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PRESENCE OF DIFFERENT GROUPS OF MICROORGANISMS DEPOSOL IN RECLAMATION AT STANARI COAL MINE

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Abstract

This paper studied the presence of certain groups of microorganisms in deposol during the phase of biological reclamation with different crops. At the site of the overburden collection site at the Stanari coal mine, in 2011, the small grains, potato, soybean, corn, grass-clover mixture were sown in previously treated deposol. Since the dominance of certain groups of microorganisms dictate the processes of synthesis and degradation and determines the quality of the soil, this paper represents the total number of bacteria, *Azotobacter sp.*, ammonifiers, oligonitrophiles, actinomycetes and fungi. In microbial community, the bacteria dominated by the quantity in the control deposol and deposol in process of reclamation. Oligonitrophiles, like ammonifiers present in significant numbers in the control deposol, and their number has increased by applying the measures of reclamation. Also, the conditions for activity of *Azotobacter sp.* have been improved by application measures of reclamation. Since the pH control deposol is acid, the number of fungi in it is higher than number of fungi in deposol in the reclamation process, except deposol under grass-clover mixture. The highest total number of bacteria (351 x 10^5) , ammonifiers (1778 x 10^3) and oligonitrophiles (1361 x 10^3) is in the soil under grass-clover mixture, while the largest concentration of *Azotobacter sp.* in the soil under soybean (133 x 10^2). The presence of actinomycetes was not recorded in control deposol. The best effect of the reclamation processes on restore deposol fertility was recorded in deposol under grass-clover mixture.

Keywords: *Overburden collection site, biological reclamation, microorganisms*

Introduction

Coal mining not only visibly disrupts the aesthetics of the landscape, but disrupts soil components such as soil horizons and structure, soil microbe populations, and nutrient cycles that are crucial to sustaining a healthy ecosystem. At the beginning of the mining process soil is completely removed and stockpiled causing soil structure and soil microbial functions to be adversely affected. This disturbance can have a range of negative impacts on soil properties and microorganisms including organic matter and a potential for impaired nutrient cycling in reclaimed surface mine soils (Insam and Domsch, 1988; Bentham et al., 1992).
Mining operations on surface mine Raškovac and future surface mine Ostružnja significantly accelerated the degradation of the environment and the entire ecosystem (Malic et al., 2012). To restore or reclaim disturbed ecosystems, functioning stable soils must be reestablished. Biological reclamation is the old technology for the restoration of lands damaged by human activity.
Soil microorganisms during reclamation of surface mine sites, are extremely important to sustainable ecosystem function. They themselves are involved in major soil processes, such as humification, recycling, and mineralization of organic residues, leading to the plant availability of nutrients. Thus, they represent an important link in the soil – plant system and

contribute to the enhancement of soil fertility (Emmerling et al., 2002; Kourtev et al., 2002; Golic et al., 2006).

The composition of the soil microbial community may be influenced by the diversity of plant species (Balser and Firestone, 2005). Soil microbial community structure is increasingly being marketed as ecologically-relevant endpoint and it can realistically be incorporated for assessing the potential risks associated with soil amendment strategies on sustainability of soil ecosystems.

The aim of this paper was to obtain information about presence of different groups of microorganisms in deposol in the process of biological reclamation with five different plant species at Stanari coal mine. Measurement of microbial communities in soil provides answers to important questions such as the success of ecosystem restoration and return to its core functions and biodiversity.

Material and method

This research with different crops was performed in three years period (2011/2013), on the experiment plot of technogenic soil of the mine, within the inside part of overburden deposition site, near Raskovac pit, which is the part of Stanari coal mine. Stanari coal mine is located in north of the Republic of Srpska and Bosnia and Herzegovina at the municipality Doboj.

Small grains were sown manually, during the autumn sowing date, with 250-280 kg/ha of seed. During sowing, starter fertilizer was used at doses of 60 kg/ha of pure nutrients (N, P_2O_5, K_2O). The nitrogen fertilizer KAN (27% N) in a dose of 200 kg/ha was used at the beginning of tillering. Potato, soybean, corn and grass-clover mixture were sown during spring sowing date. Seeding rate for potato, soybean and corn was standard, and for grass-clover mixture was 30-40 kg/ha. During the sowing period, application of fertilizers was performed through the average use of 60-80 kg/ha of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O), while during the vegetation period supplemental recharge of 50-80 kg/ha of nitrogen was conducted.

