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Soil Biology

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MICROBIOLOGICAL TRANSFORMATIONS OF N, S AND P IN DEGRADED SOIL SUBJECTED TO ONE-YEAR REMEDIATION WITH VARIOUS WASTES**

Abstract. This study was conducted on the material originating from a reclamation experiment established in the area of the former Sulphur Mine "Jeziórko", on a soil-less formation with a particle size distribution of weakly loamy sand, strongly acidified and with poor sorptive properties. 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 this manner were then sown with a mix of grasses. The control treatment in the experiment was the ground with no amendments. The analyses of the soil material comprised assays of the numbers of bacteria and fungi degrading protein, and of their enzymatic activities i.e. arylsulphatase and alkaline phosphatase. The waste materials applied to the degraded soil had a stimulating effect on the analyzed parameters. All of the wastes applied, and the sewage sludge in particular caused an increase in the numbers of proteolytic bacteria and in the activity of alkaline phosphatase and arylsulphatase. Sewage sludge applied separately or in combination with other wastes contributed also to an increase in the number of proteolytic fungi and to an intensification of the process of nitrification. The process of ammonification was also stimulated, but only under the effect of sewage sludge applied alone or together with lime. The application of mineral wool at the dose of 5 cm 50 cm^{-1} in combination with lime caused a slight decrease in the activity of that process.

Sulphur is an element that is common in nature and indispensable for the life of all living organisms. In the environment it undergoes transformations of

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both biotic (mainly with the participation of microorganisms, to a lesser extent of plants and animals) and abiotic character. However, a disturbance of the natural cycle of that element may result in a disturbance of the biological equilibrium of the environment. In consequence, sulphur becomes a significant factor limiting or even rendering impossible the correct functioning of living organisms. An example of strong anthropopressure on the natural cycle of sulphur in nature can be, for instance, sulphur mining based on the Frasch process. Exploitation of sulphur deposits with that method results in multifarious degradation of the soil environment, i.e. the geomechanical, hydrological, thermal and chemical degradation. The effect of excessive levels of sulphur in the soil is strong acidification leading to radical changes in the biological balance, destruction of the sorptive complex, increased concentration of Al^{3+} ions in the soil solution, and retrogradation of other elements, which deprives the soil of its utility values [25]. In Poland, sulphur mining occupies an area of 3.6 thousand ha, which is 8.2% of the total mining areas in the country [12]. Therefore, the problem of degradation of soils and reclamation of post-mining areas remains current and actual long after the termination of exploitation of those mines.

One of the methods of reclamation of soils degraded by sulphur mining may be the application of waste materials such as flotation lime, mineral wool or sewage sludge. Sewage sludge is a rich source of organic matter, as well as such elements as nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, and sodium, and it has valuable humus-forming properties [1, 20]. Mineral wool, a waste product in horticultural production, is also characterized by favourable sorptive properties, and, in particular, a high content of base cations and their saturation, low hydrolytic acidity and a high water retention capacity [2]. A review of the literature indicates that attempts at the reclamation of soils degraded by the sulphur industry with the use of such waste materials have already been undertaken [2, 3, 19]. These authors demonstrated that post-use mineral wool Grodan, especially when applied together with municipal sewage sludge, had a positive effect on water capacity and on the nitrogen level and balance, and thus on the productivity of the reclaimed soils [2, 3, 19]. Kaniuczak [19] noted an increase in the content of C_{org}, N_{tot}, available forms of phosphorus, potassium and magnesium, and an increase in the reaction of soils remediated with flotation lime and sewage sludge.

