# THE EFFECT OF GREEN-MANURE ON SOIL BIOLOGICAL PARAMETERS

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***Abstract***

*Actual and potential dehydrogenase, catalase and nonenzymatic catalytic and phosphatase activities were determined in the 0–10–, 10–20– and 20–30–cm layers of a preluvosoil submitted to a complex fertilisation (green-manure) experiment.*

*It was found that each activity decreased with increasing sampling depth. The fertilisation with green-manure led to a significant increase in each of the seven enzymatic and nonenzymatic activities determined.*

*The enzymatic indicators of soil quality calculated from the values of enzymatic activities depending on the kind of fertilisers, showed the order: lupinus + rape + oat > lupinus > rape + lupinus > vetch + oat + ryegrass > lupinus + oat + vetch > unfertilised plot. This order means that by determination of enzymatic activities valuable information can be obtained regarding fertility status of soils.*

**Keywords:** catalase, dehydrogenase, green-manure, phosphatase

## INTRODUCTION

Soil enzymes are the biological catalysts of innumerable reactions in soils (Dick W.A. et al, 2000). Although some enzymes (e.g. dehydrogenase) are only found in viable cells most soil enzymes can also exist as exoenzymes secreted by microorganisms or as enzymes originating from microbial debris and plant residue that are stabilised in complexes of clay minerals and humic colloides (Criquet S. et al, 2004). Since it is difficult to extract enzymes from soils, enzymes are studied indirectly by measuring the activity via assays (Angers D.A. et al, 2000; Dormaar J.F. and Sommerfeldt T.G., 2006). Nonetheless, studying soil enzyme activities provides insight into biochemical processes in soils and is sensitive as a biological index (Bőhm H. et al, 1991; Haluszczak S. et al, 1991).

The effect of green-manure on soil enzymatic activities were studied in many countries, including Romania (Chiriţă V. et al, 1980; Ştefanic G. 1991; Ştefanic G. et al, 1984). In order to obtain new data on the soil enzymological effects of soil management practices we have determined some enzymatic activities in a preluvosoil submitted to a complex fertilisation experiment at the Agricultural Research and Development Station in Oradea (Bihor county).

The first data regarding the influence of green-manure on this soil were published by (Domuța C. et al, 2004, 2005). They studied the effect of green-manure associated with mineral fertilisation on the physical and chemical properties of a preluvosoil and found that the mixture of the green-manure resulted in higher physical and chemical indicators. They published no paper on the soil enzymological effect of green-manure.

Our results are in good agreement with the literature data reviewed by (Dick R.P. et al, 1994; Kanazawa S., 1986; Ștefan M. and Radu V., 2005; Tang S., 1987; Wright A.L. and Reddy K.R., 2000) and constitute novelties for the enzymological characterization of a preluvosoil submitted to complex management practices.

**MATERIAL AND METHODS**

The ploughed layer of the studied soil is of mellow loam texture, it has a pH value of 5.5, medium humus (23.2%) and P (22 ppm) contents, but it s rich in K (83 ppm).

The experimental field was divided into plots for comparative study of green-manure fertilisation at rates of 47.8 t/ha lupinus, 29.9 t/ha vetch + oat + ryegrass, 39.7 t/ha lupinus + oat, 23.9 t/ha lupinus + rape + oat, 20 t/ha rape, 19.1 t/ha rape + lupinus.

The green-manure was maintained on the soil surface 7 days and after that the land was ploughed. The plots were installed in three repetitions.

In July 2012, soil was sampled from the 0–10–, 10–20– and 20–30– cm depths of the plots under maize crop. The soil samples were allowed to air-dry, then ground and passed through a 2- mm sieve and, finally, used for enzymological analyses.

We have determined six enzymatic activities (actual and potential dehydrogenase, catalase and phosphatase measured in unbuffered, acetate buffer and borax buffer reaction mixtures) and one nonenzymatic catalytic activity ($H_2O_2$ splitting in autoclaved samples).

Actual and potential dehydrogenase activities were determined according to the methods described in (Drăgan-Bularda M., 2000). The reaction mixtures consisted of 3.0 g soil, 0.5 ml TTC (2,3,5-triphenyltetrazolium chloride) and 1.5 ml distilled water or 1.5 ml glucose. All reaction mixtures were incubated at 37ºC for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm.

The reaction mixtures for catalase activity consisted of 3.0 soil, 2.0 ml $H_2O_2$ 3% and 10 ml buffer solution. The buffer solution was prepared as recommended by Drăgan- Bularda M., (2000).

