

Enzymatic Activity (Dehydrogenase) in Rhizospheric Soil of *Musa* AAB: Relationship with Depth, Agricultural Management Crop and Plant Age

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ABSTRACT

The objective of this work was to evaluate the dehydrogenase activity in rhizosphere of Musa AAB at different depths of the soil, agronomic management and age of the crop, and analyze the relationship between the enzyme activity and some soil biology properties. The present investigation focused on assessing the dehydrogenase enzyme in a soil *Pachic Melanudands* of Quimbaya, Quindio, Colombia. The growing practices of agricultural management were chemical, traditional and agroecological. The samplings were conducted in the rhizosphere at 0-5, 5-10,10-20 and 20-30 cm depth, and the surveys were made at 6, 12 and 18 months taking into account the plant phenology. Dehydrogenase activity was higher in the first cm of soil profile. The activity depends of the management crop being the higher in the agroecological conditions and the age of the plant. The activity was related to biological properties of the soil. This investigation constitutes the first study of enzymatic dehydrogenase activity of soil in that grow *Musa* AAB in Colombia.

Keywords: compost organic agriculture, plantain, soil enzyme

INTRODUCTION

The complexity of the soil and the need to investigate their fertility and quality, leading to this natural resource in a holistic approach, considering physical, chemical, biological, and biochemical properties. Within the later, enzymes deserve special attention for their role in the mineralization of organic matter.

Zhang *et al.* (2010), which cites several authors with reference to soil organic matter, has important effects not only soil enzymes activities, but also on microbial activities. Enzymes in soil, especially dehydrogenase, are highly associated with the microbial biomass, which in turn affects the decomposition of organic matter.

Mineralization and immobilization of nutrients such as carbon, nitrogen, phosphorus, sulfur, boron, among others, are determined by the amount and type of organisms that make up the soil biota and its contribution enzyme (Bolaños 2006). The extracellular enzymes of microbial origin degrade macromolecules in smaller molecules, which are absorbed and consumed by the microbial cells, transforming them into biomass, CO₂ and energy (Siqueira and Franco 1988). It has been determined that in the soil, the main source of enzymes is the microbial biomass, but they can also originate from residues of plants and animals (Tabatabai 1994). Dehydrogenase activity is considered as an indicator of oxidative metabolism and total microbial activity in soils (Skujins 1973 in Quilchano Marañon 2002). This led us to use the presence and activity of enzymes in the soil as an indicator of their quality and biodiversity. Also, Udawatta et al. (2008), Trasar-Cepeda et al. (2008) and Zagal et al. (2009) report that dehydrogenase activity is strongly influenced by soil management, although Lagomarsino et al. (2009) report extremely high variability of this enzyme in different systems under short-term crop rotation.

Plantain (Musa AAB) crop, at a global level, is regarded as a product that ensures food security. In Colombia is of economic importance as a source of income of about US \$ 57,000 and generates 286,000 jobs per year (Aranzazu *et al.* 2002). There is little information on plantain rhizosphere studies and therefore research is needed to better understand health and soil quality and productivity of this crop.

The objectives of this research were: 1) to estimate dehydrogenase activity in rhizosphere of plantain at different depths of the soil, agronomic management and age of the crop and 2) to establish the relationship between enzyme activity and soil properties such as respiration and microbial biomass C and N.

MATERIALS AND METHODS

The field experiment was conducted in a soil *Pachic Melanudands* medial isothermic of Quimbaya, Quindío (Colombia), in the farm Peru, under the georeferenced coordinates 4° 38' 15.6" N and 75° 44' 8" W, 1,420 masl. Average temperature of 22°C, 2.200 mm/ year, and relative humidity of 78%. The clone planted was horn cultivar ('Dominico-Hartón') (*Musa* AAB), from seed selected by health and weight (corms between 200 and 300 g). All growing practices were provided, while preserving differences in the three types of agricultural management or treatments.

a) Chemical Management (CM): application of synthetic chemical fertilizers, insecticides to control *Cosmopolites sordidus* Germar; fungicides for control of Sigatoka (*Mycosphaerella* sp.) and herbicides.

b) Traditional Management (TM): just coffee pulp was applied at the time of planting.

c) Agroecological Management (AM): includes application of compost prepared from products of banana peelings, cow manure and coffee pulp; all naturally occurring chemical fertilizers and bio-fertilizers. No agricultural chemicals were applied in any form.

