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FOREWORD

Sulfur (S) is an essential element in plant, human and animal nutrition. This publication provides an overview of the sulfur cycle and how isotopic tracers can be used to obtain unique and precise information on the fluxes and movement of S between and within different compartments of the soil–plant system. It provides information on the theory and measurement of ^{35}S and ^{34}S, safety procedures using ^{35}S, preparation of labelled S fertilizers and plant residues, estimation of the quantity of isotope required for different temporal and spatial scales, preparation (digestion/extraction) and analysis of soil and plant samples and guidelines on comparative advantages with non-nuclear techniques and applications, including case studies and references. It is a timely publication in that greater attention is now being focused on S deficiencies in agriculture, which are becoming more common due to reduced atmospheric accretions from industrial sources and to the reduced use of fertilizer materials that contain sulfur as a secondary nutrient.

The sub-programme in Soil and Water Management and Crop Nutrition is committed to the dissemination of information to Member States on the practical applications of nuclear techniques through the IAEA Training Course Series. The present publication is preceded by IAEA-TCS-14 (2001), Use of Isotope and Radiation Methods in Soil and Water Management and Crop Nutrition, and IAEA-TCS-16 (2002), Neutron and Gamma Probes: Their Use in Agronomy.

This publication was authored by two eminent scientists in the field of S cycling in the soil–plant–animal system — G. Blair and R. Till, Division of Agronomy and Soil Science, University of New England, Armidale, NSW, Australia. It brings together the accumulated experiences and specialized knowledge gained during their professional careers on the application of nuclear techniques to study the dynamics of sulfur in crop–pasture–animal systems. This publication will ensure that up-to-date practical information on the application of both radioactive (^{35}S) and stable (^{34}S) isotopic tracers is retained and disseminated. The guidelines provide a valuable educational resource for students, technicians, fellowship trainees and scientists in national agricultural and environmental research institutes. The IAEA officers responsible for this publication are P. Chalk of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and G. Hardarson of the Agency's Laboratories, Seibersdorf.

EDITORIAL NOTE

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CHAPTER 1

THE SULFUR CYCLE AND THE ADVANTAGES OF USING S ISOTOPES IN SOIL–PLANT STUDIES

1.1 INTRODUCTION

Sulfur is one of the essential elements required for the normal growth of plants (Tisdale et al. 1985; Blair, 1979). Although plant concentrations of S are lower than that of N and P, it plays an important role as a constituent of many plant processes (Tisdale et al., 1985; Blair, 1979; Morris, 1987) such as:

i) Synthesis of proteins and the essential S containing amino acids, cysteine, cystine and methionine;

ii) synthesis of coenzyme A as well as biotin, thiamine and glutathione;

iii) synthesis of chlorophyll;

iv) synthesis of volatile oils found in members of the families Cruciferae and Liliaceae; and

v) fixation of nitrogen by leguminous plants.

Spencer (1975) classified crops into three groups according to their sulfur requirement:

i) crops with high S requirement (rapeseed, lucerne and cruciferous forages);

ii) moderate S requirement (coconut, sugarcane, clover and grasses, coffee and cotton); and

iii) low S requirement (sugar beet, cereal forages, cereal grains and peanut).

For many years, little attention was paid to sulfur as a plant nutrient mainly because it has been applied to soil in incidental inputs in rainfall and volcanic emissions and as a component of nitrogen, phosphorus and potassium fertilisers. Plant metabolism depends on S and a deficiency of this nutrient will cause basic metabolic impairment which will not only reduce the crop yield but also the quality of produce (Duke and Reisenauer, 1986). Deficiency symptoms of S in plants includes a yellowing of the younger leaves as a result of a low chlorophyll production and S non-mobility (Yoshida and Chaudhry, 1979) and a marked reduction in plant height and tiller number in cereals (Blair et al. 1979; Islam and Ponnamaperuma, 1982).

The awareness of sulfur deficiency is increasing as is the development of S deficiency in previously S sufficient areas in many parts of the world.

There are many reasons of increasing sulfur deficiency but the most important have been listed by Blair et al. (1978) as follows:

i) The increasing use of high analysis, low S containing fertilisers;

ii) the increase in yields obtained as a result of other technological advances;

iii) the decreasing use of S containing pesticides and fungicides;

iv) environmental control of sulfur dioxide emissions in industrial areas; and

iv) a greater number of experiments conducted where S is studied as a nutrient in its own right.

In some early studies using fertilisers such as $(NH_4)_2SO_4$, K_2SO_4, $ZnSO_4$ and superphosphate responses attributed to the N, K, Zn and P may have been partially due to S, or its interactions with other nutrients, and were not detected. There are many commercially available sulfur fertilisers and most contain sulfate or elemental sulfur. Blair (1979) listed 40 S-containing products that are available and most of them have been evaluated in agricultural areas.

Intensification of cropping systems using high-yielding varieties has accelerated S removal from the soil, which will ultimately result in more soils becoming S-deficient (Mamaril and Gonzales, 1987). Increased use of high-analysis S-free fertilisers has aggravated the S deficiency problem in lowland rice (Blair and Lefroy, 1987; Mamaril and Gonzales, 1987) and in other cropping systems. As a

consequence of these changes, the efficient use of S and other nutrients in cropping systems must be given greater consideration.

Many alternative sources of S fertiliser are available (Bixby and Kilmer, 1975; Beaton et al. 1985, Tisdale et al. 1985). Fertiliser sulfate is immediately available to the crop (Friesen and Chien, 1986), but leaching losses may be significant from these fertilisers. Elemental S must undergo oxidation to plant available sulfate. Both moisture and aeration are interrelated factors that affect the rate of oxidation of elemental S. Generally, sulfur is oxidized most rapidly at a moisture content of approximately field capacity (Moser and Olsen, 1953), which is the optimum soil moisture for plant growth (Burns, 1968) so the SO_4 is largely released in synchrony with plant demand.

Sulfur cycling in soil is closely related to organic matter turnover. As such, S mineralisation and immobilization processes play a key role in determining the availability of S for plant growth. Consequently, knowledge about the stability and subsequent decomposition of organic matter in soil is indispensable for an understanding of the fate of plant nutrients added to soil in the form of fertilisers, crop residues and animal manure. The extent to which the S contained in a crop residue is accessed by a succeeding crop following incorporation into the soil is of considerable agronomic significance.

Although estimates of S cycling in flooded rice systems are available (Blair, 1986; Lefroy et al. 1988), information is scarce on the residual value of fertilisers and crop residues in many non-flooded and flooded cropping systems. To increase our understanding of the S requirements of crops in order to match the supply rate from applied fertiliser to crop demand, it is necessary to investigate S inputs and outputs from the system. The detailed understanding of the nutrient cycle will assist in estimating these fertiliser S requirements.

Because of the large size of the soil S pools relative to the amount of S added in fertilisers, rainfall, and crop residues the use of stable and radioactive isotopes of S has greatly assisted in understanding the S cycle.

1.2 THE S CYCLE

The sulfur cycling literature has been extensively reviewed; for example Till (1975), Metson (1979), Bettany and Stewart (1982) and Blair (1986).

The S cycle has similarities to both N and P cycles. The role of organic sources in supplying sulfate to plants is similar to both the N and P cycles and the adsorption reactions are similar to P reactions although the strength of sulfate adsorption is considerably less than for phosphate.

A simplified diagram of the S cycle is shown in Figure 1.1. Uptake by plants is from the "available SO_4^{2-}" pool, which also supplies SO_4^{2-} to, and receives SO_4^{2-} from, some of the other components in the soil-plant-animal cycle at a range of rates. In addition, there are various other environmental input and loss processes which can make significant differences to the sulfur balance of the whole system.

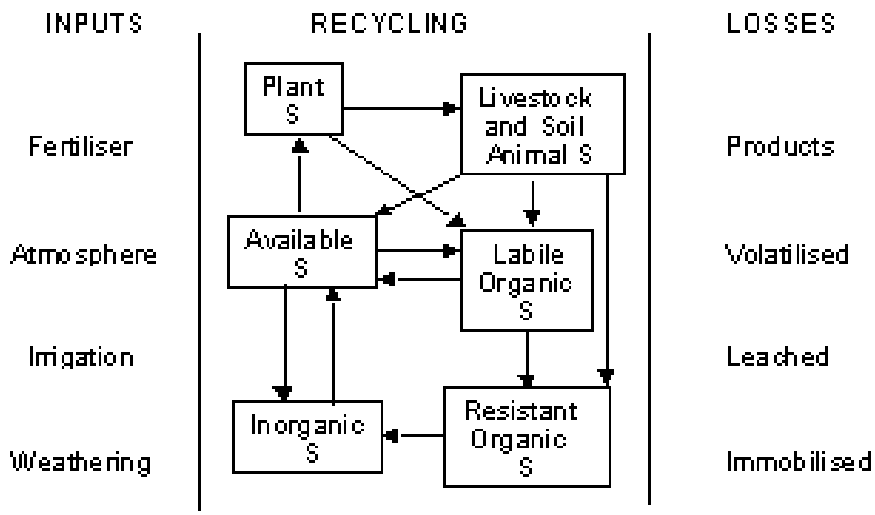

Figure 1.1. The S cycle showing pools, flow paths, inputs and outputs.

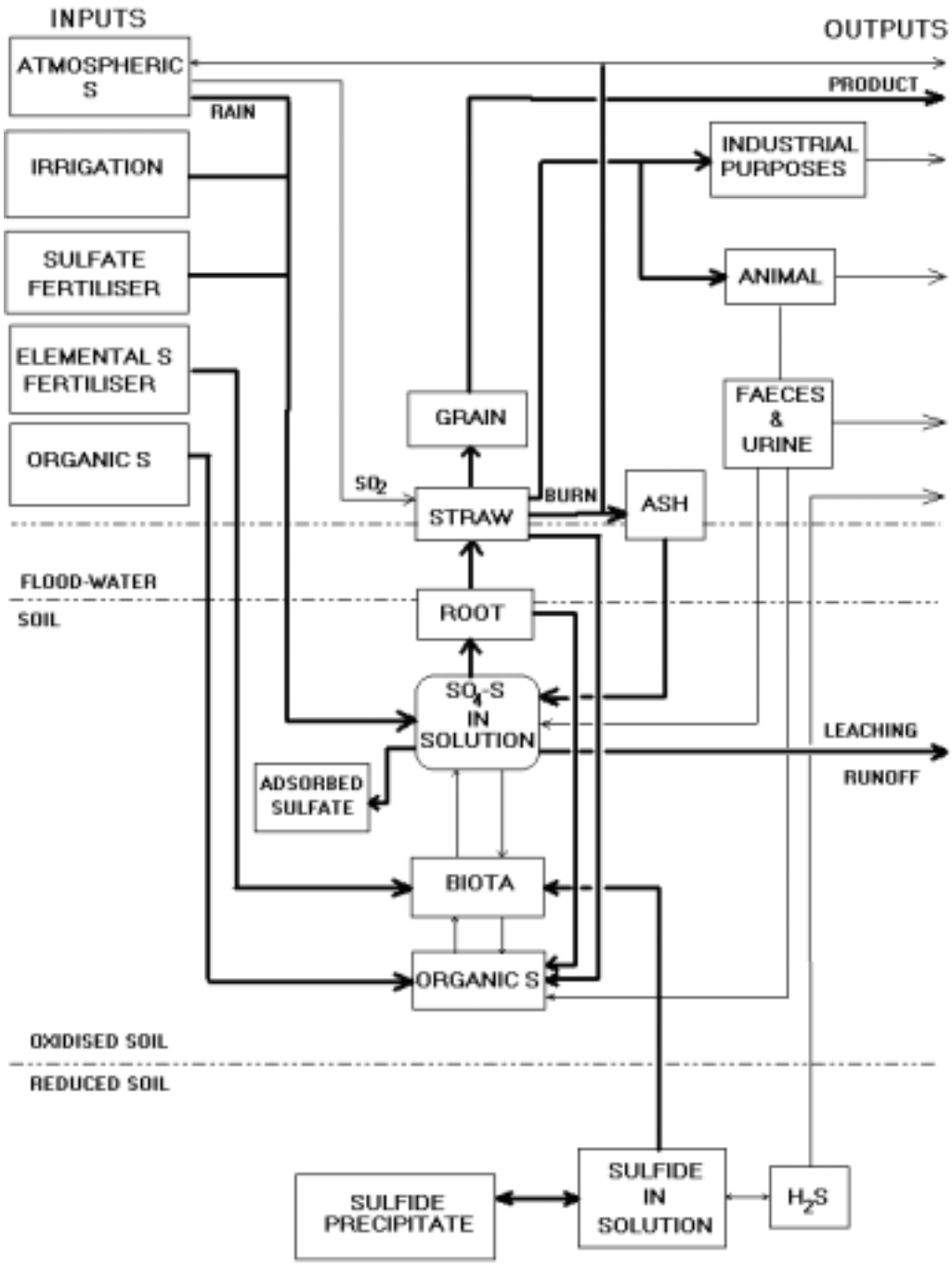

Figure 1.2. The S cycle in flooded and non-flooded soils (Chaitep, 1990).

Work on sulfur cycling related to pasture improvement in temperate conditions has been conducted by May et al. (1968) and Till and May (1970 a,b) using radiotracers. In these studies, ^{35}S applied in fertiliser could still be detected two years after the initial application, indicating a long residual effectiveness. This radiotracer work emphasized the role of organic matter as the major temporary storage pool for added fertiliser in the system and provided a basis for a simple model and simulation studies which showed the importance of process rates within the cycle (May et al. 1973).

The relationship between S inputs and outputs in the S cycle are represented in a more complex form than shown in *Figure 1.1* in *Figure 1.2* for both non-flooded and flooded soil conditions (Chaitep, 1990). Under flooded conditions, there are oxidized and reduced zones, consequently oxidation and reduction reactions can occur simultaneously in different parts of the flooded soil (Blair and Lefroy, 1987).

Sulfur can be added to the system in fertilisers, in irrigation water, through adsorption of S gases (e.g. SO_2), in additional organic matter, dry deposition, and in rainfall. Sulfate is taken up by the rice plant and converted into organic forms. Sulfur is lost from this cycle in plant and animal products, residue removal, leaching, surface water flow and gaseous emissions. When plant and animal residues are returned to the soil, microbiological conversion (mineralisation) results in the re-supply of sulfate. Estimates of S inputs and outputs in a flooded rice system have been made by Blair (1986) and Lefroy et al. (1988). These estimates suggest that in many instances much of the fertiliser S is unavailable to the crop in the short term, however little data are available on the residual value of fertilisers and crop residues in non-flooded and flooded rice cropping systems.

In many agricultural situations, crop residues are removed from the field or burnt. This can have serious long-term consequences for fertiliser management.

1.3 SOIL SULFUR

1.3.1 Organic sulfur

Data of Haque and Walmsley (1974), Neptune et al. (1975) and Zhu et al. (1984) show that in soils taken from tropical, subtropical and temperate regions, from 80 to more than 90% of total S is in organic forms, and hence unavailable to plants until mineralised. Venkateswarlu et al. (1969) reported that S in organic forms in flooded Indian rice soils varied from 14% to 84% of total S.

The distribution of organic S within a soil profile generally follows the pattern of organic matter, decreasing with depth (Probert, 1980; Freney, 1986). Exceptions may be observed in soils with buried profiles (Williams, 1974). The C:N:S ratio of agricultural soils averages approximately 130:10:1.3. Whilst individual soils may vary, the average does not vary greatly on a world-wide basis. The C:N:S ratios of virgin soils show greater variation than those for the corresponding cultivated soils. It is suggested that S in the soil is relatively more resistant to mineralisation than C and N (Probert, 1980; Freney, 1986).

Sulfur in organic fractions acts as a reserve of S. However, it must undergo mineralisation to sulfate before becoming available to plants. Since the mineralisation process is primarily microbial, factors affecting the growth of microorganisms will influence the conversion rate of organic S to plant available sulfate (Freney and Swaby, 1975). In the same way as for elemental S the released sulfate becomes available in time with plant demand, but it may also be reincorporated into organic matter, lost by leaching, or readsorbed by the soil.

Environmental conditions in the humid tropics are generally conducive to a rapid mineralisation of organic matter. Turnover rates of sulfur in these regions are therefore high (Blair, 1980). This, together with high crop removal, low S inputs and the mobility of sulfate in the soil profile are the reasons that many soils in the humid tropics contain less total sulfur than temperate soils (Freney, 1986; Blair and Lefroy, 1987).

Although organic S is the major fraction of S in most non-flooded soils, only a small part of it, as little as 10%, may easily enter the active S pool at any one time. On the other hand, organic matter may immobilize some of the sulfate added to the soil, depending on the amount of sulfate added, soil properties, and cropping and climatic conditions (Bohn et al. 1986).

Little information is available on S mineralisation in flooded soils (Freney, 1986). Villegas-Pangga et al. (2000) studied the breakdown and nutrient release from the straw of three rice varieties in a perfusion system. When perfusion was performed under N_2 gas, there were reductions, ranging from 27 to 45%, in C evolution compared to aerobic conditions, but 38% of the S was released from the straw into the perfusion solution irrespective of the conditions. These results indicate that under flooded conditions, depletion of O_2 decreases breakdown rate of straw but the rate of S release is unaffected.

Freney (1986) indicated that in the studies of the mineralisation of S in flooded soils, no attempt has been made to separate the mineralisation in the surface oxidized zone, at the soil-water interface, or in the rhizosphere, from the mineralisation in the reduced zone. Also most of the studies have been made on pre-air dried and re-wetted samples. Such treatment of soils has been found to increase mineralisation (Williams, 1967) and any sulfate produced during air-drying could be reduced to sulfide by sulfate reducers in the anaerobic environment during incubation.

Williams (1967) studied S mineralisation in a number of soils incubated under saturated conditions. During the two week incubation, only 4 μg as sulfate and 2 μg as sulfide was mineralised from 342 μg

S/g soil, indicating that S mineralisation is slow. Zhu et al. (1984) measured from 3.8 to 15.6% (mean 9.6%) of organic S mineralised in 10 weeks. These results are lower than those obtained with N_2 and perfusion Villegas-Pannga et al. (2000) and suggest that re-immobilisation may have taken place in these incubation studies, the net effect which would be only a small release of sulfate from the organic S in flooded soils.

Like other nutrients, S is needed for microbial growth. Sulfur can be released from, or transformed into, organic S, depending on the S status in soils or plant material. As long as there is less S in the organic matter than required for microbial proliferation, transformation of S into organic S (immobilization) will be dominant. When S is in excess, S will be liberated from the organic compounds. The critical C:S ratio in carbonaceous materials above which immobilization is dominant to mineralisation, is reported to be in the range 200:1 to 400:1 (Barrow, 1960; Stewart et al. 1966), or equivalent to about 0.1 to 0.2% S. Mineralisation will dominate with organic materials having lower C:S ratios or higher S concentration, but immobilization will be dominant with higher C:S ratios or lower S concentration.

As with mineralisation, little is known about the immobilization of S in flooded soils. Sachdev and Chabra (1974), using ^{35}S under aerobic conditions, found that 37.8% of the added sulfate could be recovered as organic sulfur. This was considerably greater than the incorporation under anaerobic conditions. Massoumi and Cornfield (1965) showed an increase in net sulfate S immobilization in soils treated with glucose in the first 80 days of incubation, but then a decrease with a longer period of incubation. Thus immobilization of S dominated when a readily available carbon source was present and the C:S ratio was high.

Some sulfide is produced as a result of protein decomposition (Vamos, 1964; Bloomfield, 1969), and many heterotrophic microorganisms can convert organic S to sulfide under anaerobic conditions (Kadota and Ishida, 1972).

1.3.2 Inorganic sulfur

Sulfur occurs in various oxidation states in nature, ranging from +6 in sulfate to -2 in sulfide. Sulfate is the stable species of S in aerobic conditions. In normal well-drained, well-aerated mineral soils most inorganic S is in the sulfate form, either as water-soluble, adsorbed, or insoluble sulfate (Freney and Williams, 1980; Bohn et al. 1986). Under reduced conditions sulfide is the principal stable form of S (Starkey, 1966).

Sulfate is sorbed by soils through two mechanisms. Firstly, ionic exchange of SO_4^{2-} for OH^- ions on the surfaces of Fe and Al oxides, or along the edges of kaolinite particles and positively charged radicals and secondly, through the formation of complexes with hydroxy-Al (Sanchez, 1976). The amount of adsorbed sulfate depends on soil properties and environmental conditions and not all soils adsorb sulfate readily. Many such soils are sandy and contain only small amounts of Fe and Al oxides, the colloids that react strongly with sulfate.

Adsorbed sulfate is an important S source for plants in tropical soils. The lower amounts of organic sulfur generally present in tropical soils, compared to those from temperate regions, suggest that tropical crops will be more dependent on adsorbed sulfate (Blair and Lefroy, 1987). This has been demonstrated by Neptune et al. (1975) who reported that adsorbed S constituted 58% of readily available S in a number of soils from Brazil, but only 27% in soils from Iowa, U.S.A.

The sulfate-S content in agricultural soils is variable, but non-flooded soils have often been reported to have lower extractable sulfate than flooded soils from the same regions. For example, an average available sulfur concentration of 143 μg/g for paddy soils and 64μg/g for non-flooded soils was measured by Shin (1987) in agricultural soils of Korea. Non-flooded soils from the northern region of Thailand have also been found to have lower extractable sulfate S contents than the flooded soils (Chaiwanakupt et al. 1987). The non-flooded soils contained less than 15 μg/g extractable sulfate S in the surface layer and responded to sulfur applications, whilst the flooded soils contained from 20 to 66 μg/g and were non-responsive. Similarly, extractable sulfate contents of non-flooded soils from Khon Kaen Province in the northeast of Thailand have been found to be lower than for flooded soils (Chaiwanakupt et al. 1987).

The higher sulfate content of flooded soils has often been attributed to the application of sulfur containing fertiliser to the flooded soils (Shin, 1987; Chaiwanakupt et al. 1987). Another possibility is that the higher S status of flooded soils is due to inputs from irrigation water and lower losses by leaching than in the non-flooded soils. Irrigation water can be a major source of S for flooded rice

plants (Blair et al. 1979), since it includes the S accessions from rainfall over a larger area which may become concentrated through evaporation and enriched from S inputs from fertilisers and natural sources (eg volcanic springs).

Leaching losses of S in flooded soils is generally restricted by the plow pan, however it can be a significant problem in non-flooded soils with coarse texture and low S sorption capacity.

Tropical acid soils, with their high quantities of 1:1 type clays and Fe and Al hydrous oxides, can adsorb considerable amounts of sulfate (Harward and Reisenauer, 1966). Typically the amount of adsorbed sulfate increases with depth within these soil profiles (Bromfield, 1972; Singh et al. 1980; Fox, 1982). Generally this is due to the lower pH and high exchangeable Fe and Al content of the subsoils (Harward and Reisenauer, 1966). Hue et al. (1985) suggested that the precipitation of basalanite $(Al)_4(OH)_{10}(SO_4)$ and alanite $(K(Al)_3(OH)_6(SO_4)_2$ in acid soils can be a significant factor in controlling S availability to plants and leaching losses. Although there is no experimental evidence to support this contention, solubility data lends support to this hypothesis. However, the usefulness to plants of accumulated sulfate in subsoils is not clear. Some investigators suggest that subsoil sulfate can be taken up by deep rooted plants, however, this will often depend on the tolerance of the plant roots to high acidity and Al toxicity (Bohn et al. 1986).

The inputs of sulfate from fertilisers and the removal of S by plants are two major factors affecting sulfate levels in soils. Two other important management practices which also affect sulfate content in soils are liming and application of phosphatic fertiliser (Bohn et al. 1986; Blair and Lefroy, 1987).

1.4 THE SULFUR BALANCE AND ITS IMPACT ON FERTILISER REQUIREMENTS

Estimating the S balance of a production system on a local or regional level can prove helpful in predicting S requirements. Various estimates have been made of the S input/output relationships in countries in Southeast Asia. Blair and Lefroy (1987) calculated that the net S balance of plant uptake with fertiliser input was negative in Indonesia and positive in Malaysia and Thailand. In all three countries there is a significant quantity of S contained in crop residues which suggests that residue management is important in maintaining the S balance in the long term. Although there was an overall negative balance in Indonesia in 1983 as a whole, there are differences between the food and industrial crop sectors. Calculations by Blair and Lefroy (1987) showed an overall S deficit in food crop production of 89,936 t S if all of the residue was removed. This contrasts with a surplus of 37,493 t S in the industrial crop sector.

Data from field trials in Thailand have shown that S removed in product (rice grain) from an unfertilised control was 1.2 kg/ha, with a further 1.5 kg/ha in residue. When the input of S in rainfall was taken into account the resulting S balance was + 0.9 kg S /ha per annum if the residue was returned to the field. A loss of 0.6 kg S /ha was calculated if the residue was removed (Table 1.1). When fertiliser was applied the S balance was positive irrespective of residue management. These calculations assume all S in rain and fertiliser is available to the crop. The need for fertiliser S in crop growth is dependent on the balance between addition and removal of S from the soil. Data on the removal of S therefore, highlight the importance of crop removal and residue management in determining the long term S needs of the intensive cropping system.

Table 1.1. Sulfur input/output balance sheet (kg S/ha) for rice production in Thailand (Lefroy et al. 1988)

		Unfertilised		Fertilised	
		Residue returned	Residue removed	Residue returned	Residue removed
Input	Fertiliser	0.0	0.0	8.0	8.0
	Rain	3.6	3.6	3.6	3.6
	Total	3.6	3.6	11.6	11.6
Offtake		2.7	4.2	3.2	5.6
Balance		+0.9	-0.6	+8.4	+6.0

Information on the amount of S recycled via residues is limited. Lefroy et al. (1988) reported that an average of 2.7 kg S/ha was returned to rice fields in Thailand in 3.6 t/ha of straw and stubble. Hence, crop residue management must be taken into consideration in determining the overall crop S requirement.

Nambiar (1988) summarized the S balance in intensive cropping systems under the Indian Council of Agricultural Research's all India Coordinated Research Project on long term fertiliser experiments.

Results from these experiments demonstrate that the amount of S required differed between cropping systems and according to the soil S levels. An estimate of the S balance in a rice-rice cropping system on a laterite soil (Tropaquept) showed that S uptake with applications of between 50 and 150% of the optimal N P K fertiliser rate, with S added in the superphosphate, ranged from 14 to 21 kg S/ha. The mean annual S balance at these application rates was positive and ranged from 27 to 102 kg S/ha. When no fertiliser was applied or N P K was applied at the optimal rate, but in an S free form, the mean annual S balance was negative and the concentration of available S in the soil declined by more than 5 mg/kg over 14 years.

There was little effect on the crop yield in the absence of S in the first three years. However, a decline in rice yield was recorded in the following years and was significant after 12 annual cropping cycles with the continuous use of S-free N P K fertiliser (Nambiar, 1988).

1.5 THE IMPACT OF INTENSIVE AGRICULTURE ON S LOSSES IN CROP RESIDUES

Many systems of traditional agriculture involve a complex of mixed crops in fields of shifting cultivation (Okigbo and Greenland, 1976; Karunanayake, 1983). Intensification of agriculture has become necessary because of the limited area of arable land and the inability of traditional agriculture in the developing world to satisfy the increasing demand for food. This is particularly evident where sequential cropping has moved towards double and even triple annual cropping systems that has resulted in a reduction in the recycling of crop residues.

Many workers have reported that much of the plant nutritional value of cereal straw is lost by burning (Boerner 1982; Flinn and Marciano, 1984, Ponnamperuma 1984). These results are supported by the study of Chaitep (1990), which showed that the loss of S from burning of ^{35}S labelled rice straw amounted to 60% from the low S straw and 40% from the high S straw (Figure 1.3). Although the crop recovered a greater proportion of the S released in ash, this did not offset the loss through burning. In the presence of gypsum, under non-flooded conditions, the total S content of the rice plant derived from the high S straw and high S ash were comparable, although the percentage recoveries of residue S differed significantly. These data also highlight the importance of S concentration in the plant residue to supply adequate S from the decomposition of organic matter to ensure good crop growth and to maintain soil fertility.

If the volatilised S from burning is returned to the same area in rainfall, then burning is advantageous to the S cycle. On the other hand, the first crop after burning may be adversely affected by the lower S content due to loss from burning. Therefore, a greater long term benefit in both the physical and chemical fertility of the system is likely to result from the incorporation of straw instead of ash. *Figure 1.3* illustrates the percentage of ^{35}S remaining in the soil system from straw which will provide S to the subsequent crops. These results support the contention of Ponnamperuma (1984) that rice straw has a beneficial effect in complementing fertiliser additions of N and as an important source of P and S for crops.

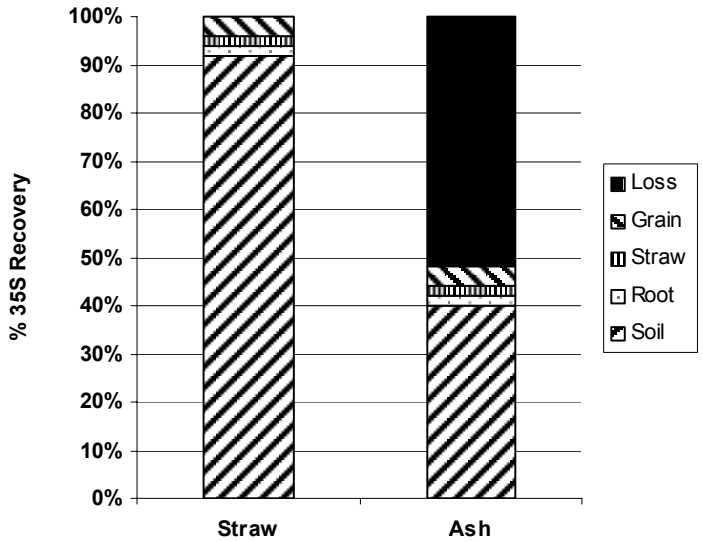

Figure 1.3. Summary diagram showing fate of S applied in ^{35}S labelled straw (0.108% S) or ash derived from that straw (0.374% S).

1.6 INCORPORATION OF S CYCLING KNOWLEDGE INTO SIMULATION MODELS TO PREDICT FERTILISER REQUIREMENTS

The world literature contains much information about various aspects of the S cycle in crop and pasture systems. Whilst this knowledge is useful in describing parts of the system and how they respond to management, they are static pieces of information that are often difficult to relate to other soil types in the same area or to other agro-ecological zones.

Simulation modelling has proven to be a valuable tool in bringing together diverse literature to overcome some of the above difficulties. McCaskill and Blair (1988) developed a simulation model of S cycling in grazed pastures as a conceptual and predictive tool to examine a range of possible fertiliser management options for improved pastures on the Northern Tablelands of New South Wales, Australia. Much of the data incorporated into the model came from experiments where ^{35}S was used. Without this it would not have been possible to separate the various pools and pathways. Because of the grass/legume nature of pastures in this environment, it was necessary to incorporate cycling of C, N and P into the model.

The model consists of interlinked organic and inorganic S cycles in the soil/plant/animal system. The model shows the dominant role of recycling of S from the organic pool and in the impact of grazing pressure on the S dynamics of the system (*Figure 1.4*). Another important output from the model has been the quantification of the impact of soil adsorption and water movement on the efficiency of utilisation of sulfate and elemental S fertiliser sources. On low adsorbing soils, in an environment where rainfall exceeds evapotranspiration for at least a portion of the year, leaching losses of S can be substantial and these can be prevented, to a major extent, by using elemental S of appropriate particle size.

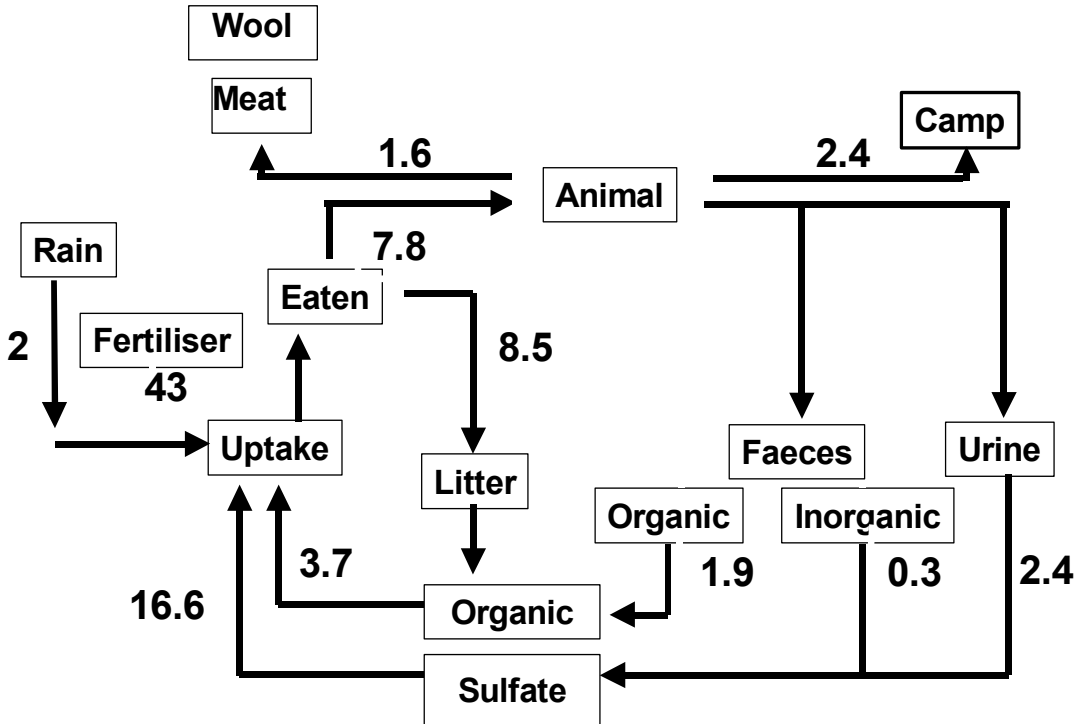

Figure 1.4. Sulfur pools and fluxes simulated by the computer model and calculated annual sulfur fluxes (kg S /ha/yr) over a 10-year period for a pasture system which received 43 kg S/ha/yr.

A sub-model developed in this simulation is concerned with the release of sulfate from elemental S (McCaskill and Blair 1989). It is known that the rate of S oxidation is affected by soil temperature, soil moisture, the S oxidizing biota present and the exposed surface area of elemental S available for microbial attack. Equations for incorporating the effects of soil temperature and moisture were developed from published data and these were incorporated into a simple model to predict sulfate supply from single superphosphate and elemental S (*Figure 1.5*).

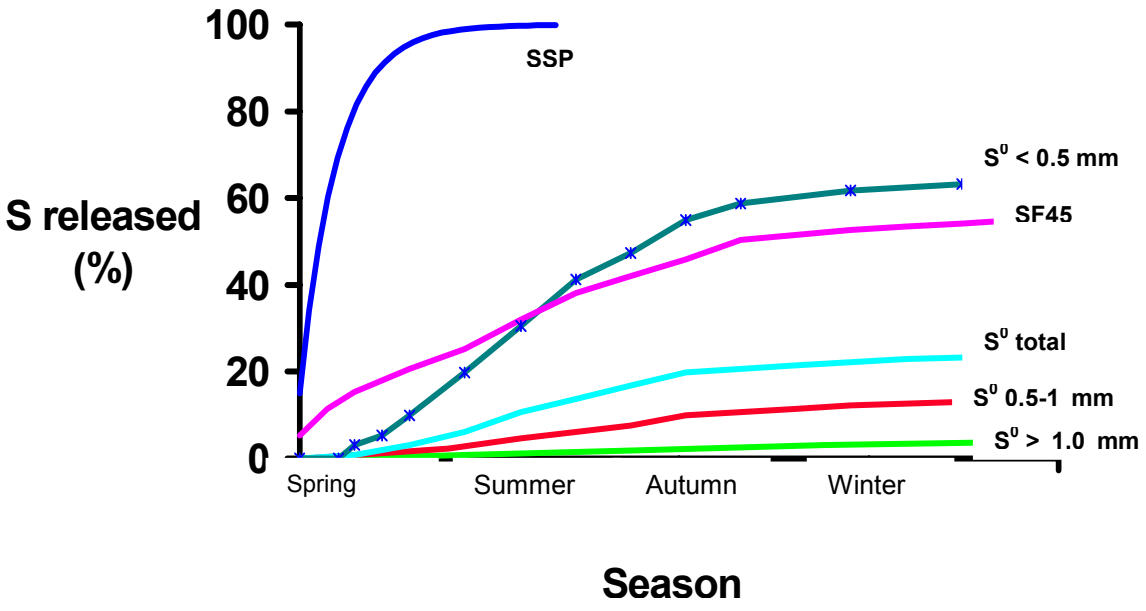

Figure 1.5. Release pattern predicted for various S fertilizers applied to a grazing trial at Armidale, NSW. SSP: Single superphosphate; SF45: sulfur-fortified superphosphate; S^0 agricultural grade crushed; S^0(total), and size fractions within it if <0.5, 0.5-1.0 and > 1.0mm diameter.

The model predicted that after 72 days 99% of the S in single superphosphate would have been released from the fertiliser granule. This compared to a release and oxidation of S from elemental S fortified single superphosphate (36% elemental S, 9% sulfate-S) of 54% after 1 year and only 23% from crushed agricultural grade elemental S over the same period.

