Plant, Soil and Environment, 71, 2025 (10): 695–707 *Original Paper*

https://doi.org/10.17221/250/2025-PSE

Contribution to the turbidimetric method for sulphur determination in arable soils

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Citation: Surleva O., Chavdarova K., Kazanlaklieva V., Angelova L., Surleva A. (2025): Contribution to the turbidimetric method for sulphur determination in arable soils. Plant Soil Environ., 71: 695–707.

Abstract: Sulphur (S) plays an important role in agriculture, being the fourth major contributor to improved quality of crops and increased yields. The applied methods for the estimation of different forms of S in soil aimed at assessing the sulphur availability to plants in various conditions. Nowadays, the wider spreading of regions with sulphur deficiency imposes optimisation of the soil testing procedures in order to increase their availability for laboratories. This study contributes to improving the analytical performance of the turbidimetric method in determining water-soluble sulphate in soil after leaching with the $CaCl_2$ reagent. The modified testing protocol showed: method limit of quantification of 5.0 mg/kg; precision as relative standard deviation less than 3%; recovery of fortified soil samples 103 ± 18%. The expanded uncertainty was 2.3 mg/kg SO_4^{2-}-S ($K = 2$, norm.). The proposed testing protocol was inexpensive, fast, used simple equipment and procedures, easily adoptable in regular laboratories, and showed characteristics suitable for the estimation of water-soluble sulfate in arable soils. A set of 546 soil samples was tested, and 74% were found to be sulphur deficient with SO_4^{2-}-S < 10 mg/kg and sulfur availability index < 6.0. Thus, the availability of laboratory analysis to a broader group of farmers could contribute to effective fertilisation programs, as the newly proposed fertiliser blending technologies are based on adequate estimation of sulfur availability in arable soils.

Keywords: available sulfur; turbidimetry; soil testing; sulphate determination; standard addition

Sulfur plays an important role in agriculture, being the fourth major contributor to high yields and increased nutritious levels of crops (TSI 2024). Together with potassium (K), nitrogen (N) and phosphorus (P), sulfur appears to be a critical plant nutrient as it influences the uptake of other macro and micro nutrients (Sharma et al. 2024). The importance of sulfur for plant nutrition was proven due to the strong connection between the plant's sulfur supply and nitrogen. Soil sulfur deficiency limits the nitrogen uptake, thus lowering the crop yield. An additional benefit of an appropriate supply of plants is the effective and intensive uptake of nitrogen, thus lowering the nitrate leaching into the soil profile and underground water (Lisowska et al. 2023, Narayan et al. 2023).

A line of studies reported continuously increasing S deficiencies of soil (Lisowska et al. 2023, Narayan et al. 2023, Magnuska et al. 2023). A recent review summarised the factors that govern the soil S deficiency (Sharma et al. 2024): strict control of industrial emissions of SO_2, widespread use of high-yielding cultivars, lowered level or even no tillage, application of highly effective NPK fertilisers with low

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Supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students-2", University of Chemical Technology and Metallurgy, Project No. #RD-15-424/29.08.2024.

content of S, leaching and erosion of topsoils, as well as continuous loss of soil organic matter. It was found that during the last decades, the atmospheric S depositions sharply declined in Europe (Seaton et al. 2023, Magnuska et al. 2023). S deficiency was recommended for addressing crop growth issues (Camberato and Casteel 2017).

Different approaches for the estimation of soil S availability to plants have been proposed. A wide variety of methods is due to the different forms of S in soil (Tabatai 1987). Only 5% of soil sulfur is in inorganic form, from which the sulphate sulfur in soil solution or adsorbed on clay minerals is available for plant uptake. Additionally, sulfate in soil exists in insoluble forms such as $BaSO_4$ and $SrSO_4$ or SO_4^2, precipitated with $CaCO_3$, as well as basic Fe and Al sulfates under certain conditions. Thus, different extraction or leaching procedures are proposed to achieve better correlations with plant response to fertilisation. However, the analytical information highly depends additionally on the analytical technique used to determine S concentration in the leachates (Kulhánek et al. 2018, Zbíral et al. 2018, Shukla et al. 2019, Zhao et al. 2020, Lisowska et al. 2023). In 2010, Esmel et al. summarised the most used procedures for soil sulfur determination and highlighted that the soil type and characteristics determine the choice of the analytical method. Moreover, the appropriate experimental procedure depends on the plant varieties. Thus, a thorough agronomic calibration is needed to ensure adequacy of the analytical method. Agronomic calibration of analytical method consists of a line of stages including: soil testing in laboratory conditions, estimation of S deficiency of soil based on laboratory analysis, development of S fertilisation program, monitoring of plant response to the fertilisation, correlation study between soil S content and crop yield, and determination of critical values. Based on these results, the appropriate method for the determination of plant available S in soil is chosen for each soil type and specific cultivars. A line of correlation studies was reported to increase the flexibility of the methods for sulphur in soil testing and the comparability of the results. Thus, the reliability and trueness of analytical methods are highly important to ensure the adequacy of the conclusions and the possibility of transferring the data from one method to another.

The turbidimetric method is well used in soil testing laboratories. It provides reproducible results with relatively simple instrumentation and operator skills requirements (Singh et al. 2011). The method is particularly appropriate when soil samples are not tested regularly for sulphate. Moreover, the working range of the turbidimetric method corresponds well to the requirement for relatively high sulphate content in arable soils.

This study aims to contribute modern approaches to the well-known turbidimetric method for soil sulphate determination, increasing the sensitivity, reliability and precision of the obtained data. A detailed study of the sources of uncertainty in the measurements is presented. The modified method is applied to various types of arable soil samples in Bulgaria, and an attempt is made to survey the sulphur status in soil.

