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APPLICATION OF BIOLOGICAL INDICATORS FOR ESTIMATION OF REMEDIATION OF SOIL DEGRADED BY SULPHUR INDUSTRY

WYKORZYSTANIE WSKAŹNIKÓW BIOLOGICZNYCH DO OCENY REKULTYWACJI GLEBY ZDEGRADOWANEJ PRZEZ PRZEMYSŁ SIARKOWY

Abstract: The study was conducted on an experiment established in the area of the former Sulphur Mine "Jeziorko." The remediation was applied to a soil-less formation with particle size distribution of weakly loamy sand, strongly acidified and with bad sorptive properties ($C_{org.}$ - 2.0 g kg^{-1}; pH_{KCl} - 4.3; T - 7.0 cmol(+) kg^{-1}). In the particular treatments of the experiment the following were applied to the soil-less formation: flotation lime and NPK; lime and sewage sludge; sewage sludge; mineral wool (5 cm 50 cm^{-1}), lime and NPK; mineral wool (5 cm 50 cm^{-1}), lime and sewage sludge; mineral wool (500 m^3 ha^{-1}), lime and NPK; mineral wool (500 m^3 ha^{-1}), lime and sewage sludge. Plots prepared in that manner were then sown with a mix of grasses. The control was the soil with no amendments. The analyses of the soil material comprised assays of the numbers of particular groups of bacteria and fungi, and of their biochemical and enzymatic activities. The study revealed that all the wastes applied for the remediation caused an increase in the numbers of the bacterial groups studied (copiotrophic, oligotrophic, cellulolytic, lipolytic), as well as in the respiration activity and rate of mineralisation of cellulose. That effect was the most pronounced in the case of sewage sludge. In treatments in which sewage sludge was applied, an increase was also observed in the numbers of the studied fungi (fungi on Martin medium, cellulolytic fungi, lipolytic fungi) and in lipase activity. Whereas, the application of the remaining wastes resulted in a slight decrease in the numbers of the fungal groups under analysis. Comparing the mean annual values of the analysed biological, physical, chemical and physicochemical properties it was found that the biological properties were as sensitive, and in the case of certain tests (numbers of cellulolytic and lipolytic bacteria, rate of cellulose mineralisation) even more sensitive indicators of positive changes taking place in the remediated soil.

Keywords: degraded soil, microbiological activity, numbers of bacteria and fungi, respiration, mineralisation of cellulose, lipase activity

Introduction

The microbiological and biochemical activity of soils is commonly accepted as a sensitive indicator of their biological status. The qualitative and quantitative composition of soil micro-biocenoses plays a highly important role in numerous biological processes, and its changes have a bearing on the correct functioning of ecosystems [1]. Therefore, the

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total number of microorganisms and the numbers of their particular physiological groups have been frequently applied for the estimation of the status of the soil environment, including the effects of various kinds of anthropopressure [2-13].

It is commonly known that determination of microbial populations with the method of culturing, though it provides valuable information on living cells, does not fully reflect the actual state of populations of soil microorganisms. That is because only a part of them are capable of growth on artificial substrates [6, 9, 13]. Other important issues include suitable choice of substrate and time of incubation, and the method of interpretation of results obtained with that method [13, 14]. The advantages are that the plate count method is relatively rapid and inexpensive and yields well-separated colonies suitable for subsequent purification and characterisation [15]. For this reason it is important to apply those methods in conjunction with other parameters defining the properties of soil, *ie* biochemical, enzymatic, or physicochemical, as complementary ones. Many of those properties are considered to be sensitive indicators of changes taking place in the soil environment. Those include, among others, the amount of evolved CO_2. The primary producers of CO_2 in soil are heterotrophic microorganisms [16]. Therefore many researchers adopted the intensity of its evolution as one of the bioindicators providing information on the processes of soil degradation or remediation, taking place under the effect of various factors [6, 8, 17-21]. That indicator has also been frequently used in the estimation of effects of soil amendment with sewage sludge [4, 5, 17, 20-22].

Another indicator of the biological activity of soil environment, related with carbon transformations, is the rate of mineralisation of cellulose, measured by the rate of CO_2 liberation. Similar to respiration, the rate of cellulose mineralisation proved to be a sensitive indicator of microbial activity in soil. That test was applied *eg* in analyses of the effects of chemical agents [23] and sewage sludge [4, 5] on the biological properties of soil.

The study of the biological activity of soils has also been based on measurements of enzymatic activity which is responsible for sustaining the biochemical processes in soil, significantly determining its fertility [24]. Soil microorganisms play the main role in the cycling of C and nutrients, and therefore their enzymatic activity has a bearing on soil fertility [25]. The enzymes include *eg* lipases (EC. 3.1.1.3.) which are a group of extracellular enzymes capable of hydrolysing triglycerides, with simultaneous liberation of free fatty acids and glycerols [26]. According to Margezin et al [6], lipase activity is an indicator that is perfectly suited for the monitoring of processes of bioremediation of degraded soils.

