CHAPTER 3

DEVELOPMENT OF A SOIL SULFUR TEST FOR CROPS AND PASTURES

3.1 Introduction

Soil and tissue testing to determine S status has met with variable success (Blair, 1979; Blair *et al.*, 1992). Several indices have been used in tissue testing, and these indices vary in their success. Whilst soil testing for S has generally met with poor success (Jones, 1986) the results are inconsist (Mahler *et al.,* 1993). Numerous methods of soil sulfur testing have been reported by many investigators and there is no general agreement as to which estimate best defines a soil's sulfur supply (Probert, 1976; Tsuji and Goh, 1979). These analytical methods for soil sulfur testing have been reviewed by Freney (1986a), and Blanchar (1986) and Anderson *et al.* (1992).

Blair (1979) stated that the major problem encountered in studies of soil sulfur is the variability in the size of soil S pools over short periods of time. Simulation modelling by Blair *et al.* (1992) pointed out that fluxes of S from the organic pool play an important role in supplying S to the plants specially in pasture systems where organic matter levels are high. Sulfur from this organic pool can undergo rapid mineralisation and or splitting to plant available forms. The main source of replenishment of the sulfate pool in soils is from labile organic S by mineralisation (Watkinson and Perrott, 1990; Boswell, 1991; Lou and Warman, 1992a,b). This labile organic S is the ester sulfates (Freney *et al.,* 1971; McLaren and Swift, 1977; Tsuji and Goh, 1979; McLaren *et al.,* 1985). In general, soil sulfur testing has concentrated on developing methods that extract soil sulfur in proportion to its availability to plants (Jones, 1986).

Plants take up sulfur in form of sulfate from the soil solution which is in equilibrium with sulfate adsorbed onto colloid surfaces (adsorbed sulfate). These sulfate pools are supplied from organic sulfur pools. The organic S pools contains pools in which S is bonded directly to carbon or indirectly via O, N or S. The latter group are ester sulfate which contains C-O-S, C-N-S, C-S-S linkages and these are the most labile fraction of soil organic matter (Freney, 1986b). Drying the soil breaks the ester linkage (Barrow, 1961) which releases plant available sulfate (Blair, 1979). Both the sulfate and organic S pools vary throughout the year because of variation in the environmental condition e.g. soil temperature and moisture which is effected the microbial activity, rate of mineralisation, plant uptake, fertiliser input, atmospheric input, leaching and surface runoff. Several researchers (Barrow, 1966; Williams, 1968; Ghani *et al.,* 1990; Anderson, 1992) have found that the soil

inorganic S is higher in spring, summer and autumn than in winter. Hence, this seasonal variation will affect the ability of the extraction method used to estimate the S availability in soils (Anderson, 1992).

The poor performance of soil S testing is believed to be related to the inability of the extractants to estimate organic sulfur forms that can mineralise, and hence they underestimate the soil sulfate supplying capacity. On the other hand some extractants may extract more organic S than that which may become available to the plant, and hence they overestimate the size of the available S pool.

It is hypothesised that the ideal soil test should measure the sulfate in solution, estimate the adsorbed sulfate which is available for plant uptake and also estimate a portion of the actively turning over organic sulfur component in the soil. The objective of this study was to develop a reliable soil sulfur test which takes into account the actively turning over organic sulfur component in the soil for crops and pastures.

3.2 Materials and Methods

3.2.1 Field calibration and verification

a) Calibration soils

This study was undertaken on soil samples and yield data obtained from a series of field experiments with pasture in northern NSW (Holford and Crocker, unpubl.). The soil samples (Table 3.1) were collected from the 0-7.5 cm horizon of soils from 18 sites on the Northwest Slopes of New South Wales with a broad range of parent materials and fertiliser histories ranging from no fertiliser in the three years prior to experimentation up to 157 kg S ha^{-1} over a 10 year period.

Table 3.1 *Details of 18 pasture soils used in the field calibration study.*

Site	Great Soil Group	pH ($CaCl_2$)	Organic Carbon (%)	Colwell P ($\mu g\ P\ g^{-1}$ soil)
Tongue	Red clay	6.8	1.36	8.0
Provan	Chocolate soil	5.6	1.70	30.0
Atkinson	Brown clay	6.7	1.72	12.8
Mayo	Red brown earth	5.7	1.47	25.4
McLean	Brown clay	5.6	2.45	28.0
Charter	Non-calcic brown soil	5.4	1.28	20.7
Wellingrove	Non-calcic brown soil	5.4	1.78	27.2
Gum Flat	Grey brown podzolic	5.2	1.26	10.2
Brooker	Brown podzolic	5.1	2.02	37.2
Chaffery	Red-brown earth	5.4	1.10	29.9
Cameron	Non calcic brown soil	5.5	1.71	15.8
Croft	Yellow earth	5.5	2.55	82.7
Davidson	Red-brown earth	5.6	0.91	25.2
Dowe	Red-brown earth	5.6	1.99	15.8
Heywood	Brown podzolic	5.6	1.51	18.2
Lee	Black earth	6.7	1.95	19.0
Thompson	Chocolate soil	6.1	2.75	111.2
Wilson	Red-brown earth	5.5	2.03	17.0

Samples were taken from each site prior to the laying out of a 2Px2S factorial experiment. In the experiment, phosphorus was applied as triple superphosphate and S as gypsum with the fertilisers being topdressed onto the pasture in May or June 1987 and 1988. Pasture yields from the 3 replicates of the +P-S and +P+S plots were used to calculate S response.

Soil samples were air dried in a glasshouse and ground to pass a 2 mm sieve. They were stored in a dry condition for up to two years prior to their use in the present study.

b) Verification soils

Soils from 30 pasture trials throughout Southern Australia were used. Some of the characteristics of these soils are given in Table 3.2.

Table 3.2 *Details of 30 pasture soils used in the verification study.*

Location	State	Year	Soil type	pH ($CaCl_2$)	C (%)	Colwell (μg P g^{-1} soil)	MCP (μg S g^{-1} soil)	KCl-40 (μg S g^{-1} soil)
Wellington	NSW	1990	Red clay loam	5.4	1.8	5.5	7.8	5.6
Coonabarabran	NSW	1990	Brown sandy loam	5.0	3.5	12.1	8.4	10.2
Coonamble	NSW	1989	Black clay loam	7.0	2.5	63.2	12.3	18.8
Dubbo	NSW	1990	Grey sandy loam	4.9	1.2	14.0	2.7	2.3
Dubbo	NSW	1990	Red sandy loam	4.4	0.8	5.9	1.6	2.0
Dubbo	NSW	1990	Red sandy clay loam	5.0	1.3	7.3	4.6	3.5
Dubbo	NSW	1990	Sandy loam	4.1	2.3	6.5	7.1	6.7
Parkes	NSW	1990	Red loam	5.0	1.2	8.7	7.1	5.7
Parkes	NSW	1990	Red loam	7.0	1.9	9.2	4.3	5.8
Parkes	NSW	1990	Red clay loam	5.0	1.5	11.9	7.4	6.7
Parkes	NSW	1990	Red loam	5.5	1.7	9.1	2.6	2.8
Parkes	NSW	1990	Red sandy loam	4.8	1.0	12.7	2.6	2.6
Duri	NSW	1989	Brown clay	6.2	1.9	10.4	6.5	4.5
Nundle	NSW	1989	Red brown earth	5.4	1.7	20.4	5.5	3.9
Bithramere	NSW	1989	Black earth	6.3	1.3	19.3	4.3	3.6
Mintaro	SA	1988	Red brown earth	6.3	2.7	45.4	20.0	14.6
Wattle range	SA	1988	White sand	4.8	4.6	14.5	2.8	4.3
Wallandue	Vic	1988	Dark grey clay loam	4.3	3.9	38.8	13.1	7.9
Millicent	Vic	1988	Black clay	7.3	6.6	39.1	10.2	8.1
Edenhope	SA	1988	Sandy loam	4.8	2.0	72.9	6.0	4.6
Victor Harbor	SA	1988	Sandy loam	4.4	6.2	58.1	19.6	18.6
Prospect Hill	SA	1991	Yellow brown podzolic	6.0	2.9	40.8	5.9	8.5
Inman	SA	1991	Solodic	6.5	3.6	25.5	9.3	5.9
Mt Pleasant	SA	1991	Brown podzolic	6.5	3.1	37.8	11.3	12.8
Ashbourne	SA	1991	Solodic	6.0	2.3	8.4	5.6	7.0
West Kendenup	WA	1990	Gravel loam	5.6	7.0	21.6	15.9	12.3
Coolup	WA	1990	Loamy sand	5.3	2.7	33.0	13.2	16.2
Chittering	WA	1990	Red clay loam	5.2	2.5	7.4	12.5	9.9
Quindanning	WA	1990	Gravelly sandy loam	6.0	5.6	16.1	14.7	10.6
Coolup	WA	1991	Loamy sand	5.3	2.3	28.0	12.8	12.5

The soils samples from the 30 sites were collected at 0-7.5 cm depth for the sites within New South Wales and 0-10 cm for the other States. Samples were taken from each site prior to application of fertilisers. The samples were air dried and ground to pass a 2 mm sieve. They were stored in the cold room (4°C) for up to six months except for the soils collected in 1988 which were stored for two years at room temperature before being analysed. In the experiments, phosphate was applied as triple superphosphate and S as single superphosphate. The fertiliser was applied in May or June in the year (Table 3.2) with pasture yield measured in the following winter and spring periods.

c) Extraction procedures

The following soil S extraction techniques were used:

i) measurement of sulfur in soil solution including inorganic and organic S

Water (Freney, 1958)

Four g of soil was shaken with 20 mL of deionised water on an end-over-end shaker at 25°C for 1 hour. The extract was filtered through a Whatman No. 42 paper and S analysed by ICP-AES.

ii) measurement of inorganic and organic sulfur in soil solution, plus adsorbed sulfur

0.01 M $Ca(H_2PO_4)_2$ (MCP) (Barrow, 1967)

Four g of soil were shaken with 20 mL of 0.01M MCP for 1 hour on an end-over-end shaker at 25 °C. The extract was filtered and S analysed by ICP-AES.

iii) measurement of inorganic and soluble organic sulfur in soil solution, adsorbed sulfur and a portion of the organic sulfur not in solution

a) *heated 0.25 M KCl (modified Gianello and Bremner, 1986).*

Three g of soil were weighed into a glass screw top vial and 20 mL of 0.25M KCl added (2M in Gianello and Bremner, 1986a,b). The vial was tightly capped and heated in an oven at temperatures of either 100, 80, 40 or 25 °C for 3 hour (instead of 4 hour in Gianello and Bremner, 1986a,b). Reducing the concentration to 0.25M KCl and time to 3 hour was found to be more applicable in a preliminary experiment. An additional treatment, where the soil was shaken at 25°C for 16 hour was also included. The extracts were filtered and S analysed by ICP-AES.

b) *0.5 M $NaHCO_3$ (Kilmer and Nearpass, 1960).*

Four g of soil and 20 mL of 0.5M $NaHCO_3$ were tumbled on an end-over-end shaker for 16 hours at 25°C. The extract was filtered 5 mL of the filtrate transferred to a 75 mL digestion tube and 1.5 mL of perchloric acid/water digestion solution (1/1 v/v) slowly added. The mixture was digested at 190°C until the solution was clear and nearly dry, 1.6 mL 1M NaOH was added to neutralise the samples and made to a volume of 7.5 mL. Sulfate was analysed by using the modified $BaSO_4$ precipitate method (Till *et al.*, 1984) on the density of the precipitate measured in a spectrophotometer at 420 nm rather than the Johnson and Nishita (1952) method used by Kilmer and Nearpass (1960).

All 8 extractants were used in the calibration study but only the MCP and KCl-40 methods were used in the verification study.

d) Statistical Analysis

In both the calibration and verification studies the relationship between the concentration of S extracted by the various extractants (μg S g^{-1} soil) and the % maximum yield was established by fitting a Mitscherlich relationship of the form

$$Y = a[1-b\ exp(-cX)]$$

where Y = % maximum yield

a = Asymptote

X = soil test value (μg S g^{-1} soil)

c = curvature coefficient, and

b = intercept

In the calibration study % maximum yield was calculated as [yield from (+P-S)/yield from (+P+S)x100]. In the verification studies the % maximum yield was calculated as [(yield from triple superphosphate/yield from single superphosphate) x 100]. When fitting the curves the asymptote (a) was set at 100%.

Soil S status (μg S g^{-1}) by the range of extractants were analysed using the statistical package NEVA (Burr, 1982) for analysis of variance. Differences between treatment means are considered to exist based on the Duncans multiple range test (DMRT) at the 5% level of probability ($P < 0.05$).

3.2.2 Pot studies

a) Soil and experimental procedures

The soil used (Table 3.3) in the experiment was an Aquic Haplustalf which was responsive to S. The soil was derived from an experiment of a comparison of S sources and placement, with two consecutive rice crops grown in the same pot (Chaitep, 1990). Soil from 2 of the non-flooded treatments were used in the study of sources of extracted S; control (no S added = unfertilised) and the gypsum treatment (fertilised). The re-applied gypsum treatments from the flooded and non-flooded treatments of this experiment were used in the study of removal of ^{35}S by plants and by different extractants.

Table 3.3 *Chemical characteristics of the soil used in the pot study (Chaitep, 1990).*

Soil characteristic	Value
Colwell P[A]	17.0 µg P^{-1}
Organic P[B]	135.8 µg P^{-1}
Total P[B]	275.0 µg P^{-1}
Extractable S[C]	6.9 µg P^{-1}
pH (1.5 0.01 M $CaCl_2$)	4.5
pH buffer capacity[D]	0.01 (mol kg^{-1} dry soil)
Organic carbon[E]	0.87%
ECEC.	32.0 m mol (p+) kg^{-1}
Exchangeable cations expressed as a % of ECEC[F]	
Ca	69.9%
Mg	10.2%
K	4.9%
Na	5.4%
Al	9.5%

A *Colwell (1963)*
B *Saunders and Williams (1955,*
C *Barrow (1967)*
D *Kirk and Nye (1985)*
E *Walkley and Black (1934)*
F *Gillman (1979)*

^{35}S labelled gypsum (0.03 M Bq ^{35}S mg S^{-1}) was added to the soil at transplanting at a rate of 18 kg S ha^{-1} (equivalent to 4.82 mg S kg^{-1} soil) and the first rice crop grown to maturity. Both the non-flooded and flooded pots were allowed to dry for 2 weeks following the first harvest, and repotted. The flooded treatments were re-flooded for 10 days before planting the second rice crop. Unlabelled gypsum was re-applied at 6 kg S ha^{-1} to the surface of the unfertilised pots and seedlings for the second crop transplanted. This rice crop was also allowed to grow to maturity.

Soil from entire pot were sampled at harvest of the second crop. These samples were air dried in a forced-draft oven at 25°C and ground to pass a 2 mm sieve prior to analysis. The extractable S from all soils was related to S uptake by that crop. There were 3 replicates of each treatment.

The % of HI reducible S removed by the extractants was determined as follows [(HI-S before extraction - HI-S after extraction)/HI-S before extraction] x 100.

