Hyphos-S^O and Rockphos-S^O fertilizers. The general observation is that all three methods produced similar S^O particle size distributions for each fertilizer material.

4.4.5.3 *Prilled forms of S^O*

In general there is little agreement between the S^O particle size analysis for sulphur 85 obtained by the three different techniques. When the wet sieving technique is employed, the dispersion of the material is greater in water than in acid due to the greater dispersion of bentonite in water. However, acid washing followed by dry sieving produced a finer S^O particle size analysis; probably due to the break down of coarser particles during the more abrasive shaking (Table 4.9).

4.4.6 Factors Affecting S^O Particle Size in Sulphurized Superphosphate

Additions of S^O in the molten form (wet mix) is preferable for the manufacture of sulphurized superphosphate than the addition of finely divided screened or ground S^O (dry mix). This is due to the fire hazard associated with S^O dust from finely screened S^O.

In the process molten S^O is metered into the mixing section where the reacting mass has a temperature in excess of 120°C (the melting point of S^O). The S^O is still in molten form leaving the mixer but soon solidifies with the rapid setting of superphosphate. This process can produce very finely-divided sulphur particles up to 80% finer than 0.15 mm (Charleston and Laing, 1985). However, the results for the samples of sulphurized superphosphate analysed in this thesis (Figure 4.4 and 4.5) show that as the S^O content of the sulphurized superphosphate increases all methods of S^O particle size measurement record a greater coarser S^O particle size (>250 μm).

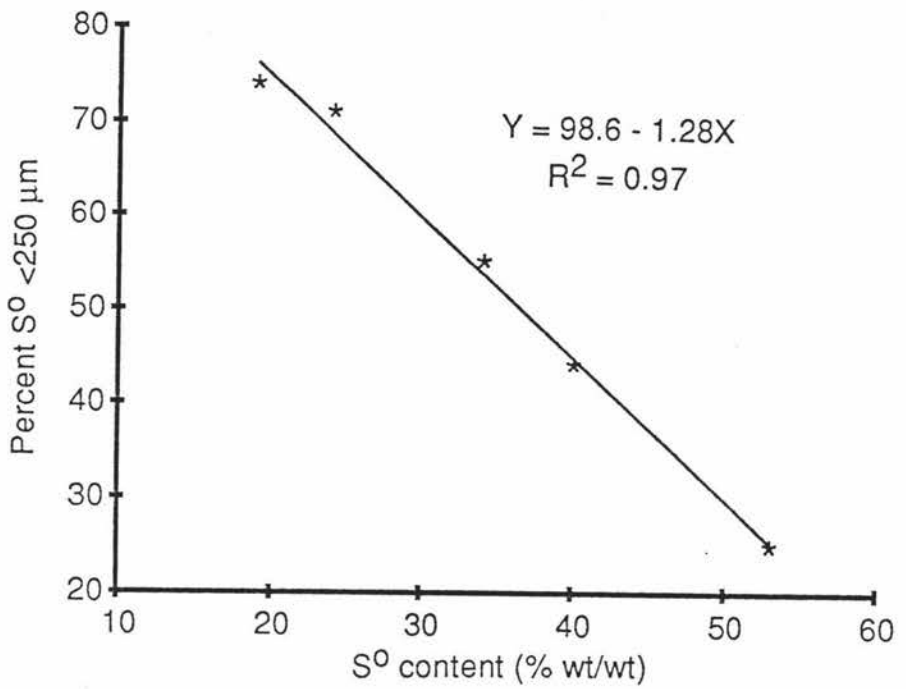


Figure 4.5 The relationship between increasing S content in sulphurized superphosphates and decreasing percentage S less than 250 µm in diameter.

Several factors influence the S^o particle size distribution of the sulphurized superphosphate fertilizer, these include the temperature of the acid and molten sulphur, the bulk reaction size and the mixing speed (Rogers and Braithwaite, 1984). The reaction temperature has a major effect, with the finer sulphur distributions being associated with higher reaction temperature. The increase of S^o particle size in the higher S^o content fertilizer (Figure 4.4), suggests that it is likely that at higher S^o additions there is less chance of S^o being separated by the PR, MCP and gypsum components of the fertilizer. Thus on solidification larger S aggregates are formed.

4.4.7 Recommended Methods

After the evaluation of the different dispersion and sieving techniques used above, the following section summarizes methodology which is recommended for determining S^o particle analysis of a wide range of fertilizer materials.

4.4.7.1 *Sampling*

Use a multi-point sampling technique to obtain a 5-10 kg sample of the fertilizer to be analysed. Obtain replicate subsamples of fertilizer (approximately 50 g), by using either a standard riffle box or a 'quartering technique' on a flat table. Subsample taken to calculate moisture content.

4.4.7.2 *Analysis*

Prior to particle size analysis determine the S^o, SO_4-S, total S and water soluble P and total P content of fertilizer.

4.4.7.3 *Dispersion*

A. *Sulphurized phosphate fertilizer*:

It is more practicable under laboratory conditions to use 10% HCl as the solvent in these fertilizer materials. The minimum amount of acid required or the fertilizer to solution ratio can be calculated from equation 4.10. If the fertilizer contains more than 60% gypsum by weight the fertilizer to solution ratio must be widened. The proposed procedures are as follows:

Weigh 25 g of each subsample into a 1 litre screw capped, plastic container. Add 1 litre (excess) of 10% HCl (1:3, HCl:water) and leave container uncapped for 1 hour to allow any gas to escape; gently stir or shake the container before shaking (end over end) the sample for 16 hours.

B. *Prilled S^o/Sodium bentonite*:

If no gypsum or P is present in the S^o fertilizer i.e., a prilled form of S^o, it is recommended that water is used for dispersion instead of acid. In acid washing the residue may become slightly sticky and difficult to remove from the plastic container and to pass through the sieves; addition of ethyl alcohol will overcome this problem.

4.4.7.4 *Sieving*:

A. *Alternative 1 - wet sieving*

Prepare a nest of stainless steel sieves (500 µm, 250 µm, 150 µm and 75 µm). Use strong elastic-bands to seal joints between sieves and to hold the rest of sieves together. Clamp sieves above a 5 litre plastic bucket.

Decant the acid:fertilizer suspension through the sieves. Immediately wash the fertilizer with a jet of water (a small pressurized glasshouse sprayer is suitable for this purpose). Lift the 500 µm sieve, leaving the other 3 in place and continue washing until the effluent from the 500 µm is clear. When the effluent is clear, rinse the sieve and the contents with a spray of 95% Ethyl alcohol. Place a square of brown paper under each sieve and transfer the sieve to a fume hood to dry before finally drying in an oven at 30°C (approximately 15 minutes). Repeat this process with the remaining sieves.

The finer particle size fraction (<75 µm) is collected in approximately 3 litres of suspension in the bucket. Allow the suspension to stand for overnight before decanting through a pre-weighed filter paper on a large Buchner funnel or through Pyrex Gooch Crucible. Wash the filter paper or Gooch Crucible and the contents with water and finally with alcohol. Dry and weigh the paper or the Gooch Crucible and the contents as above.

B. *Alternative 2 - Dry sieving*:

Collect acid dispersed fertilizer residue on a filter paper in a large Buchner funnel and thoroughly wash the collected material with water followed by alcohol. Dry the sample in an oven at 30°C.

Place the dried sample on a nest of sieves and either use a standardized Rotap shaker or a hand sieving technique. With some fertilizers e.g., sulphurized PAPR, a hard cake may form on the filter paper making this method unsuitable for determining S^O particle size.

4.4.7.5 *Estimation of S^O content*:

After acid or water washing the S^O content of each fraction can either be determined by solvent extraction or after acid washing by loss on ignition (4.3.2.4.A.)

A. *Solvent extraction*

Acetone extraction can be used as described in Section 3.4.1.9.

B. *Loss on ignition*

After drying all samples at 30°C, transfer the samples to porcelain crucibles for ignition. If a Gooch Crucible was used to collect the finer fraction, this crucible is ready for ignition after drying (The porcelain or Gooch Crucibles should have been preignited, stored in a dessicator and weighed). Return crucibles and samples to dessicator and weigh all crucibles again prior to ignition. Heat the crucibles in a muffle furnace at 550°C for 2 hours (or longer). After ignition return crucibles to dessicator prior to weighing. The percent loss in weight of each sample on ignition provides a close estimate of the S^O content (see section 4.4.2.1).

4.7 Conclusion

Methods for determining S^o Particle size in a number of S^o fertilizers were developed. The methods consist of three steps, dispersion, sieving and determination of S^o in the separate size fractions. The selection of appropriate dispersants and solid to dispersant ratio will depend on the characteristic of S^o fertilizer samples. For example, because of the cementing agents, MCP and Gypsum have greater solubility in 10% HCl than in water dispersion in 10% HCl is more practical for fertilizers containing P and gypsum such as sulphurised superphosphate and sulphurised PAPR. This allows S^o particles to be completely dispersed. Using 10% HCl also allows accurate S^o determination by loss on ignition. Water dispersion is more suitable for S^o bentonite prills because the Na-bentonite swells to a greater extent in water, and the use of alcohol during sieving is useful for dispersing pure S^o and for rapid drying.

A wet sieving technique is preferred to dry sieving because dry sieving can be more abrasive, thereby reducing the particle size of large S^o particles. In the case of dispersion with HCl, subsequent drying for dry sieving may cause caking and thereby increase the proportion of larger particle sizes.

Percentage weight loss on ignition is suitable for S^o analysis provided rock phosphate residues, gypsum and mono-calcium phosphate are removed by washing with 10% HCl prior to analysis.

CHAPTER 5

THE OXIDATION RATE OF S^0 IN INCUBATION SOILS AS INFLUENCED BY FORM OF S^0 FERTILIZER

5.1 INTRODUCTION

Elemental sulphur (S^0) represents an ideal high analysis sulphur fertilizer for use in New Zealand agriculture (Boswell 1987). Several different forms of S^0 fertilizer are available in New Zealand (see Section 5.3), to aid aerial application, granulated and prilled forms of S^0 are being manufactured. To maximize their agronomic effectiveness, agronomist recommending their use need to know the potential SO_4 release (oxidation) rates of each S^0 material.

Only a few attempts have been made to quantify differences in absolute S^0 oxidation rates of different S^0 forms. McCaskill (1984) compared absolute oxidation rates between the Mexican dark S^0 and Canadian bright S^0 in a pot experiment with maize but, he failed to find any significant differences. This was partly due to the large experimental errors involved in the technique he used to measure the amount of S^0 residue remaining in each pot. Most other work on S^0 oxidation in soils has not compared S^0 sources and in the majority of cases the source of S^0 used was not stipulated (Moser and Olson, 1953; Fox *et al.*, 1964; Li and Caldwell, 1966; Attoe and Olson 1966; Barrow, 1971; Janzen and Bettany, 1987b).

In addition to different forms of S^0 being used, much S^0 may be applied to pasture soils in the form of physical mixtures or granules of S^0 and reactive phosphate rock (RPR). The reason for this is that where RPR is recommended for used on pasture soil, many of these pastures are S responsive. Currently S^0 is the only form of S that can be applied with RPR if it is to retain a high P analysis and a cost advantage over acidulated fertilizers such as single superphosphate and Longlife Super (SSP:RPR ratio 2:1). Two pieces of information are therefore required i) the relative SO_4 release rate of the particular S^0 source to be used and ii) if combined with RPR will this rate change.

This chapter reports the results from short-term laboratory incubation studies designed to compare the relative rates of oxidation of the different sources of S^0 with that of

sulphuric acid grade sulphur (Canadian bright S^O) of a specific particle size range (100% <250 μm and 50% <150 μm). This particle size range is the one recommended by the Ministry of Agriculture and Fisheries for annual applications to pastures in warm temperate climatic zones or biennial applications in cool temperate climatic zones (Sinclair *et al.*, 1985).

5.2 OBJECTIVES

The objectives of the experiments discussed in this chapter are:

i) to examine the oxidation rates of different sources of S^O.

ii) to evaluate the effect of rock phosphate on the oxidation of S^O.

iii) to examine the effect of co-granulation of S^O with rock phosphate on the oxidation of S^O.

5.3 MATERIALS AND METHODS

5.3.1 Sources of S^O

The different sources of S^O listed in Table 5.1 are currently and/or potentially available as alternatives S^O fertilizers in New Zealand and were used in this study. These S^O sources can be categorized as follows:

5.3.1.1 *Geothermal S^O deposits*

S^O deposits occur in the Taupo and Rotorua geothermal area. A deposit at Lake Rotokawa is currently mined, on a commercial scale, for use as fertilizer. The deposit consists of several layers of different grades of material ranging from pumice overburden (20 to 50 m) to sulphur laminated silt stones and mud stones. Different grades for use as fertilizer (12, 20 and 50% S^O) material are achieved by selective mining and blending. A beneficiated material containing a minimum of 50% sulphur (fresh weight) is obtained through a flotation process. In all grades the S^O is finely divided and found to be as agronomically effective as imported agricultural grade sulphur (Bell-Booth *et al.*, 1988).

5.3.1.2 *Natural gas recovered or Screened* S^O (Bright yellow forms)

Recovery of S^O from natural gas in Canada is the major form of S^O imported into New Zealand for the manufacture of superphosphate and direct application as S^O fertilizer (Higgins 1983). It contains 100% S^O is bright yellow in colour. This sulphuric acid grade S^O is termed 'screened agricultural S^O'. Safety regulations require it to contain <20% of particles <150 μm for aerial application (Boswell and Swanney, 1986).

5.3.1.3 *Streford process* S^O

Recovery from the oil refining industry in Saudi Arabia. Damman S^O, is imported by Duraphos International (NZ). The S^O was also produced by Stretford process (H.J.Baker. Inc. L.A. California). It is brown in colour due to contamination of the S^O with trace amounts of hydrocarbons. Its S^O analysis is 100%. In this thesis it is referred to as dark S^O.

Table 5.1 Form and origins of S^O materials

Code	Fertilizer treatment[a]	Origin (process)
Ground S^O		
B	Ag.grade S^O, Bright yellow (BY)	Natural gas recovery, Canada
E	Damman S^O (BY)	Oil refineries, Saudi Arabia
C	Ground Dark S^O (Brown)	Oil refineries, ground in US
D	Ground Dark S^O (Brown)	Oil refineries, ground in NZ
F	Rotokawa S^O (pumice/S^O mixture)	Geothermal
Granule or prill		
K	Dark S^O prilled	Prilled
L	Prilled S^O rolled	Prilled then rolled to reduce particle size
M	Ground Dark S^O + Arad	granulated (this study)
N	Ag.grade S^O + Arad	granulated (this study)
O	Tiger 90	S^O bentonite prill
P	Rotokawa S^O granules	granule of > 1 mm.

[a] particle size distribution of the fertilizers are presented in Table 5.4. Granule and prill are dispersed in water before sieving.

5.3.2 Fertilizer Preparation

The different sources of S^O fertilizers and the nature of the mixtures with phosphate rocks used in the incubation are presented in Table 5.2. As received North Carolina and Arad phosphate rocks were used to make the S^O/RPR mixtures.

Ground Dark S^O (C in Table 5.2) was mixed with 'as received' Arad RPR to give a mixture (H) containing approximately 9% S. Granules (M) of this mixture were made using saturated KCl as a binder. Similar products (G, N and I, J) were made using screen agricultural grade (Ag.grade) sulphur (B) and Rotokawa S^O (F). The 0.5-1 mm particle size range of granulated products (M and N), together with the original ground dark sulphur (D) and Ag.grade sulphur (B), were used with prilled materials (K, O and P) in the soil incubation experiment.

All samples of S^O were riffled to give subsamples for physical characterization and chemical analysis (Table 5.2). The moisture contents of the material were determined after drying to constant weight at 30°C in a forced draught oven.

5.3.3 Particle Size Analysis

The particle size of the as received fertilizers were measured using both wet and dry sieving methods. Dispersion in water followed by wet sieving is considered essential for determining the 'effective particle size' of fertilizers prilled with bentonite which upon exposure to free water in field soils will disintegrate. Wet sieving was performed after each sample has been shaken overnight (end over end) with water at 1:100 (fertilizer:water) ratio. A visual end point procedure was adopted for wet sieving (Section 4.4.7.4.A.). For the ground S^O form the as received sample were dry sieved without dispersion using the end point technique on a vibratory shaker and a set of nested sieves.

Table 5.2 The fertilizer treatment and their percent S^o content.

code	Fertilizer treatment	moisture	S^o(%)	cv(%)
A	Control na		na	na
	Ground S^o			
B	Ag.grade S		100	1.6
C	Ground Dark S (US)	0.34	100	1.3
D	Ground Dark S (NZ)		100	1.9
E	Damman Saudi Arabia		100	1.8
F	Rotokawa S	9.94	54	0.8
G	Ag.grade S + Arad (dry mix)		9	0.5
H	Ground Dark S + Arad (dry mix)		9	0.3
I	Rotokawa + Arad (dry mix)		8	0.1
J	Rotokawa + NCPR (dry mix)		9	0.1
	Granule or prill			
K	Dark S prilled (1-2 mm)	0.09	100	1.8
L	Prilled S rolled (0.5-1mm)	1.31	100	1.6
M	Ground Dark S + Arad (granule)		9	0.5
N	Ag.grade S + Arad (granule)		9	0.2
O	Tiger 90	0.16	90	1.2
P	Rotokawa S granules		47	2.3

G, H, M and N = 1:10, S:RPR (wt:wt); J = 1:5.3, S:RPR (wt:wt).
na = not applicable.

5.3.4 Incubation Procedure

The objectives of the incubation were to establish the oxidation rates of the samples described in Table 5.2.

5.3.4.1 *The oxidation rate of different S^o sources*

Each fertilizer material (Table 5.2) was weighed into a 120 ml plastic container and thoroughly mixed with 41 g of moist Tokomaru silt loam (particle size <2 mm) which was taken from the top 3 cm of permanent pasture. This comprised one experimental unit. The soil contained 146 $\mu g\ S^o\ g^{-1}$ of moist soil. This is approximately equivalent to an application of 30 kg S ha^{-1} mixed with the top 2 cm of pasture soil. Moist soil,

freshly collected from the field, was used because the preliminary studies had shown that the S^0 oxidation capacity declines if the soil is kept in the laboratory for any length of time (Chapter 3).

The soil moisture content and temperature were kept constant throughout the incubation at 37% (wt/wt) and 20°C±2°C, respectively. The moisture content was very similar to the field soil moisture content found in the top 5 cm of this soil in spring.

To determine the rate of S^0 oxidation, the amount of S^0 remaining in each experimental unit was extracted with acetone as described in Section 3.4.1.9. The whole soil sample was extracted to overcome the variability caused by subsampling, particularly that associated with the larger particle size or granules at low application rate. The results are presented as the percentage of S^0 remaining at each sampling date.

5.3.4.2 *Investigation of some factors influencing the rate of S^0 oxidation*

In order to identify the possible reason for the higher oxidation rates of S^0 that were recorded in the presence of RPR (incubation experiment section 5.4.3), Ag.grade S^0 at the rate of 200 $\mu g\ g^{-1}$ soil was mixed with soil amended with various combinations of $CaCO_3$, $CaCl_2$, DCP and Arad RPR to produce a range of soil pH, Ca and P concentration (Table 5.3). Each replicate of a fertilizer treatment was weighed into a 120 ml plastic container and thoroughly mixed with 41 g of moist soil (Tokomaru silt loam of particle size <2 mm which was taken from the top 3 cm of permanent pasture as described in section 5.3.4.1) and had been stored moist at 4°C. Soil moisture and temperature were also maintained at 37% (wt/wt) and 20°C±2°C throughout the incubation respectively.

Three replicates of each treatment were destructively sampled at 4, 8 and 12 weeks for S^0 analysis as described the above section.

Table 5.3 The fertilizer treatments and the amounts of S^o, Ca and P added to the soil.

Treatment	S^o	Ca	P
		$\mu g\ g\ soil^{-1}$	
S^o	200	na	na
S^o+Arad	200	1763	271.2
S^o+DCP	200	880	271.2
S^o+$CaCl_2$	200	880	na
S^o+$CaCO_3$ (L_1)	200	880	na
S^o+$CaCO_3$ (L_2)	200	440	na
S^o+$CaCO_3$ (L_3)	200	220	na
S^o+DCP+$CaCO_3$ (L_1)	200	1760	271.2
S^o+DCP+$CaCO_3$ (L_2)	200	1320	271.2
S^o+DCP+$CaCO_3$ (L_3)	200	1100	271.2

L_1, L_2 and L_3 - The rate $CaCO_3$ at 26.6, 13.3 and 6.7 mg per experimental unit.
Arad rock 37.4% Ca, 14.4% P; DCP 18% P.
S^o/Arad 9.6% S, 90.4% P.
na = not applicable.

5.4 RESULTS AND DISCUSSION

5.4.1 S^o Concentration in Fertilizer Materials

Dark S^o (C and D) and Damman Saudi Arabian sulphur contained 100% sulphur (Table 5.1) and their dissolution characteristics in acetone were similar to the sulphuric acid grade sulphur (B). The Rotokawa sulphur granules and Tiger prills contained 54% and 90% S, respectively. Sulphate-S made up a negligible percentage of the total S content in all products.

5.4.2 Particle Size Analysis

The particle size distribution of each fertilizer material determined by both wet and dry sieving are given in Table 5.4 and the results are discussed with respect to the particle size criteria outlined by the New Zealand Ministry of Agriculture and Fisheries (MAF)

(Boswell and Swanney, 1988) for S^0 fertilizers that are to be directly applied to grazed pastures (see Chapter 2 Section 2.5 Table 2.2.b). In their simplest form they are interpreted as follows, for maximum effectiveness over a two year period S^0 particles should be <500 μm in the northern North Island and north western South Island, and <250 μm elsewhere (Boswell and Swanney, 1988).

Table 5.4 Particle size fractions of the fertilizers obtained by dry sieving as received materials and wet sieving water dispersed materials.

Code Fertilizer	Dry sieving of as received materials >500	<500 <250	<150	Wet sieving water dispersion >500	<500 <250	<150 (μm)
	% (w/w)					
Ground S^0						
C Dark S^0	9	44 20	27	14	41 17	28
D Dark S^0	44	25 12	19	37	27 14	22
E Damman S^0	21	19 14	46	25	19 17	39
B Ag.grad S^0	0	0 24	76	0	1 22	77
F Rotokawa S^0	nd	nd nd	nd	9	17 20	54
Granules or Prills						
K Dark S^0 prilled	100	0 0	0	100	0 0	0
L Prill S^0 rolled	66	17 7	10	70	14 5	11
O Tiger 90	100	0 0	0	66	13 7	14
P Rotokawa S^0 granule	98	0 0	2	92	0 1	7

nd - not determined

5.4.2.1 *Dark S^0*

Dark S^0 prills and their derived products (C and L) tended to disintegrate more readily on the 500 μm mesh opening under dry sieving than under wet sieving conditions. This led to greater percentages of these materials being retained on the 500 μm sieve during wet sieving.

Based on the wet sieve analysis (Table 5.4) dark S^0 samples (K and L), even after dispersion in water, are too coarse for the product to be an effective sulphur fertilizer

both for annual or biennial application in the cool temperate regions of New Zealand. (The bulk of the country, nearly all the South Island and the lower half of the North Island, see map of climatic region in Appendix 2.1.).

Only 63% and 36% of the dark S^0 product (D) tested was less than 500 μm and < 250 μm in particle diameter, respectively. In this form it is likely to be an extremely slow-release S fertilizer in any part of New Zealand. Only the ground dark S (C) approaches the particle size characteristics that are considered suitable for biennial application to pastoral soils in warm temperate climates (100% <500 μm, 50% <250 μm).

5.4.2.2 *Damman S^0*

After wet sieving Damman S^0 (E) has 11% more coarse material (>500 μm; Table 5.4) than the best Dark S (C), but has a higher percentage of fine particles. However, the particle size range is still coarser than the criteria for biennial application in a cool temperate climate and fails the 100% < 500 μm criterion for use biennially in a warm temperate climate. Obviously a rigid interpretation of the particle size criteria can not be applied to commercial fertilizer materials which may vary in their particle size range.

5.4.2.3 *Other S^0 forms*

Tiger 90 prills (O) and Rotokawa S^0 granules (P), even after wet sieving, did not have a satisfactory particle size range for application to pasture. However, the standard Ag.grade S^0 used in these experiments, easily satisfies the criteria for annual or biennial application in a warm temperate climate, and also for biennial (but not annual) application in a cool temperate climate. This S^0 material was used as the standard in the incubation studies.

The effectiveness of these alternative S^0 fertilizer was evaluated further by studying their oxidation rates in incubated soil.

5.4.3 Oxidation Rates

The percentages of added S^0 remaining at each sampling date are shown in Figures 5.1, 5.2 and 5.3. The results are discussed according to the different S^0 forms as follows:

5.4.3.1 *Granules and Prills S^0*

Granules of Rotokawa sulphur (P), Tiger 90 prills (O) and prilled dark S (K) had very low rates of oxidation during the incubation period (Figure 5.1).

The rate of release of SO_4-S from the prill or granules forms is affected by the rate of disintegration or dissolution of the granules as well as the surface area of the S^0 particle dispersed from them (Bosewell *et al.*, 1988a, 1988b). These materials require exposure to free water to enable dispersion. The immersion in water and shaking overnight (wet sieving) caused little disintegration of prills and had little effect on the particle size of these materials (Tiger 90 (O), Dark S prill (K), Prill S rolled (L), and Rotokawa S granule (P)), (Table 5.4). The lack of dispersion of these materials during wet sieving indicates that the reason for negligible oxidation was that the granules did not break down when incorporated into the soil. Indeed granules recovered from the soil still appeared to be the same size as those applied. Similarly McCaskill (1984), observed no disintegration of sulphur-urea melt and S-bentonite granule when these materials were incorporated into the soil. Others have also measured low oxidation rates from prilled and granular products in incubation and under field conditions (Friesen, 1988; Boswell *et al.*, 1988b).

Boswell *et al.* (1988b) found that the disintegration abilities of these materials depend on the bentonite content and the relative size or surface area exposed to the rain. The rate of S^0 oxidation can be improved if dispersion is achieved. Increased dispersion can be achieved by increasing the quantity of bentonite in the mixtures (Rothbaum *et al.*, 1983). A minimum sodium bentonite content of 10 to 15% is recommended to ensure adequate dispersion on wetting (Boswell *et al.*, 1988b). However, even when bentonite is added (e.g. Tiger 90 containing 10% bentonite) significant dispersion may only occur when the granule is on the soil surface, where there is more possibility to disintegrate than when it is incorporated into soil, which then remains unmixed.

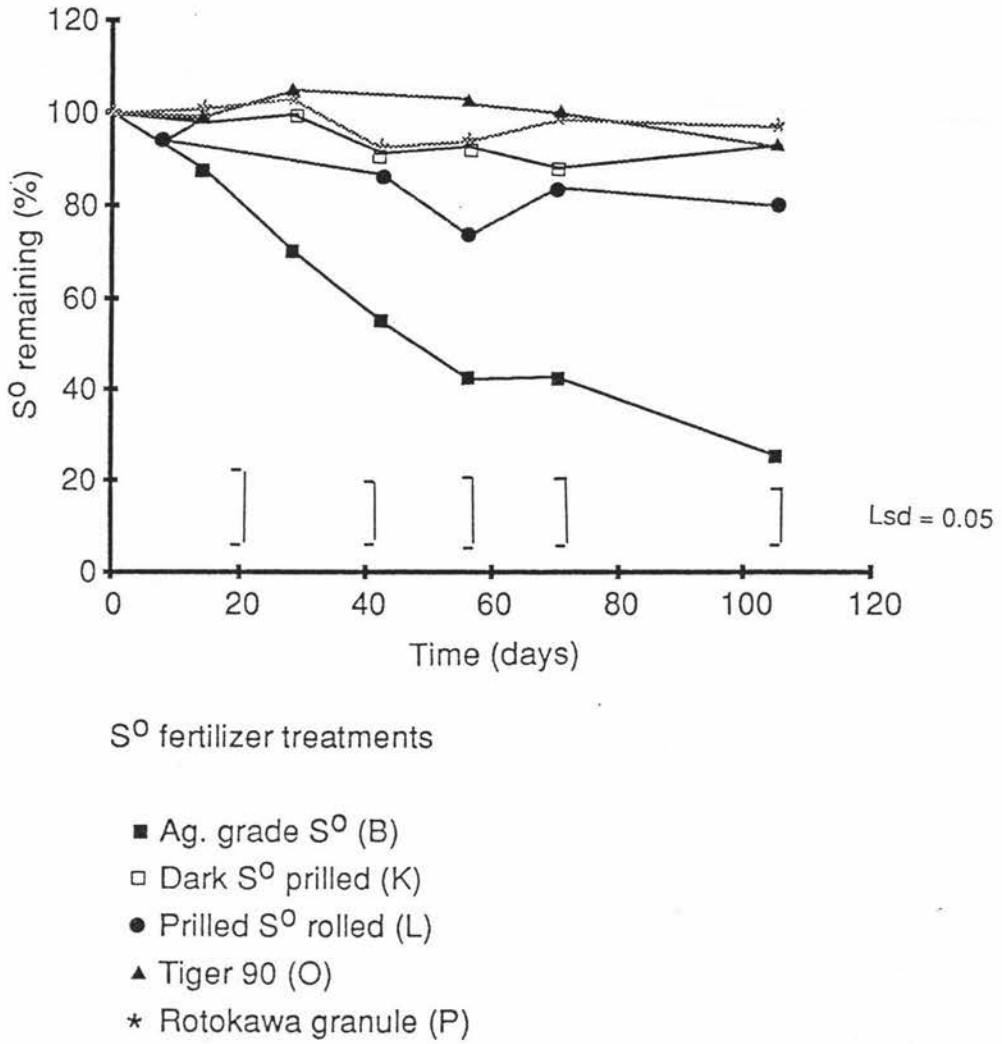


Figure 5.1 Percentage of S^0 remaining when prilled and granular forms of S^0 are incubated with soil.

At the present rate of application (146 μg S^0 g^{-1} moist soil) there was only one or two of Tiger 90 prills (O) and about 13 particles of Rotokawa granules (P) compared to 6,897 particles of Ag.grade S^0 (B) in one experimental unit. The low rate of oxidation may also be attributed to the lesser chance of S^0 particle being intercepted by an S^0 oxidisers, a factor which may become more important in soils with low populations of S^0 oxidiser (<4 g^{-1} soil in some New Zealand soil; Lee at al., 1988a). Once interception has occurred the S^0 oxidiser population will increase rapidly.

Application of S^0 in prills leads to a smaller volume of fertilized soil than if the S^0 is applied in finely divided form. The effective volume of soil available to buffer the acid produced is also smaller, Thus the slow oxidation rate of granules may be caused by the development of more acidic zones around and within the dispersed granule than around finely divided S^0 well dispersed through the incubating soil. It would appear to be important for large prills of water-dispersible materials to be surface-applied rather than incorporated. In a cropping situation (cultivated soil) it may be advisable to surface broadcast and to leave such fertilizers exposed on the soil surface for lengthy periods of time for the granules to break down before cultivation (Beaton, 1987).

5.4.3.2 *Ground S^0*

Different sources of S^0 fertilizer were incubated in their 'as received' particle size to investigate their SO_4 release characteristics and to determine whether further grinding of these materials is needed to provide rates of SO_4 release which are similar to the standard Ag.grade S^0 particle size range.

The rate of oxidation of Ag.grade S^0 was approximately twice that of the best of the alternative forms, which were Dammam S^0 (E) and dark S^0 (C and D) (Figure 5.2). After 10 weeks of incubation, only approximately 20% of the Damman S^0 and dark ground S^0 (E and D) had oxidised compared to 55% of the more finely divided Ag.grade S (B).

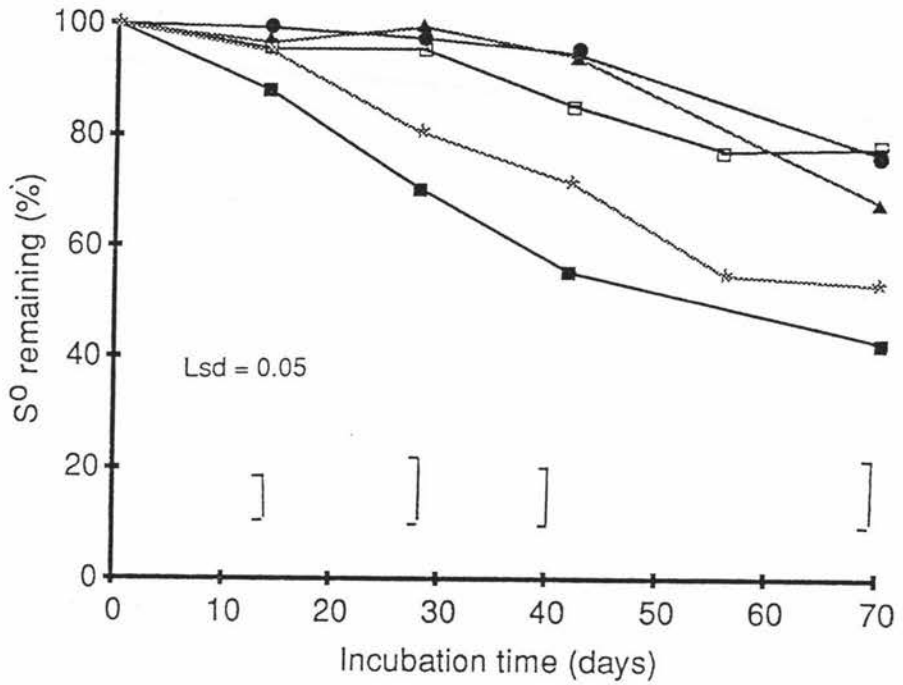


Figure 5.2 Percentage of S^0 remaining when different S^0 sources are incubated with soil.

5.4.3.3 *Comparison between all S^o forms*

The results (Figure 5.4) suggest that the rate of oxidation increased with the increase in the specific surface areas of these materials (Table 5.5), calculated from their particle size distribution (Table 5.4, dry sieving except for Rotokawa S^o). In order to compare the oxidation rate of different S^o fertilizer sources which differ in their particle size analysis, the average rate of oxidation for the incubation period was expressed per unit surface area per day ($\mu g\ S^o\ cm^{-2}\ day^{-1}$) (Table 5.5). This was calculated using the iterative procedure described in Chapter 8 section 8.2 and Appendix 8.1)

Table 5.5 Calculated surface area of S^o per g soil (cm^2), the amount of S^o oxidised and the specific oxidation rate (K, $\mu g\ S^o\ cm^{-2}\ day^{-1}$) at 10 weeks periods. Calculated using as received and dispersed particle size distributions (Table 5.4).

Code		Surface Area g^{-1} dry soil[a]	S^o Remaining	Oxidation rate per surface area		
				As received	Wet dispersion	
		cm^2	%	$\mu g\ cm^{-2}\ days^{-1}$		R^2
	Ground S^o					
B	Ag.grade S^o	0.0566	26	36.8	36.4	(0.98)
C	Ground Dark So	0.0303	64	21.6	21.9	(0.89)
D	Ground Dark S^o	0.0247	77	21.6	19.5	(0.75)
E	Damman S^o	0.0333	68	16.7	18.7	(0.71)
F	Rotokawa S^o	0.0446	40	nd	45.9	(0.95)
L	Prilled S^o rolled	0.0167	80	35.3	35.7	(0.71)
	Ground S^o + Arad rock					
G	Ag.grade S^o	0.0566	16	44.8	44.8	(0.98)
H	Ground Dark S^o	0.0303	51	37.3	37.8	(0.95)
I	Rotokawa S^o	0.0446	32	nd	51.4	(0.96)
	Granule or prill					
K	Dark S^o prilled	0.0077	92	38.8	28.9	(0.52)
O	Tiger 90	0.0184	93	19.3	5.7	(0.13)
P	Rotokawa S^o (granule)	0.0126	100	14.8	9.5	(0.19)

a Assume spherical particles size, calculated using as received and wet dispersed particle size distribution (Table 5.4) except for the prilled form the prilled diameter size were used in the as received.

The rate of SO_4 release predicted using the iterative least square fit for the different S^o sources are presented in Table 5.5. The fitted specific oxidation rate (K, $\mu g\ S^o\ cm^{-2}\ day^{-1}$) varied little between most S^o sources and were similar to that obtained from the slope (average of 24 $\mu g\ S^o\ cm^{-2}\ day^{-1}$) of the empirical linear relationship between initial surface area and total S^o oxidised (Figure 5.2). Most S^o sources (except the prilled O and P samples), has similar as received and dispersed particle sizes and therefore gave similar calculated K values. The prilled and granulated fertilizers forms (Tiger 90 (O) and granulated Rotokawa (P)) did not appear to disperse under incubation conditions. For this reason K values appear to be under estimated if the dispersed particle size distribution is used in the calculation of K by the iterative fit procedures (see discussed in chapter 8, section 8.4). However, some variation in the fitted specific oxidation rates probably arise from some error in estimating S^o surface area and the assumption that all S^o sources were spheres.

In comparing the Mexican dark S^o and the Canadian bright S^o, McCaskill (1984) found an overall (30%) higher oxidation rate from Mexican dark S^o. This was attributed to the small dust particles which stick on the Mexican dark S^o surface and hence provides a higher surface area than Canadian bright S^o.

In this present study, there were small and inconsistent differences in the specific rate of oxidation (K) of dark (C, D, L and K) and bright S^o (B, E). Despite the coarser nature of the 50 grade of Rotokawa pumice S^o (F) compared to Ag.grade S^o (B), the specific oxidation rate of this material over a 10 week incubation period was equivalent to that of the Ag.grade S^o (particle size range 100% <250 μm, 50% <150 μm). Geothermal S^o therefore appears to have similar rates of oxidation in soil as screened Ag.grad S^o. Similar agronomic effectiveness of these two sources was demonstrated by Bell-booth *et al.* (1988)

5.4.4 Effect of RPR and Granulation of S^O/RPR Mixtures

5.4.4.1 *Demonstration of effect*

The addition of Arad RPR to Ag.grade S^O (G), ground dark S^O (H) and Rotokawa S^O (I) and the addition of NCPR to Rotokawa as a simple mixtures caused small but consistent increases in the rate K and extent of S^O oxidation (Figure. 5.3 and Table 5.5). Lee *et al.* (1987) also found more S^O was oxidised in mixtures of RPR + S^O than in S^O alone treatment in incubations involving some soils selected from six major New Zealand soil groups. However, the effect was found to vary between the soil groups. No adequate reason for this variation was given.

The granulated form of S^O were found to decrease the rate of oxidation (Figures 5.1 and 5.4). The main reason for this, appear to be that the granules did not fully disintegrate under the conditions of the incubation and hindering contact between soil microbes and S^O. The extent of S^O oxidation of the prilled dark S^O (K) was markedly increased after roller crushing (L), confirming that the lack of prill dispersion is the main factor limiting the extent of oxidation of prilled dark S^O.

Several reasons have been given for the increase in the rate of oxidation due to the addition of RPR. These include:

i) increased supply of P which increases a P limited S^O oxidising microbial population (Bloomfield, 1967).

ii) Ca released from the phosphate fertilizers improves the microstructure of the soil and thereby improving water and air movement and increasing the microbial activity (Lee *et al.*, 1987) and

iii) phosphate rocks acts as a source of pH buffering in soil by consuming the protons released during the oxidation of S^O and thereby creates a more favourable condition for S^O oxidising microorganisms, especially heterotrophic S^O oxidisers which are more sensitive to decreases in soil pH than *thiobacilli* species (Germida *et al.*, 1988).

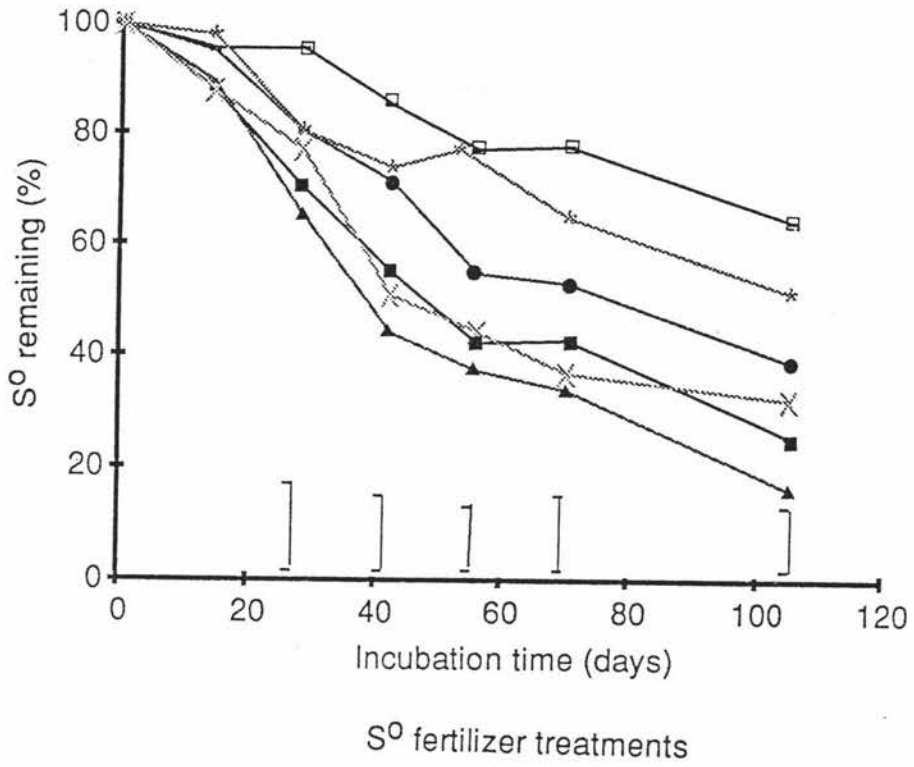


Figure 5.3 Percentage of S^0 remaining when S^0 sources are incubated in soil with and without reactive phosphate rock.

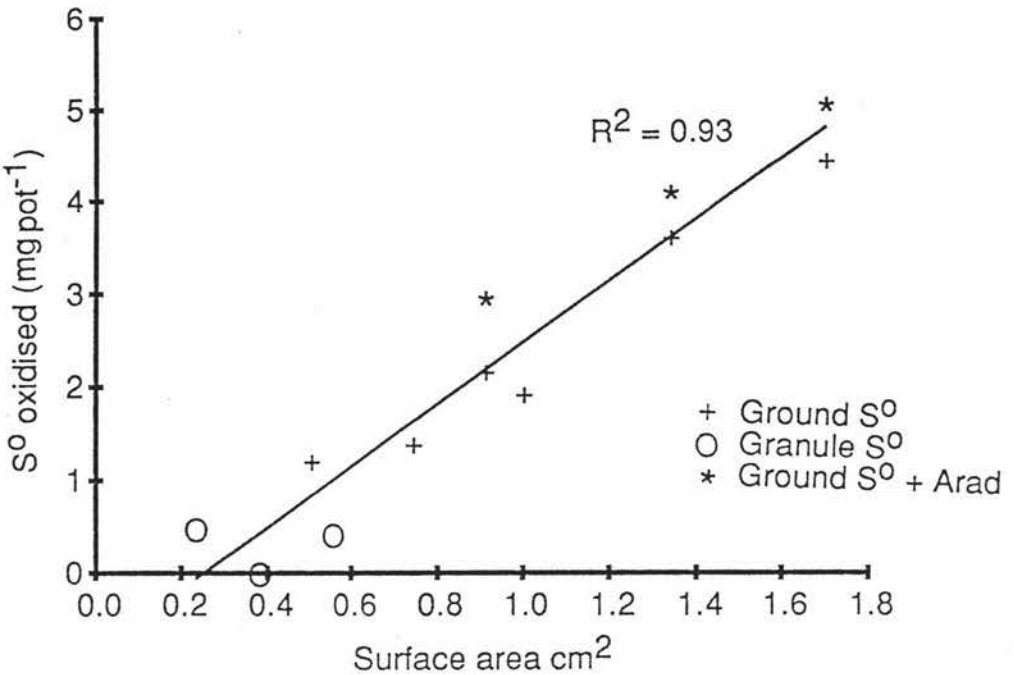


Figure 5.4 The relationship between surface area of S^o applied and total amount of S oxidized at 10 weeks.

5.4.4.2 Investigation of reasons for RPR effect

Reasons for the increased oxidation rate were further investigated in a second incubation study. The experiments included additional treatments to act as sources of P (DCP) and Ca ($CaCl_2$) and to increase pH buffering ($CaCO_3$). Ag.grade S^o was mixed with different amounts of $CaCO_3$, $CaCl_2$, DCP and Arad RPR and the mixtures were incubated in moist soil (the same soil and conditions were used as described in section 5.3.4.1) for 12 weeks period (Table 5.3).

There were no significant differences ($P > 0.05$) in the rate of S^o oxidation any of the treatments up to 8 weeks of incubation. This difference from the rates observed for the RPR treated samples in the earlier trial discussed in section 5.4.4.1, may be attributed to a longer lag period of microbial activity in this incubation. This longer lag phase may be due to the longer period of storage of the soil at 4°C before the soil was incubated. About 35 to 50% of the total S^o was oxidised after 12 weeks of incubation, but the presence of Arad RPR in this incubation did not stimulate increased S^o oxidation.

Adding $CaCO_3$, DCP or $CaCO_3$ + DCP did increase S^o oxidation rates but the increase was significantly different ($P < 0.05$) from S^o alone only at the higher rate of $CaCO_3$ addition (Table 5.6).

The results fail to explain the higher rates of S^o oxidation observed in S^o + RPR mixtures (discussed in section 5.4.4.1). However, there were higher rates of S^o oxidation when DCP and $CaCO_3$ were added (Table 5.6), which would result in a more favourable condition for the heterotrophic oxidisers. Lee *et al.* (1987) observed that for some soils, a significant decrease in the numbers of S^o oxidising heterotrophic bacteria occurred in S^o treated soil but observed no significant decrease in the RPR + S^o treatment, but there were no differences in the numbers of *thiobacilli* in S^o and RPR + S^o treated soils from the same soil groups. Thus the effect of RPR on S^o oxidation may vary depending on the soil and soil properties and the dominant type of S^o oxidisers (chemolithotrophs or heterotrophs) in the soil. Contrasting results on the effect of pH and lime were also obtained by a number of workers and they failed to establish a significant relationship between S^o oxidation and soil pH and lime addition (Kittams and Attoe 1965; Bloomfield 1967; Vitolins and Swaby 1969).

Table 5.6 The percentage of S^o oxidised and the specific oxidation rates of S^o in different lime and P levels (L_1 = 26.6 mg, L_2 = 13.3 mg, L_3 = 6.7mg).

Treatment	S^o oxidised at 4 wks	8 wks	12 wks	Specific oxidation rate+ $\mu g\ cm^{-2}\ day^{-1}$
	---------- (%) ----------			
S^o	21.7	36.0	37.7^{bc*}	15.8
S^o+Arad	19.7	35.0	34.8^{c}	14.6
S^o+DCP	23.4	29.0	43.3^{ab}	18.1
S^o+CaCl2	17.8	30.2	32.0^{c}	13.8
S^o+$CaCO_3$ (L_1)	18.7	30.4	43.0^{ab}	18.0
S^o+$CaCO_3$ (L_2)	19.5	27.4	49.2^{a}	20.7
S^o+$CaCO_3$ (L_3)	22.7	32.8	41.4^{abc}	17.4
S^o+DCP+$CaCO_3$ (L_1)	20.5	28.4	43.0^{ab}	18.1
S^o+DCP+CaCO3 (L_2)	20.8	31.0	47.2^{ab}	19.8
S^o+DCP+CaCO3 (L_3)	16.9	33.3	48.5^{a}	20.4

\+ Calculated using least square fit procedure develop in Chapter 8 siction 8.2.
* Means separation based on LSD at the 5% level.

5.5 Conclusion

In their 'as received' particle size range dark prilled products, ground S^o, Damman S^o, and Rotokawa S^o did not oxidise as fast as Ag.grade S^o which had been ground to meet the New Zealand MAF particle size criteria for application to grazed pasture.

The differences in the rate of S^o oxidation measured in incubated soil between different sources, namely Rotokawa S^o (geothermal S^o) dark S^o and Ag.grad S^o (recovered by Stretford process) can be mostly explained by their differences in particle size and the differences in surface area that this generates. When S^o oxidation was calculated per unit surface area they all had similar oxidation rates. When these materials were granulated or prilled, however, the oxidation rates in incubated soil decreased substantially due to the fact that granules or prills did not disintegrate when the granules were incorporated into soils under incubation conditions.

The results indicate that recommended strategy for effective use of these prilled or granulated S^o fertilizers is to leave the fertilizers exposed on the soil surface for lengthy periods, prior to cultivation to allow the fertilizers granules disperse.

An initial incubation provided evidence that increased sulphur oxidation occurred in S^o + RPR mixtures. However, in a second incubation it was not possible to identify the reasons for the effect of RPR on S^o oxidation and in the second incubation the extent of S^o oxidation did not increase when RPR was added but small increases occurred when $CaCO_3$ and DCP and DCP/$CaCO_3$ were added. However, this effect was not consistent with increasing rate of $CaCO_3$ added.

CHAPTER 6

THE EFFECT OF PHOSPHATE FORM ON THE OXIDATION AND PLANT AVAILABILITY OF ELEMENTAL SULPHUR

6.1 INTRODUCTION

It was observed in some cases that the rate of S^0 oxidation was enhanced when S^0 was in close contact with RPR (Chapter 5, Section 5.4.4). Similar effects had been observed by Lee *et al.* (1987). Bloomfield (1967) and Friesen (1988) had also demonstrated this effect when S^0 was incorporated within granules of diammonium phosphate and triple superphosphate. On the other hand, Kittams and Attoe (1965) found that granulation of S^0 into commercial fertilizer severely inhibited S^0 oxidation (Section 5.4.4) and the rate of sulphate release to plants.

The reasons for the variable effects of P fertilizers on the rate of S^0 oxidation remain unclear and could be a function of soil type. One of the reasons suggested to explain the increase in S^0 oxidation in the presence of RPR is that RPR neutralises acidity produced during the oxidation of S^0 and thereby maintains more favourable pH conditions for S^0 oxidation by heterotrophs (Germida *et al.*, 1985).

Several studies have examined the effect of S^0 oxidation on PR dissolution but few examined the effect of PR on S^0 oxidation. When molten S^0 or dry screened S^0 is incorporated with PR fertilizer the release of H_2SO_4 during the oxidation of S^0 has been found to induce the dissolution of PR i.e. the 'Biosuper' effect (Swaby, 1975; Rajan and Edge, 1980; Schofield *et al.*, 1981; Rajan, 1987). S^0 has also been reported able to cause slow acidulation of residual PR in partially acidulated rock phosphate fertilizers as indicated by increases in P uptake by plants (Friesen *et al.*, 1987). The extent of PR acidulation has been shown to increase when unreactive PR rather than reactive phosphate rocks were co-granulated with S^0 (Rajan, 1987; Friesen *et al.*, 1987). However, the dissolution of PR residues in different P fertilizer forms (e.g. RPR, PAPR and PAPR residue) and the mutual effect of different P fertilizer forms on the oxidation of S^0 were not examined in these experiments.

The experiments reported in this chapter aim to fill this knowledge gap by examining the effect of phosphate fertilizer form on the rate of S^0 oxidation in the presence and absence of plants. The secondary effect of S^0 oxidation on the dissolution of insoluble PR residues is also examined.

6.2 OBJECTIVES

i) To examine the influence of soluble and insoluble phosphate fertilizers on the rate of S^0 oxidation and plant uptake of S from combined P and S fertilizers.

ii) To determine whether the acidity generated from S^0 oxidation enhances the dissolution and plant uptake of P from insoluble residues in RPR and PAPR type fertilizers.

iii) To determine whether the presence of plant roots influences the rate of S^0 oxidation.

6.3 MATERIALS AND METHODS

6.3.1 Soils

Two soils (Table 6.1), Makotuku fine sandy loam and Tokomaru silt loam which contrasted in their initial rhodanese enzyme activity (S^0 oxidation potential), were collected from the top 0-3 cm of permanent pasture and sieved to <2 mm for use in the pot experiment (see also Section 3.3.1.2).

6.3.2 Fertilizers

Agricultural grade S^0 of 100% <250 μm and ground North Carolina Rock (NCPR; 100% <250 μm) were used to manufacture the fertilizer samples. The particle size distribution of these fertilizers and the chemical reactivity (solubility in dilute formic acid) of the RPR are shown in Table 6.2 and 6.3.

Table 6.1 Some physical and chemical characteristics of the Tokomaru and Makotuku soils.

