

# SULPHUR CONTENT IN TEST PLANTS AND ARYLSULFATASE ACTIVITY IN SOIL AFTER APPLICATION OF WASTE MATERIALS

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**Abstract.** Sulphur available for plants may come from organic compounds introduced to the soil with waste materials which undergoes arylsulfatase-assisted mineralisation. Studies were undertaken to assess the effect of fertilisation with sewage sludge and hard coal ash on sulphur content and uptake by test plants, and arylsulfatase activity in the soil. The experimental design was a completely randomised arrangement with three replicates. The following factors were examined: I fertilisation with organic and mineral materials: fresh sewage sludge, hard coal ash, calcium carbonate; II mineral fertilisation: no fertilisation; NPK fertilisation. The sulphur content in orchard grass was significantly higher following an application of composted sewage sludge and hard coal ash, and in maize after an application of sludge/ash mixtures. The waste materials and NPK fertilisation significantly increased sulphur uptake by both the plants tested. Arylsulfatase activity was significantly higher in fresh sludge-amended soil compared with the remaining amended units whereas an application of ash reduced the activity of the enzyme. NPK fertilisation had no significant influence on the enzyme studied.

**Keywords:** *sewage sludge, hard coal ash, manure, orchard grass, maize, enzyme*

## Introduction

Sulphur is widely found in nature as it takes part in life processes occurring in all living organisms and determines their proper functioning. Sulphur deficiency leads to a decline in plant protein, which contributes to a decline in yield quantity and quality (Millard et al., 2006). Soil sulphur has been on the decline in recent years due to an introduction of restrictions on sulphur dioxide amounts released into the atmosphere (Scherer, 2009), an application in cultivation of high-yielding cultivars and highly-concentrated mineral fertilisers, and a drop in farmyard manure production. Organic compounds in manures obtained from natural waste materials may be a source of plant-available elements, including sulphur (Ciepiela et al., 2016). The fertilisers include sewage sludge which, when introduced into the soil, undergoes arylsulfatase-assisted mineralisation. The enzyme is abundant in the soil as it takes part in the processes of oxidation of sulphur which is available for plants (Siwik-Ziomek et al., 2013). Waste materials are a valuable source of nutrients (Farasat and Namli, 2016), including sulphur which, in addition to nitrogen is the main factor determining soil fertility and hence its productive potential. It is worth considering the possibility of using sewage sludge and ashes emitted by power plants in crop fertilisation as they contain considerable amounts of sulphur (Kalembasa et al., 2008) which, however, occurs in various forms, some of them being unavailable for crop plants. When introduced into the soil, these waste materials may improve the nutrient balance in the environment (Palumbo et al., 2007; Antonkiewicz, 2010) and, at the same time, limit the effect of their excess concentration in the place where they are stored, thus solving the problem of their utilisation (Bernoud et al., 2016).

Studies were undertaken to assess the effect of fertilisation with sewage sludge and hard coal ash on sulphur content and uptake by test plants, and arylsulfatase activity in the soil.

## Materials and methods

A pot experiment was established in a glasshouse located at the experimental unit of Siedlce University of Natural Sciences and Humanities in Poland in 2007-2008. The experimental design was a completely randomised arrangement with three replicates. The following factors were examined:

I. fertilisation with organic and mineral materials:

- a) fresh sewage sludge;
- b) sludge obtained from the sewage treatment works in Siedlce and composted for three months (industrial and domestic waste water);
- c) hard coal ash obtained from the electricity distribution company in Siedlce;
- d) calcium carbonate.

Sewage sludge was applied once, adding 5% relative to soil weight. Sewage sludge and coal ash were mixed at the ratio of 2:1 when converted to dry matter.

II. mineral fertilisation:

- a) no fertilisation;
- b) NPK fertilisation.

Mineral fertilisers: urea, triple superphosphate and potassium sulphate, were applied pre-plant. The soil used in the experiment was very loamy sand obtained from the 0-20 cm layer of grey brown podzolic soil. Before the experiment was set up, soil contents of nitrogen, carbon, available phosphorus and potassium were determined (respectively: 1.10, 8.20, 0.052 and 0.071 g kg<sup>-1</sup>). Pots were filled with 10 kg of soil and kept during the growing season at the moisture level of 60% maximum water holding capacity of the soil.

