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Potential of Mehlich 3 method for extracting plant available sulfur in the Czech agricultural soils

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ABSTRACT

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Mehlich 3 is an extractant used worldwide for extracting bioavailable nutrients in soils; however, its extraction abilities for sulfur (S) are still not well described. The aim of this preliminary study was to compare the results of Mehlich 3 determined soil S fraction (S_{M3}) with the results of sulfur fractionation, mainly focusing on bioavailable S (S_{av} – sum of water-extractable (S_w) and adsorbed (S_{ads}) sulfur). Air dried soil samples from commonly used agricultural soils were chosen for the analyses. The following S fractions were determined: (i) S_w; (ii) S_{ads}; (iii) S_{av}; (iv) 1 mol/L HCl extractable (S_{HCl}); (v) estersulfate (S_{es}); (vi) organic (S_{org}) and (vii) total (S_{tot}). The median value of S_{M3} (18.3 mg/kg) was similar to S_{av} (17.9 mg/kg). From the correlation and regression analysis it is clear that S_{M3} results are in close relationship with S_{av} form. On the other hand, the relationships between S_{M3} and organic S (including S_{HCl}) were very weak. Based on the obtained results it can be concluded that Mehlich 3 method has a good potential to determine bioavailable sulfur in commonly used agricultural soils. However, especially the plant response should be further studied to confirm this theory.

Keywords: sulfur bioavailability; macronutrient; soil extractant; soil testing; soil tests comparison

Soil testing plays a vital part in providing field-based nutrient-management feedback to growers. To improve responses from nutrient inputs and to minimize environmental degradation, cost-effective, rapid soil tests are required to enable site-specific recommendations (Ostatek-Boczynski and Lee-Steere 2012). Universal soil extractants are being used routinely in soil test laboratories engaged in soil fertility evaluation, mainly to improve efficiency and applicability to a wide range of soils. Mehlich 3 method (M3) is widely used since it is capable of easily and rapidly determining the elements, phosphorus, potassium, calcium, magnesium, sodium, boron, copper, iron, manganese and zinc, and it is an extractant suitable for all acid soils and soilless mixtures (Jones 1990, Rao and Sharma 1997, Monterroso et al. 1999). Mehlich (1984) proposed a solution, M3, to expand utility to a wide range of soil types. It is widely used, particularly in the United States as well as in some European, South American, Australian and Asian countries for both, calcareous and non-calcareous soils (Gartley et al. 2002, Ring et al. 2004, Kulhánek et al. 2009, Bortolon et al. 2011, Rayment and Lyons 2012, Kulhánek et al. 2014).

However, there is only a few studies focused on M3 as an extractant for sulfur (Rao and Sharma 1997, Matula 1999, Ketterings et al. 2011, Kowalenko et al. 2014, Zbíral et al. 2018). It is probably due to the fact that sulfur was an underestimated macroelement because of a good S supply from atmospheric deposition and wide use of S-containing

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fertilizers almost until the end of the 20th century (Scherer 2001, 2009). However, the content of mineral sulfur forms in soil rapidly decreased in the last three decades (Balík et al. 2009, Scherer 2009). Therefore, mineral sulfur deficiency starts to be an actual problem in many locations (Eriksen 2005, Lehmann et al. 2008, Balík et al. 2009, Scherer 2009, Kulhánek et al. 2016). Because of these facts, relatively new methods have been developed and further improved for extracting of bioavailable and other soil sulfur forms (e.g., Shan et al. 1992, Blair et al. 1993, Morche 2008, Förster et al. 2012). Yet, most of these methods are focused only for sulfur determining and because of that, they are usually more expensive in comparison to multi-elemental methods. Therefore, it is obvious to compare the results of these methods with M3, which, in case of good correlations, can be further used for determining of bioavailable S forms with the advantage of simultaneous measurement with other macro and micronutrients.

It has been reported that anions, such as acetate and nitrate, are capable to extract S from the soils. The ability for replacing SO_4^{2-} tends to be low as compared to the phosphate (PO_4^{3-}) anion (Chao and Thomas 1963). The M3 extractant contains acetate and nitrate anions, hence, the possibility of using this extractant for S cannot be ruled out (Rao and Sharma 1997). On the other hand, some of the extractants focused on mineral or directly bioavailable sulfur can extract significant amounts of organic S (Alewell 1993). Because of this, it is important to know the relationships between M3-determined S and organic sulfur compounds.