Studies of plant-soil interactions or changes in soil nutrient pools can be carried out by simply measuring the changes in nutrient concentrations in the various plant and/or soil pools. The use of a stable or radioactive isotope label allows much more specific monitoring of the transfer of nutrients between pools and elucidation of the specific pathways (Till and Abdullah, 1983). This can be done by directly labelling added material, such as fertiliser, or using a reverse dilution technique in which soil nutrient pools are labelled and then nutrient transfers monitored as fertiliser/s are added or other treatments imposed.

The pot trial used in this experiment and those reported in Chapters 4 and 5 used the reverse dilution technique to assess the similarities between the pool/s of soil S extracted by a range of extractants and the pool/s of S from which plants obtained S.

When a labelled sulfate is added to a soil the ^{35}S enters the inorganic and organic S pool. There is initially a rapid increase in the specific activity (SA) of the soil solution, which declines over time as the labelled sulfate enters a number of pools, as unlabelled sulfate is released from these pools and as sulfate is taken up by the plant. The SA in the plant likewise increases and then decreases as the SA of the soil pool decreases. When the SA of the plant is initially high, only a small amount of S and dry matter is accumulated. The SA of the plant declines as larger amounts of S and dry matter accumulates.

The ratio of the SA (SA = the ratio of labelled to unlabelled S) of the plant at harvest to SA of the extracted soil pools, termed the specific activity ratio (SAR) (the SA of the plant/the SA of the soil extract) gives an indication as to whether the plant has been using sulfur from the same pool/s as removed by the extractant. An SAR value of 1 indicates that soil S pool/s extracted by the plant and that removed by the extractant are similar, or the extractant and the plant are drawing sulfur from the same soil pool/s.

b) Extraction procedures

In the pot soil study, six extraction methods were evaluated. These were water, MCP, $NaHCO_3$, KCl-100, KCl-80, and KCl-40 (Section 3.2.1C). Soils were first extracted with these reagents, centrifuge at 5200 g for 20 minutes and a 0.6 g subsample of the extracted sample transferred to a round-bottomed Johnson and Nishita (1952) flask. HI reducible S was then determined on the sample by the method of Dean (1966). This involved adding 2 mL of deionised water and 4 mL of reducing mixture containing a 4:1:2 mixture of hydriodic acid (SG 1.4), hypophosphorus acid (50%) and formic acic (90%), and attaching the flask to a Johnson and Nishita (1952) apparatus modified for the finish of Dean (1966). The dry weight of soil in the subsample was determined on a duplicate sample. The HI reducible S concentration in the soil prior to extraction was determined using a 0.2 g sample.

c) Measurement of ^{35}S

The ^{35}S in all soil extracts were measured by liquid scintillation counting (LSC). The sample containing the ^{35}S was added to the scintillation mixture which was prepared by using Toluene as the organic solvent and P-terphenyl (PTP) as the primary fluor (a compound that converts molecular excitation energy into light photons). A secondary fluor, 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP) was used as a wavelength shifter. A microemulsion forming agent Teric G9A8 was also added to the scintillation mixture to aid in the intimate mixing of sample and scintillator.

The scintillation mixture (cocktail) was prepared as follows: 16.92 g of PTP and 0.73 g of POPOP were dissolved in 1 L of hot Toluene, and 2 L of Teric was mixed with 1 L of Toluene. The PTP/POPOP solution was then added to the Teric/Toluene solution and the volume made up to 5 L with Toluene.

Counting was carried on 2 mL of soil extractant and 18 mL of cocktail. The samples were counted in Tri-Carb 2000 Liquid Scintillation Analyser. Total S and ^{35}S in the plant samples from the pot experiment had been determined by Chaitep (1990).

d) Statistical analysis

Data on the concentration of S in the extract (µg S g^{-1} soil), % of S removed from the HI-S pool and specific activity ratio (SAR) data were analysed using the statistical package NEVA (Burr, 1982) for analysis of variance.

The statistical differences between treatment means were considered to exist when they were different at the 5% level according to the Duncans multiple range test (DMRT) ($P < 0.05$).

3.3 Results

3.3.1 Field calibration

There were significant differences in the concentration of S in the various extractants (Table 3.4). In the 18 pasture soils, the mean concentration of S in the various extractants increased in order: KCl-25°C < MCP = KCl-40°C = KCl-16 hr < H_2O < KCl-80°C < KCl-100°C < $NaHCO_3$ (Table 3.4). As the extraction temperature with KCl was increased from 25°C to 100°C the amount of S extracted significantly increased from 5.4 μg S g^{-1} soil to 15.6 μg S g^{-1} soil.

Table 3.4 *Sulfur extracted by the various techniques (μg S g^{-1} soil) in 18 pasture soils from northern New South Wales.*

	Extractant							
Sites	H_2O	MCP	KCl-100	KCl-80	KCl-40	KCl-25	KCl-16 hr	$NaHCO_3$
Tongue	3.5	3.1	10.0	6.2	2.6	1.9	3.3	14.5
Provan	14.9	11.4	31.4	21.1	13.9	11.2	16.4	34.3
Atkinson	5.8	4.7	15.5	13.1	3.8	3.7	5.0	15.7
Mayo	5.9	4.7	14.0	10.5	4.9	4.6	6.2	16.0
McLean	8.5	7.6	18.6	13.2	7.2	4.7	6.6	37.2
Charter	6.7	6.3	11.2	9.7	5.6	6.1	6.3	24.6
Wellingrove	15.9	14.1	27.0	26.9	16.5	14.4	18.3	36.0
Gum Flat	8.1	7.5	15.7	11.3	6.7	4.9	7.8	27.5
Brooker	8.9	8.0	26.0	15.8	9.2	6.5	8.2	28.3
Chaffery	6.4	5.3	10.7	11.5	7.0	3.3	6.8	21.4
Cameron	11.6	10.6	20.9	16.0	11.1	7.0	8.3	32.7
Croft	6.6	6.0	12.0	8.8	4.1	3.5	4.3	29.5
Davidson	4.3	3.3	6.5	7.3	1.4	3.6	3.7	24.6
Dowe	5.7	4.6	8.7	7.9	2.6	3.4	4.0	30.7
Heywood	7.8	5.6	11.4	10.6	7.3	5.4	6.4	31.1
Lee	6.6	6.5	13.1	9.2	4.4	3.4	3.6	22.0
Thompson	8.0	5.8	15.6	11.0	5.9	5.7	4.7	38.0
Wilson	5.9	4.6	13.3	10.2	5.9	4.3	4.9	29.7
Mean	7.8 d[A]	6.7 e	15.6 b	12.4 c	6.7 e	5.4 f	6.9 e	27.4 a

[A] *Numbers followed by same letter are not significantly different according to DMRT.*

The coefficient of determination (r^2) for the relationship between % maximum dry matter yield from the 18 pasture soils and soil test is presented in Table 3.5.

Table 3.5 *Coefficient of determination (r^2) for the relationship between extractable sulfur and % of maximum yield in 18 pasture soils from northern New South Wales.*

Sulfur extraction method	Coefficient of determination[A]	Critical level[B]
Water	0.45	8.4
0.01M $Ca(H_2PO_4)_2$ (MCP)	0.47*	7.1
0.25M KCl heated at 100°C	0.51*	19.1
0.25M KCl heated at 80°C	0.54*	12.4
0.25M KCl heated at 40°C	0.73**	6.5
0.25M KCl heated at 25°C	0.30	6.6
0.25M KCl shaking for 16 h at 25°C	0.48	6.7
0.5M $NaHCO_3$	0.04[C]	-

*$P < 0.05$, **$P < 0.01$

[A] $n = 18$, [B] *Soil S test level at Y = 90% maximum yield,* [C] *Linear regression.*

The 0.25M KCl heated at 40°C (KCl-40) extraction method gave the best relationship between extractable sulfur and the % maximum dry matter yield with a r^2 value of 0.73. Increasing the temperature of the KCl extractant to 100°C resulted in a decrease in the r^2 value to 0.51. A r^2 value of only 0.47 was recorded for the MCP extract. The relationship was not significant with water or KCl-25°C. It was not possible to fit a Mitscherlich curve to the $NaHCO_3$ data, so a linear relationship was fitted with a very low r^2 (0.04) (Table 3.5).

The data points for the water, MCP, KCl-100, KCl-80, KCl-40 and KCl-25°C, KCl shaken for 16 h at 25°C, and $NaHCO_3$ extractions are presented in Figure 3.1. The higher correlation using the KCl-40 extraction method was most likely due to a better definition of the soils in the intermediate S range.

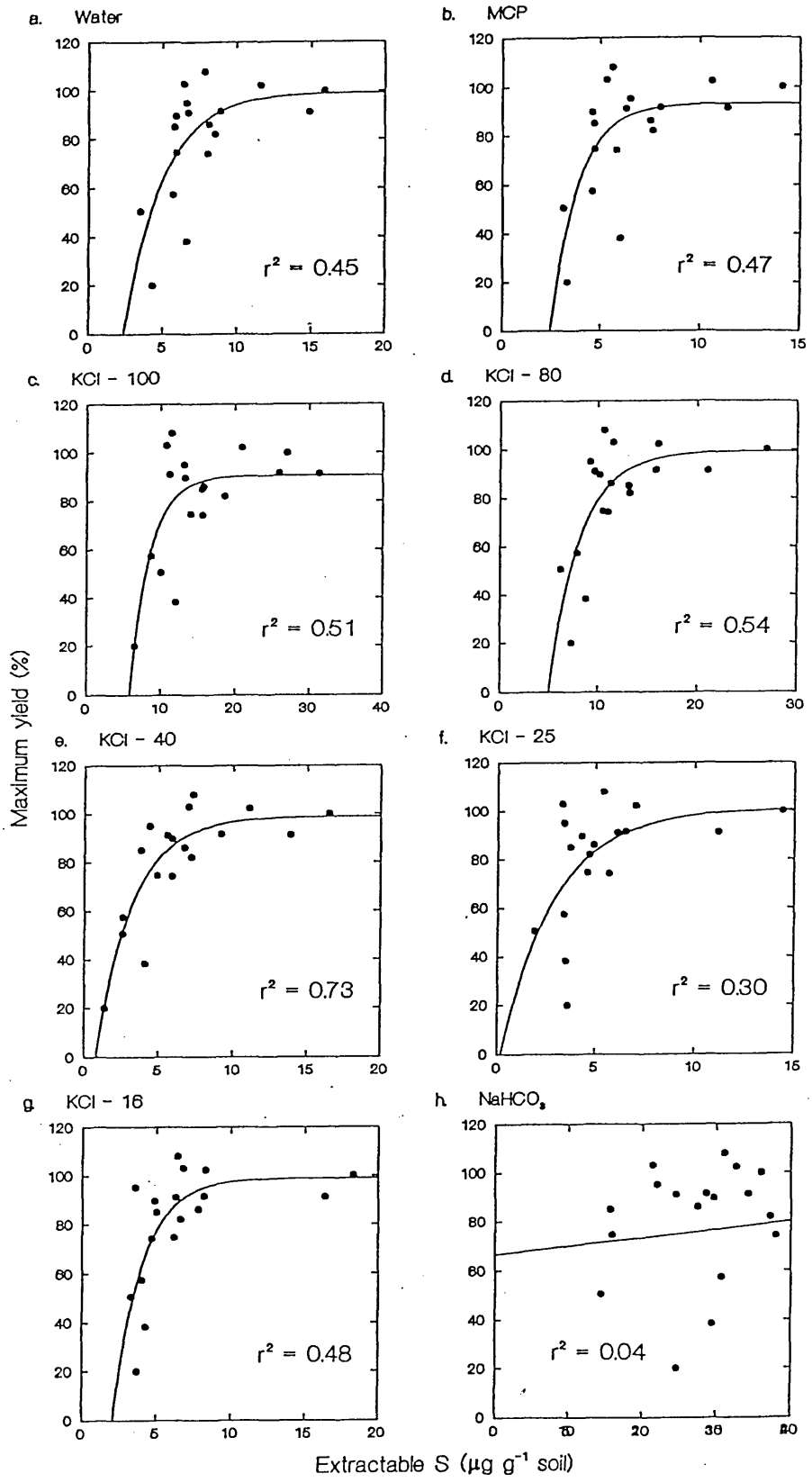


Figure 3.1 *Relationship between % maximum dry matter yield of pasture and S extracted by a) water, b) MCP, c) KCl-100°C, d) KCl-80°C, e) KCl-40°C, f) KCl-25°C, g) KCl shaking for 16 h at 25°C and h) $NaHCO_3$.*

3.3.2 Verification study

The soil sulfur test values and relative yield data from a further 30 pasture trials are presented, with data from the original 18 sites, in Figure 3.2. Mitscherlich curves of the form $Y = a[1-b\ exp(-cX)]$ were fitted to these data. The correlation between soil S test level and % maximum yield were significant for all four curves, but were higher for the KCl-40 S than the MCP S in both the calibration and verification studies. With both extractants, the correlation was lower in the verification study than in the calibration study.

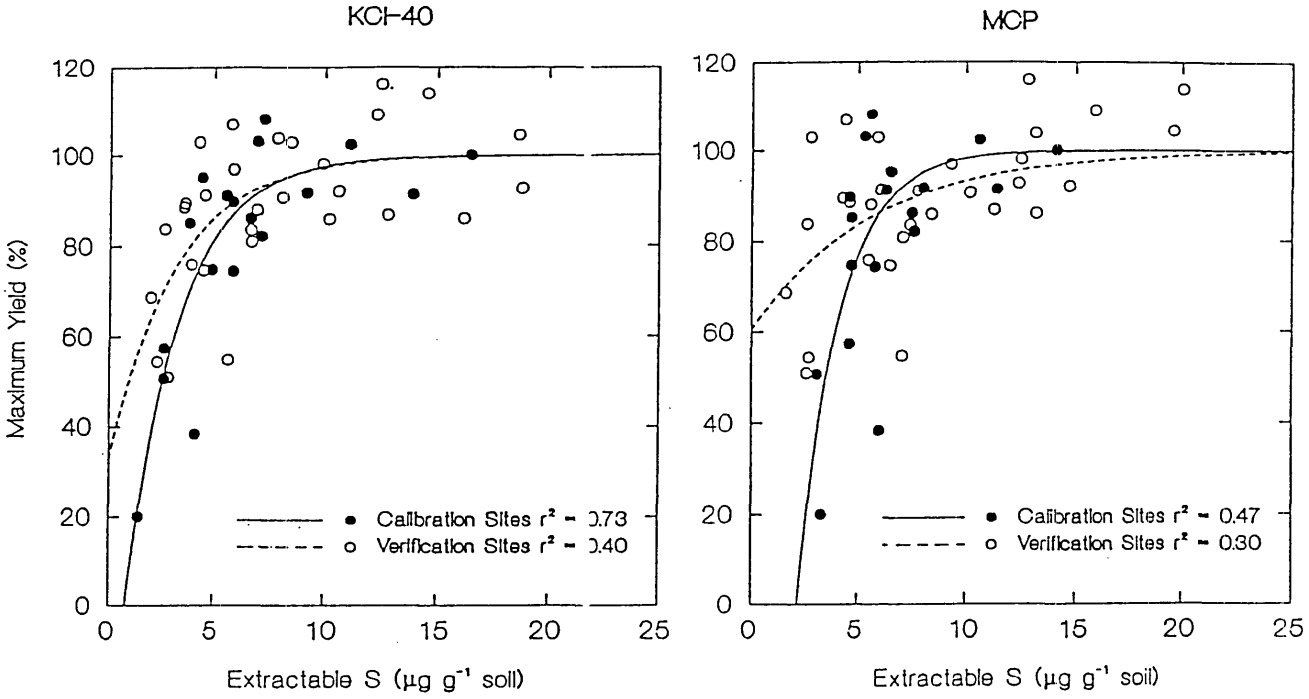


Figure 3.2 *The relationship between % maximum dry matter yield of 30 pasture sites and S extracted by the KCl-40 and MCP methods.*

Evaluation of the reduction in the residual sum of squares when curves were fitted to the two data sets (n = 18 and n = 30), as compared to a single relationship for all 48 data points, showed a significant improvement when two curves were fitted for both the KCl-40 and MCP extractions. This suggests that for both extractants, the two data sets have slightly different relationships between % maximum yield and extractable S. The improvement with fitting two curves was more significant with the MCP extraction (P = 0.012) than the KCl-40 extraction (P = 0.045).

