# ANALYSIS OF MICROBIAL COMMUNITY ON MINE TAILINGS FROM THE NATIONAL PARK OF CALIMANI MOUNTAIN (EASTERN CARPATHIANS)

# STUDIU ASUPRA MICROFLOREI HALDELOR DE STERIL DIN PARCUL NAȚIONAL CĂLIMANI (CARPAȚII ORIENTALI)

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***Abstract:*** *The mining activities from Calimani Mountain for sulphur ore between 1965 and 1997 generate a large amount of waste rocks and tailings, which get deposited in four dumps (Pinu, Puturosu, Dumitrelu and Ilva) at the surface. The degraded soils, the waste dumps are often very unstable and become sources of pollution. The direct effects was the loss of forest or grazing land, and the overall loss of production from more than 200 ha during the mining exploitation. The indirect effects included air and water pollution of rivers. The aim of this paper is to evaluate the diversity of soil microbiota from targeted areas, namely Pinu, Puturosu, Dumitrelu and Ilva waste sterile deposits. At the microscopic scale, high concentration of sulphur may have negative effects on bacteria, which are the key-players of the nutrient cycles (nitrogen, carbon, sulphur) in soils of the investigated dumps. For an easy identification of different groups of micro organisms, different specifics media cultures were used. The inseminated dishes were incubated and analyzed respecting the protocol for each micro organism groups. Collecting, isolation and identifying activity of some groups of micro organisms allowed demonstrating their presence in the sterile depositions. For example, the highest number of aerobe nitrogen-fixing bacteria Azotobacter chroococcum was counted on Dumitrelu Dump at 0-6 cm depth (29 x $14^6$ UFC/g soil), while the lower have been evidenced in Puturosu Dump (66 x $10^3$ UFC/g soil) at the same depth. The presence in the mine tailings of micro organisms, which are involved in nutrient cycles, creating the premises for an ecological reconstruction for degraded soils resulted in mining activities (vegetation cover reconstruction, waste dump stabilization).*

***Rezumat:*** *Activitățile miniere de extragere a sulfului desfăşurate în Munții Călimani în perioada 1965-1997 au generat o cantitate uriaşă de deşeuri care au fost depozitate la suprafață sub forma a patru halde de steril (Pinu, Puturosu, Dumitrelu şi Ilva). Existența acestei exploatări pe un areal de peste 200 ha a produs pierderi directe şi indirecte. Pierderile directe sunt reprezentate de neutilizarea acestor suprafețe pentru obținerea de material lemnos şi furajer, iar cele indirecte includ poluarea aerului şi a râurilor. Cercetările efectuate au urmărit evaluarea diversității microbiologice din haldele de steril Pinu, Puturosu, Dumitrelu şi Ilva. Concentrațiile ridicate de sulf pot influența negativ activitățile vitale ale bacteriilor care participă la circuitul elementelor (azot, carbon, sulf) din haldele de steril analizate. Activitățile de colectare, izolare şi identificare microbiologică au demonstrat existența unor grupe de microorganisme în deponiile miniere. Spre exemplu, în deponiile miniere de pe Halda Dumitrelul la adâncimea de 0–6 cm s-a evidențiat un număr maxim de microorganisme fixatoare de azot aerobe (29 x $10^4$ UFC/g sol), în timp ce numărul minim a fost evidențiat în Halda Puturosul (66 x $10^3$ UFC/g sol). Prezența microorganismelor implicate in deponiile miniere oferă premize pentru reconstrucția ecologică a solurilor degradate rezultate în urma activităților miniere (stabilizarea haldelor de steril, refacerea covorului vegetal).*

***Key words*****:** *microbial community, mine tailings, Calimani Mountain*

***Cuvinte cheie***: *comunitate microbiană, haldă steril, Munţii Călimani*

## INTRODUCTION

The waste resulted from Calimani mining activities for sulphur ore in the Massif with the same name is a hazardous source of pollution in the environment. Excess of sulphur in waste dumps may have deleterious effects on soil micro flora and also is dangerous for plants. Strong soil acidification causes soil chemical degradation, heavy metals mobilization, secondary deficit of P and K and changes of soil biochemical processes for example soil respiration activities. The transformation of mine tailings into an agricultural or forestry soils (technogenic soils) simultaneously with the reducing or removing of pollutants is not possible without the presence of the nutrient cycle's micro flora. This process is complex, slowly and will increase soil fertility.

