

Evaluating of soil sulphur forms changes in long-term field experiments of Látókép

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SUMMARY

The aim of this work was to evaluate the changes of different sulphur forms (soluble, adsorbed) in chernozem soil in a long-term field experiment supplied with increasing doses of NPK fertilizers for a long time. In addition, other objective of this study included the examination of the applicability of recommended extractants of the different sulphate fraction in Hungarian soils. A long-term field experiment was established at the Research Station of Látókép of the University of Debrecen in 1984. In addition to control, two levels of NPK fertilizer doses have been used with irrigated and non-irrigated variants. Winter wheat and corn were cropped in a crop rotation on plots. Soil samples were collected in three different development stages of winter wheat, at the stage of stem elongation (April), flowering (May) and ripening (June of 2018) from the topsoil (0–20 cm) of experiment plots. Water-soluble inorganic sulphate was extracted with 0.01M CaCl₂ solutions. The soluble plus adsorbed sulphate was extracted with 0.016M KH₂PO₄ solution. Sulphate was measured by turbidimetric method. 0.01M CaCl₂-SO₄²⁻ ranged between 0.293–1.896 mg kg⁻¹ and the 0.016 M KH₂PO₄-SO₄²⁻ varied between 5.087–10.261 mg kg⁻¹. The values of KH₂PO₄ SO₄²⁻ was higher than that of CaCl₂-SO₄²⁻, because KH₂PO₄ extracted the adsorbed and soluble fractions of sulphate, while CaCl₂ extracted the soluble sulphate fraction. The amount of absorbed sulphate was calculated by the differences of KH₂PO₄-SO₄²⁻ and CaCl₂-SO₄²⁻. The KH₂PO₄ characterizes mainly the adsorbed sulphate fraction much more than the water-soluble fraction. KCl is the most widely used extractant for the determination of plant available sulphate content of soil in Hungary; therefore, KCl-SO₄²⁻ fraction also was determined. The KCl-SO₄²⁻ ranged between 0.328–2.152 mg kg⁻¹. The CaCl₂-SO₄²⁻ and KCl-SO₄²⁻ fractions were compared and based on Pearson's linear correlation, moderate correlation was established ($r=0.511$) between them. In all three extractant (0.01M CaCl₂, 1M KCl, 0.016 M KH₂PO₄) higher sulphate fractions were measured in the fertilized plots where superphosphate had been supplied for ages until 2010. The arylsulphatase activity of soil also was determined and ranged between 9.284 and 26.860 μg p-nitrophenol g⁻¹ h⁻¹. The lowest value was observed in the treatment with highest NPK2 dose, both in irrigated and non-irrigated areas.

Keywords: sulphur fractions, fertilization, long-term field experiment

INTRODUCTION

Bioavailable sulphur deficiency in soils is becoming an actual problem in many sites (Eriksen, 2005; Scherer, 2009). The increased occurrence of sulphur deficiency is due to reduced concentrations of primary air pollutants, the use of concentrated-NPK low-S fertilizers, the declining use of farmyard manure and S containing fungicides and pesticides (Scherer, 2001), at a time when yields have been increasing.

Sulphur is present in soils in the form of both inorganic and organic compounds. In general, inorganic sulphur species are fractionated into water-soluble sulphate, adsorbed sulphate, dilute-HCl soluble sulphur and volatile sulphur, pyritic sulphur (Johnson et al., 1981). Organic sulphur can be divided into two distinct fractions: carbon-bonded sulphur and ester sulphur (Pirela and Tabatabai, 1988). The most important S source for plants is sulphate (SO₄²⁻) in the soil solution.

Arylsulphatases are enzymes that catalyse the hydrolysis of aromatic sulphate ester to phenols and inorganic sulphate. Sulphate esters represent a large fraction of the total S of soil and therefore, arylsulphatases may be important for mobilisation of inorganic sulphate for plant nutrition (Fitzgerald, 1976).

From the mentioned reasons it is clear that sulphur is becoming a limiting factor for the production

quality (Eriksen et al., 2004). Therefore, it is necessary to pay attention to get more information about different sulphur fractions in soil.