The model provides a means of assessing the effect of particle size of elemental S on release rates in a range of climatic environments which should allow the formulation of fertilisers that supply S at a rate similar to that required by the crop or pasture.

1.7 S SOURCES AND THEIR MANAGEMENT

The choice in alternative S fertilisers is between sulfate, elemental S (ES) or a mixture of these. While SO_4^{2-}S has an advantage in being immediately available to the crop, little can be done to prevent leaching losses from this source. On the other hand ES is not soluble (leachable) and the release rate of S from elemental S can be manipulated by changing particle size. It would be possible to provide S in the elemental form that could be released both throughout the life of the crop and over several crops.

Chaitep et al (1994) conducted a glasshouse experiment to study the effect of water regimes in successive crops (non-flooded:non-flooded, flooded:flooded and flooded:non-flooded), broadcast (B) and deep (D) placement of sulfur fertiliser and S sources (elemental S (ES) and sulfate S (SS)) on the growth of rice. A soil of granitic origin was used and ^{35}S-labelled sulfur fertilisers were used to investigate S uptake by plants and the dynamics of S in soils. There was a response in total plant yield to S source, S application method and water management (Table 1.2). The highest total dry matter yield was 83.6 g/pot obtained from the surface applied gypsum under flooded conditions. This was significantly higher than the same treatment under non-flooded conditions (71.1 g/pot). S source and placement also affected the total dry matter yield (Table 1.2). Among the sulfur sources, gypsum produced a significantly greater total yield than elemental S under flooded conditions with the treatment combinations ranked SS-B > SS-D = ES-B > ES-D > control (Table 1.2). In the non-flooded treatments there was no significant difference between ES-B, SS-B and SS-D.

Table 1.2. Plant dry matter yield of components (g/pot) and fertiliser S uptake (mg/pot) from surface or deep placement of elemental S or gypsum under non-flooded and flooded conditions from the first rice crop after application

	Control	Elemental S		Gypsum	
		Broadcast (ES-B)	Deep (ES-D)	Broadcast (SS-B)	Deep (SS-D)
			Non-flooded		
Grain yield	16.8 a[a]	18.7 a	17.9 a	18.1 a	19.6 a
Total yield	56.2 a	73.5 c	66.6 b	71.1 c	71.5 c
Fertiliser S uptake		67.7 c	51.7 a	55.0 ab	60.8b
			Flooded		
Grain yield	17.3 a	23.8 c	21.0 b	27.5 d	24.2 c
Total yield	50.4 a	66.9 c	60.2 b	83.6 e	70.5 d
Fertiliser S uptake		51.2 d	20.1 a	43.7 c	28.7 b

[a] Numbers within a row followed by the same letters are not significantly different according to Duncans Multiple Range Test.

In the study of Chaitep (1990), there was a significant effect of S source and placement on grain yield under lowland conditions. Gypsum applied at the surface produced the highest grain yield, 27.5 g/pot compared to 23.8 g/pot in the ES-B, and both surface applications were significantly greater than their respective deep applications (Table 1.2). The results under flooded conditions contrast with those under upland conditions where no significant response in grain yield to S was obtained. Grain yield in the non-flooded treatments was always significantly lower than in the corresponding flooded treatments (Table 1.2). In a companion study, Samosir et al. (1993), again using ^{35}S, found that surface applied elemental S was as effective as K_2SO_4, but that deep placement of elemental S resulted in reduced S uptake (Table 1.3) and yield compared to deep placed K_2SO_4. The use of ^{35}S allowed the calculation of fertiliser S uptake separate from soil S uptake.

Table 1.3. S uptake by flooded C4-63 rice from different sources and from different placements (Samosir 1990)

Fertiliser treatment	Total S uptake(mg/pot)		Fertiliser S uptake (mg/pot)	
	42 days	Maturity	42 days	Maturity
Broadcast Elemental	13.4	95.3	7.5	64.9
Broadcast Sulfate	24.6	88.5	20.2	59.0
Deep Elemental	5.5	32.7	0.4	5.9
Deep Sulfate	11.3	76.4	3.9	37.3

In both studies, lower plant yields and S uptake were obtained from ES compared to sulfate-S under flooded conditions. The lower S uptake from the elemental S treatments was most likely because the elemental S must be oxidized to plant available sulfate before it is available to the plant (Ponnamperuma, 1972) and the rate of this oxidation process is dependent on soil moisture conditions. Moser and Olsen (1953) and Fawzi Abed (1976) reported that S oxidation rates were most rapid at a moisture content of approximately field capacity. Smittenberg et al. (1951), Freney (1961), and Kittims and Attoe (1965) indicated that the high moisture (> 48%) inhibited the oxidation of elemental S to sulfate.

In both the studies of Chaitep (1990) and Samosir et al. (1993), broadcast application of both ES and sulfate-S resulted in significantly higher plant yields than the deep-placed fertilisers under flooded conditions. This is most likely due to reduction of sulfate to sulfide and restricted oxidation of elemental S to sulfate under the reduced conditions deep in the flooded profile (Chien and Hellums, 1985). This is possibly compounded by a lower root density and root activity in the fertilised zone when deep placed. Under non-flooded conditions there was no significant effect of placement of gypsum on yield, but the deep placement of elemental S resulted in reduced yield. Surface roots can still take up sulfate placed deep in the profile since sulfate is mobile. Elemental S however must be oxidised before it is taken up or can move to areas of greater root proliferation. The lower yield and uptake of S from deep placed elemental S under non-flooded conditions may indicate a stimulatory effect of roots on the oxidation of elemental S when placed at the surface in an area of greater root proliferation and activity. A lower population of S oxidising bacteria would also be expected deeper in the profile.

These findings support those of Samosir and Blair (1983), Blair (1984) and Chien et al. (1987) who found that deep placement of ES reduced rice yield and S uptake.

The effectiveness of different S sources for flooded rice has also been studied by Dana et al. (1994) using ^{35}S reverse dilution. Grain yields and total S uptake were found to be similar when S was applied as gypsum, elemental S, urea-S or S-coated TSP, and were lower with SCU and S bentonite (Table 1.4). The uptake of S from the fertiliser was higher with applications of elemental S mixed with the soil, urea-S melt or S- coated TSP than with gypsum, while the fertiliser S uptake from SCU and S bentonite was lower than from gypsum.

Table 1.4. Yield and S uptake relative to gypsum applied at 10 kg S/ha to flooded rice

Fertiliser	Grain yield	S uptake	Fertiliser S uptake
	Relative to gypsum		relative to gypsum [a]
S^o mixed	84	97	1.12
Urea S melt	93	104	1.12
SCU 67	67	72	0.71
S-coated TSP	88	90	1.14
S-bentonite	63	69	0.43
Gypsum	100	100	1.00

[a] Calculated from 1-SRR data as shown in Section 2.3.

Lefroy (1989) reported results from a series of field experiments conducted in Thailand where S sources were compared. In these experiments five S sources were used: gypsum, elemental S (40-100 mesh), urea-S melt (Cominco), S- bentonite prill (Degrasul) and ammonium sulfate, at two rates of application, 8 and 32 kg S /ha, and with a no-S control. The S fertilisers were applied with basal fertilisers, including urea to balance N, prior to planting.

The first experiment, which was conducted on an acid, light-textured soil at Ubon in Northeast Thailand, was planted to corn immediately following fertiliser application and a cowpea crop grown on the residual fertiliser followed this. The application of 32 kg S /ha as gypsum, elemental S or urea-S melt all significantly increased the corn grain yield over both the 8 kg S /ha applications and the control, which did not differ from each other (Table 1.5). The application of 8 or 32 kg S /ha as S bentonite or ammonium sulfate did not produce grain yields which differed significantly from the 0 kg S /ha control.

In the following cowpea crop, there were only minor treatment differences in the yield and S uptake between fertiliser sources remaining after the corn crop. The S content of the cowpea was significantly higher with the 32 kg S /ha of S-bentonite, suggesting that the release of S from this product was delayed to such an extent that it was of little value to the crop grown immediately after application but the residual value was high.

These data suggest that, for this soil, gypsum, elemental S and urea-S melt are all effective S sources. S bentonite may be appropriate in long-term fertiliser programs, but the lack of response in the first crop and high price would not favour its use.

Table 1.5. Effect of sulfur source and rate on corn grain yield (kg/ha) at Ubon, Thailand

Fertiliser	Application rate (kg/ha)		
	0	8	32
Control	488bc [a]		
Gypsum		490bc	875a
Elemental		450c	763ab
Urea/Sulfur		563bc	850a
Sulfur/Bentonite		388c	444c
Ammonium Sulfate		550bc	338c

[a] Numbers followed by the same letters are not significantly different according to Duncans Multiple Range Test.

The second experiment reported by Lefroy (1989) was with flooded rice grown at Singamerta in West Java, with the same treatments as the corn/cowpea experiment. There was a significant response in grain yield to the applications of 8 and 32 kg S /ha of gypsum, ammonium sulfate or elemental S, with no significant difference between these sources. The application of urea-S melt and S-bentonite did not produce a significant yield response.

While agronomic differences between various S sources may be shown by more long-term studies in appropriate soil types and cropping systems, the field data to date and knowledge of the manufacturing and agronomic characteristics of the potential S fertilisers does allow assessment of different S fertilisers. Appropriate S sources can be chosen with the relative P, N and S requirements of the cropping system being taken into account and based on economics, availability and farmer acceptability.

In addition to S source and placement, the timing of S application can have a marked effect on the efficiency of utilisation of the fertiliser application. R. Lefroy and J. Gaudron (pers. comm.) have found that delaying the S application until maximum tillering resulted in a lower rice grain yield compared to applications made at transplanting or active tillering (Table 1.6).

Table 1.6. The effect of timing of S application on flooded rice grain yield (g/pot)
(R. Lefroy and J. Gaudron, pers. comm)

Timing of application	Grain Yield (g/pot)
Transplanting	23.2b [a]
Active tillering	24.7b
Max. tillering	15.7a
Anthesis	14.4a
Grain fill	13.3a
Control	13.4a

[a] Numbers followed by the same letter are not significantly different according to Duncans Multiple Range Test (p=0.05).

Lefroy et al. (1995), in an experiment where gypsum and elemental were both labelled with ^{35}S, showed greater availability of elemental S to corn when it was mixed intimately with monocalcium phosphate (Table 1.7). Recovery of the elemental S by the plant was also higher when all of the elemental S was applied as a basal application compared to a split application This finding indicates that S release rate from elemental S can be manipulated, not only by altering the particle size of elemental S but also the presence of P fertiliser which stimulated the growth of S oxidising Thiobacillus bacteria..

Table 1.7. The effect of banding S (30 kg/ha) with P on fertiliser S recovery (% of applied) in corn grown for 30 days

	Basal application		Split application	
	Elemental	Gypsum	Elemental	Gypsum
S mixed with P	12.4 c [a]	16.6 d	8.4 b	13.5 bc
S separated from P	2.7 a	17.0 d	2.3 a	10.3 bc

[a] Numbers followed by the same letter are not significantly different according to Duncans Multiple Range Test (p=0.05).

These findings, together with the effects of placement, indicate that in order to maximise S utilisation by the crop the fertiliser should be applied at sowing or transplanting in particular with ES containing fertilisers where there may be a lag in oxidation while the appropriate bacteria builds up.

Many of the findings reported in this section would not have been possible without the use of labelled fertilisers or reverse dilution (See Section 2.2.2)

1.8 SOIL AND TISSUE ANALYSIS

Soil and tissue testing to determine the S status of agricultural systems has met with variable success. A number of reasons for this have been outlined in reviews by Freney (1986), Jones (1986) and Blanchar (1986). Broadly, the difficulties can be divided into two groups.

A EXTRACTION OF PLANT AVAILABLE SOIL S

The nature of the S cycle in soil, which includes four main pools (*Figure 1.1*), contributes to this poor performance. Plants take up their sulfate from the soil solution pool which receives S from both the adsorbed and organic S pools. The organic S pool contains two major sub-pools, namely ester sulfates and carbon bonded S. Soil extractants used to determine the S status have most commonly involved a measurement of the sulfate in the soil solution plus adsorbed sulfur. Amongst the extractants used, calcium dihydrogen orthophosphate containing 500 μg P/mL has been the most common.

Blair (1979) tabulated data from the world literature on critical levels of soil S. In doing this he partitioned the extractants into those which extract readily soluble sulfate, readily soluble plus portions of adsorbed sulfate, readily soluble, adsorbed and a proportion of organic sulfate. Within each of these compartments, variable critical levels have been proposed in the literature. This indicates that local calibration of a soil test is critical if sensible predictions are to be made of sulfur status.

Generally, correlations between extractable S, using these types of extractants, and plant response have been poor. Simulation modelling of agricultural systems, as outlined earlier, indicate that fluxes of S from the organic pool play a major role in supplying S to agricultural plants, particularly in pasture systems where organic matter levels are high. This has been taken into account in the development of the KCl-40 soil test procedure (Blair et al. 1991). This procedure involves the extraction of soil with 0.25 M KCl heated at 40°C for 3 hours (See Section 2.7.5). In an assessment of this extractant compared with other techniques, the developers of the method found a coefficient of determination (r^2) of 0.73 between extractable S and percent of maximum yield on a range of 18 pasture soils collected from Northern New South Wales, Australia. In a supplementary study, where radioactive S had been added to soil, it was found that the KCl extract removes a portion of the HI reducible ester sulfates (organic S compounds) which are believed to be rapidly turning over in soil systems. It is hypothesised that the greater accuracy of this test results from the extraction of soil sulfate, a portion of the adsorbed S and a portion of the actively turning over organic S components in the soil.

Although the KCl 40°C extract has been shown to be useful on the 0-7.5 cm soil samples used in the above study, it is unlikely to perform well where S leaches deeper in the profile such as reported by Probert and Jones (1977). In these soils deeper sampling would be required.

B PLANT DIGESTION AND ANALYTICAL TECHNIQUES

Freney (1986) outlined some of the losses of sulfur that can occur in open digests using nitric/perchloric acid. Both he and Blanchar (1986) point out that operator error is a major factor in these losses. Controlling temperatures and allowing the digestion to proceed for at least one hour past the acid fuming stage, eliminates both sulfur volatilisation losses and the problem of complete oxidation of methionine. The problems of volatolisation and incomplete digestion have been overcome by the development of the sealed container digest using perchloric acid and hydrogen peroxide (Anderson and Henderson, 1986) (See Section 2.4.2). This digestion procedure does not oxidise all of the S to SO_4 so it suited to analysis by inductively coupled plasma techniques (ICP).

Once the organic sulfur compounds in the plant tissue have been oxidised to sulfate, their determination has also created some analytical problems. The use of the barium sulfate precipitation method has met with variable success. However, the development of autoanalyser techniques and the use of suspending agents for the barium sulfate such as polyvinyl alcohol, have meant an increase in the reliability of these methods (Till et al. 1984).

The variable mobility of sulfur in plants and the changes in tissue concentrations that occur over time, means that standardisation of the plant part sampled and sampling time are important.

1.8.2 Conclusions

Studies under glasshouse and field conditions utilising ^{35}S have greatly increased our knowledge of key processes in S cycling in cropping and pasture systems. Separation of the fertiliser effects from those of the large background soil S pool effects would not have been possible without the use of tracers.The incorporation of this knowledge into computer simulation models has allowed extrapolation of the data to other soil types and agro-ecological zones.

Manipulation of the proportions of sulfate and elemental S in fertilisers and in the particle size of elemental S can be used to increase S efficiency. Placement and timing can also be used to influence the efficiency of utilisation of S.

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CHAPTER 2

MEASUREMENT OF S IN SOILS, PLANTS AND FERTILISERS AND ISOTOPES OF S

2.1 RADIOACTIVITY AND RADIATION

The nucleus of an atom contains two sub-atomic particles, namely protons (p) and neutrons (n). The atom of a given element has a set number of protons and this is termed the atomic number. The number of protons+neutrons is referred to as the mass number. A particular element can have differing numbers of neutrons and therefore have a different mass number.

All atoms of a particular element contain the same number of protons but may have differing numbers of neutrons. This means that an element can have several types of atoms. These different types are called **isotopes**. All isotopes of a given element are chemically identical.

Sulfur isotopes have the same atomic number (subscript) but with different mass number (superscript) eg: ${}_{16}^{32}S$, ${}_{16}^{33}S$, ${}_{16}^{34}S$, ${}_{16}^{35}S$.
A nucleus contains protons, which are positively charged so they should repel. The presence of neutrons, however, keeps the protons together and so stabilises the nucleus. Stability depends upon the neutrons:protons (n:p) ratio and this needs to be wider as the mass of the element increases.

When the ratio of neutrons to protons is outside a particular number, which varies with each atom, the nucleus becomes unstable and spontaneously emits particles and/or electromagnetic radiation and such a substance is called **radioactive**. If the ratio of (n:p) is not outside the "belt of stability" then the isotope does not spontaneously emit particles and is said to be **stable** eg. ${}^{15}N$, ${}^{34}S$, ${}^{13}C$.

Radioactive isotopes (or, more accurately, nuclides) are unstable, that is, they undergo spontaneous transformation into more stable atoms. This transformation process is called **radioactive decay** and is usually accompanied by the emission of charged particles and gamma rays.

2.1.1 Alpha, Beta and Gamma Radiation

Unstable nuclei emit radiations of 3 main types - alpha, beta and gamma.

(i) *Alpha radiation (α)* consists of 2 protons and 2 neutrons. It is a massive particle (by nuclear standards) and travels relatively slowly through matter. It thus has a high chance of interacting with atoms along its path and will give up some of its energy during each of these interactions. Consequently, alpha particles lose their energy rapidly and only travel short distances. Their range in tissue is about 0.04mm.

(ii) *Beta radiation (ß)* consists of high speed electrons which originate from the nucleus. Beta particles are small and travel quickly. They undergo fewer transactions as they travel and thus give up their energy more slowly than alpha particles. Their range in tissue is about 5mm.

(iii) *Gamma radiation (γ)* belongs to a class known as electromagnetic radiation. It consists of quanta or packets of energy transmitted in the form of wave motion. Gamma radiation travels very large distances and loses its energy by interacting with atomic electrons. It is difficult to absorb completely. It can travel through the body.

2.1.2 Units of Radioactivity

The SI unit of activity is the *becquerel* (Bq) which is defined as 1 nuclear disintegration per second. Before the introduction of SI units the most commonly used unit of radioactivity was the *curie* (Ci) defined as 3.7×10^{10} nuclear disintegrations per second. The commonly used units to express activity are as shown in Table 2.1.

Table 2.1. Units of radioactive decay

Unit	Fraction of unit	Disintegrations per second (DPS)	Disintegrations per minute (DPM)
Becquerel (Bq)	10^0	1	60
Kilobecquerel (KBq)	10^3	$1x10^3$	$60x10^3$
Megabecquerel (MBq)	10^6	$1x10^6$	$60x10^6$
Gigabecquerel (GBq)	10^9	$1x10^9$	$60x10^9$

Some useful conversion factors are:

1 becquerel (Bq)	=	60 disintegrations per minute (dpm)
1 curie (Ci)	=	3.7×10^{10} becquerels (Bq)
	=	37 gigabecquerels (GBq)
1 millicurie (mCi)	=	3.7×10^7 becquerels
	=	37 megabecquerels (MBq)
1 microcurie (μCi)	=	3.7×10^4 becquerels
	=	37 kilobecquerels (kBq)

2.2 RADIOACTIVE ISOTOPES AS TRACERS

Radioactive isotopes can be used to follow a particular element through various pathways and quantitative measurements may be made. They have the advantage of behaving in the same way as their stable counterparts, but they can be readily traced. Radioactive isotopes can be likened to a coloured dye. They have a wide range of uses and are particularly valuable in plant nutrition research.

The physical properties of a radioactive nuclide determine its usefulness as a tracer. The three most important are half-life, mode of decay and decay energy. If the half-life of a nuclide is very short compared to the duration of the study, any compound labelled with it will be difficult to prepare, use and measure within the time of decay. The mode and energy of decay determine how the nuclide will be measured.

2.2.1 Isotopes of S

Sulfur has the Atomic No. 16 and an atomic weight in a natural mixture of 32.066. This natural mixture consists of 4 isotopes, three of which are stable and one (^{35}S) which is radioactive and emits β particles (Table 2.2,). Of the stable isotopes ^{34}S is commercially available.

Table 2.2. Isotopes of S

Atomic. Mass #	Atomic Wt.	Natural Abundance (%)	Decay mode	Decay ($T_{1/2}$) days	Decay Factor (/day)	Maximum energy of Beta (MeV)
Natural Mixture	32.06600					
32	31.97207	95.02	Stable			
33	32.97146	0.75	Stable			
34	33.96787	4.21	Stable			
35	34.96903	0	100%β	87.4	0.00793	0.167
36	35.96708	0.02	Stable			

2.2.2 Techniques available to use S isotopes in soil/plant studies

A INTRODUCTION

Two isotopes of S are useful in soil/plant/animal studies. Radioactive ^{35}S has proven to be an extremely useful tool in such studies because it has a useful half-life (87.4 days) and is a relatively soft β emitter (E_{max} 0.167 MeV). The stable isotope of S, ^{34}S, occurs naturally (4.29%) and is becoming increasingly available, although still extremely expensive. The stable form has the advantage that it remains indefinitely in the system, except when lost by leaching, volatilisation and product removal. The disadvantages are that a mass spectrometer has to be used to detect ^{34}S,and a unit with appropriate sensitivity will be much more expensive to buy and run than the corresponding LSC, and is not as sensitive so it needs a very much larger amount of ^{34}S than ^{35}S to set up an experiment. For example an LSC sample only needs to contain about 0.5 Bq to double the background count rate, and 20 Bq to allow a count rate measurement with a 1%SD in 10 min. In terms of weight 20 Bq of carrier free ^{35}S would weigh about 1.3×10^{-14} g.

The total amounts of ^{34}S or ^{35}S that would be required for an experiment obviously depends on the total amounts of S in the various components of the system to be studied, the dilutions expected in those components and the relative amounts of material that end up in the samples to be analysed. Another consideration is that for the stable isotopes the amounts essentially have to be determined as a ratio to the common stable form, but for radioisotopes some useful information can be obtained without the need to measure the total amount in the particular form. The complexities of analysis of light elements (H, C, N, O and S) by isotope ratio mass spectrometry (IRMS) have been thoroughly described in the FAO IAEA Training Course Series 14 Manual (Section 1.3). If for example the sensitivity of an IRMS measurement was δ2.0 for a sample size of 100 μg S then the sample being analysed would contain about 0.2 μg ^{34}S . The corresponding amount for an LSC sample is 1.3×10^{-8} μg ^{35}S. It is comparisons like this, together with costs of the nuclides and the constraints on the use of radioactive substances that determine the ultimate feasibility of conducting any particular experiment.

B APPLICATIONS USING RADIOACTIVE ^{35}S

i *Direct labelling*

Direct labelling is where the isotope is incorporated into the fertiliser or plant material and then traced in part of, or in the whole system. Numerous studies have used ^{35}S labelled elemental S or sulfate sources to follow the fate of S supplied in fertilisers eg. Samosir et al., (1993). The fate of S added in plant residues has also been traced using ^{35}S labelled plant material (Konboon et al. 2000).

ii *Reverse dilution*

In situations where it is not possible to label particular material, such as natural mineral deposits, commercial fertilisers and plant material a reverse dilution procedure must be used. In this technique ^{35}S is used to label the “system” and the changes in SR of the system components observed as it is reduced by inputs of unlabelled S from the materials being added. Dilution of the S in the soil by sulfur released from the fertilisers or residues is monitored by measuring the changes in specific radioactivity of sulfur in plants growing in the soil. A diagrammatic representation of the reverse dilution and direct labelling procedures is presented in Figure 2.1. With direct labelling the control, which receives no isotope has a SR of zero whereas with reverse dilution the SR of the control is always higher than the fertilised treatments as the SR is reduced by the nutrient from the cold fertiliser.

The general applicability of the method depends on the fulfillment of two requirements.

The first requirement is that the radiotracer becomes distributed in the same pool from which the plant obtains its supply of sulfur. The second requirement is that the rate of movement of the tracer from the plant available pool into other soil pools is not greatly affected by the treatments (i.e. the rate of sulfate release from the fertiliser does not affect the rate of movement between soil sulfur fractions).

When both requirements are met it is possible to calculate release rates from the fertilisers. If both requirements are not met it is possible to determine relative release rates from different fertilisers provided that adequate information is available to enable suitable precautions to be taken, such as the adjustment of S application rates.

The contribution of the unlabelled S addition is measured by determining the specific radioactivity (SR) of the sample. Any contribution from the unlabelled source will reduce the SR below the control treatment, which has not received any unlabelled S source. At any particular time, the lower the SR the more S that was derived from the unlabelled fertiliser.

However, because of recycling SR is decreasing with time in all treatments, including the control, the ratio of treatment SR. to control SR termed the specific radioactivity ratio (SRR) is the parameter used to compare sulfate release from the unlabelled S source over time. Thus the SRR and the rate of release of S are inversely related to each other. This can be a difficult concept to grasp so Dana et al. (1994) used 1-SRR to directly estimate the contribution of fertiliser S to total plant S. A more detailed discussion of this procedure is presented in Section 6.8.

Models of direct labelling and reverse dilution are presented in Chapter 5.

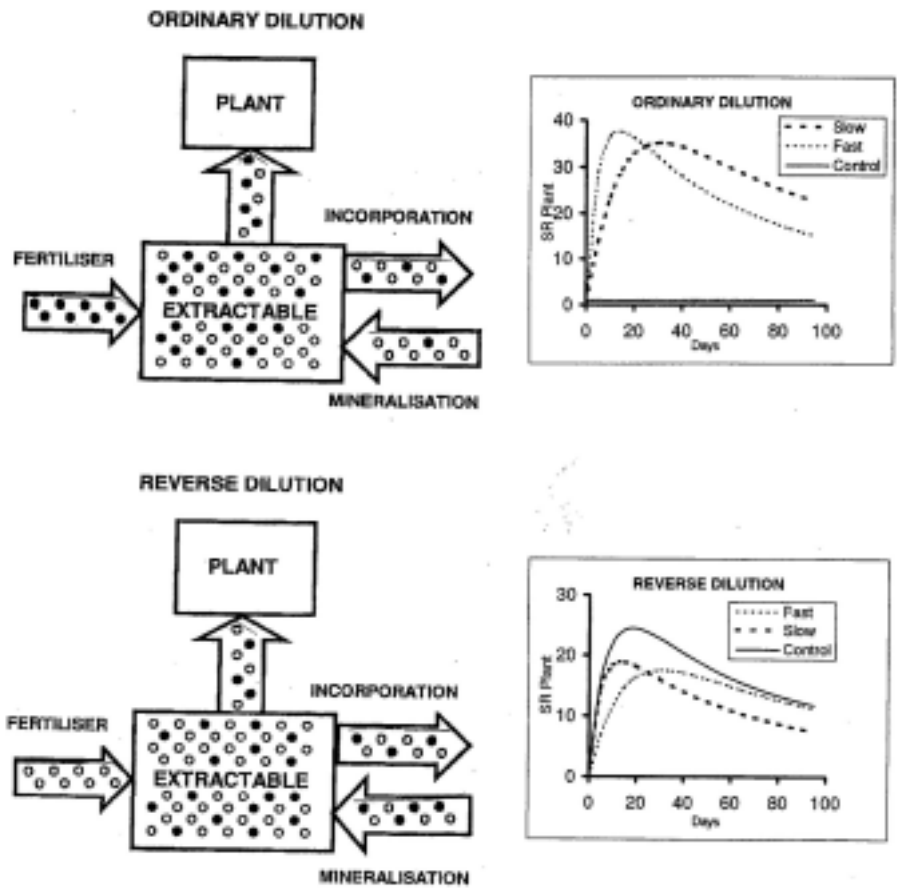

Figure 2.1. Diagrammatic representation of the reverse dilution and direct labelling procedures. Solid dots represent ^{35}S and open dots unlabelled S.

2.3 CALCULATION OF FERTILISER ^{35}S UPTAKE BY PLANTS

A ABBREVIATIONS USED IN THE CALCULATIONS

S*py*= Total S in plant yield
S*fa*= Total fertiliser S applied
S*f* = S concentration in fertiliser
^{35}S*f* = ^{35}S concentration in fertiliser
S*p* = S concentration in plant
^{35}S*p* = ^{35}S concentration in plant
S*a* = S concentration in soil "available S" pool
^{35}S*a* = ^{35}S concentration in soil "available S" pool
S*pfa* = S concentration of plant derived from the soil available s pool
S*pff* = S concentration of plant derived from the fertiliser
PS*pff* = proportion of S in the plant derived from the fertiliser
SR*p* = Specific radioactivity of plant S
SR*f* = Specific radioactivity of fertiliser S
SR*a* = Specific radioactivity of available soil S

B Defining calculations to be made

Two distinct measures of fertiliser S uptake can be made, each with their own particular merit. They are;

1. Total amount of fertiliser taken up by the plant

We need to know the total amount of fertiliser S applied (S*fa*), its specific radioactivity (SR*f*), and the concentration of sulfur in it (S*f*). Also the S in the total plant yield (Spy), the sulfur concentration in the plant (S*p*) and its specific radioactivity (SR*p*). This will allow a direct calculation of the amount of fertiliser nutrient used by the plant and will rarely be the same as other non-isotopic estimates of "fertiliser efficiency".

2. Proportion of plant nutrient derived from fertiliser.

The same measurements of nutrient concentration and amounts of tracer as those in 1 (above) are needed but it does not require quantitative measurement of total plant S yield. This is an easier measure to make, and in many ways is more meaningful when comparing the relative "efficiencies" of different fertilisers.

In radiotracer studies the specific radioactivity (SR) is used to describe the amount of radioactivity per unit of material as well as the amount of radiotracer per unit of the common element. Consequently care must be taken to avoid confusion between the SR of the plant dry matter which is expressed in units such as Bq/g DM and the SR of the sulfur in the plant (SR*p*) = $^{35}S_p/S_p$ which is expressed in units such as Bq/mg S .

2.3.1. Calculation of the total amount of fertiliser taken up by plant

In undertaking this calculation it is assumed that the tracer is uniformLy mixed in the particular 'pool' of material and that the rates with which the isotopes participate in the various processes are not substantially different from the unlabelled material; ie. isotope effects are small.

Note that in some cases even though the isotope effects are very small they can provide valuable information on system operation.

The SR of the sulfur in new plant growth reflects the integrated effective SR of the "soil available" sulfur pool over that time interval

$$SR_p = {}^{35}S_p/S_p = SR_a = {}^{35}S_a/S_a \qquad (1)$$

A ORDINARY ISOTOPE DILUTION (OD) USING ^{35}S LABELLED FERTILISER

Specific radioactivity of fertiliser $SR_f = {}^{35}S_f / S_f$ (eg Bq / g S) (2)

Specific radioactivity of plant S $SR_p = {}^{35}S_p / S_p$ (eg Bq / g S) (3)

The ^{35}S in the plant can only have come from the fertiliser and this must be accompanied by the corresponding amount of fertiliser S. The SR of the fertiliser and the ^{35}S concentration in the plant are measured so the plant S derived from the fertiliser can be calculated

$$S_{pff} = {}^{35}S_p / SR_f \qquad (4)$$

The proportion of plant S from the fertiliser $PS_{pff} = S_{pff} / S_p$ (5)

from equations 4 and 5 $PS_{pff} = {}^{35}S_p / SR_f / S_p = SR_p / SR_f$ (6)

The proportion of the S in the plants from the fertiliser is the specific radioactivity "ratio" (SRR) of the SR's in plant and fertiliser. ie. PS_{pff} = SRR(OD)

Efficiency of fertiliser utilisation.

Efficiency is calculated from the total ^{35}S uptake divided by the total ^{35}S applied in fertiliser

$$\text{Efficiency} = (SR_p \times S_{py}) / (SR_f \times S_{fa}) = SRR \times S_{py} / S_{fa} \qquad (7)$$

B REVERSE DILUTION (RD)

When it is not possible to label the fertiliser then reverse dilution must be used. In an ideal situation the tracer would be introduced into the soil and in time all the components would end up with the same SR of the tracer nutrient. Unlabelled fertiliser would then be used and the system measurements and interpretation made in a similar manner to the OD situation. However, even if enough time could be

left for this equilibrium to be established the moment any perturbation is introduced the equilibrium is upset and the SR of the nutrient in the various system components will not remain constant, thereby complicating the interpretation of some results. The problem is that we need to be able to make an estimate of the effective SR of the plant available sulfur pool at any particular time. The assumption* is that this is provided by measuring the SR of plants growing in the system without added fertiliser ie. control plants.

***Note:-** This is really the same assumption that we make about the plants echoing the SR of the soil available pool in the OD method.

In addition, for the fertiliser treatments, we have to assume that because the fertiliser treatment is usually small in relation to the total amount of S in the soil and does not mix with the available pool immediately, the flow of ^{35}S into the soil available pool is dominated by the rest of the processes in the soil and changes relatively slowly ie. in the treated plants the change in SR is due to uptake of unlabelled S from the fertiliser.

PLANT SR AND UPTAKE FROM THE SOIL AVAILABLE POOL AND APPLIED FERTILISER

The concentration of S in the fertiliser is Sf and $^{35}Sf = 0$ the $SRf = 0$

It is assumed that the values of ^{35}S and S in the control plants (^{35}Spc and Spc) give an on-going estimate of the SR of the available soil S.

$$^{35}Spc / Spc = SRpc = SRa \qquad (8)$$

For the fertiliser treated plants : -

$$SRp = {}^{35}Sp/Sp \quad \text{and} \quad Sp = Spff + Spfa \qquad (9)$$

The proportion of the total plant S derived from the fertiliser is : -

$$PSpff = Spff / Sp = (Sp - Spfa) / Sp \qquad (10)$$

The ^{35}S in the plant could only come from the labelled soil available S pool and must have been accompanied by the corresponding amount of S from that pool.

The plant S concentration derived from the soil available S pool is given by : -

$$Spfa = {}^{35}Sp / SRa \qquad (11)$$

and substituting in equation 10 for SRa (Eq 8) and $Spfa$ (Eq 11) the $PSpff$ is given by : -

$$PSpff = (Sp - {}^{35}Sp / SRa) /. Sp$$

$$= 1 - {}^{35}Sp / Sp \times 1 / SRa$$

$$= 1 - SRp / SRpc$$

In reverse dilution the SRR is defined as (SRp (fertilised plant) / $SRpc$ (control plant)

$$PSpff = 1 - SRR$$

Note **: -** This SRR is not the same as SRR used in ordinary dilution.

The efficiency of the fertiliser uptake by plant is : -

$$\text{Efficiency} = PSpff \times Spy / Sfa$$

$$\text{Efficiency} = (1 - SRR) \times Spy / Sfa$$

Even if the assumption of using the control plants to monitor the SR of the available S pool does not hold it is still possible to get valid RD comparisons between different fertilisers by using a range of rates of each and interpolating to get the application rates that give equivalent uptake of ^{35}S. See Section 6.6 for an example.

2.4 MEASUREMENT OF ^{35}S

Radioactive decay is a natural process and the probability that an atom will decay is random. The decay curve for the β emission from ^{35}S is exponential and the rate of decline of the curve is characterised by its half-life. Half-life is independent of mass and, furthermore, is independent of all prevailing conditions (e.g. temperature, pressure, concentration) and of whether it is an atom, part of a molecule, in a solution etc.

When integrated this becomes

$N = N_o e^{-\lambda t}$

where N is the number of atoms remaining after time t and N_0 the original number of atoms.

The half life ($T_{1/2}$) is when $N/N_0 = 1/2 = e^{-\lambda t}$

The half-life of a substance can be calculated from the exponential equation as follows:

$T_{1/2} = \log_e 2 / \lambda$

Rearranging this to calculate λ for ^{35}S, where $\log_e 2 = 0.6931$

$\lambda = 0.6931 / 87.4 = 0.00793$

To determine the activity of ^{35}S 20 days after its activity date, when the activity was originally 2200 Bq.

$N = N_0 e^{-\lambda t}$

$= 2200\ e^{-0.00793 \times 20}$

$= 2200 \times 0.8529$

$= 1876$ Bq

A useful rule of thumb is that 1% of radioactivity remains after 7 half-lives and 0.1% after 10 half-lives. Half life calculations for ^{35}S are shown in Table 2.3.

Table 2.3. Half-life calculations for ^{35}S

Number of half-lives	0	1	2	3	4	5
Days	87.4	174.8	262.4	349.9	437.3	524.7
Mass of ^{35}S (g)	10	5	2.5	1.25	0.625	0.3125
Mass of emissions (g)	0	5	7.5	8.25	9.375	9.6875
Fraction of original mass	1/1	1/2	1/4	1/8	1/16	1/32
Number of ^{35}S atoms	1.726×10^{23}	8.63×10^{22}	4.31×10^{22}	2.15×10^{22}	1.079×10^{22}	5.394×10^{21}
Cumulative number of β particles emitted	0	8.63×10^{22}	1.294×10^{23}	1.509×10^{23}	1.617×10^{23}	1.622×10^{23}

Liquid Scintillation Counting (LSC) is used for the measurement of beta emitting nuclides such as ^{35}S. The sample containing the radionuclide is dissolved in a suitable scintillation solution. The solution normally consists of an aromatic organic solvent (e.g. toluene) containing a primary fluor (a compound that converts molecular excitation energy into light photons, e.g. P-terphenyl) and a secondary fluor (e.g. POPOP) which acts as a wavelength shifter. An emulsifying agent (e.g. teric) is also added to the scintillation mixture or "cocktail" to aid in the intimate mixing (or miscibility) of sample and scintillator.