For determination of phosphatase activities, disodium phenylphosphate served as enzyme substrate (Drăgan-Bularda M., 2000; Öhlinger R., 1996).

Three activities were measured: phosphatase activity in unbuffered reaction mixtures, acid phosphatase activity in reaction mixtures to which acetate buffer (pH 5.0) was added and alkaline phosphatase activity in reaction mixtures treated with borax buffer (pH 9.4). The reaction mixtures consisted of 2.5 g sol, 2 ml toluene (antiseptic), 10 ml distilled water or buffer solution and 10 ml 0.5% substrate solution. Reaction mixtures without soil or without substrate solution were the controls. All reaction mixtures were incubated at 37ºC for 2 hours. After incubation, the phenol released from the substrate under the action of phosphatases was determined spectrophotometrically (at 614 nm) based on the colour reaction between phenol and 2,6-dibromoquinone-4-chloroimide.

Dehydrogenase activities are expressed in mg of triphenylformazan (TPF) produced from 2,3,5-triphenyltetrazolium chloride (TTC) by 10 g of soil in 24 hours, whereas catalase and nonenzymatic catalytic activities are recorded as mg of $H_2O_2$ decomposed by 1g of soil in 1 hour. Phosphatase activities are expressed in mg phenol/g soil/2 hours.

The activity values were submitted to statistical evaluation by the two-way *t*-test (Sachs L., 2000).

**RESULTS AND DISCUSSION**

Results of the enzymological analyses are presented in Table 1

*Variation of soil enzymatic activities in dependence of sampling depth.*

It is evident from Table 1 that each enzymatic activity and nonenzymatic catalytic activity decreased with sampling depth in all plots under maize crop. In addition, Table 2 shows that the mean values of each activities also decreased with increasing soil depth.

*Comparison of the three phosphatase activities measured*

At the same soil depths (0–10–, 10–20– and 20–30–cm) in all plots under maize crop, the activities decreased in the order: phosphatase activity measured in unbuffered reaction mixtures > acid phosphatase activity > alkaline phosphatase activity (Table 1). This decreasing order is also valid for the mean values of the three activities (Table 2).

*Enzymatic indicators of soil quality*

Significant ($p < 0.05$ to $p < 0.001$) and unsignificant ($p > 0.05$ to $p > 0.10$) differences were registered in the soil enzymatic activities depending on the type of activity and the nature of green-manure. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the seven plots:

actual dehydrogenase activity: lupinus + rape + oat > rape + lupinus > lupinus > lupinus + oat > vetch + oat + ryegrass > rape > unfertilised plot;

potential dehydrogenase activity: lupinus + rape + oat > lupinus > rape + lupinus > lupinus + oat > vetch + oat + ryegrass > rape > unfertilised plot;

catalase activity: lupinus + rape + oat > vetch + oat + ryegrass > lupinus + oat > lupinus > rape > rape + lupinus > unfertilised plot;

phosphatase activity measured in unbuffered reaction mixtures: vetch + oat + ryegrass > lupinus + oat > lupinus + rape + oat > lupinus > rape > rape + lupinus > unfertilised plot;

acid phosphatase activity: lupinus + rape + oat > vetch + oat + ryegrass > lupinus > lupinus + oat > rape + lupinus > rape > unfertilised plot;

alkaline phosphatase activity: vetch + oat + ryegrass > lupinus + rape + oat > lupinus + oat > lupinus > rape > rape + lupinus > unfertilised plot.

It is evident from these orders that seven plots presented either a maximum or a minimum value of the six soil enzymatic activities. Consequently, these orders don't make it possible to establish such an enzymatic hierarchy of the plots which takes into account each activity for each plot. For establishing such a hierarchy, we have applied the method suggested in (Samuel A.D. et al, 2005). Briefly, by taking the maximum mean value of each activity as 100%, we have calculated the relative (percentage) activities. The sum of the relative activities is the enzymatic indicator which is considered as an index of the biological quality of the soil in a given plot. The higher the enzymatic indicator of the soil, the higher position of the plots is in the hierarchy. Table 2 shows that the first positions are occupied by those plots in which enzymatic activities were the highest. The soil under the unfertilised maize plot occupying the last position can be considered as the last enzyme-active soil.

