EFFECT OF GREEN MANURE ON SOIL ENZYME ACTIVITIES IN RELATION TO SOIL PHYSICAL AND CHEMICAL PROPERTIES

EFECTUL FERTILIZĂRII CU ÎNGRĂŞĂMÂNT VERDE ASUPRA PROPRIETĂŢILOR ENZIMATICE, FIZICE ŞI CHIMICE ALE SOLULUI

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Abstract: *Soil enzyme activities (actual and potential dehydrogenizing, catalase, acid and alkaline phosphatase) were determined in the 0–10, 10–20, and 20–30 cm layers of a preluvosoil submitted to a complex fertilization experiment with different types of green manure. It was found that each activity decreased with increasing sampling depth. It should be emphasized that green-manuring of maize led to a significant increase in each of the five enzymatic activities determined. The enzymatic indicators of soil quality calculated from the values of enzymatic activities showed the order: lupinus + rape + oat > lupinus > vetch + oat + ryegrass > lupinus + oat > rape + lupinus >rape > unfertilized plot. This order means that by determination of enzymatic activities valuable information can be obtained regarding fertility status of soils. There were significant correlations of soil enzyme activities with physical and chemical properties.*

Rezumat: *Activităţile enzimatice (dehidrogenazică actuală şi potenţială, catalazică, fosfatazică acidă şi alcalină) au fost determinate la trei adâncimi: 0-10, 10-20 şi 20-30 cm într-un preluvosol supus unui experiment complex de fertilizare cu îngrăşământ verde. Activităţile enzimatice studiate scad cu adâncimea. Fertilizarea cu îngrăşământ verde a determinat creşteri semnificative ale activităţilor enzimatice studiate. Cu ajutorul indicatorilor enzimatici ai calităţii solului, care iau în considerare toate enzimele studiate, am stabilit o ierarhie a parcelelor cultivate: lupin + rapiţă + ovăz > lupin > măzăriche + ovăz + raigras > lupin + ovăz > rapiţă + lupin >rapiţă > parcelă nefertilizată. Această ierarhie furnizează informaţii valoroase privind fertilitatea preluvosolului, soluri slab fertile. S-au stabilit corelaţii semnificative între parametrii biologici, fizici şi chimici ai solului.*

Key words: *catalase, dehydrogenase, green manure, phosphatase, preluvosoil*
Cuvinte cheie: *catalaza, dehidrogenaza, fosfataza, îngrăşământ verde, preluvosol*

INTRODUCTION

Soil micro organisms, the living component of the soil, usually occupy less than 1% of the soil volume, while their number and efficiency are very high. They colonize mainly the organic matter at the micro sites (BALOTA et al., 2003). Clay minerals also serve as carrier of organisms, enzymes and metabolic products. The number and activity of soil micro organisms are dependent on plant growth (species composition, soil cover, root penetration of the soil), soil type, soil treatment, soil cultivation as well as on the macro- and microclimate at each locate (DICK, 1992). The metabolic activity of soil micro organism is essential for organic matter turnover. The mobilization and immobilization of inorganic nutrients and trace elements are also mainly a result of microbial activities (KANDELER and MURER, 1993).

Special enzymes catalyze the organic matter turnover (BANDICK and DICK, 1999). These enzymes are produced by the organisms and act intra- or extra cellular. Soil enzymes catalyze reactions in soils that are important in cycling of nutrients such as C, N, P, and S. Accumulated enzymes are primarily of microbial origin but may also originate from plant and animal residue. Soil enzymes form a part of the soil matrix as exoenzymes and as endoenzymes in viable cells. Soil enzyme activities commonly correlate with microbial parameters and have

been shown to be a sensitive index of long-term management effects such as crop rotations, animal and green manures and tillage (CANARUTTO et al., 1995).

The measurement of soil enzymes can be used as indicative of the biological activity or biochemical process (DICK et al., 1988). Soil enzyme activities have potential to provide a unique integrative biological assessment of soils because of their relationship to soil biology, easy of measurement and rapid response to changes in soil management (KIRCHNER et al., 1993).

