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# Effect of reclamation treatments on microbial activity and phytotoxicity of soil degraded by the sulphur mining industry[☆]

Jolanta Joniec [a], [*], Patryk Oleszczuk [b], Stefania Jezierska-Tys [a], Edyta Kwiatkowska [a]

[a] *University of Life Sciences in Lublin, Faculty of Agrobioengineering, Department of Environmental Microbiology, Leszczyńskiego 7, 20-069, Lublin, Poland*
[b] *Department of Environmental Chemistry, Faculty of Chemistry, 3 Maria Curie-Skłodowska Square, 20-031, Lublin, Poland*

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ABSTRACT

The aim of the work was to determine the trend, intensity and changes of selected microbial and phytotoxic parameters of degraded soil in the area of former sulphur mine reclaimed by post-flotation lime (PFL), sewage sludge (SS), mineral wool (MW- mixed with soil, MWP-pad) and mineral fertilizer (NPK). The following parameters: number of proteolytic bacteria and fungi, ammonification, nitrification, activities of alkaline phosphatase and arylsulphatase *Lepidium sativum* growth index (GI) and phenolic compounds were analysed in the soil in second and third year of the experiment. The addition of the SS separately or in combination with other remediation agents was found to be the most valuable for the number of microorganisms, intensification of nitrification process and enzymatic activities. In objects where other materials were added without sewage sludge, the inhibition of fungal growth as well as alkaline phosphatase and arylsulphatase activities was observed, however the inhibitory effect declined with time. The observed increase of GI shows the long-term, positive effect of treatments on soil properties concerning plant growth. The use of lime and lime together with sewage sludge contributed to the decrease in the content of phenolic compounds in the reclaimed soil.

## 1. Introduction

Soil degradation is a global problem that leads to a reduction in the area intended for agricultural purposes. Consequently, this leads to a deepening of the problem with the board of humanity.

This problem requires the involvement not only of governments of individual countries but also of scientists to develop methods of effective recovery of degraded lands. One of the factors affecting soil degradation is the mining industry. The consequence of this degradation is the exclusion of soils from agricultural production for many years. In addition, degraded soil does not fulfill its role, i.e. filtration and buffering, which in consequence leads to the penetration of pollutants into other components of the biosphere, i.e. water and atmosphere.

A special case is degradation driven by the sulphur mining industry. Apart from physical degradation (geomechanical, hydrological), chemical degradation of soil is also very usual, preventing growth and development of plants in areas degraded by this type of industry.

The effect of excessive sulphur in the soil is strong acidification, leading to changes in biological balance, destruction of the sorption complex, increase in the concentration of $Al^{3+}$ ions in soil solution and drop in bioavailability of, among others phosphorus, potassium and molybdenum (Karczewska, 2012). In the acidified soil, nitrosamines, mycotoxins and other phytotoxic substances are formed, which deprives the soil of functional properties. Therefore, the problems of soil degradation and reclamation of post-mining areas remain valid for a long time after the exploitation of these mines.

Economic and living activity of a human is connected with generating huge amounts of various wastes. Examples are: sewage sludge, post-flotation lime and mineral wool. These wastes contain of nutrients for plants and soil microorganisms, so they can be used to improve the quality of degraded soils (Baran et al., 2012; Joniec, 2018; Joniec, 2019). 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 use of waste for reclamation purposes contributes to the solution of the problem of their management.

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[☆] This paper has been recommended for acceptance by Bernd Nowack.
[*] Corresponding author.
*E-mail address:* jolanta.joniec@up.lublin.pl (J. Joniec).

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Due to the fact that waste organic matter in the soil undergoes processes of mineralization and humification with the participation of soil bacteria and fungi, it seems reasonable to monitor changes in their activity. The various biological, physical, and chemical parameters are used to determine general indicators of soil quality and degradation (Bastida et al., 2008; Kaczyńska et al., 2015; Obade and Lal, 2016; Orwin and Wardle, 2004). As suggested by Bastida et al. (2008), a universal indicator cannot be developed due to the limitations posed by geographical conditions.

Enzymatic activity is one of the parameters used in soil monitoring and soil quality assessment. Hydrolases are particularly important for transformations occurring in soil (Burns et al., 2013). For example, Perez-de Mora et al. (2012) demonstrated the suitability of measuring arylsulphatase and phosphatase activity to assess reclamation processes occurring in degraded soil. Frąc and Jezierska-Tys (2011) found phosphatase activity to be a good indicator of changes in sewage sludge-fertilised soils. Wei et al. (2017) used phosphatase activity in bioremediation studies of soils contaminated with pyrene and pyrene-diesel. Zaborowska et al. (2016) described the high sensitivity of these hydrolases to heavy metal-contamination in soil. Sanchez-Hernandez (2018) analysed the contribution of phosphatase and arylsulphatase activity to the biodegradation of contaminants. Joniec (2018) examined the activity of enzymes such as: protease, acid phosphatase as well as the level of FDA fluorescein diacetate hydrolysis in the study concerning the impact of wastes on processes occurring in the reclaimed soil. All hydrolases were shown to be sensitive indicators of changes induced by the application of wastes into degraded soil.

Obade and Lal (2016) claimed that the determination of soil quality based on the measurements of individual soil properties was insufficient due to the variety of functions served by soil. Therefore, it is important to consider different aspects of soil degradation in the selection of soil status indicators, e.g. chemical degradation in addition to biodiversity decline. Monitoring soil environmental status should also include the assessment of its phytosanitary condition. As shown in the literature, *Lepidium sativum*-based tests are reliable measurements of soil phytotoxicity (Alvarenga et al., 2015; Mañas and De las Heras, 2018; Masciandaro et al., 1997; Różyło et al., 2015; Stefaniuk and Oleszczuk, 2016).

