Biological parameters in technogenic soils of a former sulphur mine

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A b s t r a c t. This study was conducted on the soils originating from a reclamation area of the former sulphur mine in Tarnobrzeg, Poland. Soil was sampled 16 years after the completion of mining works with the open-pit method at Machów, as well as 7 years after sulphur mining *via* the 'smelting' method in the Jeziórko mine was abandoned. Several biological parameters were examined: soil respiration, soil microbial biomass and the activity of rhodanese and arylsulphatase enzymes taking part in sulphur transformation within the site's soils. The soils showed a high total sulphur and sulphates content. The SO_4^{2-} constituted a large fraction of total sulphur, in some cases, exceeding 80% or even 95% of total sulphur. The soil pH decreased due to the degrading effects of sulphur mining. In the soils studied from the locations with the lowest soil pH value, no activity of arylsulphatase was reported and the activity of rhodanese was lowest. The highest soil respiration values were recorded from the 0-5 cm layer in the areas covered with forest vegetation. A high soil respiration value at the waste heap at Machów wherein a very high concentration of S_{tot} and SO_4^{2-} was observed can be due to the ability of fungi to produce hyphal strands and to survive unfavourable conditions.

K e y w o r d s: arylsulphatase, microbial biomass, rhodanese, soil respiration, sulphur mine

INTRODUCTION

Since the early 1990s, the sulphur industry has suffered from the increased production of sulphur from recovered sources and a low profitability of ore extraction. In mine sites, once exploitation ceased, however, several ecological problems remained to be solved. Characteristically, when waste containing sulphur minerals is exposed to oxygen and water, sulphur is oxidised to sulphates, and strong acidification takes place: $2S+3O_2+2H_2O \rightarrow 2SO_4^{2-}+4H^+$. As a consequence, soil organic matter, nutrients, biota and plants can be dramatically destroyed. Along with the chemical pollution and the acidification of soils and waters, mining activities result in considerable transformations of the natural environment, including geo-mechanical and hydrological changes, negative shifts of natural vegetation, changes in landscapes and elimination of large areas from agricultural use (Levyk and Brzezińska, 2007).

In the 'Jeziórko' sulphur mine, sulphur had been recovered by way of the underground smelting method (Frasch method) since 1967. From 1967 to 2001 – more than 74 mln Mg of sulphur was mined. This figure accounts for more than 58% of the total amount of the mineral recovered in the Polish sulphur deposit (Czajkowski *et al.*, 2009). In 1964-1970, construction of the 'Machów' sulphur mine, together with the plant, was in progress. Sulphur deposit with open-pit mining in the 'Machów' open-cast started in 1969. During the mine operation, over 1969-1992, about 280 mln m³ of overburden was removed, about 55.8 mln m³ of sulphur ore was recovered, and about 11.1 mln Mg of refined sulphur was produced (Szmuc and Madej, 2011). Numerous technological holes were dug out and objects were removed from the fields with heavy machinery. This resulted in significant geo-mechanical transformations and triggered destruction, transition or covering over of the upper soil horizons (Gołda *et al.*, 2005). Additionally, in this territory, which holds a high natural groundwater level, the surface subsidence following deposit exploitation led

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to faulty aeration resulting from a low air capacity and insufficient air permeability; in some extreme cases, soils strongly soaked with water or ground water basins were formed (Bryk and Kołodziej, 2009; Gołda *et al.*, 2005). Devastated area restoration has been driven since exploitation was stopped, including elimination of the direct industrial objects, application of sewage sludge, fertilization, neutralization with post-flotation lime, forestation, meadow management, and other specific reclamation techniques. In the open-pit, a 500 ha reservoir called 'Lake Tarnobrzeskie' was formed for recreation purposes, with a 25m thick insulating clay layer at the bottom. Additionally, some limited areas were involved in self-restoration with natural plant succession in abandoned territories (Martyn and Jońca, 2006).

The increase in biological indicators so as to estimate changes in soil quality is a relevant research area. At present, it is accepted that soil biology is the driving force behind many soil processes, and that this supports the delivery of soil and soil ecosystem functions such as nutrient transformations (Creamer *et al.*, 2014). Soil respiration measurement gives an estimate of total microbial activity in the soil. Soil basal respiration is defined as the steady rate of soil respiration (BR) which comes from organic matter mineralization (Pell *et al.*, 2006). Basic soil respiration measurement has been applied in many different studies, and both soil microorganism respiration and organic matter mineralization are commonly accepted as indicators for measuring soil quality changes (Creamer *et al.,* 2014).