The aim of this research is to compare the results of M3-determined sulfur with different sulfur fractions in the set of soil samples taken up from the sites with a wide range of pH values as well as the sulfur contents. Due to the use of correlation analysis, it will be possible to estimate the sulfur form, which is in the closest relationship to the M3 method. Based on the results it will be possible to predict the potential of M3 to extract plant available S.

MATERIAL AND METHODS

Experimental setup. Archive, air dried (< 2 mm) soil samples from the long-term field experiments of the Czech University of Life Sciences in Prague and the Central Institute of Supervising and Testing in Agriculture in Brno were chosen for the analyses. These samples represent a common range of agronomy-used soil types, fertilizing treatments, as well as the high range of water-extractable S contents and pH values (Table 1, Figure 1). Total number of samples analysed was 98. All of them were taken up after the harvest of currently grown crops (summer/autumn) as it is usually done by collecting the samples for determining other nutrients using M3.

Laboratory analysis. Soil texture was determined using the areometric method according to Casagrande and Loos (1934). The following analyses were realized: C_{org} (total organic carbon content in soil) – oxidation with potassium dichromate and sulfuric acid (ISO 14235, 1998). N_{tot} (total nitrogen content in soil) – Kjeldahl method (ISO 11261, 1995). The pH values – 0.01 mol/L $CaCl_2$ extracts (ISO 10390, 2005).

For the contents of S_w (water-extractable (readily available) S); S_{ads} (adsorbed S) and S_{HCl} (0.1 mol/L HCl extractable S) – the fractionation after Morche (2008) was used: a soil sample was firstly extracted with demineralized water (1:10 v/w), followed with 0.032 mol/L NaH_2PO_4 (1:10 w/v) and 1 mol/L HCl (1:20 w/v) extraction. For the S_{HI} (hydroiodic acid reducible S), the method after Shan and Chen (1995 modified by Morche 2008) was used: 0.5 g of soil was weighed into a boiling flask. This was connected to the Jonshon-Nishita digestion system followed with addition of 15 mL of reducing solution (mixture of hydroiodic, formic and hypophosphoric acid in the ratio 4:1:2); this suspension was heated (in 140°C oil bath) for 50 min under nitrogen atmosphere. The developed H_2S gas was trapped in 10 mL of 0.05 mol/L NaOH solution and here converted to sulfate.

Total S (S_{tot}) content was determined in the digests obtained by the following decomposition procedure: Aliquots (0.5 g) of soil samples were decomposed with a mixture of 8 mL concentrated nitric acid, 5 mL of hydrochloric acid, and 2 mL of concentrated hydrofluoric acid. The mixture was heated in an Ethos 1 (MLS GmbH, Leutkirch im Allgäu, Germany) microwave-assisted wet digestion system for 33 min at 210°C. After cooling, the digest was quantitatively transferred in Teflon® vessel and evaporated to dryness at 160°C. The digest was then dissolved in a 3 mL nitric and hydrochloric acid mixture (1:3), transferred into a glass tube, filled up by deionized water and measured. The

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Table 1. Basic characteristics of the used soils

Site	*n*	Soil type	Altitude (m a.s.l.)	Average yearly		Soil texture					N_{tot}	C_{org}	pH
				temperature (°C)	rainfall (mm/m^2)	< 0.001	0.001 –0.01	0.01 –0.05	0.05 –0.25	0.25 –2			
						(mm)					(%)		
1	6	Arenic Luvisol	345	7.1	798	10.5	17.8	56.1	13.8	1.80	0.08	0.99	5.7
2	12	Haplic Luvisol	265	8.2	574	4.36	21.3	60.0	5.20	13.5	0.11	0.98	5.9
3	6	Slightly Gleyic Cambisol	472	7.4	573	6.70	13.7	16.6	18.8	44.0	0.12	0.80	5.8
4	14	Haplic Cambisol	525	7.0	665	5.84	24.0	25.4	9.40	41.2	0.09	1.24	5.1
5	6	Gleyic Fluvisol	425	7.5	535	18.1	20.7	38.5	14.1	8.70	0.12	1.03	6.1
6	6	Dystric Cambisol	645	6.1	605	5.90	16.6	20.5	25.6	21.4	0.14	1.09	5.7
7	6	Haplic Luvisol	460	7.6	606	10.8	16.9	16.1	19.3	37.0	0.14	0.88	7.1
8	6	Mesobasic Cambisol	505	7.7	632	7.90	17.8	24.5	20.9	28.9	0.13	0.99	5.5
9	6	Haplic Luvisol	196	9.2	551	17.3	14.8	26.4	30.4	11.5	0.12	1.06	6.6
10	6	Haplic Albeluvisol	290	8.0	650	10.1	18.0	61.8	8.70	1.50	0.12	0.97	5.9
11	6	Haplic Chernozem	300	8.4	581	16.2	16.0	40.9	23.9	3.10	0.12	1.34	7.2
12	12	Haplic Chernozem	286	9.1	495	3.18	17.4	56.6	6.14	19.9	0.12	1.11	7.4
13	6	Haplic Chernozem	247	8.3	454	21.7	16.5	26.6	24.0	11.2	0.17	1.53	6.4