The effects of green manure on soil enzymatic activities were studied in many countries (CLARHOLM and ROSENGREN-BRINCK, 1995; DENG and TABATABAI, 1997). In order to obtain new data on the soil enzymologic effects of soil management practices we have determined some enzymatic activities in a brown luvic soil submitted to a complex fertilization experiment at the Agricultural and Research and Development Station in Oradea, Bihor county, Romania.

MATERIALS AND METHODS

The ploughed layer of the studied soil is of mellow loam texture, it has a pH value of 5.5 and medium humus content (23.2%). The experimental field was divided into plots for comparative study of green manure fertilization at rates of 47.8 t / ha lupinus (*Lupinus angustifolius* L.), 29.9 t / ha vetch (*Vicea dumetorum* L.) + oat (*Avena sativa* L.) + ryegrass (*Lolium perenne* L.), 39.7 t / ha lupinus + oat, 23.9 t / ha lupinus + rape (*Brassica rapa* L.) + oat, 20 t / ha rape, and 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 2007 soil was sampled from the 0–10, 10–20 and 20–30 cm depths of the plots under maize (*Zea mays* L.) crop. The soil samples were allowed to air dry, then ground and passed through a 2 mm sieve and, finally, used for enzymologic analyses.

Two enzymatic activities (actual and potential dehydrogenase) were determined according to the methods described in (SAMUEL and KISS, 1999). 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.

Catalase activity has been determined using the permanganometric method (SAMUEL and KISS, 1999). Catalase activity is expressed as mg of H_2O_2 decomposed by 1g of soil in 1 hour.

For determination of phosphatase activities, disodium phenylphosphate served as enzyme substrate. Two activities were measured: 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 buffer solutions were prepared as recommended by (OHLINGER, 1996). Phosphatase activities are expressed in mg phenol/g soil/2 hours. Physical and chemical indicators were determined according to the methods described in (EGNER et al., 1980).

The activity values were submitted to statistical evaluation by the two *t*-test (SACHS, 2002) and the correlations between the enzymatic activities and physical indicators were determined according to the methods described in (DICK et al., 1994).

RESULTS AND DISCUSSION

Results of the enzymological analyses are presented in Table 1.

Variation of the enzymatic activities in dependence of sampling depth

It is evident from Table 1 that each enzymatic activity decreased with sampling depth in all plots under maize crop.

Table 1

The effect of different types of green manure on enzymatic activities in a brown luvic soil

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
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.
AcPA – Acid phosphatase activity.
AlkPA – 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 – Unfertilized plot.

Enzymatic indicators of soil quality

Significant ($p < 0.05$ to $p < 0.001$) and insignificant ($p > 0.05$ 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 > unfertilized plot;

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

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

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

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

It is clear from these orders that seven plots presented either a maximum or a minimum value of the six soil enzymatic activities. Consequently, these orders do not 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 and KISS, 1999). 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 soil quality, the higher position of plot 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 unfertilized maize plot occupying the last position can be considered as the last enzyme-active soil.

Table 2

Enzymatic indicators of soil quality

Position	Plot	Enzymatic indicator of soil quality
1	Lupinus + rape + oat	496.32
2	Lupinus	417.43
3	Vetch + oat + ryegrass	401.66
4	Lupinus + oat	389.11
5	Rape + lupinus	370.60
6	Rape	331.57
7	Unfertilized plot	290.48

Results of the physical and chemical analyses are presented in Tables 3 and 5. Simple correlations between enzymatic activities and physical and chemical properties in the 0-10 cm layer (Tables 4 and 6) showed that soil enzyme activities were significantly correlated with physical and chemical properties. This indicates that enzyme activities were associated with active micro organisms in soil which are the major source of soil enzymes. The activities of all five enzymes were significantly intercorrelated which suggest that green manure has similar effects on the activities of those enzymes involved in intracellular metabolism and in P cycling in soil.

CONCLUSIONS

1. The soil enzymatic activities decreased with increasing sampling depth.

2. 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 > vetch + oat + ryegrass > lupinus + oat > rape + lupinus > rape > unfertilized plot.

