PHYSICO-CHEMICAL PARAMETERS AND ENZYMATIC ACTIVITIES ON TILLED SOILS FROM JIBOU AREA, ROMANIA

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Abstract

Different soil samples from five tilled areas of Jibou (Salaj County, Romania) have been physico-chemically and enzymologically studied. Several physico-chemical parameters of tilled soil from these sulphurous areas were determined: pH, conductivity, humus and different mineral ion contents. Also, in the soil samples, the following enzymatic activities have been quantitatively determined: actual and potential dehydrogenase, phosphatase and catalase activities. In all soil samples all enzymatic activities have a uniform behavior, without big differences between the five sampling zones. Also, the physico-chemical parameters were very uniform, as compared to the other sampling sites. The enzymatic potential was appreciate by the enzymatic indicator of soil quality (EISQ), calculated on the base of these enzymatic activities. The EISQ values were good, between EISQ = 0.574 and 0.635. The granulometric analysis of the five soil samples classified them as loamy-clayey soils.

Key words: tilled soil, enzymatic activity, Enzymatic Indicators of Soil Quality, Jibou.

INTRODUCTION

Most of the processes which are essential to maintaining the terrestrial ecosystems occur at soil level (Roger-Estrade et al., 2010). The soil represents the internal lab of the ecosystems, the deposit of elements needed for their optimal functioning. The organic matter of soil accomplishes more functions, such as: contributing to the structure and porosity of soil, adjustment of cations absorption and water retention, solubility control, mobility and availability of macro and micro-elements, carbon source for microorganisms, nutrients supply for vascular plants and many other (Bieganowski et al., 2013; Paul, 2014; Bojko & Kabala, 2017). It maintains a high biodiversity, by its physico-chemical properties provides important functions and services to the ecosystems, as decomposition, nutrients cycle, sustainability of productivity, as well as resistance and flexibility to abiotic disturbances (Brussaard et al., 2007).

Microbial communities at soil level are the most susceptible indicators of the disturbances and the changes in land use (Garcia-Orenes et al., 2013). The soil quality represents a more and more essential assessment tool, important by quantitatively describing the structure of the microbial communities, along with the enzyme activities (Zelles, 1999; Zornoza et al., 2009). Due to the existence of a functionality bond between the environment and soil, elements regarding the influence of the microorganisms in soil, as microbial activity, were proposed as indicators for assessing the soil response to different management practices (Garcia-Orenes et al., 2013). It is well known that the biological activities in soil (microbial and enzyme) are largely correlated with the physico-chemical properties of soil and participate at the metabolism of organic matter, thus deriving the availability of nutrients in soil, as well as accumulation and mineralization (Zhang et al., 2018).

The soil use practices of the human activities can intervene by changing the physico-chemical and biological properties of soil and can also influence the functions of soil (Jangid et al., 2008). For instance, the sources of nutrients in the ecosystems can modify the ecological processes and can influence the global changes (Oro et al., 2013). These sources can modify the characteristics of soil and influence the biological, chemical and physical processes occurring at this level.
Yuan and Yue (2012) showed that the enzyme activities of soil are correlated with the microbial biomass and the microbial activity, because these catalyze the biochemical reactions and the cycling of elements in soil. For instance, the urease activity from soil is closely connected to the soil mineralization with nitrogen (N) and the nitrogen cycle, while the enzyme activity of soil phosphatase is involved in the metabolism and transformation of phosphorus (P) (Adamczyk et al., 2014). Different factors as humidity, light and temperature stimulate microbial and enzyme activities in soil, resulting in an increase of nutrient content in soil. Previous studies also showed that the relation between C: N: P stoichiometry and enzyme activities of different functions responsible for mineralization and C, N and P equilibrium are very close (Sinsabaugh et al., 2009; Xu et al., 2017). In conclusion, this interaction between the physico-chemical properties of soil and their biological characteristics can be an important key for revealing the processes and mechanisms of planting ecosystems restoration (Adamczyk et al., 2014). Thus, by understanding the different physico-chemical properties and the biological activities of soil while restoring it, the essential variations of the status of nutrients in soil could provide a proper environmental result (Zhang et al., 2018). However, the relation between the microbiological properties, the enzyme activities and the nutrients content of soil is not fully understood yet.

The present study consisted in correlating some physico-chemical parameters with some enzyme activities of agricultural soils, from Jibou area, where multiple sulphurous springs are present.