2.4.1 Preparation of Scintillation Mixture of LSC

There are many "cocktails" used for the preparation of radioactive samples. Most of the commercially available cocktails are good but are usually designed for a specific purpose and tend to be very costly especially if large numbers of samples are to be measured.

The following details the preparation of a cocktail that can be successfully used with a wide range of samples and is comparatively cheap.

A. REAGENTS

Toluene - scintillation grade
p-terphenyl
POPOP
Emulsifying agent (teric).

Teric is one of a range of commercial emulsifying agents, which can be used. Unfortunately such materials are not made specifically for LSC and the manufactures sometimes change their specifications which may affect their effectiveness.

A METHOD

1. Add approximately 1 litre toluene to a beaker on a heater/stirrer.
2. Add 16.92g p-terphenyl and 0.73g POPOP; heat and stir gradually until all dissolved.
3. Add 2080mL teric to a 5 litre volumetric flask and mix in the toluene/p-terphenyl/POPOP solution.
4. When cool, make to volume with toluene.

B SAMPLE/SCINTILLANT RATIO

This mixture can be used at a ratio of 3mL sample:17mL of scintillant, to give a final volume of 20mL. However, if the mixture of the sample and scintillant prove immiscible, the ratio of scintillant to sample can be varied (e.g. 18-19mL scintillant to 2-1mL sample) or the mixture acidified using a strong acid (e.g. 2mL sample, 1mL acid and 17mL of scintillant). These ratios may be altered until a clear emulsion results.

2.4.2 The Phenomenon of Quenching

Chemical constituents and colour in the sample can result in a reduction in the number of β^+ particles leaving the sample. This is termed quench. This can be accounted for by adding known quantities of the isotope to the matrix and using this count data to correct the counts measured in the samples. This is termed quench correction.

To compensate for quenching losses, a calibration curve must be prepared against which the unknown samples can be compared. The quench curve can be established by counting a series of samples containing a known constant amount of radioactivity, but varying concentrations of a quencher.

In theory, the construction of one calibration curve should suffice for all mechanisms quenching the same isotope, provided that the calibration is for the same scintillator and constant settings. However, most users calibrate for each particular sample preparation, unless using a sophisticated machine which can compensate for colour and photon quenching.

A prepared quench curve should cover the range in which the unknown samples are to be measured. The activity of the radioactive isotope used should lie between 60 and 600 mBq/vial.

Table 2.4 contains a suggested method of preparing a quench curve. When the quenched samples have been counted plot the counts against the tSie or similar measure from the LSC to calculate the efficiencies at various quench levels.

Table 2.4. Volumes used in preparing quench standards

Identifier	Volume of quenching agent (e.g. digest) (mL)	Volume of water (mL)	Volume of isotope solution (eg.6000 mBq/mL) (mL)	Volume of scintillant (mL)
Background	3.0	0	0.1	17.00
Quench 1	2.5	0.5	0.1	17.00
Quench 2	2.0	1.0	0.1	17.00
Quench 3	1.5	1.5	0.1	17.00
Quench 4	1.0	2.0	0.1	17.00
Quench 5	0.5	2.5	0.1	17.00
Quench 6	0	3.0	0.1	17.00

When running the quench curve on the LSC, set-up the measurement conditions and load the curve so that the background samples are read first, followed by the unquenched standards, then the quenched standards in increasing volumes of quench.

To measure dual labelled samples (e.g. both ^{32}P and ^{35}S), two quench curves must be prepared for each isotope, i.e. the two isotopes are not mixed together in the same standard. This is because the LSC cannot differentiate between the two different radioisotopes; it simply combines the energies. (Many modern LSCs use sophisticated software to then separate the counts for the different nuclides.) To run a quench curve for dual labelling, load the lowest energy nuclide first (e.g. ^{35}S), followed by the highest energy nuclide (e.g. ^{32}P).

2.5 APPLICATIONS USING STABLE ^{34}S

Elemental S labelled with ^{34}S is commercially available and has begun to be used in soil/plant studies (Wen *et al*. 1999). This can be added directly to the system and the enrichment of S pools measured by mass spectrometry. The contribution to the pool from the enriched fertiliser can be calculated as shown in Section 2.6.1.

A procedure has been developed at IAEA to convert ^{34}S enriched elemental S to sulfate (see Section 4.3) and this can be directly applied to the system.

2.6 MEASUREMENT OF ^{34}S

2.6.1 Calculation of $\delta^{34}S$ and ^{34}S recovery in soil and plant

From the ratios (R) of mass 50 and mass 48 ion beams measured in a reconfigured Automated Nitrogen and Carbon Analyser connected to a Mass Spectrometer (ANCA-MS).

$\delta(50)$ was calculated as follows: $\delta(50)=(R_{sample}/R_{standard}-1) \times 1000$

The laboratory standard used was methionine. The $\delta^{34}S$ value in the sample was then calculated based on the method of Eriksen (1996):

$\delta^{34}S=\delta(50) \times C + \delta^{34}S$ (lab. standard) $+1/1000 \times C \times \delta^{34}S$ (lab. standard)

where C is the correction factor for oxygen normally considered as 1.046 (Eriksen 1997 pers. comm.). The ^{34}S atom % was then calculated using the $\delta^{34}S$ value of all soil and plant samples based on the method of Peoples *et al*. (1989). Using the calculated ^{34}S atom % of all samples, the ^{34}S recovery in soil and plant samples is calculated in the same way as is done for ^{15}N.

2.6.2 Calculation of ^{34}S enrichment

The ion spectrum of SO_2 is complex with contributions from $^{34}SO_2$ / $^{32}SO_2$ and $^{34}SO/^{32}SO$ at mass 66/64 and 50/48, respectively. The spectrum is further complicated by the isotopic variation in the oxygen component of the oxides giving rise to peaks at mass 65 and 49. Since both $^{34}S^{16}O$ and $^{32}S^{18}O$ both have mass close 50 and $^{34}S^{16}O_2$ and $^{32}S^{18}O_2$ have masses of 66 and 68 correction must be made to account for these contributions.

Increasingly more sophisticated combustion furnace instruments linked to a mass spectrometer are being used to determine ^{34}S. One disadvantage of this approach is that mass 66/64 is beyond the range of the instrument so 50/48 lines are measured which results in less precision than the 66/64 lines.

Example:

^{34}S enriched elemental S was applied to a pasture (Wen, 1998) and soil samples collected from the profile over the following 21 months. The soil samples were dried and ground to < 0.5 mm and measured in a Carlo-Erba NA1500. A calibration curve was established using methionine ($^{34}\delta S=16.86$ $^{o}/_{oo}$) as shown in Figure 2.2

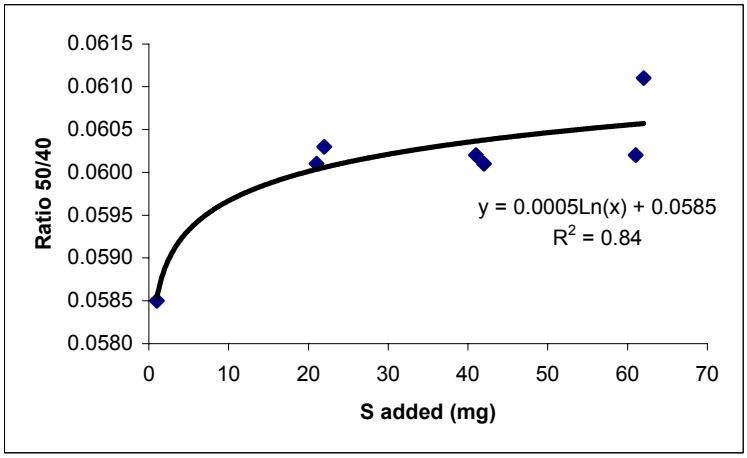

Figure 2.2. Calibration curve for methionine laboratory standard.

Soil sample measurement S(50/48) = 0.0585
Corresponding standard S(50/48) from Figure 2.2 = 0.0586
Oxygen isotope correction factor C = 1.046 (J. Eriksen pers. comm.)
$^{34}\delta S$ of methionine lab standard = 16.86 $^{o}/_{oo}$
$^{34}\delta S$ of added fertiliser = 2807.30 $^{o}/_{oo}$
$\delta(50) = (R_{sample}/R_{standard}-1) \times 1000 = ((0.0626/ 0.0603) – 1) \times 1000 = 38.1426$
$^{34}\delta S = (\delta(50) \times C) + \delta^{34}S$ (lab. standard) $+(1/1000 \times C \times \delta^{34}S$ (lab. standard))
$=(38.1426 \times 1.046) + 16.8600 + (1/1000 \times 1.046 \times 16.86)$
$=39.8972 + 16.8600 + 0.0176$
$= 56.7748$

2.6.3 Calculation of proportion and quantity of fertiliser S in the soil from ^{34}S data

The proportion of soil S derived from the added ^{34}S enriched fertiliser can be calculated from

$$\chi = \frac{(\delta - \delta)}{(\delta r - \delta s)}$$

$$= \frac{56.7748 - 14.8600}{2807.3000 - 14.8600}$$

$$= 41.9148 / 2792.4490$$

$$= 0.015 \text{ or } 1.5\%$$

Where δ is the $\delta^{34}S$ value of the soil at time t after the addition of the fertiliser, δs is the $\delta^{34}S$ of the original soil or soil of the control treatment and, δ is the $\delta^{34}S$ of the fertiliser added to the soil.

If the total S content (kg/ha) of the soil is known (C) then the absolute quantity X of sulfur from the fertiliser can be calculated as follows:

$$X = \chi \times C$$

$$= 0.015 \times 200$$

$$= 3 \text{ kg/ha}$$

2.7 MEASUREMENT OF TOTAL S IN SOIL AND PLANT SAMPLES

All the digestion methods outlined below should include digestion of some standard plant material and blank acid digests for quality control. These should later be checked against laboratory means. Aliquots of these digests can be used to count ^{35}S in a liquid scintillation counter or to determine ^{34}S in a mass spectrometer.

2.7.1 Nitric/Perchloric Acid Digest of Plant Samples for S,P, K, Ca, Mg

Method

1. Weigh out approximately 250 mg of ground oven-dried plant material into a clean and dry digestion tube or conical flask. If the samples have been stored, it will probably be necessary to dry them in the oven and then cool, preferably in a desiccator, before weighing them out. Be sure to record the exact weight and tube number; record sheets provide a convenient method for doing this.

2. To each tube or flask add 5 mL of 2:1 ratio (70%) nitric acid (70%) -perchloric acid mix with a bottle top dispenser, then place a glass bubble or small filter funnel on top of the tube. When adding the acid, avoid leaving plant material stuck to the side of the tube or flask above the acid by gently dispensing the acid down the side of the tube or flask whilst holding it at a slight angle. Slowly rotating the tube whilst adding the acid will also help.

3. Most materials benefit from a degree of pre-digestion. This can vary from a short time at room temperature in the acid mix; gentle heating prior to digestion (e.g. 50°C for 2 hours) or pre-digestion overnight at room temperature. The degree of pre-digestion will have to be decided depending on the material and the time available. The overnight pre-digestion is most commonly used.

4. Following pre-digestion, the tubes or flasks are heated at 150°C for 1 hour to evolve the brown, nitrogen dioxide fumes. Ensure that the fume hood is on during the digestion process as corrosive fumes are given off.

5. Increase the temperature to 200°C. The fuming stage of perchloric acid (white fumes) is reached at this temperature. Maintain this state for 2 hours. This stage completes oxidisation of all elements and ensures that the chemicals in the plant sample are in a soluble inorganic form.

6. Remove the digests from the heater and allow to cool to touch. Add 1mL of concentrated HCl to release any HNO_3 still present (if the tubes are not cooled sufficiently spitting occurs with the HCl addition).

7. Replace on the blocks at 200°C and fume for a further 30 minutes.

8. Remove from the blocks and allow to cool. Wash the inside walls of the tubes or flask with 20-30mL of deionised/distilled water and mix on a vortex. This washes down the digestion vessel and helps dissolve any precipitated salts. Make sure the digestion vessels are not left on the block to cool as they may crack.

9. Replace the digestion vessels on the digestion blocks at 60-70°C for 10 minutes to dissolve any perchlorate salts. (The outside of the tubes must be dry prior to placing in the blocks otherwise they may crack).

10. Following cooling make up to volume (75 mL), cover with parafilm and mix by inverting several times.

11. Allow to sit so that any silica crystals can settle out, then pour off into labelled vials for later analysis. The digests should be stored in a cold room until analysed.

12. Empty the digestion vessels and rinse several times with tap water. Soak in a 2% detergent (Decon or other suitable phosphorus-free detergent) solution. After soaking scrub with a bottle brush and rinse thoroughly with tap water, followed by 3 rinses in distilled water. Invert and place in an oven to dry.

2.7.2 Sealed Chamber Digest for Plant Samples for S,P, K, Na, Mg, Ca and Trace Elements

This sealed chamber digestion (SCD), using perchloric acid and hydrogen peroxide, was developed as an alternative to conventional procedures for plant tissue digestion. The advantages of SCD are:

- the low volume of reagents used and the ability to utilise low volume dilutions;
- low temperature of digestion;
- use of a closed system eliminating the need for a fume hood;
- micro-sample analyses capabilities;
- minimal equipment investment and, simplicity.

This digest method does not guarantee complete oxidation of the sample and so is most appropriate for ICP analyses in which final oxidation occurs in the high temperature plasma of the ICP. Analysis by other methods, such as autoanalyser, may lead to low recoveries.

The vessel used for the SCD method needs to be chemically resistant and able to withstand the pressure and temperature experienced during the procedure. The original method used 50 mL heavy-walled borosilicate glass Oak Ridge-type centrifuge tubes with 24 mm teflon-lined screw cap closures, and subsequently tried similar vessels but with a conical bottom and a 38mm screw cap closure

Method

1. Weigh out approximately 0.2 g of plant material into appropriately labelled (acid washed for micronutrient determinations) digestion vessels. Enter the exact weights and vial numbers on a record sheet. Use less material (e.g. 0.1g) for root samples. The digestion vessels can be 30 mL polycarbonate vials which are inexpensive but can only be used once because they cannot withstand the pressure build-up of subsequent digestions. Longer lasting thick glass Schott bottles fitted with a plastic lid are best.

2. Add 2 mL of a 7:3 (v/v) mixture of $HClO_4$ (70%) and H_2O_2 (30%) to each digestion vessel and cap lightly. Avoid leaving plant material stuck to the side of the tube above the acid by gently adding the acid down the side of the tube whilst holding at a slight angle.

3. After predigestion for a **minimum** of 2 hours at room temperature (longer predigestion is usually better and an overnight predigestion is often convenient), add 1 mL of H_2O_2, tightly seal the digestion vessel, and place into a warming oven at 80°C for 30 minutes.

NOTE: Some polycarbonate vials used cannot withstand the pressure that builds up during this first 30 minutes of digestion and consequently the lids blow off. To prevent this, reduce this first digestion to 5 minutes, and then continue with step 4.

4. Allow the digestion vessels to cool slightly, add a further 1 mL H_2O_2, cap tightly and digest for 1 hour longer.

5. If further digestion of the sample is necessary, digest with 1 mL aliquots of H_2O_2 at 30 minute intervals. The amounts of $HClO_4/H_2O_2$ predigestion mixture and H_2O_2 added varies with tissue type and ranges from 1 to 2 mL and 1 to 4 mL, respectively.

6. Allow the samples to cool.

7. Make to volume, or weight, using distilled/deionised water and mix thoroughly. The final volume (or weight) can be adjusted according to the sample weight used and the analyses being run. For example:

for < 0.05 g sample wt. use 10 mL total volume

for 0.05-0.1g sample wt. use 15 mL total volume

for 0.1 -0.2g sample wt. use 25 mL total volume

NOTE: Using calibrated moulds to hold the vials appropriate marks can be scored on the sides of the vial for 10, 15 and 25 mL final volumes (with an accuracy of < $\pm$3%). Alternatively, samples can be made to weight in the vials or carefully transferred to volumetric flasks.

8. Storage before analysis should be at 2°C, to reduce adsorption onto plastic and growth of microorganisms.

9. It may be necessary to filter silicate precipitates out of the sample prior to analysis. This can be done with 0.45 μm glass fibre filters and millipore filter apparatus.

NOTE: All macro and micronutrients can be analysed in the final digest mixture, which is equivalent to a 3.9% (or 0.65N) $HClO_4$ solution when made to 25 mL.

2.7.3 Nitric Acid Digest Procedure - for S,P, K, Na, Mg, Ca and trace elements

Like the SCD procedure (2.7.2), this procedure does not guarantee complete oxidation and is therefore mainly used for ICP multi-element analyses.

Method

1. Weigh 0.5-1.0g of oven-dried plant material into clean acid-washed 50 mL pyrex test tubes. Enter the exact weights and tube numbers on a record sheet.

2. Add 10mL of concentrated nitric acid (70%) with a bottle top dispenser. When adding the acid, avoid leaving plant material stuck to the side of the tube above the acid by gently dispensing the acid down the side of the tube whilst holding the tube at a slight angle. Slowly rotating the tube whilst adding the acid will also help.

3. Most materials benefit from a degree of pre-digestion. This can vary from gentle heating prior to digestion to pre-digestion overnight at room temperature. The latter is most commonly used and is aimed at avoiding excessive frothing during digestion.

4. After pre-digestion, the tubes are placed in a block digestor at 80°C, in the fumehood.

NOTE: A thermometer placed in a blank tube of acid may be used to check the temperature of the block. Also, check that the fume hood is operating properly, with the fan on, and water flowing gently down the glass at the rear and the glass panel at the front of the fume hood lowered until it is almost closed.

The temperature must not exceed 130°C or loss of B, Fe and Mn may result.

5. After 30 minutes at 80°C, increase the temperature to 125°C and continue digesting for 260 minutes, including the time taken to raise the temperature. During this time, it is important to keep a close eye on the digests as they may froth vigorously. A gentle tap to burst the froth is sometimes required to avoid loss of sample.

6. Check the digest volume of each tube and remove. When 1mL of acid remains digestion is complete, which indicates that digestion time will vary due to the different degree of oxidation required for each sample and temperature differences in the block.

7. When cool, dilute to volume (see Section 6.1) with 1% nitric acid and mix thoroughly.

Suggested dilutions are: 5 mL total volume for < 0.1 g sample weight

10 mL total volume for 0.1-0.5 g sample weight

25 mL total volume for 0.5-1.0 g sample weight

8. Stand overnight to allow amorphous silica to settle.

9. Storage before analysis should be at 2°C, to reduce adsorption onto plastic and growth of microorganisms.

Rinse the empty digestion tubes several times in tap water and leave to soak overnight in 10% HCl solution. Rinse in distilled/deionised water three times, invert tubes in a plastic tray and dry in a stainless steel oven. This procedure prevents contamination with Al, Fe, etc.

2.7.4 Combined Phosphorus and Sulfur Digest Method for Soils and Fertilisers

This method utilizes an already established analytical technique for phosphorus and combines it with sulfur, thus eliminating many of the sampling problems and equipment.

Reagents

Unless specified otherwise all chemicals are of analytical reagent grade.

Digestion Mixture - dissolve 15g potassium dichromate in 730 mL of deionised water and add to a mixture of 2170 mL of perchloric acid (70%) and 2100 mL concentrated nitric acid (70%) to make 5 litres. 50 mL bromine is finally added to the 5 litres and mixed thoroughly.

Sample Preparation

1. Weigh a 50 mL Erlenmyer flask with 3-5 anti-bumping granules; record this weight. Into this flask weigh 1.5-3g dry soil (or 0.1-0.5 g fertiliser); record the sample weight accurately.

2. Add 10 mL of the digestion mixture using a plastic syringe. Ensure this is done in a fume hood and all safety equipment, i.e. gloves, glasses, lab coat, etc. must be worn.

3. Place a small funnel in the neck of the flask to allow refluxing of the mixture.
4. After allowing the initial oxidation by nitric acid (brown fumes) to subside, the flasks are heated on a hotplate at a low temperature.
5. The temperature is gradually raised until fumes of nitrogen dioxide (white fumes) cease to be evolved and the perchloric acid begins to reflux.

   The dichromate in the mixture turns green (reduced to Cr^{3+})during the early stages and then is reconverted to orange-red as oxidation nears completion. This change is a very convenient indicator and after the change to red, the heating should be continued for about 1 hour at approximately 190°C.

   If heating is discontinued as soon as the colour change occurs, resistant materials such as methionine will not be completely oxidized. At the completion of oxidation, there should be approximately 1 mL of perchloric acid remaining.
6. Allow flasks to cool.
7. Wash glass funnels and inside of flask with distilled water using a squeeze bottle, and make volume up to 50g by weight.
8. Mix thoroughly. Allow to stand overnight and then transfer solution (minus particulate matter) to a vial ready for analysis by ICP or autoanalyser.

Washup

Rinse flask in running tap water and leave to soak overnight in a 2% Decon solution. Rinse three times in tap water, then rinse in distilled/deionised H_2O three times, invert and dry.

Safety

Bromine is corrosive or vesicant to skin and other tissues. Its vapours should not be inhaled. Instruct others to keep at a safe distance. Use fume hood or wear breathing apparatus and gloves.

Spread soda ash or sodium bicarbonate liberally if a spillage occurs, and mop up cautiously with plenty of water; run this to waste diluting greatly with running water.

2.7.5 Extractable Soil Sulfate Methods

A 0.01M CALCIUM MONOPHOSPHATE

This method is based on that of Searle (1979) and Barrow (1967)

Reagents

1. Saturated calcium hydroxide solution - weigh out approximately 1g of calcium oxide (CaO) and add to 200 mL of distilled/deionised water. Mix well for 5 minutes and filter through a Whatman No. 42. Collect filtrate and protect from the air in a sealed container.
2. 0.5% $CaCO_3$ solution (w/v) - weigh out 5 g of $CaCO_3$ and make to 1 litre with distilled/deionised water.
3. 0.01M calcium monophosphate solution ($Ca(H_2PO_4)_2$.
   (i) Weigh out 11.5175 g orthophosphoric acid (85%) and add to approximately 3.5 litre of distilled/deionised water in a 5 litre beaker.
   (ii) Slowly add with much stirring 1 litre 0.5% $CaCO_3$ solution. Mix until dissolved.
   (iii) Adjust the pH of this solution to pH 4.0 using the saturated calcium hydroxide solution ($CaOH_4$). Approximately 140 mL will be required for this.
   (iv) Make solution to 5 litres with distilled/deionised water.

Method

1. Weigh approximately 4g of soil into a 30 mL capacity vial. Record the exact weight of the sample to three decimal places.
2. Add 20 mL of 0.01M calcium monophosphate solution and tumble for 1 hour on an end-over-end shaker at 25°C. (Note: soil:solution ratio = 1:5).
3. Filter extracts through a Whatman No. 42 paper into a clean glass vial, being careful not to allow dust or particulate contamination to come through.
4. Analyse extracts on the ICP.

5. Should storage be required, store in the cold room and analyse within a week of extraction.

Standard Preparation

(i) Using 10 mL of 1000 µg/mL sulfate standard, make to 100 mL with **extraction solution**. This will give a 100 µg/mL sulfate stock.

(ii) Make a range of working sulfur standards as follows:

Standard (µg/mL)	mL of 100 µg/mL S	Final volume (mL)
0	0	200
1	2	200
2	4	200
5	10	200
8	16	200
10	20	200
12.5	25	200
15	30	200

These standards should be made to the final volume using the extracting solution.

B 0.01 M POTASSIUM DI-HYDROGEN ORTHOPHOSPHATE

This method using potassium di-hydrogen orthophossphate (pH 4.0), has been adapted to allow adjustment of the extraction solution to the pH of the soil by using potassium di-hydrogen orthophosphate.

Reagents

1. 5N potassium hydroxide (KOH) - dissolve 56.1g of potassium hydroxide in approximately 150 mL of distilled/deionised water. When cool make to 200 mL.
2. 0.01M Potassium dihydrogen orthophosphate (KH_2PO_4) - dissolve 1.3609g of potassium dihydrogen orthophosphate in approximately 800 mL of distilled/deionised water. When dissolved, adjust the pH of this solution to that of the soil (1:5 soil:0.01M $CaCl_2$) with 5N KOH. Make to 1 litre with distilled/deionised water.

Method

1. Weigh approximately 5g of air-dried soil into a 50 mL centrifuge tube. Record the weight of the sample to three decimal places.
2. Add 25 mL of 0.01M KH_2PO_4 solution and shake on an end-over-end shaker for 2 hours at 25°C.
3. Centrifuge extracts at 3000g for 10 minutes.
4. If necessary, filter using Whatman No. 42 filter papers.
5. Collect the filtrate and analyse for phosphate-extractable sulfur.

C 0.25M POTASSIUM CHLORIDE - HEATED

Reagents

(**NOTE:** In the original paper 2M KCl was used as the extractant, however, this causes "salting up" of the ICP torch during analysis. A study showed that 0.25M KCl avoided this problem without affecting the extractable sulfur recovered.)

1. 0.25M potassium chloride (KCl) - weigh out 18.64 g of KCl and make to 1 litre using distilled/deionised water.

Method

1. Weigh approximately 3 g of soil into a 30 mL capacity polycarbonate or glass screw-top vial. Record the exact weight of the sample to three decimal places.
2. Add 20 mL of 0.25M KCl and screw the lid on tightly.
3. Heat in an oven at 40°C for 3 hours.
4. Filter extracts through a Whatman No. 42 paper into a clean glass vial, being careful not to allow dust or particulate contamination to come through.
5. Analyse extracts on the ICP.
6. Should storage be required, store in the cold room and analyse within a week of extraction.

Standard Preparation

As per 0.01 monocalcium phosphate method except use 0.25M KCl to make to volume.

2.7.6 Determination of Sulfate in Solution

Introduction

The three methods of determining sulfate are based on a turbidimetric measurement of a barium sulfate precipitate. They can be used for a range of solutions containing sulfate. These include total S in plant material after digestion with nitric/perchloric acid, total S in soil after digestion with nitric/perchloric acid or potassium dichromate/nitric/perchloric, or sulfate in solution from soil or plant extracts.

A AUTOMATED METHOD

Reagents

1. Barium Chloride/Polyvinyl Alcohol (PVA): Dissolve 60 g of $BaCl_2.2H_2O$ in 500 mL distilled/deionised water; dissolve 2 g PVA in 400mL water with heat, stir continuously while heating to the boil. Allow to cool and mix both solutions. Make to the final volume of 1 litre. Filter before use with Whatman 42. This reagent will keep for 1-2 months if kept in the refrigerator when not in use.
2. Acid Mixture: To approximately 800 mL of water (distilled/ deionised), add 50 mL of glacial acetic acid, 20 mL of 10N hydrochloric acid, 20 mL of 85% orthophosphoric acid and 6 mL 1:1000 concentrated sulfuric acid/water. Make to final volume of 1 litre. This reagent will keep for 1-2 months if kept in the refrigerator when not in use.
3. Wash Solution: Mix one portion of concentrated hydrochloric acid to ten portions of distilled/deionised water, i.e. 1:10 v/v. This is an approximately 1N solution.

Manifold Design

The basic manifold design is given in Figure 2.3. An essential part of this manifold is the between sample flush, which is used to prevent the build-up of $BaSO_4$ precipitate. The flush can be achieved with the connections shown in the manifold and an extra micro-switch activator disc on the sampler. This micro-switch produces the required flush by either: (i) activating a solenoid which allows the wash solution to flow through the cell, or (ii) activating a pump which pumps wash solution through the cell.

NOTE:

1. The side arm of the D1 connector is fine bore which restricts mixing of the sample and wash streams when the flush is not operating. When activated, the flush of acid solution more than doubles the volume of solution flowing through the cell, thus removing any barium sulfate (see example traces overleaf). The flush should be timed to start when the sample trace reaches nearly to the baseline, and it should last for approximately 20 seconds - one-quarter the normal full wash cycle.
2. The length of the sample and $BaCl_2$/PVA pickup lines need to be adjusted to ensure that the sample and reagent interfaces arrive at point (A) simultaneously. Poor adjustment results in incorrect peak shapes.
3. Sample:wash times are set at 35 seconds : 85 seconds, respectively.
4. PVA is added to disperse the $BaSO_4$ precipitate removed from the flow cell, etc.
5. A low level of sulfate (6 mL of 1:1000 concentrated H_2SO_4 to 1 litre) is added to the diluent to increase the sensitivity and improve the linearity of the response of the method at low-S concentrations.

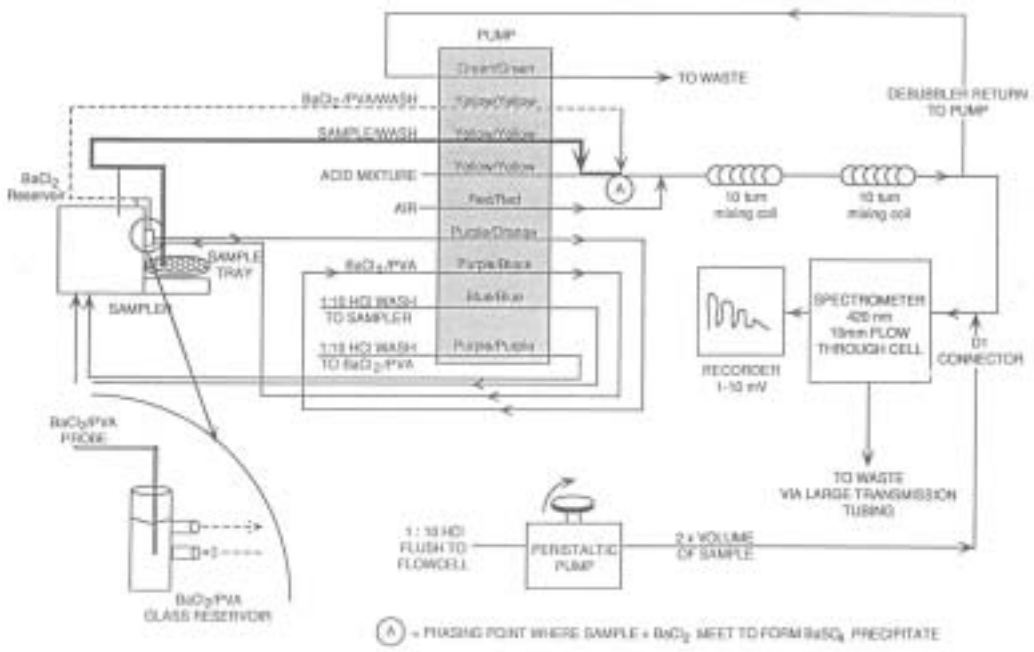

Figure 2.3. Autoanalyser manifold to measure 0-20 ppm S in solution.

B SEMI-AUTOMATED METHOD

Introduction

A semi-automated method, which should be easier and more repeatable than the manual method, can be used if a full auto-analyser system is not available but a flow cell for the spectrophotometer and a peristaltic pump with a minimum of 4 tube capacity are available; a chart recorder connected to the spectrophotometer is also useful.

Procedure

The reagents for the manual method are used with the four peristaltic pump tubes being used to pump the following:

Tube 1 - sample solution
Tube 2 - $BaCl_2$/PVA solution
Tube 3 - Sulfur diluent solution
Tube 4 - open to the air

Tubes 1, 2 and 3 should be the same size; tube 4 can be a smaller bore tube. The air bubbles are introduced to aid mixing.

Between samples, tubes 1 and 2 should be placed in a wash solution of 1:10 v/v HCl/water. This should wash the cell out effectively, but if it is not sufficient, a fine bore side arm can be put in just before the cell and a syringe used to pump a larger volume of wash solution through the cell. A debubbler and several mixing coils will be required and some form of temperature control should improve the repeatability.

C MANUAL METHOD

Reagents

1. Barium Chloride/Polyvinyl Alcohol (PVA): Dissolve 60 g of $BaCl_2.2H_2O$ in 500 mL distilled/deionised water; dissolve 2 g PVA in 400 mL water with heat, stir continuously while heating to the boil. Allow to cool and mix both solutions. Make to the final volume of 1 litre. Filter before use with Whatman 42. This reagent will keep for 1-2 months if kept in the refrigerator when not in use.

2. Acid Mixture: To approximately 800 mL of water (distilled/ deionised), add 50 mL of glacial acetic acid, 20 mL of 10N hydrochloric acid, 20 mL of 85% orthophosphoric acid and 6 mL 1:1000 concentrated sulfuric acid/water. Make to final volume of 1 litre. This reagent will keep for 1-2 months if kept in the refrigerator when not in use.

Procedure

1. Pipette 5 mL of the solution to be analysed into a vial with a sealable top.

2. Add 5 mL of the acid mixture. Mix.

3. Add 5 mL of the barium chloride/PVA solution.

4. Seal the vial.

5. Shake the vial twenty times and let stand for 30 seconds only.

6. Pour into a spectrophotometer cuvette immediately and read absorbence at 420 nm.

Standard Preparation

1. Prepare a set of standards and a reagent blank in the range 0-20 g/mL S, containing the same concentration of all other chemicals used for the unknowns.

2. Calculate the concentration of sulfur in the unknown samples from the standard curve.

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CHAPTER 3

SAFETY PROCEDURES WHEN USING ^{35}S

3.1 INTRODUCTION

Because of the weak strength of the β particle (E_{max} 0.167) only thin window Geiger tube monitors can be used to detect sources of ^{35}S and their efficiency is poor. Precautions need to be taken to ensure safety. The low β energy and hence the range of the particle means that protection from external radiation is minimal. The low β energy is a bonus when working in virtually all soil-plant systems because of the short range of the β particles in air and because of the absorption of the radiation by the bulk of the soil and plant material.

When working with nuclides, adequate warnings must be placed to remind the operator and, more importantly, others of the radiation hazard. These warnings may be as simple as a handwritten sign. Remember, the operator is aware of the hazard but others are not and they could unknowingly start using the contaminated area or equipment if no warning is given. ***This is very important.***

The standard of cleanliness in a laboratory used for radioactive work must be higher than normal. Messy, untidy and casual workers are not only a hazard to themselves, but to others. Precautions must be maintained at all times.

3.2 RADIOISOTOPE LABORATORY SAFETY RULES

The following protocols are intended to minimize internal and external hazards and to prevent contamination of the laboratory.

- Coats and other personal belongings, including books (except those required for work), should not be brought into the laboratory where they may become contaminated.
- Eating, drinking, storing or preparing food, smoking, applying cosmetics, biting fingernails or using gummed stick-on labels should be forbidden in any area where radioactive materials are stored or used. In brief, avoid any action that involves placing hands near the mouth.
- Direct contact with radioactive materials must be avoided by using protective laboratory coats and wearing rubber or disposable gloves. Such protective clothing should be reserved for this purpose and not be removed from the laboratory.
- Pipetting liquids of any type by mouth or the performance of any similar operation by mouth suction is not permitted. Use automatic pipettes or burettes instead.
- Complete records of receipts, transfers and disposals of radioactive materials must be kept.
- A film badge should be worn at all times when working with radioactive materials. The weak β particle emitted by ^{35}S means that this cannot generally be detected by such badges.
- Work should be carried out under a hood in all cases where radioactive material may be lost by volatilization, dispersion of dust, or by spraying or splattering. Dry and dusty operations, such as grinding of plant material, are inherently more hazardous than simple wet chemical operations. A disposable face mask must be worn at all times.
- All radioactive samples should be properly labelled with the isotope, activity and activity date. They should be sealed.
- Liquid wastes should not be poured into the drain or contaminated apparatus washed in the sink unless the levels of activity entering the sewer system have been calculated as permissible (see the following section on disposal).
- Every procedure must be thoroughly rehearsed using inactive material so that problems can be resolved before active materials are in use.
- Plastic or stainless steel trays, deep enough to contain all the material, lined with sufficient absorbent material to soak up all the liquid in the trary should always be used to catch spillages. Polythene sheeting should be spread on the bench top, particularly if it is porous, to prevent radioactive liquids soaking in.

- The disposal of solid wastes and contaminated articles (corks, paper, wipers, etc.) should be consigned to specific containers and, under no circumstances, into ordinary waste bins.
- The storage of all radioactive material must be in properly designated locations.
- At the close of a working period, the laboratory work surfaces should be carefully monitored.
- Before leaving the laboratory after working with active materials, each person should wash his/her hands with a "surgeon's care". If necessary, lightly scrub with a nailbrush. Avoid breaking the skin as direct radioactive contamination can be transferred to the bloodstream. Extreme care must be taken because of this danger when a person has an open wound.
- When handling apparatus or instruments, electricity, gas or water controls while performing a radioactive experiment, disposable paper towels should be used. If this is not done, maintenance staff and technicians may unknowingly become contaminated.
- Suitable contamination monitoring equipment must be available in the laboratory whenever active materials are in use, e.g. Geiger-Müller counter.

