Physico-chemical and Bacteriological Characterization of the Soil Types from Various Altitudinal Vegetation Zones in Parâng Mountains

RAHELA CARPA1*, VASILE-DANIEL GHERMAN2, MIHAIL DRĂGAN-BULARDA1, MARILENA MOTOC3, ELENA ANA PAUNCU4

1 Babeș-Bolyai University, Faculty of Biology and Geology, Experimental Biology Department, 1 Kogălniceanu Str., 400084, Cluj-Napoca, Romania
2 University of Politechnic, Faculty of Hydrotechnic, 1 George Enescu Str., 300022, Timișoara, Romania.
3 „Victor Babes” University of Medicine and Pharmacy Timisoara, 2 Piața Eftimie Murgu, 300041, Timisoara, Romania

There were collected soil samples from various vegetation sites and altitudinal vegetation zones of the Parâng Mountains, from the South-Eastern part of the Hunedoara county, and they were analysed from the physico-chemical and bacteriological point of view. The chemical analyses consisted in the appreciation of the reaction of the soil (pH), in establishing the humus and total nitrogen content. According to these analyses, the soil is generally acid and presents normal nitrogen content. In order to establish the soil type and classes existing in the Parâng Mountain, the chemical analyses were completed with physical analyses of the soil texture sampled from various altitudinal zones, determining the following soil classes: the Umbrisol Class, the Spodosol Class, the Cambisol Class and the Protisol Class. The bacteriological analyses consisted in the study of the abundance, of the dynamics, diversity and ecological significance on the groups of bacteria involved in the biogeochemical cycles of nitrogen (aerobic mesophilic heterotrophs, ammonifiers, denitrifiers, nitrate bacteria and nitrite bacteria and aerobic, free, nitrogen fixing-bacteria from Azotobacter genus) from the mountainous soils. Based on the obtained results there was also calculated the bacterial indicator of the soils biological quality (BISQ) for each type of soil in each altitudinal vegetation zones.

Keywords: soil, physico-chemical analyses, bacteriological analyses

The physical-chemical and bacteriological characterization in soils of the mountainous ecosystems is a research method in evaluation of the functional diversity of the microbiota involved in the biogeochemical cycles [21]. Though that microbial communities in soil play a central role in the productivity and health of terrestrial ecosystems [8], and the biosphere as a whole, these communities remain largely unexplored. In addition microbial communities in soil have a measurable effect on atmospheric chemistry and global climate by influencing communities in soil have a measurable effect on global climate by influencing budgetes of gases such as CO₂, CH₄, H₂, N₂O and NO [7].

The physical-chemical and bacteriological characterization in soils of the mountainous ecosystems is a research method in evaluation of the functional diversity of the microbiota involved in the biogeochemical cycles [21]. Though that microbial communities in soil play a central role in the productivity and health of terrestrial ecosystems [8], and the biosphere as a whole, these communities remain largely unexplored. In addition microbial communities in soil have a measurable effect on atmospheric chemistry and global climate by influencing budgetes of gases such as CO₂, CH₄, H₂, N₂O and NO [7].

We chose to study the soil on different altitudinal vegetation zones as on the mountain the most recent soils are succeeding, which provides information upon the processes of soil generation and of the microbial activity within it.

Studies regarding the microbial activity of the mountain soil from Romania were previously performed in the Vladeasa Mountains (The Apuseni Mountains) [17]. In the Retezat National Park there were performed the most complex physico-chemical, ecological, pedological, enzymological, microbiological studies, but also synthesis studies [18].

In order to understand the operation and composition of the nitrogen-fixing microbiota existing within these types of soil it is necessary to perform the physico-chemical and microbiological analysis of the soil specific to each mountain zones.

We specify that physico-chemical and microbiological informations related to the soils in the Parâng Mountain are not mentioned in the specialty literature. This paper analysed for the first time the evolution of microbiota activity in montainous soils from Parâng and it is necessary because the microbiological analyses pursue the knowledge of the percentage of some bacterial ecophysiological groups involved in the biogeochemical cycles of the elements and the achievement of an overall image of the biological activity in the terrestrial ecosystems. They contribute to supplying the elements needed for the mineral nutrition of the superior plants and they also prevent the toxic elements polution.