The above review of the literature indicates that the biological activity has been often used for the monitoring of the status of soils subjected to various forms of anthropopressure. An example of strong anthropopressure exerted by man on the soil environment is soil contamination with sulphur. The effect of excessive levels of sulphur in soil is acidification, leading to radical changes in biological balance, destruction of the sorptive complex, increased concentration of Al^{3+} ions in the soil solution, and to retrogradation of other elements, which deprives the soil of its utility values [27]. One of the sources of soil contamination with that element is sulphur mining. The diversity of forms of soil degradation related with sulphur mining induces the search for the best method of remediation of the soils and for suitable parameters of estimation of the degree of their regeneration.

In view of the above, a study was undertaken to provide answers to the question to what extent various waste materials (sewage sludge, mineral wool, flotation lime) can improve the relations within the microbiocenosis of soil degraded by sulphur mining and whether biological tests are more effective indicators of improvement of its quality than chemical or physicochemical ones.

The estimation of effectiveness of remediation of the soil was made with the use of microbiological and biochemical parameters related with the carbon cycle.

Materials and methods

Model of the experiment

The study was realized on the model of a remediation experiment established by the Institute of Soil Science and Environmental Engineering, University of Life Sciences in Lublin, Poland. The experiment was set up in the area of the former Sulphur Mine „Jeziórko" (Poland, Podkarpacie Region), on a soil-less formation with the particle size distribution of weakly loamy sand, strongly acidified, with bad sorptive properties and a low content of $C_{org.}$ and N total (Table 1). Sulphur mining at the Mine was conducted with the Frasch method. In the particular variants of the experiment, various remediation materials were applied to the soil-less formation: flotation lime, NPK, sewage sludge and mineral wool.

Scheme of the experiment:

1. Soil without amendments (control)
2. Soil + lime 100 Mg ha^{-1} + NPK (80; 40; 60)
3. Soil + lime 100 Mg ha^{-1} + sewage sludge 100 Mg ha^{-1}
4. Soil + sewage sludge 100 Mg ha^{-1}
5. Soil + wool 5 cm 50 cm^{-1} + lime 100 Mg ha^{-1} + NPK (80; 40; 60)
6. Soil + wool 5 cm 50 cm^{-1} + lime 100 Mg ha^{-1} + sewage sludge 100 Mg ha^{-1}
7. Soil + wool 500 m^3 ha^{-1} + lime 100 Mg ha^{-1} + NPK (80; 40; 60)
8. Soil + wool 500 m^3 ha^{-1} + lime 100 Mg ha^{-1} + sewage sludge 100 Mg ha^{-1}

Table 1

Selected properties of the degraded soil and the wastes used for remediation

Property	Unit	Degraded soil	Flotation lime	Sewage sludge	Mineral wool
Particle size distribution	[% sand] [% silt] [% fine fract.]	91 3 6	35 29 36	n.d.	n.d.
pH	[1 mol KCl]	4.3	6.8	6.4	5.3-6.6
T	[cmol(+) kg^{-1}]	7.0	122.9	54.5	60.9
$C_{org.}$	[g kg^{-1}]	2.0	2.58	193.8	28.5
N total		0.3	10.4	28.0	5.3

Mineral wool was applied in two variants, *ie* in the form of a 5 cm insert at the depth of 50 cm, and at the dose of 500 m^3 ha^{-1}, distributed within the layer of 0-20 cm. Plots (area of 30 m^2) prepared in that manner were sown with a mix of grasses. The control treatment in the experiment was the soil with no amendments.

Prior to the analyses, the particle size distribution and reaction, sorptive capacity, content of C_{org} and N total were determined in the degraded ground and in the waste materials used for the remediation. The assays were conducted at the Institute of Soil Science and Environmental Engineering, University of Life Sciences in Lublin. The results are presented in Table 1. In addition, the biological properties of the waste materials were determined (Table 2).

Table 2

Biological properties of wastes used for remediation

Biological parameter	Flotation lime	Sewage sludge	Mineral wool
Oligotrophic bacteria [cfu 10^9 kg^{-1}d.m.]	0.07	12.34	2.22
Copiotrophic bacteria [cfu 10^9 kg^{-1}d.m.]	0.35	5.18	0.91
Total fungi [cfu 10^6 kg^{-1}d.m.]	3.85	140.09	26.57
Cellulolytic bacteria [10^5 kg^{-1}d.m.]	0.00	15.00	2.50
Cellulolytic fungi [cfu 10^6 kg^{-1}d.m.]	6.30	93.66	36.32
Lipolytic bacteria [cfu 10^9 kg^{-1}d.m.]	0.04	2.72	0.63
Lipolytic fungi [cfu 10^6 kg^{-1}d.m.]	0.35	172.23	96.85
Respiration [mg C-CO_2 kg^{-1}d.m. d^{-1}]	18.88	389.28	22.53
Cellulose mineralisation [mg C-CO_2 kg^{-1}d.m. 20 d^{-1}]	94.00	1316.34	995.68
Lipase [g^{-1}d.m.]	0.08	24.98	0.16

Soil samples

Samples of soil material from the remediation experiment were taken from the layer of 0-20 cm, three times during the first year of the experiment. The first sampling time was at the beginning of May (6th May, 2011), next at the beginning of July (1st July, 2011), and then at the end of September (29th September, 2011). The microbiological and biochemical analyses were performed in suitable prepared soil samples. Those assays were complemented with physical, chemical and physicochemical analyses (Table 3) that were performed at the Institute of Soil Science and Environmental Engineering, University of Life Sciences in Lublin.

