

## MICRIBIOLOGICAL ACTIVITY IN A HIGHLY POLLUTED SOIL

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### Abstract

*To emphasize the microbiological activity in a polluted soil, the microbiological indicators (the number of bacteria; the number of fungi; and the soil respiration) had been analyzed, both from the qualitative and the quantitative point of view. The researches had been performed in Patranjeni area, in Apuseni Mountains on a slope of the Ampoi Valley. The soil is Alosol Rodic anthropic polluted (with dioxide and trioxide sulphur emissions). The results showed that total counts of bacteria showed very low ( $220 \times 10^6$  viable cells  $\times g^{-1}d.s.$ ) to medium ( $1270 \times 10^6$  viable cells  $\times g^{-1}d.s.$ ) viable cells in the plant root active layer, while the fungi total counts showed a very low number in all the pedogenetic horizons (ranging between  $2.40 \times 10^3$  cfus  $\times g^{-1}d.s.$  and  $3.70 \times 10^3$  cfus  $\times g^{-1}d.s.$ ). The global indicator of the soil microbiological activity is soil respiration, to which data pointed out low activity ( $64.20$  mg  $CO_2 \times g^{-1}d.s.$ ) in the soil profile, except the top horizon, where the activity is medium ( $11.3 - 11.4$  mg  $CO_2 \times g^{-1}d.s.$ ). The results of the taxonomic identification showed for bacteria the dominance of *Bacillus* and *Pseudomonas*, while for fungi, the dominance of *Penicillium*, *Aspergillus*, and *Fusarium*.*

**Key words:** *microbiology, microbiological indicators; micromorphology, soil pollution*

### INTRODUCTION

Microorganisms have been named „stewards of the biosphere“ (Jansson and Fredrickson, 2010; Tecon and Or, 2017).

Globally, soil microbes are the drivers of key biogeochemical cycles involving carbon, nitrogen, phosphorus, iron and more, their activity contributes to a wide variety of soil ecosystem functions, including the cycling of organic matter and nutrients, and the emergence of soil structure (Tecon and Or, 2017).

Some portray soil as an unfavorable habitat for microbial life due to its harsh and fluctuating environmental conditions, yet evidence suggests that

microorganisms thrive in soils (Stotzky, 1997, citrate by Tecon and Or, 2017).

Levi et al. (2021) showed that biological and physical indicators demonstrate the importance of the topsoil restoration method.

The micromorphological behaviour of the Alosol Rodic from Patranjeni is specific to the soils with high weathering of the skeleton grains under the influence of the high acidity.

The soils from Zlatna (as showed Manea et al., 2009; Manea et al., 2017) had a very high acidity (pH is 3.92 – 4.62) and a severe debazification (base saturation being 0.78 – 6.32 me/100 g soil) which is

associated with a high amount of Al (1.53 – 4.43 me/100 g soil) until the depth of 90 cm.

Therefore, the study objectives had been to investigate the microorganism activity in a highly polluted soil, as well as a very acid soil, which represented extreme habitat conditions for soil life.

## MATERIALS AND METHODS

The researches location was in Patranjeni area, in Apuseni Mountains (belonging to the Romanian Western Carpathians). The soil profile had been dug on the Ampoi Valley on a slope facing north and having  $\geq 25\%$ . The climate is temperate continental with an average annual temperature of  $8.0^{\circ}\text{C}$  and an average annual precipitation of 630 mm. The soil is Alosol Rodic (according to SRTS-2012; and Rhodic Alisol – according to WRB-SR-2014) formed in loamy slope deposits rich in sandstone gravels.

The profile had been dig in a young pine plantation poorly developed (50%) due to the anthropic pollution with dioxide and trioxide sulphur emissions brought by winds along the Ampoi Valley from the nearby industrial area.

Two types of sampled had been collected and analyzed according to RISSA Methodology-1987, from each pedogenetic horizon of the soil profile: disturbed samples (for the chemical analysis); and undisturbed samples for the micromorphological study.

Undisturbed soil sampled for the micromorphological research, had been impregnated with the epoxidic resins after air drayed and used to prepare thin sections (25 – 30  $\mu\text{m}$ ). Each thin section had been studied with Documator (20 X) and the optical microscope (50 - 500 X) in PPL (polarized light) and XPL (cross-

polarized light). The terminology used was from Bullock et al. (1985).

