Fractionation of sulphur with different physico-chemical properties of cultivated soils from north western Himalayas

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Abstract
Deficiencies of sulphur (S) in agricultural crops are becoming more common but comparatively little is known regarding its kinetics and relation with physico-chemical properties of cultivated soils of North Western Himalayas. Eighty surface soil samples (0-0.15 m depth) from different districts of Himachal Pradesh (India) lying in North West Himalayas were studied in present investigation. The results emanated from the present study revealed that soil pH, organic carbon (OC) and cation exchange capacity (CEC) in soil samples ranged from 5.45 to 7.18, 7.8 to 15.2 g kg\(^{-1}\) and 6.1 to 17.5 cmol (p+) kg\(^{-1}\). The textural classes varied from sandy loam to clay. Among different fractions of S; organic S was recorded as the dominant fraction in constituting total S followed by heat soluble S than available S and water soluble S. All S fractions were positively correlated with clay, OC and CEC but negatively with sand, silt and pH.

Keywords: Sulphur fractions, cultivated soils, physical and chemical properties, correlation, north western Himalayas

Introduction
Sulphur (S) is gaining considerable importance in quality crop production and recognized as fourth most important plant nutrient after nitrogen, phosphorus and potassium (Parakhia et al. 2016) \(^{18}\). S is essential for the synthesis of the amino acids like cystine, cysteine and methionine, a component of vitamin A and activates certain enzyme systems in plants. Over the last decade, S deficiency was recognized as a constraint to crop production all over the world. Removal of S by crops in India is about 1.26 mt whereas its replenishment through fertilizers is only about 0.76 mt (Tiwari and Gupta 2006) \(^{29}\). Further, the recovery of added S through external sources is also very low, being only 8 to 10% (Hegde and Murthy 2005) \(^{7}\). The demand of S by plants is not constant with time because it is regulated internally in response to the environmental conditions and stage of plant development. Sulphur pools in the soil are extremely dynamic. S occurs in the elemental form, as well as sulphides, sulphates and in organic combinations with carbon and nitrogen. In Indian soils, it ranges from 19 to 4000 mg kg\(^{-1}\) (Das 2015) \(^{3}\). Plant S nutrition depends primarily on the uptake of inorganic sulphate. The movement of sulphate in solutions through the strata of an ecosystem affects the dynamics of other elements.

Organic S in soil is a diverse mixture of soil organisms and partly decomposed plants, animals and microbial residues (Wang et al. 2006) \(^{32}\). Organic S is the main sulphur binding form in soils (Scherer 2009) \(^{21}\) and contributes up to 95% of total soil S in cultivated soils; yet little is known about the chemical characteristics of organic S compounds. The major transformations of S in the upland agriculture system are mineralization, immobilization and oxidation which govern its gains and losses in the soil-plant system through leaching, gas evolution and adsorption in various agro-climatic conditions. Consequently, a marked accumulation of residual S occurs, particularly in the soil that regularly receives liberal rates of applied S to each crop in a cropping system over a longer period of time.

The availability of sulphur in soil is not only influenced by management practices but also depends upon various forms of S present; as these different forms of S exist in dynamic equilibrium in soil.
S supplying capacity is also dependent on the status and interrelationship with some important soil characteristics which affect its release and dynamics in soil (Xiao et al. 2015) [34]. S requirement of plants has become increasingly important in India as well as in world agriculture. It is important to have site specific assessment of different S fractions, their distribution and relationships with soil properties for assessing degree of deficiency and also to suggest remedial measures. The present investigation therefore, aimed to assess the status of different forms of sulphur in relation to different physico-chemical properties of cultivated soils from Himachal Pradesh.

