

Effects of Liming and Fertilization on the Dehydrogenase and Catalase Activities

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In this paper we provide new data about the soil enzyme activity as a biological process, which is an indicator for impacts of factorial combinations of lime and fertilizers applications. Five plots divided into fifteen subplots were sampled for determination of the enzymatic indicators of soil quality, based on the actual and potential dehydrogenase and catalase activities. The research revealed that limed soil samples, in comparison with unlimed ones, resulted in significantly higher soil enzymatic activities ($p < 0.05$) in the upper (0-20 cm), while in the deeper (20-40 cm) layer, only catalase activity was significantly higher (at least at $p < 0.02$). Mineral fertilization, in comparison with its farmyard manuring, led to an insignificant increase in each of the three enzymatic activities determined, excepting catalase activity which was significantly higher ($0.05 > p > 0.02$) in the 0-20 cm layer. Based on the absolute values of the enzymatic activities, the enzymatic indicators of soil quality (EISQ) were calculated. The mineral NPK-fertilization and low dose of lime in the 0-20 cm layer, and mineral NP-fertilization and low dose of lime in the 20-40 cm layer proved to be the best variants of fertilization. The enzymatic indicators of soil quality in these variants reached the highest values: $EISQ = 0.821$ and $EISQ = 0.889$, respectively, indicating the presence of high enzymatic activities. It should be emphasized that a balanced application of lime, mineral fertilizers and farmyard manure leads to the formation of favorable conditions for the development of microorganisms, growth of plants and for an intense and lasting enzymatic activity.

Keywords: catalase, dehydrogenase, soil, fertilizers, lime

In recent decades, the intensification of human activities, the improper use of land resources, the misuse of waste of all kinds (especially of those with chemical activity: chemical residues, pharmaceutical and household waste, fertilizers and insecticide, manure, etc. [1-3]) have led, among other negative consequences, to the terrestrial ecosystems acidification [4,5] and pollution [1,6,7], with a considerable negative impact on nutrient transformations. There have been made attempts to buffer the soil chemical alterations consequent upon acid input [8]. Recent interest in defining soil quality has focused on identifying soil properties that affect soil health and quality [9]. Thus, it has been suggested that the measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality [10].

All processes in soil are inevitably connected with abundance of microorganisms, activity of which is reciprocally affected by soil qualities. The number and efficiency of soil microorganisms (even if they usually occupy less 1% of the soil volume) are very high, contributing to better plant development [11]. The metabolic activity of soil microorganisms is essential for organic matter turnover. The mobilization and immobilization of inorganic nutrients and trace elements are also mainly a result of microbial activities.

Soil enzymes catalyze the organic matter turnover, reactions in soil that are important in the cycle of nutrients such as C, N, P, and S [12]. 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 [13]. Soil enzyme activities commonly correlate with microbial parameters [14] and have proved to be a sensitive index of long-term management practices [15,16]. Among agricultural practices, ploughing, crop rotation, liming, manuring and fertilization have beneficial, harmful or neutral effects on the trinity formed by plants, soil organisms (microbes and fauna) and soil. Since in a soil-plant system, the soil's energy powerhouse is the rhizosphere, any alteration to the fertility management will have a strong impact on the soil-plant interface, and subsequently on the agricultural productivity and sustainability of the ecosystem [17].

The focus of most of the soil enzyme research has been to develop methodologies for their measurement and to provide [18] an understanding of their origin and the factors that affect their activity in soil. However, little work has been done to actually develop methods or technologies that use soil enzymatic activity data as inputs into soil fertility management decisions [19]. In the present study, we continued to research ways that relate soil enzyme activities to management practices in a preluvosoil submitted to a complex experiment at the Agricultural Research and Development Station in Livada (Satu Mare county).

Experimental part

Materials and methods

The soil was a preluvosoil one with the following physical and chemical properties in the 0-20 and 20-40 cm layers: clay 21-20.4%, humus 1.41-0.47%, soil density 1.14-1.46g/

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cm³, total porosity 58-465, pH_{water} 5.19-5.31, P11-2 ppm, K144-169 ppm.

The experiment started in 1961. The experimental field was divided into plots and subplots for the study of the soil enzymological effect of liming and mineral and farmyard-manure fertilization. The plots were annually N-fertilized at rates of 100 kg N/ha, NP-fertilized at rates of 100 kg N/ha and 70 kg P/ha, NPK-fertilized at rates of 100 kg N/ha, 70 kg P/ha and 60 kg K/ha and 5t/ha farmyard manure. The control consisted of unfertilized soil. Each plot has three subplots representing the liming variants: without lime, low dose (LD) - 0.56 t lime/ha and high dose (HD) - 1.01 t lime/ha. In October 2017, the soil was sampled from all subplots. Sampling depths were 0-20 and 20-40 cm. The soil samples were allowed to air-dry, then ground and passed through a 2-mm sieve and, finally, used for enzymological analyses.