For this study the soil samples were collected from 5 altitudinal vegetation zones in the Parâng Mountain (the alpine zone, the subalpine zone, the conifers zone, the beech zone and the Maleia flood plain). Located South-East of the Petroșani town, the Parâng Mountains form the eastern barrier of the Jiu Valley, with a large surface of 1100 km². These are the tallest mountains in the area, and the second tallest mountains in Romania after the Făgăraș Mountains in the East and the fifth highest peak (The Parângul Mare Peak) after the Peaks Moldoveanu, Negoiu, Vița Mare, Șălciu [20]. The Parângul Mare Peak (2519 m) offers spectacular landscapes of the valley and of the surrounding areas, and in the cloudless days with a clear atmosphere one may see even the Danube River, the Carpathians between the Danube and the Timoc, as well as the Balcans (Stara Planina).

Experimental part

Material and Methods: In the spring and summer of 2007, from five altitudinal vegetation zones of Parâng Massif (the alpine zone, the subalpine zone, the coniferous zone, the beech zone and from the Maleia flood plain) were collected 3 soil samples/altitudinal vegetation zones. The sampling coordinates were established by means of the GPS GEKO 201, and the sampling depth was maximum 20 cm. Thus, for the alpine zone the sample (1a, 1b, 1c) was collected from the Scurtu peak (from the altitude of 2216 m); for the subalpine zone the soil (2a, 2b, 2c) was sampled from the...
western slope of Parângul Mic, from the IEFS chalet and Parângul Mic peak (from 1871 m altitude); for the coniferous zone the sample (3a, 3b, 3c) was collected from the superior part of the chair-lift intermediary (from 1646 m altitude); for the beech zone the sampling (4a, 4b, 4c) was made in the area upstream from the Vâii Sasului stream (from 1286 m altitude), and the flood plain sample (5a, 5b, 5c) was collected from an alder flood plain on the Maleia river (from the 805 m altitude).

The samples were collected under aseptic conditions, by means of a sampling collector and with a sterile spatula and were contained in air-sealed bags. The sample were transported from the mountain in frigorific casket and subsequently kept in the refrigerator, at 4°C.

After 24-48 h these soil samples were analysed. The soil samples were conditioned in view of the physico-chemical analyses, by drying in well-ventilated rooms provided with heating systems up to a 40°C temperature, after which they were crushed up to a particle dimension smaller than 2 mm. As for these samples there was determined the humus and total nitrogen content there was made an additional sampling, by eliminating the vegetal roots residues and by fine crushing.

Initially there were established the pH and the oxide-reducing potential (Eh). The humus content was determined by the wet oxidation and titrimetrical dosage method (the Walkley-Black method, modified by Gogoaí). The total nitrogen content was determined by the Kjeldahl method which is based on the wet oxidation process (mineralization) of the nitrogen compounds in soil [13]. Then, it was determined the texture of the soil in the samples collected from various altitudinal vegetation zones, determining the soil type and class specific to each zone of vegetation.

There was established the number of bacteria belonging to the following ecophysiological groups: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers, nitrate bacteria, nitrite bacteria and nitrogen-fixing bacteria. All operations connected to the bacteriological determinations were carried under sterile conditions, there was also established the oxidizing potential (Eh). The humus content was determined by the wet oxidation and titrimetrical dosage method (the Walkley-Black method, modified by Gogoaí) after the removal of the vegetal roots residues and by fine crushing.

The samples were conditioned in view of the physico-chemical analyses, by drying in well-ventilated rooms provided with heating systems up to a 40°C temperature, after which they were crushed up to a particle dimension smaller than 2 mm. As for these samples there was determined the humus and total nitrogen content there was made an additional sampling, by eliminating the vegetal roots residues and by fine crushing.