Table 3

Selected physical, chemical and physicochemical properties of the soil (means for the year).

Item	Experimental treatments	Moisture [%]	$C_{org.}$ [g kg^{-1}]	N total [g kg^{-1}]	Sorptive capacity T [cmol (+) kg^{-1}]	pH range
1.	Soil without amendments (control)	4.26	2.03	0.32	6.97	4.1-4.3
2.	Soil + lime + NPK	3.11	2.52	0.44	14.37	7.3-7.6
3.	Soil + lime + sewage sludge	10.04	4.20	1.06	15.50	6.6-7.1
4.	Soil + sewage sludge	8.09	4.50	0.53	8.73	6.1-6.8
5.	Soil + wool 5 cm 50 cm^{-1} + lime + NPK	4.52	3.98	0.54	14.90	7.3-7.4

Item	Experimental treatments	Moisture [%]	$C_{org.}$ [g kg^{-1}]	N total [g kg^{-1}]	Sorptive capacity T [cmol (+) kg^{-1}]	pH range
6.	Soil + wool 5 cm 50 cm^{-1} + lime + sewage sludge	6.72	4.47	1.37	15.62	6.9-7.2
7.	Soil + wool 500 m^3 ha^{-1} + lime + NPK	4.16	3.40	0.35	14.60	7.3-7.4
8.	Soil + wool 500 m^3 ha^{-1} + lime + sewage sludge	8.83	5.50	0.67	15.73	6.6-7.2

Microbiological analyses

The populations of the particular groups of bacteria and fungi in the soil samples and in the waste materials were assayed with the plate count method and with the enumeration method described by Foght and Aislabie [15]. The cultures of the microorganisms were conducted at temperature of 28ºC.

The assays were made in three replicates and the results were converted to 1 kg of dry matter of soil or waste, and given in the form of colony forming units (cfu) or the most probable number (MPN) read from McCrady's Tables. The scope of the microbiological analyses included also assays of the numbers of oligotrophic bacteria, on a medium with soil extract or waste extract and K_2HPO_4; copiotrophic bacteria, on the Bunt-Rovira medium [28]; fungi on Martin medium [29]; cellulolytic bacteria on liquid medium acc. to Pochon and Tardieux [30], the most probable number of those bacteria being read from McCrady's Tables; cellulolytic fungi on mineral agar with an addition of antibiotics in amount recommended by Martin [29], covered with a circle of Whatman paper; lipolytic bacteria on agar medium with tributyrate [31] and broth instead of yeast extract; lipolytic fungi on above medium with antibiotics in amount recommended by Martin [29].

Biochemical analyses

The following parameters were analysed in the soil and waste samples: respiratory activity after addition of 1% of glucose, with the method of Ruhling and Tyler [32]; the amount of evolved CO_2 was assayed with the titration method and presented as mg C-CO_2 kg^{-1} d.m. of soil or waste d^{-1}; rate of cellulose mineralisation in 25-gram weighed portions enriched with 0.5% of powdered cellulose, where the amount of evolved CO_2 was assayed with the method of Ruhling and Tyler [32], and the results were given as mg C-CO_2 kg^{-1} d.m. of soil or waste 20 d^{-1}; lipase activity with the titration method acc. to Pokorna [33], as modified by Kuhnert-Finkernagel and Kandeler [34], the results presented as unit g^{-1} d.m. of soil or waste.

Physical, chemical and physicochemical analyses

These analyses included the following (for soil and wastes):

- particle size distribution with the method of Proszynski in Casagrande modification
- moisture, with the gravimetric method
- $C_{org.}$ with the method of Tiurin in Simakov modification
- N total with Kjeldahl method, using the Kjeltech 1002 distillation unit
- sorptive capacity (T) was calculated by summing up the hydrolytic acidity and the sum of base cations
- soil reaction, potentiometrically in 1 mol · dm^{-3} KCl.

All analyses were performed in three parallel replicates. The presented results are means from the three replicates. The results of the microbiological and biochemical analyses were processed statistically, determining the significance of differences using the Tukey test, at significance level of 0.05. Also, correlation coefficients were determined using the CORE library program for the characterisation of multi-variable samples.

Results

Microbiological indices

The data relating to the numbers of the bacterial groups studied (Tables 4 and 5) revealed a stimulating effect of all the wastes used in the study on those microbiological parameters. The positive effect was the most pronounced in the degraded soil to which sewage sludge was applied, whether alone or together with the other waste materials. The least effect on the numbers of the studied groups of bacteria was noted in the case of lime with NPK, both when applied separately or in conjunction with the insert of mineral wool (5 cm 50 cm^{-1}).

