MICROBIOLOGICAL PROPERTIES OF REKULTISOL UNDER DIFFERENT CULTURES AT STANARI COAL MINE

Zorica GOLIĆ[1]*, Nenad MALIĆ[2], Mihajlo MARKOVIĆ[3]

Original scientific paper UDK 631.427.1(497.6)

ABSTRACT

This paper studied the number of physiological and systematic groups of microorganisms in rekultisol under the different cultures at Stanari Coal Mine, and in variants of rekultisol where mineral fertilization and calcification were applied and in variants of rekultisol where only mineral fertilization was applied. The trial was set in the field conditions at the location of the internal part of overburden deposition site, near open pit Raskovac which is the part of Stanari coal mine. Mineral fertilizers were used as starter fertilizer and supplementary nutrition in the amount of 100-140 kg/ha of N and 40-60 kg/ha P_2O_5 and K_2O and calcification was completed by adding 4 t/ha $CaCO_3$. Microbiological analysis of the rekultisol determined the total number of bacteria, *Azotobacter sp*., ammonifiers, oligonitrophyls, actinomycetes and fungi. The aim of the research is to determine microbiological properties of rekultisol under small grains, potatoes, and grass-clover mixture at "Stanari" coal mine as well as to determine the effect of mineral fertilizers and calcification on the number of microorganisms in the rekultisol under different cultures. The total number of bacteria, oligonitrophyls, sporogenic ammonifiers, *Azotobacter sp.* and actinomycetes was higher in rekultisol under all of the tested cultures in the variant fertilizers + $CaCO_3$ comparing to the variant without $CaCO_3$, while the number of ammonifiers and fungi in rekultisol under all of the tested cultures was higher in the variant without $CaCO_3$ in relation to the variant mineral fertilizers + $CaCO_3$. In rekultisol under grass-clover mixture, higher total number of bacteria, oligonitrophyls and fungi was recorded in both tested variants, in relation to their number in rekultisol under potatoes and small grains. The number of actinomycetes was higher in rekultisol under potato in both variants, in relation to the number of actinomycetes in rekultisol under small grains and grass clover mixture. In rekultisol under small grains in variant mineral fertilizers + $CaCO_3$ number of

[1] Institute for Materials Testing and Construction Testing of Republic Srpska, Save Mrkalja 16, 51000 Banja Luka, Bosnia and Herzegovina
[2] EFT-Mine and Thermal Power Stanari, Stanari bb, 74208 Stanari, The Republic of Srpska, Bosnia and Herzegovina
[3] University of Banja Luka, Faculty of Agriculture, Bulevar vojvode Patra Bojovića 1A, 51000 Banja Luka, Bosnia and Herzegovina
*Corresponding author: zoricagolic@gmail.com

ammonifiers was the lowest (182 x $10^4 g^{-1}$ absolutely dry soil), while the highest number of ammonifiers was recorded in rekultisol under grass-clover mixture in the variant without $CaCO_3$ (1778 x $10^4 g^{-1}$ absolutely dry soil). *Azotobacter sp.* count was lowest in rekultisol under grass clover variant without $CaCO_3$ (68 x $10^2 g^{-1}$ absolutely dry soil), while the highest *Azotobacter sp.* count was recorded in rekultisol under small grains in the variant with fertilizers + $CaCO_3$ (123 x $10^2 g^{-1}$ absolutely dry soil).

Keywords: *rekultisol, microbiological properties, mineral fertilizers, calcification*

INTRODUCTION

Reclamation of the damaged surface mine areas represents an important part of sustainable development strategies of many countries (Sheoran *et al*., 2010). Intensification of mining operations in the Raškovac open-pit mine and future open-pit mine Ostružnja, in order to provide sufficient quantities of coal for Stanari thermal plant, significantly accelerated the degradation of the environment and the whole ecosystem. Therefore, it is necessary to conduct reclamation of the damaged area (Loncar *et al*., 2009; Malic *et al*., 2012).

Surface coal mining disrupts the primary structure and composition of land and it causes deposition of different geological materials (Resulovic, 1982). Deposols have extremely low production value, lack of or very low humus content (Dželetovic *et al*., 1995) and nutrients (Filipovic *et al*., 1981), which results in changes in the quantitative and qualitative composition of microbial populations and their biochemical activity (Golic *et al*., 2014).

Biological reclamation is the old technology for the restoration of lands damaged by human activity. During reclamation of surface mine sites, soil microorganisms are extremely important for the 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).

