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# Changes in land use practices influence soil sulfur fractions and their bioavailability

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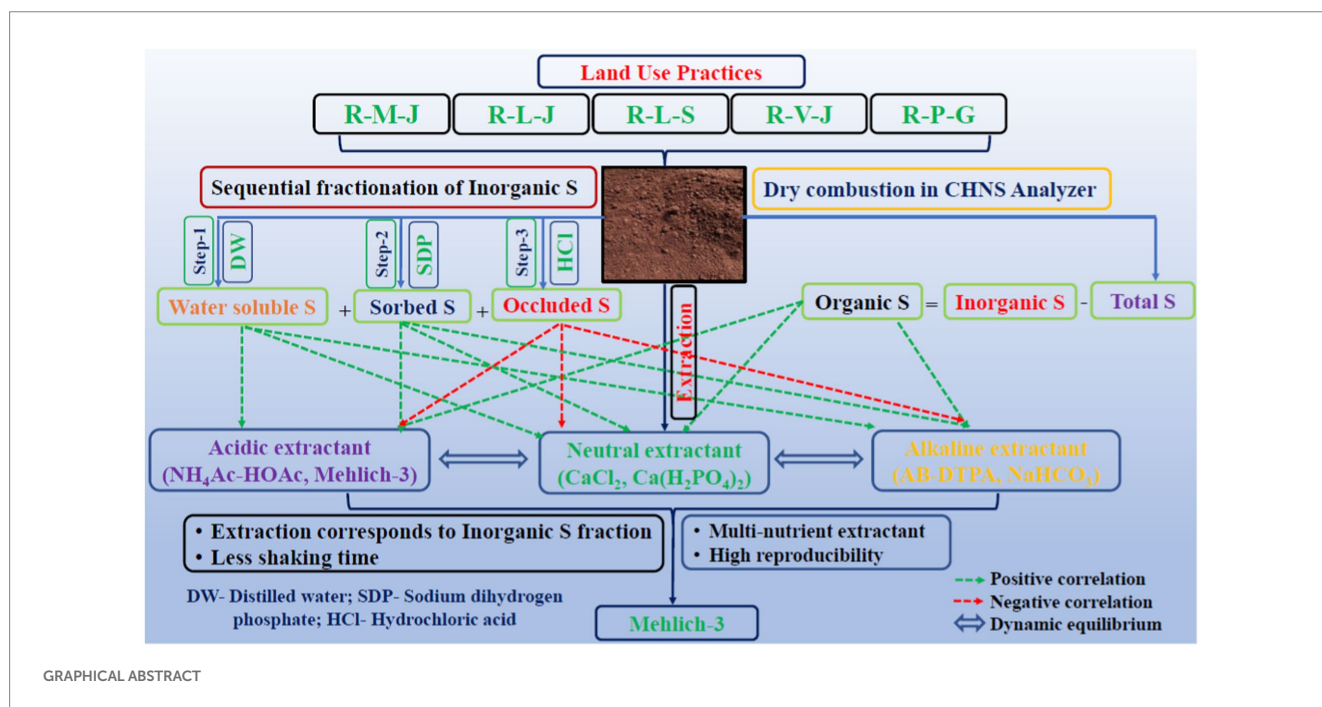
Changes in land use practices may affect the distribution of soil sulfur (S) fractions and their bioavailability. Therefore, this study was undertaken to assess the influence of different land use changes on the distribution of soil S fractions and their bioavailability for plant nutrition. Soil samples from farmers' fields with different land use practices such as rice-mustard-jute (R-M-J), rice-lentil-jute (R-L-J), rice-lentil-sesame (R-L-S), rice-vegetable-jute (R-V-J), and rice-potato-green gram (R-P-G) were collected and analyzed for different fractions of S. The bioavailability of S was assessed by extracting the soil with six different extractants (acidic, neutral, and alkaline) with different extraction modes and chemistry. The results showed that changes in land use practices could influence the distribution of soil S fractions and their bioavailability. Organic S was the dominant fraction, accounting for 93.5% of total S across land use practices. The inorganic S fraction (water-soluble, sorbed, and occluded) varied significantly among the land use practices. Among the inorganic fractions, the water-soluble fraction was the dominant fraction across the land use practices. The bioavailability of S, as assessed by different chemical extractants, was in the following order: sodium hydrogen carbonate ( $\text{NaHCO}_3$ ) > mehlich-3 > ammonium bicarbonate-diethylenetriamine penta-acetic acid (AB-DTPA) > ammonium acetate-acetic acid ( $\text{NH}_4\text{Ac-HOAc}$ ) > calcium dihydrogen phosphate  $\{\text{Ca}(\text{H}_2\text{PO}_4)_2\}$  > calcium chloride ( $\text{CaCl}_2$ ). By establishing relationships between extractable S and soil S fractions, it was observed that all the extractants could obtain S from the water-soluble, sorbed, and organic S fractions, with little extractability from the occluded fraction. Among the extractants tested, mehlich-3 extracted a similar amount of S corresponding to the inorganic fraction across the land use practices. It also maintained positive relationships with different fractions of S, and as a multi-nutrient extractant, its use in routine soil testing can be recommended.

## KEYWORDS

sulfur fractions, organic S, land use practices, bioavailability of S, extractants, mehlich-3

## 1. Introduction

Sulfur (S) is a secondary nutrient element required for the growth and development of plants. As an important constituent of different biomolecules and enzyme systems, it plays an important role in crop production (Solomon et al., 2001). Although the requirement for S is crop-specific, oilseed crops require a higher amount of S for the synthesis of glucosinolates and oils (Padhan, 2016). Therefore, an inadequate supply of S during the crop growth period affects crop production and product quality. In recent years, soil S deficiency has been found in many parts of the world. The widespread S deficiency in soils may be due to the use of chemical fertilizers with high analysis but low sulfur content (Balik et al., 2009; Eriksen, 2009; Scherer, 2009), the restricted use of organic manures, which are a major source of S for plant nutrition (Reddy et al., 2002), and



the introduction of high-yielding cultivars that remove higher amounts of S compared to traditional varieties (Kundu et al., 2020). It has been reported that approximately 58.6% of Indian soils are deficient in S (Shukla et al., 2021). Such S deficiency in soils causes significant yield reduction, and it is reported to be 50% in cereals (Zhao et al., 2001). Nearly 1.26 million tons of S are removed by different cropping systems, while only about 0.76 million tons are replenished by fertilizers (Tiwari and Gupta, 2006). Moreover, the use efficiency of the applied S is only 8–10% (Hegde and Murthy, 2005) in different crops, while it is 18% in cereals (Aula et al., 2019), which needs to be improved considering the escalating cost of S fertilizers and other environmental issues. The application of S to cereal-based cropping systems has been shown to improve yield and nutritional quality (Zhao et al., 2001). Cereal crops responded to different levels of S fertilization, and grain yield increased with increased S fertilization (Ying-Xin et al., 2017). However, contrary to this finding, Dhillion et al. (2019) observed a non-significant crop response to S fertilization, except for adequate S supply to the crop due to the mineralization of soil organic matter.

