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Radoje V. Pantovic
Zoran S. Marković

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MICROORGANISM ACTIVITY IN MINE SOILS OF BLACK LOCUST (*Robinia pseudoacacia* L.) STANDS ON COPPER MINE WASTES IN BOR, SERBIA

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ABSTRACT

Soil microorganisms represent one of the indicators of the ecosystem development and reclamation success in the revegetated mine waste areas. This research aims to evaluate microbiological activity in the surface layers under the black locust stands on copper mine wastes in Bor, and to determine contribution of the main physiological groups of microorganisms in organic matter turnover.

Key words: microorganisms, organic matter, mine soil, Bor.

INTRODUCTION

The development of plant life depends on the effective fertility of the soil, conditioned by the content of the organic matter and the activity of soil micro-fauna [8]. Soil microorganisms significantly contribute to the maintenance of the matter and energy flows in terrestrial environment by promoting organic matter turnover and nutrient cycling through their metabolic functioning [9]. Soil microorganism quantity and diversity is beneficial for plant growth in mine waste areas and can be used in evaluation of the primary ecosystem development or reclamation success in the mine waste areas. Speed and dynamics of soil processes depends on the amount and properties of organic matter produced by the plants, which represents the primary energetic material for soil microorganisms. Therefore, the quantity of microorganisms in the soil depends also on the content of the organic matter [7]. Decomposition of organic matter is a biological process, whose speed is largely determined by presence of soil microorganisms. Successive decomposition of dead material and modified organic matter results in the formation of humus [1].

Mine waste character and type influence the processes of mineralization and the formation of organic matter. When the biological processes start to take place in technogenic substrates, pedogenesis occures. Mineralogical composition of mine wastes causes differences in the number of soil microorganisms, respresenting the more

influential factor than the soil texture (Resulić, 1997). Plant-soil biota feedback accounted for more importance then abiotic factors in natural sucession at some post-industrial habitats [3].

The aim of this paper is to determine the microbiological activity of the mine waste piles and flotation tailings under the black locust stands in copper mine wastes in Bor, and therefore evaluate their effectiveness in organic matter decomposition and synthesis of humus.

MATERIALS AND METHODS

Site characteristic

Mine waste area in Bor ($44^0$04`25``N, $22^0$05`26``E, East Serbia) consists of two main waste types resulted from the copper ore exploitation and processing: flotation tailings and overburden piles (Figure 1), covering a total area of about 150 ha. Flotation tailings consist of milled rock left after the proces of ore separation. The mine waste piles mainly consist of andesite and dacite waste rocks displaced without industrial processing. Mine waste piles and flotation tailings were partially revegetated with alochtonous tree black locust (*Robinia pseudoacacia* L.) during the period 1982–1986., with limited success [4]. Flotation tailings were covered with topsoil layer of 30 cm prior to revegetation. Total of 8 revegetated fields (4 on mine waste piles and 4 on flotation tailings) under black locust were selected for the investigation (Figure 1).

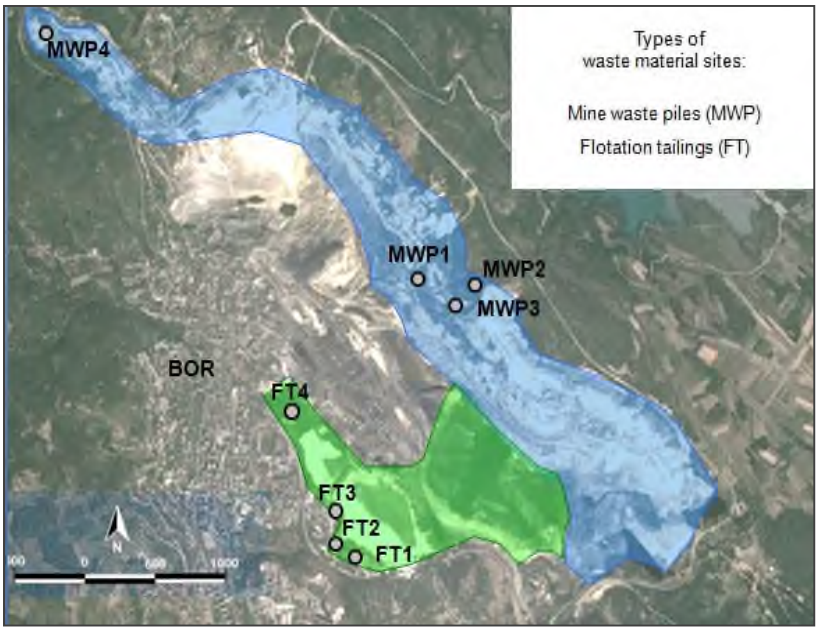

Figure 1. Mine waste sites revegetated with black locust in Bor copper mine waste area