The status of the soil environment is indicated also by the parameters informing about its microbiological and biochemical activity. In this context, a number of biological and biochemical properties can be used as early and sensitive indicators of SOM transformations and dynamics, nutrient cycling, and stress and recovery conditions in soil [5]. Indicators frequently used in the estimation of the status of the soil environment included e.g. the numbers of proteolytic bacteria and fungi, activity of alkaline phosphatase, arylsulphatase, and the rates of the processes of ammonification and nitrification [4, 6, 8–10, 15–18, 21, 26, 29, 31]. Majority of the studies were concerned with the effect of sewage sludge on the above parameters of microbial activity [4, 6, 8–10, 16, 21, 26, 29, 31]. Generally, the authors cited above noted an increase in the values of those parameters after the application of such wastes to the soil. Only Furczak and Joniec [10] did not observe any effect of sewage sludge on the numbers of proteolytic bacteria, and a slight decrease in the number of proteolytic fungi and in the rate of ammonification. However, there is a lack of research reports concerning the effect of mineral wool on the microbiological status of soil, and of both of those waste materials applied in combination with other components.

For this reason, in the study presented herein, an attempt was made at the estimation of the effect of various waste materials and their combinations on the microbiological properties of soil degraded by sulphur mining, reclaimed with these wastes. To this end, selected microbiological and biochemical parameters related to the cycle of biogenic elements of key importance for the fertility and productivity of soil, i.e. N, P and S, were analyzed.

MATERIALS AND METHODS

The 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 soilless 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_{tot} (Table 1). Sulphur mining at the Mine was conducted with the Frasch method.

TABLE 1. SELECTED PROPERTIES OF THE DEGRADED GROUND AND THE WASTES USED FOR REMEDIATION

Property	Unit	Degraded ground	Mineral wool	Sewage sludge	Flotaion lime
Particle size distribution	% sand	91	n.d.	n.d.	35
	% silt	3			29
	% fine fraction	6			36
pH	1 mol KCl	4.3	5.3–6.6	6.4	6.8
T	cmol(+) kg^{-1}	7.0	60.9	54.5	122.9
N_{total}	g kg^{-1}	0.3	5.3	28.0	10.4
C_{org}		2.0	28.5	193.8	2.6

n.d. – not determined.

In the particular variants of the experiment, various remediation materials were applied to the soil-less formation: flotation lime and NPK (80–40–60); 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. Lime was applied at the dose of l00 t ha^{-1}; sewage sludge was distributed within the soil layer of 20 cm in the amount of l00 t ha^{-1}. Mineral wool was applied in two variants, i.e. 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 prepared in that manner were sown with a mix of grasses. The control treatment in the experiment was the ground with no amendments.

Scheme of the experiment:
1. Ground (soil-less material) without amendments (control).
2. Ground + lime + NPK (80–40–60).
3. Ground + lime + sewage sludge 100 t ha^{-1}.
4. Ground + sewage sludge 100 t ha^{-1}.
5. Ground + wool 5 cm 50 cm^{-1} + lime + NPK (80–40–60).
6. Ground + wool 5 cm 50 cm^{-1} + lime + sewage sludge 100 t ha^{-1}.
7. Ground + wool 500 m^3 ha^{-1} + lime + NPK (80–40–60).
8. Ground + wool 500 m^3 ha^{-1} + lime + sewage sludge 100 t ha^{-1}.

Prior to the analyses, the particle size distribution and reaction, sorptive capacity, content of C_{org} and N_{tot} 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	Wool	Sewage sludge
Proteolytic bacteria, cfu 10^9 kg^{-1} d.m.	0.11	0.37	2.02
Proteolytic fungi, cfu 10^6 kg^{-1} d.m.	0.00	2.69	25.91
Ammonification rate, mg $N\text{-}NH_4$ kg^{-1} d.m. $3d^{-1}$	4.44	65.90	267.88
Nitrification rate, mg $N\text{-}NO_3$ kg^{-1} d.m. $7d^{-1}$	19.23	45.00	666.02
Alkaline phosphatase, mg PNP kg^{-1} d.m. h^{-1}	2.01	4.08	162.57
Arylsulphatase, mg PNP kg^{-1} d.m. h^{-1}	0.21	2.15	160.31

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, PHYSICOCHEMICAL AND CHEMICAL PROPERTIES OF THE SOIL (MEANS FOR THE YEAR)