Table 4

Numbers of oligotrophic and copiotrophic bacteria in the soil

Item	**Experimental treatments**	**Oligotrophic bacteria [cfu 10^9 kg^{-1} d.m. soil]**				**Copiotrophic bacteria [cfu 10^9 kg^{-1} d.m. soil]**			
		spring	**summer**	**autumn**	**mean**	**spring**	**summer**	**autumn**	**mean**
1.	Soil without amendments (control)	0.10	0.04	0.45	0.20	0.45	0.23	0.56	0.41
2.	Soil + lime + NPK	1.59	0.30	0.81	0.90	1.99	0.22	1.12	1.11
3.	Soil + lime + sewage sludge	3.48	0.52	1.49	1.83	2.95	1.13	1.63	1.90
4.	Soil + sewage sludge	2.21	0.81	0.70	1.24	1.81	0.87	1.02	1.23
5.	Soil + wool 5 cm 50 cm^{-1} + lime + NPK	1.25	0.13	0.97	0.78	0.21	0.21	0.83	0.41
6.	Soil + wool 5 cm 50 cm^{-1} + lime + sewage sludge	1.70	0.24	1.50	1.15	1.44	0.80	1.60	1.28
7.	Soil + wool 500 m^3 ha^{-1} + lime + NPK	1.66	0.27	1.28	1.07	1.14	0.52	1.82	1.16
8.	Soil + wool 500 m^3 ha^{-1} + lime + sewage sludge	4.37	1.07	2.14	2.52	3.60	2.06	2.10	2.58
	Mean	2.04	0.42	1.17		1.70	0.75	1.33	
	LSD* date LSD treatment LSD date x treatment	0.07 0.15 0.30				0.10 0.21 0.44			

* LSD - Least significant difference

For most of the bacterial groups studied the method of application of mineral wool proved to be significant. Generally a stronger positive effect was noted when the wool was mixed with the degraded soil (500 m^3 ha^{-1}). Among all the bacterial groups under analysis, the strongest response to the wastes applied to the soil was observed in the case of oligotrophic and cellulolytic bacteria. The stimulating effect of the wastes on those bacterial groups persisted throughout the period of the study. Statistical analysis shows that the numbers of bacteria from the particular groups were subject to periodic fluctuations (Tables 4 and 5). The largest populations of oligotrophic and copiotrophic bacteria were noted in spring, and the smallest in summer. Contrary to those bacteria, lipolytic bacteria displayed the most intensive growth in summer, and the weakest in spring. The numbers of cellulolytic bacteria were similar in spring and summer, but at a notably lower level than in autumn.

Table 5

Numbers of cellulolytic and lipolytic bacteria in the soil

Item	Experimental treatments	Cellulolytic bacteria [10^5 kg^{-1} d.m. soil]				Lipolytic bacteria [cfu 10^9 kg^{-1} d.m. soil]			
		spring	summer	autumn	mean	spring	summer	autumn	mean
1.	Soil without amendments (control)	1.00	1.00	2.00	1.00	0.06	0.12	0.25	0.14
2.	Soil + lime + NPK	3.00	3.00	10.00	5.00	0.15	0.30	0.48	0.31
3.	Soil + lime + sewage sludge	11.00	12.00	147.00	57.00	0.94	0.93	0.87	0.91
4.	Soil + sewage sludge	11.00	48.00	47.00	35.00	0.82	1.11	0.20	0.71
5.	Soil + wool 5 cm 50 cm^{-1} + lime + NPK	3.00	3.00	26.00	11.00	0.30	0.59	0.34	0.41
6.	Soil + wool 5 cm 50 cm^{-1} + lime + sewage sludge	10.00	10.00	118.00	46.00	1.16	1.47	1.23	1.29
7.	Soil + wool 500 m^3 ha^{-1} + lime + NPK	5.00	5.00	47.00	19.00	0.48	0.55	0.89	0.64
8.	Soil + wool 500 m^3 ha^{-1} + lime + sewage sludge	5.00	5.00	48.00	19.00	0.64	1.43	1.10	1.06
	Mean	6.10	10.90	55.60		0.57	0.81	0.67	
	LSD* date LSD treatment LSD date x treatment					0.05 0.10 0.21			

* LSD - least significant difference

Analysis of the data relating to the studied groups of fungi revealed that all of the waste materials applied significantly modified their numbers (Table 6), and that effect was related to the kind of remediation treatment applied. As opposed to bacteria, the fungal populations generally increased only in those treatments in which sewage sludge was applied, whether separately or in combination with the other wastes. Among the fungal groups studied, the strongest stimulation was noted in the case of cellulolytic fungi. Moreover, it was observed that in the soil remediated with wool the level of that effect was related to the method of application of the waste. Stronger growth of cellulolytic fungi and fungi on Martin medium was noted where wool was applied in the form of insert (5 cm 50 cm^{-1}), while in the case of lipolytic fungi - in the treatment with wool mixed with the soil (500 m^3 ha^{-1}).

As opposed to sewage sludge, remediation of the soil with lime and NPK, applied separately or in combination with mineral wool, resulted in a tendency towards a reduction of the numbers of the fungal groups studied. The negative effect of those wastes was the most pronounced in the case of fungi grown on Martin medium. Noteworthy is the fact that the negative effect was not related to the method of application of mineral wool.

