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RESEARCH REGARDING THE GREEN MANURES INFLUENCE ON THE ENZYMATICAL ACTIVITY OF THE SOIL

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Abstract. *Actual and potential dehydrogenase, catalase and nonenzymatic catalytic and phosphatase (measured in unbuffered, acetate buffer and borax buffer reaction mixtures) activities were determined in the 0–10–, 10–20– and 20–30–cm layers of a brown luvic soil 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

1. Introduction

Soil enzymes are the biological catalysts of innumerable reactions in soils. 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. Since it is difficult to extract enzymes from soils, enzymes are studied indirectly by measuring the activity via assays [14,15]. Nonetheless, studying soil enzyme activities provides insight into biochemical processes in soils and is sensitive as a biological index [1,9].
The effect of green-manure on soil enzymatic activities were studied in many countries, including Romania [2,17,18,19]. In order to obtain new data on the soil enzymological effects of soil management practices we have determined some enzymatic activities in a brown luvic soil 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

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by [4, 5]. 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 [13, 10, 16, 20] and constitute novelties for the enzymological characterization of a preluvosoil submitted to complex management practices.

2. Materials 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 [7]. 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 [6].

For determination of phosphatase activities, disodium phenylphosphate served as enzyme substrate [8, 12].

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 [13].

3. Results and discussions

Results of the enzymological analyses are presented in Table 1, and those of the statistical evaluation are summarised in Table 2.

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.

Tabel 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

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 [11].

Table 2. Significance of the differences between enzymatic and nonenzymatic catalytic activities in a preluvosoil submitted to a fertilisation experiment

Fertilisation experiment	Soil enzymatic activity*	Soil depth (cm)	Mean activity values in fertilisation experiment			Significance of the differences
			a	B	a-b	
1	2	3	4	5	6	7
Lupinus (a) versus vetch + oat + ryegrass (b)	ADA	0-30	7.14	4.75	2.39	0.01 > p > 0.001
	PDA		15.47	11.6	3.80	0.50 > p > 0.10
	CA		1.79	7	-0.20	0.50 > p > 0.10
	CAn		0.48	1.99	-0.06	0.50 > p > 0.10
	UPA		2.84	0.54	-0.11	0.01 > p > 0.001
	Ac PA		2.80	2.95	-0.07	0.02 > p > 0.01
	Alk PA		1.55	2.87	-0.36	0.02 > p > 0.001
				1.91		
Lupinus (a) versus lupinus + oat (b)	ADA	0-30	7.14	5.61	1.53	0.02 > p > 0.01
	PDA		15.47	11.9	3.52	0.50 > p > 0.10
	CA		1.79	5	-0.03	0.50 > p > 0.10
	CAn		0.48	1.82	-0.01	0.50 > p > 0.10
	UPA		2.84	0.49	-0.08	0.01 > p > 0.001
	Ac PA		2.80	2.92	0.05	0.01 > p > 0.001
	Alk PA		1.55	2.75	-0.14	0.02 > p > 0.001
				1.69		
Lupinus (a) versus lupinus + rape + oat (b)	ADA	0-30	7.14	9.26	-2.12	0.50 > p > 0.10
	PDA		15.47	18.7	-3.27	0.50 > p > 0.10
	CA		1.79	4	-0.44	0.01 > p > 0.0001
	CAn		0.48	2.23	0.03	p > 0.50
	UPA		2.84	0.45	-0.07	0.02 > p > 0.01
	Ac PA		2.80	2.91	-0.1	0.01 > p > 0.001
	Alk PA		1.55	2.90	-0.36	0.02 > p > 0.01
				1.91		
Lupinus (a) versus rape (b)	ADA	0-30	7.14	4.73	2.41	0.05 > p > 0.02
	PDA		15.47	9.79	4.25	0.50 > p > 0.10
	CA		1.79	1.35	0.44	0.10 > p > 0.05
	CAn		0.48	0.50	-0.02	0.50 > p > 0.10
	UPA		2.84	2.76	0.08	0.50 > p > 0.10
	Ac PA		2.80	2.56	0.24	0.50 > p > 0.10
	Alk PA		1.55	1.52	0.03	p > 0.50
Lupinus (a) versus rape + lupinus (b)	ADA	0-30	7.14	8.38	-1.24	0.50 > p > 0.10
	PDA		15.47	12.8	2.67	0.50 > p > 0.10
	CA		1.79	0	0.77	0.01 > p > 0.001
	CAn		0.48	1.02	0.00	-
	UPA		2.84	0.48	0.12	0.10 > p > 0.05
	Ac PA		2.80	2.72	0.18	0.10 > p > 0.05
	Alk PA		1.55	2.62	0.10	0.50 > p > 0.02
				1.45		
Lupinus (a) versus unfertilised plot (b)	ADA	0-30	7.14	4.25	2.89	0.02 > p > 0.001
	PDA		15.47	9.29	6.18	0.50 > p > 0.10
	CA		1.79	0.81	0.98	0.01 > p > 0.001
	CAn		0.48	0.48	0.00	-
	UPA		2.84	2.64	0.20	0.05 > p > 0.02
	Ac PA		2.80	2.45	0.35	0.10 > p > 0.05
	Alk PA		1.55	1.42	0.13	0.50 > p > 0.10