Sulfur fractions in soils are highly dynamic (Dutta et al., 2013). Soil S primarily occurs in organic and inorganic fractions, with the organic fraction being dominant and accounting for more than 90% of total S in agricultural soils (Wang et al., 2006; Sharma et al., 2014; Padhan et al., 2023). Plants uptake sulfate (SO<sub>4</sub><sup>2-</sup>)-S for their nutrition, which is supplied from the native soil reserves in addition to external fertilizer inputs. However, the availability of SO<sub>4</sub><sup>2-</sup>-S for plant uptake is governed by several soil properties. The presence of iron and aluminum oxides and/or soils with high clay content adsorb the SO<sub>4</sub><sup>2-</sup> ions through specific adsorption mechanisms that influence their availability to plants (Padhan, 2018; Das et al., 2020). Interestingly, this mechanism is more pronounced in soils with a pH below 6.5 (Williams and Steinbergs, 1962). A similar one operates when the soil is rich in CaCO<sub>3</sub>, which could bind the SO<sub>4</sub><sup>2-</sup> as occluded S (Hu et al., 2005). Murthy et al. (2002) reported that changes in land use practices could affect the distribution and availability of soil nutrients by altering soil properties and influencing biological transformations in the rooting zone. Soil S fractions and their bioavailability varied under different land uses, for

example, in maize-wheat (Gourav et al., 2018), rice-wheat (Sharma et al., 2014; Meena et al., 2022), and finger millet-maize (Lavanya et al., 2019). The mineralization of organic S to an inorganic fraction or bioavailable form varies with changes in land use and types of crop species within the land use (Padhan et al., 2016). Suri et al. (2021) studied the relationship between the different fractions of soil S and their bioavailability under various land uses, viz., maize-wheat, paddy-wheat, vegetable-based cropping sequence, sugarcane-based cropping sequence, and orchard; the occurrence of S fractions followed the order of organic S > non-sulfate S > available S > exchangeable S > water-soluble S. Land use change has a strong influence on carbon storage by controlling the amount and quality of litter addition, decomposition rate, stabilization of soil organic carbon, etc. (Gelaw et al., 2014; Huang et al., 2018), which in turn influences the nutrient dynamics in soil. As the lion's share of total soil S is associated with the organically bound form, bioavailability is influenced by land use management (Padhan et al., 2016). Information on the influence of land use changes on soil S fractions, extractable S, and their bioavailability for crop nutrition is scarce. We hypothesized that changes in land use could alter the distribution of different fractions of soil S by changing the proportion of inorganic and organic S fractions, further influencing its bioavailability. Thus, the present study was designed to ascertain the influence of land use changes on soil S fractions and their bioavailability for crop nutrition.

## 2. Materials and methods

Surface soil samples (0–15 cm) were collected from farmers' fields in Nadia district of West Bengal with five different land uses, viz., rice-lentil-jute (R-L-J), rice-lentil-sesame (R-L-S), rice-mustard-jute (R-M-J), rice-vegetable-jute (R-V-J), and rice-potato-green gram (R-P-G), which were more than 10 years old. In total, 90 geo-referenced soil samples were collected after the rice crop was harvested using a simple random technique. The sampling site falls under the agroecological sub-region 15.1 of India and is characterized by a tropical, moist, sub-humid climate. It receives an annual rainfall of ~1,400 mm, and more than 70%

of the rainfall occurs from June to September. In winter, the minimum temperature goes down to 8°C, while in summer, the maximum temperature goes up to 41°C. Different fertilizer schedules and doses are followed for all land uses. On average, R-L-J systems receive N:P:K at a rate of 94:60:80 kg/ha annually, while the R-L-S, R-M-J, R-V-J, and R-P-G systems receive 135:89:85, 175:120:125, 249:210:200 and 250:260:220 kg/ha/year, respectively. For lentils and green grams, no fertilizers are used. Canal and/or groundwater sources are used for the irrigation of rice, jute, vegetables, and potatoes, while lentils and green grams are cultivated with residual soil moisture. The collected samples were air-dried, ground, sieved with a 2.0 mm sieve, and stored in moisture-proof bags for further analysis of soil properties. Different chemical properties and available nutrients in soils of all land uses were analyzed following standard protocols as outlined in Table 1.

## 2.1. Sequential fractionation of sulfur in soils

Fine soils (<2 mm) were used for the analysis of S fractions as per the method outlined by Morche (2008). The first step in the sequential fractionation was the extraction of the soil sample with demineralized water at 1:10 (w/v). After 30 min of shaking in a rotary shaker, the samples were centrifuged at 10,000 rpm for 10 min. In the second step, the samples were extracted with 0.032 M NaH<sub>2</sub>PO<sub>4</sub> at a 1:10 (w/v) ratio by shaking for 30 min followed by centrifugation at 10,000 rpm for 10 min. The residual soil sample was extracted with 1 M HCl, maintaining the extraction ratio of 1:20 (w/v). After shaking for 60 min, the samples were centrifuged for 10 min at 10,000 rpm. The extracted S fractions were measured by following the method of Chesnin and Yien (1950). The sum of all three fractions was considered the total inorganic S fraction of the soil. The total S of soil samples was analyzed using the CHNS VarioEL cube analyzer (Elementar, Germany). Organic S was calculated as the difference

between the total and inorganic S of soil samples. The extractable S from soils of all land uses was extracted with six different chemical extractants (two with a neutral solution pH, two with an acidic solution pH, and two with a slightly alkaline to alkaline solution pH) having different extraction modes and chemistry (Table 2).

Schematic representation of the sequential fractionation of sulfur in soil (Morche, 2008).

## 2.2. Statistical analysis

Statistical analysis was performed using the Windows-based SPSS program (ver. 22.0, SPSS Inc./IBM, Armonk, United States). Duncan's Multiple Range Test (DMRT) was used to compare means. Pearson correlation was performed between S fractions and extractable S and between soil properties to establish their relationships. A multiple linear regression equation was also computed with extractable S as the dependent variable and the main soil properties as the independent variables.

## 3. Results

### 3.1. Influence of land use on soil properties and nutrient availability

Changes in land use significantly influenced soil properties and nutrient availability (Table 3). The soil pH, measured with a 0.01 M CaCl<sub>2</sub> solution, ranged from 7.18 to 7.54. The highest soil pH was observed in the R-M-J system and the lowest in the R-P-G system. Soil organic carbon content (g kg<sup>-1</sup>) varied between 6.68 and 7.36 across different land use systems. Compared with the R-V-J system, the R-L-S,