Materials and Methods
Himachal Pradesh (India) lying in North West Himalayas has vastly dissected mountain ranges interspersed with deep gorges and valleys. Altitude of the state ranges from 350 m to 6975 m above mean sea level. The total geographical area of Himachal Pradesh is 55,673 sq km, whereas, net cultivated area of only 5,820 sq km (10.5%). This state has been divided into four distinct agro-climatic zones viz., Sub-mountain low hills subtropical zone, Mid-hills sub humid subtropical zone, Mid-hills wet temperate zone and High-hills temperate dry zone. It is located between latitudes from 30°22′40″N to 33°12′40″ N and longitudes from 75°45′55″E to 79°04′20″ E. State has different kinds of soil due to variations in climate, parent material, vegetation, topography etc. and different textured soils have different effect on S behavior. Owing to these variations, soil samples from almost all the agro climatic situations across the state had used for conductance of present study. One hundred ten soil samples (0.0-0.15 m depth) were collected randomly across different districts of the state. Collected samples were air dried and lightly crushed in wooden pestle and mortar to break clods and then subsequently passed through a 2 mm sieve. The processed soil samples were analyzed for particle size distribution, pH, organic carbon (OC) and cation exchange capacity (CEC). Particle size distribution was done by the standard Bouyoucos hydrometer method (Day 1965) [4]. Soil pH was estimated by glass electrode with calomel as standard (Jackson 1973) [8]. OC was determined by wet digestion method of Walkley and Black (1934) [31]. The CEC was worked out by leaching the soil with 1N NH4OAC and subsequently displacing the adsorbed NH4 following the methods of Schollenberger and Simon (1945) [22]. Out of these collected samples, twenty soil samples (0.0-0.15 m depth) varying in pH, OC, CEC and clay content, in each characteristic were separated out for S fractionation purpose and further analysis (Fig 1).

Sulphur Fractions
Different forms of sulphur were determined in the soil samples following standard procedures. The sulphate sulphur in soil extract was observed colorimetrically by developing BaSO4 turbidity in the presence of sodium acetate-acetic acid buffer (Chesnin and Yien 1950) [2]. For heat soluble S; Soil samples were hydrolyzed with the addition of distilled water and then evaporated to dryness on a gently boiling water bath. Thereafter, soil was dried in oven at 102°C for 1 hour before extraction by suitable reagent. The sulphur in the solution was determined turbidimetrically (Williams and Steinbergs 1959) [33]. Water soluble S was estimated turbidimetrically using de-
ionized water as extracting solution (Chesnin and Yien 1950) [2]. Organic S was calculated by subtraction of sulphate sulphur from total S. Total S was estimated turbidimetrically using BaCl₂ from extract obtained after digesting the soil with HNO₃ and HClO₄ di-acid mixture (Johnson and Nishita 1952) [10]. The data generated during the course of the present investigation was subjected to simple and multiple correlation coefficients (Gomez and Gomez 1984) [3] to determine relationships of important soil properties with different forms of sulphur, through the requisite statistical computation.

Results & Discussions
Physical Characteristics

Soil samples varied quite considerably with respect to different soil separates i.e. sand, silt and clay contents (Table 1). A close look on data revealed that sand fraction in these selected soils varied from 15.4 to 76.1 per cent. About 62 per cent samples had more than 50 per cent sand, whereas, 23 per cent samples had more than 60 per cent sand. Silt content ranged between 5.10 to 53.8 per cent. About 68 per cent samples had silt content either equal to or lower than 25 per cent. Similarly, the clay content of samples varied from 4.70 to 45.1 per cent. Around 67 per cent samples recorded less than 25 per cent clay content. The textural classes of the soils were determined on the basis of relative proportion of different soil separates. The texture of the soils under study varied from sandy loam to clay. About 41 per cent samples were sandy loam, whereas, 42 per cent samples were sandy clay loam and clay in texture, 12 per cent were sandy clay and clay in texture and loam, 5 per cent were loam in texture. Low amount of clay fraction in the soils of North-West Himalayan region had also been reported earlier by Kaistha and Gupta (1994) [11]. Such dissimilarity in soil texture and separates could very well be estimated due to the development of these soils under different climatic conditions, vegetation, topography and having varied parent materials. The irregular distribution of sand and silt that indicated discontinuities in the study area was also reported by Kaistha and Gupta (1993) [12], Mahajan et al. (2007) [16], Kumar et al. (2017) [14] and Salve et al. (2018) [20].

