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SOIL ENZYMES - BIOINDICATORS OF SOIL HEALTH

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Abstract

Nowadays, soil health is a key element in achieving agriculture sustainability. Worldwide organic farming is being increasingly promoted as a sustainable alternative to conventional farming because it can solve the problems associated with the usage of agrochemicals by long-term use of soil resources. Soil enzymes have been suggested as bioindicators of soil health because they are a measure of soil microbial activity, strictly related to organic matter decomposition and nutrient cycles and easy to measure. The purpose of this study was to measure the enzymatic activity (alkaline and acid phosphatase, amylase, cellulase and catalase) from the rhizosphere of organic and conventional soils. These biological indicators were correlated with some physio-chemical parameters such as humidity, pH, total nitrogen and organic carbon. The results showed a significant difference between the two soils with different management systems regarding the enzymatic activity, long-termed pesticide application having a negative effect on the soil enzymes. The results were strongly correlated with pH, total nitrogen and organic carbon.

Key words*: amylase, cellulase, organic soil, phosphatase.*

INTRODUCTION

Nowadays, organic farming has an important role in producing healthy food through exclusion of applications of synthetic chemicals. It is more suited to human metabolism, in full correlation with environment preservation. One of the main goals of organic farming is the production of fresh and authentic agri-food products, respecting the natural and environmental factors (Srinivasa Rao et al., 2017). One important aspect in obtaining healthy products consists in monitoring and measuring the ecosystem status of soils in order to maintain and increase the soil quality. Studies have shown that a long-term soil organic management practices with wastes application led to an increase in the urease, dehydrogenase, polyphenol oxidase and peroxidase activities in soil (Senicovscaia, 2014).

On the other hand, continuous application of pesticides can lead to soil pollution threatening, influencing the soil microorganisms and enzymes and thereby, affecting soil fertility (Lopez et al., 2002; Cycon et al., 2006).

Therefore, soil management has an important impact on the chemical, physical and biological properties of the soil and on the subsequent growth of plants. In addition, soil management affects microorganisms and microbiological processes by changing the quantity and quality of plant remains, which are the primary source of soil organic matter (Błońska et al., 2017).

A better understanding of the dynamics of microbial activity and mineralization of soil nutrients from plant residues is essential to quantify the potential benefits for soil and crop quality due to the changes introduced in the soil-plant system by using organic fertilizers (Mihalache et al., 2015).

Compost application is important in establishing and maintaining soil organic matter to a certain level in organic farming. Chang et al. (2007) found that soil enzymes activities, as well as other microbial properties increased significantly in compost-treated soil compared with chemical fertilized soils.

Soil fertility and productivity depend on the content of organic matter, which is an important reservoir of nutrients in the nutrient cycle (Steiner et al., 2007) and can improve the physical, chemical and biological properties of soils (Bhattacharya et al., 2010). The process of decomposition of organic matter in the soil takes place with the participation of soil

microorganisms and enzymes (Schimel and Bennett, 2004).
The suggested bio-indicators for monitoring soil quality were: soil microbial biomass, nutrient cycling, community structure and biodiversity, soil animals, plants and soil enzymes (Killham, 2002).
Soil enzymes are necessary catalysts for decomposition of soil organic matter and nutrient cycling, influencing the energy transformation, environmental quality and agronomic productivity. Further, soil enzymes can provide early detection of changes in soil health because they respond to soil management changes and environmental factors faster than other soil quality parameters (Srinivasa Rao et al., 2017).
A better understanding of the role of this soil enzymes activity in the ecosystem can provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management practices (Sherene, 2017).
Phosphatases are a group of enzymes that are capable to catalyze the hydrolysis of esters and anhydrides of phosphoric acid. In soil, these enzymes are believed to play an important role in phosphorus cycles because they can be correlated to phosphorus stress and plant growth. Besides being good indicators of soil fertility, phosphatases play key roles in the soil system (Dick et al., 2000).
Amylase is a starch hydrolyzing enzyme, consisting of α-amylase and β-amylase. The activity of amylase can be influenced by some factors such as cultural practices, type of vegetation, environment, soil types (Rose and Roberts, 1970) and plants by directly supplying enzymes from their residues or indirectly providing substrates for the synthetic activities of microorganisms (Sherene, 2017).
Cellulases are the group of enzymes that catalyze the degradation of cellulose, the most abundant organic compound on the Earth. The activities of cellulases in agricultural soils are affected by some factors such as temperature, soil pH, humidity, the chemical structure and the location of organic matter in the soil profile horizon, the quality of organic matter/plant debris and soil mineral elements and the trace elements from fungicides (Srinivasulu and Rangaswamy, 2006).
Catalase decomposes peroxide and its activity depends on organic oxygen concentration, microbe biomass, changes in carbon dioxide, and dehydrogenase, amidase, glucosidase and esterase activities in soils. Thus, they are an important indicator of soil fertility and aerobic microorganisms (Burns, 1982). Catalase stability is very high in soil and the enzymatic activity is significantly correlated with the organic carbon content (Alef and Nannipieri, 1995).
The aim of this study was to examine the influence of different management systems (organic and conventional) on the enzymatic activities registered in soil. The enzymes analysed were acid and alkaline phosphatase, catalase, amylase and cellulase.
These enzymatic parameters were correlated with some physio-chemical parameters such as humidity, pH, total nitrogen and total carbon.

