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Original Research Article

New Technique for Sequential Fractionation of Soil Sulphur

N. Y. Azmi[1*], Seema[2] and Manish Kumar[3]

[1]Department of Soil Science, NCOH, Noorsarai, Nalanda, Bihar-803113, India
[2]Department of Botany & Plant Physiology, NCOH, Noorsarai, Nalanda, Bihar-803113, India
[3]Department of Agriculture Engineering, NCOH, Noorsarai, Nalanda, Bihar-803113, India
**Corresponding author*

A B S T R A C T

A new technique for sequential fractionation of soil sulphur was developed over traditional independent fractionation scheme from a long-term experiment which is being carried out since Kharif, 1985 under micronutrient scheme at RAU Research farm, Pusa. The result indicates that like independent fractions, all the fractions except residual-S extracted by sequential extraction schemes increased significantly with increasing fertility levels under the influence of long-term intensive cropping and fertility levels. Superimposition of organic manure with different fertility levels also increased all these forms except residual-S. Major contribution to the total-S was accounted for by the organic-S while distilled water soluble-S, sulphate-S and inorganic-S contributed very little to total-S. The behaviour of residual-S with respect to fertility level, superimposition of treatment and cropping sequences was very much similar to that of non sulphate –S except that the amount non sulphate-S was higher than residual-S. Residual-S (extracted by sequential fractionation scheme) produced negative and significant effect however, non-sulphate-S (extracted by traditional independent fractionation procedure) has negative and non-significant effect. Dynamic equilibria were found among different fractions of S fractionated by any of the fractionation scheme under both rotations. However, non-sulphate-S and residual-S showed negative but significant correlation with other fractions. The overall results obtained from regression studies suggested that sequential fraction procedure for the fractionation of soil S could be successfully utilized in place of independent extraction procedure to reduce labour cost as well as chemical cost. Among the fractions, sulphate-S followed by organic-S was the most important Sulphur fraction with respect to crop production and S-utilization by crops under both rotations.

Keywords

Sequential fractionation, fertility levels, intensive cropping, residual-S, non-sulphate-S, dynamic equilibria

Introduction

Sulphur, one of the essential nutrients for the proper growth and development of plants, is required by growing plants in amount similar to phosphorus and 1/3rd of nitrogen. Sulphur performs many important functions in plants such as synthesis of proteins, oils, vitamins etc. S transformation in soil involves complex mineralogical, chemical and biological processes. When sulphur is added to soil through mineral fertilizers or any other sources, a part of it remains in soil solution and some part is adsorbed on soil colloids to a variable extent depending upon the nature of soil and after sometimes, a new equilibrium is established between the solution and sulphur on solid phase. Therefore, the availability of sulphur is governed by the simultaneous equilibria of several mechanisms including downward movement and the distribution pattern into different forms of sulphur. Sulphur concentration in different forms varies greatly which may be positively and significantly correlated with most of the soil properties. Knowledge of different forms of sulphur in such soils is essential in improving the sulphur nutrition of plants. The traditional common procedure followed for the fractionation of soil sulphur into different forms is tedious and costly affairs besides more time consumption because of extraction of different form of sulphur are being done following different independent procedures. The extraction of Organic-S is itself a lengthy procedure consisting of removal of different forms of loosely bound sulphur by using different chemicals. These individual chemicals remove individual soil sulphur fractions e.g. washing with distilled water remove distilled water soluble sulphur and washing with HCl followed by distilled water remove both sulphate-S as well as inorganically complexed sulphur. Hence, it was thought that this procedure could be utilized for sequential extraction of different forms of sulphur with slight modification to separate out sulphate sulphur and inorganically complexed sulphur. Keeping this in view, sequential extraction procedure was developed for the fractionation of soil sulphur into distilled water soluble sulphur, SO_4-S, inorganically complexed sulphur, organic-S, residual-S and total-S.