Mineral fertilization has the strongest influence on field crops yield. However, results of trials carried out by Barabasz *et al*. (2001) have shown that mineral fertilization also strongly affects a number of microorganisms and qualitative selection of whole communities of soil microorganisms. The universal system of fertilization is very difficult to achieve on acid soils due to numerous problems with nutrition, as well as the application of mineral nutrients, because it is difficult to specify needs of plants for certain nutrients in acid soils, as a result of very uneven both physical and chemical properties of soil (Bennett, 1993).

Adequate application of limestone material in combination with mineral and organic nutrients is the most effective way of avoiding adverse characteristics of acid soil (Jelić *et al*., 2008).

The aim of the research is to determine microbiological properties of rekultisol under small grains, potatoes and grass-clover mixture at "Stanari" coal mine as well as to determine the effect of mineral fertilizers and calcification on the number of microorganisms in the rekultisolunder different cultures.

MATERIAL AND METHODS

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.

Small grains were sown manually, during the autumn sowing date, with 250-280 kg/ha of seed. Seeding rate for potato and for grass-clover mixture was 40-45 kg/ha.

In variants of rekultisol where mineral fertilization and calcification were used, mineral fertilization used as starter fertilizer and supplementary nutrition in the amount of 100-140 kg/ha of N and 40-60 kg/ha P_2O_5 and K_2O, and calcification was completed by adding 4 t/ha $CaCO_3$. Same amount of mineral fertilization were used variants of rekultisol where only mineral fertilization were applied.

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), 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

Chemical properties

Results of the basic chemical properties analysis of deposol (control) and deposolin the reclamation process are shown in Table 1.

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

Nº	Species	Phase of deposol (substrate) and fertilizing		pH		Organic matter	Humus (%)	Plant available	
				H_2O	KCl			P_2O_5 mg/100g	K_2O mg/100g
1.	-	Deposol (control)		5.2	4.1	1.60	0.0	0.0	1.0
2.	Small grains and potato	Deposol in the reclamation process	Mineral fertilizer	6.3	5.0	3.98	0.2	2.3	5.9
3.			Mineral fertilizer + $CaCO_3$	6.7	5.9	4.70	1.0	1.1	7.4
4.	Grass-clover mixture		Mineral fertilizer	6.1	5.0	3.01	0.0	1.0	5.4
5.			Mineral fertilizer + $CaCO_3$	7.2	6.2	3.5	0.1	1.6	5.5

Deposol (control) belongs to very acid soils (pH in KCl 4,1) with organic matter content of 1.6%, while the humus content and available phosphorus is below the level of detection. The content of readily available potassium is 1 mg/100 g soil.

Deposol in the reclamation process, under all cultures in the variant with the mineral fertilizer, had the 5.0 pH value in KCl, whereas in the variant with fertilizers + $CaCO_3$ pH value of the soil under small grainsand potatoes was higher 5.9 and in the soil under grass-clover mixture 6.2.

The content of organic matter in deposols in the process of reclamation has increased in relation to deposols-control. The land under small grainsand potatoes, the variant with mineral fertilizer, had the organic matter content of 3.98%, and in variant with fertilizers + $CaCO_3$ it was 4.7%. In soil under the grass-clover mixture the organic matter content was much lower with 3.01% in the variant with mineral fertilizer, and in variant mineral fertilizers + $CaCO_3$, the content of organic matter was 3.5%.

The humus content is very low in both variations, and it ranges from 0%, in the soil under grass-clover mixture, and in variants with mineral fertilizers up to 1% on the land under small grainsand potato crops in variant with mineral fertilizer + $CaCO_3$.

In all the tested variants the soil is poorly provided with readily available phosphorus. The content of readily available potassium is slightly higher in relation with the contents of available phosphorus, but is still poorly provided with potassium regarding the plant production.

Microbiological properties

Microbiological properties of this deposol (control) and deposol in the process of reclamation are shown in Table 2. The number of microbial groups is much lower in deposols (control) comparing to the deposols in the reclamation process.

The total number of bacteria and their composition is one of the indicators of the biological value of the land. The minimum total number of bacteria was recorded in soil under small grains and in the variant withthe mineral fertilizer only (54 x 10^6 g^{-1} of absolutely dry soil), while a slightly higher number of bacteria was recorded in the variant with the mineral fertilizer + $CaCO_3$ (97 10^6 g^{-1} absolutely dry soil). In the soil under the grass-clover mixture, the total number of bacteria was the highestin the variant with fertilizers + $CaCO_3$ and was 351 x 10^6 g^{-1} of absolutely dry soil.