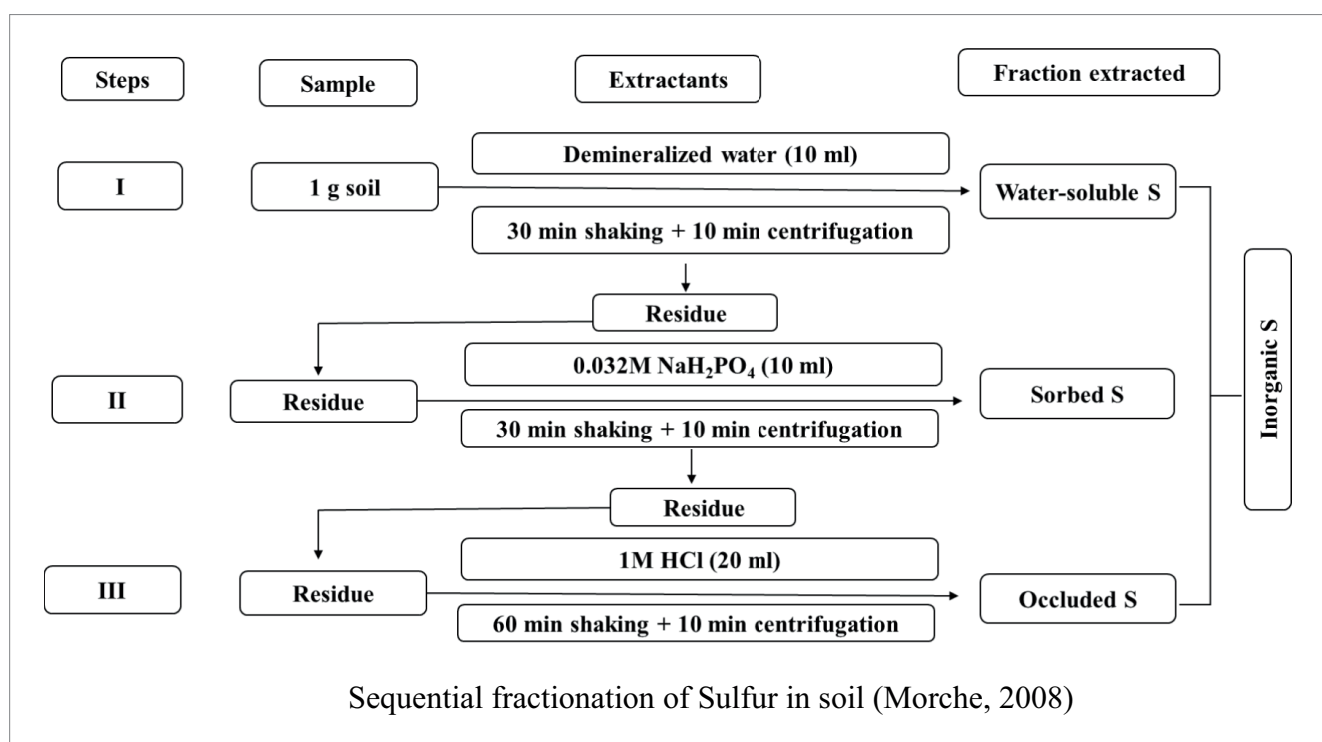


TABLE 1 Methods used for analysis of soil properties and available nutrients.

Soil properties	Abbreviation	Unit	Method
pH	-	-	Soil: 0.01 M CaCl <sub>2</sub> suspension of 1:2 (Jackson, 1973)
Soil organic carbon	SOC	g kg <sup>-1</sup>	Walkley and Black (1934)
Calcium carbonate	CaCO <sub>3</sub>	g kg <sup>-1</sup>	Page et al. (1982)
Iron oxide	Fe <sub>o</sub>	g kg <sup>-1</sup>	McKeague and Day (1966)
Aluminum oxide	Al <sub>o</sub>	g kg <sup>-1</sup>	McKeague and Day (1966)
Manganese oxide	Mn <sub>o</sub>	g kg <sup>-1</sup>	McKeague and Day (1966)
Available nitrogen	Av. N	kg ha <sup>-1</sup>	Alkaline-KMnO <sub>4</sub> method (Subbiah and Asija, 1956)
Available phosphorus	Av. P	kg ha <sup>-1</sup>	Sodium bicarbonate extraction method followed by colorimetric determination (Olsen et al., 1954)
Available potassium	Av. K	kg ha <sup>-1</sup>	Ammonium acetate extraction method followed by analysis in flame photometer (Jackson, 1973)
Available iron	Av. Fe	mg kg <sup>-1</sup>	DTPA extraction method followed by analysis in Atomic absorption spectrophotometer (Lindsay and Norvell, 1978)
Available manganese	Av. Mn	mg kg <sup>-1</sup>	DTPA extraction method followed by analysis in Atomic absorption spectrophotometer (Lindsay and Norvell, 1978)
Available copper	Av. Cu	mg kg <sup>-1</sup>	DTPA extraction method followed by analysis in Atomic absorption spectrophotometer (Lindsay and Norvell, 1978)
Available zinc	Av. Zn	mg kg <sup>-1</sup>	DTPA extraction method followed by analysis in Atomic absorption spectrophotometer (Lindsay and Norvell, 1978)

TABLE 2 Extractants used for estimation of bioavailable S in soils under different land uses.

Extractants used	Extractants composition	Soil: extractant ratio	Shaking time	References
CaCl <sub>2</sub>	0.15% CaCl <sub>2</sub> (pH 7.0)	1:5	30 min	Williams and Steinbergs (1959)
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	(500 ppmP) Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> · 2H <sub>2</sub> O (pH 7.0)	1:5	30 min	Fox et al. (1964)
AB-DTPA	1.0 M NH <sub>4</sub> HCO <sub>3</sub> + 0.5 M DTPA (pH 7.6)	1:2	15 min	Soltanpour and Schwab (1977)
NaHCO <sub>3</sub>	0.5 M NaHCO <sub>3</sub> (pH 8.5)	1:4	40 min	Kilmer and Neary (1960)
Mehlich-3	0.2 MHOAc + 0.25 M NH <sub>4</sub> NO <sub>3</sub> + 0.015 M NH <sub>4</sub> F + 0.013 M HNO <sub>3</sub> + 0.001 M EDTA (pH 2.5 ± 0.1)	1:10	5 min	Mehlich (1984)
NH <sub>4</sub> Ac-HOAc	0.5 mol L <sup>-1</sup> NH <sub>4</sub> Ac + 0.25 mol L <sup>-1</sup> HOAc (pH 4.5)	1:2.5	30 min	Bardsley and Lancaster (1960)

TABLE 3 Chemical properties of soils under different land use practices.

Land use	pH	SOC	CaCO <sub>3</sub>	Fe-oxide	Al-oxide	Mn-oxide
R-M-J	7.54 <sup>a</sup>	7.23 <sup>b</sup>	2.86 <sup>a</sup>	0.800 <sup>c</sup>	0.503 <sup>b</sup>	0.342 <sup>d</sup>
R-L-J	7.45 <sup>ab</sup>	7.36 <sup>a</sup>	2.61 <sup>b</sup>	0.842 <sup>d</sup>	0.518 <sup>b</sup>	0.365 <sup>c</sup>
R-L-S	7.32 <sup>bc</sup>	7.11 <sup>c</sup>	2.56 <sup>b</sup>	0.945 <sup>c</sup>	0.522 <sup>b</sup>	0.388 <sup>b</sup>
R-V-J	7.26 <sup>c</sup>	6.68 <sup>d</sup>	2.22 <sup>c</sup>	0.987 <sup>b</sup>	0.546 <sup>a</sup>	0.403 <sup>a</sup>
R-P-G	7.18 <sup>c</sup>	6.78 <sup>d</sup>	2.15 <sup>c</sup>	1.063 <sup>a</sup>	0.562 <sup>a</sup>	0.408 <sup>a</sup>

Different letters (a–e) in each column indicate significant differences between the treatments according to Duncan's multiple range test ( $p < 0.05$ ).

R-M-J: Rice-Mustard-Jute; R-L-S: Rice-Lentil-Jute; R-L-S: Rice-Lentil-Sesame; R-V-J: Rice-Vegetable-Jute; R-P-G: Rice-Potato-Green gram.

R-M-J, and R-L-J systems showed an increase in SOC content of 6.4, 8.2, and 10.2%, respectively. Calcium carbonate content showed an increasing trend with rising soil pH, while oxides of Fe, Al, and Mn decreased with increasing pH (Table 3; Supplementary Figure S1). The iron oxide content (g kg<sup>-1</sup>) ranged from 0.800 (R-M-J) to 1.063 (R-P-G). Similarly, Al<sub>o</sub> and Mn<sub>o</sub> contents (g kg<sup>-1</sup>) ranged from 0.503 to 0.562 and

from 0.342 to 0.408, respectively. Soils under all land uses had medium levels of available nitrogen content except for the R-M-J system, while they had high levels of available phosphorus and potassium. The lowest available N was found in the R-M-J system, while the highest available N was reported in the R-P-G system. However, the availability of cationic micronutrients (iron, manganese, copper, and zinc) was

TABLE 4 Available nutrient status of soils under different land use practices.

