# SOIL ENZYMATIC ACTIVITIES UNDER THE INFLUENCE OF GREEN MANURE AND MANURE ON ERODED SOIL FROM ORADEA

Domuța Cristian\*, Samuel Alina Dora\*\*, Domuța Cornel\*, Șandor Maria\*, Borza Ioana\*, Vușcan Adrian\*

 \* University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048, Oradea, Romania, e-mail: <u>cristian\_domuta@yahoo.com</u>
\*\* University of Oradea, Faculty of Science, 1 Universitii st., Oradea, Romania, e-mail: <u>samuelalina@rdslink.ro</u>

#### Abstract

The paper is based on the research results carried out in a long term trial placed on an eroded soil in 1999 at the Station for Agricultural Research and Development from Oradea. The experiment had three monitored factors (crop rotation, organic fertilization, annual chemical fertilization) and in 2012 after winter wheat harvesting there were prelevated soil samples from the depth of 0 - 10 cm, 10 - 20 cm, 20 - 30 cm. The actual and potential dehydrogenase activity, the catalase activity, the acid and alkaline phosphatase were determined. The usual methods were used for determination. Enzymological activity decressed acording with increse of the depth. In the variant without organic fertilization were determind the smallest values of the enzymological parameters, the biggest values were determined in the variant with manure 50 t/ha. Both manure and green manure determined bigger values of the enzymological parameters studied than the etalon. Using a mixture of Lupinus angustifolius + oat + rape as green manure there were determined bigger values of the enzymological parameters compared to the the use of Lupinus angustifolius in pure crop. Also using the mixture recommanded by the biology school (vetch + oat + ryegrass) the enzymological parameters were bigger then Lupinus angustifolius in pure crop but smaller than the values determined in the variant with the mixture of Lupinus angustifolius + oat + rape. The mixture of Lupinus angustifolius + oat + rape is recomanded.

Key words: green manure, manure, dehydrogenase, catalase, phosphatase.

#### **INTRODUCTION**

The use of green manure (leguminous especially) is known from antiquity in the Roman Empire and Greece as a main crop or secondary crop (Eliade et al., 1983). Many researches carried out during 1947 - 1963 established the explosive development of the microbiological process due to the small value of the C/N ratio; as consequence the rapid decrease of the soil hummus reserve is registred (Broadbent, Norman, 1947; Stotzky, Mortensen, 1958; Domsch, 1963; quoted by Domuţa, 2005). Of course there were different opinions regarding this subject. But generally speaking it is considered that the effect of green manure is short lived and the process of humus accumulation is not intensified (Eliade Gh. et al., 1983; Neamţu T., 1996).

Bogouslavski, 1997 quoted by Domuţa (2005) established that on a long period of time the effect of green manure had a small influence on the

dehydrogenase activity. Samuel and Domuta determined dehydrogenase activity bigger in the soil fertilized with lupin than in the unfertilized soil with 32% more on 0 - 10, with 24% more on 10 - 20 cm, with 43% more on 20 - 30 cm; the use of the mixture of lupin + oat + rape determined the biggest differences: 114% on 0 - 10 cm, 126% on 10 - 20 cm, 112% on 20 - 30 cm.

The first research regarding the green manure technology and their effect on the fields physical and chemical properties of the soils from the North-Vest of Romania was realised by Domuta starting from 1988 at Pocola. The research continued at Beius from 1990 to 1994 and starting from 1999 in Oradea; The research established the similar effect of the green manure (mixture of lupin + oat + rape or lupin + oat) and manure 25 t/ha; the use of lupin in pure culture determined a smaller harvest of maize and winter wheat and also they have noticed a decrese in water use efficiency. The physical and chemical properties of the soil were improved in the same conditions.

From the microbiological and enzymologic point of view the processes studied by Samuel were in the same trend registered in the physical and chemical soil properties.