*Table 1*

The effects of soil management practices on enzymatic and nonenzymatic catalytic activities in a preluvosoil under maize crop

| Soil enzymatic activity* | Soil depth (cm) | Type of green – manure** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ | $V_7$ |
| ADA | 0-10 | 9.01 | 6.95 | 7.31 | 11.82 | 6.10 | 11.56 | 5.52 |
| | 10-20 | 7.31 | 4.59 | 5.61 | 10.20 | 4.70 | 8.50 | 4.52 |
| | 20-30 | 5.10 | 2.72 | 3.91 | 5.76 | 3.40 | 5.10 | 2.72 |
| PDA | 0-10 | 22.78 | 16.66 | 14.28 | 24.28 | 11.22 | 16.32 | 10.60 |
| | 10-20 | 15.30 | 10.20 | 11.22 | 16.66 | 9.50 | 12.24 | 9.41 |
| | 20-30 | 8.33 | 8.16 | 10.37 | 15.30 | 8.67 | 9.86 | 7.88 |
| CA | 0-10 | 1.98 | 2.07 | 1.96 | 2.44 | 1.79 | 1.09 | 0.89 |
| | 10-20 | 1.79 | 1.95 | 1.85 | 2.23 | 1.33 | 1.07 | 0.83 |
| | 20-30 | 1.60 | 1.95 | 1.67 | 2.03 | 0.95 | 0.92 | 0.71 |
| CAn | 0-10 | 0.51 | 0.56 | 0.54 | 0.55 | 0.56 | 0.51 | 0.51 |
| | 10-20 | 0.52 | 0.54 | 0.51 | 0.46 | 0.50 | 0.49 | 0.48 |
| | 20-30 | 0.41 | 0.54 | 0.44 | 0.36 | 0.45 | 0.44 | 0.45 |
| UPA | 0-10 | 2.87 | 2.97 | 2.94 | 2.96 | 2.83 | 2.80 | 2.77 |
| | 10-20 | 2.84 | 2.96 | 2.92 | 2.91 | 2.79 | 2.76 | 2.61 |
| | 20-30 | 2.81 | 2.93 | 2.90 | 2.87 | 2.67 | 2.60 | 2.55 |
| AcPA | 0-10 | 2.85 | 2.94 | 2.81 | 2.96 | 2.81 | 2.79 | 2.69 |
| | 10-20 | 2.81 | 2.87 | 2.75 | 2.89 | 2.69 | 2.75 | 2.38 |
| | 20-30 | 2.74 | 2.81 | 2.69 | 2.85 | 2.20 | 2.32 | 2.30 |
| AlkPA | 0-10 | 1.72 | 1.97 | 1.90 | 1.94 | 1.85 | 1.71 | 1.67 |
| | 10-20 | 1.53 | 1.93 | 1.67 | 1.84 | 1.38 | 1.35 | 1.31 |
| | 20-30 | 1.40 | 1.83 | 1.51 | 1.76 | 1.34 | 1.31 | 1.29 |

* ADA – Actual dehydrogenase activity
PDA – Potential dehydrogenase activity
CA – Catalase activity
CAn – Nonenzymatic catalytic activity
UPA – Phosphatase activity measured in unbuffered reaction mixtures
Ac PA – Acid phosphatase activity
Alk PA – Alkaline phosphatase activity

$V_1$ - Lupinus
$V_2$ – Vetch + oat + ryegrass
$V_3$ – Lupinus + oat
$V_4$ – Lupinus + rape + oat
$V_5$ - Rape
$V_6$ – Rape + lupinus
$V_7$ – Unfertilised plot

*Table 3*

Enzymatic indicators of soil quality

| Position | Plot | Enzymatic indicator of soil quality |
|---|---|---|
| 1 | Lupinus + rape + oat | 594.93 |
| 2 | Lupinus | 441.49 |
| 3 | Rape + lupinus | 421.69 |
| 4 | Vetch + oat + ryegrass | 421.36 |
| 5 | Lupinus + oat | 425.86 |
| 6 | Rape | 370.66 |
| 7 | Unfertilised plot | 347.29 |

## CONCLUSIONS

The soil enzymatic activities decreased with increasing sampling depth.

The soil phosphatase activities decreased in the order: phosphatase activity measured in unbuffered reaction mixtures > acid phosphatase activity > alkaline phosphatase activity.

The enzymatic indicators of soil quality calculated from the values of enzymatic activities determined in the plots under maize crop showed the order: lupinus + rape + oat > lupinus > rape + lupinus > vetch + oat + ryegrass > lupinus + oat > rape > unfertilised plot.