	Tokomaru	Makotuku
Texture	silt loam	fine sandy loam
pH	5.8	5.4
P-retention %	25.5	32.3
Total S %	0.023	0.039
Ca-P ext.SO_4^{2-} $\mu g\ g^{-1}$	4.0	8.4
Olsen P $\mu g\ g^{-1}$	9.8	8.5
CEC me $100g^{-1}$	20.1	14.3
Exch.Ca me $100\ g^{-1}$	6.3	4.7

Table 6.2 Particle size of the S^o and RPR[1] used. The amount of material collected on each sieve is expressed as a percentage of the total amount of material recovered from all sieves. The method used was a dry sieving method.

	>500	500-250	250-150	150-75	<75
	---------- sieve size (μm) ----------				
Ag.grade S^o	0	0	13	40	47
NCPR[1]	0	0	2	24	74

1 NCPR particle size analysis from Officer, 1990

Table 6.3 Solubility in 2% formic acid (in a 30 min. extraction) of the ground NCPR (Officer, 1990).

	Total P	Formic P	
	% wt/wt	% wt/wt	% of total P
NCPR (ground)	13.1	9.2	70.2

6.3.3 Fertilizer Manufacture

The PAPR, containing 16.0% P was prepared using the method described by Harrison and Hedley (1987). North Carolina phosphate rock was acidulated with Texas Gulf commercial phosphoric acid (22% P) at an acid to rock ratio of 0.48. For the manufacture of S^o/PAPR, the S^o was added prior to acidulation, and thoroughly mixed with the phosphate rock, to give 16.0% P and 7.2% S. After acidulation and denning for 25 min. the fertilizer was cut out through a 1 mm sieve and granulated in a rotating drum, where it was sprayed with water until granulating freely. The granules were subsequently oven dried (65°C), and sieved to give granule sizes between 0.2-0.5 mm in diameter.

The non-water soluble PAPR residue (PAPR-res) was obtained by sequentially extracting PAPR with water for three times at 1:100, solid:solution ratio.

The S^o/PAPR residue and (S^o/RPR) were prepared by granulating a mixture of S^o and PAPR residue or S^o and NCPR with 1% agar and saturated KCl solution. Similarly, monocalcium phosphate (MCP) and S^o/MCP granules with 9.4% P and 5.5% S were prepared from A.R. grade MCP and S^o.

The same number of granules (approximately 30 granules) were applied in each fertilizer treatment to ensure that similar volumes of soil were fertilized. This was achieved by including finely sieved (<150 μm) Tokomaru silt loam as a filler prior to granulation of the S^o, NCPR, PAPR residue and S^o/PAPR residue fertilizers. The complete list of fertilizer materials and their analysis is given in Table 6.4.

6.3.4 Experimental Procedure

The effect of phosphate fertilizer forms and the presence of plants on the rate of S^o oxidation were examined in pot incubation trials. Complete details of the experimental procedure are described in Section 3.3.3.2. Each fertilizer was applied to the soil in the fertilizer zone (see Figure 3.1) at rate of 120 μg S^o g^{-1} soil (Table 6.4). Both with and without white clover plants, were watered frequently (approximately every second

day) and they were allowed to drain freely. Over 98 day period, from seeding to final harvest, seven harvests of herbage and soil were taken for measurement of dry matter yield, herbage S and P content and measurement of residual S^o, extractable SO_4-S and residual P fractions remaining in soil sampled from the fertilized zone.

Table 6.4 The rate of P and S applied to the fertilized zone in each treatment and the distribution of P within different soil P fractions at the beginning of the experiment.

Treatment	P	S	NaCl-P[a]	NaOH-P[a]	HCl-P[a]
	$\mu g\ g^{-1}$ soil		(% of total P added)		
Tokomaru soil					
control	0	0	0	0	0
S^o	0	120	0	0	0
RPR	158	0	3.6	25.0	71.4
S^o/RPR	158	120	3.6	25.0	71.4
PAPR	307	0	20.1	47.5	32.4
S^o/PAPR	307	120	20.1	47.5	32.4
PAPR-res	145	0	1.2	18.7	80.2
S^o/PAPR-res	145	120	1.2	18.7	80.2
MCP	171	0	28.9	71.1	0.0
S^o/MCP	171	120	28.9	71.1	0.0
Makotuku soil					
control	0	0	0	0	0
S^o	0	120	0	0	0
RPR	161	0	1.6	17.3	81.6
S^o/RPR	161	120	1.6	17.3	81.6
PAPR	319	0	16.7	42.4	41.0
S^o/PAPR	319	120	16.7	42.4	41.0
PAPR-res	147	0	0.2	7.3	92.5
S^o/PAPR-res	147	120	0.2	7.3	92.5
MCP	165	0	26.4	71.2	2.3
S^o/MCP	165	120	26.4	71.2	2.3

[a] Calculated as: e.g.

NaCl-P(%) = 100 x (NaCl-P Treated soil - NaCl-P control soil)/P added

6.3.5 Soil Analysis

6.3.5.1 *Elemental and sulphate sulphur*

The pots were destructively sampled at fortnightly intervals and the soil samples from the fertilized zone were air dried and ground with a coffee grinder to provide a uniform subsample for S^0 and SO_4-S analyses (Chapter 3 section 3.4.1.9 and 3.3.4.2, respectively).

6.3.5.2 *Inorganic P fractionation*

Samples of soil from the fertilized zone, taken at the beginning and the end of experiment, were ring ground to reduce soil subsampling errors. The following sequential extraction was carried out on the ring ground soil to measure the amounts of the different inorganic P fractions present in fertilized and unfertilized soil.

Three replicate samples of one g ring ground soil were prewashed with 0.5 M NaCl for 30 minutes as described by Mackay *et al.* (1986) before sequential extraction firstly with 1 M NaOH and secondly with 1 M HCl, using shaking times of 16 hours (Apthorp *et al.*, 1987). All extractions were carried out at soil: solution ratios of 1:40 in 50 ml screw capped, polypropylene centrifuge tubes. After each extraction the tubes were centrifuged at 8000 rpm for 10 minutes on a Sorvall RC5C centrifuge using a SS-34 head. A 20 ml sample was taken from the supernatant solution and stored for later analysis. The remainder of the supernatant was discarded between each step. By knowing the weight of extractant entrapped in the soil pellet after the supernatants were discarded, a correction could be made to account for the contribution of P in the entrapped solution to the subsequent extract. The inorganic P concentration in the extracted solution was measured using the method of Murphy and Riley (1962).

6.3.5.3 *Estimation of PR dissolution*

Sequential P fraction results were used to calculate the extent of dissolution of the RPR components in the RPR and PAPR treatments. After pre-extraction with NaCl and NaOH, dissolved PR residues contribute P to the HCl extraction only (Bolan and

Hedley, 1989). The extent of dissolution of the RPR residue was calculated as follows:

Dissolution of RPR residue (%) = 100 x (A - B) / A
where

$A = HCl\text{-}P_{t0}$ (fertilized soil - unfertilized control)
$B = HCl\text{-}P_{tn}$ (fertilized soil - unfertilized control)

$HCl\text{-}P_{t0}$ is the amount of P extracted by HCl at the beginning and the $HCl\text{-}P_{tn}$ is the amount of P extracted by HCl at the n^{th} harvest of the incubation.

6.3.5.4 *Rhodanese enzyme activity*

Rhodanese enzyme activity was determined as described in Chapter 3, Section 3.3.4.4.

6.3.6 Herbage analysis

6.3.6.1 *Dry matter yield*

In all, seven harvests were taken approximately at fortnightly intervals. Plants were cut to a level of 2 cm above the soil surface to minimize damage to the stolons. After drying at 65°C for 4-hr, the herbage was weighed and ground for P and S analysis.

6.3.6.2 *Herbage P and S*

Herbage S was analysed by alkaline wet oxidation, a modification of the method of Tabatabai and Bremner (1970). Finely ground herbage samples, 0.02-0.05 g (10-50 µg S), were placed in digestion tubes (2.5 x 20 cm) and four ml of freshly prepared sodium hypobromite (NaOBr) was added. The tubes were heated at 250-260°C until the contents were evaporated to dryness. This step was repeated if digestion appeared incomplete. After digestion the tube was removed from the digestion block and allowed to cool, and 1.00 ml of formic acid added. The tube's contents were diluted to a desired volume with deionized water, mixed thoroughly with a vortex mixer and left

until the contents settled down. An appropriate aliquot was taken for S analysis (Section 3.3.4.2).

For the analysis of P, the herbage sample was digested using a Kjeldahl digestion procedure and the P concentration in the digest was determined using a Technicon Autoanalyser II system (Twine and Williams, 1971).

6.4 RESULTS AND DISCUSSION

6.4.1 Effect of Phosphate Fertilizer Form on S^o Oxidation

Sulphur oxidation (Figures 6.1 and 6.2) was initially rapid over the first six weeks period when RA's were at their highest values (Appendix 6.1). Over the whole experimental period, however, there was no effect of phosphate fertilizer form on RA, either in the presence or absence of plants. The reasons for the lack of a consistent relationship between RA and S^o oxidation were discussed in Chapter 3, Section 3.4.2.

After the initial six week period the rate of S^o oxidation decreased with time. A decrease in the rate of S^o oxidation with time was also observed by Lee *et al.*, 1987 and was associated with a decrease in *thiobacilli* numbers. It is expected that the decrease will be due to a number of factors which include the decreasing surface area of S^o available for oxidation as oxidation proceeds. Also when the particles are very small the core of the residual S^o particles may be inaccessible (perhaps because of steric hindrance) to the oxidising organisms (Laishley *et al.*, 1986). Another possible explanation is that as S^o oxidation proceeds the immediate soil pH decreases, which may inhibit some S^o oxidisers, especially heterotrophs (Wainwright, 1984). Later in Chapter 8 it is demonstrated that a S^o oxidation model, which assumes a constant rate of oxidation per unit area of S^o, adequately explained the pattern of S^o oxidation in these incubation studies.

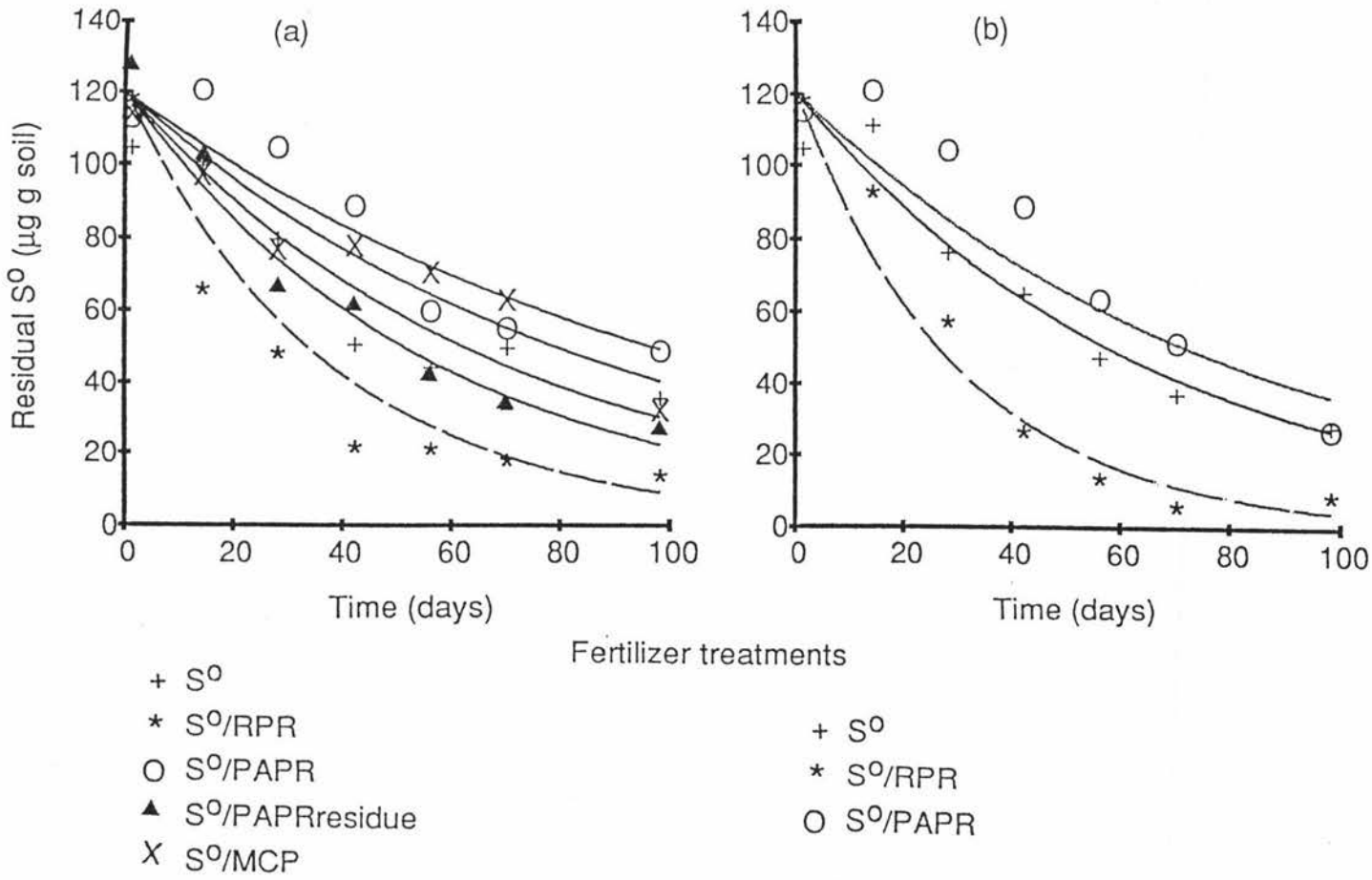


Figure 6.1 The amounts of S^0 remaining in Tokomaru soil fertilized with various S^0 fertilizers in the presence (a) and absence (b) of plants.

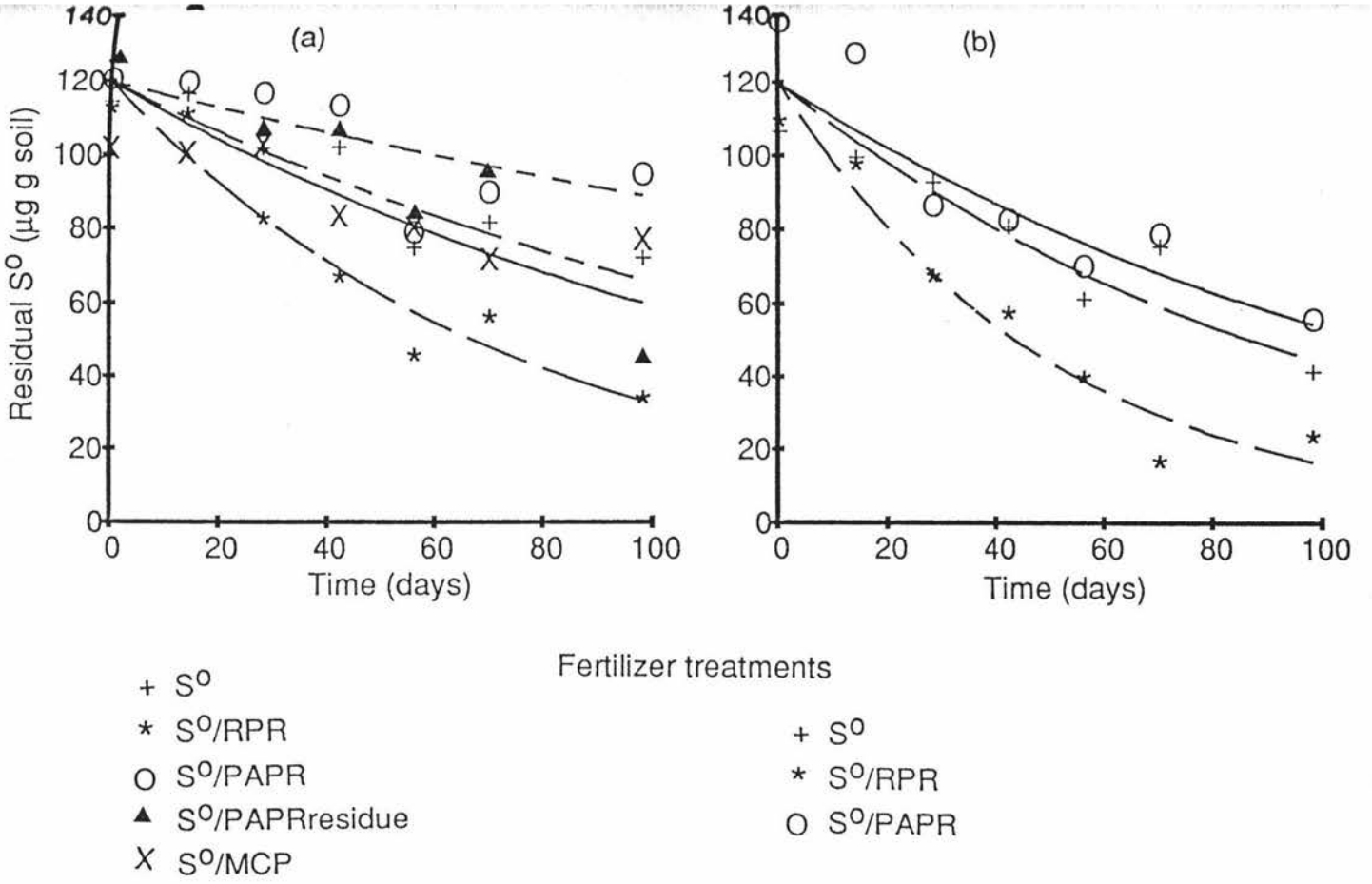


Figure 6.2 The amounts of S^o remaining in Makotuku soil fertilized with various S^o fertilizers in the presence (a) and absence (b) of plants.

In order to compare the rates of oxidation of S^o in different fertilizers in this chapter the rate of S^o oxidation for each treatment was modelled by the following equation :

$$Y = A \times \exp(B \times t) \quad (6.1)$$

$$lnY = lnA + B \times t \quad (6.2)$$

Where Y = residual amount of S^o remaining at each sampling time; A = initial amount of S^o added; B = slope of the *ln* transformed line; t = time (days).

Lines of best fit are presented in Figures 1a, b and 2a, b. In the present study coefficient **A** was equal to 120 μg S^o g^{-1} soil (Table 6.4). Over the 7 sampling times, the above equation, in general, adequately described the measured data, Coefficients of determination for the fitted models (R^2) ranged from 84-98% (Table 6.5). However, the equation was less satisfactory in representing the oxidation pattern of 3 treatments (S^o/PAPR, R^2=0.55; S^o/PAPR residue, R^2=0.66; and S^o/MCP, R^2=0.45) in Makotuku soil with plant growth. The latter result could be attributed mainly to the high variability of the residual S^o measurements in these treatments.

The rates of S^o oxidation of different fertilizers were compared by comparing the fitted **B** values. T-tests were carried out to compare the **B** (slope of log transformed function) values between each pair of the treatments (Gomez and Gomez, 1984). The values for the slope (**B**) and the coefficient of determination (R^2) are presented in Table 6.5 and the T test in Appendix 6.2.

The rate of oxidation of S^o (**B**, slope) was higher for the Tokomaru soil (mean of 0.018) than for the Makotuku soil (mean of 0.01) (Table 6.5). The rates of oxidation of S^o, in the presence of plants, for both soils were ranked as follows: ($p < 0.05$) S^o/RPR = S^o/PAPR residue > S^o = S^o/PAPR = S^o/MCP; and in the absence of plants it followed: S^o/RPR > S^o = S^o/PAPR. The data indicate that the addition of P as insoluble sources such as RPR and PAPR residue increases the rate of oxidation of S^o both in the presence and absence of plants. This result was similar to the effect of RPR on the oxidation of S^o in the laboratory incubations reported in Chapter 5, Section 5.4.4. The addition of P as soluble sources, such as MCP and PAPR, had no significant effect on the oxidation of S^o. However, others have found that the addition of P to soil as both an insoluble source, such as Jordan rock (Lee *et al.*, 1987), and soluble P sources such as diammonium phosphate and triple superphosphate (Bloomfield, 1967) increase the rate of S^o oxidation.

Table 6.5 The B^ parameter of the exponential decay function fitted to observed amounts of S^o remaining at each harvest and the amounts of S^o oxidised calculated from the equation.

Treatment	Exponential decay model -B^	R^2	S^o oxidised after 98 days ($\mu g\ S\ g^{-1}$ soil)	(%)	day^{-1}
Tokomaru-plant					
S^o	0.015	98	92.4	77	0.79
S^o/RPR	0.033	88	115.0	96	0.98
S^o/PAPR	0.012	87	83.0	69	0.70
Mean	(0.020)		(96.8)		(0.82)
Makotuku-plant					
S^o	0.010	86	75.0	63	0.64
S^o/RPR	0.020	86	103.0	86	0.88
S^o/PAPR	0.008	85	65.2	54	0.55
Mean	(0.013)		(81.1)		(0.69)
Tokomaru+plant					
S^o	0.014	86	89.6	75	0.77
S^o/RPR	0.026	84	111.0	92	0.94
S^o/PAPR	0.009	89	70.3	59	0.60
S^o/PAPR-res	0.017	98	97.3	81	0.83
S^o/MCP	0.011	89	79.2	66	0.67
Mean	(0.015)		(89.5)		(0.76)
Makotuku+plant					
S^o	0.006	86	53.3	45	0.46
S^o/RPR	0.013	92	86.4	72	0.73
S^o/PAPR	0.003	55	30.6	26	0.27
S^o/PAPR-res	0.007	66	59.6	50	0.51
S^o/MCP	0.006	45	53.3	45	0.46
Mean	(0.007)		(56.6)		(0.49)

To facilitate comparison of the results with published data using similar sized S^o (but different application rates), the data were also expressed as proportional oxidation rates, or the percent of applied elemental S^o oxidisied per day. The mean oxidation rates (0.76% day^{-1} and 0.48% day^{-1} in Tokomaru and Makotuku with plant, respectively) were similar to rates obtained using Australian soils in pot experiments (Shedley, 1982) and in a field study (Barrow, 1971) but were approximately 4 times the rates reported by Li and Caldwell (1966) (0.13% day^{-1} for 0.2 mm S) and by Kittams and Attoe (1965) (0.14% day^{-1} for 0.32 mm S) in incubation experiments. In the latter two experiments S^o oxidation was measured from the amounts of SO^4-S produced. Use of sulphate measurement can underestimate the rate of oxidation (Chapter 3, section 3.4.3.2) which could explain why the rates measured by Li and Caldwell (1966) and Kittams and Attoe (1965) were low.

6.4.2 Effect of Plants on S^o Oxidation

All treatments (S^o, S^o/RPR, and S^o/PAPR) had higher S^o oxidation rates without plants than with plants for both soils. The mean oxidation rates (all treatments) in the absence of plants were 0.82 and 0.69% per day compared to 0.76 and 0.48% per day in the presence of plants for Tokomaru and Makotuku soils, respectively (Table 6.5). However, the difference in S^o oxidation rate (calculated as the slope of the exponential function) between plus plant and minus plant treatments was significant ($P < 0.05$) for the S^o/PAPR treatment only (Appendix. 6.2). As watering regimes were similar for both planted and unplanted pots, the only explanation for higher rates in the unplanted pots is that the bare soil reached higher day time temperatures than soils with plant cover. Soil temperatures were not measured but such an increase can be expected to increase the rate of S^o oxidation (Janzen and Bettany, 1987a).

Little is known about the effect of plants on S^o oxidation, only some indirect evidence is available on the effect of plants. Skiba and Wainwright (1984), in an incubation using non-rhizosphere and rhizosphere soils and sand samples, found that the rate of oxidation generally increased with increasing soil C and N content, increasing vegetation cover and decreasing soil or sand pH.

The metabolic activity of plant roots is able to modify the rhizosphere. For example, excretion of CO_2, organic acids or other substrates which stimulate microbial activity could be expected to influence microbial S^o oxidation. It was expected that, because rhizosphere microbial populations are higher than those of the bulk soil (Stevenson, 1968), the presence of plants may stimulate S^o oxidation. Legumes (clover) actively fixing nitrogen are expected to acidify their rhizosphere (Bolan *et al.*, 1989) and soil pH's in planted pots were, in general 0.2 units lower than the average initial soil pH values of 5.8 and 5.4 in Tokomaru and Makotuku soils, respectively. This small decrease in bulk soil pH, however, is unlikely to cause a significant reduction in S^o oxidation rate.

6.4.3 Effect of Granulation

In Chapter 5, Section 5.4.4 it was demonstrated that granulation of S^o/Arad phosphate rock mixtures resulted in lower rates of S^o oxidation (Figure 5.3). In this pot incubation study all forms of S^o were added in granular form. Although day time temperatures were higher in the glasshouse study than those of the incubation (Chapter 5, Section 5.4.4) rates of S^o oxidation (see Chapter 8 Section 8.2) were lower in the glasshouse study, probably due to the addition of S^o in granular form. However, when S^o is applied in a granular form the effect of RPR is considerably greater (Chapter 6) than when S^o is applied as a mixture (Chapter 5). Under these circumstances granulated mixtures of S^o/RPR had significantly higher S^o oxidation rates than when S^o was added alone or in any other form (Table 6.5). Possible reasons for this are that the H^+ produced around a S^o/RPR granule can be better neutralised by the intimate presence of RPR. Furthermore, the finer particle size used in this chapter (Table 6.3) and the higher extent of carbonate substitution of NCPR than Arad (Officer, 1990), may also contribute to a highly efficient pH buffering system providing suitable conditions near the fertilizer granules for heterotrophic oxidisers.

6.4.4 Agronomic Effectiveness of S^0 Fertilizer

6.4.4.1 *Dry matter yield*

Although the amount of S^0 added in each treatment was kept constant, the level of P applied varied between the different fertilizer forms (Table 6.4). For this reason the effect of S^0 addition on dry matter yields, and other plant growth parameters, can only be compared within the same phosphate fertilizer treatment. The dry matter yields accumulated over time were modelled by least squared fitting the following logistic function:

$$Y = y_0 / (1 + \exp (- (A + B \times t))) \qquad (6.3)$$

or in the linear form of:

$$ln (Y / y_0 - Y) = A + B \times t \qquad (6.4)$$

where A = a constant representing a theoretical intercept at t = 0.
B = initial change rate,
Y_0= upper asymptote, and
t = days.

The equation described the data adequately and accounted for 95-99% of variation in dry matter yields (Appendix 6.3a). The statistical significance between rates of dry matter accumulation was tested by T-testing the slopes of the log transformed function as described in section (6.4.1 and Appendix 6.3b).

On average the Tokomaru soil produced higher dry matter yield than the Makotuku soil. The S contents of the herbage from all pots receiving no S^0 were similar to or above the critical level for white clover plant (0.26 % S; McNaught and Christofels, 1961) throughout the experiment, and except for the PAPR residue treatment there were no significant ($P > 0.05$) yield responses to the application of S^0 (Table 6.6 and Appendix 6.3b). This indicated that the soils used in this pot experiment were not deficient in S.

Phosphorus fertilizer application rather than S^0 fertilizer application stimulated the major yield responses in both soil (Table 6.6). Despite the higher application rate of P in the PAPR treatments (Table 6.4), however, these did not produce yields significantly higher than those of the RPR and S^0/RPR treatments.

Table 6.6 The accumulated dry matter, plant P and S concentration and accumulated P and S uptake from Tokomaru and Makotuku soils fertilized with various S and P fertilizers.

	Cumulat. Dry matter	Average S conc.	Average P conc.	Cum.S uptake	Cum.P uptake
	---- g ---	------- % -------		------ mg ------	
Tokomaru soil					
Control	8.57	0.26	0.24	18.86	17.29
S^o	9.39	0.38	0.24	27.78	19.74
RPR	11.17	0.27	0.26	23.04	26.15
S^o/RPR	13.09	0.34	0.26	37.83	28.64
PAPR	13.72	0.24	0.28	23.97	30.62
S^o/PAPR	14.95	0.32	0.30	37.09	37.07
PAPR-res	7.98	0.24	0.26	23.39	22.37
S^o/PAPR-res	11.31	0.34	0.26	33.06	26.43
MCP	11.45	0.24	0.31	23.18	31.21
S^o/MCP	11.63	0.32	0.30	29.55	29.98
Makotuku soil					
Control	5.65	0.34	0.21	17.61	11.07
S^o	6.05	0.36	0.23	21.32	14.33
RPR	7.55	0.32	0.23	22.14	16.78
S^o/RPR	6.96	0.41	0.26	26.37	18.28
PAPR	8.54	0.31	0.27	22.87	22.31
S^o/PAPR	8.49	0.38	0.29	30.53	24.27
PAPR-res	6.55	0.32	0.23	18.10	14.30
S^o/PAPR-res	8.17	0.34	0.24	22.45	16.10
MCP	7.17	0.31	0.27	19.56	17.93
S^o-MCP	7.83	0.35	0.29	25.36	21.71

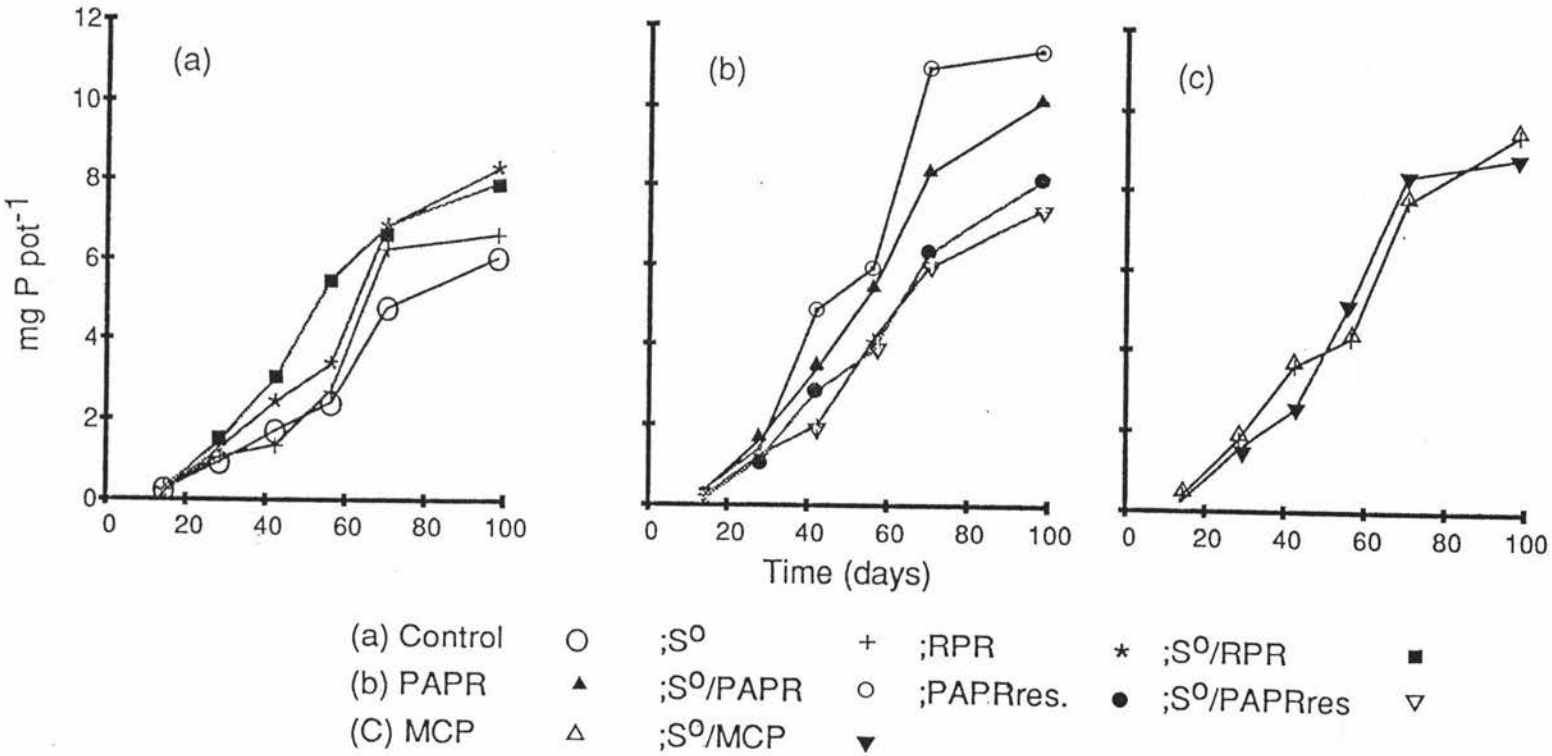


Figure 6.3a The plant uptake of P from Tokomaru soil fertilized with various P and S fertilizers.

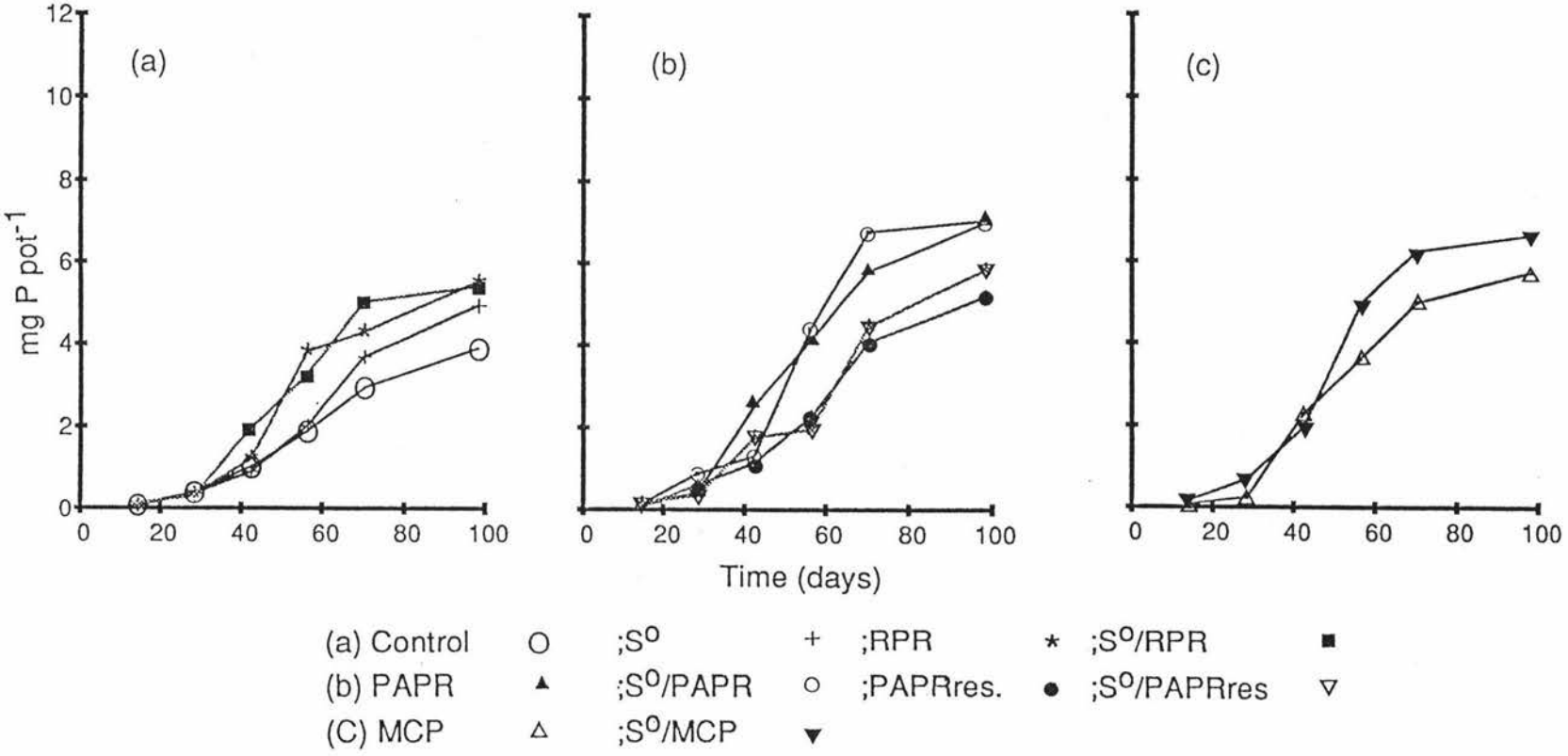


Figure 6.3b The plant uptake of P from Makotuku soil fertilized with various P and S fertilizers.

The PAPR residues alone produced similar dry matter yields to the no P treatments (control and S^0 alone). A marked increase in dry matter yield and to a lesser extent an increase in plant P uptake occurred when PAPR residue was sulphurised (Figure 6.3a and b). This increase in plant yield and P uptake was probably caused by increased P release from the residue and to a lesser extent improved S nutrition. Similar increases in plant P uptake were observed when RPR and PAPR's were cogranulated with S^0 rather than when applied alone. The only sulphurised treatment not to have higher P

uptake than the non-sulphurized treatment was S^0/MCP in Tokomaru soil. In all other cases addition of S, even to MCP in the Makotuku soil, increased P uptake. Plant P uptake in the (S^0/PAPR) treatment increased by an average of only 12%. Using similar cogranulated fertilizer materials but for longer plant growth periods (over 2 years under glasshouse conditions), Friesen *et al.*, 1987, found that cogranulation of PAPR, prepared from Christmas Island or Duchess PR, and S^0 increased P uptake by lucerne by 40% over the PAPR treatment alone. However, this effect did not appear until 6 months after application. Under glasshouse conditions it is clear that sulphurization of PAPRs can lead to increased P uptake by plants, probably partly caused by S oxidation enhancing dissolution of insoluble P. This effect is investigated further in section 6.4.5.

6.4.4.2 *Herbage S concentration and S uptake*

The mean herbage S concentration (%) and S uptake at each sampling date were consistently higher in the presence of S^0 than in the absence of S^0 (Table 6.6 and Figures 6.4 a and b), particularly in the Tokomaru soil. As discussed earlier these differences (S% and S uptake) did not result in major increases in dry matter production, indicating that the increased uptake of S in the S^0 treatments was mainly due to luxury uptake of S. These effects were more evident in Tokomaru soil than Makotuku soil which is consistent with the higher rates of S^0 oxidation in the Tokomaru soil (Table 6.5). There was greater plant uptake of S when RPR, PAPR and MCP fertilizers were cogranulated with S^0 rather than when either component (P or S) was applied alone (Table 6.6). However, the appropriate comparison between the effect of cogranulated P on S uptake could not be made. Increased uptake of S in S^0 versus P-source/S^0 comaparison is due to the response to P application (Table 6.6). Additional mixed (but not co-granulated) treatments (S^0 + RPR, etc.) would be necessary for this comparison in terms of S uptake.

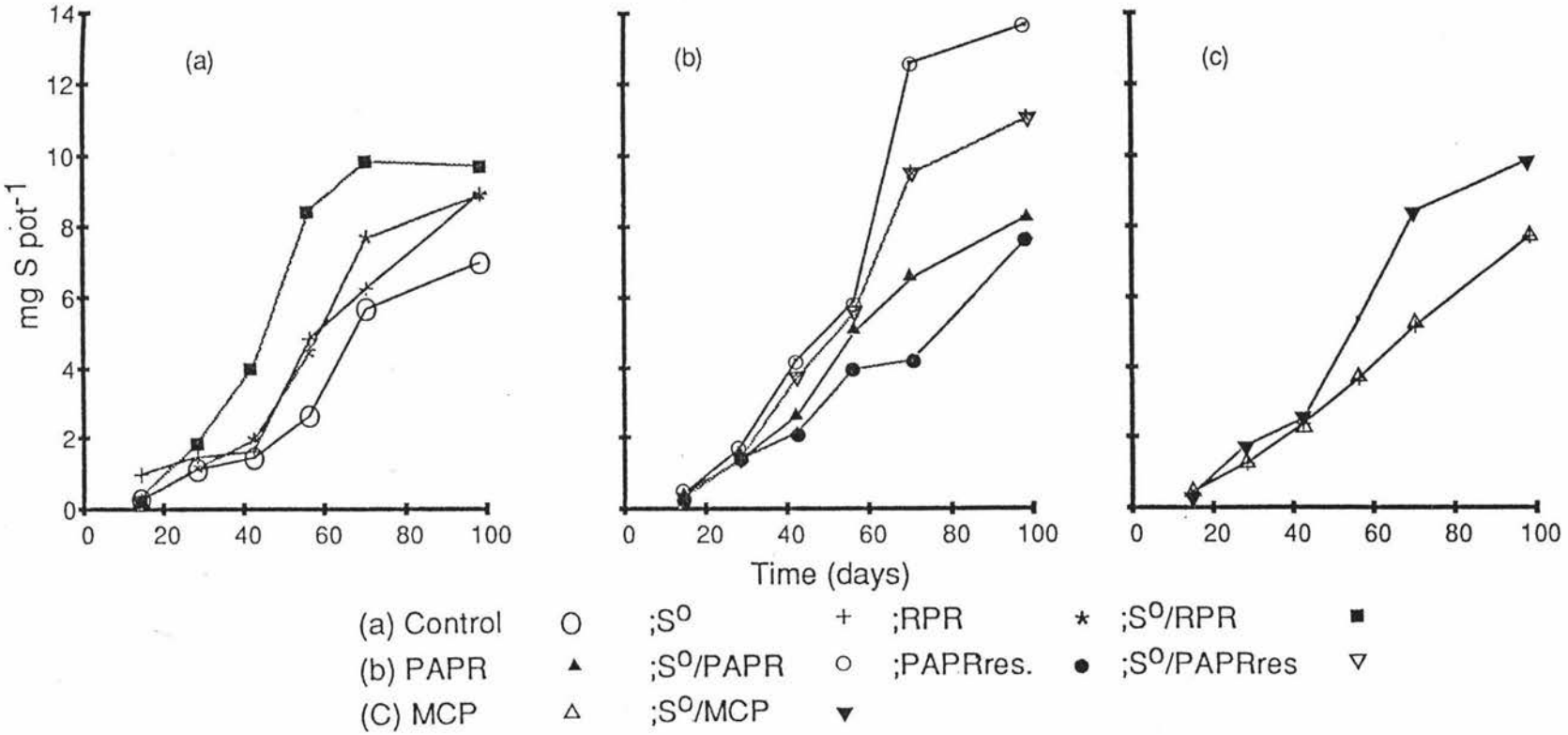


Figure 6.4a The plant uptake of S from Tokomaru soil fertilized with various P and S fertilizers.

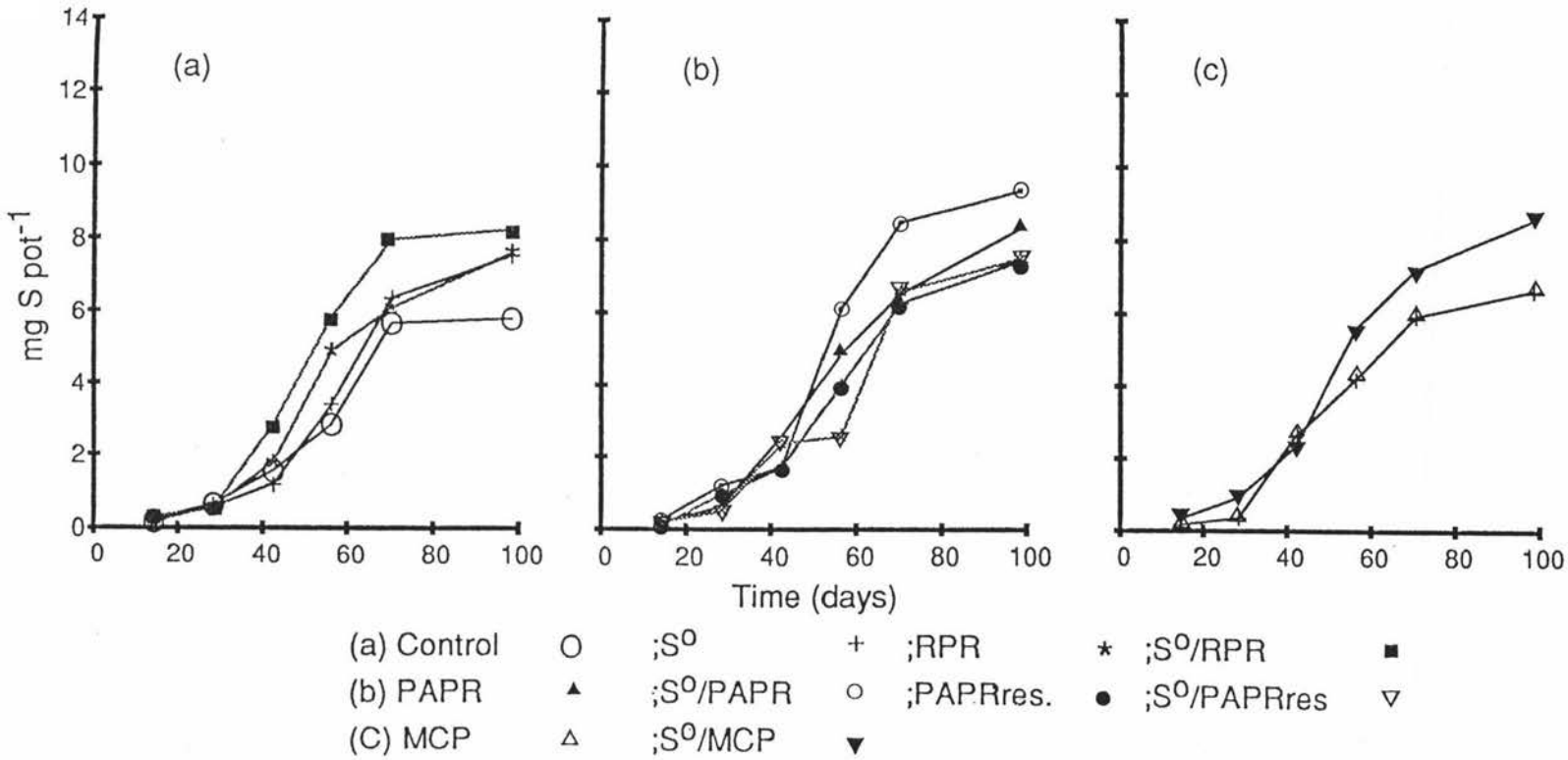


Figure 6.4b The plant uptake of S from Makotuku soil fertilized with various P and S fertilizers.

6.4.4.3 *Sulphate level in soil*

The extractable soil sulphate (see Chapter 3 for definition) levels measured at each harvest (Figure 6.5a, b and Figures 6.6a, b) reflect the balance between SO_4 production (i.e., soil organic S mineralization, S^o oxidation) and above ground plant uptake, SO_4 immobilization (microbial immobilization and plant root uptake) plus leaching from the zone of fertilizer application. All soils fertilized with S^o had higher extractable SO_4 levels than the control soils or soils receiving P treatments only. The highest amounts of extractable SO_4 were recorded in pots containing Tokomaru soil which had the highest rates of S^o oxidation (Table 6.5)

By 28 days after application, SO_4 levels in the S^o treated soils began to decrease both in the presence and absence of plants, which was due to both plant uptake, immobilization and leaching. Greater decreases were observed in the planted pots, presumably due to above ground plant uptake and increased immobilization in the rhizosphere (root plus soil).

The differences between the amounts of S^o oxidised and the amounts of SO_4-S remaining in soil plus S taken up by plants give the amounts of S immobilised and leached. It can be shown that on an average 50 and 90% of the S^o oxidised is immobilised and/or leached in the presence and absence of plants, respectively. In another pot experiment Shedley (1982) also found that a large proportion of the sulphate released from the fertilizers was immobilized. On average, at an application rate equivalent to 60 kg ha^{-1} for S^o particle size of 100 μm, 49% of the sulphur released from S^o fertilizers was immobilised into soil organic form.

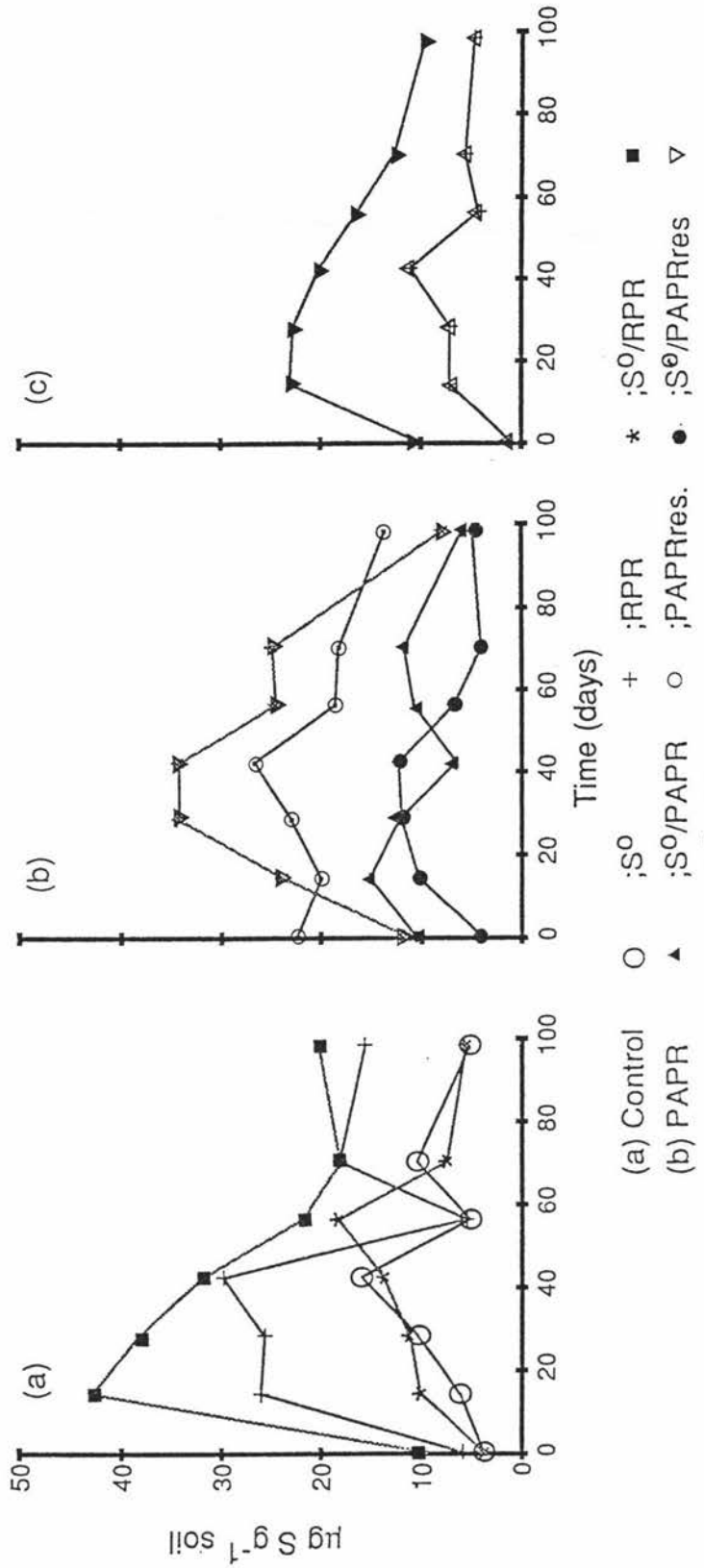


Figure 6.5a The amounts of soil sulphate extracted from Tokomaru soil (plant with clover) fertilized with various P and S fertilizers.