Studies of soil enzyme activity provide information on the biochemical processes which occur in soils. Thus, biochemical soil condition has been proposed as an indicator of the real biological state of natural ecosystems (Trasar-Cepeda *et al.,* 2008), as well as that of polluted soils (Tejada *et al.,* 2015). Rhodanese (EC 2.8.1.1.) cleaves the S-S bond of thiosulphate, forming S and sulphite, and then S is subsequently oxidized into sulphite by sulphur oxygenase, while sulphite is further oxidized into sulphate by sulphite oxidase. Arylsulphatase (EC 3.1.6.1) catalyzes the hydrolysis of organic sulphate esters $R\text{-}O\text{-}SO_3^- + H_2O \rightarrow ROH +$

$H + SO_4^{2-}$ (Tabatabai and Bremner, 1970). The enzymatic activity of soil can be an indicator for monitoring the pollution of soils due to their important role in soil ecosystems.

The aim of this study was to measure several biological soil parameters that are indicators of microbial activities and soil health, in recultivation areas in Tarnobrzeg (Poland) 16 years after the end of mining works *via* the open-pit mining method in the Machów mine, as well as 7 years after the completion of sulphur mining by way of the 'smelting' method in the Jeziórko mine. Among such parameters, we investigated basic respiration, microbial biomass and the activities of rhodanese and arylsulphatase.

MATERIAL AND METHODS

The study involved soil sampled from the 'Machów' Sulphur Mine and the Sulphur Processing Plant at 'Machów', as well as in the vicinity of the 'Jeziórko' mine, which, together with 'Piaseczno' mine (not covered by the study), constituted the Siarkopol plant complex located near Tarnobrzeg city, south-eastern Poland (50°34'22''N 21°40'45''E) (Fig. 1). When cheap sulphur, a petroleum processing by-product, appeared on the global markets, such mines had to be closed down. 'Machów' Sulphur Mine and Sulphur Production Plant, built in 1964-1970, were then liquidated. However, the mining fields, where sulphur was pumped out onto the surface, with time, changed into devastated areas and became almost completely deprived of vegetation.

The tested soils showed a typical post-industrial character. Technogenic soils (Technosols) are a relatively young soil group in the WRB soil system (Word Reference Base for Soil Resources); they include a variety of extremely heterogeneous soils in urban, industrial, traffic, mining and military areas (Charzyński *et al.*, 2013). Technosols are strongly influenced by human activity and human-made materials, with properties dominated by their technical origin (Uzarowicz, 2011). Their evolution significantly depends on the duration of the pedogenesis (Ciarkowska *et al.*, 2016), as well as on post-mine soil properties and plant cover (Józefowska *et al.*, 2016).
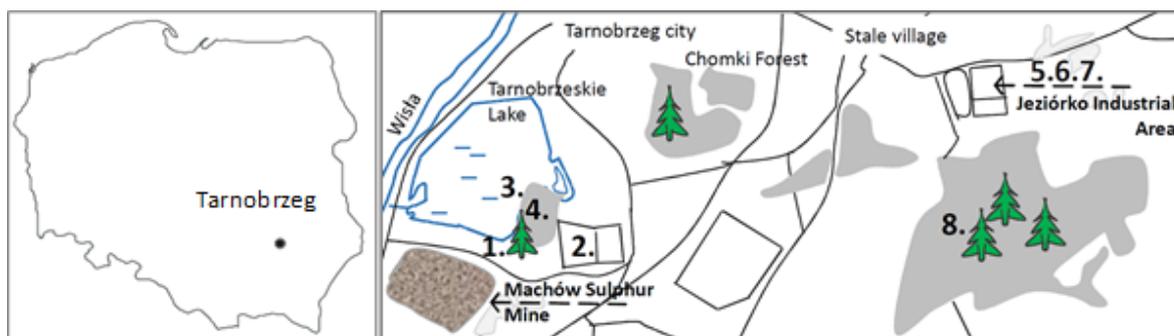
Fig. 1. Map of the studied area (left - location of Tarnobrzeg city in Poland). 1 to 4 – Open-cast mine Machów (1 – Waste-heap; 2 – Clarifier; 3 – Open-pit excavation area, *ca.* 200 m from the current border of Lake Tarnobrzeskie; 4 – Re-forested external dump). 5 to 7 – Underground melting mine Jeziórko (5, 6 and 7 – sites located 10, 20, and 40 m, respectively, from a boring well; parcel No. XXI. 8 – Control stand (Unaffected forest ecosystem).

