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To cite this article: X. Y. Cui , S. P. Wang , Y. F. Wang , X. R. Xing , Z. Z. Chen , S. Haneklaus , J. Fleckenstein & E. Schnug (2006) Suitability of Extractants for Predicting Available Sulfur in Natural Grassland in the Inner Mongolia Steppe of China, Communications in Soil Science and Plant Analysis, 37:05-06, 721-732, DOI: [10.1080/00103620600563895](https://doi.org/10.1080/00103620600563895)

To link to this article: <https://doi.org/10.1080/00103620600563895>



Published online: 05 Feb 2007.



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Suitability of Extractants for Predicting Available Sulfur in Natural Grassland in the Inner Mongolia Steppe of China

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Abstract: It was the objective of this study to compare the suitability of different extractants for predicting the availability of sulfur (S) in natural grassland in a sulfur response trial on three different soil types in the Inner Mongolia steppe of China. For soil analysis, seven different extractants have been employed. The inorganic $\text{SO}_4\text{-S}$ concentration was determined by ion chromatography. Additionally, in the $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extract the total soluble S was determined employing turbidimetry. Weak salt solutions (0.15% CaCl_2 , $\text{Ca}(\text{H}_2\text{PO}_4)_2$, and KH_2PO_4) extracted similar

Received 14 March 2003, Accepted 20 July 2005

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amounts of $\text{SO}_4\text{-S}$. Extraction with 0.025 M KCl provided the lowest $\text{SO}_4\text{-S}$ values. Deionized water dissolved significantly more $\text{SO}_4\text{-S}$ in the control plots than most weak salt extractants. The concentration of soluble organic S decreased in the control plots after 100 days of plant growth, indicating that the organic S pool contributed significantly to the S nutrition of the forage crops. Significant relationships among the $\text{SO}_4\text{-S}$ in the soil determined in different extracts and crop yield, sulfur content in the forage, and total sulfur uptake were only found for the $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extract. In general, the correlation coefficients proved to be unsatisfactory for field experimentation.

Keywords: Inner Mongolia steppe of China, soil analysis, sulfur

INTRODUCTION

The cultivation of crops with a high sulfur (S) requirement, the use of S-free fertilizers, low atmospheric S depositions, and low organic S reserves in soils are the major reasons for the occurrence of severe S deficiency (Fan and Messick 1997; Schnug and Haneklaus 1998). Plant-tissue analysis allows a direct and accurate evaluation of S nutritional status. However, the problem of using plant analysis as a diagnostic tool is the short available time span between sampling and fertilization and the fact that yield losses may only be partly compensated because of the late application (Haneklaus and Schnug 1994). Soil tests are commonly used to predict the fertilizer demand. There are numerous reviews and comparisons of methods for the determination of plant available S in soils (Blair et al. 1991; Anderson et al. 1992; Gowrisankar and Shukla 1999; Tabatabai 1982).

The suitability of a soil analytical method for determining the S nutritional status depends on their physical and chemical characteristics (Blair et al. 1991; Anderson et al. 1992; Gowrisankar and Shukla 1999; Tabatabai 1992; Lisle et al. 1994) and its correlation with crop yield and plant S status (Haneklaus and Schnug 1994; Anderson et al. 1994). Plants take up S from soils in the form of SO_4^{2-} , with the exception of some marsh plants, which appear to be able to use S in the form of sulfide (Carlson and Forrest 1982). Therefore, soil analytical methods determine the $\text{SO}_4\text{-S}$ content as an indicator for the S supply. But the $\text{SO}_4\text{-S}$ content shows a high spatial and temporal variability under humid conditions (Bloem 1998; Haneklaus, Bloem, and Schnug 2003) so that the suitability of soil analysis needs to be questioned principally in these climatic regions. Here, S balances that include soil hydrological and soil physical parameters are preferable diagnostic tools. In the Inner Mongolia steppe of China there are no $\text{SO}_4\text{-S}$ losses by leaching because of the arid/semi-arid climatic conditions so that soil analytical data supposedly reflect the S nutritional status of a crop and thus may be suited for prognosis of the S demand, particularly if tested in S response trials.