Materials and Methods

A long-term experiment is being conducted since kharif, 1985 at R.A.U. research farm, PUSA with four fertility levels (0, 50, 100 and 150 % of the recommended NPK levels) and six replications. The design of the experiment is RBD where two crop rotations 1^{st} –rice-wheat- sorghum and 2^{nd} –rice-mustard-moong were followed. After 10 complete cycles of rotation, i.e. 30^{th} crop, all the treatments under different replications were superimposed with the following sub treatments: R_1 – 10 kg Zn ha^{-1}, R_2 -10 kg Zn + 2 kg B + 40 Kg S ha^{-1}, R_3-100q FYM $ha^{-1,}$ R_4-100q FYM + 10 kg Zn + 2 Kg B + 40 Kg S ha^{-1} and R_5, R_6 were kept as such. After the harvest of 36^{th} crop, surface soil of all the treatments superimposed with different replications were separated out and replication five and six (i.e. R_5 and R_6) were pooled for the fractionation of different forms of sulphur.

Hypothesis

An attempt was made for standardizing sequential extraction scheme based on organic-S estimation procedure as given by Evans and Rost (1945) with some modification as given in sequential extraction scheme in fig-I. As in case of sequential fractionation of cations here also, more soluble fractions were eliminated after estimation and least soluble fraction was estimated. Total-S was estimated by acid digestion method of Tabatabai (1982) and by difference residual-S was calculated.

Results and Discussion

The results obtained on content of sequential forms of sulphur as influenced by long-term effect of fertility levels and management practices are presented in Table-1

Distilled water soluble sulphur (DWS-S)

The DWS-S in surface soil varied from 5.19 to 18.44 mg kg^{-1} under rice –wheat-sorghum and 3.89 to 13.21 mg kg^{-1} under rice-mustard-moong rotation. While this fraction vary from 3.68 to 10.27 mg kg^{-1} under dummy plot. This indicated that higher crop production retained the applied as well as native S into DWS-S which might be due to exploitation of root zone soil by crop roots while under low crop yield or without crop condition the added as well as native S might have been transformed into less soluble form.

Sulphate sulphur (SO_4 –S or NaCl-S)

The content of SO_4-S as influenced by long-term effect of fertility levels and management practices was found to vary from 3.19 to 19.98 mg kg^{-1} which was comparable with that of DWS-S. Increasing fertility levels increased the SO_4 -S content in soil which was due to addition of graded level of S through SSP at increasing fertility levels. It was also noticed that the contribution of SO_4-S in soil with respect to T-S was comparatively more in 2nd rotation as compared to Ist rotation. This indicated the transformation of S by roots of oilseed and pulse crop into sulphate form of S. Superimposition of organic manure alone or along with $ZnSO_4$ was also effective and in transformation of S under both crop rotations whose effect was more pronounced at higher fertility levels. The high value of SO_4-S form under dummy condition especially at lower fertility level might be due to non-utilization by plant.

Inorganically bound sulphur (Inorganic-S or HCl-S)

The amount of HCl-S was found to vary from 3.29 to 18.21 and 3.77 to 16.58 mg kg^{-1} under 1st and 2nd rotation, respectively. The contribution of this fraction was almost similar to that of SO_4-S. This fraction was also found to be increased with increasing fertility levels as well as due to superimposition of different treatments. However, the effect of superimposition of treatment was not much marked especially at higher fertility levels, when its proportion with respect to T-S was considered.

Organically bound sulphur (0-S or H_2O_2-S)

The data in table 1 indicated that organic-S was the major fraction of S in soil whose extent and distribution was further increased with continuous use of S through SSP under different fertility levels and superimposition of organic manure. Although increasing rate of S application through SSP at different fertility levels increased the 0-S content in soil to a large extent but the per cent contribution towards total-S did not vary much. The distribution of organic sulphur in these soils is mainly influenced by the organic matter treatment.