Locations specific to both mining activities and the control stand were selected so as to collect soil samples. These are:

the open-cast 'Machów' mine:

- Waste-heap – a post-refining waste (called 'cake') disposal site without plant vegetation, non-reclaimed with apparent sulphur lumps up to a few cm in size; area of 42 ha (depth: 0-20 cm);

- Clarifier – clarifier of deposit water, a site with initial vegetation (Poaceae family); after reclamation by application of post-flotation lime; area of 13 ha (depth: 0-15 and 15-25 cm);

- Open-pit excavation area - the crater formed as a result of open-cast excavation. This was eliminated by filling it up with post-refining waste and covering it with a 25 m thick insulating clay layer obtained from an external dump (Szmuc and Madej, 2011); now with a common reed (Phragmites australis) cover, the soil was collected about 200 m away from the present border of Lake Tarnobrzeskie (total area of 500 ha), before the reservoir was fully filled with water, in 2009 (depth: 0-15 and 15-25 cm);

- Re-forest external dump – south-eastern scarp covered by spontaneously developed forest with natural succession of trees and shrubs, self-restoration area of 15 ha with a domination of *Betula* sp., *Sorbus* sp., *Frangula* sp.; at present on Lake Tarnobrzeskie; these heavy grounds are composed mainly of Miocene Tertiary formations (Krakowiec loams) with hard limestone, marl and marl shale intrusions, loams, and granulometric composition of clay or heavy loam (Kowalik, 2008) (depth: 3-5 and 5-30 cm).

Underground melting, borehole Jeziórko mine:

- 10, 20, and 40 m – post-eruption sites located 10, 20, and 40 m, respectively, from a faulty boring well (No. T164) with uncontrolled leakage of water (parcel No. XXI with area of 100 ha); *Phragmites australis* plants present only around the well (at 10 m), while bare ground was found 20 m and 40 m away from the well. Post-industrial soil-less formation with the particle size distribution of weakly loamy sand or sandy loam (Joniec *et al.*, 2015; Knap *et al.*, 2016); (depth: 0-10 and 10-20 cm).

Control stand:

- Unaffected forest ecosystem – the control stand is located in natural deciduous forest dominated by *Betula* sp., *Corylus* sp., *Populus* sp., *Quercus robur*, and situated in the vicinity of the sulphur mine, but outside the area of direct mining activities (Nowa Dęba Forest District). The soil in this region was classified as a Haplic Gleysol developed on loamy sand (Bryk and Kołodziej, 2009); (depth: 0-10 and 10-20 cm).

In each location, a soil pit (1 x 1 x 0.5 m) was made in the representative position. Since all collected technosols were evidently post-industrial and lost their original distribution of horizons, the depth of soil sampling depended on apparent differences observed in situ in the profiles. Soil was collected in three points with a specific depth, mixed, and taken to the laboratory. Next, a soil sample of about 3 kg for each horizon was air-dried and sieved with a 2 mm sieve. Basic soil properties were assayed in air-dry soils. Total sulphur (S_{tot}) and $S-SO_4^{2-}$ were determined according to Bardsley-Lancaster (1960). Organic carbon (C_{org}) was assayed with a TOC-V_{CPH} analyzer (Shimadzu, Japan). Soil pH in H_2O was measured potentiometrically after 24 h stabilization in the soil: water ratio of 1:2.5 (w/w).

The biological properties were determined in soil samples rewetted to 60% WHC and preincubated for 2 days at 20ºC. The amount of soil microbial biomass (C_{mic}) was defined applying the substrate induced respiration (SIR) method based on the initial respiratory response of the microbial population to amendment with an excess of glucose as an easily available carbon and energy source (Anderson and Domsch, 1973). Microbial biomass was calculated based on CO_2 evolved during 2 h incubation with glucose (5 mg g^{-1} dry soil) in a water bath at 25ºC (Šimek and Kalčik, 1998). Basal respiration rate (BR) was measured based on CO_2 evolved during 24 h incubation at 20ºC without the addition of C sources.

