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SOIL ENZYMATIC ACTIVITY IN THE IMPACT ZONE OF SULPHUR COMPOUNDS EMISSION

AKTYWNOŚĆ ENZYMATYCZNA GLEB W STREFIE ODDZIAŁYWANIA EMISJI ZWIĄZKÓW SIARKI

Abstract: Activity of various enzymes is used for the assessing the effect of anthropogenic fsctors on soil microbiological activity. Dehydrogenase activity is considered a measurement of total soil microbiological activity. A crucial element of anthropogenic transformations of the natural environment is acid soil degradation caused by the storage of acid forming substances, such as *eg* elementary sulphur and atmospheric pollution with its compounds. In Poland the largest areas strongly polluted with sulphur are situated in the Tarnobrzeg Sulphur Basin, where pulverized sulphur (transported by wind from the minefields and places of storage) and the products of its oxidation, such as acid rains or dry deposit have found its way not only to the soils covering the mining area but also to the soils beyond the boundary of the mine area.

The research aimed to assess the influence of pollution emitted during the thirty-year-long exploitation of sulphur deposits on the microbiological activity of soils situated in the vicinity of "Grzybow" Sulphur Mine.

The investigated soils taken from the areas situated in neighbouring of mine are characterized by low, as a rule below 1, level of degydrogenase activity and acid soil reaction and generally a high share of sulphate sulphur in the total sulphur content. The conducted assessment of the activity of studied enzymes shows, that the pollution of the soil environment around the mine remains on the level which may pose a threat to living organisms. The cause of the considerable decrease of the level of dehydrogenase activity of studied soils may be their acid reaction which is the result of long-term emission of sulphur compounds. In the surface soil layer sulphur is oxidized to sulphuric acid, which cause a strong soil acidification.

Keywords: dehydrogenase activity, acid soils, sulphur mine

Activity of various enzymes is used for determining the effect of anthropogenic factors on soil microbiological activity [1, 2]. Dehydrogenase activity is considered a measure of total microbiological activity in soils and it is often determined in order to assess the soil anthropogenic transformations. A crucial element of anthropogenic transformations of the natural environment is acid soil degradation caused by the storage of acid forming substances, such as *eg* elementary sulphur and atmospheric pollution with its compounds. In Poland the largest areas strongly polluted with sulphur

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are situated in the Tarnobrzeg Sulphur Basin, where extraction and processing of sulphur have led to a considerable pollution of the atmosphere, soils, surface and underground waters. Pulverized sulphur (transported by wind from the minefields and places of storage) and the products of its oxidation, such as acid rains or dry deposit have found its way not only to the soils covering the mining area but also to the soils beyond the boundary of the mine area. In the soil surface layer sulphur is oxidized to sulphuric acid, which causes strong soil acidification [3–5].

The research aimed to assess the influence of pollution emitted during the thirty-year-long exploitation of sulphur deposits on the microbiological activity of soils situated in the vicinity of "Grzybow" Sulphur Mine.

Materials and methods

The material for analyses was collected from mineral surface and subsurface horizons of 9 representative surfaces located about 3 km from the boundaries of the former "Grzybow" sulphur mine, which was the first mine in Poland as well in Europe where sulphur deposits were exploited using the borehole Frash method. Later, exploitation with the borehole Frash method was also led on areas of mines "Jeziorko", "Osiek" and "Basznia". Although the method of underground smelting of sulphur was regarded less harmful to the environment than an opencast mining, geochemical, hydrological and chemical transformations are spread through not only areas where mining works were led but also on areas occurring beyond borders of mine. The cause of chemical transformations (acidity) of these areas was a significant degree of the atmospheric air pollution by sulphur compounds entering different components of ecosystems. As Levyk and Brzezinska [5] say mining activity within Roztocze region contributed to impoverishment of the species, landscape and ecosystems diversities. Soils of areas beyond the border of Sulphur Mine "Grzybow", in contrast with the soil of mining fields weren't subjected to reclamation treatments aiming at an improvement of their physical and chemical properties.

On selected surfaces (wastelands, overgrown with grasses) remaining under impact of long-lasting emission of sulphur compounds field research was carried on during which soil was sampled for laboratory analyses,

In air dried soil material pH in H_2O and in 1 mol $\cdot$ dm^{-3} KCl solution was estimated potentiometrically, soil texture using the Casagrande areometric method in Proszynski's modification, organic C content by means of the Tiurin method modified by Oleksynowa, total N using the Kjeldahl method and total S and S-SO_4 using the modified Barsley-Lancaster methods [6]. Sulphur forms were determined in the obtained extracts using the gravimetric method according to Polish standards PN-75 C-04566/21 and PN-74 C-04566/09. The soil dehydrogenase activity was assessed by colorimeter after 24 hour incubation of the soil samples at 37 ^{o}C with TTC solution (2,3,5-Triphenyltetrazolium Chloride) used a substrate for measuring the investigated enzymes with the Casidy et al [7] method and stated in cubic centimeters of hydrogen per 1 kilogram of soil per day [$cm^3H_2 \cdot kg^{-1} \cdot d^{-1}$].The intensity of colouration was measured using the Beckman DU 600 spectrophotometer at the wavelength 485 nm.