Before seeding these crops, implementation of sudangrass growth (one year) was performed with the green manure fertilization of deposol and it represents an agro-technical phase of reclamation process within the mine.

Samples were collected from deposol without measures of biological reclamation (deposol control) and deposol in the reclamation process. Chemical analysis of deposol determined the parameters as follows: pH values (determined in a 1:25 ratio of soil/distilled water and in KCl), humus content (dichromate method by Tjurin in modification by Simakov), total content of nitrogen (determined by burning with the Kjeldahl procedure), availability of potassium and phosporous (Al-method by Egner-Riehm-Domingo).

Microbiological analyses involved determination of the number of particular systematic and physiological groups of microorganisms using the method of dilution on specific solid media. The following was determined: total number of bacteria (on the 1/10 strength Trypton Soya agar), soil fungi (on Czapek-Dox agar), actinomycetes (on the synthetic agar, Krasilljnikov, 1965), ammonifiers (on the nutrient agar), oligonitrophilic bacteria (Fyodorov's medium, Anderson and Domasch, 1958), count of *Azotobacter sp.* (Fyodorov's medium by the fertile drop method, Anderson, 1965). Incubation was followed by identification and counting of the colony forming units per 1 g of absolutely dry soil.

Results and discussion

Chemical properties of deposol

Since there is a very close connection between the microorganisms in the soil and their environment, the main chemical properties of deposol (without measures of biological reclamation) and deposol in the process of reclamation were determined (Tab. 1).

Chemical analysis of this technogenic soil were carried out before the start of reclamation (sample no. 1) and sowing, and then in the process of reclamation at the end of 2013 (sample no. 2). Based on the control results of active and potential soil reactions, deposols samples are classified into a category of highly acidic and moderately acidic reaction. The content of humus, total nitrogen and available phosphorus was recorded under detection level. According to the readily available potassium content, deposols are classified into a category of very poor content of these elements.

Tab. 1. Results of chemical analysis of the deposol (control) and deposol in the reclamation process

N°	Variant (phase of deposol)	pH		Humus (%)	N (%)	Plant available	
		H_2O	KCl			P_2O_5 mg/100g	K_2O mg/100g
1.	Deposol (control)	5,2	4,1	0,0	0,0	0,0	1,0
2.	Deposol in the reclamation process	6,3	5,0	0,2	0,01	2,3	5,9

The results of repeated chemical analysis of deposols in the reclamation process at the end of 2013 showed to a certain extent improvement of basic chemical properties. Precisely these changes are specific for the previously determined meliorated deposol (Malic, 2010). A decrease in the acidity at sample no. 2 was noticed, compared to the state before the start of reclamation process. The prominent changes were noted in the increase of humus and content of available phosphor and potassium.

Microbial properties of deposol

Microbial activity is a key factor affecting the functioning of all terrestrial systems. It has an important role in decomposition and nutrient cycling. Measurement of process rates governed by the soil microflora and general metabolic activities of these organisms is used to evaluate the reclamation efforts (Mummey et al., 2002; Izquierdo et al., 2003). The total number of bacteria in control deposol was lower than the total number of bacteria in deposol in the reclamation process, especially in deposol under potato, soybean and grass-clover mixture (tab 2). In deposol in the reclamation process, the total number of bacteria was the highest in the soil treated with grass-clover mixture. Somewhat lower number of bacteria was recorded with potato and soybeans, and the lowest one with corn.

Tab. 2. The number of the microbial groups in deposol in the reclamation process and control deposol

Species of microorganisms		Phase of the deposol					
		Small grains	Potat o	Soybean	Corn	Grass-clover mixture	Deposol (control)
Total number of bacteria x 10^5 g^{-1}		54	194	153	55	351	47
Ammonifiers	total x 10^3 g^{-1}	296	1120	200	350	1778	960
	sporogenic x $10^3 g^{-1}$	63	167	96	24	53	7
Oligonitrophiles x 10^3 g^{-1}		751	1171	697	587	1361	961
Azotobacter x 10^2 g^{-1}		120	93	133	19	68	5
Actinomycetes x $10^3 g^{-1}$		70	548	41	57	21	0
Fungi x 10^3 g^{-1}		168	248	294	220	391	365

