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SOIL FERTILITY ASSESSMENT THROUGH ENZYME ACTIVITY

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Abstract

Soil is an important component of all terrestrial ecosystems as well as fundamental resource in the agricultural production system that why monitoring of soil fertility is an important objective for the sustainable agro-ecosystems development. Changes in its physical, chemical and biological properties must be taken into account, in order to evaluate soil fertility. Biological indicators are suitable tools for predicting and assessing soil changes that are caused by both natural and anthropogenic factors. Among the biological features, soil enzymes are often used as indicator of soil fertility because they are very sensitive and respond to changes in soil management more quickly than other soil variables. In this respect, the objective of this paper was to determine enzyme activity of the soil from USAMV – Bucharest orchard in order to evaluate its fertility.

Key words*:soil enzyme activity, dehydrogenases, phosphatases, catalases,soil fertility*

INTRODUCTION

To understand the functioning of soils and to prevent soil damage due to both natural and anthropogenic factors, it is important to have suitable tools for predicting and assessing soil changes that are caused by environmental factors and management practices. Strategies based on biological indicators would be a suitable tool to evaluate the sustainability of the soil ecosystem. Studies of soil enzymes are important since they indicate the potential of the soil to support the biochemical processes that are essential for the maintenance of soil fertility. Soil enzymes regulate the functioning of the ecosystem and play key biochemical functions in the overall process of organic matter transformation and nutrient cycling in the soil system. They also take part in the detoxification of xenobiotics, such as pesticides, industrial wastes, etc. .

Soil enzyme activities have been suggested as sensitive indicators of soil fertility since they catalyze the principal biochemical reaction that involves nutrient cycles in soil, are very sensitive and respond to soil changes and can be easily analyzed within a few days using a small amount of soil

The total enzyme activity in soil consists of distinct intracellular and extracellular enzymes that originate from microorganisms (e.g., bacteria, fungi), from plants and animals (e.g., plant roots or residues, digestive tracts of small invertebrates). The same enzyme can originates from different sources and the exact origin as well as the temporal and spatial variability of their activity is difficult to identify. Intracellular enzymes exist in different parts of living cells, while extracellular enzymes are produced by living cells and secreted outside the cell as free enzymes in the soil solution. Some of these enzymes remain associated with the external surface of the root epidermal or microbial cell wall. The rest of the extracellular enzymes are either free in soil solution or adsorbed by argilo - humic complex. The amounts of free enzyme in soil are very low compared with enzymes adsorbed, due to their short life span [8]. Adsorbed enzymes are usually protects against

degradation but they reveal less activity than free enzymes.

Numerous factors can influence enzyme activity in soil. Natural parameters like seasonal changes, geographic location, in situ distribution, physical-chemical properties, content of clay and organic matter usually affect the enzyme activity level by influencing both the production of enzymes by organisms and their persistence under natural conditions.

The physical and chemical properties of a soil are involved in the immobilization and stabilization processes of most extracellular enzymes. A high content of clay or humus colloids is usually associated with stable but less active enzymes.

Enzyme activities often provide a unique integrative biological assessment of soil function, especially those that catalyze a wide range of soil biological processes, such as dehydrogenase, urease and phosphatase.

MATERIALS AND METHODS

Enzyme assays were performed on soil samples collected in spring (March 2015) and consist of eight experimental variants: soil planted with fruit trees (apple, plum, apricot) and control (meadow) from a neighboring orchard area, the depths of the sampling were 0-20 cm, and 20-40 cm.

Phosphatase activity (PA)

In order to determine PA was used Kramer and Erdei method (1995). Reaction mixture consisted of: 3 g soil, 2 ml of toluene, 10 ml of 0.5% disodium phenylphosphate solution, 0.3% ammonium alum solution, borax buffer and Gibbs solution. Incubation took place at 37 ° C for 24 hours. Measurements were performed using a spectrophotometer at a wavelength of 620 nm. PA is expressed in mg phenol / g dry weight soil.

Catalase activity (CA)

The action of catalase refers to the decomposition of hydrogen peroxide (H_2O_2) with the production of molecular oxygen and water. Catalase activity and enzymatic cleavage of H_2O_2 in the samples were determined by the method Kappen.

The reaction mixtures for the determination of CA containing: 3g soil, 10 mL of distilled water, 2 ml of 3% H_2O_2 solution, 10 ml of 4M H_2SO_4, distilled water were titrated with a solution of potassium permanganate ($KMnO_4$) 0.05M. Incubation was done at room temperature for one hour. Expression of CA is in mg H2O2 / g soil dry soil.

Dehydrogenase activity (DHA)

DHA was determined with and without glucose added by the Casida method (Casida et al, 1964).