Considering seasonal changes of the enzymatic activity samples of the soil for estimating the dehydrogenase activity were taken twice a year: in May and October, from both depths of every surface. Values of the dehydrogenase activity, presented in the paper, in the soil of the given surface are mean values from estimations in the spring and autumn terms.

Results and discussion

Sulphur is ranked among elements widely spread in the biosphere, the nutrients essential for the normal growth and the development of plants. It is also one of pollutants occurring most often which are contributing to the ground acidity, the same constituting a considerable risk for the balance of the entire ecosystem [4]. A considerable diversification of the assessed sulphur compound content was found in the analyzed soils (Table 1).

Table 1

Selected physicochemical properties of analysed soils

Profile No.	Depth [cm]	pH		C_{org}	N_{tot}	S_{tot}	S-SO_4	< 0.02 [%]
		H_2O	KCl	[g · kg^{-1}]				
1	0–18	4.92	3.75	13.85	1.20	0.129	0.002	6
	18–32	5.33	4.19	7.44	0.84	0.113	0.003	7
2	0–17	4.72	3.59	70.36	5.80	0.893	0.015	9
	17–33	4.75	3.74	25.92	2.27	0.215	0.006	9
3	0–24	4.35	3.25	6.12	0.65	0.046	0.003	5
	24–41	5.09	3.65	0.69	0.11	0.010	0.006	5
4	0–25	6.31	5.25	11.23	2.09	0.144	0.015	8
	25–42	5.89	4.72	11.49	0.77	0.022	0.012	8
5	0–20	5.21	4.51	15.15	2.20	0.223	0.018	24
	20–35	5.44	4.47	5.82	0.97	0.071	0.015	22
6	0–16	4.70	3.91	11.22	1.03	0.129	0.008	26
	16–29	4.77	3.98	9.37	1.02	0.105	0.009	28
7	0–22	5.92	5.13	7.68	0.95	0.127	0.005	8
	22–35	5.21	4.16	0.54	0.22	0.044	0.010	3
8	0–28	4.86	3.71	4.10	0.64	0.036	0.007	5
	28–41	4.85	4.02	1.57	0.23	0.020	0.006	3
9	0–23	4.72	3.55	5.57	0.65	0.049	0.011	6
	23–33	4.61	3.45	0.51	0.25	0.045	0.014	4

S_{tot} content fell within the range from 0.01 to 0.893 g · kg^{-1} of soil and was diminishing with the soil depth. The assessed content of S-total was approximate to the quantities of this element in the mineral soils of Poland [8]. Apart from the total sulphur

also sulphate sulphur was assessed in the analysed soils (Table 1). Their amount indicates a hazard posed to the soil environment by this element excessive emission due to anthropopressure. The share of $S\text{-}SO_4$ in the total sulphur content in the mineral soils of Poland does not exceed 10 % and higher concentrations are considered the result of anthropopressure [8]. In the surface horizons of the analysed soils the percentage share of sulphate sulphur in the total sulphur content was the lowest (it did not as a rule exceed 10 %) and was increasing with depth, reaching even 60 % (Fig. 1) in the subsurface horizons. In view of limited inflow of elementary sulphur pollutants (due to the mine close-down) to the soils of the investigated area, it was mainly caused by sulphate translocation from surface horizons to lower situated ones. On the basis of limit numbers of total sulphur and sulphate sulphur content in the soil surface horizons suggested by IUNG [8], the researched soil with texture of loamy sands and light loams

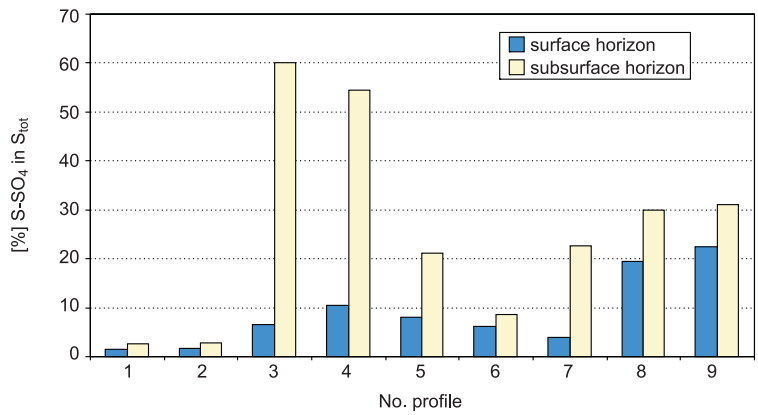

Fig. 1. Participation of $S\text{-}SO_4$ [%] in S_{tot} content

were counted among the soils with natural content of the analysed sulphur forms.