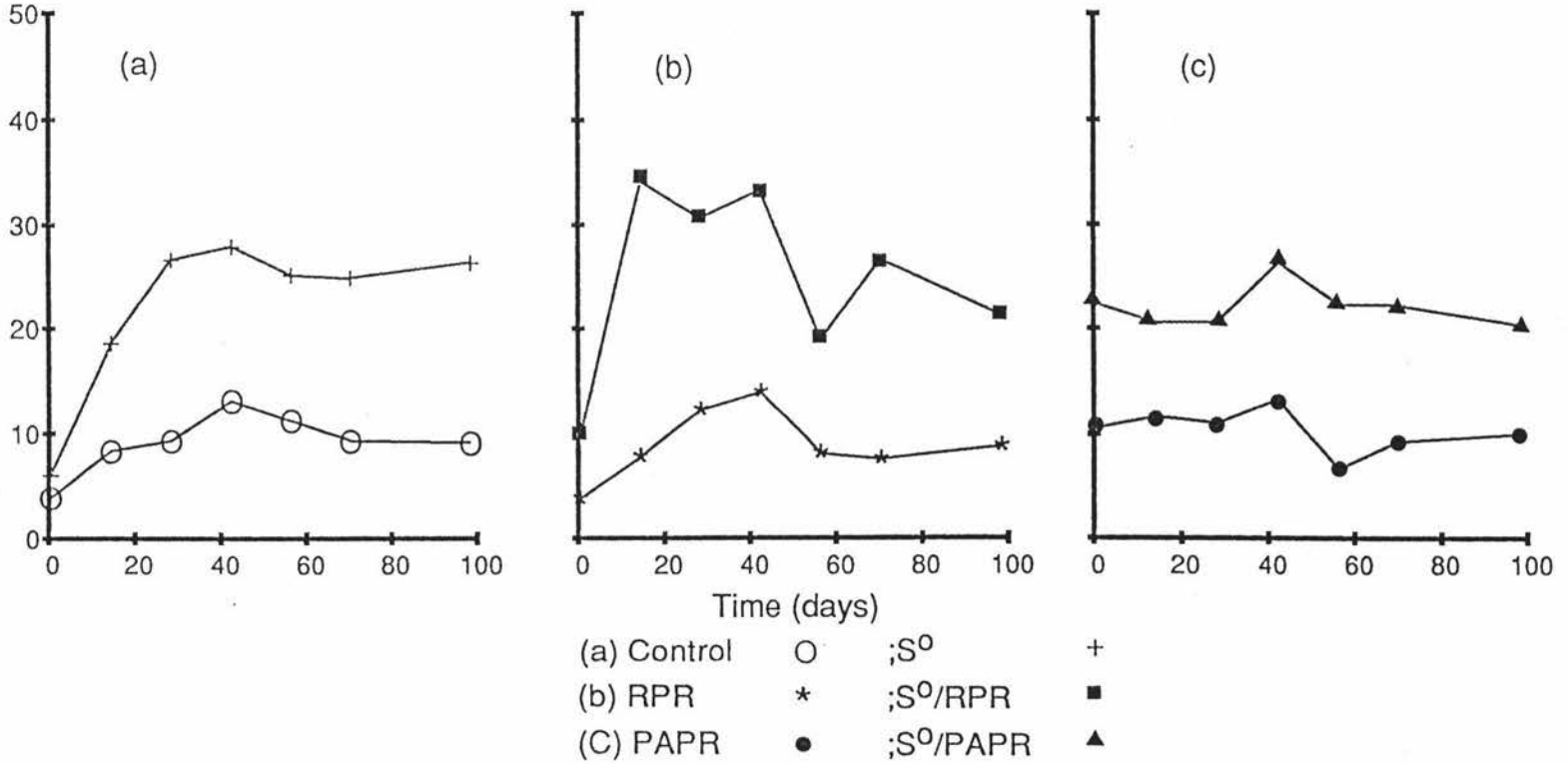


Figure 6.6a The amounts of soil sulphate extracted from Tokomaru soil (without plants) fertilized with various P and S fertilizers.

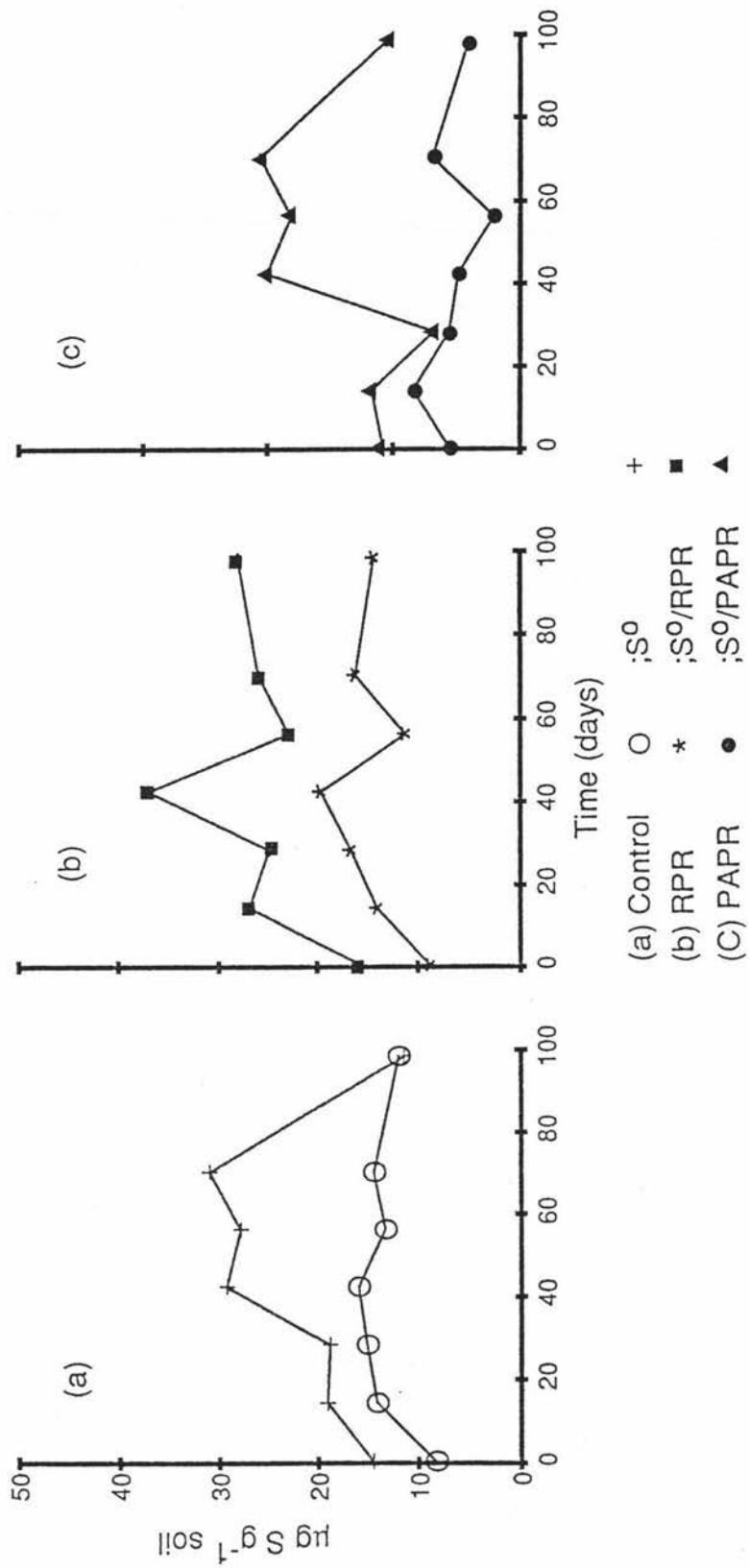


Figure 6.6b The amounts of soil sulphate extracted from Makotuku soil (without plants) fertilized with various P and S fertilizers.

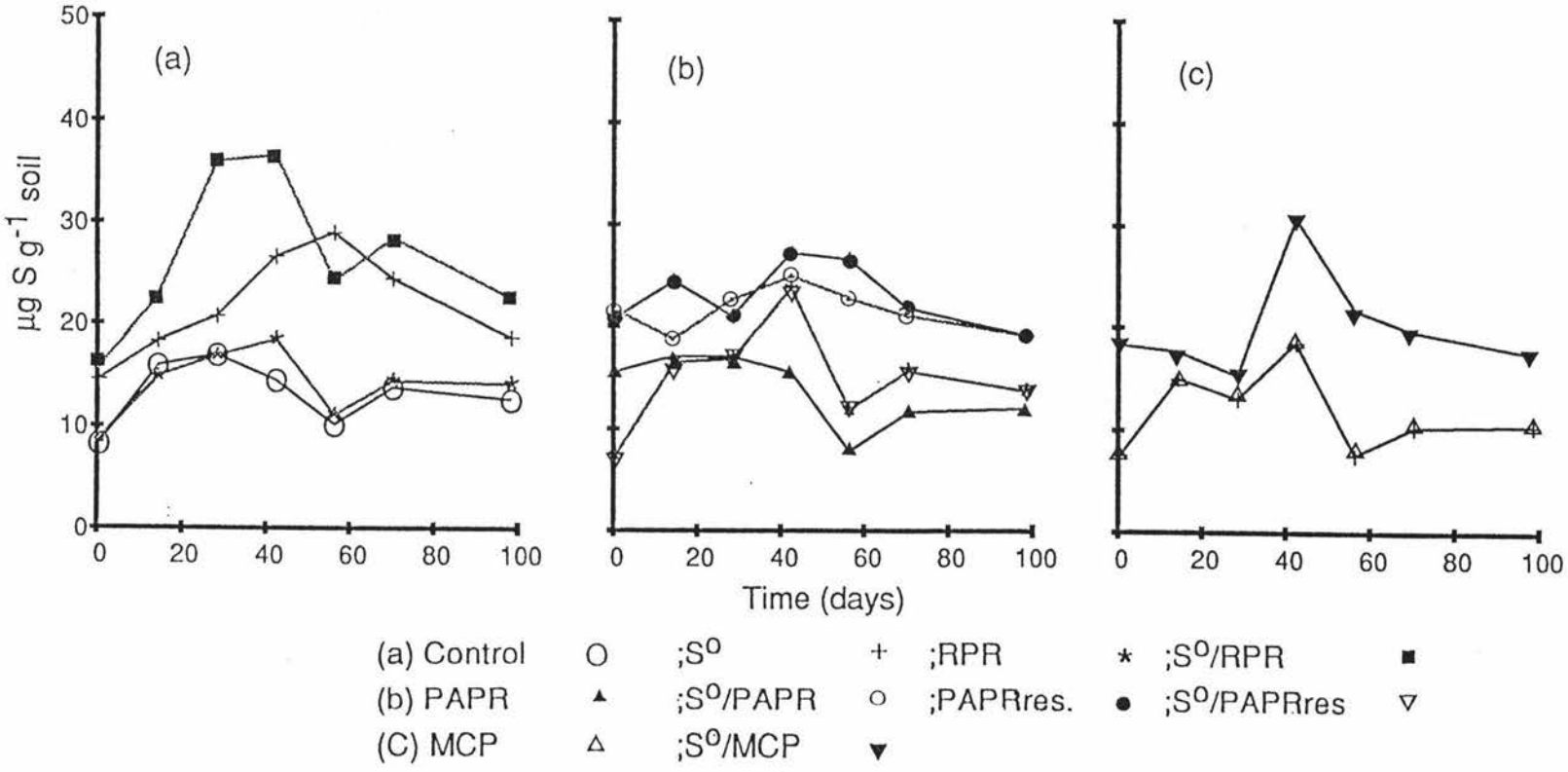


Figure 6.5b The amounts of soil sulphate extracted from Makotuku soil (planted with clover) fertilized with various P and S fertilizers.

6.4.5 Effect of S^o Oxidation on the Effectiveness of Phosphate Fertilizers

The uptake of plant P from RPR and PAPR was found to be enhanced by oxidation of S^o to sulphuric acid and the subsequent reaction of acid with the RPR and PAPR granules (Swaby, 1975; Rajan and Edge, 1980; Rajan, 1987; Friesen *et al.*, 1987). In most of these studies the effect of S^o oxidation on increasing the dissolution of insoluble phosphate forms has only been measured indirectly from the increase in plant P uptake by the S/P fertilized plants over the control. Until recently, the direct measurement of dissolution of PR residue as enhanced by S oxidation has not been extensively studied except by Rajan (1987), who used a sequential P fraction procedure to demonstrate that dissolution of a Florida PR residue in field soil increased when the PR was cogranulated with S^o. In this field trial, however, the dissolution of more reactive rocks, North Carolina and Chatham Rise, was not increased significantly by granulation with S^o.

By isolating the zone of fertilized soil in this present study, the opportunity arises to fractionate soil and fertilizer P by the method of Bolan and Hedley (1989). This has permitted a comparison of the effects of S^o oxidation on the dissolution of RPR and PAPR residues.

6.4.5.1 *Phosphate rock dissolution as influenced by cogranulation with S^o*

Inorganic phosphate in all soils (with and without plants) was fractionated into water soluble P (NaCl extractable), soluble and adsorbed P (NaOH extractable) and acid soluble P (HCl extractable). The results of this fractionation are expressed as μg P extracted g^{-1} soil (Table 6.7).

Dissolution of phosphate rock in soil can be measured from the increases in the amount of NaOH extractable P (dissolved P) or from decreases in the amount of HCl extractable P (Δ HCl method). In the presence of plants, however, some of the dissolved P is taken up by the plants and hence the increase in NaOH-P may not given a accurate estimate of the dissolved P (Bolan and Hedley, 1989). The effect of S^o oxidation on P dissolution in this study was estimated by the difference between the HCl extractable P fractions in the P treated and non P treated soils (e.g. treatment and

control treatment pairs were control vs RPR, S^o/PR vs S^o, S^o/PAPR vs S^o, S^o/PAPRres vs S^o.) at the initial and the last soil sampling (Table 6.7, and 6.8).

6.4.5.2 *Soluble and adsorbed P*

Apart from soils receiving PAPR residues, the NaCl and NaOH extractable P fractions of planted soils decreased with time (Table 6.7). There was no effect of S^o oxidation on the water soluble P remaining at the final sampling. NaOH-extractable P was either unaffected or increased by co-granulation of a particular P-source with S^o, both in presence and absence of plants. However, changes in the amounts of the NaOH-P fractions in planted soils were not consistent nor showed patterns which reflected the magnitude of RPR or RPR residue dissolution (Table 6.8). This result is similar to the results obtain by Rajan (1987) from field trials, where the amount of NaOH-P at any one time is the net result of P dissolved from the fertilizer, plant uptake and its reaction with the soil. The change in the amount of NaOH-P was found to under estimate the extent of PR dissolution when compared with the change in the amount of HCl-P (or 0.5M H_2SO_4) (Bolan and Hedley, 1989).

6.4.5.3 *The PR residue fraction*

The HCl extractable P fraction which includes apatite residues, was considered to be the best estimate of undissolved PR remaining in soil (Rajan, 1987., Δ HCl P, Bolan and Hedley, 1989).

There was a significant decrease in the HCl extractable P fractions in RPR, PAPR and PAPR-res treatments between day 0 and 98 days. When S^o was added to these treatments the decreases in HCl-P between day 0 and day 98 increased markedly in all planted soils (Table 6.7). The results clearly show that the oxidation of S^o increased the extent of dissolution of the insoluble P residues in all planted soils and in the unplanted Tokomaru soil. Consequently S^o addition increased plant P uptake and dry matter yields in these treatments (Table 6.6). It is unclear why S^o amendment did not increase PR dissolution in the unplanted Makotuku soil. In general PR residue dissolution was greater in planted soils, presumably resulting from the fact that plant uptake removes dissolution products allowing dissolution to continue at higher pH values (Kirk and Nye, 1986).

Table 6.7 The amounts of inorganic P fractions at the initial (t = 0 days) and final sampling (t = 98 days) in Tokomaru and Makotuku soil fertilized with various S and P fertilizers in the presence and absence of white clover plants.

	P fractions extracted by								
	NaCl	NaOH	HCl	NaCl	NaOH	HCl	NaCl	NaOH	HCl
	At t=0 day			At t=98 days					
						($\mu g\ g^{-1}$)			
Tokomaru soil				-------(+plant)-------			-------(-plant)-------		
Control	1.8	171.5	75.5	1.7	167.7	57.1	2.2	195.3	63.9
S^o	1.9	174.3	71.3	1.5	165.5	63.9	2.3	199.8	66.9
RPR	6.6	225.3	171.8	4.5	204.2	118.6	5.2	254.1	150.0
S^o/RPR	8.2	227.4	172.1	4.2	205.0	101.3	5.5	279.4	129.8
PAPR	63.3	326.1	175.5	5.9	279.4	147.4	7.1	376.3	179.3
S^o/PAPR	63.6	312.1	170.3	6.7	296.6	126.0	9.4	411.3	175.9
PAPR-res	3.9	201.5	184.5	3.6	201.2	133.9			
S^o/PAPR-res	3.1	198.0	195.0	2.6	205.0	116.3			
Makotuku soil				-------(+plant)-------			-------(-plant)-------		
Control	3.7	169.4	57.1	2.1	161.0	55.2	1.8	187.1	65.7
S^o	4.1	172.9	63.1	2.1	168.5	60.1	1.9	184.1	51.1
RPR	6.2	201.5	192.0	4.2	181.9	159.8	4.4	239.2	141.8
S^o/RPR	6.8	196.7	188.2	4.0	196.0	135.4	4.2	266.0	147.0
PAPR	53.9	312.1	192.4	4.4	275.0	173.6	6.8	363.6	178.5
S^o/PAPR	59.2	298.8	187.5	5.7	292.8	147.4	6.7	380.0	156.0
PAPR-res	4.5	182.7	194.6	3.3	187.8	166.9			
S^o/PAPR-res	3.9	181.3	197.2	4.0	199.0	134.3			

Table 6.8 The dissolution of P (%) from different residual P forms as affected by S^o oxidation in Tokomaru and Makotuku soils.

	Percentage of PR residue +plant	P dissolved[a] -plant
Tokomaru soil	(%)	
RPR	36	11
S^o/RPR	63	38
PAPR	10	0
S^o/PAPR	37	0
PAPR-res	30	-
S^0/PAPR-res	58	-
Makotuku soil		
RPR	22	44
S^o/RPR	40	23
PAPR	12	16
S^o/PAPR	30	16
PAPR-res	19	-
S^o/PAPR-res	45	-

a PR residue P dissolved = $[1 - (\text{PR residue P} / \text{PR residue } P_{t0}] \times 100$

where

PR residue P $= HCl\ P_{t98}$ [P treated - non P treated]

PR residue P_{t0} $= HCl\ P_{t0}$ = [P treated - non P treated]

6.4 CONCLUSION

The oxidation rate of S^o was found to be higher in Tokomaru than in Makotuku soil. The difference may be attributed to the inherent S^o oxidation potentials of each soil and may simply reflect the higher water holding capacity of the finer textured Tokomaru soil. The effect of available water on S^o oxidation is discussed more fully in chapter 8.

The presence of plants in both soils was found to result in lower S^o oxidation rates. This is in contradiction to the generally accepted belief that the presence of plants increases the rate of oxidation due to rhizosphere effects. However, the effect of plants

on S^o oxidation must be dependent on the type of plant and type of S^o oxidising organisms in the soil. The present study used white clover which can fix atmospheric N generating acidity in the process. The generated acidity may cause a decrease in rhizosphere pH which may reduce the activity of heterotrophic oxidizers in the rhizosphere. Several other interactions between plants, available water and O_2% in the soil air may also influence the S^o oxidiser activity.

In both soils, the granulation of RPR with S^o increased the rate of S^o oxidation. Furthermore when S^o was granulated with insoluble P, namely RPR and PAPR residues the S^o oxidation rate was faster than when S^o was granulated with soluble P (i.e. MCP and PAPR). The relative increase in S^o oxidation caused by RPR addition was greater in this experiment where granular fertilizers were used than when S^o/PR physical mixes were used in the incubation experiments described in Chapter 5.

The oxidation of S^o increased the dissolution of the RPR, PAPR and PAPR residues as indicated by the P uptake and the HCl extractable P.

CHAPTER 7

THE EFFECT OF THE PARTICLE SIZE AND FORM OF FERTILIZER ON THE RATE OF OXIDATION OF SURFACE APPLIED S^0

7.1 INTRODUCTION

A number of studies have examined the relationship between particle size and sulphur oxidation rates in soil (Fox *et al.*, 1964; Attoe and Olson, 1966; Li and Caldwell, 1966; Barrow, 1971; Weir, 1975; Shedley, 1982; Lee *et al.*, 1987). Most experiments have been laboratory incubations, in which the S^0 particles were mixed thoroughly with soil, and held under constant temperature and moisture conditions for short periods of time in the absence of plants (Attoe and Olson, 1966; Li and Caldwell, 1966; Lee *et al.*, 1987). Few studies have examined how appropriate laboratory incubation studies are for explaining the SO_4 release characteristics of S^0 applied to field soils (Shedley, 1982; McCaskill, 1984), particularly surface applications to pasture soils.

Most field studies that have been carried out have either examined the influence of S^0 on soil SO_4 levels or S uptake by plants, but because no measurements of residual S^0 were made, the maximum S^0 oxidation potentials of the soil systems could not be defined accurately (e.g. Sinclair *et al.*, 1985; Boswell and Swanney, 1986; Swanney *et al.*, 1988). Large sampling errors due to environmental and soil factors which arise in field trial work may prevent accurate field measurement of S^0 oxidation rates (Barrow, 1971; Lee *et al.*, 1987). In a previous chapter (Chapter 4) it has been shown that accurate rates of S^0 oxidation can only be obtained if amounts of residual S^0 are measured at several time intervals. Strictly speaking, under field conditions measured rates of S^0 oxidation are valid only if they can be demonstrated that S^0 is not lost from trial sites by other mechanisms such as runoff and vertical movement of particles in soils.

In Chapter 5, oxidation rates per unit surface area were found to be similar for different particle sizes of S^0, although in these studies prilling and granulation reduced S^0 oxidation rates. In all but one incubation study the addition of RPR and RPR residues (from PAPR) increased S^0 oxidation, particularly when granules of S^0 and RPR (or residue) were placed in soils. Under field conditions S^0/RPR fertilizers would be applied to soil surfaces, where there is an opportunity for granule breakdown prior to

incorporation into the soil. After granule breakdown the RPR and S^0 may become separated. No published study has shown whether the effect of granulation and the effect of RPR on the S^0 oxidation remain significant in these circumstances.

Surface applied fertilizers are subject to diurnal temperature fluctuations, which may either increase the susceptibility of S^0 to oxidation by physical fracturing, or decrease the activity of the sulphur oxidisers. It is important to define the maximum potential oxidation rate of S^0 in field soils and the relationships between oxidation rates and different particle sizes under field conditions. To achieve this, undisturbed cores of pasture soil were transferred to a glasshouse where they were kept under optimum moisture conditions for S^0 oxidation and at higher temperatures than similar cores that remained at field sites.

7.2 OBJECTIVES

i) to determine the effect of granulation and particle size on S^0 oxidation when S^0 fertilizers are surface applied.

ii) to evaluate the effects of P form on S^0 oxidation when fertilizers are added to soil surfaces in glasshouse and field trials.

iii) to use a radiotracer (^{35}S) to determine the effect of S^0 fertilizer form on the efficiency of S uptake by pasture plants.

7.3 MATERIALS AND METHODS

7.3.1 Soils

Two examples of different soils, the Tokomaru silt loam and the Ramiha silt loam, contrasting in their organic matter content, S content and P retention were chosen for investigation of the effect of P forms and S^0 particle size on the rate of S^0 oxidation in undisturbed soil core experiments.

Tokomaru soil is a moderately leached and moderately acid, classified as a yellow-grey earth (Typic fragiaqualf). It was formed on fairly thick deposits of loess of fine sandy loam texture. Drainage is impeded during the wet season which results in the common occurrence of pale-coloured horizons bearing iron/manganese concretions lying between the top and sub soils (Cowie, 1978). The field site was located at the Massey No.4 Dairy farm 5 km from Palmerston North.

Table 7.1 Location and soil description of the Ramiha and Tokomaru trial sites (Data from P. Sornsri-vichai, 1985 who used the same trial sites)

	Ramiha	Tokomaru
Location	10 km.east of Palmerston North. (Tararua range)	5 km.south of Palmerston North. (High river terrace)
Rainfall (mm)	1270-1520	890-1140
Soil type	Ramiha silt loam.	Tokomau silt loam.
NZ soil group	Yellow-brown earth/ Yellow-brown loam intergrade.	Yellow-grey earth.
Bulk density (0-5 cm; Kg m^{-3})	963	1035
Soil pH (water)	5.5	5.7
Total Carbon (%)	7.6	4.5
N (%)	0.69	0.39
CEC (me.100 g^{-1} soil)	15.5	24.2
Ca (me.100 g^{-1} soil)	4.8	4.3
Organic P (µg g^{-1})	585	420
P retention (%)	88	20
Extractable P (µg g^{-1})		
Water	1.9	6.0
Olsen	10.4	8.6

Ramiha soil is derived from similar parent materials to the Tokomaru soil, but was formed under higher rainfall and the soil has been more strongly leached. The higher P retention is due to the weathering of volcanic ash present in the loess which produces amorphous type clays which are absent in Tokomaru silt loam. Naturally, these soils are low in exchangeable bases and acid in reaction. These soils are classified as

yellow-brown-earths (Andic dystrochrepts). The site used to collect undisturbed soil cores and the field core experiment was a hill soil (intergrade between yellow-brown earth/yellow-brown loam) at Tuapaka farm at an elevation of 300 m, 10 km east of Palmerston North. The climate is cool-temperate with an evenly distributed rainfall through out the year, normally peaking in June and December (Pollok and Mclaughlin, 1986).

Both sites were under established pastures which had regularly been grazed by sheep. The pasture composition on the Tokomaru site were predominantly clover (*Trifolium repens*) and ryegrass (*Lolium perenne*). On the Ramiha site there was a significant amount of browntop (*Agrostis tenuis*), together with ryegrass and white clover. The Ramiha pasture had received superphosphate at the rate of 200 Kg ha^{-1} (1983-84) and 300 Kg ha^{-1} (1984-85). In 1985-86 it received 200 Kg ha^{-1} of Hyphos-S + Se (6% S) and no fertilizer was applied during the 1986-87 trial period. The Tokomaru pasture had not been fertilized for at least 13 years. The locations and soil descriptions are given in Table 7.1.

7.3.2 Preparation of Soil Cores

Galvanised steel cylinders (1 mm wall thickness diameter of 15 cm and length 10 cm) were driven into pasture of uniform sward content at both field sites. The cores were then removed and the bottoms were sealed with nylon mesh. The cores were placed in a glasshouse and maintained at a moisture content of approximately 90% of field capacity by weighing and watering every 3 days. This provided a simulated pasture situation, with soil microflora and fauna, top-soil profiles and plants virtually undisturbed.

Before fertilizer application, herbage on cores at both the field site and in the glasshouse were cut to about 2.5 cm height and the herbage discarded. At the field site the cores were also fenced to prevent access by stock.

Under glasshouse conditions, a minus N, P and S nutrient solution (Middleton and Toxopeus, 1973) was applied regularly to supply other nutrients to the soil cores.

7.3.3 Fertilizer Manufacture

7.3.3.1 *Isotopic labelling*

Isotopically labelled elemental sulphur (S^o) was prepared by Mr. S. Phimsarn (Ph.D. student) (Phimsarn and Hedley, 1988) by adding carrier-free $^{35}S^o$ dissolved in toluene to agricultural grade sulphur (<0.500 mm particle size) contained in an aluminium foil covered porcelain crucible. The mixture was uniformly melted on an electric hotplate at 115-120°C for five to eight minutes. To reduce volatilisation of S during melting, a watch-glass containing 2.00 ml acetone was placed on top of the crucible for cooling and condensing the remaining toluene. The melt material was agitated carefully to ensure even mixing. After overnight cooling in a fume cupboard, the solidified S^o was crushed with a pestle and mortar and sieved to different particle sizes using nylon sieve cloth.

Table 7.2 Fertilizer treatments and the amounts of S and ^{35}S applied to the surfaces of undisturbed soil cores.

Fertilizer Treatments (part. size μm)	Form	P (%)	S (%)	Rate of application mg core^{-1}	$S^{35}S$ MBq core^{-1}	Spec.Activity. MBq g^{-1} S
S^o<150	NG	na	100	53	9.429	177.91
S^o150-250	NG	na	100	53	9.429	177.91
S^o250-500	NG	na	100	53	9.429	177.91
S^o/RPR	G*	10.6	8.9	53	8.797	165.98
S^o grn$^+$	G*	na	8.7	53	9.429	177.91
S^o/PAPR	G*	14.5	7.8	53	na	na
RPR	G*	10.6	na	na	na	na

NG loose particle material. G granulated.
* soil filler used. na not applicable.
grn$^+$ granule.

There was about 2-4 percent loss of S^o during crushing and sieving to three different particle size ranges of 100% <150 μm including 16% <75 μm); 150-250 μm and 250-500 μm fraction.

7.3.3.2 *Granular S^o, S^o/RPR and S^o/PAPR*

Finely ground North Carolina reactive phosphate rock (NCPR, 13.2% P; 100% <150 μm; 80% <75 μm) and its partially acidulated product (30% acidulation) were granulated with S^o (100% <150 μm particle size) to give fertilizers granules containing 9% S^o as described in Section 6.3.3. The S and P contents of these fertilizers are presented in Table 7.2. Samples of uniform granule size (0.5 to 1 mm in diameter) were produced by sieving and these were used in the experiments. A soil filler was used so that the same number of granules were added per core (see Chapter 6, Section 6.3.3).

7.3.4 Fertilizer Application

The fertilizers were applied on the surface of undisturbed soil cores at 53 mg of S^o per core (30 kg S ha^{-1}). The same rates of application were also applied to the surface of the undisturbed soil cores at both field sites. For the glasshouse trial, a total of 18 replicates of each treatment were prepared to give cores (number in parenthesis after sampling date) that could be destructively sampled at the following dates 15(1), 30(3), 45(1), 60(3), 90(3) and 180(7) days. For the field trial 3 replicates of each treatment were prepared which were sampled once at 180 days after fertilizer application. Herbage was harvested from field cores at approximately 30 day intervals.

7.3.5 Leaching

In addition to regular watering the cores were leached on five occasions during the first few weeks with an amount of water equivalent to 10 mm of rainfall to simulate early winter rain events. The leachates were collected for the analysis of SO_4-S and ^{35}S the data are reported in another study (S. Phimsarn Ph.D study). No more than 1% of the ^{35}S added as S^o was leached from any fertilized core.

7.3.6 Soil and Plant Analysis

In the glasshouse six harvests were taken at approximately fortnightly intervals. Herbage was removed and the soil cores were destructively sampled and cut into three sections (0-3 cm; 3-6 cm and 6-10 cm). Soil samples from non-radioactive treatments were air dried at room temperature (20°C). All soil samples from radioactive treatments were frozen and freeze-dried to stop microbial activity before grinding to <1 mm particle size using a large hammer mill.

7.3.6.1 *S^o recovery*

Three replicated samples of 40 g of the top layer of soil (0-3 cm) were extracted with 200 ml acetone on an end-over-end shaker for 16 hours. The soil-acetone suspensions were allowed to settle overnight. Appropriate aliquots (0.2 - 1 ml) were taken for S^o analysis as described in Section 3.4.1 9.

7.3.6.2 *Extractable SO_4-S*

Replicate 5 g soil samples were extracted with 40 ml of 0.04 M calcium dihydrogen phosphate $Ca(H_2PO_4)_2$ on an end-over-end shaker for two hours as described by Searle (1979).

The hydriodic acid reducible S in the soil extract was determined by using the auto analyser method of CSIRO Division of Forest Research (Method No PS17).

7.3.6.3 *Herbage analysis*

Herbage samples were dried at 65°C, weighed and the dry matter yield recorded. The dried herbage was then ground finely and the S concentration in the herbage was measured using the method described in section 6.3.6 2. The samples were also digested using a Kjeldahl-type digestion for N and P analysis using an Technicon AA II, auto analyser (Twine and Williams, 1971).

7.3.7 Determination of ^{35}S in Soil and Plant Samples

Radioactive sulphate sulphur ($^{35}SO_4^{2-}$) has been used in several studies to trace the fate of applied fertilizer S (Chao *et al.*, 1962; Gregg and Goh, 1978; Goh and Gregg, 1982) and to determine the nature of the sulphur cycle in soils (Freney *et al.*, 1971; McLaren *et al.*, 1984). Radioactive $^{35}S^{o}$ has also been utilised in evaluation of the effectiveness of elemental sulphur (S^{o}) fertilizers (Shedley, 1982; Phimsarn and Hedley, 1988.)

Acetone has proved to be effective for extracting elemental sulphur from soil samples (Chapter 3, Section 3.4.1). However, when the activity of $^{35}S^{o}$ is detected by liquid scintillation systems, the presence of acetone will lower the counting efficiency because of it's quenching ability.

Quenching is defined as any process that reduces the overall quantum efficiency of the energy transfer between the β^- particle and light output and hence reduces the scintillation pulse heights and counting efficiency (L'annunziata, 1987). Polar compounds with high electron affinity are potential quenching agents, carbon tetra chloride (CCl_4) and chloroform (used for the measurement of S^{o} oxidation; Barrow, 1968; Watkinson *et al.*, 1987) are the examples of these compounds.

It is expected that the diluted alkaline digests of soil and herbage material will cause some chemical quenching while some soil extracts will be coloured and cause colour quenching. Therefore, for the measurement of $^{35}S^{o}$ activity in acetone extracts, herbage digests and soil extracts will require quench correction curves.

7.3.7.1 *Establishing quench curves for liquid scintillation counting.*

Standard curves were prepared to relate counting efficiency to $H^{\#}$ (see Appendix 7.1 for $H^{\#}$ description). To achieve this a set of $^{35}S^{o}$ and $^{35}SO_4^{=}$ standards, were prepared in liquid scintillation vials containing varying amounts of quenching agent (i.e. varying volumes of acetone (from 0-2 ml) for ^{35}S counting and varying volumes of herbage digest mixtures or soil extracts for $^{35}SO_4^{=}$ counting) in the scintillant fluor. These standards were then counted at optimal window settings and the percent

counting efficiency, E, was given by observed cpm / dpm added x 100. The counting efficiencies of unknown samples were determined from their $H^{\#}$ and the standard curves relating $H^{\#}$ to counting efficiency.

The following quenching curves were generated:

i) **For soil SO_4 extracts**

$$Y = 101.9 - 0.02 \times H \qquad (7.2)$$

$$R^2 = 0.90$$

ii) **For herbage digests**

$$Y = 104.6 - 0.06 \times H \qquad (7.3)$$

$$R^2 = 0.95$$

where

Y = counting efficiency (%)

H = H number (The Beckman quench correction system which uses the 'Compton edge effect' see Appendix 7.1).

One ml of a diluted, herbage digested (digests were diluted to 10 or 25 ml with deionised water) or soil SO_4 extract was mixed thoroughly with 12 ml of the scintillation cocktail solution in twenty ml glass scintillation vials. The mixtures were left overnight in a dark room in order to limit chemiluminescence before being counted using a Beckman 3801, Bench-top liquid scintillation counter. The cocktail recipe was 2,5-diphenyloxazole (8 g) and 1,4-di-[2-(5-phenyloxazolyl]-benzene dissolved in a mixture of 2:1 Toluene: Triton X (volume basis).

All ^{35}S activity data were normalized to the day when the fertilizers were applied (29/11/1987) using the relationship:

$$A_o = A_t \times \exp(t \times 0.693 / t_h) \qquad (7.4)$$

where,

A_o = radioactivity at day 0

A_t = radioactivity at day t

t = time from day 0

t_h = half life (87.4 days)

Some of related herbage ^{35}S calculations were presented as follows: (Phimsarn and Hedley, 1988).

Percent recovery of radioactivity in plant materials

$$Rc(h)_n\ (\%)\ (\text{or}\ \Sigma_1\ Rc\ \%) = Ac(h)_n \times Dm(h)_n / B \times 100 \qquad (7.5)$$

where

$Rc(h)_n$ = recovery percentage at harvest $(h)_n$, or

(Σ_1 Rc(h) % = accumulated percent recovery)

Ac = activity of ^{35}S per g of plant (or accumulated uptake, Σ_1 Ac) (Bq g^{-1} plant)

Dm = dry matter (or accumulated dry matter, Σ_1 Dm) (g)

B = Total radioactivity applied in fertilizer (Bq)

Specific radioactivity of ^{35}S in herbage

$$SA = Ac / Sc \qquad (7.6)$$

where

SA = specific activity of the radioactive nutrient (^{35}S / ^{32}S, Bq mg^{-1} S)

Ac = activity of ^{35}S in plant (or accumulated plant material, Σ_1 Ac) (Bq g^{-1} plant)

Sc = herbage S content (or accumulated S uptake (Su); Σ_1 Su) (mg S g^{-1} plant)

Percent of plant S derived from fertilizer (SDFF %)

$$SDFF(\%) = SAp / SAf \times 100 \qquad (7.7)$$

where SAp = specific activity of ^{35}S in plant (Bq mg^{-1} S)

SAf = specific activity of ^{35}S in fertilizer(Bq mg^{-1} S)

or $SDFF(\%) = Fs / Sc$

where Fs = Fertilizer S in plant;

$= \Sigma_1$ Ac / SAf, and

Sc = herbage S content (or accumulated S uptake, Σ_1 Su) (mg S g^{-1} plant)

Percent of oxidised S taken up by plant (OSUP %)

$$OSUP(\%) = \Sigma_1 \, Rc(h)\,(\%) \times 100 / (100 - {}^{35}S^{o} \text{ recovered}(h)_n) \qquad (7.8)$$

where

$(h)_n$ = harvest no. (n)
$^{35}S^{\circ}$ = acetone extractable $^{35}S^{o}$ remaining at harvest (n).

7.3.7.2 *Investigation of methods for improving the counting efficiency of acetone extracts*

As $^{35}S^{o}$ labelled fertilizer is oxidised, the $^{35}S^{o}$ activity in the extracts from soil containing residual $^{35}S^{o}$ fertilizer may decrease. The activity for counting can be increased by concentrating a large volume of acetone extract prior to the addition of scintillation cocktail. Removal of acetone by evaporation may also overcome the necessity for quench correction. However, evaporation may also cause a loss of ^{35}S. An investigation was carried out to examine the effect of evaporation of acetone on the loss of ^{35}S activity.

Two sets (no in set = 10) of scintillation vials were prepared containing various amount of ^{35}S labelled S^{o} (specific activity 0.06 MBq mg^{-1} S) dissolved in acetone (1 ml) giving a range of activities (1×10^4 to 6×10^4 cpm). The acetone in both sets was allowed to evaporate. In one set a fresh aliquot (1 ml) of acetone ($^{35}S^{o}$ carrier free) was added. Scintillation cocktail was added to both sets and ^{35}S activity counted. Removal of acetone gave increased counting rate (Figure 7.1). Drying followed by the addition of fresh acetone, however, gave counts similar to that of undried samples. This indicates that there was no loss of $^{35}S^{o}$ during the drying of acetone. Presence of acetone caused quenching and thereby reduced the counting rate. In the subsequent analyses it was decided to dry the acetone extracts before the addition of the scintillation cocktail. Repeated evaporation of 1 ml aliquots of acetone caused a minor reduction in $^{35}S^{o}$ activity (Figure 7.2). It was not necessary, however, to use repeated evaporation as a method for concentrating $^{35}S^{o}$ activity in this study.

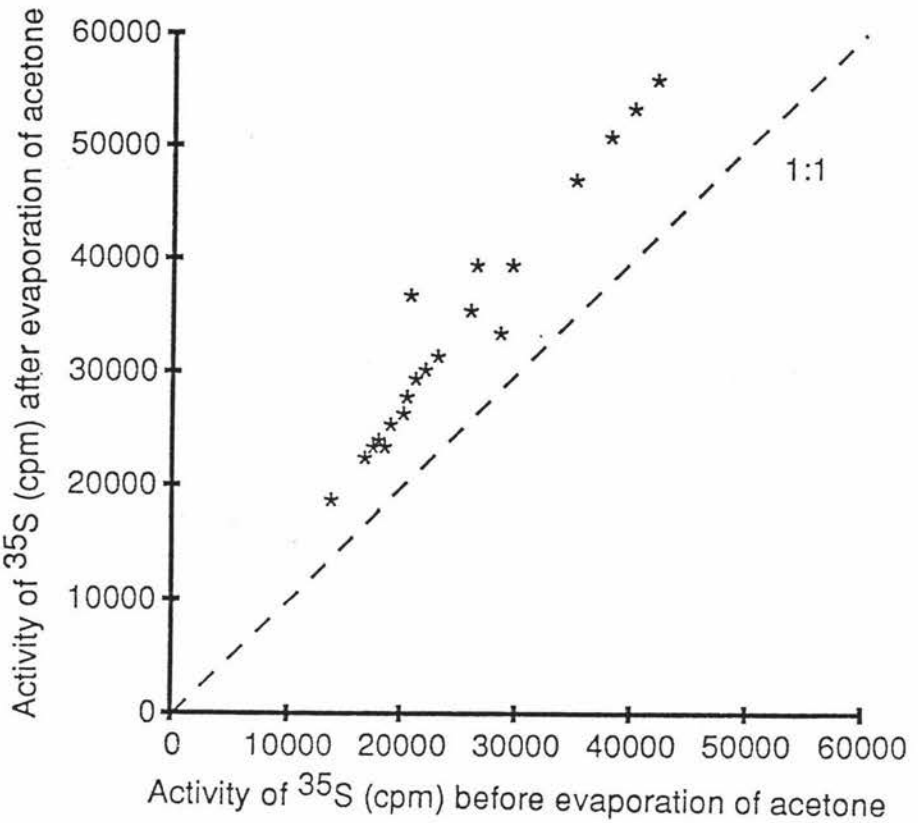


Figure 7.1 The relationship between the activity of ^{35}S counted before and after the removal of acetone by evaporation.

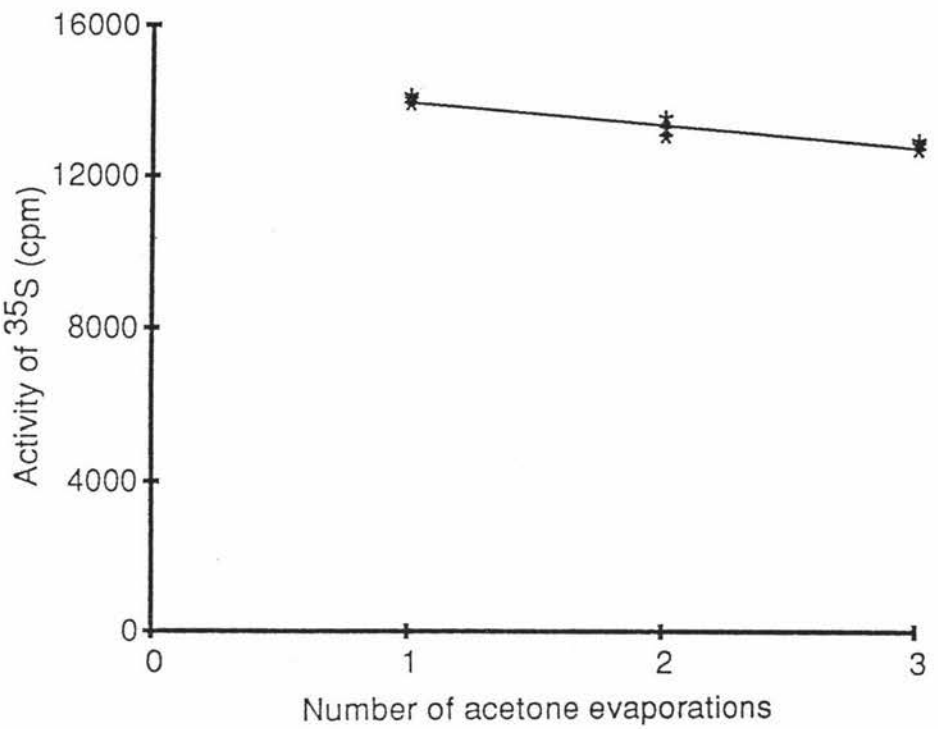


Figure 7.2 Relationship between the number of acetone evaporations and the activity of ^{35}S cpm remaining in the sample.

7.3.8 Statistical Analysis

At 15 day and 45 day harvests only single replicate cores were sampled for the purpose of curve fitting and not for statistical analysis. At the other harvests triplicate cores were sampled except for the last harvest (180 days) in which 7 cores were sampled from each glasshouse treatment. Each set of treatments within a soil type was subject to analysis of variance to determine the significance of treatment effects. Both sets of soil data were also pooled to produce the analysis shown in Table 7.4. Differences between treatment means were compared using Duncan's Multiple Range Test.

7.4 RESULTS AND DISCUSSION

The rates of S^o oxidation as measured by the amounts of residual S^o remaining in the soil and the recovery of $^{35}S^o$ activity from soil in acetone extracts were compared for the following fertilizer effects.

7.4.1 Effect of S^o Particle Size

7.4.1.1 *S^o remaining*

For the purposes of comparison between treatments it is assumed that any removal of S^o from the top 0-3 cm soil layer (e.g. vertical movement of particles) other than by oxidation, is similar for all treatments. Negligible S^o was detected in acetone extracts of some selected glasshouse soils samples taken at the 3-6 cm depth.

The percentages of added S^o (53 mg S^o $core^{-1}$) remaining in the top layer (0-3cm) of soil cores fertilized with different S^o particle size ranges and granulated with different P forms are presented in Tables 7.3 and 7.8. Such was the variability in the measurement of S^o recovered by acetone extraction that in the Tokomaru soil at least 60 days of incubation were required before differences in the amounts of S^o remaining in the three particle size treatments were evident. In the Ramiha soil these differences were evident at 30 days, but not significantly different.

For both soils the rates of oxidation of S^o (estimated from the amount of S^o remaining at 180 days) in the glasshouse and field trials appeared to be similar (Table 7.3). At the last harvest (180 days), the amount of S^o oxidised increased significantly ($P < 0.05$) with a decrease in particle size (Table 7.4 treatment means averaged over two soils). Both the glasshouse and field results are generally consistent with effects of particle size on S^o oxidation observed in Chapter 5 and the results of previous workers (e.g. Fox *et al.*, 1964; Shedley, 1982; McCaskill, 1984 and Watkinson 1989) who reported linear relationships between the calculated particle surface area and initial S^o release rates.

It is noted that the S^o measurement technique did not appear sufficiently sensitive to detect the small changes in the amounts of the large particle size (250-500 μm) of S^o remaining at the early samplings (0-45 days) but sufficient S^o had oxidised by the final sampling dates (180 days).

Table 7.3 Effect of S^o particle size on the percentage of added S^o remaining in the 0-3 cm soil depth at each harvest (Non-granulated materials).

Particle size (μm)	days after application 15	30	45	60	90	180	180
	S^o remaining (%)						
			Glasshouse trial				Field trial
Ramiha							
S^o<150	97.0	61.7	72.6	57.5[a]	54.2[a]	22.5[a]	20.7[a]
S^o250-500	102.8	93.2	122.5	115.1[b]	100.8[b]	48.7[b]	41.5[a]
Tokomaru							
So<150	79.2	79.1	86.0	68.9	55.5	35.5[a]	38.5[a]
S^o150-250	99.2	100.0	95.3	76.6	68.3	58.4[b]	54.8[a]
S^o250-500	94.0	102.3	74.3	80.6	81.1	77.7[b]	73.2[b]

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.

Table 7.4 Mean recovery of S^o (%) at 180 days from the 0-3 cm depth of undisturbed soil cores of the glasshouse and field trial with both Ramiha and Tokomaru soils (A. group means for all S^o treatments, B. individual treatment means for both soils).

A.

	Soil	S^o Recovered %		S^o Oxidised (100 -%recovered)
Glasshouse trial				
	Ramiha	29.3^a	(2.4)	70.7
	Tokomaru	48.1^b	(3.0)	52.9
Field trial				
	Ramiha	22.9^a	(3.5)	77.1
	Tokomaru	41.5^b	(4.5)	56.3

B.

Treatment (particle size μm)	Physical form	S^o Recovery %		S^o Oxidised (%) (100 -%recovered)
Glasshouse trial				
S^o<150	NG	29.0^a	(2.4)	71.0
S^o150-250	NG	58.4^c	na	42.6
S^o250-500	NG	63.1^d	(4.4)	36.9
S^o/RPR	G	29.3^a	(4.5)	70.7
S^ogranule	G	32.1^{ab}	(3.9)	67.9
S^o/PAPR	G	40.0^b	(2.8)	60.0
Field trial				
S^o<150	NG	29.6^a	(4.8)	70.4
S^o150-250	NG	54.8^b	na	45.2
S^o250-500	NG	57.3^b	(7.4)	42.7
S^o/RPR	G	25.2^a	(4.8)	74.8
S^ogranule	G	28.8^a	(4.7)	71.2
S^o/PAPR	G	20.6^a	(4.4)	79.4

Mean separation based on Duncan's Multiple Range Test at the 5% level.
Standard error of the means in brackets
NG = Non granulated, G = granulated.
na = not applicable.

Irrespective of particle size used in the glasshouse trial, the rate of oxidation was significantly higher in the Ramiha soil than in the Tokomaru soil ($P < 0.01$; Table 7.4) which may be related partly to the higher water holding capacity in the Ramiha soil. After watering to 'core capacity' glasshouse cores of the Tokomaru and Ramiha soils drained to 71.5% and 87.9% of core capacity in the 0-3 cm soil depth, respectively and hence the Ramiha soil provided a more suitable moisture condition at the soil surface for the S^o oxidisers (see Chapter 8, section 8.2).

Shedley (1982) concluded from the published data of Moser and Olson (1953) and Kittams and Attoe (1965) that the rate of oxidation increases with increases in water holding capacity ranging from 30% up to 90% of the maximum water holding capacity.

The effect of particle size on S^o oxidation rates as measured by the amounts of $^{35}S^o$ recovered in acetone extracts (Table 7.5) showed similar trends to the amounts of S^o remaining (Table 7.3). At earlier sampling times (90 and 60 days in Tokomaru and Ramiha soil, respectively), however, the $^{35}S^o$ provided a more sensitive (less variability in replicate data) measure of S^o oxidation than measuring the amounts of S^o residues (Table 7.3). In general, the recovery of $^{35}S^o$ (Table 7.5) from the soil in acetone extracts was lower than the recovery of S^o (Table 7.3). This trend is unexplainable but must result from a consistent analytical error in either the measurement of S^o or $^{35}S^o$ activity.

Table 7.5 The effect of S^o particle size on the percentage of $^{35}S^o$ recovered in acetone from the 0-3 cm of soil cores of Tokomaru and Ramiha soils in the glasshouse and field trial (Non-granulated materials).

Particle size (μm)	days after S^o application 15	30	45	60	90	180	180
			$^{35}S^o$ recovery (%)				
			Glasshouse trial				Field trial
Ramiha							
S^o<150	70.5	58.7	50.4	45.4[a]	42.4[a]	21.4[a]	21.7[a]
S^o250-500	98.4	82.7	81.9	94.6[b]	84.6[b]	75.6[b]	63.2[b]
Tokomaru							
S^o<150	70.1	64.8	66.3	61.8[a]	50.6[a]	30.1[a]	32.4[a]
S^o150-250	87.0	86.5	76.0	69.9[ab]	62.0[ab]	69.8[b]	47.9[b]
S^o250-500	87.5	90.4	68.1	76.0[b]	80.0[b]	69.9[b]	64.3[c]

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.

7.4.1.2 *Extractable soil Sulphate*

The amounts of phosphate extractable SO_4 in the whole ranged from 13.6-65.5 mg $core^{-1}$ (approximately equivalent to 10-50 μg g^{-1} soil) which is considered to be non-limiting of plant growth in pasture soil (Saunders *et al.*, 1988). At earlier harvests there was no significant effect of S^o particle size on the amounts SO_4 in all soils (Table 7.6). Although it was assumed that S^o oxidation would occur immediately with the <150 μm particle size, increased SO_4 levels were not evident due to the high native level of soil SO_4 in both soils which was sufficient to meet the immediate S requirement of the plant. At later times (90 and 180 days in Tokomaru and Ramiha soil, respectively), however, a significant effect of particle size on extractable SO_4 levels was evident. This presumably resulted from the reduction of native soil SO_4 levels in the control pots by continued plant uptake while the amount of SO_4 derived from S^o oxidation had increased.

The effect of particle size on S^o oxidation was more noticeable when only the top 3 cm layer in each core was considered (Table 7.6). This is due to the presence of S^o in this zone and probably the active root uptake in this soil layer caused depletion of native soil SO_4. Both would accentuate the differences between fertilized and unfertilized pots as well as differences in the rates of S^o oxidation of two S^o treatments. The <150 μm particle size produced much higher SO_4 levels than the larger particles indicating greater rate of oxidation for the smaller particle size.