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

Species of Microorganisms		Phase of the deposol						
		Deposol (control)	Deposol in the reclamation process					
			Small grains		Potato		Grass-clover mixtures	
			MF	MF+ $CaCO_3$	MF	MF+ $CaCO_3$	MF	MF+ $CaCO_3$
Total number of bacteria x 10^6 g^{-1}		4.7	54	97	128	194	158	351
Ammonifying bacteria	**Totalx 10^4 g^{-1}**	96	296	182	1120	766	1778	1069
	Sporogenicx 10^3 g^{-1}	7	63	126	167	233	34	53
Oligonitrophilic bacteriax 10^4 g^{-1}		96.1	751	1292	1171	1291	1361	1600
***Azotobacter sp.* x 10^2 g^{-1}**		5	83	123	93	109	68	110
Actinomycetes x 10^3 g^{-1}		0	47	70	306	548	21	38
Fungi x 10^4 g^{-1}		36.5	233	168	337	248	461	391

The number of ammonifiers in deposols in the reclamation process under all tested cultures is lower in the variant with fertilizers + $CaCO_3$ compared to the variant with only mineral fertilizers. The highest number of ammonifiers was recorded in soil under grass-clover mixture in variant only with mineral fertilizers (1778 x 10^4 g^{-1} of absolutely dry soil), and the lowest in the soil under small grains variant with fertilizers +$CaCO_3$ (182 x 10^4 g^{-1} of absolutely dry soil).

In the soil under all of the cultures, the number of sporogenic ammonifiers was lower in variant with only mineral fertilizer in relation to the variation with mineral fertilizers + $CaCO_3$. Minimum number of sporogenic ammonifiers was recorded in soil under grass-clover mixture in variant with mineral fertilizer and was 34 x 10^3 g^{-1} of absolutely dry soil, while the highest number of sporogenic ammonifiers was

recorded in the soil under potato, variant with fertilizers and $CaCO_3$ and it was 233 x 10^3 g^{-1} of absolutely dry soil.

Oligonitrophyls represent a dominant physiological group of microorganisms in the analyzed samples. Presence ofoligonitrophyls in soil under all of the cultures is greater in variation with mineral fertilizers + $CaCO_3$ compared to variants with only mineral fertilizers. The soil under grass-clover mixture contains higher number of oligonitrophyls in both variations, compared to the number of oligonitrophyls in soil under other cultures.

Applying calcification with mineral fertilizer, the number of *Azotobactersp.* and actinomycetes in the soil under all the tested cultures increased. The highest number of *Azotobactersp.* is recorded in the soil under small grains (123 x 10^2 $g^{-1}$of absolutely dry soil - a variant with fertilizers + $CaCO_3$), and the lowest number is in the soil under grass-clover mixture (68 x 10^2 g^{-1}absolutely dry soil - variant with mineral fertilizer). The number of actinomycetes is the highest in the soil under potato (548 x 10^3 g^{-1} absolutely dry soil - variant mineral fertilizers + $CaCO_3$), and the lowest in the soil under grass-clover mixture (21 x 10^3 g^{-1} absolutely dry soil - variant with mineral fertilizers).

The number of fungi in the soil under all tested cultures was higher in variant with only mineral fertilizer in relation to the number of fungi in variant with fertilizers + $CaCO_3$. The highest number of fungi, in both variations, is recorded in the soil under grass-clover mixture in variant with mineral fertilizer where the recorded number of fungi was 461 x 10^4 g^{-1} of absolutely dry soil, and in variant with fertilizers +$CaCO_3$ it was 391 x 10^4 g^{-1} absolutely dry soil. The minimum number of fungi in both variations was recorded in the soil under small grains. In this soil, invariant with mineral fertilizer, the number of fungi was 233 x 10^4 g^{-1} of absolutely dry soil, and in variant with fertilizers + $CaCO_3$ in the amount of 168 x 10^4 g^{-1} of absolutely dry soil.

DISCUSSION

The number of microorganisms in certain ecosystem is a definite indicator of soil fertility (Tabatabai, 1982). Soil fertilization with mineral fertilizers has a positive effect on increasing the biological productivity of various ecosystems, as well as the microbial activity in the soil (Barabasz, 2002). Results of basic chemical characteristics point to an acidic reaction of the tested deposol. The pH values directly affect the mobility of nutrients and enable their accessibility for the plants, but also cause the composition of soil microbial populations and affect the formation of certain species of plant-covers (Tintor *et al*., 2008). As most of cultivated plants require a slightly acidic, neutral to slightly alkaline soil reaction, a small number of crops can tolerate the conditions in acid soils. Neutralizing the acid soils by introducing limestone material is a measure that is regularly recommended in order to improve the physical, chemical and biological properties and to increase the level of soil fertility (Jelic *et al*., 2008).

