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Nature of the HCl-soluble sulfate in the sequential extraction for sulfur speciation in soils

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Abstract Thirty soils collected from different regions of China were used to investigate the nature of HCl-soluble sulfate (HCl-S) and to evaluate the importance of HCl-S for sulfur speciation in soils. The soils were first extracted with NaH_2PO_4 solution to remove water-soluble and adsorbed sulfate, followed by extraction in 1 mol/l HCl at room temperature, minimizing the hydrolysis of organic sulfur into sulfate. Excellent linear correlations ($r = 0.986$) were found between the HCl-S and the carbonate contents in these soils, indicating HCl-S was mainly the carbonate-occluded inorganic sulfate in calcareous soils, averaging for 39% of total sulfur. However, the traditionally recognized major form of soil sulfate (water-soluble and adsorbed sulfate) only account for 7.5% of total sulfur. The non-calcareous soils were also found to contain some of HCl-S (6.9% of total sulfur). Large errors will occur in the determination of ester sulfur if HCl-S is not subtracted from the HI-reducible sulfur, especially in calcareous soils and soils containing little organic matter, which was the common practice in sulfur speciation studies. The reasonableness of including HCl-S in the subtraction method to determine ester sulfur was further verified by the good correlations between ester sulfur and organic carbon content in the soils

Introduction

Sulfur occurs in soils in organic and inorganic forms. The organic sulfur can be subdivided into two distinct fractions: ester sulfur (organic sulfates containing C-O-S linkages, e.g. choline sulfate, phenolic sulfates, sulfated polysaccharides) and carbon-bonded sulfur, in which sulfur is directly bonded to carbon, consisting mainly of sulfur containing amino acids. The inorganic sulfur fraction may

be present as sulfate and compounds of lower oxidation state such as sulfide, polysulfide, pyrite, etc.

Detailed and accurate information on sulfur speciation in soils and sediments is necessary for the study of sulfur dynamics and biogeochemistry [1, 2]. This information is currently provided by chemical extraction methods, with each extraction step designed to remove a particular fraction of sulfur [3–5]. However, the extractions employed often lack specificity and selectivity, making it difficult to identify the nature of the extractable sulfur and determine accurately the desired form of sulfur. For example, NaH_2PO_4 was widely used to extract adsorbed sulfate [3, 5, 6], however, organic sulfur can be dissolved in the extract [3]. The specificity of HI reduction for ester sulfur is good relative to carbon-bonded sulfur, but most inorganic sulfur is reactive to HI reduction. A remedy for the non-selectivity of NaH_2PO_4 extraction is to use an ion chromatographic determination [5, 7], whereas the determination of ester sulfur has to rely upon difference methods, by subtracting HI-reducible inorganic sulfur from HI-reducible sulfur (a mixture of ester sulfur and inorganic sulfur) in soils and sediments [3–5].

It appears that the accuracy of the determination of ester sulfur depends on the complete estimation of HI-reducible inorganic sulfur. However, different understandings exist in the literature on the content of HI-reducible inorganic sulfur when using subtraction methods. For example, some authors thought NaH_2PO_4 -extractable sulfate and HCl-volatile sulfur (sulfide) should be subtracted [3], while others added HCl-Zn-reducible sulfur (pyritic sulfur) in their subtraction formula [6, 8]. In well-drained, well aerated soils where reduced inorganic sulfur compounds are unlikely to be present, the subtraction can be simplified to include only water-soluble and adsorbed sulfate [2, 9, 10].

The largest uncertainty in the determination of ester sulfur, as well as in sulfur speciation in soils, is the nature of HCl-soluble sulfur. Traditionally, water-soluble and adsorbed sulfate were considered to constitute the bulk of soil inorganic sulfate [3, 9]. But when this fraction of sulfate was removed from soils and HCl extraction was per-

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formed subsequently, a substantial amount of sulfate was detected [5]. Krairapanond et al. [1] also found HCl-soluble sulfur to be an important sulfur fraction. However, the nature of the HCl-soluble sulfur was not investigated, and the necessity of including HCl-soluble sulfate in the subtraction methods to obtain ester sulfur was not verified. Large errors may occur in the determination of ester sulfur and also in the estimation of the status and distribution of soil inorganic sulfate from the neglect of HCl-soluble sulfur due to its relatively large content. Therefore, the objective of the present work is to make clear the nature of HCl-soluble sulfur and evaluate the importance of HCl-soluble sulfate for sulfur speciation and ester sulfur determination in soils.