The aim of this study was to evaluate the changes of different sulphur forms (soluble, adsorbed) in chernozem soil in a long-term field experiment supplied with increasing doses of NPK fertilizers for a long time, considering that S containing P fertilizer (as superphosphate) was not used since 2010. Furthermore, other objective of this study was to examine and compare of different extractants that are recommended for measuring of the different sulphate fraction.

MATERIALS AND METHODS

A long-term field experiment was established at the Research Station of Látókép of the University of Debrecen in 1984, by Ruzsányi László. It is located 15 km from Debrecen in Hungary. The soil is a calcareous chernozem with 2.8–3.0 % humus content. The depth of the humus layer is 70–90 cm. Main parameters of the experimental soil are pH (CaCl₂) = 7.2; Hu% = 2.8; K_A = 37.5; AL-P₂O₅ = 394.1 mg kg⁻¹; AL-K₂O = 190.8 mg kg⁻¹; AL-Ca = 5130 mg kg⁻¹; AL-Mg = 403.0 mg kg⁻¹.

The experiment consists of treatments with increasing NPK doses with irrigated and non-irrigated variants (Table 1).

Table 1

Rates of mineral fertilizers applied at the experiment (Látókép)

Treatments		N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	K ₂ O (kg ha ⁻¹)
irrigated	control	0	0	0
	NPK1	100	70	80
	NPK2	200	140	160
non-irrigated	control	0	0	0
	NPK1	100	70	80
	NPK2	200	140	160

Each treatment consists of 46 m² plots, arranged in a randomized block design with four replications. Besides control two levels of NPK fertilizer doses have been used. Winter wheat and corn were cropped in a crop rotation on plots.

At the first part of the experiment, between 1984 and 2010, the N, P K were supplied as NH₄NO₃, superphosphate and KCl, respectively. The superphosphate is a sulphur containing fertilizer, with 10.9% sulphur content. From 2010 the superphosphate was replaced by monoammonium dihydrogen phosphate (MAP) and sulphur was not supplied any more in this area. Solid N, P and K fertilizers were mixed into the soil before sowing.

In the soil sampling year (2018) there was 25 mm rain in April, 55 mm in May and 55 mm in June, and there was no need to irrigate in the experimental area. However, irrigation of the previous years make it possible to distinguish irrigated and non-irrigated areas.

Soil samples were collected in three different development stages of winter wheat, at the stage of stem elongation (April), flowering (May) and ripening (June of 2018) from the topsoil 0–20 cm of experimental plots. Soil samples were air dried, ground and passed through a 2 mm sieve.

Water-soluble inorganic sulphate was extracted with 1M KCl and 0.01M CaCl₂ solution. The soluble plus adsorbed sulphate was extracted with 0.016M KH₂PO₄ solution. 10g soil was shaken with 25ml extractants. Extracts were filtered and sulphate was measured by turbidimetric method (Zhao and McGrath, 1994). This method based on the forming of BaSO₄ precipitate in a slightly acid medium when excess BaCl₂·2H₂O is added to a solution containing sulphate-S. For the determination of absorbed soil sulphate 0.01M CaCl₂ soluble sulphate also was determined and the absorbed sulphate was calculated with the difference of KH₂PO₄-SO₄ and CaCl₂-SO₄. The soil pH was determined in a 1:2.5 soil to 1M KCl and distilled water.

The activity of arylsulphatase was determined using *p*-nitrophenol sulphate as a substrate (Tabatabai and Bremner, 1970b). This method is based on conversation of *p*-nitrophenyl sulphate to *p*-nitrophenol and sulphate, which can be quantified by turbidimetrically.

For statistical analysis of experimental results IBM SPSS Statistics 22 and Microsoft Excel 2016

programs were used. The mean values of each treatment group were subjected to comparisons analysis using the One-Way ANOVA (significance level of $p < 0.05$) with post hoc comparisons using Tukey test and Pearson's linear correlation was used to determine the relationship between extractants. The measured values were analysed separately in three different development stages.