## MATERIAL AND METHODS

The samples necessary for microbiological analysis regarding the diversity of soil microbiota were harvested from targeted areas (Pinu, Puturosu, Dumitrelu and Ilva waste sterile deposits) in 2008. The samples were put in sterile bags for transportation to laboratory and used in the same day for analysis. The soil dilutions were prepared using the successive dilutions method, and the medium from the Petri dishes was contaminated using the inclusion method.

For an easy identification of different groups of micro organisms, different specifics media cultures were used. The inseminated dishes were incubated and analyzed respecting the protocol for each micro organism groups.

Laboratory analysis results of microbial community from mine tailings involved in carbon and sulphur cycle are showed only at qualitative level.

## RESULTS AND DISCUSSIONS

The laboratory analysis confirms in the collected samples from all four waste dumps (Pinu, Puturosu, Dumitrelu and Ilva) the presence of aerobic (*Azotobacter chroococcum*) and anaerobic (*Clostridium pasteurianum*) nitrogen-fixing bacteria. A close examination on the biological activity of nitrogen-fixing bacteria shows differences in soil profile. So, at 3 cm depth the number of aerobic nitrogen-fixing bacteria overcomes the number of anaerobic nitrogen-fixing bacteria, but at 8 cm depth the anaerobic nitrogen-fixing bacteria density is greater. These observations are valid for all waste dumps with one exception, Puturosu Dump, where at 3 cm depth the *Clostridium pasteurianum* colony density is greater that *Azotobacter chroococcum* (table 1, 2).

The collected and analyzed samples (figure 1) from 3 cm depth show the highest aerobic nitrogen-fixing bacteria density (294 x $10^3$ UFC/g soil) and the smallest number of anaerobic bacteria (66 x $10^3$ UFC/g soil) in case of Ilva Dump and vice versa at Puturosu Dump: 121 x $10^3$ UFC/g soil for *Clostridium pasteurianum* and 39 x $10^3$ UFC/g soil for *Azotobacter chroococcum*.

A distribution more uniform for aerobic (*Azotobacter chroococcum*) and anaerobic (*Clostridium pasteurianum*) nitrogen-fixing bacteria in all four analyzed waste dumps encountered in the samples collected at 8 cm depth (figure 2). Thus, the highest density of aerobic bacteria was counted in samples from Pinu and Dumitrelu Dumps (78 x $10^3$ UFC/g soil) followed by Puturosu Dump (72 x $10^3$ UFC/g soil) and with the lowest density was Ilva Dump (43 x $10^3$ UFC/g soil). The highest density of anaerobic bacteria is found on Dumitrelu Dump (208 x $10^3$ UFC/g soil) followed by Pinu Dump (110 x $10^3$ UFC/g soil), Puturosu Dump (86 x $10^3$ UFC/g soil) and Ilva Dump (78 x $10^3$ UFC/g soil).