The effect of the wastes applied for the remediation on the populations of the fungal groups under study was observed in all periods of study and it was subject to seasonal fluctuations (Table 6). The greatest numbers of cellulolytic and lipolytic fungi were noted in spring, and the smallest in summer and autumn. Whereas, the numbers of fungi on Martin medium were the highest in autumn and the smallest in spring.

Comparing the annual mean values it was noted that among the microbiological indicators the most pronounced response to the positive changes taking place in the soil was that of cellulolytic bacteria, followed by lipolytic bacteria.

Table 6

Numbers of selected groups of fungi in the soil

Item	Experimental treatments	Fungi (Martin medium) [cfu 10^6 kg^{-1} d.m. soil]				Cellulolytic fungi [cfu 10^6 kg^{-1} d.m. soil]				Lipolytic fungi [cfu 10^6 kg^{-1} d.m. soil]			
		spring	sum-mer	au-tumn	mean	spring	sum-mer	au-tumn	mean	spring	sum-mer	au-tumn	mean
1.	Soil without amendments (control)	30.7	52.2	50.2	44.4	26.0	46.0	29.9	34.0	29.5	31.3	24.9	28.6
2.	Soil + lime + NPK	15.4	22.7	40.5	26.2	22.0	22.3	32.2	25.5	18.1	17.5	27.0	20.9
3.	Soil + lime + sewage sludge	47.1	34.7	62.7	48.2	216.1	26.3	46.4	96.3	27.1	21.4	36.4	28.3
4.	Soil + sewage sludge	55.7	60.3	86.8	67.6	231.2	69.9	58.6	119.90	58.2	60.4	35.6	51.4
5.	Soil + wool 5 cm 50 cm^{-1} + lime + NPK	26.1	33.8	35.4	31.8	23.5	23.8	27.8	25.0	23.0	26.2	22.9	24.0
6.	Soil + wool 5 cm 50 cm^{-1} + lime + sewage sludge	68.5	70.5	85.8	74.9	102.0	43.5	50.4	65.3	42.5	37.6	45.3	41.8
7.	Soil + wool 500 m^3 ha^{-1} + lime + NPK	11.9	21.8	48.8	27.5	26.2	28.6	26.8	27.2	17.1	16.6	31.0	21.6
8.	Soil + wool 500 m^3 ha^{-1} + lime + sewage sludge	44.2	38.5	92.8	58.5	55.8	36.8	42.4	45.0	73.0	54.4	28.6	52.0
	Mean	37.46	41.81	62.88		87.9	37.1	39.3		36.05	33.20	31.46	
	LSD* date LSD treatment LSD date x treatment	1.96 4.21 8.65				3.70 7.90 16.2				1.86 3.98 8.18			

* LSD - least significant difference

Biochemical indices

The data relating to the rate of respiration and the rate of cellulose mineralisation indicate that all of the wastes applied caused an increase of those biochemical parameters (Table 7). The positive effect was more pronounced in the case of the rate of cellulose mineralisation. As in the case of the populations of the particular groups of bacteria, the strongest effect on both processes was caused by the sewage sludge, both when applied separately and in combination with the other wastes. Lime and NPK, applied separately or together with mineral wool, also stimulated those biochemical parameters, but notably less strongly than the sewage sludge. The method of application of mineral wool had a certain effect on the level of the stimulation, but that relationship was not a clearly oriented one.

Lipase activity analysed in the study was stimulated only in treatments with sewage sludge, applied both separately and in combination with the other wastes (Table 7). Application of that waste separately or together with lime proved to be the most beneficial. Whereas, a weaker effect was noted after the application of sewage sludge in combination with lime and mineral wool, and especially when wool was in the form of insert (5 cm 50 cm^{-1}). In the remaining treatments lipase activity was at a level similar to that observed in the control treatment.

Table 7

Biochemical activity in the soil

Item	Experimental treatments	Respiration [mg C-CO_2 kg^{-1} d.m. soil d^{-1}]				Cellulose mineralisation [mg C-CO_2 kg^{-1} d.m. soil (20d)$^{-1}$]				Lipase activity [g^{-1} d.m. soil]			
		spring	sum-mer	au-tumn	mean	spring	sum-mer	au-tumn	mean	spring	sum-mer	au-tumn	mean
1.	Soil without amendments (control)	86.82	66.03	64.21	72.36	497.09	238.03	172.00	302.38	0.42	0.25	0.08	0.25
2.	Soil + lime + NPK	76.96	67.43	102.2	82.20	498.06	451.93	650.45	533.48	0.16	0.12	0.08	0.12
3.	Soil + lime + sewage sludge	200.9	169.2	137.5	169.2	1821.8	1415.2	511.04	1249.3	4.81	1.67	0.23	2.24
4.	Soil + sewage sludge	192.6	164.7	120.8	159.3	1625.1	1285.0	793.47	234.52	4.95	3.44	0.59	2.99
5.	Soil + wool 5 cm 50 cm^{-1} + lime + NPK	87.03	55.31	110.4	84.24	333.86	457.22	613.84	468.31	0.23	0.17	0.06	0.15
6.	Soil + wool 5 cm 50 cm^{-1} + lime + sewage sludge	151.8	152.0	195.8	166.6	1376.9	1101.7	1357.1	1278.5	0.15	1.19	0.33	0.56
7.	Soil + wool 500 m^3 ha^{-1} + lime + NPK	71.37	78.94	96.97	82.43	325.60	465.86	460.82	417.43	0.04	0.20	0.19	0.14
8.	Soil + wool 500 m^3 ha^{-1} + lime + sewage sludge	161.3	185.7	122.4	156.5	2170.3	1232.9	651.45	1351.6	1.70	1.21	0.34	1.08
	Mean	128.6	117.4	118.8		1081.1	830.97	651.27		1.56	1.03	0.24	
	LSD* date LSD treatment LSD date x treatment	2.22 4.77 9.80				17.71 3.00 78.06				0.08 0.17 0.35			