Actual and potential dehydrogenase activities were determined according to the methods described in a previous paper [20]. Dehydrogenase activities were expressed in mg of triphenylformazan (TPF) produced (from 2, 3, 5-triphenyltetrazolium chloride, TTC) by 10 g soil in 24 hours. Catalase activity was determined using the permanganometric method [21]. The activity values were submitted to statistical evaluation by the two-way-t-test [22].

Results and discussions

Results of the enzymological analyses are presented in Tables 1-3.

The effect of liming on the enzymatic activities in soil

Each of the three enzymatic activities determined was significantly higher ($p < 0.05$) in the upper (0-20 cm) layer of the limed subplots than in the same layer of the unlimed subplots, while in the deeper (20-40 cm) layer, only catalase activity was significantly higher (at least at $p < 0.02$) in the limed than in the unlimed subplots. Low dose of liming (LD) in comparison with high dose (HD) led to a significant (at least at $p < 0.05$ and $p < 0.01$) increase in each activity, excepting actual and potential dehydrogenase activities in the 20-40 cm layer, in which, these activities were insignificantly higher ($p > 0.10$). Application of lime to soils normally leads to significant increases in pH and, thus in the chemical and biological reactions and in microbiological processes. Such treatments result in changes in the solubility of many chemical compounds and improvement in the plant roots and development environment, increasing soil microbial biomass, including microbial dynamic and diversity, and, therefore, significant changes in enzyme activities [23].

Previous studies with limed soil have focused mostly on the changes of the activity of acid phosphatases in forest soils, because of the positive correlation between phosphate availability and pH of the soil. Even though pH is considered one of those important properties affecting soil health and quality, its role in modifying enzymatic reactions in soils has not been demonstrated clearly, i.e. with many enzymes involving a range of soil pH [24]. The results of statistical evaluation are summarized in Tables 4 and 5.

Table 1
ACTUAL DEHYDROGENASE ACTIVITY (mg TPF/10g soil/24 h)

Fertilization*	Lime					
	0 t/ha		0.56 t/ha		1.01 t/ha	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
Control	1.736	1.392	2.016	1.792	1.288	2.464
N ₁₀₀	1.120	1.736	3.752	3.080	1.568	2.464
N ₁₀₀ P ₇₀	1.624	2.408	2.632	7.000	2.352	2.576
N ₁₀₀ P ₇₀ K ₆₀	1.736	2.184	3.640	4.200	3.136	2.576
FYM	1.568	2.184	2.016	1.952	1.960	2.016

*FYM – farmyard-manured.

Table 2
POTENTIAL DEHYDROGENASE ACTIVITY (mg TPF/10g soil/24 h)

Fertilization FYM	Lime					
	0 t/ha		0.56 t/ha		1.01 t/ha	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
Control	9.520	9.520	6.384	9.576	5.600	4.592
N ₁₀₀	9.576	4.872	5.656	8.456	4.704	8.064
N ₁₀₀ P ₇₀	5.476	4.984	5.476	9.240	7.168	6.552
N ₁₀₀ P ₇₀ K ₆₀	6.328	5.096	6.328	5.040	5.320	5.528
FYM	7.840	5.376	7.840	3.568	5.824	7.896

Table 3
CATALASE ACTIVITY (mg H₂O₂/soil/h)

Fertilization FYM	Lime					
	0 t/ha		0.56 t/ha		1.01 t/ha	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
Control	1.70	5.40	3.80	6.30	5.60	5.00
N ₁₀₀	2.70	6.20	5.30	7.10	5.80	5.20
N ₁₀₀ P ₇₀	1.70	5.40	4.60	5.00	5.50	6.80
N ₁₀₀ P ₇₀ K ₆₀	6.60	5.30	5.50	4.60	5.60	5.00
FYM	4.80	6.10	6.0	4.80	4.40	4.50