#### MATERIAL AND METHOD

The paper is based on the researches carried out at the Agricultural Research and Development Station Oradea. The experiment was placed on an eroded soil with a slope of 10%. The experimental field contains three factors:

Factor A: crop rotation a<sub>1</sub>: winter wheat – maize  $a_2$ : oat + clover - clover - maize - winter wheat Factor B: organic fertilization b<sub>1</sub>: control without fertilization b<sub>2</sub>: lupin  $b_3$ : lupin + oat + rape  $b_4$ : vetch + oat + ryegrass b<sub>5</sub>: manure, 25 t/ha  $b_6$ : manure, 50 t/ha Factor C: annual chemical fertilization  $c_1: N_0 P_0$ c<sub>2</sub>: N<sub>120</sub>P<sub>90</sub> Number of repetitions: 4 The plot surface:  $100 \text{ m}^2$ Total surface of the experiment:  $9600 \text{ m}^2$ 

The soil samples was prelevated in 2012 after the winter wheat harvesting for the first crop rotation. The depths of prelevation were: 0 - 10 cm, 10 - 20 cm, 20 - 30 cm. The soil samples were allowed to air dry, then grinded and passed trough a 2mm sieve and finally, used for enzymological analyses.

Actual dehydrogenase and potential dehydrogenase were determined according to the method described by Kiss S. and D. Paşca et al, 1990. The reaction mixture consists of 3.0 g soil, 0.5 ml TTC (2,3,5 triphenyltetrazolium clorhide) and 1.5 ml distilled water or 1.5 glucose solution respectively, for potential dehydrogenase. All reaction mixtures incubated at 37° C for 24 hours. After incubation the were triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm. Dehydrogenase activities are expressed triphenylformazan (TPF) of produced from in mg 2.3.5 triphenyltetrazolium clorhide (TTC) by 10 g of soil in 24 hours.

For the determination of catalase activity the permanganatic method was used according the procedure recommanded by Kiss et al, 1991. The reaction mixture consists of 3.0 g soil, 2.0 ml H<sub>2</sub>O<sub>2</sub> 3% and 10 ml phosphate buffer. It sufferd incubation at 37° C for 1 hour. Catalase activity is expressed as mg of H<sub>2</sub>O<sub>2</sub> decomposed by 10 g of soil in 1 hour.

Disodium phenylphosphate was used as enzyme substrate for determining of phosphate activity. Two activities were mesured: acid phosphatase in reaction mixtures to wich acetate buffer (pH 5.0) was added and alkaline posphatase activity in reaction mixture treated with borax buffer (pH 9.4). The buffer solutions were prepared as recomanded by Ohlinger R, 1996. The reaction mixture consisted of 2.5 g soil, 2 ml toluene (antiseptic), 10 ml buffer solution and 10 ml 0.5% substrate solution. All reaction mixtures were incubated at 37° C for 2 hours. After incubation, the phenol released from the substrate under the action of posphatases was determined spectrophotometrically (at 614 nm) based on the colour reaction betwen phenol and 2,6-dibromoquine-4-chloromide. Phosphatase activities are expressed in mg phenol/g soil/2 hours.

The results were calculated by variance analysis methods (Domuta, 2006).

## **RESULTS AND DISCUSSION**

#### Actual dehydrogenase activity

The values of the actual dehydrogenase activity are bigger on the depth of 0 - 10 cm in comparison with the one on 10 - 20 cm and 20 - 30 cm. The smallest value of the actual dehydrogenase activity were determined in the variant without fertilizer: 5.50 mg TPF/24 hours on 0 - 10 cm, 4.51mg TPF/24 hours on 10 - 20 cm and 2.72 mg TPF/24 hours on 20 -

30 cm The use of manure and green manure determined a bigger value of the actual dehydrogenase activity, in average on the depth of 0 - 30 cm, in comparison with the variant without fertilizers, the use of Lupinus angustifolius used as a green manure determined an increase with 66%, unsignificant from a statistical point of view. In the variant with a mixture of Lupinus angustifolius + oat + rape the actual dehydrogenase activity increased with 116%, significantly from a statistic point of view; the value determined in the variant with mixture recomanded by the biological school (vetch + oat + raigrass) was bigger than the control value with 66%, unsignificantly statistically. The manure, 25 t/hectare, determined an increase of 113%, significantly from a statistic point of view and manure with 50 t/hectare detemined an increase of 150%, quite significantly statistic speaking (Table 1).