Chemical Characteristics

A perusal of data in Table 1 revealed that soil pH in different soil samples ranged from 5.45 to 7.18 with a mean of 6.44 ± 0.37. Around 13 per cent soil samples were acidic in nature (pH<6.0), 80 per cent samples had pH between 6.0 to 7.0 and 7 per cent of soil samples possessed alkaline soil reaction (pH>7.0). There was no specific trend in pH values of areas under study. Similar results have been also reported by Kaistha and Gupta (1993) [12] and Salve et al. (2018) [20]. Data pertaining to carbon indicated that OC content ranged from 7.80 to 15.2 g kg⁻¹. A cursory look on the data revealed that 59 per cent of the soil samples were high in OC and 41 per cent samples were in medium range. Most of the selected soil samples were medium to high in OC which might be attributed due to temperate and subtropical conditions prevailing in the Himalach Pradesh, causing reduction in oxidation/decomposition of accumulated organic matter. These results supported the findings of Minhas et al. (1997) [17], Mahajan et al. (2007) [16] and Kumar et al. (2018) [15]. The values of cation exchange capacity in different soils ranged from 6.10 to 17.5 cmol (p+) kg⁻¹ with an average of 11.6 cmol (p+) kg⁻¹ and standard deviation of ± 2.37 cmol (p+) kg⁻¹. This wide variation in CEC across different location might be due to differences in soil texture and organic matter content observed in the current study. The higher CEC in the North Western Soils having higher organic matter were also reported by Minhas et al. (1997) [17] and Mahajan et al. (2007) [16].

Sulphur Fractions
Available sulphur

A glance at data in Table 2 illustrated that the available sulphur ranged between 7.82 and 22.3 mg kg⁻¹ with an average of 13.8 ± 2.78 mg kg⁻¹. The content of available S was the highest in fine textured soils followed by medium textured and coarse textured soils. The values varied between 8.1 to 17.6 mg kg⁻¹ (with an average of 12.9 ± 2.40 mg kg⁻¹) in coarse textured soils, 7.8 to 21.2 mg kg⁻¹ (with a mean value of 14.2 ± 2.85 mg kg⁻¹) in medium textured soils and 12.2 to 22.3 mg kg⁻¹ (with an average of 15.5 ± 2.85 mg kg⁻¹) in fine textured soils, respectively. Higher concentration of available S in some soils might possibly be due to a greater plant and microbial activities resulted in the subsequent accumulation of organic matter. These results were in lines with the findings of Saharan et al. (2001) [19].

Water soluble sulphur

A close look at the data embodied in Table 2 indicated that the water soluble sulphur ranged from 6.30 to 21.5 mg kg⁻¹ with a mean of 12.1 ± 2.64 mg kg⁻¹. The water soluble S for coarse, medium and fine textured varied as 6.30 to 16.3 mg kg⁻¹ (a mean value of 11.6 ± 2.30 mg kg⁻¹), 7.41 to 17.9 mg kg⁻¹ (mean value of 11.9 ± 2.64 mg kg⁻¹) and 11.4 to 21.5 mg kg⁻¹ (average of 14.4 ± 2.84 mg kg⁻¹), respectively. The reason for having higher amount of water soluble S in fine textured soils could be explained on the basis that this fraction of S is adsorbed specifically onto clay surface and higher the clay content more will be the water soluble S. Water soluble S content in the soils during the present study was found to be minimum compared to all other forms of sulphur. These findings were in conformity with the findings of Sharma et al. (1986) [24] and Sen et al. (2017) [23].

