

THE ENZYMATIC ACTIVITY OF SOIL'S DEHYDROGENASES IN TASCA AND TARCAU AREAS

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Keywords: dehydrogenases, soil, microorganisms

Abstract: The present study aimed at the comparison of potential and actual dehydrogenase's activity in soil samples from Tarcau and Tasca areas of Neamt county, in May and August 2013, the material's harvest being made both from the overlay (0-10cm), as from the deepness (20-30 cm, respectively, 40-50 cm). The experimental results evidenced the existence of a different behavior of these oxidoreductases, higher values registering in the case of the samples derived from the Tasca area, lied in the immediate vicinity of cement factory.

INTRODUCTION

The enzymatic activity of the soil can be strongly influenced on the one hand by natural disturbances, and on the other hand by the anthropic disturbances, indicating a quick answer to the changes induced by these ones (Dick, 1997). Of the enzymes existing in the soil, dehydrogenases represent a main component of the microorganisms' activity existing in this level, taking part and assuring the right order of all anabolic and catabolic reactions of the biogeochemical cycles of the soil (Ladd, 1985; Kumar *et al.*, 2013).

The specialty literature mentions the fact that a series of biotic and abiotic factors like the time and the temperature of incubation, the type of soil, the season, the soil's aeration degree, its pH, the humidity, the management and maintenance practices, the soil's fertilization, the soil's depth profile, the presence of different heavy-metal etc. have a significant effect on dehydrogenase's activity in soil (Januszek, 1993; Leirós *et al.*, 2000; Stepniewski *et al.*, 2000; Subhani *et al.*, 2001; Agnelli *et al.*, 2004; Ghaly and Mahmoud, 2006; Levyk *et al.*, 2007; Trasar-Cepeda *et al.*, 2007; Xie *et al.*, 2009; Błońska, 2010; Fernandez-Calviño *et al.*, 2010; Macci *et al.*, 2012; Yuan and Yue, 2012). In fact, the dehydrogenase is often used like a measure instrument of pesticide application, of oligoelements or of soil's processing management, as well as a direct measure of the soil's microbial activity (Pitchel and Hayes, 1990; McCarthy *et al.*, 1994).

The soil's microbiologic activity, as a biomarker of degradation and reparation processes, influence directly the ecosystem's stability and its fertility, being largely accepted the fact that a good level of microbiologic activity is essential for the maintenance of the soil's quality (Visser and Parkinson, 1992; Pascual *et al.*, 2000).

The soil's quality and its degradation depend on a high number of physical, chemical, biological, microbiological and biochemical factors, the last two being the most sensitive, because, they answer as quickly as possible to changes (Kumar *et al.*, 2013).

Therewith, the soil's enzymatic activity, determined by the microbiologic activity, plays a key-role in the nutrients' cycle, being essential both in mineralization, as in the transformation of organic matter and of the nutrients necessary to the plants (Dick and Tabatabai, 1993).

Consequently, the enzymatic activity can be considered an efficacy indicator of the soil's quality changes, due to the stress condition from the environment and to the maintenance practices (Kiss *et al.*, 1975 quote by Kumar *et al.*, 2013).

This study was represented by the dehydrogenase's analyze from soil samples derived from two areas of Neamt county (Tarcau and Tasca) and harvested both from the overlay (0-10 cm) as from the deepness (20-30 cm, respectively 40-50 cm), in order to evidence the season's influence and the soil's profile on the microbial enzymatic behavior, given the fact that the activity of those oxidoreductases serves as an indicator of microbial redox systems, being considerate a good measures instrument of microbial oxidative activity in soils (Tabatabai, 1982) and representing a main part of enzymatic system of all microorganisms (the respirator metabolism's enzymes, the Krebs cycle and the nitrogen's metabolism).

MATERIAL AND METHODS

The researches were effectuated on samples soil derived from two areas of Neamt County – Tarcau and Tasca, harvested from five different points of each location taken into study, in May and August 2013, at different deepnesses. The dehydrogenase's activity was determined with 2,3,5 chloride-triphenil-tetrazolium, after incubation at 30°C, the formed triphenil-formazan being extracted with an ethanol- acetic acid blend and spectrophotometric evaluated on ripple

length of 540nm, the obtained experimental results being expressed in triphenil-formazan micrograms/ soil gram (**Kiss and Boaru, 1965**; Casida *et al.*, 1964 quoted by **Dragan-Bularda, 2000**).