Table 7.6 The effect of S^o particle size on the $Ca(H_2PO_4)_2$ extractable SO_4 (mg $core^{-1}$) in whole soil cores (A) and SO_4 concentration ($\mu g\ g^{-1}$ soil) of the top 0-3 cm soil depth (B) of Tokomaru and Ramiha soil (Non-granulated materials)

A

Particle size (μm)	Days after S^o application 15	30	45	60	90	180	180
	Phosphate extractable SO_4 (mg $core^{-1}$)						
			Glasshouse trial				Field trial
Ramiha							
S^o<150	53.8	48.1	44.7	57.3	52.8	65.5[a]	50.3[a]
S^o250-500	42.6	37.2	63.7	52.0	36.8	53.6[b]	41.7[b]
Control	42.8	46.9	39.1	54.2	46.5	36.5[c]	40.9[b]
Tokomaru							
S^o<150	36.3	32.9	23.9	32.8	43.4[a]	39.8[a]	32.0[ab]
S^o150-250	28.5	29.3	23.6	36.3	25.1[b]	33.7[b]	25.0[b]
S^o250-500	33.3	27.6	13.6	25.9	22.6[b]	33.0[b]	26.5[b]
Control	20.8	23.8	24.2	25.8	19.0[b]	35.1[b]	33.3[a]

B

	Phosphate extractable SO_4 of top 0-3 cm soil depth ($\mu g\ g^{-1}$ soil)						
Ramiha							
S^o<150	57.6	54.4[a]	51.2	54.4[a]	46.9[a]	36.1	27.6[a]
S^o250-500	26.5	20.8[b]	32.0	26.6[b]	19.2[b]	21.0	19.7[b]
Control	33.0	26.1[b]	25.6	29.3[b]	17.6[b]	22.6	19.2[b]
Tokomaru							
S^o<150	33.6	33.1	30.4	39.5[a]	45.9[a]	33.8[a]	15.5[a]
S^o150-250	16.0	17.6	16.0	29.3[a]	15.5[b]	15.4[b]	12.3[b]
S^o250-500	19.2	17.1	11.2	20.3[b]	14.9[b]	11.2[b]	10.7[b]
Control	17.6	14.9	16.0	18.1[b]	11.7[b]	13.7[b]	11.7[b]

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.

7.4.1.3 *Herbage dry matter yield, S concentrations, S uptake*

Changes in the particle size of S^o did not affect the accumulated pasture dry matter yield on the glasshouse or field cores (Figures 7.3a, 7.3b and Appendix 7.2a). Dry matter yield of the herbage grown on the glasshouse undisturbed soil cores were consistently higher than on the field cores. This is due to the more favourable growth conditions (temperature and moisture) in the glasshouse than in the field.

In terms of S concentrations in the herbage on glasshouse cores, the smaller particle size consistently, but not always significantly, increased the herbage S concentrations (Table 7.7). At 30-day sampling time, the S concentration in herbage in all treatments appeared to be above the deficiency S level (0.24%) reported by McNaught *et al.*, (1961). The low herbage S concentrations measured at 60-150 day in the glasshouse reflect S deficiency despite the fact that extractable SO_4 levels in the lower parts of each soil core remained high (see Table 7.6) and the pots were fertilized with S^o. It was visually observed that the proportion of the legume plants in the sward was low particularly in Tokomaru soil. At 180-day, all herbage sampled was above the critical S level probably due to the higher supply of N from mineralisation.

The S concentrations in the herbage on field cores were much lower than that in the glasshouse. This may be attributed to the low proportion of clover plants and N deficiency in soil of the field cores. The total N concentration (2.7-3.0%; Appendix 7.3) in the herbage measured was much lower than the reported levels of adequacy (4.5-5.0%; Cornforth and Sinclair, 1982). This was also observed by McNaught *et al.* (1961) who found that in severely deficient white clover plants, with total S concentration below about 0.13%, the herbage N concentration may fall to as low as 2%, compared with a normal value of 4.5% to 5%. While it is well known that S deficiency limits clover growth and therefore N fixation, it is unclear whether initially low N availability leads to low herbage S concentration.

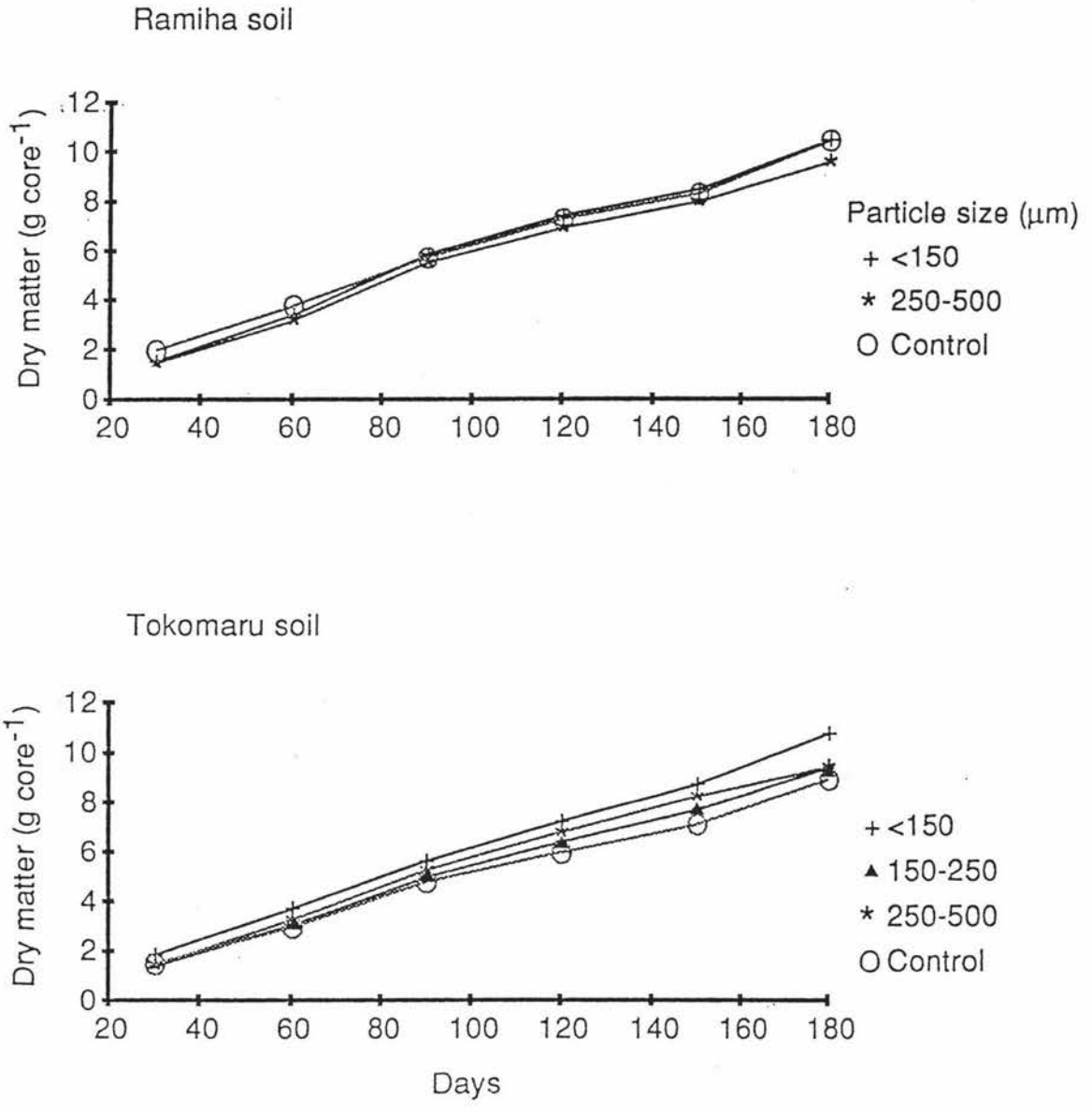


Figure 7.3a The effect of S^o particle size on the dry matter yield of herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions.

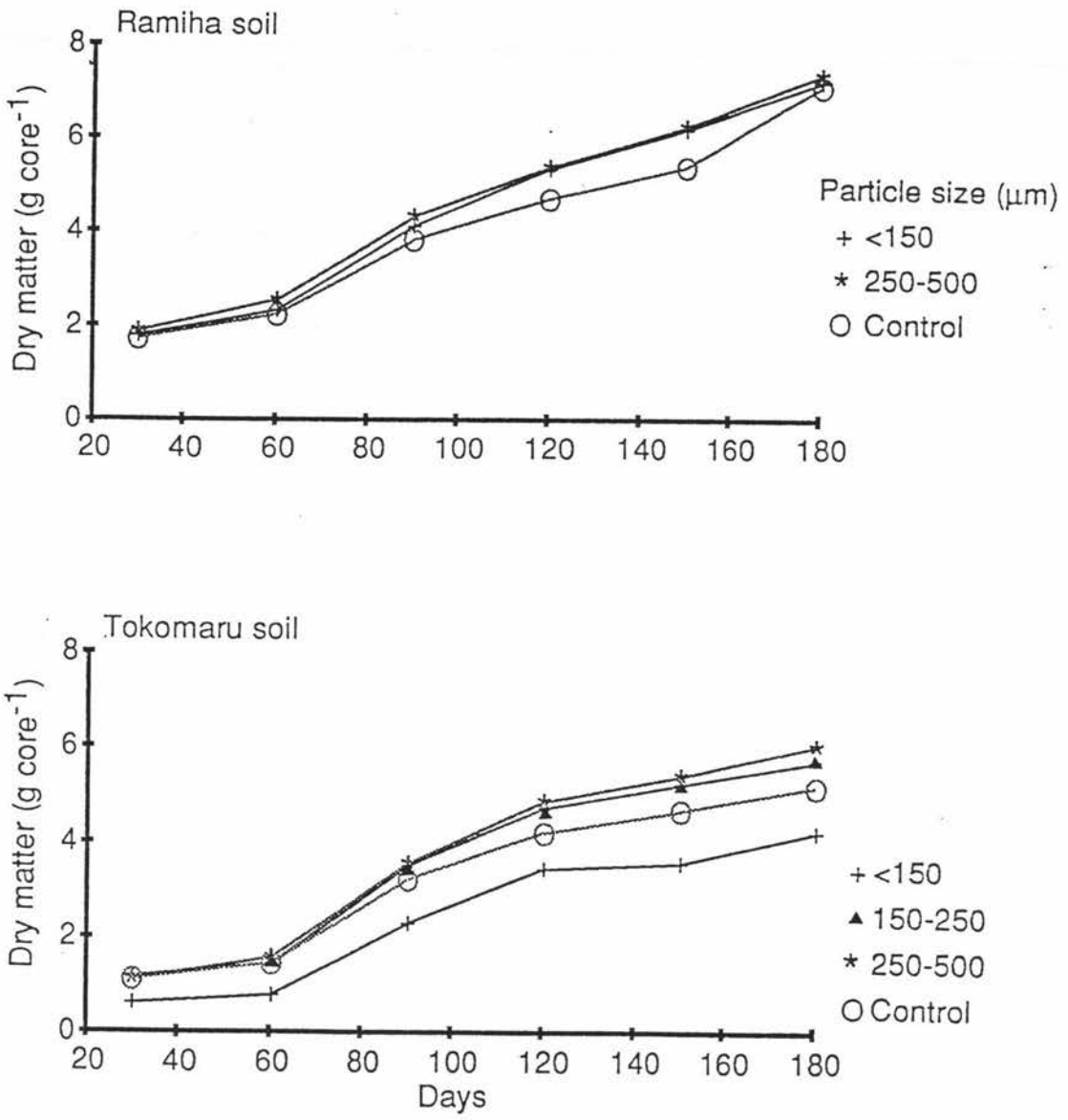


Figure 7.3b The effect of S^0 particle size on the dry matter yield of herbage grown in Ramiha and Tokomaru soils under field conditions.

Table 7.7 The effect of S^o particle size on herbage S concentration (%) grown on undisturbed cores of Tokomaru and Ramiha soil (Non-granulated materials).

Particle size (μm)	Days after S^o applied 30	60	90	120	150	180
	Herbage S concentration (%)					
	Glasshouse trial					
Ramiha						
S^o<150	0.47[a]	0.23[a]	0.20[a]	0.25	0.26[a]	0.39[a]
S^o250-5000.38[b]	0.14[b]	0.17[b]	0.25	0.21[b]	0.34[b]	
Tokomaru						
S^o<150	0.36[a]	0.18	0.20	0.24	0.17	0.32[a]
S^o150-2000.33[ab]	0.17	0.19	0.18	0.15	0.26[b]	
S^o250-5000.31[b]	0.15	0.19	0.16	0.14	0.26[b]	
	Field trial					
Ramiha						
S^o<150	0.15	0.15	0.15	0.22	0.17	0.17[a]
S^o250-5000.14	0.13	0.16	0.18	0.14	0.13[b]	
Control	0.13	0.13	0.15	0.17	0.13	0.12[b]
Tokomaru						
S^o150	0.14	0.11	0.17	0.17	0.17	0.16[a]
S^o150-2000.12	0.13	0.14	0.14	0.13	0.14[b]	
S^o250-5000.12	0.12	0.15	0.14	0.12	0.13[b]	
Control	0.12	0.11	0.13	0.14	0.12	0.12[b]

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.

The accumulative S uptake by the herbage on glasshouse and field cores are shown in Figures 7.4a, 7.4b and Appendix 7.4a, respectively. The smaller particle size significantly increased S uptake only in soil in the glasshouse which could be due to the higher rate of S^o oxidation. The larger particle sizes (<250, <500 μm) resulted in a similar herbage S uptake as the control. Thus the differences in accumulated S uptake were caused by the enrichment of the herbage with S and not by increased dry matter yield. These results were not found in the field which suggest that other factors might have limited S uptake.

There was an significant effects of S^o particle size on the accumulated uptake of ^{35}S by herbage on glasshouse and field cores (Figures 7.5a, 7.5b and Appendix 7.5a) which is contrasting to that of accumulated S uptake (Figures 7.4a, 7.4b). It was noted that at 180-days plant recovery of $^{35}S^o$ was much higher (20% of the applied S) than in the field (10%) in Ramiha soil while the same data for Tokomaru soil was only 14% and 4%.

From these two sets of data it was possible to calculate (see Section 7.3.7.1) the percent of plant S derived from the applied fertilizer and percentage of oxidised S^o taken up by herbage. The calculated results, shown in Figures 7.6a, 7.6b, 7.7, 7.12b and Appendix 7.6a demonstrated that S^o of finer particle size supplied substantially more S to plants than the coarser fractions in both soils. When these results are expressed as the percent of oxidised S entering the plant it is evident that the S oxidised from the finer particle size S was taken up more efficiently by the plant. This is probably because at the same level of S^o application, finer S^o particles fertilize a greater soil volume and thereby created a greater chance for root interception of the SO_4 produced from S^o oxidation.

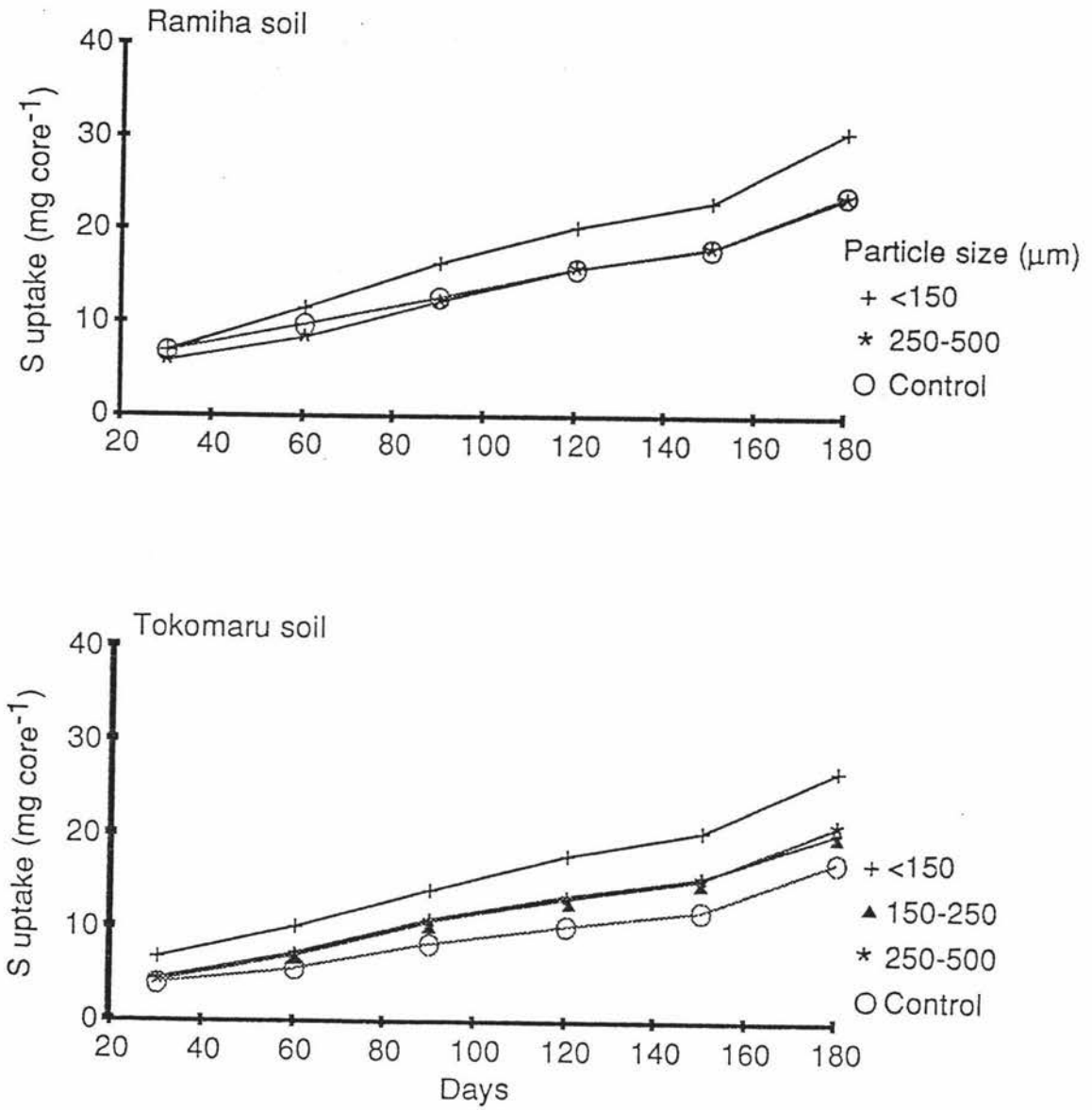


Figure 7.4a The effect of S^0 particle size on cumulative uptake of S by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions.

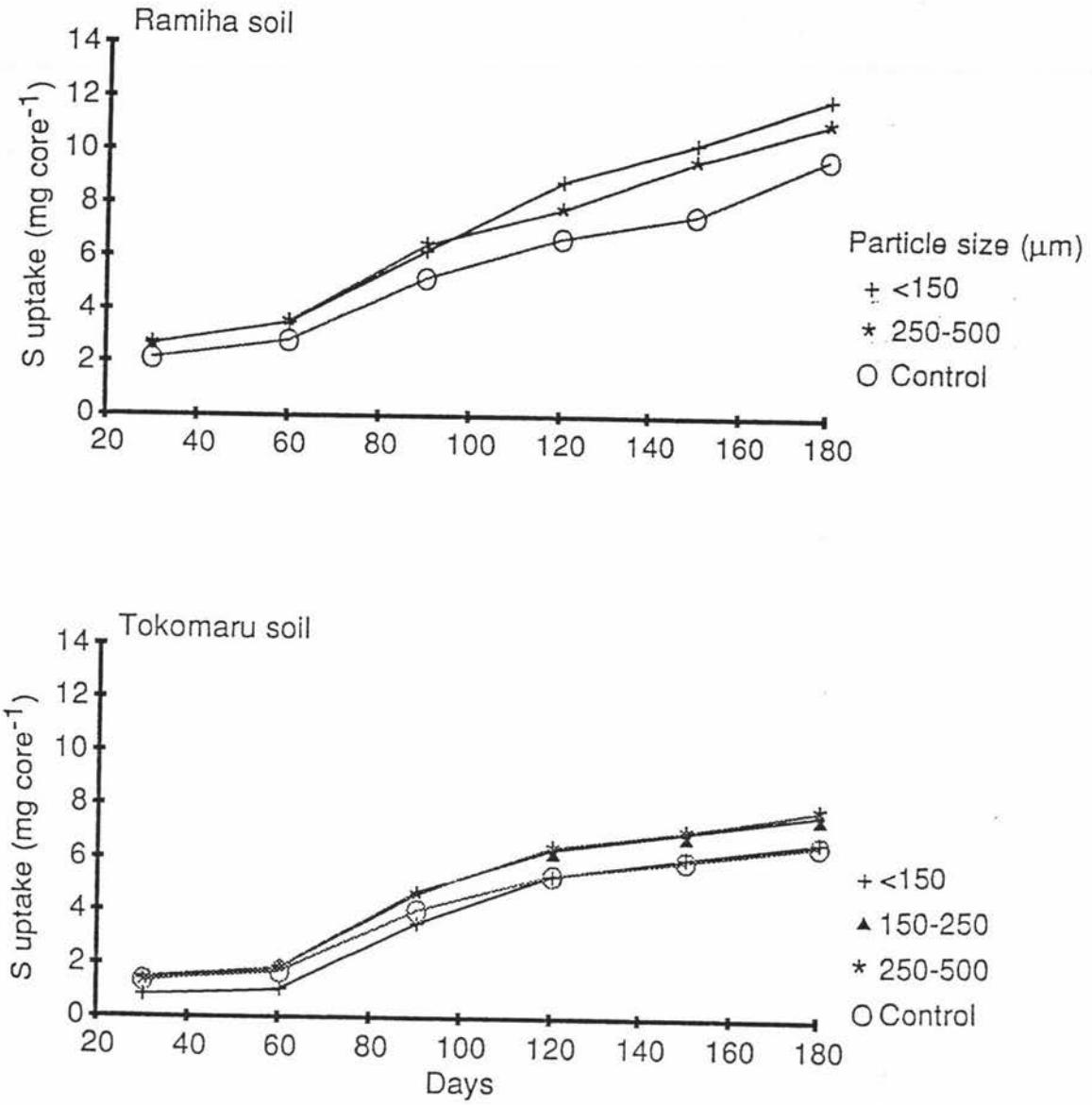


Figure 7.4b The effect of S^0 particle size on cumulative uptake of S by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under field conditions.

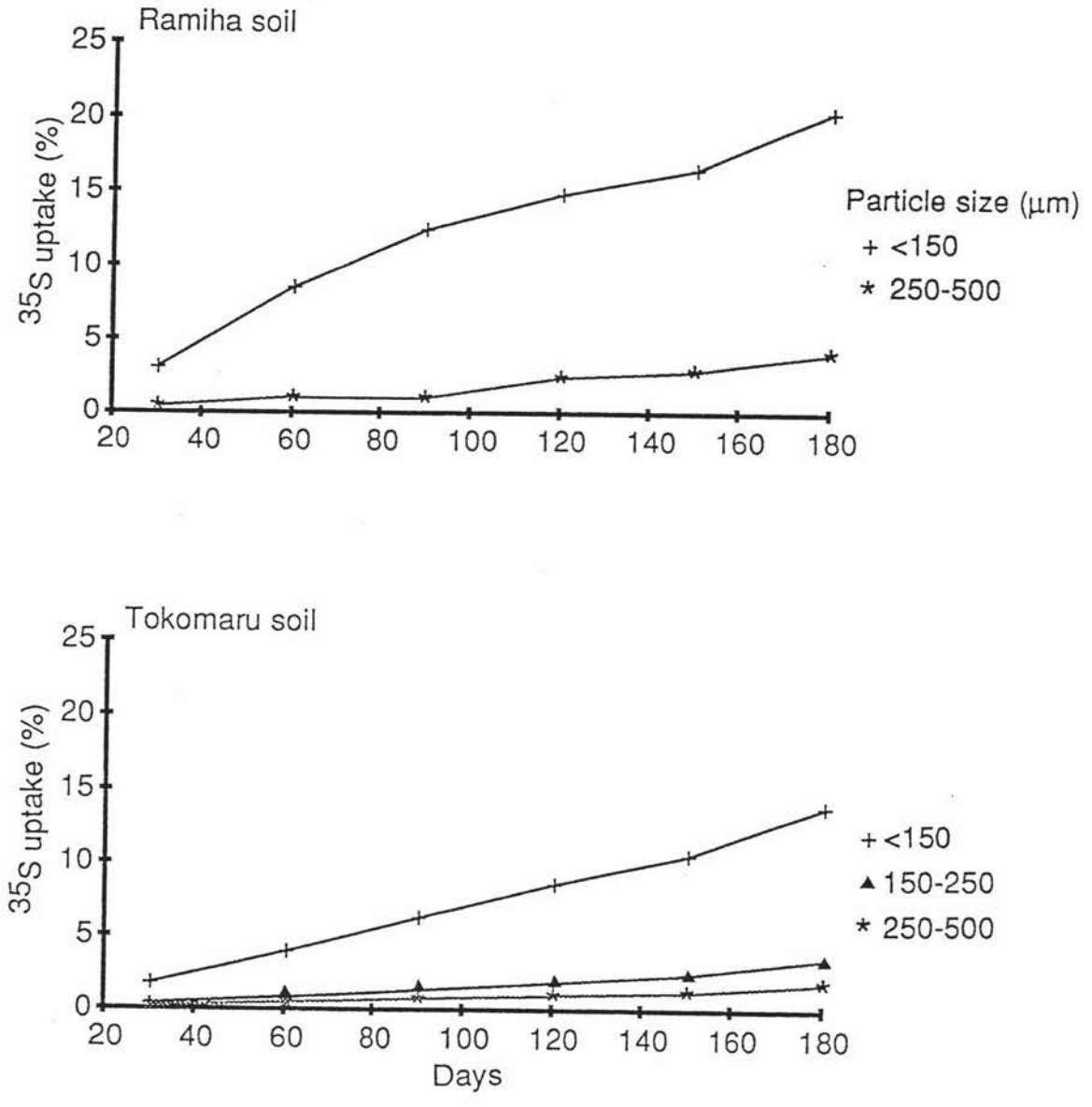


Figure 7.5a The effect of S^0 particle size on the percentage of ^{35}S taken up by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions.

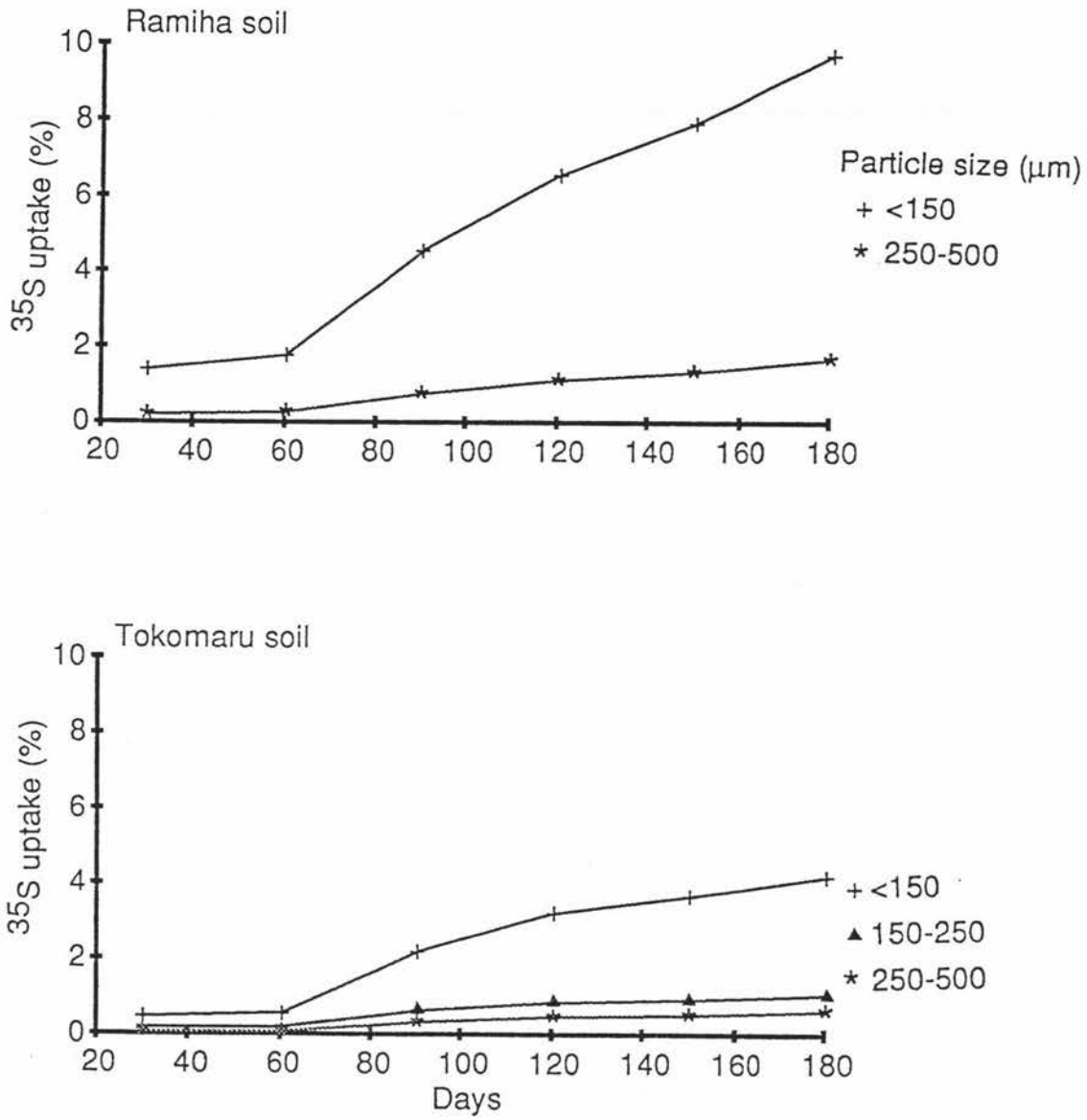


Figure 7.5b The effect of S^o particle size on the percentage of ^{35}S by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under field conditions.

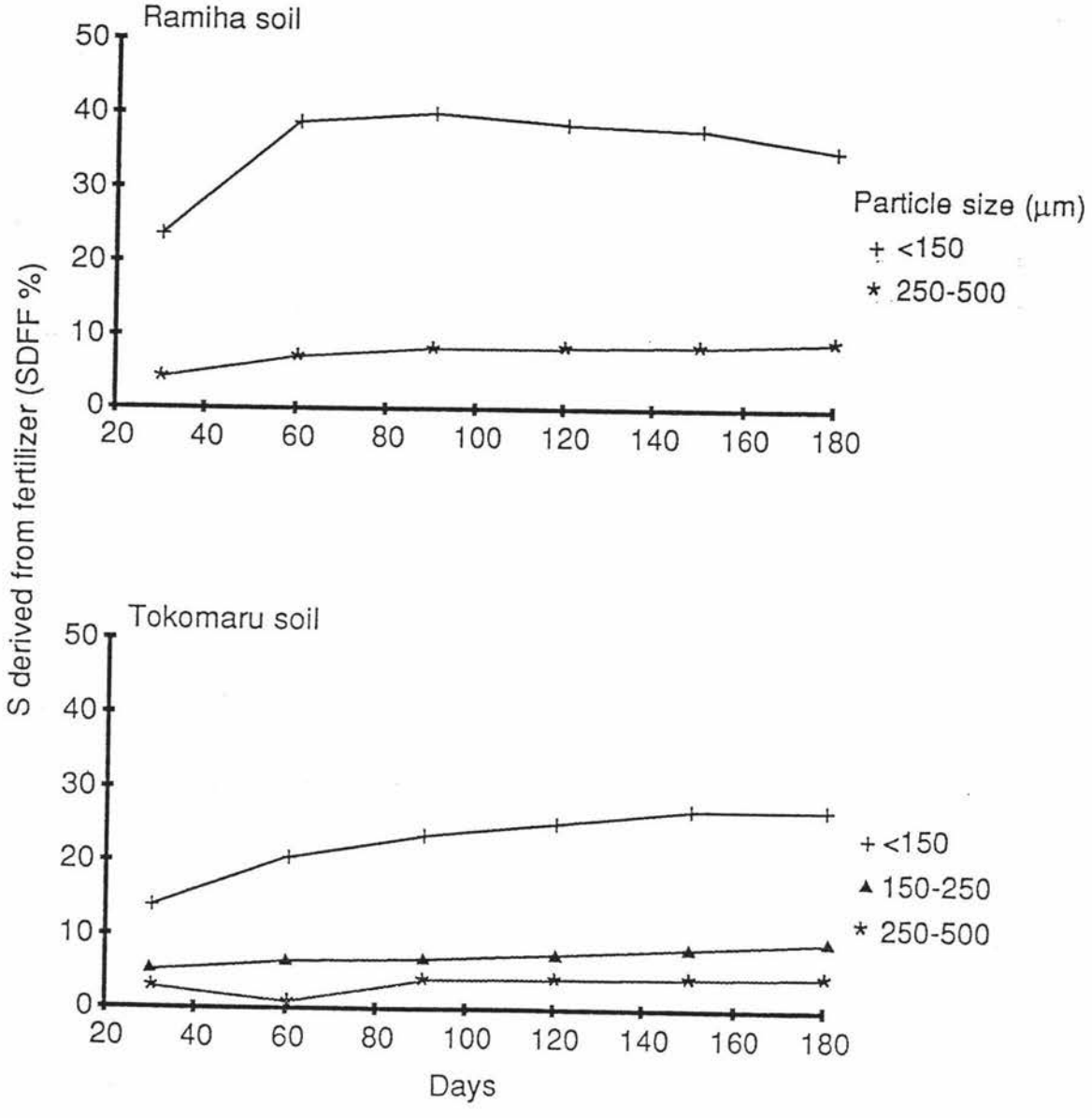


Figure 7.6a The effect of S^0 particle size on the percent of plant S derived from fertilizer S (SDFF) for herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions.

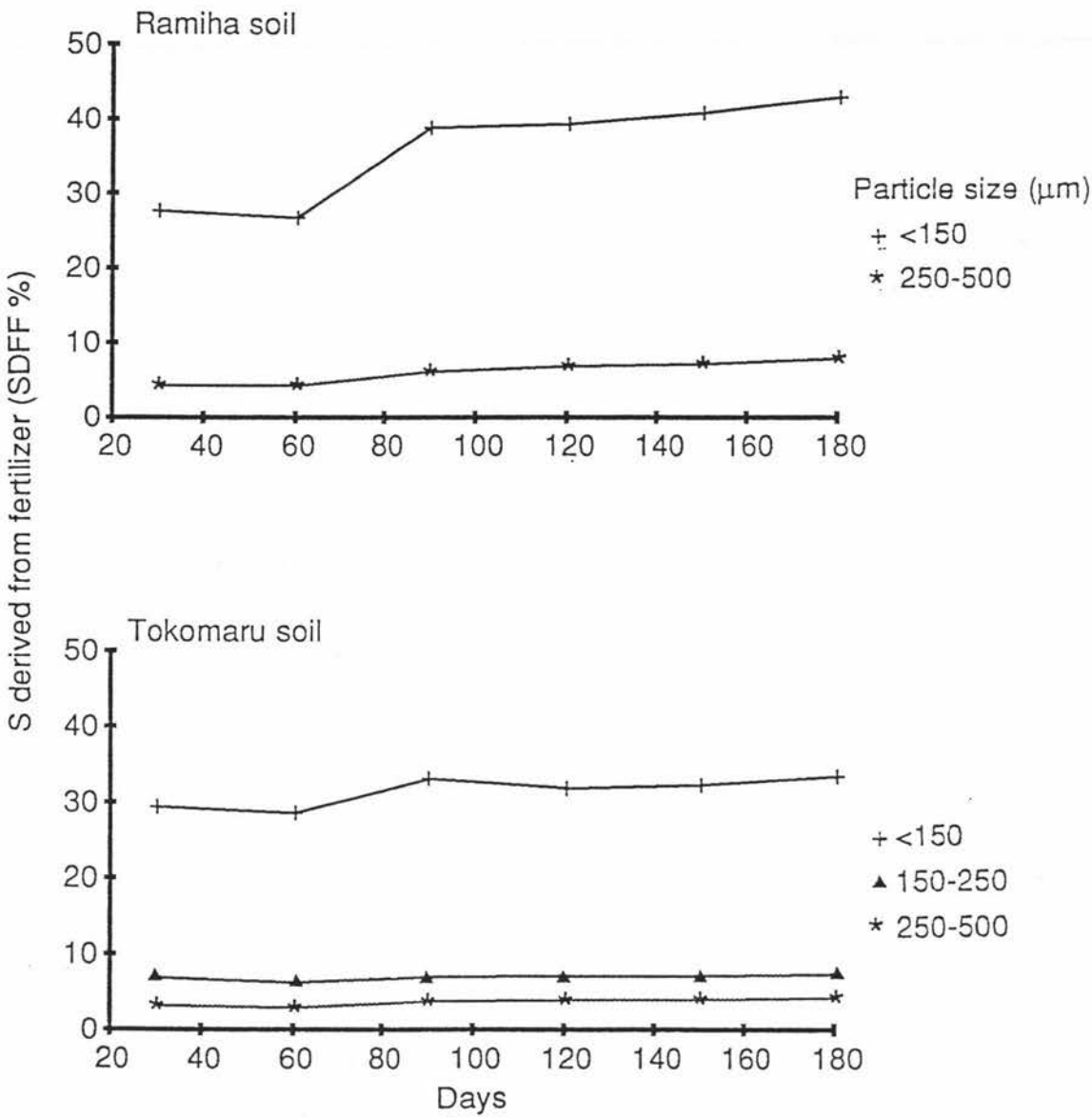


Figure 7.6b The effect of S^0 particle size on the percentage of plant S derived from fertilizer S (SDFF) for herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under field conditions.

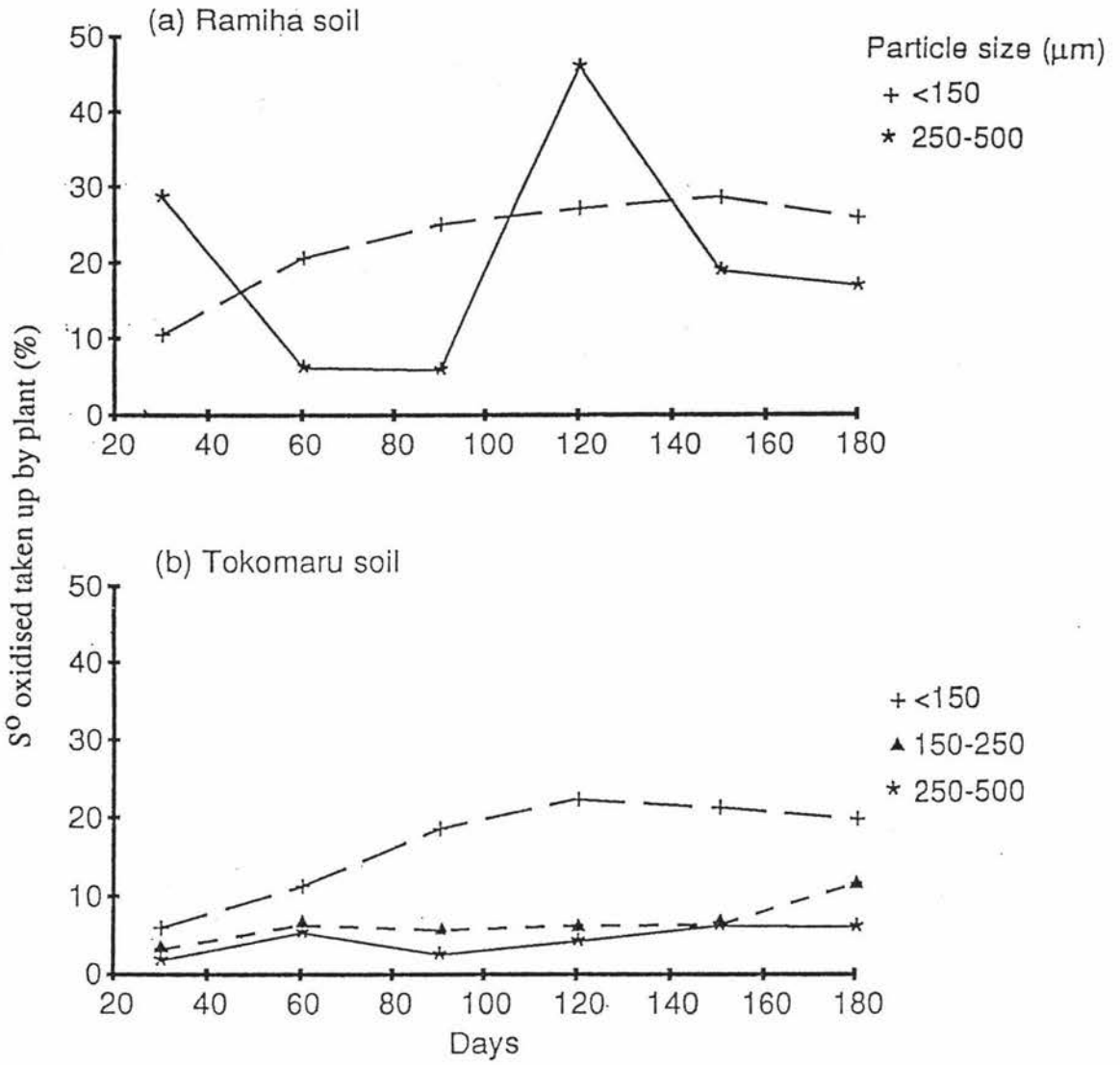


Figure 7.7 The effect of S^0 particle size on the percentage of oxidized S^0 taken up by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions at 180 days.

7.4.2 Effects of S^o Granulation and P Fertilizers Co-granulated with S^o

7.4.2.1 *S^o remaining in soil*

In general, the form of P fertilizer did not significantly influence the extent of S^o oxidation in this experiment when fertilizers were surface applied (Table 7.8 and Table 7.9). Small differences did occur in the Ramiha soil (glasshouse) at 90 and 180 days, but fertilizer effects were not consistent at both dates. This is in contrast to earlier results in the glasshouse and soil incubations that demonstrated S and P fertilizer forms significantly influenced the rate of S^o oxidation of fertilizers placed in the soil.(see Chapter 6, Section 6.4.1). The lack of effect of RPR on S^o oxidation when S^o/RPR granular fertilizers are surface applied may be due to the fact that the granules have more opportunity to breakdown resulting in less intimate contact between the RPR and the S^o particles.

Table 7.8 Effect of fertilizer form on the percentage of added S^o remaining in the 0-3 cm soil depth at each harvest (Granulated materials).

Fertilizer treatments		days after S^o application 15	30	45	60	90	180	180
		S^o remaining (%)						
				Glasshouse trial			Field trial	
Ramiha								
S^o<150	NG	90.7	61.7	72.6	57.5	54.2[a]	22.5[a]	20.7
S^ogran	G	98.3	80.0	67.7	81.3	79.1[b]	22.5[a]	15.0
S^o/RPR	G	94.0	70.6	56.0	66.4	64.2[ac]	18.1[a]	15.8
S^o/PAPR	G	87.2	82.5	93.8	79.4	70.9[bc]	34.7[b]	24.3
Tokomaru								
^{o}<150	NG	79.2	79.1	86.0	68.9	55.5	35.5	38.5
S^ogran	G	79.8	72.6	86.4	81.3	48.7	41.7	34.3
S^o/RPR	G	74.3	87.7	77.9	68.7	63.4	40.4	32.7
S^o/PAPR	G	88.3	86.0	85.5	74.7	71.3	45.1	28.8

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.
NG = Non-granulated, G = granulated.

Table 7.9 The effect of fertilizer form on the percentage of $^{35}S^{o}$ recovered in acetone from the 0-3 cm of soil cores of Tokomaru and Ramiha soils in the glasshouse and field trial.

Fertilizer treatments		days after S^{o} application 15	30	45	60	90	180	180
		$^{35}S^{o}$ recovery						
				Glasshouse trial			Field trial	
Ramiha								
S^{o}<150	NG	70.5	58.7	50.4	45.4	42.4[a]	21.4	21.7
S^{o}gran	G	88.4	67.0	51.2	53.8	63.4[b]	25.5	18.7
S^{o}/RPR	G	78.0	63.4	45.2	52.6	52.1[a]	21.2	22.3
Tokomaru								
S^{o}<150	NG	70.1	64.8	66.3	61.8[a]	50.6	30.1	32.4
S^{o}gran	G	73.9	68.1	69.6	75.6[b]	43.9	35.6	29.2
S^{o}/RPR	G	75.9	69.5	60.0	66.3[a]	65.5	34.3	28.3

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.
NG = Non-granulated, G = granulated.

Concentrations of S in herbage fertilized with S^{o} and S^{o}/RPR granules were similar to that of <150 μm particle size but generally both were higher than the control treatment (Table 7.11). These similar herbage S concentration indicate that surface applied granules did indeed break down releasing S in a manner similar to the <150 μm powder form.

Adding RPR to the fertilizer granules did not significantly enhance the rate of S^{o} oxidation and as expected there were no effects on the amounts of phosphate extractable SO_4 (Table 7.10), percent herbage S concentration (Table 7.11) nor the accumulated herbage S and ^{35}S uptake (Figures 7.9, 7.10 and Appendix 7.5b).

Table 7.10 The effect of fertilizer form on the total $Ca(H_2PO_4)_2$ extractable SO_4 (mg $core^{-1}$) in whole soil cores (A) and extractable SO_4 concentration ($\mu g\ g^{-1}$ soil) of the top 0-3 cm depth (B) of Tokomaru and Ramiha soil.

Fert Treatment	Physical form	Days after S^o applied 15	30	45	60	90	180	180
		Phosphate extractable SO_4 (mg $core^{-1}$)						
	A			Glasshouse trial				Field trial
Ramiha								
S^o<150	NG	53.8	48.1	44.7	57.3^a	52.8^a	65.8^{ab}	50.3^{ab}
S^ogran	G	50.1	46.9	44.5	57.8^a	44.3^{ab}	68.1^a	45.5^{bc}
S^o/RPR	G	45.2	50.8	56.3	68.6^b	47.3^{ab}	75.2^a	54.1^a
RPR	G	44.4	39.8	37.0	61.6^{ab}	35.0^b	57.1^b	44.0^{bc}
Control	-	42.8	46.9	39.1	54.2^a	46.5^{ab}	36.5^c	40.9^c
Tokomaru								
S^o<150	NG	36.3	32.9	23.9	32.8	43.4^a	39.8^a	32.0
S^ogran	G	34.6	49.0	23.3	32.4	31.4^b	41.2^a	33.1
S^o/RPR	G	34.7	40.3	33.7	37.8	25.3^{bc}	43.3^a	35.1
RPR	G	33.2	30.0	21.6	29.6	23.5^{bc}	34.5^b	30.9
Control	-	20.8	23.8	24.2	25.8	19.0^c	35.1^b	33.3
	B	Phosphate extractable SO_4 concentration ($\mu g\ g^{-1}$ soil)						
Ramiha								
S^o<150	NG	57.6	54.4^a	51.2	54.4^a	46.9^a	36.1^b	27.6
S^ogran	G	32.0	41.6^{ab}	32.0	64.5^a	29.3^{ab}	54.2^a	25.6
S^o/RPR	G	40.0	57.1^a	62.4	56.0^a	44.3^a	44.8^{ab}	22.9
RPR	G	32.0	32.5^{ab}	22.4	36.3^b	19.6^b	21.7^b	24.0
Control	G	33.0	26.1^b	25.6	29.3^b	17.6^b	22.6^b	19.2
Tokomaru								
S^o<150	NG	33.6	33.1^a	30.4	39.5^a	45.9^a	33.8^{ab}	15.5^b
S^ogran	G	22.4	36.3^a	30.4	25.6^{ab}	30.4^b	28.1^b	17.6^{ab}
S^o/RPR	G	36.8	41.1^a	46.4	38.4^a	20.8^c	35.4^a	19.2^a
RPR	G	24.0	25.6^a	19.2	19.2^b	19.2^c	12.6^c	11.2^c
Control	-	17.6	14.9^b	16.0	18.1^b	11.7^c	13.7^c	11.7^c

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.

NG = Non-granulated, G = granulated.

Both soils fertilized with S^o granules (S-grn), S^o/RPR granules (S^o/RPR), RPR granules and the standard S^o particle size (<150 μm) produced similar plant yields which were not significantly higher than yields on the unfertilized control soil (Figures 7.8a and 7.8b and Appendix 7.2b). This demonstrated that pasture growth on both soils was unresponsive in the short term to both P and S fertilizer application. This lack of short term responsiveness is the norm for soils where annual fertilizer applications are maintained at maintenance levels (i.e. to balance nutrient loss) or on sites which are extremely N deficient.

The <150 μm S^o powder form produced significantly higher S uptake than the granulated S^o, RPR added and unfertilized control soils under glasshouse conditions on both Ramiha and Tokomaru soils (Figures 7.9a and Appendix 7.4b). However, this difference was not evident under field conditions in both soils (Figure 7.9b) due to the higher experimental variability in the field conditions.

In the glasshouse, the herbage in Ramiha soil recovered significantly less ^{35}S from the S^o granule and the S^o/RPR granules than from the <150 μm S^o particle size. This difference was not observed in the Tokomaru soil.

By calculating the percent of plant S derived from fertilizer and the percent oxidised S^o taken up by plant (Figures 7.11a, 7.11b; 7.12a, 7.12b and Appendix 7.6b), it can be demonstrated that at earlier times of sampling, S^o in <150 μm powder form tended to supply a greater proportion of herbage S than other fertilizer forms. At later sampling times, however, the proportion of plant S derived from fertilizer in both glasshouse and field conditions was not affected by the <150 μm S^o fertilizer form Figures 7.11a and 7.11b.

Interestingly in all soils fertilized with the <150 μm particle size the proportion of plant S derived from fertilizer lay between 24% and 35% despite the fact that several of these treatments did not stimulate increased plant yield or increased plant S uptake. This appears to indicate that the fertilized plants used fertilizer S at the expense of soil derived S.

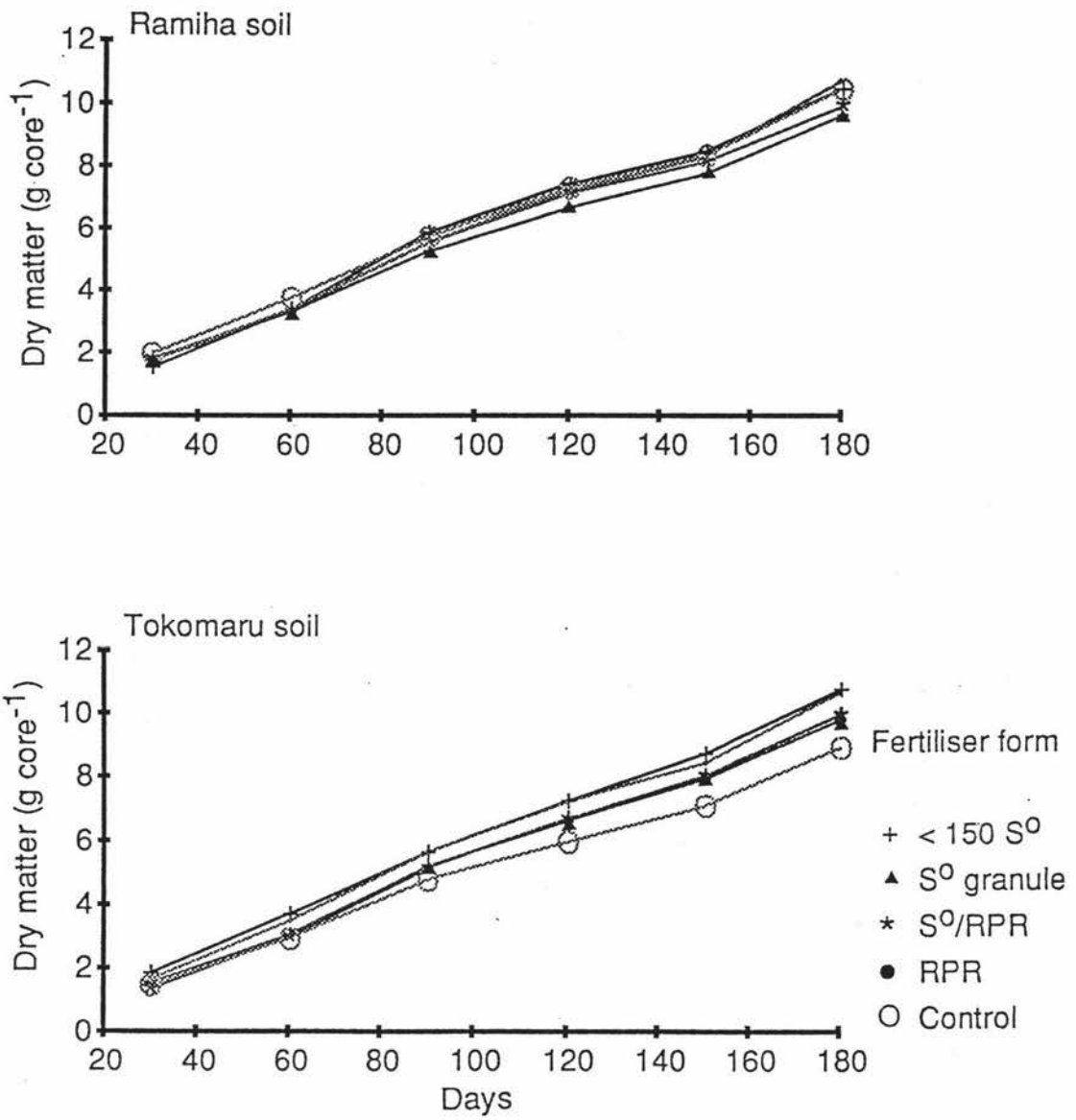


Figure 7.8a The effect of S^{o} granulation and S^{o}/RPR granulation on the dry matter yield of herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions.