3. Each of the five enzymatic activities was positively correlated with the physical and chemical indicators.

Table 3

The effect of different types of green manure on physical properties in a brown luvic soil

Physical properties	Soil depth (cm)	Type of green manure*						
		V_1	V_2	V_3	V_4	V_5	V_6	V_7
Soil density (g/cm^3)	0-10	1.41	1.40	1.39	1.38	1.43	1.42	1.44
Porosity (%)	0-10	12.7	13.2	14.0	14.5	11.4	12.0	10.9
Resistance to penetration (kg/cm^2)	0-10	20.6	20.1	20.5	19.5	21.7	21.5	25.6
Coefficient of filtration (mm/h)	0-10	15.97	16.19	16.91	16.57	14.32	14.36	13.85

*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 – Unfertilized plot.

Table 4

Simple correlations (r) between soil enzyme activities and physical properties in the 0-10 cm depth

Vari-ables***	ADA	PDA	CA	AcPA	AlkPA	SD	Po	RP
ADA	-	-	-	-	-	-	-	-
PDA	0.758*	-	-	-	-	-	-	-
CA	0.248**	0.646**	-	-	-	-	-	-
AcPA	0.645*	1.559**	0.909**	-	-	-	-	-
AlkPA	0.034**	0.457**	0.815*	0.824**	-	-	-	-
SD	0.509**	0.689**	0.833**	0.690**	0.644**	-	-	-
Po	0.623**	0.684*	0.848**	0.868**	0.820**	0.981**	-	-
RP	0.505**	0.754**	0.862**	0.938**	0.837**	0.836**	0.824**	-
CF	0.277**	0.793*	0.870**	0.832**	0.903**	0.927**	0.950**	0.797*

* Significantly at $P \leq 0.05$. ** Significantly at $P < 0.001$.

*** ADA – Actual dehydrogenase activity. PDA – Potential dehydrogenase activity. CA – Catalase activity. AcPA – Acid phosphatase activity. AlkPA – Alkaline phosphatase activity. SD – Soil density. Po – Porosity. RP – Resistance to penetration. CF – Coefficient of filtration.

Table 5

The effect of different types of green manure on chemical properties in a brown luvic soil

Chemical properties	Soil depth (cm)	Type of green manure*						
		V_1	V_2	V_3	V_4	V_5	V_6	V_7
Available P (mg P_2O_5/ 100g soil)	0-10	37.4	38.7	38.8	38.9	37.5	37.6	34.1
Available K (mg K/100g soil)	0-10	210.1	213.1	213.9	214.0	211.4	212.0	209.2
N-NO_3 (mg N/kg soil)	0-10	1.03	0.80	1.05	1.08	0.70	0.71	0.68
N-NH_4 (mg N/kg soil)	0-10	4.25	4.41	4.61	4.80	3.12	3.30	2.50

*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 – Unfertilized plot.

Table 6

Simple correlations (r) between soil enzyme activities and chemical properties in the 0-10 cm depth

Variables***	ADA	PDA	CA	AcPA	AlkPA	Available		$N\text{-}NO_3$
						P	K	
ADA	-	-	-	-	-	-	-	-
PDA	0.758*	-	-	-	-	-	-	-
CA	0.248**	0.646**	-	-	-	-	-	-
AcPA	0.645*	1.559**	0.909**	-	-	-	-	-
AlkPA	0.034**	0.457**	0.815*	0.824**	-	-	-	-
Available P	0.460**	0.522**	0.804**	0.843**	0.809*	-	-	-
Available K	0.404**	0.328**	0.660**	0.713**	0.863**	0.881**	-	-
$N\text{-}NO_3$	0.419**	0.750**	0.764**	0.580**	0.557**	0.621**	0.507**	-
$N\text{-}NH_4$	0.424**	0.230**	0.280**	0.850*	0.856**	0.397**	0.761**	0.876**

* Significantly at $P \leq 0.05$.

** Significantly at $P < 0.001$.

*** ADA – Actual dehydrogenase activity. PDA – Potential dehydrogenase activity. CA – Catalase activity. AcPA – Acid phosphatase activity. AlkPA – Alkaline phosphatase activity.

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