Concentrations of CO_2 in the soil headspace were measured with a gas chromatograph Shimadzu GC-14A (Japan) equipped with a thermal conductivity detector (TCD) (Brzezińska *et al.*, 2012). Arylsulphatase and rhodanese were determined colorimetrically according to Tabatabai and Bremner (1970), and Tabatabai and Singh (1976), respectively.

Based on microbial biomass C and respiration rate, an eco-physiological indicator was derived *i.e.* the $C_{mic}:C_{org}$ ratio (percent of C_{mic} in C_{org}).

All measurements were done in three replications and the results were expressed on an oven-dry weight basis (105°C, 24 h). Simple regression procedure was used to describe the correlations between measured soil properties, and one-way analysis of variance (ANOVA, post-hoc Tukey test) was used to determine significant differences between all the samples (STATISTICA 10.0 program, StatSoft Inc.).

RESULTS

The content of organic carbon in soils depended on their location and depth. Soil sampled from the Machów Waste-heap showed the lowest content of C_{org} at 0.44 ±0.12%, and in soils from the Clarifier and the Open-pit, C_{org} was from 0.70 to 1.28%. The highest content of C_{org} was found in the surface layer of the Forested external dump, where it accounted for 12.49±0.06% (Table 1). With regard to the soil sampled in the vicinity of the underground melting Jeziórko mine, the content of C_{org} was lower in the deeper layer in soils 10 and 20 m away from the boring well

(0.65-0.74 and 1.20-1.33%, respectively). In the soils 40 m away and in the Unaffected forest, a higher content of C_{org} was found in the 0-10 cm layer than in the deeper layer of 10-20 cm, and it accounted for 1.36% and 4.68 ±0.011%, respectively. The soils showed high sulphur content. The Waste-heap site soils contained 69.98 g S_{tot} kg^1, which was 230- and 390-fold the values observed in the Unaffected forest soil *i.e.* 0.30 and 0.18 g S_{tot} kg^{-1} (at 0-10 and 10-20 cm depths, respectively). In other sites of the Machów open-cast mine, there was up to 4.41 g S_{tot} kg^{-1} in the *Clarifier*. In the Jeziórko Frasch method mine, the highest S_{tot} of 2.68 g kg^{-1} was at the 10 m site (9 times more than in the Unaffected forest soil, p<0.05).

SO_4^{2-}-S showed similar tendency of distribution in all the experimental sites, and it ranged from 29.8 to 14.6 mg kg^{-1} in the Unaffected forest*,* to 4 3584.5 in mg kg^{-1} in the Waste-heap. A relatively low sulphate value was measured in Re-forest (84.2-66.7 mg kg^1), while SO_4^{2-}-S in soils of the Clarifier and the 10m sites reached greater values of 3 704.5 and 919.5 mg kg^{-1}, respectively.

Both, S_{tot} and sulphate-S were more abundant in the top-soil than in deeper layers, except for the Open-pit, as well as the 20 and 40 m sites. SO_4^{2-}-S constituted a large fraction of S_{tot}, in some cases, exceeding 80% or even 95% of S_{tot} (Clarifier, 10 and 20 m). In turn, in the Re-forest site, this ratio was relatively low (8-34%), and was close to that observed for the Unaffected forest soil *i.e.* 8-10%.

The mine-heap at the open-cast Machów mine showed very strong acidity and very little organic carbon (C_{org}) content. Waste accumulation in the Machów mine resulted in soil degradation in the place of storage and a decrease in soil pH in that place to the value of 0.91±0.02. Similarly, the soil sampled 7 years after sulphur mining in the Jeziórko mine was very highly acid (pH 1.75-2.95) and highly acid (pH 3.9-4.28) or acid (pH 4.60-4.61). Only the soils from the vicinity of the open-cast Machów mine Clarifier and Open-pit excavation area demonstrated a slightly alkaline reaction.

T a b l e 1. Organic carbon, total sulphur, sulphate sulphur content, and pH in the investigated soils