Since the two lowest relative yields of the calibration sites were much lower than the lowest of the verification sites, it can be argued that these low points be excluded when comparing separate curves for the two data sets to a single curve for all the data. When these points are removed and the comparison is carried out between the verification and calibration data with maximum yields over a similar range (n = 16 and n = 30), there is no significant improvement when the two data sets are fitted separately for either the KCl-40 (P = 0.372) or MCP (P = 0.167) extractions.

The b and c parameters were not significantly different between the calibration and the verification curves for either extractant, however, the absolute and relative changes in these parameters between the two data sets were larger and the average standard errors (ASE) were greater for the MCP S than for the KCl-40 S (Table 3.6). The greater error associated with these parameters in the MCP S curves is reflected in the lower correlation between % maximum yield and extractable S and in a larger change in the critical soil test value. The soil S test value at 90% maximum yield changed from 6.6 to 6.0 μg S g^{-1} for the KCl-40 method and from 6.6 to 7.8 μg S g^{-1} for the MCP method.

Table 3.6 *Coefficient of determination (r^2), b and c parameters, with average standard errors (ASE-b and -c), and the soil S test value at 90 % maximum yield for the relationship between S extracted by the KCl-40 and MCP methods and % maximum yield in calibration (18 pasture soils) and verification (30 pasture soils) studies.*

		r^2	b	ASE-b	c	ASE-c	Critical value[A]
MCP	Calibration (n=18)	0.46*	3.039	1.966	0.513	0.160	6.6
	Verification (n=30)	0.31*	0.394	0.149	0.175	0.078	7.8
KCl-40	Calibration (n=18)	0.73**	1.367	0.316	0.398	0.079	6.6
	Verification (n=30)	0.40*	0.671	0.276	0.316	0.115	6.0

[A]= Soil S test level at Y = 90% maximum yield. ** P < 0.05, ** P < 0.01*

3.3.3 Pot study

a) Sources of S extracted.

The fraction of sulfur removed by the various extractants from unfertilised and fertilised non-flooded rice soil are presented in Table 3.7. The lowest concentration of S was in the H_2O and MCP extracts whilst the $NaHCO_3$ extractant had the highest concentration of S. The KCl-100 extract also had a high S concentration. As the temperature of extraction with KCl was reduced from 100°C to 40°C, the concentration of S in the extract decreased.

$NaHCO_3$ removed the highest amount of S from the HI reducible S fraction. Extraction with KCl 100°C also removed a significant amount of the HI-reducible S fraction. This amount declined as the extraction temperature was reduced to 40°C. The amount of S removed from the HI-reducible S did not significantly differ among the water, KCl-40 and MCP methods. These patterns of extraction were similar in both the unfertilised and fertilised soils. However, there was no significant difference between the unfertilised and fertilised soils in the amount of S removed from HI-reducible S fraction.

Table 3.7 *S in extract (µg S g^{-1} soil) and % S of removed from HI fraction in unfertilised and fertilised, non-flooded soil.*

Extractant	S in extract (µg S g^{-1} soil)		% of S removed from HI-S pool	
	Unfertilised	Fertilised	Unfertilised	Fertilised
H_2O	2.6 j	3.3 h	7.1 c	6.0 c
MCP	2.6 j	2.9 i	6.6 c	5.9 c
KCl-40	2.9 i	3.6 g	7.0 c	5.9 c
KCl-80	5.8 f	6.3 e	11.4 c	11.5 c
KCl-100	17.3 d	18.8 c	32.2 b	32.6 b
$NaHCO_3$	23.0 b	24.8 a	42.9 a	38.8 a

Numbers within each of the two parameters, which are followed by the same letter, are not significantly different according to DMRT.

b) Relationship between S removed by extractants and plants.

Data on SAR from both non-flooded and flooded rice are presented in Table 3.8.

Table 3.8 *Specific activity ratio (SAR) from rice grown in non-flooded and flooded soils.*

Extractant	System	
	Non flooded	Flooded
H_2O	1.21 fg	1.25 ef
MCP	1.14 h	1.18 gh
KCl-40	1.07 i	1.08 i
KCl-80	1.23 f	1.27 e
KCl-100	2.12 d	2.53 c
$NaHCO_3$	3.66 b	5.24 a

Numbers followed by the same letter are not significantly different according DMRT.

The lowest SAR value was recorded in the KCl-40 treatment and this was close to 1.00 in both the non-flooded and flooded soils. The SAR with the water extract was not significantly different from the KCl-80 treatment, and higher than MCP. The highest SAR value was found in the KCl-100 and $NaHCO_3$ treatments, with the SAR value greater than 1.00 in both the non-flooded and flooded soils.

3.4 Discussion

3.4.1 Comparison of extractants

In this study the total amount of S extracted by the various extractants was measured by ICP-AES except in the $NaHCO_3$ which underwent digestion to oxidise extracted organic S prior to turbidimetric determination. On the basis of previous studies the analysis would include soluble organic S (Maynard *et al.*, 1987; Vendrell *et al.*, 1990; Holmberg, 1991; Watkinson and Perrott, 1990; Boswell, 1991). The heated KCl method was modified from the method of Gianello and Bremner (1986a,b) who used a 2M KCl extract heated at 100°C to assess potentially available N and nitrate plus ammonia. This method was found to be highly correlated to the incubation methods used for assessing potentially mineralisable organic N in soil. The heated KCl method was originally used to extract soil S by Anderson (personal communication) who indicated that the 2M KCl method extracted S from the organic S pool. Anderson (1992) modified the method by reducing the concentration to 0.25M KCl heated in the oven at 98°C and measured with the ICP-AES. The reduction of the concentration from 2M KCl to 0.25M KCl was more applicable for the ICP-AES measurement and it had no affect to the amount of S extracted (Anderson, personal communication).

In the field calibration study the amount of S extracted by H_2O was higher than the MCP or the KCl solution at temperatures up to 40°C (Table 3.4). This result is similar to that reported by several workers. Spencer and Freney (1960) found that H_2O extractable S was greater than MCP for 37% of the soils they examined. Williams and Steinbergs (1959) also observed that H_20 extracted more soil organic S than $CaCl_2$ solution. Maynard *et al.* (1987) found that H_2O extracted significantly more organic S than many salt solutions because of the greater dispersion of organic matter by H_2O.

The results of this study differ from the findings of Santoso (1989) who found that the same amount of S was removed by either water, MCP or $CaCl_2$ solutions in the low sorbing granitic soil and MCP extractant removed more S than H_2O or $CaCl_2$ in a high sorbing soil. Recently, Anderson (1992) found that the amount of S extracted by H_20 was equal to or greater than the MCP (total) on granitic soils but not on the basaltic soils he studied.

Water dissolves more organic S than these extractants due to the higher pH of H_2O. A similar effect was shown by Kilmer and Nearpass (1960) who found that the higher amounts of organic S were extracted when the pH of $NaHCO_3$ solution was increased from 8.5 to 10.0.

Increasing the temperature from 25°C to 100°C resulted in a higher amount of extracted S (Table 3.4). Williams and Steinbergs (1959) suggested the use of "heat soluble sulfur" and indicated that heat is the main factor leading to the release of additional sulfur. Spencer and Freney (1960) found that the amount of S extracted by hot water were almost three times the amount extracted by cold water. Fox *et al.* (1964) also found that the heat-soluble and autoclave methods extracted from twice to four times as much as S, respectively as did water or phosphate. Recently Anderson (1992) observed that the heated KCl method extracted greater amounts of S than either MCP or water. The results of this study supports these findings.

In the present study, the $NaHCO_3$ method removed more S than the H_2O, MCP and KCl methods (Table 3.4). This result is similar to that found by Anderson (1992) who found the $NaHCO_3$ method extracted a greater amount of S than the H_2O, MCP and the heated KCl methods. Nguyen and Goh (1992a) also found the $NaHCO_3$ extracted more S than MCP. The different amounts of S extracted by these extraction methods is due to the different amounts of inorganic and organic S extracted. It is not possible to determine from these studies the amount of adsorbed sulfate removed by 0.5M $NaHCO_3$ and 0.01M $Ca(H_2PO_4)_2$. On the basis of adsorption strength $H_2PO_4^-$ would be expected to desorb more SO_4^{2-} than HCO_3^{2-}. On the other hand the higher concentration of HCO_3^{2-} ions than $H_2PO_4^-$ ions would result in a greater mass action effect. The $NaHCO_3$ would be expected to extract more soil organic S due to the high pH (Kilmer and Nearpass, 1960). Watkinson and Perrott (1990) also found that solutions of increasing alkalinity extracted increasing amounts of organic S, i.e. $NaHCO_3$ pH 8.5 > $Ca(H_2PO_4)_2$. pH 4. The results reported here confirm the findings of Rehm and Caldwell (1968) Probert (1976) and Nguyen and Goh (1992a) who found that $NaHCO_3$ extracted S from both adsorbed sulfate and a fraction of the organic S with amount of S extracted greater than that extracted by phosphate solution.

In the pot study the amount of S extracted by water and MCP was equal in both the unfertilised and fertilised soils (Table 3.7). Soils used in this study had a low S sorbing capacity (Triana, 1991) thus the water and MCP methods removed equal amounts of S. This result is similar to the findings of Santoso (1989). Anderson (1992) also found that the amount of S extracted by water was equal to the MCP method in the same soil type as used in this study. The amount of S extracted by the KCl methods was of the similar pattern to that of the field calibration study (Table 3.4). The $NaHCO_3$ also removed more S than the other extractants.

The field correlation study (Table 3.5) showed a poor correlation between MCP or water extractable S and pasture response to S across the 18 sites used. This result is similar to that reported for MCP in several field studies. Spencer and Glendinning (1980) found that there was no relationship between phosphate-extractable sulfur and the relative yield of the pasture. Hoque *et al.* (1987) also found that sulfate extracted by MCP did not correlate with field response to S in grass.

The r^2 value of 0.47 found for the MCP extract in this study is the same as that found by Rayment (1983) using soil from Queensland. Similarly, a low r^2 for the MCP has been found by many workers. A very low r^2 of 0.11 was found by Jones *et al.* (1983 cited in Jones, 1986). Hoeft *et al.* (1973), Probert and Jones (1977) and Vaughn *et al.* (1987) found the r^2 of 0.32, 0.34 and 0.40 for the MCP extract respectively in their studies. An r^2 of 0.43 in corn was reported by Reneau and Hawkins (1980 cited in Jones, 1986). These r^2 values are considerably lower than the r^2 found in this study.

The concentration of S extracted by water was found to be correlated well (r^2 = 0.77) with pasture response to S of legume and grass by Walker and Doornenbal (1972). Westermann (1974) also found a good correlation between water extractable S and the yield response of alfalfa with a r^2 value of 0.62. The r^2 value of 0.45 found for the water extractant in this study is lower than that found by these workers.

The KCl-100 extract, which was adapted from the soil N extractant of Gianello and Bremner (1986a,b) and $NaHCO_3$ were found to be poorly correlated with pasture response to S. This result differs from the findings of Anderson (1992) who found that heated to 98°C KCl and $NaHCO_3$ were more highly correlated to plant response than MCP and H_2O in both early winter and spring. Similarly, William and Steinbergs (1964) found the $NaHCO_3$ extractable S was highly correlated to S content of oats. Some other researchers (Kilmer and Nearpass, 1960; Freney *et al.*, 1971; Nguyen and Goh, 1992a) have also found that $NaHCO_3$ was a reasonable predictor of soil S status. However the heated $NaHCO_3$ and KCl methods were not consistent in Anderson's (1992) experiment.

The coefficient of determination between KCl-40 extractable sulfur and pasture response in the present field study is high for such a study (Table 3.5). This value compares with an r^2 of 0.61 for the best P extraction method (Bray No 1) reported by Holford and Crocker (1988). It is also higher than that reported for six S extraction techniques in the glasshouse experiment of Anderson (1992).

In the verification study, the relationships between % maximum yield and extractable S were different from those in the calibration study. In both studies, the KCl-40 method was more highly correlated with yield response to S than the MCP method and the improvement in fitting separate relationships to the data sets from each study, compared to a single relationship, was greater for the MCP method, indicating that this relationship had changed more (Figure 3.2, Table 3.6). The higher correlation, smaller change in b and c parameters, lower average standard errors and smaller change in the critical value between the two data sets, strongly suggests that the KCl-40 S extraction is a more robust soil test.

In the verification study, soil samples were collected in May or June and pasture yield measured in the following winter and spring (Section 3.2.1b). The level of S would be expected to be high in autumn, to decline over winter and to increase due to mineralisation over spring (Barrow, 1966; Williams, 1968; Ghani *et al.,* 1990, Nguyen and Goh, 1992b). The KCl-40 extract appears to extract the ester sulfate fraction which is mineralised during spring. This ester sulfate fraction is potentially available to plants, hence the S extracted by KCl-40 was found to be better correlated with pasture response to S compared to the MCP method.

The critical level for the KCl-40 method in the verification study was found to be less than the calibration study. In contrast, the MCP method had a higher critical level in the verification study than in the calibration study (Table 3.6). This was probably due to the depth of soil sampling used. In the calibration study, soil samples were collected from the 0-7.5 cm while soil samples in the verification study were taken from depth of both 0-7.5 cm and 0-10 cm. Bolland (1992) found that when soil samples were collected to either 5 or 10 cm that the soil test P was similar on a low P sorbing soil but soil P test was consistently higher for the 5 cm samples on a high sorbing P soil, compared to the 10 cm sample. This would be likely in the soil S test. A 7.5 cm soil sampling depth is probably not deep enough for mobile SO_4^{2-} which can be leached and accumulate at the variable depth in the profile such as the weathered soils or light textured soils (Blair, 1979). In the verification study, the change of the critical value for the KCl-40 and MCP methods was related to the extraction of different forms of S. The decrease in the critical level for the KCl-40 method was most likely related to the decrease in the organic S level down the profile. Whilst the increase in the critical level of the MCP method was most likely due to the increase in the inorganic S level down the profile.