Residual sulphur (Res-S)

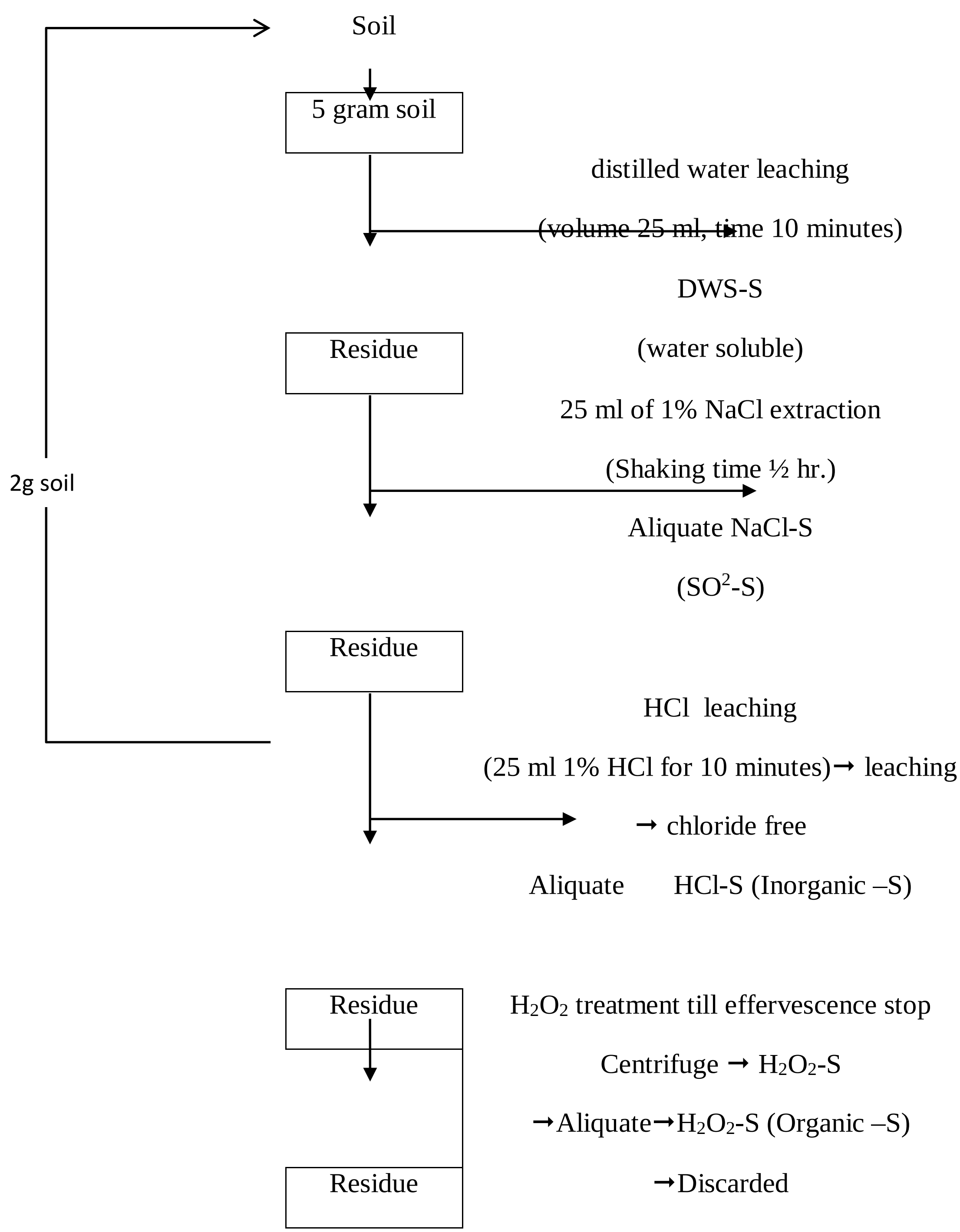
The residual fraction of soil S represents the unaccounted S not extracted by any of the previous sequential extractants, hence, this fraction was calculated from the difference between T-S and sum of all fractions estimated.