Localization	Depth (cm)	C_{org} (%)	S_{tot} (g kg^{-1})	SO_4^{2-}-S (mg kg^{-1})	SO_4^{2-}-S (% in S_{tot})	pH (H_2O)
Open-cast mine Machów						
Waste-heap	0-20	0.44 e ±0.12	69.98 a ±0.087	43584.5 a ±331	62	0.91 i ±0.02
Clarifier	0-15	1.14 c ±0.01	4.41 b ±0.122	3704.5 b ±0.45	84	7.75 b ±0.13
	15-25	0.70 d ±0.09	0.75 gh ±0.005	716.7 def ±12.9	95	7.73 b ±0.10
Open-pit excavation area	0-15	1.23 c ±0.03	1.61 d ±0.052	754.7 def ±73.8	47	8.18 a ±0.14
	15-25	1.28 c ±0.01	1.76 d ±0.108	956.4 d ±8.14	54	8.20 a ±0.03
Re-forested external dump	0-5	12.49 a ±0. 11	0.99 ef ±0.035	84.2 h ±2.14	8	3.09 f ±0.07
	5-30	0.85 d ±0.06	0.20 i ±0.007	66.8 h ±2.12	34	4.4 1cd ±0.08
Distance from the boring well (m)			*Underground melting mine Jeziórko*			
10	0-10	0.65 de ±0.03	2.68 c ±0.037	1548.2 c ±46.9	58	1.75 h ±0.09
	10-20	0.74 d ±0.12	1.05 e ±0.013	919.5 de ±58.9	88	2.12 g ±0.01
20	0-10	1.20 c ±0.06	0.84 fgh ±0.026	601.2 ef ±40.9	71	2.16 g ±0.01
	10-20	1.33 c ±0.04	0.70 h ±0.014	676.3 def ±3.50	96	2.95 f ±0.03
40	0-10	1.36 c ±0.01	0.30 i ±0.004	216.4 gh ±11.0	72	3.90 e ±0.04
	10-20	0.80 d ±0.11	0.91 efg ±0.020	495.2 fg ±6.91	54	4.28 d ±0.01
Control stand						
Unaffected forest ecosystem	0-10	4.68 b ±0.011	0.30 i ±0.016	29.8 h ±0.1	10	4.61 c ±0.07
	10-20	1.36 c ±0.02	0.18 i ±0.001	14.6 h ±1.2	8	4.60 c ±0.08

Data pairs in each of the column which do not differ statistically were marked with the same letter.

Microbial biomass C was more abundant in the upper soil layers. The C_{mic} equalled to 1.275 ± 0.16 mg g^{-1} in Unaffected forest topsoil, and it was even higher in Re-forest topsoil, 2.524 ± 0.16 mg g^{-1} (Table 2). Other sites expressed significantly lower C_{mic} content, especially in the Waste-heap, Open-pit and 10 m sites.

The $C_{mic}:C_{org}$ ratio in the topsoils ranged from $0.58\pm0.07\%$ in Open-pit, to $3.42\pm0.12\%$ in Clarifier. Such figures are even higher than in the Unaffected forest and Re-Forest topsoils (2.75 ± 0.33 and $2.02\pm0.13\%$, respectively). The C_{mic} of the deeper layer showed similar or some lower percentage of C_{org}, except for the Re-Forest site, wherein we observed a slightly higher $C_{mic}:C_{org}$ ratio of $2.30\pm0.07\%$.

The Re-forest soil, especially in the organic layer, was characterized by a high respiration rate of 73.27 ± 6.14 mg CO_2-C kg^{1} d^{-1}, which was almost 3.5 higher than in the Unaffected forest (21.29 ± 1.13 mg CO_2-C kg^{1} d^{-1}). Additionally, the topsoils of Waste-heap and Clarifier were cha-racterized by elevated basal respiration rates of 13.96 ± 2.55 and 7.80 ± 0.25 mg CO_2-C kg^{1} d^{-1}, respectively. The subsoil of the forested profile also expressed relatively high BR of 8.33 ± 0.18 mg CO_2-C kg^{1} d^{-1}, however, that value was lower than that in the natural forest ecosystem (by 30%, p<0.05).

The soil close to the Open-cast sulphur mine at Machów showed arylsulphatase activity ranging from 0.022 to 0.19 mM pNPg^{-1}h^{-1}. The activity of such enzymes in the soils near the underground melting mine Jeziórko ranged from 0.014 to 0.176 mM pNP g^{-1}h^{-1}. The Waste-heap and Open-pit excavation area at Machów and the area of 10 m from the underground melting mine Jeziórko were characterized by undetected arylsulphatase activity. The highest activity of this hydrolase was observed in the upper horizon of soils located on the Re-forest and Unaffected forest. The activity of rhodanese as detected in all the samples of soils was very diverse and depended on the sampling locations. The highest activity of rhodanese was determined in topsoil near the