Recultivation treatments were applied to improve the conditions for the development of microorganisms responsible for soil fertility. The literature review shows that used Grodan mineral wool, especially with municipal sewage sludge, has a positive effect on water capacity and the formation of nitrogen content and balance, and thus the production capacity of reclaimed soils. The use of lime along with mineral wool and sewage sludge resulted in soil density reduction, proportional to the dose of wool and the addition of organic matter (Baran et al., 2011; Baran et al., 2012).

The model of reclamation experiment described in this study was used to carry out extensive research on the soil condition recultivated using various wastes (Joniec, 2013; Joniec et al., 2015; Joniec and Frąc, 2017; Joniec, 2018; Joniec, 2019). The authors, using soil microorganism activity parameters, i.e. abundance, catabolic diversity, intensification of biochemical processes and enzymatic activity, showed that the waste used, and in particular sewage sludge, positively affected biological life in degraded soil.

The presented research constitutes a new component in the comprehensive assessment of the soil condition recultivated using waste. The study expands the current state of knowledge by providing further valuable information on the aspects of this issue that are different from those presented so far. The demonstrated research may be helpful in making decisions related to waste management and selecting the method of soil remediation.

The aim of the presented work was to evaluate the changes of the microbiological activity and phytotoxicity in soil degraded by sulphur mining industry during different remediation steps. Number of proteolytic bacteria and fungi, ammonification, nitrification, activity of alkaline phosphatase and arylsulphatase and plant (*Lepidium sativum*) growth index and count of phenolic compounds were used to measure quality and health of the soil during remediation.

## 2. Materials and methods

### 2.1. Field experiment

The experiment was set up in the area of the former 'Jeziórko' Sulphur Mine (Poland, Podkarpacie Region, N50°33′09″, E21°46′40″). Sulphur mining was based on the Frash method, where sulphur was extracted from post-gypsum limestones with the use of hot water at a temperature of 120 °C and compressed air, until 2001. From 1967 to 2001 – more than $74\,10^6$ Mg of sulphur was mined. Total sulphur content ($S_T$) was 5002 mg $kg^{-1}$ in the 0–20 cm soil layer (Likus-Cieślik et al., 2017; Siwik-Ziomek et al., 2018).

The remediation experiment consisted of eight treatments. The particular plots were established in three replicates (the size of each plots was 30 $m^2$). Post-flotation lime (PFL), sewage sludge (SS), mineral wool (MWP or MW) and mineral fertilisation (NPK) were used as remediation agents in different combinations. The experiment included the following objects: (1) degraded soil without changes, control (UNP); (2) lime with NPK (PFL-NPK); (3) lime with sewage sludge (PFL-SS); (4) sewage sludge (SS); (5) lime with NPK and wool in the form of a 5-cm pad at a depth of 50 cm (PFL-NPK-MWP); (6) lime with sewage sludge and wool in the form of a 5-cm pad at a depth of 50 cm (PFL-SS-MWP); (7) lime with NPK and wool 500 $m^3$ $ha^{-1}$ (PFL-NPK-MW); (8) lime with sewage sludge and wool 500 $m^3$ $ha^{-1}$ (PFL-SS-MW).

Lime (PFL) was applied at a dose of 100 t $ha^{-1}$. Sewage sludge was distributed in the 20-cm soil layer in an amount of 100 t $ha^{-1}$. Mineral wool was applied to the plots in two ways: (1) in the form of a 5-cm pad at a depth of 50 cm (MWP) and (2) a dose of 500 $m^3$ $ha^{-1}$ distributed within the 0–20 cm layer (MW). The main purpose of using mineral wool was to restore proper water and air conditions in the reclaimed soil. The improvement of these properties will contribute to the restoration of biological life in degraded soils, among others, by stimulating the activity of microorganisms responsible for the processes of mineralization, humification or detoxification. Mineral fertilisation (NPK) was used in the following proportions: 80 (N), 40 (P) and 60 (K) kg $ha^{-1}$. NPK was applied to eliminate the effect of a nutrient deficiency in the experiment with mineral wool without sewage sludge compared to the experiment with the mineral wool and sewage sludge.

The remediation agents were spread to the soil surface. The homogenization of agents with soil was performed using a disc harrow and rototiller. Plots prepared in this manner were sown with a grass mixture (*Festuca pratensis*—41%, *Festuca rubra*—19.2%, *Lolium perenne*—14.7%, *Lolium multiflorum*—12.4%, *Dactylis glomerata*—6.5% and *Trifolium pratense*—6%). Characterization of the soil and remediation agents important for the development and activity of soil microorganisms was presented in Table 1. The sewage sludge used in the experiment did not exceed the permissible contamination polish standards (heavy metals), (Regulation of the Minister of the Environmental Journal of Laws, 2015).

### 2.2. Soil sampling

Soil samples from the experiment were taken from the 0–20 cm layer in the second and third year of the experiment. The samples were taken in July (Term I and III) and in October (Term II and IV)

**Table 1**
Selected properties of the degraded ground and the wastes used for remediation (Joniec, 2018).

| 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 |

each year.

The soil samples were collected randomly from each plot. Average soil sample from each plot consisted of a mixture of 10 soil cores of 3-cm diameter each. The samples were sieved through a 2-mm mesh sieve and stored in a refrigerator at +4 °C.

Selected soil properties at particular experimental time points published in an earlier work (Joniec, 2018) are presented in Table 2.

### 2.3. Microbiological analyses

The numbers of proteolytic bacteria and fungi were assayed using the plate method according to the procedure described by Foght and Aislabie (2005). Microorganisms were cultured on Frazier gelatine medium (Rodina, 1968). In the case of fungi, antibiotics were added to the medium in the amounts recommended by Martin (1950). Microbial cultures were incubated at 28 °C for 3 days. The results were expressed as colony forming units (cfu $kg^{-1}$ d.m. soil).