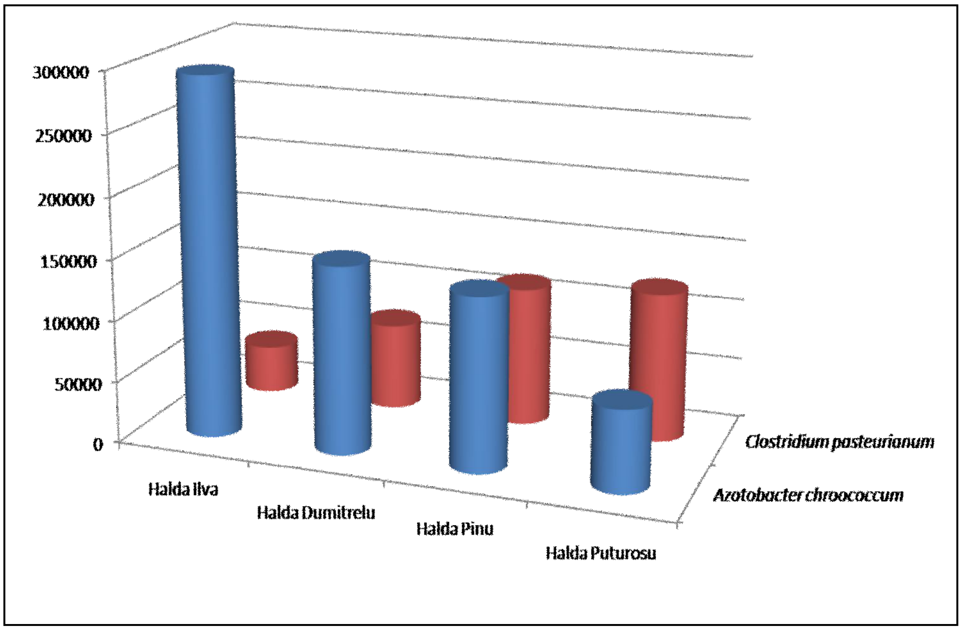



Figure 1. Number of aerobe and anaerobe nitrogen-fixing bacteria (UFC/g sol) at 3 cm depth

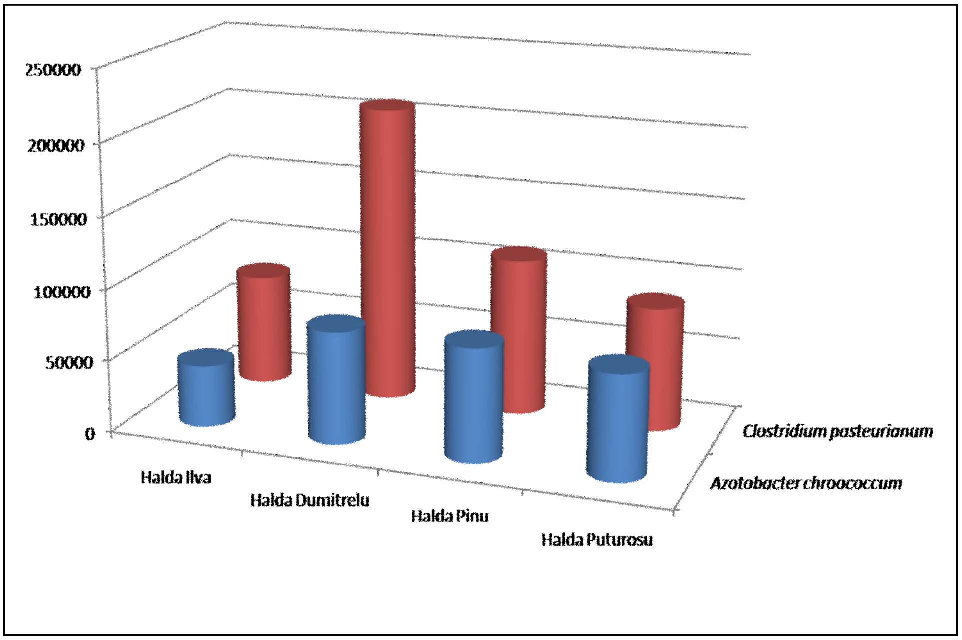
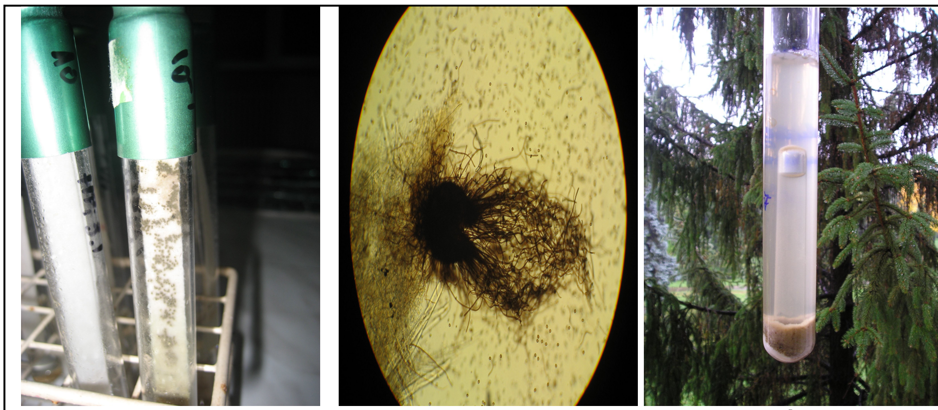