RESULTS AND DISCUSSION

CaCl₂ soluble SO₄²⁻ content of soil

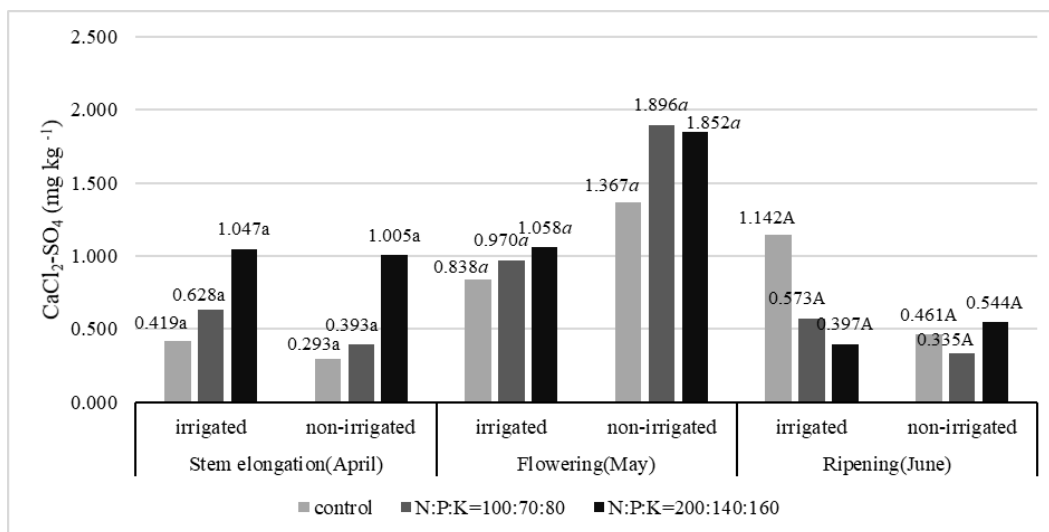
The *Figure 1* shows the result of 0.01M CaCl₂ soluble SO₄²⁻ content of soil. This extractant is a weak salt solution and it solves the water soluble inorganic fraction (Hu et al., 2005).

At the stage of stem elongation of wheat the CaCl₂ soluble SO₄²⁻ content of soil ranged between 0.419–1.047 mg kg⁻¹. The sulphate content of irrigated and non-irrigated area were almost the same. At this stage higher sulphate was measured in fertilized plots and the highest fertilizer dose (NPK2) caused the most increased CaCl₂ soluble sulphate in soil. It can be also establish, that there was no big difference between the control and NPK1 treatment at this development stage.

From April to May there was an increment in soluble sulphate of soil. At the stage of flowering the sulphate values varied between 0.838–1.896 mg kg⁻¹. The increment of sulphate in time can be explain by the fact, that comparing to May there was more than 50 mm of rain average (Net1) in April and the solubility of sulphate changed. At this time the lowest value was observed in control treatments, and the highest value was detected in NPK2 treatments in the irrigated area and in NPK1 treatment in the non-irrigated area. The sulphate content of non-irrigated area was a little bit higher than that of irrigated area. In this case, the increased fertilizer doses still enhanced the sulphate content a little bit compared to control.

From May to June, the sulphate content tended to decrease in the soil, which might be due to the more intensive nutrient uptake of plant after flowering. At the stage of ripening (measured in June), the sulphate content in fertilized plots was lower mostly in the irrigated area that can be explained by the fact, that higher yield probably resulted in higher sulphur uptake and lower sulphate content of soil compared to the control.