Vetch + oat + ryegrass (a) versus lupinus + oat (b)	ADA	0-30	4.75	5.61	-0.86	0.10 > p > 0.005
	PDA		11.67	11.95	-0.28	p > 0.50
	CA		1.99	1.82	0.17	0.10 > p > 0.005
	CAn		0.54	0.49	0.05	0.50 > p > 0.010
	UPA		2.95	2.92	0.03	0.02 > p > 0.01
	Ac PA		2.87	2.75	0.12	0.01 > p > 0.001
	Alk PA		1.91	1.69	0.22	0.50 > p > 0.10
Vetch + oat + ryegrass (a) versus lupinus + rape + oat (b)	ADA	0-30	4.75	9.26	-4.51	0.05 > p > 0.02
	PDA		11.67	18.74	-7.07	0.01 > p > 0.001
	CA		1.99	2.23	-0.24	0.50 > p > 0.10
	CAn		0.54	0.45	0.09	0.50 > p > 0.10
	UPA		2.95	2.91	0.04	0.10 > p > 0.05
	Ac PA		2.87	2.90	-0.03	0.10 > p > 0.05
	Alk PA		1.91	1.84	0.07	0.10 > p > 0.05
Vetch + oat + ryegrass (a) versus rape (b)	ADA	0-30	4.75	4.73	0.02	p > 0.50
	PDA		11.67	9.79	1.88	0.02 > p > 0.01
	CA		1.99	1.35	0.64	p > 0.50
	CAn		0.54	0.50	0.04	0.50 > p > 0.10
	UPA		2.95	2.76	0.19	0.05 > p > 0.02
	Ac PA		2.87	2.56	1.24	0.50 > p > 0.10
	Alk PA		1.91	1.52	0.39	0.10 > p > 0.05
Vetch + oat + ryegrass (a) versus rape + lupinus (b)	ADA	0-30	4.75	8.38	-3.63	0.05 > p > 0.02
	PDA		11.67	12.80	-1.13	0.50 > p > 0.10
	CA		1.99	1.02	0.97	0.01 > p > 0.001
	CAn		0.54	0.48	0.06	0.10 > p > 0.05
	UPA		2.95	2.72	0.23	0.05 > p > 0.02
	Ac PA		2.87	2.62	0.25	0.50 > p > 0.10
	Alk PA		1.91	1.45	0.46	0.05 > p > 0.002
Vetch + oat + ryegrass (a) versus unfertilised plot (b)	ADA	0-30	4.75	4.25	0.50	0.10 > p > 0.05
	PDA		11.67	9.29	2.38	0.50 > p > 0.10
	CA		1.99	0.81	1.18	0.10 > p > 0.05
	CAn		0.54	0.48	0.06	0.05 > p > 0.02
	UPA		2.95	2.64	0.31	0.05 > p > 0.02
	Ac PA		2.87	2.45	0.42	0.05 > p > 0.02
	Alk PA		1.91	1.42	0.49	0.05 > p > 0.02
Lupinus + oat (a) versus lupinus + rape + oat (b)	ADA	0-30	5.61	9.26	-3.65	0.10 > p > 0.05
	PDA		11.95	18.74	-6.79	0.10 > p > 0.05
	CA		1.82	2.23	-0.41	0.01 > p > 0.001
	CAn		0.49	0.45	0.04	p > 0.50
	UPA		2.92	2.91	0.01	0.50 > p > 0.10
	Ac PA		2.75	2.90	-0.15	0.01 > p > 0.001
	Alk PA		1.69	1.84	-0.15	0.50 > p > 0.10
Lupinus + oat (a) versus rape (b)	ADA	0-30	5.61	4.73	0.88	0.05 > p > 0.002
	PDA		11.95	9.79	2.16	0.05 > p > 0.002
	CA		1.82	1.35	0.47	0.10 > p > 0.05
	CAn		0.49	0.50	0.01	p > 0.50
	UPA		2.92	2.76	0.16	0.10 > p > 0.05
	Ac PA		2.75	2.56	0.19	0.50 > p > 0.010
	Alk PA		1.69	1.52	0.17	0.50 > p > 0.010
Lupinus + oat (a) versus rape + lupinus (b)	ADA	0-30	5.61	8.38	-2.77	0.10 > p > 0.05
	PDA		11.95	12.80	-0.85	0.50 > p > 0.010
	CA		1.82	1.02	0.80	0.50 > p > 0.010
	CAn		0.49	0.48	0.01	0.05 > p > 0.002
	UPA		2.92	2.72	0.20	0.05 > p > 0.002
	Ac PA		2.75	2.62	0.13	0.50 > p > 0.10
	Alk PA		1.69	1.45	0.24	0.05 > p > 0.002

