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ORIGINAL PAPER

Enzymatic activity as an indicator of regeneration processes in degraded soil reclaimed with various types of waste

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Abstract Soil material sampled from a reclamation experiment established in a former "Jeziórko" Sulphur Mine was analysed. The reclamation was carried out on a soil-less substrate with a particle size distribution of slightly loamy sand characterised by high acidity and poor sorption capacity. The different variants of the experiment consisted in the addition of post-flotation lime, mineral fertilisation, sewage sludge, and mineral wool to the reclaimed soil-less substrate. Next, the plots prepared in this way were sown with a mixture of grasses. A plot without any reclamation treatments served as a control. The analyses consisted in the determination of soil enzymatic parameters. The results obtained revealed a positive effect of the reclamation treatments on the analysed properties. All wastes and combinations thereof introduced into the degraded substrate stimulated catalase, protease, and urease activity. The activity of the other enzymes, i.e. dehydrogenases and acid phosphatase, as well as the level of fluorescein diacetate hydrolysis increased only in objects treated with sewage sludge. In turn, in objects receiving mineral fertilisation, a decline in the acid phosphatase activity was noted. In objects treated with mineral wool, the level of stimulation was dependent on the mode of application of this additive. In general, a mixture of 500 m^3 ha^{-1} of mineral wool with the substrate proved more beneficial (with the exception of the acid phosphatase activity and fluorescein diacetate hydrolysis). A higher increase in the analysed enzymatic parameters was also found in objects treated with sewage sludge combined with post-flotation lime than in objects where sewage sludge was used alone.

Keywords Bacteria and fungi · Degraded soil · Enzymatic activity · Mineral wool · Post-flotation lime · Reclamation · Sewage sludge

Introduction

Soil is a specific environment, in which synthesis of chemical compounds as well as their decomposition and transformation takes place. These processes ensure degradation of plant and animal residues, element cycle, formation of humus, and a proper soil structure. A majority of these enzymatic processes are primarily carried out by soil microorganisms, whose enzymatic activity has an impact on soil fertility (Nannipieri et al. 2002; Wolińska and Stępniewska 2011). Therefore, the qualitative and quantitative composition of soil microbiocoenoses has a significant effect on the course of many biological processes, and changes therein affect the proper function of ecosystems (Cavigelli and Robertson 2000). In this context, reclamation of degraded soils combined with recycling of waste substances is primarily a biological process. It is well known that microorganisms and metabolic enzyme-based processes carried out by them are primarily involved in mineralisation and humification of organic matter introduced with wastes and in reclamation of soils and upgrading their fertility. Enzymes from the class of oxidoreductases (dehydrogenases, nitrate reductase, polyphenol oxidase, catalase, peroxidase) and hydrolases (esterases, phosphatases, proteases, cellulases, urease, and

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invertase) play the most important role in transformations occurring in soil (Tabatabai 1994). FDA hydrolysis is widely accepted as an accurate and simple measurement of total microbial activity in soils (Adam and Duncan 2001). Therefore, biological properties, including enzymatic activities, can serve as sensitive early indicators of the dynamics of organic matter transformation, nutrient cycle, stress, and soil regeneration processes (Burns et al. 2013; Perez-de-Mora et al. 2012).

Literature shows that enzymatic activity has been repeatedly used in investigations of the condition of the soil environment affected by various degrees of anthropopressure (Cele and Maboeta 2016; Joniec et al. 2012, 2015; Li et al. 2008, 2009; Margesin et al. 2000; Taylor et al. 2002; Zaborowska et al. 2016). In their investigations, Li et al. (2009) focused on the potential of analysis of FDA hydrolytic activity and the activity of eight hydrolases for assessment of a long-term impact of heavy metals on soil. They concluded that the tests applied in the investigations ensured reliable and comprehensive evaluation of the condition of the soil environment.

Margesin et al. (2000) monitored the processes of bioremediation of hydrocarbon-contaminated soils with the use of, e.g. dehydrogenase, catalase, lipase, and urease activity. Li et al. (2008) analysed the enzymatic activity of urease and catalase to assess changes induced by long-term mineral and organic fertilisation in soil. They found that the assays were sensitive bio-indicators of the condition of soils. Taylor et al. (2002) analysed the microbiological and biochemical activity in the top layer of arable soil and subsoil. They noted differences, e.g. in the dehydrogenase, urease, phosphatase, arylsulfatase, and β-glucosidase enzymatic activity as well as the FDA hydrolytic activity. Furthermore, strong correlations of the above-mentioned enzymatic activities with microbial abundance and organic matter content were found. Many studies have evidenced the suitability of enzymatic tests for evaluation of the effects of application of sewage sludge into soils (Franco-Otero et al. 2012; Joniec et al. 2015; Oszust et al. 2015). The study results demonstrated a generally positive effect of waste on the enzymatic activity in soil.

The literature cited indicates that enzymatic parameters are sensitive indicators of changes in soil induced by different types of anthropopressures.

Exploitation of sulphur deposits is one of the examples of human activity that causes substantial and diverse degradation of the soil environment. A result of sulphur excess in soil is strong acidification leading to dramatic changes in the biological balance, destruction of the sorption complex, increased Al^{3+} ion concentrations in the soil solution, and reduction of other elements, which deteriorates soil quality (Motowicka-Terelak and Terelak 2000).

Reclamation of highly degraded soils requires a number of various revitalisation treatments. Given the scale of another problem, i.e. the growing amounts of a variety of wastes, application of some of them for reclamation of degraded soil environments seems advisable.