Figure 1: Change of 0.01M CaCl₂ soluble SO₄²⁻ of soil at different development stage of wheat



Note: data marked with the same letter is not significantly different at the significant level of p <0.05

KH₂PO₄ soluble SO₄²⁻ content of soil

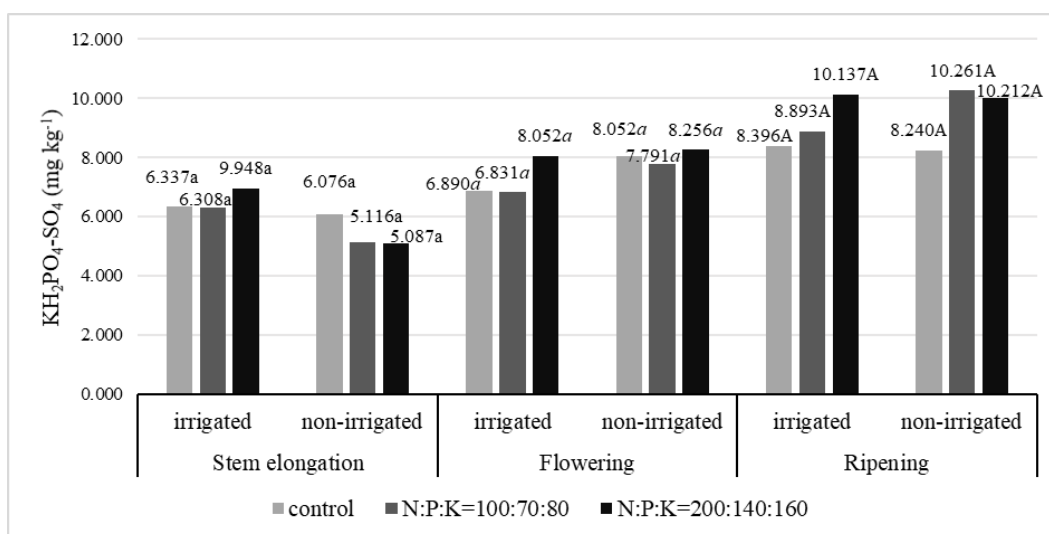
Figure 2 shows the result of KH₂PO₄ soluble SO₄²⁻ values. Based on our results, the values of KH₂PO₄-SO₄²⁻ were higher than that of 0.01M CaCl₂-SO₄²⁻, because KH₂PO₄ may extracts not only the soluble SO₄²⁻ but adsorbed fraction as well, while CaCl₂ extract soluble sulphate fraction of soil.

The KH₂PO₄ -SO₄²⁻ values was higher than values of CaCl₂-SO₄²⁻ and ranged between 5.087–10.261 mg kg⁻¹. A slight increment in the soil’s sulphate content was observed in time, which was probably

caused by the optimal condition for mineralization of the organic sulphur sources.

At the stage of stem elongation the sulphate-values varied between 5.087–6.948 mg kg⁻¹. In this time, the sulphate-content of irrigated area was a little bit higher than that of non-irrigated area. There was no high differences between the control and fertilized treatments, only the highest fertilizer dose caused a little increased sulphate value in the irrigated area. However, in the non-irrigated areas, the treatments with increasing fertilizer doses resulted in decreased sulphate-values.

Figure 2: Change of 0.016M KH₂PO₄ soluble SO₄²⁻ of soil at different development stage of wheat



Note: data marked with the same letter is not significantly different at the significant level of p <0.05

At the stage of flowering, measured in May, the sulphate-content ranged between 6.890–8.256

mg kg⁻¹. The values of the non-irrigated area was a little bit higher, than the results of irrigated area. In



this time, the value of NPK1 treatment did not differ from value of control, but the NPK2 treatment (the highest fertilizer dose) resulted a little bit higher sulphate content of soil.

The KH_2PO_4 soluble SO_4^{2-} measured at the ripening stage ranged between 8.240–10.261 mg kg^{-1} . The lowest sulphate-content was measured in control treatments. The effect of increasing fertilizer doses clearly prevailed compared to the control treatment, because this treatments resulted higher sulphate-content both in the irrigated and the non-irrigated area.

The calculated adsorbed sulphate fraction of soil

The adsorbed sulphate was calculated with the difference of values of $\text{KH}_2\text{PO}_4\text{-SO}_4$ and values of $\text{CaCl}_2\text{-SO}_4^{2-}$. The results of adsorbed sulphate content of soil is summarised in Table 2.