n – number of soil samples per locality (archive samples from different fertilizing treatments and years); N_{tot} – total nitrogen content in soil; C_{org} – total organic carbon content in soil

certified reference material RM 7003 Loam was applied for the quality assurance of the analytical data. For the comparison with other fractions, M3 extraction (Mehlich 1984) was used to obtain S_{M3} (Mehlich 3 extractable S), where the soil samples were extracted using 0.2 mol/L CH_3COOH, 0.25 mol/L NH_4NO_3, 0.015 mol/L NH_4F, 0.013 mol/L HNO_3, 0.001 mol/L $(HOOCCH_2)_2NCH_2CH_2N$ (EDTA) in the ratio 1:10 (w/v) with 5 min of shaking.

The concentrations of S in soil digests and extracts were determined using the optical emission spectroscopy with inductively coupled plasma

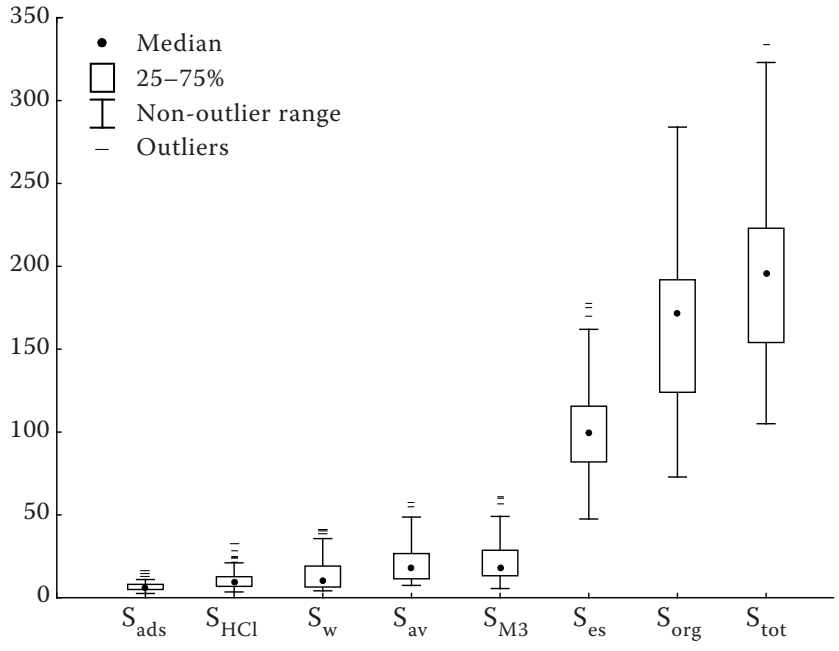


Figure 1. Basic statistical characteristics of the results (values in mg/kg) (*n* = 98). S_{ads} – adsorbed S; S_{HCl} – 0.1 mol/L HCl extractable S; S_w – water-extractable (readily available) S; S_{av} – bioavailable S; S_{M3} – Mehlich 3 extractable S; S_{es} – estersulfate S; S_{org} – organic S; S_{tot} – total S

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(ICP-OES) with axial plasma configuration, Varian, VistaPro, equipped with autosampler SPS-5 (Mulgrave, Australia). Operating measurement wavelength for ICP-OES was 180.7 nm for S. The measurement conditions were: power 1.2 kW, plasma flow 15.0 L/min, auxiliary flow 0.75 L/min, nebulizer flow 0.9 L/min.