As indicated by the research presented by Baran et al. (2012) and Joniec et al. (2015), such wastes as sewage sludge, post-floatation lime, and mineral wool exert a positive impact on a number of properties of soils degraded by sulphur mining industry. After treatment of degraded soils with the aforementioned wastes, Baran et al. (2012) reported improved total, ammonium and nitrite N. Furthermore, Joniec et al. (2015) have demonstrated a positive impact of wastes, in particular sewage sludge, on many microbiological and biochemical properties of soil degraded by sulphur mining industry, e.g. microbial abundance, respiration, increased cellulose mineralisation, and lipase activity. The considerable effect of sewage sludge on the biological life in the soil environment is associated with the strong positive impact of this type of waste on organic matter, nutrient content, soil porosity, bulk density, aggregate structure, and water capacity (Singh and Agrawal 2008).

The publications cited above indicate great interest in the use of the parameters of soil microbial activity for assessment of the condition of the soil environment. Simultaneously, there are no reports on comprehensive multiyear investigations of enzymatic activity in soils degraded by sulphur mining industry and reclaimed with sewage sludge, mineral wool, and post-floatation lime.

The present investigations were undertaken due to the importance of waste recycling in environmental protection and the scale of the soil degradation problem. The aim of the study was to evaluate the effectiveness of the applied reclamation treatments based on enzymatic assays. Additionally, we attempted the assessment of the suitability of the enzymatic tests for monitoring processes of reclamation of soil degraded by sulphur mining industry.

This study follows the current trend in soil enzymatic research, which, as suggested by Burns et al. (2013), should provide an answer to the question of the enzymatic activity potential as an indicator of the fertility and condition of soils and for reclamation and regeneration of a degraded environment.

This study was conducted in the years 2011–2013 in the Department of Environmental Microbiology of the University of Life Science in Lublin, Poland.

Materials and methods

Experimental design

The investigations were carried out in a reclamation experiment design established by the Institute of Soil Science, Environment Engineering, and Management,

University of Life Sciences in Lublin, Poland. The experiment was set up in the area of a former "Jeziórko" Sulphur Mine (Poland, Podkarpacie region) on a soil-less substrate with a particle size distribution of slightly loamy sand characterised by high acidity, poor sorption capacity, and low contents of C_{org} and total N (Table 1). Sulphur had been extracted with the Frasch method, i.e. borehole smelting. The experimental variants consisted in application of different reclamation agents into the soil-less substrate. These included post-flotation lime and mineral fertilisation NPK (80; 40; 60), (PFL + NPK); post-flotation lime and sewage sludge (PFL + SS); sewage sludge (SS); mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime, and NPK (MWP + PFL + NPK); mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge (MWP + PFL + SS); mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK (MW + PFL + NPK); and mineral wool (500 m^3 ha^{-1}), post-flotation lime, and sewage sludge (MW + PFL + SS). Post-flotation lime (PFL) was applied at a level of 100 t ha^{-1} and NPK at doses of 80, 40, and 60 kg/ha. 100 t ha^{-1} of sewage sludge (SS) obtained from the municipal wastewater treatment plant in Stalowa Wola was applied in a 20-cm soil layer. Mineral wool was applied in two variants, i.e. as a 5-cm pad at the depth of 50 cm (MWP) and at a dose of 500 m^3 ha^{-1} applied into a 0–20-cm layer (MW). The plots prepared in this way were sown with a mixture of grasses composed of *Festuca pratensis*—41%, *Festuca rubra*—19.2%, *Lolium perenne*—14.7%, *Lolium multiflorum*—12.4%, *Dactylis glomerata*—6.5%, and *Trifolium pratense*—6%. An untreated plot served as a control (C). The substrate analysed in this research will be thereafter referred to as degraded or reclaimed soil.

Experimental design:

1. Untreated plot (control), (C)
2. Post-flotation lime + NPK (80; 40; 60) (PFL+NPK)
3. Post-flotation lime + sewage sludge 100 t ha^{-1} (PFL + SS)
4. Sewage sludge 100 t ha^{-1} (SS)
5. Mineral wool pad (5 cm·50 cm^{-1}) + post-flotation lime + NPK (80; 40; 60) (MWP + PFL + NPK)
6. Mineral wool pad (5 cm·50 cm^{-1}) + post-flotation lime + sewage sludge 100 t ha^{-1} (MWP + PFL + SS)
7. Mineral wool 500 m^3 ha^{-1} + post-flotation lime + NPK (80; 40; 60) (MW + PFL + NPK)
8. Mineral wool 500 m^3 ha^{-1} + post-flotation lime + sewage sludge 100 t ha^{-1} (MW + PFL + SS)

Prior to the investigations, the particle size distribution, pH, sorption capacity, and the contents of C_{org} and total N were determined in the degraded soil and waste used for reclamation (Table 1). Additionally, selected biological properties of the waste material applied were specified (Table 2).

Soil samples

Soil material from the reclamation experiment was sampled from the 0–20-cm layer on the following days: three times during the 1st year of the experiment, i.e. in early May (6.05.2011), summer (01.07.2011), and autumn (29.09.2011); twice in the 2nd year, i.e. in summer (22.06.2012) and autumn (04.11.2012); and twice in the 3rd year, i.e. in summer (20.07.2013) and autumn (26.10.2013).

The samples were sieved through a 2-mm-mesh sieve and stored in a refrigerator at + 4 °C.