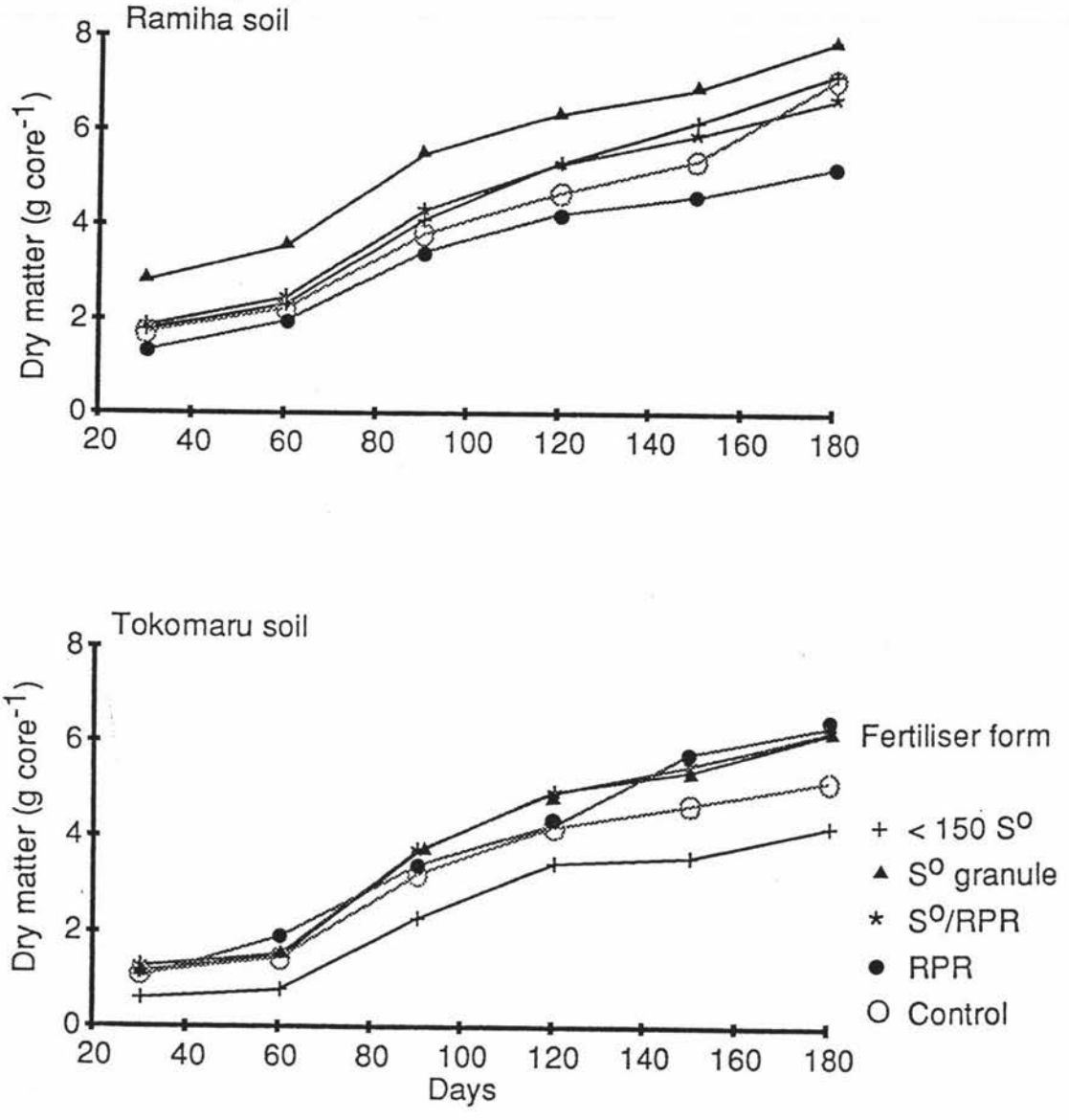


Figure 7.8b The effect of S^{o} granulation and S^{o}/RPR granulation on the dry matter yield of herbage grown in (a) Ramiha and (b) Tokomaru soils under field conditions.

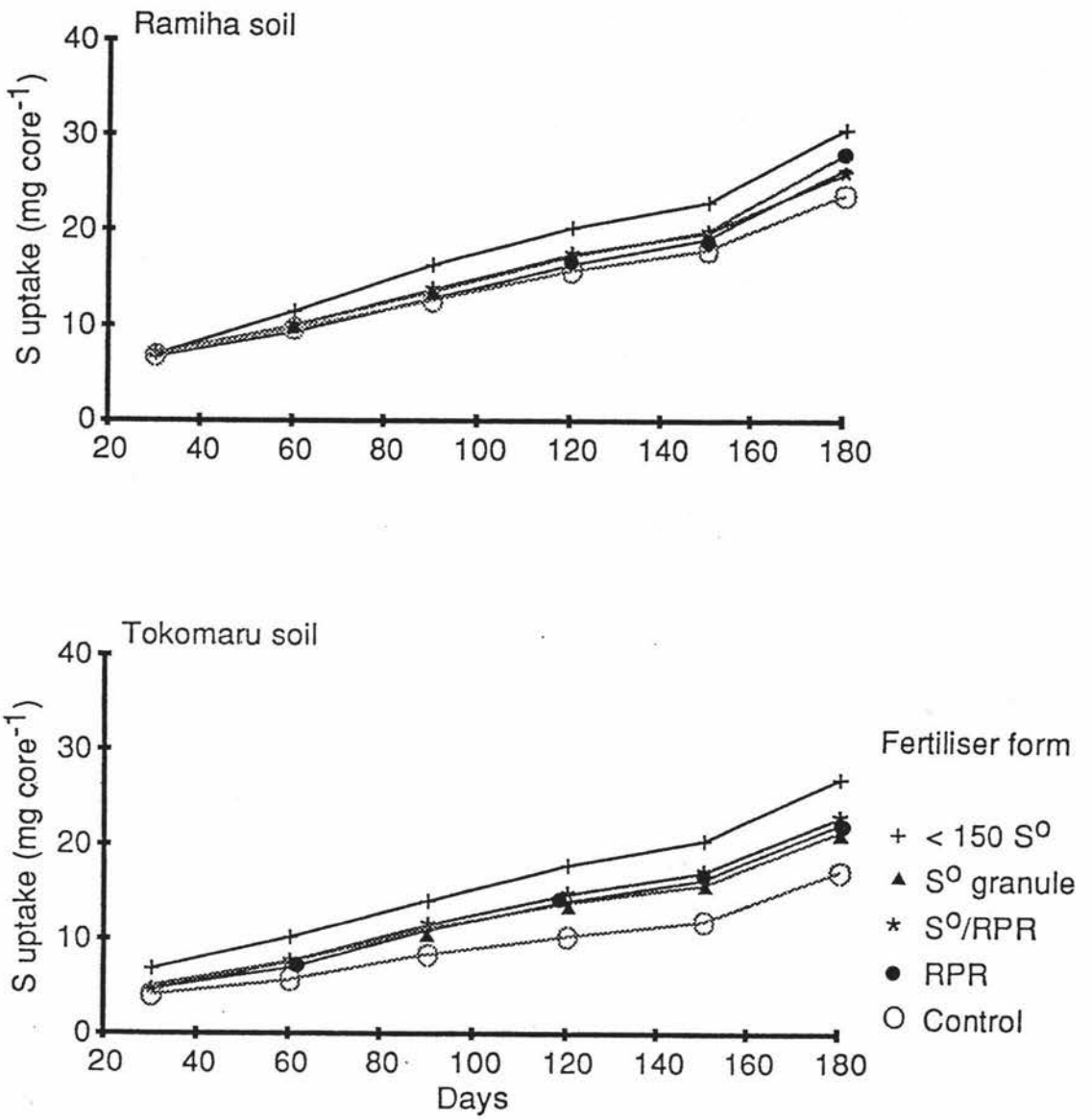


Figure 7.9a The effect of S^{o} granulation and S^{o}/RPR granulation on the cumulative S uptake by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions.

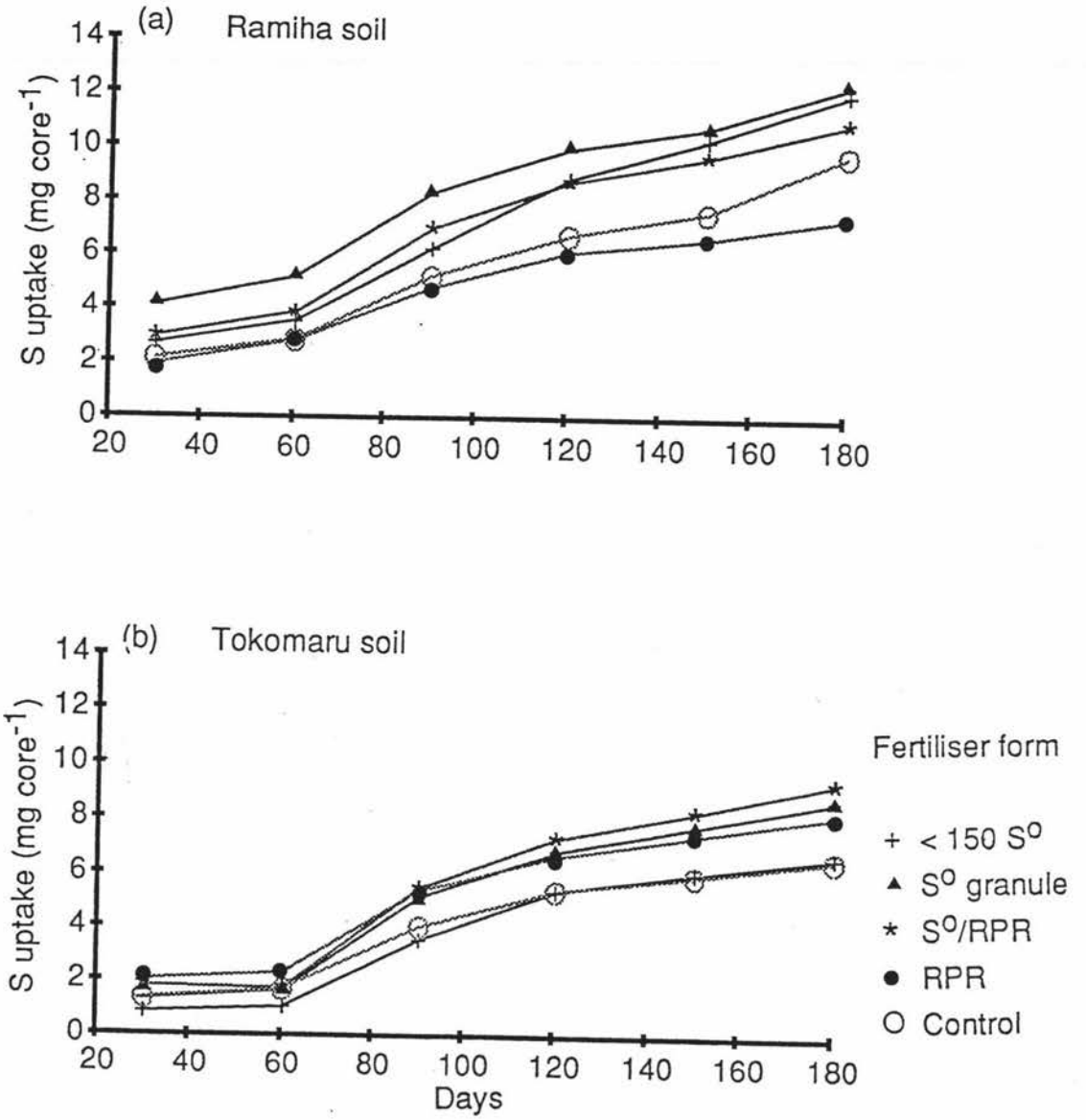


Figure 7.9b The effect of S^{o} granulation and S^{o}/RPR granulation on the cumulative S uptake by herbage grown in (a) Ramiha and (b) Tokomaru soils under field conditions.

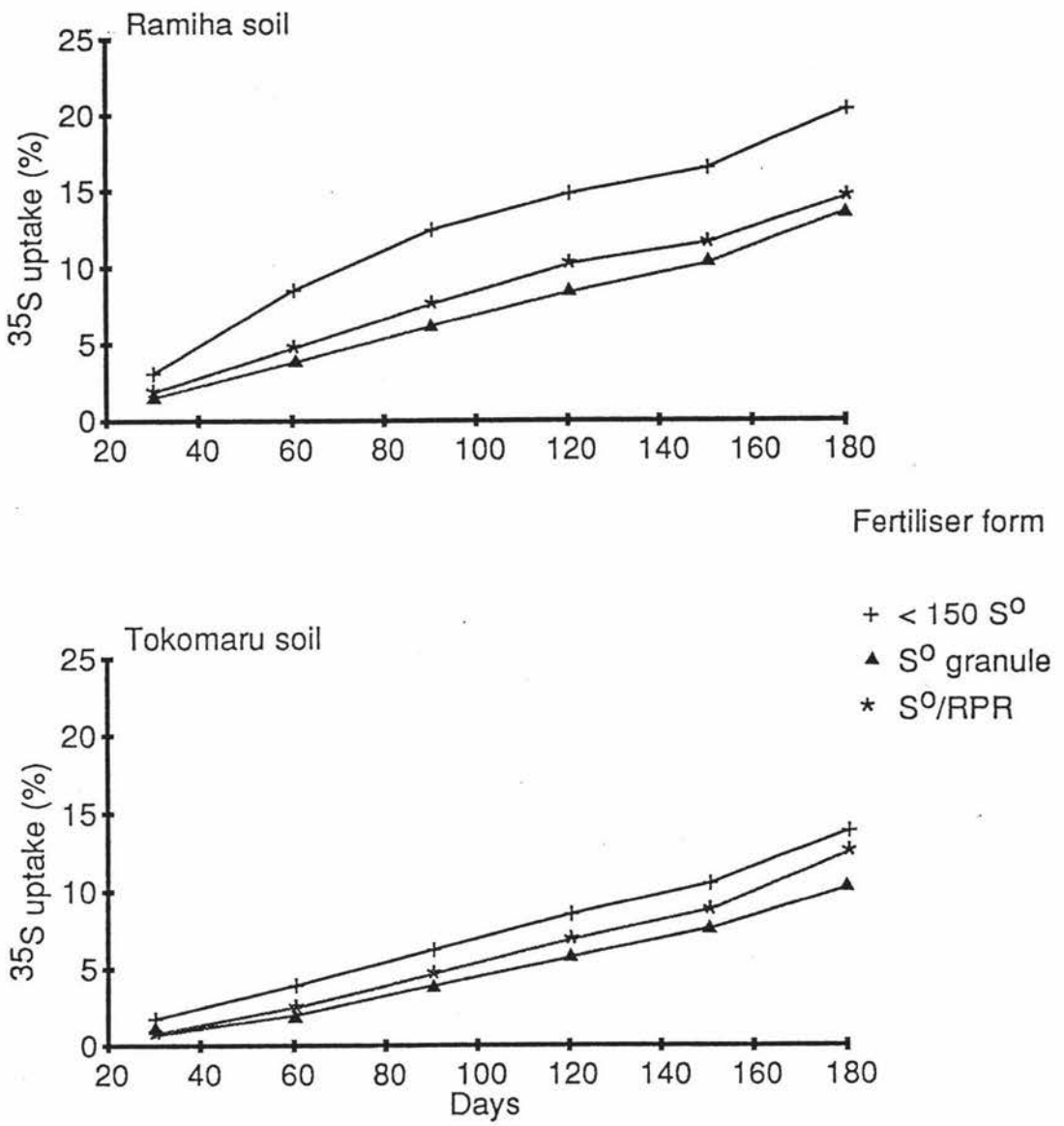


Figure 7.10a The effect of S^o granulation and S^o/RPR granulation on the percentage uptake of ^{35}S by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions.

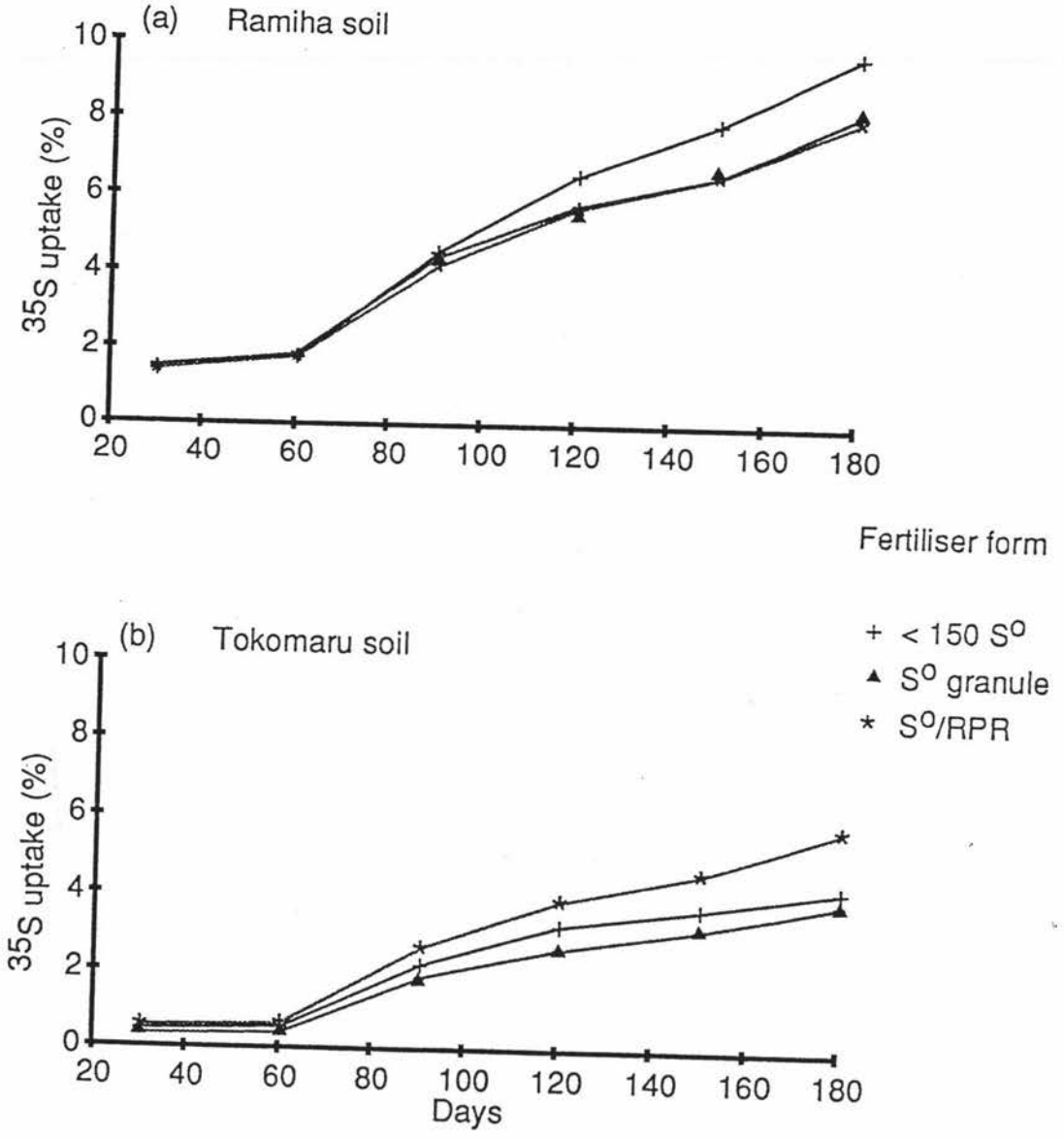


Figure 7.10b The effect of S^{o} granulation and S^{o}/RPR granulation on the percentage uptake of ^{35}S by herbage grown in (a) Ramiha and (b) Tokomaru soils under field conditions.

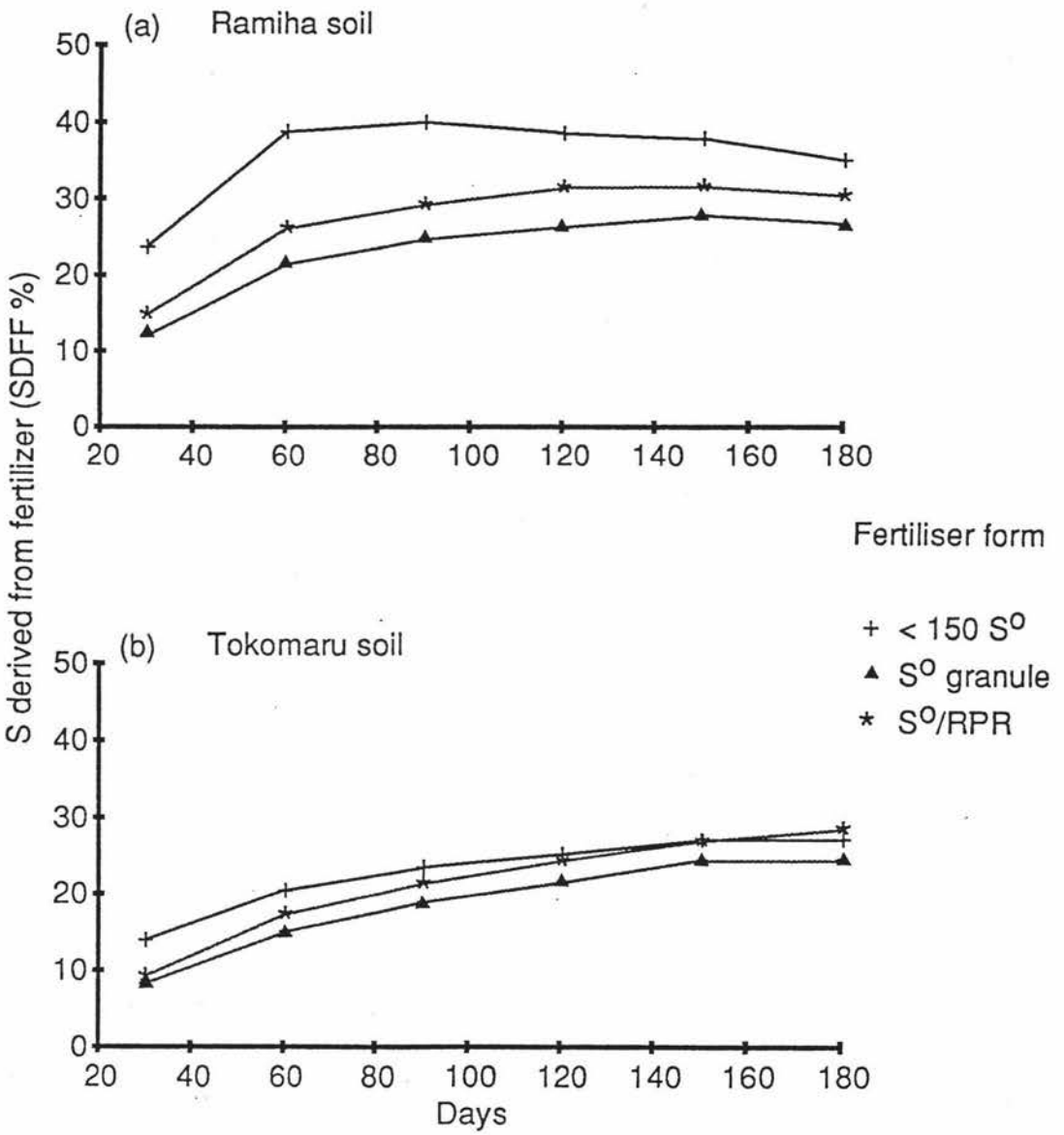


Figure 7.11a The effect of S^0 granulation and S^0/RPR granulation on the percentage of plant S derived from fertilizer S (SDFF) for herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions. The <150 μm S^0 particle size served as the standard treatment.

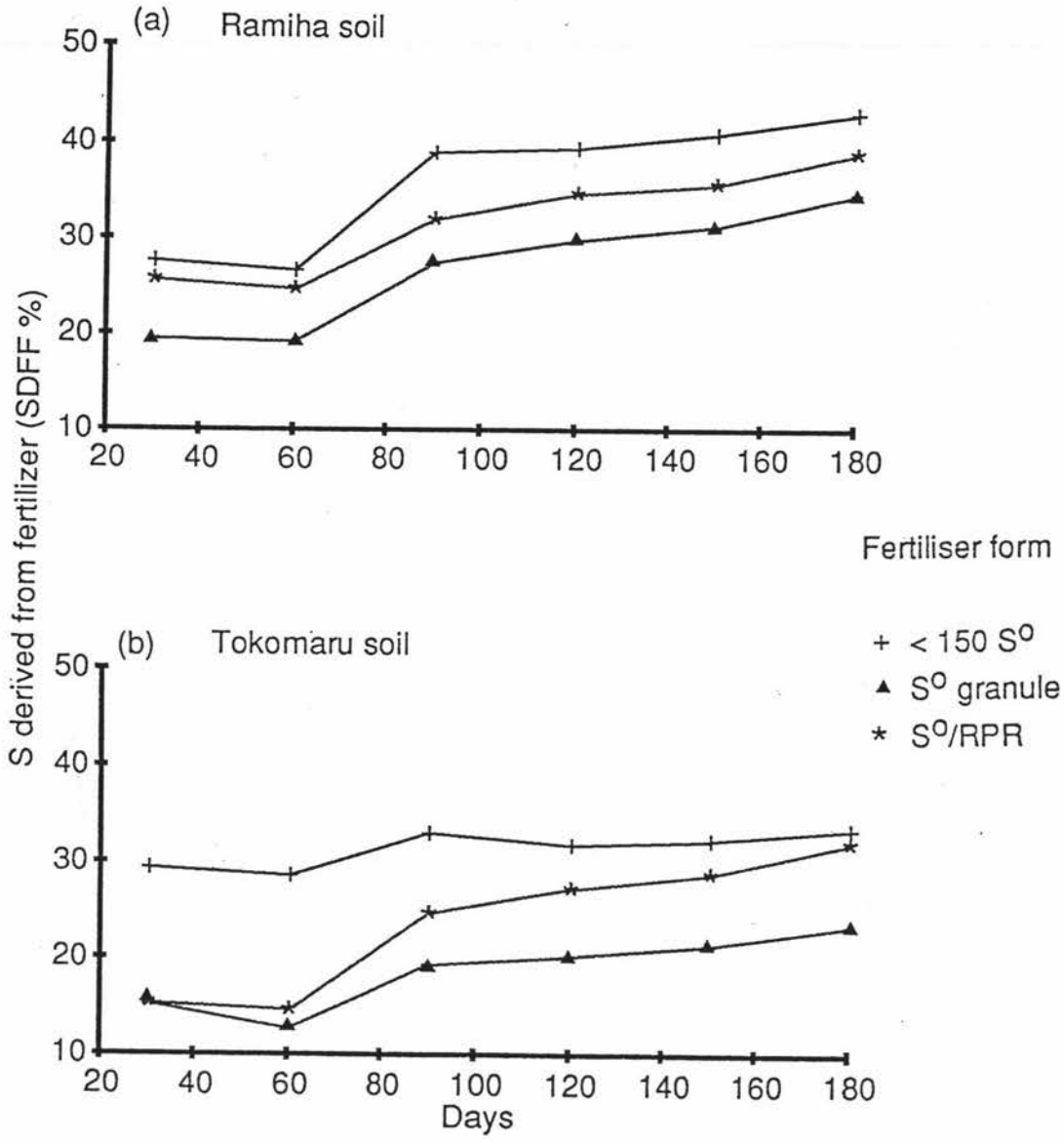


Figure 7.11b The effect of S^o granulation and S^o/RPR granulation on the percentage of plant S derived from fertilizer S (SDFF) for herbage grown in (a) Ramiha and (b) Tokomaru soils under field conditions. The <150 um S^o particle size served as the standard treatment.

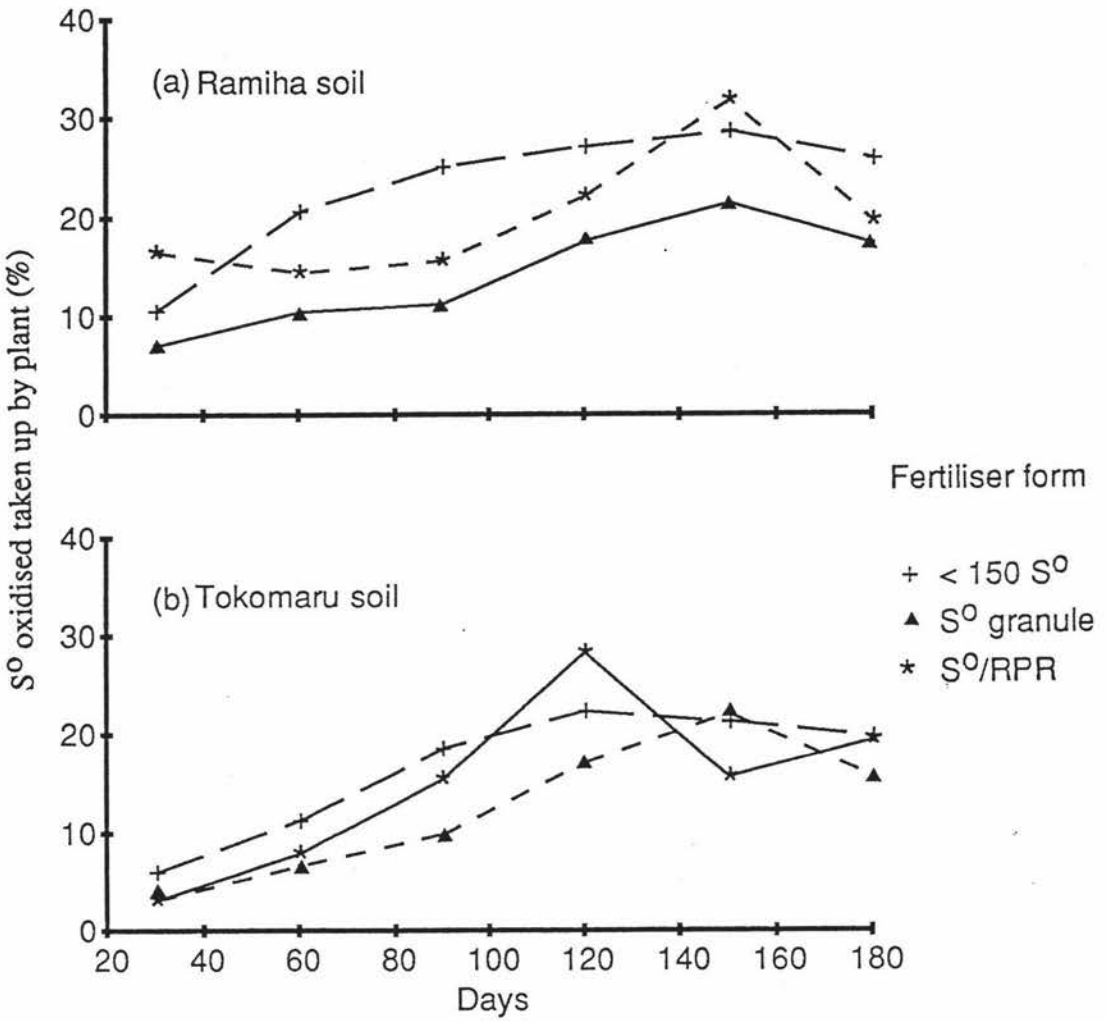


Figure 7.12a The effect of S^0 granulation and S^0/RPR granulation on the percentage of oxidised S^0 taken up by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under glasshouse conditions. The <150 um S^0 particle size served as the standard treatment.

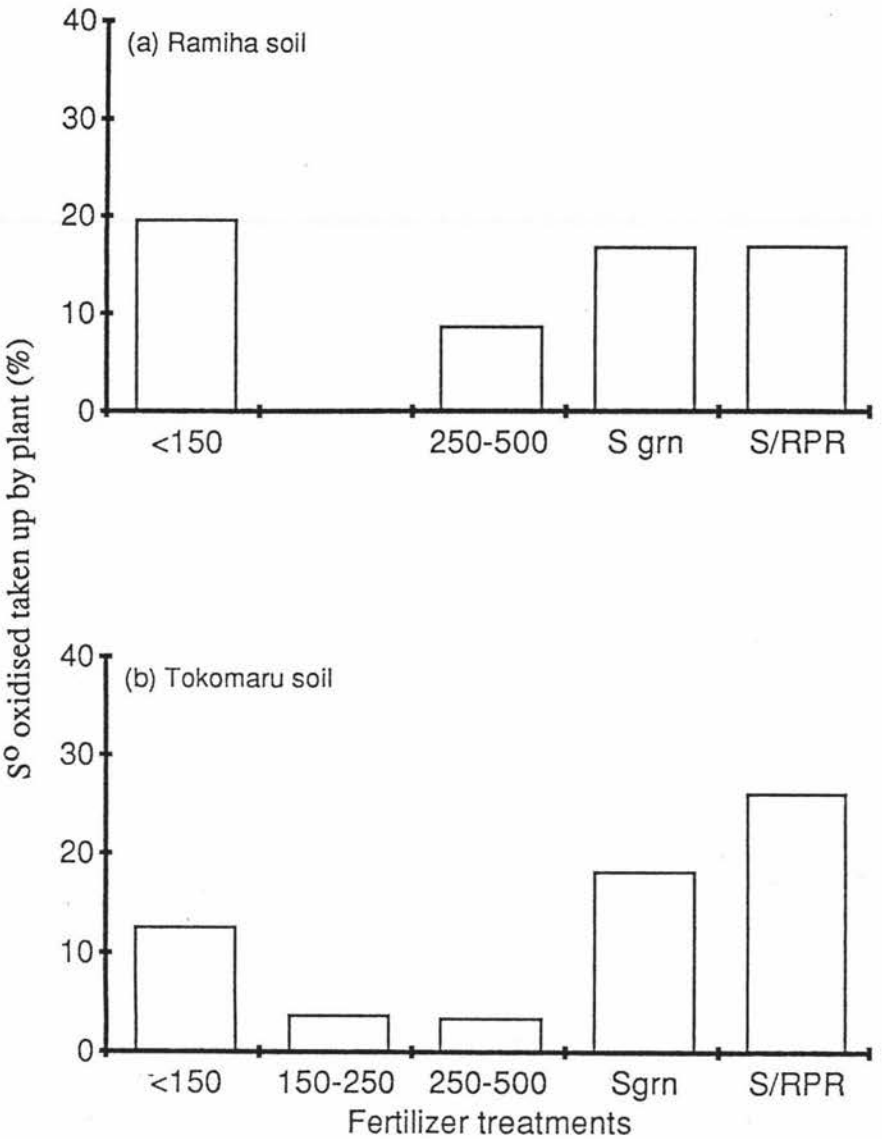


Figure 7.12b The percentage of oxidised $^{35}S^{o}$ taken up by herbage grown in undisturbed soil cores of (a) Ramiha and (b) Tokomaru soils under field conditions.

Table 7.11 The effect of fertilizer form on herbage S concentration (%) in Tokomaru and Ramiha soil.

Fertilizer Treatments		days after S^o application 30	60	90	120	150	180
		Herbage S concentration (%)					
		Glasshouse trial					
Ramiha							
S^o<150	NG	0.47[a]	0.23[a]	0.20[a]	0.25[a]	0.26[a]	0.39[a]
S^ogran	G	0.38[bc]	0.17[b]	0.18[b]	0.25[a]	0.25[a]	0.40[a]
S^o/RPR	G	0.41[b]	0.19[ab]	0.17[b]	0.23[a]	0.23[ab]	0.36[a]
RPR	G	0.40[b]	0.18[ab]	0.17[b]	0.23[a]	0.24[ab]	0.33[a]
Control	G	0.34[c]	0.17[b]	0.15[c]	0.19[b]	0.21[b]	0.27[b]
Tokomaru							
S^o<150	NG	0.36[a]	0.18[a]	0.20[a]	0.24[a]	0.17[a]	0.32[a]
S^ogran	G	0.32[b]	0.14[b]	0.19[ab]	0.22[a]	0.18[a]	0.31[ab]
S^o/RPR	G	0.35[a]	0.17[a]	0.19[ab]	0.21[a]	0.18[a]	0.31[ab]
RPR	G	0.32[b]	0.14[b]	0.17[b]	0.17[b]	0.15[ab]	0.26[c]
Control	G	0.29[c]	0.14[b]	0.14[c]	0.16[b]	0.14[b]	0.29[bc]
		Field trial					
Ramiha							
<150	NG	0.15	0.15	0.15	0.22	0.17	0.17[a]
Sgrn	G	0.16	0.14	0.16	0.19	0.14	0.16[b]
S/RPR	G	0.16	0.15	0.17	0.18	0.14	0.16[b]
RPR	G	0.14	0.14	0.13	0.17	0.14	0.13[bc]
Control	G	0.13	0.13	0.15	0.17	0.13	0.12[c]
Tokomaru							
<150	NG	0.14	0.11	0.17	0.17	0.17	0.16[a]
Sgrn	G	0.11	0.12	0.16	0.16	0.15	0.14[ab]
S/RPR	G	0.15	0.11	0.16	0.15	0.16	0.15[ab]
RPR	G	0.13	0.13	0.13	0.13	0.13	0.13[b]
Control	G	0.12	0.11	0.13	0.14	0.12	0.12[b]

Mean separation based on Duncan's Multiple Range Test at the 5 % level for comparison made within a soil and a harvest. Letters have been added only where significant treatment differences occur.

NG = Non-granulated, G = granulated.

The results in Table 7.8 and the calculated specific oxidation rates (K) presented in Table 7.12 indicate that the rates of oxidation from surface applied S^o granules and S^o/RPR granule were not significantly different from those of S^o in the powder form in terms of the following parameters: amounts of S^o residue, plant dry matter yields and accumulated S uptake. This was true in both soils under glasshouse and field conditions. This contradicts previous results (see Chapter 5) show that when the S^o was granuled and incorporated to the soil, under laboratory and pot incubation conditions, there were decreases in S^o oxidation rates. These constrasting results indicate that there is more opportunity for the break down of the fertilizer granules when surface applied which leads to oxidation rates comparative to S^o applied in the powdered form.

Results in this chapter also disagree with the expected increase in S^o oxidation due to S^o/RPR granulation as has been reported the pot incubation study (Chapter 6) and by Lee *et al.* (1987). However, surface applied S^o fertilizers (Table 7.12) were found to have a slower rates (13 $\mu g\ S^o\ cm^{-2}\ day^{-1}$) of oxidation than when it was incorporated to soil (average of 30 $\mu g\ S^o\ cm^{-2}\ day^{-1}$). Slower oxidation rates can be attributed to less intimate contact between the S^o granule and soil particles which may provide less opportunity for microbes to attach to the S^o surface. Also fluctuations in soil surface moisture and temperature conditions may reduce microbial populations and the rates of microbial oxidation. This aspect is discussed again in Chapter 8 section 8.3. The absence of an effect of RPR on S^o oxidation rate from surface applied S^o/RPR granules may also be due to a lower rate of RPR dissolution at the soil surface as a result of inadequate moisture.

Table 7.12 The calculated (best fit) specific oxidation rates (K, μg S^o cm^{-2} day^{-1}) of surface applied S^o fertilizers in the glasshouse and field trials.

S^o fertilizer form	Simulated oxidation rate (μg S^o cm^{-2} day^{-1})	
	Glasshouse trial	**Field trial**
Tokomaru soil		
S^o<150	14.1 (0.85)[a]	11.8 (0.84)
S^o/RPR	12.7 (0.97)	13.4 (0.66)
S^o Grn	10.7 (0.88)	12.9 (0.50)
S^o/PAPR	10.7 (0.95)	14.8 (1.75)
Mean	12.1	13.2

a value in brackets are coefficient of determination.

7.5 CONCLUSIONS

The oxidation rate of surface applied S^o either in the glasshouse or in the field was found to increase with decreasing particle size of S^o irrespective of the type of soil used. S^o granules were found to have similar oxidation rates as finely divided S^o forms. This result is in contradiction to previous findings in the laboratory and pot incubation studies (Chapters 5 and 6). The lack of effect of S^o granulation on oxidation rate suggests that granular S^o fertilizers can be surface applied and could provide an advantage over the finely divided S^o forms in terms of aerial application convenience provided the granules disperse readily once added to soil surfaces. Granulation of RPR and PAPR with S^o and surface applied in glasshouse and field did not increase the oxidation rate of S^o as found in the pot and laboratory incubation studies (see Chapters 5 and 6).

The efficiency of $^{35}S^o$ fertilizer use by the herbage in the glasshouse was found to be higher with the finely divided particles than the S^o granules and S^o/RPR in Ramiha soil. This was however not found to be the case in the field situation. The opposite trend was observed in Tokomaru soil i.e. higher efficiency with the finely divided

particles in the field than S^o granule and S^o/RPR but a similar efficiency in the glasshouse.

The potential oxidation rates of 17 and 14 μg S^o cm^{-2} day^{-1} were calculated for the fine S^o particle (<150 μm) applied to undisturbed cores of Ramiha and Tokomaru soil, respectively. These values agreed very closely to those measured under field conditions in the same soils. The oxidation rate of S^o applied to undisturbed cores was found to be significantly lower than of S^o incorporated into soils in laboratory and glasshouse incubations.

The implications of the findings in this chapter suggest that the efficiency of plant use of S^o fertilizers can be improved by decreasing S^o particle size. Form of S^o fertilizer, either granulated alone or with RPR and PAPR, will not significantly influence S^o oxidation rates or the efficiency of plant use provided that complete granule dispersion is achieved after application. It is recommended that other experiments are carried out on S responsive sites to confirm this data. Data already obtained by Boswell and Swanney (1988) supports these conclusions.

CHAPTER 8

MODELLING S^o OXIDATION RATES IN THE FIELD SOILS

8.1 INTRODUCTION

With the increasing use of S^o as a fertilizer for pasture, there is a need to accurately predict under all relevant conditions the rate at which it oxidises and supplies sulphate to plants. To predict S^o oxidation rates, it is essential to establish the maximum potential oxidation rate of a S^o fertilizer and the site specific factors that may prevent this potential being realised (McCaskill and Blair, 1989). For such prediction of S^o oxidation there is a prerequisite to establish whether there is agreement in the literature over the rate at which S^o oxidises and then determine whether the major factors that influence this rate can be reasonably explained by mathematical functions.

A wide range of oxidation rates have been reported in the literature, even when experiments have been conducted under similar conditions (Li and Caldwell, 1966; Attoe and Olson, 1966; Shedley, 1982; McCaskill, 1984; Jansen and Bettany, 1987b; Lee at al., 1987). According to Janzen and Bettany (1987b) the traditional way of expressing oxidation rate, as the proportion of S oxidized over a given time (weight per unit time), has the following two inherent major limitations:

i) oxidation rates expressed in this traditional manner are specific to the particle size used, and therefore the rate of oxidation of one particle size is not applicable to particles of a different size.

ii) the oxidation rate expressed as the amount of S^o oxidised per unit time is biased by the confounding effects of diminishing substrate during oxidation. To avoid these limitations these authors proposed that an expression independent of particle size and time should be used and oxidation rate expressed on the basis of S oxidised per unit surface area per unit time ($\mu g\ S^o\ cm^{-2}\ day^{-1}$). In this Chapter this expression is termed as 'specific oxidation rate' (K). There is much experimental evidence to support the use of a specific oxidation rate (McCaskill and Blair, 1987; Janzen and Bettany, 1987b; Watkinson, 1989).

Mathematical models of S^o oxidation using specific oxidation rates are seen as a convenient means of assessing the release pattern of S^o fertilizers (Janzen and Bettany,

1987b; Watkinson, 1988; McCaskill and Blair, 1989). The following assumptions have been made in order to model S^o oxidation by the above workers:

i) Oxidation of S^o proceeds as a function of its surface area. It has been found that S^o oxidation is dependent upon the attachment of microbes to S^o particle (Bryant *et al.*, 1984; Laishley *et al.*, 1986) suggesting thereby that oxidation is a surface dependent process. Linear relationships between the amount of S^o oxidised and the surface area of S^o applied to soil incubations and field trials has also been established by several researchers (Fox *et al.*, 1964; Janzen *et al.*, 1982; Koehler and Roberts, 1983; Laishley *et al.*, 1983; Janzen and Bettany, 1987b. McCaskill and Blair, 1989; Watkinson, 1988, 1989) but may be less dependent on other soil chemical properties.

ii) Most of these models assumed that the S^o takes the form of spheres, although models can be derived for irregular shaped particles (Watkinson 1989). Both types of model produce similar outputs for particles of similar sieve size ranges (Watkinson 1989).

iii) The specific oxidation rate, (K, $\mu g\ S^o\ cm^{-2}\ day^{-1}$), per unit area was found to be essentially constant when measured over long periods of time (Watkinson, 1989). McCaskill and Blair (1989) have shown that the value of K depends on environmental variables such as moisture, temperature and microbial growth rate, but is not influenced by soil texture (McCaskill and Blair, 1987). Oxidation rates are constant where appropriate environmental conditions were held constant (McCaskill and Blair, 1989; Watkinson, 1989). Thus to model S^o oxidation in field soils, K must include the effects of temperature and moisture which strongly influence microbial activity and nutrient and oxygen diffusion rates in soil (Watkinson, 1989) but may be less dependent upon other soil properties.

iv) In soils containing populations of both heterotrophic and chemolithotrophic S^o oxidisers it appears that rates of S^o oxidation are not limited by the build up of the oxidation products H^+ and SO_4^{2-} (Watkinson, 1989). Thus S^o oxidation can be considered to be a first order reaction. In most well fertilized New Zealand pasture soils nutrient limitations to microbial growth are not expected to differ significantly between soils. During pasture establishment, however, more wide ranging nutrient limitation may occur.

From the above assumptions, it requires that firstly, initial particle diameter and the potential oxidation rate of S^o are known. In the literature researchers have measured

oxidation rates that vary considerably, ranging from 48 to 76 μg S^o cm^{-2} day^{-1} in incubated New Zealand soil (Watkinson, 1989) and an average of 54 μg S^o cm^{-2} day^{-1} in incubated Australian soil (Blair, 1987). These values are 10-15 times greater than values for incubated Canadian soils, which were reported by Janzen and Bettany (1987b) to explain S^o oxidation in short term incubations (6 days). The limitations of Janzen and Bettany's (1987b) short term experimental method of measuring S^o oxidation were discussed in Section 2.6.2 and also by Watkinson (1989). In short incubation periods the rate of oxidation may also be dependent upon microbial growth rates and the time taken for organisms to attach to the surfaces of S^o particles.

The data used in the formulation of most simulation models (McCaskill and Blair, 1989; Watkinson, 1989) were drawn from soil incubation studies, which may not be applicable to field conditions where S^o is applied to soil surfaces and not incorporated. No experiments to test the validity of applying potential oxidation rates, measured in incubation experiments to field based models have been published, except those described in Chapter 7 of this thesis.

The aim of this chapter is to select an appropriate S^o oxidation model and parameterize it to simulate S^o oxidation in New Zealand field soils by drawing on the most appropriate information derived from literature and experiments conducted in this thesis.

In setting up the discussion for this chapter, firstly it is assumed that the specific oxidation rate for S^o would be constant over time periods greater than 2 months given constant environmental conditions. Secondly it is hypothesized that the specific S^o oxidation rate (K) in New Zealand pasture soils is influenced only by moisture availability and temperature; i.e. other soil physical and chemical properties have negligible effect. Inherent in this hypothesis is belief that in well developed pastoral soils in New Zealand the availability of other nutrients will not limit S^o oxidation. The hypothesis is structured as follows: at optimum soil moisture and temperature conditions for S^o oxidation a maximum specific S^o oxidation rate (Kmax) will occur which will be common to all New Zealand pastoral soils. The assumption and hypothesis are examined in developing a S^o oxidation model to explain data obtained from various glasshouse and field trials.

8.2 DEFINING THE POTENTIAL OXIDATION RATE (Kmax)

8.2.1 Simulation Models Using Constant K Values.

The model of McCaskill and Blair (1989) assumed that S^o particles were spheres and defined S^o release rate in terms of the decrease in radius (Δr) for each time increment (Δt), i.e. $\Delta\rho/\Delta\tau$. This allows for easier comparison of release rates between different particle sizes. For spherical particles, $\Delta r/\Delta t$ can be calculated from a known diameter (d, mm) and the amount of S^o remaining after t days. Since the proportion of added S remaining (S_t/S_o, dimensionless) after t days is the fraction of current mass / initial mass:

$$S_t/S_o = 4/3\ \Pi\ r_t^3\ \rho\ N\ /\ (4/3\ \Pi\ r_o^3\ \rho\ N) \qquad (8.1)$$

where r_o (mm) is the initial radius, r_t (mm) the radius after t days, N the number of particles applied and ρ is the S^o density (2.07 g cm^{-3}). By rearranging equation (8.1) we get;

$$r_t = r_o\ (S_t/S_o)^{1/3} \qquad (8.2)$$

and when$\Delta r/\Delta t$ is expressed as

$$\Delta r/\Delta t = (r_o - r_t)\ /\ t \qquad (8.3)$$

and by substituting r_t from equation 8.2

$$\Delta r/\Delta t = r_o\ (1 - (S_t/S_o)^{1/3})\ /\ t \qquad (8.4a)$$

McCaskill and Blair (1989) established, using time steps of 1 day, that Δr remains essentially constant until S_t/S_o equal approximately 0.05. This model is essentially the same cubic mathematical function derived by Swartzendruber and Barber (1965) to explain the dissolution of limestone particles. The merits of using such function to explain S^o oxidation has been discussed by Watkinson (1989).

McCaskill and Blair's (1989) S^o oxidation model considered only equally sized spherical particles. Watkinson (1989) considered the general case of particles of different sizes and mass distributions. In a special case he showed that provided there was uniform mass distribution within particle size separates, then a geometric mean size calculated from the extreme sizes could be used in such cubic models to estimate the decrease in mass of a particle size separate caused by S^o oxidation. Use of a geometric mean for such models is restricted to situations where the extreme particle sizes limits (upper (c) and lower (b)) of a distribution vary by less than a factor of 2,

i.e. 1<c/b<2 as pointed out by Swartzendruber and Barber (1965) and Watkinson (1989). In practice agricultural grade S^o does not have equal mass distribution amongst sieve size separates (e.g. Table 6.2). The effect of varying mass distribution is discussed more fully in Section 8.4 (Table 8.4).

Unfortunately at the time of writing this chapter it was not possible to retrieve S^o samples used in the previous laboratory, glasshouse and field trials and sieve them to produce a description of particle size separates which conform to the size and mass distribution criteria described above. With insufficient size data, geometric means can not be calculated for the smaller particle sizes of S^o used (e.g. <150 μm, see Table 6.2) because the upper and lower size limits do not conform to the size criteria given above. For this reason an arithmetic particle size mean has been used. The accuracy of the iterative procedures used to calculate K values and the oxidation model subsequently developed may be improved with more accurate descriptions of particle size and mass distribution.

8.2.2 Calculating K Values from Sequences of Experimental Data

An iterative procedure was developed to calculate the specific S^o oxidation rate (K) by least square fitting $\Delta r/\Delta t$ values to experimentally determined amounts of S^o remaining in incubated and glasshouse soils (Chapter 5, 6 and 7). Two S^o oxidation models were used in the fitting procedures, the first assumes a constant $\Delta r/\Delta t$ (Equation 8.4a; Appendix 8.1.A) and the second uses equation 8.4c to calculate the new daily radii of S^o particles (Appendix 8.1.B).

In the first procedure the specific oxidation rate K ($\mu g\ S^o\ cm^{-2}\ day^{-1}$) can be calculated from Δr (mm day^{-1}) as follows:

$$K = (\Delta r/\Delta t) \times \rho \times 100000 \qquad (8.4b)$$

given the special relationship between surface area and volume of a sphere.