### 2.4. Biochemical analyses

The rate of ammonification was analysed in 25-g soil samples containing 0.1% of asparagine. After 3 days of incubation, ammonium ions were extracted using 2% KCl and their concentration was assayed with the Nessler method (Nowosielski, 1974). The result was expressed in mg N-$NH_4^+$ $kg^{-1}$ d.m. soil 3 $d^{-1}$. The rate of nitrification was analysed in 25-g soil samples containing 0.1% of monobasic ammonium phosphate. After 7 days of incubation, nitrate ions were extracted using 2% KCl and their content was measured with the brucine method (Nowosielski, 1974). The result was expressed as N-$NO_3$ $kg^{-1}$ d.m. soil 7 $d^{-1}$.

### 2.5. Enzymatic analyses

Alkaline phosphatase activity was determined in 1-g soil samples using p-nitrophenyl phosphate disodium as a substrate and incubated in a modified universal buffer (pH 11) at 37 °C for 1 h (Tabatabai and Bremner, 1969). The enzyme activity was expressed as mg PNP $kg^{-1}$ d.m. soil $h^{-1}$. Arylsulphatase activity was analysed in 1 g soil samples using p-nitrophenyl sulphate (PNS) as a substrate and incubated in a modified universal buffer (pH 5.8) at 37 °C for 1 h (Tabatabai and Bremner, 1970). The enzyme activity was expressed as mg PNP $kg^{-1}$ d.m. soil $h^{-1}$.

### 2.6. *Lepidium sativum L.* growth index (GI%)

Soil phytotoxicity was evaluated based on the *Lepidium sativum* growth index. Aliquots of fresh soil (50 g) with a moisture content of 60% total water capacity were placed on Petri dishes and 100 seeds were sown in each dish. The plates were incubated at a temperature of approx. 22 °C at a constant moisture level. After 4 days, the number of germinated seeds was calculated and their weight was determined. The experiment was carried out in triplicate. The growth index (GI%) was calculated based on these parameters using the formula proposed by Masciandaro et al. (1997):

$$GI\% = P\ (T/C)$$

P – mean % of germinated seeds in the reclaimed soil relative to the value for the control soil; T – mean weight of fresh *L. sativum* sprouts in the reclaimed soil; C – mean weight of fresh *L. sativum* sprouts in the control soil.

### 2.7. Chemical analyses

The content of phenolic compounds was analysed in the study. Phenols were extracted from the soil using the methodology developed by Hruszka (1982). In order to obtain the extract, 300 g air-dried soil was extracted (2 × 24 h) with working solution (70% ethyl alcohol + 28% redistilled water + 2% acetic acid); subsequently, the solution was evaporated and the pellet was transferred with redistilled water into a 50 ml volumetric flask. The amount of

**Table 2**
Selected physical, physicochemical and chemical properties of the soil (means for the year) (Joniec, 2018).

| 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 |

Explanations: 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.

phenolic compounds in the solution was determined by the method proposed by Swain and Hillis (1959) using the Folin-Denis reagent and a saturated $Na_2CO_3$ solution.

### 2.8. Statistical analysis

All microbiological, biochemical, enzymatic and phytotoxic analyses were carried out in triplicate. The results were statistically analysed using the Statistica 13 software with ANOVA models and multiple Tukey's T-tests at a significance level of $\alpha = 0.05$. The results are shown in the graphs with standard deviation indicated. The relationships between the analysed microbial activities, phytotoxicity in each object and time point were determined using the principal component analysis (PCA method).

The microbiological results from present experiment were additionally correlated with the results on chemical, physical and physicochemical analysis obtained concurrently in the same experimental model and presented in the earlier publication (Joniec, 2018).

## 3. Results

### 3.1. Number of bacteria and fungi

The application of all remediation agents to degraded soil significantly affected the growth of proteolytic bacteria and fungi in the 2nd and 3rd year of the experiment (Fig. 1A and B). The character and intensity of the effect depended on the agent type and investigation period. The growth of bacteria was significantly ($P < 0.05$) stimulated in the Term II and III with the exception of the PFL+SS+MWP object (Fig. 1A). The highest abundance values of these microorganisms were observed in objects treated with sewage sludge only (SS) or with sewage sludge and other agents (PFL-SS, PFL-SS -MWP). The stimulation of the growth of proteolytic bacteria was most pronounced in the Terms I and II. Differences between the first and second Term and the remaining ones were statistically significant in most objects ($P < 0.05$).

The effect of remediation agents on the growth of proteolytic fungi was not as apparent as for bacteria (Fig. 1B). Increase or decrease of number of fungi were observed depending on the experimental variant and investigation period. Significant stimulation ($P < 0.05$) was noted in objects treated with sewage sludge (PFL-SS, SS, PFL-SS-MWP, PFL-SS-MW). The application of other agents but without of SS (PFL-NPK, PFL-NPK-MWP, PFL-NPK-MW) had the least favourable effect on fungal growth. As regards proteolytic fungi, a more beneficial option was the use of sewage sludge alone (SS) than in combination with lime (PFL-SS). There was a significantly ($P < 0.05$) higher development of these microorganisms from the 2nd Term in the object with sewage sludge (SS) than in the object with lime and sewage sludge (PFL-SS). Similarly, the application of mineral wool pad (PFL-SS-MWP) caused a considerably higher ($P < 0.05$) fungal development than in the wool-soil mixture (PFL-SS-MW).

### 3.2. Biochemical activity (ammonification and nitrification)

Fig. 2A presents the results of intensification of the ammonification process. The process of the ammonification in the majority of the objects with addition of remediation agents was significantly ($P < 0.05$) inhibited compared to control soil (UNP). Over the period of experiment, an inhibition of organic nitrogen mineralization to $N\text{-}NH_4^+$ (Fig. 2A), was observed in the lime-treated object (PFL-NPK) and in the object with sewage sludge and lime (PFL-SS).