Table 2. Amount of soil microbial biomass (C_{mic}), ratio of $C_{mic}:C_{org}$, basal soil respiration rate (BR), and rhodanese (RDN) and aryl-sulphatase (AR) activities in the investigated soils (n=45, p < 0.001)

Localization	Depth (cm)	C_{mic} (mg g^{-1})	$C_{mic}: C_{org}$ (%)	BR (mg CO_2 kg^{-1} d^{-1})	AR (µM pNP g^{-1} h^{-1})	RDN (µM SCN g^{-1} h^{-1})
			Open-cast mine Machów			
Waste-heap	0-20	0.077 e ±0.01	1.77 de ±012	13.96 c ±2.55	n.d.	0.122 i ±0.014
Clarifier	0-15	0.389 c ±0.01	3.42 a ±0.12	7.80 cde ±0.25	0.022 de ±0.001	1.479 d ±0.025
	15-25	0.067 e ±0.01	0.96 fgh ±0.17	1.43 ef ±0.39	n.d.	0.621 g ±0.026
Open-pit excavation area	0-15	0.071 e ±0.01	0.58 h ±0.07	3.25 def ±0.09	n.d.	1.810 c ±0.075
	15-25	0.067 e ±0.01	0.52 h ±0.01	2.00 def ±0.17	n.d.	1.932 b ±0.015
Re-forested external dump	0-5	2.524 a ±0.16	2.02 cd ±0.13	73.27 a ±6.14	0.190 a ±0.005	5.885 a ±0.02
	5-30	0.195 cde ±0.01	2.30 bcd ±0.07	8.33 cd ±0.18	0.036 cd ±0.001	0.723 g ±0.033
Distance from the boring well (m)			Underground melting mine Jeziórko			
10	0-10	0.081 e ±0.01	1.25 efg ±0.04	1.30 f ±0.09	n.d.	0.867 f ±0.005
	10-20	0.060 e ±0.01	0.81 gh ±0.05	1.34 ef ±0.03	n.d	0.340 h ±0.016
20	0-10	0.163 de ±0.01	1.36 efg ±0.08	4.89 def ±0.28	0.045 c ±0.002	1.026 e ±0.015
	10-20	0.074 e ±0.01	0.56 h ±0.01	2.06 def ±0.35	n.d.	0.677 g ±0.04
40	0-10	0.190 cde ±0.04	1.40 ef ±0.27	4.23 def ±0.19	0.018 e ±0.001	0.977 ef ±0.025
	10-20	0.108 e ±0.02	1.34 efg ±0.24	2.54 def ±0.12	0.014 e ±0.002	0.671 g ±0.06
			Control stand			
Unaffected forest ecosystem	0-10	4.68 b ±0.011	0.30 i ±0.016	29.8 h ±0.1	10	4.61 c ±0.07
	10-20	1.36 c ±0.02	0.18 i ±0.001	14.6 h ±1.2	8	4.60 c ±0.08

Data pairs in each of the column which do not differ statistically were marked with the same letter, n.d. – not detected.

Ta b l e 3. Relation between investigated parameters. Correlation coefficients (R) with bold-face are significant at $p \leq 0.05$ (n=45)

Treatment	C_{org}	S_{tot}	SO_4^{2-}-S	pH	C_{mic}	C_{mic}:C_{org}	BR	AR	RDN
C_{org}	1								
S_{tot}	-0.150	1							
SO_4^{2-}-S	-0.165	0.999	1						
pH	-0.105	-0.378	-0.371	1					
C_{mic}	0.976	-0.130	-0.143	-0.102	1				
C_{mic}:C_{org}	0.248	0.075	0.083	0.006	0.419	1			
BR	0.962	0.043	0.029	-0.185	0.952	0.352	1		
AR	0.722	-0.313	-0.417	-0.211	0.778	0.286	0.723	1	
RDN	0.943	-0.239	-0.253	0.125	0.896	0.180	0.889	0.641	1

BR – basal respiration, AR – arylsulphatase activity, RDN – rhodanese activity.

open-cast Machów Re-forest (5.885 µM SCN$^-$ g^{-1}h^{-1}) and it was 3-fold higher than was rhodanese from the upper horizon soil from the Unaffected forest *(*1.822 µM SCN$^-$ g^{-1}h^{-1}). Rhodanese activity of the upper horizon (0-10 cm) of the profile at the Jeziórko mine was 30% higher than soil from the depth of 10-20 cm (20 and 40 m) and 60% higher when 10 m away from the mine.