3.3 ACCIDENTS AND EMERGENCY PROCEDURES

In the event of exposure of any person to ionizing radiation, the Radiation Safety Officer must be informed immediately. In the event of a radioactive spill where permanent structures are contaminated, such as laboratory benches and floors, the Radiation Safety Officer must also be informed. Great care must be taken to avoid spreading the contamination.

3.4 DECONTAMINATION

Accidental contamination will occur despite the strictest precautions and adherence to the appropriate Radioisotope Laboratory Safety Rules. Hazards from radiotracers are primarily contamination of the laboratory rather than threat to the health of the radioisotope user.

3.4.1 Laboratory Glassware/Plasticware

Implements that have become contaminated must be thoroughly washed to eliminate both the radiation hazard and the possibility of cross-contamination of successive experiments. There is a considerable tendency for the absorption of radioactive materials onto glass. To reduce this problem, never allow radioactive solutions to dry on glass surfaces. In case of unavoidable contamination of glassware, treatment with chromic acid or 5% detergent solution is necessary. A 10% solution of EDTA is especially effective for removing radioactive metal contaminants, while 6N HCl is preferable for sulfate and phosphate radioisotopes.

Having used laboratory glassware/plasticware for radioactive work, it must be decontaminated. Initially, rinse well in large amounts of running water, then soak in a 5% detergent/water solution at least overnight. Keep the soaking tubs for radioactive work separate from the general washing-up area. Having soaked the contaminated utensils, rinse thoroughly in running water. A general procedure is 4 times in tap water and 4 times in distilled water before returning it to general circulation.

3.4.2 Laboratory Equipment

Laboratory equipment, such as balances, hotplates etc. that cannot be immersed in chemical solutions should be protected as much as possible by aluminium foil or plastic sheeting that can be discarded. Hand-held automatic pipettes, bottle top dispensers, etc., can also become contaminated during radioactive work and must be thoroughly cleansed by repeated flushing with a 5% detergent/water solution. Renew this solution a couple of times while cleaning to ensure that contamination is removed. All equipment should be thoroughly checked for contamination before it is returned to general use. This can be done by squeezing the liquid from the tissue and counting it in a scintillation counter. This swab should read no more than twice background before the equipment is returned to general use.

3.4.3 Minor Spills

For minor spills, where the spill is contained in a safety tray and provided there are no other complications, the operator (wearing rubber or plastic gloves) should proceed as follows:

- *Wet spill*: the liquid should be absorbed by blotting paper or similar material.

- *Dry spill*: the material should be carefully wiped up with absorbent tissue moistened with water.
- After all visible signs of the radioactive material have been removed, the tray must be monitored.

Contamination of surfaces with ^{14}C, ^{3}H and ^{35}S can be readily detected by repeatedly pressing cellulose tape, sticky side down, onto the surface, placing the tape in a counting vial with the appropriate solution, and counting in a scintillation counter.

3.4.4 Solid or Liquid Radioactive Material in the Eyes

Irrigate with saline (0.9% common salt solution). If this solution is not available, use tap water.

3.4.5 Decontamination of Hands

- Scrub lightly with soap and water. Do NOT use abrasive materials and/or vigorous scrubbing.
- If this fails, titanium dioxide paste may be applied and subsequently washed off with soap and water.
- If this fails, try EDTA/soap mixture.
- As a last resort, immerse the hands in saturated potassium permangate solution, rinse in water and remove stain with a 5% solution of sodium bisulphite.

The decontamination procedures should be halted if there is a risk of radioactive material entering the bloodstream through roughened or broken skin.

3.4.6 Decontamination of the Skin other than Hands

- Rub the contaminated area gently with "Cetavolon" (or similar antiseptic). Cetavolon contains cetrimide, a surface active quaternary ammonium compound with antiseptic, emulsifying and detergent properties.

3.4.7 Radioactive Materials in the Mouth

- Wash out several times with diluted hydrogen peroxide solution (20 mL of 3 % [10 volume] hydrogen peroxide in 250 mL of water). **Take care**: the laboratory stock of hydrogen peroxide may be 30% ("100 volumes") strength.

3.4.8 Decontamination of a Wound

- Wash under a tap with copious quantities of water and encourage bleeding.
- If the wound is on the face, take care not to contaminate the eyes, mouth or nostrils.
- Next, wash the wound with soap and water and apply a first-aid dressing.
- Seek medical advice.

3.5 DISPOSAL OF RADIOACTIVE WASTE

Laboratory use of radioactive isotopes inevitably results in radioactive wastes (i.e. paper towelling, disposable implements, soil, plant material, plastic sheeting, plant and soil digest solutions, etc.) that must be disposed of without endangering the public. Possible routes of disposal are into sewers, by incineration, or by ground burial. Disposal of radioactive waste at public rubbish dumps must be organised through the appropriate authorities. Hopefully, the means of disposal required will be reasonably convenient and economical.

Two general policies are usually followed in waste disposal: (i) maximum dilution, or (ii) maximum concentration. Disposal by dilution usually involves release into the sewer or air, or incineration of the wastes. Concentration and storage of radioactive wastes may be necessary for materials that cannot be disposed of by dilution, such as carcasses, soils and other solid wastes.

Short-lived radioactive materials can be stored until their activity has decayed to a sufficiently low level for disposal as inactive waste. It can easily be calculated that after ten half-lives, the original activity will be reduced by a factor of 1000.

3.5.1 Disposal of liquid wastes

Aqueous waste can be disposed of down the sink with running water, provided the levels comply with the relevant local regulations. An indication of the amounts of ^{35}S that can be disposed of in this way is presented in Table 3.1.

Table 3.1. An indication of the permissible levels of the disposal of some radioactive isotopes.

Form of ^{35}S	Concentration (Bq/cm^3)
Soluble	75
Insoluble	375

The following can be used to calculate how may days until waste may be disposed of safely.

Time to safe disposal (t)= $-1/\lambda \ln (C_s/C_c)$ where C_s is the safe disposal concentration and C_c the current concentration.

3.5.2 Disposal of Solid Waste

- ***Gloves, masks, plastic bags, paper towels, ground plant material (in vials):*** should be placed in two strong plastic bags, labelled clearly as radioactive, and taken to an approved disposal site for burial after making arrangements with the appropriate authorities.
- ***Scintillation counting vials:*** the solvent in counting vials is usually toluene; it is flammable and hazardous in its own right. Vials should be emptied into strong plastic containers (e.g. 20L drum with lid) and both the contents and vials taken to the dump for burial.
- ***Soil:*** should be placed in 2-3 strong plastic bags, each being individually sealed, then taken to the dump for burial.
- ***Sharp objects (e.g. needles, pasteur pipettes, etc.):*** should be placed in a sealed plastic bottle, then taken to the dump for burial.

3.6 MEDICAL EXAMINATIONS

All persons who are handling potentially hazardous radioactive material should have medical examinations. The frequency of these medical examinations will depend on the activity and type of radioactivity handled.

CHAPTER 4

PREPARATION OF LABELLED SULFUR FERTILISERS AND PLANT MATERIAL

4.1 PREPARATION OF RADIOACTIVELY-LABELLED GYPSUM

Gypsum ($CaSO_4.2H_2O$) is prepared by mixing calcium chloride and sulphuric acid. A thick white precipitate is formed which is dried and ground to a fine white powder.

If care is taken with precipitate transferral and rinsing, a recovery of > 95 % can be achieved.

Before attempting to make the labelled gypsum it is best to have a trial run with 'cold' chemicals. This allows you to become familiar with the method and recovery.

Safe working practices must be adhered to at all times.

4.1.1 Equipment/materials

- $CaCl_2.2H_2O$.
- H_2SO_4 (98%).
- Beakers.
- Stirring rods.
- Evaporating dish.
- Wash bottle.
- ^{35}S - as sulphate in aqueous solution.
- Trays.
- Geiger Muller counter.
- Pasteur pipettes and bulbs.
- Plastic bags.
- Plastic sheeting.
- Radiation stickers.
- Elastic bands.

4.1.2 Method

- If the reaction would proceed stochiometrically then the quantities of calcium chloride and sulfuric acid required can be calculated from the following equation:

$CaCl_2.2H_2O + H_2SO_4 \rightarrow CaSO_4.2H_2O + 2HCl$
Molecular weights 147.02 g + 100.08 g $\rightarrow$ 172.17g
If 10 g $CaSO_4.2H_2O$ is to be prepared,
then the amount of $CaCl_2.2H_2O$ required = 147.02/172.17x10 g = 8.54 g
The amount of H_2SO_4 required = 100.08/172.17 x 6.885 g = 4.00 g H_2SO_4
Experience has shown that it is very difficult to dry $CaCl_2.2H_2O$ so an excess of 10 to 30 % is required

- Weigh $CaCl_2.2H_2O$ into a beaker and dissolve in a small amount of distilled/deionised H_2O.

- Weigh H_2SO_4 into a beaker. H_2SO_4 needs to be diluted approximately 1:6 with H_2O otherwise the precipitate forms too quickly when mixed with solution of $CaCl_2$.

- Divide the H_2SO_4 into two containers, one containing the majority. Mix the ^{35}S with the H_2SO_4. This can be done by adding the ^{35}S into the larger of the two amounts of H_2SO_4 and then rinsing the hot vial several times with the other H_2SO_4 into the now labelled H_2SO_4. If the reaction proceeds too fast the product will be a mixture of $CaSO_4$ and $CaSO_4.1/2H_2O$, not $CaSO_4.2H_2O$.

- Slowly add the labelled H_2SO_4 to the $CaCl_2.2H_2O$ with gentle stirring. The precipitate should develop gradually. Rinse the $CaCl_2.2H_2O$ into the H_2SO_4 with H_2O.

- Mix well to get a smooth, sloppy paste.
- Separate the precipitate by filtration and wash the precipitate with a minimum amount of water to remove excess $CaCl_2$.
- Carefully transfer the precipitate to a pre-weighed evaporating dish. The precipitate is best dried at room temperature in a vacuum descicator, or can be placed in a fan-forced oven at 25-30°C to speed up drying.
- While gypsum is drying, stir regularly to prevent precipitate drying into a hard lump. As it dries, crush up finely.
- When the gypsum is completely dry, weigh dish and contents to determine % recovery.
- Store gypsum in a clearly labelled vial stating:

  (i) contents
  (ii) user
  (iii) activity
  (iv) date

  The labelling is important. It should alert other persons to the content's radioactive danger.

4.2 PREPARATION OF ^{35}S LABELLED ELEMENTAL SULFUR

Elemental sulfur is prepared by dissolving cold crystalline elemental sulfur in toluene, adding ^{35}S, then recrystallising the sulfur. Once dry, the sulfur is sieved to size (150 -250 μm).

If care is taken with sulfur preparation and sieving, a recovery of >90% can be achieved.

Before preparing the labelled elemental S, a trial run should be carried out.

4.2.1 Equipment/materials

- S-free toluene
- ^{35}S - as elemental sulfur.
- Beakers.
- Hotplate.
- Foil.
- Pasteur pipettes and bulbs.
- Trays.
- Plastic bags.
- Plastic sheeting.
- Radiation stickers.
- Elastic bands.
- Geiger-Müller counter.

4.2.2 Method

- Determine amount of elemental S fertiliser required. Add 10-20% to allow for sample loss during preparation.
- Weigh cold, crystalline elemental S into a small beaker.
- Take a small portion of this elemental S and transfer into another beaker. This portion will be used for rinsing the ^{35}S into the larger portion.
- To both beakers of elemental S add just enough toluene to dissolve crystals. Generally, 1mL toluene/150 mg elemental S. A third container of approximately 5-10mL toluene is also required.
- On a foil-covered hotplate, gently heat the larger elemental S portion to dissolve. Agitate gently and avoid splashing crystals up the sides of the beaker.
- When the larger portion is almost dissolved, start heating the smaller portion. This should dissolve very quickly. The extra toluene can also be heated at this stage.

- Keep both portions warm but do not boil. Do not allow to cool, otherwise the sulfur will recrystallise.
- Using a pasteur pipette, transfer one addition of the warmed smaller portion to the ^{35}S crystals. The ^{35}S vial may be placed upon the hotplate to aid dissolution if the warmed addition is not effective. Once dissolved, transfer the ^{35}S to the larger portion. Continue rinsing the ^{35}S vial by adding the warmed smaller portion and transfering to the larger portion. Rinse the ^{35}S vial several times with the third container of warmed toluene to ensure maximum transferral.
- Mix the final solution well. Remove from heat. Crystals should start developing quickly but it may take several days for the excess toluene to evaporate. To speed up evaporation, leave sample uncovered in fume hood with fan operating.
- When crystals are completely dry sieve to size. Any crystals which fall outside the required dimensions may be redissolved and sieved again to boost recovery.
- Keep a check of the recovery by noting (i) final weight of elemental S liberated, and (ii) activity of the elemental S. This can be done by taking a known weight, dissolving in scintillation fluid, heating if necessary, and counting by liquid scintillation.
- Store crystals in a clearly labelled vial stating:

  (i) contents
  (ii) user
  (iii) activity
  (iv) date

4.3 PREPARATION OF ^{34}S LABELLED H_2SO_4 OR GYPSUM

^{34}S elemental S is commercially available but $^{34}SO_4$ sources are not. The sulfate form is required in many studies and the following method has been developed to convert elemental S to sulfate.

4.3.1 Principle

The reaction $6HNO_3 + S \rightarrow H_2SO_4 + 6NO_2 + 2H_2O$

4.3.2 Method

- Weigh 0.5 g of elemental S into a digestion tube.
- Add 2.0 mL of 65% HNO_3 (SG 1.4).
- Place a small stemmed funnel in the top of the tube.
- Heat in an aluminium digestion block in a fume hood to exhaust the NO_2 evolved at 80°C for 1hr. Raise the temperature of the block to 130°C and maintain this for 7 hours.
- Add a further 0.5 mL of 65% HNO_3 (SG 1.4) and continue heating overnight.

Theoretically the 0.5 g of elemental S should be completely oxidised by 1.79 mL of 65% HNO_3. When only this amount of acid was added complete oxidation did not result and it was found necessary to add a total of 2.5 mL.

The low temperature at the start of the oxidation was necessary to prevent the elemental S from being pushed up the tube on top of the evolved NO_2.

The H_2SO_4 can be converted to gypsum by neutralising the solution and adding excess $CaCl_2$ to the cooled solution. The solution and precipitate of $CaSO_4.2H_2O$ are filtered through a Whatman 42 paper and washed several times with distilled water. The paper and precipitate is dried in an oven at 40°C. The temperature of drying must be kept low to avoid loss of water of hydration.

4.4 PRODUCTION OF ^{35}S LABELLED PLANT MATERIAL

^{35}S labelling of plant material for use in organic matter turnover experiments has successfully been accomplished by Basilio-Sanchez et al. (2000). This was achieved by adding 10 mL of solution 93 KBq ^{35}S/mL every 4 days for 6 weeks to the surface of the pot with a syringe before watering. The pots had bases below them to catch any solution that inadvertently leaked through the pot and this

was returned to the pot. The S concentrations and specific activity of the two materials produced by this method are presented in Table 4.1.

Table 4.1. Dry matter, nutrient concentration and specific activity of flemingia leaves and medic hay after 6 weeks exposure to $^{14}CO_2$.

Plant Material	Dry matter yield (g)	S (%)	Specific Activity (MBq^{35}S/g S)
Flemingia leaves	184.25	0.20	27.80
Medic hay	154.27	0.35	38.64

4.5 PRODUCTION OF ^{34}S LABELLED PLANT MATERIAL

Because of the cost of ^{34}S it is necessary to recover as much applied ^{34}S in the plant as possible. This has been achieved by growing the plants hydroponically in the solution listed in Tables 6.8 and 6.9 in these guidelines. Elemental S with 99.5% enrichment was oxidised by the method shown in Section 4.3. and 12mg S/L was added to the nutrient solution. Because the addition was as a mixture of sulfuric and nitric acid the pH of the solution had to be adjusted. This was achieved by adding 0.079g NaOH /L of solution.

REFERENCE

Basilo-Sanchez, P, Blair, GJ, Till AR and Faint, M (2000). Production of Labelled plant materials to trace the fate of Residue-Derived Carbon, Nitrogen and Sulfur. Pp 39-40 in “Proceedings International Symposium on Nuclear Techniques in Integrated Plant Nutrient, Water and Soil Management”, IAEA, Vienna, October 2000.

CHAPTER 5

ESTIMATING THE QUANTITY OF ISOTOPE TO ADD TO EXPERIMENTS

5.1 INTRODUCTION

Some of the difficulties faced when planning experiments utilizing ^{35}S is to understand the system in which you are working and to decide how much isotope is required for the study. This chapter aims to assist in overcoming these problems.

5.2 SELECTION OF APPROPRIATE ISOTOPIC TRACERS

Many elements have naturally occurring stable and radioactive isotopes and the relative abundances of these may change due to a range of physical, chemical and biological processes. As the development of sensitive analytical tools progresses there is increasing scope to make better use of these naturally available isotopic tracers.

Isotopic tracers can be used in various ways such as:-

a) Just to trace where an element goes.

b) By simple isotope dilution to estimate volumes and flows.

c) Use dilutions and chemical forms to show precursor/product relationships and rate processes. These will include the 'direct method' where labelled material is added and its use in other components studied, and the 'reverse dilution' where changes in labelled systems, as a result of treatment with non-labelled materials, are used to study processes.

d) Various other combinations of the above including changes in introduced and natural isotope ratios.

Where possible radiotracers are determined using Liquid Scintillation Counting (LSC) or Gamma (γ) Spectrometry

There is a wide range of radioactive tracers available for use in soil/plant/animal studies.

1) Having defined the particular problem make sure that it is **absolutely essential** to use isotopic methods.

2) If essential, establish what potentially suitable stable or radioactive isotopes are available, and their cost etc.

3) Unfortunately the estimation of requirements demands starting with the available analytical techniques and working backwards through experimental options to see what is feasible in terms of SAFETY and COST.

In the case of S the primary choice is between ^{35}S and ^{34}S. Because of the cost difference between the two isotopes and the sensitivity of measurement (see Section 2.2.2) ^{35}S would generally be the preferred isotope. Restrictions on the use of radioactive isotopes may mean that ^{34}S has to be used. Where dual labels are required 34S should be used to label the component, which has to be traced over the longest time period or in the component with the highest activity.

Because of the relative low sensitivity of ^{34}S analyses enriched sources of ^{34}S will be easier to trace than sources with natural abundance.

5.3 EXPERIMENTS USING RADIOISOTOPES

All proposed studies must be discussed with supervisors/colleagues and have the approval of the Radiation Safety Officer. Before starting any experiments users must have had training in SAFE handling and disposal of residual material. Whenever working with radioactive material users must wear a film badge and appropriate protective clothing. No one should ever work alone in the 'high level' laboratory, or use it for storage of low level samples.

Radioactive decay must be allowed for from the time of ordering to the completion of all radioassays.

5.3.1 Radio-Assay

Much useful information can be gained simply by measuring the amount of nuclide in a particular material but a great deal more important information can usually be derived from comparisons of Specific Radioactivity (SR) in the different parts of the system being studied.

Where possible counting is performed on a portion of the same solution that is used for chemical analysis. This is very important in keeping errors to a minimum as it eliminates the errors that arise from variation in concentration in the original sample ie. the determination of SR does not require quantitative recovery so it is not affected by the variation in the amount of component in sub-samples.

5.3.2 Observed counts

Radioactive decay is a random process and for any particular nuclide the $T_{1/2}$ is constant, but the probability of a particular atom undergoing decay is random and the observed count rate is not regular.

A consequence of the random nature of radioactive decay is that the variance of any observed total count is the square root of the total count. Therefore to obtain a count rate with a 1% standard deviation it is necessary to count a sample until at least 10,000 counts are registered

5.4 A MODEL TO EXAMINE THE SPECIFIC RADIOACTIVITY OF S WHEN ORDINARY DILUTION IS USED

Model structure

A simple S cycle (*Figure 5.1*) was used to develop a model, written in EXCEL, to track changes in total S and its specific radioactivity in various S pools in plants and soil.
In the cycle and model the large capitals represent the pools. The flows, such as FFA, represent a flow from F to A. It is assumed that the pools are uniformly mixed and there are no isotope effects in the flow processes. In the model as used here the individual flows are taken as first order.

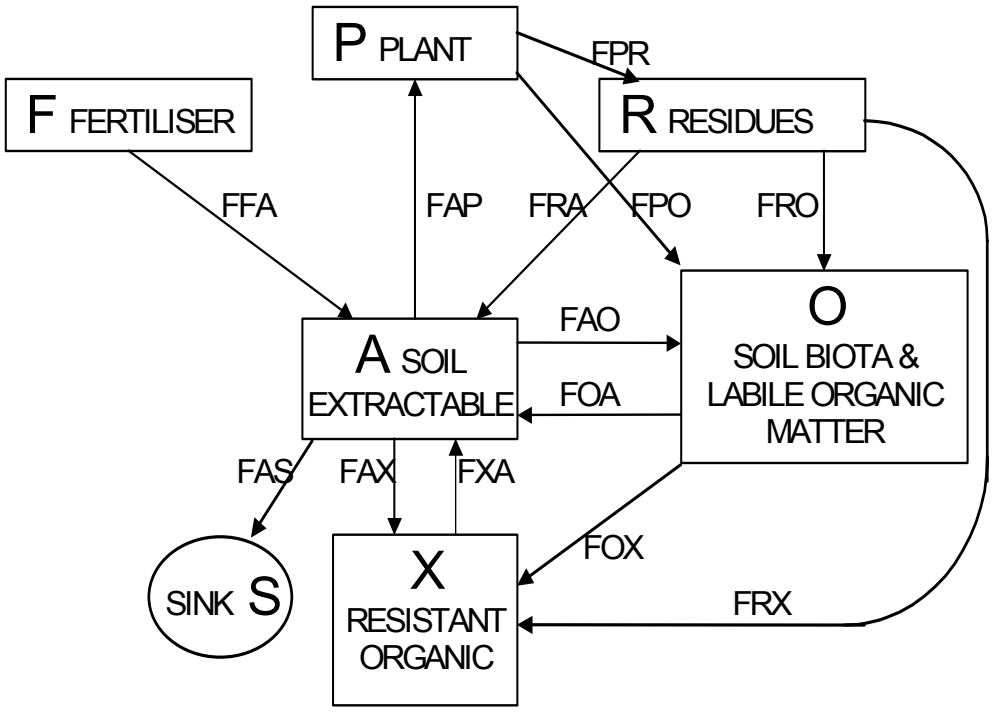


Figure 5.1. Pools and flows of the S cycle used in the computer model. The boxes represent pools and the arrows flows.

The program allows the same model to be run with the ability to introduce the tracer into any pool. The EXCEL version is constrained to run for 100 intervals with the interval length controlled by the TOTAL run-time required.

In the original model the X pool was just put in to allow simulation of leaching or some other pool such as inorganic P if you wanted to turn it into a P model. However in developing the model it became necessary to use it as pool of resistant OM and the original O pool as more labile material (biota and ester fractions) to allow sufficient transfer of tracer into the soil.

The Ordinary Dilution model can be accessed at:
http://sciences.une.edu.au/c-sfs/isotopic_tracer.asp

In the working model different colours are used to place emphasis on various operations e.g.-

Miscellaneous notes and/or action to be taken are in **RED**

Entries that can be changed are shown in **BLUE**

Do not change values shown in **BLACK** as they are frequently linked to other parts of the model.

Model parameters and output

An example of the model inputs and outputs is presented below (Table 5.1 and *Figure 5.2*). The printout cannot be used as a working model and only serves to give an example of its operation. Unfortunately the different colours used in the model cannot be shown in this example.

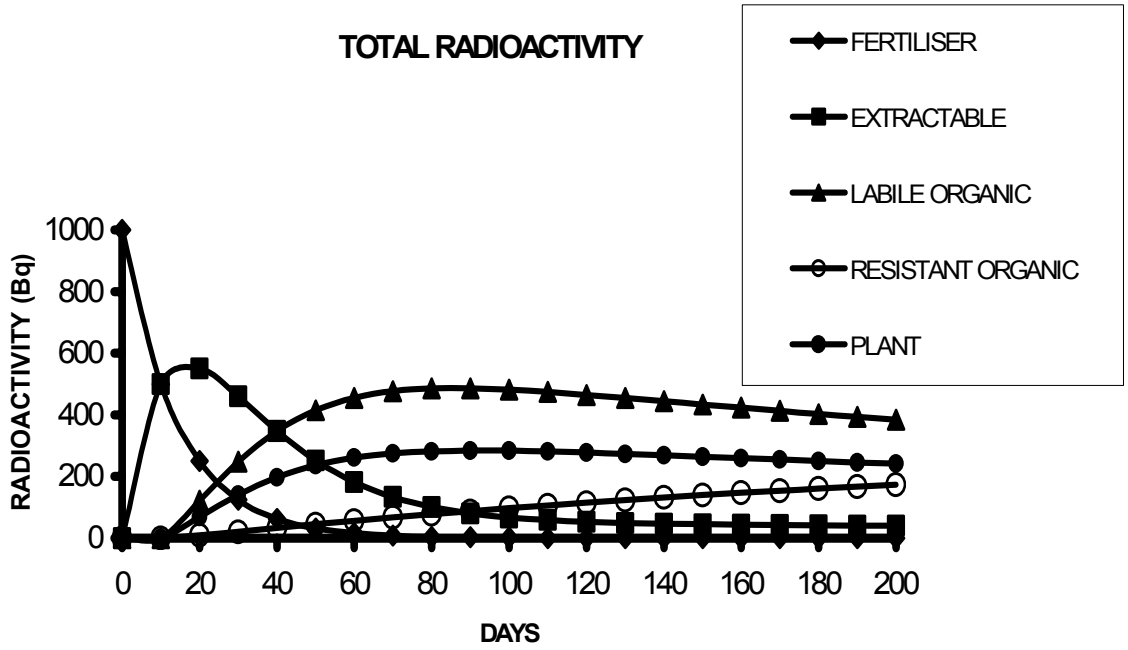
Table 5.1. Computer screen image of the ordinary dilution model. The letters refer to the pools and pathways in *Figure 5.1*.

SULFUR CYCLE ORDINARY DILUTION MODEL

POOLS		FERTILISER	EXTRACTABLE	LABILE ORGANIC	PLANT	RESIDUE	RESISTANT ORGANIC	SINK
		F	A	O	P	R	X	S
	Kg/ha	20.0	15.0	100.0	1.5	20.0	200.0	0.0
	MBq	1000	0	0	0	0	0	0
PATH	F—A	A--P	A--O	A--X	A--S	R--A	R--O	R--X
T-1/2	10	35	25	250	1000	150	200	500

POOLS		PLANT	RESIDUE	RESISTANT ORGANIC		
		P	R	X	RUNNING TIME (days)	
	DM (Kg/ha)	500	8000	200000	TOTAL	INTERVAL
	S Conc.(%)	0.30	0.25	1.00	200.0	10.0

PATH	O--A	O--X	P--R	P--O	X--A
T-1/2	200	350	200	1000	600



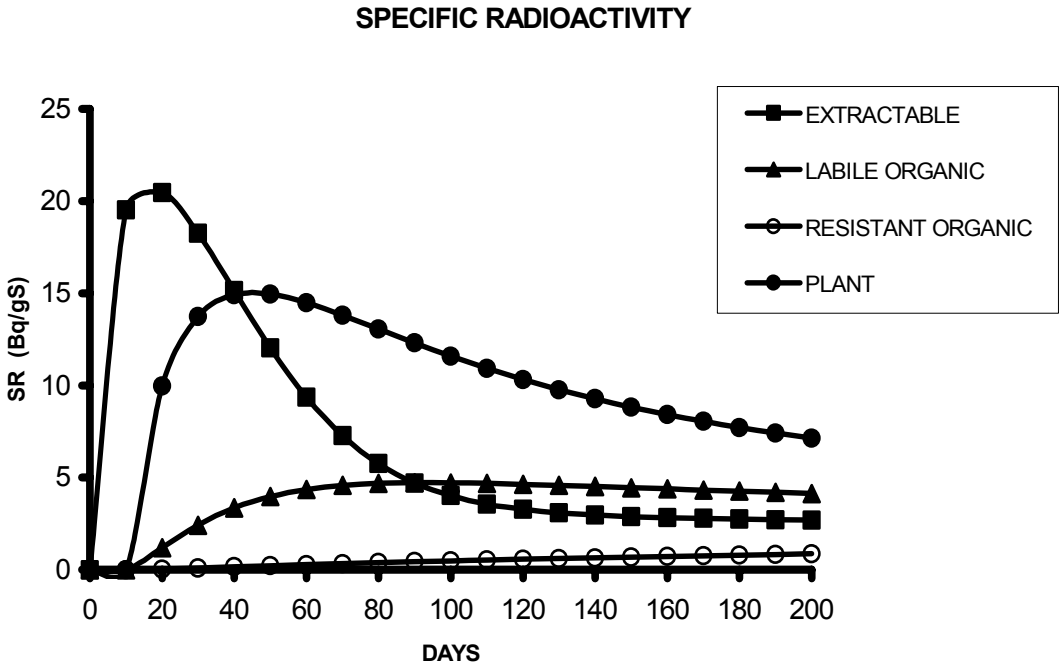


Figure 5.2. Examples of outputs of specific and total radioactivity from the ordinary dilution model.

5.5 A MODEL TO EXAMINE THE SPECIFIC RADIOACTIVITY OF S WHEN REVERSE DILUTION IS USED

Model structure and output

The reverse dilution model is identical to the ordinary dilution except that it uses a preliminary incubation period to allow the incorporation of varying amounts of tracer into the different pools. The Reverse Dilution model can be accessed at www.une.edu.au/c-sfs

An example of the output is shown in *Figure 5.3* and *Figure 5.4*.

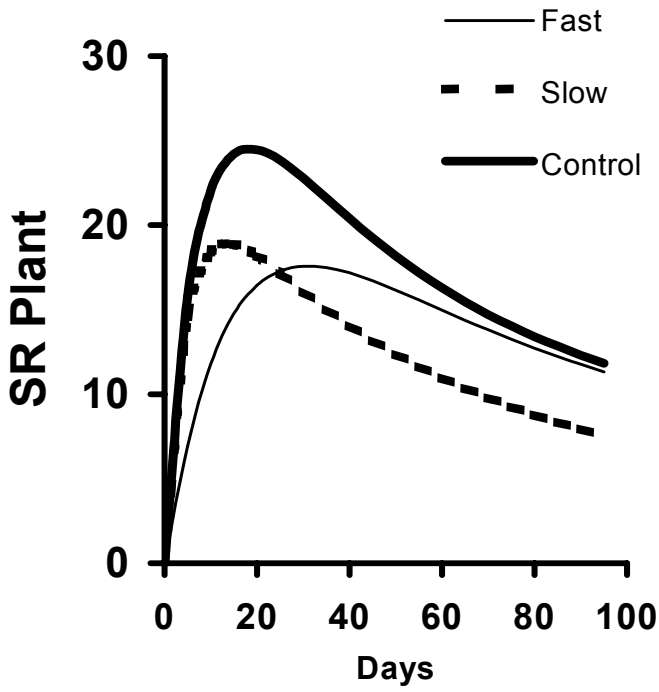


Figure 5.3. An example of plant specific radioactivity output from the reverse dilution model with a –S control treatment and fertilisers with fast and slow release rates.

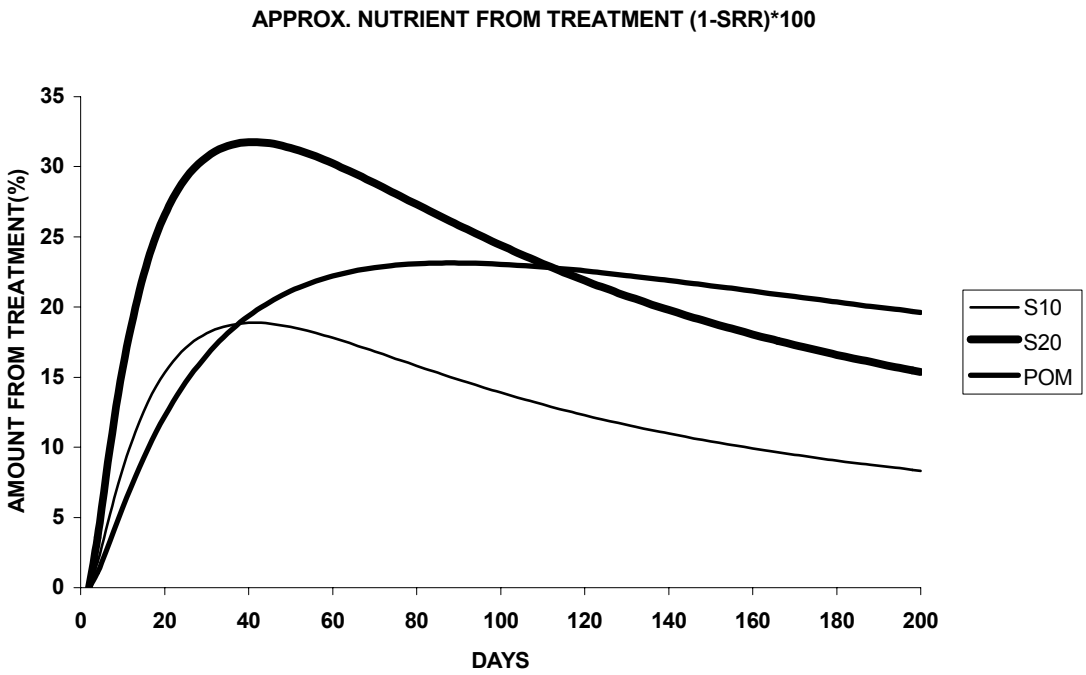


Figure 5.4. An example of output from the reverse dilution model of the percentage of S in the plant from S applied at 10 kg/ha (S10), 20 kg/ha (S20) and plant organic matter (POM).

5.6 A PROGRAM TO "GUESTIMATE" THE AMOUNT OF ^{35}S TO BE USED IN EXPERIMENTS

The 'Guestimates' presented in this program are based on previous experience and/or data from the literature. The Guestimates program can be accessed at:
http://sciences.une.edu.au/c-sfs/isotopic_tracer.asp
Miscellaneous notes and/or action to be taken are in **RED**

Entries that can be changed are shown in **BLUE**

Do not change values shown in **BLACK** as they are frequently linked to other parts of the model.

The program uses an example taken from a study conducted by an IAEA Fellow. This was a pot study but the same method is readily adaptable to field studies.

In the EXCEL program there are 10 sheets, which contain the following:

Index

Listing of the contents of the following pages.

Background

A general background to the planning and use of isotopes in soil/plant studies.

Radiotracers

Lists the properties of isotopes commonly used in soil/plant studies. This data is also provided in Table 5.1 above.

Experiment pt 1

In the program presented there are 5 levels of 6 factors. It is not necessary to use all levels or all factors. If a field is left vacant it is subsequently identified as "not used."

Space is provided to specify the time of the various operations to be undertaken in the experiment. Information is also presented on the preparation of starting materials and the methods to be used in sampling, analyses and data processing.

Experiment pt 2

This sheet calculates the amount of soil required for the specified pot dimensions and the quantities of the specified factors to be applied.

Experiment pt 3

Contains information on the influence of selecting various soils and pot shapes on the calculation of material application rates.

Tracer Guestimates

In Section 4.1 the estimated concentrations in the various S pools has to be made and in Section 4.2 plant production has to be estimated.

In Section 4.3 details of liquid scintillation counting have to be entered to provide estimates of the overall counting efficiency.

Section 4.4 relates counting efficiency to assay procedures to allow estimation of the radioactivity required in various samples at time of assay.

Section 4.5 is used for ordinary dilution and Section 4.6 is used for reverse dilution.

In Section 4.5.1 Guestimates of the fate of the applied tracers are made and these related to the SR of the various components required for LSC. Section 4.5.2 is automatically loaded from the previous inputs and calculates the application rates required.

Section 4.6 contains a similar set of tables modified for use with reverse dilution.

Section 4.7 outlines the approach to be taken with the preparation of labelled organic matter, which may be used in the experiment. If this treatment is not included in the experiment skip this section.

Shipment

This section is to keep track of the delivery and use of the isotope.

Preparation

This section details the amounts needed to prepare the labelled materials. Examples are given for gypsum, plant organic matter and reverse dilution solution.

LSC

This sheet contains the counting details including the preparation of standards, calculation of efficiency, quench correction and routine counting of samples

5.7 ESTIMATION OF THE AMOUNT OF ^{34}S TO BE USED IN EXPERIMENTS

The amount of ^{34}S used in an experiment depends on which components of the system are to be measured and the sensitivity of the measuring equipment. If for example the sensitivity of an IRMS measurement was δ2.0 for a sample size of 100 μg S then the sample being analysed would need to contain about 0.2 μg ^{34}S .

CHAPTER 6

APPLICATIONS OF ^{35}S AND ^{34}S. CASE STUDIES

6.1 STUDY OF S FERTILISER USE EFFICIENCY IN RICE

Source: Samosir SSR, Blair GJ, Lefroy RDB (1993). Effects of placement of elemental S and sulfate on the growth of two rice varieties under flooded conditions. Australian Journal of Agricultural Research, 44, 1775-1788.

6.1.1 Background

If S fertilisers are to be added to rice crops then we must know which is the best form to apply (elemental or sulfate) and where is the best placement (surface or deep). Once we have this information then we can decide on the best application strategy.