In China, the S nutrition of crops is widely ignored though severe S deficiency is increasing rapidly. Response of crops to S fertilization was reported for field experiments carried out in southern and northern parts of China (Fan and Messick 1997; Wang et al. 1998; 2001; 2002a; 2002b; 2003; 2004). The total area of the Inner Mongolia autonomous region is 1.18 million km², which represents 12.3% of the total area of China. Animal farming is the main agricultural activity in the Inner Mongolia steppe. Sulfur fertilization increased yield and quality of grassland and improved wool quality. Besides water stress and a limiting N and P supply, S deficiency proved to be a major limiting factor in crop and livestock production (Fan and Messick 1997; Wang et al. 1998; 2001; 2002a; 2002b; 2003; 2004). However, little information is available about the suitability of soil analysis data to predict the S nutritional status of natural grassland in the Inner Mongolia steppe of China, which therefore was the aim of this study.

MATERIALS AND METHODS

The field experiments were conducted in 1999 at the Inner Mongolia Grassland Ecosystem Research Station, Xilinhot, managed by Chinese Academy of Sciences, located at 43°37'N, 116°43'E. The regional climate is continental. The 30-year mean annual precipitation is 350 mm (250–450 mm), with 60–70% of the total rainfall occurring from June to August. The annual mean temperature is –0.4°C, with 150 to 180 plant-growing days per year. The mean monthly temperature is –21.5°C in January and 19°C in July. Minimum- and maximum-recorded temperatures were –43°C in 1981 and 35.1°C in 1973. Typical steppe plants such as *Stipa grandis*, *Leymus chinensis*, and *Artemisia frigida* characterize vegetation in this region. Chestnut soils, Calcustepts according to the U.S. soil taxonomy classification system, are the dominant type of soil in Inner Mongolia grasslands (Wang et al. 2002a; Wang and Cai 1988).

Sulfur (0 and 30 kg S ha⁻¹) was applied as gypsum to the natural grassland on a dark chestnut (Udic Calcustepts), a typical (Typic Calcustepts), and a sandy chestnut soil (Aridic Calcustepts) on May 6, 2000. The plot size was 10 by 10 m, arranged in a randomized block design with three replicates. Each plot received 92 kg N ha⁻¹ as urea and 15 kg P ha⁻¹ as triplephosphate. The soil samples (0–20 cm) were taken before fertilization on May 4, 2000, and at the end of the experiment on August 15, 2000. Together with the soil samples, the whole aboveground plant material was taken. Each plant and soil sample was a mixture of five core samples, taken in an area of about 1 m².

Plant samples were dried at 65°C in a ventilated oven until constancy of weight and ground to a particle size <1 mm. All soil samples were air-dried and sieved to a particle size of <2 mm. Basic soil characteristics are summarized in Table 1.

Table 1. Basic soil characteristics of three chestnut soils in the Inner Mongolia steppe of China before start of the experiment

Soil type	Dark chestnut	Typical chestnut	Sandy chestnut
pH (CaCl ₂) (1 : 2.5)	6.9	7.4	6.9
pH (H ₂ O) (1 : 5)	7.6	8.4	7.6
Organic carbon (%)	1.60	1.53	0.95
Total nitrogen (%)	0.18	0.16	0.10
Inorganic CaCO ₃ (%)	0.96	3.59	0.39
Clay (%)	14.4	15.0	8.6
Silt (%)	24.6	24.1	11.9
Sand (%)	61.0	60.9	79.5
P-CAL ^a (mg kg ⁻¹)	1.39	1.10	0.98

^aCAL method (Schueller 1969).

Plant Analysis

Plant samples were digested with H₂O₂ + HNO₃ (65%) in a ratio of 1 : 4, and total S in plants was determined by ICP-AES.

Soil Analysis

The total carbon content in soils was measured by dry combustion using an EC-12 Carbon Determinator (LECO Corp., USA). Inorganic carbon was determined by adding 25 mL HCl + FeCl₂ solution to 1.0 g soil and measuring the volume of emitted CO₂ after shaking for 4 min, employing the Scheibler equipment. Soil organic carbon was calculated by subtracting inorganic carbon from total carbon. Soil pH was determined potentiometrically in a 1 : 2.5 suspension with 0.01 M L⁻¹CaCl₂ and in a 1 : 5 suspension with distilled water, respectively. The soil available P content was determined by the CAL method (Schueller 1969).

The extractants used to determine SO₄-S are listed in Table 2. SO₄-S was determined by IC (761 Compact IC), whereas turbidimetry was used for the final determination of the dissolved inorganic and organic S fraction. The extractable organic S content was calculated by subtracting the SO₄-S content from the total S content determined by turbidimetry in the Ca(H₂PO₄)₂ extract according to Fox et al. (1964).