However, the data presented in Fig. 2B indicated that the introduced remediation agents significantly ($P < 0.05$) intensified the nitrification process. The most beneficial significant ($P < 0.05$) effect was noted for treatments with sewage sludge combined with lime (PFL-SS) and in combination with mineral wool pad and lime (PFL-SS-MWP). The positive significant ($P < 0.05$) impact of wastes persisted over the study period. However, this biochemical parameter was stimulated in some objects (PFL-NPK, PFL-SS, SS, PFL-SS-MWP) in the Term II.

### 3.3. Enzymatic activity

All remediation agents applied for reclamation exerted a positive significant ($P < 0.05$) effect on alkaline phosphatase and arylsulphatase activities (Fig. 3A and B). This effect depended on the type of agent and investigated period. From October in 2nd year (Term II), phosphatase activity (Fig. 3A) was significantly ($P < 0.05$) stimulated in the objects treated with sewage sludge in combination with other components (PFL-SS, PFL-SS-MW). However, the phosphatase activity was significantly ($P < 0.05$) inhibited in most terms in the object where sewage sludge (SS) was applied alone. It should be emphasised that the inhibitory effect of sewage sludge declined over time (Fig. 3A). The significant ($P < 0.05$) stimulation of alkaline phosphatase activity compared to control soil was recorded in all objects in the last experimental period.

The negative significant ($P < 0.05$) impact of tested materials was more pronounced for arylsulphatase activity (Fig. 3B). Decline of this parameter was observed in more objects than for phosphatase activity. Over the experimental period the inhibition of arylsulphatase activity decreased. The application of sewage sludge in combination with lime was found to be especially advantageous (PFL-SS) in the case of this enzymatic parameter. In this object, in the majority of terms, the highest values of this activity were recorded significantly ($P < 0.05$).

### 3.4. Phytotoxicity

Fig. 4 presents the growth index of *L. sativum* in the subsequent years of the experimental period. The positive impact of all remediation agents was observed in almost all experimental periods. In the Term I (the second year of the experiment), the GI values were at a similar level to the control soil (without amendments). In this case, no significant differences ($P < 0.05$) were found between the control soil and individual experimental variants (Fig. 4). At the Term II (second year of the experiment), a significant ($P < 0.05$) increase in the GI value was observed, demonstrating a positive impact of reclamation regardless of treatment type. The highest values were observed for the experiments with PFL-NPK, SS and PFL-NPK-MW. The GI values were 41% (PFL-NPK), 57% (SS) and 49% (PFL-NPK-MW) higher than for the control soil. In the following year of the experiment (Term III and IV), the GI values decreased slightly in these treatments, but still were higher compared to the control soil. At the same time (Term III and IV), however, an increase in GI was observed for the PFL-SS and PFL-SS-MW treatments, showing a long-term positive impact of these treatments on soil properties concerning plant growth. The GI values for PFL-SS and PFL-SS-MW were in the range (depending on Term) from 27% to 53% and from 41% to 48% higher than for the control soil, respectively.

### 3.5. Content of phenolic compounds

Over time, i.e. in the 2nd and 3rd year of the experiment, the content of phenolic compounds declined in the reclaimed soil (Fig. 5). A significant ($P < 0.05$) reduction in the content of these compounds was recorded in the combinations with lime (PFL-NPK), sewage sludge and lime (PFL-SS) and mineral wool mixed

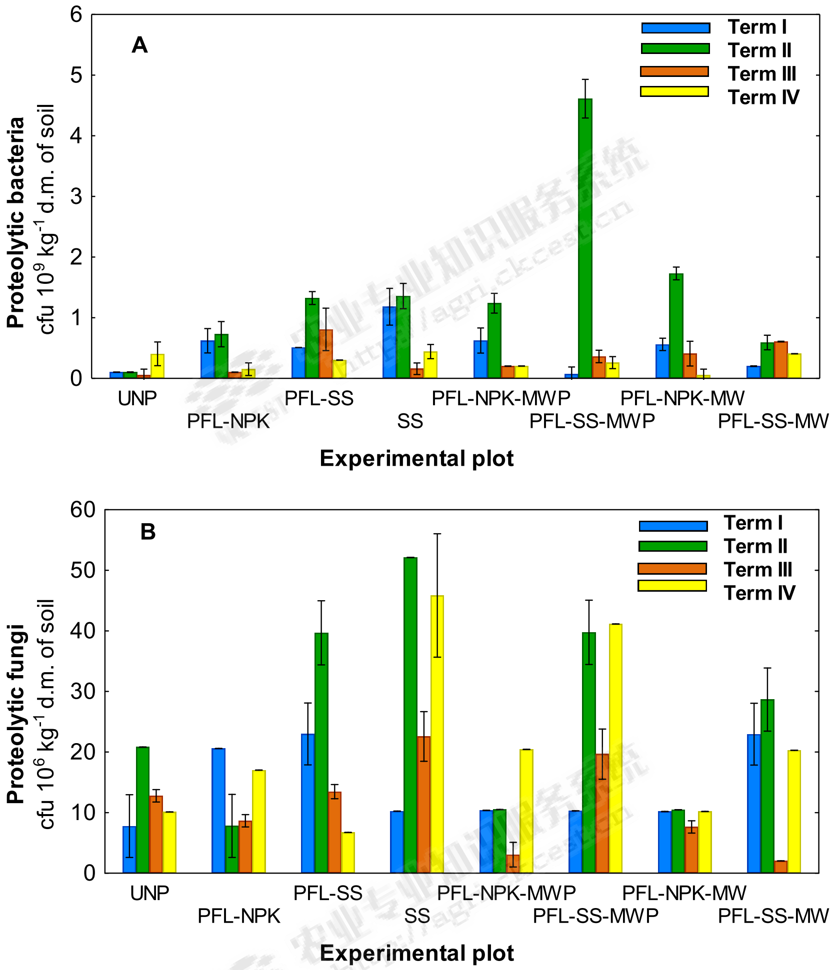

**Fig. 1.** Numbers of proteolytic bacteria and fungi in the control soil and soil under different treatment strategies. (1) degraded soil without changes, control (UNP); (2) lime with NPK (PFL-NPK); (3) lime with sewage sludge (PFL-SS); (4) sewage sludge (SS); (5) lime with NPK and wool 5 cm 50 cm$^{-1}$ (PFL-NPK-MWP); (6) lime with sewage sludge and wool 5 cm 50 cm$^{-1}$ + (PFL-SS-MWP); lime with NPK and wool 500 m$^3$ ha$^{-1}$ (PFL-NPK-MW); lime with sewage sludge and wool 500 m$^3$ ha$^{-1}$ (PFL-SS-MW). Term I- July/2nd year, Term II – October/2nd year, Term III-July/3rd year, Term IV- October/3rd year.