The adsorbed sulphate values of chernozem soil ranged between 4.333 and 9.468 mg kg^{-1} . Kulhánek et al. (2016) estimated similar values (3.80–10.6 mg kg^{-1}) on chernozem soil in a long term-field

experiments. The changes of adsorbed sulphate depending on different treatments showed almost the same tendencies as the changes of values of 0.016M KH_2PO_4 soluble SO_4 . In this case of adsorbed sulphate a slightly increment was observed in time.

By the effect of growing rate of soil fertilizer the rate of adsorbed sulphate increased. According to Förster et al. (2012), SO_4^{2-} - adsorption is strongly pH-dependent and increases with decreasing pH. In the long term fertilization experiment the pH decreased with increasing fertilizer doses. Highest $\text{pH}_{(\text{KCl})}$ -values were measured in control treatments ($\text{pH}_{(\text{KCl})}=4.97\text{--}5.51$) and lowest ones were observed in the NPK2 treatments ($\text{pH}_{(\text{KCl})}=4.42\text{--}4.80$). This phenomenon can be observed in our results as well.

Based on Pearson's correlation analyzis high correlation was observed between values of adsorbed sulphate and KH_2PO_4 soluble sulphate ($r=0.949$). Summarizing this results, it can be concluded that the KH_2PO_4 extract better characterizes the adsorbed sulphate fraction than the water-soluble fraction.

Table 2

The adsorbed sulphate fraction of soil at different development stage

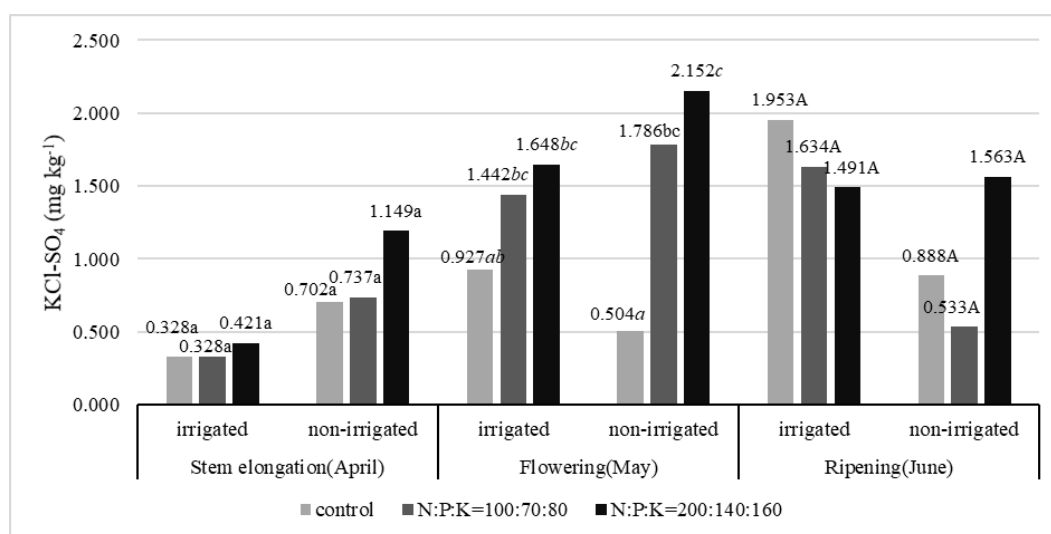
Treatment	Stem elongation		Flowering		Ripening	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated
control	5.709	5.531	6.052	6.685	7.999	7.779
N:P:K=100:70:80	6.057	4.321	5.861	5.895	8.320	9.926
N:P:K=200:140:160	6.236	4.333	6.994	6.404	8.517	9.468

KCl soluble SO_4^{2-} -content of soil

Potassium-chloride is the most widely used extractant for the determination of plant available sulphate content of soil in Hungary, therefore this extractant was also investigated and the results of KCl-SO_4^{2-} were compared to the result of 0.01M $\text{CaCl}_2\text{-SO}_4^{2-}$.