Ammonification, as a significant process of crude protein decomposition and their transformation into mineral or organic forms is carried out by ammonifiers (Jarak et al, 2003). Since these compounds are common microbial origin in the soil, the number and activity of ammonifiers in the soil represents an index of its general biogenity. Based on these results, we can notice a significant presence of total number of ammonifiers in control deposols (960 x 10^3 g^{-1}). The higest number of ammonifiers in deposol under different vegetation cover was recorded in the deposol under grasses-clover mixture (1778 x 10^3). In contrast to that, the lowest number of ammonifiers was recorded in deposol under soybeans (200 x 10^3). Compared to the total number of bacteria, a relatively high number of the ammonifiers was manifested, suggesting the organic matter decomposition processes as being normally proceeding, whereas the mineralisation process was slowed down. The number of sporogenic ammonifiers was lower in control deposol (7 x 10^3) than the number of sporogenic ammonifiers in deposol in the reclamation process. The lowest number of sporogenic ammonifiers in deposol in the reclamation process was recorded in deposol under corn (24 x10^3) and the highest number of sporogenic ammonifiers was in deposol under potato (167 x 10^3).

Oligonitophiles, as fixation factors of the atmospheric nitrogen for meeting their own needs and suppliers of plants with accessible forms of nitrogen (Bogdanovic, 1990), represented the dominant physiological group of microorganisms in deposol in the phase of reclamation, except in deposol under grass-clover mixture. This indicates unfavourable nitrogenous regime of deposol. Oligonitrophiles, like ammonifiers, are present in significant number in the control deposol (961 x 10^3 g^{-1}), and their number increased by applying the measures of reclamation. In deposol in the reclamation process, the lowest number of oligonitrophiles was found in the soil under corn (587 x 10^3 g^{-1}) while the highest number of oligonitrophiles was found in the soil under grass-clover mixture (1361 x 10^3 g^{-1}).

Azotobacter sp. as indicator of fertile soil and the strongest associative fixation factor of the atmospheric nitrogen showed low presence in the control deposol in comparison with the number of *Azotobacter sp.* in deposol in the reclamation process. The application measures of reclamation have improved conditions for activity of this nitrogen fixation factor. The lowest number of *Azotobacter sp.* was found in the soil under corn (19 x 10^2 g^{-1}), while the highest number of *Azotobacter sp.* was found in the soil under soybeans (133 x 10^2 g^{-1}).

Actinomycetes are able to degrade hardly degradable compounds such as lignin and chitin, but also participate in the formation of humus. The number of actinomycetes in deposoil in reclamation process ranged from 21 x 10^3 g^{-1} in deposol under grass-clover mixture to 548 x 10^3 g^{-1} in deposol under potato. The presence of actinomycetes was not recorded in deposol - control. Milošević et al. (2003) points out that the presence of actinomycetes is highest in slightly alkaline soil. Deposol pH value increased after reclamation measures, which led to a higher number of actinomycetes in deposol.

Fungi are responsible for the important process of decomposition in terrestrial ecosystems as they degrade and assimilate cellulose, the component of plant cell walls. Fungal growth was maximal at pH 4.5, and decreased by a factor of more than 5 toward the high pH end (Rousk et al, 2010). Because pH control deposol is acidic, the number of fungi in this control deeposol is higher than number of fungi in deposol in the reclamation process, except deposol under grass-clover mixture.

The number of fungi in deposol in reclamation process ranged from 168 x 10^3 g^{-1} in deposol under small grain to 391 x 10^3 g^{-1} in deposol under grass-clover mixture.

Conclusion

Reclamation is an essential part in developing mineral resources in accordance with the principles of ecologically sustainable development. In addition to the chemical properties of soil, reclamation of overburden dumps can be managed effectively when microbiological properties of soil are accurately determined. The application of reclamation measures increased the biological activity in deposol. Acidic deposol reaction is probably influenced on the higher number of fungi in control deposol than in deposol under small grains, potato, soybeans and corn. The highest total number of bacteria, total number of ammonifiers, number of oligonytrophils and fungi was found in deposol under grass-clover mixture. The presence of actinomycetes was not recorded in control deposol. Reclamation of the deposol with small grains, potato, soybeans, corn, and grass-clover mixture will be able to restore the deposol fertility and accelerate ecological succession. The best effect of the reclamation processes on restore deposol fertility was recorded in deposol under grass-clover mixture.

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