with soil and lime (PFL-NPK-MW), and the tendency persisted in all four time points. Other objects (SS, PFL-NPK-MWP, PFL-SS-MWP, PFL-SS- MW) showed a decline ($P < 0.05$) or no differences in the phenolic content relative to the control soil. The use of sewage sludge with lime (PFL-SS) was more favourable ($P < 0.05$) for the environment than the application of sewage sludge alone (SS).

### *3.6. Relationships between microbial activity, phytotoxicity and phenolic content in the objects at different time points*

As revealed by the principal component analysis (PCA), soil in particular objects and time points exhibited certain variability in the level of parameters analysed in this study. This was confirmed by data distribution on the score plot in Fig. 6A, corresponding to individual objects and time points in 4 groups. Group 1 included objects treated with sewage sludge from time point II, group 2 – objects with sewage sludge applied in combination with other agents from time points II and III, group 3 – objects treated with other remediation agents from time points III and IV, and group 4 – mainly the control object from all experimental periods and objects with sewage sludge applied separately or in combination with wool mixed with soil and with lime.

This distribution suggests differences in the analysed parameters between the objects treated with sewage sludge in combination with other agents and objects. Differences in the analysed activities were determined by component 1 (PC1) in 30.76% and by component 2 (PC2) in 19,61%. Moreover, the loading plot (Fig. 6B) demonstrated a number of correlations within the analysed microbiological activities, phytotoxic city and phenolic content as well as between these parameters and total N, TOC, cation exchange capacity and pH. Data regarding the aforementioned soil parameters at specific time points are provided in Table 2. There were strong positive correlations between the number of proteolytic bacteria and proteolytic fungi and moisture content, while slightly weaker correlations with total N. Furthermore, strong correlations were found between enzymatic activities, nitrification, and GI and their dependence on total N, TOC, cation exchange capacity, and pH. In turn, the content of phenolic compounds and the intensity of ammonification was negatively correlated with microbiological and biochemical, enzymatic activities.

Distances between cases and their coordinates (Fig. 6A and B) indicated favourable growth conditions for proteolytic microorganisms in objects from group 1 and for enzymatic activities, nitrification, and *L. sativum* growth in objects from group 2 and, to a lesser extent, group 3. An adverse effect of the treatments on microbiological and enzymatic activities, nitrification, and GI was

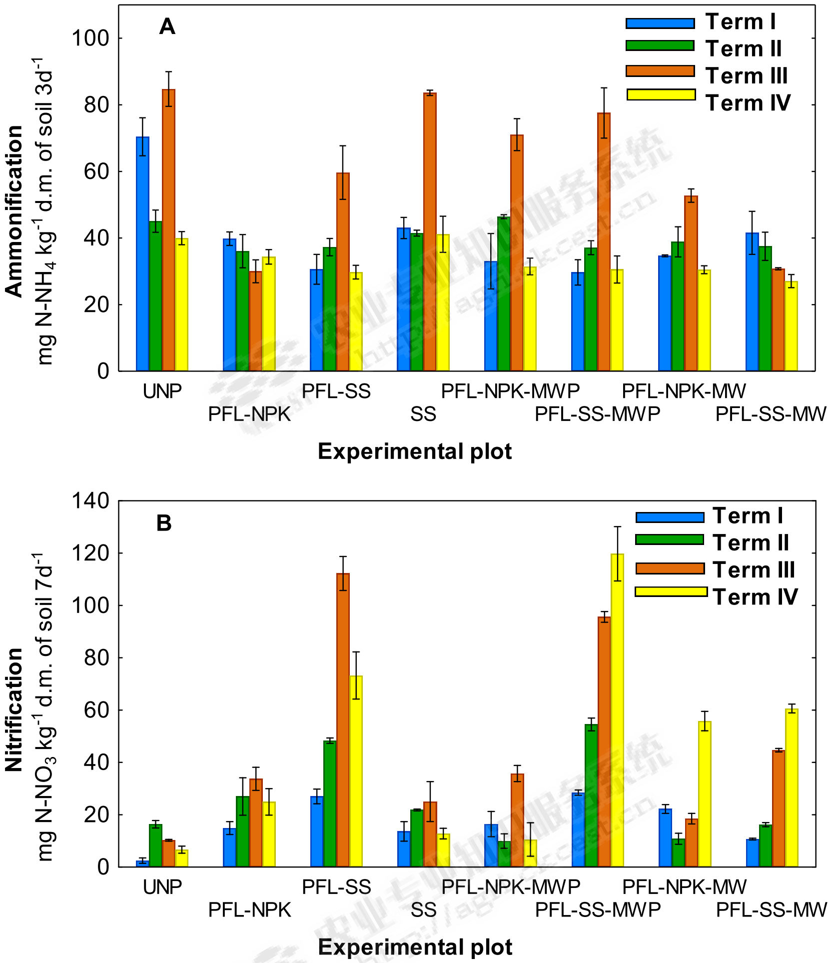

**Fig. 2.** Ammonification and nitrification in the soil. Description is given in Fig. 1.

demonstrated in the control (non-reclaimed) object and those treated with sewage sludge alone or in combination with mineral wool mixed with soil and lime. In turn, the overlapping distribution of these objects with the content of phenolic compounds and ammonification indicated high values of these parameters in these objects.