Experimental section

Apparatus

A Jarrell-Ash model 1155V Atomcomp polychromator (Jarrell-Ash Division, Fish Scientific Company, Waltham MA, USA) was used for the determination of sulfur at SI 180.74 nm. The spectral interferences were corrected and the accuracy of sulfur determination was checked by using NIST standard reference materials [5]. The detection limit (2σ of 10 replicate measurements) was found to be $11 \mu\text{g L}^{-1}$.

A Dionex model 2000i ion chromatograph equipped with an injection valve, a sample loop, guard, separator and suppressor columns, a temperature compensated CDM-II conductivity detector and a model 4270 integrator was used for the determination of sulfate concentrations in the solutions.

All chemicals used in this study were of analytical reagent grade.

Soils

Thirty soil samples collected from several major geological regions of China were used to avoid incomplete conclusions. Of these soils eight were from North China. These soils developed in a humid, frigid-temperature zone. Ten were from middle China located in temperate semi-humid climate areas. Most of these soils were calcareous soils with high pH values and low organic matter content. The other twelve soils were collected from South China, in subtropical climates with abundant precipitation. These soils generally have low pH values (< 6) and contain little calcite. Some basic properties of these soils are listed in Table 1.

The soils were air-dried at room temperature, ground to pass a 2-mm sieve and stored in air-tight bottles. The pH, organic carbon content and calcium carbonate equivalent of the soils were determined by standard methods [11].

Sequential extraction of water-soluble, adsorbed and HCl-soluble sulfate

HCl-extraction was performed after water-soluble and adsorbed sulfate was removed by NaH_2PO_4 solution. The sequential extraction procedure is carried out as described below.

Table 1 Some properties of the soils used

Soil	Location	Geological description	Texture	pH (Soil: $\text{H}_2\text{O} = 1:1$)	Organic C (%)	CaCO_3 (%)	Total S ($\mu\text{g/g}$)
1	Mundanjiang	North China, frigid-temperate zone, humid climate	Sandy silt	8.0	1.6	0.10	663
2	Changchun		Sandy silt	7.5	1.72	1.01	1205
3	Bajia		Clay loam	7.9	1.46	5.98	1063
4	Miaopu		Sandy loam	7.7	0.89	2.58	742
5	Yuanmingyuan		Loamy sand	7.7	2.04	4.18	1027
6	Shanyin		Sandy loam	7.2	0.47	7.61	853
7	Shanyin		Loamy sand	7.9	0.36	12.5	1458
8	Shanyin		Loamy Sand	7.6	0.14	12.5	1018
9	Anguo	Middle China, temperate zone, semi-humid climate	Sandy loam	7.0	0.40	3.32	880
10	Anguo		Sandy loam	6.9	0.21	4.92	668
11	Anguo		Loamy sand	7.0	0.11	3.43	492
12	Anguo		Loamy sand	6.8	0.09	3.16	470
13	Huanxian		Silty clay loam	7.8	0.48	11.2	1050
14	Nanle		Sandy loam	7.8	0.48	7.43	748
15	Guantao		Sandy loam	7.2	0.28	6.59	678
16	Liaocheng		Clay loam	7.8	1.62	14.4	2032
17	Shandong	South China, subtropic zone, humid climate	Silty clay loam	6.4	0.80	0.04	682
18	Linyi		Silty clay loam	6.6	0.80	0.04	310
19	Changsha		Sandy loam	7.3	2.39	0.05	872
20	Hangzhou		Sandy loam	5.6	3.74	ND ^a	1301
21	Jinhua		Loamy sand	6.5	0.36	0.04	1636
22	Sichuan		Loamy sand	5.6	0.55	1.08	1986
23	Sichuan		Loamy sand	5.5	1.04	ND	2081
24	Nanjing		Clay loam	7.6	2.60	1.56	1836
25	Daicun		Loamy sand	5.5	1.19	ND	1543
26	Haikou		Sandy loam	5.4	0.82	0.02	202
27	Gukou		Sandy loam	5.4	0.84	0.08	392
28	Hushan		Loamy sand	5.6	1.46	0.04	1361
29	Zhongzhou		Loamy sand	5.6	1.41	0.04	1795
30	Boyang		Loamy sand	5.5	0.95	0.05	213

^aNot detected

NaH₂PO₄-extractable sulfate. An accurately weighed soil sample (~ 1.00 g) was transferred to a 50-ml centrifuge tube and 10 ml of 0.032 mol/L NaH₂PO₄ was added to the sample. The mixture was shaken on a reciprocal shaker for 30 min. The resulting suspension was centrifuged and the supernatant was subjected to IC (ion chromatography). Water-soluble and adsorbed sulfate were extracted by this procedure [5].