Initially there were established the pH and the oxide-reducing potential (Eh). The humus content was determined by the wet oxidation and titrimetrical dosage method (the Walkley-Black method, modified by Gogoaí). The total nitrogen content was determined by the Kjeldahl method which is based on the wet oxidation process (mineralization) of the nitrogen compounds in soil [13]. Then, it was determined the texture of the soil in the samples collected from various altitudinal vegetation zones, determining the soil type and class specific to each zone of vegetation.

There was established the number of bacteria belonging to the following ecophysiological groups: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers, nitrate bacteria, nitrite bacteria and nitrogen-fixing bacteria. All operations connected to the bacteriological determinations were carried out under sterile conditions. There was also established the content of the soil in dry substance, by the drying of some samples at 105°C, for three days.

The number of the aerobic mesophilic heterotrophs was determined on a plates bullion agarized medium [11]. After incubation the number of colonies in each Petri dish was read, the reverse value of the respective dilution. For all the studied soil samples, the bacterial indicators of the soil quality (BISQ) were calculated [16].

The aerobic, free, nitrogen-fixing bacteria from the Azotobacter genus were isolated on Ashby agarized selective medium [3]. Electronical microscopy (T.E.M.) observation. have also been done. The samples for the electronical microscopy have been done by the standard method [15].

Results and discussions

In order to chemically characterize these soil samples, there was determined first the pH and the Eh (oxide-reducing potential) in water slurry in ration soil/solution of 1:1 by means of the Multi parameter 340i/SET. According to the criteria established by the National Research Institute for Pedology, Agrochemistry and Environmental Protection (INCDPAPM) [4] the reaction of the soil (pH) was evaluated and the results are presented. The oxide-reducing processes have a significant influence upon the solidification processes and upon the fertility of the soil. The change of the organic substances is connected to these processes, the bioaccumulation rhythm and the composition of the organic substances (especially that of the humus).

According to the contribution of the three granulometric fractions (loam, dust and sand) to the composition of the soil, each pedogenetic horizon may be classified in different textural groups, classes and subclasses [12]. Based on the analyses performed in the Pedology Laboratory of the Agency of Pedological and Agrochemical Studies (OSPA) Cluj-Napoca with the analyse code SC II 22, it was established the texture and type of soil coming from the five altitudinal vegetation zones of the Parâng Mountains (table 1).

The humus content is considered the most important component of each soil and the most quantitative index of the potential and effective fertility. The nutritive and hydric regime, the agrochemical and physical properties of the soils depend on the quantity and composition of the organic substance. The largest humus content in the soil is found in the upper shallow layers of the soil. This is related to the fact that in the upper layer there are vegetal residues, nutritive elements and a certain amount of organic matter, of which the humic substances were partially formed.

The humus content in the soil samples from the altitudinal vegetation zones of Parâng Massif was determined by the Walkley-Black method, modified by Gogoaí (wet oxidation and titrimetrical dosage method).