Enzymatic analyses

Dehydrogenases activity was determined in 5-g soil samples using 2,3,5-triphenyl tetrazolium chloride as substrate, incubating in 0.2 M trishydroxymethyl-aminomethane-HCl buffer (Tris-HCl pH 7.4) for 48 h, at 30 °C (Thalmann 1968). Enzyme activity is expressed as mg TPF kg^{-1} d.m. of soil d^{-1}. Catalase activity was determined in 2-g soil samples using H_2O_2 aqueous solution as substrate, mixing for 20 min at 21 °C (Johnson and Temple 1964). Enzyme

Table 1 Selected properties of the degraded ground and the wastes used for remediation

Property	Unit	Degraded ground	Mineral wool	Sewage sludge	Flotation lime
Particle size distribution	% sand	91	n.o.	n.o.	35
	% silt	3			29
	% fine fract.	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
Corg.		2.0	28.5	193.8	2.6

Table 2 Biological properties of wastes used for remediation

Biological parameter	Flotation lime	Wool	Sewage sludge
Dehydrogenase (mg TPF kg^{-1} d.m. d^{-1})	1.63	2.86	63.44
Catalase (mmol H_2O_2 kg^{-1} d.m. min^{-1})	207.96	887.78	1509.29
Acid phosphatase (mg PNP kg^{-1} d.m. h^{-1})	1.63	5.61	91.20
Protease (mg tyrosine kg^{-1} d.m. h^{-1})	38.4	0.0	293.46
Urease (mg N-NH_4 kg^{-1} d.m. 18 h^{-1})	1.38	5.98	2239.46
The FDA hydrolytic activity (mg fluorescein kg^{-1} d.m. h^{-1})	2.39	10.56	292.11

activity is expressed as mmol H_2O_2 kg^{-1} d.m. of soil min^{-1}. Protease activity was determined in 2-g soil samples using casein as substrate, incubating in 0.2 M Tris-HCl buffer (pH 8.0) for 1 h, at 50 °C (Ladd and Butler 1972). Enzyme activity is expressed as mg tyrosine kg^{-1} d.m. of soil h^{-1}. Urease activity was determined in 10-g soil samples using urea solution as substrate, incubating for 18 h, at 37 °C (Zantua and Bremner 1975). Enzyme activity is expressed as unit: mg N-NH_4 kg^{-1} - d.m. of soil 18 h^{-1}. Acid phosphatase activity was determined in 1-g soil samples using p-nitrophenyl phosphate disodium as substrate, incubating in modified universal buffer (pH 6.5) for 1 h, at 37 °C (Tabatabai and Bremner 1969). Enzyme activity is expressed as mg PNP kg^{-1} d.m. of soil h^{-1}. The level of fluorescein diacetate (FDA) hydrolysis was determined in 1-g soil samples using fluorescein diacetate as a substrate, incubating in 60 mM sodium phosphate buffer (pH 7.6) for 2 h, at 25 °C (Schnurer and Rosswall 1982). Enzyme activity is expressed as mg fluorescein kg^{-1} d.m. of soil h^{-1}.

Supplementary analysis

The examinations were accompanied by physical, chemical, and physico-chemical analyses (Table 3). The following parameters were determined: moisture content with the gravimetric method; the content of organic carbon (C_{org}) determined according to the modified Tiurin method (Arinuškina 1961); total nitrogen content (N total) with the modified Kjeldahl method using a unit from the Tecator company (oven 2006 and distillation unit 1002); sorption capacity (T) calculated by adding the total hydrolytic acidity and the sum of alkaline cations determined with method described by Ostrowska et al. (1991); soil pH measured electrometrically in 1 mol dm^{-3} KCl using a pH metre.

Statistical analysis

All analyses were performed in three parallel repetitions and presented as a mean of these repetitions. The results obtained were statistically analysed in the Statistica programme with ANOVA models and multiple Tukey's t tests at a significance level $\alpha = 0.05$. The correlations between the analysed variables were tested with Pearson's correlation coefficients at significance levels $p < 0.01$ and $p < 0.05$.

Results and discussion

The data presented in Tables 4 and 5 indicate that the reclamation agents exerted a significant impact on the activity of enzymes involved in oxidoreductive transformations in the soil, i.e. dehydrogenases and catalase. The intensity of this effect depended on the type of the waste applied and the study period.

Dehydrogenase activity was stimulated only in objects treated with sewage sludge alone or in combination with the other types of wastes. However, the positive effect in all the objects treated with this waste was only noted in the 2nd year of the study. In the 1st and 3rd years, stimulation of dehydrogenase activity was only noted in the variant of sewage sludge combined with post-flotation lime (PFL + SS) as well as post-flotation lime and mineral wool mixed with soil at a dose of 500 m^3 ha^{-1} (MW + PFL + SS). A greater effect on the dehydrogenase activity was exerted by sewage sludge combined with post-flotation lime (PFL + SS) rather than by sewage sludge alone (SS).

Throughout the study period, an increase in the catalase activity was noted as well. It was evident in a greater number of the objects than in the case of dehydrogenases. In the 1st and 3rd years of the investigations, stimulation of catalase activity was reported from nearly all the objects. In all study years, the effect was the greatest in plots treated with sewage sludge alone or in combination with the other wastes. In turn, in the 2nd year of the study, the stimulation of this parameter was detected in a majority of the objects with sewage sludge (SS; PFL + SS; MWP + PFL + SS). It should be noted that the mode of application of mineral wool into the objects had a significant impact on the magnitude of the stimulation. Greater effects were achieved by mixing this agent with soil at a dose of

Table 3 Selected physical, physico-chemical and chemical properties of the soil (means for the year)