No.	Experimental treatments	Sorptive capacity T (cmol(+)kg^{-1}	C_{org}	N_{total}	Moisture (%)	pH range
1.	Ground without amendments (control)	6.97	2.03	0.32	4.26	4.1–4.3
2.	Ground + lime + NPK	14.37	2.52	0.44	3.11	7.3–7.6
3.	Ground + lime + sewage sludge 100 t ha^{-1}	15.50	4.20	1.06	10.04	6.6–7.1
4.	Ground + sewage sludge 100 t ha^{-1}	8.73	4.50	0.53	8.09	6.1–6.8
5.	Ground + wool 5 cm 50 cm^{-1} + lime + NPK	14.90	3.98	0.54	4.52	7.3–7.4
6.	Ground + wool 5 cm 50 cm^{-1} + lime + sewage sludge 100 t ha^{-1}	15.62	4.47	1.37	6.72	6.9–7.2
7.	Ground + wool 500 m^3 ha^{-1} + lime + NPK	14.60	3.40	0.35	4.16	7.3–7.4
8.	Ground + wool 500 m^3 ha^{-1} + lime + sewage sludge 100 t ha^{-1}	15.73	5.50	0.67	8.83	6.6–7.2

Microbiological Analyses

The numbers of bacteria and fungi in the soil samples and in the samples of the waste materials were measured with the plate method according to the procedure described by Foght and Aislabie [7]. Microbial cultures were conducted at temperature of 28°C.

The assays were carried out in three replicates and the results were converted to values per 1 kg of dry matter of soil or waste and given in colony forming units (cfu). The microbiological analyses included determinations of the numbers of bacteria degrading protein, on a Frazier gelatine medium [30], protein-degrading fungi, on a Frazier gelatine medium [30], with an addition of antibiotics in amounts recommended by Martin [24].

Biochemical Analyses

The analyses on samples of soil and the wastes included assays of the rate of ammonification, in 25-gram weighed portions of soil containing 0.1% of asparagine (after 3 days of incubation ammonium ions were extracted and their content was assayed with the method of Nessler [27], and the result was given in the form of mg N-NH_4^+ kg^{-1} d.m. of soil $3d^{-1}$), the rate of nitrification, in 25-gram weighed portions containing 0.1% of monobasic ammonium phosphate (after 7 days of incubation nitrate ions were extracted and their level was measured with the brucine method [27], and the result was given in the form of N-NO_3 – kg^{-1}d.m. of soil $7d^{-1}$), activity of alkaline phosphatase, with the method of Tabatabai and Bremner [32], the results given in mg PNP kg^{-1} d.m. of soil h^{-1}, and the activity of arylsulphatase, with the method of Tabatabai and Bremner [33], the results presented in mg PNP kg^{-1} d.m. of soil h^{-1}.

RESULTS AND DISCUSSION

The results concerning the numbers of proteolytic bacteria in the soil subject to one-year reclamation showed that all of the components studied caused an increase in the values of this microbiological parameter (Table 4). The largest numbers of those microorganisms were noted in the treatments where the reclamation was conducted with the application of sewage sludge, alone or in combinations with the other wastes. The lowest increase in the number of protein-degrading bacteria was caused by the mineral wool applied in the dose of 500 m^3 ha^{-1} in combination with lime and NPK.

As in the case of bacteria in the treatments with sewage sludge applied alone or in combination with other wastes, there was also an increase in the numbers of proteolytic fungi (Table 4). The greatest stimulation of the numbers of those microorganisms was caused by sewage sludge applied alone. In the other treatments, i.e. with no addition of sewage sludge, no significant changes in the values of that parameter were observed.

Statistical analysis of seasonal changes in the numbers of proteolytic bacteria and fungi revealed that the highest values were attained in spring, and the lowest in autumn (Table 4).