* LSD - least significant difference

The biochemical parameters under study were stimulated in all the periods of study, but with varied intensity (Table 7). The rate of cellulose mineralisation and lipase activity most often attained the highest values in spring. Whereas, in the case of respiration this kind of regularity was noted only for three treatments.

Summing up the results obtained one should conclude that among the biochemical tests studied the rate of cellulose mineralisation was the most sensitive indicator of improvement in the properties of the degraded soil.

Correlations

The data presented in Table 8 indicate the existence of positive correlations both within and among almost all of the microbiological and biochemical parameters analysed in this study. Almost all of the correlations were at significance level of $p < 0.01$. The lowest number of significant correlations was noted in the case of the numbers of cellulolytic bacteria that correlated only with the number of lipolytic bacteria, total fungi, and respiration. Whereas, almost all of the microbiological and biochemical parameters correlated positively with the content of $C_{org.}$ (except cellulolytic fungi) and with moisture (except cellulolytic bacteria). Most of the parameters under analyses were also correlated with the content of N total (except total number of bacteria, number of cellulolytic and lipolytic fungi, and lipase activity). Whereas, reaction was correlated only with the numbers of oligotrophic and lipolytic bacteria, and sorptive capacity only with total number of oligotrophic and macrotrophic bacteria, and with the number of lipolytic bacteria.

Table 8

Coefficients of correlation

	Copio-trophic bacteria	**Total fungi**	**Cellulo-lytic bacteria**	**Cellulo-lytic fungi**	**Lipolytic bacteria**	**Lipolytic fungi**	**Respiration**
Oligotrophic bacteria	0.8914**	-	-	0.5278**	0.2432*	0.4574**	0.5179**
Copiotrophic bacteria		-	-	0.4455**	0.4032**	0.5060**	0.6171**
Total fungi			0.5634**	0.2634*	0.4868**	0.4412**	0.4829**
Cellulolytic bacteria				-	0.3360**	-	0.3317**
Cellulolytic fungi					0.2717*	0.3816**	0.6129**
Lipolytic bacteria						0.4764**	0.7370**
Lipolytic fungi							0.6563**
Respiration							
Mineralisation of cellulose							
Lipase							

	Cellulose minerali-sation	**Lipase**	**pH**	**Moisture**	**T**	**$C_{org.}$**	**N total**
Oligotrophic bacteria	0.6598**	0.4621**	0.2383*	0.6824**	0.2821*	0.5009**	-
Copiotrophic bacteria	0.6936**	0.4330**	-	0.6752**	0.3362**	0.5412**	0.2965*
Total fungi	0.3061**	-	-	0.2353*	-	0.5857**	0.5719**
Cellulolytic bacteria	-	-	-	-	-	0.4177**	0.5881**
Cellulolytic fungi	0.6178**	0.8486**		0.8232**	-	-	-
Lipolytic bacteria	0.5732**	0.3460**	0.3694**	0.4677**	0.4873**	0.6298**	0.6542**
Lipolytic fungi	0.7086**	0.4891**	-	0.4850**	-	0.5074**	-
Respiration	0.8816**	0.6877**	-	0.7960**	-	0.6732**	0.6034**
Mineralisation of cellulose		0.7112**	-	0.7979**	-	0.5965**	0.4375**
Lipase				0.8341**	-	0.2492*	-

- no correlation; ** significance level $p = 0.01$; * significance level $p = 0.05$