Table 1

<table>
<thead>
<tr>
<th>Soil sample</th>
<th>pH</th>
<th>Eh (mV)</th>
<th>Humus</th>
<th>Total N</th>
<th>Texture</th>
<th>Soil type</th>
<th>Soil class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpine</td>
<td>4.4</td>
<td>146</td>
<td>32.96</td>
<td>1,058</td>
<td>Medium coarse, loamy sand</td>
<td>Humosiosol (HS) (humic-silicate)</td>
<td>Umbriol Class (UMB)</td>
</tr>
<tr>
<td>Subalpine</td>
<td>4.1</td>
<td>160</td>
<td>24,36</td>
<td>0.529</td>
<td>Coarse, sandy-loam</td>
<td>Cryptopodzolic (CP)</td>
<td>Spodisol Class (SPO)</td>
</tr>
<tr>
<td>Conifers</td>
<td>3.7</td>
<td>181</td>
<td>28,50</td>
<td>1,020</td>
<td>Middle, sandy-loam</td>
<td>Podzolic (PD)</td>
<td>Spodisol Class (SPO)</td>
</tr>
<tr>
<td>Beech</td>
<td>5.3</td>
<td>110</td>
<td>4.93</td>
<td>0.270</td>
<td>Middle, medium loam</td>
<td>Eutricambosol (EC)</td>
<td>Cambisol Class (CAM)</td>
</tr>
<tr>
<td>Flood plain</td>
<td>6.2</td>
<td>75</td>
<td>4.18</td>
<td>0.133</td>
<td>Coarse fine sandy-loam</td>
<td>Pelic Fluvisol (AS)</td>
<td>Protisol Class (PRO)</td>
</tr>
</tbody>
</table>
The largest amount of humus was found in the sample collected from the alpine area, with a percentage of 32.96. According to the data in the specialty literature [4, 12], this percentage corresponds to the coarse sandy-loam type of the textural class, the soil in the alpine zone is classified as the Humosiosol type, the Umbrisol class. In the sample form the subalpine zone, there was found 24.36 humus which corresponds to the coarse loam-sandy category, this soil is classified in the type of Cryptopodzolic soil, Spodosol class. In this class there is also classified the soil in the coniferous zone, but in the Podzolic type. A low percentage of humus was found in the soil from the sample in the beech zone and from the flood plain, belonging to the Eutricambosol type (Cambisol class) and respectively the Fluvisol type (Protisol class) [12].

In order to establish the level of nitrogen supply it was used the direct method, Kjeldahl method. This method for the determination of the total nitrogen content is based on the procedure of wet oxidation (mineralization) of the nitrogen compounds in soil [11]. The principle of the method is the following: by boiling the soil with concentrated H2SO4 in the presence of a catalyst, the nitrogen in the organic substances is transformed in ammonia sulphite. The latter, treated with an excess of NaOH or KOH, frees the nitrogen as NH3, which then binds in a H2SO4 solution, with a known concentration. The sulphuric acid left unbound with the ammonia is titrated with a NaOH solution of known concentration [6]. By the difference, the ammonia amount was determined and thus the nitrogen content of the analysed soil was calculated. The data were interpreted according to the following periods: very small < 0.100; small with values ranging between 0.100 and 0.140; medium with values between 0.141 and 0.270; large with values between 0.271 and 0.600; very large > 0.600 [14].

The bacteriological analyses consisted in determination of bacteria belonging to the following ecophysiological groups taken in study in the spring of 2007 (table 2) and in the summer of the same year (table 3).

From the mountainous soil samples there were isolated Azotobacter strains on Ashby selective medium. The Azotobacter species fixe the molecular nitrogen to the organic N which latter will be amonified. Due to the vegetal cover from the conifers vegetation zone on the soil samples from there the pH is highly acid (3.7). Normally Azotobacter can not grow at such pH, because these species have the optimal interval between 4.5 and 7.5. Initially in the soil samples from this zone there were not isolated Azotobacter strains. After the pH of the culture medium was ajusted to 3.7 Azotobacter strains were obtained (fig. 1a). At electronic microscopy (T.E.M.) the specific cyst with poly-β-hydroxybutyrates (PHB) granules were evidenced, produced by Azotobacter species (fig. 1b).

---

**Table 2**

THE RESULTS OF THE BACTERIOLICAL ANALYSES OF THE SPREING SOIL SAMPLES

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Number of the bacteria/ g soil of dry substance</th>
<th>BISQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic mesophilic heterotrophs</td>
<td>Amonifiers</td>
</tr>
<tr>
<td>Alpine</td>
<td>25184772</td>
<td>45287</td>
</tr>
<tr>
<td>Subalpine</td>
<td>18337853</td>
<td>40520</td>
</tr>
<tr>
<td>Conifers</td>
<td>7339027</td>
<td>1350</td>
</tr>
<tr>
<td>Beech</td>
<td>17619239</td>
<td>42675</td>
</tr>
<tr>
<td>Flood-plain</td>
<td>30162846</td>
<td>123164</td>
</tr>
</tbody>
</table>