The data were analyzed using one-way analysis of variance (ANOVA) (SPSS 1998). The simple correlation coefficients between S extracted by different extractants and plant parameters were determined using the Pearson algorithm.

Table 2. Soil analytical methods employed to assess the S status of a forage crop in the Inner Mongolia steppe of China

No.	Extractant	Soil: extractant ratio	Shaking time (h)	Determination ^a	Reference
1	Deionized water	1 : 5	1	IC	Williams and Stein- berg (1959)
2	Ca(H ₂ PO ₄) ₂	1 : 5	1	IC/TM	Fox et al. (1964)
3	0.005 M P	1 : 5	1	IC	Blair et al. (1991)
	Ca(H ₂ PO ₄) ₂	1 : 5	1	IC	Ensminger (1954)
4	KH ₂ PO ₄	1 : 5	1	IC	Little (1958)
5	0.001 M HCl	1 : 5	1	IC	Williams and Stein- berg (1959)
6	0.15% CaCl ₂	1 : 5	3	IC	Bloem (1998)
7	0.025 M KCl				

^aIC: ion chromatography; TM: turbidimetry.

RESULTS

Extraction Force of Different Soil Analytical Methods

The various extractants used in this study differed widely in the amount of S extracted (Table 3). The concentration of SO₄-S extracted by KCl was 3 to 10 times lower than that in other extractants. Independent of the soil type, water dissolved significantly more SO₄-S in the control plots than most of the other extractants. Similar amounts of SO₄-S were extracted by weak salt extractants. Samples extracted by HCl and KH₂PO₄ showed the highest variation of the SO₄-S content (Table 3). The total dissolved S extracted by Ca(H₂PO₄)₂ and measured by turbidimetry delivered the highest values for plant available S. Sulfur fertilization increased the overall variability of SO₄-S two to eightfold (Table 3).

Plant Response to Sulfur

On all soils S fertilization significantly increased herbage S concentration and S uptake (Table 4). The S supply significantly increased the aboveground biomass on the dark and sandy chestnut soils, whereas differences proved not to be significant on the typical chestnut soil (Table 4). The plant parameters crop yield, S concentration, and total S uptake correlated significantly and positively with the S and SO₄-S content in the soil extracted by different extractants with the exception of HCl (Table 5). The closest relationships were found between S uptake and S/SO₄-S in soils. Only SO₄-S in the

Table 3. Sulfur extraction force of different extractants (mg kg⁻¹) on three soil types in the Inner Mongolia steppe of China

Soil type	Treatment	H ₂ O 1 ¹		Ca(H ₂ PO ₄) ₂ 2 ²		Ca(H ₂ PO ₄) ₂ 2 ³		Ca(H ₂ PO ₄) ₂ 3		KH ₂ PO ₄ 4		HCl 5		CaCl ₂ 6		KCl 7	
		Mean	CV ⁴	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Dark chestnut	BT ⁵	28.5 ^d	9.5	28.3 ^d	16.3	38.5 ^e	12.5	22.2 ^c	10.4	24.7 ^{cd}	4.9	17.0 ^{bc}	7.6	25.9 ^c	8.5	7.1 ^a	11.2
	+S	50.7 ^a		48.2 ^a		68.3 ^a	33.0	49.0 ^a	46.5	45.3 ^a	35.1	45.7 ^a	44.9	50.7 ^a	37.0	22.0 ^a	56.4
	-S	28.2		33.4		24.6 ⁱ	6.1	11.0 ^c	5.5	16.0 ^e	5.0	12.6 ^{cd}	2.4	14.1 ^{de}	7.1	3.2 ^a	3.1
		19.1 ^g	5.0	14.7 ^{de}	10.2												
Typical chestnut	BT	28.2 ^{ef}	7.8	19.2 ^{cd}	5.2	34.4 ^f	5.5	23.9 ^{de}	18.8	22.9 ^{de}	11.8	28.2 ^{ef}	15.9	23.9 ^{de}	7.5	6.9 ^a	10.0
	+S	57.7 ^a	25.1	46.3 ^a		69.8 ^a	29.3	47.2 ^a	30.9	50.8 ^a	23.9	88.2 ^a	31.6	53.2 ^a	33.3	17.8 ^a	34.3
	-S	18.0 ^{cde}		30.5		24.6 ^{ef}	8.9	9.6 ^{abc}	4.2	19.6 ^{de}	3.1	21.4 ^{de}	28.5	14.0 ^{bcd}	1.4	3.2 ^a	3.1
		8.3		14.2 ^{bcd}	2.1												
Sandy chestnut	BT	32.2 ^a	27.3	18.1 ^a	1.1	29.7 ^{ab}	7.9	20.1 ^{ab}	5.9	57.7 ^c	37.4	27.6 ^{ab}	46.0	19.6 ^{ab}	8.7	6.3 ^a	15.8
	+S	25.7 ^{bc}		24.6 ^{bc}	21.9	42.8 ^d	24.1	24.2 ^{bc}	11.5	22.3 ^{abc}	6.5	35.1 ^c	34.4	21.3 ^{abc}	12.6	5.6 ^a	16.1
	-S	13.2		12.7 ^{abc}	1.6	18.4 ^{bcd}	7.6	10.3 ^{ab}	4.8	25.6 ^{cd}	22.5	33.9 ^e	26.5	11.8 ^{ab}	10.2	2.4 ^a	8.3
		15.9 ^{abc}	4.4														