Land use	Av. N	Av. P	Av. K	Av. Fe	Av. Mn	Av. Cu	Av. Zn
R-M-J	270.3 <sup>c</sup>	42.8 <sup>c</sup>	220.6 <sup>d</sup>	8.68 <sup>b</sup>	6.44 <sup>b</sup>	6.02 <sup>a</sup>	0.98 <sup>b</sup>
R-L-J	285.2 <sup>d</sup>	50.3 <sup>d</sup>	228.5 <sup>c</sup>	9.02 <sup>a</sup>	6.59 <sup>a</sup>	6.12 <sup>a</sup>	1.05 <sup>a</sup>
R-L-S	304.5 <sup>c</sup>	58.2 <sup>c</sup>	240.3 <sup>b</sup>	8.34 <sup>c</sup>	6.23 <sup>c</sup>	5.93 <sup>ab</sup>	0.87 <sup>c</sup>
R-V-J	328.3 <sup>b</sup>	68.7 <sup>b</sup>	253.5 <sup>a</sup>	7.78 <sup>c</sup>	5.87 <sup>c</sup>	5.64 <sup>c</sup>	0.68 <sup>c</sup>
R-P-G	346.2 <sup>a</sup>	74.2 <sup>a</sup>	260.2 <sup>a</sup>	8.04 <sup>d</sup>	6.06 <sup>d</sup>	5.71 <sup>bc</sup>	0.75 <sup>d</sup>

Different letters (a–e) in each column indicate significant differences between the treatments according to Duncan’s multiple range test ( $p < 0.05$ ).  
 R-M-J: Rice-Mustard-Jute; R-L-S: Rice-Lentil-Jute; R-L-S: Rice-Lentil-Sesame; R-V-J: Rice-Vegetable-Jute; R-P-G: Rice-Potato-Green gram.

TABLE 5 Changes in soil S fractions under different land use practices.

Land use	Water	Sorbed	Occluded	Inorganic	Organic	Total
R-M-J	7.1 <sup>c</sup>	1.0 <sup>d</sup>	6.6 <sup>a</sup>	14.7 <sup>d</sup>	216.1 <sup>c</sup>	230.8 <sup>c</sup>
R-L-J	8.3 <sup>d</sup>	1.1 <sup>d</sup>	5.4 <sup>b</sup>	14.8 <sup>d</sup>	230.5 <sup>b</sup>	245.3 <sup>b</sup>
R-L-S	9.4 <sup>c</sup>	1.5 <sup>c</sup>	4.9 <sup>c</sup>	15.8 <sup>c</sup>	209.5 <sup>c</sup>	225.3 <sup>c</sup>
R-V-J	10.2 <sup>b</sup>	1.8 <sup>b</sup>	4.6 <sup>d</sup>	16.6 <sup>b</sup>	233.6 <sup>b</sup>	250.2 <sup>b</sup>
R-P-G	11.2 <sup>a</sup>	2.0 <sup>a</sup>	4.1 <sup>c</sup>	17.3 <sup>a</sup>	249.1 <sup>a</sup>	266.4 <sup>a</sup>

Different letters (a–e) in each column indicate significant differences between the treatments according to Duncan’s multiple range test ( $p < 0.05$ ).  
 R-M-J: Rice-Mustard-Jute; R-L-S: Rice-Lentil-Jute; R-L-S: Rice-Lentil-Sesame; R-V-J: Rice-Vegetable-Jute; R-P-G: Rice-Potato-Green gram.

TABLE 6 Extractable S in soils under different land use practices.

Land use	CaCl <sub>2</sub>	Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	Mehlich-3	NH <sub>4</sub> Ac-HOAc	NaHCO <sub>3</sub>	AB-DTPA
R-M-J	8.4 <sup>c</sup>	9.8 <sup>c</sup>	15.4 <sup>d</sup>	10.9 <sup>d</sup>	23.4 <sup>c</sup>	12.8 <sup>d</sup>
R-L-J	9.3 <sup>d</sup>	10.5 <sup>d</sup>	16.8 <sup>c</sup>	11.7 <sup>c</sup>	26.5 <sup>d</sup>	13.2 <sup>d</sup>
R-L-S	10.2 <sup>c</sup>	11.6 <sup>c</sup>	17.2 <sup>c</sup>	10.6 <sup>c</sup>	28.4 <sup>c</sup>	14.5 <sup>c</sup>
R-V-J	11.1 <sup>b</sup>	12.4 <sup>b</sup>	18.9 <sup>b</sup>	12.9 <sup>b</sup>	32.4 <sup>b</sup>	16.8 <sup>b</sup>
R-P-G	12.6 <sup>a</sup>	13.8 <sup>a</sup>	20.2 <sup>a</sup>	14.2 <sup>a</sup>	36.5 <sup>a</sup>	18.1 <sup>a</sup>

Different letters (a–e) in each column indicate significant differences between the treatments according to Duncan’s multiple range test ( $p < 0.05$ ).  
 R-M-J: Rice-Mustard-Jute; R-L-S: Rice-Lentil-Jute; R-L-S: Rice-Lentil-Sesame; R-V-J: Rice-Vegetable-Jute; R-P-G: Rice-Potato-Green gram.

significantly higher in the R-L-J system compared to other land uses (Table 4).

### 3.2. Total and organic S fractions in soils under different land use practices

The data presented in Table 5 show that soil organic and total S contents varied significantly among the land uses. The highest organic and total S concentrations (249.1 and 266.4 mg kg<sup>-1</sup>, respectively) were observed in the R-P-G system, while the lowest (209.5 and 225.3 mg kg<sup>-1</sup>, respectively) were observed in the R-L-S system. Compared to the R-L-S system, the R-V-J and R-P-G systems showed an increase in total S content to the magnitude of 11.1 and 18.2%, respectively. The organic S followed a similar pattern of occurrence as the total S content in all land uses.

### 3.3. Inorganic soil S fractions under different land use practices

A significantly higher inorganic S fraction was observed in R-P-G land use, while the lowest amount was found in the R-M-J system. The

water-soluble, sorbed, and occluded S fractions together constituted the inorganic fraction. Across all land uses, the inorganic S fraction constituted 6.5% of total soil S. The water-soluble S fraction was lowest in the R-M-J system (7.1 mg kg<sup>-1</sup>) and highest in the R-P-G system (11.2 mg kg<sup>-1</sup>). Similarly, the sorbed S fraction ranged from 1.0 to 2.0, being lowest in the R-M-J system and highest in the R-P-G system. On the other hand, R-P-G land use witnessed the lowest amount of occluded S fraction, while R-M-J land use showed the highest value. Across the land uses, inorganic S fractions were found in the order of water-soluble > occluded > sorbed S.

### 3.4. Extractable S in soil under different land use practices

Land use change significantly influenced the extractability of all six extractants (Table 6). The CaCl<sub>2</sub> extractable S ranged from 8.4 mg kg<sup>-1</sup> in the R-M-J system to 12.6 mg kg<sup>-1</sup> in the R-P-G system. Irrespective of the extractants used, the lowest amount of extractable S was observed in the R-M-J system, while the highest amount was observed in the R-P-G system. Interestingly, the mehlich-3 and NaHCO<sub>3</sub> extractants obtained on average 1.7 and 2.8 times more S than those extracted with CaCl<sub>2</sub> solutions, respectively. Across all land uses, the order of extractability was: NaHCO<sub>3</sub> > mehlich-3 > AB-DTPA > NH<sub>4</sub>Ac-HOAc > Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> > CaCl<sub>2</sub>.