**MATERIALS AND METHODS**

**Soil samples.** Five soil samples were collected in autumn of 2018 from Jibou area. The collection sites were: sample 1: N 47°15’04 and E 23°14’16; sample 2: N 47°15’06 and E 23°14’15; sample 3: N 47°15’07 and E 23°14’14; sample 4: N 47°15’08 and E 23°14’14 sample 5: N 47°15’09 and E 23°14’14. The soil samples were passed in a portable storage box and transported on ice into the laboratory for different analyses. The soil samples used for measuring enzymatic activity and physico-chemical properties were air-dried and then temporarily stored at 4°C, for further analyses.

**Physical and chemical properties of soils.** Soil samples were air-dried, ground and sieved (2 mm) prior to determination of available nutrients and soil characteristics. The pH and electrical conductivity (EC) of the soils were potentiometrically measured in aqueous fraction (1: 5) (ISO 10390, 2005). The humus quantity in the soil samples was determined by Walkley-Black method, while total nitrogen by Kjeldahl method. The availability of nutrients in soil was also tested. Mobile phosphorus was determined by colorimetric method (with ammonium molybdate) and with Metertech 830 Plus spectrophotometer, while mobile potassium was calculated by flame photometer method, by extraction with ammonium lactate-acetate using Sherwood flame photometer. Carbonates were determined by Scheibler method with Scheibler calcimeter. SO₄ anion was determined by precipitation with BaCl₂ and titration with sodium thiosulfate from aqueous extract aqueous extract. At all the soil samples a granulometric analysis by Kacinski method was done. All these analyses were done in the lab of the Office of Pedology and Agrochemical Studies from Cluj and in the pedology lab of the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca.

**Enzymatic activities in soil samples.** Activities of the following four enzymes in soil were measured in triplicate: phosphatase, catalase, actual and potential dehydrogenase (Alef & Nannipieri, 1995; Carpa et al., 2014). These analyses were done in the microbiology lab of Babes-Bolyai University Cluj-Napoca. Dehydrogenase activity (actual and potential) was determined after 24 h incubation of the soil samples at 37°C, with TTC solution, and expressed by the amount of the formed 2, 3, 5-triphenylformazan (mg formazan/g soil). Enzymatic activity of dehydrogenases was determined at λ=485 nm by Able Jasco V530 spectrophotometer. Phosphatase activity was determined after 24 h incubation of the soil samples at 37°C, with phenyl phosphate disodium solution, and it is
expressed in mg phenol/g soil. Phosphatase activity was determined at $\lambda = 600$ nm by using an Able Jasco V530 spectrophotometer. Catalase activity was determined after 1 h incubation of the soil samples at room temperature. The residual $H_2O_2$ is determined by titration with $KMnO_4$ in the presence of $H_2SO_4$. Catalase activity was expressed in mg splitting $H_2O_2$/g soil.

The analytical data serves as the base for calculating the enzymatic indicator of soil quality (EISQ) (Muntean et al., 1996).

RESULTS AND DISCUSSIONS

The earth of each soil type is made of clay, dust and sand. Dependent on the share of the three granulometric fractions in the soil formation, each pedogenetic horizon can be classified in different texture groups, classes and subclasses. (Blaga et al., 2005). Because sampling was done at 5-15 cm depth, all the soil samples belong to the pedogenetic horizon A. The texture (granulometric composition) of these soil samples was determined in the pedology lab of the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca, by pipette method and was concluded that all the samples have loamy-clayey texture.

According to the criteria set by the National Institute of Research-Development for Pedology, Agrochemistry and Environmental Protection (Blaga et al., 2005), it was valued that the reaction of the analyzed soils (pH) is weak alkaline (Table 1).

Assessing the soil pH is important not only for the pedology studies but also for the microbiology and enzymology ones, because the pH influences the physiological groups of bacteria, which reach an optimum at specific pH values.

The humus content in the soil samples from Jibou zone was assessed by wet oxidation and by titration method (Walkley-Black) in the lab of the Office of Pedology and Agrochemical Studies from Cluj-Napoca.

According to the methodology for pedological studies (Florea et al., 1987), the data obtained show a low humus content, no higher than 1.7% (Table 1).