Heat soluble sulphur

Heat soluble sulphur, which gives a measure of sulphate sulphur plus a fraction of organic S (Williams and Steinbergs 1959) [35], is an important indication for evaluating sulphur status of soils. Heat soluble S varied from 9.11 to 23.1 mg kg⁻¹ with a mean value of 16.8 ± 2.94 mg kg⁻¹ in different textured soils (Table 2). The values of heat soluble S for the coarse, medium and fine textured soils varied from 9.11 to 22.5, 11.0 to 23.1 and 14.9 to 22.5 mg kg⁻¹, respectively. The mean values of heat soluble S for the coarse, medium and fine textured soils ranged from 15.9 ± 3.16 mg kg⁻¹, 17.0 ± 2.41 mg kg⁻¹ and 19.1 ± 2.97 mg kg⁻¹, respectively. In present study, the content of heat soluble S on average constituted 8 to 10 per cent of total sulphur for fine, medium and coarse textured soils.

A perusal of the mean values of heat soluble S, it may be conjectured that heat soluble S fraction was more as compared to available and water soluble S indicated the release of sulphur by wet and dry heating of soil during the extraction and also be due to liberation of sulphate sulphur during heat treatment. In some of the soils the heat soluble S was less as compared to available and water soluble S where organic matter was low. Similar findings were reported earlier by Gowrisankar and Shukla (1999) [6].
### Table 1: Physico-chemical characteristics of soil samples

| S. No. | District       | Coarse textured (n = 9) | Fine textured (n = 9) | Total S  |
|--------|----------------|-------------------------|-----------------------|----------|
|        |                | Mean                    | Mean                  | Mean     |
|        |                | SD(±)                   | SD(±)                 | SD(±)    |
| 1      | Shimla         | 56.5                    | 20.8                  | 6.56     | 10.3     | 11.3-14.5 |
|        | SD(±)          | 10.3                    | 3.87                  | 0.23     | 0.82     | 1.14      |
| 2      | Hamirpur       | 53.4                    | 15.8                  | 6.73     | 12.1     | 12.5      |
|        | SD(±)          | 13.2                    | 10.0                  | 0.36     | 1.86     | 1.70      |
| 3      | Kangra         | 44.1                    | 26.1                  | 6.01     | 11.9     | 11.5      |
|        | SD(±)          | 17.0                    | 12.2                  | 0.34     | 1.67     | 2.33      |
| 4      | Mandi          | 41.2                    | 25.7                  | 6.31     | 11.3     | 11.4      |
|        | SD(±)          | 8.68                    | 8.81                  | 0.20     | 1.09     | 1.45      |
| 5      | Una            | 40.3                    | 25.0                  | 6.47     | 11.6     | 12.8      |
|        | SD(±)          | 7.24                    | 9.30                  | 0.43     | 1.94     | 1.40      |
| 6      | Chamba         | 54.0                    | 22.4                  | 6.29     | 11.6     | 11.9      |
|        | SD(±)          | 4.40                    | 5.79                  | 0.15     | 1.05     | 3.40      |
| 7      | Kullu          | 51.7                    | 22.9                  | 6.50     | 10.8     | 11.6      |
|        | SD(±)          | 5.56                    | 9.49                  | 0.26     | 1.25     | 2.20      |
| 8      | Kinnaur        | 53.2                    | 15.2                  | 6.47     | 11.6     | 11.2      |
|        | SD(±)          | 7.24                    | 9.30                  | 0.43     | 1.94     | 1.40      |
| 9      | Solan          | 47.5                    | 27.5                  | 6.57     | 11.3     | 11.2      |
|        | SD(±)          | 15.9                    | 16.9                  | 10.9     | 0.30     | 1.05      | 3.40    |
| 10     | Bilaspur       | 56.4                    | 20.7                  | 6.61     | 10.7     | 11.3      |
|        | SD(±)          | 5.56                    | 9.49                  | 0.42     | 2.12     | 2.20      |
| 11     | Lahaul & Spiti | 53.5                    | 19.4                  | 6.44     | 11.0     | 8.16      |
|        | SD(±)          | 10.2                    | 6.09                  | 0.57     | 1.67     | 1.62      |
| Total Samples | Range | 45.1-63.1 | 10.0-20.0 | 6.45-6.50 | 10.0-14.0 | 12.0-17.0 |