3.4.2 Source of S removed by extractants and plants

The pot study, where ^{35}S was used to trace S dynamics, revealed that the KCl-100 and $NaHCO_3$ extractants, removed a considerable amount of HI-reducible S from the soil (Table 3.7). The high amount of S extracted by the KCl-100 and $NaHCO_3$ methods was most likely related to the amount of S removed from the HI-reducible S pool by these extractants.

Reduction of the temperature of the KCl extractant to 40°C resulted in a higher correlation with plant response. This was most likely due to the extractant removing a portion of S from the HI-S pool more closely related to that available to plants. As the temperature of extraction with KCl decreased from 100°C to 40°C the critical level decreased (Table 3.5). This was most likely related to a reduction in the amount of HI-reducible S removed by the extractant.

The data on specific activity ratio (SAR) showed that the SAR of the KCl-40 extract was close to 1.00 (Table 3.8). This means that the KCl-40 extract had the closest specific activity to that of the plant. This indicates that the KCl-40 extract and plants are drawing S from similar pools. The KCl-40 extract removed S from soil solution and adsorbed sulfate plus the portion of HI-reducible S (Section 3.3.3a). The HI-reducible S is believed to be composed mainly of ester sulfates (Freney, 1986a,b) which are rapidly turning over in the soil system (Blair *et al.,* 1992). This ester sulfate is considered a labile form of organic S and thus potentially available to plants (Freney *et al.,* 1971, 1975; Tsuji and Goh, 1979; McLaren *et al.,* 1985; Schnitzer, 1991). This suggests that plants were utilising S from the soil solution sulfate and adsorbed sulfate and some portion of labile HI reducible ester sulfate. The other extractants had the high SAR (greater than 1.00) indicating that these extractants are removing S from soil pools not taken up by the plant.

The poor correlation with the pasture response in the KCl-100 and $NaHCO_3$ methods in the calibration study (Table 3.5) suggested that these extractants correlate poorly with plant response to S because they over-estimate the contribution of S from this labile S pool. This was supported by the data of the SAR. The KCl and $NaHCO_3$ extracts had the high SAR. This finding is similar to that of Anderson (1992) who found that the KCl-100 and $NaHCO_3$ methods tended to overestimate the size of the available S pool with only 6 to 55% of the extracted S being take up by pasture. In a previous study Probert (1976) reported that $NaHCO_3$ removed some soil S that was not available to the plant.

The KCl-40 method has also been found to correlate with the yield of canola (Lefroy *et al.,* 1993). A more predictive soil S test should be obtained for annual crops when the depth of sampling is measured especially on light textured soils (Lefroy *et al.*, 1993). On these soils, with low S sorption capacity, the intensity and frequency of drainage is a major factor governing sulfate movement (Ghani *et al.,* 1990). Losses of sulfate would be relatively high and not accounted for by the soil S test.

In the calibration and verification studied the KCl-40 method was found to be correlated well with plant response on 0-7.5 cm and 0-10 cm soil samples. Although the KCl-40 method performed well in this study, it is unlikely to be as predictive in soils where the S leaches deeper in the profile such as that reported by Probert and Jones (1977) and Rayment (1983) unless deeper sampling is practised. However, it is suggested that the KCl-40 method will still perform well on these deeper samples due to its ability to extract both sulfate and labile organic S. This needs to be confirmed.

Though the KCl-40 method performed well for the pasture and rice crop in this study. The question remains as to the utility of this extractant for crops and pastures with different growth times. This is the subject in Chapter 4.

CHAPTER 4

SOURCES OF SULFUR TAKEN UP BY RYEGRASS AND MEASURED BY CHEMICAL EXTRACTANTS

4.1 Introduction

The soil sulfur test developed in Chapter 3 is based on KCl heated at 40°C for 3 hours. The KCl-40 extract was found to be the most highly correlated with pasture yield response. This extractant also tended to remove more S from the HI reducible (ester sulfate) fraction than did the most commonly used extractant, monocalcium phosphate (MCP) in the rice pot experiment. Specific activity ratio (SAR) data confirmed that the KCl-40 test removed S from soil S pools similar to those accessed by the plant.

The data used to establish these correlations was based on the total S content of the extract as measured by ICP-AES. The question arises as to the source of that sulfur and to how much transformation of organic to sulfate took place during extraction, how much organic sulfur was present in the extract and what was the source of the organic S extracted. This has important implications as to the method of sulfur determination used. This aspect is studied in this chapter.

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.

4.2 Materials and Methods

4.2.1 Experimental design

The experiment was conducted in a glasshouse at the Department of Agronomy and Soil Science, the University of New England, Armidale. A factorial experiment, consisting of three replicates of ryegrass (*Lolium perenne* L.) grown in two soils (granite and basalt) with two rates of S fertiliser 0 and 17.7 mg kg^{-1} soil (equivalent to 0 and 30 kg S/ha), in a randomised block design.

4.2.2 Soil

An Aquic Haplustalf of granitic origin and Ultic Haplustalf of basaltic origin was collected from unfertilised pasture sites on the Northern Tablelands of New South Wales from Uralla and Walcha respectively. Some of the characteristics of the soils are given in Table 4.1.

Table 4.1 *Soil characteristics of Uralla and Walcha.*

Soil Characteristic	Uralla	Walcha
Clay content <2 um (%)	10.0	39.0
pH (1:5 0.01M $CaCl_2$	4.9	5.2
pH (1:5 water)	5.6	5.7
Organic Carbon (Walkley and Black) (C) %	0.7	2.5
Nitrate-Nitrogen (N) mg kg^{-1}	10.2	11.7
Phosphorus Bicarb (Cowell) (P) mg kg^{-1}	10.0	36.0
Sulfur (Phosphate extractable) (S) mg kg^{-1}	4.6	10.4
Potassium (Exchangeable) (K) $cmol_c$ kg^{-1}	0.09	0.96
Calcium (Exchangeable) (Ca) $cmol_c kg^{-1}$	1.36	7.79
Magnesium (Exchangeable) (Mg) $cmol_c$ kg^{-1}	0.30	2.83
Sodium (Exchangeable) (Na) cmol kg^{-1}	<0.01	0.02
Effective CEC $cmol_c$ kg^{-1}	1.74	11.60
Chloride (Water extractable) (Cl) mg kg^{-1}	5.0	10.0
Conductivity mS cm^{-1}	0.04	0.07
Copper (DTPA) (Cu) mg kg^{-1}	0.3	3.0
Zinc (DTPA) (Zn) mg kg^{-1}	0.7	1.3
Manganese (DTPA) (Mn) mg kg^{-1}	28.0	119.0
Iron (DTPA) (Fe) mg kg^{-1}	40.0	128.0
Boron (Hot water extractable) (B) mg kg^{-1}	0.07	0.10
Buffer pH	6.2	5.8

Source: Triana (1991).

The soils were air dried and processed through a Royer shredder to achieve good mixing, a relatively uniform size, and to allow the removal of the vegetative material, then passed through a 2 mm sieve before being used.

4.2.3 ^{35}S labelling and basal nutrients.

The ^{35}S labelling technique used in this study was the reverse dilution method. This technique was used to allow the calculation of specific activity (SA) for the soil S fractions and plant sulfur in the same way as for the rice pot experiment data in chapter 3.

Specific activity (SA) is the ^{35}S activity per unit of S (KBq mg S^{-1}) and the ratio of the SA in the plant at harvest to the SA of the extracted soil pool, termed the specific activity ratio (SAR), gives an indication as to whether the plant has been utilising nutrient from the same pool/s as the extractant. An SAR value of 1 indicates that the extractant and the plant are drawing sulfur from the same soil pool/s.

Three kilograms of air dried soils was weighed into plastic bags. ^{35}S obtained from Amersham Australia Pty. Ltd., was added with K_2SO_4 solution (2.091 g K_2SO_4 L^{-1}) to give a solution containing 0.325 MBq mL^{-1}. A 60 mL aliquot of this was added to each 3 kg of soil which resulted in an addition of 19.5 MBq pot^{-1}. An addition of 498 mL and 920 mL of distilled water was then applied to Urala soil and Walcha soil respectively to bring them to field capacity. These soils were 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 in 4 mL aliquots and micronutrients in 2 mL aliquots at the rates given in the Table 4.2. Each bags was again mixed thoroughly and approximately 200 g soil sample collected prior to cropping.

4.2.4 Pot management

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 with distilled water.

Perennial ryegrass (*Lolium perenne* L.) seeds were directly sown in the pots, and thinned to 10 plants per pot after emergence.

Pots were re-positioned every week to minimise any effects of uneven environmental factors such as light and temperature and watered to field capacity with distilled water by weighing daily. The temperature of the glasshouse was maintained with minimum and maximum temperatures of 15°C and 25°C throughout the period of the experiment.

Table 4.2 *Basal nutrients applied to the pots.*

Nutrient	Chemical form used		Application
	-S	+S	(mg nutrient kg^{-1})
N	$Ca(NO_3)_2$, $Mg(NO_3)_2$, NH_4NO_3	$Ca(NO_3)_2$, $Mg(NO_3)_2$	49.5
P	KH_2PO_4	$NH_4H_2PO_4$	34.2
K	KH_2PO_4	K_2SO_4	43.0
Ca	$Ca(NO_3)_2$	$Ca(NO_3)_2$	23.6
Mg	$Mg(NO_3)_2$	$Mg(NO_3)_2$	14.1
Mn	$MnCl_2.4H_2O$	$MnCl_2.4H_2O$	0.6
Zn	$ZnCl_2$	$ZnCl_2$	1.2
Cu	$CuCl_2.2H_2O$	$CuCl_2.2H_2O$	0.1
Mo	$Na_2MoO_4.2H_2O$	$Na_2MoO_4.2H_2O$	0.01
B	H_3BO_3	H_3BO_3	0.03

4.2.5 Plant and soil analysis

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°C for 48 hours. The dry plant samples were weighed and ground to pass a 1 mm screen. A sub-sample of 0.2 g for tops and 0.1 g for roots was digested using the sealed chamber digestion (SCD) 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 the cold room at 4°C prior to S analysis.

a) Soil S fractionation

Four test extractants were evaluated. These were MCP (Barrow, 1967; Searle, 1979), 0.25M KCl heated at 40°C or 100°C (Chapter 3, Section 3.2.1c) and Bray-1 (Bray and Krutz, 1945). The Bray-1 extract was used in a preliminary study and had shown that on many soils this extractant removed approximately the same amount of S as the KCl-40 method.

The S concentration in each of these extracts 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 4.1.

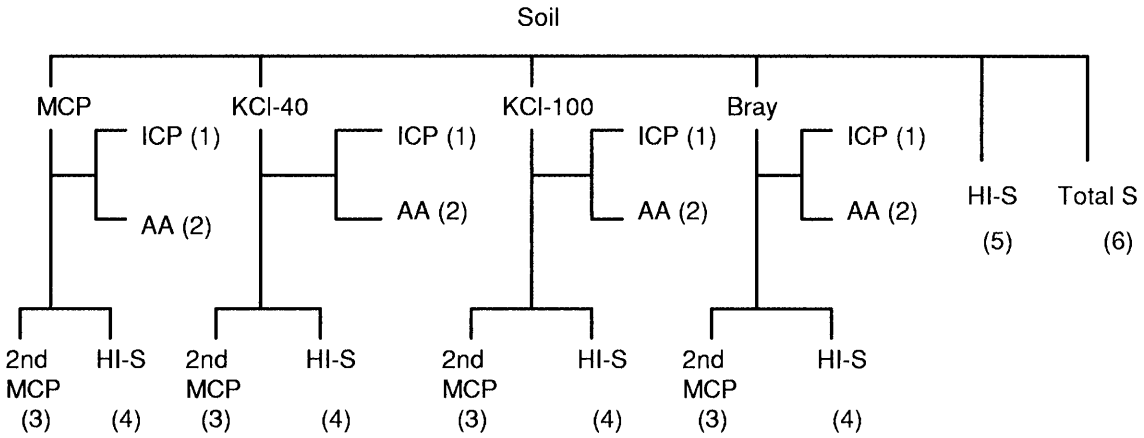
Soils (Figure 4.1) were first extracted with one of the four extractants, centrifuged at 5200 g for 20 minutes and the supernatant was filtered through a Whatman No 42 paper and S in the supernatant from the four test extracts measured by ICP. A sub-sample of 10 mL of the supernatant was treated with 0.02 g of activated charcoal, 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:

i) 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 subsample was determined on a duplicate sample of approximate 0.2 g, HI reducible S was then determined on the sample by the method of Dean (1966) as described in Section 3.2.2b.

ii) 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 4.1).

The total S pool was determined by the method of Till *et al.* (1984) and the HI-S pool was determined by method of Dean (1966) as described in Section 3.2.2b.



(1) = Total S in extract

(2) = Sulfate S in extract

(3) = Sulfate S not removed by first extraction (referred to as 2nd MCP)

(4) = Ester sulfate remaining in soil after first extracion

(5) = Total ester S pool

(6) = Total S pool

Figure 4.1 *Flow diagram for the fractionation of soil sulfur*

This sequence of extractions allowed the estimation of the following soil S pools for each of the four extraction methods The terms in brackets are those used in subsequent text, tables and figures.

a) The total S concentration in the extract (ICP-S).

b) The sulfate in the extract (SO_4-S) as measured by the autoanalyzer after removing the organic S in solution with charcoal.

c) The organic S compounds in solution (Organic S), was calculated by subtracting S measured by the autoanalyzer (SO_4-S) from S measured by ICP.

d) The HI reducible S pool (HI-S) or ester S pool was calculated by subtracting sulfate S (SO_4-S) from the S measured after reduction.

e) The HI reducible S pool remaining after the initial extraction (HI-S remaining).

f) The HI-S lost during extraction with the four test extractants (HI-S lost) was estimated by the difference between the HI-S pool in the original soil and the HI-S remaining after each of the four extraction.

g) Mineralised sulfate (Mineralised SO_4) is the amount of organic S measured as sulfate after extraction and was estimated by subtracting the soluble organic S (Organic S) from the HI-S removed during the extraction (HI-S lost).

g) The original sulfate (Original SO_4) is the sulfur that existed as sulfate in the soil before extraction, and is estimated by subtracting the soluble organic S (Organic S) from the mineralised sulfate and S by ICP.

h) The remainder of the soil S (Remainder) which was not extracted by the different test extractions or HI extraction, was calculated by subtracting the ICP-S and HI-S remaining from the total S.

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 relies on the precipitation of sulfate with Ba^{+2} and the measurement of the suspension turbidity on an autoanalyzer 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

This method involves the reduction of organic S compounds to hydrogen sulfide (H_2S) by a mixture containing hydriodic, hypophosphorus and formic acids. The H_2S formed is trapped in sodium hydroxide and reacted with bismuth nitrate, gelatin, acetic acid reagent to develop colloidal

bismuth sulfide. The colour was then measured at 400 nm. (Dean, 1966). This acid mixture reduces sulfate and a variety of organic S compounds in which the S is not bonded directly to carbon (ester sulfate), to sulfide (Johnson and Nishita, 1952; 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.

c) Measurement of ^{35}S

The ^{35}S in all plant and soil sample was measured by liquid scintillation counting (LSC) technique as described in Chapter 3 (Section 3.2.2.c). Counting was carried out on the plant and soil digests by adding 3 mL of the digest to 17 mL of cocktail. The supernatant samples from the four test extracts was counted on a mixture of 2 mL of extract and 18 mL of cocktail. The samples were counted in a Tri-Carb 2000 Liquid Scintillation Analyzer.