Table 1

r	010	ded son nom onded 2	012		
Variant	ADA		Diffrence	Sstatistically	
, unun	mgTPF/24 ł	n %	%	significant	
		Depth 0 – 10 cm			
1.Control	5.50	100	-	control	
2. Lupin	9.18	167	67	Х	
3.Lupin +oat + rape	11.62	212	112	Х	
4. Vetch + oat + ryegras	9.15	167	67	Х	
5. Manure 25 t/ha	11.50	209	109	Х	
6. Manure 50 t/ha	13.90	253	153	XX	
LSD 5% 3.27	7	LSD 1% 6.74	LS	LSD 0.1% 10.90	
		Depth 10 – 20 cm			
1.Control	4.51	100	-	control	
2. Lupin	7.06	157	57	-	
3.Lupin +oat + rape	10.20	227	127	Х	
4. Vetch + oat + ryegras	7.26	161	61	Х	
5. Manure 25 t/ha	10.18	226	126	Х	
6. Manure 50 t/ha	11.02	245	145	Х	
LSD 5% 3.60		LSD 1% 6.90	LSD 0.1 11.20		
		Depth 20 – 30 cm			
1.Control	2.72	100	-	control	
2. Lupin	4.90	181	81	-	
3.Lupin +oat + rape	5.76	212	112	х	
4. Vetch + oat + ryegras	4.70	173	73	-	
5. Manure 25 t/ha	5.40	199	99	х	
6. Manure 50 t/ha	6.88	253	153	х	
LSD 5% 2.28		LSD 1% 4.70	LS	SD 0.1% 6.76	
Average Deapth 0 – 30 cm					
1.Control	4.25	100	-	control	
2. Lupin	7.05	166	66	-	
3.Lupin +oat + rape	9.20	216	116	Х	
4. Vetch + oat + ryegras	7.04	166	66	-	
5. Manure 25 t/ha	9.03	213	113	х	
6. Manure 50 t/ha	10.60	250	150	XX	
LSD 5% 3.05		LSD 1% 6.14	LSD 0.1% 9.62		

Green manure and manure influence on actual dehydrogenase activity (ADA) on the eroded soil from Oradea 2012

### Potential dehydrogenase activity (PDA)

The values of the potential dehydrogenase activity were bigger than the value of the actual dehydrogenase activity and decreased together with the increase of the determination depth.

The smallest set of values of the potential dehydrogenase activity were determined in the variant without fertilizers: 10.56 TPF/24 h on 0 -10 cm, 9.31 mg TPF/24 h on 10 - 20 cm and 7.88 mg TPF/24 h on 20 - 30 cm. In average on the 0 – 30 cm depth the value of potential dehydrogenase activity was of 9.25 mg TPF/24 h; the green manures and manure 25 t/ha determined a significant increase compared with the control. The increases were of 67% in the variant with Lupinus angustifolius; of 78% in the variant with vetch + oat + ryegrass; 95% in the variant with manure 25 t/ha and 98% in the variant with Lupinus angustifolius + oat + rape. The biggest difference in comparison with the control was the value of 128% registred in the variant with manure 50 t/ha (Table 2).