Measurement using ICP-OES was chosen intentionally in this research because it is commonly used for analysis of M3 extracts allowing simultaneous extraction of bioavailable macro and micronutrients in soil. This is the main advantage of M3 against other extractants specialized on S only. On the other hand, ICP-OES measures also some part of organic S in extracts focused on inorganic S only. The ratio of organic S in S_w and S_{ads} extracts is usually very low, but in S_{HCl} extracts on non-calcareous soils it is usually significant. Because of that, ion chromatography (IC) (Kowalenko and Grimmett 2007) or capillary electrophoresis (Zbíral 1998, 1999) are commonly used for the precise measurement of the inorganic S.

Derived measures and data analysis. The content of S_{av} (bioavailable S) was calculated as the sum of S_w and S_{ads}, because water soluble and adsorbed S are generally believed to be immediate S source for plants (Förster et al. 2012), the content of S_{es} (estersulfate S) sulfur as $S_{es} = S_{HI} - S_{av}$ and the approximate content of S_{org} as $S_{org} = S_{tot} - (S_{av} + S_{HCl})$, respectively.

For the basic statistical evaluation (medians, averages, linear regression), Excel (2016) was used and for further analyses (box-plots, normality of distribution and Pearson's correlation analysis) Statistica ver. 13.2 (Dell software 2016) was used.

RESULTS AND DISCUSSION

Descriptive statistics. Figure 1 represents the basic statistical evaluation of the obtained results. The median values of extracted S increased in the order $S_{ads} < S_{HCl} < S_w < S_{av} < S_{M3} < S_{es} < S_{org} < S_{tot}$, and the contents reached 6.15, 9.41, 10.3, 18.0, 18.2, 99.7, 171 and 196 mg S/kg, respectively. The interesting fact is that the value determined using the M3 extractant corresponds closely with the content of bioavailable sulfur, where the value obtained with M3 was only by 0.2 mg S/kg higher in comparison to S_{av}. The content of S_{av} represented 9.19% of S_{tot}. The sum of all determined S forms (except S_{tot}) reached the higher values than S_{tot}. It was caused by the extraction methods overlapping; e.g. S_{es} represents the significant part of S_{org}.

Correlation analysis. The most important part of this research was to evaluate the relationships between M3 and other determined S forms. For this purpose, the correlation and regression analysis was carried out. The results of the Pearson's correlation analysis are mentioned in Table 2. From the obtained results, it is clear that the S_{M3} values corresponded well with the contents of S_{ads} and S_w, where the correlation coefficients reached 0.73 (with S_{ads}) and 0.886 (with S_w), respectively. Therefore the correlations between S_{M3} and S_{av} were also very high (0.882). On the other hand, the relationships between the S_{M3} and organic S compounds were weak. The correlation coefficient between S_{M3} and S_{org} was 0.349 (with lower significance $P \leq 0.01$) and with S_{es} reached the nonsignificant value 0.112, respectively. The significance increased by comparison of S_{M3} with

Table 2. Pearson correlation coefficients between the sulfur (S) fractions (n = 98)

	S_w	S_{ads}	S_{av}	S_{HCl}	S_{M3}	S_{org}	S_{es}
S_{ads}	0.809***						
S_{av}	0.992***	0.877***					
S_{HCl}	0.274**	0.297**	0.288**				
S_{M3}	0.886***	0.731***	0.882***	0.391***			
S_{org}	0.248*	0.066	0.217*	0.406***	0.349**		
S_{es}	0.076	–0.099	0.040	0.413***	0.112	0.761***	
S_{tot}	0.436***	0.255*	0.411***	0.515***	0.517***	0.975***	0.728***

*P < 0.05; **P < 0.01; ***P < 0.001; S_{ads} – adsorbed S; S_{av} – bioavailable S; S_{HCl} – 0.1 mol/L HCl extractable S; S_{M3} – Mehlich 3 extractable S; S_{org} – organic S; S_{es} – estersulfate S; S_{tot} – total S; S_w – water-extractable (readily available) S