The samplings were conducted in the rhizosphere at 0.5, 5-10, 10-20 and 20-30 cm depth, according to the distribution of the plantain root system the soil profile. The surveys were made at 6, 12 and 18 months, taking into account the phenology of the plant.

To quantify the activity of dehydrogenase (EC 1.6.99.3) the TTC reduction technique (triphenyl tetrazolium chloride) was used (Thalmann 1968 cited by Alef 1995). The method is based on the rate of reduction of TTC to TPF (triphenyl formazan) after incubation at 30°C for 24 hrs in soil. Each sample (108) had three replicates. After incubation, the TPF formed was extracted with acetone. The samples were centrifuged and the intensity of red or optical density of the supernatant (TPF) was estimated at 546 nm by a spectrophotometer (Biorad Smartspec 3000). The concentration of TPF μ g.g⁻¹ in dry soil was determined by calibration curve (Casida *et al.* 1964).

Statistical analysis

The information was analyzed using the SAS statistical program (Statistical Analysis System) Version 8. Data variables that are not normally distributed were transformed before performing analysis of variance and Tukey's test. After three treatments (CM, TM, AM), three ages, four depths and three replicates a total of 108 samples were processed under a split design under randomized block. In this arrangement, the main plot was the crop and soil management and the sub plot were the three ages of plants.

Results between the enzyme activity – dehydrogenase and microbial biomass were analyzed using Pearson's correlation (P < 0.05).

RESULTS AND DISCUSSION

The activity of the enzyme varied significantly with depth of sampling, age and agronomic management of the crop.

Dehydrogenase and depth of soil

The highest activity occurred in the first five centimeters of soil depth independent of the age of the plant. It decreased significantly as occurred in the profile, 75% of the dehydrogenase activity was concentrated in the top 10 cm of soil, and this value was 58.44% of 0 to 5 cm. These results may be related to soil moisture and activity of soil biota.

Quilchano and Marañón (2002) found that dehydrogenase activity was higher in rainy season than in dry season, and was related to soil moisture and increased biological activity. Similarly, they found significant correlation with pH, Ca, Mg, K and water content in the soils studied, but not with C and N total.

Enowashu *et al.* (2009) also reports that the enzyme activities were higher in the upper soil layer and decreased within the soil profile.

Dehydrogenase and agronomic management practices

The activity of this enzyme was significantly different (P < 0.01) in the rhizospheric soil of plants that were environmentally sustainable practices 50.2 µg TPF.g dry weight (dw)⁻¹, followed by 28.5 mg TPF.g dw⁻¹ in chemical management and traditional 1.19 µg TPF.g dw⁻¹ (**Fig. 1**).

In the agricultural management treatment it was observed the activity of the dehydrogenase decreased significantly along with depth, being higher in the ecological management at different depths (**Fig. 2**).

The increased activity of dehydrogenase in the ecological management vs. the other may be associated with the presence and effect of organic amendments (compost) applied in this operation, as described in the methodology.

Similar results were found by Bolton *et al.* (1985), in the countryside, land under sustainable management, with input from green manures. There, the activity of dehydrogenase, urease and phosphatase showed highly significant differences in soil which had received regular applications of chemical fertilizers. In the chemical management, in addition to the applications of synthetic fertilizers are also applied insecticides, fungicides and herbicides, which explain the activity of this enzyme was significantly lower than in the ecological management. Chang *et al.* (2007) found that



Fig. 1 Dehydrogenase activity, at a depth of 0 to 5 cm under different agricultural management practices. Bars with different letters indicate statistical differences according to Tukey's test (P < 0.01).



Depth (cm)

Fig. 2 Dehydrogenase activity in three agricultural management practices under to four depths of the soil profile. Bars with different letters indicate statistical differences according to Tukey's test (P < 0.01).

the soil microbial biomass, populations of bacteria, fungi and actinomycetes, as well as soil enzyme activities (dehydrogenase, cellulase, glucosidase, protease, urease, arysulphatase, and acid and alkaline phosphatases) increased significantly in the compost-treated soils compared with the chemical fertilizer-treated soil.