Lupinus + oat (a) versus unfertilised plot (b)	ADA PDA CA CAn UPA Ac PA Alk PA	0-30	5.61 11.95 1.82 0.49 2.92 2.75 1.69	4.25 9.29 0.81 0.48 2.64 2.45 1.42	1.36 2.66 1.01 0.01 0.28 0.30 0.27	0.05 > p > 0.002 0.05 > p > 0.002 0.05 > p > 0.002 0.50 > p > 0.10 0.05 > p > 0.002 0.50 > p > 0.10 0.05 > p > 0.002
Lupinus + rape+ oat (a) versus rape (b)	ADA PDA CA CAn UPA Ac PA Alk PA	0-30	9.26 18.74 2.23 0.45 2.91 2.90 1.84	4.73 9.79 1.35 0.50 2.76 2.56 1.52	4.53 8.95 0.88 -0.05 0.15 0.34 0.32	0.10 > p > 0.05 0.001 > p > 0.001 0.02 > p > 0.01 0.50 > p > 0.10 0.05 > p > 0.002 0.50 > p > 0.10 0.10 > p > 0.05
Lupinus + rape + oat (a) versus rape + lupinus (b)	ADA PDA CA CAn UPA Ac PA Alk PA	0-30	9.26 18.74 2.23 0.45 2.91 2.90 1.84	8.38 12.80 1.02 0.48 2.72 2.62 1.45	0.88 5.94 1.21 -0.03 0.19 0.28 0.39	0.50 > p > 0.10 0.05 > p > 0.02 0.01 > p > 0.001 p > 0.50 0.01 > p > 0.001 0.50 > p > 0.10 0.05 > p > 0.002
Lupinus+ rape + oat (a) versus unfertilised plot (b)	ADA PDA CA CAn UPA Ac PA Alk PA	0-30	9.26 18.74 2.23 0.45 2.91 2.90 1.84	4.25 9.29 0.81 0.48 2.64 2.45 1.42	5.01 9.45 1.42 -0.03 0.27 0.45 0.42	0.05 > p > 0.02 0.05 > p > 0.02 0.01 > p > 0.001 p > 0.50 0.50 > p > 0.10 0.05 > p > 0.02 0.05 > p > 0.02
Rape (a) versus rape + lupinus (b)	ADA PDA CA CAn UPA Ac PA Alk PA	0-30	4.73 9.79 1.35 0.50 2.76 2.56 1.52	8.38 12.80 1.02 0.48 2.72 2.62 1.45	-3.65 -3.01 0.33 0.02 0.04 -0.06 0.07	0.10 > p > 0.05 0.50 > p > 0.10 0.50 > p > 0.10 0.50 > p > 0.10 0.10 > p > 0.05 0.50 > p > 0.10 0.50 > p > 0.10
Rape (a) versus unfertilised plot (b)	ADA PDA CA CAn UPA Ac PA Alk PA	0-30	4.73 9.79 1.35 0.50 2.76 2.56 1.52	4.25 9.29 0.81 0.48 2.64 2.45 1.42	0.48 0.50 0.54 0.02 0.12 0.11 0.10	0.10 > p > 0.05 0.50 > p > 0.10 0.50 > p > 0.10 0.50 > p > 0.10 0.10 > p > 0.05 0.50 > p > 0.10 0.50 > p > 0.10
Rape + lupinus (a) versus unfertilised plot (b)	ADA PDA CA CAn UPA Ac PA Alk PA	0-30	8.38 12.80 1.02 0.48 2.72 2.62 1.45	4.25 9.29 0.81 0.48 2.64 2.45 1.42	4.13 3.51 0.21 0.00 0.08 0.17 0.03	0.10 > p > 0.05 0.10 > p > 0.05 0.01 > p > 0.001 - 0.50 > p > 0.10 0.50 > p > 0.10 0.05 > p > 0.02

