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BIOCHEMICAL INDICATORS IN SOME MICROBIOTA SOILS CULTIVATED WITH CUCUMBERS

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Abstract. The study discusses some biochemical investigations (dry matter and water contents, calcined residue, pH, the current and potential dehydrogenase) devoted to a soil (uncultivated and cultivated, unfertilized and fertilized with natural fertilizer at different stages of culture development) harvested from a greenhouse intended for the production of cucumbers. The analysis of the obtained results highlights the complexity of the processes taking place in the soil. These processes depend on the growth and development of plant organisms. Involved in biological oxidation processes, soil dehydrogenase activity provides correlated information related to its health.

INTRODUCTION

Soil is a dynamic, living system whose function is dependent on its condition. The biological component of soil health depends on the number, diversity and health of the present macro-, meso- and microflora. Lately, there is a particular interest in the idea of "functional redundancy" of the microorganisms existing in the soil (Shepherd *et al.*, 2000). The hypothesis is that the number of species of organisms required for the proper functioning of soil processes is inferior to what occurs naturally in most soils (Wardle and Giller, 1996).

From a biological point of view, the soil is an enzymatic system in which the enzymes accumulated together with the enzymes released by the soil microorganisms participate in the biological cycles of the elements thus contributing to soil fertility and implicitly to the creation of conditions for plant nutrition. Enzymes may be indicators of soil quality because they can indicate the microbial activity and implicitly the degree of degradation. Plants provide organic substrates (carbohydrates, amino acids etc.) to the microorganisms involved in the microbiological degradation and they, in turn, produce and excrete their own oxidoreductive enzymes that play an active role in biodegradation (Cunningham and Ow, 1996; Radwan *et al*., 2000).

Enzymes in the soil are subject to complex biochemical processes involving integrated and ecologically connected syntheses that determine soil metabolism. The level of enzymatic activity in the soil system varies due to the fact that in each soil type the amount of organic matter, the composition and activity of living organisms and the intensity of biological processes differ.

Dehydrogenases play an important role in the biological oxidation of organic matter in the soil by the transfer of protons and electrons between substrate and acceptors as well as in the microbial activity measurement (Garcia et al., 1997; Garcia et al., 1998; Garcia et al., 2000). Dehydrogenase activity also provides correlative information on microbial populations in the soil. These enzymes are among the most important endocellular enzymes involved in ATP-producing metabolic reactions and are thought to not accumulate extracellularly in the soil, but exist in intact cells (Denton, 2009).

The aim of the paper is to evaluate the activity of dehydrogenases, in correlation with other biochemical indicators, from soils fertilized with organic fertilizers and non - fertilized, taken from a greenhouse for the cultivation of cucumbers. The dehydrogenase assessment provides information on the biological state of the soil. Assessment of dehydrogenases provides information on the biological state of the soil because, while using oxygen and other electron acceptors, soil dehydrogenase activity is intensified under anaerobic conditions.

MATERIAL AND METHODS

The biological material to be analysed is represented by the soil harvested from a personal property garden in the commune of Matca, located in the central area of Galați County, greenhouse intended for the production of cucumbers. Soil harvesting is a particularly important step in the analysis process because the harvested samples must be representative and at the same time should not introduce changes in the soil composition and qualities due to a faulty technique or improper material preparation conditions.

Considering the fact that the area from which soil samples were collected for biochemical determinations is between 2000 – 5000 m^2, the number of samples required for the average was 2-3 sub-samples. Soil samples were harvested

when they had a low degree of moisture. To this end, we removed the surface layer of soil to a depth of 15 - 20 cm and we harvested 500 g of soil sample from three different points located in relation to the other in the form of chess. Then we removed the vegetal remains (roots, herbs, leaves) and the impurities (splinters, splinters) and we mixed the samples for homogenization. Of the total amount, we have retained about 500 g of soil representing the average sample.

Samples were harvested from unfertilized soils and fertilized with natural fertilizer at different stages of culture development:

1. uncultivated, unfertilized soil;
2. uncultivated soil, fertilized with natural fertilizer;
3. cultivated soil, unfertilized, plants in the vegetative stage;
4. cultivated soil, fertilized with natural fertilizer, plants in the vegetative stage;
5. cultivated, unfertilized soil, plants in the flowering stage;
6. cultivated soil, fertilized with natural fertilizer, plants in the flowering stage;
7. cultivated, unfertilized soil, plants in fruiting stage;
8. cultivated soil, fertilized with natural fertilizer, plants in the fruiting stage.

After sampling, the soil was transported to the laboratory. After the determination of dry matter and water content, it was distributed in Petri dishes where it was maintained to dry under physiological conditions. After drying, the soil was shredded and sifted.