The protocol reported here is designed to study the effect of placement of ^{35}S-labelled elemental S and potassium sulfate as S sources on the growth of rice and of the fate of the applied fertilisers. The original paper (Samosir et al., 1993) reported results from two rice varieties but only one is presented here to demonstrate the principles.

6.1.2 Procedures

A DESIGN AND APPLICATION OF TRATMENTS

A pot experiment was set up to study a complete factorial of the following treatments on rice production:

2 fertiliser sources x 2 placements x 2 harvests x $\pm$ plants x 3 replicates.

The top layer (0-25 cm) of a soil which had been air dried and passed through a 2 mm sieve was used. The pots used were made from 15 cm diameter PVC tube in 31 cm lengths, sealed at the bottom with an end cap. A mass of 6 kg soil was mixed with 9 g of dry chopped straw (S concentration 0.05%) prior to potting. The treatments detailed below were applied at the required times.

The pots were arranged in a factorial design to include the complete combination of the following factors:

Fertiliser sources (^{35}S labelled): a) K_2SO_4 in solution and b) elemental S (size 425 to 142 μm).

Fertiliser placement: a) Broadcast onto the soil surface in the presence of 0.5 cm of surface water soon after transplanting and b) Mixed with the bottom layer soil (depth 7-21 cm) 30 days before transplanting (total soil depth was 21 cm).

The fertiliser treatments were denoted as:

ES-B = elemental S broadcast
SS-B = K_2SO_4 broadcast
ES-D = elemental S deep placed in the bottom soil layer
SS-D = K_2SO_4 deep placed in the bottom soil layer

Pots without plants were also prepared. The planted and unplanted treatments are referred to as (+) and (-) plant treatments, respectively.

^{35}S labelled K_2SO_4 was prepared by adding carrier free ^{35}S to a solution of K_2SO_4. Elemental S was labelled by dissolving elemental S in toluene, adding the carrier free material and re-crystallizing (Section 4.2). Both S sources were added at 120 mg S/pot with the ^{35}S added at 2.97 MBq/pot in K_2SO_4 and 0.72 MBq/pot in elemental S.

The plants were harvested from the (+) plant treatment and the soils from the pots sampled at 42 days after transplanting (42 dat) and at maturity (114 dat). The (-) plant treatment was sampled only at 114 dat.

For deep placement (D), the soil was taken from the pot and 4 kg of this soil was mixed thoroughly with the required K_2SO_4 solution or powdered elemental S, then returned to the pots as the bottom layer of soil. KCl was added to the elemental S pots to balance the K application and mixed with the bottom layer of soil to make a paste and the soil was then stirred thoroughly. Excess water on the

surface of the bottom layer of soil was left to dry by evaporation. A small amount of dry soil was added to the surface of the bottom soil to absorb the excess water at the surface. A coarse (1 cm) plastic mesh of approximately the same size as the surface area of the pot was used to mark the interface between the top and the bottom soil, and the remaining 2 kg soil, which had been stirred to a paste was added. This procedure was followed to prevent mixing of the bottom layer soil or solution with the upper soil layer. Finally, distilled water was added to produce a 5 cm depth of surface water.

The same procedures were followed for the surface treatment (B) and the (–) plants treatment, but without the addition of fertilisers to the bottom layer. All pots were incubated in the greenhouse under continuous flooding for 30 days before transplanting. The temperature of the glasshouse was controlled at approximately 31°C during the day (12 hours) and 25°C at night (12 hours). The pots without plants were treated in the same ways as those with plants.

Seven days before transplanting, the depth of the surface water was gradually allowed to decrease by evaporation until only about 0.5 cm remained at transplanting. For the surface treatment, the KCl to balance K was added to the surface water 3 days before transplanting.

Phosphorus at a rate of 30 kg P/ha as a solution of $Ca(H_2PO_4)_2$, nitrogen 60 kg/ha as urea, and micronutrients were added to the surface water. Following fertiliser addition one 20-day-old seedling of rice var. C4-63 was transplanted into the centre of the pot, to depth of 3 cm. After transplanting, distilled water was added to give 3 cm of surface water. K_2SO_4 in solution or powdered elemental S was then added to the surface water. As the plants grew the depth of the surface water was increased to 6 cm. All pots were kept continuously flooded during this experiment with the depth surface water fluctuating from 3 to 6 cm during the day.

Additional nitrogen was applied, as urea, to the surface water at 3, 6, 9 and 12 weeks after transplanting, at rates of 30, 60, 60 and 30 kg/ha, respectively. The number of tillers was monitored weekly.

At 42 days after transplanting half of the pots were harvested. Plant tops were cut close to the soil surface and all the surface water and algae in it were siphoned into containers. Soil samples were taken from 0 - 5 cm and 7 - 21 cm layers. The 5 - 7 cm layer was not sampled. The remaining pots were harvested at 114 dat (maturity of the C4-63).

B SOIL SAMPLING AND ANALYSIS

The bottom of the pots were removed and the soil was pushed from the pots with a plastic coated wooden plunger from the top of the pots. The soil column was then cut into the 0 - 7 cm top layer and the 7 - 21 cm bottom layer and representative samples taken for chemical analysis. The soil samples were packed into 50 mL vials, flushed with nitrogen to prevent oxidation, and then tightly sealed.

Another 300 g representative soil sample was taken and centrifuged for 10 minutes at 5000 rpm to collect the soil solution. The soil solution was then filtered through 22 μm millipore filter paper. A few drops of concentrated HCl were added to this extract to prevent iron precipitation. The S concentration in the soil solution was measured by ICP-spectrometry.

Soil analyses included the extraction of 3.5 g (equivalent dry weight) of soil with deoxygenated 0.02 M KH_2PO_4 solution at pH 6.5 in a nitrogen environment with a soil solution ratio of 1:10. The extraction time was 24 hours with continuous shaking. After centrifugation for 10 minutes at 5000 rpm followed by filtration through 22 μm millipore filter paper the S concentration of the solutions were measured by ICP-spectrometry.

Another 3.5 g soil sample was used for the determination of sulfide-S content. This soil sample was placed in a Johnson and Nishita (1952) distillation apparatus and acidified with HCl. The sulfide was carried by N_2 gas into a solution of NaOH and this was used to determine the sulfide-S concentration in the soil.

The radioactivity of the extracts (soil solution, KH_2PO_4 extracts, and the NaOH that had absorbed the sulfide) was measured by liquid scintillation according to the method of Till et al. (1984). Unfortunately, S in the sulfide extracts could not be measured by ICP-spectrometry, probably because of the high concentration of Na in the extract, and so the concentration of sulfide S was calculated from liquid scintillation counting data. The amount of ^{35}S counted in each component of the system (corrected for counting efficiency quenching and decay) was compared to the amount added per pot.

In calculating the content of S in the various soil layers the concentrations were multiplied by the soil mass in the particular layer.

6.1.3 Results

A DRY MATTER YIELD

A significant interaction of time of harvest x fertiliser sources x placement was measured. At 42 dat, the highest dry weight of shoots was recorded in the SS-B treatment, with significantly lower yields in the ES-B, SS-D and ES-D treatments. Similar differences due to fertiliser treatments were also found in the straw weight at maturity, although the differences between treatments were not always significant. Data of the bottom layer root weight showed no interaction effect of time x fertiliser source x fertiliser placement, but, differences in the weight of topsoil roots were measured.

B PLANT SULFUR UPTAKE

In the 0 - 42dat period both total and fertiliser S uptake was highest in the SS-B treatment and lowest in the ES-D treatment (Table 6.1). In the 42 dat - maturity period total and fertiliser S uptake was highest in the ES-B treatment and again lowest in the ES-D treatment. At 42 dat 82.1 % of the S in the plant was from the added sulfate in the SS-B treatment and this was higher than in the other treatments. By maturity, this had declined to 66.8 %, which was not different to that in the ES-B treatment. At both sampling times the % of S in the plant derived from the fertiliser was higher with broadcast than with deep placement for each source.

Table 6.1. Plant sulfur uptake (mg/ pot) and % of plant S derived from the labelled fertiliser

Fertiliser treatment	Total S uptake		Fertiliser S uptake		% S in plant derived from fertiliser	
	0-42 days	42d - maturity	0-42 days	42d - maturity	At 42 dat	At maturity
ES-B	13.4	81.9	7.5	57.3	56.0	68.0
SS-B	24.6	63.9	20.2	38.9	82.1	66.8
ES-D	5.5	27.2	0.4	5.5	7.3	18.0
SS-D	11.3	65.1	3.9	33.4	34.5	48.8

An example calculation of the % S in plant derived from fertiliser for the ES-B and SS-B treatments at 42 dat is presented in Section 6.1.7.

6.1.4 Soil sulfur

A S IN THE SOIL SOLUTION

There was no difference between S sources, and no significant fertiliser interaction terms in the amount of S in the soil solution in any layer or sampling time so the data in Table 6.2 is averaged over S sources. Deep placement resulted in a lower amount of S in the soil solution at both harvests. The presence of plants reduced the amount of S in the soil solution in the top layer in all treatments at both sampling times (Table 6.2) and in the deep layer at 42 dat and the surface layer at maturity.

Table 6.2. The effect of plants on the content of S in the soil solution of the top and bottom soil layers

Harvest time	Placement	Soil solution-S (mg/layer)				% contribution of fertiliser S to soil solution S	
		Top layer		Bottom layer		Top layer	Bottom layer
		- Plants	+ Plants	- Plants	+ Plants	- Plants	+ Plants
42 days	Broadcast	1.4	0.9	3.5	3.6	30.4	9.7
	Deep	0.9	0.4	4.1	2.8	1.0	20.3
Maturity	Broadcast	0.8	0.6	4.7	3.2	54.8	16.2
	Deep	0.5	0.3	4.4	4.6	2.0	18.4

There was a decline in the amount of S in the soil solution in the top layer in all treatments between 42, dat and maturity. Changes in the bottom layer from 42 dat to maturity were inconsistent with a decrease measured in the broadcast treatment and an increase in the deep application treatment (Table 6.2).

A significant time x fertiliser placement x layer x plant interaction on the concentration of fertiliser S in the soil solution was recorded. The contribution of fertiliser S in the soil solution was always higher in the soil layer where the fertiliser was applied (Table 6.2).

B S EXTRACTED BY KH_2PO_4

There was a significant time x fertiliser placement x soil layer interaction on the pool size of S extracted with KH_2PO_4 (S-KP) as shown inTable 6.3. At both 42 dat and maturity, in each fertiliser placement, less S-KP was measured in the top layer than in the bottom layer. Fertiliser placement had no significant effect on the pool size of S-KP in either the top or the bottom soil layers at 42 dat or at maturity. A significant reduction in S-KP in each fertiliser placement between 42 dat to maturity was observed in the top layer, but not in the bottom soil layer.

Table 6.3. The amount of S extracted by KH_2PO_4 from the top and bottom soil layers (mg S /layer)

Harvest time	Placement	KH_2PO_4 -S (mg/layer)		% contribution of fertiliser S to KH_2PO_4 –S	
		Top layer	Bottom layer	Top layer	Bottom layer
42 days	Broadcast	16.9	25.1	24.8	3.6
	Deep	17.1	23.5	2.3	9.8
Maturity	Broadcast	8.4	24.5	36.9	9.0
	Deep	7.6	24.6	10.5	23.9

There was a significant time x fertiliser placement x soil layer x variety interaction recorded in the pool size of fertiliser S extracted by KH_2PO_4 (^{35}S-KP). As for fertiliser S in the soil solution, there was always a higher proportion of fertiliser S in the ^{35}S-KP pool in the soil layer where the fertiliser was applied.

A significant decrease in the % contribution of fertiliser S to ^{35}S-KP was measured in the top layer in the broadcast application between 42 dat and maturity. With deep placement, the % contribution of fertiliser S to ^{35}S-KP of the top layer increased from 42 dat to maturity.

C FERTILISER S RECOVERED AS SULFIDE

There was a significant time x fertiliser placement x soil layer interaction on the pool size of fertiliser S recovered as sulfide (Table 6.4). As with sulfate in the soil solution (Table 6.2) and that extracted with KH_2PO_4 (Table 6.3).Table 6.3), a larger sulfide S pool size was found in the layer where the fertiliser was applied, both at 42 dat and at maturity (Table 6.4). The application of sulfate resulted in the higher sulfide levels compared to elemental S in both soil layers at 42 dat but a lower sulfide level at maturity.

Table 6.4. The pool size of fertiliser S recovered as sulfide in the top and bottom soil layers (mgS/layer)

Placement	42 days		Maturity	
	Top layer	Bottom layer	Top layer	Bottom layer
ES-B	3.4	0.0	14.8	2.0
SS-B	18.4	0.3	6.6	1.0
ES-D	0.0	9.4	5.2	30.2
SS-D	0.9	10.6	1.9	26.1

Fertiliser source also affected the ^{35}S-sulfide content of the top layer soils. In the absence of the plant, higher sulfide contents were found in the application layer in the SS-B than in the ES-B treatment both at 42 dat and at maturity (Table 6.5). In the presence of the plant, SS-B was higher than ES-B in the application layer at both samplings, while ES-D was higher than SS-D in both layers at maturity.

A similar effect of fertiliser source on the sulfide level was also found in the bottom layer of pots without plants. Again, at maturity, in the presence of the plant, deep placed sulfate (SS-D) resulted in a lower sulfide level compared to elemental S (ES-D). The two treatments showed no difference in the sulfide levels of the soils at 42 dat, both in the absence and in the presence of plants.

Table 6.5. Pool size of fertiliser S recovered as sulfide in the top and bottom soil layers (mg S /layer).

Harvest	Fertiliser treatment	Top layer		Bottom layer	
		- Plant	+ Plant	- Plant	+ Plant
42 dat	ES-B	0.1	3.4	0	0
	SS-B	22.1	18.4	0	0.3
	ES-D	0.9	0	8.1	9.4
	SS-D	2.7	0.9	9.0	10.6
Maturity	ES-B	11.2	14.8	3.2	2.0
	SS-B	34.1	6.6	1.0	1.0
	ES-D	3.5	5.2	31.3	30.2
	SS-D	2.2	1.9	38	26.1

5% LSD: 3.7

6.1.5 The amount of fertiliser S in algae and surface water

Data in Table 6.6 shows that the fertiliser S present in the surface water plus algae was highest where a broadcast fertiliser application had been made. This amount declined from 42 dat to maturity in both placements. The presence of plants reduced the amount of fertiliser S present in the algae plus surface water in the broadcast application treatments (Table 6.6) which is consistent with observations of algae growth. Both the lower concentration of nutrients in the floodwater and the shading by plants would be expected to contribute to this.

Table 6.6. The amount of fertiliser S recovered (mg/S pot) in the algae plus surface water as affected by fertiliser source and placement.

Fertiliser treatment	unplanted	planted
ES-B	6.35 b[a]	2.77 cd
SS-B	11.95 a	2.06 d
ES-D	0.06 e	0.04 e
SS-B-D	0.06 e	0.05 e

[a] Numbers followed by the same letter are not significantly different according to Duncans Multiple Range Test (p=0.05).

Table 6.7. The amount of fertiliser S recovered (mg S/pot)in the algae plus surface water as affected by S placement and the presence or absence of plants.

Harvest	Broadcast placement		Deep placement	
	unplanted	planted	unplanted	planted
42 days	14.32	6.14	0.04	0.05
Maturity	6.64	0.33	0.10	0.06

6.1.6 Balance sheets of recoveries of fertiliser S

At maturity (*Figure 6.1*), in the presence of plants, 34 to 74 % of the fertiliser S added was accounted for in those four pools. Lower recoveries (20 to 39% of the fertiliser S added) were found in the absence of plants.

6.1.7 Example calculation

A DATA

A 250 mg sample of plant material was digested and the digest made up to 25 mL.

3mL of this digest was counted in an LSC and a count of 7500 cpm was recorded when the sample was counted 87.4 days (1 half life) after T_0. The counting efficiency was 60 %. The plant material had an S concentration of 0.1 %.

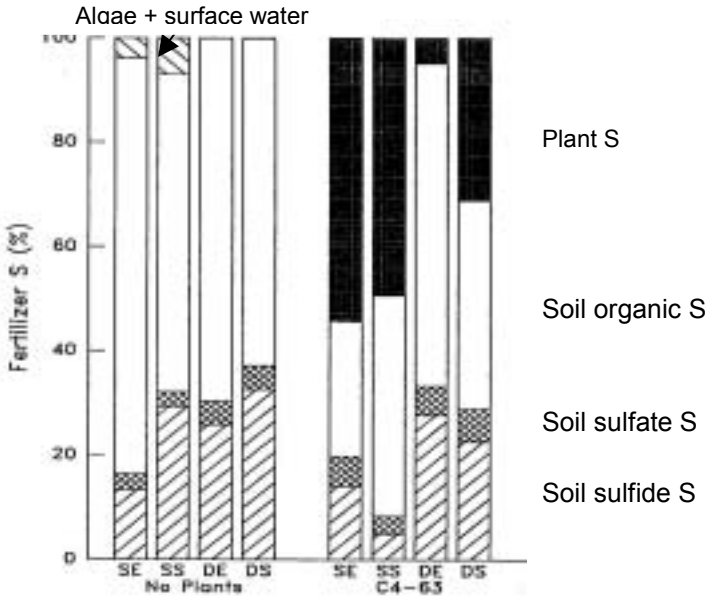


Figure 6.1. Fate of ^{35}S labelled fertiliser applied in different forms and different placements to flooded rice SE = Broadcast elemental, SS = Broadcast sulfate, DE = Deep elemental, DS = Deep sulfate.

B CALCULATION OF SPECIFIC RADIOACTIVITY OF S

= Counts x Counting efficiency x Proportion of digested sample counted/mg S in digested sample
= 7500 x (100 / 60) x (25 / 3) / (0.1 % S x 250 mg)
= 104167 / 0.25
= 416668 cpm /mg S
= 6944 Bq / mg S (i.e. 416688 / 60)
Correction for decay (Counted after 1 half-life)
SR when counted x decay correction
= 6944 x 2
= 13888 Bq / mg S

REFERENCES

Samosir SSR, Blair GJ, Lefroy RDB. (1993). Effects of placement of elemental S and sulfate on the growth of two rice varieties under flooded conditions. Australian Journal of Agricultural Research, 44,1775-1788

Johnson CM, Nishita H (1952) Microestimation of sulfur in plant materials, soils, and irrigation waters. Analytical Chemistry 24, 736-742

Till AR, McArthur GS, Rocks RL (1984) An automated procedure for the simultaneous determination of sulfur and phosphorus and of radioactivity in biological samples. Proceedings of Sulphur-84. Sulphur Development Institute of Canada (SUDIC)

6.2 USE OF ^{35}S TO DETERMINE THE ABILITY OF PLANTS TO ACQUIRE S FROM THE ATMOSPHERE

Adapted from Hoeft, R.G., Keeney D.R., and Walsh L.M. (1972) Nitrogen and sulfur precipitation and sulfur dioxide in the atmosphere in Wisconsin. Journal of Environmental Quality 1,203-208.

6.2.1 Background

Atmospheric concentrations of SO_2 vary both spatially and temporally as does the ability of plant species to utilize this form of S. There were three principal sources of atmospheric S namely, sulfate in sea spray, H_2S and volatile forms of S released as a result of organic matter decomposition in marshlands and in the sea, and SO_2 resulting from the burning of sulfurous fuels. In recent years the use of low S containing coals, improved burning efficiencies, and the scrubbing of flue gases in power stations has substantially reduced emissions.

The contribution of atmospheric S to plant S uptake has been measured by the dilution of ^{35}S taken up from a nutrient solution by non-labelled S from SO_2.

6.2.2 Procedures

Lucerne *(Medicago sativa)* was grown in containers filled with 3.5 kg of acid washed silica sand. $K_2{}^{35}SO_4$ was added to the sand at rates of 0, 5, 10, 20 and 40 mg S/container. This can be done by adding a constant amount of $K_2{}^{35}SO_4$ equivalent to the 10 mg/container and additional unlabelled K_2SO_4 to the other treatments or by adding different amounts of $K_2{}^{35}SO_4$. In the first instance the specific radioactivity of the S will decrease with increasing application rate and in the second case the specific radioactivity will remain constant. Each method will require different calculation procedures. See calculation in Section 6.2.4.

Table 6.8. Concentration of the stock solutions and the dilutions made for the nutrient solutions used in the study

Compound	Amount added	Conc.	+All	-N	-P	-K	-S	-Ca	-Mg	-Zn
Macronutrients	(g/ L)	(M)	----------mL /L ----------							
$Ca(NO_3)_2.4H_2O$	236.10	1.00	3.0	-	3.0	3.0	3.0	-	3.0	3.0
KNO_3	101.10	1.00	2.0	-	2.0	-	-	4.0	0.5	2.0
$NH_4H_2PO_4$	115.00	1.00	2.0	-	-	2.0	-	2.0	2.0	2.0
$MgSO_4.7H_2O$	184.80	0.75	1.0	1.0	1.0	1.0	-	1.0	-	1.0
KCl	149.00	2.00	1.0	1.0	1.0	-	1.0	-	1.0	1.0
KH_2PO_4	136.00	1.00	-	2.0	-	-	2.0	-	-	-
K_2SO_4	87.10	0.50	-	-	-	-	-	-	1.5	-
$CaCl_2.6H_2O$	109.50	0.50	-	-	-	-	-	-	-	-
NH_4NO_3	40.00	0.50	-	-	2.0	-	2.5	2.0	1.5	-
$Mg(NO_3)_2.6H_2O$	128.20	0.50	-	-	-	-	1.5	-	-	-
$MgCl_2.6H_2O$	101.65	0.50	-	-	-	-	-	-	-	-
NH_4Cl	106.80	2.00	-	-	-	1.0	-	1.0	-	-
$Ca(CH_3COO)_2$	158.20	1.00	-	3.0	-	-	-	-	-	-
$K(CH_3COO)$	98.10	1.00	-	-	-	-	-	-	-	-
$(NH_4)_2SO_4$	132.10	1.00	-	-	-	-	-	-	-	-
Micronutrients		(mM)								
Fe sequestrene	64.360	150.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$MnCl_2.4H_2O$	2.969	15.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$ZnCl_2$	0.204	1.50	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-
$CuCl_2$	0.134	1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
H_3BO_3	0.031	0.50	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$(NH_4)_6Mo_7O_{24}.4H_20$	0.012	0.01	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Note : The Fe, Mn, Zn, Cu, and B and Mo can be combined into one stock solution if a minus trace elements treatment is to be used.

Table 6.9. Nutrient concentrations in the solutions

Nutrient	+All	-N	-P	-S	-K	-Ca	-Mg	-Zn
	----------mM ----------							
N	10.00	0.00	10.00	10.00	10.00	10.00	10.00	10.00
P	2.00	2.00	0.00	2.00	2.00	2.00	2.00	2.00
K	4.00	4.00	4.00	4.00	0.00	4.00	4.00	4.00
S	0.75	0.75	0.75	0.00	0.75	0.75	0.75	0.75
Ca	3.00	3.00	3.00	3.00	3.00	0.00	3.00	3.00
Mg	0.75	0.75	0.75	0.75	0.75	0.75	0.00	0.75
Cl	2.04	2.04	2.04	2.04	2.04	2.04	2.04	2.04
Acetate	0.00	6.00	0.00	0.00	0.00	0.00	0.00	0.00
	---------	----------	---------	µM	---------	------	------	------
Fe	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00
Mn	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Zn	1.50	1.50	1.50	1.50	1.50	1.50	1.50	0.00
Cu	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
B	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mo	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07

A sulfur free nutrient solution was routinely added to each container and the plant tops were harvested at various times and analyzed for total S and ^{35}S. In the experiment conducted by Hoeft et al. (1972) a S-containing fungicide was applied and the amount of S contained in it subtracted from total S in the plant tops. This assumes that all of the S in the fungicide was taken up by the plant. It is preferable not to use S-containing fungicides to avoid this problem. In the calculations presented below it is assumed that no fungicide or sources of S other than from the $K_2{}^{35}SO_4$ and K_2SO_4 and atmospheric S was available to the plant.

A nutrient solution has been developed by Lisle et al. (2000), which has proven to be very good for a range of plant species and is recommended for studies such as this. The composition of the solution is shown in Table 6.8 and Table 6.9.

The plants were grown from December 31 with harvests of the tops on March 5, April 6 and May 12. It appears from the paper that each harvest was analysed separately for total S and ^{35}S, and the data presented as the sum of the 3 harvests.

6.2.3 Results

The percentage of S in the tops that was obtained from the atmosphere declined from 73 % when 5 mg S/pot was added down to 44 % when 40 mg S/pot was added (Table 6.10)

Table 6.10. Sources of S taken up by lucerne

S applied (mg/pot)	Total yield (g/pot)	Total S [a] (mg/pot)	S from $K_2{}^{35}SO_4$ (mg/pot)	S from atmosphere (mg/pot)	% S from atmosphere
0	7.32	14.6	0	14.6	100
5	7.70	15.9	1.7	14.2	73
10	9.21	20.4	4.2	16.2	67
20	9.57	23.3	9.2	14.1	52
40	10.00	36.2	18.5	17.7	44

[a]Excludes the 3.6 mg/pot that was applied in the fungicide.

6.2.4 Calculations

A USING $K_2{}^{35}SO_4$ WITH CONSTANT SR

If the pot contained 2 kg of silica sand you would need to add a minimum of 1 MBq of ^{35}S/pot. The activity added and the specific radioactivity of S added in each treatment is presented in (Table 6.11).

Table 6.11. Calculation of % of S taken up from the atmosphere following addition of fertiliser $K_2{}^{35}SO_4$ with a constant specific radioactivity

S added (mg/pot)	Activity added (MBq/pot)	Specific radioactivity (MBq/mgS)	SR of plant material (MBq/mgS)	% S from fertiliser	% S from atmosphere
0	-	-	-	-	100
5	1	0.2	0.056	28	73
10	2	0.2	0.066	33	67
20	4	0.2	0.096	48	52
40	8	0.2	0.112	56	44

The fertiliser added had a SR of 0.2 MBq/mg S. If the plant took up all of its S from the fertiliser then the SR of the plant would be the same as the fertiliser because the labelled fertiliser S had not been diluted by cold S. Had the plant taken up equal amounts of S from the fertiliser and the atmosphere then the SR in the plant would have been reduced to half. In the present case the SR of the plant was 0.056 MBq/mgS

% of plant S derived from the fertiliser = (SR of plant / SR of fertiliser) x 100

= (0.056 / 0.20) x 100

= 28%

B USING $K_2{}^{35}SO_4$ WITH VARIABLE SR

This is often the easiest option as it means making up only one solution of labelled fertiliser and adding different volumes to the pots to obtain the different application rates. The activity added and the specific radioactivity of S added in each treatment is presented in (Table 6.12).

Table 6.12. Calculation of % of S taken up from the atmosphere following addition of fertiliser $K_2{}^{35}SO_4$ with a variable specific radioactivity

S added (mg/pot)	Activity added (MBq/pot)	Specific radioactivity (MBq/mgS)	SR of plant material (MBq/mgS)	% S from fertiliser	% S from atmosphere
0	-	-	-	-	100
5	1	0.2	0.056	28.0	73
10	1	0.1	0.033	33.0	67
20	1	0.05	0.024	48.0	52
40	1	0.025	0.014	56.0	44

% of plant S derived from the fertiliser = (SR of plant / SR of fertiliser) x 100

= (0.056 / 0.20) x 100

= 28%

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Lisle LM, Lefroy RDB, Blair GJ (2000) Method for rapid assessment of nutrient supply capacity of soils. Communications in Soil Science and Plant Analysis. 31, 2627-2633.

6.3 USING ^{35}S TO TRACE THE SULFUR RELEASED FROM PLANT RESIDUES IN A SOIL–PLANT SYSTEM

Source: Konboon, Y., Blair, G.J., Lefroy, R. and Whitbread, A. (2000). Tracing the nitrogen, sulfur and carbon released from plant residues in a soil/plant system. Australian Journal of Agricultural Research. 38, 699-710.

6.3.1 Background

There is a need to study the fate of S released from crop residues to enable them to be managed effectively. A glasshouse experiment, using drained pots, was undertaken to examine the fate of S applied in residues of Flemingia *(Flemingia macrophylla)* leaf, medic *(Medicago truncatula)* hay and wheat (*Triticum aestivum*) straw which were labelled with ^{35}S. Japanese millet (*Echinochloa frumentocea*) was used as the test plant to follow plant uptake of ^{35}S mineralised from the labelled plant residues.

6.3.2 Procedures

A PRODUCTION OF LABELLED PLANT RESIDUES

Plastic pots (30 cm diameter for Flemingia and 20 cm diameter for wheat and medic), lined with a plastic bag to prevent contamination of the pot with radioisotope and prevent drainage, were filled with a 1:1 by volume of vermiculite and sand. The pots were maintained near field capacity with distilled water and a complete nutrient solution was applied at intervals.

Once the plants were well established, the Flemingia was heavily defoliated and the wheat and medic thinned to 10 and five plants per pot, respectively. Applications of ^{35}S solution were then commenced. Every two days 5 mL of carrier-free $Ca^{35}SO_4$ containing approximately 0.068 MBq/mL was applied from a syringe over the pot surface making sure that no solution contacted the plant. Repeated applications are necessary to ensure uniform labelling.

B SOIL AND POT PREPARATION

The soil, which contained 93.7 mg/kg total S and 6.4 mg/kg KCl-40 extractable S, was air-dried and passed through a 2 mm sieve before being used in the experiment.

Pots, 30 cm deep, were made from 15 cm internal diameter polyvinyl chloride (PVC) pipe fitted and silicone sealed with PVC end caps which had several holes (2 mm diameter) to allow drainage. A fine (1 mm mesh) nylon screen was placed above the holes to prevent the loss of soil particles. The pots were then placed on top of a modified end cap, to enable the collection of leachate. Before the soil was added to the pots they were marked into depths of 0 – 8, 8 – 16, and 16 – 24 cm (hereafter referred to as top, middle, and bottom parts, respectively). Each part was filled with 2.033 kg of air-dried soil and a plastic mesh (5 mm gutter guard) was used to separate the top and middle soil layers.

C EXPERIMENTAL DESIGN AND LAYOUT

Treatments were laid out according to a split-plot design with 3 replicates. The main-plot factor was harvest time and the subplot factors consisted of plant residue and fertiliser. Japanese millet (*Echinochloa frumentocea*) was used.

In summary the design was:

Main-plot factor : 3 harvests [27 and 48 days after planting, and at maturity (91 days)]

Sub-plot factors: 4 residues [non-residue control, Flemingia leaf, barrel medic hay, and wheat straw]

Fertilisers: 2 [low, high] x 3 replicates

The experiment was conducted over 12 weeks under controlled temperature (20 ^{o}C night – 30 ^{o}C day).

Before application the plant residues were cut into 2 - 3 cm pieces and dried in an oven at 65 °C. The residue was applied at 5.4 g/pot, which is equivalent to approximately 3 t/ha on the basis of pot surface area. The plant residues and fertiliser applications, consisting of either low or high, were incorporated into the top 8 cm soil layer before potting. The two fertiliser treatments applied were equivalent to 100 kg/ha of 10 : 0 : 0 : 0 and 30 : 25 : 20 : 8 (N : P K : S). These are hereafter referred to as low and high treatments.

The millet showed symptoms of nitrogen deficiency, so urea solution was applied at 10 and 30 kg/ha to the low and high fertiliser treatments respectively, 49 and 63 days after planting.

D MANAGEMENT OF THE JAPANESE MILLET CROP

Approximately 10 seeds of millet were planted into each pot and these were thinned to four healthy seedlings. The pots were then placed in the glasshouse. The pots were weighed periodically to maintain the soil moisture near field capacity, except during the weekly period of leachate collection when each pot was watered to 25 % above field capacity. Leachate was collected one day after excess watering, weighed to record the volume and stored in plastic bottles in a freezer prior to analysis.

At each harvest the millet plants were cut at the soil surface, and at the third harvest the tops were separated into grain and stem plus leaf. The millet roots were removed from each soil layer and cleaned. The plant shoot, grain and root samples were then oven-dried at 80 °C, weighed, ground to pass a 1 mm sieve and stored for subsequent chemical analyses.

E SAMPLE COLLECTION AND MEASUREMENT

The soil from the pots which were destructively sampled at each harvest time was pushed out of the pot, laid on trays and separated into the top, middle and bottom sections. The soil within each section was mixed thoroughly and visible roots removed from each soil layer. A 500-g sub-sample of each soil layer was then taken, air-dried, ground to pass a 1 mm sieve, and stored in a plastic bottle for chemical analyses.

The ^{35}S activity of the leachate was determined on a 3-mL sample which was placed into a vial and mixed with 17 mL of scintillation fluid consisting of toluene, p-terphenyl, POPOP and teric. The ^{35}S activity in the sample was then determined in a Liquid Scintillation Counter.

Extractable S in soil samples was determined using the KCl-40 soil test developed by Blair et al. (1991). A 3-mL sub-sample of extract was taken for determination of ^{35}S activity by LSC.

The ground millet plant parts and plant residues were sub-sampled (approximately 0.2 g) for digestion in a sealed container (Anderson and Henderson 1986not in references) and the digest measured for cations, P and S by ICP-AES. A 3 mL sub-sample of the plant extract was also taken for measurement of ^{35}S.

The proportion of ^{35}S derived from a plant residue was calculated using:

$$\text{Proportion of residue } ^{35}S = \sigma \frac{(\text{radioactivity of } ^{35}\text{S of each component})}{(\text{radioactivity of added } ^{35}\text{S from residue})}$$

In order to calculate the amount of residue S in each component using ^{35}S data, the following formula was used:

$$\text{Specific Radioactivity of residue (SR)} = \frac{\text{radioactivity of } ^{35}\text{S in residue}}{\text{total S in residue}}$$

Amount of residue S in each component = SR x radioactivity of the component.

6.3.3 Results

A MILLET TOPS YIELDS

At the 27-day harvest, there were no significant differences in plant top yield between the residue treatments in the N alone treatment (Table 6.13). Application of Flemingia leaf and wheat straw in the high fertiliser treatment produced the highest plant top yields. The plant tops yields in the Flemingia leaf and wheat straw treatments were significantly higher in the high fertiliser than in the low fertiliser treatment, but there was no difference between the medic hay and control treatments (Table 6.13).

At the 48-day harvest, application of medic hay, Flemingia leaf and wheat straw in the low treatment produced increased plant top yield compared to the non-residue control. In the high fertiliser treatment the application of Flemingia leaf or wheat straw produced a higher plant top yield compared to the control (Table 6.13)

The response of plant top yield (including grain) to the treatments at the 91-day harvest was similar to that at the second harvest. In the low fertiliser treatment the highest plant tops yield of 13.40 g/pot was measured in the medic hay treatment followed by Flemingia leaf, wheat straw and the control treatments (Table 6.13). An interaction between plant residue and fertiliser treatment was again found with high fertiliser enhancing the crop yield in the Flemingia leaf, wheat straw and control treatments compared to the low fertiliser treatment, but there was no significant yield increase in the medic hay treatment.

Table 6.13. Effect of plant residue and fertiliser rate on plant top yield (g/ pot) of millet. Within each harvest, means followed by a common letter are not significantly different at P = 0.05 by DMRT

Harvest	Residue	Plant tops yield (g)/pot)	
		Low fertiliser	High fertiliser
27 days	None	0.19 b	0.25 b
	Flemingia leaf	0.22 b	0.42 a
	Medic hay	0.19 b	0.24 b
	Wheat straw	0.21 b	0.41 a
48 days	None	0.94 d	1.48 cd
	Flemingia leaf	2.35 b	3.40 a
	Medic hay	2.51 b	1.96 bc
	Wheat straw	1.91 bc	2.55 a
91 days	None	5.54 e	10.05 cd
	Flemingia leaf	10.16 cd	17.16 a
	Medic hay	13.40 bc	13.92 ab
	Wheat straw	7.68 de	15.30 ab

B UPTAKE OF ^{35}S BY JAPANESE MILLET

There was no significant difference between the residue treatments in the percentage of residue S contained in the millet when the crop was harvested at day 27 and day 48. At the 91-day harvest the application of wheat straw and medic hay resulted in greater uptake of residue S by the millet and these were significantly higher than that observed in the Flemingia leaf treatment (Table 6.14).

C ^{35}S CONCENTRATION IN THE KCl-40 EXTRACT

At the 91 day harvest the highest KCl-40 extractable S concentrations were found in the bottom soil layer. The highest concentration of residue ^{35}S in the KCl-40 extract, in each soil layer, was found in the medic and wheat straw treatments. The highest recovery of ^{35}S in the KCl-40 extract in the middle and bottom soil layers was also in these two treatments.