MATERIAL AND METHODS

Reagents. Potassium sulphate K_2SO_4 (p.a., Fluka, Buchs, Switzerland), $CaCl_2$ (p.a., Merck, Darmstadt, Germany) and activated charcoal powder (Valerus, Bulgaria) were used in this study. A sulphate reagent (SulfaVer 4, Hach Lange, Loveland, USA) containing 40–50% barium chloride dihydrate and 50–60% anhydrous citric acid was used as supplied. All solutions were prepared with distilled water.

Samples. The soil samples were collected from several agricultural regions in Bulgaria. All samples were collected from 0–30 cm depths by an automatic soil sampler. The samples were air-dried at room temperature, ground by a mechanical grinder (Polymix PX-MFC 90 D, Kinematica AG, Luzern, Switzerland) equipped with a 2 mm sieve. Thirteen soil samples with different characteristics were chosen for the estimation of the performance of the analytical procedure (Table 1). Plant available (exchangeable) P and K were determined following the calcium lactate/ammonium acetate extraction at a 1:25 solid-to-liquid ratio for 1 h; the P and K in leachates were measured by a molybdenum blue method and flame atomic emission spectrometry, correspondingly (Angelova et al. 2021, 2022). Mineral nitrogen was determined by leaching in an acid mixture (0.05 mol/L HCl and 0.013 mol/L H_2SO_4) for 5 min at a 1:10 ratio, followed by spectrophotometric measurements applying Nitraver 5 and Nessler methods for nitrate and ammonium ions, respectively (Hach Lange, Loveland, USA). Organic carbon was determined by back titration of an excess of $K_2Cr_2O_7$ with Morh salt standard solution (Tyurin method), soil active acidity was determined potentiometrically at a 1:2 solid-to-liquid ratio in d. H_2O.

Table 1. Characteristics of representative soil samples

No. sample	Clay content (%)	pH	Organic carbon (%)	Mineral nitrogen	Exchangeable potassium	Exchangeable phosphorus
				(mg/kg)		
1	0.75	8.00	2.10	28	54.8	12.4
2	0.72	7.84	2.35	29	50.6	7.22
3	2.33	8.28	4.55	17	271.4	29.1
4	3.58	8.14	2.78	19	171.0	24.2
5	5.45	4.73	0.61	12	126.2	29.0
6	16.4	7.19	3.36	13	148.6	9.59
7	16.5	6.94	3.90	8	136.1	3.72
8	7.67	6.07	3.18	15	193.4	24.8
9	6.52	7.23	2.25	11	292.2	36.5
10	12.9	6.69	2.78	15	293.8	3.45
11	16.5	6.37	2.94	10	268.9	3.12
12	15.4	5.71	3.04	12	239.9	3.17
13	16.3	6.68	2.83	12	247.3	23.7

Procedures

The sulphate in soil leachates was determined turbidimetrically using the DR3900 Hach Lange spectrophotometer (Loveland, USA) at 450 nm wavelength.

Calibration. The calibration curve was prepared daily by working standards in the 10–70 mg/L concentration range. A stock standard sulphate solution at a concentration of 1 000 mg/L was prepared by dissolving 0.4533 g dry to constant weight K_2SO_4 in a 250 mL volumetric flask. Calibration standards were also prepared daily by appropriate dilution of the stock sulphate solution with leaching solution 0.15 % (0.014 mol/L) $CaCl_2$. Each solution's absorbance at 450 nm was measured in triplicate against distilled water.

Sulphate leaching. Sulphate in soil was leached following the procedure: 10 g of dried soil were mixed with 0.15% $CaCl_2$, at a solid to liquid ratio of 1:5 and agitated at a reciprocal shaker for 30 min at 100 rpm/min. Approximately 0.10 g of charcoal powder was added to the sample and agitated for 1–2 min. The mixture was filtered through filter paper K4 (Papirna, Perstejn, Czech Republic) to remove the residues. The leachate was subjected to turbidimetric or ICP-OES measurement of the concentration of SO_4^{2-}. Each soil sample was analysed in duplicate. blank that did not contain a soil sample was prepared in triplicate daily following the same procedure.

Measurement of sulphate concentration. A new procedure based on standard addition (spiking) was developed for the determination of sulphate in soil leachates. A diluted standard solution was prepared from the stock solution at a concentration of 200 mg/L SO_4^{2}. A volume of 1.00 mL of the diluted standard solution was added to 10 mL of blank or soil leaching solution. Approximately 0.2 g of the sulphate reagent was homogenised with the samples. A 5-min reaction time was applied according to Hach Lange (Loveland, USA). After gentle mixing, absorption was measured in triplicate at 450 nm in a 2 cm cuvette. Three aliquots of each leaching solution were analysed following the procedure described above. The results were calculated following the Eq. (1):

$$C_{SO_4^{2-}-S} = \frac{A_1 - A_2}{slope} \times \frac{V_l}{m_s} \times \frac{1}{3}, \frac{mg}{kg} \qquad (1)$$

where: A_1 – measured absorbance of spiked sample leachate, mg/L; A_2 – measured absorbance of spiked blank solution, mg/L; V_l – volume of the extracting solution, L; m_s – weight of the dry soil sample, kg; slope of the calibration curve.

Method characterisation. The method's performance characteristics were estimated using MS Excel statistics tools (Redmond, USA). The obtained experimental data were statistically treated to assess key verification parameters: working range and linearity, trueness (bias and recovery), precision in conditions of repeatability and within-lab reproducibility, as well as uncertainty of measurement (Magnusson and Örnemark 2014). Linearity was evaluated using the external standard method, followed by the linear regression and plot of residuals approach. The software calculated the correlation coefficient without forcing through the origin.