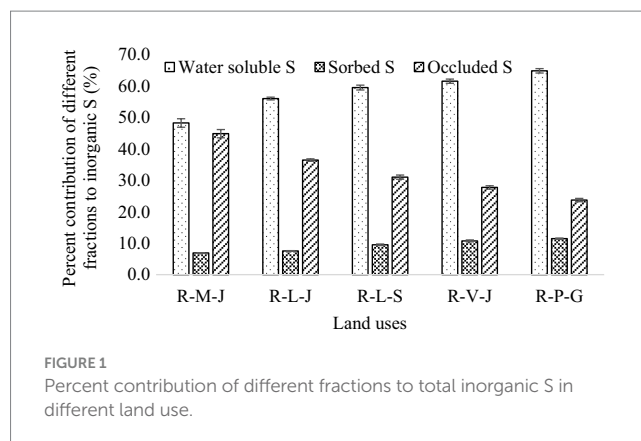
## 4. Discussion

### 4.1. Influence of land use changes on soil properties

Changes in land use significantly influence soil properties and nutrient availability. The low pH value in the R-P-G system could be due to the higher content of amorphous  $Fe_0$  and  $Al_0$ . The presence of  $Fe_0$  and  $Al_0$  generally lowers the soil pH due to their oxidation, which releases  $H^+$  ions into the soil system (Yang et al., 1997). Furthermore, the high quantity of chemical fertilizers applied in the R-P-G system compared to other land uses might be the reason for low soil pH. Higher SOC content in the R-M-J and R-L-J systems may be caused by the slow decomposition of organic matter due to the prevailing anaerobic environment during rice and jute cultivation. Manlay et al. (2002) also reported that anaerobic conditions could slow down the organic matter decomposition rate during rice cultivation. A high calcium carbonate content in the R-M-J system could be associated with the soil pH, which was supported by their positive correlations (S1). Similarly, the decrease of  $Fe_0$ ,  $Al_0$ , and  $Mn_0$  in all land uses with the increase in soil pH can be attributed to their strong negative relationships (S1). The highest amount of available nutrient content, especially N, P, and K, may be due to the high amount of fertilizer applied in the R-P-G system compared to other land uses. The increased availability of cationic micronutrients in R-L-J land use compared to the others may correspond to the increased organic carbon content, which was observed from their significant positive correlations (S1). A reduced soil environment in rice and jute cultivations could have altered the availability of cationic micronutrients.

### 4.2. Influence of land use on soil S fractions

The highest organic and total S were found in the R-P-G system, which might be due to the lower crop removal compared to oilseed-based land use. The sulfur requirement is high for oilseed crops compared to cereals and other crops, and the inclusion of crops with high S requirement in the land use could deplete the soil S. Meena et al. (2022) reported that the inclusion of pulses in the rice-wheat land use system could influence the availability of S. Shifting the virgin soils to cultivable lands with different agricultural management could change the equilibrium status of soil organic S and affect its distribution in surface layers (Solomon et al., 2003). Organic S accounted for an average of 93.5% of the total S, representing the dominant fraction in the soils. A similar magnitude of the organic S fraction under different land uses has been reported by other researchers (Padhan et al., 2016; Meena et al., 2022). The inorganic S fraction represented the pool of S available for plant uptake. The inorganic S present in soils is readily available (extracted with distilled water or any weak salt solution), adsorbed S (extracted with phosphate-containing solution), and sulfate co-precipitated with  $CaCO_3$  or  $BaCO_3$  (solubilized and extracted with HCl solution). Therefore, we employed the sequential fractionation procedure as described by Morche (2008), which includes demineralized water, 0.032 M  $NaH_2PO_4$ , and 1 M HCl solution. Among the three fractions, water-soluble S accounted for an average of 58% of the total inorganic S in the soils (Figure 1). This signifies that a higher proportion of inorganic S is readily available for all land uses. On average, the



magnitude of water-soluble S was 1.9 times that of occluded and 6.4 times that of sorbed S. Increased amounts of water-soluble S in the R-P-G system compared to other land uses might be due to lower crop removal.

The sorbed S varied among land uses. Among all land uses, the R-P-G system had the highest amount of sorbed S, which suggests that this particular land use had a high amount of inorganic S bound to the colloidal matrix. The presence of high amounts of  $Fe_0$  and  $Al_0$  in R-P-G could offer a specific adsorption site for  $SO_4^{2-}$  (Singh, 1984; Das et al., 2020). This was confirmed by the positive relationships between the sorbed S and the oxides of Fe and Al (Supplementary Figure S2). A highly sorbed S could also suggest a high amount of bound  $SO_4^{2-}$  in the soil system, as water extracts the readily available  $SO_4^{2-}$  and leaves behind the adsorbed fraction. The low amount of sorbed S in the R-M-J system could be due to the organic anions released from the decomposition of soil organic matter, which could have displaced the adsorbed  $SO_4^{2-}$  in the colloidal matrix of  $Fe_0$  and  $Al_0$  into the soil solution. Previous research has also shown that  $SO_4^{2-}$  and low-molecular-weight organic anions compete for the same adsorption site (Evans, 1986) and that removal of organic matter can increase the adsorption capacity of soils (Das et al., 2002). Moreover, the organic anions make chelation with oxides of Fe and Al (which preferentially adsorb  $SO_4^{2-}$ ) resulting in reduced sorption of  $SO_4^{2-}$ . The magnitude of occluded S was higher than that of sorbed S for all land uses. A significant positive relationship between occluded S and  $CaCO_3$  content of soils confirmed that the presence of a high amount of carbonate could adsorb  $SO_4^{2-}$ , rendering it unavailable for plant nutrition (Supplementary Figure S3). It was also observed that, on average, S co-precipitated with carbonate accounted for 32.8% of the total inorganic S. Such co-precipitated S could be released and made available to the plant, which is primarily governed by rhizosphere pH. In addition, nitrogenous fertilizers applied to the soil may have a favorable effect on the solubilization of  $CaCO_3$ -occluded S, and this solubilized fraction may be partially transformed into a soluble organic form (Hu et al., 2005).

### 4.3. Extractable S

The  $CaCl_2$  solution extracted the lowest amount compared to other extractants across land uses, accounting for 4.2% of total soil S. Suri et al. (2021) reported similar observations in soils from different land use practices. On average,  $Ca(H_2PO_4)_2$ , Mehlich-3,  $NH_4Ac-HOAc$ , AB-DTPA, and  $NaHCO_3$  extracted 4.8, 7.3, 4.9, 12.0, and 6.2% of total soil S,

respectively. The highest amount of  $\text{NaHCO}_3$ -extractable S across land uses compared to other extractants could be due to the extraction of a portion of the organically-bound S (mostly ester sulfate) due to its high pH besides the readily available S (Kilmer and Neary, 1960). This was confirmed by the positive relationship between  $\text{NaHCO}_3$ -extractable S and the organic S content of the soil (computed across different land uses). In addition, the presence of  $\text{HCO}_3^-$  in AB-DTPA and 0.5 M  $\text{NaHCO}_3$  displaced the sorbed S from the colloidal complex (Tabatabai, 1996), while also solubilizing partly the labile insoluble minerals in the soil (Soltanpour, 1985). Acidic extractants, particularly mehlich-3, showed higher extractability compared to neutral solvents  $\{\text{CaCl}_2$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2\}$  and slightly alkaline solvents (AB-DTPA) across land uses. Low pH can solubilize the oxides of Fe and Al, thereby releasing the bound sulfate into the soil solution. Moreover, the acetate and nitrate anions in Mehlich-3 favored the extraction of S from the soil (Seth et al., 2018). The lowest amount of S extracted by a 0.15%  $\text{CaCl}_2$  solution in soils of all land uses could be due to its efficiency in extracting only the readily available or water-soluble S. Hu et al. (2005) reported that  $\text{Cl}^-$ -based extractants can mobilize only the water-soluble  $\text{SO}_4^{2-}$  and also have lower extraction power due to their tendency to form slowly soluble  $\text{CaSO}_4$  (Matula, 1999). Phosphate ions in  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  solution could displace the sorbed S, thereby showing increased extractability compared to  $\text{CaCl}_2$  solution across land uses.