Discussion

Microbiological indices

The increase noted in the numbers of all bacterial and fungal groups studied, observable especially in treatments with sewage sludge, was surely caused by supplying, together with that waste, nutrients necessary for the growth and development of heterotrophic microorganisms. The analysis of the content of C_{org}. and N total in the remediated soil indicates that the values of those parameters increased notably after the application of sewage sludge (Table 3). The data presented in Table 8 indicate that the numbers of almost all of the microbial groups under study were positively correlated with the content of $C_{org.}$, and most of them also with the content of N total. An increase in the numbers of analogous microbial groups as a result of enrichment of soils with nutrients is also reported by other authors [4, 6, 9-11, 35]. Moreover, Whitelaw-Wecker et al [10] demonstrated significant positive correlations between the content of $C_{org.}$ and the numbers of oligotrophic and copiotrophic bacteria, fungi, and cellulolytic bacteria and fungi. Also Czaban et al [3] observed a positive correlation between soil organic matter and the number of oligotrophic bacteria. Noteworthy is the fact that also bacteria with low nutrient requirements responded to the increase in the content of nutrients for heterotrophic microorganisms. Such a phenomenon was observed earlier by numerous authors [4, 9, 10, 35]. Fierer et al [35] suggest that there may be an additional factor that determines the growth of those microorganisms. Under the conditions of this experiment, it could have been the reaction of the soil, for which a positive correlation was found with the number of oligotrophic bacteria. A positive correlation between the total number of bacteria and soil reaction was noted also by other authors [3, 7]. The increase of the reaction of the remediated soil could have also had an additional effect on the number of lipolytic bacteria which was positively correlated with that physicochemical parameter. Moreover, the positive effect of the waste materials on the numbers of the microbial groups under study could have been related with improvement of other soil properties, such as sorptive capacity or moisture, as an improvement of those parameters in the soil amended with the waste materials was demonstrated (Table 3). The data given in Table 8 prove the existence of positive correlations between moisture and nearly all of the microorganisms analysed in this study (except cellulolytic bacteria). In addition, a positive correlation was shown between the total numbers of oligotrophic and macrotrophic bacteria and lipolytic bacteria, and the sorptive capacity (Table 8). A positive correlation of moisture with the number of oligotrophic bacteria and with the number of total fungi was also noted by Czaban et al [3].

The periodic increase in the numbers of microorganisms in soil remediated with the wastes, and especially with the sewage sludge, could have been contributed to by the introduction of a certain number of microorganisms together with the wastes (Table 2). However, studies by Joniec and Furczak [5] indicate that microorganisms of sludge origin do not tend to inhabit a soil environment.

The application of wool and lime alone could be conducive to a stimulation of competition between bacteria and fungi, with consequent slight decrease in the numbers of fungi, though the relationships observed between the numbers of the fungal and bacterial groups under study had either a positive character or were totally absent. A lack of correlations between the numbers of oligotrophic bacteria and the numbers of fungi on Martin medium was also noted by Czaban et al [3].

Biochemical indices

The introduction of nutrients for microorganisms together with the waste materials (Table 1), and the improvement of other conditions for microbial life in the remediated soil, *ie* moisture, sorptive capacity, reaction (Table 3), caused not only an increase in the numbers of the microorganisms but also a stimulation of their biochemical activity. The above statement is supported by numerous positive correlations between the microbial populations and respiration, rate of cellulose mineralisation, and lipase activity (Table 8). The stimulating effect was the most pronounced in treatments where sewage sludge was applied for soil remediation. That phenomenon was accompanied by the greatest increase in the content of $C_{org.}$ and N total (Table 3). This indicates that the main cause of the stimulation of the rate of respiration, cellulose mineralisation and lipase activity was the introduction of nutrients together with that waste, that intensified the growth and activity of microorganisms responsible for those processes and for lipase production. This is supported by the results given in Table 8 which show that all biochemical parameters under analysis were positively correlated with the content of $C_{org.}$, and in addition almost all of them (except lipase) were also correlated with the content of N total. Similar conclusions were reached also by other authors who noted an increase in the values of the biochemical parameters in question after the introduction of an additional source of nutrients for heterotrophic microorganisms into soil [4-6, 16-18, 22, 26]. Most of those authors demonstrated also a higher stimulation of respiration or of lipase activity under the effect of organic fertilisation relative to mineral. Likewise, in this study a stronger stimulation of respiration, cellulose mineralisation and lipase activity was noted in soil amended with sewage sludge relative to soils in which wastes of mineral character were applied.

The increase of biochemical activity in the remediated soil enriched with waste organic matter was probably helped also by its protective function towards extracellular enzymes, which was also noted by other authors [22].

Under the conditions of this experiment soil moisture also had a significant effect on the biochemical and enzymatic activities. Significant correlations were observed between that parameter and respiration, rate of cellulose mineralisation, and lipase activity (Table 8). No such correlations were shown with reaction, though other authors noted a positive correlation between lipase and soil reaction [6].

The improvement of living conditions for the microorganisms under the effect of the wastes applied, noted in the form of increase of sorptive properties (Table 3), could also have an effect on the stimulation of respiration processes, rate of cellulose mineralisation and lipase activity. However, the data relating to the correlations (Table 8) did not show any relationships between those parameters.

It is also possible that in the initial period a certain role in increasing the level of those parameters could have been played by microorganisms and extracellular enzymes introduced in the soil together with sewage sludge (Table 2). Similar conclusions were reached also by other authors [4, 5, 36, 37].

In the analysed soil positive correlations were noted between respiration, cellulose mineralisation and lipase activity (Table 8). A positive correlation between respiration and lipase activity was noted also by Margesin et al [6].

