AGRONOMICAL SIGNIFICANCE OF THE PEDOENZYMICAL TESTS

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Biological signification of accumulated enzymes in soil was formulated and commented, first of all, by Kiss *et al.* (1972), reffering to the research from abroad and from Romania, which attested the existence of accumulated enzymes in soil, their origin and potential activities.
In the present papers, the authors are offering the new concept: *Agronomical signification of pedoenzymes,* by which the effects of some agrophytotechnical measures on pedoenzymic potential in different agricultural soils are interpreted. Pedoenzymic data are evidencing some linear, positive and significant correlations between the Chemical Synthetic Indicator (CSI%) of soils and the Indicator of Enzymic Potential Activity (IEAP%) in the same soils and also between the humus contents and different hydrolases as: saccharase, urease and total phosphatase potentials in the researched soils. These reflect, also, the possibility to control the evolution of the fertility state of land subjected to different agricultural measures (chemical fertilization or ecological one).

Key words: Enzymic activity; Pedoenzymes; Agronomical signification of pedoenzymes.

INTRODUCTION

Enzymic activity of soils results from accumulated enzymes and also from those of microorganisms which proliferate in soil. By definition, accumulated enzymes, so named pedoenzymes, are considered as present and active, even in the soil where doesn't exist any microbial proliferation. The sources of accumulated enzymes are, first of all, the microorganism cells, but they result even from the plant and animal refuses. The enzymes are accumulated in soil as free enzymes (exoenzymes from living cells plus endoenzymes released from the desintegrated cells) and as enzymes bound to cell constituents (enzymes present in cell fragments as well as in viable but nonproliferating cells). The free enzymes occur, in majority, in adsorbed state on the soil mineral particles and/or adsorbed on the humus. The quantity of enzymes in soil solution is much smaller.

Components of the enzyme activity of soil are classified by Kiss *et al.* (1972) as shown in Table 1. A comprehensive review dealing with this problems was published by Kiss *et. al.* (1971), renderring evident *the biological significance of the accumulated enzymes in soil.* An exhaustive synthesis of the researches about the enzymatic activity of agricultural soils, in Romania, was published by Kiss *et al.* (1974; 1975). In this synthesis one mentions three characteristic stages of the soil research.

First stage can be delimited between the years 1901–1932, when some analytical proofs were furnished about so named "*catalytic power of soil*", expressed by soil capacity of splitting the oxygenated water, releasing oxygen. This catalytical power was named "catalase" and was then registered in the class of the oxydoreductase enzymes (Loew, 1901; König, Hasenbäumer and Copperath, 1906).

In the second stage (1929–1949), the papers about soil enzymes are quoted: about the catalase activity (Dobrescu and Radu, 1929 and Radu, 1933), about pyrophosphatase and urease activities (Rotini, 1933 and 1935); about phosphatase activity (Ionescu, 1939; Pavlovschi and Ionescu, 1940) and about glycerophosphatase and nuclease (Rogers, 1942). These papers related about origin, kinetics and accumulation of enzymes in soil.

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Table 1

Components of enzymatic activity in soil

Enzyme activity of soil	Acumulated enzymes	Free enzymes	Exoenzymes released from living cells	in adsorbed state and/or in complexes with humic substance
				in soil solution
			Endoenzymes released from disintegrated	in adsorbed state and/or in complexes with humic substance
				in soil solution
		Enzymes bound to cell constituents Enzymes present in:	Disintegrated cells	in adsorbed state and/or in complexes with humic substance
				in soil solution
			Dead but not disintegrated cells	in adsorbed state
				in suspension
			Viable but not proliferating cells	in adsorbed state
				in suspension
	Enzymes of proliferating cells	Free enzymes	Exoenzymes from living cells	in adsorbed state and/or in complexes with humic substance
				in soil solution
		Enzymes bounded to cell constituents	Proliferating cells	in adsorbed state
				in suspension

In the third stage, it can say that, the research series were opened, regarding the hydrolase enzymes: carbohydrases, proteinases, amidases, phosphatases; and in a small measure transferases and oxydoreductases. Kiss *et al.*(1974) found the hydrolases: lichenase, dextranase and levanase and also the transferases: levansucrase and dextransucrase, first time, in soils from Romania.

The year of relaunching of complex research of soil enzymes can be considered 1951, by Hoffman and Seegerer's papers about saccharase (β-h-fructosidase, and by Kuprevichs one, about urease. Dynamics of the world number of the pedoenzyme publications was presented in a graph, by Kiss and Boaru (1965), which from we are relating, by approximation: in 1951 – 7 papers; in 1955 – 36 papers, in 1960 – 42 papers; in 1964 – 45 papers. Pedoenzymic researches, since 1951 to 1965 period, put in evidence: enzymic activity which develops in soil; their specificity as against the enzymic substrate; utilization of antiseptics in enzymatic mixture for preventing the simultaneous activity of microflora during the analyse; whether it must rectify the pH of enzymic mixture by optimum of enzymic system; optimal temperature of the enzyme reaction *etc*.