In the second iterative procedure (Appendix 8.1.B) the following equation was used to calculated new r values (r_{n+1}) at time t+1 day.

$$r_{n+1} = (r_n^2\ (r_n - 3K / \rho))^{1/3} \qquad (8.4c)$$

where

r_n = current daily radius
r_{n+1} = radius after a further day (t+1) of oxidation
K = specific S^o oxidation rate ($\mu g\ S^o\ cm^{-2}\ day^{-1}$)
ρ = density of S^o ($2.07\ g\ cm^{-3}$)

Both model procedures fitted well the amounts of S^o remaining in the Tokomaru and Ramiha undisturbed soil cores used in the glasshouse experiment in Chapter 7. (Table 8.1). Interestingly both procedures produced a narrow range of K values for different particle sizes of S^o and for S^o in the presence of different P fertilizer forms.

Table 8.1 The least square fitted K values calculated using the constant change in Δr or using K in equation (8.4c) of the surface applied S^o to the Tokomaru and Ramiha undisturbed soil cores of glasshouse trial.

Fertilizer	Mean radius	Δr model		Equation 8.4c	
	μm	K ($\mu g\ S^o\ cm^{-2}\ days^{-1}$)			
Tokomaru soil					
S^o<150	75	14.1	(0.85)[a]	13.1	(0.84)
S^o150-250	200	7.5	(0.65)	12.0	(0.65)
S^o250-500	375	12.7	(0.77)	19.0	(0.77)
S^o/RPR	75	12.7	(0.88)	12.6	(0.88)
S^o Grn	75	10.7	(0.97)	10.7	(0.97)
S^o/PAPR	75	10.7	(0.95)	10.7	(0.95)
	Mean	11.4	±2.32[b]	13.02	±3.08
Ramiha soil					
S^o<150	75	17.4	(0.97)	16.1	(0.97)
S^o250-500	375	16.5	(0.52)	22.7	(0.52)
S^o/RPR	75	18.9	(0.79)	18.9	(0.79)
S^o Grn	75	12.9	(0.79)	12.1	(0.79)
S^o/PAPR	75	11.2	(0.91)	10.3	(0.91)
	Mean	16.4	±2.57	17.46	±4.49

a R^2 values.
b standard deviation of the means.

The iterative procedure using equation 8.4a gave a better approximation of a single mean K value than the equation 8.4c for the size ranges of S^o considered (Table 8.1). The former procedure was then selected to calculate the specific S^o oxidation rates (K values) for S^o applied to the soil surfaces in the glasshouse trial described in Chapter 7, S^o incorporated to the soil (glasshouse trial described in Chapter 6) as well as that of S^o in incubated soils (Chapter 5). The particle size ranges of the fertilizers used in the model (Appendix 8.1.A) are given in the respective Chapters. The K values obtained (Data from Chapter 5, 6, 7 are given in Tables 8.1 and 8.2) and the reasonable fits of the predicted data (R^2) support the evidence for constant specific oxidation rate over long periods of time. Larger granules (Tiger 90 and Rotokawa (P) oxidised very slowly and their extent of oxidation was difficult to measure (Chapter 5). Partly for this reason little confidence can be placed in the K values determined. Notably, higher K values are obtained if the as-received granule sizes (Tiger 90 = 5 mm, Rotokawa (P) = 1 mm) are used to calculate K instead of the dispersed particle size range (Table 5.4 Chapter 5 and Table 8.2.)

On average the oxidation rates were lower for surface applied (Table 8.2; C) than for incorporated S^o (Table 8.2; B) measured in the same soil, Tokomaru, at approximately similar temperature and moisture contents. The fertilizer form (i.e. granular or non granular and nature of P additive) does not markedly influence the long term K provided fertilizers are applied to the surface of the soil (Table 8.2; C). These aspects were discussed more fully in Chapter 7.

The oxidation rates (K) for surface applied S^o were determined for the Ramiha and Tokomaru soils under optimum conditions of soil moisture content (see Section 7.4.1.1) and known temperature in the glasshouse trial (Table 8.2; C). The oxidation rate (K) was faster in the Ramiha than the Tokomaru soil, possibly due to the higher water holding capacity of the Ramiha compared to the Tokomaru soil. The effect of moisture on K has been observed by Moser and Olson (1953) and was emphasized by McCaskill and Blair (1989) in their model. The relationship which related fractional specific S^o oxidation rate to fractional soil moisture content, derived by McCaskill and Blair (1989), was used to correct the <150 μm S^o K value (17.4 μg S^o cm^{-1} day^{-1}) to the theoretical Kmax values for the Ramiha soil, which was at an average soil moisture content of 88% of field capacity throughout the glasshouse trial (Chapter 7, Section 7.4.1.1 Table 7.4). This correction procedure is discussed more fully in the following section (Section 8.3).

Table 8.2 The best fit (least square fit) specific S^o oxidation rates (K, $\mu g\ S^o\ cm^{-2}\ day^{-1}$) of different S^o forms and particle sizes from the incubation, glasshouse and field experiments carried out in Chapters 5, 6 and 7. Mean diameters used in model were derived from Tables 5.4, 6.2 and 7.2 in Chapter 5, 6 and 7 respectively.

S^o fertilizer form		Simulated oxidation rate $\mu g\ S^o\ cm^{-2}\ day^{-1}$	
A.	Incorporation (laboratory incubation, Chapter 5)		
Code	**Ground S^o**		
B	Ag.grade S^o	31.0	(0.98)[1]
C	Ground Dark S^o	21.1	(0.89)
D	Ground Dark S^o	21.6	(0.75)
E	Damman S^o	18.7	(0.71)
F	Rotokawa S^o	34.2	(0.95)
L	Prilled S^o rolled	35.3	(0.71)
	Granule or prill		
O	Tiger 90	19.3	(0.13)
P	Rotokawa S^o (granule)	14.8	(0.19)
	S^o + Arad rock		
N	Ag.grade S^o	44.8	(0.98)
M	Ground Dark S^o	37.8	(0.95)
I	Rotokawa S^o	51.4	(0.96)

B.	Incorporation (pot incubation, Charter 6)	
	$\mu g\ S^o\ cm^{-2}\ day^{-1}$	
	+Plant	-Plant
Tokomaru		
S^o Grn.	25.9 (0.90)	29.0 (0.91)
S^o/PR	68.3 (0.99)	59.7 (0.93)
S^o/PAPR	17.7 (0.86)	15.8 (0.90)
S^o/PAPRres	33.6 (0.97)	
S^o/MCP	21.5 (0.88)	
Makotuku		
S^o Grn.	10.9 (0.87)	17.6 (0.86)
S^o/PR	25.2 (0.92)	36.5 (0.90)
S^o/PAPR	6.6 (0.63)	15.8 (0.90)
S^o/PAPRres	14.1 (0.68)	
S^o/MCP	12.4 (0.52)	

Table 8.2 continued

C.	Surface applied (undisturbed soil core, Chapter 7)	
	Glasshouse	Field
Tokomaru soil		
S^o<150	14.1 (0.85)	11.8 ± 0.84[2]
S^o/PR	12.7 (0.88)	13.4 ± 0.66
S^o Grn.	10.7 (0.97)	12.9 ± 0.50
S^o/PAPR	10.7 (0.95)	14.8 ± 1.75
Mean	12.1 (1.66)	12.2[a]± 1.24
Ramiha soil		
S^o<150	17.4 (0.97)	18.2 ± 2.4
S^o/PR	18.9 (0.79)	21.1 ± 1.4
S^o Grn.	12.9 (0.79)	17.4 ± 3.3
S^o/PAPR	11.2 (0.91)	21.7 ± 1.1
Mean	15.1 (3.54)	19.5[b]± 2.12

All S^o particle sizes used to calculate K values were those measured after dispersion e.g. Table 5.4 or those of the screened S^o used to manufacture the granular and non granular fertilizers.

1 R^2 values of the linear regression between the observed and predicted amount of S^o remaining in the soil.

2 Standard error was determined from the best fit K values for each replicate. Each K is mean of 3 replicate observations.

a,b LSD at 0.05.

Assuming the corrected Kmax value for the Ramiha soil (18 μg S^o cm^{-2} day^{-1}) in the glasshouse to be near optimal, this was then used to predict the oxidation rates observed in the Tokomaru soil, by accounting for the differences in the observed fractional moisture contents of the two soils. The Tokomaru soil was at an average soil moisture content of 72% of field capacity throughout the glasshouse trial. Accounting for this, by using Equation 8.5, the predicted mean K for the <150 μm S^o particle size for the Tokomaru soil was 14.9 μg S^o cm^{-2} day^{-1} which compares favorably with the observed mean of 14.1 μg S^o cm^{-2} day^{-1} (Table 8.1). The results show that by incorporating the effects of soil moisture on S^o oxidation the predicted S^o oxidation rates of all three particle sizes in the Tokomaru soil glasshouse trial became closer to the observed values than predicted by S model using surface area alone (R^2 of 0.89 and 0.86, respectively; Figures 8.1 and 8.2).

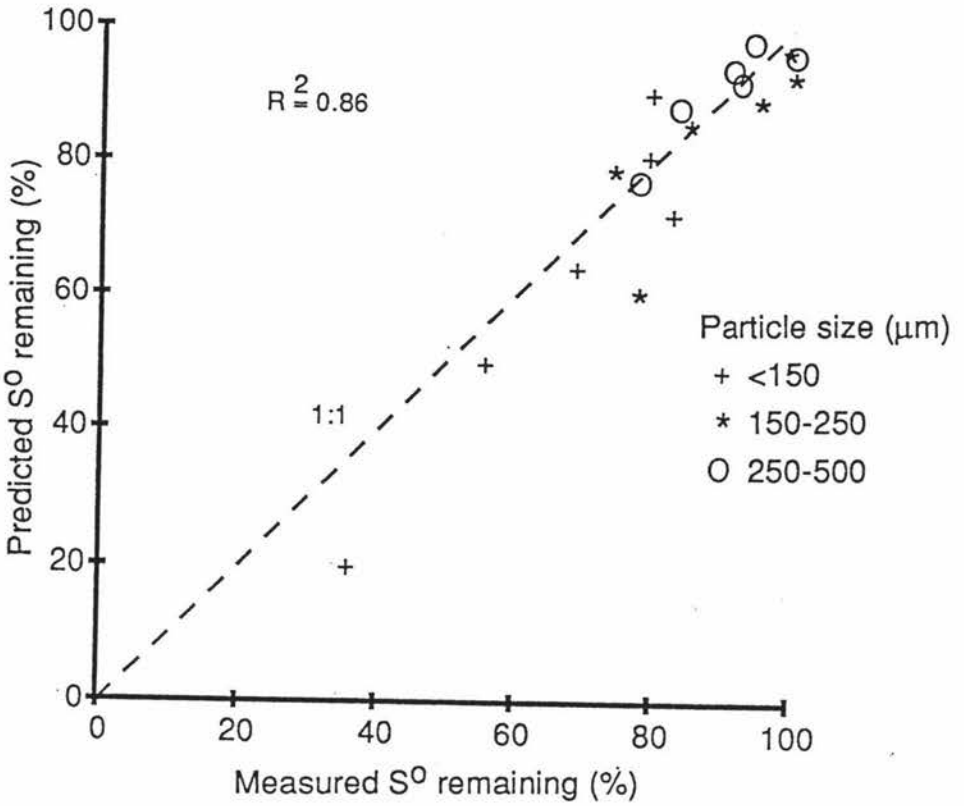


Figure 8.1 The relationship between the predicted and measured percentage of S^0 remaining in undisturbed cores of Tokomaru soil (glasshouse experiment, Chapter 7). The predicted values were calculated using Kmax measured on the Ramiha soil and account for the effect of surface area alone.

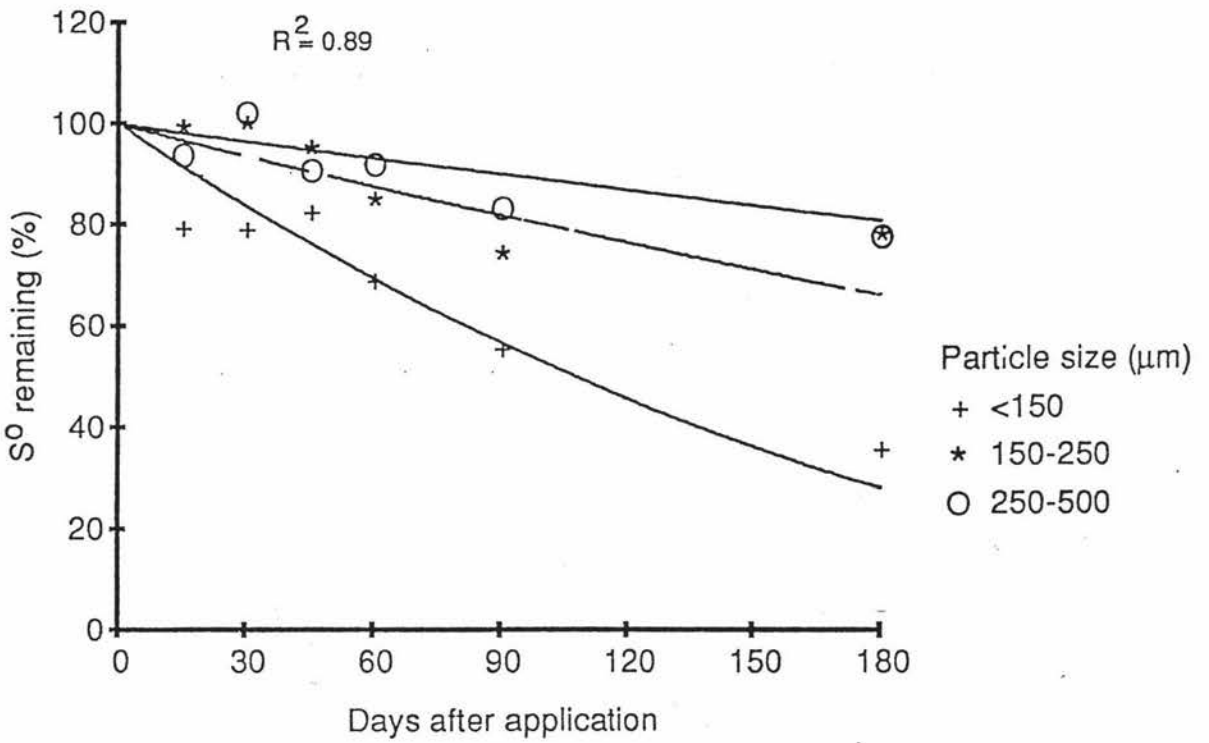


Figure 8.2 The measured and predicted percentage S^o remaining in Tokomaru soil cores (glasshouse experiment, Chapter 7). The predicted values were calculated using Kmax measured on the Ramiha soil (Kmax = 18 µg S^o cm^{-2} day^{-1}) and adjusted for the moisture content.

8.3 Construction of a S^o Oxidation Simulation Model to Explain S^o Oxidation Rates Observed in Field Trials

Arguments were discussed in Section 8.1 which suggest that the major influences on the rate of S^o in most well fertilized, pasture soils in New Zealand may be purely physical. The important soil physical properties considered to have strong influences on the rate of oxidation, are available water (by transporting organisms to and products away) and temperature. These influence the rate of S oxidation through increased micro-organism activity.

In support of these ideas it was demonstrated (Section 8.2 above) that by accounting for moisture differences between soils the largest specific oxidation rate (K for Ramiha soil) could be used to calculate a Kmax value which adequately predicted the rate of S^o oxidation in the Tokomaru soil in glasshouse experiments.

In the light of this preliminary evidence the 'simple' hypothesis, discussed in Section 8.1 is more specifically defined. The optimum S^o oxidation rate for surface applied S^o can be explained by a single Kmax value which is approximated by the Kmax calculated (Section 8.2) for S^o oxidation in the Ramiha soil in the glasshouse experiments in Chapter 7.

The discussion in this chapter evaluates whether this hypothesis holds, i.e. can an optimal Kmax value of 18 $\mu g\ S^o\ cm^{-2}\ day^{-1}$ be applied to explain the rate of S^o oxidation measured in field trials.

In this section environmental factors are incorporated into a modified simulation model, in a manner similar to that of Shedley (1982) and McCaskill and Blair (1989), in an attempt to explain observed rates of S^o oxidation in field experiments. It is assumed that under ideal conditions, a maximum oxidation rate is achieved, and any departure of the moisture and temperature from this optimum level, would cause a reduction in the oxidation rate. As mentioned above the relationships between K and these factors were expressed as the degree to which the departure from the optimum level of each factor caused a fractional reduction in the potential maximum oxidation rate (Kmax), with a value of 1 representing the maximum rate. As discussed above the initial hypothesis is that Kmax calculated for the Ramiha soil in the glasshouse

experiments (Section 8.2) is a suitable Kmax to explain the optimal rate of S^o oxidation in naturally structured pastoral soils in the cool temperate climatic zone which represent most parts of New Zealand (Region 1; appendix 2.1).

8.3.1 Accounting for Moisture Effects

Moser and Olson (1953) measured the effect of soil moisture on the rate of S^o oxidation in soil incubation experiment. The scalars used to account for below-optimal moisture conditions were derived from the regression relationship computed from the data of Moser and Olson (1953) by McCaskill and Blair (1989) using the following relationship:

$$S_m = - 0.386 + 2.37\ F - 0.945\ (F)^2 \qquad (8.5)$$

where, S_m is the fractional specific oxidation rate (K/Kmax) and varies between 0 to 1 (with 1 being the optimal condition), depending on F, the fractional soil moisture content ($F\Theta/FC$, i.e. the proportion of soil moisture content relative to 'field capacity', FC). The value of FC for all field soils (Ramiha, Tokomaru and Warepa) was assumed to equal 0.5 $cm^3\ cm^{-3}$, which approximates the mean of measured values in the Tokomaru top soil (0-5 cm) (Horne, 1985).

In the case of most field soils it is assumed that moisture contents above FC will only be transient and therefore the second relationship of McCaskill and Blair (1989), which explains the effect of moisture contents above FC on S^o oxidation, was not used.

After application, most of the applied S^o appears to remain in the top 2 cm of field soils (Chapter 7). Thus the fractional soil moisture contents were estimated for the top 2 cm of soil only. S^o oxidation rates should be influenced only by the moisture content of the immediate soil zone. This moisture content was estimated by using a daily water balance calculated for the top 2 cm of soil using daily rainfall records (plus irrigation if applicable) as water input and evapotranspiration (ET) and soil water storage as outputs. Mean daily air temperature and sunshine hours were used to calculate ET (Priestley and Taylor, 1972)

The percentage of total evapotranspiration derived from the top 2 cm of soil was assumed to be proportional to the percentage of total pasture root in that soil zone. Williams (1988) measured 21% of the total pasture root weight in the top 2 cm of soil at a site adjacent to the Tokomaru field site used in this study. Thus 0.21 x ET was assumed to be derived from the top 2 cm of the soil. Daily water content ($F\Theta$) in the top 2 cm of soil was calculated as:

$$F\Theta = W + R - 0.21\ ET \qquad (8.6)$$

where W is the equivalent depth of water in the top 2 cm (mm) and R is rainfall (mm). The soil water content can not exceed FC for the 0-2 cm zone, which was taken as 10 mm in these soils (Appendix 8.3a shows an example calculation).

8.3.2 Accounting for Temperature Effects

A linear relationship between the fractional specific rate of S^o oxidation (S_t = K/Kmax) and soil temperature was derived from the data of an undisturbed soil core experiment conducted by Shedley (1982). Two S^o particle sizes (100 and 400 μm) were surface applied and the air temperatures were set ranging from 14/8 to 30/24 °C day/night (Figure 8.3a). The slope of the change of S_t in relation to change in temperature was derived from Shedley's data using the 400 μm particle size. No useful relationship could be derived from the 100 μm particle size because, essentially the S^o had been completely oxidised before the end of Shedley's experimental period.

To use the relationship between temperature and K derived from Shedley (1982), his K values were normalized (K/Kmax) by his maximum observed K to give values between 0 to 1 in a mean daily temperature range from 11 to 27 °C (Figure 8.3a). At the highest mean temperature Shedley (1982) noted that plant growth was limited and S^o oxidation appear to be decreased, but probably as a result of soil drying and not a single effect of temperature. There is insufficient data therefore to warrant using a curvilinear relationship to explain the variation of S^o oxidation with temperature.

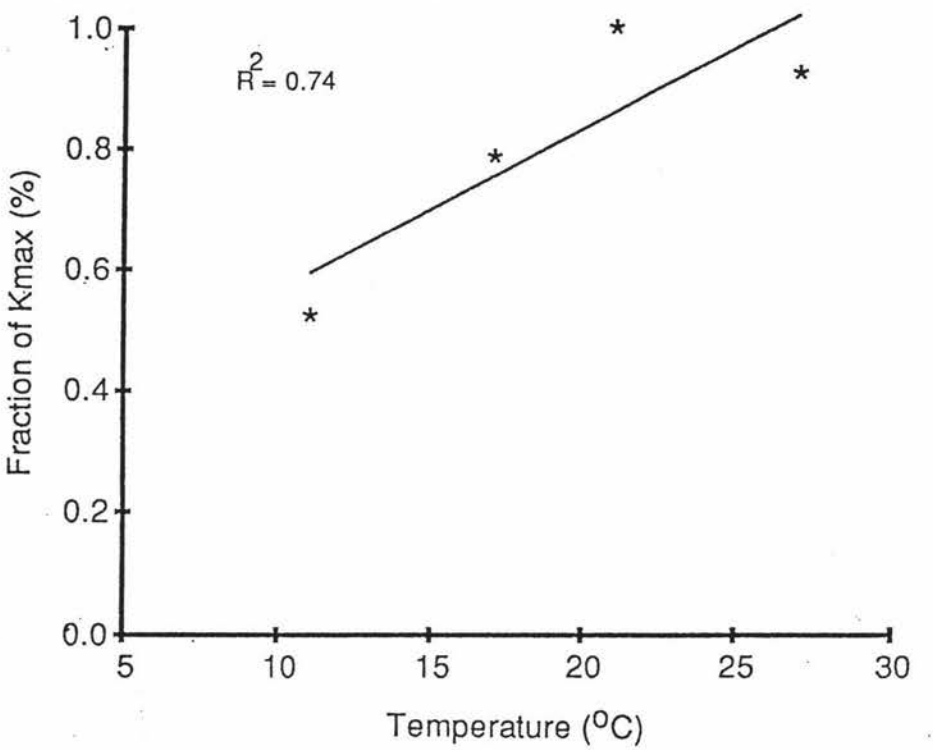


Figure 8.3a The simple relationship between the rate of S^o oxidation (fraction of Kmax and temperature, derived from the experiment of Shedley (1982) using S^o of 400 um particle size applied to surfaces of undisturbed soil cores.

For the purposes of deriving a useful relationship from Shedley's data, it is assumed that K/Kmax ≈ 1 in the mean daily temperature range of 21-27 °C. Unfortunately the linear relationship between K/Kmax and temperature does not allow Kmax or T_{opt} (the temperature at which Kmax occurs) to be defined. Provided, however, mean daily temperatures do not lie outside the range of 11-27 °C then the linear relationship shown in Figures 8.3a and equation 8.7 estimates the effect of temperature (T) on specific S^o oxidation rate.

$$K/Kmax\ (S_t) = 0.3 + 0.027\ T \qquad (8.7)$$

From this equation the slope of the change of S_t in relation to change in temperature was

$$dS_t / dT = 0.027$$

$$dS_t = 0.027\ dT \qquad (8.8)$$

The specific oxidation rates (K) calculated from the glasshouse trial (Table 8.2c) in this study were obtained with a mean daily glasshouse temperature of 24 °C (Chapter 7), which falls within the range where K/Kmax ≈ 1 in Figure 8.3a. Thus for New Zealand conditions where daily mean temperature rarely exceeds 24 °C Kmax is considered to occur at T_{opt} of 24 °C. Therefore, for modelling purposes in this chapter, the temperature effect on the fractional specific rate of S^o oxidation (S_t) was approximated by the linear relationship in equation 8.9.

$$S_t = 1 - 0.027\ (T_{opt} - T_d) \qquad (8.9)$$

Where, S_t is the fractional specific S^o oxidation rate (K/Kmax) and values vary from 0 to 1 depending upon T_d which is the difference between field soil temperature at 30 cm and T_{opt}, the average temperature (24 °C) measured for the glasshouse experiment described in Chapter 7.

Soil temperature at a 30 cm depth was used as an input in the model because it was found to be less sensitive to the time of daily measurement than the maximum and minimum air temperature yet represents daily mean soil surface temperature well. As shown in Figure in 8.3b, soil temperature at 30 cm is closely related to the calculated mean soil surface temperature (after Scotter and Horne, 1985).

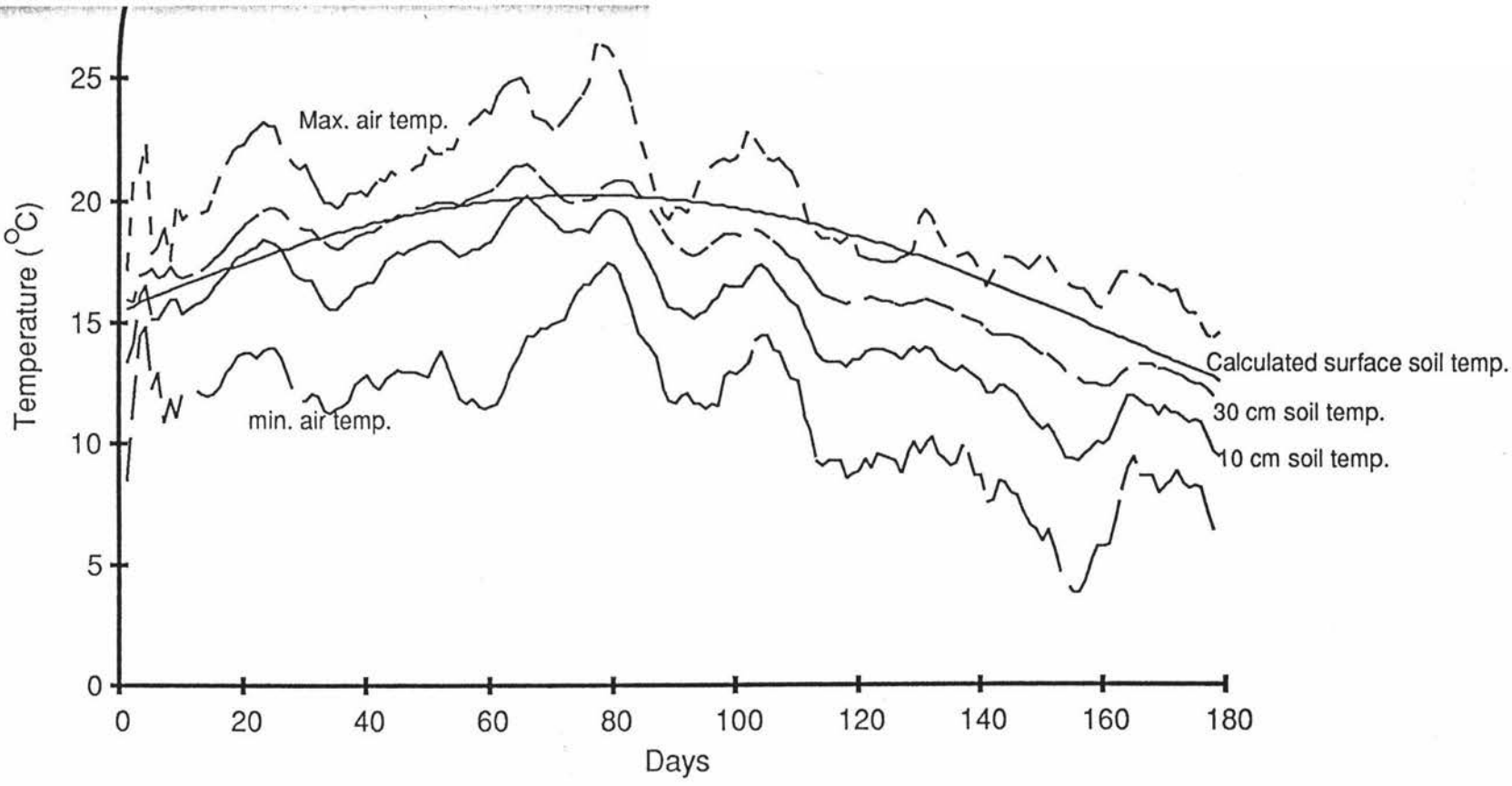


Figure 8.3b The calculated field soil surface temperature and maximum air temperature, minimum air temperature, soil temperature at 10 and 30 cm depth in Tokomaru soil over the experimental period.

8.3.3 Accounting for Combined Changes in Soil Moisture and Temperature

In the absence of information in the literature which describes a relationship reflecting changes in oxidation rate with combined moisture and temperature change, a relation was constructed by multiplying the moisture (S_m) and temperature (S_t) scalars together to produce a combined scalar ($S_m \cdot S_t$). Kmax was adjusted using this combined scalar:

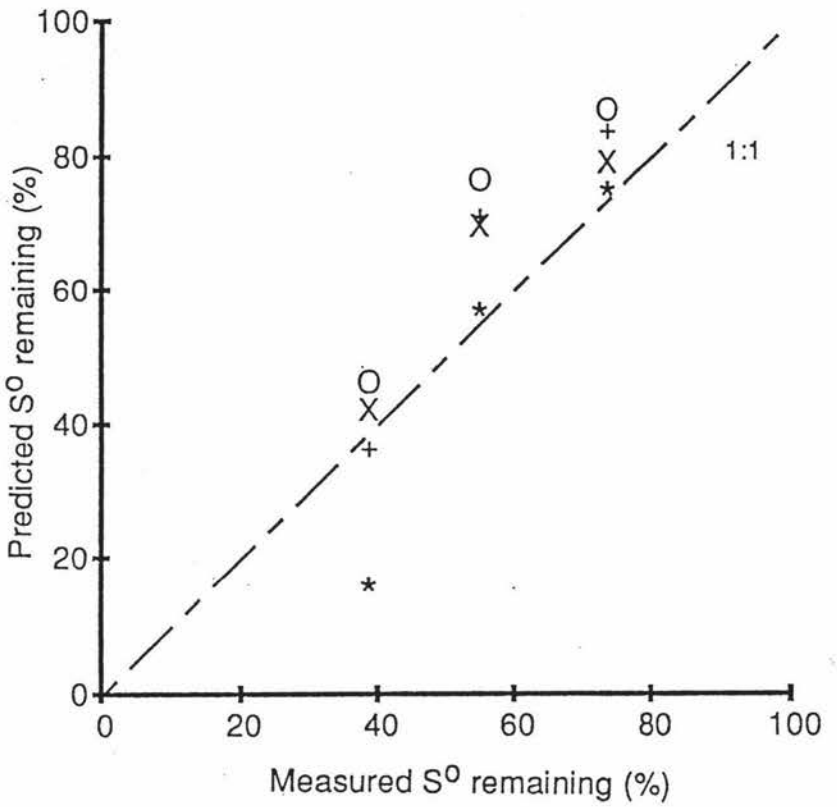
$$K = Kmax\ (S_m \cdot S_t) \qquad (8.10)$$

Janzen and Bettany (1987a) derived moisture and temperature relationships from short term laboratory incubation but, as discussed earlier (Section 8.1) these were not considered appropriate for long term S^o oxidation in undisturbed soil cores.

8.4 RESULTS AND DISCUSSION

The complete model (Appendix 8.2) was tested to give the predicted daily oxidation rate of a range of S^o particle sizes under the field conditions in Tokomaru (Chapter 7) and Warepa soils at Invermay, South Island (Boswell and Swanney, 1988) for 180 and 340 days, respectively (I am grateful to Dr. C. Boswell for providing the climatic data for the Warepa soil). The Ramiha field data was modelled accounting for soil moisture changes only because no temperature data was available. Inputs to the model were S^o particle size and the various environmental data collected from the field sites. The environmental data for calculating moisture and temperature which were ultimately used in the model are included in Appendix 8.3a and 8.3b.

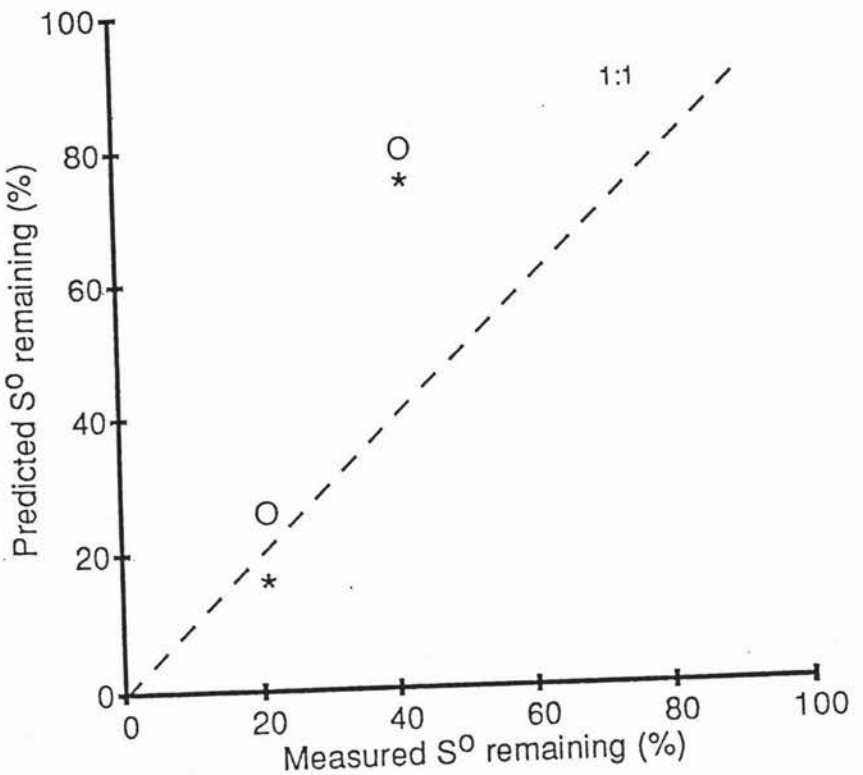
The amounts of S^o remaining were initially predicted using a constant Kmax of 18 μg S cm^{-2} day^{-1} and accounting only for the specific surface area of the applied S^o. The effect of including moisture, and moisture x temperature relationships (complete model) are shown in Figure 8.4a for the Tokomaru soil; Figure 8.4b for the Ramiha soil and Figure 8.5 for the Warepa soil, respectively.



Factor accounted for (R^2)

* Surface area (0.81)
\+ Surface area x Moisture (0.79)
O Surface area x Moisture x Temperature (0.44)
X Chromite, Surface area x Moisture x Temperature (0.75)

Figure 8.4a The relationship between the predicted and measured percentage of S^o remaining in the Tokomaru soil at 180 days (under glasshouse conditions). The predicted values were calculated using a Kmax of 18 ug S^o cm^{-2} day^{-1} and accounting for different climate effects (R^2 in brackets).

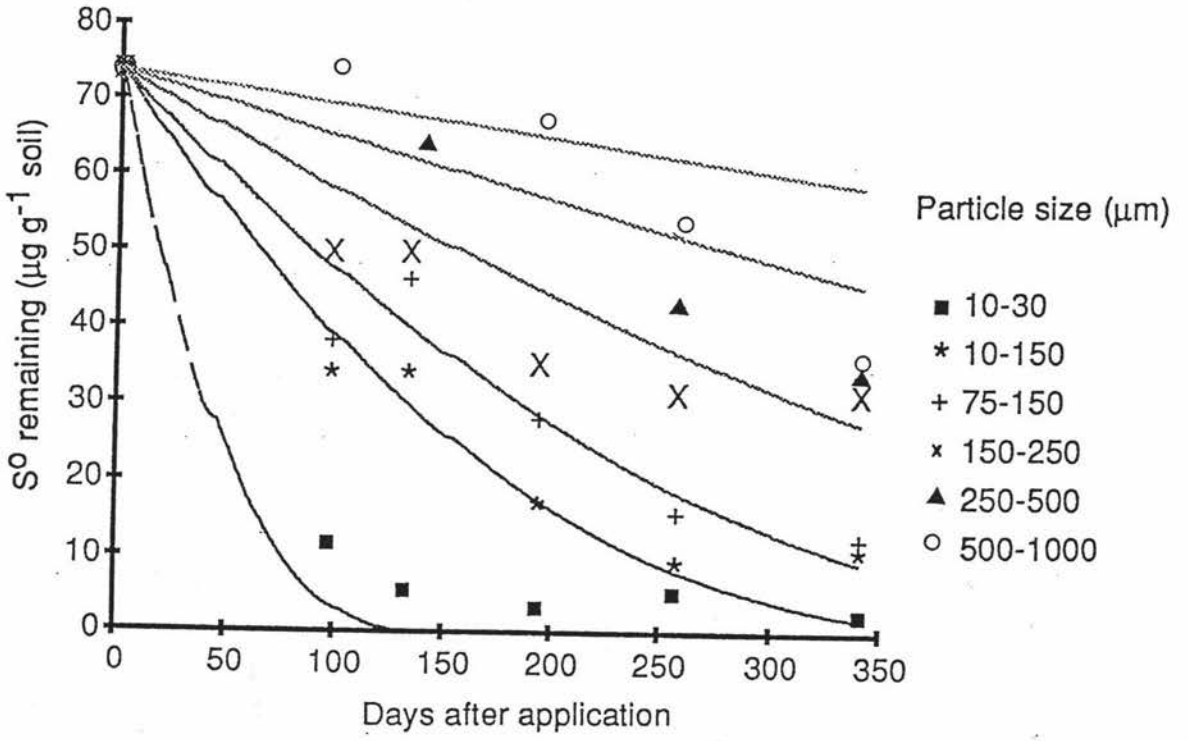


Factor accounted for

* Surface area

O Surface area x Moisture

Figure 8.4b The relationship between predicted and measured S^o remaining (percent) in the Ramiha soil at 180 days (under glasshouse conditions). The predicted values were calculated using a Kmax of 18 ug S^o cm^{-2} day^{-1} and accounting for different climate effects.



Figures 8.5 The measured and the predicted amounts of S^o remaining in Warepa soil (field experiment; Lee et al., 1987) using the surface area S^o oxidation models (see Appendix 8.3b for data input to model)

In Tokomaru soil, when the specific surface area of the S^o only is considered, the predicted amounts of S^o remaining were similar ($R^2 = 0.81$) to the observed amounts remaining for the larger particle size but predicted oxidation rates were too fast for the <150 μm S^o particle size. When the effects of S^o surface area and soil moisture content were considered the observed and predicted amounts of S^o remaining for the <150 μm sizes were close although the prediction for larger particle sizes was overestimated (overall particle sizes $R^2 = 0.79$; Figure 8.4a). The improved prediction for the smaller particle sizes through inclusion of moisture effects can be attributed to the drought conditions for the period between 35 and 70 days which slowed down the rate of oxidation (Appendix 8.3a). However, when the complete model was used, the predicted amounts of S^o remaining were much higher than that observed in the field ($R^2 = 0.44$; Figure 8.4a). In the Ramiha soil the predicted amounts of S^o remaining either by surface area and surface area x moisture were close only for the <150 μm S^o particle size (Figure 8.4b) but not for the 250-500 μm particles.

The faster than predicted oxidation rate of the larger particle sizes (150-250 and 250-500 μm) observed in the Tokomaru soil and Ramiha soil field conditions were inconsistent with the observations of McCaskill and Blair (1989). In their work, they observed slower specific oxidation rates for larger particle sizes (>400 μm). Reasons for these differences between these findings are unclear. In this field study, the higher rates of oxidation of the larger particle size ranges may have been a result of a low recovery of S^o particles due to the losses of the S^o particles from the top soil sampling zone (0-3 cm) by vertical movement induced by earthworm activities. In a concurrent study (Fertilizer and Lime Research Centre; Massey University. unpublished data), there was an evidence that approximately 0.09% day^{-1} of an inert chromite tracer of similar particle size (100% <250 μm) was lost when surface applied to the same field sites (Ramiha and Tokomaru soils) over the same time period. Thus, the low recovery of larger particle sizes of S^o may cause some misinterpretation of the extent of S^o oxidation data. In addition, the chance of loss due to this mechanism of the larger particle sizes is greater than the smaller particle sizes because of the fact that they oxidise at a slower rate and hence tend to persist longer in the field.

When the complete model was modified by the inclusion of particle loss factor (based on the rate of chromite loss), the prediction of amounts of S^o remaining was markedly improved ($R^2 = 0.75$; Figure 8.4a).

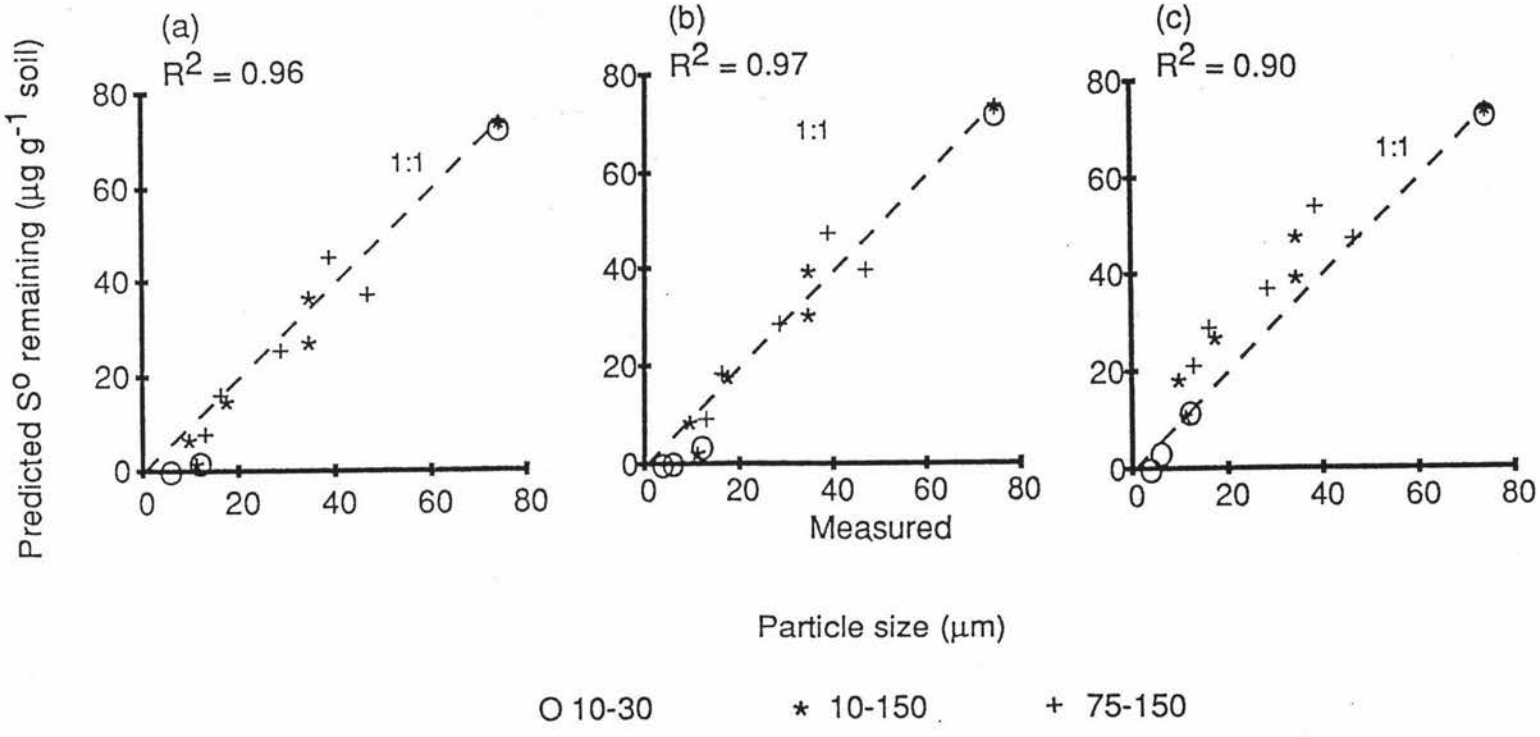


Figure 8.6a The relationship between the measured amounts of S^o remaining for the <150 um particle size range in Warepa soil (field experiment; Lee *et al.*, 1987) and that predicted by surface area (a) surface area x moisture (b) and surface area x moisture x temperature (c) S model.

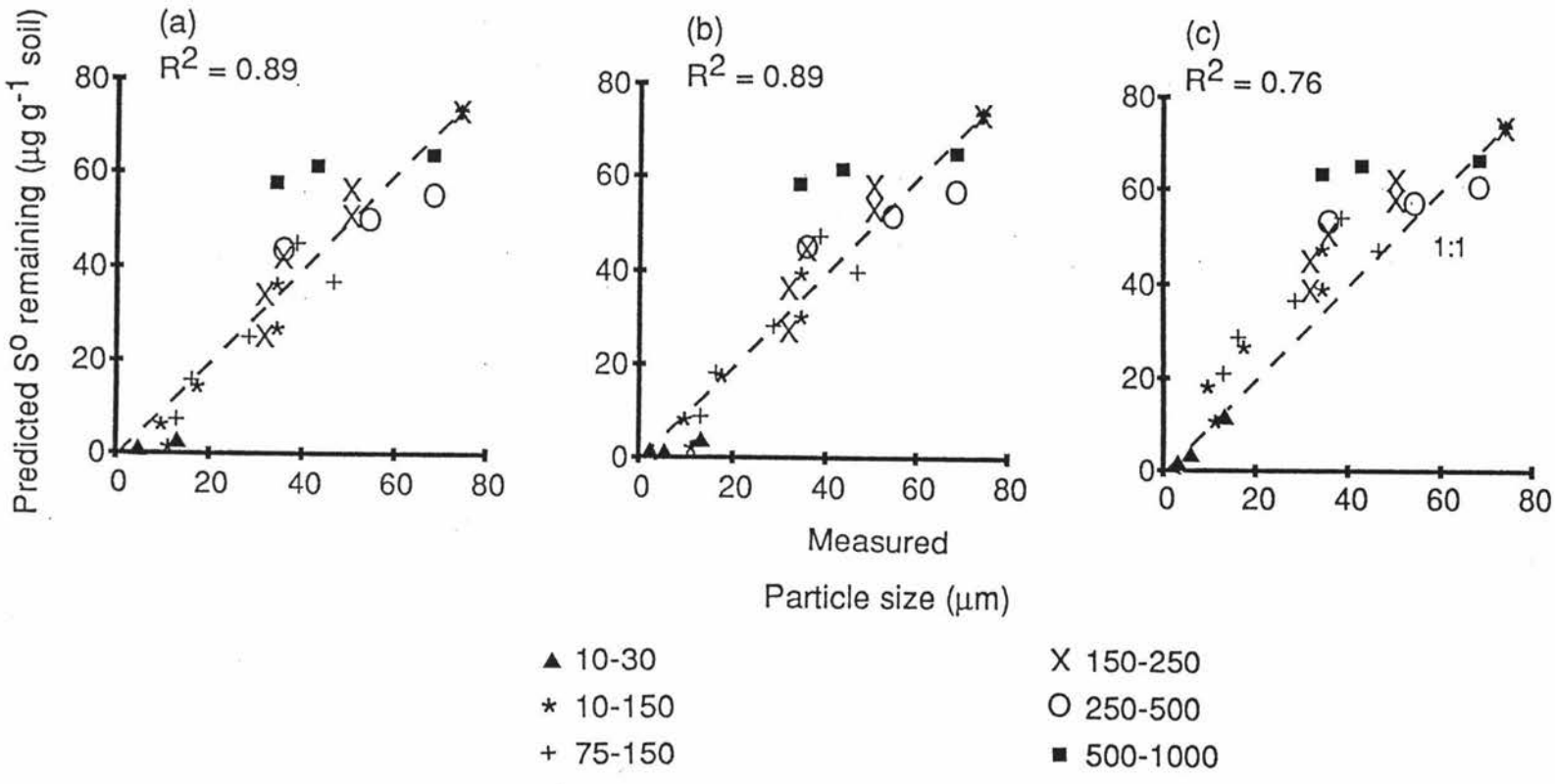


Figure 8.6b The relationship between the measured amounts of S^o remaining for all particle size range in Warepa soil (field experiment; Lee *et al.*, 1987) and that predicted by surface area (a) surface area x moisture (b) and surface area x moisture x temperature (c) S model.

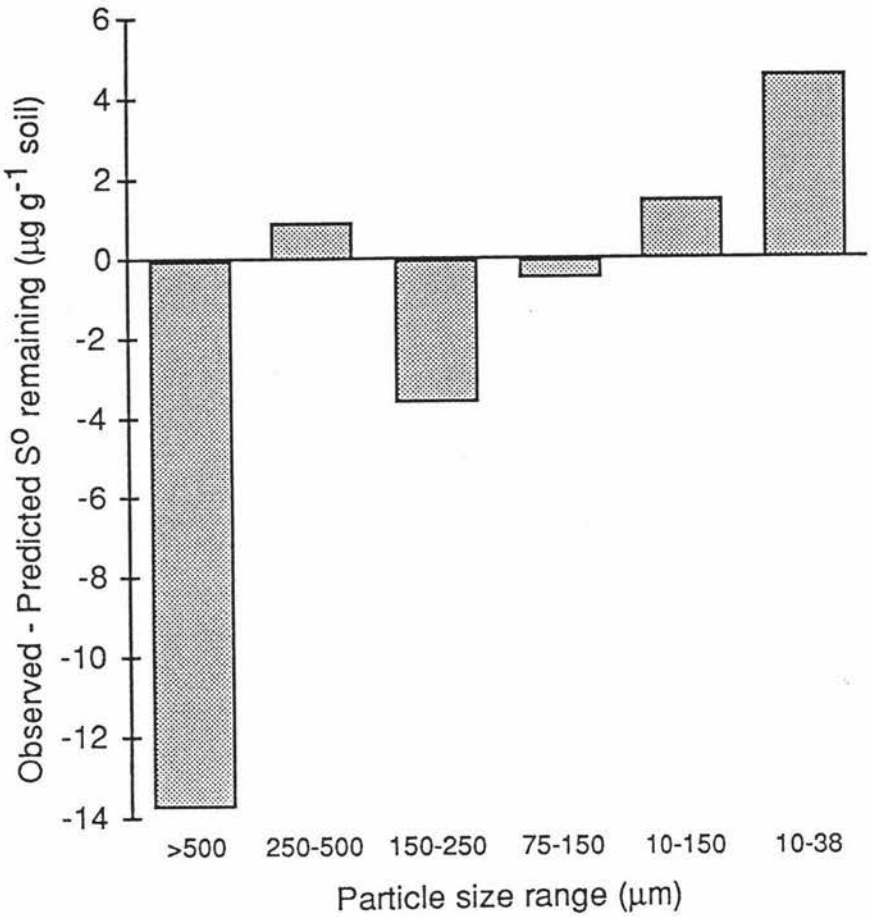


Figure 8.7 The difference between the observed and predicted amount of S^o remaining using the surface area S model for all particle size ranges in Warepa soil (calculated over all sampling dates).

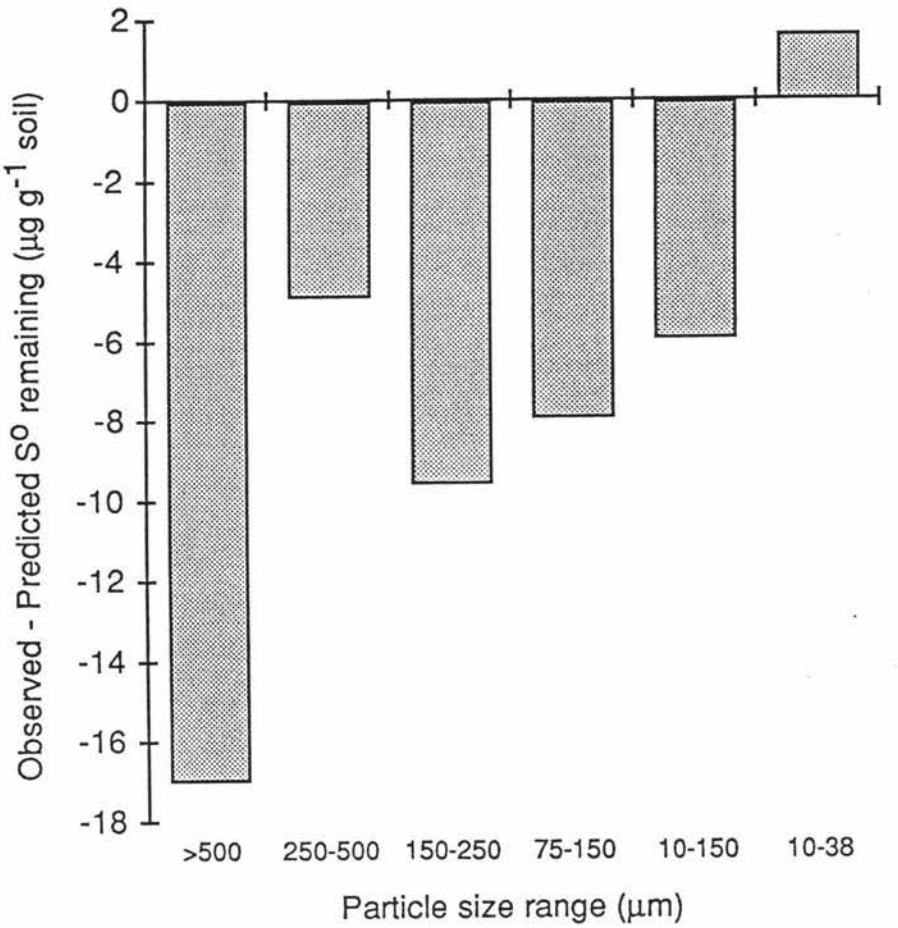


Figure 8.8 The difference between the observed and predicted amount of S^o remaining using the surface area x moisture x temperature S model for all particle size range in Warepa soil (calculated over all sampling dates).