Table 6.14. Recovery of ^{35}S in millet tops, in the soil layers and loss through leaching as affected by harvest times and plant residues (% of ^{35}S added in residue). Within a column, means followed by a common letter are not significantly at P = 0.05 by DMRT

Plant residue	Recovery of ^{35}S in millet at 3 harvests (%)			Recovery of ^{35}S in KCl-40 extracts of 3 soil layers (%)			^{35}Sleached (%)
	27 days	48 days	91 days	Top	Middle	Bottom	
Flemingia leaf	0.7 a	5.0 a	10.0 b	5.2b	1.6b	0.7b	2.3b
Medic hay	0.9 a	7.4 a	21.6 a	11.6a	4.4a	2.0a	7.4a
Wheat straw	1.5 a	8.1 a	21.4 a	11.3a	4.4a	2.4a	8.7a

D LOSSES OF SULFUR IN LEACHATE

The greatest loss of S by leaching over the 12 week period, 21.4 mg, was observed from the wheat straw treatment followed by 18.7, 18.2 and 16.9 mg from medic hay, Flemingia leaf and the control treatments, respectively.

Leaching of residue S, as measured by ^{35}S, gradually increased from the beginning of the experiment and reached a maximum at week five in all residues. There was a significantly higher (29.7 %) loss of residue S from the high fertiliser treatment compared to the low fertiliser treatment when averaged over residue additions. After 12 weeks the highest loss of residue S by leaching was from the wheat straw and medic hay treatments, 8.7 and 7.4 % respectively, which was significantly different from the 2.3 % of residue S that was leached from the Flemingia leaf treatment (Table 6.14). These higher leaching losses were associated with a higher recovery of ^{35}S from the residues present in the bottom soil layers of these treatments.

Losses of S through leaching were high in week 1 with the highest value observed from the control, followed by *Flemingia* leaf, medic hay and wheat straw treatments, respectively (Figure 6.2). At week 2, leached S decreased in all treatments and this was generally followed by a gradual increase for the following 3 to 5 weeks. The maximum S lost from the control (2.0 mg / week), medic hay (2.0 mg / week), wheat straw (2.3 mg / week) and *Flemingia* leaf treatments (1.9 mg / week) were observed at weeks 1, 5, 6 and 7, respectively. After the leaching peak was reached leached S gradually decreased through to week 12 (Figure 6.2). On average, increasing fertiliser application enhanced the leaching of S by approximately 13.6 %.

The greatest loss of S by leaching over the 12 week period, 21.4 mg, was observed from the wheat straw treatment followed by 18.7, 18.2 and 16.9 mg from medic hay, *Flemingia* leaf and the control treatments respectively.

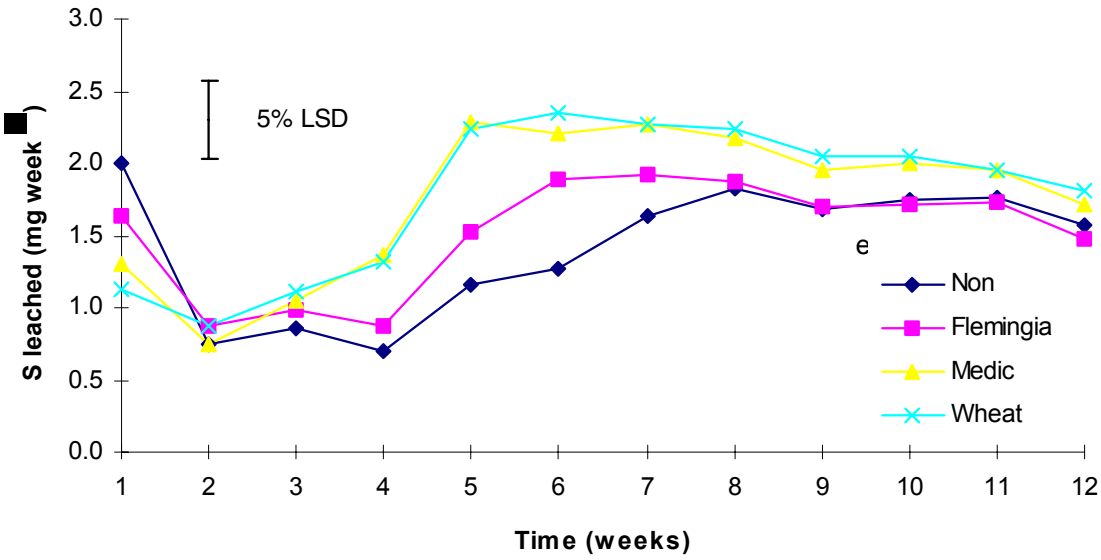

Figure 6.2. S leaching rate from different residue treatments.

Leaching of residue S, as measured by ^{35}S, gradually increased from the beginning and reached a maximum at week 5 in all residues (Figure 6.3). At week 5 the highest amount of residue S leached, 1.04 % of total residue S, was observed from the wheat straw, followed by 0.87 and 0.30 % from medic hay and wheat straw treatments, respectively. After the peak, the percentage of residue S lost in leachate declined gradually (Figure 6.3). Increasing fertiliser application led to an increase of 29.7 % in residue S leached.

The cumulative leaching of residue S is shown in Figure 6.4. After 12 weeks, 8.7, 7.4 and 2.3 % of residue S was leached from wheat straw, medic hay and *Flemingia* leaf treatments, respectively. There was no significant difference between wheat straw and medic hay treatments, but these treatments were significantly different from the *Flemingia* leaf treatment.

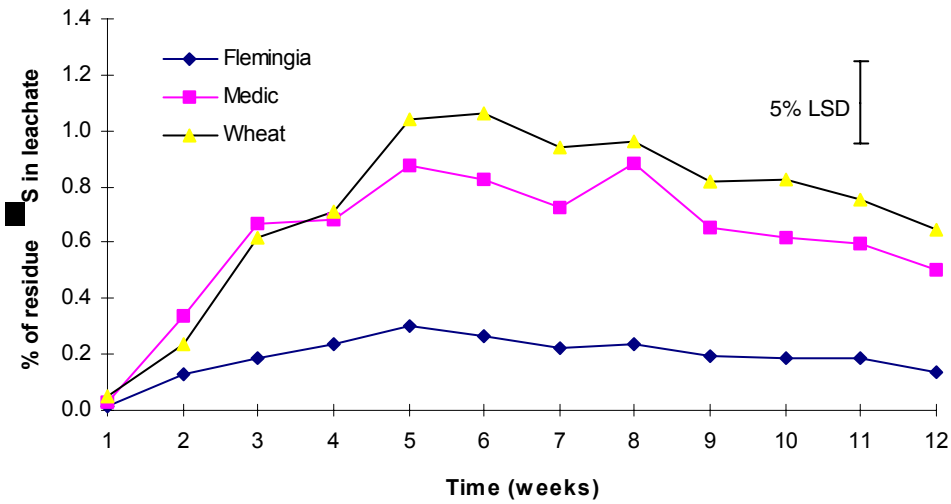

Figure 6.3. Percentage of residue S leached.

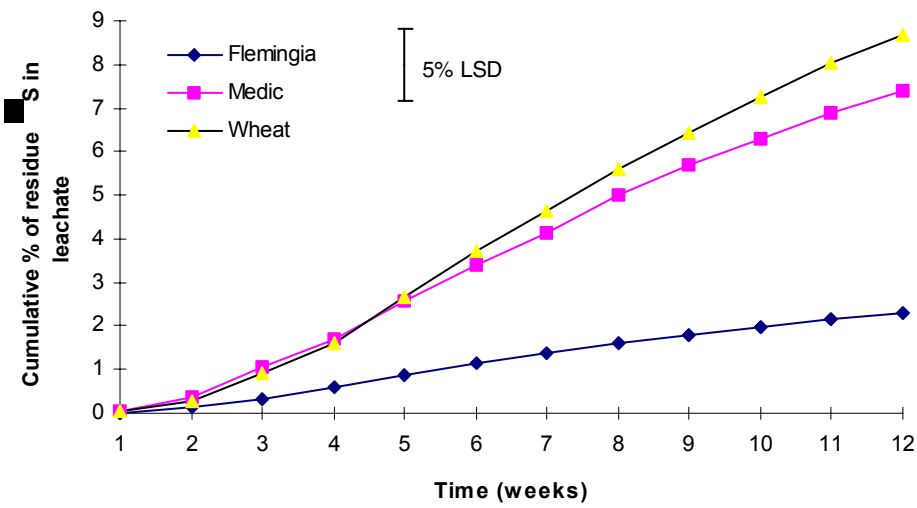

Figure 6.4. Cumulative loss of residue S in leachate.

E SUMMARY OF ^{35}S IN EACH COMPONENT

A summary of residue ^{35}S expressed as a percentage of ^{35}S input contained in each component of the system at the 91-day harvest is shown in Figure 6.5. Increasing fertiliser application rate led to increasing leachate S in all residue treatments, with the greatest increases, 2.5 and 2.0 %, in the medic hay and wheat straw treatments. The effect of fertiliser rate on extractable and non-extractable soil S varied with residue treatment. Generally, application of *Flemingia* leaf resulted in a higher amount of non-extractable S compared to the medic hay and wheat straw treatments. In contrast, *Flemingia* leaf application led to a lower concentration of extractable S and leached residue S compared to medic hay and wheat straw application. Increasing fertiliser enhanced the concentration of extractable S in the medic hay treatment, but had no significant effect on other residues.

Uptake of residue S by millet had a similar pattern to that of the leachate S with a lower uptake in the *Flemingia* leaf treatment, while the application of medic hay and wheat straw led to a higher uptake of residue S. Increasing fertiliser significantly enhanced the uptake of S by millet in the wheat straw treatment but no such effect was recorded in the *Flemingia* leaf and medic hay treatments (Figure 6.5).

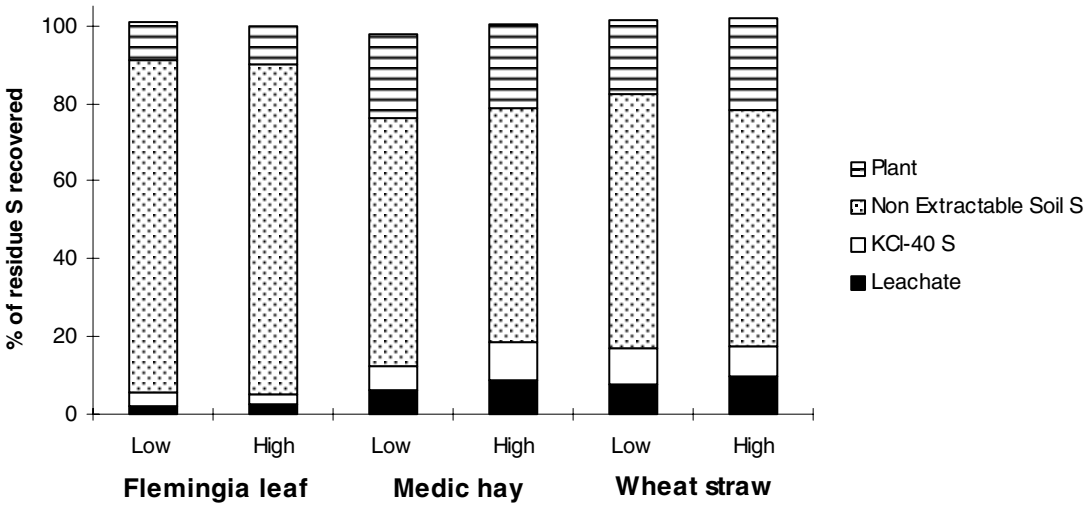

Figure 6.5. ^{35}S recovered in each component of the planting system at low and high fertiliser at the 91-day harvest (% of ^{35}S input).

6.3.4 Interpretation

A CROP YIELDS

The differences in decomposition rate (medic> Flemingia> wheat) of the three residues used in this study as measured by Lefroy et al. (1995) were not reflected in the millet yields measured here. At the early stage of growth, the poor millet yields from the treatment receiving medic hay compared to Flemingia leaf and wheat straw was most likely the results of higher organic acid production during the rapid decomposition period of the medic hay. Moreover, during the leachate collection period the pots were temporary subjected to waterlogging (approximately 5 - 6 h) and this may have stimulated the accumulation of organic acids from rapidly decomposing residue (Cannell and Lynch 1984).

Despite the low contribution of residue N to the millet in the Flemingia leaf and wheat straw treatments millet yields were quite high in these treatments. This suggests that the N from the applied inorganic fertiliser had compensated for the lower N supplied from residues, especially during the early stage of crop growth. By maturity there were no differences in crop yields among the treatments receiving the three residues, but they produced a higher yield than that of the control treatment. Generally, application of residues improved crop yields over the control by approximately 44 to 70 %. Results from this study support the findings of many workers that application of plant residues improves crop yield (Broadbent 1984;Ta and Faris 1990; Bremer and van Kessel 1992; Wonprasaid et al., 1995).

B MOVEMENT OF S

The recovery of ^{35}S in the plant, soil and leachate, was near by 100 %. A higher percentage of residue S was found in the leachate and in plant uptake from treatments receiving medic hay. Compared to the Flemingia leaf treatment, leachate S from the medic hay treatment were three and six fold higher, respectively, while plant uptake of N and S from the medic hay treatment were three and two fold higher than uptake from the Flemingia leaf treatments. The high S concentration of medic hay (0.29 %), in addition to its rapid decomposition rate, resulted in a greater amount of S and the higher percentages of total S released during the decomposition process. Once released, the S was readily available for plant utilisation. However, the high amount of S released led to an S supply greater than plant demand and this resulted in the movement of excess S down the soil column, with subsequent loss through leaching, as found by Becker et al. (1994). As a consequence of greater plant uptake and S losses through leachate, the total soil S was lower with the application of medic hay than that with the Flemingia leaf treatment. This suggests a greater residual value with the slower breakdown material.

Application of wheat straw led to a high residue S recovery in leachate with a significant amount of both residue S utilised by the millet. The high S concentration in the wheat straw (0.23 %) resulted in a high loss of residue S through leaching, which was similar to that observed from medic hay. This

confirms the results from the perfusion study of Lefroy et al. (1995) which showed that the release of S was high from wheat straw.

In contrast to medic hay and wheat straw, application of Flemingia leaf resulted in the highest percentage of residue S remaining in the soil, with a lower amount of S found in the leachate and in the plant (*Figure 6.5*). The residue S in soil resulting from Flemingia leaf application was 1.25 times higher than that observed from the medic hay treatment. This resulted from the slow decomposition of this residue as shown in the perfusion study of Lefroy et al. (1995). The slow decomposition rate resulted in a slower release of nutrients and, as a result, a significant amount of nutrients remained in un-decomposed residue and soil microbial biomass and less extractable soil S. Subsequently, the loss of S through leaching was reduced significantly compared to the rapid breakdown residue.

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6.4 MEASURING THE AVAILABILITY OF SUBSOIL SULFATE USING $Ca^{35}SO_4.2H_2O$

6.4.1 Labelling different depths of soil

Source: Bole JB, Pittman UJ (1984) Availability of subsoil sulphates to barley and rapeseed. Canadian Journal of Soil Science, 64: 301-312.

A INTRODUCTION

The radioisotope ^{35}S has been used in several research studies to measure directly the uptake of fertiliser S and to study the dynamics of S in the soil. Bentley et al. (1955) found that cereal crops derived an average of 44 % of their S from surface broadcast $Na_2{}^{35}SO_4$ but only 23% from the fertiliser when it was placed at 25-cm depth. Alfalfa, however, utilized $^{35}SO_4$-S from surface, 25-cm and 55-cm depths with equal effectiveness.

The objective of this study was to determine the effect of subsoil reserves on S nutrition of barley (*Hordeum vulgare*) and rapeseed (*Brassica napus*), and to measure the effectiveness of these crops in taking up SO_4-S from known depths.

B PROCEDURES

A loam surface soil low in available S (2.0μg $S0_4$-S / g soil) was used to prepare lysimeters for the controlled environment studies. Calcium sulfate (25 μg $S0_4$-S / g soil) was mixed with layers of soil in the lysimeters so that soil high in available S would be present from the depth of 0, 18, 36, and 54cm from the bottom of the lysimeter. The 25 x 25 cm lysimeters were 72 cm deep and contained 63 kg of soil (bulk density 1.4 g / cm^3). The $CaSO_4.2H_2O$ was labelled with ^{35}S at an initial specific radioactivity of 0.1 GBq ^{35}S / g S so that the uptake of added S could be determined. This specific radioactivity equates to 1 dpm corrected to T_0 representing 0.00017 μg fertiliser S (ie. 0.1 x 10^9 Bq = 10^6 μg S or 0.1 x 10^9 x 60 dpm = 10^6 μg S)

The commercial S source containing bentonite was crushed to pass through a 2 mm sieve for these treatments. The control treatment had no added S. The seven treatments were replicated five times for a total of 35 lysimeters. Adequate N and P were supplied by mixing 40 μg N / g soil (NH_4NO_3) throughout the lysimeter and 10 μg P / g soil (KH_2PO_4) throughout the surface 18 cm.

Three successive crops, barley (*Hordeum vulgare* "Galt") followed by two crops of rapeseed (*Brassica napus* "Galt"), were grown in each lysimeter without further addition of S. Crops were watered as required with deionized water to minimize SO_4-S inputs. Water was added at the surface to minimize upward rnovement of SO_4-S. Mature barley plants were divided into grain and straw, and rapeseed plants into seeds, stems and pods, and leaves. The plant material was dried at 60°C, weighed, ground, dry-ashed (Sanford and Lancaster 1962), and analysed turbidimetrically for total S (Blanchar et al., 1965) and for ^{35}S in a liquid scintillation spectrophotometer.

Lysimeters were overseeded in the first two crops and plants were removed from each lysimeter at approximately 2-weekly intervals to determine when labeled SO_4-S at depth began to contribute to S nutrition. Cores of soil 2 cm in diameter were removed from each lysimeter prior to planting the second and third crop. The soil cores were divided into four 18 -cm segments and extracted with 0.002 M $CaCl_2.2H_2O$ to determine the distribution of SO_4-S (Carson et al., 1972). Lysimeters were rewetted to field capacity for successive studies by adding deionised water to the core hole to minimize sulfate leaching. The hole was refilled with original soil. Additional NH_4NO_3 (25 μg N / g soil) was added to the surface of the lysimeters prior to the subsequent crops. The lysimeters were randomised in a 3.3 x 5.0 m growth room with a 16 / 8 hr and 20 / 16^0C day / night regime.

C RESULTS AND DISCUSSION

Added S did not increase the yields of barley grain or straw. The 2 μg / g SO_4-S initially in the soil, in addition to mineralized organic S, adequately supplied the S requirement of the barley.

When rapeseed was grown on the same soil, marked interveinal chlorosis of leaves was evident prior to stem elongation on the control and the treatment which had SO_4-S only in the 54-72 cm layer. All treatments other than the control recovered and showed no deficiency symptoms at maturity. The leaves of the control treatment were cupped and the leaves, stems, and seed pods turned purple. Maturity was delayed and seed yield was reduced. Maynard et al. (I983) and Ukrainetz (1983) reported similar symptoms on S-deficient rapeseed. The third crop (rapeseed 2) showed marked S-

deficiency symptoms on all treatments except where SO_4-S was initially added to within 36 cm of the surface (T-4,5). Seed yield was greatly reduced in the control and elemental-S treatments and to a lesser extent where the SO_4-S was initially placed 36 cm from the surface or deeper (T- 2,3). Seeds formed in the pods but failed to develop. Growth habit was indeterminate and rapeseed plants continued to produce new leaves, stems, and pods until harvested. Plants deficient in S thus produced a greater weight of stems and pods than the treatments having sufficient S so that total yield was only reduced on the control treatment. The initial barley crop derived about 80% of its S from the labelled SO_4-S when the high SO_4-S soil was placed in the bottom half (36-72 cm), three-quarters (18-72 cm), or throughout the entire lysimeter (0-72cm) (Figure 6.6A). The soil thus contributed 20% of the S (non-labelled) taken up by the barley. In T-5, the soil contributed one-quarter (20/80) as much S as the 25 µg ^{35}S / g. Isotope dilution concepts would suggest that the available soil S pool was about 6 µg S / g soil. Since the soil only contained 2 µg SO_4-S / g before the $^{35}SO_4$- S was added, mineralization of organic S contributed twice as much unlabelled S as was originally present. This may have been intensified because surface soil was used throughout the entire root zone. When the SO_4 was placed in the bottom quarter of the lysimeter (54 - 72 cm) the barley derived about one-half of its S from the high SO_4- S soil (Figure 6.6A). The SO_4-S at that depth contributed little to the plant S supply until 30 - 40 days after planting.

The second crop (rapeseed) grown in the lysimeters showed a pattern of uptake of labelled SO_4-S from depth (Figure 6.6B) similar to that of the barley, despite the lower mineral-S reserves and the redistribution of SO_4-S within the lysimeter. Although the added SO_4-S contributed a somewhat lower proportion of the total uptake, it still provided 60 - 80% of S where SO_4-S was initially mixed with soil from the 18 - 72 cm depth, or throughout the entire lysimeter. The delay in ^{35}S uptake when the SO_4-S was initially placed at the 54-72 cm depth was again evident. For some crops, or under growth conditions which would restrict rooting at that depth, the delayed SO_4-S uptake may limit production. The yields were unaffected under the conditions studied.

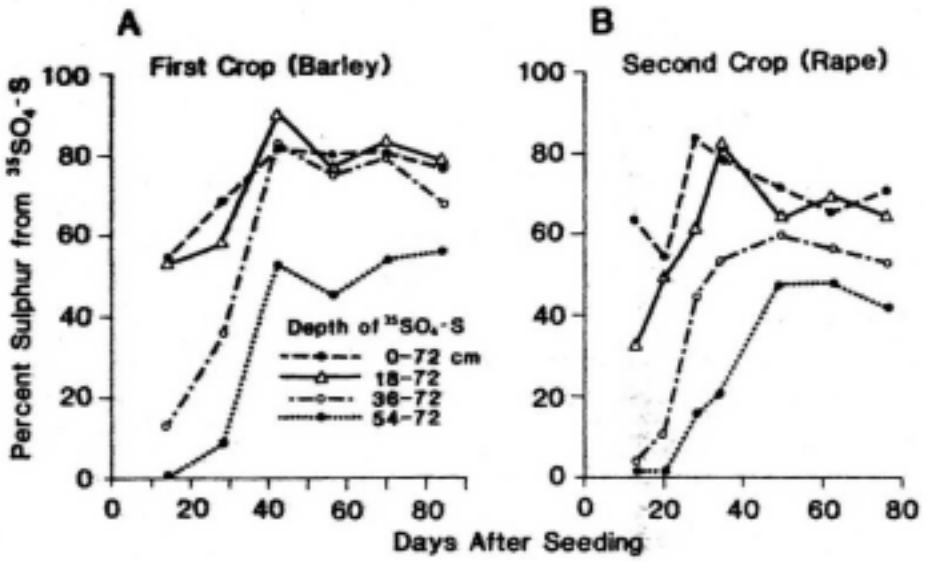

Figure 6.6. Recovery of fertiliser ^{35}S placed at various depths by successive crops of (A) Barley and (B) rapeseed.

6.4.2 Labelling bands of soil

Source: McRae DI (1994). The Effect of Applying Sulfur at Various Depths and at Delayed Times on the Yield of Canola. B.Rur.Sc. Thesis, University of New England, Armidale, NSW 2351, Australia.

A INTRODUCTION

It is important to determine the availability of subsoil S as it is potentially a large source of S for deep rooted crops such as canola. Bole and Pittman (1984) found that sulfate at a depth of 54 cm or more was available to canola plants. Mahler et al. (1993) supported this finding as they found that sulfates at depths of up to 75 cm were important in the contribution of S to the growth of canola. However,

Bole and Pittman (1984) expressed some concern that a delay in sulfate uptake may limit seed production.

The aims of this experiment were to evaluate the availability of S placed at depth in the profile to canola on the yield, S distribution within the plant tops, and maturity time of canola.

B PROCEDURES

The soil used was an S responsive Vertisol clay that had grown several canola crops, was mildly acidic (pH $CaCl_2$ 6.4) and had a low concentration of organic carbon (1.0 %) and a low (KCl-40 S (Blair et al., 1991) of 3.7 mg / kg.

Plastic PVC pipe of 15 cm diameter and 1m high was used to make the pots. The soil was packed into the pots in 2.0 kg portions to a bulk density of 1.2 g / cm^3, then 600 mL of water was added to bring the soil up to field capacity (30 % moisture content). This was repeated ten times. Sulfur for the placement treatments was applied at the appropriate depth during the soil packing process. All pots received 69.6 mg S / pot (equivalent to 40 kg S / ha) as $Mg^{35}SO_4$ and a basal application of P, K, Mo and Zn at 70.7, 44.2, 3.53, 0.88 and 8.84 mg / pot respectively. The basal nutrients were mixed into the top 5 cm of soil.

Four-week-old seedlings of canola cv. Barossa, were transplanted from an S-deficient nutrient solution into the pots. Because the seedlings were transplanted the S application and harvest times are referred to as days after transplanting (DAT) rather than from sowing.

The experiment consisted of the following treatment combinations:

4 depths of S placement	3 harvests	x 4 replicates
0 cm (Surface)	26 DAT	
5 cm	41 DAT	
35 cm	159 DAT	
60 cm		

At 26 and 41 DAT, one representative sized plant was harvested from each pot, whilst at 159 DAT the two remaining plants were harvested. At the 26 and 41 DAT harvests the tops were dried immediately after harvest at 80 °C for greater than 48 hours. The tops at the 159 DAT harvest were cut at the root/ shoot interface and placed intact in paper bags for two weeks. This was done to allow the seeds to fully mature prior to oven drying. The tops were divided into residue and seed. The residue comprised the leaves that matured during the course of the experiment, and stems, pods and leaves that were collected at the final harvest.

All plant material was ground to less than 2 mm and then digested in a sealed chamber using $HClO_4$ and H_2O_2 (Anderson and Henderson 1986). The plant samples were analysed by ICP-AES. A 3-mL sub-sample of the digested plant sample was mixed with a scintillation fluid (Till et al. 1984), and the ^{35}S activity was measured by liquid scintillation counting and the counts corrected to the day of ^{35}S application.

The S content of the tops (mg / plant), the specific radioactivity of the tops (Bq / mg S), the proportion (%) of S in the tops derived from the fertiliser, and the recovery (%) of fertiliser S in the tops was calculated for each harvest.

C RESULTS AND DISCUSSION

D Dry matter yield

Depth of S placement had no significant effect on DM yield at 26 DAT (Table 6.15). At 41 and 159 DAT, DM yield was significantly higher in the 5 cm treatment than in the 0, 35 or 60 cm treatments, which were not significantly different from each other. The DM yield in the 5 cm treatment at 159 DAT was 17 times the yield in the other three treatments.

Table 6.15. The effect of S fertiliser placement on canola yield and S uptake. Means followed by the same letter in each category are not significantly different according to DMRT.

Harvest time (Days after transplanting, DAT)	Depth of S placement (cm)			
	0	5	35	60
	Tops dry matter yield (g)			
26	0.11a	0.19a	0.10a	0.14a
41	0.16a	0.96b	0.19a	0.27a
159 [A]	1.83c	24.16d	1.94c	1.23bc
	S content of tops (mg)			
26	1.0a	2.2b	1.0a	1.0a
41	0.9a	8.3c	1.3a	1.3a
159 [A]	8.3c	193.2d	7.9ce	4.6e
	S concentration of tops (%)			
26	0.91c	1.16d	1.00d	0.71bc
41	0.56a	0.86c	0.68d	0.48a
159 [A]	0.45a	0.80c	0.41a	0.37a
	Recovery of fertiliser S in tops (%)			
26	0.00a	1.04b	0.01a	0.00a
41	0.00a	4.01c	0.03a	0.00a
159 [A]	0.41a	61.65d	0.16a	0.02a
	Proportion of S in tops from fertiliser (%)			
26	0.00a	34.29b	0.49a	0.02a
41	0.32a	33.41b	1.48a	0.21a
159 [A]	3.38a	44.81c	1.61a	0.32a

[A] S content, % fertiliser S in tops and % of S in tops from fertiliser at 159 DAT is comprised of both S in the residue and seed.

E Recovery of fertiliser S in the tops

At each harvest the recovery of fertiliser S was significantly higher in the 5 cm placement treatment than the other 3 placements. The recovery of fertiliser S increased significantly at each harvest in only the 5 cm placement treatment (Table 6.15). The recovery (%) of fertiliser S in the tops (62 %) in the 5 cm treatment was over 60 times greater than in the 3 other treatments.

F Proportion of S in tops from fertiliser

The 5 cm treatment was the only treatment which had a significant quantity of fertiliser S in the tops (Table 6.15). Fertiliser S accounted for 44.8 % of S in the plant tops at 159 DAT and this was 13 times greater than the fertiliser S contribution in the 0 cm treatment.

G Rate of fertiliser S accumulation.

The 5 cm treatment accumulated fertiliser S at an appreciable rate (Table 6.16). In this experiment, the placement of S fertiliser at 35 cm depth in the soil profile resulted in a severe reduction in dry matter and seed yields. This result differs from the findings of Bole and Pittman (1984), who reported that canola plants absorbed 60 - 80 % of their S requirements from the 18 - 72 cm depth and barley derived 55 % of its S requirement from the 54 - 72 cm depth. The value of S deep in the soil profile was also highlighted by the high correlation ($r^2 = 0.78$) found by Mahler et al. (1993) between sampling (check alignment) depth to 75 cm and canola yield. The apparent difference in the value of S deep in the soil profile between experiments can be largely accounted for by the different S concentration of the topsoils and the effect of disease on the S stressed plants in the present experiment. Plants which accessed S did not suffer disease problems whereas plantsdied where S was placed on the surface or deep in the profile.

Table 6.16. Rate of fertiliser S accumulation in tops as affected by fertiliser S placement (μg fertiliser S /day) calculated from ^{35}S data.

	Depth of S placement (cm)			
Period of S uptake	0	5	35	60
0-26 DAT	0.0	27.8	0.3	0.0
26-41 DAT	0.0	137.8	0.9	0.0
41-159 DAT	2.4	340.0	0.8	0.1

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6.5 THE STUDY OF SULFUR RECYCLING PROCESSES IN A GRAZING SYSTEM USING ^{35}S

Source Till AR (1981) Chapter 3 (pp. 33-54) in World Animal Science, B1, Grazing Animals, Elsevier, Amsterdam, The Netherlands.

6.5.1 Introduction

The majority of studies in grazing systems have been aimed at evaluating management practices such as fertiliser application, and the effects of fodder conservation on animal production. They are frequently of the input-output type where the major effort is devoted to relating the particular treatments to liveweight gain or some other desired final product. Such studies are essential for the provision of useable information in the short term, but the results may only apply to the particular area and system being studied, and extrapolation to other situations may be hazardous. This is particularly true of nutrient studies because the experiments give little information about the production processes involved. Detailed studies of complete grazing systems are costly in labour and resources, and because of the uncontrolled environmental inputs and biological variability, they have to be run for many years to obtain *reliable* results. These difficulties have stimulated studies of parts of the system under controlled conditions, which aim at evaluating key variables and processes. However, such studies will only be of real value when the influences of other processes in the complete system are known and can be allowed for, and the dynamics of the system are monitored.

The dynamics of systems can be studied by either 'classical' or tracer methods which are then frequently combined with computer techniques that enable systems to be analysed or synthesised depending on the data available.

6.5.2 'Classical' methods

In the 'classical' approach changes in biomass of various components are measured and from these, carbon or energy flows are calculated (Wiegert and Evans, 1964; Hutchinson, 1971). However, where there is extensive recycling, such as with many nutrients, these techniques are difficult to apply. Nevertheless, if the concentrations of nutrients are measured in the various pools and nutrient budgets are set up, the net amounts of transfer (and recycling) can sometimes be estimated (Wilkinson and Lowrey, 1973). The major disadvantage of classical techniques is their inability to allow the investigation of specific pathways in the system. This inability prevents the true effects of competing processes and other interactions from being measured directly.

6.5.3 Radiotracer techniques

By using isotopic tracers the pathways of the different isotopes can be followed by measuring changes in the isotope ratios. Isotope techniques do not provide an alternative method of nutrient study but do provide a valuable extension of the classical methods. In fact, for studies of the dynamics of grazing systems, classical, isotopic and computer systems analysis and simulation techniques complement one another.

Radioactive isotopes are usually much easier to measure than stable elements and consequently are usually preferred. The application of radiotracers to plant and soil studies is not new (e.g. Hevesy, 1923) but their use in fairly large field experiments presents some problems. May et al. (1968) showed that manageable amounts of ^{35}S could be used to measure the uptake and movement of fertiliser sulfur in a 0.4-ha pasture grazed by sheep.

There are three main ways that radiotracers are used:

(1) Simple direct measurements. These show the location of the material and an example is the movement of a fertiliser nutrient through the soil profile.

(2) Isotope dilution analysis. Changes in the specific radioactivity (SR or isotope concentration) in the various pools following the introduction of a tracer, allow the nutrient flow rates, and sometimes the size of the functional pool, to be determined.

(3) Precursor-product relationships. In this application the tracing of the isotopes through various compounds and materials is used to show the pathways and reactions that take place in the system.

Various combinations of these are used in the techniques developed for field experiments. In field experiments, the labelled (radioactive) material, e.g. fertiliser, plant material or excreta, is introduced into the system and its appearance in, or loss from, the appropriate components is measured.

The amount of radioactive material that can be used in an experiment is determined by major factors of safety, sensitivity of measuring equipment and cost. For example, the values given in Table 6.17 show the estimated minimum radioactivity application rates required for the study of the various sulfur components in a grazed pasture. As about three animals is the minimum number for a grazing experiment, the area required to study grazing sheep is of the order of 0.3 ha. The cost and safety aspects dictate that it is not feasible to uniformly label the whole of the grazing area in large-scale experiments if one is to trace the ^{35}S to parts of the system other than wool. Consequently a 'hot spot' method has been developed in which the labelled material is applied to a portion of the total area of the pasture. For studies of sulfur cycling in pastures grazed by sheep, the amounts of radioactivity required and the proportions of the areas treated for particular experiments are shown in Table 6.18. Uniform or hot spot application of fertiliser gave similar patterns of incorporation of ^{35}S into wool and a typical wool SR curve ('transient') is shown in *Figure 6.7*. The shape of this curve can give considerable information about interactions between the pools and processes in the cycle. If the plateau portion of the wool transient (*Figure 6.7*) represents the stage when the applied fertiliser sulfur has become mixed with the other pools of sulfur then the amount of sulfur taking part in the production processes (cycling sulfur pool) can be calculated from the isotope dilution. May et al. (1972) proposed an S recycling network and used it and the wool transients in simulation studies to estimate some of the transfer rates that cannot be directly measured by other techniques. These authors consider that modelling and simulation studies should form an integral part of such a research program.

Table 6.17. Estimated quantities of ^{35}S required[1] for studies of S cycling in pastures grazed by sheep

^{35}S traced to part of cycle	Sulfur concentration of material (% S)	Radioactivity required (MBq/ha)
Wool	3.3	222
Plants	0.2	22 000
Total soil S	0.02	74 000
'Available' soil S	0.002	440 000

[1]These calculations are based on the sulfur concentration, size of the sample, and the minimum specific radioactivity for detection. These values are corrected for one-year radioactivity decay, but not for non-uniform mixing of the tracer.

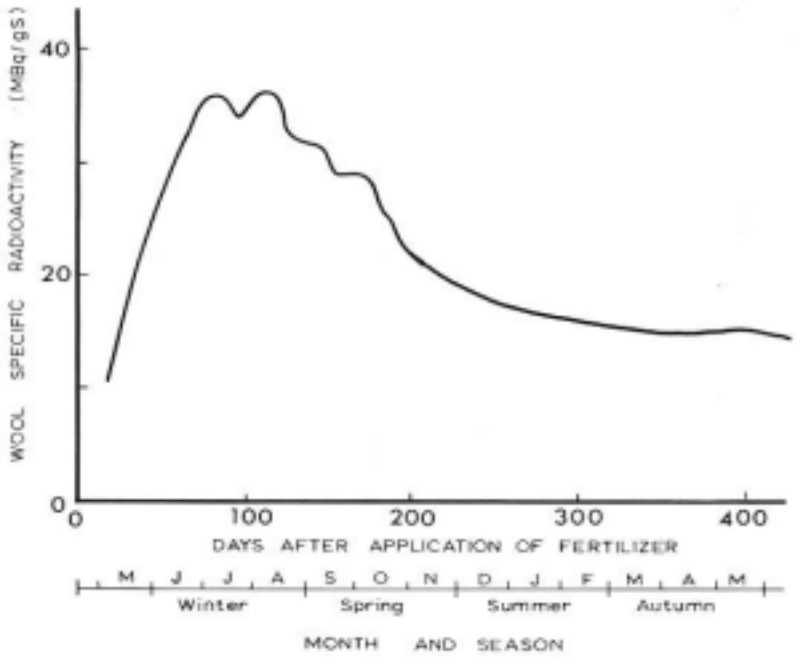

Figure 6.7. Specific radioactivity of wool from sheep grazing ^{35}S labelled pasture.

Table 6.18. The effect of applying ^{35}S to different proportions of the experimental areas on the length of time that various parts of the system can be studied

% Area labelled	Radioactivity		Duration of measurable radioactivity (days)			
	Total (MBq)	Rate (MBq/ha)	Wool	Plant	Soil (total)	Soil (available)
100	222	720	440	*	*	*
3.3	222	22000	440	400	*	*
0.04	370	2300000	650	650	600	400

* Time too short for meaningful observations.