## 4. Discussion

Biological reclamation is based mainly on the stimulation of native microflora (Embar et al., 2006). The increase in the growth of proteolytic bacteria and fungi recorded in this experiment was induced by the supplementation of nutrients contained in sewage sludge (Table 1). Furthermore, the improvement of such environmental parameters as pH, moisture content and cation exchange capacity had an impact on this microbiological parameter in other objects as well. The suitability of mineral wool for the improvement of soil physical parameters was also shown previously by some researchers (Baran et al., 2012; Joniec, 2018). Moreover, the application of mineral wool and lime into soil may improve soil sorption capacity, increase and stabilize total nitrogen and organic carbon content as well as regulate the soil pH which all affect microbiological activity and plant growth. Cluster analysis revealed that the number of bacteria and fungi was strongly correlated with the moisture content and the total N and OC content. Data on soil moisture at particular time points are shown in Table 2. The parallel investigation conducted previously (Joniec, 2018) on the same experimental model demonstrated a steady increase in the values of total N and OC in the 2nd and 3rd year (Table 2). These observations were consistent with those reported by other authors, who showed a significant effect of such factors as nutrient content, moisture level, pH and cation exchange capacity on the growth of soil microflora (Czaban et al., 2010; Joniec et al., 2015; Oleszczuk et al., 2014). According to Baran et al. (2011), the use of lime in combination with mineral wool, for reclamation purposes is more beneficial than lime alone. Applying lime alone for de-acidification can lead under dry conditions to soil petrification. Wool, due to the positive influence on water and air properties, used together with lime, prevents this negative phenomenon. The authors have not recorded, under the conditions of the present experiment, directional differences in the activities studied between the object with lime alone and objects where lime was applied together with wool.

Sewage sludge applied in combination with other remediation agents was the major growth activator of proteolytic microorganisms. The strongest stimulation of their growth was noted in objects treated with this agent. This was associated with the positive effect of sewage sludge on organic matter, nutrient content, soil porosity, bulk density, aggregate structure and water capacity (Singh and Agrawal, 2008).

Changes in the growth of proteolytic bacteria and fungi, persisting with varying intensity in the 2nd and 3rd year, resulted from the selection of the microorganism associated with new conditions.

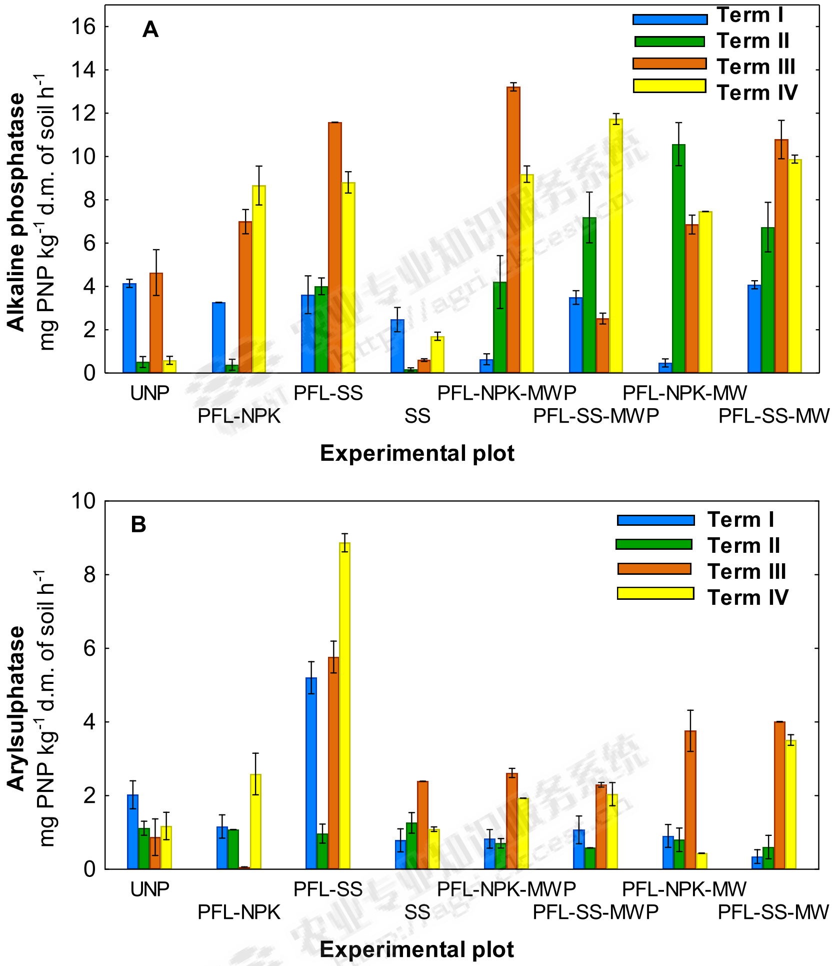

**Fig. 3.** Enzymatic activity in the soil. Description is given in Fig. 1.

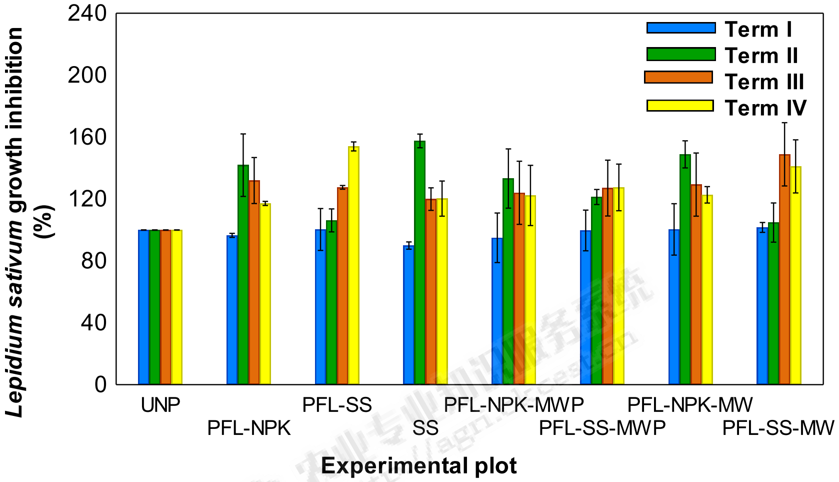

**Fig. 4.** Growth index *Lepidium sativum* in the soil. Description is given in Fig. 1.

Changes in the soil environment trigger a diverse response of native microorganisms, i.e. death of sensitive microbes and multiplication of resistant species, which leads to changes not only in their abundance, but also biodiversity (Ravikumar et al., 2017; Sun et al., 2017; Wu et al., 2014).