The microbiological samples had been analyzed as follows: the number of heterotrophic bacteria was determined using the method of dilutions – soil suspensions by dispersion on Topping nutrient medium. The results are expressed in  $\times 10^6$  viable cells  $\times \text{g}^{-1}$  dry soil. The total number of fungi was determined by dispersing soil dilutions-suspensions on the Czapek-Dox nutrient medium. The calculation takes into account the number of colonies that resulted from inoculation, dilution and soil moisture. The results are expressed in  $\times 10^3$  cfus  $\times \text{g}^{-1}$  dry soil. Soil respiration as a global indicator of soil microbial activity was determined by the Ștefanic method. The results are expressed in  $\text{mg CO}_2 \times \text{g}^{-1}$  dry soil.

The taxonomic identifications had been made on the basis of cultural, morphological and/or physiological characteristics, according to the manual of determinative bacteriology (Bergey, 1994) and fungi in agricultural soils (Domsch et Gams, 1972).

## RESULTS AND DISCUSSIONS

The general background of the studied soil is highly polluted (fig. 1) and strongly acidified (fig. 2 and 3) by the anthropic pollution.

The soils from Zlatna area, are polluted by heavy metals (Cu, Pb, Zn, Cd), under the influence of non-ferrous smelter of copper ores; the impact of atmospheric emissions from these metallurgical plants was detected at great distances from the emission point (Manea et al., 2009; Manea et al., 2017).

The main pollutants being (in decreasing order) Pb, Zn, Cu, and Ni,

The pollutants values far exceeded the alert threshold (fig. 1).

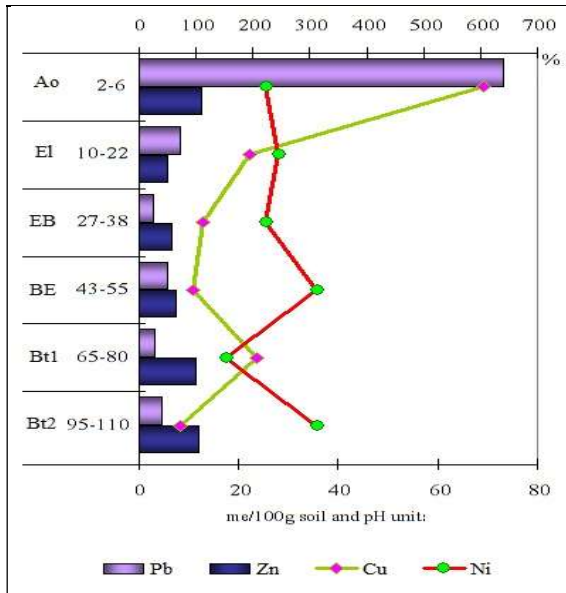


Figure 1. The pollutant with the highest values.

Despite the fact that from the chemical point of view, the studied soil is more developed, older and strongly acidic, the micromorphological image (fig. 2) emphasised a young soil, due to the continuous supply with weathering products: clay, Fe, etc. (Raducu, 2006; 2015).

Consequently, the extended areas in the matrix appear enriched with plasmic material composed of secondary clay impregnated with high amount of Fe, that give to the soil a reddish colour.

Even if many voids are filled with weathered products (fig. 2), the high amount of the skeleton grains (rock fragments of different sizes) makes the texture sandy loam.

From this image it follows that the soil environment is aerated and favourable to soil life development.

On this general soil background, there were created the premises for the

quantitative results of the microbiological analysis.