4.2.6 Statistical Analysis

Dry matter yield (g pot^{-1}), plant uptake (mg S pot^{-1}), specific activity (KBq mg S^{-1}), S concentration in plant (%), soil S status (µg S g^{-1} soil or Bq g^{-1} soil) by the range of extractants, the recovery of ^{35}S, and the specific activity ratio (SAR) was analysed separately for each soil using the NEVA statistical package (Burr, 1982) for analysis of variance.

The statistical differences between treatment means were considered to exist when they were different according to Duncans multiple range test at the 5% level of ($P < 0.05$) probability.

4.3 Results

4.3.1 Yield

Plant yield increased with S application in both soils (Tables 4.3 and 4.4). In the Uralla granitic soil S application resulted in a significantly higher dry weight of the tops, roots and total plants (top+root) (Table 4.3). A similar response was found in the yield in the Walcha basaltic soil (Table 4.4).

Table 4.3 *Dry matter yield (g pot^{-1}), S concentration (%) ,S content (mg pot^{-1}), and the specific activity (SA) (KBq mg S^{-1}) of ryegrass grown in the Uralla soil.*

	Top		Root		Total (top+root)	
	-S	+S	-S	+S	-S	+S
Yield	6.44 b[A]	7.40 a	2.02 b	2.76 a	8.46 b	10.16 a
S conc	0.19 b	0.33 a	0.11 a	0.15 a	0.30 b	0.48 a
S content	12.29 b	24.62 a	2.27 a	4.04 a	14.56 b	28.66 a
SA	612.8 a	197.9 b	474.3 a	235.8 b	586.4 a	204.2 b

[A] *Numbers followed by the same letter within the same plant part and component are not significantly different according to DMRT at P <0.05.*

4.3.2 Total S uptake and plant S concentration.

The S application resulted in increased S concentration and content in the plants grown in both soils (Tables 4.3, and 4.4). In the Uralla soil, the S content of tops and total plants (top+root) was significantly higher when S was applied (+S) than when no S was applied (-S) treatment. However, there were no significant difference between treatments in the S content of the roots (Table 4.3).

A significantly higher S concentration was recorded in the tops and total plant from the Uralla soil in the +S treatment than the -S treatment. However, the difference in the S concentrations in the roots between the +S and -S treatments was not significant (Table 4.3).

Table 4.4 *Dry matter yield (g pot^{-1}), S concentration (%) ,S content (mg pot^{-1}), and the specific activity (SA) (KBq mg S^{-1}) of ryegrass grown in the Walcha soil.*

	Top		Root		Total (top and root)	
	-S	+S	-S	+S	-S	+S
Yield	6.82 b[A]	9.52 a	2.51 b	2.88 a	9.33 b	12.40 a
S conc	0.20 b	0.37 a	0.08 a	0.09 a	0.28 b	0.46 a
S content	13.79 b	35.43 a	2.02 a	2.48 a	15.81 b	37.91 a
SA	510.6 a	218.9 b	409.8 a	214.6 b	497.9 a	218.7 b

[A]*Numbers followed by the same letter within the same plant part and component are not significantly different according to DMRT at P <0.05.*

In the Walcha soil, the response to S in S concentration and content in the tops, root, and total plants was similar to that in the Uralla soil (Table 4.4).

4.3.3 The specific activity (SA) of the plants

There were significant differences between the -S and +S treatment in the specific activity (SA) of the plants. The SA of the tops, roots and total plants in the -S treatment was significantly higher than that the +S treatment in all plant parts on both soils (Tables 4.3, 4.4).

4.3.4 Major soil S pools

a) Total S

There was a significant difference between the -S and +S treatment in the total S concentration in the Uralla and Walcha soils before planting and after cropping (Tables 4.5 and 4.6).

The specific activity (SA) of the total S pool was significantly higher in the -S than that the +S treatment both Uralla granite and Walcha basalt soil before planting. By contrast, the SA was lower in the -S than the +S treatment after cropping in both soils (Tables 4.5, 4.6).

Table 4.5 *The total S pool concentration (μg S g^{-1} soil), HI-S pool (μg S g^{-1} soil) and the specific activity (KBq mg S^{-1}) in the Uralla soil, before planting and after cropping.*

	Total S pool		HI-S pool	
	-S	+S	-S	+S
S concentration				
Before planting	91.4 b[A]	167.0 a	46.6 b	53.0 a
After cropping	70.8 b	93.4 a	34.9 b	41.0 a
SA				
Before planting	86.6 a	43.9 b	91.8 a	45.4 b
After cropping	29.7 b	58.4 a	28.8 a	34.9 a

[A] *Numbers followed by the same letter within the same soil pool and cropping are not significantly different according to DMRT at P <0.05.*

b) HI-S

In both soils, the concentration of S in the HI-S pool was significantly higher in the +S than the -S treatment both before planting and after cropping (Table 4.5). Prior to planting the SA of the HI-S pool was significantly higher in the -S than the +S treatment in both the Uralla granite and the Walcha basalt soils. By contrast, the SA in the -S treatment was lower than the +S treatment after cropping in the Uralla granite but not different in the Walcha basalt soil. In both soils the S concentration and SA declined with cropping although this difference was not analysed statistically.

Table 4.6 *The total S concentration (μg S g^{-1} soil), HI-S pool (μg S g^{-1} soil) and the specific activity (SA) (KBq mg S^{-1}) in the Walcha soil, before and after cropping.*

	Total S		HI-S pool	
	-S	+S	-S	+S
S concentration				
Before planting	271.1 b[A]	341.2 a	152.7 b	168.7 a
After cropping	230.1 b	262.4 a	121.4 b	140.7 a
SA				
Before planting	25.1 a	19.5 b	25.4 a	19.9 b
After cropping	13.9 a	14.5 a	10.3 a	14.9 a

[A] *Numbers followed by the same letter within the same soil pool and cropping are not significantly different according to DMRT at P <0.05.*

4.3.5 Components of soil S pools

a. Uralla granite soil, -S treatment

i) Components of extracted S

ICP-S

The total S concentration in the extractants as measured by ICP increased in the order Bray<MCP<KCl-40<KCl-100 (Table 4.7).

Significantly more percentage of ^{35}S was recovered in the KCl-100 and KCl-40 extracts than in MCP and Bray (Table 4.8). The KCl-40 extracts had a significantly higher SA than the other extracts (Table 4.9).

Table 4.7 *S concentration (μg S g^{-1}) in a range of extractants in the -S treatment, ryegrass before planting, Uralla soil.*

	MCP	KCl-40	KCl-100	Bray
ICP- S	6.1 c[A]	9.1 b	16.5 a	5.1 c
SO_4-S	5.3 c	7.7 b	12.3 a	3.7 d
Organic S	0.8 b	1.4 b	4.2 a	1.4 b
2nd MCP	1.2 b	2.5 a	0.3 c	2.4 a
HI-S remaining	42.3 a	40.8 a	34.9 b	42.7 a
Remainder	42.9 ab	41.5 ab	40.1 b	43.6 a

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT.*

Components of ICP-S

Approximately 13% of the S in the MCP extract was present as Organic S. This compared to 26% in KCl-100 and 27% in the Bray extracts (calculated from Table 4.7). 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 4.8).

The SA of the SO_4-S pool was highest in the KCl-40 and KCl-100 extracts (Table 4.9). The SA in the soluble organic S pool was lower than in the SO_4-S pool in all extracts with the greatest difference in the KCl-100 extract.

Sulfate not removed by first extraction

The additional S removed from al previously extracted soils by MCP (2nd MCP) showed significant differences between extractan s. The lowest concentration was in the KCl-100 and the highest in KCl-40 extract (Table 4.7). Th e amount of ^{35}S recovered in this fraction was less than 10% of that added, with the highest amount found in the Bray and KCl-40 extracts (Table 4.8). The SA of the second extract was of a similar magnitude to that of the organic S component of the first extract for KCl-40 and Bray, higher for KCl-100 and lower for the MCP extract (Table 4.9).

Table 4.8 *The percentage of ^{35}S recovered in a range of extractants in the -S treatment, ryegrass before planting, Uralla soil.*

	MCP	KCl-40	KCl-100	Bray
ICP-S	33.6 c[A]	63.1 b	82.5 a	26.5 c
SO_4-S	29.2 c	58.5 b	78.0 a	21.6 d
Organic S	4.4 a	4.6 a	4.5 a	4.9 a
2nd MCP	2.5 b	7.2 a	1.2 b	9.9 a
HI-S remaining	18.3 ab	23.8 ab	12.0 b	31.7 a
Remainder	48.2 a	13.1 b	5.6 b	41.8 a

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT.*

HI-S remaining

The concentration of HI-S remaining in the soil after the first extraction was lowest following KCl-100 extraction (Table 4.7). The HI-S remaining following the KCl-40 and Bray extractions contained in the excess of 23% of ^{35}S recovered (Table 4.8) and the SA in these fractions was higher than that following the KCl-40 and Bray extracts (Table 4.9).

Remainder

Significantly less S remained after the KCl-100 extraction and following after HI-S extraction, than with the Bray extraction. The other two were intermediate and not significantly different (Table 4.7). A significantly lower % ^{35}S recovery was found following KCl-40 and KCl-100 extraction (Table 4.8) and the SA of the remainder S was also lower in these extracts (Table 4.9).

There were significant differences between extractants in the amount of SO_4-S and Organic S measured with the highest amount in the KCl-100 extract. The concentration of HI-S remaining in the soil after the first extraction was also significantly lowest following the KCl-100 extract (Table 4.7).

Table 4.9 *The specific activity (KBq mg S^{-1}) by a range of extractants in the -S treatment, ryegrass before planting, Uralla soil.*

	MCP	KCl-40	KCl-100	Bray
ICP-S	429.1 b[A]	546.5 a	393.7 b	405.5 b
SO_4-S	433.3 b	595.3 a	498.8 ab	462.5 b
Organic S	424.9 a	276.7 b	86.0 c	267.4 b
2nd MCP	163.4 b	224.7 ab	369.7 a	326.8 ab
HI-S remaining	34.4 a	45.8 a	26.7 a	59.5 a
Remainder	89.8 a	26.4 b	12.4 b	76.1 a

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT.*

Summary of components of soil S

A summary of the S components in the various extractants is presented in Figure 4.2.

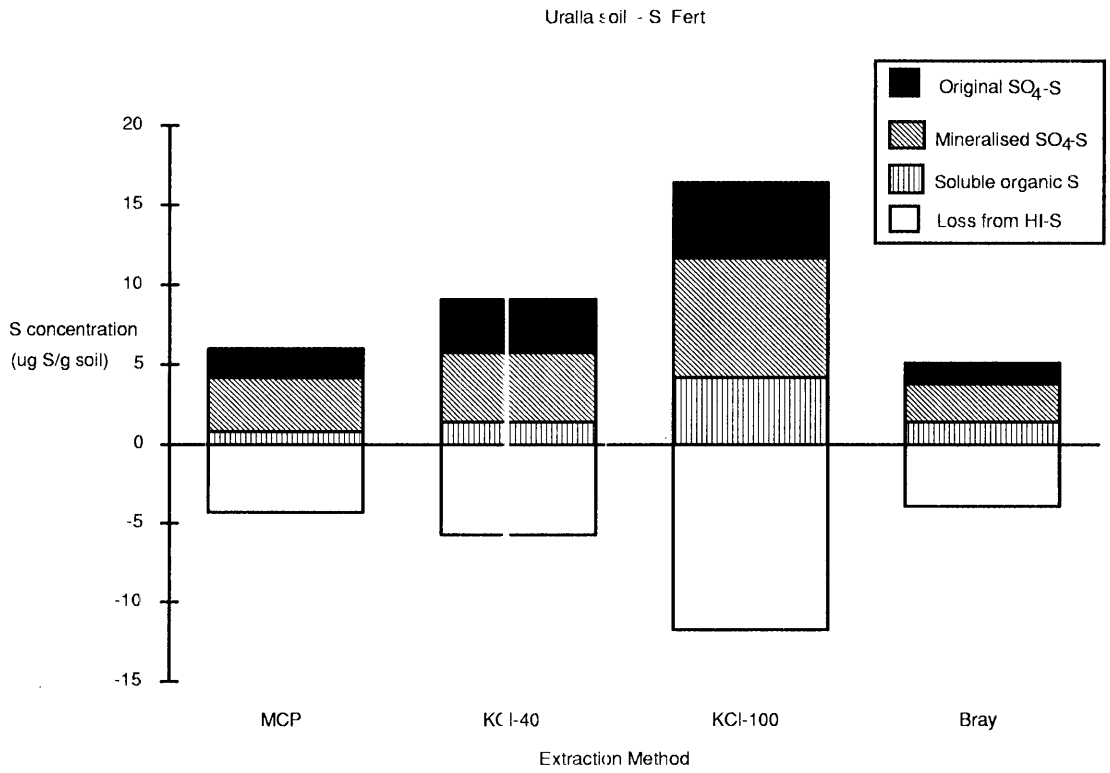


Figure 4.2 *S fractions in a range of extractants in the -S treatment Uralla soil, before planting .*

Before planting, the highest total S concentration (ICP-S) was recorded in the KCl-100 extract. This extract also removed most S from the HI-S pool. The converse of this was found in the Bray extract (Table 4.10).

The highest concentration of ICP-S was recorded in the KCl-100 extract and the lowest was in the Bray extract prior to planting. By contrast, the KCl-100 extract had the lowest concentration of HI-S after extraction and the highest HI-S concentration was found in the Bray extract. The concentration of S and HI-S remaining after the four test extractants after cropping was of similar magnitude to that before planting (Table 4.10).

ii) Specific activity (SA)

Before planting, the KCl-40 extract had the highest SA in the ICP-S fraction and the SA in the HI-S remaining fraction was also high. By contrast, the KCl-100 extract had lowest SA in the ICP-S and the HI-S remaining fractions. There were no significant difference in the SA of the ICP-S fraction between the Bray and KCl-100 extracts. The SA of HI-S remaining fraction following the Bray and KCl-40 extracts was higher than that of the MCP and KCl-100 extracts (Table 4.10).

After cropping, the SA of the ICP-S fraction was significantly lower in the KCl-100 and MCP extracts than that in the KCl-40 and Bray extracts. The SA of the ICP-S fraction tended to decrease during cropping. There was no significant difference between extracts in the SA of the HI-S remaining fraction after cropping (Table 4.10).