TABLE 4. NUMBERS OF PROTEOLYTIC BACTERIA AND FUNGI IN THE SOIL

No.	Experimental treatments	Proteolytic bacteria (cfu 10^8 kg^{-1} d.m. of soil)				Proteolytic fungi (cfu 10^6 kg^{-1} d.m. of soil)			
		spring	summer	autumn	mean	spring	summer	autumn	mean
1.	Ground without amendments (control)	1.0	0.2	1.2	0.8	5.2	20.7	5.3	10.4
2.	Ground + lime + NPK	3.4	1.9	2.2	2.5	3.4	36.1	5.2	14.9
3.	Ground + lime + sewage sludge 100 t ha^{-1}	7.7	3.8	3.7	5.0	64.8	58.8	36.9	53.5
4.	Ground + sewage sludge 100 t ha^{-1}	14.1	6.1	2.2	7.5	116.8	81.8	47.1	81.9
5.	Ground + wool 5 cm 50 cm^{-1} + lime + NPK	3.6	0.5	5.1	3.1	3.5	27.6	5.3	12.1
6.	Ground + wool 5 cm 50 cm^{-1} + lime + sewage sludge 100 t ha^{-1}	12.7	5.6	5.9	8.1	90.3	21.2	54.0	55.2
7.	Ground + wool 500 m^3 ha^{-1} + lime + NPK	0.5	1.4	2.9	1.6	5.2	3.5	5.3	4.6
8.	Ground + wool 500 m^3 ha^{-1} + lime + sewage sludge 100 t ha^{-1}	12.2	6.3	3.5	7.3	92.1	18.3	17.7	42.7
	Mean	6.9	3.2	3.3		4.0	33.4	22.1	
	LSD date	0.3				3.9			
	LSD treatment	0.6				8.4			
	LSD date x treatment	1.3				17.2			

The increase in the numbers of protein-degrading bacteria in fungi, observed primarily in treatments with sewage sludge, was surely related to the introduction of nutrients necessary for the growth and development of those microorganisms, as the results presented in Table 3 show an increase in the levels of C_{org} and N_{tot} under the effect of all of the wastes applied, but the strongest under the effect of the sludge. The above supposition is supported by the positive correlations obtained in this study between the numbers of protein-degrading bacteria and fungi and the levels of C_{org} and N_{tot} (Table 7). An increase in the numbers of proteolytic microorganisms caused by soil enriching in nutrients, including those introduced with sewage sludge, was also noted by Emmerling *et al.* [6], Joniec *et al.* [16], and by Niewiadomska *et al.* [26]. Studies conducted by Hattori and Mukai [13] demonstrated that sewage sludge is also a rich source of protein. Therefore, the stimulation of the growth of proteolytic bacteria and fungi, noted especially in soil reclaimed with this waste, was probably also caused by the soil being enriched with protein brought in with the sludge, being a principal source of nutrients for those microorganisms.

Another factor contributing to the increase in the numbers of these microorganisms could have been an improvement of their living conditions, i.e. increase in soil reaction, moisture, and sorptive capacity (Table 3). Soil moisture, with which the numbers of proteolytic bacteria and fungi displayed positive correlations, proved to be of particular importance (Table 7). Moreover, studies by Baran *et al*. [2], Jaroszuk-Sierocińska and Słowińska-Jurkiewicz [14] indicate that mineral wool has a beneficial effect on the water-air relations in reclaimed soil. Improvement of those properties under the conditions of our experiment could also have a positive effect on the growth of the microbial groups under analysis.

Another contribution to the growth of the numbers of proteolytic microorganisms in soil reclaimed with the wastes, and especially with the sewage sludge, could have been the introduction of a certain number of microorganisms together with these wastes (Table 2). Studies by Joniec *et al.* [16] indicate that protein-degrading fungi introduced together with sewage sludge are capable of surviving in the soil for a certain period of time.

As can be seen from the data presented in Table 5, the process of ammonification in the reclaimed soil was subject to varied changes in relation to the waste material applied. A significant positive effect was caused only by the sewage sludge, applied alone or in combination with lime, higher stimulation of the process being observed in the case of sewage sludge applied together with lime, whereas the mineral wool (5 cm 50 cm^{-1}), applied together with lime, caused even a slight decrease of ammonification.

Analyses of the effect of the waste materials used in the study on the process of nitrification revealed that sewage sludge caused a strong stimulation of the process (Table 5). The application of sewage sludge together with lime or with mineral wool in the form of 5cm-50 cm^{-1} turned out to be the most beneficial.