Among the five land uses, the R-P-G system always had the highest amount of extractable S compared to other land uses (Table 6). Moreover, the influence of the R-P-G system over the R-M-J system was more pronounced when S was extracted with 0.5 M  $\text{NaHCO}_3$  (56.0), followed by  $\text{CaCl}_2$  (50.0), AB-DTPA (41.4),  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  (40.8), Mehlich-3 (31.2), and  $\text{NH}_4\text{Ac-HOAc}$  (30.3; Figure 2). High organic S content in the R-P-G system (Table 5), which increases the availability of S in soils (by mineralizing the labile S), was captured by the extractants used in the present study.

#### 4.4. Extractable S and S fractions

By establishing the relationship between extractable S (neutral extractants) and S fractions across the land uses, it was observed that  $\text{CaCl}_2$

extractable S was positively correlated with water-soluble S ( $r=0.968^{**}$ ), sorbed S ( $r=0.950^{**}$ ), and organic S ( $0.696^{**}$ ), while negatively correlated with occluded S ( $r=-0.927^{**}$ ; Table 7; Figures 3A–D). A similar relationship was found between  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ -extractable S and S fractions. Both extractants are highly correlated with the water-soluble S fraction, which is considered the readily available S in the majority of agricultural soils. Acidic extractants mehlich-3 and  $\text{NH}_4\text{Ac-HOAc}$  maintained significant positive correlations with water-soluble, sorbed, organic, and total S fractions while they were negatively correlated with occluded S (Table 7; Figures 4A–D). Interestingly, the  $\text{NH}_4\text{Ac-HOAc}$ -extractable S showed a high degree of correlation with organic ( $r=0.914^{**}$ ) and total S ( $0.928^{**}$ ) compared to mehlich-3 indicating that it could extract a higher proportion from the organic S fraction. A similar relationship existed between the S extracted by weakly alkaline to alkaline extractants ( $\text{NaHCO}_3$  and AB-DTPA) and the S fractions (Table 7; Figures 5A–D). Notably, the inorganic fraction closely corresponds to the amount of S extracted by Mehlich-3 and AB-DTPA. Occluded S was the only fraction of S that maintained a negative correlation with S obtained by the extractants across all land uses. From the relationships established between extractable S and S fractions, it can be inferred that all extractants primarily extracted the water-soluble, sorbed, and partially organic S fractions, with little extractability from the occluded S fraction.

#### 4.5. Comparison of extractants

Acidic extractants, particularly mehlich-3, were able to extract 1.7 and 1.5 times more S than  $\text{CaCl}_2$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , respectively. Notably, the  $\text{NH}_4\text{Ac-HOAc}$  extracted higher amounts of S than  $\text{CaCl}_2$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ . Across all land uses, the order of extractability was:  $\text{NaHCO}_3 > \text{mehlich-3} > \text{AB-DTPA} > \text{NH}_4\text{Ac-HoAc} > \text{Ca}(\text{H}_2\text{PO}_4)_2 > \text{CaCl}_2$ . A Pearson correlation was constructed among the extractable S to establish their relationships. The  $\text{CaCl}_2$ -extractable S showed a strong positive correlation with  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ;  $r=0.988^{**}$ ), mehlich-3 ( $r=0.967^{**}$ ),  $\text{NH}_4\text{Ac-HoAc}$  ( $r=0.846^{**}$ ),  $\text{NaHCO}_3$  ( $r=0.980^{**}$ ), and AB-DTPA ( $r=0.956^{**}$ ; Table 8). Such dynamic relationships among the extractable S suggested that S could be obtained from similar pools, which was further supported

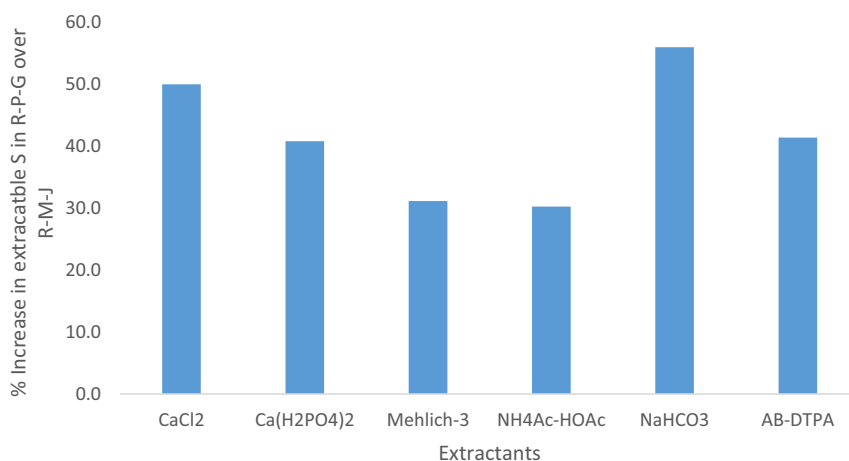
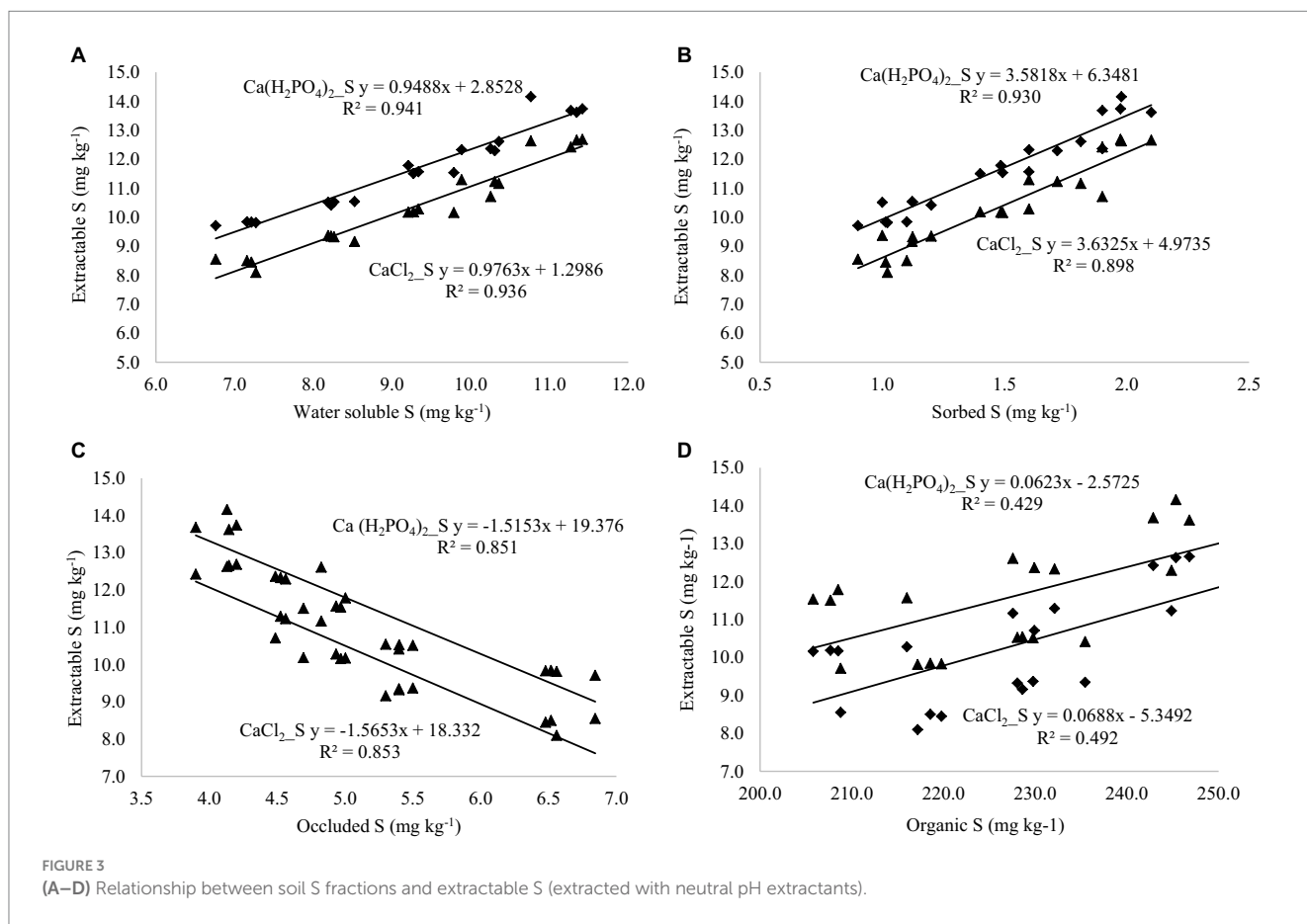