The precision was estimated in repeatability conditions and presented as standard deviation in mg/kg and relative standard deviation (RSD, %). The precision in reproducibility conditions was estimated by analysing a pooled test soil sample for 3 months by two analysts using different reagent solutions but the same instrument. Samples were analysed in duplicate each day following the entire testing procedure. The mean values were used for within-lab precision estimation. The precision was presented as s_{RW} (mg/kg), and RSD_{RW} (%).

The limit of detection (LOD) and the limit of quantification (LOQ) of the measurement were determined as 3.3- and 10-times the standard deviation of the spiked analytical blank at different concentration levels: 4, 12 and 18 mg/L SO_4^{2-}. The method limit of detection (MLOD) and the method limit of quantification (MLOQ) corresponded to the lowest concentration of sulphate in the soil sample that could be detected or quantified by the proposed experimental procedure. MLOD and MLOQ account for the soil to liquid ratio in the leaching procedure and were presented in mg/kg SO_4^{2-}-S.

Trueness was estimated by bias and recovery of spiked leachate as well as by fortifying soil samples. The spiking with standard sulphate solution (standard additions) was made at different steps of the protocol: (1) spiking of soil leachate before measurement according to the protocol (1.00 mL of 200 mg/L SO_4^{2-} standard solution were added to 10.0 mL of soil leachate); (2) spiking/fortifying of soil samples before leaching step: the air-dried soil samples (weight of 5 g) were fortified with 2.50 mL of 200 mg/L standard solution of SO_4^{2-}, homogenised and left to equilibrate for 20 days. The fortified samples were then analysed according to the newly proposed protocol for exchangeable sulfate determination, described above (see the section Measurement of sulphate concentration*)*. Each soil sample was spiked in duplicate, each leachate was analysed in triplicate, and the absorbance of each sample was measured in triplicate.

The bias was calculated as Eq. (2):

$$\text{bias} = C_1 - C_2 \tag{2}$$

where: C_1 – determined concentration of the spike; C_2 – theoretical concentration of the spike.

The recovery (R, %) of the spike was calculated using the Eq. (3):

$$R = \frac{C_{ss} - C_s}{C_s} \times 100 \tag{3}$$

where: C_{ss} – sum of concentrations of the sample and the spike; C_s – concentration of the sample.

The second series of experiments included fortification of the original soil samples by the addition of a sulfate standard solution. Recovery of the fortification was estimated using the Eq. (4):

$$R = \frac{C_{fs} - C_s}{C_f} \times 100 \tag{4}$$

where: C_{fs} – concentrations of the fortified sample (sum of originally presented and added sulphate in the sample); C_s – concentration of the sample; C_f – final concentration of the added sulphate standard solution for sample fortification.

Uncertainty was estimated applying two approaches: (1) a mathematical model and (2) a single laboratory validation and control chart (Magnusson and Örnemark 2014, Kruve et al. 2015).

(1) Mathematical model approach: a soil sample containing 32 mg/kg sulphate sulfur was analysed in duplicate, applying the proposed protocol. The mathematical model was presented in Eq. (1).

The equation calculated the uncertainty:

$$\begin{aligned} u^2(C_{S-SO_4}) &= u^2(A, Spiked\ sample) + \\ &+ u^2(A, Spiked\ blank) + u^2(slope) + \\ &+ u^2(V) + u^2(m) + u^2(C_{DC}) \end{aligned} \tag{5}$$

The standard uncertainty of the spiked sample and spiked blank was calculated as standard deviation in repeatability conditions following the full protocol from sulphate extraction to absorbance measurement and concentration calculation according to the abovementioned equations. The Kragten approach was applied for combined standard uncertainty calculation.

(2) Single laboratory and control chart approach:

The combined standard uncertainty was estimated as Eq. (6):

$$u_c = \sqrt{u_{RW}^2 + u_{bias}^2} \tag{6}$$

The experimental data from a control chart were obtained over 4 months. A spiked soil sample was tested in duplicate on each day of the experiment, and the data for precision in reproducibility conditions (estimated as standard deviation) were used as a standard uncertainty component (u_{RW}). The standard bias uncertainty was estimated using data from a spiking leachate by standard sulphate solutions before absorbance measurements. Each spike was made in triplicate, and the precision in repeatability conditions (standard deviation) was used as the standard uncertainty of bias.

As bias was estimated using a single reference standard solution, the standard uncertainty was calculated using the Eq. (7):

$$u_{bias} = \sqrt{RMS_{bias}^2 + u_{C_{ref}}^2 + \frac{s}{n}} \qquad (7)$$

where: RMS_{bias} – used for averaging the bias values obtained on different days spread over four months; s – standard deviation of bias obtained from n = 11 measurements; u (C_{ref}) was calculated as Eq. (8):

$$u_{C_{ref}} = \sqrt{u_{mass}^2 + u_{volume}^2 + u_{purity}^2} \qquad (8)$$

Standard uncertainties included in Eq. (8) were estimated as B-type uncertainties assuming a quadratic distribution model. Expanded uncertainty was calculated at a 95 % confidence level (coverage factor 2, normal distribution).