Amendments*	Soil enzymatic activity**	Soil depth (cm)	Mean activity values in different amendments			Significance of the differences
			a	b	a-b	
Unlimed (a) vs. LD limed soil (b)	ADA	0-20	1.55	3.03	-1.48	0.05>p>0.02
		20-40	1.97	3.60	-1.63	0.10>p>0.05
	PDA	0-20	6.63	6.33	0.30	0.02>p>0.01
		20-40	5.96	7.17	1.21	p>0.10
	CA	0-20	3.50	5.04	-1.54	0.01>p>0.001
		20-40	5.68	5.56	0.12	0.01>p>0.001
Unlimed (a) vs. HD limed soil (b)	ADA	0-20	1.55	2.05	-0.50	0.05>p>0.02
		20-40	1.97	2.41	0.44	0.10>p>0.05
	PDA	0-20	6.63	5.72	0.91	0.05>p>0.02
		20-40	5.96	6.52	-0.56	0.05>p>0.02
	CA	0-20	3.50	5.38	-1.88	0.05>p>0.02
		20-40	5.68	5.30	0.38	0.02>p>0.02
LD limed soil (a) vs. HD limed soil (b)	ADA	0-20	3.03	2.05	0.98	0.05>p>0.02
		20-40	3.60	2.41	1.19	p>0.10
	PDA	0-20	6.33	5.72	0.61	0.01>p>0.001
		20-40	7.17	6.52	0.65	p>0.10
	CA	0-20	5.04	5.38	-0.34	0.01>p>0.001
		20-40	5.56	5.30	0.26	0.05>p>0.02

*LD- Low dose of lime; HD- High dose of lime.

**ADA - Actual dehydrogenase activity; PDA - Potential dehydrogenase activity; CA - Catalase activity.

Table 4
SIGNIFICANCE OF THE DIFFERENCES BETWEEN ENZYMATIC ACTIVITIES IN A PRELUVOIL SOIL SUBMITTED TO DIFFERENT AMENDMENTS

Table 5

SIGNIFICANCE OF THE DIFFERENCES BETWEEN ENZYMATIC ACTIVITIES IN A PRELUVOIL SOIL SUBMITTED TO DIFFERENT FERTILIZERS

Fertilization FYM	Soil enzymatic activity**	Soil depth (cm)	Mean activities in fertilization practices			Significance of the differences
			a	b	c	
Control (a) vs. N fertilization (b)	ADA	0-20	1.68	2.14	-0.46	p>0.10
		20-40	1.88	2.42	-0.54	p>0.10
	PDA	0-20	7.16	6.64	0.52	p>0.10
		20-40	7.89	7.12	0.77	0.10>p>0.05
	CA	0-20	3.70	4.60	-1.90	p>0.10
		20-40	5.56	6.16	-0.60	0.10>p>0.05
Control (a) vs. NP fertilization (b)	ADA	0-20	1.68	2.20	-0.52	p>0.10
		20-40	1.88	3.99	-2.11	p>0.10
	PDA	0-20	7.16	5.81	1.35	p>0.10
		20-40	7.89	6.91	0.98	p>0.10
	CA	0-20	3.70	3.93	-0.23	p>0.10
		20-40	5.56	2.26	3.36	0.05>p>0.02
Control (a) vs. NPK fertilization (b)	ADA	0-20	1.68	2.83	-1.15	p>0.10
		20-40	1.88	2.98	-1.10	p>0.10
	PDA	0-20	7.16	5.42	1.74	p>0.10
		20-40	7.89	5.21	2.68	0.10>p>0.05
	CA	0-20	3.70	5.90	-2.20	p>0.10
		20-40	5.56	4.96	0.60	p>0.10
Control (a) vs. FYM (b)	ADA	0-20	1.68	1.84	-0.16	p>0.10
		20-40	1.88	2.04	-0.16	p>0.10
	PDA	0-20	7.16	6.10	1.06	p>0.10
		20-40	7.89	5.60	2.29	0.05>p>0.02
	CA	0-20	3.70	5.06	-1.36	0.10>p>0.05
		20-40	5.56	5.13	0.43	p>0.10
N fertilization (a) vs. NP fertilization (b)	ADA	0-20	2.14	2.20	-0.06	0.05>p>0.02
		20-40	2.42	3.99	-1.57	p>0.10
	PDA	0-20	6.64	5.81	0.83	p>0.10
		20-40	7.12	6.91	0.21	p>0.10
	CA	0-20	4.60	3.93	0.67	0.10>p>0.05
		20-40	6.16	2.26	3.90	0.10>p>0.05
N fertilization (a) vs. NPK fertilization (b)	ADA	0-20	2.14	2.83	-0.69	p>0.10
		20-40	2.42	2.98	-0.56	p>0.10
	PDA	0-20	6.64	5.42	1.22	p>0.10
		20-40	7.12	5.21	1.91	p>0.10
	CA	0-20	4.60	5.90	-1.30	p>0.10
		20-40	6.16	4.96	1.20	p>0.10
N fertilization (a) vs. FYM(b)	ADA	0-20	2.14	1.84	0.30	p>0.10
		20-40	2.42	2.04	0.38	0.10>p>0.05
	PDA	0-20	6.64	6.10	0.54	p>0.10
		20-40	7.12	5.60	1.52	p>0.10
	CA	0-20	4.60	5.06	-0.46	0.05>p>0.02
		20-40	6.16	5.13	1.03	p>0.10
NP fertilization (a) vs. NPK fertilization (b)	ADA	0-20	2.20	2.83	-0.63	p>0.10
		20-40	3.99	2.98	1.01	p>0.10
	PDA	0-20	5.81	5.42	0.39	p>0.10
		20-40	6.91	5.21	1.70	p>0.10
	CA	0-20	3.93	5.90	-1.97	p>0.10
		20-40	2.26	4.96	-2.7	p>0.10