### Table 2: Sulphur fractions (mg kg⁻¹) of soils in mechanical separates

| Category              | Available S | Water Soluble S | Heat Soluble S | Organic S | Total S |
|-----------------------|-------------|-----------------|----------------|-----------|---------|
| Coarse textured (n = 33) | 8.10-17.6   | 6.30-16.3       | 9.11-22.5      | 141-199   | 151-214 |
| Mean                  | 12.9        | 11.6            | 15.9           | 173       | 186     |
| SD(±)                 | 2.40        | 2.30            | 3.16           | 15.5      | 15.8    |
| Medium textured (n = 38) | 7.82-21.2  | 7.41-17.9       | 11.0-23.1      | 156-225   | 169-241 |
| Mean                  | 14.2        | 11.9            | 17.0           | 183       | 198     |
| SD(±)                 | 2.85        | 2.64            | 2.41           | 13.5      | 15.4    |
| Fine textured (n = 9)  | 12.2-22.3   | 11.4-21.5       | 14.9-22.5      | 166-230   | 181-242 |
| Mean                  | 15.5        | 14.4            | 19.1           | 201       | 216     |
| SD(±)                 | 2.85        | 2.84            | 2.97           | 19.9      | 19.9    |
| Overall               | 7.82-22.3   | 6.30-21.5       | 9.11-23.1      | 141-230   | 151-242 |
| Mean                  | 13.8        | 12.1            | 16.8           | 181       | 195     |
| SD(±)                 | 2.78        | 2.64            | 2.94           | 17.0      | 18.3    |

### Table 3: Relationship of sulphur fractions with mechanical separates and soil chemical properties

| Sulphur Fractions | Sand | Silt | Clay | Soil pH | OC | CEC |
|-------------------|------|------|------|---------|----|-----|
| Available S       | -0.24 | -0.01 | 0.38** | -0.30** | 0.12 | 0.18 |
| Water Soluble S   | -0.12 | -0.14 | 0.36** | -0.28*  | 0.20 | 0.27*|
| Heat Soluble S    | -0.22* | -0.04 | 0.31** | -0.19  | 0.19 | 0.23*|
| Organic S         | -0.15 | -0.22* | 0.47** | -0.09  | 0.25*| 0.16 |
| Total S           | -0.18 | -0.21 | 0.49** | -0.13  | 0.28*| 0.19 |

**Significant at 1% level of significance
*Significant at 5% level of significance

### Table 4: Relationship among different sulphur fractions

| Available S | Water Soluble S | Heat Soluble S | Organic S | Total S |
|-------------|-----------------|----------------|-----------|---------|
| Available S | 0.81**          | 1              |           |         |
| Water Soluble S | 0.36**  | 0.33**         | 1         |         |
| Heat Soluble S   | 0.39** | 0.44**         | 0.32*     | 1       |
| Organic S         | 0.52** | 0.53**         | 0.36*     | 0.99**  | 1      |

**Significant at 1% level of significance
*Significant at 5% level of significance
Organic sulphur
The organic sulphur in different textured soils varied from 141 to 230 mg kg⁻¹ with mean value of 181 ± 17.0 (Table 2). The values of organic S varied from 141 to 199 mg kg⁻¹ having mean of 173 ± 15.5 mg kg⁻¹ for coarse textured soil, from 156 to 225 mg kg⁻¹ with an average value of 183 ± 13.5 mg kg⁻¹ for medium textured soil and from 166 to 230 mg kg⁻¹ with a mean value of 201 ± 19.9 mg kg⁻¹ for fine textured soil. Organic S accounted for 91 to 93 percent of total S in all the different textured soils. This form of S follows the same trend to organic matter, and this distributional trend might be due to its intimate relation with organic carbon content. Similar results were also reported by Sharma and Jaggi (2001) [25], Solomon et al. (2001) [26] and Borkotoki and Das (2008) [1].