Reaction mixtures consisted of: 3 g soil, 3% TTC solution (chloro-triphenyl-tetrazolium), 1 ml of distilled water, 3% glucose solution, acetone. Incubation took place at 37 ° C for 24 hours. The color intensity directly correlating with DHA was spectrophotometrically determined at a wavelength of 440 nm. DHA is expressed in mg formazan / g soil dry soil.

RESULTS AND DISCUSSIONS

Soil dehydrogenases are the major representatives of the oxidoreductase enzymes class. The activity of the DHA reflects the total range of the oxidative activity of soil microorganisms and may be considered a good indicator of the oxidative metabolism in soils, and therefore, of microbiological activity. Dehydrogenases oxidize soil organic matter by transferring protons and electrons from organic substrates to inorganic acceptors. Many specific dehydrogenases transfer hydrogen on either the nicotinamide adenine dinucleotide (NAD) or the nicotinamide adenine dinucleotide phosphate (NADP). Throughout mentioned co-enzymes hydrogen atoms are involved in the reductive processes of biosynthesis. These processes are part of the respiration pathways of soil microorganisms and are closely related to the type of soil and air-water conditions.

The DHA of investigated orchard and meadow soils are repressent in the table 1. The values indicated a moderate activity of DHA for each soil cultures at each soil depths. This situation is corelate with the unexpected fluctuation of temperatures for this season of year (from 5.3 ^{0}C to 16 ^{0}C at the moment of samplings). These temperatures inhibit for certainly the normal microorganism developments.

Table 1. Enzyme activities (PA, CA, DHA) of orchard and meadow soils from University of Agronomic Sciences and Veterinary Medicine- Bucharest

Experimental variant	PA (µg phenol / g soil)	CA (mg H_2O_2 / g soil)	DHA (µg formazan / g soil)
Soil Depth 0-20 cm			
Meadow	245	3.2	4.3
Apple trees	210	0.42	4.5
Apricot trees	265	0.54	4.0
Plum trees	240	0.86	4.3
Soil Depth 20-40 cm			
Meadow	275	1.54	4.8
Apple trees	220	0.48	4.5
Apricot trees	225	1.84	4.5
Plum trees	250	1.28	4.3
Low activity values*	**< 100**	**< 0.5**	**< 1**
Moderate activity values*	**100-150**	**0.5-1**	**1-5**
High activity values*	**> 150**	**> 1**	**> 5**

Phosphatases are a group of enzymes that are of great agronomic value because they catalyze the hydrolysis of organic phosphorus compounds and transform them into an inorganic form of P, which is then assimilated by plants and microorganisms. Agricultural soils contain phosphatases in varying amount depending on the microbial count, the amount of organic materials, mineral and organic fertilizers, tillage and other agricultural practices. The relationship between the available P content and phosphatase activity in soil is complex. A positive, negative or no relationship can be observed between these properties.

Our study revealed a high PA (Table 1), and that could be correlated with the lock of fertilization in the period of sampling. Generally, a significant and positive relationship between phosphatase activity and P availability is obtained in soils that are not fertilized and/or those that have small amounts of nutrients in which a P deficiency occurs. An inverse relationship between these two parameters is usually observed in soils that are fertilized with P and/or those with a sufficient content of available P. There are studies that show that phosphatase activity is inversely proportional to the plant available P content, which confirms the thesis that the production and activity of soil phosphatases is connected with the demand of microorganisms and plants for P. Phosphatases are typical adaptive enzymes and their activity increases when the plant available P content decreases. Kinetics studies indicate that orthophosphate ions, which are the product of the reaction that is conducted by the phosphatases, are competitive inhibitors of their activity in soil.

Catalaseis an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert approximately 5 million molecules of hydrogen peroxide to water and oxygen each second. Catalase is universal among plants, fungi, and almost all aerobic microorganisms.

Our results showed a low to moderate catalase activity (Table 1), that is correlated well with DHA, and therefore with moderate microbial activity in soils at the sampling times (at 5.3

^{o}C air temperature). In an old article, Heinicke considered that the catalase activity would eventually prove as good an indicator of metabolism as do the temperature and pulse rate in the human body. The highest catalase activity was registered at the tree plantations that budding earlier (e.g. apricot and plum tress).

CONCLUSIONS

The DHA values indicated a moderate activity for each soil cultures at each soil depths. This situation is correlate with the unexpected fluctuation of temperatures for this season of year (from 5.3 ^{0}C to 16 ^{0}C at the moment of samplings). These temperatures inhibit for certainly the normal microorganism developments.

The high PA could be correlated with the lock of fertilization in the period of sampling. Generally, a significant and positive relationship between phosphatase activity and phosphorus availability is obtained in soils that are not fertilized (like the University investigated soils).

The moderate catalase activity is correlated well with moderate DHA, and therefore with moderate microbial activity in soils at the sampling times.

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