Microorganism sampling and analysis

Mine soil from the surface horizon layer was taken with sterile spatula in spring and autumn season at all selected mine waste sites. The number of microorganisms was determined by the plate dillution method. Diluted soil suspension is introduced into adequate selective media for each physiological group. Following physiological groups of microorganisms were determined: the number of ammonifiers on meso-peptonic agar; the number of oligonitrofiles on Esbhi's agar; the number of actinomyces on synthetic agar; the number of fungi on Čapek's agar; the total number of microorganisms (total microbial content) on soil agar. Nutrient media were sown by soil suspension 0.1 ccm in 10^{-3} dilution. The media were sterilized in an autoclave at the temperature of 120ºC and transferred to petri dishes. The sowing was repeated three times and petri dishes were placed in a thermostat at the temperature of 22ºC. The determination of the total number of fungi, bacteria and actinomyces, developed on nutrient media, was performed after 7 and 14 days. The number of microorganisms was calculated as the average in thousand per 1g of absolutely dry soil.

Data analysis

Non-parametric *Spearman* rank *correlation* was used to test the association between ranked variables. Non-parametric Mann-Whitney U-test was used in order to compare means of physiological groups of microorganisms for spring and autumn seasons. All analysis were performed in Statistica 8.0. Statsoft software.

RESULTS

The average number of soil microorganisms in mine soils of black locust stands at flotation tailings and mine waste piles is presented in Figure 2.

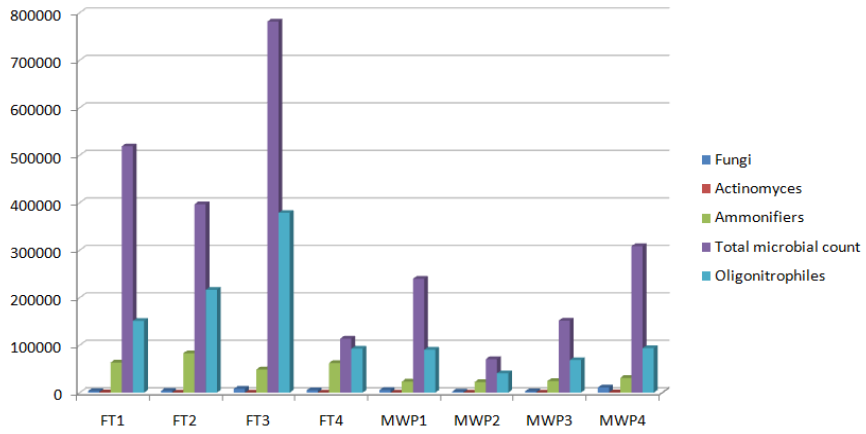

Figure 2. Average number of soil microorganism groups in black locust stands on flotation tailings (FT 1-4) and mine waste piles (MWP 1-4) in Bor

As visible in Figure 2, total content of microorganisms dominates at all investigated black locust sites, especially at flotation tailings sites (FT1-FT3). Number of oligonitrophiles follows the same trend. Average content of ammonifiers is also higher in flotation tailings sites (FT1-FT4). Lowest content or even total absence from surface soil layers of investigated sites is noticed for the physiological group of actinomyces.

Content of main physiological groups of microorganisms in spring and autumn season is reported in Table 1. Number of fungi, actinomyces, oligonitrophiles and total microbes on investigated sites varies among seasons, while number of ammonifiers show lower values for the autumn season.

Table 1. Number of microorganism physiological groups in spring and autumn season on Bor flotation tailings and mine waste piles revegetated with black locust

Group	Season	FT 1	FT 2	FT 3	FT 4	MW P 1	MW P 2	MW P 3	MW P 4
Fungi	spring	3774	1989	9163	5243	4712	2790	3747	8955
	autumn	3174	5475	6864	4746	6292	1616	1717	12554
Actinomyces	spring	1153	0	0	107	0	0	0	52.5
	autumn	113	0	0	116	0	0	0	1315
Ammonifiers	spring	79679	117267	72284	99503	29317	33265	41748	41106
	autumn	46929	48725	24854	25117	17129	11313	7189	21136
Oligonitrophiles	spring	45815	175900	671937	88804	71197	54727	78144	71893
	autumn	256181	257312	85214	96071	109529	26935	59015	115362
Total microbial count	spring	430893	299449	1262427	155140	68056	121257	229079	226381
	autumn	606447	494093	300615	72459	411317	19932	74037	390569

DISCUSSION

Mann-Whithey U-test showed statistically significant differences between the number of amonificators in spring and autumn season for the flotation tailings and mine waste piles sites ($Z=2.3$, $p=0.02$). The other groups of soil microorganisms didn`t exibit significant differences in their number between spring and autumn season at the investigated groups of sites.