The 1M KCl soluble SO_4^{2-} values are summarized in Figure 3. Hu et al. (2005) stated that the KCl soluble SO_4^{2-} represents the water-soluble inorganic sulphate fraction of soil.

Figure 3: 1M KCl soluble SO_4^{2-} of soil at different development stage of wheat



Note: data marked with the same letter is not significantly different at the significant level of $p < 0.05$



The changes of KCl soluble sulphate values showed similar tendency as the results of 0.01M CaCl₂ soluble SO₄. The values of KCl-SO₄²⁻ tended to increase in time in irrigated plots, but a little drop was appeared after flowering stage in non-irrigated plots. In the most cases, the effect of increasing fertilizer doses appeared in the increased sulphate content of soil, although this increase was not statistically proven. At the stage of ripening the tendency of change was the same as the results of 0.01M CaCl₂-SO₄²⁻ that is sulphate concentration slightly decreased with increased fertilizer doses.

Based on Pearson's correlation analysis moderate correlation was observed between values of 1 M KCl soluble sulphate and the 0.01M CaCl₂ soluble sulphate ($r=0.511$).

The arylsulphatase activity at the stage of ripening

The relative proportions of inorganic and organic S forms in soils vary widely. However, the organic S compounds are dominant and a big proportion of the organic S is present as ester sulphates. Although ester sulphates are considered to be the most labile form of soil organic S, they are unavailable to plants and must be hydrolysed to inorganic SO₄²⁻ before plant uptake. In this process arylsulphatase is involved by cleaving the O-S bond and is believed to make a major contribution to the mineralization of ester sulphate in soils (Tabatabai, 1994).

The arylsulphatase activity of soil was measured in June and the results in different treatments are summarised in *Table 3*.

Table 3

Arylsulphatase activity of chernozem soil (Látókép) in June

Treatments	Arylsulphatase activity ($\mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$)		KH ₂ PO ₄ -SO ₄ (mg kg ⁻¹)	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
control	23.031	24.433	8.396	8.240
NPK1	26.860	22.420	8.893	10.261
NPK2	9.284	16.713	10.137	10.012

The arylsulphatase activity of soil ranged between 9.284 and 26.860 $\mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$. The lowest value was observed in NPK2 treatment, both in irrigated and non-irrigated areas. In general, it can be stated, that the higher sulphate-content of soil resulted lower arylsulphatase activity. The observed negative correlations between arylsulphatase activity and inorganic sulphate sulphur in soil was observed also by Gupta et al. (1988). The decline in arylsulphatase activity could also be attributed to the decrease in its source along with the end product inhibition caused by the higher SO₄-S concentration in soil (Wainwright, 1980).

CONCLUSIONS

The amount of KCl and CaCl₂ soluble sulphate of calcareous chernozem soil was approximately equal and varied between 0.3–2.2 mg kg⁻¹. Based on Pearson's correlation analysis, moderate correlation was observed between values of 1 M KCl soluble sulphate and the 0.01M CaCl₂ soluble sulphate ($r=0.511$).

The KH₂PO₄ soluble sulphate was the highest and ranged between 5.1–10.2 mg kg⁻¹. The adsorbed sulphate was calculated with the difference of values of KH₂PO₄-SO₄ and values of CaCl₂-SO₄.

The adsorbed sulphate content of soil was typically higher at the highest fertilizer dose treatment compared to the control treatment and in the adsorbed sulphate content a slightly increment was observed in time.

Based on our results it can be concluded that the effect of sulphur present in superphosphate is nowadays still measurable. In most cases, the increasing fertilizer doses resulted higher sulphate content of soil compared to the control treatment.

It was confirmed by our measurements that the arylsulphatase enzyme activity was decreased with increasing sulphate content of the soil. The arylsulphatase activity of calcareous chernozem soil ranged between 9.284–26.860 $\mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$.

It is important to mention that the content of studied sulphur fractions in the soil was influenced by the local climatic conditions as well.

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