The plate method used in the present research for determining the number of microorganisms may cause overestimation or underestimation of results (Hill et al., 2000; Taylor et al., 2002). Underestimation results from the fact that only selected microorganisms present in the natural environment are able to

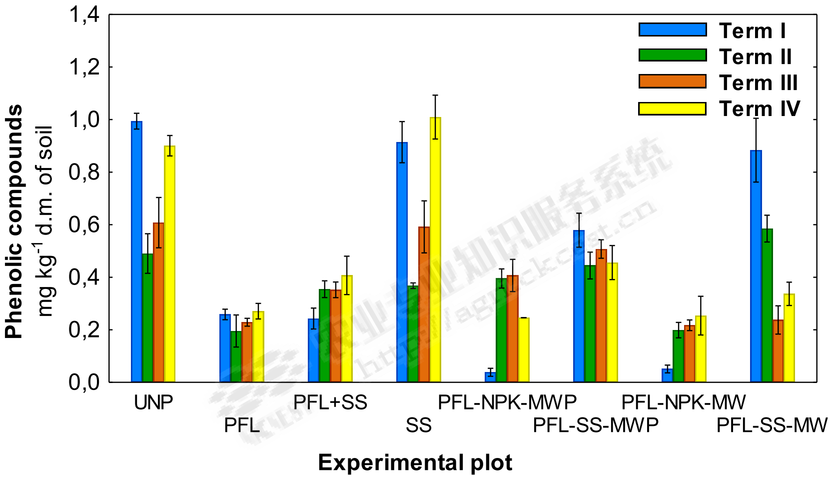

**Fig. 5.** Content of phenolic compounds in the soil. Description is given in Fig. 1.

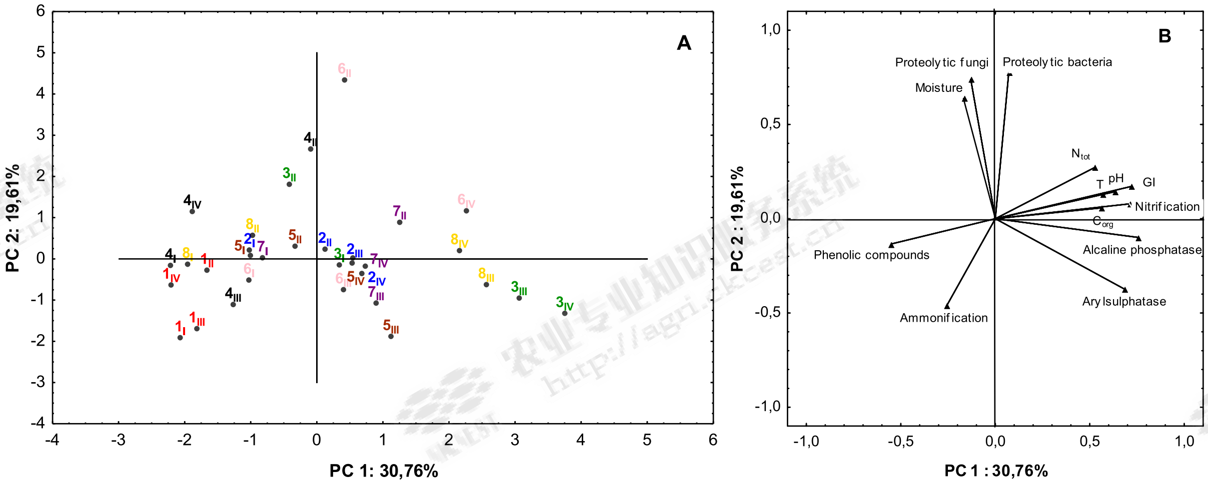

**Fig. 6.** PCA score plot (A) and loading plot (B), for the results of analysed parameters in the soil. 1-(UNP, red); 2- (PFL, blue); 3- (PFL – SS, green); 4- (SS, black); 5- (MWP – PFL, brown); 6- (MWP – PFL – SS, pink); 7- (MW – PFL, violet); 8- (MW – PFL – SS, yellow). I, II, III, IV - terms (the 2nd and 3rd year). T – cations exchange capacity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

grow on culture media. It is assumed that the method based on DNA extracted from soil is more precise (Taylor et al., 2002). However, Wolińska et al. (2013) showed positive relationship between the DNA content and the number of microorganisms determined by plate method. Our previous studies (Joniec, 2019) confirmed these observations for difficult matrices such as sewage sludge, lime and mineral wool. In such a case, even if this parameter is underestimated or overestimated (when the plate method is used), it is possible to observe differences between the various experimental variants, which allows to assume which of the variants was more favourable for the microorganisms growth and development.

Wastes used for reclamation exerted an effect not only on the abundance, but also biochemical and enzymatic activity of native microflora. Ammonification and nitrification processes are two important stages in the N cycle in the environment. However, the accumulation of mineral nitrogen forms derived from the mineralization of waste organic matter may be an environmentally adverse phenomenon. This is associated with the susceptibility of mineral nitrogen form to leaching, which in turn contributes to the risk of water contamination and losses of this element from the soil (Sierra et al., 2012).