FIGURE 2  
Relative increase in extractable S content in R-P-G over R-M-J land use.

TABLE 7 Pearson correlation between S fractions and extractable S.

	CaCl <sub>2</sub>	Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	Mehlich-3	NH <sub>4</sub> Ac-HOAc	NaHCO <sub>3</sub>	AB-DTPA
Water-soluble	0.968**	0.970**	0.948**	0.785**	0.968**	0.940**
Sorbed	0.950**	0.967**	0.918**	0.778**	0.943**	0.937**
Occluded	-0.927**	-0.926**	-0.923**	-0.715**	-0.921**	-0.862**
Inorganic	0.949**	0.955**	0.915**	0.806**	0.949**	0.959**
Organic	0.696**	0.651**	0.736**	0.914**	0.724**	0.665**
Total	0.729**	0.686**	0.765**	0.928**	0.756**	0.700**

\* and \*\* significant at 0.05 and 0.01 level, respectively.



by their relationship with different S fractions. Comparing the procedure involved during the extraction process, it can be noted that the shaking time for mehlich-3 and AB-DTPA was restricted to 5 min and 15 min respectively, and since they are primarily used as multi-nutrient extractants, they can be employed in routine laboratory analysis. However, the unavailability of NH<sub>4</sub>NO<sub>3</sub> (banned in many countries), which is one of the primary chemicals in the mehlich-3 composition, calls into question the use of mehlich-3 in routine laboratory analysis in the future. The presence of Ca in Ca(H<sub>2</sub>PO<sub>4</sub>) and CaCl<sub>2</sub> extractants flocculates the soil particles, and a clear extract can be obtained, which is highly essential for the turbidimetric determination of S. On the other hand, in the case of NaHCO<sub>3</sub>, the high pH of the extractant could partially solubilize the organic matrix of the soil, resulting in a

turbid solution that needs to be clarified prior to S determination. To test the reproducibility of extractable S by all six extractants, the relative standard deviation (RSD) value was calculated. A high RSD value indicates poor reproducibility of the data, while a low RSD value shows greater reproducibility. Across all land uses, mehlich-3 had the lowest RSD value, while NaHCO<sub>3</sub> showed a high RSD value signifying its poor reproducibility.

### 4.6. Influence of soil properties on the extractability of extractants

The results presented in Table 9 indicate that the extractability of different extractants is influenced by soil properties. In the



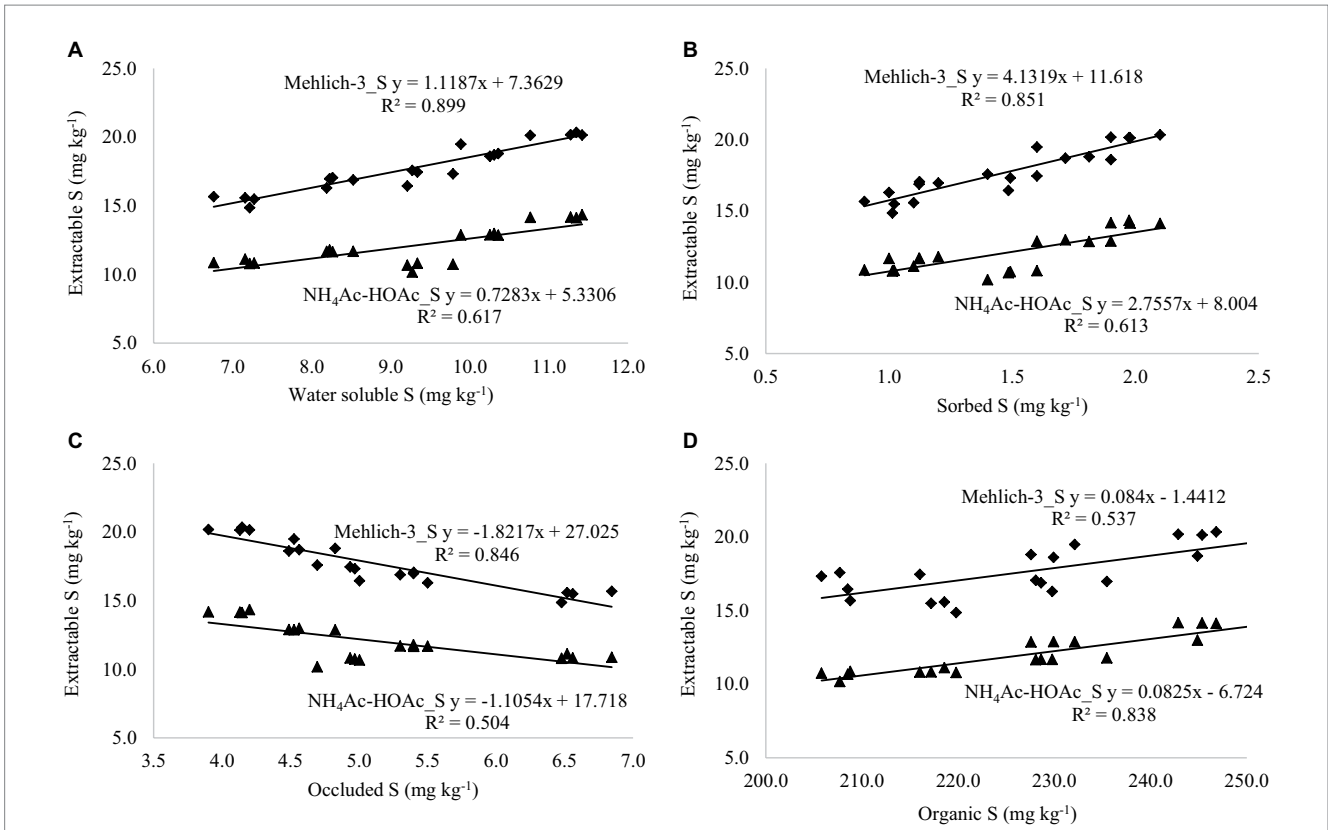


FIGURE 4 (A–D) Relationship between soil S fractions and extractable S (extracted with acidic pH extractants).