For the Invermay field conditions, which remained at almost optimum moisture content (S_m >0.9) for about 280 days of the 340-day experimental period, surface area alone and surface area x moisture effects accounted for approximately the same amount (R^2 = 0.97) of the variance in the observed data for the amounts remaining of the finer S^o particle size ranges of 10-38, 10-150 and 75-150 μm (Figure 8.6a). But the prediction was poorer when temperature effects are accounted for (R^2 = 0.90). However, when the larger particle size range was also included in the predictions, the R^2 values of the linear regression relating the predicted and observed oxidation rates decreased to 0.89 for the surface area and surface area x moisture and R^2 has only 0.76 when the complete model was considered (Figures 8.6b, 8.6c). The reduction in R^2 values was due to greater underestimation of the rate of oxidation of the larger particle sizes (Figures. 8.7, 8.8). This may be partly associated with the very large sampling errors with the larger S^o particle sizes (Lee *et al.*, 1987; see Chapter 3 Section 3.3.1 H).

Surface area alone and surface area x moisture effects seem to provide better R^2 than inclusion of temperature. This indicates that the Kmax used in the model which is estimated from the Ramiha soil was slightly lower than the observed Kmax at Invermay. This is also confirmed by the iterative fit of K's to all the Invermay data in Table 8.3A.

The K values determined for the 10-500 μm particle size ranges have K's similar to or slightly lower than the Ramiha Kmax value of 18 μg S^o cm^{-2} day^{-1}. Thus little or no improvement can be made by using climatic scalars.

The most reliable experimental data for each site is for the <150 μm size fraction (10-150 μm at Warepa). It is apparent that the oxidation rates observed in the Ramiha soil, Tokomaru soil and Warepa soil field sites for the <150 μm particle size range (or 10-150 μm at Warepa) are all in the range of 11 to 20 μg S^o cm^{-2} day^{-1} (Table 8.2c and 8.3a). At the Tokomaru site oxidation was measured from November to April and the optimum soil moisture content was approximately optimum (S_m >0.9) at this site for only 49% of the experimental period (days) compared to 67 and 82% at the Ramiha and Warepa field sites. This would partly account for the lower K at the Tokomaru site. All 3 sites are in the cool temperate zone defined by Boswell and Swanney (1988).

Table 8.3 The best least square fit of specific S^o oxidation rates (K, μg S^o cm^{-2} day^{-1}) of different S^o particle sizes in field experiments in (A) the cool temperate region at Invermay, South Island (Lee *et al.*, 1987) and (B) the warm temperate region of the North Island at Huntly (Lee *et al.*, 1988). R^2 value in parentheses calculated by comparing the observbed and predicted S^o remaining at each sampling date.

A

S^o particle size (μm)	Mean diameter use (μm)	Simulated oxidation rate μg S^o cm^{-2} day^{-1}	
10-38	24	11.4	(0.99)
10-150	80	15.8	(0.96)
75-150	113	16.7	(0.94)
150-250	200	19.4	(0.91)
250-500	375	13.9	(0.56)+
>500	750	33.3	(0.58)+
Means (excluding >500 μm particle size)		15.4	
Means (for all particle size)		18.4	

B.

		Autumn application		Spring	
First application					
<150	75	27.1	(0.94)	58.3	(0.82)
150-250	200	20.5	(0.60)	21.6	(0.45)
500-1000	750	+			
means				40.0	
Second application					
<150	75	23.0	(0.49)	77.3	(0.95)
150-250	200	22.2	(0.63)	33.1	(0.74)
500-1000	750	+			
means				55.2	

\+ large sampling error involved in the observed data.

Iteratively fitted K values were also calculated for a pasture field trial, on a yellow-

brown pumice soil near Huntly, carried out by Lee *et al.* (1988)(Table 8.3B). Lee *et al.* (1988) measured amounts of S^o remaining after two consecutive annual spring and autumn fertilizer applications. This trial is in the warm temperate zone defined by Boswell and Swanney, (1988).

The iteratively fitted K values for the two autumn first applications 20-27 $\mu g\ S^o\ cm^{-2}\ day^{-1}$ (Table 8.1c for the Ramiha soil and Table 8.3b are not significantly greater than those measured at the Ramiha and Warepa field sites (Table 8.2c and 8.3a). This relatively narrow spread of K values across these 3 field sites and all particle sizes supports the assumption that a single Kmax value (not necessarily the Kmax defined in the Ramiha soil under glasshouse conditions) could be used as an input to predict the rates of S^o oxidation in similar climatic zones.

The fitted K values for spring application at Huntly site which varied greatly between the two particle sizes (<150 and 150-250 μm), however, are much higher than that of K values calculated for other field or glasshouse trials mentioned in this chapter. This could suggest a different Kmax depending on particle sizes as reported by McCaskill and Blair (1989). However, there was a high variation in the field data of Lee *et al.* (1988) and this can not be concluded. Also modelling errors may occur because as the S^o mass distribution within the particle size separate is not known (see discussion below).

Therefore, at this stage insufficient field information is available to conclude that a single Kmax value for S^o oxidation plus climatic scalars can be used to explain field rates of S^o oxidation in different climatic zones. It can only be explained if less variable experimental data for the rate of S^o oxidation in soil is obtained. With accurate field observations, however, will come the need to have narrower size descriptions of the S^o for calculating K values. In table 8.4 the effect of varying the S^o particle size distribution on the K values calculated from the data obtained from the oxidation of <150 μm S^o at the 4 field sites discussed above. The data shows that calculated K values can be inaccurate if the proportion of the finer S^o in a sample is not accurately known, i.e. if equal mass distribution is assumed within a particle size separate (Section 8.2). After demonstrating these effects, it is clear that similar differences in S^o particle size within large size fractions could be an additional reason why accurate prediction of S^o oxidation rates at the field sites remained illusive.

Table 8.4 K values calculated from the observed amounts of the <150 μm S^o size fraction remaining at the 4 field sites using 3 different particle size distributions. R^2 value in parentheses.

Mean radius (μm)	25	75	100	75	25	75	100
% of sample	60	30	10	100	10	30	60
Glasshouse							
Ramiha	9.4	(0.86)		18.5 (0.84)	19.8	(0.36)	
Tokomaru	6.9	(0.84)		13.9 (0.83)	14.6	(0.85)	
Field trial							
Invermay	8.1	(0.98)		14.8 (0.96)	16.5	(0.97)	
Huntly	16.1	(0.98)		27.2 (0.94)	30.7	(0.96)	

8.5 CONCLUSIONS

Specific S^o oxidation rates were in general lower when non-granulated forms of S^o were applied to soil surfaces rather than incorporated into incubated soils. Unlike, when granulated or P and S^o forms were incorporated into incubated soils there was also no significant effect of S^o fertilizer form on S^o oxidation rates when fertilizers were surface applied.

Despite the differences in observed and predicted Kmax values for the field sites, the computer model simulating S^o oxidation adequately predicted the oxidation rates of S^osurface applied to field soil in the cool temperate climatic region in New Zealand. In most cases the model predicted closely the rate of oxidation of surface applied S^o under long term field conditions by accounting for S^o surface area alone. The reason for this was that the maximum oxidation rate (Kmax) (measured in the Ramiha soil in the glasshouse) chosen for the model is approximately equivalent to the observed oxidation rate (iteratively fitted to field data) occurring in the field. When the effects of moisture and temperature were included in the model, the predicted oxidation rate was slightly slow compared to that observed. This was due to the large reduction in the predicted oxidation rate caused by using climatic scalars for S^o oxidation rate obtained by multiplying together the scaled effects of temperature and moisture when both factors departed significantly from their optimum level (Shedley, 1982).

At this stage insufficient field information is available to conclude that a single Kmax value for S^o oxidation plus climatic scalars can or can not be used to explain field rates of S^o oxidation in different climatic zones.

The lack of information available for formulating relationships between environmental effects (moisture and temperature) the rate of S^o oxidation could be remedied by more in depth research of these factors. Before this detailed research work is conducted, however, simple field experiments should be performed to improve field sampling techniques in order to decrease the variability in measuring oxidation rates of S^o. In addition, physical movement of S^o out of the soil sampling zone, which was observed at the Ramiha soil and Tokomaru soil field sites needs to be quantified. Only then can a S^o oxidation model be validated for subsequent use as a tool for improving recommendations for the form, rate and frequency of application of S^o containing fertilizers.

Despite the shortcomings of the S^o oxidation model examined in this chapter, it provides a useful basis for designing future research projects aimed at evaluating S^o fertilizers in other climatic regions, particularly with respect to choosing appropriate S^o particle sizes for application and the extent of particle size description required for useful knowledge to be gained from field experiments.

CHAPTER 9

SUMMARY

9.1 INTRODUCTION

Current interest in using high analysis phosphorus (P) and sulphur (S) fertilizers in New Zealand pasture has led to the need to evaluate the agronomic effectiveness of these new fertilizers materials. Many of these fertilizers contain S^o either in a pure form or in mixtures with high analysis P fertilizers (e.g. RPR and PAPR).

Some important aspects of S^o oxidation e.g. the effective S^o particle size range for each climatic region in New Zealand have been investigated for powdered (screened) S^o forms by recent Ministry of Agriculture and Fisheries (MAF) trials. Most S^o, however, is generally surface applied to pastures in granulated or prilled forms.

The literature review (Chapter 2) indicated that little information exists on the influence of fertilizer form and placement of fertilizers on the rate of S^o oxidation.

9.2 METHODOLOGY

This study examined and developed methodology for measuring the effect of fertilizer form, and particle size of S^o in pure and compound S^o fertilizers on the rate at which S^o in these materials oxidises in laboratory incubated, glasshouse and field soils.

A method of measuring the residual S^o in soils was developed in the present study (Chapter 3). The acetone (40 g:200 ml acetone, using a 16 h shaking period) extraction of S^o from soils and subsequent measurement of S^o in the extract by a modification of the Johnson and Nishita S determination (tin/HCl reduction) proved suitable for measuring amounts of residual S^o in soil at concentrations above 5 $\mu g\ S\ g^{-1}$ soil and below 200 $\mu g\ S\ ml^{-1}$ acetone.

Measurement of residual S^o (Chapter 3) in soils was found to be the method suitable for obtaining accurate estimates of S oxidation rates in both field and laboratory studies. Provided care is taken preparing representative samples, extraction with

acetone is simple and the determination of S concentration in the acetone or chloroform extracts by colorimetric assay or HPLC, respectively is readily achieved.

Preliminary incubations were conducted to determine the influence of soil type and fertilizer history on the potential of soils to oxidise S^0 (Chapter 3). Soils with contrasting rhodanese enzyme activity (RA) levels ranging from 167 to 1536 nmole SCN g^{-1} h^{-1} were selected in the study. The soil that had previously received S^0 had higher initial RA and higher S^0 oxidation rates than the soil that received nil S^0. However, there was no simple relationship between RA and initial S^0 oxidation rate. Furthermore RA decreased with time of incubation both in S^0 amended and non-amended soils. It was concluded that neithere RA nor fertilzer history were reliable indices of a soil's potential to oxidise S^0.

Methods for measuring the particle size of S^0 in pure and compound S^0 containing fertilizers were evaluated (Chapter 4). A range of S^0 containing fertilizers were dispersed in water or 10% HCl; then sieved by wet or dry methods; and their particle sizes determined by acetone or loss of ignition methods. Of the methods, dispersion in 10% HCl followed by wet sieving proved the most successful for SSP, RPR and PAPR based fertilizers because of the solubilizing effect of the acid on the gypsum content of the fertilizers. Further advantage of using 10% HCl is that S^0 content of residual material could be accurately determined by loss of ignition. However, S^0/Na-bentonite fertilizers dispersed greater in water than in acid.

9.3 FERTILIZER FORM AND S^0 OXIDATION RATE

Further laboratory incubations (Chapter 5) showed that different sources of S^0, namely Rotokawa S^0 (geothermal S^0) dark S^0, Damman S^0, and agricultural grade S^0 (recovered by Stretford process) had similar oxidation rates in incubated soil. When these materials were granulated or prilled, the oxidation rates in incubated soil decreased substantially due to the fact that granules or prills did not disintegrate when the granules were incorporated into soils under incubation conditions.

The oxidation rates of Ag.grade, Rotokawa and dark S^0 were increased when the S^0 was mixed with reactive phosphate rocks (Arad and North Carolina rocks). It was

postulated that this increase was due to either increased pH buffering or increased P supply by the RPR component. Further incubation of S^o in the presence of various combinations $CaHPO_4$, $CaCl_2$ and $CaCO_3$ demonstrated that the presence of $CaHPO_4$ and $CaCO_3$ could increase S^o oxidation rates. In this second set of incubations RPR treatment did not increase oxidation rates and therefore the effect of RPR on S^o oxidation was not always reproducible.

The effect of P fertilizer form and the effect of plant on the rate S^o oxidation were further investigated in a glasshouse pot incubation study in the presence and absence of white clover plants using Motokutu and Tokomaru soils (Chapter 6). The soluble and insoluble P forms were granulated with S^o to produce fertilizers with S:P ratios ranging from 0.4-0.8 When S^o was granulated with insoluble P forms (e.g. PR and PAPR residues) there was an increase in the rate of S^o oxidation in both soils. However, no effect of soluble P forms (e.g. MCP and PAPR) was found. Therefore the enhancing effect of RPR on S^o oxidation was consistent when only S^o/RPR was granulated and incorporated in soils.

A range of fertilizer materials, S^o of various particle sizes, S^o/RPR and S^o/PAPR were subsequently used to fertilize undisturbed pasture cores in the field and similar cores removed to the glasshouse (Chapter 7). When these fertilizers were surface applied to pasture soils, granulation of RPR and PAPR with S^o did not significantly increase the oxidation rate of S^o both in the glasshouse and field trials.

Under surface application conditions, granules of S^o alone also had a similar oxidation rates to ungranulated S^o forms of the same particle size. The lack of effect of S^o granulation on oxidation rates suggested that, when surface applied, dispersion of the granules occurred. Reduced oxidation rates of granulated S^o in the incubation studies (Chapter 5) were probably because the granules incorporated into soil could not be dispersed.

9.4 MODELLING S^o OXIDATION

An iterative computer programme (involving a least square fitting routine) was developed to calculate specific oxidation rates (K, $\mu g\ S\ cm^{-2}\ day^{-1}$) from S^o

remaining in soils. The average rate of oxidation of the standard S^o material (<150 μm particle) surface applied to the undisturbed soil cores in the glasshouse was significantly lower than that previously measured in laboratory and glasshouse incubated soils (Chapter 8).

Specific oxidation rates of different particle sizes (<150, 150-250 and 250-500 μm) of S^o applied to undisturbed pasture soils in the glasshouse were similar for Ramiha soil (11.2 - 17.4 μg S^o cm^{-2} day^{-1}) and for Tokomaru soil (7.5 - 14.1 μg S^o cm^{-2} day^{-1}). Correction of oxidation rates for the different soil moisture contents (using relationship derived by Australian workers), however, provided evidence that the <150 μm S^o particle size had similar maximum potential K values in both soils (Kmax, 18 μg S^o cm^{-2} day^{-1}) and on average explained 86-89% of variance in the amounts of S^o remaining for the S^o of <150, 150-250 and 250-500 μm particle sizes in the glasshouse trial.

This suggested, with other evidence from the literature, that S^o oxidation in soil could be effectively modelled by knowing S^o particle size and the effects of soil moisture and temperature, on S^o oxidation.

Accordingly a S^o oxidation simulation model was constructed using the Kmax observed in the glasshouse trial and information from literature on the effects of soil moisture and temperature on S^o oxidation.

The model was validated by attempting to predict the rate of S^o oxidation in 2 field trials (the Tokomaru field trial and one carried out at Invermay by Lee et al., 1988; the Ramiha soil field was not included due to an insufficient number of observations and no soil temperature were recorded) using the appropriate climatic data. For most soils and S^o particle sizes, the best fit of observed data was obtained by using only S^o particle size information in the simulation model. This explained 81% and 89% of the variance in the observed field trial data at the Tokomaru and Invermay sites, respectively. At both sites inclusion of moisture and temperature effects did not improve the predictions. In general, predicted oxidation rates were slower than observed.

Several factors could account for the observed S^o oxidation being more rapid than predicted. For example, loss of S^o from the soil sampling zone will cause an overestimation of S^o oxidation rates, some evidence for this is provided.

Results from this thesis provide evidence that providing S^o containing fertilizers disperse upon application to soil, fertilizer form need not be considered as a factor influencing S^o oxidation rate. However, P deficient soils were not among those examined in this thesis. P deficiency may affect S oxidation rates and is expected to influence the plant availability of oxidised S.

Within experimental error the simulation model predicted S^o oxidation in field soil well and provides a useful basis for designing future research projects. There is evidence provided in this thesis that Kmax for surface applied S^o may be independent of soil type. Further research should test this hypothesis by evaluating the simulation model using S^o oxidation data measured in other climatic regions of New Zealand. More research should also be conducted to better define the value of Kmax for surface applied S^o.

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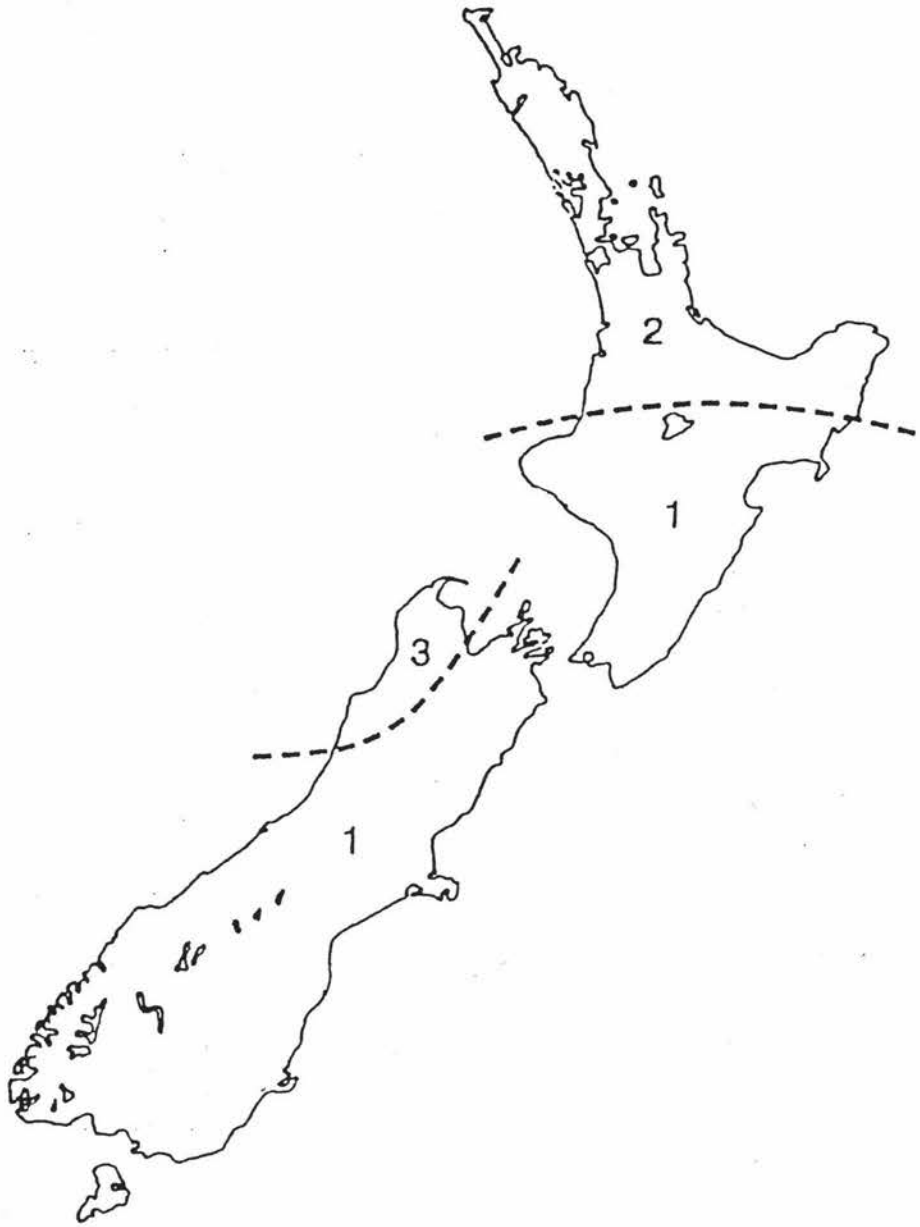
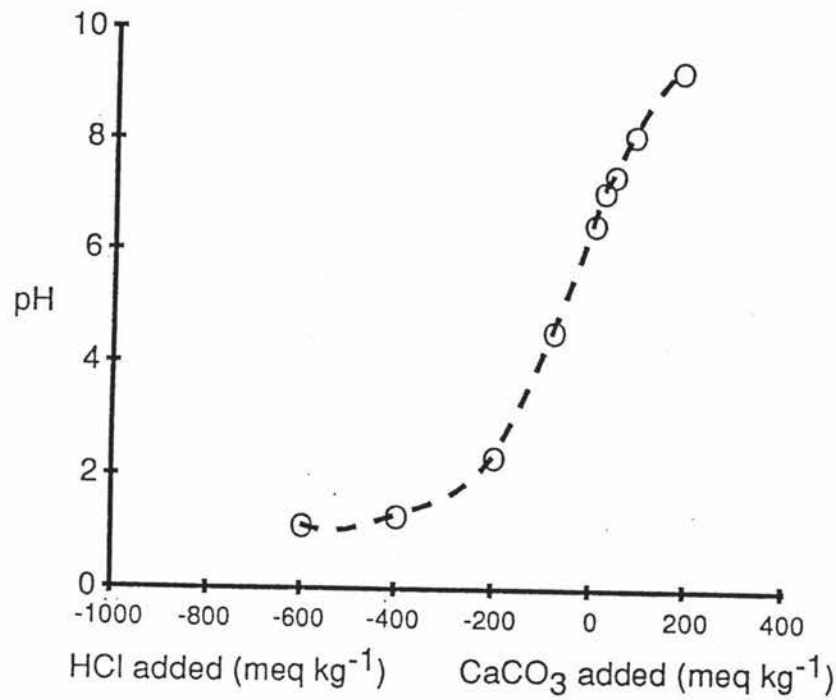
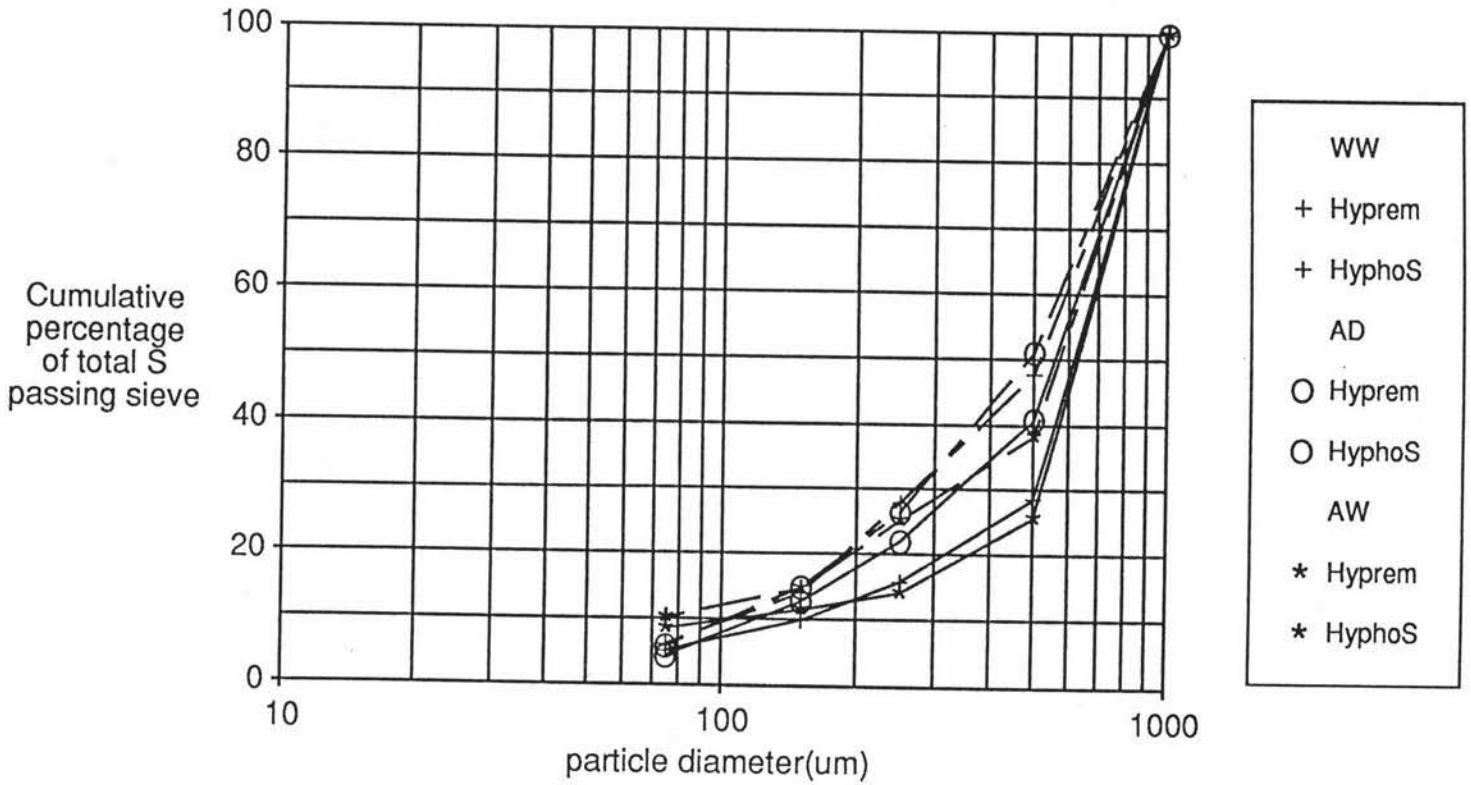


Appendix 2.1 Map of New Zealand showing broad climate zones which affect recommended elemental fertilizer particle size ranges.

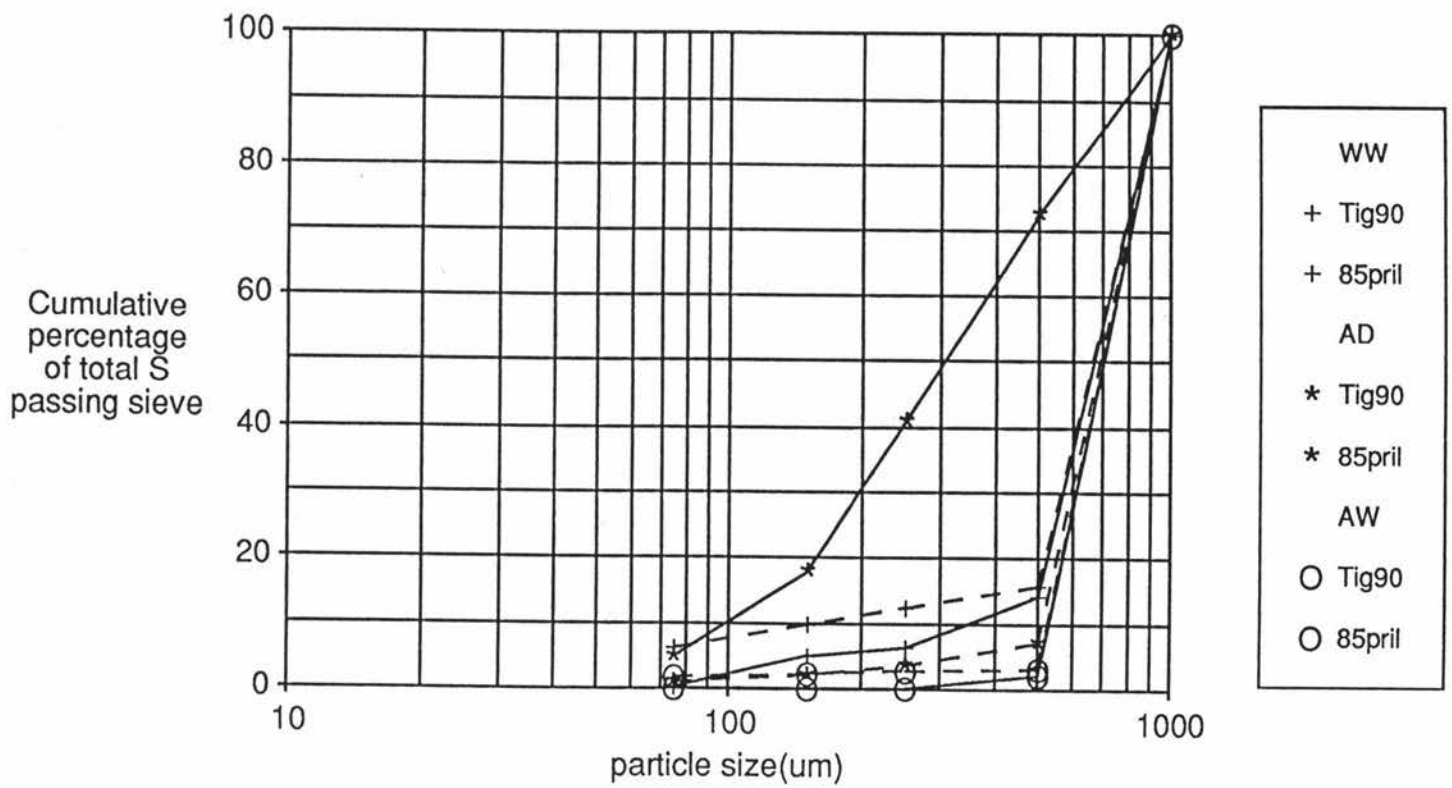


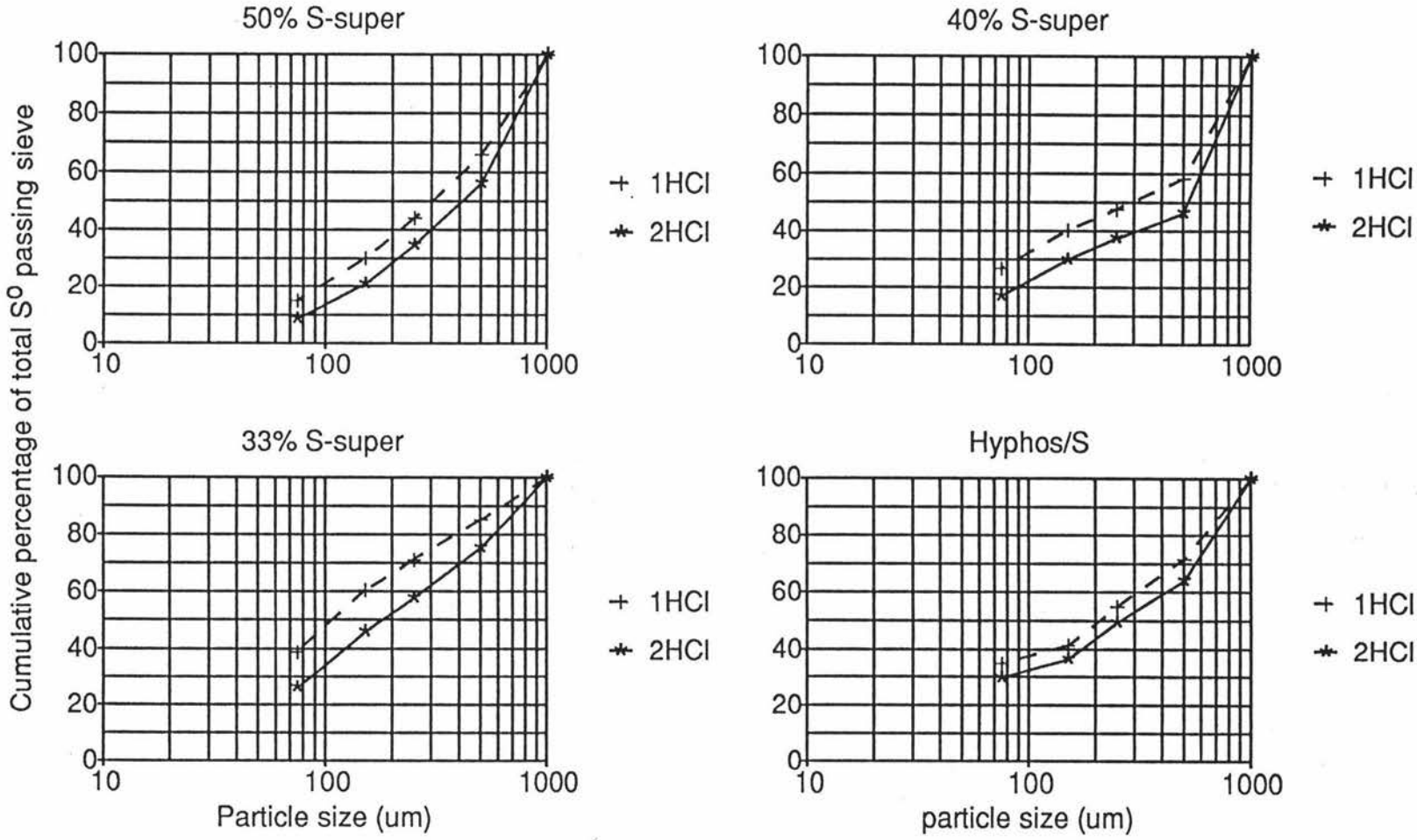
Appendix 3.1 Effect of adding HCl or $CaCO_3$ on the pH of Tokomaru soil measured in 0.01 M of NaCl (Bolan et al., 1986).

Appendix 4.1 S particle size distribution in Hyphos products



Appendix 4.2 S particle size distribution in Prilled S fertilizers





Appendix 4.3 S^o particle size distribution in sulphurised phosphate fertilizers after dispersion by single (1) and double (2) washing in 10% HCl. S^o determination by loss on ignition.

Appendix 6.1 The effect of fertilizer form on rhodanese activity (RA) in the presence of white clover in both Tokomaru and Motokutu soils at each sampling date.

Fertilizer	Days after sowing 16	30	43	59	72	98
			nmole SCN^- g^{-1} h^{-1}			
Tokomaru						
S^o	285	438	253	261	95	473
S^o/RPR	264	427	162	238	360	452
S^o/PAPR	324	455	184	217	206	348
Control	246	600	150	308	202	284
LSD 5%	15	22	68	42	45	53
Makotuku						
S^o	403	600	595	594	330	552
S^o/RPR	445	648	562	694	391	580
S^o/PAPR	482	894	578	430	435	756
Control	520	753	745	538	448	432
LSD 5%	26	43	74	42	43	34

Appendix 6.2 The T^1-tests of the slopes (B) between each pair of treatments within the Motokutu and Tokomaru with and without planted soils.

With plant

	S^o	S^o/RPR	S^o/PAPR	S^o/PAPRres
Makotuku				
S^o/RPR	3.82**			
S^o/PAPR	0.98	4.13**		
S^o/PAPR-res	1.85	0.99	2.46*	
S^o/MCP	1.16	4.66**	0.12	2.66*
Tokomaru				
S^o/RPR	2.64*			
S^o/PAPR	0.54	3.08*		
S^o/PAPR-res	2.24	1.48	3.23*	
S^o/MCP	0.21	2.08*	0.32	2.57*

Without plant

	S^o	S^o/RPR
Makotuku		
S^o/RPR	2.65*	
S^o/PAPR	0.06	2.73*
Tokomaru		
S^o/RPR	3.31**	
S^o/PAPR	0.97	2.96*

Appendix 6.3a The parameter of the logistic function fitted to the accumulated dry matter yield.

Treatment	Logistic function (ln (Y/Y_0) = A + B x t)) A	B	R^2	A	B	R^2
		Tokomaru			Makotuku	
Control	-5.37	0.078	0.99	-5.31	0.077	0.99
S^o	-5.98	0.088	0.99	-6.01	0.087	0.99
RPR	-6.00	0.088	0.99	-5.62	0.080	0.99
S^o/RPR	-5.72	0.087	0.99	-5.38	0.078	0.99
PAPR	-5.47	0.079	0.99	-5.49	0.078	0.99
S^o/PAPR	-5.43	0.081	0.99	-5.72	0.080	0.99
PAPRres	-4.09	0.048	0.95	-5.22	0.076	0.99
S^o/PAPRres	-5.55	0.089	0.99	-6.87	0.103	0.99
MCP	-5.30	0.077	0.99	-5.40	0.078	0.99
S^o/MCP	-5.60	0.081	0.99	-5.12	0.077	0.99

Appendex 6.3b Estimated t statistics for differeces amongst pairs of fitted dry matter b^(regression coefficients) between treatments.

	Control	S^o	PR	S^o/RPR	PAPR	S^o/PAPR	PA.res	S^o/PA.res	MCP
Tokomaru									
S^o	0.61								
RPR	2.13	1.16							
S^o/RPR	3.15*	2.35*	0.11						
PAPR	1.18	0.54	1.21	1.83					
S^o/PAPR	1.57	1.03	0.59	0.88	0.58				
PAPRres	2.75*	3.02*	3.81**	3.35**	3.32**	3.49**			
S^o/PAPRres	0.75	0.26	1.19	1.60	0.18	0.65	3.00*		
MCP	0.03	0.52	2.01	2.84*	1.06	1.46	2.71*	0.69	
S^o/MCP	1.12	0.56	1.06	1.51	0.08	0.47	3.26*	0.23	1.02
Makotuku									
S^o	1.21								
RPR	2.49*	1.27							
S^o/RPR	0.05	1.25	2.52*						
PAPR	1.00	0.06	0.93	1.17					
S^o/PAPR	0.86	2.03	3.29*	0.79	1.66				
PAPRres	0.34	0.44	1.26	0.37	0.43	0.89			
S^o/PAPRres	2.19	1.53	0.83	2.21	1.36	2.64*	1.59		
MCP	0.73	0.17	0.56	0.77	0.20	1.36	0.25	1.48	
S^o/MCP	0.13	1.42	3.07*	0.20	0.51	1.21	0.30	2.27	0.74

Appendix 7.1 H-number quench correction

The H-number is a popular method for quench correction in liquid scintillation counting. The $H^{\#}$ method offers certain advantages over other conventional quench correction methods, the major one are:

i) any sample can have only one $H^{\#}$ value, contrary to the channels ratio method (L'Annunziata, 1987) where one sample may produce many different channel ratios depending on the channel width and gains selected

ii) the $H^{\#}$ method results in less variable quench correction curves over a wider range of counting efficiency

iii) the $H^{\#}$ may be used to determine correctly the disintegration rate of a sample regardless of the sample volume or geometry and

iv) quench correction curves using $H^{\#}$ are identical for all fluor cocktails, and all chemical and colour quenching agents.

The method uses an external radionuclide source (e.g. ^{137}Cs or ^{226}Ra) to generate compton electrons which, in turn, activate the scintillant. The energies of the electrons are constant from sample to sample. However, the pulse heights produced by the electrons through scintillation can vary and are a function of the amount and type of quenching agent in each sample. The shift of the 'compton edge' of the pulse height caused by quenching to lower energies can be accurately monitored electronically (L'Annunziata, 1987).

Appendix 7.2a Effect of S^o particle size on the accumulated dry matter yield (g $core^{-1}$) of herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (non-grnaulated materials).

Particle size (μm)	Glasshouse trial days after S^o application						Field trial
	30	60	90	120	150	180	180
	Dry matter yield (g $core^{-1}$)						
Ramiha							
<150	1.56	3.42	5.90	7.46	8.51	10.50	7.23
250-500	1.48	3.19	5.55	6.96	8.02	9.63	7.38
Tokomaru							
<150	1.88	3.73	5.66	7.27	8.76	10.80	4.23
150-250	1.40	3.07	5.01	6.42	7.71	9.43	5.71
250-500	1.44	3.29	5.29	6.81	8.27	9.46	6.04

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

Appendix 7.2b Effect of fertilizer form on the accumulated dry matter yield (g core^{-1}) of herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (Granulated materials).

Particle size (μm)	Physical form	Glasshouse trial days after S^o applied 30	60	90	120	150	180	Field trial 180
		Dry matter yield (g core^{-1})						
Ramiha								
<150	NG	1.56	3.42	5.90	7.46	8.51	10.50	7.23
Sgrn	G	1.79	3.34	5.29	6.69	7.78	9.65	7.92
S/PR	G	1.84	3.41	5.58	7.15	8.18	9.95	6.70
PR	G	1.77	3.44	5.59	7.25	8.34	10.77	5.25
control	-	2.01	33.79	5.77	7.33	8.36	10.50	7.13
Tokomaru								
<150	NG	1.88	3.73	5.66	7.27	8.76	10.80	4.23
Sgrn	G	1.58	3.08	5.22	6.66	7.97	9.84	6.21
S/PR	G	1.37	3.01	5.17	6.71	8.06	9.99	6.25
PR	G	1.68	3.50	5.66	7.25	8.47	10.70	6.36
Control	-	1.52	2.97	4.81	6.00	7.13	8.96	5.19

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

NG = Non-granulated, G = granulated.

Appendix 7.3 Cumulative and the average herbage concentration of N and P of herbage grown on undisturbed soil cores in both Tokomaru and Ramiha.

Treatment	Phys. forms	Cumul. uptake N (mg)	Cumul. uptake P (mg)	Conc. N (%)	Conc. P (%)	Cumul.uptake N (mg)	Cumul.uptake P (mg)	Conc. N (%)	Conc. P (%)
		Ramiha soil				Tokomaru soil			
S^0<150	NG	2959	30	2.81	0.28	332	24	3.08	0.22
S^0gran	G	276	27	2.86	0.28	301	22	3.06	0.22
S^0/RPR	G	308	29	3.09	0.29	278	27	2.78	0.27
RPR	G	295	33	2.74	0.31	313	27	2.92	0.25
S^0/PAPR	G	258	30	nd	nd	298	35	nd	nd
Control	-	303	29	2.88	0.27	254	19	2.83	0.21
lsd 5%		ns	ns			ns	5.9		

NG = Non-granulated, G = granulated.
nd = not determine.

Appendix 7.4a Effect of S^o particle size on the ccumulative S uptake (mg $core^{-1}$) by herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (non-granulated materials).

Particle size (μm)	Glasshouse trial days after S^o application 30	60	90	120	150	180	Field trial 180
	Cumulative S uptake (mg $core^{-1}$)						
Ramiha							
<150	7.05	11.60	16.47	20.34	23.07	30.76[a]	12.04
250-500	5.90	8.41	12.30	15.84	18.08	23.65[b]	11.12
Tokomaru							
<150	6.99[a]	10.26[a]	14.18[a]	17.92[a]	20.50[a]	27.03[a]	6.72
150-250	4.75[b]	7.49[b]	11.12[b]	13.62[b]	15.59[b]	20.41[b]	7.74
250-500	4.46[b]	7.21[b]	10.85[b]	13.32[b]	15.42[b]	21.14[b]	7.93

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

Appendix 7.4b Effect of fertilizer form on the cumulative S uptake (mg core^{-1}) by herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (Granulated materials).

Particle size (μm)	Physical form	Glasshouse trial days after S^o applied 30	60	90	120	150	180	Field trial 180
		Cumulative S uptake (mg core^{-1})						
Ramiha								
<150	NG	7.05	11.60	16.47	20.34	23.07	30.76^a	12.04
Sgrn	G	6.73	9.41	12.97	16.50	19.21	26.56^b	12.37
S/PR	G	7.15	10.14	13.87	17.52	19.88	26.09^b	10.95
PR	G	7.04	10.17	13.65	17.42	19.99	28.14^b	7.46
control	-	6.94	9.84	12.76	15.88	18.05	23.88^b	9.81
Tokomaru								
<150	NG	6.99^a	10.26^a	14.18^a	17.92^a	20.50^a	27.03^a	6.72
Sgrn	G	4.86^b	7.06^b	11.06^b	14.19^b	16.47^b	22.21^b	8.77
S/PR	G	4.73^b	7.71^b	11.65^b	14.89^b	17.22^b	22.98^b	9.39
PR	G	5.16^b	7.73^{bc}	11.27^b	13.99^b	15.85^b	21.51^{bc}	8.23
Control	-	4.22^b	5.79^c	8.49^c	10.41^c	12.00^c	17.28^c	6.61

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

NG = Non-granulated, G = granulated.

Appendix 7.5a Effect of S^o particle size on the cumulative ^{35}S uptake (% applied) by herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (non-granulated materials).

Particle size (μm)	Glasshouse trial days after S^o application						Field trial
	30	60	90	120	150	180	180
	Cumulative ^{35}S uptake (%)						
Ramiha							
<150	3.14[a]	8.59[a]	12.51[a]	14.89[a]	16.57[a]	20.47[a]	9.76[a]
250-500	0.46[c]	1.09[c]	1.09[c]	2.49[c]	2.93[c]	4.15[c]	1.69[b]
Tokomaru							
<150	1.83[a]	4.01[a]	6.30[a]	8.60[a]	10.59[a]	13.95[a]	4.22[a]
150-250	0.43[c]	0.87[c]	1.39[c]	1.91[c]	2.46[c]	3.54[c]	1.08[b]
250-500	0.23[c]	0.52[c]	0.82[c]	1.04[c]	1.26[c]	1.85[c]	0.64[b]

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

Appendix 7.5b Effect of fertilizer form on the cumulative ^{35}S uptake (% applied) by herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (Granulated materials).

Particle size (μm)	Physical form	Glasshouse trial days after S^o applied 30	60	90	120	150	180	Field trial 180
		Cumulative ^{35}S uptake (%)						
Ramiha								
<150	NG	3.14[a]	8.59[a]	12.51[a]	14.89[a]	16.57[a]	20.47[a]	9.76[a]
S/PR	G	1.92[b]	4.80[b]	7.67[b]	10.27[b]	11.67[b]	14.70[b]	8.09[a]
Sgrn	G	1.57[b]	3.87[b]	6.23[b]	8.41[b]	10.31[b]	13.71[b]	8.27[a]
Tokomaru								
<150	NG	1.83[a]	4.01[a]	6.30[a]	8.60[a]	10.59[a]	13.95[a]	4.22[a]
S/PR	G	0.84[b]	2.57[b]	4.73[b]	6.93[ab]	8.87[ab]	12.59[ab]	5.77[a]
Sgrn	G	0.77[b]	2.06[b]	3.98[b]	5.81[b]	7.66[b]	10.38[b]	3.88[a]

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

NG = Non-granulated, G = granulated.

Appendix 7.6a Effect of S^0 particle size on the amount of plant S derived from fertilizer (%) by herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (Non-granulated materials).

Particle size (μm)	Glasshouse trial days after S^0 application						Field trial
	30	60	90	120	150	180	180
	S derived from fertilizer (%)						
Ramiha							
<150	23.79[a]	38.99[a]	40.17[a]	38.77[a]	38.08[a]	35.24[a]	43.06[a]
250-500	4.23[c]	7.13[c]	8.30[c]	8.41[c]	8.63[c]	9.29[c]	7.94[b]
Tokomaru							
<150	14.12[a]	20.63[a]	23.66[a]	25.39[a]	27.26[a]	27.31[a]	33.52[a]
150-250	5.26[c]	6.54[c]	6.88[c]	7.60[c]	8.47[c]	9.32[c]	7.52[c]
250-500	3.01[c]	1.00[c]	4.03[c]	4.18[c]	4.37[c]	4.61[c]	4.38[c]

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

Appendix 7.6b Effect of fertilizer form on the amount of plant S derived from fertilizer (%) by herbage grown on undisturbed soil cores of Ramiha and Tokomaru soils (Granulated materials).

Particle size (μm)	Physical form	Glasshouse trial days after S^o applied 30	60	90	120	150	180	Field trial 180
		S derived from fertilizer (%)						
Ramiha								
<150	NG	23.79[a]	38.99[a]	40.17[a]	38.77[a]	38.08[a]	35.24[a]	43.06[a]
Sgrn	G	12.10[b]	21.54[b]	24.80[b]	26.39[b]	27.88[b]	26.81[b]	34.65[a]
S/PR	G	14.89[b]	26.22[b]	29.30[b]	31.53[b]	31.72[b]	30.52[ab]	38.86[a]
Tokomaru								
<150	NG	14.12[a]	20.63[a]	23.66[a]	25.39[a]	27.26[a]	27.31[a]	33.52[a]
Sgrn	G	8.45[b]	15.18[ab]	19.06[a]	21.62[a]	24.60[a]	24.63[a]	23.38[b]
S/PR	G	9.41[b]	17.46[ab]	21.47[a]	24.49[a]	27.10[a]	28.65[a]	32.05[a]

Means separation based on Duncan's Multiple Range Test at the 5 % level for comparison made for comparisons made across all treatments (particle sizes and fertilizer forms) within a soil and a harvest. Letters have been added only where significant treatment differences occur.

NG = Non-granulated, G = granulated.