RESULTS AND DISCUSSIONS

In the soil there are a multitude of enzymes belonging to the oxidoreductases', transphases', hydrolases', izomerases', liases' and ligases' class, each of these ones playing a key role in the biochemical transformations which occur in the majority conversion processes of substances and energy (**Gu *et al.*, 2009**), the dehydrogenases representing the most important enzymes, belonging to the oxidoreductases' class and being used as indicator in the soil's microbial activity (**Quilchano and Marañon, 2002**; **Salazar *et al.*, 2011**), because these ones have intracellular localization, being located in all living microbial cells (**Moeskops *et al.*, 2010**; **Zhao *et al.*, 2010**; **Yuan and Yue, 2012**). What is to retain is the fact that the dehydrogenases reflect the soil's oxidative power, are localized on mitochondria level and implied in the derulation of tricarboxylic acids cycle, being a measure of the total viability microbial's cells, failing to hoard in soil, on extracellular level. They participate at the metabolic reactions generative of ATP, playing a significant role in biological oxidation processes of the organic matter in soil, through the transfer of protons from organically substratum to inorganic acceptants (**Zhang *et al.*, 2010**), a lot of the specific dehydrogenases operating either in the separated transfer's sense of hydrogen, or in then sense of forming the nicotinamid-adenin-dinucleotid or the nicotinamid-adenin-dinucleotid-phosphate (**Subhani *et al.*, 2001**).

The dehydrogenases' activity, as well as the one of microbial proteolitical enzymes varies in function of the prelevation location, of the harvest date and of the type of soil (**Heininger and Tippman, 1995**; Piotrowska and Dlugosz, 2012 quote by **Wolińska and Stepniewska, 2012**; **Tutu *et al.*, 2012**), the dehydrogenasic activity increasing along with the degree pollution, in contrast with the specific activity of proteolitical enzymes, considerably higher in unpolluted places, which can be interpreted, in authors' opinion like a possible enzymatic inhibition (**Nisteriuc, 2012**).

In what concerns the dehydrogenazic activity from the soil samples, harvested from the Tarcau area (Fig.1), we can observe that there is a significant difference, on the one hand in function of the deepness from which were prelevated the soil samples, and on the other hand in function of the period when it was done the harvest. Hereby, as regards the potential dehydrogenase, in the samples harvested in May, it is point out a higher activity in the soil segment 0-10 cm (25.87 µg formazan/g soil), so as to the deepness of 20-30 cm this one being equal with 17.34 µg formazan/g soil, and in the deepest layer (40-50 cm) to represent only 27.82% from the registered value on the surface. In what concerns of the actual dehydrogenase, this one registers values more moderate, presenting the same decreasing aspect from the surface towards the depth of the soil. Thus, if in the harvested samples from the shallow layer, the activity gets to the valoric threshold of 8.34µg formazan/g soil, on 20-30 cm the registered value is of 6.37µg formazan/g soil, and in the bottom layer of 4.98 µg formazan/g soil (of 1.7 times smaller than in the surface samples).

As regards the samples harvested in August, it is remarked the same tendency of decreasing the activity, strongly correlated with the deepness of the layer from where was done the sampling, with higher values in the overlay and diminished in the deep layers in what concerns the potential dehydrogenase (15.39 µg formazan/g soil in the light layer and 6.18 µg formazan/g soil in the deepest layer), while, the actual dehydrogenase presents a certain

uniformity of the activity, no matter the level from where were done the harvests (approximately 4μg formazan/g soil).

Our results concords with those from the specialty literature which underlines the fact that the deepness of the soil is one of the best known and popular factors that diminish the dehydrogenase’s level from the soil, the biggest abundance of microorganisms being on the layer’s surface (up to an deepness of 30 cm), while, in its deepness, the number of microbial cells is limited and, consequently the dehydrogenase’s activity level present a tendency of decreasing (Agnelli *et al.*, 2004; Levyk *et al.*, 2007; Wolińska, 2010).

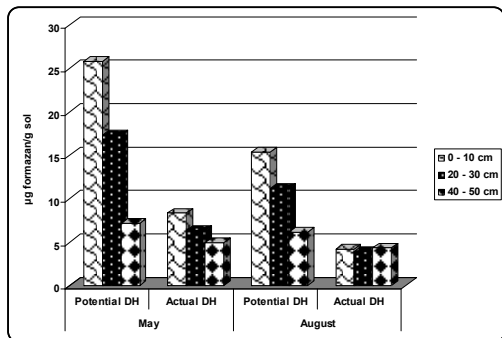


Fig.1. The dehydrogenase activity of the soil in May and August - the Tarcau area

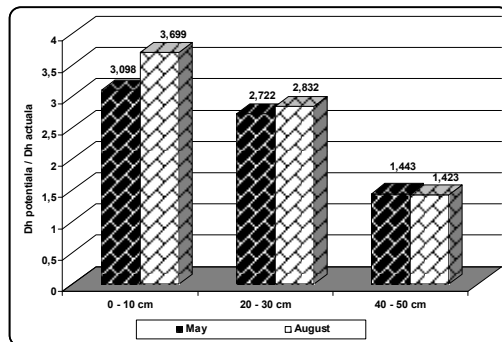


Fig.2. The rapport of potential dehydrogenase / actual dehydrogenase - the Tarcau area

As well, Wolińska and Stępniewska (2012) indicates that the highest values of dehydrogenases’ activity were identified in the overlay (0-20cm), while in the deeper parts (40-60cm) the activity of those enzymes was diminished with 95% in close connection with the deepness from where was harvested.