Using direct soil measurements Till and May (1971) found that after 300 days, 38 % of an original application was still present in the top 20 cm of the hot spot soil. This suggests that, in the absence of leaching or other losses, 62 % of the applied ^{35}S had been redistributed over the experimental area by grazing and other processes. An alternative indirect estimate of the proportion of the dose remaining at the site can be made using the formula given by Till et al. (1970)

$$R = \frac{fP}{w}$$

where R = proportion of dose remaining at site

f = fraction of area originally labelled

P = SR of plants in the originally labelled area

w = SR of wool at plateau.

Application of this formula to the results from the Till and May (1971) experiment indicated that 51 % of the applied material remained at the site of application. The apparent 13 % discrepancy between the direct and indirect measurements in this experiment probably represents redistributed nutrients that had still not re-entered the cycling pool.

Other direct measurements of ^{35}S in soil have shown only limited movement of fertiliser sulfur through the profile following application at rates of 8-15 kg S / ha. However, it is inevitable that large proportions of the sulfur will be moved by leaching and surface flows following high application rates. This is because there is no mechanism for the retention of large amounts of inorganic S in the root zones, and the capacity for the biological processes to convert sufficient sulfur to, and retain it as, organic materials will be exceeded.

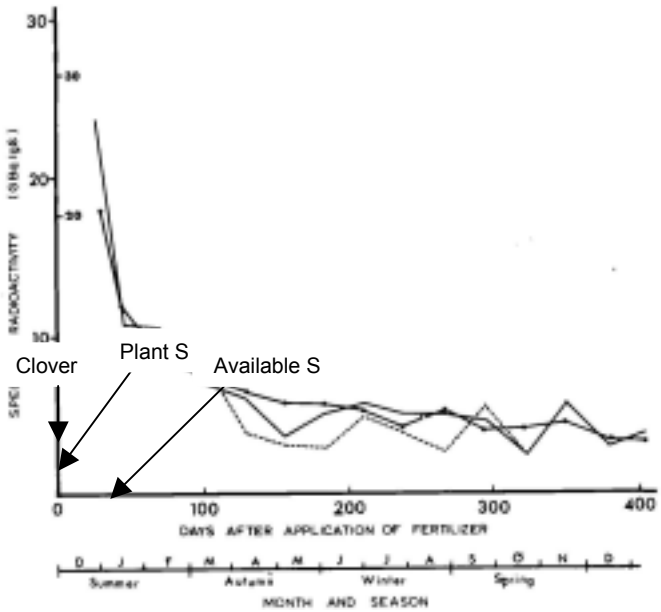

Figure 6.8. Specific radioactivity of plant S, clover and available S versus days after application of fertiliser. The axis using small numbers shows the percentage of the plant sulfur that was derived directly from the fertiliser.

The precursor-product relationships can be used to provide very valuable information on the use of nutrients, and examples of the soil-plant and fertiliser-plant relationships are given below.

The relationship between the SR of plant S and the SR of the S in the available pool shows that after about 150 days the plants were obtaining their nutrients from the top 7.5 cm of soil (*Figure 6.8*). The SR of available S decreases with depth and this is reflected in the higher values of clover SR (shallow roots) than phalaris (deeper roots) in the early stages of the experiment. As time progresses the SR of available S becomes more uniform over the root depth.

This technique allows the study of fertiliser-plant relationships and estimation of the relative inputs of S from the fertiliser and the recycling processes, and also show the time over which the fertiliser is making a significant direct contribution to production. It has been suggested (Till, 1976) that these fertiliser-soil-plant relationships could form the basis for specification of fertiliser release rates and application times to obtain maximum efficiency of nutrient uptake.

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6.6 THE USE OF THE ^{35}S REVERSE DILUTION TECHNIQUE FOR STUDYING THE RELEASE OF S FROM ELEMENTAL SULFUR AND SULFATE SOURCES

Source: Shedley CD, Till AR, Blair GJ (1979). A radiotracer technique for studying the nutrient release from different fertilizer materials and its uptake by plants. Communications in Soil Science and Plant Analysis 10, 737-745.

6.6.1 Background

There are two common methods used for studying nutrient release and uptake from fertilisers. In the most widely used method, fertiliser applied is related to nutrient recovery and product response in the particular plant or animal system being studied (Weir et al., 1963). Such studies are simple and necessary but give little insight to the mechanisms involved and factors determining the different recoveries and responses.

Radioactive fertilisers can be used to provide similar information to the first method plus valuable information on the mode of action and fate of the fertiliser (Till and May,1971). The major disadvantage with this method is the difficulty of producing radioactive fertiliser with exactly the same physical and chemical form as that available commercially.

6.6.2 Procedures

PVC tubes, 16 cm diameter and 12 cm long were driven into a uniform *Poa annua* grass sward. The tubes containing the cores were then removed from the pasture, the bottoms sealed with tight fitting PVC covers, and the intact cores used in the experiment. The pasture cores were placed in a glasshouse, and watered to 60 % of field capacity.

Carrier-free $Na_2\ ^{35}SO_4$ was diluted with 0.1 M KH_2PO_4, to give a solution containing 122 kBq/mL and 3.1 mg P/mL. The phosphate was added to ensure that the carrier-free sulfate was not left adsorbed onto the glass container used for dilution, to aid penetration by reducing adsorption of the sulfate ion onto soil components, and to provide adequate phosphate for plant growth. A syringe was then used to apply 20 mL of the radioactive solution evenly to the surface of each pot.

After fourteen days, the plant tops were clipped to 1 cm above the soil surface to remove any foliage which had been contaminated during the application of radioactivity. Fertiliser treatments shown in Table 6.19 were applied to the pots, which were arranged in a randomized block design with four replicates.

Table 6.19. Sulfur application rates and mean particle diameter of Mexican dark elemental S used in the experiment

Fertiliser	Particle diameter	S Application Rate (kg/ha)		
	(mm)	Level 1	Level 2	Level 3
Elemental sulfur	0.05	2	4	16
Elemental sulfur	0.1	4	8	32
Elemental sulfur	0.2	16	32	128
Elemental sulfur	0.4	30	60	240
Elemental sulfur	1	200	400	1600
Na_2SO_4 in solution		2	4	16
Control		0	0	0

Plant tops were harvested every 14 days by clipping 1 cm above the soil surface. Ten harvests were taken, and the specific radioactivity (SR) of the S in each plant sample was measured. This SR measurement was used to determine the amount of dilution of the S in the plant available sulfur pool. At any particular time, the lower the plant SR the more S that was in the plant derived from the unlabelled fertiliser.

However, because SR was decreasing with time in all treatments, the ratio of treatment SR to control SR (SRR) was the parameter used to compare sulfate release from fertilisers in various treatments over time. Thus the SRR and the rate of release of sulfate are inversely related to each other.

After the tenth harvest, soil samples from each pot were analysed for elemental sulfur by extraction with acetone, for available sulfur by extraction with 0.01 M mono calcium orthophosphate, and for total soil sulfur by digestion with nitric and perchloric acid.

6.6.3 Results

Some results have been used to demonstrate the effectiveness of the technique for comparing the rates of nutrient release from different fertilisers and the subsequent uptake of these nutrients by plants.

By the time of the first harvest the sodium sulfate fertiliser had caused a large depression in SR relative to the control (*Figure 6.9*), indicating that the fertiliser was supplying a large proportion of the plant sulfur. By comparison, the two elemental sulfur treatments did not release appreciable amounts of sulfate until 42 days after the start of the experiment. The similar shapes of the SRR curves for the two elemental sulfur treatments during the first 70 days indicates that sulfate release from both particle sizes was similar over this period, even though they were applied at different application rates (16 kg / ha for 0.05 mm and 32 kg / ha for 0.1 mm particles).

After decreasing for 70 days the SRR from the 0.05 mm sulfur treatment began to increase in a manner similar to the sulfate treatment, indicating that the 0.05 mm sulfur had finished oxidising after 70 days.

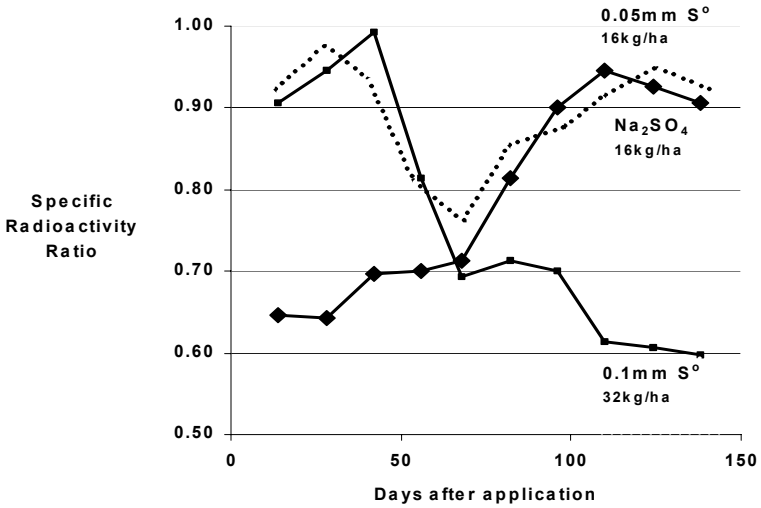

Figure 6.9. Effect of form and particle size of sulfur on its availability to plants.

At the end of the experiment neither treatment was making a significant contribution to the sulfur available to the plants. Conversely, the continuing decline in the SRR curve for the 0.1 mm treatment indicated that sulfur was beinq released and taken up by the plants throughout the experiment.

Data from the experiment has been used to demonstrate the relationships between application rate, particle size and sulfate release from fertilisers. Table 6.20 contains mean plant SRR's for some selected treatments.

Table 6.20. The effect of particle size and application rate on the supply of sulfur to plants

Particle Size	Application Rate	Mean SRR x 100	
(mm)	(kg/ha)	0-140 day	126-140 day
0.1	32	75	57
0.2	32	87	66
0.4	30	92	85
0.4	60	82	68
0.4	240	74	58

For treatments with similar application rates (0.1 mm, 0.2 mm and 0.4 mm at about 30 kg/ha), the plant SRR increased as particle size increased. This in in accordance with the inverse relationship between particle size and oxidation rates of sulfur reported in the literature (Kittams and Attoe, 1965; Li and Caldwell 1966; Barrow, 1971)

The three 0.4 mm treatments show the relationship between application rate and sulfate release for one particle size.

It is interesting to note that 240 kg/ha of 0.4 mm particles supplied the same amount of plant S as 32 kg/ha of 0.1 mm particles, indicating the agronomic importance of particle size distribution in elemental sulfur fertilisers.

6.6.4 Discussion

Results show that the technique is capable of detecting different patterns of sulfur release from fertilisers and its uptake by plants. The general applicability of the method depends on the fulfillment of two requirements.

The first requirement is that the radiotracer becomes distributed in the same pool from which the plant obtains its supply of sulfur. In this experiment, the phosphate added to the radioactive sulfur solution reduced the likelihood of the $^{35}SO_4^{2-}$ being adsorbed on surface layers of organic matter and soil before it could mix in the available pool.

The initial size of the available sulfur pool as measured by extraction with mono calcium orthophosphate, was 22 mg of sulfur per pot. The theoretical SRR of the available pool at the time of S application was calculated as 111 MBq/g S assuming that all radioactivity had moved into the available pool. Plant sulfur SR could not be measured at this time, but a semilog plot of the SR of plant sulfur in the control pots was linear for the first seven harvests. Extrapolation of this line to the time of application of the radioactivity gave a zero time SR of sulfur of 113 MBq/g S, which agreed with the theoretical value, indicating that the first requirement was satisfied.

The second requirement is that the rate of movement of the tracer from the plant available pool into other soil pools is not greatly affected by the treatments (i.e. the rate of sulfate release from the fertiliser does not affect the rate of movement between soil sulfur fractions). At the commencement of the experiment we were not confident that this condition could be met for all treatments. Consequently the application rates were adjusted using data available in the literature (Moser and Olsen, 1953; Fox et al., 1964. Kittams and Attoe,1965; Li and Caldwell,1966; Barrow,1971; Bloomfield, 1967) to give about the same sulfate release rates for all treatments in the same application level (levels 1, 2 or 3 from Table 6.19). Thus, even if the rate of sulfate release did influence tracer movement between soil sulfur pools, the influence should be the same for all treatments enabling valid comparisons to be made.

The net movement of S from the available to other soil sulfur pools over the whole experiment was calculated for all level 3 treatments (Table 6.21). These data show that although these treatments affected plant uptake of the tracer, they appear to have no net effect on its movement into other soil pools. The mean net movement into other pools for all level 3 treatments was the same as the control value, indicating that the second requirement was satisfied.

Table 6.21. Effect of treatments on the movement of ^{35}S between soil sulfur pools

Treatment Material	Size (mm)	Rate (kg/ha)	^{35}S in soil available pool at day 140 (kBq)	Total plant uptake of ^{35}S over 140 days (kBq)	^{35}S which moved into other soil pools over 140 days (kBq)
Control			120	511	1829
Sulfate		16	180	444	1818
Sulfur	0.05	16	127	500	1815
Sulfur	0.1	32	141	451	1850
Sulfur	0.2	128	132	451	1859
Sulfur	0.4	240	173	440	1829
Sulfur	1	1600	212	429	1801

When both requirements are met it is possible to calculate release rates from the fertilisers. If both requirements are not met it is possible to determine relative release rates from different fertilisers provided that adequate information is available to enable suitable precautions to be taken, e.g. the adjustment of application rates in this experiment. The technique will be valuable in assessing the availabilities of commercial fertilisers which are difficult to label with radiotracer, yielding valuable agronomic information on optimum application rates and frequencies.

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6.7 THE USE OF ^{34}S TO STUDY SULFUR DYNAMICS OF CONTRASTING GRAZED PASTURES

Source: Chen W, Blair GJ, Scott J, Lefroy R (1999). Nitrogen and sulfur dynamics of contrasting grazed pastures. Australian Journal of Agricultural Research 50, 1381-1392.

6.7.1 Background

Sown perennial pastures are an important grazing resource. As a result of drought, overgrazing and variable fertiliser inputs, many sown perennial pastures have 'degraded' to a state where they are dominated by short-lived perennial and annual species (Lees and Reeve 1994). Sown perennial grasses, with their deeper and more permanent root systems, can make better use of nutrients and water deeper in the profile than shallow rooted plants, thus enhancing production and reducing nutrient loss (Anon 1992). There is uncertainty about changes in sulfur (S) dynamics, and potential S leakage from grazed systems as these sown perennial pastures degrade to short-lived perennial and annual species based pastures. It is hypothesised that, as the sown perennial pastures degrade to pastures with low year-round dry matter production and shallow root systems, the systems could become more 'open' and thus nutrients especially N and S are likely to be leached.

Either the radioactive ^{35}S or the stable isotope ^{34}S can be used to monitor the movement of S in the soil-plant system. This study outlines the ^{34}S technique used by Chen et al. (1999) and the results obtained.

6.7.2 Procedures

A ESTABLISHMENT OF THE ^{34}S LABELLED AREAS

Till and May (1971) successfully studied the fate of S in grazed pastures following the application of ^{35}S to a small area-known as a 'hot spot'. In the study reported here the approach of not in references Till and May (1971) was used with a small area labelled with ^{34}S.

There were three pasture treatments each with two replicates (degraded, phalaris dominant and phalaris/white clover. Each of the 6 experimental plots was divided into three strata, namely the top, the middle and the lower slope areas. Two 1 m by 0.5 m areas were selected in each stratum, by visual assessment of pasture biomass and species, to be representative areas within each stratum. Elemental S, 90 % of which was present as ^{34}S, was ground to pass a 250 μm sieve, and 50 mg of the sieved sulfur was mixed with 30 g of similarly sieved sand. This mixture was evenly applied by hand on each labelled area. The S applications were equivalent to 1 kg S/ha and thus would have had only a negligible effect on S dynamics, but provided a pulse of ^{34}S which could be measured as it moved through the soil-plant system under grazing.

B PLANT, SOIL AND WATER SAMPLING

A frame with 32 (8 rows x 4 columns) 12 x 12 cm squares was placed over the labelled area using permanently located pegs to identify the locations within the labelled area to be sampled each time (figure 6.10). Two sampling locations within the frame at each sampling time were determined randomly (not in references Chen 1998). Plant and soil samples were taken from the labelled areas in May 1995 (prior to application of ^{34}S) and at approximately two monthly intervals to July 1996, and then at about one monthly intervals to February 1997.

Soil cores were taken from each labelled area with a 3 cm diameter hand auger and the holes were re-packed with the soils collected from the same site. Whole soil cores were carefully laid on a PVC tube cut longitudinally in half to avoid contamination between depths and then sectioned into 0–5, 5–10 and 10–20 cm soil layers. After significant summer rainfall events the sampling depth was extended to 60 cm in February 1996 and 1997.

Pasture tops were harvested from the two sampling locations within the frame at the same time as the soils were sampled by cutting the plants with clippers. Samples were not sorted into component species. Pasture roots were separated from the soil cores and washed.

Figure 6.10. Grid frame for sampling.

C SAMPLE PREPARATION AND ANALYSIS

Total soil sulfur was determined following digestion with nitric-perchloric acid and measured by ICP-AES .

A 0.2 g subsample of tops was taken and digested in a sealed container with $HClO_4$ and H_2O_2, and the S in the digest determined by ICP-AES (Anderson and Henderson 1986). Sulfate was precipitated as $BaSO_4$ and $\delta\ ^{34}S$ determination was carried out in the same way as used for the soil samples.

6.7.3 Calculation of $\delta^{34}S$ and ^{34}S recovery in soil and plant

From the ratios (R) of mass 50 and mass 48 ion beams measured in the ANCA-MS, $\delta(50)$ was calculated as follows:

$$\delta(50)=(R_{sample}/R_{standard} - 1) \times 1000$$

The laboratory standard used was methionine. During the running of the modified ANCA-MS, apart from the top and bottom standards, there was one standard for every 10 samples, thus the $R_{standard}$ value corresponding to each sample was estimated based on the regression of all standards over the total run. The $\delta^{34}S$ value in the sample was then calculated based on the method of not in references Eriksen (1996):

$$\delta^{34}S = (\delta(50) \times C + \delta^{34}S\ (\text{lab. standard}) + 1) / (1000 \times C \times \delta^{34}S)\ (\text{lab. standard})$$

where C is the correction factor. In this study only SO was introduced into the mass spectrometer thus the correction factor for oxygen of 1.046 (Eriksen pers. comm.) was used. The laboratory standard had a $\delta^{34}S$ value of 186 ‰. The ^{34}S atom % was then calculated using $\delta^{34}S$ value of all soil and plant samples based on the method of Peoples et al. (1989). Using the calculated ^{34}S atom % of all samples, the ^{34}S recovery in soil and plant samples was calculated in the same way as for ^{15}N.

6.7.4 Results

A TOTAL SOIL S

There were no significant differences between pasture types in total S, but the effects of time and depth were significant without any interactions between them. Total soil S varied significantly over time, generally being higher in the winter than in the summer. Total S concentration declined from 292 mg/kg in the 0 - 5 cm layer to 186 mg / kg in the 5 – 10 cm layer

B KCl-40 S

There was a gradual decrease in KCl-40 extractable S, without significant differences between pasture types in the 0 - 5 to the 10 - 20 cm soil layers. There was a significant increase in KCl - 40 extractable between the 20 - 40 cm and the the 40 - 60 cm layer for all three pastures. In the 40 - 60 cm soil layers extractable S in the phalaris/white clover pasture was significantly higher than in the other two pastures, but there were no significant differences between the degraded and the phalaris dominant pastures (*Figure 6.11*).

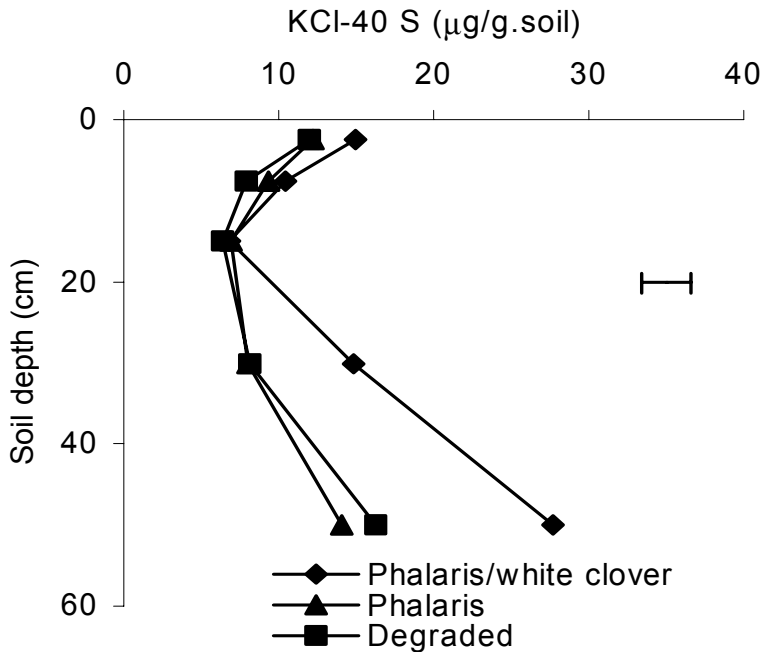

Figure 6.11. Soil extractable S (KCl-40) through the soil profile in the three pastures averaged over the two sampling times. Horizontal bar is lsd (P = 0.05). delete the dot in figure g.soil!

C S SORPTION

Soil samples from the 0 - 5 cm and 40 - 60 cm soil layers in the phalaris/white clover pasture taken on 5th February 1997 were analysed for their S sorption. Sulfur sorption increased significantly as soil depth increased with no significant difference between pasture types (Table 6.22).

Table 6.22. Soil S sorption from a solution of 25 µg / mL in soils from the three pastures. Data within a column, followed by the same letter are not significantly different at P = 0.05.

Soil depth (cm)	S sorption (µg/g soil)
0-5	1.7 a
5-10	15.7 bc
10-20	22.6 c
20-40	30.0 c
40-60	45.7 d

D ^{34}S RECOVERY IN SOIL

The effect of pasture type on ^{34}S recovery in soil was not significant but there were significant effects of time and soil depth with astrong interaction between them (Table 6.23). ^{34}S was recovered from the top 0-5cm soil layer at all samplings but only in the 5-10cm layer in August 1995 and February 1996. The result from further soil analysis for the samples down to 10 - 20cm indicated no further movement of ^{34}S down the soil profile as δ ^{34}S in December 1995 and February 1996 samplings at 10 - 20cm soil layer were not significantly different to the background value (*Figure 6.12*).

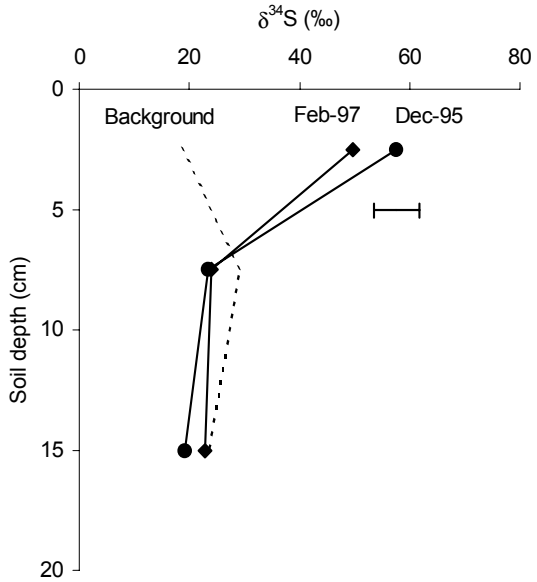

Figure 6.12. $\delta^{34}S$ over three samplings at three depths averaged over pasture types. Horizontal bar is l.s.d (P = 0.05).

Table 6.23. ^{34}S recovery in soil over time at two depths averaged over pasture types. Data followed by the same letter are not significantly different at P = 0.05

	Period					
Soil depth	Aug 95	Dec 95	Feb 96	Jul 96	Dec 96	Feb 97
	Recovery (%)					
0-5 cm	45.5 a	30.0 b	41.1 a	20.3 c	11.0 d	22.1 c
5-10 cm	6.4 de	0.0 e	6.0 de	0.0 e	0.0 e	0.0 e

E ^{34}S RECOVERY IN PASTURE TOPS

^{34}S recovery in pasture tops varied from 0.5 to 1.6 % of that applied throughout the experiment without any difference between the three pastures. There was an effect of time with ^{34}S recovery being higher in the year of application than in the second year, and generally higher in summer than winter.

F ^{34}S IN DRAINAGE WATER AND RUNOFF

Enrichment was too low in these samples to be detected in the ANCA-MS?

G ^{34}S BALANCE

The balance of applied ^{34}S over time averaged over the three pastures, is presented in *Figure 6.13*. There was a general decline of total ^{34}S recovery from 1995 / 96 to 1996 / 97. Three months after ^{34}S application (August 1995), 53 % of the fertiliser ^{34}S was recovered in soil and plant and 47 % was unaccounted for. By the end of this experiment, only 23 % was recovered whilst 77 % was unaccounted for (*Figure 6.13*).

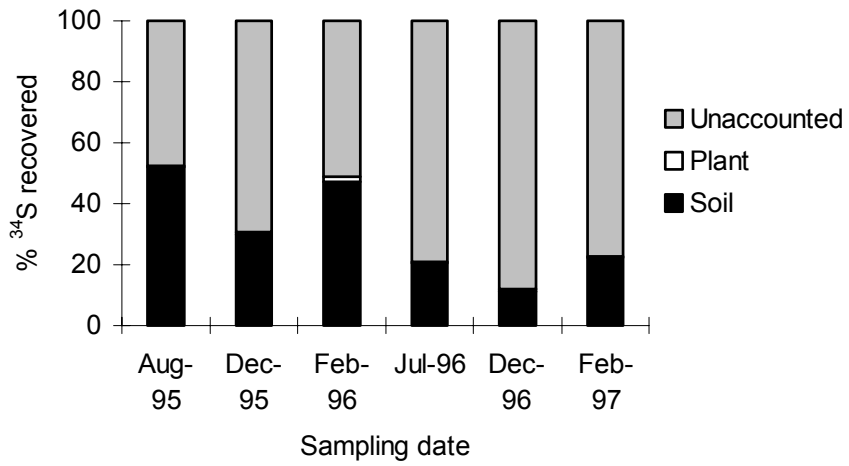

Figure 6.13. Balance of applied ^{34}S over time averaged over the three pastures.

6.7.5 Interpretation

A SOIL S

In this experiment, the KCl-40 soil sulfur test (Blair et al., 1991) used to measure 'plant available' S for the three pastures was well above the critical level (approximately 6 μg/g soil) for this soil test. This high S soil test is the result of the long-term application of single superphosphate in these plots.

KCl-40 S generally decreased with soil depth in the top 20 cm soil layer. However, beyond this depth there was a significant increase that was related to the significant capacity for SO_4^{2-}-S sorption in the 40-60 cm soil layer.

Most of the recovered ^{34}S was in soil although this recovery gradually declined from 1995/96 to 1996/97 (Table 6.23). Only a small proportion of the applied ^{34}S (approximately 1 %) was recovered in pasture tops (Table 6.23). δ ^{34}S values at the different soil depths (*Figure 6.12*) indicate no movement of the applied ^{34}S down to the 20 cm soil layer. Based on the results from an unpublished experiment on an adjacent site the elemental sulfur should have been readily oxidized to sulfate and hence be free to move. Continuous uptake by the plants would remove sulfate from the soil available pool and minimise the chance of leaching, in the short term.

The possible reasons why there was a relatively high ^{34}S recovery in the soil and a low recovery in the plant are the presence of sulfate in the soil available pool and that incorporated into soil organic matter. In addition, the residual root material remaining in the soil would contribute to the high ^{34}S recovery in the soil and also the periodic removal of pasture from the labelled area by the grazing sheep resulted in reduced pasture dry matter on offer thus resulting in a low recovery of ^{34}S in the pasture.

The majority of the applied ^{34}S could not be accounted for in the soil and pasture. The mean proportion of ^{34}S unaccounted for was 58 and 73 % for 1995 and 1996, respectively. These values are comparable to the estimates of pasture S utilisation by grazing sheep using the alkane technique. In the early experiment using ^{35}S, Till and May (1971) suggested that as much as 80 % of the dose applied could be removed by grazing sheep from the "hot spots" to the remainder of the pasture.

Whilst the tracing of ^{34}S allowed the movement of fertiliser S down the profile to be monitored the lack of sensitivity using ^{34}S in an open grazing system due to isotope dilution and animal grazing needs to be considered and the alternative of using ^{35}S considered (See section 6.5). The 50 mg of 90 % enriched ^{34}S used here is equivalent to 7.32 x 10^{10} MBq of ^{35}S.

6.7.6 Summary

High KCl-40 extractable S concentration in the top 20cm soil layers was associated with the history of superphosphate application. The results of this study reveals that in this environment there is significant translocation of S applied to grazed pastures. In some cases other losses, particularly leaching and run-off, may occur when high applications of soluble or readily mineralisable materials are made. Better fertiliser management with regular soil testing is needed to minimise the risks of such losses.

6.7.7 Interpretations

A WITHOUT ISOTOPE

The increase in KCl-40 extractable S down the profile is evidence of the downward movement of S over some period of time at this site. Long-term application of superphosphate, together with the difference in sulfate retention capacity between the soil layers, have resulted in a large amount of sulfate stored at depth.

B WITH ISOTOPE

The movement of recently applied S down the profile is extremely slow as evidences by the location of the applied ^{34}S. This suggests that S moves down the soil profile through dynamics adsorption/desorption reactions with the "new" S replacing the old or that movement only occurs when there is a flush of mineralisation at a time when plant demand is low such as occurs after a drought when a significant amount of the pasture has died.

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6.8 USE OF ^{35}S REVERSE DILUTION TO DETERMINE THE SOURCES OF SULFUR TAKEN UP BY RYEGRASS AND MEASURED BY CHEMICAL EXTRACTANTS

Source: Chinoim N, Lefroy RDB, Blair GJ (1997). The effect of crop duration and soil type on the ability of soil sulfur tests to predict plant response to sulfur. Australian Journal of Soil Research 35, 1131-1141.

6.8.1 Background

The aim of this experiment was to evaluate the sources of sulfur extracted by a range of extractants and the forms in which the sulfur was present in the extracted solution.

6.8.2 Procedures

The ^{35}S labelling reverse dilution method of Shedley et al. (1979) was used to allow the calculation of specific activity (SR) for the soil S fractions and plant sulfur. Specific radioactivity (SR) is the ^{35}S activity per unit of S (KBq / mg S), and the ratio of the SR in the plant at harvest to the SR of the extracted soil pool, termed the specific rarioactivity ratio (SRR), gives an indication as to whether the plant has been utilising nutrient from the same pool(s) as the extractant. It should be noted that this SRR calculation is the inverse of that presented by Blair et al. (1991). This has been done because the resultant whole numbers are easier to interpret without changing the meaning of the ratio. An SRR value of 1 indicates that the extractant and the plant are drawing sulfur from the same soil pool(s).

Three kilogram of air-dried soils was weighed into plastic bags. Carrier free ^{35}S (6.5 MBq / kg soil) was mixed thoroughly by rolling the plastic bags, and incubated for three weeks to allow the equilibration of ^{35}S with the native S in the soil. Each week, the plastic bags were opened and soils mixed thoroughly to ensure that the ^{35}S was mixed as homogenously as possible with the soils and that they remained well aerated. After incubation, basal macronutrients were applied and the bags again mixed thoroughly. Approximately 200 g of soil was sampled for analysis prior to cropping.

Pots of 12 cm height were made from 15 cm diameter polyvinyl chloride (PVC) pipe fitted with a PVC end cap and sealed with silastic silicone to prevent drainage. The plastic bags containing the ^{35}S labelled soils were placed into these pots. Pots were watered to field capacity daily with distilled water.

Perennial ryegrass (*Lolium perenne* L.) seeds were directly sown into the pots, and thinned to 10 plants per pot after emergence. The temperature of the glasshouse was maintained with minimum and maximum temperatures of 15 ^{o}C and 25 ^{o}C, respectively, throughout the period of the experiment.

Ryegrass was harvested at 50 days by cutting plants at the soil surface. All soil from each pot was pushed out and laid out in a plastic tray and roots carefully removed and washed. Plant material was dried in an oven at 80 ^{o}C for 48 hours and ground to pass a 1 mm screen. A sub-sample of 0.2 g of tops was digested using the sealed chamber digestion method utilizing perchloric acid and hydrogen peroxide (Anderson and Henderson, 1986). Total S in the digests was measured by ICP-AES spectrometry.

Soils from each pot were thoroughly mixed and dried in a forced-draft oven at 25°C, ground to pass a 2 mm sieve and kept in a cold room at 4 ^{o}C prior to S analysis.

A SOIL S EXTRACTION

Two test extractants were evaluated. These were MCP (Barrow, 1967; Searle, 1979) and 0.25M KCl heated at 40°C (KCl-40, Blair et al., 1991).

The S concentration in each extract, and that remaining in the soil after extraction was determined using the four methods described below. A flow diagram of the extraction procedure is presented in *Figure 6.14*.

Soils were first extracted with one of the two extractants, centrifuged at 5200 G for 20 minutes and the supernatant filtered through a Whatman No 42. paper. S in the supernatant from the two extracts was measured by ICP.

A sub-sample of 10 mL of the supernatant was treated with 0.02 g of activated charcoal to remove organic S, the solution stirred and the charcoal allowed to settle for 1 hour. These samples were filtered through 0.45 μm Gelman glass filter and S measured turbidimetrically on the autoanalyzer (AA).

The soil remaining after the first extraction was then treated as follows:

3) HI-S : A 0.6 g subsample of the remaining soil was transferred to a round-bottomed Johnson and Nishita (1952) flask. The dry weight of soil in the sub-sample was determined on a duplicate sample of approximate 0.2 g. HI reducible S was then determined on the sample by reduction using the Dean (1966) finish.

4) A duplicate sub-sample of the soil remaining after the first extraction was re-extracted with MCP and the S contained in the extractant determined by ICP-AES (*Figure 6.14*).

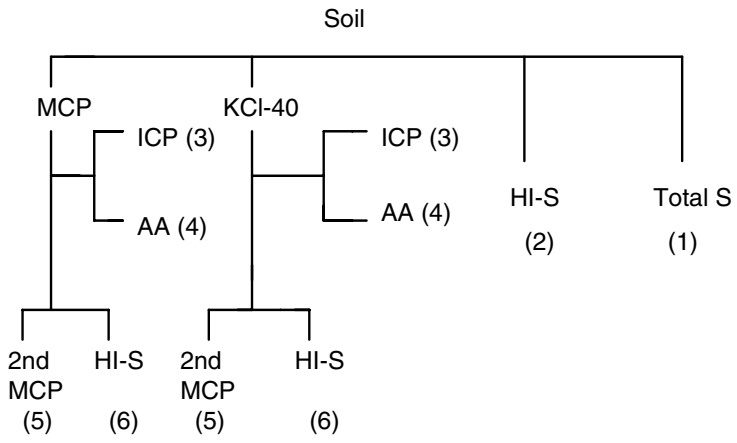

Figure 6.14. Schematic of the fractionation of soil sulfur used in the study.

This sequence of extractions together with total S and HI-S on the un-extracted soil, allowed the estimation of the 6 pools shown in *Figure 6.14* for each of the two extraction methods. The terms in brackets are those used in subsequent text, tables and figures and the method of measurement are as follows;

(Total S) = The total S pool was determined by the digestion method of Till et al. (1984) (1 in *Figure 6.14*).

(HI-S) = The total ester S or HI reducible S pool was determined by HI reduction using the Dean (1966) finish. The concentration of sulfate-S (SO_4-S) in the primary extract was subtracted (2 in *Figure 6.14*).

(ICP-S) = The total S concentration in the primary extract as measured by ICP (3 in *Figure 6.14*)

(SO_4-S) = The sulfate in the primary extract as measured by the autoanalyzer after removing the organic S in solution with charcoal (4 in *Figure 6.14*).

(2nd MCP) = The sulfate S not removed by the first extraction was determined by re-extracting the soil with MCP after the primary extraction (5 in *Figure 6.14*).

(HI-S remaining) = The ester sulfate remaining in the soil after the primary extraction was determined by HI reduction using the Dean (1966) finish (6 in *Figure 6.14*).

From the above measurements the following fractions were estimated;

a) The concentration of organic S compounds in the primary extract (Organic S) was calculated by subtracting the S measured by the autoanalyzer after charcoal treatment of the extract (SO_4-S) from the S in the extract as measured by ICP (ICP-S).

b) The amount of HI-S removed by the primary extractant (HI-S lost) was estimated by subtracting the HI-S remaining after the primary extraction (HI-S remaining) from the HI reducible S pool (HI-S) in the original soil.

c) Mineralised sulfate (Mineralised SO_4) is the amount of S that was mineralized during the primary extraction. It was estimated by subtracting the concentration of organic S compounds in the primary extract S (Organic S) from the amount of HI-S removed by the primary extractant (HI-S lost).

d) The original sulfate (Original SO_4) is an estimation of the sulfur that existed as sulfate in the soil before extraction. This is estimated by subtracting mineralised sulfate (Mineralised SO_4) from (SO_4-S) as determined by the autoanalyzer after charcoal treatment.

e) The S which was not removed by any extractant (Remainder) was calculated as Total S - (ICP-S + HI-S remaining) .