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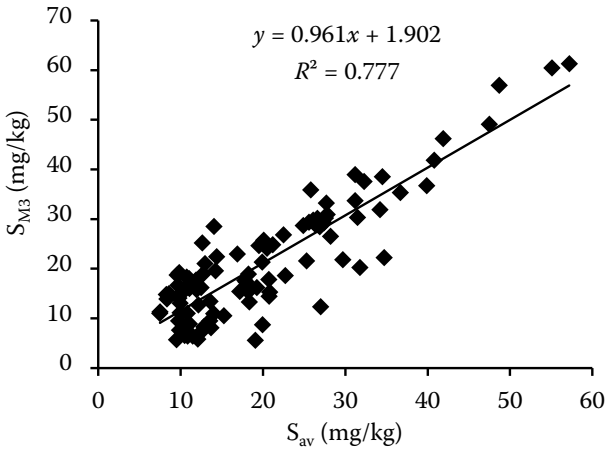


Figure 2. Regression analysis between S_{M3} (Mehlich 3 extractable S) and S_{av} (bioavailable S) (n = 98)

S_{tot} (r = 0.517). It was probably caused due to the fact that S_{tot} contains also inorganic S forms, which showed a close relationship with S_{M3}. The correlations between S_{M3} and S_{HCl} were significant at $P < 0.001$, but the r value was only 0.391. It has been described in many studies that a significant part of organic S is extracted by hydrochloric acid (discussed further). Further analyses confirmed highly significant relationships of S_{tot} with S_{org}, as well as with S_{es}. The correlations between S_{org} and S_{es} were also very high.

Regression analysis. It was carried out to confirm the results of correlation analysis (Figure 2, Table 3). Based on the main aim of this study, the relationships between S_{M3} and other soil S fractions are shown here. From the comparison of exponential, logarithmic, polynomic and linear regression, the last one showed the best results in most of the cases. Figure 2 shows the relationship between S_{M3} and S_{av}. This confirmed the results from the previously mentioned correlation analysis. The regression coefficient R^2 = 0.777 confirms very close relationships between M3-determined S and bioavailable S. With 77.7% of reliability it can be also calculated that $S_{M3} = 0.961 \times S_{av} + 1.902$.

The following results of regression analysis between S_{M3} and other S fractions are presented in Table 3. The results correspond with the data obtained by correlation analysis. It confirms the ability of M3 extractant to determine the bioavailable S forms due to the close relationships with both, S_w and S_{ads}, respectively. On the other hand, regression relationships with organic S forms and S_{HCl} are very weak.

DISCUSSION

According to the previous studies, readily available sulfur is in form of the SO_4^{2-} ions dissolved in the soil solution. The main ratio of these ions can be extracted with distilled water and other weak solutions, e.g., $CaCl_2$ or $LiCl_2$ (Walker and Doomenbal 1972, Tabatabai 1982). However, this form is not the only one that can be taken up with plants. There are also sulfates bound with the weak binds on the soil particles – adsorbed S. These can be usually easily released into the soil solution and subsequently taken up with plant roots. Therefore, these ions are also believed to be plant available (Förster et al. 2012). Because of that, the plant available sulfur content represents the sum of S_w and S_{ads}, which also corresponds with many literature sources (Bohn et al. 1986, Tisdale et al. 1993). The majority of the sulfur taken up by plants is derived from the soil organic S pool (Boye et al. 2010), from which S is transformed in adsorbed and water-extractable forms as well. Because of that, organic S pool is also very important (especially from the long-term point of view); however, it could not be directly taken as a bioavailable pool.

From the obtained data it is clear that S_{av} represents only a small part of the total sulfur. As

Table 3. The results of regression analysis between S_{M3} (y value) and other sulfur (S) fractions

	a	b	R^2		a	b	R^2
S_{M3}:S_W	1.184	5.274	0.784	S_{M3}:S_{es}	0.043	16.94	0.013
S_{M3}:S_{ads}	3.659	–3.021	0.534	S_{M3}:S_{org}	0.081	7.782	0.122
S_{M3}:S_{HCl}	0.827	12.43	0.153	S_{M3}:S_{tot}	0.106	0.083	0.267

Linear regression parameters $y = ax + b$; a – slope; b – intercept; R^2 – regression coefficient; S_{M3} – Mehlich 3 extractable S; S_w – water-extractable (readily available) S; S_{ads} – adsorbed S; S_{HCl} – 0.1 mol/L HCl extractable S

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confirmed e.g. by Tisdale et al. (1993), Wang et al. (2006), Balík et al. (2009) or Förster et al. (2012) bioavailable (mineral sulfur) forms represents less than 10% of S_{tot}. In our case, the average value of 9.19% was obtained. The relatively higher value is probably caused due to the fact that using the ICP-OES some part of dissolved organic S is measured (Shan et al. 1992, Kowalenko and Grimmett 2007).