**Table 3**

THE RESULTS OF THE BACTERIOLICAL ANALYSES OF THE SUMMER SOIL SAMPLES

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Number of the bacteria/ g soil of dry substance</th>
<th>BISQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic mesophilic heterotrophs</td>
<td>Amonifiers</td>
</tr>
<tr>
<td>Alpine</td>
<td>91721852</td>
<td>145332</td>
</tr>
<tr>
<td>Subalpine</td>
<td>55395192</td>
<td>85513</td>
</tr>
<tr>
<td>Conifers</td>
<td>9263191</td>
<td>10531</td>
</tr>
<tr>
<td>Beech</td>
<td>52536193</td>
<td>109171</td>
</tr>
<tr>
<td>Flood-plain</td>
<td>73287822</td>
<td>131034</td>
</tr>
</tbody>
</table>

---

Fig. 1.a. Azotobacter chroococcum colony isolated from the mountainous soil on Ashby medium; b. Azotobacter chroococcum cyst with PHB granules
There is a very large number of the aerobic mesophilic heterotrophs of order $10^6 - 10^7$ cells/g soil of dry substance, in the soil of the alpine altitudinal vegetation zone and in the samples of the Maleia flood plain in the spring (fig. 2), and of order $10^7 - 10^8$ cells/g soil of dry substance in the summer, in the same altitudinal zones (fig. 3). The number of bacteria belonging to the other ecophysiological groups involved in nitrogen cycle is much smaller.

The presence of the amonifiers which do the reduction of the organic compounds with nitrogen to NH$_3$, was of order $10^5 - 10^6$ cells/g soil of dry substance. Latter ammonia is subjected to nitrification processes which are realised in two stages: in the first one ammonia is oxidated to nitrite (nitrit bacteria) and in the second stage the nitrite to nitrate (nitrate bacteria).

During the two seasons in all the montainous soil samples nitrite bacteria were the least represented ($10^2$ cells/g soil of dry substance). The reactions through which nitrite bacteria (= nitrosifyers) oxidate ammonia to nitrite are:

\[
\text{NH}_4\text{OH} + \frac{1}{2}\text{O}_2 \rightarrow \text{NH}_2\text{OH (hydroxylamin) + H}_2\text{O + E} \quad [9]
\]

\[
\text{NH}_2\text{OH} + \text{O}_2 \rightarrow \text{HNO}_2 + \text{H}_2\text{O + E}
\]

Nitrate bacteria (= nitrite-oxidizing bacteria, nitrifying bacteria) which oxidate nitrite to nitrate, were present during the seasons studied in high numbers of order $10^3 - 10^4$ cells/g soil of dry substance. The oxidation reaction is catalysed by nitrite-oxidoreductase:

\[
\text{HNO}_2 + \frac{1}{2}\text{O}_2 \rightarrow \text{HNO}_3 + \text{E} \quad [9]
\]

Denitrifiers which realise the reduction of nitrates to nitrites, NO, N$_2$O or N$_2$, were present in high numbers ($10^4 - 10^5$ cells/g soil dry substance) in all the analysed samples:

\[
2\text{H}_2 + 2\text{HNO}_3 \rightarrow \text{N}_2 + 6\text{H}_2\text{O + E}
\]

The stages of reaction are:

\[
\text{NO}^-_3 \text{ (nitrate)} \rightarrow \text{NO}^-_2 \text{ (nitrite)} \rightarrow \text{NO} \text{ (nitric oxide)} \rightarrow \text{N}_2 \text{ (nitrogen)}
\]

Similar to the aerobic mesophilic heterotrophs in the samples from alpin and flood plain soils the number of ammonifiers, denitrifiers, nitrate and nitrite bacteria is much bigger than the number registered in the other three soils (Tables 2 and 3).

Based on the data obtained from the microbiological analyses, the bacterial indicators of the soil quality (BISQ) were calculated, according to formula [16]:

\[
\text{BISQ} = \frac{1}{n} \sum \log_{10} N
\]

where:

- BISQ = the bacterial indicator of the soil quality;
- $n$ = the number of the physiological groups considered within the calculation;
- $N$ = the number of the bacteria belonging to each ecophysiological group.