This fraction was found to vary from as low as 1.87 to as high as 66.51 mg kg^{-1} as influenced by long-term effect of fertility levels and management practices which constitute 0.51 to 26.50% of T-S. The behaviour of Res-S with respect to fertility levels, superimposition of treatments and cropping sequence was very much similar to that of NS-S except that the amount of NS-S was higher than Res-S. This suggested that a portion of NS-S was also extracted by sequential extraction procedure probably in the form of HCl-S.

Total Sulphur (T-S)

Total sulphur content in surface soil as influenced by long-term effect of fertility levels and management practices varied from 231.21 to 397.26 mg kg^{-1} (Table 1).

Fig.1 Sequential Extraction scheme for sulphur



Total –S extraction procedure → Total-S

Residual – S = Total-S – (Σall above fraction)

Table.1 Sequential extraction of soil sulphur (mg kg^{-1}) in post-harvest soil of a long-term experiment

Treatment		DWS-S	NaCl-S	HCl-S	H_2O_2-S	Res-S	Total-S
Superimposition	**Sub-treatment**						
Rice-wheat-sorghum rotation							
Zn	Control	6.36	4.44	6.03	179.76	49.91	246.50
	Low fertility	10.96	7.10	8.07	215.46	42.25	283.84
	Medium fertility	12.47	9.93	12.33	254.54	29.91	319.18
	High fertility	16.79	13.81	16.44	294.15	22.92	364.11
OM.	Control	5.99	3.81	3.29	186.19	40.72	240.00
	Low fertility	10.27	9.00	5.25	221.26	30.80	276.58
	Medium fertility	13.56	12.31	7.53	257.67	18.66	309.73
	High fertility	15.93	16.47	12.47	299.64	11.65	356.16
Zn + OM	Control	8.93	4.12	7.62	200.18	31.48	252.33
	Low fertility	11.64	9.66	11.92	234.92	20.90	289.04
	Medium fertility	14.04	14.16	13.85	270.64	8.68	321.37
	High fertility	18.44	18.26	18.21	338.45	3.90	397.26
Control	Control	5.19	3.19	6.85	167.81	55.32	238.36
	Low fertility	7.53	6.20	9.18	188.44	53.03	264.38
	Medium fertility	9.19	10.41	11.51	217.81	48.34	297.26
	High fertility	11.92	15.39	14.23	249.75	41.59	332.88
Dummy	Control	3.68	6.42	3.67	170.68	66.51	250.96
	Low fertility	5.71	7.39	8.08	187.65	61.17	270.00
Table 1(contd.)	Medium fertility	7.00	9.97	10.36	204.17	59.59	291.09
	High fertility	10.27	12.31	14.79	227.49	54.32	319.18
Rice-mustard-moong rotation							
Zn	Control	5.49	4.11	3.90	174.77	47.21	235.48
	Low fertility	7.47	6.82	8.14	211.18	40.36	273.97
	Medium fertility	10.33	8.81	11.92	252.55	31.46	315.07
	High fertility	11.23	11.65	16.27	284.93	22.42	346.50
OM.	Control	6.13	5.07	4.39	181.18	34.44	231.21
	Low fertility	6.37	9.43	9.08	216.12	25.58	266.58
	Medium fertility	7.53	15.88	12.04	259.13	17.75	312.33
	High fertility	9.32	19.98	15.78	294.81	6.14	346.03
Zn + OM	Control	7.74	7.91	5.01	204.17	26.68	251.51
	Low fertility	9.27	9.93	10.36	233.43	17.97	280.96
	Medium fertility	10.67	14.83	13.15	279.15	6.86	324.66
	High fertility	13.21	17.89	16.58	320.45	1.87	370.00
Control	Control	3.89	4.01	3.77	169.35	57.34	238.36
	Low fertility	5.69	5.38	6.18	187.20	55.55	260.00
	Medium fertility	7.23	7.98	9.03	210.75	45.69	280.68
	High fertility	10.27	11.57	14.66	240.92	39.43	316.85

Table.2 Correlation coefficient between various soil sulphur and plant parameters for both crop rotations