On the investigated fields of Bor mine wastes (flotation tailings and mine waste pills) under black locus stands the total microbial content of soil was the most abundant in both seasons. Their higher content during the autumn season is a result of the increased presence of the end-products of decomposed organic matter, that consists of the mineral form of plant asimilatives released from soil organic layer during the year. Total microbial content of soil indicates nutritional value of the environment, but at the same time these microorganisms are the competitors to the vegetation as they use the same form of assimilatives as higher plants for their metabolic processes [2].

Total micobial content prevails over the number of ammonifiers in both seasons on all fields, indicating the decomposition of the majority of organic materials to their end-products. Plant asimilatives are therefore predominantly in the mineral form, and the synthesis of humus from the inter-products of organic matter decomposition is slow. Greater abundance of oligonitrophiles in relation to ammonifiers indicates that the synthesis of low molecular weight fraction of humic substances (fulvic acids) is ongoing, but according to a relatively high total number of microorganisms in relation to these two groups it can be concluded that the nature of the synthesized humic substances is not aggressive. The large number of ammonifiers on all investigated fields during the spring season is a result of fresh organic matter load, which presents a suitable material for these organisms. This is reflected in positive correlation (ρ=0.67) of their number with the content of organic matter at investigated sites. Content of ammonifiers in both seasons also positively correlates with total nitrogen content (ρ=0.8 in spring season, ρ=0.68 in autumn season).

Actinomyces actively tranform organic matter in later stages of it`s decomposition [10]. Small number or total absence of actinomyces in the investigated fields of Bor mine wastes indicates that either there is no decomposition of humic material to the end-products, or that the process is very slow. Fungi prevails over the actinomyces at all investigated sites, and have dominant role in decomposition of organic matter. Fungi exibit inverse correlation with mine soil pH (ρ=-0.64 in spring season, ρ=-0.82 in autumn season) and organic matter content (ρ=-0.62 in spring season, ρ=-0.67 in autumn season).

Contaminants of air and soil reduce the number and vitality of the soil fauna that decompose organic substances and provides its circulation in an ecosystem [11]. One of the effects of reduced microbial activity and slow decomposition of the plant residues is manifested by an increased depth of organic layer due to the accumulation of non-decomposed organic matter (Figure 3-5).

Figure 3. Soil profile on flotation tailing site FT 1

Figure 4. Soil profile on mine waste piles site MWP 3

Figure 5. Soil profile on mine waste piles site MWP 4

Research on the impact of gaseous pollutants and aerosols from the copper mine and metallurgical plant in Bor on the surrounding soil microorganisms [5] revealed that their effect is negative, and it increases with the proximity of the pollution sources. Thus, the number and diversity within the different groups of microorganisms in the soil decreased from locust stands outside of the pollution zone to the locations that are closer to the mining and smelting facilities.

The relatively small total content of soil microbes found in this investigation is a consequence of the direct and indirect effects of present pollution: the oxidation of the sulfur dioxide and the precipitation of atmospheric particulate matter of heavy metals on the surface of investigated mine soils, and contamination of the plant tissues which becomes a source of energy for the microorganisms after the decay.

CONCLUSION

The content of microorganisms in mine soils of black locust stands on copper mine wastes in Bor generally shows similar trends in spring and autumn season. Total micobial content prevails over the other groups of microorganisms, but their relatively small content reveals the negative effect of the pollution at the mine waste sites. While small number or absence of actinomyces results from a low humus content, increased number of ammonifiers on all investigated fields during the spring season results from fresh organic matter input.

Based on the the ratio of the number of different of soil microorganisms at the investigated sites following conclusions can be made: a) major part of the organic matter decomposes to the end-products, b) synthesis of humus out of the inter-products of organic matter decomposition is slow, c) synthesis of of fulvic acids occures, but their nature is not aggressive, d) further decomposition of the synthesized humic substances to the end products is either very slow, or was not identified. However, the overall picture of soil microflora on Bor mine soils clearly shows that the transformation process of organic matter under the black locust stands has been eastablished, wich significantly contributes to the development and ecological stability of revegetated sites.

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