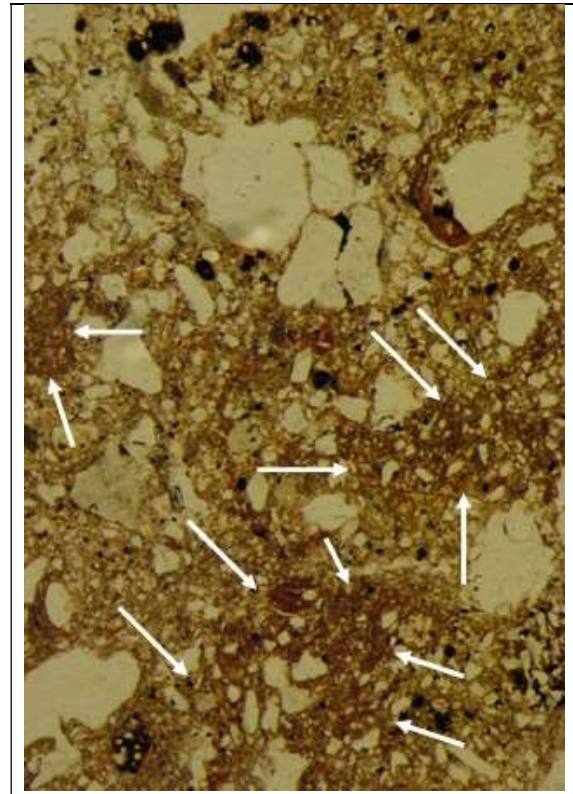


Figure 2. The extended areas in the matrix with weathering products.

In this respect, the results of the quantitative evaluation of the microbiological analysis showed very low ( $220 \times 10^6$  viable cells  $\times g^{-1}$ d.s.) total counts of **bacteria** (Figure 1) in the Ao horizon, which highly increased in the underlined pedogenetic horizons to medium ( $1270 \times 10^6$  viable cells  $\times g^{-1}$ d.s.) total counts into the EB and high in the deeper horizons (ranging between  $2570 \times 10^6$  viable cells  $\times g^{-1}$ d.s. to  $3470 \times 10^6$  viable cells  $\times g^{-1}$ d.s.).

The lower total count of bacteria had been registered in the top Ao horizon, while the highest total count had been reached into the EB horizon, where the weathering process is very active.

In what concerning the distribution of the viable cells into the soil profile, their total

count showed higher values in the deeper horizons (fig. 3).

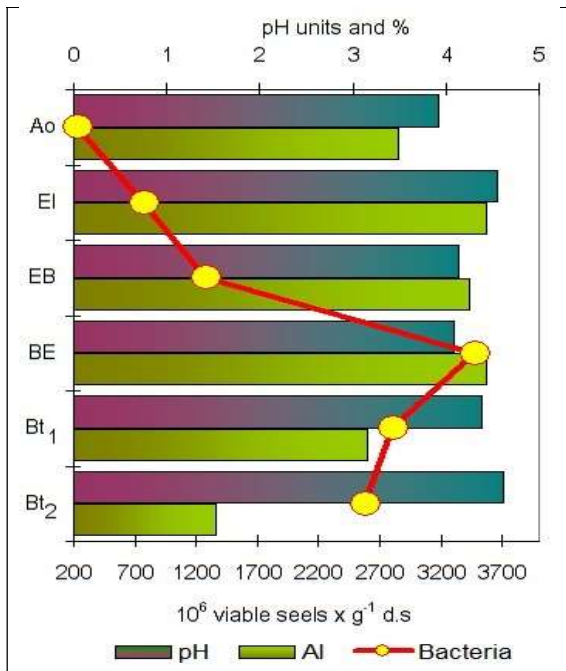


Figure 3. The total count of bacteria in relation to pH and AI content

It is important to underline that the total count of bacteria matched neither with pH, nor with the organic matter, which showed a relatively hostile environment for them.

The increased total count of bacteria with depth, where the horizons enriched in clay and others weathering products (as for example the different types of cations) pointed out a struggle of the microorganisms to find vital conditions to survive, even in this hostile highly polluted soil.

In what concerning the total count of **fungi** (fig. 4), the data showed a very low number in all the pedogenetic horizons, ranging between  $2.40 \times 10^3$  cfus  $\times$   $g^{-1}$  d.s. and  $3.70 \times 10^3$  cfus  $\times$   $g^{-1}$  d.s.

Their distribution into the soil profile is different comparing to the bacteria, the lowest number ( $2.4 \times 10^3$  cfus  $\times$   $g^{-1}$  d.s.) was registered into the EB horizon, while the highest value ( $3.7 \times 10^3$  cfus  $\times$   $g^{-1}$  d.s.) of the total count of fungi had been reached into the eluvial (El horizon).