Experimental treatments	Sorptive capacity T (cmol(+) kg^{-1})			Corg. (g kg^{-1})			N total (g kg^{-1})			Moisture (%)			pH (range)		
	I year	II year	III year	I year	II year	III year	I year	II year	III year	I year	II year	III year	I year	II year	III year
C	7.0	7.8	7.0	2.03	1.83	2.28	0.32	0.32	0.40	4.26	3.90	1.83	4.1–4.3	4.6–4.7	4.4–4.6
PFL + NPK	14.4	15.0	15.6	2.52	2.18	3.40	0.44	0.35	0.45	3.11	3.46	1.94	7.3–7.6	7.3–7.6	7.6
PFL + SS	15.5	20.5	17.7	4.20	4.43	6.20	1.06	1.05	1.30	10.36	3.97	0.95	6.6–7.1	6.8–6.9	7.0–7.4
SS	8.7	8.7	6.8	4.50	4.65	5.40	0.53	0.63	0.59	8.09	3.39	2.23	6.1–6.8	5.1–5.6	4.0–5.4
MWP + PFL + NPK	14.9	19.7	18.2	3.98	3.88	5.65	0.54	0.55	0.71	4.52	4.5	1.94	7.3–7.4	7.1–7.4	7.3–7.4
MWP + PFL + SS	15.6	17.8	16.4	4.47	3.90	4.45	1.37	1.33	1.55	6.72	4.53	3.16	6.9–7.2	6.8–7.1	7.2–7.6
MW + PFL + NPK	14.6	16.3	16.2	3.40	3.45	4.15	0.35	0.36	0.37	4.16	3.38	1.89	7.3–7.4	7.2–7.4	7.4–7.6
MW + PFL + SS	15.7	17.4	19.2	5.50	4.85	6.20	0.67	0.63	0.95	8.83	3.1	3.1	6.6–7.2	6.6–6.9	7.2–7.4

C, control soil; PFL + NPK, post-flotation lime and NPK; PFL + SS, post-flotation lime and sewage sludge; SS, sewage sludge; MWP + PFL + NPK, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and NPK; MWP + PFL + SS, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge; MW + PFL + NPK, mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK; MW + PFL + SS, mineral wool (500 m^3 ha^{-1}), post-flotation lime and sewage sludge

Table 4 Activity of dehydrogenase in the soil

Experimental treatments	mg TPF kg^{-1} d.m. of soil d^{-1}									
	I year				II year			III year		
	Spring	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean
C	0.644	0.666	0.367	0.559	0.557	0.196	0.376	0.795	1.033	0.914
PFL + NPK	0.358	0.360	0.333	0.351	0.387	0.419	0.403	0.521	1.317	0.919
PFL + SS	12.879	1.493	1.501	5.291	0.695	0.701	0.698	1.448	2.115	1.782
SS	1.559	1.510	0.337	1.135	1.543	0.243	0.893	1.025	1.863	1.444
MWP + PFL+NPK	0.336	0.835	0.511	0.561	0.614	0.320	0.467	0.786	1.071	0.929
MWP + PFL + SS	0.688	1.453	1.509	1.217	0.701	1.311	1.006	0.997	0.719	0.858
MW + PFL + NPK	0.254	0.280	0.639	0.391	0.274	0.319	0.297	0.439	0.630	0.534
MW + PFL + SS	3.269	2.778	0.855	2.301	0.895	0.560	0.728	2.552	1.182	1.867
Mean	2.498	1.172	0.756	1.475	0.708	0.509	0.608	1.070	1.241	1.156
HSD year	0.209									
HSD date	0.234									
HSD treatment	0.436									
HSD year × treatment	I year—0.971; II year—0.203; III year—0.551									
HSD year × date	0.396									
HSD year × date × treatment	1.556									

C, control soil; PFL + NPK, post-flotation lime and NPK; PFL + SS, post-flotation lime and sewage sludge; SS, sewage sludge; MWP + PFL + NPK, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and NPK; MWP + PFL + SS, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge; MW + PFL + NPK, mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK; MW + PFL + SS, mineral wool (500 m^3 ha^{-1}), post-flotation lime and sewage sludge

500 m^3 ha^{-1} (MW). A more pronounced effect was also noted upon application of sewage sludge combined with post-flotation lime (PFL + SS) than sewage sludge alone (SS).

The activity of dehydrogenases and catalase varied over time. The enzymes exhibited the highest activity in the 1st year of the study, and the lowest in the 2nd year. In the 3rd year, the parameters increased; however, they were still lower than at the beginning of the experiment. The analysed enzymatic activities also displayed periodic fluctuations, particularly evident in the 1st year of the experiment (Tables 4, 5). Typically, their level was the highest in spring but declined in the subsequent periods.

Table 5 Activity of catalase in the soil

Experimental treatments	mmol H_2O_2 kg^{-1} d.m. of soil min^{-1}									
	I year				II year			III year		
	Spring	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean
C	167.92	79.55	123.66	123.71	106.37	22.91	64.64	101.02	111.54	106.28
PFL + NPK	236.54	124.73	182.76	181.34	90.55	45.78	68.16	134.69	235.91	185.30
PFL + SS	717.44	446.52	239.64	467.87	172.27	174.58	173.42	266.67	377.44	322.05
SS	546.83	336.32	149.62	344.25	157.87	137.65	147.76	113.40	134.48	123.94
MWP + PFL + NPK	149.20	113.89	186.01	149.70	79.97	23.13	51.55	202.04	191.11	196.57
MWP + PFL + SS	455.77	365.39	490.64	437.27	124.66	233.27	178.96	135.99	328.13	232.06
MW + PFL + NPK	220.25	205.89	150.04	192.06	44.83	115.35	80.09	149.43	216.81	183.12
MW + PFL + SS	721.52	435.31	338.24	498.35	44.88	114.63	79.76	177.78	335.06	256.42
Mean	401.93	263.45	232.57	299.32	102.67	108.41	105.54	160.13	241.31	200.72
HSD year	6.50									
HSD date	7.26									
HSD treatment	13.55									
HSD year × treatment	I year—24.94; II year—22.34; III year—22.60									
HSD year × date	12.32									
HSD year × date × treatment	48.36									