TABLE 5. AMMONINIFICATION AND NITRIFICATION IN THE SOIL

No.	Experimental treatments	Ammonification rate (mg N-NH^4 kg^{-1} d.m. of soil $3d^{-1}$)				Nitrification rate (mg N-$N0^3$ kg^{-1} d.m. of soil $7d^{-1}$)			
		spring	summer	autumn	mean	spring	summer	autumn	mean
1.	Ground without amendments (control)	44.26	41.95	39.76	41.99	24.48	16.48	4.96	15.30
2.	Ground + lime + NPK	37.57	39.39	32.17	36.38	51.61	38.33	20.51	36.82
3.	Ground + lime + sewage sludge 100 t ha^{-1}	198.39	30.52	32.12	87.01	61.61	445.17	221.68	242.82
4.	Ground + sewage sludge 100 t ha^{-1}	85.09	32.88	41.79	53.25	242.40	313.13	77.44	210.99
5.	Ground + wool 5 cm 50 cm^{-1} + lime + NPK	32.85	31.41	38.92	34.39	28.82	19.47	17.29	21.86
6.	Ground + wool 5 cm 50 cm^{-1} + lime + sewage sludge 100 t ha^{-1}	57.51	27.62	27.15	37.43	376.69	561.47	428.00	455.39
7.	Ground + wool 500 m^3 ha^{-1} + lime + NPK	34.94	34.79	36.11	35.28	48.37	42.43	55.44	48.75
8.	Ground + wool 500 m^3 ha^{-1} + lime + sewage sludge 100 t ha^{-1}	76.09	29.35	37.65	47.70	491.03	413.35	105.13	336.50
	Mean	70.84	33.49	35.71		165.63	231.23	116.91	
	LSD date LSD treatment LSD date x treatment	3.41 7.31 15.02				12.92 27.72 56.93			

In addition, the intensification of the process of nitrification was noted also in the treatment with mineral wool (500 m^3 ha^{-1}) applied together with lime and NPK. However, this effect was at a definitely lower level than in the treatments with sewage sludge.

The statistical analysis shows that the intensity of the processes of ammonification and nitrification was subject to seasonal variation (Table 5). The process of ammonification attained the highest values in spring, and at subsequent dates of analyses it remained at a fairly stable lower level. The process of nitrification, on the other hand, attained the highest values in summer and the lowest in autumn.

The stimulating effect of the sludge on the process of ammonification, and especially on the process of nitrification, was probably a result of introducing with that waste to the soil a certain amount of substrates used in those processes, as well as microorganisms (Table 2). This supposition is supported by studies of Wielgosz [35] which concerned the numbers of those microorganisms and the content of ammonium ions in sewage sludge. Stimulation of those processes by sewage sludge was also observed by Frąc and Jezierska [9]. However, in a soil amended with sewage sludge, Furczak and Joniec [10] observed an inhibition of ammonification with a simultaneous intensification of the process of nitrification. The weaker stimulation of ammonification observed in this study, or its absence, could have been the result of simultaneous strong stimulation of the activity of nitrifiers, which, especially in the soil with sewage sludge, mineral wool and lime, found favourable growth conditions. Changes in the intensity of the processes of ammonification and nitrification in the environment depend on the presence and composition of populations of soil microorganisms and on the availability of nitrate and ammonium ions and other forms of nitrogen [11, 28]. Under the conditions of this experiment, a certain role in the described stimulation could have also been played by an improvement of soil moisture, reaction, and sorptive capacity (Table 3), and possibly also oxygenation, which is of particular importance in the case of nitrifiers. Mineral wool is known to contribute to the improvement of the physical parameters of soil, such as water-air relations [2, 14]. Both the process of ammonifcation and that of nitrification showed positive correlation with soil moisture (Table 7). Moreover, the process of nitrification was positively correlated with the sorptive capacity (Table 7).