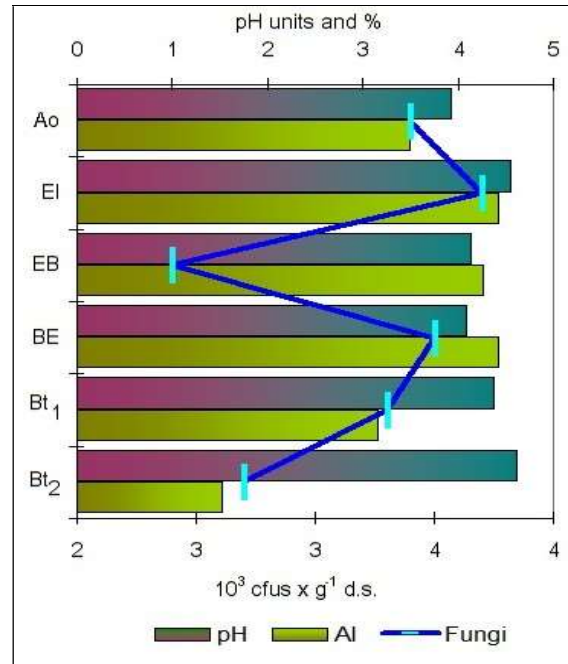


Figure 4. The total count of fungi in relation to pH and AI content

Even if the fungi are more tolerant to acidity, their number and genus decreased, as the bacteria, which are more sensitive to the low pH.

According to the *soil respiration* data, the soil microbiological activity is medium ( $64.20 \text{ mg CO}_2 \times g^{-1} \text{ d.s.}$ ) in the top horizon, the value attended the middle of the values range ( $30 - 80 \text{ mg CO}_2 \times g^{-1} \text{ d.s.}$ ). In the underlined horizons, the values drastically decreased to  $11.3 - 11.4 \text{ mg CO}_2 \times g^{-1} \text{ d.s.}$

In what concerning the qualitative results, the *taxonomic identification* of the microorganisms (fig. 5) reveal reduced biodiversity and respectively low level of development of the colonies (genera and species).

The taxonomic identification of *bacteria* (fig. 5) showed the dominance of the *Bacillus* and *Pseudomonas* groups.

The taxonomic identification of the microbial community of *fungi* (fig. 5) showed the dominance of the *Penicilium*, *Aspergillus* and *Fusarium* genus.

The microbiological activity in the soil drastically decreased as a consequence of the

severe reduction of the group and genus diversity.

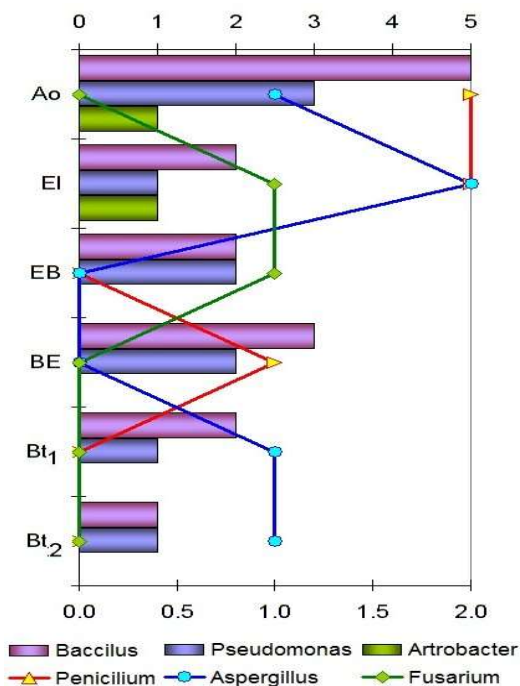


Figure 5. The taxonomic identification of microbial community of bacteria and fungi.

On the other hand, the bacteria tolerant to acidity developed together with the fungi, creating a modest habitat in the studied soil, either for themselves, or for the tree roots.

## CONCLUSIONS

To emphasize the microbiological activity in a highly polluted soil, the microbiological indicators (the number of bacteria; the number of fungi; and the soil respiration) had been investigated.

The qualitative results of the taxonomic identification of the microorganisms reveal reduced biodiversity and respectively low level of development of the colonies (genera and species).

The fungi, even if prove to be more tolerant to acidity, their number and genus decreased, as the bacteria, which are more sensitive to the low pH.

The microbiological activity in the soil drastically decreased as a consequence of the severe reduction of the group and genus diversity.

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