C, control soil; PFL + NPK, post-flotation lime and NPK; PFL + SS, post-flotation lime and sewage sludge; SS, sewage sludge; MWP + PFL + NPK, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and NPK; MWP + PFL + SS, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge; MW + PFL + NPK, mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK; MW + PFL + SS, mineral wool (500 m^3 ha^{-1}), post-flotation lime and sewage sludge

The data presented in Tables 6, 7, and 8 indicate that the activity of enzymes involved in transformations of such biogens as N and P, i.e. protease, urease, and acid phosphatase, also underwent distinct changes induced by the reclamation agents applied. This interaction varied between the different enzymes and proceeded at varying intensity, depending on the type of wastes used and the duration of their impact on the soil environment.

All the wastes and their combinations stimulated the activity of enzymes involved in nitrogen transformations in soils, i.e. protease and urease. As in the case of dehydrogenases and catalase, the effect was most pronounced in objects where sewage sludge alone or in combination with the other wastes was applied for reclamation. Similarly, application of sewage sludge with post-flotation lime (PFL + SS) rather than alone (SS) proved to be more efficient in the case of these enzymatic parameters. As in the case of oxidoreductases, the mode of mineral wool application was relevant. In general, greater stimulation of protease and urease activity was noted in objects where mineral wool was mixed with soil at a dose of 500 m^3 ha^{-1} (MW).

In turn, the impact of the wastes used for reclamation on the activity of acid phosphatase was multidirectional and its character and intensity varied over time. In the 1st year of the study, an increase in the activity of this enzyme was noted although, in contrast to most of the analysed enzymes, only in objects treated with sewage sludge alone or in combination with the other wastes. In the other objects (without sewage sludge), the activity of acid phosphatase was inhibited. In the subsequent years of the study, the positive effect of sewage sludge was clearly reduced and noted only in some of the objects (PFL + SS; SS). In this period, the inhibition was simultaneously more pronounced in a majority of the other objects (PFL + NPK; SS; MWP + PFL + NPK; MW + PFL + NPK; MW + PFL + SS). Furthermore, the results also revealed that the application of the 5 cm·50 cm^{-1} mineral wool pad (MWP) was more beneficial to the activity of acid phosphatase over a greater part of the period.

The analysis of protease, urease, and acid phosphatase activity observed over the 3 experimental years revealed that it was the highest in the 1st year, significantly declined in the subsequent year, and increased again in the 3rd year. These parameters exhibited seasonal fluctuations as well (Tables 6, 7, 8). In the 1st year, protease and urease activity was the highest in spring while in the consecutive years the highest levels of the enzymes were noted in summer. In contrast, acid phosphatase activity was the highest in summer in the 1st and 2nd years of the study and in autumn in the 3rd year.

The results presented in Table 9 show significant changes in the FDA hydrolytic activity induced by

Table 6 Activity of protease in the soil

Experimental treatments	mg tyrosine kg^{-1} d.m. of soil h^{-1}									
	I year				II year			III year		
	Spring	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean
C	7.578	1.446	3.356	4.127	1.668	0.620	1.144	3.464	0.351	1.908
PFL + NPK	15.172	7.264	11.848	11.428	9.531	2.876	6.204	5.143	5.505	5.324
PFL + SS	78.824	30.883	8.584	39.430	11.844	3.111	7.478	11.407	7.559	9.483
SS	94.219	39.519	11.108	48.282	4.521	4.282	4.401	2.922	2.852	2.887
MWP + PFL + NPK	11.940	9.084	9.840	10.288	3.526	0.196	1.861	11.143	7.225	9.184
MWP + PFL + SS	23.129	19.542	22.082	21.584	4.255	8.127	6.191	4.760	13.440	9.100
MW + PFL + NPK	11.780	11.962	11.849	11.864	3.186	0.991	2.088	5.564	5.233	5.398
MW + PFL + SS	69.494	60.558	33.276	54.443	3.784	3.185	3.485	16.107	9.548	12.827
Mean	39.017	22.532	13.993	25.181	5.289	2.924	4.106	7.564	6.464	7.014
HSD year	0.610									
HSD date	0.683									
HSD treatment	1.273									
HSD year × treatment	I year—2.868; II year—0.760; III year—1.382									
HSD year × date	1.158									
HSD year × date × treatment	4.544									

C, control soil; PFL + NPK, post-flotation lime and NPK; PFL + SS, post-flotation lime and sewage sludge; SS, sewage sludge; MWP + PFL + NPK, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and NPK; MWP + PFL + SS, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge; MW + PFL + NPK, mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK; MW + PFL + SS, mineral wool (500 m^3 ha^{-1}), post-flotation lime and sewage sludge

application of the wastes. This effect was dependent on the type of the reclamation agent applied into the soil. Throughout the study period, stimulation of FDA hydrolysis was only noted in objects treated with sewage sludge alone or in combination with the other wastes. As in the case of the other enzymes, the combination of sewage sludge with post-flotation lime (PFL + SS) proved to enhance the activity more efficiently. The increase in the FDA hydrolysis level was also influenced by the mode of mineral wool application. Greater stimulation of this enzymatic parameter was achieved upon the use of a 5 cm × 50 cm^{-1} mineral wool pad (MWP) rather than the mixture thereof with soil at a dose of 500 m^3 ha^{-1} (MW), which was more efficient only in the 1st year of study. In the other objects where sewage sludge was not applied (PFL + NPK; MWP + PFL + NPK; MW + PFL + NPK), there was no effect of the wastes on the analysed activity, or slight inhibition of the activity, followed by disappearance in the last year, was observed.