As we can notice in figure 2, the microbial potential of soils, defined by the values of the bacterial indicators of soil quality is different. Thus, the soil in the alpine altitudinal vegetation zone and in the flood plain from spring and summer, has the highest bacterial potential and the soil in the conifers altitudinal vegetation zone has the lowest potential. The differences are not very high. Only the soil in the confiers zone has lower value of the BISQ both in spring and summer (tables 2 and 3).

In the summer samples the bacterial potential is generally higher compared to the spring samples, the greatest value was on the alpine soil and the lowest potential on the conifers soil (fig.3).

The bacterial indicators of the analysed habitats quality offers an overall image on the intensity of the microbial activity and the general biological activity in the analysed soils. Based on the results and in comparison with the data in the specialty literature [17, 18], we may consider that the analysed soils have an appreciable biological potential; only the soil in the conifers altitudinal vegetation zone have lower values of the two quality indicators, bacterial and enzymatic, which set the basis for this appreciation (IBCS=3.964 in the spring and IBCS=4.376 in the summer).

**Conclusions**

Physico-chemical analyses were done on the soil samples from each altitudinal vegetation zones of Parâng Massif and the types and classes of analysed soil were established.

All those five types of soil analysed are fit into the acid reaction class, but the soil in the coniferous zone presents a highly acid character ($\text{pH} = 3.7$), while the soil in the water river flood plain belongs to the weakly acid soils class ($\text{pH}=6.2$).
The values of Eh (of the oxide-reducing potential) are closely connected to the pH values, as the formers decrease with the increase of the pH values.

The values of the total nitrogen in the analysed soil are classified as normal nitrogen values, these are very high at the samples in the alpine, subalpine and coniferous zone, and for the soil in the beech zone and in the Maleia flood plain soil, the values were several times lower.

The humus quantity in the five types of soil analysed varies very much both from the qualitative and quantitative point of view; very high values were recorded in the alpine, subalpine and coniferous zone soil as compared to the other samples from the beech zone and Maleia flood plain.

The presence of all the five studied bacterial ecophysiological groups studied was noticed in all the soil samples. Their number decreases in the following order: aerobic mesophilic heterotrophs (10^7 - 10^8 cells/g soil of dry substance) > ammonifiers (10^5 – 10^6 cells/g soil of dry substance) > denitrifiers (10^4 – 10^5 cells/g soil of dry substance) > nitrate bacteria (10^2 - 10^3 cells/g soil of dry substance).

The microbial potential of soils, defined by the values of the bacterial indicators of soil quality decreases in the following order: flood plain soil > soil from the alpine altitudinal vegetation zone > soil from the subalpine altitudinal vegetation zone > soil from the beech zone > soil from the conifers altitudinal vegetation zone. The differences among them are not significant. Only the soil in the conifer forests has a value of the bacterial indicator of quality smaller than four (IBCS=3.964 in the spring and IBCS=4.376 in the summer).

Poly-β-hydroxybutyrates (PHB) producing Azotobacter strains were isolated from montainous soils samples even from the conifers vegetation zones where the soil pH is 3.7.

References
2. ALLEN, O.N. Experiments in Soil Bacteriology, Third Ed., 1957, p.31, Burgess, Minneapolis
5. BORLAN, Z., HERA, C. Method de apreciere a starii de fertilitate a solului in vederea folosirii raționale a îngrășămintelor, Ed. Ceres, 1973, București
6. BORLAN, Z., RĂUA, C., (Red. coord), Metodologie de analiză agrochimică a solurilor în vederea stabilirii necesarului de amendamente și îngrășăminte, 1981, Seria Metode, Rapoarte Indrumări; ICPA nr. 3
11. DRĂGAN-BULARDA, M. Microbiologie Generală, Lucrări Practice, Univ. Babeș-Bolyai, 2000, Cluj-Napoca

Manuscript received: 20.12.2007