RESULTS AND DISCUSSION

The choice of leaching reagent is crucial for interpreting the analytical information and estimating sulphur availability in arable soils. The inorganic fraction, such as sulphate ion, is available to plants (Padhan et al. 2016). Sulphate ions could be found in soil solution or adsorbed on colloidal matrices (Combs et al. 2011). The soil pH, type of clay, and presence of other cations governed the leaching and adsorption of sulphate in soils (Tabatabai 1987). The sulfur deficiency is more likely to occur in sandy-textured soils with a low organic matter content (van Bijon et al. 2004). The adsorbed fraction increased especially in soils with a pH lower than 6.5 and high clay content, and is negligible at pH > 6 (Tabatabai 1987, Combs et al. 2011, Singh et al. 2011). Thus, the sulphur availability is a very complex issue due to the numerous factors that govern sulphur uptake by plants. This fact originates from a challenging question of soil testing procedure choice, data treatment, and interpretation (Padhan et al. 2016, Zbiral et al. 2018). To estimate the stability of the method behaviour, soils with different characteristics were used: clay content from 1–17%; pH 4.73–8.28; organic carbon 0.6–4.5%; mineral N 8–18 mg/kg; exchangeable K 55–300 mg/kg; exchangeable P 3–37 mg/kg (Table 1).

Analytical performance of the proposed protocol

Linear concentration range and sensitivity. The calibration function (Figure 1A) obtained by the method of the external standard was:

$A = -0.0587 + 0.0236 \times C(SO_4^{2-})$ ($r^2 = 0.9993$; $N = 11$). The plot of residuals (Figure 1B) showed the homoscedastic nature of residuals; hence, the linear concentration range was defined as 2–70 mg/L SO_4^{2-}. Sensitivity presented the gradient of the calibration function and was determined from the slope of the linear calibration curve: 0.017 L/mg.

Precision. Firstly, the precision of the spiked analytical blank was studied at two concentration levels: 4 and 12 mg/L. The results showed that the precision of the spiked blank samples depended on the concentration at the studied levels. The precision in the conditions of repeatability of the spiked blank was RSD 12% and 0.8% ($N = 3$) at 4 and 13 mg/L, respectively. The precision in the reproducibility conditions, as RSD, was 14% and 3.6% ($N = 6$) at 4 and 13 mg/L, respectively. Thus, spiking at a 13 mg/L concentration level was chosen to study the method characteristics further. Additionally, the precision of the proposed analytical protocol was estimated by analysing soil samples according to the proposed protocol under conditions of repeatability. Each soil

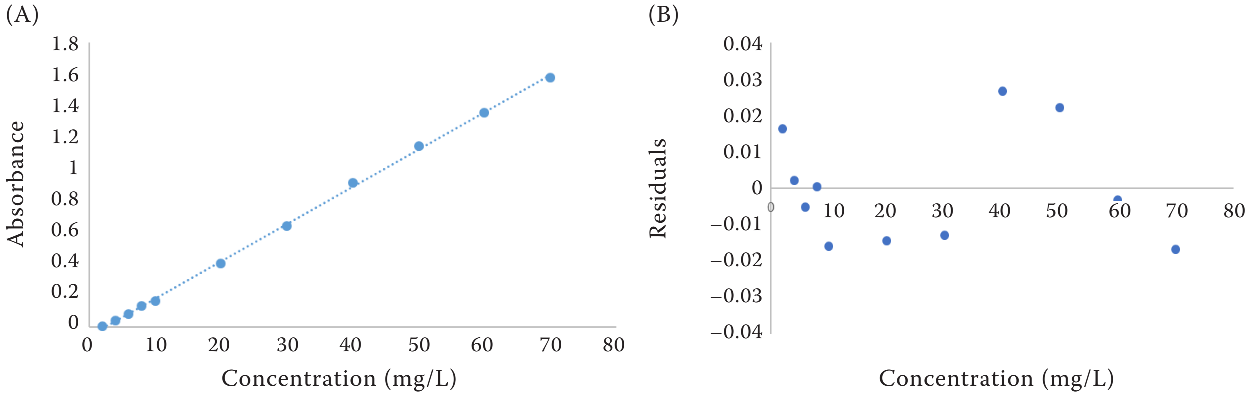

Figure 1. Calibration curve of SO_4^{2-} at λ = 450 nm, b = 2 cm (A) and plot of residuals (B)

sample was analysed in duplicates, and each leachate was analysed in triplicate: RSD = 2.5 and 0.5% (*N* = 9) in the concentration range 12–55 mg/kg. Precision in reproducibility conditions RSD varied from 3% to 1% (*N* = 12) in the 20–55 mg/kg concentration range.

Limit of detection and limit of quantification. The unknown concentration was calculated as a difference between the results of the spiked analytical blank and the spiked soil leachate (Eq. 1). The same spike was used for blank and soil samples. This approach enabled the determination of lower sulphate content in soil samples. It was because of the specificity of the turbidimetric reaction. Formation of $BaSO_4$ crystals at low activities of sulphate ions was limited by the initialisation of the crystallisation (Combs et al. 2011). Introducing additional quantities of sulphate ions into solutions supports the precipitation reaction and enhances the formation of $BaSO_4$ crystals. In our previous experiments, it was observed that sulphate content in soils lower than 5 mg/kg (found by ICP-OES measurements of leachates) did not initiate precipitation reaction and resulted in an underestimation of sulphate content in soil samples. Thus, we proceeded by adopting the idea of Combs et al. (2011) to the Sufaver 4 Method (Hach Lange, Loveland, USA). Additionally, to minimise the interference of organic sulphur in the soil leachate, the samples were pretreated with activated charcoal. Thus, the precision of the spiked analytical blank determined the lowest detectable and quantifiable concentrations by the proposed protocol.