The *dry matter* and *water contents* are determined by the gravimetric method. This basically consists of evaluating the indicator by keeping the biological material at a temperature of 105°C to constant weight. The results are expressed in g of dry matter per 100 g of freshly analysed material. By difference, the amount of water contained in the biological material to be analysed is evaluated.

The method for determining the *calcined residue at 550°C* consists, in principle, in maintaining the sample to be analysed, a determined time at 525 ± 25°C (Mănescu et al., 1978). Keeping the sample to be analysed at the calcination temperature leads to the loss of organic substances and some of the volatile mineral substances.

The results, representing the average of three consecutive determinations, are expressed in g of calcined residue/100 g of soil to be analysed.

To determine the *pH*, we used the electrometric method, basically based on measuring the potential difference between a glass electrode and a reference electrode. The recorded difference varies linearly with the sample pH. The determinations were performed at 20 ± 0.5°C resulting in the average of three repetitions for each experimental variant.

In principle, the method of determining *the current and potential dehydrogenase* activity is based on the ability of these enzymes to transfer hydrogen from different substrates (carboxylic acids, alcohols, carbohydrates) to 2,3,5-triphenyltetrazolium chloride (TTC) which is reduced and switches to red coloured triphenylformazan. Triphenylformazan is extracted with acetone and the colour intensity of the obtained solution, proportional to the dehydrogenase activity, is determined spectrophotometrically (Kiss and Boaru, 1965). To determine the activity of actual dehydrogenases, pre-existing organic substances in the soil will serve as hydrogen donors. To determine the potential dehydrogenase activity, glucose is added to the soil samples, which together with the pre-existing organic substances in the soil will serve as hydrogen donor. Calculation of the current and potential dehydrogenase activity is performed in relation to a calibration curve constructed with TTC reduced to formazan with Zn powder.

RESULTS AND DISCUSSION

In our experimental model, we considered the 1* and 2* samples as control samples for unfertilized and fertilized soils. The analysis of the experimental results indicates the decrease of the dry matter (DM) content of the cultivated soils in both variants, unfertilized and fertilized with organic fertilizer (fig. 1).

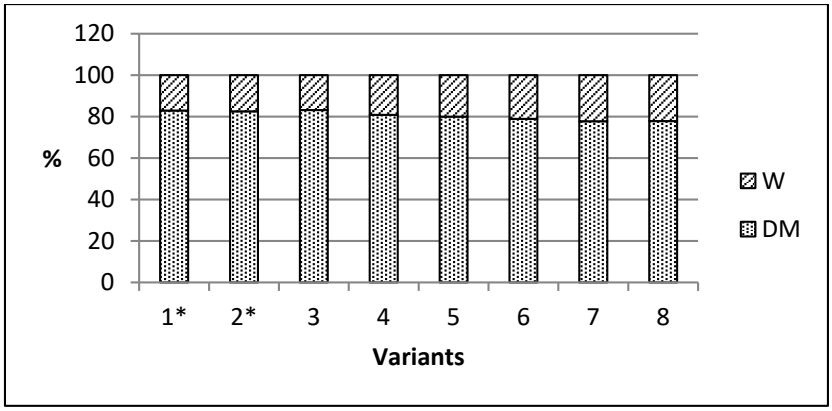

Fig. 1. Dynamics of dry matter (DM) and water content (W) in the soil samples, unfertilized and fertilized with natural fertilizer, harvested at different stages of development of a cucumber culture

In the dynamics of the investigated phenophases, we find the diminishing of the value of the indicator to the fruiting phenophase both in the unfertilized soil version and in the fertilized soil. Considering each phenophase in part, we find the decrease of the dry matter content in the fertilized soils; the diminution becomes almost insignificant in the fruiting phenophase.

The water content of the soils taken to evaluate this biochemical marker has inverse amplitudes to those discussed in the analysis of the dry substance content results.

The results from the determination of the amount of mineral substances are approximate because at the temperature of 525 ± 25°C not only organic substances, but also some of the inorganic substances such as carbonates, nitrates, chlorides or ammonium salts are decomposed. The analysis of the results on unfertilized soils highlights amplitudes describing the trend of diminishing the values of the indicator to fructification phenophase compared to control. The minimum value recorded in fruiting phenophase is 94.09 g% (fig. 2).