Table 4.10 *The concentration of ICP-S and HI-S remaining after extraction (μg S g^{-1} soil) and the specific activity (SA) (KBq mg S^{-1}) of extractants in the -S Uralla soil before planting and after cropping.*

	MCP	KCl-40	KCl-100	Bray
S concentration				
ICP-S before	6.1 c	9.1 b	16.5 a	5.1 d
ICP-S after	3.0 e	1.7 ef	6.7 c	0.9 f
HI remaining before	42.3 a	40.8 a	34.9 b	42.7 a
HI remaining after	34.7 b	35.8 b	28.6 c	36.6 b
SA				
ICP-S before	429.1 b	546.5 a	393.7 c	405.5 c
ICP-S after	103.3 e	254.9 d	110.2 e	322.8 cd
HI remaining before	34.4 ab	45.8 ab	26.7 b	59.5 a
HI remaining after	29.9 ab	27.6 b	27.5 b	26.9 b

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT at P <0.05.*

iii) Specific activity ratio (SAR)

Prior to planting, the lowest SAR was recorded in the SO_4-S fraction. In the ICP-S and SO_4-S fractions of the KCl-40 extract the SAR was close to 1 (Table 4.11).

Table 4.11 *The specific activity ratios (SAR) in components of a range of extractants in the -S Uralla soil before planting.*

	MCP	KCl-40	KCl-100	Bray
ICP-S	1.44 a[A]	1.12 a	1.56 a	1.62 a
SO_4-S	1.43 a	1.03 a	1.23 a	1.43 a
Organic S	1.45 b	2.27 b	7.37 a	2.56 b
2nd MCP	3.83 a	2.74 ab	1.82 b	1.89 b
HI-S remaining	19.11 a	13.39 a	23.54 a	12.74 a
Remainder	6.94 a	28.36 a	86.51 a	8.30 a

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT.*

After cropping, the SO_4-S pool had the highest SAR. However the KCl-40 and Bray had a lower SAR than the other extractants (Appendix 4.1).

b). Uralla granite soil, +S treatment

There were significant differences in the concentration of S in the various extractants and S fractions (Appendix 4.2) in this treatment Significant differences were recorded in the recovery of ^{35}S (Appendix 4.3) and in the SA (Appendix 4.4) of the various fractions.

The addition of S to the Uralla soil resulted in a similar pattern to that in the -S treatment in the S concentration in the various extractants and S fractions, the % ^{35}S recovered and SA of the various fractions.

There were significant differences between extractants in the amount of Organic S with the highest amount in the KCl-100 extract. The KCl-100 extract also had the highest amount of HI-S lost, mineralised SO_4 and original sulfate SO_4. By contrast, the Bray extract had the lowest value in these fractions (Figure 4.3).

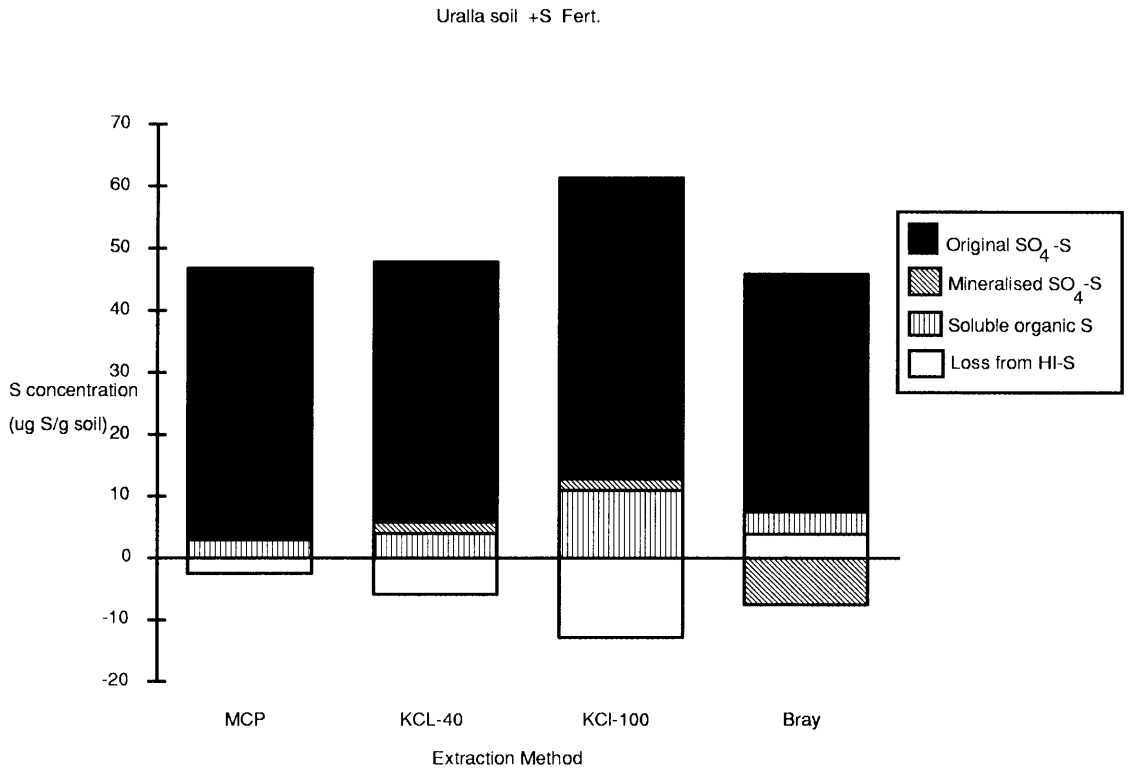


Figure 4.3 *The S fractions in a range of extractants in the +S treatment Uralla soil, before planting.*

Before planting in the +S treatment, the KCl-100 extract had the highest concentration of ICP-S and lowest concentration in the HI-S remaining fraction (Table 4.12). In contrast, the reverse was found in the Bray extract. The MCP extractant had a significantly higher concentration of HI-S remaining than the KCl-40 extract. After cropping, the pattern in the S concentration and HI-S remaining fractions in the four extractants was similar to that before planting. However, the difference in the concentration of the HI-S remaining between the MCP and KCl-40 extracts was not significant (Table 4.12).

Table 4.12 *The concentration of ICP-S and HI-S remaining (μg S g^{-1} soil) and the specific activity (KBq mg S^{-1}) in a range of extractants in the +S Uralla soil, before planting and after cropping.*

	MCP	KCl-40	KCl-100	Bray
S concentration				
ICP-S before	45.7 b[A]	47.9 b	61.4 a	34.5 c
ICP-S after	12.8 e	13.6 e	22.6 d	6.8 f
HI remaining before	50.5 b	47.1 c	40.2 d	56.9 a
HI remaining after	39.1 ce	37.3 e	31.2 f	44.9 c
SA				
ICP-S before	65.8 e	84.3 d	91.9 d	62.1 e
ICP-S after	181.4 c	211.9 b	168.9 c	240.1 a
HI remaining before	40.4 ab	43.7 a	27.3 bc	45.6 a
HI remaining after	22.1 c	24.3 c	12.4 d	26.9 c

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT at P <0.05.*

The highest SA in the ICP-S fraction were found in the KCl-100 and KCl-40 extracts prior to planting (Table 4.12). In addition, the KCl-100 extract had the lowest SA in the HI-S remaining fraction, which did not differ from the HI-S remaining fraction of the MCP extract. The highest SA of this fraction where the Bray extract was used. After cropping, the SA of the ICP-S was lower than that before planting. The lowest SA was recorded in the MCP and KCl-100 extracts. The lowest SA in the HI-S remaining fraction was also recorded in the KCl-100 extract. There were no significant differences in the SA of this fraction recorded among the Bray, KCl-40 and MCP extracts (Table 4.12).

Before planting, the SAR data showed that all fractions had a high SAR value. The SO_4-S fraction had a lower SAR than the other fractions (Appendix 4.5). The KCl-40 and KCl-100 extracts had a lower SAR in the ICP-S and SO_4-S fractions than the MCP and Bray extracts. The SAR data was reversed after cropping (Appendix 4.6), with the lowest SAR in the sulfate pool and these were all close to 1 in all four extractants. The ICP-S fraction of the KCl-40 extract was closest to 1.00.

c) Walcha basalt soil, -S treatment

i) Components of extracted S

ICP-S

The concentration of the S in the extractants as measured by ICP increased significantly in the order Bray<KCl-40<MCP<KCl-100 (Table 4.13). The recovery of the ^{35}S in this component was significantly higher in the KCl-100 and MCP extracts than in the KCl-40 and Bray extracts (Table 4.14). The highest SA was in the KCl-40 extract and the lowest in the KCl-100 extract (Table 4.15).

Table 4.13 *S concentration (μg S g^{-1}) in a range of extractants in the -S treatment, before planting, Walcha soil.*

	MCP	KCl-40	KCl-100	Bray
ICP-S	13.7 b[A]	8.2 c	18.9 a	6.8 d
SO_4-S	11.2 b	5.9 c	13.6 a	4.8 d
Organic S	2.5 b	2.3 b	5.3 a	2.1 b
2nd MCP	4.4 c	14.2 a	7.8 b	13.6 a
HI-S remaining	148.0 ab	142.2 bc	136.3 c	153.9 a
Remainder	109.3 b	120.7 a	115.9 ab	110.4 b

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT.*

Components of ICP-S

In the extracts, approximately 18% of the S in the MCP extract was present as Organic S fraction. This compared to 28% in KCl-40 and KCl-100 extracts and 30% in Bray extract (Table 4.13). The recovery of the ^{35}S was higher in the SO_4-S than in the Organic S fraction, with less that 10% of the ^{35}S recovered in the Organic S fraction (Table 4.14).

Table 4.14 *The percentage of ^{35}S recovered in a range of extractants in the -S treatment, before planting, Walcha soil.*

	MCP	KCl-40	KCl-100	Bray
ICP-S	84.1 a[A]	58.8 b	83.8 a	43.7 c
SO_4-S	73.8 b	51.3 c	80.9 a	42.6 d
Organic S	10.3 a	7.5 a	2.9 b	1.1 b
2nd MCP	6.1 b	46.7 a	14.9 b	44.8 a
HI-S remaining	11.1 b	25.9 a	9.5 b	35.8 a
Remainder	4.7 b	15.3 ab	6.7 b	20.5 a

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT.*

The SO_4-S component had the highest SA. A higher SA was recorded in the KCl-40 and Bray extractants than in the MCP and KCl-100 extracts (Table 4.15). In all extracts the SA in the Organic S fraction was lower than in the SO_4-S fraction, indicating that only a small amount of ^{35}S had entered this pool.

Table 4.15 *The specific activity (KBq mg S^{-1}) in a range of extractants in the -S treatment, before planting, Walcha soil.*

	MCP	KCl-40	KCl-100	Bray
ICP-S	416.0 c[A]	486.7 a	301.2 d	435.8 b
SO_4-S	446.4 b	594.0 a	403.2 b	609.1 a
Organic S	283.4 a	221.2 a	38.1 b	32.2 b
2nd MCP	94.4 b	225.1 a	128.8 b	225.8 a
HI-S remaining	5.1 b	12.3 a	4.7 b	15.9 a
Remainder	2.9 b	8.6 ab	3.9 ab	9.0 a

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT*

Sulfate not removed by first extraction

Additional S from previously extracted soils was removed by the second extraction (MCP). There were significant differences between extractants in the concentration of this S (Table 4.13). The S concentration in the second extract was lowest where MCP was used as the primary extractant and highest in the KCl-40 treatment. ^{35}S recovery was significantly higher following the KCl-40 and Bray extracts than in the KCl-100 and MCP extracts (Table 4.14). The SA of the

second MCP extract was of a similar magnitude to that of the S-org fraction in the first extract for the KCl-40, higher for Bray and KCl-100 and lower for MCP (Table 4.15).

HI-S remaining

The concentration of HI-S remaining in the soil after the first extraction was in the order Bray>MCP>KCl-40>KCl-100 (Table 4.13). A significantly higher recovery of ^{35}S was found in the Bray and KCl-40 extracts which contained in excess of 25% of the ^{35}S recovered (Table 4.14). The SA of HI-S remaining fraction following the Bray and KCl-40 extractions was significantly higher than that following the MCP and KCl-100 extracts (Table 4.15).

Remainder

There were significant differences between extractants in the concentration of S in the remainder fraction (Table 4.13). In all extractants the lowest recovery of ^{35}S was recorded following the MCP and KCl-100 extracts and the highest in the KCl-40 and Bray extracts (Table 4.14). The SA of the remainder fraction was also lower than that in the other fractions (Table 4.15).

Summary of components of soil S

Significant differences were recorded in the concentration of Organic S and HI-S remaining after the first extraction (Table 4.13). The highest concentration of Organic S and lowest concentration of HI-S remaining was found in the KCl-100 extract. Extraction with KCl-100 was also found to result in the highest amount of HI-S lost and mineralised sulfate (Figure 4.4). This contrasted with the Bray extractant. The amount of original sulfate was higher in the MCP than that of the other extractants. Conversely, the KCl-40 extract resulted in the lowest amount of the original sulfate (Figure 4.4).

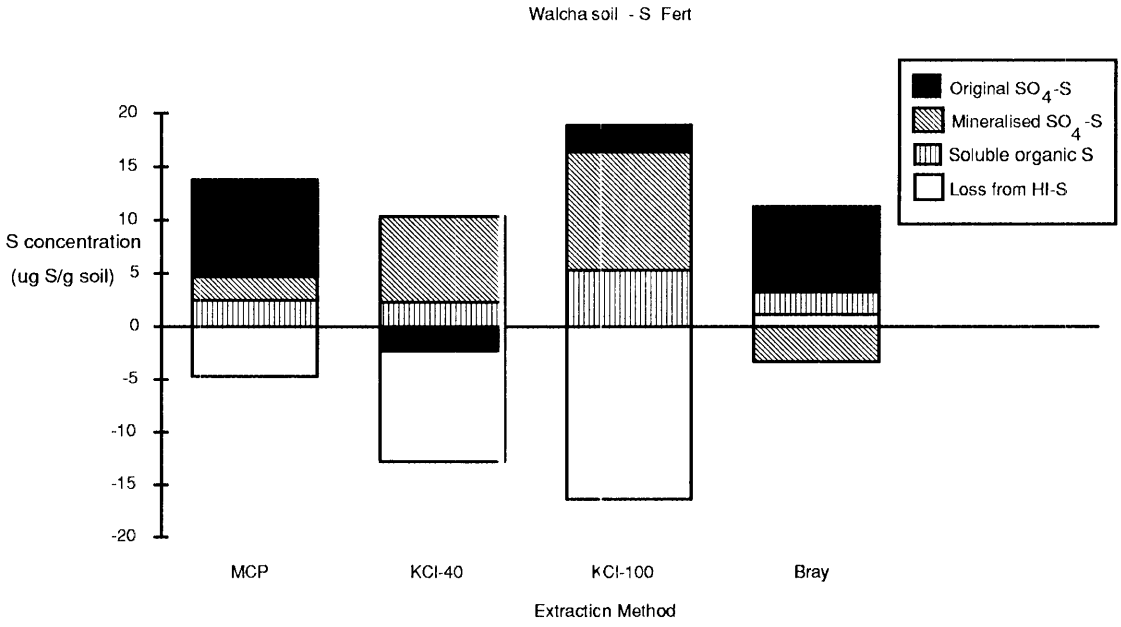


Figure 4.4 *S fractions in a range of extractants in the -S treatment Walcha soil, before planting.*

In the Walcha soil -S treatment before planting, the highest concentration of the ICP-S was found in the KCl-100 extract and the lowest S concentration where the Bray extract was used (Table 4.16). The MCP extract had a significantly higher S concentration than did the KCl-40 extract. The concentration of the HI-S remaining was lowest following KCl-100 extraction while the concentration of the HI-S following the Bray extract was the highest. The concentration of HI-S remaining following MCP extraction was higher than that following KCl-40 extraction (Table 4.16).