The nitrogen uptake level was also assessed in the lab of the Office of Pedology and Agrochemical Studies, by direct Kjeldahl method and the obtained data were interpreted according to the next intervals: very low <0.100; low between 0.100-0.140; medium between 0.141-0.270; big between 0.271-0.600; very big >0.600 (Paulette, 2007).

We can say that the nitrogen uptake is very small in all the soil samples analyzed. Also, the nutrients from the tested soil, as phosphorus and potassium, did not surpass the value of 129 ppm and 612 ppm, respectively, and the carbonate level reach a maximum of 6.72, in sample 5.

The soil can be viewed as a biological entity in which a complex of biochemical reactions occur.

The action of microorganisms on the substrates from the environment is carried out in an enzymatic way, thereby assessing the enzyme activities offers suggestive data and in a shorter time than the microbiological analyses, regarding the soil processes.

The enzyme activities were quantitatively determined: phosphatase activity, actual and potential dehydrogenases activities and catalase activity (Table 2).

The enzyme activities were assessed using UV-VIS Jasco-V530 spectrophotometer.

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### Table 1. Physico-chemical parameters in tilled soils from Jibou area

<table>
<thead>
<tr>
<th>Soil samples</th>
<th>pH</th>
<th>Electrical Conductivity (mS)</th>
<th>Humus (%)</th>
<th>$SO_4$ mg/mc/100 g soil</th>
<th>$N_2$ (%)</th>
<th>P (ppm)</th>
<th>K (ppm)</th>
<th>Carbonates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.02</td>
<td>0.24</td>
<td>1.6</td>
<td>19.20/0.40</td>
<td>0.093</td>
<td>129</td>
<td>612</td>
<td>6.70</td>
</tr>
<tr>
<td>2</td>
<td>7.98</td>
<td>0.21</td>
<td>1.4</td>
<td>19.02/0.36</td>
<td>0.091</td>
<td>122</td>
<td>600</td>
<td>6.51</td>
</tr>
<tr>
<td>3</td>
<td>8.05</td>
<td>0.26</td>
<td>1.5</td>
<td>19.15/0.39</td>
<td>0.099</td>
<td>127</td>
<td>610</td>
<td>6.66</td>
</tr>
<tr>
<td>4</td>
<td>8.03</td>
<td>0.27</td>
<td>1.7</td>
<td>19.21/0.40</td>
<td>0.098</td>
<td>126</td>
<td>611</td>
<td>6.69</td>
</tr>
<tr>
<td>5</td>
<td>8.04</td>
<td>0.28</td>
<td>1.6</td>
<td>19.23/0.41</td>
<td>0.095</td>
<td>128</td>
<td>612</td>
<td>6.72</td>
</tr>
</tbody>
</table>
Actual dehydrogenase activity (ADHA) (reduction of 2, 3, 5-triphenyltetrazolium chloride in samples without added glucose) and potential dehydrogenase activity (ADHP) (with added glucose) of soil samples was expressed in mg formazan/g soil by measuring the absorbance at 485 nm (Carpa et al., 2014). As illustrated by Figure 1, ADHA was more intense in soil samples 3 and 5, followed by samples 4, 1 and 2. The least actual dehydrogenase activity was displayed by the soil from sample 2, where pH was also lower and probably influenced the development of microorganisms. The values are low, but they fall within the general tendency for enzyme activities of agricultural soils at which the actual dehydrogenase activity presents highest values in spring and lowest in autumn and summer (Drăgan-Bularda & Kiss, 1986).

Phosphatase activity was detected in all soil samples. Phosphatase activity was more intense in sample 3, and weakest activity was recorded in sample 2 (Figure 2). Phosphatase activity was fairly good in the analyzed soils and this is supported also by the samples being taken in the autumn, when this activity grows due to the increase of organic matter in soil (Maheshwari, 2011). However, phosphatase activity is limited by availability of organic carbon in soil.