Green manure and manure influence on potential dehydrogenase activity (PDA on the
eroded soil from Oradea 2012

Variant	PDA		Diffrence	Sstatistically	
v ar fant	mgTPF/24 h	%	%	significant	
Depth 0 – 10 cm					
1.Control	10.56	100	-	control	
2. Lupin	22.68	215	115	XX	
3.Lupin +oat + rape	24.18	229	129	XX	
4. Vetch + oat + ryegras	23.12	219	119	XX	
5. Manure 25 t/ha	23.92	227	127	XX	
6. Manure 50 t/ha	26.94	256	156	XXX	
LSD 5% 4.20	)	LSD 1% 9.72	LSD 0	.1% 14.20	
		Depth 10 – 20 cm			
1.Control	9.3	100	-	control	
2. Lupin	15.30	165	65	х	
3.Lupin +oat + rape	16.66	179	79	х	
4. Vetch + oat + ryegras	15.98	172	72	х	
5. Manure 25 t/ha	16.20	174	74	Х	
6. Manure 50 t/ha	19.20	207	107	х	
LSD 5% 3.60	LSD 5% 3.60 LSD 1% 7.85 LSD 0.1		.1% 12.40		
Depth 20 – 30 cm					
1.Control	7.88	100	-	Control	
2. Lupin	8.33	107	7	-	
3.Lupin +oat + rape	14.30	182	82	х	
4. Vetch + oat + ryegras	10.14	129	29	-	
5. Manure 25 t/ha	13.76	175	75	Х	
6. Manure 50 t/ha	17.01	216	116	XX	
LSD 5% 3.52	2	LSD 1% 7.60	LSD 0	.1% 12.02	
Average Deapth 0 – 30 cm					
1.Control	9.25	100	-	control	
2. Lupin	15.44	167	67	х	
3.Lupin +oat + rape	18.38	198	98	X	
4. Vetch + oat + ryegras	16.42	178	78	x	
5. Manure 25 t/ha	17.96	195	95	х	
6. Manure 50 t/ha	21.05	228	128	XX	
LSD 5% 3.78 LSD 1% 8.39		LSD 0	.1% 12.88		

### Catalase activity (CA)

Catalase activity decreases according with the increase of the depth determination.

All the types of green manure and manure determined bigger values of the catalase activity in comparison with the control; all the differences were statistically assured. In average on the 0 - 30 cm depth the value of the catalase activity was of 0.72 mg H<sub>2</sub>O<sub>2</sub>/1 h. In the variant with *Lupinus angustifolius* the catalase activity increased with 145%. In the variant with a mixture of vetch + oat + ryegrass the difference was of 81. The diffrence in the variant with manure 25 t/ha was of 96%. In the variant *Lupinus angustifolius* + oat + rape the difference in comparision with the control was of 107%. The biggest diffrence was registred in the variant with manure 50 t/ha and the value registred was 243% (Table 3).

Green manure and manure influence on catalase activity (CA) on the eroded soi	l from
Oradea 2012	

Variant	СА		Diffrence	Sstatistically
v ariant	mg H2O2/1 h	%	%	significant
		Depth 0 – 10 cm		1
1.Control	0.80	100	-	control
2. Lupin	1.96	245	145	XX
3.Lupin +oat + rape	2.40	300	200	XXX
4. Vetch + oat + ryegras	2.20	275	175	XXX
5. Manure 25 t/ha	2.32	290	190	XXX
6. Manure 50 t/ha	2.66	333	233	XXX
LSD 5% 0.25	5	LSD 1% 0.41	LSD	0.1% 0.96
		Depth 10 – 20 cm		
1.Control	0.73	100	-	control
2. Lupin	1.70	233	133	XX
3.Lupin +oat + rape	2.20	302	202	XXX
4. Vetch + oat + ryegras	1.99	273	173	XXX
5. Manure 25 t/ha	2.09	287	187	XXX
6. Manure 50 t/ha	2.32	318	218	XXX
LSD 5% 0.31		LSD 1% 0.72	LSD	0.1% 1.15
		Depth 20 – 30 cm		
1.Control	0.61	100	-	control
2. Lupin	1.60	263	163	XX
3.Lupin +oat + rape	2.01	330	230	XXX
4. Vetch + oat + ryegras	1.87	307	207	XX
5. Manure 25 t/ha	1.96	322	222	XXX
6. Manure 50 t/ha	2.27	373	273	XXX
LSD 5% 0.33	3	LSD 1% 0.76	LSD	0.1% 1.27
Average Deapth $0 - 30$ cm				
1.Control	0.72	100	-	control
2. Lupin	1.76	245	145	XX
3.Lupin +oat + rape	2.21	307	207	XXX
4. Vetch + oat + ryegras	2.02	281	181	XXX
5. Manure 25 t/ha	2.13	296	196	XXX
6. Manure 50 t/ha	2.47	343	243	XXX
LSD 5% 0.30		LSD 1% 0.63	0.63 LSD 0.1% 1.13	