The spiking of blank samples at a 12 mg/L concentration level resulted in lower LOD (0.33 mg/L) and LOQ (1.0 mg/L), as well as lower MLOD (1.7 mg/kg SO_4^{2-}-S) and MLOQ (5.0 mg/kg SO_4^{2-}-S) of the proposed protocol. It was chosen in the recommended protocol (Table 2). The critical values of 9–10 mg/kg as SO_4^{2-}-S were reported (Sahrawat et al. 2009, Kulhanek et al. 2018, Zbiral et al. 2018). Thus, MLOD and MLOQ were lower than critical values, satisfying the agronomic application of the protocol proposed in this study.

It should be emphasised that the proposed protocol was limited to concentrations lower than 55 mg/L sulfate in soil leachates, and the samples should be appropriately diluted before sulfate measurement.

Trueness. The method's trueness was estimated by the bias and recovery of spiked samples at different concentration levels and soil types (Table 3). The mean recovery of the leachate spike was 98 ± 2% (*N* = 20, P = 95%) and presented the bias of the turbidimetric measurement. The mean recovery of fortified samples was 103 ± 18% (*N* = 4, *P* = 95%). It should be mentioned that fortifying the original sample, although recommended as a bias estimation approach in case of lack of appropriate CRM, suffered from some limitations (Kruve et al. 2015). The state of added sulphate to the soil sample could significantly differ from the state of the indigenous sulphate in the soil, even after prolonged exposure.

For comparison, the concentration of extractable sulphates in the soil leachates was determined by ICP-OES. The results (Figure 2) showed a positive high correlation between the data, with a difference of 0–5 mg/kg and R^2 = 0.997 (*N* = 12). However, it should be noted that the mean values statistically coincided within the confidence limits (Table 4).

Table 2. Precision and limit of detection and limit of quantification of the proposed protocol for turbidimetric determination of sulfate in soil

Concentration (mg/L)	Standard deviation (*N*)		LOD	LOQ	MLOD	MLOQ
	repeatability	reprodcubility	(mg/L)		(mg/kg)	
Spiked blank						
4	0.58 (3)	0.27 (12)	1.9	5.7	9.5	28
12	0.10 (3)	0.45 (6)	0.33	1.0	1.7	5.0
18	0.17 (3)	na	0.5	1.7	2.9	8.7
Soil samples						
32.0	0.8 (9)	0.95 (17)				
12.6	0.8 (9)	0.53 (12)				
20.8	0.61 (9)	0.66 (10)				
51.8	0.27 (6)	0.53 (12)				

N – number of measurement; na – not available; LOD – limit of detection; LOQ – limit of quantification; MLOD – method limit of detection; MLOQ – method limit of quantification

Table 2. Recovery study of the proposed protocol using standard addition before (fortification) and after (spiking) the sample preparation step

No. of sample	SO_4^{2-} in soil leachate	SO_4^{2-} in spiked soil leachate	Spike concentration	Recovery (%)	s (*N*)
	(mg/L)				
Spiking of leachate before measurement					
4	12	32	20	100	3.8 (8)
5	32	50	20	90	1.3 (4)
6	3	20	20	85	1.8 (8)
7	4.2	23	20	90	4.9 (8)
12	3	22	20	97	2.6 (6)
	SO_4^{2-}-S in soil	SO_4^{2-}-S in fortified soil	fortification level	Recovery (%)	s (*N*)
	(mg/kg)				
Fortifying of soil samples					
4	20.8	46.6	26.9	96	3(6)
5	51.8	93.6	52.8	80	2 (6)
7	5.6	21.2	13.2	118	2 (6)
12	5.6	21.0	12.9	119	0 (3)

s – standard deviation of recovery values; *N* – number of measurements

Stability. The method's robustness presented the stability of the method's performance with minor deliberate variations of the conditions (Magnussen and Örnemark 2014). The precision in repeatability conditions of the volume of spikes was crucial for the method's performance. To estimate the robustness of the method against variation of the spike, the following parameters were varied: volume of the spike (0.200 and 1 000 mL), concentration of spiking solution (560, 200 and 112 mg/L), and analyst (3 persons). Two soil samples at concentration levels from 12 and 32 mg/kg SO_4^{2-}-S were analysed in duplicates, and each leachate was analysed in triplicate during the stability study over 30 days. The RSD was between 2.7% and 5.7% for 12 and 32 mg/kg concentrations. Hence, the RSD varied relatively narrowly, and the method performance could be considered stable. However, the repeatability of the spike volume should be carefully controlled. The control charts for spiked blanks could be applied to keep critical parameters under control.

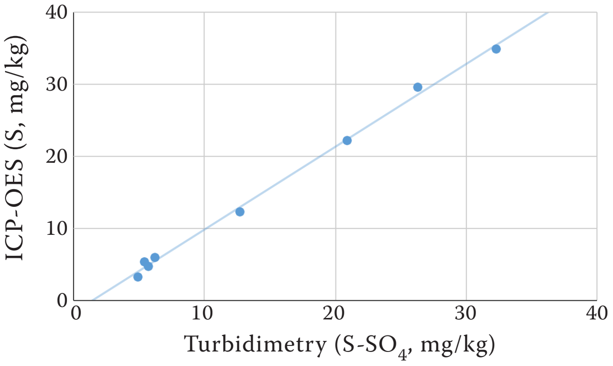

Figure 2. Correlation between results from turbidimetry and inductively coupled plasma optical emission spectrometry (ICP-OES) methods for exchangeable sulfur determination

Uncertainty. Figure 2 presents the cause-and-effect diagram following the mathematical model's approach. Standard uncertainty of the spiked sample and spiked blank was calculated as standard deviation in repeatability conditions following the full protocol from sulphate leaching to absorbance measurement and concentration calculation according to Eq. (1).