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

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 + lupines	421.69
4	Vetch + oat + ryegrass	421.36
5	Lupinus + oat	425.86
6	Rape	370.66
7	Unfertilised plot	347.29

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 3 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.

Conclusions

(1)The soil enzymatic activities decreased with increasing sampling depth.
(2)The soil phosphatase activities decreased in the order: phosphatase activity measured in unbuffered reaction mixtures > acid phosphatase activity > alkaline phosphatase activity.
(3)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] Bőhm, H., Grocholl, J., Ahrens, E., 1991, Mikrobiologiche Beurteilung von Bodenbearbeitungssystem am Beispiel dreier Bodentypen, *Z. Kulturtechn. Landentwicklung.*, 32, 114-120.

[2] Chiriţă, V., Eliade, G., Ştefanic, G., 1980, Modificarea unor indici biologici şi chimici ai fertilităţii solului sub acţiunea îngrăşămintelor, *An. Inst. Cercet. Cereale Plante Tehn* .(Fundulea), 46, 379-386.

[13] 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, 123-131.

[4] Domuţa, 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: 15th Int. Symp. of the Sci. Center of Fert. (Pretoria), pp.115-126.

[5] Domuţa, C., Şandor, M., Bandici, G., Domuţa, 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.

[6] Drăgan-Bularda, M., 2000a, Determinarea activităţii catalazice a solului, In : *Lucrări practice de microbiologie generală*, Drăgan-Bularda, M. (ed.), Univ. Babeş-Bolyai, Cluj-Napoca, pp. 175-176.

[7] Drăgan-Bularda, M., 2000b, Determinarea activităţii dehidrogenazice a solului, In : *Lucrări practice de microbiologie generală*, Drăgan-Bularda, M. (ed.), Univ. Babeş-Bolyai, Cluj-Napoca, pp. 176-178.

[8] Drăgan-Bularda, M., 2000c, Determinarea activităţii dehidrogenazice a solului, In : *Lucrări practice de microbiologie generală*, Drăgan-Bularda, M. (ed.), Univ. Babeş-Bolyai, Cluj-Napoca, pp. 176-180.

[9] Haluszczak, S., Schrőder, W., Vetter, L.,1991, Dehydrogenaseaktivität in biologisch und konventionell bewirtschafteten Bőden Schleswig-Holsteins, *Schr. Naturwiss. Ver Schleswig-Holstein,* 61, 55-79.

[10] 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.

[11] Kiss, S., Paşca, D., Drăgan,-Bularda, M., Blaga, G., Crişan, R., 1991, Enzymological analysis of lead and zinc mine spoils submitted to biological recultivation, *"Stud. Univ. Babeş-Bolyai", Biol.*, 35 (2), 70-79.

[12] 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.

[13] Sachs, L. , 1968, *Statistiche Auswertungsmethoden*, Springer, pp. 384.

[14] Samuel, A. D., Kiss, S., Sandor, M., 2000, Phosphatase activities in a brown luvic soil, *"Stud. Univ. Babeş-Bolyai", Biol*. 45, (2), 91-99.

[15] Samuel, A. D., Drăgan-Bularda, M., Domuţa, C., 2005, Enzymatic activities in a brown luvic soil, *"Stud. Univ. Babeş-Bolyai", Biol*. 50 (1), 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, Ceres, Bucureşti, pp. 65, 71, 78, 84.

[18] Ştefanic, G., Eliade, G., Chirnogeanu, I., 1983, Influenţa aplicării îndelungate a îngrăşămintelor chimice asupra unor însuşiri biologice ale solului, "Lucr. Conf. Naţ. Ştiinţa Solului" (Brăila, 1982), *Public. Soc. Naţ. Rom. Ştiinţa Solului*, Bucureşti. No. 21B, 87-93.

[19] 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, 35-45.

[20] Tang, S., 1987, Microbiological characteristics and biological activity of albic soils, *Acta Pedol. Sin.*, 24, 239-247.