The results presented in Table 9 demonstrate that FDA hydrolytic activity was the highest in the 1st year of the experimental period and gradually declined in the other study years. Seasonal variations (Table 9), which varied in the different years, were also reported. In the first year, the highest FDA activity was noted in spring, in the 2nd year in summer, and in the 3rd year in summer.

The results obtained (Tables 4, 5, 6, 7, 8, 9) indicate that urease and protease levels underwent the most substantial changes induced by addition of the wastes into the degraded soils. The stimulation of the activity of these enzymes persisted at the highest level even in the 3rd year of study. In turn, the acid phosphatase activity exhibited the weakest response to the reclamation treatments applied. The initial increase in the level of this parameter declined substantially already in the 2nd year of the experimental period and almost entirely disappeared, turning into slight inhibition, in the 3rd year.

The slight inhibition of phosphatase activity persisted throughout the experimental period.

The data presented in Table 10 indicate that all enzymatic activities were positively correlated at a significance level of $p < 0.01$. Additionally, the analysed biochemical parameters were positively correlated at a significance level of $p < 0.01$ with the physical and chemical properties, i.e. moisture content and C_{org} and total N contents (with the exception of protease). No such correlations were noted between sorption capacity and pH. Only catalase activity was positively correlated with the reaction at a significance level of $p < 0.05$.

The increased activity of all the enzymes analysed in these investigations, i.e. dehydrogenases, catalase, urease, protease, acid phosphatase, and FDA hydrolytic activity

Table 7 Activity of urease in the soil

Experimental treatments	mg $N\text{-}NH_4$ kg^{-1} d.m. of soil 18 h^{-1}									
	I year				II year			III year		
	Spring	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean
C	0.687	2.721	3.123	2.177	17.395	4.456	10.925	18.139	15.739	16.939
PFL + NPK	1.364	39.365	8.888	16.539	20.661	7.306	13.984	46.356	34.291	40.323
PFL + SS	1241.552	421.989	534.164	732.569	126.196	86.381	106.288	162.824	85.601	124.213
SS	734.190	551.050	531.552	605.597	126.549	59.559	93.054	65.330	66.961	66.146
MWP + PFL + NPK	13.051	67.487	33.169	37.902	35.784	4.154	19.969	68.302	38.353	53.328
MWP + PFL + SS	361.978	262.438	184.725	269.714	93.375	129.850	111.612	105.090	104.631	104.861
MW + PFL + NPK	23.865	93.566	40.297	52.576	78.931	10.013	44.472	89.664	78.308	83.986
MW + PFL + SS	646.935	660.410	562.311	623.219	117.185	27.101	72.143	148.747	134.812	141.780
Mean	377.953	262.378	237.279	292.537	77.009	41.102	59.056	88.057	69.837	78.947
HSD year	5.667									
HSD date	6.336									
HSD treatment	11.818									
HSD year × treatment	I year—24.923; II year—13.200; III year—15.686									
HSD year × date	10.745									
HSD year × date × treatment	42.177									

C, control soil; PFL + NPK, post-flotation lime and NPK; PFL + SS, post-flotation lime and sewage sludge; SS, sewage sludge; MWP + PFL + NPK, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and NPK; MWP + PFL + SS, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge; MW + PFL + NPK, mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK; MW + PFL + SS, mineral wool (500 m^3 ha^{-1}), post-flotation lime and sewage sludge

was certainly associated with the application of organic matter into the degraded soil. The data presented in Table 3 indicate that application of the wastes increased the content of C_{org} and total N in the reclaimed soil. The wastes applied, and in particular sewage sludge, were a source of nutrients for microorganisms. This is supported by the positive correlations between all the analysed enzymatic parameters and the C_{org} content and between almost all parameters and the total N content (Table 10). Similar correlations, although in different experimental conditions, were reported by other authors (Oszust et al. 2015; Taylor et al. 2002). It should be emphasised, however, that the studies cited here were conducted in different experimental conditions, i.e. they did not involve reclaimed soil and were rather short-term investigations. The correlations found in the present research are additionally interesting given the multiyear span of the study. Dehydrogenases are regarded as indicators of the total microbial activity in soil, as they are contained exclusively in living cells, where they catalyse oxidoreductive processes (Wolińska and Stępniewska 2011). This has been confirmed by these and parallel investigations conducted in this model, which indicate that the wastes applied (in particular sewage sludge) not only stimulated dehydrogenase activity but also increased the abundance of many bacterial and fungal groups (Joniec et al. 2015).

Catalase is another oxidoreductase enzyme analysed in this study, which is associated with the activity of aerobic microorganisms. Therefore, the increase in its activity may have been caused by not only the supply of nutrients for these microorganisms but also improved soil aeration. As suggested by Garcia-Gil et al. (2000), the increase in catalase activity may have resulted from the improved oxidation of the soil achieved by the application of organic matter. In the conditions of this experiment, the wastes applied into the soil, in particular the sewage sludge, may have contributed to the increase in oxygenation of the reclaimed soil by increasing its porosity and reconstruction of the aggregate structure. The results obtained indicate multiple positive effects of sewage sludge on the properties of degraded soils and, hence, the suitability of this type of waste for reclamation.