Orchard grass (*Dactylis glomerata*) was tested in the first study year. It was sown at 1.0 g pot<sup>-1</sup> and harvested three times during the growing season. In the second year, maize was grown at a density of 3 plants per pot. After harvest, plants were dried and ground to determine the total sulphur content by ICP – AES after dry mineralisation. The plant material was incinerated in a muffle furnace at 450°C, temperature being increased gradually, and HCl solution (1:1) was added to mineralised samples which were then evaporated to dryness in order to decompose carbonates and precipitate silica. 10% HCl was added to the resultant crude ash which was moved to volumetric flasks by pouring it through a hard filter paper. The total sulphur content in sewage sludge and hard coal ash were determined by ICP-AES following mineralisation of material to dryness. Arylsulfatase activity was determined by colorimetry of *p*-nitrophenol released when soil samples were incubated one hour at 37<sup>0</sup> C with *p*-nitrophenyl sulfate (Tabatabai and Bremner, 1970). All colorimetric data was determined with a spectrophotometer UV-VIS Lambda 25 (Perkin Elmer, Waltham, USA).

The program STATISTICA (data analysis software system), version 12 (www.statsoft.com) was used to statistically analyse the results. Significance of differences between means for the experimental factors were checked using Tukey's test at the significance level of  $\alpha \leq 0.01$ .

## Results and discussion

In addition to nitrogen and phosphorus, sulphur is an element which markedly affects crop yields. Sulphur content in sewage sludge and hard coal ash used in this experiment was, respectively, 8.47 and 5.45 g kg<sup>-1</sup> in d.m. This high sulphur content in sewage sludge and coal ash is several times higher compared with farmyard manure (Kalembasa and Godlewska, 2010).

Sulphur content in the biomass of orchard grass in the first study year (*Table 1*) was affected by all the experimental factors and averaged 3.46 g kg<sup>-1</sup> d.m. Such an amount of sulphur is believed to be high from the standpoint of livestock nutritional needs (Falkowski, 2000). An application of either fresh or composted sewage sludge reduced sulphur content in orchard grass compared with the control grass. The fact can be indicative of an occurrence in sewage sludge of barely soluble sulphur forms which are unavailable for plants and undergo mineralisation in the soil. An application of hard coal ash increased sulphur content in control plants and sludge-manured plants. However, significant differences in sulphur content were found between plants harvested from hard coal ash-manured plots and plants manured with composted sewage sludge. An addition of ash to composted sludge contributed to an increase in sulphur content in orchard grass biomass. NPK fertilisation only slightly increased sulphur content in the plants tested but the differences were not statistically significant.

**Table 1.** The content of S (in g kg<sup>-1</sup> DM) in orchard grass

NPK fertilization		0				NPK				Mean
Organic and mineral materials	Cuts	I	II	III	Mean	I	II	III	Mean	
control object		3.90	3.21	3.83	3.65	3.98	3.21	3.86	3.68	3.67bcd
sewage sludge		2.86	3.18	4.00	3.35	3.57	3.01	4.38	3.65	3.50abcd
composted sewage sludge		3.26	2.91	3.84	3.34	3.32	2.68	2.72	2.91	3.12ab
hard coal ash		3.90	3.35	3.35	3.60	4.70	3.84	3.98	4.17	3.85cd
sewage sludge/ash		2.09	2.66	3.59	3.04	4.16	2.79	3.77	3.57	3.18abc
composted sewage sludge/ash		3.38	3.51	4.38	4.76	3.09	2.80	4.64	3.51	3.63bcd
liming		4.74	3.78	3.96	4.16	5.42	2.85	3.26	3.84	4.00d
liming/sewage sludge/ash		2.89	2.13	3.18	2.73	4.06	2.07	2.95	3.03	2.88a
liming /composted sewage sludge/ash		2.70	2.76	3.70	3.05	3.78	2.07	4.68	3.51	3.28abc
Mean		3.30	3.05	3.76	3.37	4.01	2.81	3.80	3.54	3.46

a,b,c,d – groups of means which do not differ significantly at  $\alpha < 0.01$

Maize needs average amounts of sulphur but it produces high dry matter content so it requires intensive fertilisation. Maize cultivated in the second study year (*Table 2*) contained on average 1.02 sulphur per kg<sup>-1</sup> d.m. The organic materials and coal ash

markedly influenced the plant content of sulphur. The effect of fertilisation with sewage sludge was visible in the second study year, which can indicate that the sludge organic compounds underwent mineralisation and sulphur was transformed into forms available for plants. Nutrients in sewage sludge occur mainly in organically-bound forms, which means they are available only when these materials have undergone mineralisation (Fijałkowski and Kacprzak, 2009). The biomass of maize harvested from sewage sludge-amended units and sewage/coal ash-amended units contained significantly more sulphur compared with control plants. Research conducted by Gondek (2010a) demonstrated that plant biomass content of sulphur increased following an application of sewage sludge. Also Gondek and Filipek-Mazur (2008) reported a significant increase in sulphur content in the biomass of sewage sludge/peat-fertilised maize. An addition of hard coal ash to composted sewage sludge contributed to an increase in the plant biomass content of sulphur but the differences were not substantial, which seems obvious as the mineral fertilisers that had been applied contained sulphur (triple superphosphate and potassium sulphate).