MATERIALS AND METHODS

Soil

For this study, three types of soil with different management systems were collected from the same area from a depth of 0-20 cm: conventional soil, organic soil and soil under conversion management. On the soil during the conversion period (year I) were cultivated: autumn cabbage and pumpkin. The ecological soil is certified, on its surface being cultivated basil, beans and okra. These cultures were organically treated with milk and garlic. Conventional soil was used for growing beans (current year), celery and flowers.

Enzyme assays

The soil samples were analyzed fresh and were kept in plastic bags in the refrigerator at 4°C.
Acid and alkaline phosphatase method was based on the spectrophotometric determination of p-nitrophenol released after incubation of the soil with an artificial substrate, p-nitrophenyl phosphate (p-NPP) and Modified universal buffer (MUB) (pH = 11 for alkaline phosphatase and pH = 6.5 for acid phosphatase), for 1 h at 37°C (Tabatabai and Bremner, 1969). $CaCl_2$ 0.5 M and NaOH 0.5 M were added for colour development, after

incubation. The samples were homogenized, filtered and the yellow colour was measured spectrophotometrically at the wavelength of 400 nm.

The method used to evaluate cellulase activity was based on the determination of reducing sugars by the method of Deng and Tabatabai (1994) using 3,5-dinitrosalicylic acid (DNS) as a reagent at the wavelength of 640 nm. The samples were incubated 24 h at 50°C in the presence of 0.05 M acetic acid - acetate buffer, pH 5.5 and 1% carboxymethylcellulose solution (CMC).

The assay used for soil amylase measurement was developed by Cole in 1977. The soil samples were incubated with acetic acid acetate - buffer 2 M (pH = 5.5) and 2% starch at 37°C for 24 h. After incubation the samples were centrifuged, and the reducing sugars were determined using 3,5-dinitrosalicylic acid (DNS). The absorbance was read at the wavelength of 546 nm.

Catalase activity was measured by back-titrating residual H_2O_2 with $KMnO_4$ (Johnson and Temple, 1964; Roberge, 1978). The soil samples were homogenized with distilled water and 0.3% hydrogen peroxide solution. The mixture was shaken for 20 min and then 1.5 mol/L H_2SO_4 were added. Afterwards the solution was filtered and titrated using 0.02 mol/L $KMnO_4$.

Physio-chemical methods

The soil samples were dried in the atmosphere and sieved through a 1 mm sieve. The pH assay was performed using 10 g soil and 25 ml of distilled water, to obtain a 1:2.5 (w/v) soil-water extract. The conductivity was measured using a 1:5 (w/v) soil-water. The soil moisture was determined in the oven at 105°C for 24 h.

Total nitrogen from soil was measured using the Kjeldahl method (Saez-Plaza et al., 2013). The Kjeldahl method consisted of three steps: digestion (the decomposition of nitrogen in organic samples utilizing 98% H_2SO_4 and catalysts), distillation (the ammonia content of the digest was determined by distillation with excess 35% NaOH in 25 ml of 4% solution of H_3BO_3) and titration (with 0.1 M HCl in presence of 2-3 drops of indicator).