The factors that mainly contributed to the uncertainty were found to be the uncertainty of slope (61%), blank repeatability (25%) and volume measurement (12%). The standard uncertainty was 1.16 mg/kg SO_4^{2-}-S. The expanded uncertainty of the proposed protocol for determination of exchangeable sulphate in soil samples was 2.3 mg/kg SO_4^{2-}-S (K = 2, norm.). Relative expanded uncertainty was 6.8%. Based on the approach of single-laboratory validation and control charts, the expanded uncertainty was found to have the same value.

In summary, the newly proposed protocol based on $CaCl_2$ extraction and turbidimetric detection offered a working range from 2–55 mg/L SO_4^{2-}-S, MLOD

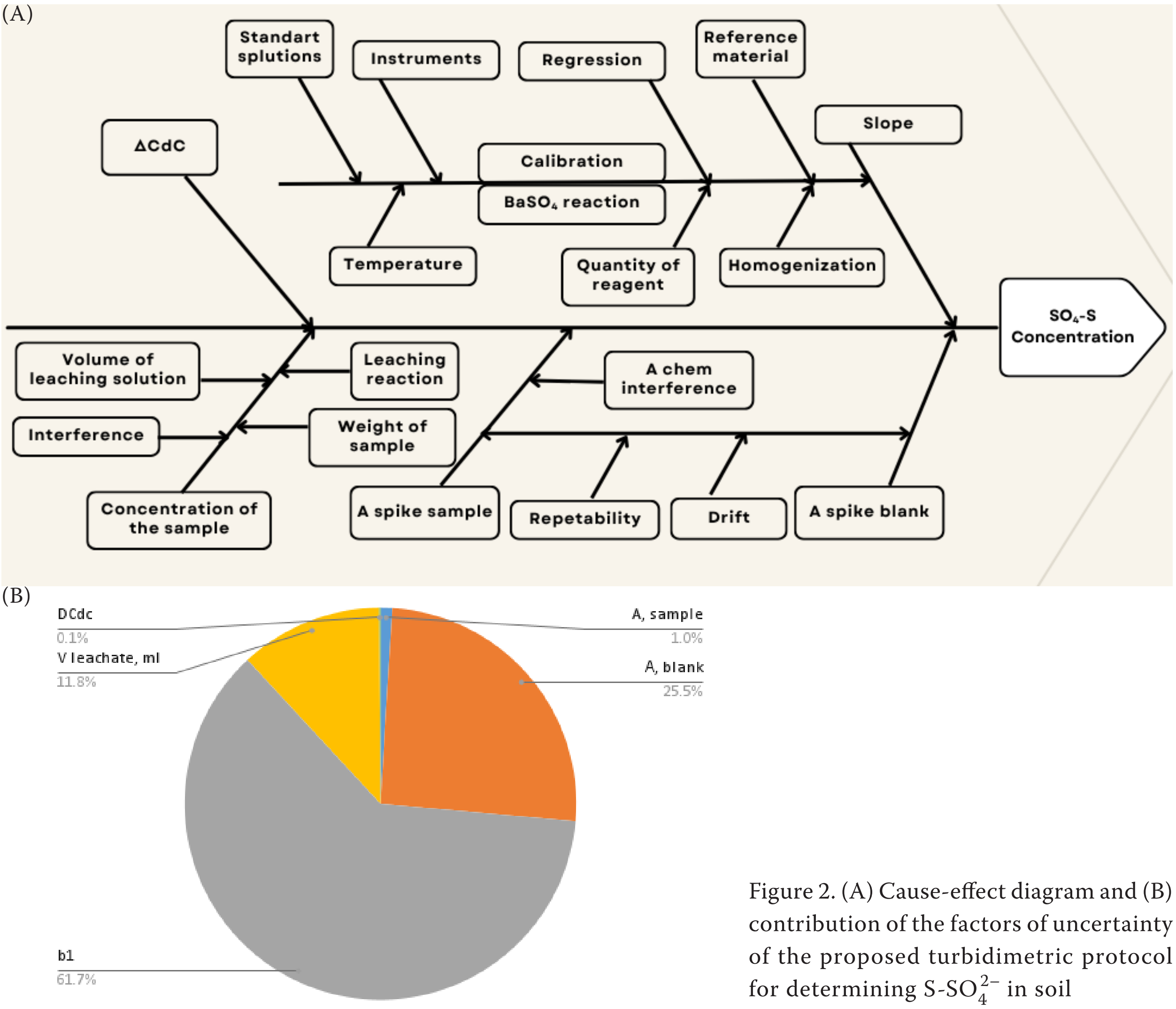

Figure 2. (A) Cause-effect diagram and (B) contribution of the factors of uncertainty of the proposed turbidimetric protocol for determining S-SO_4^{2-} in soil

Table 4. Comparison of sulfur (as water leachable sulfate) concentrations in the studied soil samples, obtained by the proposed turbidimetric protocol and inductively coupled plasma optical emission spectrometry (ICP-OES)

No.	S-SO_4^{2-} (mg/kg)		
	Turbidimetry (SD, *N* = 9)	ICP-OES (SD, *N* = 2)	bias= $C_T - C_{ICP\text{-}OES}$
1	32.3 (0.1)	34.9 (0.8)	–2.6
2	12.6 (0.8)	12.3 (1.1)	0.3
3	26.2 (0.8)	29.6 (1.5)	–3.4
4	20.8 (0.6)	22.2 (0.9)	–1.4
5	51.8 (0.3)	94.4 (2.1)	–42
6	4.9 (0.8)	3.26 (0.1)	1.6
7	5.6 (0.8)	4.73 (1.1)	0.9
8	5.3 (0.7)	5.36 (1.3)	–0.06
9	6.0 (0.5)	5.97(0.9)	0.03
10	14.3	17.2 (1.7)	–2.9
11	2	7.45 (3.9)	–5.45
12	6	7.25 (0.9)	–1.25
13	6	4.28 (0.2)	1.72

https://doi.org/10.17221/250/2025-PSE

Table 5. Summary of methods for bioavailable sulfur determination (published after 2009)