NP fertilization (a) vs. FYM (b)	ADA	0-20	2.20	1.84	0.36	p>0.10
		20-40	3.99	2.04	1.95	p>0.10
	PDA	0-20	5.81	6.10	-0.29	p>0.10
		20-40	6.91	5.60	1.31	p>0.10
	CA	0-20	3.93	5.06	-1.13	0.10>p>0.05
		20-40	2.26	5.13	-2.87	p>0.10
NPK fertilization (a) vs. FYM (b)	ADA	0-20	2.83	1.84	0.99	p>0.10
		20-40	2.98	2.04	0.94	p>0.10
	PDA	0-20	5.42	6.10	-0.68	p>0.10
		20-40	5.21	5.60	-0.39	p>0.10
	CA	0-20	3.93	5.06	-1.13	0.10>p>0.05
		20-40	4.96	5.13	-0.20	p>0.10

Continuated table 5

The effect of fertilization on the enzymatic activities in soil

The studied plots could serve for comparing the enzymological effect on the soil of mineral fertilization and farmyard manuring. When the three doses of lime were considered together (Table 4), each of the three enzymatic activities was found to be insignificantly higher ($0.10 > p > 0.05$) in the minerally fertilized plots than in the farmyard-manured plot, excepting catalase activity in the 0-20 cm layer, which was significantly higher ($0.05 > p > 0.02$) in the farmyard-manured plot than in the N-fertilized plot. Each of the three enzymatic activities determined was generally higher in fertilized plots than in the unfertilized ones, excepting potential dehydrogenase activity which was insignificantly higher ($p > 0.10$) in the unfertilized plot than in the mineral fertilized plot and significantly higher ($0.05 > p > 0.02$) in the unfertilized plot than in the deeper layer of the farmyard manured one and catalase activity in the deeper layer which was significantly higher ($0.05 > p > 0.02$) in the unfertilized plot than in the mineral NP-fertilized plot and insignificantly higher ($p > 0.10$) in the unfertilized plot than in the mineral NPK-fertilized and farmyard manured plots.

Our results are in a good agreement with the literature data reviewed by [25, 26]. Studies have revealed that the application of balanced amounts of nutrients and manures improved the organic matter which corresponded with higher enzyme activities. Dehydrogenase activity is influenced more by the quality, than by the quantity of organic matter incorporated into soil [27]. Thus, stronger effects of FYM on dehydrogenase activity might be due to more easily decomposable components of crop residues on the metabolism of soil microorganisms.

Enzymatic indicators of soil quality

To establish a hierarchy of the plots admitting equal importance for the three enzymatic activities, we have used the formula, referred to in [28], to calculate the enzymatic indicators of soil quality:

$$EISQ = \frac{1}{n} \times \sum_{i=1}^n \frac{Vr(i)}{Vmax(i)}$$

where EISQ - the enzymatic indicators of soil quality; n - number of activities; Vr(i) - real individual value of the activity; Vmax(i) - maximum theoretical individual value of the activity.

The maximum individual values, calculated from the composition of the reaction mixtures are: 13.45 mg triphenylformazan for the actual and potential dehydrogenase activities and 60 mg H₂O₂ for the catalase activity. The enzymatic indicator can theoretically have values between 0 (when there is no activity in the studied samples) and 1 (when the real individual values are equal to the maximum theoretical individual values of all activities).