The determination of organic carbon in the soil was measured based on the Walkley-Black method of humid oxidation of chromic acid. The oxidizable material from the soil was oxidized by the solution of $K_2Cr_2O_7$ 1 N. The reaction was favored by the heat generated by the addition of two volumes of H_2SO_4 over the volume of bichromate. The rest of the bichromate was titrated with iron sulphate solution in the presence of indicator Ferroin, the titrated volume being inversely proportional to the amount of C present in the soil sample (Barlett et al., 1994).

Enzymatic and chemical parameters were statistically evaluated by the determination of variance and correlation analysis, using Microsoft Excel 2016 tools. The linear correlation was considered significant for a significance level p = 0.05.

RESULTS AND DISCUSSIONS

The chemical and biochemical parameters considered in this study provided information on differences in soil quality and fertility between organically and conventionally managed systems. In fact, organic soils have been characterized by higher carbon mineralization, higher enzymatic activity, increased soil nutrients (N) and energy (higher total carbon content). These findings suggested that environmentally managed soils could be considered more conservative systems.

The highest activities of acid and alkaline phosphatase, cellulase, amylase and catalase were recorded in organic soil, which were characterized by the highest accumulation of soil organic matter (Table 1).

Table 1. Physio-chemical parameters of soils

Soil Type	Moisture (%)	Conductivity (μS/cm)	pH	C (%)	N (%)
Conventional soil	15.9	195.6	8.8	1.63	0.519
Soil in conversion	11.5	144.8	8.2	2.00	0.801
Organic soil	13.9	159.9	7.4	2.86	0.943

The content of organic carbon and total nitrogen were higher in the soil with organic management, compared to those in the soil under conventional agriculture.

The obtained results for total organic carbon were similar with the results obtained by Hábová et al. (2019), which comparing soils under organic and conventional farming systems pointed out that total organic carbon values were higher in organic farming (2%). These findings were in concordance with Marriott and Wander (2006) study, where total organic carbon was higher in organic soil and this increase was strongly correlated with the values obtained for total nitrogen, which were as well higher compared with conventional farming.

Conventional soil was characterized by a moderately alkaline pH compared with the organic soil were the pH was weakly alkaline.

From the point of view of the enzymatic activity, significantly higher values were observed in the organic soils compared to the conventional one for all the enzymes analyzed (Table 2).

Cellulase activity in soil was strong correlated with total carbon ($r = 0.99$, $p<0.05$) and total nitrogen ($r = 0.85$, $p<0.05$). The same observation were noticed regarding the amylase activity, having a strong correlation with the two chemical parameters ($r = 0.96$, respectively $r = 0.98$; $p<0.05$).

Table 2. Enzymatic activity for the three types of soil with different management systems

Enzymes analysed	Soil type	Enzymatic activity
Cellulase (µg glucose/ g soil/24h)	Conventional soil	0.184 ± 0.013
	Soil in conversion	0.281± 0.002
	Organic soil	0.750 ± 0.146
Amylase (µg maltose/ g soil/24h)	Conventional soil	0.796 ± 0.148
	Soil in conversion	1.372 ± 0.054
	Organic soil	1.892 ± 0.158
Acid phosphatase (µg p-nitrophenol/g soil/h)	Conventional soil	124.46 ± 7.54
	Soil in conversion	177.63 ± 9.86
	Organic soil	287.99 ± 10.92
Alkaline phosphatase (µg p-nitrophenol/g soil/ h)	Conventional soil	331.61 ± 16.06
	Soil in conversion	376.41 ± 14.38
	Organic soil	499.39 ± 22.72
Catalase (mmol H_2O_2/ g soil/h)	Conventional soil	0.105 ± 0.010
	Soil in conversion	0.156 ± 0.010
	Organic soil	0.262 ± 0.015

Cellulase and amylase activities were significantly higher ($p<0.05$) in the soil with organic management compared with conventional soil (Figure 1).