Soil parameter	Rice-wheat-sorghum					Rice-mustard-moong					
	Rice		Wheat		Sorghum	Rice		Mustard		Moong	
	Grain yield	Straw yield	Grain yield	Straw yield	Straw yield	Grain yield	Straw yield	Grain yield	Straw yield	Grain yield	Straw yield
Independent fraction											
0.15%$CaCl_2$ extractable-S	0.893**	-0.320	0.932**	0.874**	0.883**	0.898**	0.918**	0.871**	0.840**	0.913**	0.950**
Monocalcium phosphate extractable-S	0.836**	-0.269	0.898**	0.795**	0.829**	0.777**	0.850**	0.761**	0.709**	0.831**	0.890**
Exchangeable-S	0.602*	-0.124	0.704**	0.521*	0.607*	0.542*	0.671**	0.540*	0.469	0.637**	0.715**
Total water soluble-S	0.900**	-0.308	0.936**	0.882**	0.910**	0.796**	0.904**	0.841**	0.770**	0.882**	0.905**
Organic-S	0.896**	-0.243	0.910**	0.832**	0.780**	0.583*	0.722**	0.704**	0.650**	0.680**	0.606*
Non sulphate-S	-0.348	-0.018	-0.417	-0.333	-0.330	-0.119	-0.238	-0.215	-0.028	-0.163	-0.371
Total-S	0.921**	-0.301	0.949**	0.885**	0.916**	0.922**	0.971**	0.896**	0.902**	0.969**	0.942**
Sequential fractions											
DWS-S	0.868**	-0.322	0.896**	0.855**	0.851**	0.794**	0.830**	0.756**	0.777**	0.840**	0.825**
Sulphate-S	0.923**	-0.300	0.956**	0.894**	0.942**	0.744**	0.869**	0.811**	0.707**	0.836**	0.873**
Inorganic-S	0.826**	-0.414	0.872**	0.767**	0.783**	0.936**	0.961**	0.929**	0.914**	0.964**	0.955**
Residual-S	-0.602*	0.121	-0.670**	-0.570*	-0.582*	-0.501*	-0.644**	-0.564*	-0.465	-0.610*	-0.702**

Table.3 Correlation coefficient among various sulphur fractions (independent) under two relations

	Monocalcium phosphate	**Exchangeable sulphur**	**Total water soluble sulphur**	**Organic sulphur**	**Non sulphate sulphur**	**Total Sulphur**
Rice-wheat-sorghum						
0.15%$CaCl_2$ extractable-S	0.979**	0.802**	0.981**	0.962**	-0.642**	0.948**
Monocalcium phosphate extractable-S		0.906**	0.964**	0.966**	-0.732**	0.923**
Exchangeable-S			0.796**	0.842**	-0.821**	0.745**
Total water soluble-S				0.961**	-0.821**	0.954**
Organic-S					-0.605**	0.976**
Non sulphate-S					-0.659**	-0.482
Rice-mustard-moong						
0.15%$CaCl_2$ extractable-S	0.957**	0.796**	0.924**	0.720**	-0.422	0.953**
Monocalcium phosphate extractable-S		0.937**	0.949**	0.749**	-0.606*	0.907**
Exchangeable-S			0.842**	0.697**	-0.758**	0.749**
Total water soluble-S				0.725**	-0.566*	0.933**
Organic-S					-0.361	0.695**
Non sulphate-S						-0.300

Table.4 Correlation coefficient among various sulphur fractions (sequential extraction) under two relations

	Sulphate	Inorganic sulphur	Organic sulphur	Residual sulphur	Total Sulphur
Rice-sheat-sorghum					
Water soluble-sulphur	0.914**	0.813**	0.984**	-0.864**	0.957**
Sulphate-sulphur		0.831**	0.940**	-0.746**	0.956**
Inorganic-sulphur			0.828**	-0.572*	0.891**
Organic-sulphur				-0.864**	0.976**
Residual-sulphur					-0.738**
Rice-mustard-moong					
Water soluble-sulphur	0.730**	0.883**	0.903**	-0.746**	0.894**
Sulphate-sulphur		0.868**	0.926**	-0.871**	0.882**
Inorganic-sulphur			0.950**	-0.717**	0.977**
Organic-sulphur					0.977**
Residual-sulphur					-0.728**