¹Method (see Table 2).²Ion chromatography.³Turbidimetry.⁴CV: coefficient of variation (%); values in each row followed by the same letter did not differ significantly (p < 0.05).⁵BT: before S fertilization treatment in May.

Values in each row followed by the same letter did not differ significantly (p < 0.05).

Table 4. Effect of S fertilization on yield, S concentration, and total S uptake of herbage

Soil type	Treatment	Yield (g m ⁻² d.w.)	S concentration (%)	S uptake (g m ⁻²)
Dark chestnut	+ S	182.6 ^a	1.60 ^a	2.93 ^a
	- S	146.0 ^b	1.27 ^b	1.85 ^b
Typical chestnut	+ S	133.1 ^a	1.80 ^a	2.40 ^a
	- S	121.0 ^a	1.47 ^b	1.78 ^b
Sandy chestnut	+ S	116.7 ^a	2.03 ^a	2.38 ^a
	- S	80.1 ^b	1.53 ^b	1.24 ^b

Values in each column for +S and -S treatments on three soils followed by the same letter did not differ significantly ($p < 0.05$).

Ca(H₂PO₄)₂ extracts showed a significant correlation with all plant parameters (Table 5).

Changes in Organic Sulfur

In the control treatments, the SO₄-S content decreased by 25% to 50% after 100 days of experimentation. The total extractable S content (organic S and SO₄-S) decreased by 30% to 35% on the dark chestnut and typical chestnut soils but by only 5% on the sandy chestnut soil (Table 3). Net changes in the organic S fraction shown in Figure 1 reveal that the organic S pool

Table 5. Correlation coefficients for the relationship between S and SO₄-S extracted by different methods and plant parameters of a grassland community (n = 18)

Extractant ^a	Correlation coefficient (r)		
	Yield	S concentration	S uptake
Deionized water	0.580*	0.451	0.788**
Ca(H ₂ PO ₄) ₂ (IC)	0.599**	0.469*	0.815**
Ca(H ₂ PO ₄) ₂ (TM)	0.643**	0.432	0.829**
0.005 M P Ca(H ₂ PO ₄) ₂	0.577*	0.471*	0.797**
KH ₂ PO ₄	0.350	0.393	0.636**
0.01 M HCl	0.172	0.456	0.431
0.15% CaCl ₂	0.588*	0.425	0.781**
0.025 M KCl	0.620**	0.335	0.763**

^aIC: ion chromatography, TM: turbidimetry.

*, **Significant differences at the 0.05 and 0.01 level, respectively.

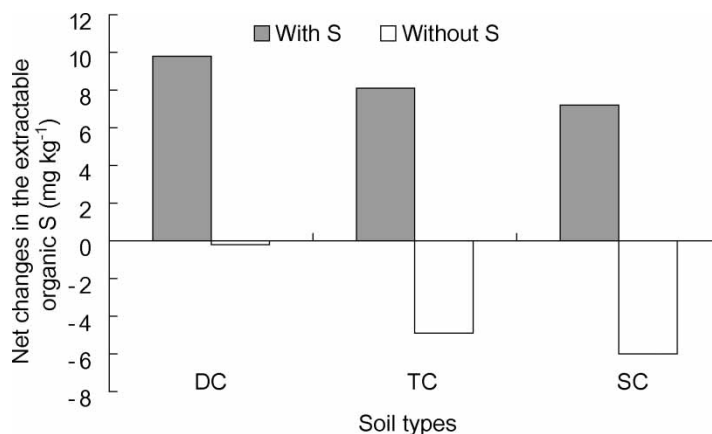


Figure 1. Net changes in the extracted organic S fraction determined in the $\text{Ca}(\text{H}_2\text{PO}_4)_2$ extract according to Fox et al. (1964) after 100 days of herbage growth. DC: dark chestnut soil, TC: typical chestnut soil, and SC: sandy chestnut soil.

decreased by 0.24 (dark chestnut soil), 4.92 (typical chestnut soil), and $6.03 \text{ mg S kg}^{-1}$ (sandy chestnut soil) if no S was applied but increased significantly after S fertilization. The results showed that the labile pool of organic S contributed significantly to the S nutrition if no S was applied.