The waste materials applied caused also a significant stimulation of the activity of alkaline phosphatase and arylsulphatase (Table 6). The exception was lime which, when applied separately, did not have any effect on the activity of arylsulphatase (Table 6). As in the case of the numbers of proteolytic bacteria, the greatest increase of the enzymatic activities under study was noted under the effect of sewage sludge, whether applied alone or in combination with other wastes. The strongest stimulation of these parameters appeared in the treatments with sewage sludge applied together with lime, as well as with lime and mineral wool, at the dose of 500 m^3 ha^{-1}. In treatments without sewage sludge the stimulation of enzymatic activity was at a notably lower level.

TABLE 6. ENZYMATIC ACTIVITY IN THE SOIL

No.	Experimental treatments	Alkaline phosphatase (mg PNP kg^{-1} d.m. of soil h^{-1})				Arylsulphatase (mg PNP kg^{-1} d.m. of soil h^{-1})			
		spring	summer	autumn	mean	spring	summer	autumn	mean
1.	Ground without amendments (control)	1.34	2.87	1.77	1.99	0.37	0.41	0.23	0.33
2.	Ground + lime + NPK	3.25	0.98	6.56	3.59	0.63	0.47	1.24	0.78
3.	Ground + lime + sewage sludge 100 t ha^{-1}	24.41	16.91	2.67	14.66	21.07	4.17	1.55	8.93
4.	Ground + sewage sludge 100 t ha^{-1}	12.34	14.23	2.84	9.80	17.90	2.73	0.64	7.09
5.	Ground + wool 5 cm 50 cm^{-1} + lime + NPK	2.90	8.63	6.93	6.15	0.86	0.89	1.45	1.07
6.	Ground + wool 5 cm 50 cm^{-1} + lime + sewage sludge 100 t ha^{-1}	9.79	9.18	15.54	11.50	3.70	2.17	5.00	3.62
7.	Ground + wool 500 m^3 ha^{-1} + lime + NPK	2.02	1.11	6.63	3.26	1.39	0.26	1.07	0.90
8.	Ground + wool 500 m^3 ha^{-1} + lime + sewage sludge 100 t ha^{-1}	14.60	13.01	10.74	12.78	9.14	14.15	4.40	9.23
	Mean	8.94	8.36	6.71		6.88	3.16	1.94	
	LSD date LSD treatment LSD date x treatment	0.41 0.88 1.81				0.24 0.51 1.04			

TABLE 7. COEFFICIENTS OF CORRELATION

Parameters	pH	Moisture	C_{org}	N_{total}	T
Proteolytic bacteria	–	0.6800**	0.4722**	0.3547**	–
Proteolytic fungi	–	0.6082**	0.4211**	0.3132**	–
Ammonification	–	0.8225**	–	–	–
Nitrification	–	0.3635**	0.5379**	0.6224**	0.2895*
Arylsulphatase	–	0.9492**	0.4042**	–	–
Alkaline phosphatase	–	0.8210**	0.6283**	0.4646**	0.2873*

– no correlation; ** significance level p = 0.01; * significance level p = 0.05

The statistical analysis of the seasonal variation of the enzymatic parameters revealed that they attained the highest values in spring, and the lowest in autumn (Table 6).

The literature review indicates that the application of sewage sludge to soil stimulates the activity of arylsulphatase and alkaline phosphatase [4, 17, 21, 31] The increase of the activity of those enzymes in this study was probably caused mainly by the supply, together with the waste materials, of nutrient substrates used by the microorganisms, as well as enzymes, which is suggested by studies of other authors [4, 17, 21, 23, 31, 34]. The above supposition is supported by the results given in