The enzymatic potential of soils defined by the values of the quality enzymatic indicators is represented in Figures 1 and 2. It can be seen from both figures that the intensity of enzymatic activities varies within large limits. The enzymatic potential of these fertilized and limed plots was not close to the control soil. In the limed soil samples with low dose of lime, the enzymatic potential exceeded the enzymatic potential of the soil which had received high dose of lime and was far higher than the enzymatic potential of the control plots. These findings, like those registered in all variants of fertilization, prove the role played by the application of lime, fertilizers and farmyard manure in soil, in the creation of the enzymatic potential in soil.

In the 0-20 cm layer, the NPK-fertilization proved to be a good treatment from the enzymological point of view. The enzymatic indicator of soil quality reached the highest values: EISQ=0.821 for low dose of lime and EISQ=0.746 for high dose of lime. These values indicate the presence of high enzymatic activities as compared to the other treatments analyzed. In the 20-40 cm layer, the NP-fertilized subplots had the highest values of enzymatic indicators with EISQ=0.889 for low dose of lime and EISQ=0.669 for

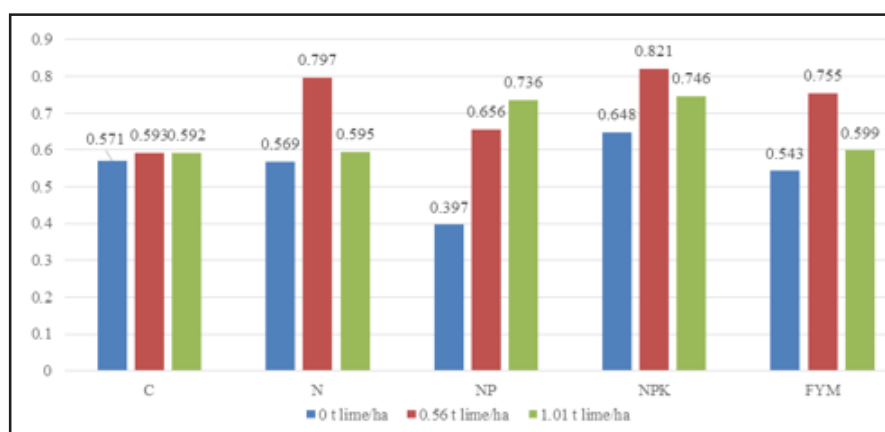


Fig. 1. Enzymatic indicators of soil quality in the 0-20 cm layer

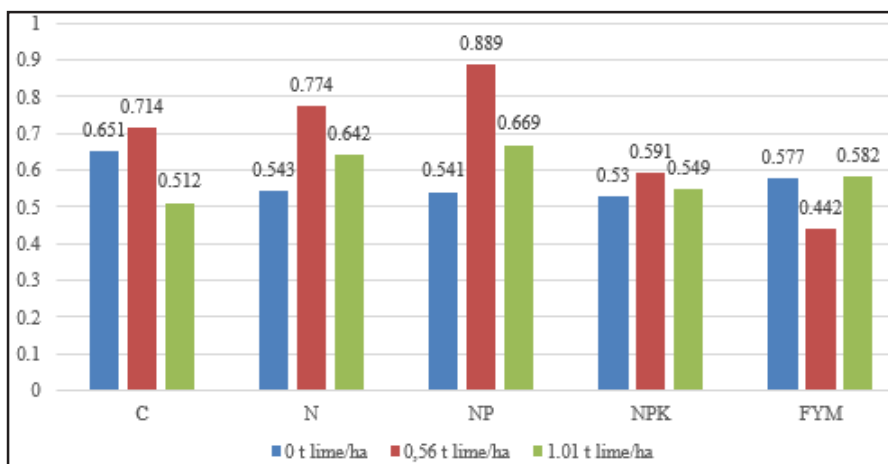


Fig. 2. Enzymatic indicators of soil quality in the 20-40 cm layer

high dose of lime. Based on the results and in comparison with several literature data [29-31] we may consider that the agricultural analyzed preluvsol has an appreciable enzymatic potential. This means that by determining enzymatic activities, valuable information can be obtained regarding fertility status of soils.

Conclusions

The obtained results confirm the usefulness of assessing the enzyme activity to evaluate the managed soil. The results of the present study demonstrated the strong relationship between enzyme activity and the management practices. Since dehydrogenase and catalase activity is only present in viable cells, it is thought to reflect the total range of oxidative activity of soil microflora and consequently may be considered to be a good indicator of the total metabolic activity of soil microorganisms. Biological approaches are being sought for assessing soil processes related to crop production, soil quality and overall soil sustainability.

Sustainable soil management practices and the maintenance of soil quality are central issues to our agricultural soil. This study has shown that the long-term application of liming and fertilization leads to the formation of favorable conditions for the development of microorganisms, in creating the enzymatic potential in soil.

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