Like dehydrogenase activity, FDA hydrolysis is regarded by some researchers as a reliable measure of total microbial activity although, unlike dehydrogenases, these enzymes can function outside the cell and form stable complexes with soil colloids (Schnurer and Rosswall 1982). Protease, urease, and phosphatase are important hydrolytic enzymes catalysing the processes of mineralisation of nitrogen and organic phosphorus and, as commonly known, are induced by the presence of available substrates in the environment (Burns 1982).

Table 8 Activity of acid phosphatase in the soil

Experimental treatments	mg PNP kg^{-1} d.m. of soil h^{-1}									
	I year				II year			III year		
	Spring	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean
C	2.095	2.679	5.079	3.284	4.266	2.350	3.308	5.851	5.423	5.637
PFL + NPK	1.499	2.924	1.361	1.928	1.653	0.349	1.001	2.448	3.758	3.103
PFL + SS	8.844	9.840	5.788	8.157	5.077	4.873	4.975	5.463	7.674	6.569
SS	13.646	8.132	2.170	7.982	5.659	4.358	5.009	2.474	5.551	4.013
MWP + PFL + NPK	1.930	2.336	3.224	2.497	2.439	1.329	1.884	3.631	3.585	3.608
MWP + PFL + SS	4.990	7.200	8.426	6.872	3.791	3.041	3.416	2.870	7.676	5.273
MW + PFL + NPK	1.133	1.015	2.689	1.612	1.213	0.127	0.670	4.578	4.105	4.341
MW + PFL + SS	2.292	7.444	5.541	5.092	2.489	1.622	2.056	3.635	6.916	5.276
Mean	4.554	5.196	4.284	4.678	3.323	2.256	2.790	3.863	5.586	4.727
HSD year	0.189									
HSD date	0.211									
HSD treatment	0.394									
HSD year × treatment	I year—0.715; II year—0.626; III year—0.709									
HSD year × date	0.359									
HSD year × date × treatment	1.408									

C, control soil; PFL + NPK, post-flotation lime and NPK; PFL + SS, post-flotation lime and sewage sludge; SS, sewage sludge; MWP + PFL + NPK, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and NPK; MWP + PFL + SS, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge; MW + PFL + NPK, mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK; MW + PFL + SS, mineral wool (500 m^3 ha^{-1}), post-flotation lime and sewage sludge

The organic matter applied with the wastes not only was a source of nutrients but also introduced extracellular enzymes and microorganisms that synthesise these enzymes. This is confirmed by the data presented in Table 2; they indicate that the reclamation wastes exhibited certain enzymatic activity, which was especially high in the case of sewage sludge. The wastes applied, in particular sewage sludge, contained microorganisms, as shown in the investigations conducted by Joniec et al. (2015). Other authors report that extracellular enzymes and enzyme-producing microorganisms are introduced into soils through organic matter (Joniec et al. 2012; Tejada et al. 2012). As reported by Joniec et al. (2012), some sewage sludge microorganisms can colonise soil to some extent and introduce extracellular enzymes, thereby contributing to a periodic increase in soil enzymatic activity. Importantly, these investigations were carried out in the laboratory condition over a short time span. The research conducted in field conditions over a multiyear period provided information from the analysis of changes in the enzymatic activities occurring in reclaimed soil.

Additionally, organic matter not only is a source of nutrients but also serves a protective function towards extracellular enzymes (Ceccanti et al. 2008). Formation of complexes between humic substances and extracellular enzymes is a mechanism to stabilise and protect enzymes in soil (Burns 1982). Similarly, in the present experiment, the increased hydrolase activity in the reclaimed soil may have been caused by the protective effect of the organic matter applied into the soil with sewage sludge. Another probable cause of the increase in the activity of the analysed oxidoreductases and hydrolases, mainly produced by microorganisms, was the improved living conditions, i.e. moisture content and pH (Table 3). This assumption was confirmed by the reported correlations (Table 10). Positive correlations between all the analysed enzymes and moisture content and, additionally, between catalase and pH were noted. However, the investigations in this field are not unanimous. Perez-de-Mora et al. (2012) noted positive correlations of dehydrogenases and proteases with soil reaction and a negative correlation between acid phosphatase and this physico-chemical parameter. The relationship between the increase in the enzymatic activity and the total microbiological activity has also been confirmed by the positive correlations between dehydrogenase activity and that of all the other enzymes, as shown in this research (Table 10). The recorded increase in dehydrogenase activity only in the objects treated with sewage sludge used alone or in combination with other wastes was probably caused by the fact that dehydrogenases are enzymes specific only to living cells. As reported by Joniec et al. (2015) and Joniec (2013) in investigations conducted on this experimental model, the sewage sludge introduced the greatest amounts of microorganisms, compared with

Table 9 FDA hydrolytic activity in the soil

Experimental treatments	mg fluorescein kg^{-1} d.m. of soil h^{-1}									
	I year				II year			III year		
	Spring	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean
C	42.801	4.703	29.986	25.830	23.347	18.012	20.680	18.194	14.791	16.493
PFL + NPK	16.859	12.827	32.696	20.794	21.156	14.051	17.603	24.852	13.715	19.284
PFL + SS	183.781	82.752	26.873	97.802	47.503	81.002	64.253	77.406	27.106	52.256
SS	134.069	64.656	32.387	77.037	69.612	48.974	59.293	30.975	22.338	26.657
MWP + PFL + NPK	9.974	11.076	24.538	15.196	29.391	10.372	19.882	46.453	17.136	31.795
MWP + PFL + SS	71.659	59.360	82.366	71.129	33.767	81.176	57.471	51.745	38.554	45.149
MW + PFL + NPK	32.138	21.065	17.513	23.572	9.972	15.277	12.625	21.568	9.978	15.773
MW + PFL + SS	167.837	88.907	61.622	106.122	32.039	40.562	36.301	26.747	19.876	23.311
Mean	82.390	43.168	38.498	54.685	33.348	38.678	36.013	37.242	20.437	28.840
HSD year	1.586									
HSD date	1.774									
HSD treatment	3.307									
HSD year × treatment	I year—6.749; II year—3.792; III year—5.078									
HSD year × date	3.007									
HSD year × date × treatment	11.801									