Table.5 Multiple regression equation showing contribution of different soil parameters on crops yield under rice-wheat-sorghum rotation

Y Factor	Regression Equation	R^2 Value
1. Rice grain yield (Y_1)	**(a)** $-134.3235 + 0.0144X_{13} -0.0205X_{14} +0.0613X_{15} -0.0110X_{16}$ (-0.01105) (0.0878) (-0.5749) (-0.5749) $+0.2198X_{17} -0.2577X_{18} +0.0410X_{19} -0.8974X_{20} -0.8956X_{21}$ (0.1613) (-0.6369) (0.0259) (-0.0320) (-0.08974) $+0.8984X_{27}$ (0.0314)	0.942**
	(b) $-37.0898 +0.4481X_{13} +1.1531X_{14} -0.0531X_{15} +0.0045X_{22}$ (0.2844) (0.5925) (-0.0760) (0.0013) $+0.0045X_{23}$ $+0.0045X_{24} +0.0045X_{25} +0.0045X_{26} +0.0045X_{27}$(0.0016) (0.0014) (0.0016) (0.0054) (-0.0002)	0.927**
2. Wheat grain yield (Y_2)	**(a)** $-66.0758 + 0.5905X_{13} -0.3111X_{14} +0.1156X_{15} -0.7204X_{16}$ (0.6503) (-0.3212) (0.3330) (-0.7522) $+0.3144X_{17}$ $-0.3318X_{18} +0.6311X_{19} -0.4043X_{20} -0.4046X_{21}$ (0.4636) (-0.1649) (0.8044) (-0.0290) (-0.8152) $+0.4049X_{27}$ (0.0284)	0.967**
	(b) $-40.4756 +7.3391X_{13} -0.0207X_{14} +0.0983X_{15} -0.0933X_{22}$ (0.0937) (-0.0214) (0.2831) (0.0545) $+0.0932X_{23} +0.0931X_{24} +0.0931X_{25} -0.0931X_{26} +0.0931X_{27}$ (0.0669) (0.0584) (0.0067) (0.0023) (-0.6539)	0.966**

Figures in parentheses indicates the standard regression coefficient

X_{13}	Organic carbon	X_{21}	Non-sulphate-Sulphur
X_{14}	P_2O_5	X_{22}	Distilled water soluble-S
X_{15}	K_2O	X_{23}	SO_4^{2-}-Sulphur
X_{16}	0.15% $CaCl_2$ extractable-S	X_{24}	Inorganic-Sulphur
X_{17}	Monocalcium Phosphate extractable-S	X_{25}	Organic sulphur
X_{18}	Exchangeable-S	X_{26}	Residual sulphur
X_{19}	Total water soluble sulphur	X_{27}	Total sulphur
X_{20}	Organic sulphur		

Similar values of T-S in surface soil of Calciorthents have been reported by Bhogal *et al.*, (1996a), however, a little higher value was reported by Rakesh (1999). As expected, increasing fertility level increased the T-S probably due to addition of sulphur through SSP. The rate of increase was higher when these fertility levels were superimposed with $ZnSO_4$ and organic manure either alone or together as compared to control. This indicated that organic-manure increased the sulphur retaining power of surface soil. Secondly, the sub-soil sulphur which moved upward was probably retained in the surface soil.

Correlation studies

The correlation coefficient values of different forms of S with respect to crops yield under both rotations have been presented in table-2. Different fractions of soil S as estimated by two fractionation scheme were also significantly correlated with crops yield in both the rotations. Rice straw (cv. Rajshree) failed to produce significant correlation with any of the sulphur fractions. Among different fractions, Res-S produced negative but significant correlation with crops yield. However, the effect of NS-S was non-significant but negative. Among different fractions, the value of correlation coefficients of SO_4-S with crops yield was found to be highest followed by TWS-S and CC-S indicating importance of these fractions in crop production. Total-S was also highly correlated with crops yield indicating thereby not only different fractions of S but T-S is also equally important in the crop production.