Figure 1. Actual and potential dehydrogenases activities (ADHA and PDHA) in tilled soil from Jibou

<table>
<thead>
<tr>
<th>Soil samples</th>
<th>EISQ</th>
<th>Dehydrogenase activities (mg formazan/g soil)</th>
<th>Catalase activity (mg splitting H₂O₂/g soil)</th>
<th>Phosphatase activity (mg phenol/g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual</td>
<td>0.282</td>
<td>0.989</td>
<td>48.62</td>
<td>9.354</td>
</tr>
<tr>
<td>1</td>
<td>0.278</td>
<td>2.144</td>
<td>46.58</td>
<td>6.275</td>
</tr>
<tr>
<td>2</td>
<td>0.499</td>
<td>1.110</td>
<td>43.52</td>
<td>10.796</td>
</tr>
<tr>
<td>3</td>
<td>0.303</td>
<td>1.782</td>
<td>47.94</td>
<td>9.128</td>
</tr>
<tr>
<td>4</td>
<td>0.363</td>
<td>1.671</td>
<td>48.28</td>
<td>9.456</td>
</tr>
<tr>
<td>5</td>
<td>0.625</td>
<td></td>
<td></td>
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</tbody>
</table>

It can be noted that PDHA presents higher values in all samples, as compared to ADHA. The glucose addition has a stimulative effect on dehydrogenase activity. These enzymes which catalyze the oxidation of many organic compounds by exchange of electrons and protons are localized only in live, intact cells. Phosphatase activity of the soil samples was expressed in mg phenol/g soil by measuring absorbance at 600 nm (Carpa et al., 2014).
Catalase activity is expressed by mg H$_2$O$_2$/g soil. Catalase activity is determined by expressing the breakdown intensity of peroxide (Carpa et al., 2014). Catalase activity was detected in all the soil samples analyzed (Figure 3). Especially in samples 1 and 5 catalase activity was more intense. Catalase is an enzyme which accumulates in soil and thus keeps its activity for a long time. Also, catalase is correlated with the humus quantity in soil and pH. This explains the fact that in samples 1, 4 and 5 catalase activity was more intense, these spots being characterized by a higher humus content and a more alkaline pH (Table 1).

The quality of the soil enzymologically studied is characterized by the intensity of enzyme activities, defined by the values of the enzymatic indicator of soil quality (EISQ).

The greater the enzymatic indicator is the higher the enzymatic potential of that soil. Enzymatic indicator of soil quality (EISQ) offers a general image on the enzyme potential of it, being calculated based on computing formula developed by Muntean et al. (1996). Theoretically, the enzymatic indicator can reach values in the range 0 (when there is no activity in the studied samples) to 1 (when all the individual real values are equal to theoretical individual maxima of all activities). The enzymatic indicator of soil quality values are presented in Table 2.
The studied soils have a fairly high enzymatic activity. EISQ values have ranged between 0.574 and 0.633 (Figure 4). The quality of soil is better as the EISQ is higher (Muntean et al., 2001).

The enzymatic potential of a soil directly or indirectly reflects the microbiota activity, the influence of different physical, chemical, anthropogenic and even of the intensity of the different enzymatic activities in soil. Thus, the functioning of an ecosystem can not be understood without the active participation of enzymatic processes (Drăgan-Bularda et al., 2004). Based on the results obtained and compared with the data in published studies (Pasca et al., 1993; Carpa, 2007) we can consider that the analyzed soils have a wide biological potential.

CONCLUSIONS

Physico-chemical and enzymological properties of soils from Jibou area, where there are sulphurous springs, were the subject of this study. Physico-chemical analyses showed that all the five analyzed soil types have a loamy-clayey texture and are alkaline. The weakest alkaline character was displayed by the soil in sample 2 (pH = 7.98). The humus quantity does not pass 1.7% and the nitrogen supply is small in all the analyzed soil samples. Also, the nutrients from the tested soil, as phosphorus and potassium, did not surpass the 129 ppm and 612 ppm, respectively. The carbonates value was maximum 6.72% in sample 5.

The enzymological research on tilled soils from Jibou area encompassed assessing 4 quantitative enzymatic activities, all of these displaying intensity fluctuations, according to the sampling place and the substrates needed for enzyme synthesis by microorganisms. PDHA was greater than ADHA in all the studied soils, reflecting the stimulating effect of the easy available carbon source on synthesis of enzymes by microorganisms. The values of dehydrogenase activity prove the existence of a microbial potential and a medium respiratory activity in the analyzed soils. Phosphatase activity showed medium values which can be caused by the accumulation of plant debris in soil at the end of the growing season. Catalase activity is correlated with the level of the humus quantity, being very intense in soil samples where the humus is more abundant. Based on the EISQ values, the soil in Jibou area has a fairly good enzymatic activity (values above 0.57). Based on these values it can be stated that the analyzed soils have a wide biological potential.

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