Dehydrogenase activity in the surface horizons of the studied soils ranged between 0.13 and 1.85 $cm^3H_2 \cdot kg^{-1} \cdot d^{-1}$ and was decreasing with increasing soil depth (in the subsurface horizons it fluctuated from 0.11 to 0.28 $cm^3H_2 \cdot kg^{-1} \cdot d^{-1}$, Fig. 2). On the basis of many experiences a diversified level of the dehydrogenase activity depending on the depth of the soil profile was stated. Their activity achieves the maximum values in top horizons, and along with the increase in the depth of the soil profile, a decline in the activity of these enzymes is observed [9].

According to Bielinska et al [10] such regularity is mainly connected also with the profile distribution of humus in soil. Higher values of the analyzed enzymes in the surface horizons of the analyzed soils were accompanied by higher values of organic C. In the surface horizons the range of organic carbon content was from 4.10 to 70.36 $g \cdot kg^{-1}$ of soil and in the subsurface horizons between 0.54 and 25.92 $g \cdot kg^{-1}$ of soil. However, statistical analysis conducted on the obtained results did not reveal any significant dependency between the assessed enzymatic activity and the content of total

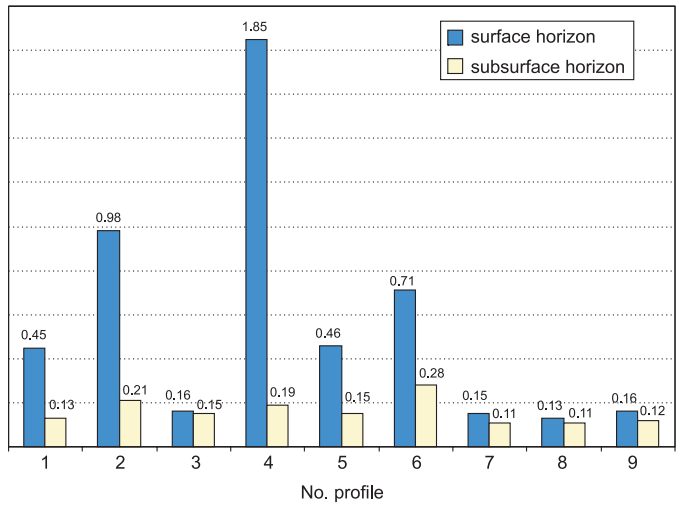

Fig. 2. Level of dehydrogenase activity in horizons of analysed soils [$cm^3H_2 \cdot kg^{-1} \cdot d^{-1}$]

nitrogen, organic carbon and sulphur compounds. Levyk and co-authors [9] stated the lack of the statistical relation between the dehydrogenase activity and the degree of the sulphur content, examining the activity of these enzymes in the chemically degraded soils, located soil in areas of the sulphur mine of Jaworow and Nemyriv (Ukraine).

The obtained results of deydrogenase activity (Table 1) point to high inactivation of the studied enzymes in the soils collected from the areas situated in the vicinity of former Grzybow sulphur mine. Similar values of dehydrogenase activity (below one) were also assessed by Bielinska et al [10–12] while determining these enzymes activity in the soils of the industrialized city agglomerations of the upper Silesia and in the soils transformed as a result of the mining operation of the sulphur mine in Piaseczno, but also in the soil which for a long time remained under nitrogen emission, situated in the immediate neighbourhood of the Pulawy S. A. fertilizer factory. According to Bielinska et al [10, 11] low dehydrogenase activity in soils evidences a lowered Total Microbiological Activity (TMA) of the environment and its cause in the investigated soils may be their reaction.