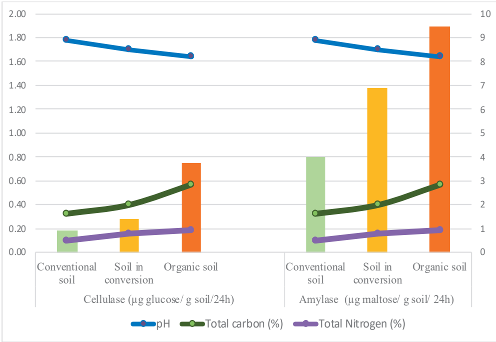

Figure 1. Activity of cellulase and amylase in soil

Similar results were obtained by Balota et al (2004), where was highlighted that amylase and cellulase activity in soil were lower in conventional farming system compared with organic management.

Regarding acid and alkaline phosphatase, the results were higher in organic management and were strongly correlated with total carbon ($r = 0.99$; $r = 0.99$, $p<0.05$) and total nitrogen ($r = 0.92$; $r = 0.89$, $p<0.05$) (Figure 2).

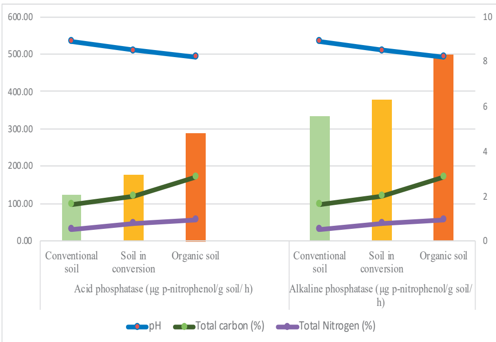

Figure 2. Activity of acid and alkaline phosphatase in soil

Several studies have compared soil enzymatic activities responsible with nutrient cycle under organic and conventional system. They suggested that organically managed fields had a greater enzymatic activity that conventionally managed fields (Garcia-Ruiz et al., 2008; Fliessbach et al., 2007; Melero et al., 2008). These results were in concordance with the results obtained in this study, demonstrating that long-term application of agrochemicals on soil can lead to a strong decrease of enzymatic activity. They have shown that organic soils were characterized by a greater acid and

alkaline phosphatase activity compared with the conventional management where the phosphatase activity was lower.
Catalase activity (Figure 3) from conventional soil was significantly lower ($p<0.05$) than the activity registered in organic management, being positive correlated with the other parameters (total C with $r = 0.92$ and total N with $r = 0.99$; $p<0.05$).

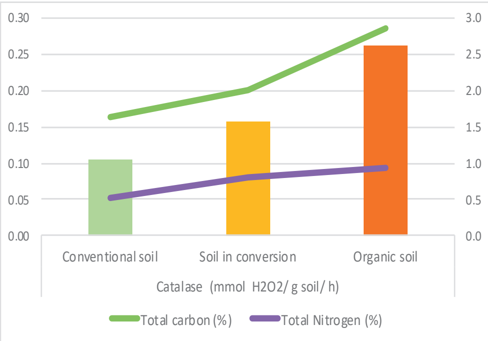

Figure 3. Activity of catalase in soil

Alef and Nannipieri (1995) reported as well in their study that catalase activity was very stable in organic soil and showed a significant correlation with the content of total organic carbon, resulting a higher activity in organic soil.

CONCLUSIONS

The results of this study confirmed that activities of cellulases, amylase, acid and alkaline phosphatase and catalase can be used to obrain a preliminary indication of some of the physical and chemical properties of soil, thus, improving agricultural soil management strategies. Significant differences between organic and conventional soils enzymatic activity were noticed.
Conventional soils contained significantly less organic matter, which could lead in weaker soil structure and lower enzymatic activity. Low activities of the cellulases, amylase, acid and alkaline phosphatase and catalase were observed in the conventional cultivated soils, whereas higher activities of these enzymes were obtained in organic soils.
Due to their importance in soil organic matter degradation, nutrients cycle, environmental quality and agronomic productivity, enzymes can be suggested as bio-indicators of soil health. On the other hand, the results from this study confirmed the usefulness of assessing enzymes activities in order to evaluate the differently managed soils.

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