The correlation coefficient values as given in table 3 and 4 represents dynamic equilibria among different fractions of S fractionated by any of the fractionation scheme under both crop rotations as evidence by highly significant and positive correlation among different fractions. However, NS-S in the Ist (Independent) scheme and Res-S in the 2^{nd} (sequential) scheme showed negative but significant correlation with other fractions.

The existence of dynamic equilibrium among different sulphur fractions as estimated by independent fractionation scheme suggested that some common forms of S are being extracted by all the independent extraction procedure. However, the existence of dynamic equilibrium among different S fractions as estimated by sequential extraction procedure suggested the transformation of different forms of sulphur among themselves to maintain S equilibrium

Multiple regression studies

The relative contribution of some soil properties and different S fractions as estimated by two separate fractionation procedure was evaluated on different plant parameters as dependent variables such as grain yield of rice, (Y_1), grain yield of wheat (Y_2) under 1^{st} rotation through multiple regression analysis and the results obtained in the form of regression equation are presented in table 5. The independent variables were organic carbon (X_{13}), available P_2O_5(X_{14}), available K_2O (X_{15}) DWS-S (X_{22}), SO_4-S (X_{23}), I-S (X_{24}), O-S (X_{25}), R-S (X_{26}) and T-S (X_{27}). It was noticed that under the rice-wheat-sorghum rotation, the contribution of different soil S fractions as estimated by two separate procedures towards plant parameters was comparable as evidenced from almost similar R^2 values for both regression equations with respect to grain yield of rice and wheat. It was observed that all the soil S fractions as estimated by sequential extraction procedure contributed positively except T-S towards all the plant parameters studied, however, when independent fractions were considered, the contribution of different fractions was fluctuating for different plant parameters. It was surprising to note that the contribution of O-S was negative when considered along with individual soil S fraction, while the effect was positive when considered along with sequential fractions. The positive effect seems to be realistic indicating thereby superiority of sequential fractionation over individual fractionation procedure. Comparing the relative importance of different fractions though standard regression coefficient, it was found that SO_4-S is the most important soil S fraction followed by DWS-S and Res-S with respect to crops yield.

Similar regression equations were also developed under rice-mustard- moong rotation. The result indicated that soil-S fraction, either estimated independently or by sequential extraction, along with some soil properties contributed significantly to the plant parameters like grain yield of rice, mustard and moong with R^2 values ranging from 0.950 ** to 0.999***. The R^2 values were comparatively more in case of 2nd rotation as compared to 1st rotation. As in case of first rotation, in 2nd rotation also the R^2 values obtained involving two fractionation schemes was comparable suggesting thereby the usefulness of sequential extraction procedure. Hence, sequential extraction procedure could be utilized successfully for the estimation of S-fractions in soil.

Among the independent fractions, the contribution of MCP-S, Ex-S and T-S was found to be positive, and the relative importance of T-S was found to be more followed by Ex-S and MCP-S as evidenced by the higher standard regression coefficient values for T-S. Among the soil sulphur fractions as estimated by sequential extraction procedure, all the fractions except T-S contributed positively towards grain yield as well as total S-uptake by rice, mustard and moong, suggesting thereby the superiority of sequential extraction procedure over independent procedure. The relative importance of different fractions as shown by standard regression coefficient suggested that O-S is the most important fraction followed by inorganic-S. It was further noticed that SO_4-S and residual-S were equally important and DWS-S was found least important fraction with respect to crops yield and S-uptake. The overall results suggested that sequential extraction procedure for the fractionation of soil S could be successfully utilized in place of individual extraction procedure to reduce labour as well as chemical cost. Among the fractions, sulphate-S followed by organic S was the most important S fraction with respect to crop production and S-utilization by crops under rice-wheat-sorghum and rice-mustard-moong rotations.

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