## Acid phosphatase activity (AcPA)

The values of the acid phosphatase activity decreases according with the increase of the determination depth but the differences are smaller than the differences registred in the previous enzymological parameters.

The smallest values of the acid phosphatase activity were registered in the control: 2.60 mg phenyl/ g soil/ 2h on 0 -10 cm; 2.30 mg phenyl/ g soil/ 2h on 10 - 20 cm and 2.2 mg mg phenyl/ g soil/ 2h on 20 - 30 cm. In average on the 0 - 30 cm in comparison with the control 2.38 mg phenyl/ g soil/ 2h, a signifiant result from a statistical point of view is Lupinus angustifolius (15%). In the variant with vetch + oat + ryegrass and manure 25 t/ha diffrence was 18%. The variant with Lupinus angustifolius + oat + rape registred 20%. The biggest diffrence was the variant with manure 50 t/ha and it was 24%, very signifiant statistically (Table 4).

Green manure and manure influence on acid phosphatase activity (CA) on the eroded soil
from Oradea 2012

Variant			Diffrence	Sstatistically		
varian	mg phenol/g	%	%	significant		
	soil/2 h	,0				
		Depth 0 – 10 cm		1		
1.Control	2.60	100		control		
2. Lupin	2.80	108	8	Х		
3.Lupin +oat + rape	2.92	105	5	XX		
4. Vetch + oat + ryegras	2.86	110	10	X		
5. Manure 25 t/ha	2.89	112	12	Х		
6. Manure 50 t/ha	3.02	117	17	XX		
LSD 5% 0.19	)	LSD 1% 0.34	LSD (	0.1% 0.60		
		Depth 10 - 20 cm				
1.Control	2.30	100	-	control		
2. Lupin	2.74	120	20	XX		
3.Lupin +oat + rape	2.85	124	24	XX		
4. Vetch + oat + ryegras	2.79	122	22	XX		
5. Manure 25 t/ha	2.80	122	22	XXX		
6. Manure 50 t/ha	2.94	128	28	XXX		
LSD 5% 0.17		LSD 1% 0.29	LSD (	LSD 0.1% 0.57		
	•	Depth 20 – 30 cm				
1.Control	2.22	100	-	control		
2. Lupin	2.68	121	21	XX		
3.Lupin +oat + rape	2.73	123	23	XX		
4. Vetch + oat + ryegras	2.70	122	22	XX		
5. Manure 25 t/ha	2.71	122	22	XX		
6. Manure 50 t/ha	2.86	129	29	XXX		
LSD 5% 0.20 LSI		LSD 1% 0.34	LSD (	0.1% 0.62		
Average Deapth 0 – 30 cm						
1.Control	2.38	100	-	control		
2. Lupin	2.74	115	15	XX		
3.Lupin +oat + rape	2.84	120	20	XX		
4. Vetch + oat + ryegras	2.79	118	18	XX		
5. Manure 25 t/ha	2.80	118	18	XX		
6. Manure 50 t/ha	2.94	124	24	XXX		
LSD 5% 0.19		LSD 1% 0.33 LSD 0.1% 0.6		0.1% 0.60		

### Alkaline phosphatase activity (AlkPa)

The values of alkaline phosphatase activity were smaller than the values of the acid phosphatase activity and decreased with the depth of the collected sample.