**Table 2.** The content of S (in g kg<sup>-1</sup> DM) in maize

Organic and mineral materials	NPK fertilization		Mean
	0	NPK	
control object	0.620	0.858	0.739a
sewage sludge	0.820	1.52	1.17cd
composted sewage sludge	0.705	1.25	0.978bc
hard coal ash	0.615	1.28	0.948ab
sewage sludge/ash	0.760	1.30	1.03bcd
composted sewage sludge/ash	0.890	1.35	1.12bcd
liming	0.828	1.09	0.959abc
liming/sewage sludge/ash	1.01	1.43	1.22d
liming/composted sewage sludge/ash	0.98	1.07	1.03bcd
Mean	0.803a	1.24b	1.02

See explanation in *Table 1*

Sulphur uptake by plants in the first study year differed and depended on the waste materials applied (*Table 3*). Statistical analysis demonstrated that sulphur uptake by plants was significantly higher following an application sewage sludge (fresh and composted) and its mixture with hard coal ash. Sulphur uptake was the highest after an application of composted sewage sludge and hard coal ash, the increase being over 140% compared with control plants. Also grasses grown in pots where only liming or hard coal ash had been applied took up more sulphur compared with control but the differences were insignificant. Hard coal ash is a waste material which contains substantial amounts of calcium and magnesium (Kalembasa et al., 2008), which might contribute to an increase in sulphur uptake by plants. Moreover, a tendency was

observed for orchard grass to take up less sulphur in successive cuts. However, in pots without NPK fertilisation where soil had been amended with sewage sludge and its mixture with coal ash, sulphate uptake was the highest in the second cut. The fact can indicate how fast the rate of mineralisation was.

**Table 3.** The total sulphur uptake ( $\text{mg pot}^{-1}$ ) by orchard grass

NPK fertilization	0				NPK				Mean	
	Cuts	I	II	III	Mean	I	II	III		Mean
<b>Organic and mineral materials</b>										
control object		31.2	13.9	12.2	19.1	19.5	10.9	23.5	18.0	18.5a
sewage sludge		48.3	54.4	45.6	49.4	32.6	49.7	29.5	37.3	43.4b
composted sewage sludge		47.3	49.8	32.3	43.1	24.3	43.4	30.2	32.6	37.9b
hard coal ash		34.6	14.6	8.81	19.3	63.5	50.3	14.4	42.7	31.0ab
sewage sludge/ash		26.2	43.6	39.1	36.3	49.9	46.2	32.4	42.8	39.6b
composted sewage sludge +ash		49.1	59.6	36.8	48.5	62.0	33.2	27.2	40.8	44.7b
liming		53.7	19.8	26.3	33.3	77.0	17.8	5.97	33.6	33.4ab
liming/sewage sludge/ash		31.3	18.4	18.8	22.8	74.7	40.2	24.9	46.6	34.7b
liming/composted sewage sludge/ash		35.6	35.2	30.3	33.7	64.6	35.1	28.1	42.6	38.2b
Mean		39.7	34.3	27.8	33.9a	52.0	36.3	24.0	37.4a	35.7

See explanation in *Table 1*

Analysis of data revealed that mineral (NPK) fertilisation had an insignificant effect on sulphur uptake by orchard grass.

In the second study year, differences in sulphur uptake by maize remained significant (*Table 4*). An application of hard coal ash increased sulphur uptake by maize compared with control, the differences being insignificant. A significantly lower amount of sulphur was taken up by control maize compared with plants grown in all the remaining experimental units. However, the greatest increase in sulphur uptake by maize (by almost 130% compared with control) was observed in fresh sewage sludge-amended units. Liming increased sulphur uptake by plants, which agrees with findings reported by Gondek (2010b). NPK fertilisation increased sulphur uptake by maize by 239%, the differences being significant.