Figure 2. Number of aerobe and anaerobe nitrogen-fixing bacteria (UFC/g sol) at 8 cm depth

The microbiological activity, and especially bacteria activity, can be easy influenced by hostile factors at 3 cm depth. Because of these facts we consider that obtained results at 8 cm depth are more close to the real biological activity on mine tailings and especially on the nutrient cycles (nitrogen, carbon, sulphur) in soils.

The relatively high density of nitrogen-fixing bacteria indicates indirectly an acceptable supply of sterile tailings with carbon compounds and, in the same time, the presence of a microflora able to decompose complex substances on carbon-basis to glucose. This is demonstrated by showing the results from the process of aerobic and anaerobic (gas accumulation in Durham tubes) cellulose degradation (figure 3a, b). Cellulose degradation began relatively quickly (after 8 days) and this fact confirmed a high concentration of cellulolytic microflora in all four waste dumps.



a. b.

Figure 3. Aerobic (a. - *Chaetomium globosum*) and anaerobic (b.) cellulose degradation

In the collected samples from all four dumps (Pinu, Puturosu, Dumitrelu and Ilva) the presence of bacteria involved in sulphur cycle was also confirmed (figure 4).

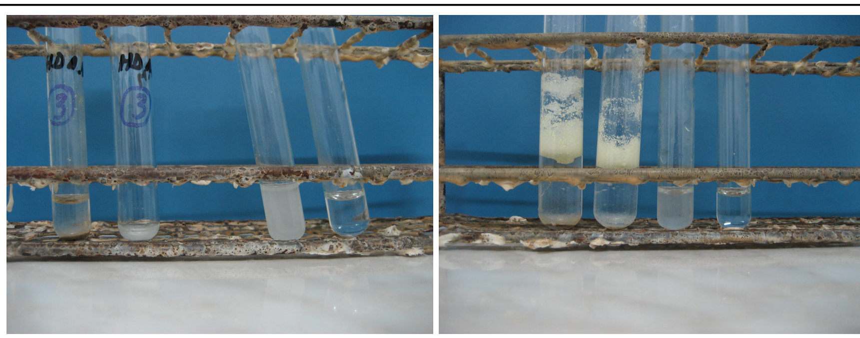


Figure 4. Indication of sulphide-oxidizing bacteria

The presence in the mine tailings of microorganisms, which are involved in nutrient cycles, creating the premises for an ecological reconstruction for degraded soils resulted in mining activities (vegetation cover reconstruction, waste dump stabilization).

## CONCLUSIONS

1. In all four waste dumps (Pinu, Puturosu, Dumitrelu and Ilva) the presence of nutrient cycles (nitrogen, carbon, sulphur) microflora, especially bacteria, was confirmed.

2. The highest density of aerobic nitrogen-fixing bacteria density (*Azotobacter chroococcum*) at 8 cm depth was counted on samples from Pinu and Dumitrelu Dumps (78 x $10^3$ UFC/g soil) and the lowest on samples from Ilva Dump (43 x $10^3$ UFC/g soil).

3. The highest amount of anaerobic nitrogen-fixing bacteria (*Clostridium pasteurianum*) at 8 cm depth was found on Dumitrelu Dump (208 x $10^3$ UFC/g soil) followed by Pinu Dump (110 x $10^3$ UFC/g soil), Puturosu Dump (86 x $10^3$ UFC/g soil) and Ilva Dump (78 x $10^3$ UFC/g soil).

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