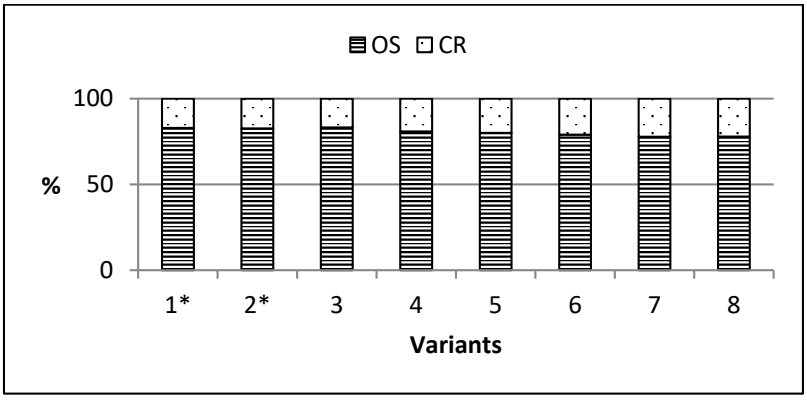

Fig. 2. Dynamics of the calcined residue (CR) and organic substances (OS) in the soil samples, unfertilized and fertilized with natural fertilizer, harvested at different stages of development of a cucumber culture

Compared to uncultivated soil, in the fertilized soils, the values of the amount of mineral substances show a tendency to increase. This is a normal behaviour, considering the higher consumption of organic substances by the plants during their growth and development.

The content of organic substances presents dynamically an inverse aspect to that discussed in relation to the variation in the content of inorganic substances (fig. 2).

The pH, measure of acidity or basicity, is a particularly important feature because it controls many chemical processes that take place in the soil. The value specifically affects the availability of nutrients by controlling their chemical forms. The pH variation curve in unfertilized soil samples records a maximum value in the vegetative stage and a minimum in the flowering phenophase (fig. 3). By comparison with the control, the value of the investigated indicator is superior to the soil taken in the vegetative phenophase of the cucumber culture and diminishes in the case of the soils taken in the flowering and fruiting phenophases.

Fertilized soils have a similar behaviour to non-fertilized soils regarding pH dynamics (fig. 3). The difference is that, although the control samples have identical pH, the fertilized soil samples show in all phenophases higher values of the studied indicator. This behaviour is due to the use of a fertilizer of natural provenance.

The literature mentions that cucumber culture soil should have a slightly acidic to neutral reaction, which requires a pH of 6.5-7.5 (Ghehsareh and Samadi, 2012). The pH value influences the plant's ability to feed the nutrients needed for growth and development.

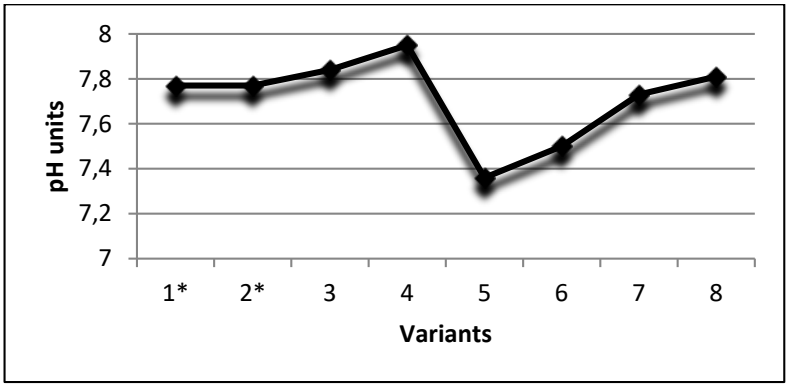

Fig. 3. PH dynamics in the soil samples, unfertilized and fertilized with natural fertilizer, harvested at different stages of development of a cucumber culture

Determination of dehydrogenase activity is a way to immediately assess the metabolic activities of soil microorganisms. Several environmental factors, including soil moisture, oxygen availability, oxidation-reduction potential, pH, organic matter content, depth of the soil profile, temperature, season of the year, heavy metal contamination and soil fertilization or pesticide use can significantly affect the dehydrogenase activity in the soil environment (Wolińska and Stępniewska, 2012; Pandely and Singh, 2006; Brzezinska *et al.*, 1998)

The analysis of current dehydrogenase activity evaluated in unfertilized and fertilized soils, not cultivated and cultivated with cucumbers, generally reveals higher amplitudes of enzyme activity in fertilized soils (fig. 4). The explanation for this behaviour is that the enzyme activity is potentiated by the natural fertilizer that has been applied to the analysed soil. Pesticide studies have

highlighted that, in most cases, they exhibit a temporary inhibitory effect on soil enzymes (Pandely and Singh, 2006). For this reason, we find, in the case of unfertilized soils, a trend of increasing the activity of the investigated enzymes to the fructification stage of the cucumber culture. The maximum enzyme activity is recorded in the final stage of the experiment when the current dehydrogenase activity is 101.81 μg formazan/g analysed soils.

Regarding the fertilized soil, we find that in the vegetative phenophase the activity of the investigated enzyme increases by about 10%, because in the flowering and fructification phenophases the activities recorded for the present dehydrogenases have comparable values of 138.3μg formazan/g and 137.52μg formazan/g, respectively, which mean a decrease of about 34%.