Table 2 which indicate that the wastes applied for soil reclamation were a source of microorganisms and of the enzymes studied – sewage sludge in particular. Moreover, the results concerning the content of C_{org} and N_{tot} in the reclaimed soil (Table 3) demonstrated an increase in the levels of C_{org} and N_{tot} under the effect of all the waste materials, the effect being the strongest in treatments with sewage sludge. This was reflected in increased activity of arylsulphatase and alkaline phosphatase, most strongly observable in the treatments were sewage sludge was applied for soil remediation. Positive correlations were noted between the activity of those enzymes with the content of C_{org}, and in the case of alkaline phosphatase also with N_{tot} (Table 7). Also other authors observed correlations of the activity of arylsulphatase with C_{org} and N_{tot} [22]. Taking into account that the experiment was established on a degraded soil-less formation that was characterized by strong acidification, poor sorptive properties and low levels of C_{org} and N_{tot} (Table 1), the protective role of organic matter with relation to the enzymes also appears to be important. This function of organic matter relative to extracellular enzymes was also noted by other authors [8]. Ros *et al.* [31] reported that another factor contributing to an increase in the activity of alkaline phosphatase can be the production of those enzymes by plant roots, which could have also taken place in this study.

However, the weaker stimulation of the activity of alkaline phosphatase noted in the treatments with NPK could possibly be a result of an increase in the content of mineral phosphorus in the soil, which can be a factor inhibiting the activity of this enzyme, a phenomenon also noted by other authors, e.g. Pascual *et al.* [29].

An additional element stimulating the activity of the enzymes under study could have been the improvement of living conditions of microorganisms which are the primary producers of these enzymes, i.e. soil reaction, moisture and sorptive capacity (Table 3). Positive correlations were found between the activity of arylsulphatase and alkaline phosphatase and soil moisture, and additionally between phosphatase and the sorptive capacity of the soil (Table 7).

The observed seasonal variations in the numbers of proteolytic bacteria and fungi and in the values of the biochemical parameters under study demonstrated that they attained the highest levels in spring. This was probably related with the high availability of nutrients introduced into the soil several months earlier together with the waste materials, and with the increase of temperature after the winter period.

CONCLUSIONS

1. The results obtained demonstrated that the waste materials applied for reclamation purposes had a positive effect on the studied microbiological and biochemical properties of the degraded soil. That effect was observed the most strongly in the treatments in which sewage sludge was applied, both alone and in combination with other wastes.
2. Sewage sludge caused distinct stimulation of all the studied biological properties of the soil. The level of that effect in treatments where mineral wool was additionally applied was dependent to a certain degree on the form in which the wool was applied in the soil.
3. For the growth of the microbial groups under study and for the stimulation of nitrification the most favourable was the form of 5 cm 50 cm^{-1}, while for the enzymatic activities – 500 m^3 ha^{-1}.
4. The microbiological and biochemical parameters used in this study proved to be good indicators of the effectiveness of the undertaken reclamation measures.

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MIKROBIOLOGICZNE PRZEMIANY N, S I P W GLEBIE ZDEGRADOWANEJ PODDANEJ ROCZNEJ REKULTYWACJI RÓŻNYMI ODPADAMI

Badania przeprowadzono na materiale pochodzącym z doświadczenia rekultywacyjnego, założonego na terenie byłej Kopalni Siarki „Jeziórko", na utworze bezglebowym o składzie granulometrycznym piasku słabo gliniastego, silnie zakwaszonym i o złych właściwościach sorpcyjnych. W poszczególnych wariantach 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 stanowił grunt bez usprawnień. W materiale glebowym określano liczbę bakterii i grzybów rozkładających białko, nasilenie procesu amonifikacji i nitryfikacji oraz aktywność enzymatyczną, tj. arylosulfatazy i fosfatazy zasadowej. Wprowadzone do gleby zdegradowanej odpady wpłynęły stymulująco na analizowane parametry. Wszystkie zastosowane odpady, a w szczególności osad ściekowy, spowodowały wzrost liczby bakterii proteolitycznych oraz aktywności fosfatazy zasadowej i arylosulfatazy. Osad ściekowy wprowadzony oddzielnie lub łącznie z innymi odpadami przyczynił się także do wzrostu liczby grzybów proteolitycznych i nasilenia procesu nitryfikacji. Proces amonifikacji również podlegał nasileniu, ale tylko pod wpływem osadu ściekowego dodanego oddzielnie i łącznie z wapnem. Natomiast zastosowanie wełny mineralnej w dawce 5 cm 50 cm^{-1} łącznie z wapnem spowodowało niewielki spadek aktywności tego procesu.