C, control soil; PFL + NPK, post-flotation lime and NPK; PFL + SS, post-flotation lime and sewage sludge; SS, sewage sludge; MWP + PFL + NPK, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and NPK; MWP + PFL + SS, mineral wool pad (5 cm·50 cm^{-1}), post-flotation lime and sewage sludge; MW + PFL + NPK, mineral wool (500 m^3 ha^{-1}), post-flotation lime and NPK; MW + PFL + SS, mineral wool (500 m^3 ha^{-1}), post-flotation lime and sewage sludge

Table 10 Coefficients of correlation

	Protease	Acid phosphatase	Urease	Catalase	FDA	pH	moisture	Corg.	N total	T
Dehydrogenase	0.5992**	0.3877**	0.7176**	0.6337**	0.6615**	–	0.6744**	0.2156**	0.2339**	–
Protease		0.5825**	0.8633**	0.8478**	0.8266**	–	0.8393**	0.2233**	–	–
Acid phosphatase			0.5760**	0.6490**	0.5382**	–	0.4576**	0.3520**	0.4214**	–
Urease				0.7877**	0.7969**	–	0.8245**	0.3086**	0.2478**	–
Catalase					0.8326**	0.1918*	0.7321**	0.4069**	0.4063**	–
FDA						–	0.8038**	0.2986**	0.3604**	–

** Significance level $p < 0.01$; * significance level $p < 0.05$; – no correlation

the other wastes used. Additionally, the abundance of different groups of microorganisms was the greatest in the combinations with sewage sludge. Among the analysed enzymes, acid phosphatase exhibited the weakest stimulation by the reclamation treatments applied. Moreover, it was the only enzyme with decreased activity in objects fertilised with NPK. As shown by other authors, phosphatase activity may be inhibited by the presence of mineral phosphorus in the soil (Perez-de-Mora et al. 2012). Perez-de-Mora et al. (2012) reported negative correlations between phosphatase activity and the content of available mineral phosphorus. The results obtained in our study highlight the greater suitability of the wastes used than that of NPK mineral fertiliser for reclamation of soils in these experimental conditions. The analysis of the seasonal variations in the enzymatic activities showed their highest values primarily in spring and summer, which was probably associated with the higher temperatures prevailing in these periods than those in autumn. Temperature is one of the factors influencing the growth and activity of microorganisms. The increase in temperature after the winter period probably contributed to stimulation of the growth and enzymatic activity of microorganisms. This is confirmed by observations reported in other studies

conducted on this model regarding the abundance of the groups of bacteria and fungi and their biochemical and enzymatic activity (Joniec et al. 2015; Joniec 2013). The authors noted significant fluctuations in the parameters mentioned above. As in this study, the highest values were recorded in spring.

It should be emphasised that the positive effect of the reclamation treatments on the analysed enzymatic parameters was not only reported in the 1st year but also persisted, with slightly lower intensity, in the 2nd and 3rd years. This probably resulted from the positive alterations in the physical, chemical, and physico-chemical properties of the reclaimed soil (Table 3). This phenomenon was particularly pronounced in objects treated with sewage sludge, with which organic matter was introduced into the degraded soil. The results indicate that a single application of greater amounts of organic matter into soil results in increased microbial activity persisting even for a few years, which is explained by improved soil fertility. An especially strong effect of sewage sludge on the biological life in the soil environment is the considerable positive impact of this type of waste on organic matter, nutrient content, soil porosity, bulk density, aggregate structure, and water capacity (Singh and Agrawal 2008).

Conclusion

The reclamation treatments used resulted in increased of all analysed enzymatic activity. The activity of catalase, protease, and urease increased upon application of all the wastes and combinations. The greatest impact was noted in objects treated with sewage sludge alone or in combination with the other wastes. While the activity of dehydrogenase, acid phosphatase, as well as the FDA hydrolytic activity was stimulated only in this objects. For a majority of the analysed enzymatic activities, application of sewage sludge combined with post-flotation lime (PFL + SS) proved more beneficial than the use of sewage sludge alone (SS).

The mode of mineral wool application was also important for the analysed enzymatic activities. In general, the mixture of mineral wool with soil at a dose of 500 m^3 ha^{-1} (MW) yielded better results. Stronger stimulation was achieved upon application of the 5 cm·50 cm^{-1} mineral wool pad (MWP).

All the analysed enzymes, i.e. dehydrogenases, catalase, urease, protease, acid phosphatase, and the FDA hydrolytic activity were shown to be sensitive indicators of changes induced by application of wastes into degraded soil. Therefore, the activity of these enzymes can be helpful in monitoring reclamation processes that take place in degraded soil. However, given the intensity and persistence of the changes in the analysed parameters, the activity of enzymes involved in nitrogen transformations in soils, i.e. urease and protease, proved to be the most sensitive indicator.

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Abbreviations

FDA	Fluorescein diacetate
NPK	Mineral fertilisation

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