Total sulphur
A cursory look into the Table 2 indicated that total sulphur content in different textured soils varied from 151 to 242 with a mean of 195 ± 18.3 mg kg⁻¹. The values of total S ranged from 151 to 214 with an average of 186 ± 15.8 mg kg⁻¹ in coarse textured soils, from 169 to 241 with an average of 198 ± 15.4 mg kg⁻¹ in medium textured soils and from 181 to 242 with an average of 216 ± 19.9 mg kg⁻¹ in fine textured soils. Generally total S showed similar trend as that of organic matter content in different textured soils. It may be concluded from the above said discussion that the mean values of all the S fractions was highest in fine textured soils as compared to medium and coarse textured soils because fine textured soils contained more content of organic matter which might have led to more availability of S. These results supported the findings of Singh et al. (2006) [26].

Relationship of sulphur fractions with mechanical separates
The correlation of different sulphur fractions with mechanical separates (sand, silt and clay) has been illustrated in table 3. All fractions of sulphur viz.; available S (r=0.38**), water soluble S (r=0.36**), heat soluble S (r=0.31**) organic S (r=0.47**) and total S (r=0.49**) correlated positively and significantly with clay. Available (r=0.24*) and heat soluble S (r=0.22*) had negative and significant relationship with sand but water soluble S (r=0.12), organic S (r=0.15) and total S (r=0.18) had non-significant and negative correlation with sand. In case of silt, organic S (r=-0.22) had negative and significant correlation, whereas, with other S fractions viz.: available, water soluble, heat soluble and total S, the relationship was found to be negative and non-significant. On the contrary, clay fraction of soils showed significant and positive relationship with all S fractions. These results supported the findings of Sharma and Jaggi (2001) [25] and Singh et al. (2009) [27].

Relationship of sulphur fractions with soil chemical properties
A perusal of data in Table 3 revealed that soil pH showed negative and significant relationship with available S (r=-0.30**) and water soluble S (r=-0.28*) but non-significant and negative relationship with heat soluble S (r=-0.19), organic S (r=-0.09) and total S (r=-0.13). The relationship of soil organic carbon with organic S (r=0.25*) and total S (r=0.25*) showed positive and significant relationship. Sulphur fractions viz.: water soluble (r=0.27*) and heat soluble S (r=0.23*) showed significant and positive relationship with CEC whereas available S (r=0.18), organic S (r=0.16) and total S (r=0.19) showed positive and non-significant relationship. Positive association of organic carbon and cation exchange capacity with S fractions and negative with pH was also reported by Tripathi and Singh (1992) [30]. In correlation studies sulphur availability was found significantly and positively affected by organic matter, CEC, and finer soil particles. Indeed quite a substantial amount (63.2 to 76.45 per cent, this study) of total S came from organic source. These results were in accordance with those of Kotur and Jalali (2008) [13] and Sen et al. (2017) [23]. Jaggi (2004) [9] also reported such significant negative correlation with pH and positive relation with organic carbon.

Relationship among different sulphur fractions
A close look at data presented in the Table 4, revealed that all the five sulphur fractions were significantly and positively correlated with each other in different textured soils. The results indicated that water soluble S and total S had strong correlation with available S (0.81**) and organic S (0.99**). Significant correlations between different forms of S suggested that some sort of equilibrium exists among these forms. All S fractions were directly proportional to each other and in all the textured soils, organic S was strongly associated with total S fraction. These findings were in line with Saharan et al. (2001) [19] and Sen et al. (2017) [23].

Conclusion
The cultivated soils of Himachal Pradesh are variable in texture, acidic in reaction and have medium to high organic matter, and CEC in the soils are generally moderate. Distribution of different forms of sulphur in soils is strongly dependent upon soil characteristics especially pH, OC and CEC. Correlation studies revealed that organic S was the dominant fraction; constituting 92.8 per cent of total S followed by heat soluble S than available S and water soluble S. All S fractions were positively correlated with OC, clay, and CEC and negatively correlated with pH, sand and silt. All S fractions showed significant and positive correlation with each other.

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