Arylsulfatase activity in the soil studied (*Table 5*) averaged  $18.5 \mu\text{g PNP g}^{-1} \text{h}^{-1}$ . Koper and Siwik-Ziomek (2004) obtained similar results ranging from  $16.9$  to  $30.8 \mu\text{g PNP g}^{-1} \text{h}^{-1}$ , for soils amended with farmyard manure and NPK. According Balota and Chaves (2010), arylsulfatase activity cited in literature varies widely from  $4$  to  $770 \mu\text{g PNP g}^{-1} \text{h}^{-1}$  and depending on different factors. Statistical analysis demonstrated a varied significant effect of waste materials on arylsulfatase activity in the soil. The greatest increase in the activity of the enzyme (by 33.9%) was found in soil amended with fresh sewage sludge compared with the control. The findings confirm that organic matter significantly affects enzyme activity (Bielńska and Mocek-Płóćiniak, 2009;

Siwik-Ziomek and Lemanowicz, 2014). When carbon compounds are present, they stimulate the biosynthesis of enzymes by soil microorganisms so fresh organic matter incorporated into the soil in the form of sewage sludge significantly increased arylsulfatase in the soil studied.

**Table 4.** The total sulphur uptake ( $\text{mg pot}^{-1}$ ) by maize

Organic and mineral materials	NPK fertilization		Mean
	0	NPK	
control object	14.8	75.7	45.3a
sewage sludge	47.6	158	102.8c
composted sewage sludge	33.0	101	67.0b
hard coal ash	20.1	104	62.1ab
sewage sludge/ash	50.2	102	76.1b
composted sewage sludge/ash	38.9	113	76.0b
liming	21.2	121	71.1b
liming/sewage sludge/ash	37.6	124	80.8b
liming/composted sewage sludge/ash	33.6	111	72.3b
Mean	33.0a	112b	72.6

See explanation in Table 1

**Table 5.** Arylsulfatase activity in soil ( $\mu\text{g PNP g}^{-1} \text{ h}^{-1}$ )

Organic and mineral materials	NPK fertilization		Mean
	0	NPK	
control object	13.8	24.5	19.2cd
sewage sludge	27.1	24.3	25.7e
composted sewage sludge	16.6	15.8	16.2bc
hard coal ash	17.3	7.00	12.2ab
sewage sludge/ash	10.1	10.0	10.1a
composted sewage sludge/ash	17.6	13.0	15.3abc
liming	19.5	21.0	20.3cde
liming/sewage sludge/ash	21.4	23.2	22.3de
liming/composted sewage sludge/ash	25.8	24.2	25.0e
Mean	18.8a	18.1a	18.5

See explanation in Table 1

An application of hard coal ash and its addition to fresh and composted sewage sludge reduced soil enzymatic activity compared with control. Soil liming increased arylsulfatase activity in the soil but the differences were insignificant. According to Bielinska and Baran (2009) that an addition of fluidal ashes from hard coal increased the enzymatic activity. Mineral fertilisation only slightly reduced arylsulfatase activity in the soil, which may be indicative of an inhibitory effect of mineral fertilisers on the activity of the enzyme, which has been confirmed by other authors, too (Siwik-Ziomek and Koper, 2008). Vong et al. (2004) has pointed out that high rates of nitrogen fertilisation contribute to a decline in soil enzymatic activity. Also Sun et al. (2016) observed sufficient N increased the enzyme activity, but excess N did not stimulate activity.

In the present work, the linear correlation coefficient was calculated for the comparison between sulphur content and uptake by the biomass of maize and orchard grass harvested in individual cuts. The correlation (mean values) between these parameters determined in orchard grass biomass was significant and positive for the first cut only and amounted to  $r = 0.54^{**}$ . The value of the coefficient of linear correlation between sulphur content and uptake in maize biomass was  $r = 0.89^{***}$ .

## Conclusions

The sulphur content in orchard grass was significantly higher following an application of composted sewage sludge and hard coal ash, and in maize after an application of sludge/ash mixtures. The waste materials and NPK fertilisation significantly increased sulphur uptake by both the plants tested. Arylsulfatase activity was significantly higher in fresh sludge-amended soil compared with the remaining amended units whereas an application of ash reduced the activity of the enzyme. NPK fertilisation had no significant influence on the enzyme studied.

In summary, it should be noted that the degree of organic matter decomposition and origin has a considerable effect on the characteristics discussed in the work. Further studies are needed to develop more comprehensive results and evaluate the impact of different rates of organic and mineral materials.

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