HCl-soluble sulfate. The residual soil from the above step was rinsed with 5 ml of deionized-distilled water which was discarded after centrifugation. Then 20 ml of 1 mol/L HCl was added to the centrifuge tube. Because the HCl-extraction was a gas-yielding (mainly CO₂) procedure, the centrifuge tube was placed upward on the shaker and covered with a lid bearing a small hole produced by a needle in order to release the gas. The hole was small enough to prevent any leaking of the solution from the tube. The shaking was continued for 1 h followed by the centrifugation to obtain the supernatant.

Determination of total sulfur and HI-reducible sulfur. To evaluate the importance of HCl-soluble sulfate for sulfur speciation and determination of ester sulfur, total sulfur and HI-reducible sulfur in the soils were determined using the method described by Shan et al. [5].

Results and discussion

Nature of HCl-soluble sulfate

HCl-soluble sulfur is an important constituent of sedimentary sulfur due to its high proportion of the total sulfur [4]. As for the nature of HCl-soluble sulfur, it could include any of the following: i) pore water SO₄²⁻ and soluble organics; ii) labile organic sulfur compounds, such as ester

sulfates and the S-containing amino acids [4]. Obviously, this explanation is suitable only for sediments or water-logged soils, but cannot be applied to aerated soils with drastically different conditions.

In a previous work [5] we found that HCl-soluble sulfur consists of two kinds of sulfur which can be distinguished by IC determination, i.e., SO₄²⁻ and organic sulfur. Because the organic sulfur may be either ester sulfates or carbon-bonded sulfur soluble in HCl, only the nature of HCl-soluble SO₄²⁻ was considered. This task was complicated by the fact that organic sulfur may be oxidized and hydrolysed to sulfate in hot HCl [12], which was used previously [4, 5]. To minimize this negative effect, a mild reaction mode was employed in which the HCl-extraction was performed at room temperature. Several standard organic sulfur compounds were subjected to this procedure and the resulting SO₄²⁻ was determined by IC to test the hydrolysis degree (HD) of these compounds. The HD was further compared with that obtained by HCl-extraction under boiling conditions. The results are shown in Table 2. In both cases the carbon-bonded sulfur (methionine, cystine and taurine) showed a very low HD of < 1%. But things are quite different with respect to ester sulfate, a low HD of 4.2% and a high HD of 57% were obtained for heparin when the extraction was performed at room temperature and under boiling temperature, respectively. It is well known that soil organic matter mainly exists in the form of humus, and the native soil organic sulfur compounds are much more stable than simple, small molecule compounds [13–16]. From the stability exhibited by the amino acids and heparin, it is reasonable to infer that soil organic sulfur will not undergo significant hydrolysis when the HCl-extraction was performed at room temperature.

Considering water-soluble sulfate and adsorbed sulfate had been removed by NaH₂PO₄ extraction, a further judgement can be made that HCl-soluble sulfate may be occluded in one or several solid phases in soils which can be dissolved or partly dissolved by HCl. Calcite was one of the probable candidates for its insolubility in water or neutral salt solution and ease of dissolution in acid medium.

The concentration of HCl-soluble sulfate of the thirty soil samples is correlated with the CaCO₃ content of these soils (Fig. 1). A very good linear relationship was found between the two variables with $r = 0.986$. Because these soils were collected from across China with varying chemical and physical properties, the consistent association between HCl-soluble sulfate and carbonate content should not be regarded as an accidental result. The most reasonable explanation is that carbonate minerals such as calcite and dolomite are important deposits for soil inorganic sulfate which will be released during HCl-extraction accompanying the dissolution of these minerals. It has been realized that in addition to water-soluble and adsorbed sulfates, several forms of insoluble sulfates including sulfate coprecipitated with CaCO₃ are likely to exist in soils [9], however, no further investigation is made. The carbonate-bound sulfate may occur as minerals such as

Table 2 Hydrolysis of several organic sulfur compounds in 1 mol/L HCl under different extraction conditions

Extraction condition	Hydrolysis degree (HD) ^a (%)			
	Taurine	Cystine	Methionine	Heparin
At room-temperature	0.15	0.20	0.23	4.2
Under boiling condition	0.50	0.67	0.88	57

^aPercent of organic sulfur converted to sulfate-S

Fig. 1 Simple linear correlations between HCl-soluble sulfate and calcium carbonate content of the thirty soils

gypsum, anhydrite, celestite, barite or other insoluble sulfates coprecipitated with CaCO_3 [17].