DISCUSSION

Some researchers reported that phosphate salts extracted more S than other extractants, and KH_2PO_4 more than $\text{Ca}(\text{H}_2\text{PO}_4)_2$ as they dissolve adsorbed and a part of the organic S fraction besides soluble, inorganic $\text{SO}_4\text{-S}$ (Williams and Steinberg 1959; Ajwa and Tabatabai 1993). In comparison, in 0.15% CaCl_2 and in deionized water, the soluble $\text{SO}_4\text{-S}$ is determined exclusively (Blair et al. 1991; Spencer and Frency 1960; Saha and Singh 1992; Eriksen et al. 1995). Principally, on agricultural soils with soil pH values >5 the adsorption of $\text{SO}_4\text{-S}$ is negligible (Haneklaus, Bloom, and Schnug 2003; Harward and Reisenauer 1996). In the present study, differences in the extraction force between the extractants tested were only minor. Blair et al. (1991) found similar results. The extraction force of different extractants in extracting plant-available S is related to soil characteristics (Mehta, Singh, and Singh 1988). Maynard, Kalra, and Radford (1987) determined that water extracted more organic S than salt extractants in the surface organic horizons of forest soils (pH 4.3–6.3) in Canada. The results of Ribeiro et al. (2001) showed that on soils with a low S adsorption capacity, the extraction with 0.01 M CaCl_2 reflected the plant-available S concentrations best and therefore proved to be the preferable method for predicting the S supply.

In contrast, on soils with a high adsorption capacity 0.5 M NH_4OAc and 0.25 M HOAc (0.25 M) provided the best results with a view to extracting the plant-available S content.

Grassland soil methods, which extracted a part of the organic S fraction besides inorganic $\text{SO}_4\text{-S}$, revealed the plant-available S pool best (Blair et al. 1991), and the extracted amount of organic S was directly related to the total mineralizable organic S pool (Watkinson, Perrott, and Thorrold 1991). Results from this study showed decreases in the concentration of organic S in soils extracted with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ after 100 days of herbage growth if no S was fertilized. When S was fertilized the amount of organic S increased. These findings indicate that fertilizer S is immediately incorporated in the organic matter fraction (Zhao et al. 1996). The correlation coefficients for the relationship between S extracted by different methods and forage yield and S concentration were unsatisfactory, whereas those for S uptake were distinctly higher. This could possibly be due to the fact that the plant species *Leymus chinensis* and *Artemisia frigida* have a significantly higher S demand than the dominant species *Stipa grandis*, which showed no response in terms of yield to S fertilization (Wang et al. 2004).

Instrumental methods, such as ion chromatography and inductively coupled plasma atomic emission spectrometry (ICP-AES), are widely used among others for the determination of $\text{SO}_4\text{-S}$ and total S (Tabatabai 1992), but the equipment is too expensive for developing countries. However, turbidimetry may also provide satisfactory results (see Table 3), though it is thought to be tedious and to suffer from the effects of serious chemical interferences (Pasricha and Fox 1993; Singh et al. 1993; Gupta, Gupta, and Kala 1995). Interferences by Cl when employing ion chromatography can be avoided by diluting the extraction solution (Bloem 1998).

In this study, S fertilization significantly increased the total S concentration, S uptake, and crop yield (Table 4). Soil analysis employing extraction with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (Williams and Steinberg 1959) provided the closest relationship with these plant parameters (Table 5), but the outcome is not satisfying for an S response trial. The inadequacy of soil analysis for plant-available S, even on soils where soil hydrological parameters and S adsorption are not relevant factors contributing to the S supply, stresses the need for alternative methods to assess the S nutritional status.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support by the Key Program of Knowledge Innovative Engineering, Chinese Academy of Sciences (KSCX2-SW-107), DAAD-K. C. WONG Postdoctoral Fellowship, the President Funds of the Chinese Academy of Sciences, and Chinese-German Bilateral Agricultural Program.

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