Singh and Singh (2005) assessed the activity of dehydrogenase in soil to which was applied protectant (insecticide) for seeds and found that decreased activity in samples taken 15 and 90 days after applying protectant. The adverse effect of insecticides can be derived from the toxicity of these products in the soil microbial population and activity. However, gradual increases over time, the activity of enzymes can be explained by increases in the populations of microbes resistant to such toxic.

According the results obtained by Pajares *et al.* (2009) the addition of compost and farm manures (organic management), or fertilizer inputs adjusted to crop demand and combined with mulching had positive effect on the soil organic C content and soil biological activity in the short-term period in volcanic soil studied (Acrisol). In another study, Moeskops *et al.* (2010), comparing the effect of organic and conventional farming practice on soil microbial dynamics; a strong negative impact of intensive chemical fertilizer and pesticide use on soil enzyme activities was demonstrated.

Dehydrogenase and plantain cultivation age

The analysis of variance detected highly significant differences in dehydrogenase activity in rhizosphere of plantain according age of plantain (**Fig. 3**).

According to the comparison of means test, was higher (40.1 μ g TPF.g dw⁻¹) in plantain flowering plants (12 months), followed by plants at harvest, 18 months (33.6



Fig. 3 Dehydrogenase activity rhizospheres of plantain in different age (depth of 0 to 5 cm). Different letters indicate statistical differences according to Tukey's test (P < 0.01).

Fig. 4 Dehydrogenase activity in rhizosphere of plantain plants of different ages and four deep. Different letters indicate statistical differences according to Tukey's test (P < 0.01).

TPF.g dw⁻¹) and finally plants flower differentiation, sampled at six months (24.1 μ g TPF.g dw⁻¹) (**Fig. 4**).

In the cultivation of plantain, at an altitude between 1300 and 1500 m, 12 months of age that correspond to the physiological development of flowering period, may to explain the increased enzyme activity, when you consider that flowering is a phenological phase, in this case was increased the soil rhizospheric microbial activity and therefore the enzyme (Barea and Azcón-Aguilar 1982; Sánchez 2003).

The values found in this enzyme ranged between 4 and 51 µg TPF.g dw⁻¹, which can be considered high when compared with those found by Bolton *et al.* (1985). However, these values are within the range recorded by Casida *et al.* (1964).

Dehydrogenase activity is considered an appropriate measure of the total soil microbial activity, as there is high ratio (r = 0.84) between the consumption of O₂ by microorganisms and dehydrogenase activity in soils, but found no relationship between activity of this enzyme and the number of organisms, specifically bacteria (Stevenson 1959). In this sense, Casida *et al.* (1964) found similar results evaluating actinomycetes, bacteria and fungi.

Dehydrogenase enzyme activity was found to positively and significantly correlated with BMC (0.3, P < 0.05). These results confirm the reason for the close relationship between mineralization and soil enzymatic activity.

CONCLUSIONS

The dehydrogenase activity was higher in the first 10 cm soil profile and greater 0 to 5 cm. With regard to agricultural management practice, the soil dehydrogenase indicator was valid and sensitive to changes related to practices in the management of each evaluated; in the environmental management practice, the activity increased significantly compared with traditional management practice and with the soil that received agrochemicals. In the rhizosphere of plants in bloom (12 months) was higher dehydrogenase activity compared with the rhizosphere of plants found in six or 18 months (floral differentiation and harvest, respectively). The dehydrogenase activity was related to biological activity measured in terms of BMC. Changes in this enzyme are important in the mineralization of carbon and are a reliable diagnostic parameter, easy and inexpensive. It is in this research corroborates the importance of studying enzymes in the soil as indicators sensitive to changes, which become the monitoring tools can be used in making management decisions agronomic cultivation of plantain.