Within the total number of microorganisms in the soil, the largest share belongs to bacteria. Bacteria are the most numerous in the neutral soils. By reducing the acidity, the conditions for increasing bacterial activity is created (Jarak *et al*., 2003), which was confirmed in this study. The total number of bacteria in the soil under all tested cultures is increased by application of $CaCO_3$ with mineral fertilizer.

Ammonifiers, as consumers of organic nitrogen and protein decomposers, are the most abundant group of microorganisms in the soil (Bogdanović, 1990). The size of this group of microorganisms is least sensitive to changes in physical and chemical parameters in soil and negative impacts (Tintor *et al*., 2009). Number of ammonifiers in soil under all tested cultures was higher in the variant with only mineral fertilizer in relation to the variant of mineral fertilizers + $CaCO_3$.

Free nitrogen-fixing bacteria, that can fix atmospheric nitrogen, play an important role in maintaining soil fertility. In the tested soils, the number of oligonitrophylsin the soil under all tested cultures was greater in variations with fertilizers + $CaCO_3$ compared to variants with only mineral fertilizer. In the soils under all tested cultures, there are modest reserves of nutrients, which is more acceptable for oligonitrophyles in comparison to the other groups of microorganisms. Dominance of oligonitrophilic bacteria compared to ammonifiers indicates the presence of mineral nitrogen.

Actinomycetes require alkaline environment and sufficient organic matter (Jarak and Govedarica, 2003), while the number of actinomycetes in the acid soil is low (Williams and Wellington, 1982). With the decrease of soil acidity, by calcification with mineral fertilizers, there was an increase in the number of actinomycetes in the soil under all tested cultures.

Azotobacter sp. is one of several soil bacteria important as nitrogen fixers that convert atmospheric nitrogen into forms available to plants and is very important parameter for determination of soil biogeny. This group of bacteria is very sensitive to all changes occuring in the soil and responds to them by its abundance and vigorous activities. According to Jarak and Čolo (2006), *Azotobacter sp*. in acid soils is either absent or present in very small numbers. Based on the results of this study, it can be concluded that increasing the pH value of the soil in the tested soil samples, due to the calcification with mineral fertilizer, had influenced on increase of *Azotobactersp*. number in the soil.

Fungi live in communities with a wide range of pH value (1.7-3.0 to 9.0). In environments with a low pH value, which is not suitable for bacteria and actinomycetes, fungi are the dominant microbial flora, which in such circumstances is particularly active in biochemical transformations. Due to the increased pH of the tested soil by applying calcification measures, a lower number of fungi are expected in variants with fertilizers + $CaCO_3$.

CONCLUSIONS

Based on the study of the effect of the mineral fertilizer applications and calcification on the number of microorganisms in deposols under small grains, potato crops and grass-clover mixtures, we can conclude the following:

- The application of mineral fertilizers and calcification showed improvement of chemical properties of deposol in the reclamation process.
- In the soil undergrass- clover mixtures, the total number of bacteria, the total number of ammonifiers, oligonitrophyls and fungi in both variants was higher than in soils under other tested cultures.
- Number of actinomycetes is the highest in the soil under potato in both studied variants.
- Number *Azotobacter sp*. was the lowest in soil under grass-clover mixtures in the variant with only mineral fertilizer, while the highest number of *Azotobacter sp*. was recorded in soil under small grains in variant with mineral fertilizer + $CaCO_3$.
- The total number of bacteria, oligonitrophyls, sporogenic ammonifiers, *Azotobacter sp*. and actinomycetes was higher in deposols in the reclamation process under all tested culture in variants with fertilizers + $CaCO_3$ compared to variant without $CaCO_3$, while the number of ammonifiers and fungi in deposols in the reclamation process under all tested cultures was higher in the variant without $CaCO_3$ compared to variant with fertilizers +$CaCO_3$.

REFERENCES

Anderson, G. R. 1965. Ecology of Azotobacter in soil of the Palouse region I. Occurrence. Soil Science, 86:57-65.

Anderson, J.P.E., Domasch, K.H. 1958. A physiological method for the quantitative measurement of microbial biomas in soil. Soil Boil. Biochem. 10: 215-221.

Barabasz, W., Albińska1, D., Jaśkowska, M., Lipiec, J. 2002. Biological Effects of Mineral Nitrogen Fertilization on Soil Microorganisms. Polish Journal of Environmental Studies, 11: 193-198.

Bennet, W. F. 1993. Nutrient deficiencies and toxicities in crop plants. APS Press, St. Paul, Minnesota. Pages 202.