But carbonate is not the only source of HCl-soluble sulfate as indicated by the regression equation $y = 49.5x + 61.3$, in which non-calcareous soils ($x = 0$) were found to have an average of $61.3 \mu\text{g/g}$ of HCl-soluble sulfate. It can be inferred that some other HCl-soluble minerals such as Fe-Al oxides or hydroxides may account for this part of sulfate, as evidenced by the high concentration of Fe and Al in HCl extracts. Basic Fe and Al sulfates are also likely to occur [17].

Based on the above discussion, HCl-soluble sulfate can be regarded as inorganic sulfate occluded in carbonate or other HCl-soluble minerals. Because these minerals are readily dissolvable in HI reduction solution, HCl-soluble sulfate must be subtracted from the HI-reducible sulfur to gain an accurate value of ester sulfates, especially in case of calcareous soils.

Importance of HCl-soluble sulfate to sulfur speciation in soils

The thirty soil samples were classified as calcareous soils ($\text{CaCO}_3 > 1\%$) and non-calcareous soils ($\text{CaCO}_3 < 1\%$). The proportions of NaH_2PO_4 -extractable sulfate ($\text{NaH}_2\text{PO}_4\text{-S}$), HCl-soluble sulfate (HCl-S) and ester sulfur in terms of

Fig. 2 Distribution of NaH_2PO_4 -extractable sulfate, HCl-soluble sulfate, and ester sulfur in the calcareous and non-calcareous soils

total sulfur in the two groups of soils are schematically shown in Fig. 2.

HCl-S is the predominant form of sulfur in calcareous soils, averaging for 39.0% of total sulfur, whereas the $\text{NaH}_2\text{PO}_4\text{-S}$ makes up only 7.5% of total sulfur. These results demonstrated that inorganic sulfate mainly exists in occluded forms in calcareous soils, water-soluble and adsorbed sulfate only represent a minor fraction of soil inorganic sulfate. Taking this into account, it could be found that ester sulfur will be greatly overestimated if HCl-S was not subtracted. The value of ester sulfur would be 46.9% instead of being 7.9%. Considering most of the calcareous soils contain little organic matter, the former content of ester sulfur is questionable. The reasonableness of the current subtraction method is further tested by correlation analysis (Fig. 3). No significant correlation ($r = 0.244$) exists between the content of ester sulfur and organic carbon in these soils if only $\text{NaH}_2\text{PO}_4\text{-S}$ is subtracted. In the contrast, highly significant correlation is observed if HCl-S is also subtracted ($r = 0.943$).

The content of HCl-S in non-calcareous soils is not so large as in the calcareous soils, as can be expected. $\text{NaH}_2\text{PO}_4\text{-S}$ restored its status as the major form of soil inorganic sulfate, averaging 16.4% of total sulfur, whereas the average content of HCl-S accounted for 6.9% of total sulfur. The much higher content of $\text{NaH}_2\text{PO}_4\text{-S}$ in these non-calcareous soils was understandable because most of these soils were rich in Fe-Al oxides, which are the main factors responsible for sulfate adsorption [18, 19]. The proportion of ester sulfur also increased significantly (from 7.9% to 17.4% of total sulfur), which might be attributed to the more quantities of organic matter present in the non-calcareous soils. The average content of organic carbon in the calcareous and non-calcareous soils were

Fig. 3A, B Simple linear correlations between ester sulfur obtained by different subtraction methods and organic carbon content in the calcareous soils. **A** Ester S = HI-S - $\text{NaH}_2\text{PO}_4\text{-S}$. **B** Ester S = HI-S - ($\text{NaH}_2\text{PO}_4\text{-S}$ + HCl-S)

found to be 0.82% and 1.49%, respectively. Similarly, the correlation coefficients of 0.848 between the ester sulfur obtained by subtracting both $\text{NaH}_2\text{PO}_4\text{-S}$ and HCl-S from HI-S and organic carbon content was superior to the value of 0.791 obtained by subtracting only $\text{NaH}_2\text{PO}_4\text{-S}$.

It is clear from the above results that $\text{HCl-soluble sulfate}$ must be included in any programs for sulfur speciation in soils, especially in case of calcareous soils and soils containing little organic matter, otherwise large errors will be introduced. However, this problem did not cause serious attentions in the past. Most authors ignored $\text{HCl-soluble sulfate}$ in their speciation studies [2, 3, 6, 8–10, 20–22]. This work revealed that $\text{HCl-soluble sulfate}$ is an important sulfur fraction in soils and carbonate minerals have large capacity to incorporate sulfur, but the process and mechanism of the incorporation and the role of $\text{HCl-soluble sulfate}$ in sulfur dynamics and sulfur biogeochemistry is not well understood. Further studies are needed to clarify these uncertainties.

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