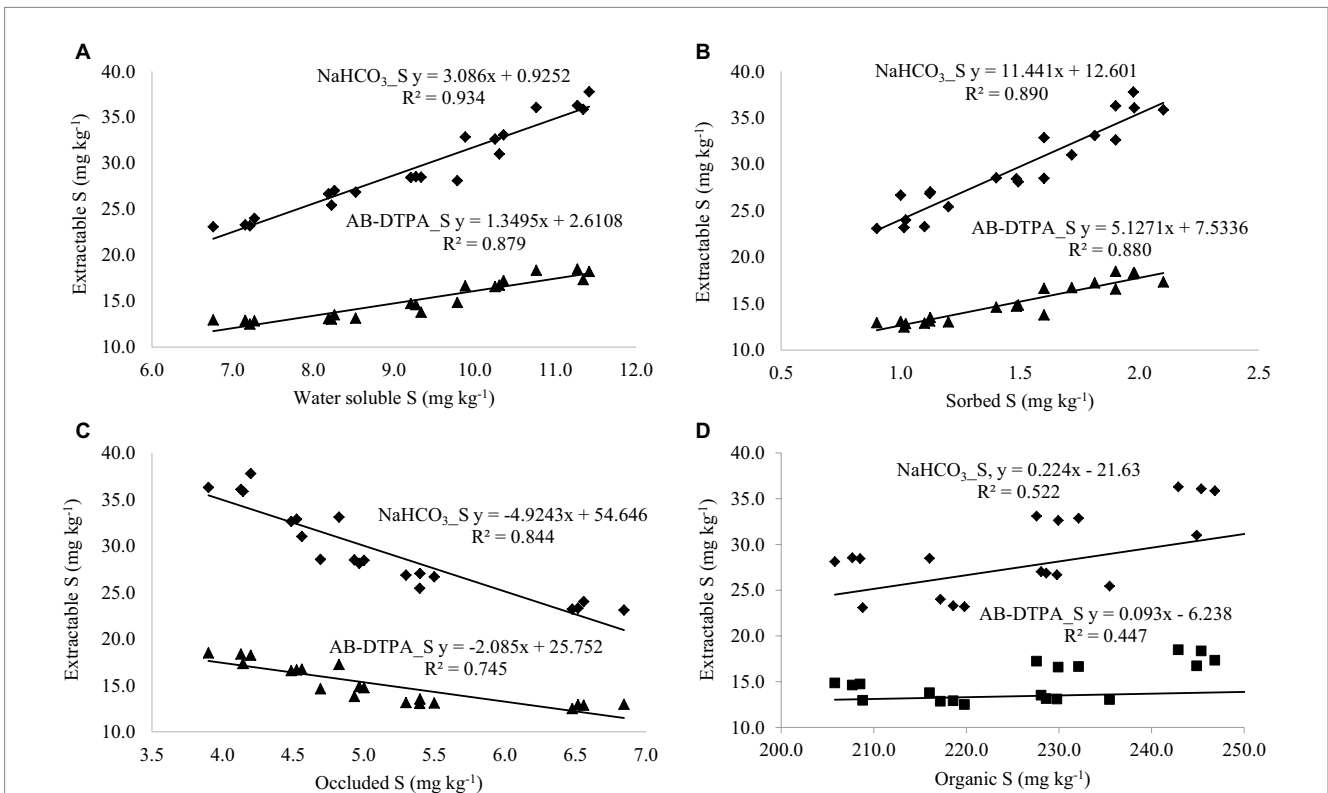


FIGURE 5 (A–D) Relationship between soil S fractions and extractable S (extracted with slightly alkaline to alkaline pH extractants).

TABLE 8 Pearson correlation coefficients among the extractable S.

	CaCl <sub>2</sub>	Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	Mehlich-3	NH <sub>4</sub> Ac-HOAc	NaHCO <sub>3</sub>	AB-DTPA
CaCl <sub>2</sub>	1					
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	0.988**	1				
Mehlich-3	0.967**	0.950**	1			
NH <sub>4</sub> Ac-HOAc	0.846**	0.827**	0.880**	1		
NaHCO <sub>3</sub>	0.980**	0.981**	0.972**	0.877**	1	
AB-DTPA	0.956**	0.968**	0.943**	0.863**	0.967**	1

\* and \*\* significant at 0.05 and 0.01 level, respectively.

TABLE 9 Step-wise multiple regression between extractable S and soil properties.

Extractable S	Regression equation	R <sup>2</sup>
CaCl <sub>2</sub>	-2.978+ 14.348 Fe-oxide***	0.934
	6.991+ 9.172 Fe-oxide***-2.085 CaCO <sub>3</sub> **	0.958
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	-1.334+ 13.955 Fe-oxide***	0.918
	-4.388+ 9.790 Fe-oxide*** + 18.148 Mn-oxide*	0.944
	-8.506+ 7.622 Fe-oxide*** + 16.322 Mn-oxide* + 12.872 Al-oxide*	0.958
Mehlich-3	32.613-6.013 CaCO <sub>3</sub> ***	0.887
	21.081-3.812 CaCO <sub>3</sub> ** + 6.553 Fe-oxide*	0.911
NH <sub>4</sub> Ac- HOAc	22.843-4.347 CaCO <sub>3</sub> ***	0.743
	46.188-7.806 CaCO <sub>3</sub> ***-38.747 Mn-oxide**	0.828
NaHCO <sub>3</sub>	-12.217+ 44.913 Fe-oxide***	0.903
	-37.867 + 29.916 Fe-oxide*** + 76.620 Al-oxide***	0.949
AB-DTPA	33.130-7.283 CaCO <sub>3</sub> ***	0.867
	16.010-4.016 CaCO <sub>3</sub> * + 9.728 Fe-oxide*	0.902

\*, \*\* and \*\*\* significant at 0.05, 0.01 and 0.001 probability levels respectively.

case of CaCl<sub>2</sub>-extractable S, Fe oxide alone caused a variation of 93.4%, which was improved to 95.8% with the inclusion of CaCO<sub>3</sub>. Similarly, the variation in Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>-extractable S caused by Fe oxide alone was 91.8%, and it was improved to 94.4 and 95.8% with the inclusion of Fe oxide and Mn oxide and oxides of Fe, Mn, and Al, respectively. Both CaCO<sub>3</sub> and Mn oxide could explain 82.8% of the variability in NH<sub>4</sub>Ac-HOAc-extractable S, and the exclusion of Mn oxide from the regression model decreased the predictability by 8.5%. The amount of S extracted by NaHCO<sub>3</sub> was found to be influenced by the oxides of Fe and Al. In general, the oxides of Fe, Al, Mn, and CaCO<sub>3</sub> are the main soil constituents that are highly responsible for predicting the variability of extractable S in soil under different land uses.

## 5. Conclusion

Land use practices significantly influence the distribution of S into different fractions and its bioavailability. Land use practices with high oxides of Fe and Al and CaCO<sub>3</sub> content showed a high amount of sorbed and occluded S, respectively. Extractants used to capture the bioavailable S removed S from water-soluble, sorbed,

and organic fractions, with little extractability from the occluded fraction. The dynamic relationships among the extractants and between extractable S and S fractions suggest that all extractants are efficient enough to extract the bioavailable S. However, the use of multi-nutrient extractants such as Mehlich-3 can save time and cost and, thus, can be recommended for routine laboratory analysis. Further research must focus on the replacement of NH<sub>4</sub>NO<sub>3</sub> in Mehlich-3 with a suitable chemical compound.

## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

## Author contributions

DP: conceptualization, and manuscript preparation and analysis. PR: statistical analysis and manuscript editing. AS: writing review and editing. All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2023.1233223/full#supplementary-material>

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