Extractant	Solid: liquid	Time	Detection	S Forms	Advantages	Limitation	Note	Reference
Mehlich 3	1:10	5 min	ICP-OES	bioavailable sulfur (Sav)	simultaneous extraction of macro- and micronutrients, suitable a large-scale soil testing, where high sample throughput is demanded	limited data on S forms other than bioavailable sulfur	critical limits varies based on soil type, generally 15–20	Kulhánek et al. (2018)
			turbidimetric (Bardsley and Lancaster method)	sulfate				Magnucka et al. (2023)
Distilled or deionised water	1:20	12 h	colourimetry (turbidimetric)	soluble sulfates	suitable for field-based analysis; simple equipment needed	not suitable for precise laboratory quantification; longer preparation time than other methods	this test will only read concentrations from 5–200 mg/L (100–4 000 ppm)	Tex-145-E (2005), Asmarlaili et al. (2018)
0.15% $CaCl_2$	1:5	10 min	colourimetric (manual)	organic sulfur fractions	focus on organic sulfur mineralisation	limited field applicability; lacks robustness	depends on organic matter content	Narayan et al. (2022)
0.15% $CaCl_2$	1:10	30 min	ICP-OES	sulfate and some organic S	for India case, lower cost and easy availability	extractability depends on the soil pH	indian case, can replays MCP for soil with neutral and alkaline pH	Sahrawat et al. (2009), Shirisha et al. (2010)
0.01 mol/L $Ca(H_2PO_4)_2$ (MCP)	1:5	30 min	ICP-OES	extractable sulfur (sulfates)	cost-effective, widely used; strong correlation with crop response	limited to available sulfur; cannot quantify total sulfur		Sahrawat et al. (2009)
0.15% $CaCl_2$	na	na	na	extract mainly water soluble sulphate	not explicitly mentioned	not explicitly mentioned	extracted nearly similar amount of sulphur in black soils as $Ca(H_2PO_4)_2$	Padha et al. (2016)
0.01 mol/L Ca $(H_2PO_4)_2$ (MCP)	na	na	na	for both water soluble and adsorbed sulphate	not explicitly mentioned	not explicitly mentioned	extracted higher amount sulfur in red soils compared to $CaCl_2$	Padha et al. (2016), Sheler et al. (2012)

https://doi.org/10.17221/250/2025-PSE

Continuous Table 5. Summary of methods for bioavailable sulfur determination (published after 2009)

Extractant	Solid: liquid	Time	Detection	S Form	Advantage	Limitation	Note	Reference
0.01 mol/L Ca $(H_2PO_4)_2$ (MCP)	na	na	na	for both water soluble and adsorbed sulphate	not explicitly mentioned	not explicitly mentioned	extracted higher amount sulfur in red soils compared to $CaCl_2$	Padhan et al. (2016), Sheler et al. (2012)
0.25 mol/L H_2SO_4	1:10	1 h	turbidimetry	sulfates	simple preparation; cost-effective	limited to lab setups with spectrophotometric tools	not explicitly mentioned	Shukla et al. (2019)
Mehlich 3	1:10	5 min	ICP-OES	bioavailable sulfur (Sav)	multi-nutrient extraction; fast analysis; bioavalable forms; strong correlation with crop uptake	not suitable for low sulfur soils; some inconsistencies in correlation with plant tissue results	Mehlich 3 extractant proved its capability to reveal long-term changes in soil S	Zbíral et al. (2018), Lisowska et al. (2023)
No	pressed pellet	na	WD-XRF	total sulfur	non-destructive; rapid multi-element analysis; precise for geological samples	total sulfur, interference from other minerals; calibration required for different matrices	MLOD = 5.43, RSD < 5%, bias < 10%	Zhao et al. (2020)
Combustion	na	na	elemental analyser, IR spectrometry	total sulfur	no sample preparation, easy operation	total sulfur	RSD >10%, bias < 8%	Zhao et al. (2020)
No	na	na	WD-XRF	sulfate sulfur	non-destructive; rapid multi-element analysis	sofisticated instruments, well trained personal, high instrumental cost	in combination of PLA regression	Sverchkov and Gvozdetskaya (2024)
0.03 mol/L CH_3COOH	1:10	30 min	ICP-OES	sulfate sulfur	bioavailable forms, total sulfur determination after appropriate sample pre-treament, fast measurement	relatively high instrumental cost, well trained personal, high salt effect	na	Tabak et al. (2019)
0.25 mol/L KCl	na	3 h, 40 °C	ICP-OES	sulfate and organic S	suitable for low S concentrations	total concentration in soil leachates	compared to MCP method measure higher quantities of labile organic S	Andreson et al. (2013)
0.01 mol/L Ca $(H_2PO_4)_2$ (MCP)	na	3 h, 25 °C	ICP-OES	sulfate and organic S	suitable for low S concentrations	total concentration in soil leachates	compared to KCl-40 method extract smaller `uanties of organis S	Andreson et al. (2013)
0.15% $CaCl_2$	1:5	30 min	turbidimetric	water soluble sulfates	bioavailbale sulfur, simple, cost effective, easy available equipment, basic trained personal	working range 2–55 mg/L sulfate in soil leachate, limited to lab setups	MLOD = 1.7 mg/kg; MLOQ = 5.0 mg/kg	this method