B DETERMINATION OF S

Three analytical procedures were used to determine the S concentration in the various soil extracts namely;

i) The turbidimetric method following treatment with charcoal. This method measures only the sulfate in the extract which may have been derived from either sulfate in the soil or which was converted to sulfate during extraction.

ii) Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The ICP-AES method involves the injection of the extract into a plasma at high temperature. This determines the total S (inorganic and organic) in the extracts at a wavelength of 182.04 nm.

iii) Reduction method (Johnson and Nishita, 1952) using the Dean (1966) finish. The acid mixture used in this procedure reduces sulfate and a variety of organic S compounds in which the S is not bonded directly to carbon (ester sulfate), to sulfide (Freney 1961). This method measured HI reducible sulfur (HI-S). The HI reducible component was obtained by after subtraction of the sulfate-S component as measured in the charcoal treated MCP extract from the S measured after reduction.

The ^{35}S in all plant and soil sample was measured by liquid scintillation counting (LSC) (Till et al 1984).

6.8.3 Results

Plant yield increased with S application in both soils (Table 6.24). S application also resulted in increased S concentration, S content and a decrease in SR in the plants. There was a decrease in the S concentration and in the SR of the total S and HI-S pools in the soil with cropping (Table 6.25).

Table 6.24. Dry matter yield, S concentration, S content, and the specific radioactivity (SR) of ryegrass.

Parameter	-S	+S
Yield (g / pot)	6.44 b[a]	7.40 a
S concentration (%)	0.19 b	0.33 a
S content (mg / pot)	12.29 b	24.62 a
SR (KBg / mg S)	612.8 a	197.9 b

[a] Data followed by the same letter within the same soil and parameter are not significantly different according to DMRT at P = 0.05.

Table 6.25. The total S concentration, HI-S pool and the specific radioactivity (SR) in the soil before and after cropping.

	Total S	HI-S pool
S concentration (µg S/g soil)		
Before planting	91.4 b[a]	46.6 b
After cropping	70.8 b	34.9 b
SR (KBq/ mg S)		
Before planting	86.6 a	91.8 a
After cropping	29.7 b	28.8 a

[a] Data followed by the same letter within the same soil, soil pool and cropping are not significantly different according to DMRT at P = 0.05.

A COMPONENTS OF EXTRACTED S

B ICP-S

The ICP-S and SO_4-S concentration in the KCl-40 extract were higher than MCP. Similarly organic-S and 2nd MCP were higher following extraction with KCl-40 (Table 6.26). There was a greater recovery

of ^{35}S in the ICP-S and SO_4-S fractions in the KCl-40 extract than with MCP. The ICP-S and SO_4-S fractions of the KCl-40 extract had a significantly higher SR than the MCP.

C Components of ICP-S

Approximately 13% of the S in the MCP extract was present as Organic S. This compares to 15 % in KCl-40 extract (calculated from Table 6.26). Most of the ^{35}S recovered was in the SO_4-S (inorganic SO_4) fraction, with less than 5 % of the ^{35}S added recovered in the Organic S pool (Table 6.26).

The SR of the SO_4-S pool was highest in the KCl-40 extract (Table 6.26). The SR in the Organic S pool was lower than in the SO_4-S pool in the KCl-40 extract indicating a greater removal of organic S by the KCl-40 extractant.

Table 6.26. S concentration, recovery of ^{35}S, and specific radioactivity in two extractants in the soil before planting

	S concentration (μg S/g)		Recovery of ^{35}S (%)		Specific radioactivity (KBq/mg S)	
	MCP	KCl-40	MCP	KCl-40	MCP	KCl-40
ICP- S	6.1 a[a]	9.1 b	33.6 c[A]	63.1 b	429.1 b[A]	546.5 a
SO_4-S	5.3 a	7.7 b	29.2 c	58.5 b	433.3 b	595.3 a
Organic S	0.8 a	1.4 a	4.4 a	4.6 a	424.9 a	276.7 b
2nd MCP	1.2 a	2.5 b	2.5 b	7.2 a	163.4 b	224.7 ab
HI-S remaining	42.3 a	40.8 a	18.3 ab	23.8 ab	34.4 a	45.8 a
Remainder	42.9 a	41.5 a	48.2 a	13.1 b	89.8 a	26.4 b

[a] Data followed by the same letter within the same row within a component are not significantly different according to DMRT.

D Sulfate not removed by the first extraction

The additional S removed from all previously extracted soils by MCP (2nd MCP) showed significant differences between extractants. The lowest concentration was in the MCP and the highest in KCl-40 extract (Table 6.26). The amount of ^{35}S recovered in this fraction was less than 10 % of that added, with the highest amount found in the KCl-40 extract. The SR of the second extract was of a similar magnitude to that of the Organic S component of the first extract for KCl-40 and lower for the MCP extract.

E HI-S remaining

The HI-S remaining following the KCl-40 extraction contained in excess of 23 % of ^{35}S recovered (Table 6.26) and the SR in this fraction was higher in the KCl-40 treatment than that following the MCP extraction.

F Remainder

There was no difference between extractants in the S remaining following HI-S extraction (Table 6.26). A significantly lower % ^{35}S recovery was found following KCl-40 extraction and the SR of the remainder S was also lower in this extract (Table 6.26).

G SUMMARY OF COMPONENTS OF SOIL S

A summary of the S components in the two extractants is presented in Figure 6.15.

Before planting, the highest total S concentration (ICP-S) was recorded in the KCl-40 extract. This extract also removed most S from the HI-S pool (Table 6.27). Cropping resulted in a greater decline in ICP-S than MCP. The concentration of HI-S remaining declined with cropping and was similar in the two extractants.

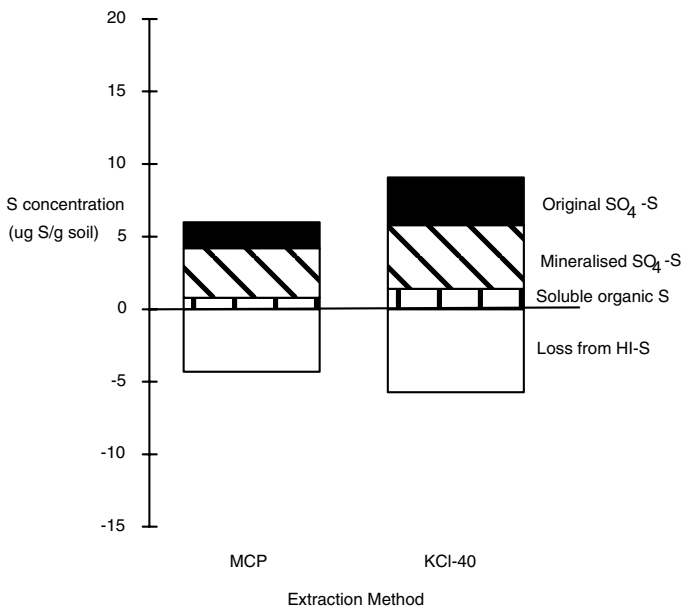

Figure 6.15. S fractions in a range of extractants in the soil before planting. Correct SO4 in Fig

Before planting, the KCl-40 extract had the highest SR in the ICP-S and HI remaining fractions. After cropping, the SR of the ICP-S fraction was significantly lower in the MCP extract than that in the KCl-40 extract. The SR of the ICP-S fraction decreased during cropping. There was no significant difference between extracts in the SR of the HI-S remaining fraction after cropping (Table 6.27).

Table 6.27. The concentration of ICP-S and HI-S remaining after extraction and the specific radioactivity (SR) of extractants before planting and after cropping.

	S concentration (μg S / g soil)		Specific radioactivity (KBq / mg S)	
	MCP	KCl-40	MCP	KCl-40
ICP-S before planting [a]	6.1 c	9.1 b	429.1 b	546.5 a
ICP-S after cropping	3.0 e	1.7 ef	103.3 e	254.9 d
HI remaining before planting	42.3 a	40.8 a	34.4 ab	45.8 ab
HI remaining after cropping	34.7 b	35.8 b	29.9 ab	27.6 b

[a] Data followed by the same letter within the same row are not significantly different according to DMRT at P = 0.05.

H SPECIFIC RADIOACTIVITY RATIO (SRR)

Prior to planting, the lowest SRR was recorded in the SO_4-S fraction (Table 6.28). The SRR in the ICP-S and SO_4-S fractions was closest to 1 in the KCl-40 extract (Table 6.28). After cropping, the SO_4-S pool had the highest SRR. However the KCl-40 extract had a lower SRR than the MCP (Data not presented).

Table 6.28. The specific radioactivity ratios (SRR) in components of two extractants in the Uralla soil before planting.

	MCP	KCl-40
ICP-S	1.44 a[a]	1.12 a
SO_4-S	1.43 a	1.03 a
Organic S	1.45 b	2.27 b
2nd MCP	3.83 a	2.74 ab
HI-S remaining	19.11 a	13.39 a
Remainder	6.94 a	28.36 a

[a] Data followed by the same letter within the same row are not significantly different according to DMRT (P = 0.05).

6.8.4 Discussion

A YIELD

This study showed that S application increased the yield and S uptake of the ryegrass. The S concentration in plant tops was 0.19 % in the -S treatment compared to 0.33 % in the +S treatment.

The SR of the plant was greater in the -S treatment because of no dilution from applied fertiliser. This agrees with the findings of Shedley (1982) who found that the SR of plants in his 50 g S/g soil treatment was less than in the 10 g S/g soil treatment, due to the greater dilution of ^{35}S by the unlabelled Na_2SO_4.

B SOIL

C HI-reducible S

Approximately 50 % of the total S was HI-S both before and after cropping. This result agrees with those of Williams (1975) who reported that the HI reducible sulfur (ester sulfate) fraction ranged from 30 to 70 % in a wide range of soils from temperate areas. Kurmarohita (1973) found that the ester sulfate fraction (HI-S) constituted an average of 37 % of total S in 13 Thai surface soils. Biederbeck (1978) concluded that HI-S was the dominant form of organic S, constituting between 33 and 78 % of the total soil organic S in most mineral soils. Nguyen and Goh (1990) also found that the HI reducible S was one of the major organic sulfur fractions constituting 40 – 50 % of the total S in New Zealand soils.

The size of the HI-S pool reduced with cropping (Table 6.25) indicating that this pool supplied S to the plants and/or soil pools. This result supports the finding of Tsuji and Goh (1979) and Blair et al. (1994) who found that S uptake by plant was related to decreases in extractable and the HI-reducible S during cropping. Blair et al. (1994) also found that there was a movement of a mean of 18.6 % of ^{35}S into the plant plus plant available soil sulfate fraction after 70 days, with almost all this coming from the HI-reducible organic fraction.

D The components of extracted S

In both extractants, the sulfate fraction accounted for 5.7 to 8.4 % of the total S and the HI-reducible S remaining in the soil was approximately 45 % of the total S before planting. This result supports the findings of Freney (1961) who studied 24 topsoils from eastern Australia and found that, on average, only 6 % of the total S occurred as adsorbed plus soluble sulfate and that an average of 59 % of the total S was converted to hydrogen sulfide by the reducing mixture containing hydriodic acid. This reducible S fraction also included the inorganic fraction. Neptune et al. (1975) found that in Brazilian soils 5 to 23 % of total S was inorganic sulfate S and from 20 to 65 % of total S was ester sulfate. This compares with 2 to 8 % of inorganic sulfate and from 43 to 60 % ester sulfate S found in soils from Iowa (Neptune et al., 1975). Fitzgerald (1976) also reported that only an average of 5.2 % of the total S of 208 different soils was present as SO_4^{2-} (copy for use through out?) and of 112 different soils investigated, ester sulfate represented an average of 40.8 % of the total S.

The concentration of S in the extractants, as measured by ICP, was lower in the MCP than in the KCl-40 (Table 6.26). The KCl-40 extract removed a greater amount of S than MCP. The granitic soil used has a low S sorption capacity and the KCl-40 extract removed the solution and adsorbed sulfate fraction, and some portion of labile HI reducible S, while the MCP extracted the soil solution and adsorbed sulfate fraction and a lower proportion of the labile HI-S fraction. This was confirmed by the concentration of S from the second extraction (MCP) and the HI-S remaining in the soil after the first extraction (Table 6.26). The lowest S concentration in the second extracted fraction was recorded when the soil had been previously extracted by MCP. This extract had a higher concentration of S in the HI-S remaining fraction than did the KCl-40 extract. It was shown that MCP removed a greater amount of soil soluble and adsorbed sulfate, but a lower amount of the HI-reducible S, than KCl-40. The % of ^{35}S recovered in these fractions by these two extracts also confirmed this result. The KCl-40 extract had a higher % of ^{35}S recovery in the ICP-S and HI-S remaining fractions.

The KCl- 40 extract had a higher concentration of S, as measured by both ICP and AA, than MCP. This is because the KCl-40 extract had the ability to remove a significant amount of the soil solution sulfate and adsorbed sulfate, and a portion of HI reducible S. The KCl-40 extract, where heat was applied, extracted both inorganic and organic S fractions, as reported by Williams and Steinbergs (1959), and Spencer and Freney (1961), Fox et al. (1964) and Anderson (1992). Most of the S released by the heat treatment was found by Williams and Steinbergs (1959) to be organic. The

highest amount of soluble organic S, the loss of HI-S and mineralised sulfate, and the lowest amount of the HI-S remaining was recorded in the KCl-40 extract. The % of ^{35}S recovery by the KCl-40 also confirmed these results. The recovery of ^{35}S showed that the KCl-40 extract had the highest % of ^{35}S recovery in the ICP-S and the lowest % of ^{35}S recovery in the HI-S remaining fraction. By contrast, the MCP extract had the lowest recovery of ^{35}S in the total S and highest ^{35}S recovery in the HI-S remaining fraction.

Data on the % ^{35}S recovery in the various fractions of the extracts showed that most of the ^{35}S was recovered in the sulfate pool, with less that 10 % of the ^{35}S recovered in the soluble Organic S pool . This indicates that a large amount of the ^{35}S that was added entered the sulfate pool rather than the soluble Organic S pool. The recovery of ^{35}S was investigated by Probert (1976), who found near full recovery of the added ^{35}S labelled sulfate obtained initially by 0.01M MCP. Similarly, Santoso et al. (1995) also found almost full recovery of the added ^{35}S in the MCP extractant in a Krasnozem soil. Generally, the MCP extractant is believed to measure S from the soil solution and the adsorbed sulfate pool (Fox et al., 1964; Barrow, 1967; Searle, 1979; Blakemore et al., 1981; Sinclair et al., 1985; Anderson, 1992). The results of this study supports these findings but also shows a contribution from the HI-S pool.

The data from the ^{35}S recovered in the HI-S remaining fraction following the KCl-40 extraction showing that in excess of 23 % of ^{35}S was recovered in this fraction indicating that ^{35}S rapidly entered the HI-S pool. This result supports by the finding of Blair et al. (1994) who reported a rapid incorporation of S into the reducible organic fraction. Maynard et al. (1985) also found that in both the control and sulfate treatments of an uncropped soil, more than 64 % and 42 % respectively, of the incorporated ^{35}S was in the HI-reducible S fraction.

Both before and after cropping, KCl-40 removed a consistently higher concentration of ICP-S and the HI-S than MCP. The KCl-40 extract removed more S than the MCP extract. The reason for this has been discussed above. After cropping, lower concentrations of ICP-S and HI-S remaining were recorded in both extractants because of S uptake by the plant.

Data on the specific radioactivity (SR) of the ICP-S of the two extractants showed that the highest SR was recorded in the sulfate pool , indicating that the ^{35}S in the sulfate pool was more highly labelled than the ^{35}S in the other pools. This result was confirmed by the recovery of ^{35}S, where most of the ^{35}S recovered was in the sulfate pool (Table 6.26). There was a higher SR in the sulfate pool in the KCl-40 than in the MCP extract. This suggests that the ^{35}S was removed from the highly labile soluble and adsorbed sulfate pools and a portion of the HI-S pool.

After cropping the SR of the ICP-S in both extractants was reduced . This was because plants took up a high proportion of their S in the form of $^{35}SO_4^{2-}$. The data of the SR in the plant supports this contention. The increase in SR following cropping in the ICP-S was more marked in the KCl-40 extract than in the MCP extract in both S treatments, and the SR of the ICP-S fraction was higher than that of the HI-S remaining. This indicates that ^{35}S remained in the soil soluble and adsorbed sulfate pools and the HI-reducible S pool after cropping. Similarly, May et al. (1968) found that a large percentage of the added S was still cycling in the system after plant growth.

Data on the specific radioactivity ratio (SRR) showed that the SRR was lower in the sulfate pool than that the other pools prior to planting (Table 6.28). Within the sulfate pool, the KCl-40 extract had the SRR closest to 1.00 or the KCl-40 extract had the SR closest to the SR of the plant. This indicated that the KCl-40 was removing S from similar pools as the plant and that the plant was utilising S from the soil solution and adsorbed sulfate and some portion of highly labile HI-S pool. This result is supported by the findings of Till and May (1971) who showed, by the use of ^{35}S, that the soil sulfate extracted with MCP was a precursor of the plant S and the organic S fractions in the soil were the source of replenishment of the extractable sulfate pool. Similarly the results of the experiment reported by Blair et al. (1991) showed that the KCl-40 extract removed S from similar soil pools as did plants. Some of this S came from the ester sulfate fraction which is important in supplying S to plants.

After cropping, the SRR of the sulfate pool was greater than 1.00 in both extractants (Data not presented). This was most likely due to a higher proportion ^{35}S being taken up by the plant, and a lesser proportion of ^{35}S remaining in the soil. Both extractants had a lower SR than the SR of the plant.

This experiment has identified the reasons why the KCl-40 extract is better able to predict S supply to ryegrass than MCP and this would not have been possible without the use of an isotope of S.

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6.9 USE OF ^{35}S LABELLED LEAF SAMPLES TO DETERMINE THE TIME COURSE OF S UPTAKE BY CROPS

Source: Yasmin N (2001) Availability of Fertilizer S and Suitable Time of Application for Rice, Mustard and Cotton. Ph. D Thesis, University of New England, Armidale, Australia.

6.9.1 Background

When comparing fertilisers it is often necessary to study the time course of S uptake. This is generally done by harvesting whole plants at various growth stages and determining S content. By adding a tracer it is possible to partition the S uptake into that from the fertiliser and from the soil.

6.9.2 Methods

In the original study a comparison was made between gypsum and S coated urea. Because it was not possible to label the S coated urea the reverse dilution procedure of Shedley et al., (1979) was used. Carrier free $K_2{}^{35}SO_4$ was added at the rate of 1.44 MBq to each 2 kg pot of soil and the labelled soil was maintained at field capacity. The ^{35}S was allowed to react with the various soil S pools for two weeks before sowing. Three crops were investigated namely, mustard, rice and cotton and plants sampled at 3 growth stages (see below).

At each sampling stage, the youngest fully expanded leaf (YFEL) was removed from each plant. The YFEL samples from within each pot were bulked, cut into small pieces and placed into dried, pre-weighed digestion bottles and dried at 80 ^{o}C.

After removal of the YFEL, the remainder of the plants was cut above the surface of the soil. Following drying of this sample the plants were ground to pass a 1 mm sieve and stored for analysis. The YFEL and remaining tops samples were digested using the sealed chamber digestion technique with $HClO_4$ and H_2O_2 (Anderson and Henderson, 1986). Total sulfur was determined by ICP-AES and ^{35}S in a Liquid Scintillation Counter (LSC) (Till et al. 1984). A 3 mL sub sample of the digested plant sample was mixed with 17 mL of LSC fluid (a mixture of toulene, P-terphenyl, POPOP and teric). Radioactivity data was corrected for decay and the specific radioactivity (SR) expressed as Becquerels (Bq) per unit mass of material.

6.9.3 Results

A CORRELATION BETWEEN SPECIFIC RADIOACTIVITY (SR) OF YFEL AND TOPS AT DIFFERENT GROWTH STAGES

Mustard

Correlation data (Table 6.29) showed that at the all growth stages, there was a positive significant correlation (r^2= 0.90, 0.79 and 0.91 respectively) between the SR of the YFEL and the SR of the tops sampled at the same growth stage.

Table 6.29. Linear correlation (y = a + bx) of YFEL with the SR of tops at different growth stages. Where Y= SR of YFEL and X= SR of tops.

Sampling Stage	b	A	r^2
5-6Leaf stage	0.96	0.57	0.90 **
Bud Visible Stage	0.91	-0.32	0.79 **
Stem Elongation	1.19	-3.47	0.91 **

Rice

Correlations between the SR of single YFEL and the SR of the tops at all growth stages was positive and significant (Table 6.30).

Table 6.30. Linear correlation (y = a + bx) of YFEL with the SR of tops at different growth stages. Where Y= SR of YFEL and X= SR of tops.

Sampling stage	b	a	r^2
Active Tillering	1.03	-1.06	0.87 **
Maximum Tillering	1.11	-11.80	0.90 **
Panicle Initiation	0.88	+9.12	0.87 **

Cotton

Correlation between SR of YFEL and the SR of the tops were positive and significant at all the three growth stages (Table 6.31).

Table 6.31. Linear correlation (y = a + bx) of at YFEL sampling with the SR of tops at different growth stages. Where Y= SR of YFEL and X= SR of tops.

Sampling Stage	b	a	r^2
Three bud stage	1.32	-12.83	0.94 **
First flower	1.40	-8.61	0.84 **
Cutout	0.62	3.31	0.38 **

B ESTIMATION OF TIME COURSE OF FERTILISER S UPTAKE

These data show that the leaf sampling technique correlates well with whole plant data. This means that considerable savings in experimental costs can be obtained by using this technique. The results obtained when gypsum was applied are presented in Table 6.32, showing marked differences between crops in the timing of acquisition of fertiliser S. This information could not have been obtained without the use of isotopes.

Table 6.32. Fertiliser S uptake (mg/pot) and % of total S uptake in the growth period ( ) determined by radioassay of leaf samples from gypsum fertilised pots.

Crop	Stage	Fertiliser S uptake (mg/pot)	% of total uptake
Mustard	Sowing – 5/6 leaves	1.72	9
	5/6 leaves – Bud Visible	6.79	38
	Bud Visible – Stem Elongation	0	0
	Stem Elongation – Maturity	9.60	53
Rice	Sowing – Active Tillering	7.31	9
	Active Tillering – Max. Tillering	1.20	5
	Max. Tillering – Panicle Initiation	0	0
	Panicle Initiation – Maturity	14.13	63
Cotton	Sowing – 3 Bud	6.39	77
	3 Bud – First flower	2.12	26
	First flower – Cut out	0	0
	Cut out – Maturity	0	0

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6.10 USE OF STABLE SULFUR ISOTOPE RATIOS TO DETERMINE LONG TERM CHANGES IN SULFUR DEPOSITION IN THE BROADBALK EXPERIMENT, UK

Source: Zhao FJ,. Knights JS, Hu ZY, McGrath SP.(2003). Stable Sulfur Isotope Ratio Indicates Long-term Changes in Sulfur Deposition in the Broadbalk Experiment Since 1845. Journal of Environmental Quality, January 2003 (in press)

6.10.1 Background

There have been marked changes in atmospheric S concentrations through time. Prior to the industrial revolution atmospheric S concentrations were low and as industry developed atmospheric S concentrations rose substantially to the point where large areas of Europe received acid rain. With the clean up of the atmosphere atmospheric S concentrations have fallen substantially.

The stable S isotope ratio ($\delta^{34}S$) has been used extensively to identify sources and the fate of S in the environment (e.g. Krouse, 1977; Nriagu et al. 1991; Mayer et al. 1995; Ohizumi et al. 1997; Zhao et al. 1998; Alewell et al. 1999; Novák et al. 2001a,b). This approach requires that the S isotope ratios of the different sources are known. With this it is possible to apportion the contributions of S from the sources.

Archived samples from the Broadbalk Experiment at Rothamstead, UK have been utilized to follow the atmospheric contributions to the agricultural system.

6.10.2 Methods

c chose 44 samples of wheat (*Triticum aestivum*) grain and 38 samples of wheat straw from the archived Rothamstead Broadbalk samples spanning a period in excess of 150 years. The samples were from the continuous wheat section, where wheat straw was removed after harvest, of the control plots where no fertiliser had been applied. The experiment commenced in 1843 and has been run continuously for 159 years.

Winter wheat is normally sown in October and harvested in August, and yields of grain and straw determined. Samples of straw and grain were dried at 80°C for 16 hours before being archived in sealed glass jars or tin boxes. In this study, samples were selected in approximately 5-year intervals, starting from 1845. In addition, the yearly samples between 1970 and 1978 and between 1990 and 1999 were also included. The majority of the grain and straw samples were from the same year, except in a few cases when either straw or grain samples were missing from the archive and samples from the next available year were used.

The concentration of total S and other elements were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES), following a digestion with $HNO_3/HClO_4$ (Zhao et al., 1994). Full details of the methods used is presented in Zhou et al. (2003). The S isotope ratio was determined using a continuous flow isotope ratio mass spectrometer (20-20 IRMS, Europa Scientific Ltd, Crewe, UK), which was coupled to an elemental analyser (ANCA-SL sample converter).

6.10.3 Results

The concentrations of total S in wheat grain and straw samples fluctuated between 1.1 and 2.0 mg/g, and between 0.6 and 3.2 mg/g, respectively (*Figure 6.16*a). Despite large changes in the SO_2 emissions in the UK over the last 150 years (*Figure 6.16*b), there was no clear trend in the concentration of total S in either grain or straw from this site. Zhang et al. (2003) suggest that the lack of a response in wheat may be because the crop grown on the control plot was always limited by nitrogen.

The stable S isotope ratio ($\delta^{34}S$) in wheat grain and straw decreased rapidly from 6 – 7‰ in 1845 to between -2 and -5‰ in the early 1970s, and since then had increased to 0.5 – 2‰ in the late 1990s (*Figure 6.16*). The pattern of changing $\delta^{34}S$ in wheat straw and grain mirrored the pattern of the SO_2 emissions in the UK (*Figure 6.16*b), indicating that the soil indigenous S (derived from the parent material) and the S from anthropogenic sources had very different isotopic signatures at the experimental site.

Regression analyses showed a strong and negative correlation (P<0.001) between grain or straw $\delta^{34}S$ and annual SO_2 emissions in the UK over the last 155 years (*Figure 6.17*). The data sets before and including 1970 (the peak of SO_2 emissions) and after 1970 were used separately in the regression analyses, because it is evident that they did not follow the same regression line. For both grain and straw, the intercept and slope for the post-1970 data set were smaller that those for the pre-1970 data

set. This can be explained by a "memory effect" of anthropogenic S (with a low $\delta^{34}S$) in the soil-plant system, as a result of cycling of S between inorganic and organic pools in the soil, which caused a delay in the "recovery" of plant $\delta^{34}S$. Even if UK SO_2 emissions were to decrease to zero in the future, plant $\delta^{34}S$ would not return to the high values recorded at the beginning of the experiment. This is because a proportion of the soil S has been derived from anthropogenic sources, which will remain in the soil-crop system for a long time.

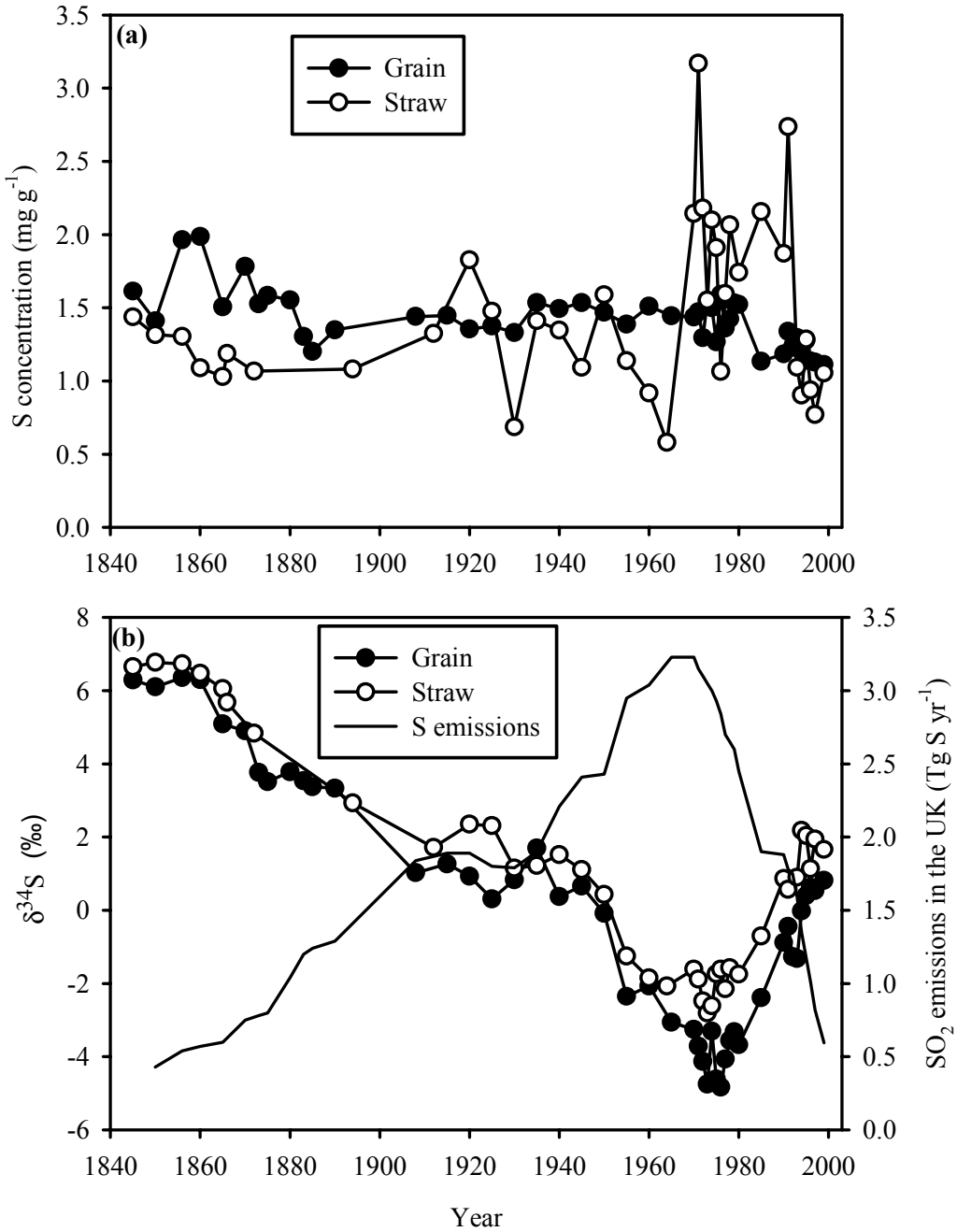

Figure 6.16. Concentrations of S (a) and $\delta^{34}S$ (b) in wheat straw and grain from the control plot of the Broadbalk experiment and UK SO_2 emissions (Zhao et al. (2003).

The strong relationships between plant $\delta^{34}S$ at the experimental site and national SO_2 emissions over the last 155 years are striking, and imply that the changes in the inputs of anthropogenic S at the site were parallel to the changes in the national SO_2 emissions. This is not unreasonable considering the proximity of the experimental site to London. Extrapolation of the regression lines for the pre-1970 data sets to zero emissions of SO_2 gave 7.2 and 7.8‰ for grain and straw $\delta^{34}S$, respectively, with a

mean of 7.5‰ for the whole plant. This value can be considered as an estimate of plant $\delta^{34}S$ growing at the site before any anthropogenic influence. Assuming that S uptake by wheat is not associated with isotopic fractionation, but that mineralization of organic S to sulfate in soil leads to 2–3‰ depletion (see above), the total S in the soil of the experimental site before any anthropogenic influence would have a $\delta^{34}S$ of about 10‰. This value represents an estimate of the mean isotopic signature for the S derived from soil parent material at the site and from other non-anthropogenic sources, e.g. S from marine aerosols, biogenic and volcanic emissions. Marine aerosol-derived S was less than 1 kg/ha/yr in the region where the experimental site is located (United Kingdom Review Group on Acid Rain, 1983; National Expert Group on Transboundary Air Pollution, 2001), and thus constituted only a small proportion of the total atmospheric deposition recorded at the site, which varied from 10 to 70 kg ha^{-1} yr^{-1} over the last 150 years (Sverdrup et al., 1995).

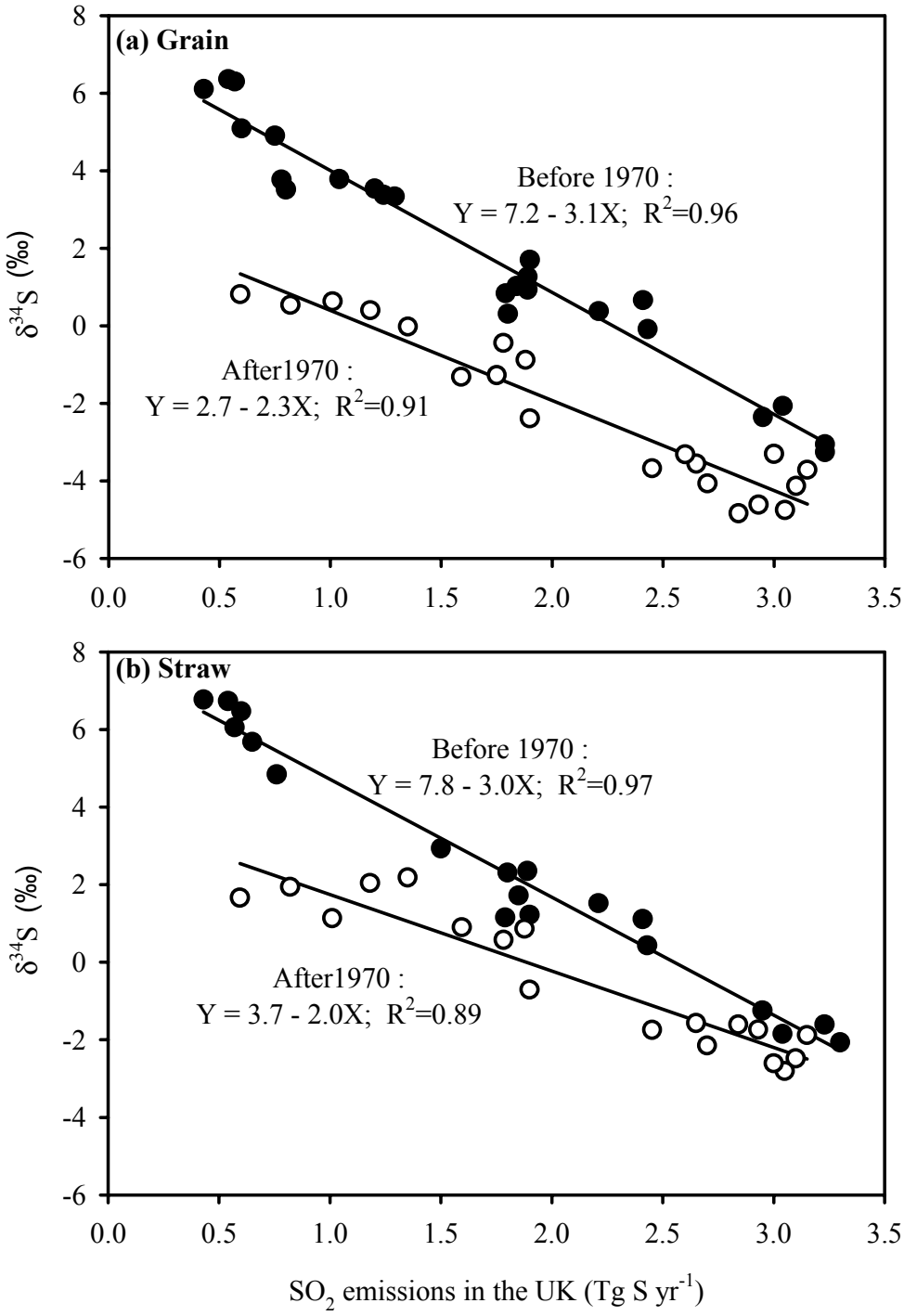

Figure 6.17. Relationships between UK SO_2 emissions and wheat grain $\delta^{34}S$ (a), or straw $\delta^{34}S$ (b) in the Broadbalk experiment.

The linear relationship between plant $\delta^{34}S$ and national SO_2 emissions, particularly in the period before 1970 (*Figure 6.17*), suggests that the anthropogenic S deposited at the experimental site had a relatively constant isotope ratio. Mixing models described by Krouse (1980) could not be used to estimate the isotope ratio of anthropogenic S for the pre-1970 period, because plant S concentration was independent of S emissions (*Figure 6.16*). However, anthropogenic S deposited at the experimental site in the pre-1970 period must have had a negative $\delta^{34}S$ to explain the negative values of plant $\delta^{34}S$ during the most polluted phase from 1950 to 1970. In the post-1970 data set, there was a tendency that the data for recent years, when national SO_2 emissions fell below 1.5 Tg S/yr^{-}, deviated from the linear trend, and the plant $\delta^{34}S$ approached a stable level of between 1–2‰. This is consistent with a $\delta^{34}S$ value of about 2‰ for the atmospheric S recorded in recent lichen samples. It appears that atmospheric $\delta^{34}S$ changed from a negative value in the early 1970s to a current value of about 2‰.

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