Bogdanović, V. 1990. Zastupljenost mikroorganizama u deponiji pepela. Zemljište i biljka, 39 (2): 139-145.

Dželetović Ž., Filipović R., Stojanović D., Vučković M., Djurdjević M., Lazarević M. 1995. Agrohemijska ispitivanja odlagališta jalovine "Petka" ugljenokopa "Đirikovac" radi rekultivacije njegove površine. Rudarski glasnik, 3-4: 23-30.

Emmerling, C., Schloter, M., Hartmann, A., Kandeler, E. 2002. Functional diversity of soil organisms-a review of recent research activities in Germany. Journal Plant Nutrient and Soil Science, 165: 408–420.

Filipović R., Kotlajić, M., Simić, S. 1981. Uticaj mineralnih djubriva na prinos nekih ratarskih kultura na zemljištima oštećenim rudarskim adovima. Zb.gozdarstva in lesarstva, 19: 273 - 290.

Golic, Z., Malic N., Raicevic, V. 2014: Presence ofDifferent Groups of Microorganisms Deposol in Reclamation at Stanari Coal Mine. Book of Proceedings – Fifth International Scientific Agricultural Symposium "Agrosym 2014" B&H – Jahorina, pp. 777–782.

Golić, Z., Raičević, V., Jovanović, Lj., Antić-Mladenović, S., Kiković, D. 2006. Number and activity of microorganisms in bauxite mine soil. Zemljište i biljke, 55: 203-210.

Jarak, M, Belić, M., Govedarica, M., Milošević, N., Đurić, S. 2003. Effect of phosphogypsum and peat on microbiological and chemical properties of arenosol. Zemljište i biljka, 52: 1-6.

Jarak, M., Čolo, J. 2007. Mikrobiologija zemljišta, Univerzitet u Novom Sadu, Poljoprivredni fakultet.

Jarak, M., Govedarica, M. 2003. Mikrobiologija, Univerzitet u Novom Sadu, Poljoprivredni fakultet.

Jelić, M., Đalović, I, Živanović-Katić, S. 2008. The effect of application of different systems fertilization on the changes of chemical properties of soil and grain yield of spring small grains. Proceedings Ecological Truth 2008, UB-Technical faculty in Bor, Serbia, pp. 261-267.

Kourtev, P.S., Ehrenfeld, J. G. M., Häggblom, M. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology, 83: 3152-3166.

Krasiljnikov, N.A., 1965. Biologija otedeljnih grup aktinomicetov, Nauka, Moskva.

Lončar S., Đurović M., Trbić M., Malić N. 2009.Presjek dugoročnog plana rekultivacije površinskih kopova ugljenog basena Stanari. Zbornik radova VIII međunarodne konferencije "Nemetali 2009". Srbija – Vrujci, pp. 126–133.

Malić, N., Matko-Stamenković, U., Nožinić, M. 2012. Tehnogena zemljišta stanarskog ugljenog basena u funkciji ekološkog i poljoprivrednog resursa. Zbornik radova Međunarodnog kongresa ekologa "Ekološki spektar 2012" Republika Srpska – BiH, Banja Luka, Vol: 1, pp. 651–669.

Resulović, H. 1982. Neke specifičnosti procesa pedodinamike i pedogeneze u deposolima. Zemljište i biljka, 31: 357 - 363.

Sheoran, V, Sheoran, A.S., Poonia, P. 2010. Soil Reclamation of Abandoned Mine Land by Revegetation. International Journal of Soil, Sediment and Water, 3: 1-20.

Tabatabai, M. 1982. The role of soil enzymes in the degradation of Organic in the Tropics, Subtropics and Temperature zones. 12th International Congress of Soil Science, New Delhy.

Tintor, B., Milošević, N., Sekulić, P., Pucarević, M. 2008. Mıcrobıologıcal properties of soil in Pančevo inudstrial areas. Proceedings Ecological Truth, UB-Technical faculty in Bor, Serbia, pp. 297-301.

Tintor, B., Milošević, N., Vasin, J. 2009. Mikrobiološka svojstva černozema Južne Bačke u zavisnosti od načina korišćenja zemljišta. Zbornik radova Naučnog instituta za ratarstvo i povrtlarstvo, 46: 189-198.

Williams, S.T., Wellington, E.M.H. 1982. Principles and problems of selective isolation of microbes, in: J.D. Bullock, L.J. Nisbet, D.J. Winstanley (Eds.), Bioactive Microbial Products 1: Search and Discovery, Academic Press, UK pages 9-26.