The inter-relations between the measured soil properties are provided in Table 3. Organic C is positively and significantly correlated with C_{mic} (R = 0.976, p < 0.001). Organic C also shows positive correlation with BR (R = 0.962, p< 0.001). Organic carbon promotes microbial activity so it was significantly correlated between C_{org} and enzymes activity: arylsulphatase (R = 0.722, p < 0.001) and rhodanese activity (R = 0.942, p < 0.001).

DISCUSSION

Soil pH is an important parameter influencing soil surface chemistry and determining cation and anion adsorption onto oxide minerals. Soil pH near the open-cast Machów mine and Jeziórko mine varied with depth and distance from the mine. Elementary sulphur is chemically inactive, only when it oxidises and bonds, it forms sulphuric acid contributing to degrading soil acidification. At all sites near the Jeziórko mine, pH increased with soil depth (Table 1). The model studies performed in the 1980s by Siuta (1982) in the Jeziórko mine demonstrated that as little as 2% addition of sulphur after 14 days results in a decrease in soil pH from 6.0 to 5.3, and after the third month of the experiment, down to 1.9. According to Urlich *et al.* (1980), one can assume that natural acidification is caused in soils by weak acids, mostly carbonic acid and various organic acids. It is assumed that they can decrease the soil reaction to the value pH KCl >5.0. However, the occurrence of strong acids, *e.g.* H_2SO_4, in soil due to anthropogenic reasons decreases the reaction pH KCl<5.0. One can, therefore, assume that even

in the forest, the soil pH decreased due to the degrading effect of sulphur mining by smelting, which miners consider an eco-friendly method, as compared to its traditional open-pit recovering. The recultivated areas around the Machów mine, on the other hand, show higher, slightly alkaline (pH 7.73-7.75) and alkaline (pH 8.18-8.20) pH values. Liming in the Machów region was common and involved a successive supply of inconsiderable calcium fertiliser rates (CaO and $CaCO_3$) within a few years or introducing a homogeneous single preventive rate.

According to the scale of the limit, in the sulphur contents within the surface soil layers (Motowicka-Terelak and Terelak, 1998) both as (S) and (SO_4^{2-}-S), in all the cases studied, its excess is recorded due to human impact. A long persistence of easily leaching sulphur compounds in soils, namely 16 years after mining in the Machów mine was neglected and 7 years after the Jeziórko mine was closed down, accounts for the formation of sulphate minerals. González *et al.* (2011) found that in the periods of high evaporation, when exposed to low pH values and a relatively high concentration of soluble salts present in mining waste, mostly ions (SO_4^{2-}, Al^3 and Mg^2), as well as trace elements (Zn^2, Cd^2, Pb^2), more or less complex sulphate minerals get formed. During precipitation, secondary salt deposits dissolve, thus contributing to a very low pH value and a release of ions Mg, Al and Zn, and mostly SO_4^{2-}. The theory is confirmed by the results reported by Martyn and Jońca (2006), who found that waters from the mine area showed that the presence of sulphates in the waters under study exceeds the norms for their economic use potential. In the former mine waters, high amounts of total iron were identified (Martyn and Jońca, 2006). The percentage share of SO_4^{2-}-S in the total sulphur content in most soils (except for 40 m -site and Unaffected Forest control soil) also increased gradually with depth and accounted for even 96% of the total sulphur content (at the 20 m-site from the

underground Jeziórko mine) (Table 1). Today, due to a lack of influx of elementary sulphur to the soils under study, the accumulation of sulphates in deeper layers must have been due to their leaching from the upper profile part.