Appendix 8.1.A The least square fit procedure for calculating the specific oxidation rate ($\mu g\ S^o\ cm^{-2}\ day^{-1}$) from the data input as S^o remaining at different sampling date using the constant change in Δr of the surface applied S^o.

```
CLOSE
'QuickBASIC  Program Title : LSRFIT.BAS
'Simulation of Specific Oxidation Rate of S° using Least Square Iteration Procedure
'Units and Symbols:
'                Rate  = mg
'                   D  = mm,
'                  dR  = mm
'                   ρ  = particle density, 2.07 mg mm-3
'                  No  = number of particles,
'                   z  = number of samplings,
'                   m  = number of size fractions
'                   D  = diameter (mm)
'                   R  = radius (mm)
'                   A  = area (mm2)
'                   V  = volume (mm3),
'               Mpart  = Mass per particle (g)
'                   S  = days
'                  Th  = predicted S° remaining (%)
'                  Ob  = observed S° remaining (%)
'                   K  = dRmax x ρ x 100000 (ug S cm-2day-1)

'Initial Section
CLS
INPUT "Enter ele-S oxidation inputfile :", inputfile$
INPUT "Enter output filename :", outputfile$
OPEN inputfile$ FOR INPUT AS #1
OPEN outputfile$ FOR OUTPUT AS #2
INPUT "Enter # samplings : ", z
INPUT "Enter # size fractions : ", m
INPUT "Enter Rate of Application, mg : ", Rate
pi = 3.14159: ρ = 2.07: dRmax(1) = .01 / 1000: L = 0
Obm = 0: Obs = 0: Smdiffs = 0: mdiffs = 0: mdiff = 0: IN = .2: h = 0
DIM S(z), Ob(z), Th(z), SS(z)
DIM D(m), R(m), A(m), V(m), Mpart(m), Mtot(m), No(m), TotalA(m), percent(m)
INPUT "Enter eleS oxidation inputfile : ", inputfile$
INPUT "Enter output filename : ", outputfile$
OPEN inputfile$ FOR INPUT AS #1
OPEN outputfile$ FOR OUTPUT AS #2

'Read observed data file and calculate mean (Obm) and total sum of squares (Smdiffs)
FOR n = 1 TO z
        INPUT #1, S(n), Ob(n)
        Obs = Obs + Ob(n)
NEXT
        Obm = Obs / z
FOR n = 1 TO z
        mdiff = Ob(n) - Obm
        mdiffs = mdiff * mdiff
        Smdiffs = Smdiffs + mdiffs
NEXT
```

Appendix 8.1.A continued

```
FOR n = 1 TO m
        PRINT
        PRINT "Enter particle diameter & percent of total S^O by weight"
        INPUT "diameter (mm) "; D(n)
        INPUT "percent "; percent(n)
NEXT
1850 h = 1: L = L + 1
FOR n = 1 TO m
        R(n) = D(n) / 2
        A(n) = 4 * pi * R(n) ^ 2
        V(n) = 4 / 3 * pi * R(n) ^ 3
        Mparticle(n) = ρ * V(n)
        Mtot(n) = Rate * percent(n) / 100
        No(n) = Mtot(n) / Mparticle(n)
NEXT

'Dynamic Section
FOR Day = 1 TO S(z)
        TotMnet = 0: TotMoxid = 0
        FOR n = 1 TO m
            dR(n) = dRmax(L)
            R(n) = R(n) - dR(n)
            IF R(n) < 0 THEN R(n) = 0
            Mnet(n) = 4 / 3 * pi * (R(n) ^ 3) * ρ * No(n)
            Moxid(n) = Mtot(n) - Mnet(n)
            TotMnet = TotMnet + Mnet(n)
            TotMoxid = TotMoxid + Moxid(n)
            PerMnet = TotMnet / Rate * 100
            PerMoxid = TotMoxid / Rate * 100
NEXT
  IF INT(Day) = INT(S(h)) THEN
    Th(h) = PerMnet
    h = h + 1
    IF h > z THEN h = h - 1
  END IF
NEXT

'Calculates 3 Residual Sum of Squares on First Cycle,
'2 Calculated on subsequent cycle from 3(2) estimates of dRmax(L)
S = 0
FOR i = 1 TO z
        Diff = Th(i) - Ob(i)
        diffs = Diff * Diff
        S = S + diffs
NEXT
SS(L) = S
IF L = 3 THEN 3000
2800 dRmax(L + 1) = dRmax(1) * (1 + IN)
        IN = -IN
        GOTO 1850
```

Appendix 8.1.A continued

```
'Choosing the Smallest Residual Sum of Squares and Best dRmax Estimate
'If Best dRmax is the Previous Value (ie. SS(1) is selected) then
'Line 3035 Reduces Change in dRmax(1)

3000    IF SS(1) < SS(2) AND SS(1) < SS(3) THEN
            IN = IN * .5: GOTO 3800
        END IF
        IF SS(2) < SS(3) THEN
            SS(1) = SS(2)
            dRmax(1) = dRmax(2)
        ELSE
            SS(1) = SS(3)
            dRmax(1) = dRmax(3)
        END IF

3800    L = 1
        IF ABS(IN) < .01 THEN
        GOTO 4000
        ELSE
        GOTO 2800
        END IF

4000 CLS
        PRINT "Input Filename: "; inputfile$
        PRINT ; "K = ";
        PRINT USING "####.##"; dRmax(1) * ρ * 100000
        PRINT "Day  Predict%  Observed% "
        FOR n = 1 TO z
            PRINT USING "###"; S(n),
                PRINT USING "   ###.## "; Th(n), Ob(n)
        NEXT
        PRINT : PRINT "R sq.= ";
        PRINT USING "#.##"; (1 - SS(1) / Smdiffs)
        PRINT #2, "Input Filename : ", inputfile$
        PRINT #2, "K = ";
        PRINT #2, USING "####.##"; dRmax(1) * ρ * 100000
        PRINT #2, "Day  Predicted %  Observed % "
        FOR n = 1 TO z
                PRINT #2, USING "###"; S(n),
                PRINT #2, USING "   ###.## "; Th(n), Ob(n)
        NEXT

'Print unadjusted R square value
        PRINT #2, : PRINT #2, "R sq.= ";
        PRINT #2, USING "#.##"; (1 - SS(1) / Smdiffs)
CLOSE : END
```

Appendix 8.1.B The least square fit procedure for calculating the specific oxidation rate ($\mu g\ S^o\ cm^{-2}\ d^{-1}$) from the data input as S^o remaining at different sampling date using equation 8.4c.

```
CLOSE
'Quick BASIC Program Title : KFIT.BAS
'Calculation of Specified Oxidation Rate of S° using Least Square Iteration Procedure
'Units and Symbols:
'              Rate = mg
'                 K = µg S° cm-2 d-1
'                 ρ = particle density, 2.07 mg mm-3
'                No = no. particles
'                 z = no. samplings
'                 m = no. size fractions
'                 D = diameter, mm
'                 R = radius, mm
'                 A = area, mm2
'                 V = volume, mm3
'             Mpart = mass per particle, mg
'                 S = days
'                Th = predicted S° remaining (%)
'               Obs = observed S° remaining (%)

'Initial Section
CLS
INPUT "Enter # samplings : ", z
INPUT "Enter # size fractions : ", m
INPUT "Enter Rate of Application, mg : ", Rate
INPUT "Enter Approximate K (µg S cm-2day-1): ", Ka
pi = 3.14159: ρ = 2.07: K(1) = Ka/100000: L = 0
Obm = 0: Obs = 0: Smdiffs = 0: mdiffs = 0: mdiff = 0: IN = .2: h = 0
DIM S(z), Ob(z), Th(z), SS(z)
DIM D(m), R(m), A(m), V(m), Mpart(m), Mtot(m), No(m), TotalA(m), percent(m)
INPUT "Enter eleS oxidation inputfile : ", inputfile$
INPUT "Enter output filename : ", outputfile$
OPEN inputfile$ FOR INPUT AS #1
OPEN outputfile$ FOR OUTPUT AS #2

'Read observed data file and calculate mean (Obm) and total sum of squares (Smdiffs)
FOR n = 1 TO z
        INPUT #1, S(n), Ob(n)
        Obs = Obs + Ob(n)
NEXT
Obm = Obs / z

FOR n = 1 TO z
        mdiff = Ob(n) - Obm
        mdiffs = mdiff * mdiff
        Smdiffs = Smdiffs + mdiffs
NEXT
```

Appendix 8.1.B continued

```
FOR n = 1 TO m
        PRINT
        PRINT "Enter diameter & percent "
        INPUT "diameter (mm) "; D(n)
        INPUT "percent "; percent(n)
NEXT
1850 h = 1: L = L + 1
FOR n = 1 TO m
        R(n) = D(n) / 2
        A(n) = 4 * pi * R(n) ^ 2
        V(n) = 4 / 3 * pi * R(n) ^ 3
        Mparticle(n) = ρ * V(n)
        Mtot(n) = Rate * percent(n) / 100
        No(n) = Mtot(n) / Mparticle(n)
NEXT

'Dynamic Section
FOR Day = 1 TO S(z)
        TotMnet = 0: TotMoxid = 0
        FOR n = 1 TO m
            K(1) = K(L)
        R(n) = ((R(n) ^ 2) * (R(n) - (3 * K(1) / 2.07))) ^ (1 / 3)
2000 IF R(n) < 0 THEN R(n) = 0
        Mnet(n) = 4 / 3 * pi * (R(n) ^ 3) * ρ * No(n)
        Moxid(n) = Mtot(n) - Mnet(n)
        TotMnet = TotMnet + Mnet(n)
        TotMoxid = TotMoxid + Moxid(n)
        PerMnet = TotMnet / Rate * 100
        PerMoxid = TotMoxid / Rate * 100
NEXT
IF INT(Day) = INT(S(h)) THEN
        Th(h) = PerMnet
        h = h + 1
        IF h > z THEN h = h - 1
END IF
NEXT

'Calculates 3 Residual Sum of Squares on First Cycle
'2 Calculation on Subsequent Cycle from 3(2) estimates of K(L)
S = 0
FOR i = 1 TO z
        Diff = Th(i) - Ob(i)
        diffs = Diff * Diff
        S = S + diffs
NEXT
SS(L) = S
IF L = 3 THEN 3000
2800 K(L + 1) = K(1) * (1 + IN)
        IN = -IN
        GOTO 1850
```

Appendix 8.1.B continued

```
'Choosing the Smallest Residual Sum of Squares and Best K Estimate
'If Best K is the Previous Value (ie. SS(1) is selected) then
'Line 3035 Reduces Change in K(1)

3000  'LPRINT SS(1), SS(2), SS(3)
    'LPRINT K(1), K(2), K(3)
          IF SS(1) < SS(2) AND SS(1) < SS(3) THEN
               IN = IN * .5: GOTO 3800
          END IF
          IF SS(2) < SS(3) THEN
               SS(1) = SS(2)
               K(1) = K(2)
          ELSE
               SS(1) = SS(3)
               K(1) = K(3)
          END IF

3800      L = 1
          IF ABS(IN) < .01 THEN
               GOTO 4000
          ELSE
               GOTO 2800
          END IF

4000  CLS : LPRINT
    PRINT "Input Filename: "; inputfile$
    PRINT ; "K (K) = ";
    PRINT USING "####.##"; K(1) * 100000
    PRINT "Day  Predict%  Observed% "
    FOR n = 1 TO z
     PRINT USING "###"; S(n),
     PRINT USING "   ###.## "; Th(n), Ob(n)
    NEXT
    PRINT : PRINT "R sq.= ";
    PRINT USING "#.##"; (1 - SS(1) / Smdiffs)
    PRINT #2, "Input Filename : ", inputfile$
    PRINT #2, "K (K) = ";
    PRINT #2, USING "####.##"; K(1) * 100000
    PRINT #2, "Day  Predict%  Observed% "
    FOR n = 1 TO z
     PRINT #2, USING "###"; S(n),
     PRINT #2, USING "   ###.## "; Th(n), Ob(n)
    NEXT

'Print unadjusted R square value
    PRINT #2, : PRINT #2, "R sq.= ";
    PRINT #2, USING "#.##"; (1 - SS(1) / Smdiffs)
CLOSE : END
```

Appendix 8.2 The predictive S^o model using the potential maximum oxidation rate obtained from the glasshouse trial and the scalar of moisture and temperature effects as the input.

```
CLOSE
'QuickBASIC  Program Title: EleSOxid.BAS
'Effect of particle sizes, moisture and temperature on field oxidation
'           of elemental S, using Kmax =18 µg S° cm-2 day,
'           or dRmax of.087/1000 mm
'Units and Symbols:
'              Rate  =  mg
'                D  =  mm
'               dR  =  mm
'                ρ  =  density,  = mg mm-3
'               No  =  # particle
CLS

'Initial Section
'Open file for daily scalars and input for predicted data
INPUT "enter inputfile: ", inputfile$
INPUT "enter outputfile: ", outputfile$
INPUT "enter rate applied: ", Rate
OPEN inputfile$ FOR INPUT AS #1
OPEN outputfile$ FOR OUTPUT AS #2
pi = 3.14159: ρ = 2.07: dRmax = .087 / 1000
INPUT "Enter # Days Experiment carried out : "; z
INPUT "Enter # size fractions : ", m
DIM D(m), R(m), A(m), V(m), Mpart(m), Mtot(m), No(m), TotalA(m)

FOR n = 1 TO m
          PRINT
          PRINT "Enter particle diameter & percent of total EleS"
          INPUT "diameter (mm) "; D(n)
          INPUT "percent "; percent(n)
          R(n) = D(n) / 2
          A(n) = 4 * pi * R(n) ^ 2
          V(n) = 4 / 3 * pi * R(n) ^ 3
          Mparticle(n) = ρ * V(n)
          Mtot(n) = Rate * percent(n) / 100
          No(n) = Mtot(n) / Mparticle(n)
NEXT
CLS

PRINT "InputFile : "; inputfile$
PRINT "OutputFile : "; outputfile$
PRINT #2, "Modelling Tokomaru Field Elemental S Oxidation"
PRINT #2, "InputFile : "; inputfile$
PRINT #2, "OutputFile : "; outputfile$
COLOR 3
```

Appendix 8.2 continued

```
        PRINT "Day totMn totMo  %Mn   %Mo    K          scalar"
        PRINT "    mg   mg    %    %   µg/cm2/d   daily scalar"
        PRINT #2, "Day totMn totMo  %Mn   %Mo    K          scalar"
        PRINT #2, "    mg   mg    %    %   µg/cm2/d   daily scalar"
VIEW PRINT 4 TO 25

'Dynamic Section
FOR day = 1 TO z
        INPUT #1, scalar
        k = dRmax * ρ * 100000 * scalar
        TotMnet = 0: TotMoxid = 0

FOR n = 1 TO m
                dR(n) = dRmax * scalar
                R(n) = R(n) - dR(n): IF R(n) < 0 THEN R(n) = 0
                Mnet(n) = 4 / 3 * pi * (R(n) ^ 3) * ρ * No(n)
                Moxid(n) = Mtot(n) - Mnet(n)
                TotMnet = TotMnet + Mnet(n)
                TotMoxid = TotMoxid + Moxid(n)
                PerMnet = TotMnet / Rate * 100
                PerMoxid = TotMoxid / Rate * 100
NEXT
        PRINT USING "###"; day;
        PRINT #2, USING "###"; day;
        PRINT USING "  ##.#"; TotMnet; TotMoxid;
        PRINT USING " ###.#"; PerMnet; PerMoxid; k;
        PRINT USING " #.##"; scalar
        PRINT #2, USING "  ##.#"; TotMnet; TotMoxid;
        PRINT #2, USING " ###.#"; PerMnet; PerMoxid; k;
        PRINT #2, USING " #.##"; scalar

NEXT: CLOSE : END
```

Appendix 8.3a The daily evapotranspiration, rain fall, soil temperature at 30 cm depth and the calculated moisture and temperature scalar

Daily ET	ET top 2 cm (mm)	Rain fall	Moist. Scalar	Temp.at 30 cm °C	Temp. Scalar	Rain fall mm	Moist. Scalar
		Tokomaru soil				Ramiha soil	
3.1	0.7	0.0	1.00	16.0	0.80	5.1	1.00
3.4	0.7	0.0	0.96	15.9	0.80	1.4	1.00
2.2	0.5	0.5	0.96	17.0	0.81	1.3	1.00
3.4	0.7	7.2	1.00	17.1	0.81	0.0	1.00
4.3	0.9	0.0	0.99	17.3	0.81	0.1	1.00
3.9	0.8	3.6	1.00	16.9	0.82	18.9	1.00
4.0	0.8	0.0	0.99	17.0	0.82	0.8	0.96
2.1	0.4	19.1	1.00	17.4	0.82	0.1	0.90
3.1	0.7	12.1	1.00	17.0	0.82	0.1	1.00
2.4	0.5	3.0	1.00	17.1	0.83	16.2	1.00
3.9	0.8	0.0	0.99	16.9	0.83	29.4	1.00
5.0	1.1	0.0	0.92	17.6	0.83	3.4	0.99
6.1	1.3	0.0	0.79	17.9	0.84	0.0	0.96
4.3	0.9	0.2	0.71	19.0	0.84	0.0	0.95
4.2	0.9	0.2	0.63	19.7	0.84	0.0	0.91
4.7	1.0	1.6	0.71	19.6	0.84	0.4	0.84
2.4	0.5	0.0	0.64	20.2	0.85	0.5	0.76
5.1	1.1	0.2	0.52	19.8	0.85	3.2	0.85
3.5	0.7	12.8	1.00	20.3	0.85	0.8	0.78
4.5	0.9	0.0	0.99	19.8	0.85	0.2	0.69
5.2	1.1	0.0	0.90	19.0	0.85	14.6	0.62
2.7	0.6	2.4	1.00	19.7	0.86	0.0	0.54
2.7	0.6	15.2	1.00	20.0	0.86	0.0	1.00
4.3	0.9	0.0	0.99	19.7	0.86	0.0	1.00
2.4	0.5	3.8	1.00	19.5	0.87	11.9	0.97
3.3	0.7	11.3	1.00	18.8	0.87	6.2	0.94
5.5	1.2	0.0	0.97	17.5	0.87	1.6	1.00
5.6	1.2	0.0	0.88	17.6	0.87	4.2	1.00
4.0	0.8	0.0	0.79	18.7	0.87	2.9	0.99
2.2	0.5	1.4	0.88	18.8	0.88	0.0	0.94
2.1	0.4	1.2	0.95	18.3	0.88	0.0	1.00
6.1	1.3	0.0	0.84	17.2	0.88	0.9	1.00
5.9	1.2	0.0	0.70	17.5	0.88	7.4	1.00
3.6	0.8	0.0	0.60	18.1	0.88	0.0	0.99
5.8	1.2	0.0	0.42	18.8	0.89	0.0	1.00
2.1	0.4	0.9	0.49	19.8	0.89	0.0	0.99
5.8	1.2	0.0	0.29	18.8	0.89	0.6	0.96
4.1	0.9	0.0	0.13	19.9	0.89	5.7	0.87
3.5	0.7	0.3	0.05	19.8	0.89	0.0	0.78
2.7	0.6	0.0	0.00	19.4	0.89	0.1	0.67
4.4	0.9	0.0	0.00	18.4	0.90	0.0	0.52

Appendix 8.3a continued

5.2	1.1	0.3	0.00	19.2	0.90	2.1	0.41
2.1	0.4	0.2	0.00	19.7	0.90	0.0	0.27
4.7	1.0	0.0	0.00	19.5	0.90	0.1	0.22
2.4	0.5	0.0	0.00	20.7	0.90	0.0	0.10
4.8	1.0	0.0	0.00	19.8	0.91	0.0	0.94
4.6	1.0	0.0	0.00	20.3	0.91	0.0	0.88
4.1	0.9	0.0	0.00	20.8	0.91	0.1	0.88
2.9	0.6	1.5	0.00	20.6	0.91	0.1	0.83
2.6	0.5	2.0	0.12	20.4	0.91	0.0	0.90
4.3	0.9	0.0	0.00	19.2	0.91	0.0	0.81
4.2	0.9	0.0	0.00	19.0	0.91	6.1	1.00
4.1	0.9	0.0	0.00	19.3	0.91	0.0	1.00
3.9	0.8	0.0	0.00	19.6	0.91	0.1	1.00
3.0	0.6	0.0	0.00	20.4	0.92	0.0	1.00
3.7	0.8	0.0	0.00	21.0	0.92	0.0	1.00
3.6	0.8	0.0	0.00	21.5	0.92	0.1	1.00
2.9	0.6	0.0	0.00	21.6	0.92	0.0	0.99
3.4	0.7	0.0	0.00	22.0	0.92	0.0	1.00
3.3	0.7	0.0	0.00	21.2	0.92	0.0	1.00
3.2	0.7	0.0	0.00	21.8	0.92	0.0	1.00
3.1	0.7	0.0	0.00	22.0	0.92	0.0	0.94
3.0	0.6	0.0	0.00	21.6	0.92	0.0	0.84
2.9	0.6	0.0	0.00	21.4	0.92	0.0	0.73
2.9	0.6	0.0	0.00	20.7	0.92	0.0	0.76
2.0	0.4	6.0	0.64	22.0	0.92	0.0	1.00
2.5	0.5	12.6	1.00	20.0	0.92	0.0	1.00
3.2	0.7	9.5	1.00	17.8	0.92	19.6	0.97
2.5	0.5	19.7	1.00	19.4	0.93	4.2	0.90
1.9	0.4	8.1	1.00	19.3	0.93	25.5	1.00
4.1	0.9	0.0	0.99	19.0	0.93	1.3	1.00
4.0	0.8	0.2	0.95	19.8	0.93	22.1	1.00
2.6	0.5	27.0	1.00	20.7	0.93	0.0	1.00
4.6	1.0	14.0	1.00	21.2	0.93	2.2	1.00
4.1	0.9	7.0	1.00	21.8	0.93	0.7	1.00
3.3	0.7	5.9	1.00	21.8	0.93	29.8	1.00
3.7	0.8	7.6	1.00	21.5	0.93	6.2	1.00
3.6	0.8	1.5	1.00	21.3	0.93	0.0	0.96
2.0	0.4	3.0	1.00	21.7	0.93	5.2	1.00
1.7	0.4	3.1	1.00	20.4	0.93	7.6	1.00
3.9	0.8	0.0	0.99	19.1	0.93	4.2	1.00
2.8	0.6	7.1	1.00	19.5	0.93	6.1	1.00
2.4	0.5	10.5	1.00	19.2	0.93	0.1	1.00
3.6	0.8	1.0	1.00	17.9	0.92	9.9	0.94
2.4	0.5	0.0	1.00	18.1	0.92	11.0	1.00
4.1	0.9	3.6	1.00	18.1	0.92	0.9	1.00
3.0	0.6	1.7	1.00	18.0	0.92	0.2	1.00
3.8	0.8	0.0	0.99	17.1	0.92	0.0	0.95
2.9	0.6	0.0	0.95	17.3	0.92	6.0	0.94
2.3	0.5	0.2	0.93	17.6	0.92	0.3	1.00
2.2	0.5	0.6	0.94	18.4	0.92	0.0	1.00
3.5	0.7	0.0	0.88	17.5	0.92	0.0	1.00

Appendix 8.3a continued

4.6	1.0	0.0	0.78	17.6	0.92	1.1	0.96
4.7	1.0	0.0	0.67	19.6	0.92	0.0	0.93
4.5	0.9	0.0	0.54	19.5	0.92	0.0	0.85
1.8	0.4	0.0	0.48	20.4	0.92	0.0	0.73
1.5	0.3	1.0	0.58	19.5	0.92	0.0	0.59
1.4	0.3	0.2	0.57	19.1	0.92	0.0	0.45
1.3	0.3	0.4	0.58	17.6	0.91	3.1	0.80
1.5	0.3	0.5	0.61	17.3	0.91	3.7	0.73
2.2	0.5	0.8	0.66	18.3	0.91	0.0	0.67
3.2	0.7	0.3	0.61	18.7	0.91	8.4	0.56
1.4	0.3	0.0	0.56	19.4	0.91	7.5	0.42
1.4	0.3	7.7	1.00	18.5	0.91	0.2	0.94
3.5	0.7	0.0	1.00	17.6	0.91	2.4	0.91
2.6	0.5	3.3	1.00	17.8	0.91	7.9	1.00
2.2	0.5	3.8	1.00	17.3	0.90	0.0	1.00
1.8	0.4	3.4	1.00	16.7	0.90	2.6	1.00
1.1	0.2	5.2	1.00	15.6	0.90	8.0	1.00
2.2	0.5	0.0	1.00	15.8	0.90	9.2	1.00
1.6	0.3	0.0	0.99	14.9	0.90	0.4	1.00
3.7	0.8	0.0	0.94	15.0	0.89	3.7	1.00
1.9	0.4	0.0	0.91	15.4	0.89	0.0	1.00
2.7	0.6	0.0	0.86	15.7	0.89	0.0	1.00
2.3	0.5	0.0	0.81	16.3	0.89	0.0	1.00
2.4	0.5	0.0	0.75	16.9	0.89	0.1	0.95
1.4	0.3	0.0	0.72	16.8	0.89	0.0	1.00
2.3	0.5	0.0	0.66	15.6	0.88	0.0	1.00
1.1	0.2	0.0	0.63	16.1	0.88	0.2	1.00
1.4	0.3	0.4	0.64	15.8	0.88	0.1	1.00
3.0	0.6	0.0	0.55	16.0	0.88	0.1	1.00
0.8	0.2	0.0	0.53	15.9	0.88	3.0	1.00
1.1	0.2	0.0	0.50	15.0	0.87	3.2	0.99
1.5	0.3	0.0	0.45	15.0	0.87	0.0	0.90
2.4	0.5	0.0	0.37	15.5	0.87	0.0	0.82
2.5	0.5	0.0	0.28	16.1	0.87	0.0	0.71
2.5	0.5	0.0	0.18	15.8	0.87	0.1	0.93
1.5	0.3	0.0	0.12	16.5	0.86	0.1	0.98
3.0	0.6	0.0	0.00	16.6	0.86	0.0	1.00
2.2	0.5	0.0	0.00	16.5	0.86	0.0	1.00
0.8	0.2	44.7	1.00	16.7	0.86	0.0	0.99
1.5	0.3	0.0	1.00	15.7	0.85	0.0	0.92
1.5	0.3	0.0	1.00	14.0	0.85	51.1	0.82
0.7	0.1	0.0	1.00	13.8	0.85	0.0	1.00
0.9	0.2	0.0	0.98	13.8	0.85	0.0	1.00
0.9	0.2	1.4	1.00	14.4	0.84	0.0	1.00
1.9	0.4	0.0	1.00	15.2	0.84	0.0	0.98
2.4	0.5	0.0	0.99	15.3	0.84	0.8	1.00
2.4	0.5	0.0	0.95	15.6	0.84	0.1	1.00
1.7	0.4	0.0	0.92	15.0	0.83	8.6	1.00
0.6	0.1	0.0	0.91	13.8	0.83	0.0	0.99
0.8	0.2	0.1	0.91	14.0	0.83	0.0	0.97

Appendix 8.3a continued

1.0	0.2	0.0	0.89	14.4	0.82	0.0	1.00
2.2	0.5	0.0	0.85	13.9	0.82	0.3	0.99
2.2	0.5	0.0	0.80	13.3	0.82	0.0	0.97
2.4	0.5	0.0	0.74	13.7	0.82	0.0	0.92
2.3	0.5	0.0	0.69	13.7	0.81	0.1	0.87
2.2	0.5	0.0	0.63	13.5	0.81	0.0	0.78
2.2	0.5	0.0	0.56	13.3	0.81	0.0	0.67
2.0	0.4	0.0	0.50	13.2	0.81	0.0	0.58
0.5	0.1	0.0	0.48	13.5	0.80	0.0	0.49
1.5	0.3	0.0	0.44	12.6	0.80	0.0	0.38
1.5	0.3	0.0	0.39	12.0	0.80	0.7	0.22
1.8	0.4	0.0	0.32	11.4	0.79	0.1	0.51
0.4	0.1	0.0	0.31	11.3	0.79	0.0	0.86
0.4	0.1	0.1	0.31	12.0	0.79	0.1	0.82
0.8	0.2	0.0	0.28	12.6	0.79	0.0	1.00
0.7	0.1	0.0	0.25	13.2	0.78	0.1	1.00
1.1	0.2	0.0	0.21	13.4	0.78	0.0	1.00
0.4	0.1	15.7	1.00	12.4	0.78	0.0	1.00
0.4	0.1	6.6	1.00	13.4	0.77	0.0	1.00
0.4	0.1	4.5	1.00	14.3	0.77	5.5	1.00
1.5	0.3	9.2	1.00	14.5	0.77	14.9	1.00
0.6	0.1	9.0	1.00	14.3	0.77	23.7	1.00
1.6	0.3	0.0	1.00	13.1	0.76	6.4	1.00
0.8	0.2	0.2	1.00	11.9	0.76	10.3	1.00
0.5	0.1	0.8	1.00	12.4	0.76	0.4	1.00
0.9	0.2	0.0	1.00	13.0	0.75	0.1	0.97
0.6	0.1	11.8	1.00	11.9	0.75	2.6	1.00
0.3	0.1	0.2	1.00	12.0	0.75	0.0	1.00
0.3	0.1	11.4	1.00	12.5	0.74	0.0	1.00
0.3	0.1	30.9	1.00	13.6	0.74	14.5	1.00
0.3	0.1	0.3	1.00	13.4	0.74	12.5	1.00
0.3	0.1	0.5	1.00	12.6	0.74	28.2	0.97
0.9	0.2	8.4	1.00	12.0	0.73	0.3	1.00
1.3	0.3	0.0	1.00	11.8	0.73	2.0	1.00
1.3	0.3	0.0	1.00	10.2	0.73	9.3	0.99
1.4	0.3	0.0	0.99	9.7	0.72	0.1	0.97
1.4	0.3	0.0	0.97	9.8	0.72	0.0	0.93
1.0	0.2	0.7	1.00	10.8	0.72	0.1	0.99

Appendix 8.3b The daily evapotranspiration, rainfall, soil temperature at 30 cm depth at Invermay, South Island.

Daily ET	ET top 2 cm (mm)	Rain fall	Moist. Scalar	Temp.at 30 cm °C	Temp. Scalar	Moist. x Temp. Scalar
1.5	0.3	2.5	1.00	8.0	0.60	0.60
0.8	0.2	10.1	1.00	8.1	0.60	0.60
2.0	0.4	0.2	1.00	9.0	0.62	0.62
2.1	0.4	0.0	1.00	9.2	0.63	0.63
1.3	0.3	4.3	1.00	9.8	0.64	0.64
1.9	0.4	0.0	1.00	8.6	0.61	0.61
0.9	0.2	0.0	1.00	8.5	0.61	0.58
0.9	0.2	5.7	1.00	8.8	0.62	0.55
0.8	0.2	0.8	1.00	8.0	0.60	0.60
0.8	0.2	2.4	1.00	10.0	0.65	0.65
1.1	0.2	2.6	1.00	10.5	0.66	0.66
2.0	0.4	0.0	1.00	10.0	0.65	0.64
1.6	0.3	6.0	1.00	10.0	0.65	0.62
1.8	0.4	0.0	1.00	9.4	0.63	0.60
1.5	0.3	0.0	1.00	9.3	0.63	0.57
1.3	0.3	0.2	1.00	9.2	0.63	0.53
1.4	0.3	0.1	0.98	9.3	0.63	0.48
2.1	0.4	0.0	0.95	10.3	0.66	0.56
2.1	0.4	0.0	0.92	10.2	0.65	0.51
1.1	0.2	11.4	1.00	10.7	0.67	0.46
1.5	0.3	9.3	1.00	11.1	0.68	0.42
2.4	0.5	0.0	1.00	11.4	0.69	0.37
1.6	0.3	1.0	1.00	12.0	0.70	0.70
2.3	0.5	0.4	1.00	11.4	0.69	0.69
1.0	0.2	7.4	1.00	11.4	0.69	0.67
1.2	0.3	6.5	1.00	12.4	0.71	0.67
1.4	0.3	0.9	1.00	12.0	0.70	0.70
1.3	0.3	0.9	1.00	12.6	0.72	0.72
1.9	0.4	0.0	1.00	12.6	0.72	0.71
2.1	0.4	5.4	1.00	12.8	0.72	0.68
3.0	0.6	0.2	1.00	13.2	0.74	0.74
2.0	0.4	4.7	1.00	13.2	0.74	0.74
1.8	0.4	4.6	1.00	12.3	0.71	0.71
3.1	0.7	0.0	1.00	12.2	0.71	0.70
3.4	0.7	0.0	0.96	11.5	0.69	0.69
3.1	0.7	0.0	0.90	12.0	0.70	0.70
2.0	0.4	7.5	1.00	12.6	0.72	0.69
2.7	0.6	1.1	1.00	13.2	0.74	0.64
1.4	0.3	0.0	1.00	13.7	0.75	0.58
2.4	0.5	0.0	0.99	13.6	0.75	0.50
2.2	0.5	0.0	0.96	13.8	0.75	0.39
2.5	0.5	0.4	0.95	14.2	0.76	0.31

Appendix 8.3b continued

2.9	0.6	0.0	0.91	14.4	0.77	0.21
3.5	0.7	0.0	0.84	14.8	0.78	0.17
3.6	0.8	0.0	0.76	14.5	0.77	0.08
2.2	0.5	1.3	0.85	14.0	0.76	0.71
3.1	0.7	0.0	0.78	13.8	0.75	0.66
3.5	0.7	0.0	0.69	13.0	0.73	0.64
2.6	0.5	0.0	0.62	13.5	0.74	0.62
2.7	0.6	0.0	0.54	13.8	0.75	0.68
1.7	0.4	14.0	1.00	13.8	0.75	0.61
2.2	0.5	0.2	1.00	13.0	0.73	0.73
4.1	0.9	0.0	0.97	13.0	0.73	0.73
2.3	0.5	0.0	0.94	13.7	0.75	0.75
2.8	0.6	3.5	1.00	12.2	0.71	0.71
3.3	0.7	9.2	1.00	12.8	0.72	0.72
4.1	0.9	0.0	0.99	13.3	0.74	0.74
4.2	0.9	0.2	0.94	13.1	0.73	0.73
2.1	0.4	1.6	1.00	13.0	0.73	0.73
1.7	0.4	17.3	1.00	13.0	0.73	0.73
2.1	0.4	0.5	1.00	13.6	0.75	0.75
3.9	0.8	0.0	0.99	13.7	0.75	0.70
2.6	0.5	4.4	1.00	14.2	0.76	0.64
3.8	0.8	0.0	0.99	15.1	0.79	0.57
3.9	0.8	0.3	0.96	16.4	0.82	0.62
5.3	1.1	0.0	0.87	14.5	0.77	0.77
4.2	0.9	0.0	0.78	13.9	0.75	0.75
4.2	0.9	0.0	0.67	14.6	0.77	0.75
5.1	1.1	0.0	0.52	15.3	0.79	0.71
3.4	0.7	0.0	0.41	16.3	0.82	0.82
4.0	0.8	0.0	0.27	16.2	0.82	0.82
4.3	0.9	0.6	0.22	15.5	0.80	0.80
3.4	0.7	0.1	0.10	14.0	0.76	0.76
3.5	0.7	6.9	0.94	12.4	0.71	0.71
3.3	0.7	0.0	0.88	13.4	0.74	0.74
3.6	0.8	0.7	0.88	13.6	0.75	0.75
2.1	0.4	0.0	0.83	14.2	0.76	0.76
2.1	0.4	1.1	0.90	13.7	0.75	0.72
4.5	0.9	0.0	0.81	14.3	0.77	0.77
3.4	0.7	21.0	1.00	14.3	0.77	0.77
2.7	0.6	0.2	1.00	14.6	0.77	0.77
2.2	0.5	0.1	1.00	14.8	0.78	0.78
2.6	0.5	0.5	1.00	14.9	0.78	0.78
2.0	0.4	1.1	1.00	14.2	0.76	0.72
2.3	0.5	0.1	1.00	15.9	0.81	0.81
2.2	0.5	0.0	0.99	15.7	0.80	0.80
4.3	0.9	2.8	1.00	14.7	0.78	0.78
2.0	0.4	0.0	1.00	14.1	0.76	0.72
2.2	0.5	0.1	1.00	14.5	0.77	0.72
3.8	0.8	0.0	0.94	15.0	0.78	0.78
5.2	1.1	0.0	0.84	13.5	0.74	0.74
5.0	1.1	0.0	0.73	13.3	0.74	0.74

Appendix 8.3b continued

2.5	0.5	0.8	0.76	13.7	0.75	0.72
3.0	0.6	23.5	1.00	14.6	0.77	0.72
3.3	0.7	0.0	1.00	14.5	0.77	0.65
3.3	0.7	0.2	0.97	14.7	0.78	0.57
4.2	0.9	0.0	0.90	15.7	0.80	0.47
3.3	0.7	5.5	1.00	16.7	0.83	0.37
2.3	0.5	21.7	1.00	17.7	0.86	0.69
1.9	0.4	14.0	1.00	15.7	0.80	0.59
1.9	0.4	6.2	1.00	14.8	0.78	0.52
1.9	0.4	0.3	1.00	15.4	0.79	0.45
1.9	0.4	0.0	1.00	16.5	0.82	0.35
2.3	0.5	0.3	1.00	17.8	0.86	0.81
3.1	0.7	0.8	1.00	16.7	0.83	0.76
3.5	0.7	0.0	0.96	16.4	0.82	0.82
3.2	0.7	2.9	1.00	15.7	0.80	0.80
2.3	0.5	0.0	1.00	14.7	0.78	0.78
4.7	1.0	0.9	1.00	14.2	0.76	0.76
2.2	0.5	1.1	1.00	13.8	0.75	0.75
3.5	0.7	0.0	1.00	14.0	0.76	0.76
4.8	1.0	0.2	0.94	14.3	0.77	0.77
4.9	1.0	4.3	1.00	14.6	0.77	0.77
4.6	1.0	1.4	1.00	14.5	0.77	0.77
3.8	0.8	0.1	1.00	14.4	0.77	0.77
3.8	0.8	0.0	0.95	15.2	0.79	0.75
2.8	0.6	0.5	0.94	16.4	0.82	0.82
3.2	0.7	26.4	1.00	15.0	0.78	0.78
2.4	0.5	0.8	1.00	14.4	0.77	0.77
3.5	0.7	0.1	1.00	15.3	0.79	0.79
3.0	0.6	0.0	0.96	16.0	0.81	0.81
2.0	0.4	0.0	0.93	14.7	0.78	0.78
4.5	0.9	0.0	0.85	14.7	0.78	0.77
5.3	1.1	0.0	0.73	15.3	0.79	0.71
5.1	1.1	0.0	0.59	16.3	0.82	0.67
5.0	1.1	0.1	0.45	17.1	0.84	0.60
2.0	0.4	3.1	0.80	18.1	0.87	0.81
3.0	0.6	0.0	0.73	17.8	0.86	0.84
2.8	0.6	0.1	0.67	17.3	0.85	0.85
3.8	0.8	0.0	0.56	16.2	0.82	0.82
4.6	1.0	0.0	0.42	15.3	0.79	0.78
3.2	0.7	5.1	0.94	15.3	0.79	0.73
2.7	0.6	0.2	0.91	15.6	0.80	0.66
1.9	0.4	12.6	1.00	16.6	0.83	0.83
2.0	0.4	7.0	1.00	17.2	0.84	0.84
2.2	0.5	3.1	1.00	16.4	0.82	0.82
1.8	0.4	0.8	1.00	16.1	0.81	0.80
2.9	0.6	0.0	1.00	16.7	0.83	0.83
2.6	0.5	1.4	1.00	18.0	0.87	0.87
2.7	0.6	4.8	1.00	17.0	0.84	0.84
2.0	0.4	9.9	1.00	15.5	0.80	0.79
2.9	0.6	16.0	1.00	16.2	0.82	0.79

Appendix 8.3b continued

2.9	0.6	0.2	1.00	16.7	0.83	0.83
5.1	1.1	0.0	0.95	15.2	0.79	0.78
2.8	0.6	17.2	1.00	14.0	0.76	0.73
3.2	0.7	1.4	1.00	14.9	0.78	0.72
3.9	0.8	1.0	1.00	15.1	0.79	0.68
2.6	0.5	6.9	1.00	15.6	0.80	0.62
2.3	0.5	23.3	1.00	16.4	0.82	0.55
2.7	0.6	0.5	1.00	17.0	0.84	0.49
4.3	0.9	0.1	0.99	17.0	0.84	0.41
5.4	1.1	0.0	0.90	16.6	0.83	0.31
4.4	0.9	0.0	0.82	17.0	0.84	0.18
4.7	1.0	0.0	0.71	17.5	0.85	0.43
2.5	0.5	2.8	0.93	16.5	0.82	0.71
2.5	0.5	1.2	0.98	16.2	0.82	0.67
1.8	0.4	4.5	1.00	17.0	0.84	0.84
1.7	0.4	0.0	1.00	16.8	0.83	0.83
2.3	0.5	0.0	0.99	17.3	0.85	0.85
4.4	0.9	0.0	0.92	17.3	0.85	0.85
5.5	1.2	0.0	0.82	16.1	0.81	0.81
4.9	1.0	6.8	1.00	15.5	0.80	0.80
1.9	0.4	14.9	1.00	15.1	0.79	0.79
1.6	0.3	0.0	1.00	15.2	0.79	0.79
3.6	0.8	0.0	0.98	15.1	0.79	0.79
5.1	1.1	2.2	1.00	15.0	0.78	0.78
2.5	0.5	5.5	1.00	15.0	0.78	0.78
2.0	0.4	0.1	1.00	15.3	0.79	0.77
2.8	0.6	0.0	0.99	15.8	0.81	0.81
4.1	0.9	0.6	0.97	16.1	0.81	0.81
2.2	0.5	2.7	1.00	15.4	0.79	0.79
3.8	0.8	0.0	0.99	14.5	0.77	0.77
2.6	0.5	0.2	0.97	13.6	0.75	0.75
3.3	0.7	0.0	0.92	13.7	0.75	0.73
2.7	0.6	0.0	0.87	14.1	0.76	0.76
4.1	0.9	0.0	0.78	14.3	0.77	0.77
4.4	0.9	0.0	0.67	13.7	0.75	0.74
3.2	0.7	0.0	0.58	13.8	0.75	0.73
2.9	0.6	0.0	0.49	13.6	0.75	0.69
4.0	0.8	0.1	0.38	13.6	0.75	0.74
4.4	0.9	0.0	0.22	14.1	0.76	0.76
2.0	0.4	2.2	0.51	14.7	0.78	0.76
1.4	0.3	3.1	0.86	15.4	0.79	0.77
1.5	0.3	0.0	0.82	15.3	0.79	0.75
1.4	0.3	2.8	1.00	15.1	0.79	0.79
2.4	0.5	0.4	1.00	15.3	0.79	0.79
2.4	0.5	1.4	1.00	14.3	0.77	0.77
1.4	0.3	34.8	1.00	13.5	0.74	0.72
1.3	0.3	4.8	1.00	13.3	0.74	0.69
2.2	0.5	1.4	1.00	13.2	0.74	0.71
1.4	0.3	16.0	1.00	13.2	0.74	0.70
2.2	0.5	22.2	1.00	13.1	0.73	0.73

Appendix 8.3b continued

1.2	0.3	0.4	1.00	13.2	0.74	0.74
1.6	0.3	0.1	1.00	11.0	0.68	0.68
1.8	0.4	0.0	1.00	11.3	0.68	0.68
2.9	0.6	0.0	0.97	10.8	0.67	0.67
2.9	0.6	7.2	1.00	11.2	0.68	0.67
1.3	0.3	9.2	1.00	11.7	0.69	0.67
1.1	0.2	26.5	1.00	11.9	0.70	0.65
1.4	0.3	0.7	1.00	12.3	0.71	0.65
3.0	0.6	0.1	1.00	12.5	0.72	0.64
3.2	0.7	0.0	0.97	12.6	0.72	0.63
2.8	0.6	1.5	1.00	12.5	0.72	0.60
0.9	0.2	0.0	1.00	12.4	0.71	0.71
1.9	0.4	0.0	0.99	12.8	0.72	0.72
1.3	0.3	0.0	0.97	12.7	0.72	0.72
2.7	0.6	0.0	0.93	11.8	0.70	0.70
3.1	0.7	1.5	0.99	11.4	0.69	0.67
2.0	0.4	0.5	1.00	11.0	0.68	0.64
1.6	0.3	0.1	0.98	11.8	0.70	0.64
1.0	0.2	0.0	0.97	10.5	0.66	0.66
1.3	0.3	0.0	0.95	11.5	0.69	0.69
1.1	0.2	8.9	1.00	11.5	0.69	0.69
1.8	0.4	0.2	1.00	11.4	0.69	0.69
1.8	0.4	0.0	1.00	10.2	0.65	0.65
2.6	0.5	0.0	0.97	10.8	0.67	0.67
2.1	0.4	0.0	0.94	8.7	0.61	0.61
1.6	0.3	0.6	0.96	8.5	0.61	0.61
1.6	0.3	0.2	0.95	9.0	0.62	0.62
2.6	0.5	1.1	0.99	9.4	0.63	0.63
1.2	0.3	2.5	1.00	9.8	0.64	0.64
1.3	0.3	0.3	1.00	9.0	0.62	0.62
1.7	0.4	0.2	1.00	9.0	0.62	0.62
2.3	0.5	0.0	1.00	9.0	0.62	0.62
1.7	0.4	0.0	0.98	9.3	0.63	0.63
1.3	0.3	0.0	0.96	9.0	0.62	0.62
2.1	0.4	0.0	0.93	8.7	0.61	0.61
0.8	0.2	0.0	0.92	8.8	0.62	0.62
1.0	0.2	0.0	0.90	9.1	0.62	0.62
0.7	0.1	0.0	0.88	9.1	0.62	0.62
2.2	0.5	0.0	0.84	8.6	0.61	0.61
1.0	0.2	4.8	1.00	8.4	0.61	0.61
0.7	0.1	0.5	1.00	8.1	0.60	0.60
2.0	0.4	0.0	1.00	8.2	0.60	0.60
1.6	0.3	0.0	1.00	8.0	0.60	0.60
2.0	0.4	0.0	0.97	7.5	0.58	0.58
1.7	0.4	0.0	0.94	6.4	0.55	0.55
1.3	0.3	0.0	0.92	6.0	0.54	0.54
0.8	0.2	3.4	1.00	6.1	0.54	0.54
1.6	0.3	3.1	1.00	7.7	0.59	0.59
0.4	0.1	0.9	1.00	8.3	0.60	0.60
0.5	0.1	1.9	1.00	8.3	0.60	0.60

Appendix 8.3b continued

0.8	0.2	10.4	1.00	7.8	0.59	0.59
0.6	0.1	8.1	1.00	7.0	0.57	0.57
0.5	0.1	5.3	1.00	7.5	0.58	0.58
0.6	0.1	0.1	1.00	7.1	0.57	0.57
0.5	0.1	0.0	1.00	7.1	0.57	0.57
0.4	0.1	15.8	1.00	7.0	0.57	0.57
1.6	0.3	0.2	1.00	6.8	0.56	0.56
1.3	0.3	0.1	1.00	6.5	0.55	0.55
0.4	0.1	0.5	1.00	7.2	0.57	0.57
0.6	0.1	3.8	1.00	7.0	0.57	0.57
0.4	0.1	0.0	1.00	6.8	0.56	0.56
1.2	0.3	0.0	1.00	6.9	0.57	0.57
0.8	0.2	0.0	1.00	7.4	0.58	0.58
0.8	0.2	0.3	1.00	7.2	0.57	0.57
0.6	0.1	1.5	1.00	7.0	0.57	0.57
0.7	0.1	0.5	1.00	6.6	0.56	0.56
1.1	0.2	0.2	1.00	6.9	0.57	0.57
1.2	0.3	0.0	1.00	6.5	0.55	0.55
0.5	0.1	3.4	1.00	5.7	0.53	0.53
0.3	0.1	1.8	1.00	5.7	0.53	0.53
1.0	0.2	0.0	1.00	6.0	0.54	0.54
0.8	0.2	0.0	1.00	7.4	0.58	0.57
0.9	0.2	0.0	1.00	6.5	0.55	0.54
0.3	0.1	0.0	1.00	6.5	0.55	0.54
0.3	0.1	14.0	1.00	6.0	0.54	0.52
0.3	0.1	5.8	1.00	5.5	0.53	0.50
0.2	0.0	0.7	1.00	5.7	0.53	0.51
0.4	0.1	1.7	1.00	4.9	0.51	0.48
0.5	0.1	2.0	1.00	4.2	0.49	0.47
0.4	0.1	4.5	1.00	4.8	0.51	0.47
0.3	0.1	0.7	1.00	5.0	0.51	0.48
0.4	0.1	0.2	1.00	5.2	0.52	0.48
0.8	0.2	0.0	1.00	5.0	0.51	0.47
0.2	0.0	0.0	1.00	6.5	0.55	0.55
0.2	0.0	0.6	1.00	8.0	0.60	0.60
0.2	0.0	11.5	1.00	8.0	0.60	0.60
0.5	0.1	0.8	1.00	8.0	0.60	0.60
1.0	0.2	0.3	1.00	8.2	0.60	0.60
0.7	0.1	0.0	1.00	8.0	0.60	0.60
0.7	0.1	0.0	1.00	8.2	0.60	0.60
0.8	0.2	0.0	1.00	8.3	0.60	0.60
0.9	0.2	0.6	1.00	8.2	0.60	0.60
0.9	0.2	0.0	1.00	8.1	0.60	0.60
0.7	0.1	0.4	1.00	8.2	0.60	0.60
0.4	0.1	0.0	1.00	8.2	0.60	0.60
0.9	0.2	0.0	1.00	8.0	0.60	0.60
1.0	0.2	0.0	1.00	8.0	0.60	0.60
0.7	0.1	0.0	1.00	8.1	0.60	0.60
0.4	0.1	0.0	1.00	8.1	0.60	0.60
0.6	0.1	0.0	0.99	8.0	0.60	0.60

Appendix 8.3b continued

0.6	0.1	0.0	0.98	8.0	0.60	0.60
0.6	0.1	0.0	0.98	8.0	0.60	0.60
0.9	0.2	0.0	0.96	8.0	0.60	0.60
0.5	0.1	0.0	0.95	7.5	0.58	0.58
0.4	0.1	0.0	0.95	7.6	0.58	0.58
0.7	0.1	0.0	0.94	7.5	0.58	0.58
0.3	0.1	0.2	0.95	7.5	0.58	0.58
0.8	0.2	0.0	0.93	7.5	0.58	0.58
0.3	0.1	0.0	0.93	7.5	0.58	0.58
0.8	0.2	0.0	0.92	7.5	0.58	0.58
0.5	0.1	0.0	0.91	7.5	0.58	0.58
0.2	0.0	23.8	1.00	7.4	0.58	0.58
0.2	0.0	13.0	1.00	7.4	0.58	0.58
0.2	0.0	1.4	1.00	7.5	0.58	0.58
0.4	0.1	0.1	1.00	7.5	0.58	0.58
0.8	0.2	0.1	1.00	7.1	0.57	0.57
0.2	0.0	0.1	1.00	7.0	0.57	0.57
0.2	0.0	5.5	1.00	6.5	0.55	0.55
0.2	0.0	0.0	1.00	6.4	0.55	0.55
0.3	0.1	0.1	1.00	5.4	0.52	0.52
0.3	0.1	0.1	1.00	5.0	0.51	0.51
0.9	0.2	0.1	1.00	4.6	0.50	0.50
0.9	0.2	0.9	1.00	5.0	0.51	0.51
0.3	0.1	3.8	1.00	4.7	0.51	0.51
0.5	0.1	8.6	1.00	4.5	0.50	0.50
0.3	0.1	5.3	1.00	4.8	0.51	0.50
0.2	0.0	8.5	1.00	5.1	0.52	0.51
0.2	0.0	1.2	1.00	5.5	0.53	0.52
0.2	0.0	0.2	1.00	7.0	0.57	0.57
1.0	0.2	0.1	1.00	7.0	0.57	0.57
1.0	0.2	0.0	1.00	7.5	0.58	0.58
0.3	0.1	6.9	1.00	7.8	0.59	0.59
0.2	0.0	3.0	1.00	8.2	0.60	0.59
0.2	0.0	3.8	1.00	7.9	0.59	0.57
0.7	0.1	0.1	1.00	8.0	0.60	0.60
0.4	0.1	0.1	1.00	7.5	0.58	0.58
1.1	0.2	0.0	1.00	7.5	0.58	0.58
0.9	0.2	0.0	1.00	8.0	0.60	0.60
1.0	0.2	0.0	1.00	6.5	0.55	0.55
0.4	0.1	0.0	1.00	6.5	0.55	0.55
0.3	0.1	5.9	1.00	6.5	0.55	0.55
0.7	0.1	0.2	1.00	7.0	0.57	0.56
0.4	0.1	0.0	1.00	7.0	0.57	0.55
0.9	0.2	4.2	1.00	7.5	0.58	0.56
0.4	0.1	2.5	1.00	7.5	0.58	0.58
1.0	0.2	6.8	1.00	8.0	0.60	0.60
1.2	0.3	5.2	1.00	7.9	0.59	0.59
0.4	0.1	0.0	1.00	8.0	0.60	0.60
0.8	0.2	0.0	1.00	8.5	0.61	0.60
0.6	0.1	0.6	1.00	8.0	0.60	0.56

Appendix 8.3b continued

1.0	0.2	0.0	1.00	8.0	0.60	0.60
0.8	0.2	0.0	1.00	8.0	0.60	0.60
0.9	0.2	0.0	1.00	8.5	0.61	0.61
1.5	0.3	0.0	0.99	8.5	0.61	0.60
0.6	0.1	0.0	0.98	7.9	0.59	0.57
0.6	0.1	0.2	0.99	8.5	0.61	0.57
0.5	0.1	2.5	1.00	8.5	0.61	0.54
0.5	0.1	0.2	1.00	9.0	0.62	0.62
0.5	0.1	0.0	1.00	9.0	0.62	0.62
1.4	0.3	0.0	1.00	8.5	0.61	0.61
2.0	0.4	0.0	0.99	8.0	0.60	0.60
1.9	0.4	0.0	0.97	8.9	0.62	0.62
1.5	0.3	1.8	1.00	8.5	0.61	0.60
1.4	0.3	0.7	1.00	8.0	0.60	0.57
1.5	0.3	8.3	1.00	8.0	0.60	0.54
1.7	0.4	0.6	1.00	8.0	0.60	0.50
0.8	0.2	0.1	1.00	8.0	0.60	0.46
1.1	0.2	0.0	1.00	8.2	0.60	0.41
1.2	0.3	0.0	1.00	8.5	0.61	0.37
1.3	0.3	0.0	0.99	8.9	0.62	0.33
1.3	0.3	0.0	0.97	9.5	0.64	0.64
0.6	0.1	0.0	0.97	8.5	0.61	0.61
0.9	0.2	1.7	1.00	8.5	0.61	0.59
0.8	0.2	2.4	1.00	8.0	0.60	0.56
2.1	0.4	0.0	1.00	8.8	0.62	0.62
0.9	0.2	0.0	1.00	7.8	0.59	0.59