It is one of the crucial soil features which is an important factor of their productivity because it directly affects the development of microorganisms and higher plants. Martyn et al [3] state that pH value in KCl ranging from 4.6 to 5.0 in the surface horizons of soils located in the vicinity of sulphur mines points to a negative effect of sulphur extraction and storage. The main reason for this might be spreading sulphur dust throughout the neighbourhood by wind and precipitations. The investigated soils situated under many-year influence of Grzybow borehole sulphur mine revealed pH values within the range from 3.25 to 4.75 and on this basis they were counted among acid and very acid soils, except the subsurface horizon of the profile 4 soil where pH value in KCl was the highest 5.25. The soil of this horizon was also characterised by the highest activity of the investigated enzymes – 1.85 $cm^3H_2 \cdot kg^{-1} \cdot d^{-1}$, which

corroborates the results obtained by Frankenberger and Johanson [13], who on the basis of conducted research found that even small changes of pH values may considerably change the activity of soil enzymes. The influence of the soil reaction on the enzymatic activity was noted also by Levyk and collaborators [9] when comparing the dehydrogenase activity of the anthropogenic soil transformed as a result of the exploitation of sulphur deposits. On the basis of conducted estimations authors stated several times lower the level of the activity of examined enzymes in acid soils than in alkaline ones. According to many authors, the most beneficial reaction conditions for the activity of dehydrogenases are close to neutral [14, 15].

Conclusions

1. Many-year extraction and storage of sulphur in the area of "Grzybow" Sulphur Mine had a negative influence on the state of the natural environment of the neighbouring areas including the analysed soils. These soils are characterized by a low, as a rule below one, level of soil dehydrogenase activity and acid reaction, and a generally high share of sulphate sulphur in total sulphur content.

2. The cause of the considerable decrease of the level of dehydrogenase activity of studied soils may be their acid reaction which is the result of long-term emission of sulphur compounds.

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AKTYWNOŚĆ ENZYMATYCZNA GLEB W STREFIE ODDZIAŁYWANIA EMISJI ZWIĄZKÓW SIARKI

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Abstrakt: Aktywność różnych enzymów wykorzystuje się do oszacowania wpływu antropogenicznych czynników stresowych na aktywność mikrobiologiczną gleb. Za miarę całkowitej aktywności mikrobiologicznej w glebie uznaje się aktywność dehydrogenaz, których oznaczenie jest często stosowane jako ocena stopnia antropogenicznych przekształceń gleb. Istotnym elementem antropogenicznych przekształceń środowiska przyrodniczego jest kwasowa degradacja gleb, której przyczyną jest składowanie kwasotwórczych substancji, np. siarki elementarnej i zanieczyszczenie atmosfery jej związkami. W Polsce największe obszary gleb silnie zasiarczonych występują w Tarnobrzeskim Zagłębiu Siarkowym, gdzie rozpylona siarka (transportowana eolicznie z pól górniczych i miejsc składowania) oraz produkty jej utlenienia w postaci kwaśnych deszczy lub suchego opadu dostawała się nie tylko do gleb położonych na terenie kopalń, ale także do gleb znajdujących się poza ich granicami.

Celem badań była ocena wpływu zanieczyszczeń emitowanych podczas 30-letniej eksploatacji złóż siarki na aktywność mikrobiologiczną gleb położonych w sąsiedztwie Kopalni Siarki Grzybów. Badane gleby, pobrane z terenów położonych w sąsiedztwie kopalni charakteryzują się niskim, z reguły poniżej jedności, poziomem aktywności dehydrogenaz glebowych oraz kwaśnym odczynem i z reguły wysokim udziałem siarki siarczanowej w zawartości siarki ogółem. Aktywność dehydrogenaz w poziomach powierzchniowych badanych gleb mieściła się w granicach od 0,13 do 1,85 $cm^3H_2 \cdot kg^{-1} \cdot d^{-1}$ i zmniejszała się wraz ze wzrostem głębokości gleby (w poziomach podpowierzchniowych kształtowała się w zakresie od 0,11 do 0,28 $cm^3H_2 \cdot kg^{-1} \cdot d^{-1}$). Przeprowadzone oznaczenia aktywności badanych enzymów wskazują, że wielkość zanieczyszczenia środowiska glebowego wokół kopalni pozostaje na poziomie, który może zagrażać organizmom żywym. Przyczyną znacznego obniżenia poziomu aktywności dehydrogenaz badanych gleb może być ich kwaśny odczyn, który jest wynikiem długoletnich emisji związków siarki. Siarka w powierzchniowej warstwie gleby ulega utlenieniu do kwasu siarkowego, który powoduje silne zakwaszenie gleb. Badane gleby znajdujące się w zasięgu długoletniego oddziaływania otworowej kopalni siarki Grzybów charakteryzowały się wartościami pH w KCl w zakresie od 3,25 do 4,75 i na tej podstawie zostały zaliczone do gleb kwaśnych i bardzo kwaśnych.

Słowa kluczowe: aktywność dehydrogenaz, gleby kwaśne, kopalnia siarki