Soil physical conditions have a strong indirect influence on dehydrogenase activity by the changes they make to the soil aeration state. We make these claims because in literature it is known that the dehydrogenase activity is in reverse relation with the air penetration into the pores, the oxygen diffusion rate and the redox potential. As a result, the activity of the enzyme increases with the increase of aerobic activity, which can be translated by the fact that anaerobic or optionally anaerobic members of the microbial association become more important in the total respiratory process of the soil. An important aspect that must be taken into account is that the dehydrogenase activity is in a positive relationship with the soil moisture.

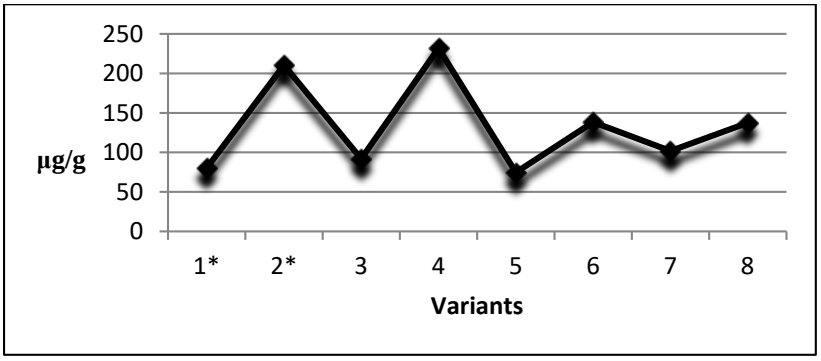

Fig. 4. Current dehydrogenase dynamics in soil samples, unfertilized and fertilized with natural fertilizer, harvested at different stages of development of a cucumber culture

Potential dehydrogenase activity, both in the case of control samples and experimental variants of cultivated soil, shows higher values in cases where the soils are untreated with natural fertilizers (fig. 5). We explain this behaviour by the fact that the fertilization is a factor in disrupting the oxido-reduction reactions involved in the metabolism of microorganism populations as long as the dehydrogenase activity is inhibited by the presence of the products with which the treatment is performed.

In both cases - unfertilized soils and fertilized soils – the samples of cultivated soils have higher values of potential dehydrogenases. The variation curves show increasing trends from vegetative phenophase to fructification, with a slight decrease in flowering phenophase in unfertilized soils, as well as in fruiting phenophase in fertilized soils.

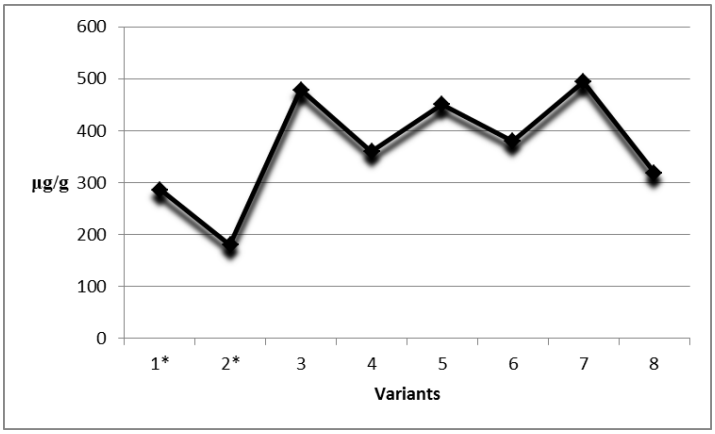

Fig. 5. Potential dehydrogenase dynamics in soil samples, unfertilized and fertilized with natural fertilizer, harvested at different stages of development of a cucumber culture

CONCLUSIONS

The comparative analysis of the results recorded for dry matter quantity in fertilized and non-fertilized soils harvested in different phenophases of growth and development of a cucumber culture, indicates the decrease of the values compared to the control represented by the uncultivated soil. In vegetative and flowering phases, the amount of dry substance diminishes in relation to the control soil.

The amount of mineral substances in non-fertilized soils tends to decrease to fruiting phenophase compared to the control sample. In the case of naturally fertilized soils the tendency is to increase the values of the investigated indicator.

The pH variation curve in unfertilized soil samples records a maximum in vegetative stage and a minimum in flowering phenophase. The pH dynamics in fertilized soils is comparable to that resulting from indicator analysis in unfertilized soils. Recorded amplitudes are superior in fertilized soil samples. According to data from the literature we find optimal levels of pH throughout the experimental model.

The obtained results allow us to appreciate that the current and potential dehydrogenase activities are good indicators of soil biological activity. Enzymes are active in all investigated experimental variants, the particular manifestations being dependent on the fact that the analysed soils are cultivated or not, are treated with natural fertilizer or are taken in a certain phenophase of the cucumber culture. Organic fertilizer, the oldest tool used to improve soil fertility, has to be seen from the point of view of its action due to nutrients as well as soil improvers because it enhances the physical, chemical and biological soil features and thus stimulates many factors of production.

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