Table 6. Sulfate sulphur in arable soil from different regions in Bulgaria

	N	% of samples			SO_4^{2-} (mg/kg)			SAI		
		< 10 mg/kg	10–20 mg/kg	> 20 mg/kg	max	min	mean	max	min	mean
2024	146	53	35	12	35	1	9	14	0.7	4.4
2023	228	86	14	0	17	1	5	9.8	1.7	3.7
2022	59	64	18	18	36	4	11	18	1.9	7.4
2021	113	92	5	3	24	2	3	14	1.9	3.9

N – number of samples; critical levels: 10 mg/kg deficient; 10–20 mg/kg adequate; > 20 mg/kg high; SIA – sulphur availability index, calculated according to Padhan et al. (2016); soil status: SAI < 6.0 low; SAI between 6.0 and 9.0 medium; SAI > 9.0 high (Brajendra and Sarma 2016)

and MLOQ were lower than critical values of available sulphate, the expanded uncertainty was 2.3 mg/kg. Thus, the proposed protocol was estimated to be "fit-to-purpose" for determining water-soluble sulphate sulphur in arable soils. The limitations of the proposed protocol are related to the repeatability of conditions of turbidimetric reaction and measurements (Singh et al. 2011). However, it should be noted that RSD of only 6% was observed in 30 days, demonstrating the stability of the method performance in routine laboratory work.

Methods comparison

An in-depth review of methods for determining bioavailable sulphur in soils was presented in Esmel et al. (2010). Until then, different extractants, procedures and detection techniques have been applied to estimate the sulphur status in arable soils and establish adequate critical limits for improved fertilisation programs (Table 5). Due to the well-recognised importance of adequate monitoring programs for soil diagnostics, as well as governmental support for sustainable agriculture, more farmers are interested in soil analysis, and the number of soil samples continuously increases. Thus, ICP-OES after Mehlich 3 extraction has been frequently used (Kulhánek et al. 2018, Zbiral et al. 2018, Lisowska et al. 2023). The method's main advantages are simultaneous extraction and determination of macro-, mezzo- and micronutrients, as well as a high sample throughput. The main drawbacks could be mentioned: the high cost of the equipment and the determination of the sulphur pool. In some cases, detailed agronomical calibration or specific correlation studies are needed. Compared with the ICP-OES method, the turbidimetric method with $CaCl_2$ leaching appeared flexible, easy to use, and cost-effective. Moreover, the water-soluble sulphate and partially adsorbed sulphate could be determined. It should be pointed out that in some soil types, leaching by $CaCl_2$ reagent (known to extract mainly sulphate in soil solution) and by $Ca(H_2PO_4)_2$ (MCP) (known to extract in addition adsorbed sulphate) showed the same results (Padhan et al. 2016). One of the advantages of the turbidimetric protocol presented here is that the approach could be applied to different leaching reagents, including MCP, after appropriately adjusting the sulphate quantity of the spike.

The latest innovation in soil analysis for sulphate combines wave-dispersed X-ray fluorescence spectroscopy (WD-XRF) with appropriate statistical data treatment (Sverchkov and Gvozdetskaya 2024). The authors demonstrated that WD-XRF could be applied for non-destructive speciation of sulfate-sulfur in soil samples. However, only the total sulphate in soil could be estimated. The novel method has not been agronomically calibrated, but combined with different machine learning approaches could propose a versatile tool for the future estimation of bioavailable sulphur in soil samples. The important advantages of the turbidimetric method with $CaCl_2$ extraction, especially for low-scale analysis of bioavailable sulphate, are the low initial and operational costs. Considering the initial cost of acquiring equipment and training personnel, turbidimetry has a 10 times lower cost than the ICP-OES technique. The turbidimetry does not need additional instrumental costs, unlike ICP-OES, which requires regular supply with high-purity gases. If we consider the costs of reagents for sulphate leaching from soil samples, calcium chloride appeared the same as for the KCl reagent, 2.5 times lower than the MCP and 7 times lower than the Mehlich 3 reagent. Thus, turbidimetry with $CaCl_2$ extraction appeared to be the cost-effective method for determining bioavailable sulphate in arable soils.

Application to real samples

The proposed testing protocol was applied to monitor the water-soluble sulphate in arable soils from different agricultural regions in Bulgaria. In four years, 546 soil samples were analysed.

The results (Table 6) showed that, on average, 74% of the soil samples showed a deficiency of available sulphur; 18% were at a moderate level, and only 8% had high sulphur availability to plants. The mean sulphur availability index (SAI) was lower than 6.0, indicating a low sulphur nutrient status of the studied soils in the stated period. The exception was SAI in 2022, where the mean value was between 6.0 and 9.0, indicating medium sulphur availability; however, it should be noted that the number of samples was low. The trend of high sulphur deficiency in the studied samples was well seen. However, based on the critical values approach over the years, it could be seen that the percentage of deficient samples decreased and that of adequate status increased. It may be due to an appropriate fertilisation program using appropriate sulphur-containing fertilisers.

Thus, the presented results demonstrate the need for external supplementation through appropriate fertilisers. However, the blending technology is based on the estimation of current needs of each region and specific field through regular laboratory testing. Thus, the availability of laboratory analysis to a broader group of farmers could contribute to effective fertilisation programs. The proposed method was cheap and fast, used simple equipment and procedures, and was easily adopted in the regular laboratory. At the same time, it showed characteristics suitable for the estimation of water-soluble sulphate in arable soils.

Acknowledgement. We thank Mr Yoan-Chritian Nekov and Mr Vencislav Nekov ("Sembodja" Ltd, Bulgaria) for providing soil samples and valuable support for this study.

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Received: June 6, 2025
Accepted: September 9, 2025