Sulphur recovery results in unfavourable geomorphological transformations, in changes in the existing hydrological systems (Bryk and Kołodziej, 2009) and in chemical pollution. Chemical contamination with sulphur compounds occurs not only in the place of open-cast mining or boring, but also, in the case of sulphur smelting from deposits, the compounds of that element penetrating into waters and the ground in an uncontrolled way. The highest accumulation of sulphates in the waste zone at the open-cast Machów mine decreased the soil reaction to pH 0.91. Such low soil pH results in a loss in capacity for growth and development of living organisms, and this was confirmed in practice as a lack of any vegetation in that area. In our studies, from the point with such low soil pH, no activity of arylsulphatase was assayed and the activity of rhodanese was lowest. Soil enzyme activities are sensitive to stress in ecosystems and have the potential to serve as an indicator of the health and sustainability of ecosystems (Trasar-Cepeda *et al.,* 2008). Enzymatic activities can reflect the biological state in the soil (Igbinosa, 2015). Indeed, Wainwright (1979) shows that rhodanese activity was higher in polluted than in unpolluted soils. The results indicate that microbial S-oxidation occurs in soils exposed to heavy atmospheric pollution. According to Wainwright (1979), polluted soils contained more fungi (total count) than unpolluted soils, as well as fewer heterotrophic bacteria (total count). Thus, the different reaction of rhodanese, as compared with the activity of arylsulphatase, may come from the fact that it is produced by fungi (Szajdak, 2003), and the metabolic processes of such microorganisms can express a different pattern than that of the bacteria which are a source of arylsulphatases in soils (Knauff *et al.*, 2003).

According to Anderson (2003), the C_{mic}:C_{org} of agricultural and forest soils at neutral pH is very similar and in the range between 2.0 and 4.4%. Values below 2.0 for the C_{mic}:C_{org} could be considered as critical for soils with a neutral soil pH. Under acidic conditions, the cell-internal pH (which needs to be kept around 6.0) diverges from the surrounding pH conditions, and, accordingly, little biomass can be produced. This is reflected in low C_{mic}:C_{org}. In our studies, the lowest C_{mic}:C_{org} were reported in soils sampled from the areas where we observed low pH (the waste-heap in open-cast mine Machów and the 10, 20, 40 m sample sites in the underground melting mine Jeziórko) but also where we saw high pH (Clarifier- at 0-15cm and in the Open-pit excavation area). The area with for-est vegetation (Re-forest, External dump and Unaffected forest ecosystems) have C_{mic}:C_{org} above 2%, whereby the carbon is available for soil microbial community growth (Anderson, 2003). A lack of significant correlation between the C_{mic}:C_{org} and C_{org} (Table 3) confirms the disturbance of

soil habitats in the tested technogenic soils. Nevertheless, microbial biomass C_{mic} showed a high positive relationship with arylsulphatase and rhodanese activities.

The major factors influencing soil respiration include soil moisture affecting the overall microbial activity as a source of CO_2-C efflux, soil diffusion kinetics and the existing relationship between efflux diffusion through the soil (Goupil and Nkongolo, 2014). According to Kuzyakov and Gavrichkova (2010), underground root and microbial respiration are greatly promoted by aboveground photosynthesis. Li *et al.* (2004) estimated the contribution of aboveground litter to total soil CO_2-C. In our studies, the highest BR values were reported in soil 0-5 cm layer sampled from the areas covered with forest vegetation. The Machów forested external dump was a three and a half times less unaffected forest ecosystem than that of Jeziórko. Ramesh *et al.* (2008) in their studies, revealed that water holding capacity depended on the level of organic carbon. Presumably, the highest organic carbon content (12.49 in Machów and 4.68 in Jeziórko) and the highest C_{mic} in both places (2.524 and 1.275 mg g^{-1}) influenced the basic respiration by increasing holding capacity and microbial biomass and enzyme activity. In both places, we found the highest activity of arylsulphatase and rhodanese (Table 2). Soil depth is a soil parameter which can be considered to account for soil respiration. Indeed, soil depth could control the root zone water availability (Cable *et al.*, 2008), and, consequently, affect the plant, microbial and enzyme activity. Environmental stresses brought about by mining activities and contamination by sulphur in the forest sites, as compared to places near the mine, could be responsible for the reduction in microbial population and diversity (Monson *et al.,* 2006). Only the waste-heap site in the open-cast mine Machów surprised us with its high basal respiration (13.96 mg CO_2 kg^{-1} d^{-1}). This may be due to abiotic organic carbon mineralization. Presumably in the soil with the highest total sulphur and sulphate content where the activity of rhodanese was observed, the soil fungi have the ability to produce hyphal strands. Such adaptability is to help survival under unfavourable conditions.

CONCLUSIONS

1. This study has shown that the kind of sulphur mining has a long-term influence on soil quality and enzyme activity.

2. Soil acidity is a major challenge in the study sites and requires liming or wood ash application.

3. The microbial stress associated with acidity, and high sulphur content lower the activity of arylsulphatase and rhodanese. The basic respiration is correlated to C_{org}, C_{mic} and enzyme activities, which suggests that the effect of microorganisms on respiration should be significant and direct.

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