After cropping, the KCl-100 extract had the highest concentration of ICP-S followed by the MCP (Table 4.16). In contrast, the KCl-40 and Bray extracts had the lowest S concentration. The lowest concentration of HI-S remaining was also recorded following KCl-100 extraction. The Bray and KCl-40 resulted in a higher concentration of HI-S remaining than that the MCP and KCl-100 extracts (Table 4.16).

Table 4.16 *The concentration of ICP-S and HI-S remaining (μg S g^{-1} soil) and the specific activity (SA) (KBq mg S^{-1}) in a range of extractants in the -S Walcha soil before and after cropping.*

	MCP	KCl-40	KCl-100	Bray
S concentration				
ICP-S before	13.7 b[A]	8.2 d	18.9 a	6.8 e
ICP-S after	5.8 f	3.0 g	9.2 c	3.0 g
HI remaining before	148.0 ab	142.2 bc	136.3 c	159.9 a
HI remaining after	110.9 de	113.8 d	107.6 e	113.9 d
SA				
ICP-S before	416.0 b	486.7 a	301.2 c	435.8 b
ICP-S after	125.2 e	170.7 d	101.0 e	165.0 d
HI remaining before	5.1 c	12.3 ab	4.7 c	15.9 a
HI remaining after	11.5 ab	11.7 ab	11.2 ab	9.0 b

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT at P <0.05.*

ii) Specific activity (SA)

Before planting, the SA of the ICP-S was highest in the KCl-40 extract followed by the Bray and MCP with the lowest SA was in the KCl-100 extract (Table 4.16). The KCl-100 also resulted in the lowest SA in the HI-S remaining fraction which did not differ significantly from the MCP. The highest SA of the HI-S remaining fraction was in the Bray and KCl-40 extracts.

After cropping, the SA of the ICP-S was lower than that before planting. The highest SA was recorded in the KCl-40 extract and the lowest in the KCl-100 extract. No significant differences were recorded between extracts in the SA of the HI-S remaining fraction (Table 4.16).

iii) The specific activity ratio (SAR)

The lowest SAR was found in the sulfate fraction, and the highest SAR in the HI-S remaining and the remainder fractions before planting (Table 4.17). In the ICP-S fraction the KCl-40 extract had the lowest SAR and it was closest to 1.00. The SAR of the SO_4-S fraction for the KCl-40 and Bray were less than 1.00, whlist the MCP and KCl-100 fractions had SAR values greater than 1.00.

After cropping, the lowest SAR for the ICP-S and SO_4-S pools, were found with the KCl-40 and Bray extracts, with all values greater than 1.00 (Appendix 4.7). The SAR of the ICP-S, SO_4-S

pools and remainder prior to cropping (Table 4.17) were less than after cropping (Appendix 4.7), whilst the SAR of the 2nd MCP were higher after cropping.

Table 4.17 *The specific activity ratios (SAR) in a range of extractants in the -S Walcha soil before planting.*

	MCP	KCl-40	KCl-100	Bray
ICP-S	1.23 b[A]	1.04 c	1.70 a	1.17 c
SO_4-S	1.14 b	0.86 c	1.26 a	0.83 c
Oganic S	1.84 b	2.45 b	17.86 a	20.74 a
2nd MCP	5.84 a	2.304 c	3.97 b	2.45 c
HI-S remaining	105.25 a	43.33 b	108.16 a	26.94 b
Remainder	189.06 a	80.98 b	368.14 a	58.27 b

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT.*

d) Walcha basalt soil, +S treatment

Significant differences were recorded in the concentration of S (Appendix 4.8), % ^{35}S recovered (Appendix 4.9), and the SA (Appendix 4.10) between the various extractants for the different S fractions. Generally, the S concentrations for the +S treatments were higher than for the -S treatments, whilst the % ^{35}S and SA were lower for the +S treatments. Whilst the magnitudes of the S concentrations, % ^{35}S and the SA of all S fractions for the various extractants were very different between the -S and -S treatments, their relative magnitudes for the different extractants were similar (Appendices 4.8, 4.9, 4.10 cf Tables 4.13, 4.14, 4.15).

Significant differences between extractants were recorded in the amount of Organic S in the extracts, with the highest amount found in the KCl-100 extract and lowest amount in the MCP extract. The amount of HI-S lost, the mineralised SO_4, and the original SO_4 was also higher in the KCl-100 extract. The Bray extractant had the lowest amounts of the HI-S lost and mineralised SO_4. The lowest amount of original SO_4 was found in the KCl-40 extract (Figure 4.5).

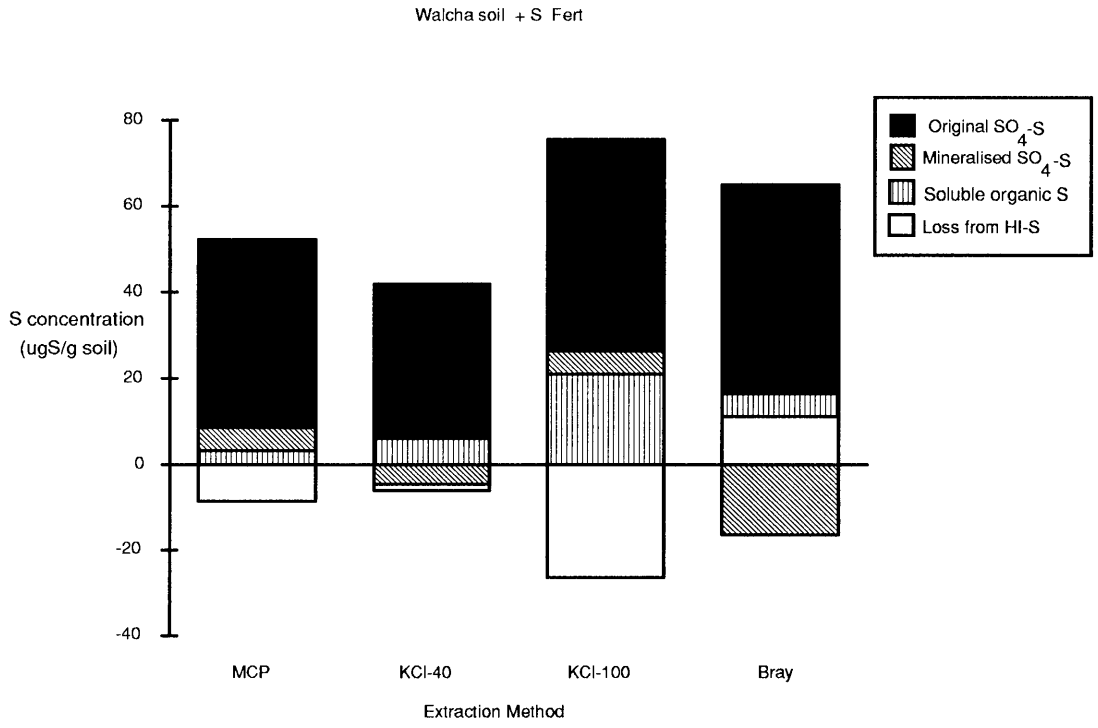


Figure 4.5 *S fractions in a range of extractants in the +S treatment Walcha soil, before planting.*

Before planting in the Walcha +S treatment, the highest concentration of ICP-S and lowest concentration of HI-S remaining was recorded in the KCl-100 extract whilst the reverse was found in the Bray extractant. After cropping, similar patterns were observed in the concentration of S in the HI-S remaining fraction in the four test extractants (Table 4.18).

In the +S treatment, before planting, the KCl-40 extract tended to have a higher SA in the ICP-S fraction than the MCP extract although no significant differences were recorded between these extractants. The SA of the HI-S remaining fraction tended to be lower in the KCl-100 extract, however this did not differ significantly from the MCP extracts. The Bray extractant had the highest SA in the HI-S remaining fraction.

Table 4.18 *The concentration of ICP-S and HI-S remaining (μg S g^{-1} soil) and the specific activity (SA) (KBq mg S^{-1}) in a range of extractants in the +S Walcha soil before planting and after cropping.*

	MCP	KCl-40	KCl-100	Bray
S concentration				
ICP-S before	52.4 b[A]	37.5 c	75.6 a	37.5 c
ICP-S after	18.9 e	12.2 f	25.9 d	9.0 f
HI remaining before	160.1 c	167.2 b	142.2 d	179.9 a
HI remaining after	119.1 f	125.1 ef	111.9 g	128.7 e
SA				
ICP-S before	82.9 e	84.5 e	70.9 f	61.4 g
ICP-S after	119.5 c	145.2 b	98.5 d	205.0 a
HI remaining before	8.3 bc	9.6 b	6.6 c	12.9 a
HI remaining after	9.6 b	11.9 ab	8.5 bc	6.3 c

[A] *Numbers followed by the same letter within the same row are not significantly different according to DMRT at P <0.05.*

After cropping, the SA of the ICP-S fraction was higher than that before planting. The Bray extractant had the highest SA in the ICP-S fraction. There was a higher SA in the ICP-S fraction in the KCl-40 than in the MCP and KCl-100 extracts. The KCl-100 extract had the lowest SA for this fraction. The KCl-40 extract tended to have the highest SA in HI-S remaining fraction but this did not differ significantly from the MCP and KCl-100 extracts. Significant differences between the KCl-40 and Bray extracts were observed in the SA of HI-S remaining fraction (Table 4.18).

Prior to planting, the highest SAR was found in all S fractions in the +S Walcha soil, with the SAR value greater than 1.00 (Appendix 4.11). The lowest SAR was with the SO_4-S fraction, compared to the other fractions. The KCl-100 and KCl-40 extracts had a lower SAR than the MCP and Bray extracts. After cropping, the SO_4-S pool had a lower SAR than the other pools, with the SAR value greater than 1.00 (Appendix 4.12). The lowest SAR was in the KCl-40 and Bray extracts.

4.4 Discussion

4.4.1 Plant

The results of this study showed that S application increased the yield and S uptake of the ryegrass on both the Uralla and Walcha soils (Tables 4.3 and 4.4). This result was similar to the findings of other investigators (Samosir, 1989; Chaitep, 1990 and Dana 1992).

A higher dry matter yield and total S uptake was recorded in the Walcha basalt than the Uralla granite soil. This was most likely due to the higher organic matter and the other nutrient content in the Walcha soil (Table 4.1). McCaskill (1984) reported that basaltic soils of the New England Tablelands had higher contents of most nutrients including phosphorus, sulfur and organic carbon, and that greater pasture vigour was found on basalt soils compared to those of granitic origin.

The S concentration in plant tops in the -S treatment was 0.19% and 0.20% in Uralla and Walcha soils respectively, compared to 0.33 and 0.37% in the +S treatment (Tables 4.3 and 4.4). Reuter and Robinson (1986) indicated that the critical S concentration for perennial ryegrass was 0.22% and that a high concentration was > 0.32%.

The SA 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 SA of plants in the 50 μg S g^{-1} soil treatment was less than the 10 μg S g^{-1} soil treatment, due to the greater dilution of ^{35}S by the unlabelled Na_2SO_4.

4.4.2 Soil

a) Total S

The total S concentration ranged from 91 to 167 μg S g^{-1} soil in the Uralla soil and 271 to 341 μg S g^{-1} soil in the Walcha soil (Tables 4.5 and 4.6), This result is similar to that of Chaitep (1990) who found that total S concentration in the Uralla soil under non flooded conditions, ranged from 94 to 99 μg S g^{-1} soil in the nil S treatment after the first rice crop. Anderson (1992) reported that the total S in the -S and +S treatment, was 220 to 330 μg S g^{-1} soil after the eleven growth periods of a field experiment on the Walcha basalt site.

The total S concentration of the +S treatment was higher than that of the -S treatment on both soils before cropping due to the application of S fertiliser (Tables 4.5 and 4.6). Similar result was recorded in the total S concentration on both soils after cropping.

Before cropping, the SA of the total S pool was less in the +S treatment than that the -S treatment in both soil. By contrast, the SA was lower in the -S treatment after cropping (Tables 4.5 and 4.6). This was due to the greater dilution of ^{35}S by the S added as fertiliser (unlabelled S) in the +S treatment. This was confirmed by the SA of the plant (Tables 4.3, 4.4), with a higher SA in the -S than in the +S treatment.

b) HI-reducible S

According to Tabatabai (1982), the amount of ester sulfate fraction can be estimated by subtracting the amount of inorganic sulfate from the amount of HI reducible S.

The amount of HI-reducible S in the Uralla and Walcha soils was approximately 32-51% and 49-56% of the total S respectively (Tables 4.5, 4.6). 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 the temperate areas. Kurmarohita (1973) found that the ester sulfate fraction 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 highest amount of HI-reducible S was recorded in the +S treatment in both soils (Tables 4.5, 4.6). Application of S fertiliser resulted in an increased HI-S pool size. A similar result was found by Gupta *et al.,* (1988) who studied the impact of elemental S fertilisation on the two Grey Luvisolic soils and reported that the application of elemental S fertiliser significantly increased the total S and HI-S concentration in both soils. Nguyen and Goh (1990) similarly found that the concentration of HI-reducible S increased when superphosphate was applied.

A higher concentration of HI-S was recorded in the Walcha than in the Uralla soil. This was most likely related to the high clay content and high total S content in the Walcha basalt soil (Tables 4.1, 4.5 and 4.6). Bettany and Stewart (1983) reported that the HI-reducible S fraction was generally concentrated in the clay fraction of soils.

The size of the HI-S pool reduced with cropping (Tables 4.5, 4.6) indicating that this pool supplied S to the plants and/or soil pools. This result supports the finding of Tsuji and Goh (1979)

who found that S uptake by plant was related to decreases in extractable and the HI-reducible S during cropping. Shedley (1982) 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.

Before planting, there was a lower SA in the HI-S fraction in the +S, compared to the -S treatment (Tables 4.5, 4.6). The reverse was recorded on both soils after cropping. The reason for this is similar for total S as discussed above.

c) The components of extracted S

In all extractants, the sulfate fraction accounted for approximately 1.8-13.5 % of the total S (Digestion) and the HI-reducible S remaining in the soil was approximately 50.9-56.3% of the total S in the -S treatment in both the Uralla and Walcha soils before planting (Tables 4.5, 4.6, 4.7 and 4.13). This result was supports the findings of Freney (1961) who studied 24 top soils 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 5.2% (average value) of the total S of 208 different soils, was present as SO_4^{2-} and of 112 different soils investigated, ester sulfate represented an average of 40.8% of the total S.

Before planting, all extractants had a higher S concentration in the ICP-S and HI-S remaining fraction in the +S treatment than in the -S treatment in both the Uralla and Walcha soils (Tables 4.10, 4.12, 4.16 and 4.18) because of the addition of S fertiliser. The lower S concentration in these fractions after cropping in both treatments and soils was expected because of S uptake by the plant.