The intensification of the ammonification process in the experimental conditions was lower than in control. Simultaneously, nitrification process was intensified in the agents-treated objects, especially in those with the separate or combined addition of sewage sludge. This demonstrated regular utilisation of ammonification products by nitrifiers. Cluster analysis revealed a negative correlation between the intensification of ammonification and nitrification which can means a decrease of the ammonification intensity with increase of nitrification process.

Alkaline phosphatase (EC 3.1.3.1) and arylsulphatase (EC 3.1.6.1) are exoenzymes from the hydrolase group involved in S and P transformations in the environment (Kang and Freeman, 1999; Pascual et al., 2002). The activity of these enzymes was previously found to be positively correlated with organic matter content or soil moisture (Scelza et al., 2007; Siwik-Ziomek et al., 2016). Pajares, Gallardo and Etchevers (2012) demonstrated a negative correlation of phosphatase activity with mineral phosphorus content in soil. In the present experiment, the increase in the activity of these enzymes resulted from the application of waste-based organic amendments into the soil. This was confirmed by correlation circle plot, which revealed correlations of these activities with total N, TOC, cation exchange capacity and pH. The decrease in the activity, in particular in the case of arylsulphatase, observed in some objects in the 2nd year, may imply the sensitivity of this enzyme to contaminants introduced with remediation agents. Analyzing the sludge, the authors found the presence of phenolic compounds, the content of which was 5.05 mg $kg^{-1}$ d.m. (unpublished data). However, the obtained results did not show the accumulation of phenolic compounds in the soil reclaimed by waste. Such an unfavourable effect of the application of sewage sludge was probably evoked by the introduction of a certain pool of phenolic compounds into the soil together with this waste. Our unpublished data indicated that the concentration of phenolic compounds in sewage sludge was 5.05 mg $kg^{-1}$ d.m. Moreover, waste matter may have been transformed into phenolic compounds. As suggested by Sierra et al. (2012), organic contaminants, including phenols, are introduced into the soil as a result of human pressure or emerge as an effect of transformations of, e.g. sludge matter. Ontañon et al. (2018) found that microorganisms degrading phenolic compounds were introduced into the environment together with wastes containing high concentrations of these contaminants. Oleszczuk (2007) demonstrated a reduction in the concentration of phenols over time, which was explained by the enhanced ability of microorganisms to degrade these compounds. Microorganisms utilize these pollutants as a C source, which is reflected in the intensification of respiratory processes (Nwankwegu et al., 2016). Own research conducted on the same model also showed an increase in respiratory activity (Joniec, 2019). This was also confirmed by the results, which did not show the accumulation of phenolic compounds in the soil reclaimed by waste, although a certain amount of them was introduced into this environment together with waste.

The rate of biodegradation of contaminants is determined by such environmental factors as moisture and oxygenation (Abioye et al., 2012). As suggested by Oleszczuk (2007), the sorption complex has a significant influence on the susceptibility of phenols to biodegradation. Phenols become more available to microorganisms along with the deterioration of this parameter in soil. Margesin (2007) reported a pivotal role of the availability of nutrients, especially nitrogen and phosphorus, in the microbiological degradation of organic contaminants. The correlation circle plot carried out in this study confirmed these observations. There was a negative correlation between the concentration of phenolic compounds and N and C content, pH and sorption capacity.

Soil recovered with wastes, especially with sewage sludge should be monitored for phytotoxicity. The latter is determined by the content of different kind of contaminants that inhibit plant growth and development, e.g. phenols, heavy metals or polycyclic aromatic hydrocarbons (Alvarenga et al., 2015; Mañas and De las Heras, 2018; Oleszczuk, 2007). A study conducted by Alvarenga et al. (2015) demonstrated that sewage sludge exerted different effect on soil phytotoxicity measured with *L. sativum* GI, depending on its origin and degree of processing. At other time points and years, *L. sativum* GI increased, which was probably caused by the presence of a certain pool of important plant nutrients introduced to the soil with remediation agents. As shown by cluster analysis, GI correlated strongly with the content of total N, TOC and nitrate ions as well as pH and phosphatase and arylsulphatase activity. These enzymes catalyse reactions that lead to the formation of available phosphorus and sulphur forms. A positive effect of organic fertilisation on *L. sativum* GI was also noted by Masciandaro et al. (1997) and Alvarenga et al. (2015). The measurement of *L. sativum* root growth is another parameter of phytotoxicity. Using this parameter, Różyło et al. (2015) demonstrated gradual disappearance of phytotoxicity in waste-enriched soil, as in the present study. Stefaniuk and Oleszczuk (2016) observed an increase in soil phytotoxicity after sewage sludge application, and the addition of biochar abolished this negative effect.

## 5. Conclusions

The study results indicated that all remediation agents used for reclamation had a generally positive impact on microbiological, biochemical, and enzymatic activity of the soil. This effect persisted with varying intensity throughout the study period. The inhibition of alkaline phosphatase and arylsulphatase activity noted in some objects decline over time. The analysis of the impact of materials used allowed to conclude that the use of sewage sludge separately or in combination with other materials proved most advantageous. The form in which mineral wool was applied was important only for fungal development and nitrification process. The observed increase of GI shows the long-term, positive effect of treatments on soil properties concerning plant growth. Despite the introduction of a certain amount of phenolic compounds along with sewage sludge, their accumulation in soil was not recorded, and even their decrease was observed in the case of objects with lime, and lime and sewage sludge.

The proposed reclamation treatments affect soil microorganisms activity in positive way what can lead directly to soil fertility improving. The remediation of degraded soils will not only lead to minimizing their negative impact on the surrounding environment but will also contribute to the recovery of soils that can potentially be used in agricultural production. In the situation of progressing degradation of soils and their depletion, this aspect is particularly important.

## Conflicts of interest

The authors declare no conflict of interest.

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