The smallest values of the alkaline phosphatase activity were registered in the variant used as control. In average the value of the alkaline phosphatase activity on the depth of 0 - 30 cm was 1.29 mg phenyl /g soil/ 2 h. In the variants with green manure and manure the values were bigger than the control. The difference for Lupinus angustifolius in pure crop was 19%, signifficant statistically; the difference registred in the variants with vetch + oat + ryegrass, manure 25 t/ha and *Lupinus angustifolius* + oat + rape were very close (32%; 33%; 34). The biggest diffrence was registred at variant with manure 50 t/ha (Table 5).

Green manure and manure influence on alkaline phosphatase activity (CA) on the eroded	
soil from Oradea 2012	

	S	on from Oraclea 2012			
	AlkPa		Diffrence	Sstatistically	
Variant	mg phenol/g soil/2 h	%	%	significant	
		Depth $0 - 10 \text{ cm}$			
1.Control	1.36	100	-	control	
2. Lupin	1.68	124	24	х	
3.Lupin +oat + rape	1.96	144	44	XXX	
4. Vetch + oat + ryegras	1.94	143	43	XXX	
5. Manure 25 t/ha	1.95	143	43	XXX	
6. Manure 50 t/ha	2.03	149	49	XXX	
LSD 5% 0.18	3	LSD 1% 0.37	LSD	0.1% 0.61	
		Depth 10 - 20 cm			
1.Control	1.32	100	-	control	
2. Lupin	1.52	116	16	-	
3.Lupin +oat + rape	1.69	128	28	XX	
4. Vetch + oat + ryegras	1.64	125	25	XX	
5. Manure 25 t/ha	1.68	128	28	XX	
6. Manure 50 t/ha	1.76	134	34	XX	
LSD 5% 0.17		LSD 1% 0.29	LSD	0.1% 0.54	
		Depth 20 - 30 cm			
1.Control	1.20	100	-	control	
2. Lupin	1.39	116	16	Х	
3.Lupin +oat + rape	1.52	127	27	Х	
4. Vetch + oat + ryegras	1.50	125	25	х	
5. Manure 25 t/ha	1.51	126	26	Х	
6. Manure 50 t/ha	1.67	140	40	XX	
LSD 5% 0.15		LSD 1% 0.27	LSD 0.1% 0.58		
Average Deapth 0 – 30 cm					
1.Control	1.29	100	-	control	
2. Lupin	1.53	119	19	Х	
3.Lupin +oat + rape	1.73	134	34	XX	
4. Vetch + oat + ryegras	1.70	132	32	XX	
5. Manure 25 t/ha	1.72	133	33	XX	
6. Manure 50 t/ha	1.82	141	41	XX	
LSD 5% 0.17		LSD 1% 0.31	LSD	LSD 0.1% 0.58	

#### CONCLUSIONS

The research carried out in a long term trial from the Agricultural Research and Development Station Oradea permeted the following conclusions:

- The use of green manure (Lupinus angustifolius, Lupinus angustifolius + oat + raped, vetch + oat + ryegras) and manure (25 t/ha and 50 t/ha) determined the increase of dehydrogenase activity, catalase activity, acid and alkaline phosphatase activity.
- Enzymological activity decreases according with the increase of the determination depth.
- The smallest values of the enzymological parameters studied were determined in the variant without organic fertilizers and the biggest value was determined in variant with manure 50 t/ha.
- The use of *Lupinus angustifolius* in mixture with oat and rape determined bigger values then Lupinus angustifolius in pure culture. The mixture of vetch + oat + ryegrass determined bigger values of the enzymatical parameters in comparision with Lupinus angustifolius pure crop but smaller than the values determinated data the variant with Lupinus angustifolius + oat + raped.
- The data regarding the enzymological activity sustain the need of green manure and recommend the use of the mixture Lupinus angustifolius + oat + raped as green manure.

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