In both the -S and +S treatments, the concentration of S in the various extractants as measured by ICP decreased in the order KCl-100>KCl-40>MCP>Bray in the Uralla granitic soil (Tables 4.10 and 4.12). By contrast, the concentration of S in these extractants decreased in the order KCl-100>MCP>KCl-40>Bray for the Walcha basaltic soil (Tables 4.16 and 4.18). A difference in the S concentration in the MCP and KCl-40 extracts was recorded between the two soils. This was probably due to the relative size of the adsorbed S and ester-S pools in the soils. Several workers (Samosir, 1989; Santoso, 1989; Triana, 1991) found that the basaltic soils had a

higher S adsorption capacity than the granitic soils of the Northern Tablelands of NSW. Triana (1991) reported a difference between the S sorbing capacity of the Uralla and Walcha soils and indicated that this was most likely due to a lower clay content and organic matter content of the Uralla soil.

The MCP extracted a greater concentration of S than KCl-40 from the Walcha soil which had a higher S sorption capacity. By contrast, the KCl-40 extract removed a greater amount of S than MCP from the Uralla soil. In the high S sorbing Walcha soil the MCP solution had a high sulfate desorbing power which displaced most of the adsorbed sulfate (Ensminger, 1954; Fox *et al.,* 1964; Barrow, 1967 and Santoso, 1989). Aymore *et al.,* (1967) also found that solution containing KH_2PO_4 desorbed some 20% more sulfate from clay and oxide surfaces than did water. In contrast, the KCl solution did not extract more adsorbed sulfate because chloride is a poor desorber of sulfate (Chao, 1964, and Curtin and Syers, 1990).

In the Uralla soil, which had less capacity to adsorb sulfate, 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 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 by these two extractants (Tables 4.7 and 4.13). The lowest S concentration in the second extraction 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 in both soils and S treatments. 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 on both soils also confirmed this result (Tables 4.8 and 4.14). The KCl-40 extract had the highest % of ^{35}S recovery in the ICP-S and HI-S remaining fractions in the Uralla soil (Table 4.8 and Appendix 4.3). On the other hand, the MCP extractant had the highest recovery of ^{35}S in the ICP-S and lowest recovery of ^{35}S in the HI-S remaining fraction in the Walcha soil (Table 4.14 and Appendix 4.9). This result supports the findings reported in Chapter 3 where the KCl-40 extract was found to remove more S from the HI reducible (ester sulfate) fraction than MCP.

The KCl-100 extract had the highest concentration of S in the fraction as measured by both ICP and AA, conversely the Bray had the lowest S concentration in these fractions in both soils and treatments (Tables 4.7, 4.13 and Appendices 4.2, 4.8). This is because the KCl-100 extract had the ability to extract a large amount of the soil solution sulfate and adsorbed sulfate and large portion of HI reducible S. This amount declined as the temperature of the KCl extract was reduced to 40°C. The KCl-100 and KCl-40 extracts, where heat was applied, extracted both inorganic and organic S fractions, as reported by Williams and Steinbergs (1959), Spencer and Freney (1960),

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 acidic Bray extractant containing fluoride, removed readily soluble and adsorbed sulfate and a portion of the HI reducible fraction (Vaughn, *et al.,* 1987). The data from the soluble organic S, loss of the HI-S, and mineralised sulfate (Figures 4.2, 4.3, 4.4 and 4.5) and the amount of HI-S remaining in the soil after the first extraction (Tables 4.7, 4.13 and Appendices 4.2, 4.8) indicates that the Bray extract removed less S from the readily soluble and adsorbed sulfate and the HI-reducible S pools. The highest amount of soluble organic S, the loss of HI-S and mineralised sulfate (Figures 4.2, 4.3, 4.4, 4.5) and the lowest amount of the HI-S remaining (Tables 4.7, 4.13 and Appendices 4.2, 4.8) was recorded in the KCl-100 extract. Whilst the reverse was found in the Bray for these fractions. The % of ^{35}S recovery by these extractants also confirmed these results. In both soils and S treatments, the recovery of ^{35}S showed that the KCl-100 extract had the highest % of ^{35}S recovery in the ICP-S and lowest % of ^{35}S recovery in the HI-S remaining fraction. By contrast, the Bray extract had the lowest recovery of ^{35}S in the total S and highest ^{35}S recovery in the HI-S remaining fraction (Tables 4.8, 4.14 and Appendices 4.3, 4.9).

In both S treatments the concentration of S extracted by the various extractants from the Walcha basaltic soil was higher than from the Uralla granitic soil except in the KCl-40 extract (Tables 4.10, 4.12, 4.16 and 4.18). A similar result was recorded by Triana (1991) and Anderson (1992). The amount of S removed by the KCl-40 extract in the Walcha soil was less than that of the Uralla soil, due to the higher adsorption capacity of the Walcha soils.

Data on the % ^{35}S recovery in the various fractions of the extracts in both soils and S treatments 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 (Tables 4.8, 4.14 and Appendices 4.3, 4.9). 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 as measured by the various extractant 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 (1989) 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 and Bray extractions showing that in the excess of 23% of ^{35}S was recovered in the fraction indicates that ^{35}S rapidly entered the HI-S pool. This result supports by the finding of Shedley (1982) 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. The ^{35}S recovered in the Bray extractant was higher in the HI-S remaining than in the sulfate pool. This was because the Bray extractant appears to remove only a small amount of S from the soluble and adsorbed sulfate pool and the HI-S pool. The lowest concentration of S in the ICP-S fraction and highest concentration of HI-S remaining following the Bray extraction confirmed this result (Tables 4.7, 4.13 and Appendices 4.2, 4.8).

Both before and after cropping, KC -100 removed a consistently higher concentration of ICP-S and the HI-S in both treatments and soils than other extracts. By contrast, the Bray extract removed the least concentration of ICP-S (Tables 4.10, 4.12, 4.16 and 4.18). The concentration of S extracted by these two extractants was higher in the Walcha soil than the Uralla soil. In both S treatments, the KCl-40 extract removed more S than the MCP extract in the Uralla soil (Tables 4.10, and 4.12) while the MCP removed a higher concentration of S than the KCl-40 extract in the Walcha soil (Tables 4.16 and 4.18). The reason for this has been discussed above. After cropping, lower concentrations of ICP-S and HI-S remaining were recorded in all both soils and S treatments in all four test extractants (Tables 4.10, 4.12, 4.16 and 4.18) because of S uptake by the plant.

Data on the specific activity (SA) of the ICP-S of the four test extractants showed that the highest SA was recorded in the sulfate pool in both soils (Tables 4.9 and 4.15), 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 (Tables 4.8 and 4.14). Prior to planting, the SA of this fraction in the -S treatment was higher than that in the +S treatment in both soils (Tables 4.9, 4.15, and Appendices 4.4, 4.10), due to a greater dilution of ^{35}S by sulfate from the addition of S fertiliser in the +S treatment. In the Uralla soil, the highest SA in the sulfate pool was in the KCl-40 and KCl-100 extracts in both S treatments (Tables 4.9 and Appendix 4.4). On the other hand, the KCl-40 and Bray extracts had a high SA in the -S treatment (Table 4.15), and the KCl-100 and KCl-40 had a high SA in the +S treatment in the Walcha soil (Appendix 4.10). All these extractants removed the readily soluble and adsorbed sulfate and some labile organic sulfate fractions as discussed above. 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 SA of the ICP-S in the four extractants was less in the -S than that the +S treatment in both the Uralla and Walcha soils (Tables 4.10, 4.12, 4.16 and 4.18). This was because plants took up a high proportion of their S in the form of $^{35}SO_4$ in the -S treatment, compared to the +S treatment. As a result, there was a greater decline in the SA in the -S

treatment. The data of the SA in the plant supported this result (Section 4.4.1), The increase in SA following cropping in the ICP-S was more marked in the Bray and KCl-40 extracts than in the MCP and KCl-100 extracts in both soils and S treatments and the SA of the ICP-S fraction was higher than that of the HI-S remaining (Tables 4.10, 4.12, 4.16 and 4.18). 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 removal.

Data on the specific activity ratio (SAR) showed that the SAR was lower in the sulfate pool than that the other pools in both soils and S treatments prior to planting (Tables 4.11, 4.17, Appendices 4.5 and 4.11). Within the sulfate pool, the KCl-40 extract had the SAR closest to 1.00 in the -S treatment (Tables 4.11 and 4.17) or the KCl-40 extract had the SA closest to the SA 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 calcium phosphate 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 in Chapter 3 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.

The SAR of the sulfate pool in the +S treatment was greater than 1.00 in all extractants prior to planting in both the Uralla and Walcha soil (Appendices 4.5, 4.11). In this situation all the extractants could not access the S pool used by plant because the greater dilution of ^{35}S in the +S treatment by the addition of S fertiliser. All the extractants under-estimated the contribution of S from this pool when compared to the plant pool. This was support by the data of the SA of the total S (ICP-S) (Tables 4.12, 4.18) and the SA of the plant (Tables 4.3, 4.4), in all extractants where the SA of the soil was lower than the SA of the plant (Tables 4.3, 4.4, 4.12 and 4.18).

After cropping, the SAR of the sulfate pool in the -S treatment was greater than 1.00 in all extractants in both Uralla and Walcha soils (Appendices 4.1, 4.6, 4.7, 4.12). 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 soils. All four test extractants had a lower SA than the SA of the plant (Tables 4.3, 4.4, 4.10 and 4.16).

In the +S treatment, the SAR in the sulfate pool was less than the other pools in both soils (Appendices 4.6 and 4.12). In the Uralla soil, the KCl-40 extract had an SAR close to 1.00 in the ICP-S fraction. All extractants had an SAR greater than 1.00 in the Walcha soil.

4.5 Summary

The increase in yield and S uptake of the ryegrass on both the Uralla and Walcha soils was due to the application of S fertiliser. The S concentration in tops in the -S treatment was 0.19% and 0.20% in Uralla and Walcha soils respectively. These concentrations were below the critical value for perennial ryegrass reported by Reuter and Robinson (1986). The SA of the plant was greater in the -S treatment than +S treatment, because of the absence of dilution from applied fertiliser.

Total soil S pool ranged from 91 to 167 μg S g^{-1} soil in the Uralla soil and 271 to 341 μg S g^{-1} soil in the Walcha soil. The addition of S fertiliser increased the total S concentration in both soils before planting. The size of the total S pool was reduced by cropping. Before planting, the SA of the total S pool was less in the +S treatment than that the -S treatment, in both soils. By contrast, the SA was lower in the -S treatment after cropping

A similar pattern was found in the HI-reducible S pool. The size of HI-reducible S pool in the Uralla and Walcha soils was approximately 32-51% and 49-56% of the total S, respectively. A higher concentration of HI-S was found in the Walcha than in the Uralla soil. The application of S fertiliser resulted in an increased HI-S pool size, which was reduced by cropping, indicating that this pool supplied S to the plants and/or other soil pools. Before planting, there was a lower SA in the HI-S pool in the +S, compared to the -S treatment. The reverse was recorded on both soils after cropping.

In the two S treatments for both soils, the KCl-100 extract had the highest concentrations of S (ICP-S) and SO_4-S in solution. These fractions were lowest in the Bray extract. This is because the KCl-100 extract had the ability to extract a large amount of the soil solution sulfate and adsorbed sulfate as well as a large portion of HI reducible S, whilst the Bray extractant removed less S from all three of these pools. The KCl-100 extract had the highest % ^{35}S recovery in the ICP-S fraction and lowest recovery in the HI-S remaining fraction. Conversely, the Bray had the lowest recovery of ^{35}S in the ICP-S and highest recovery in the HI-S remaining, in both soils and S treatments. A higher S concentration was founded in the KCl-40 extract than in the MCP extract, in both S treatment in the Uralla soil. Conversely, the MCP had a higher S concentration in the Walcha soil. This was most likely because of the relative size of the adsorbed S and ester-S pools in the soils. In the Uralla soil, which had less capacity to adsorb sulfate, the KCl-40 extract removed the solution and adsorbed sulfate fraction and some portion of the 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 sulfate

extraction and the HI-S remaining in the soil after the first extraction by these two extractants. The % ^{35}S recovered in these fractions by these two extracts on both soils also confirmed this result.

Reduction of the temperature of the KCl extractant from 100°C to 40°C resulted in lower amounts of S extracted. This was due primarily to a reduction in the amount of HI-reducible S removed at the lower temperature.

Before cropping, all extractants had a higher S concentration in the ICP-S and HI-S remaining fraction in the +S treatment than in the -S treatment in both soils, due to the addition of S fertiliser. The S concentration in all the extractants reduced with cropping because of S uptake by the plant.

Most of the ^{35}S was recovered in the sulfate pool, with less that 10% of the ^{35}S recovered in the Organic S pool, indicating that a large amount of the ^{35}S that was added entered the sulfate pool rather than the Organic S pool. The recovery of ^{35}S in the HI-S remaining fraction following the KCl-40 and Bray extractions, with excess of 23% of ^{35}S recovered, indicates that ^{35}S also entered the HI-S pool.

Data on the specific activity (SA) of the ICP-S fraction of the four test extractants showed that the highest SA was recorded in the sulfate pool in both soils, indicating that the ^{35}S in the sulfate pool was more highly labelled than the ^{35}S in the other pools. Prior to cropping, the SA of ICP-S fraction in the -S treatment was higher than that in the +S treatment in both soils. This was due to the greater dilution of ^{35}S by sulfate from the addition of S fertiliser in the +S treatment. The highest SA in the ICP-S pool was in the KCl-40 extract in both soils and S treatments. 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, there was a greater decline in the SA of the ICP-S fraction in the four extractants in the -S treatment, in both soils, because plants took up a higher proportion of their S in the form of ^{35}S in the -S treatment, compared to the +S treatment. The data of plant SA in supports this contention. The increase in SA following cropping in the ICP-S fraction was more marked in the Bray and KCl-40 extracts than in the MCP and KCl-100 extracts, in both soils and S treatments, and the SA of the ICP-S was higher than that of the HI-S remaining fraction. This suggests that the ^{35}S remained in the soil soluble, adsorbed sulfate and HI-reducible S pools after cropping.

The specific activity ratios (SAR) were lower where the sulfate pool was the denominator than for the other pools in both soils and S treatments prior to cropping. The SAR using the ICP-S and sulfate fractions were closest to 1.00 for the KCl-40 extract compared to the other extractants in the -S treatment (i.e. the SA in the KCl-40 extract was close to the SA of the plant). This

indicates that the KCl-40 was removing S from the same as similar pools as the plant and that the plant was utilising S from the soil solution and adsorbed sulfate pools and a portion of the highly labile HI-S pool. The SAR of the ICP-S and sulfate pools in the +S treatment were greater than 1.00 for all extractants prior to planting, in both the soils. After cropping, the SAR with these pools, for the -S treatment, were greater than 1 00 in all extractants in both soils. The SAR in the these pools were less than the other pools in the +S treatment in both soils. In the Uralla soil, the KCl-40 extract had an SAR close to 1.00 in the ICP-S fraction and all extractants had an SAR greater than 1.00 in the Walcha soil.

This experiment has identified the reasons why the KCl-40 extract is better able to predict S supply to ryegrass. The question remains as to whether this holds true for crops with different relative growth rates and S demands. This is the subject of the experiment reported in Chapter 5.