The interesting fact is that the average value determined by the M3 extractant (18.2 mg/kg) corresponds closely with the average content of S_{av} (18.0 mg/kg). The higher S_{M3} values were expected because the M3 is one of the strongest extractants to determine bioavailable forms of phosphorus (Kulhánek et al. 2009, Wuenscher et al. 2015) and other macroelements (Zbíral and Němec 2005, Bortolon et al. 2011, Ostatek-Boczynski and Lee-Steere 2012).

Similar relationships between S_{M3} and S_w were found in the study of Kowalenko et al. (2014). These authors evaluated 109 contrasting soils, where the average values of S_{M3} and S_w were almost the same. Based on their results it is possible to assume that water extracts similar soil S pool as M3 and multiple-element soil extraction methods show a considerable promise for soil S testing.

The order of $S_{es} < S_{org} < S_{tot}$ is logical, because S_{org} represents the main part of S_{tot} and S_{es} a large part of S_{org}. Similar distribution of soil sulfur fractions was confirmed e.g. by Morche (2008), Scherer (2009), Kulhánek et al. (2011), Förster et al. (2012).

The correlation analysis shows close relationships ($r = 0.883$) between both bioavailable S forms (S_w and S_{ads}) and S_{M3}. Therefore it is clear, that M3 extractant determines a significant amount of bioavailable S. The relationship between S_{HCl} and S_{M3} is much lower ($r = 0.391$). It is probably due to the extraction abilities of 1 mol/L HCl. This extractant was developed to determine occluded S form that is bound in Ca and Mg co-precipitated forms. This form is only possible to measure using the ion chromatography (Förster et al. 2012). Yet, in the case of ICP measurement, a significant amount of organic S-compounds is determined especially in low-carbonates soils (Shan et al. 1992). The results of Morche (2008) show that in soils with less than 1% $CaCO_3$, most of the S extracted with 1 mol/L HCl is organically bonded S. In our case, 79.6% of the tested soils had less than 1% of $CaCO_3$. Therefore, it is possible to assume that a significant ratio of the ICP-measured S_{HCl} was organically bonded S. The lower correlation coefficient between S_{HCl} and S_{M3} also shows that M3 extractant probably does not extract the significant part of organic S. It is confirmed also by lower correlations between S_{M3} and S_{org} and no significant correlation between S_{M3} and estersulfate S.

Additional observations confirmed close relationships between S_{ads} and S_w, S_{ads} and S_{av} and S_w and S_{av}. The close correlations between S_{org} and S_{tot} as well as between S_{es} and S_{tot} confirm that significant ratio of soil sulfur is organically bonded (Morche 2008, Scherer 2009, Kulhánek et al. 2011, Förster et al. 2012 and others).

As mentioned before, M3 is a widely used extractant allowing to determine simultaneously many important macro and micronutrients in different soils (Jones 1990, Rao and Sharma 1997 and others). Therefore, determination of S using M3 could represent a cost-saving way to evaluate bioavailable sulfur together with other nutrients. In this preliminary study, close relationships between S_{M3} and soil sulfur forms were found, which are described as bioavailable in the literature. In the other set of plant and soil samples taken up from fields with a wide range of different soils ($n = 152$) during vegetation, significant correlations ($r > 0.350$; $P < 0.001$) between S_{M3} content and total S content in winter wheat and winter rape were found. However, the correlations were not confirmed for maize. These data are not published here, because the further research of plant response is needed.

The study of the set of 98 samples from different commonly used agricultural soils showed that S content determined using the M3 method closely corresponds with the amounts of bioavailable S fractions. That was confirmed with both, correlation and regression analyses as well by the fact that relationships between S_{M3} and organic S fractions were usually very weak. M3 can be also evaluated as a suitable extractant for bioavailable sulfur for the commonly used arable soils. However, this manuscript is only a preliminary study and brings a lot of new questions. To confirm this preliminary study, further research of S_{M3} and S content and uptake by different plant species is needed. It is also important to further study the relationships between M3-determined soil sulfur and soil pH, organic matter content and enzymatic activity.

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