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REFERENCES

- Alef K (1995) Dehydrogenase activity. In: Alef K, Nannipieri P (Eds) Methods in Applied Soil Microbiology and Biochemistry, Academic Press Ltd., London, pp 228-231
- Aranzazu LF, Arcila MI, Bolaños MM, Castellanos P, Pérez JC, Rodriguez JL, Valencia JA (2002) Manejo Integrado del Crecimiento del Banano, Manual técnico, CORPOICA (Ed) Litoas, Manizales, Colombia, 114 pp
- Barea JM, Azcon-Aguilar C (1982) Rhizosphere: Microbe interactions plant. Proceedings of Soil Science and Agrobiology 41, 1517-1532
- **Bolaños BMM** (2006) Evaluación de la actividad enzimática (deshidrogenasa, proteasa, fosfatasa y arilsulfatasa) en rizosfera de *Musa* AAB: relación andisol. PhD thesis, Universidad Nacional de Colombia, Palmira Colombia, 240 pp
- Bolton J, Elliot LF, Papendick RI, Bezdicek C (1985) Soil microbial biomass and selected soil enzyme activities: Effect of fertilization and cropping practices. *Soil Biology and Biochemistry* **17**, 297-302
- Casida LE, Klein DA, Santoro T (1964) Soil dehydrogenase activity. Soil Science 98, 371-376
- Chang Ed-H, Chung R, Tsai Y (2007) Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Sci*ence and Plant Nutrition 53, 132-140
- Enowashu E, Poll C, Lamersdorf X, Kandeler E (2009) Microbial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. *Applied Soil Ecology* **43**, 11-21
- Lagomarsino A, Moscatelli MC, Di Tizio A, Mancinelli R, Grego S, Marinari S (2009) Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic in a Mediterranean environment. *Ecological Indicators* 9, 518-527
- Moeskops B, Sukristiyonubowo, Buchan D, Sleutel S, Herawaty L, Husen E, Sarawati R, Setyorini D, De Neve S (2010) Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. *Applied Soil Ecology* 45, 112-120
- Pajares S, Gallardo JF, Masciandaro G, Ceccanti B, Marinari S, Etchevers JD (2009) Biochemical indicators of carbon dynamic an Acrisol cultivate under different management practice in the central Mexican highlands. Soil and Tillage Research 105, 156-163
- Quilchano C, Marañón T (2002) Dehydrogenase activity in Mediterranean forest soils. *Biology and Fertility of Soils* 35, 102-107
- Sánchez M (2003) Actividad biológica en rizosfera de frutos (*Pasiflora edulis* var. Flavicarpa), sistemas de manejo en diferentes estados de desarrollo y condiciones fitosanitarias. Doctoral Thesis, Universidad Politécnica de Madrid – Madrid, Spain, 261 pp
- Singh J, Singh DK (2005) Dehydrogenase and phosphomonoesterase activities in groundnut (*Arachis hypogaea* L.) field after diazinon, imidacloprid and lindane treatments. *Chemosphere* 60, 32-42
- Siqueira JO, Franco AA (1988) Just do Biotecnologia. Fundamentals and Prospects, MEC Ministry of Education, Brasilia, ABEAS, Lavras, 235 pp
- Stevenson IL (1959) Dehydrogenase activity in soils. Canadian Journal of Microbiology 5, 229-235
- Tabatabai MA (1994) Soil enzymes. In: Pag Al, Miller EM, Keeney DR (Eds) Methods of Soil Analysis, Chemical and Microbiological Properties (Part 2), American Society of Agronomy, Madison, WI, pp 294-347

- Trasar-Cepeda C, Leirós MC, Gil-Stores F (2008) Hydrolitic enzyme activities in agricultural and Forest soil. Some implications for their use as indicators of soil quality. Soil Biology and Biochemistry 40, 2146-2155
- Udawatta RP, kemer RJ, Adamson BW, Anderson SH (2008) Variations in soil aggregate stability and enzyme activities in a temperate agroforestry practice. *Applied Soil Ecology* **39**, 153-160

Zagal E, Muñoz C, Quiroz M, Córdova C (2009) Sensitivity of early indica-

tors for evaluating quality changes in soil organic matter. Geoderma 151, 191-198

Zhang N, He X-D, Gao Y-B, Li Y-H, Wang H-T, Ma D, Zhang R, Yang S (2010) Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in *Artemisia ordosica* community. *Pedosphere* 20, 229-235