## REFERENCES


1. Angers, D. A., Bissonnette, N., Légére, A., Samson, N., 2000, Microbial and biochemical changes induced by rotation and tillage in a soil under barley production, Can. J. Sci., 73, pp. 39-50.
2. Bőhm, H., Grocholl, J., Ahrens, E., 1991, Mikrobiologiche Beurteilung von Bodenbearbeitungssystem am Beispiel dreier Bodentypen, *Z.* Kulturtechn. Landentwicklung., 32, pp. 114-120.
3. Chiriță, V., Eliade, G., Ştefanic, G., 1980, Modificarea unor indici biologici şi chimici ai fertilității solului sub acțiunea îngrăşămintelor, An. Inst. Cercet. Cereale Plante Tehn. (Fundulea), 46, pp. 379-386.
4. Criquet, S., Ferre, E., Farnet, A.M., Le petit, J., 2004, Annual dynamics of phosphatase activities in an evergreen oak litter: influence of biotic and abiotic factors., Soil Biol. Biochem., 36, pp. 1111-1118.
5. Dick, W.A., Cheng, L., Wang, P., 2000, Soil acid and alkaline phosphatase activity as a pH adjustment indicator, Soil Biol. Biochem., 32, pp. 1915-1919.
6. Dick, R. P., Sandor, J. A., Eash, N. S., 1994, Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru*,* Agric. Ecosyst. Environ., 50, pp. 123-131.
7. Domuța, C., Ciobanu, G., Ciobanu, C., Şandor, M., Sabău, N. C., 2004, Research regarding the green-manure technology and the influences in maize yield in the contest of the sustainable agriculture practicing in Western Romania, In: 15$^{th}$ Int. Symp. of the Sci. Center of Fert. (Pretoria), pp.115-126.
8. Domuța, C., Şandor, M., Bandici, G., Domuța, C., 2005, The influence of green-manure on soil properties and on maize yield in conditions of the Crişurilor Plain,

In: Tehnologii de cultură pentru grâu şi porumb în condiţiile sistemului de agricultură durabilă, Ciobanu, G., Domuţa, C., Lazányi, J. (eds.), Ed. Univ. Oradea, pp. 261-275.
9. Dormaar, J. F., Sommerfeldt, T. G., 2006, Effect of excess feedlot manure on chemical constituents of soil under non-irrigated and irrigated management, Can. J. Soil Sci. 66, pp. 303-313.
10. Drăgan-Bularda, M., 2000, Lucrări practice de microbiologie generală, Univ. Babeş-Bolyai, Cluj-Napoca, pp. 175-179.
11. Haluszczak, S., Schrőder, W., Vetter, L., 1991, Dehydrogenaseaktivität in biologisch und konventionell bewirtschafteten Bőden Schleswig-Holsteins, Schr. Naturwiss. Ver Schleswig-Holstein, 61, pp. 55-79.
12. Kanazawa, S., 1986, Seasonal changes of the numbers, biomass and activities of microorganisms in paddy soils under different fertilizer management, In: 13th Congr. Int. Soc. Soil Sci. (Hamburg), 2, pp. 592-593.
13. Őhlinger, R., 1996, Phosphomonoesterase activity with the substrate phenylphosphate, In: Schinner, F., Őhlinger, R., Kandeler, E., Margesin, R., (eds.), Methods in Soil Biology, Springer, Berlin, pp. 210-213.
14. Sachs, L.,2000, Statistiche Auswertungsmethoden, Springer, pp. 384.
15. Samuel, A. D., Drăgan-Bularda, M., Domuţa, C., 2005, Enzymatic activities in a brown luvic soil, Stud. Univ. Babeş-Bolyai, Biol. 50 (1), pp. 119-125.
16. Ştefan, M., Radu, V., 2005, Researches concerning the influence of organic and chemical fertilizers upon the chemical composition at the cultivated maize in the conditions of the brown-reddish soil from the central area of Oltenia, In: Bul. U.S.A.M.V., vol. 61, Marghitaş, L.A. (ed.), Academic Press, Cluj-Napoca, pp. 231-234.
17. Ştefanic, G., 1991, Enzimologia solurilor cultivate, In: Kiss, S., Ştefanic, G., Paşca, D., Drăgan-Bularda, M., Zborovschi, E., Crişan, R., Enzimologia mediului înconjurător, vol. 1, Ed. Ceres, Bucureşti, pp. 65-71.
18. Ştefanic, G., Eliade, G., Chirnogeanu, I., 1984, Researches concerning a biological index of soil fertility, Fifth Symp. on Soil Biology (Iaşi, 1981), Rom. Natl. Soc. Soil Sci., Bucharest, pp. 35-45.
19. Tang, S.,1987, Microbiological characteristics and biological activity of albic soils, Acta Pedol. Sin., 24, pp. 239-247.
20. Wright, A.L., Reddy, K.R., 2001, Phosphorous loading effects on extracelullar enzyme activity in Everglades Wetland soils. Soil Sci. Soc. Am. J., 65, pp. 588-595.