Under the conditions of this experiment, the existence of positive correlation (Table 8) between the rate of cellulose mineralisation and the number of cellulolytic fungi, and a lack of such correlation with the number of cellulolytic bacteria, suggests that fungi were more active in the processes of cellulose mineralisation in the soil under analysis.

The results relating to the seasonal changes showed that almost all of the microbiological and biochemical parameters studied attained their highest values in spring. That was probably related with high availability of nutrients brought in several months earlier with the wastes, and with temperature increase after the winter period. Only the numbers of cellulolytic bacteria and total fungi were the highest only in autumn.

The existence of numerous positive correlations within and among the microbiological and biochemical activities under study suggests that the processes of remediation involve participation of various mutually related groups of microorganisms. The existence of such relationships was demonstrated earlier also by Furczak and Joniec [4]. Moreover, the occurrence of positive correlations between all studied microbiological and biochemical parameters and respiration, accepted as a sensitive indicator of activity of soil microorganisms [16], suggests that those parameters are also sensitive indicators of changes taking place in remediated soil.

Conclusions

The study demonstrated that the wastes applied for soil remediation purposes significantly and most often positively modified the studied microbiological (numbers of oligotrophic, copiotrophic, cellulolytic, lipolytic bacteria; numbers of fungi on Martin medium, cellulolytic and lipolytic fungi) and biochemical (respiration, rate of cellulose mineralisation, lipase activity) parameters of the soil. The strongest stimulation was caused by the application of sewage sludge, both separately and in combination with other wastes. That waste caused a notable stimulation of the biological properties of the degraded soil. Moreover, the level of that stimulation in treatments where mineral wool was additionally applied depended to a degree on the method of application of that waste. For most of the parameters analysed, mixing the wool with the soil proved to be more beneficial (500 m^3 ha^{-1}). Only in treatments where lime, mineral wool and NPK were applied a slight decrease was noted in the numbers of the analysed groups of fungi. The biological parameters used in the experiment proved to be, in their majority, sensitive indicators of positive changes taking place in the analysed soil after the application of the waste materials. Noteworthy is the fact that the response of some of them (numbers of cellulolytic and lipolytic bacteria, cellulose mineralisation) to the remediation measures was stronger than that of the chemical and physicochemical properties. The above observations indicate their high applicability for the estimation of the effectiveness of undertaken remediation operations, and for the monitoring of the status of the soil environment.

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WYKORZYSTANIE WSKAŹNIKÓW BIOLOGICZNYCH DO OCENY REKULTYWACJI GLEBY ZDEGRADOWANEJ PRZEZ PRZEMYSŁ SIARKOWY

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Abstrakt: Badania wykonano na doświadczeniu, założonym na terenie byłej Kopalni Siarki „Jeziorko”. Rekultywacji poddano utwór bezglebowy o składzie granulometrycznym piasku słabogliniastego, silnie zakwaszony i o złych właściwościach sorpcyjnych ($C_{org.}$ - 2.0 g kg^{-1}; pH_{KCl} - 4.3; T - 7.0 cmol(+) kg^{-1}). W poszczególnych kombinacjach doświadczenia do rekultywowanego utworu bezglebowego wprowadzono: wapno poflotacyjne i NPK; wapno i osad ściekowy; osad ściekowy; wełnę mineralną (5 cm 50 cm^{-1}), wapno i NPK; wełnę mineralną (5 cm 50 cm^{-1}), wapno i osad ściekowy; wełnę mineralną (500 m^3 ha^{-1}), wapno i NPK; wełnę mineralną (500 m^3 ha^{-1}), wapno i osad ściekowy. Tak przygotowane poletka obsiano następnie mieszanką traw. Kontrolę doświadczenia stanowiła gleba niepoddana zabiegom ulepszającym. W ramach analiz w materiale glebowym określano liczebność poszczególnych grup bakterii i grzybów oraz ich aktywność biochemiczną i enzymatyczną. Przeprowadzone badania wykazały, że wszystkie zastosowane do rekultywacji odpady spowodowały wzrost liczby badanych grup bakterii (kopiotroficzne, oligotroficzne, celulolityczne, lipolityczne) aktywności oddechowej i tempa mineralizacji celulozy. Oddziaływanie to najsilniej uwidoczniło się pod wpływem osadu ściekowego. W obiektach, gdzie wprowadzono osad ściekowy, odnotowano również wzrost liczby badanych grup grzybów (grzyby na pożywce Martina, celulolityczne, lipolityczne) i aktywności lipazy. Natomiast dodatek pozostałych odpadów skutkował niewielkim spadkiem liczby analizowanych grup grzybów. Porównując średnie roczne wartości badanych właściwości biologicznych, fizycznych, chemicznych i fizykochemicznych, stwierdzono, że właściwości biologiczne okazały się równie czułymi, a nawet w przypadku niektórych testów (liczba bakterii celulolitycznych i lipolitycznych, tempo mineralizacji celulozy) czulszymi wskaźnikami pozytywnych zmian zachodzących w rekultywowanym gruncie.

Słowa kluczowe: gleba zdegradowana, aktywność mikrobiologiczna, liczebność bakterii i grzybów, oddychanie, mineralizacja celulozy, aktywność lipazy