In the next stage we had recourse to the rapport calculation between the potential and actual dehydrogenase activity. Thus, as we can observe also from the graphic representation (Fig. 2), in the overlay, the potential dehydrogenase is of 3 times higher than the actual dehydrogenase on harvested samples in May and of 3.7 times higher on those harvested in August, while, in the deeper layers, the potential dehydrogenase is approximately of 2.8 times, respectively, of 1.4 times higher than the actual. It can be ascertain, so, the fact that the difference between the potential dehydrogenase and the actual one is significant high, which can be explain through the sequence of an intense respiratory process in the soil, taking into account also the supplementary flux of nutritive material (the ad of glucose to determine the activity of potential dehydrogenase), namely the challenging action of carbon assimilation by the microorganisms in the enzymatic synthesis’ process (Crișan *et al.*, 2001; Treitli *et al.*, 2011).

The literature data report the employment of potential enzymatic activity as a index of soil’s productivity or of microbial activity (Alef *et al.*, 1995; Dick *et al.*, 1996), in the soil samples harvested from Tasca area (Fig. 3), in May, highlighting a potential dehydrogenase activity a little more important, with values between 29.57μg formazan/g soil (in 0-10 cm layer) and 8.26 μg formazan/g soil (in 40-50 cm layer), while, in August, varies from 18.17 μg formazan/g soil in the overlay, to 15.46 μg formazan/g soil in the medium layer and 6.48 μg formazan/g soil in the bottom one.

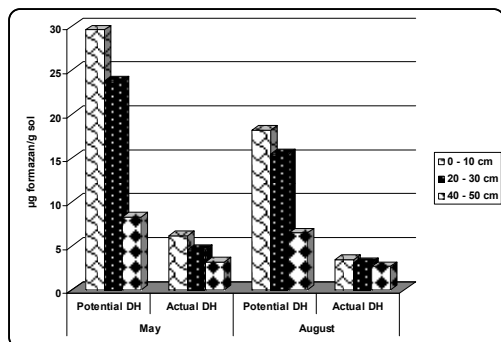


Fig.3. The dehydrogenase activity of the soil in May and August - the Tasca area

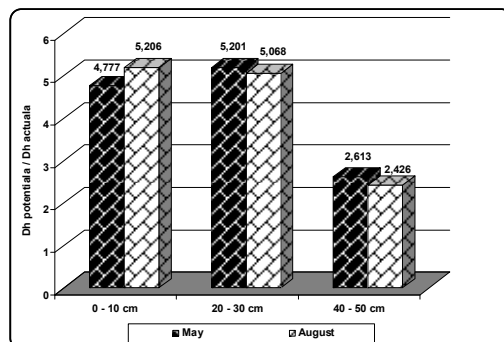


Fig. 4. The rapport of potential dehydrogenase / actual dehydrogenase - the Tasca area

In what concerns the actual dehydrogenase, we can remark values a little more considerable in the case of harvested samples from the shallow layer of the soil, in May (6.19 µg formazan/g soil), while, on the rest of samples, the enzymatic activity was uniform, varying in the interval 2.67 - 4.56 µg formazan/g soil.

From the rapport’s analyze potential dehydrogenase/ actual dehydrogenase – enzymes marker of global biological activity, we can emphasize the existence of a significant difference between the maximum action capacity of microbial biomass and the actual enzymatic activity, on the samples from Tasca area the difference between the two types of activity being significant, the potential dehydrogenase registering an approximately 5 times higher activity than the actual one in the first two layers taken into study and of 2.5 times higher on 40-50 cm deepness (Fig. 4).

CONCLUSIONS

The analyze of experimental results regarding the dehydrogenases’ activity from soil samples harvested from Tarcau and Tasca area shows the existence of some significant differences in the behavior manner of those oxidoreductases, explicable, probably, through the different influence of some factors like the type of soil and its humidity degree, the temperature and the soil’s pH, its aeration degree, the organic matter’s dissonibility and the influence of anthropic activities, any exploitation activity or pollutant source, disturbing the ecosystem’s equilibrium and, consequently the microbial oxido-reductant capacity.

The higher values of the rapport between the potential dehydrogenase and the actual dehydrogenase, registered in Tasca area could due to not necessary to a higher pollution degree (the existence of the cement factory), but to some possible differences in the bacterial physiology (including the cellular wall or the dehydrogenase system, signaling different dehydrogenase systems, in different microorganisms - **Praveen-Kumar, 2003**).

In the interpretation of experimental results should be taken into account on one hand of the bacteria type that colonize the soils (the development of some of them may requiring another genre of nutritive substratum than that of hydro-carbonic nature, like the glucose used by us), and on the other hand by the fact that the investigations were done in lab conditions and not *in situ* and there are affected by the method’s limit used for the evaluation and the testing of enzymatic activity’s levels.

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