In the fourth stage (1965-2007, the pedoenzymic researches passed from the identification of enzymes in soil and elaboration of adequated method of the analyses to the characterization of the accumulation level of each enzyme in different soil type; to put in evidence of the agricultural intervention role and even of the anthropic interventions (pollution, technogenic soils, landed improvement etc. (Dick *et al*., 1988; Bandick and Dick, 1999; Trasar-Cepeda *et al.*, 2000; Gheorghiță, 2004; Gheorghiță *et al.*, 2007); the elaboration of some synthetic indicators (Ştefanic, 1981; Ştefanic 1994; Ştefanic *et al.*, 1997, 1998, 2001, 2006).

The characteristic of the majority of those researches is that in interpretation of results, one considers that those represent enzyme activity even in soil. That is an impossibility, because the analytical methods are conceived to determine the enzymatic potential of soil, not that of activity *in situ.*

In 1972, Kiss *et al.* presented the concept *"Biological significance of accumulated enzymes in soil"*. From another mode of tackling, in this papers, we are presenting "*the agronomical significance of pedoenzymic potential"*, that contributes to understanding the anthropic influence on the soil fertility level.

MATERIAL AND METHODS

The enzymic analyses were performed with soil samples gathered from different soil types, from long-term experiments with farmyard manure + mineral NP of the agricultural research stations: Research Institute for Cereals and Industrial Crops - Fundulea, Calarasi (cambic chernozem); SCA Albota – Arges (albic luvosol); SCA Caracal – Olt (cambic chernozem); SCA Simnic – Dolj (reddish preluvosol); SCA Podu Iloaiei – Iasi, (cambic chernozem) and SCA Livada – Satu Mare (albic luvosol). They were pursued to determin the enzymic potential of soils. We are presenting the principle of the pedoenzymic analyses: one prepares enzymic reaction mixture using: 5 g fresh soil + 10 ml of specific enzymical substrate with antiseptic. So, indifferently of enzyme quantity it supplies a maximum quantity of specific substrate (according with Michaelis – Menten constant). After 24 hours of enzyme reaction at 28^0C, it makes the extraction with kalium alum solution 0,3% and after the filtration, in filtrate one determins the enzymic produce by spectrophotometry or other methods. The analytical methods where published by Ştefanic (1994; 1999 and 2007).

Analytical results were statistical worked by variance analysis and Turkey test (Snedecor, 1968).

RESULTS AND INTERPRETATION

Methods used for obtaining *agronomical signification of the pedoenzymical test* are the same with those for obtaining *biologic signification of enzyme activity in soil.* The difference, very important, consists in the interpretation of results. In the biological interpretation is important to find that in soil, different enzymes exist and they are active and to what potential level, but in the agronomical interpretation, we must find under which agrotechnical measures and climatic or pedological conditions the pedoenzyme potential are manifesting. For this purpose we have created some indicators by which we can control the soil fertility evolution.

Table 2

Modification of some pedoenzymical parameters under the influence of organo-mineral fertilization for a long time in several agricultural soils from Romania

Soil enzyme potential		a_1–Chernozem	a_2–Reddish preluvosol	a_3–Albic luvosol
Catalase O_2 cm^3/ 100g soil	b_1	a 800 a	a 313 c	a 364
	b_2	b 655 a	b 214 c	b 296
	LD P0,1%	Factor A =55	Factor B = 39	
Saccharase mg glucose/ 100g soil	b_1	b 1836 a	b 699 b	b 964
	b_2	a 2020 a	a 1067 c	a 1559
	LD P0,1%	Factor A =77	Factor B = 33	
Urease mg NH_4^+/ 100g soil	b_1	b 57 a	b 18 c	b 30
	b_2	a 77 a	a 23 c	a 36
	LD P0,1%	Factor A =4	Factor B = 1	
Phosphatase mg P/ 100g soil	b_1	b 2,6 a	a 2,4 b	a 3,1
	b_2	a 6,6 a	a 2,5 c	a 3,4
	LD P0,1%	Factor A =0,6	Factor B = 0,3	
Indicator of Enzymic Activity Potential %	b_1	b 37 a	b 14 c	b 20
	b_2	a 40 a	a 17 c	a 24
	LD P0,1%	Factor A =4	Factor B = 2	

*b_1 – unfertilized; b_2 – organo-mineral fertilized and limed of 3 folds at 10 years;
**the letters placed anterior of numbers marked the same group of values statistical significative of factor A;
*** the letters placed after the numbers marked the same group of values statistical significative of factor B;

In the Table 2, the analytical data, concerning pedoenzyme potentials of: catalase, saccharase, urease and total phosphatase, from long-term experiments with mineral and organic manures, from only three soils in different pedo-climatic zones in Romania, are presented. Because the supplying of soils with manures determins the best modifications in chemical, biochemical and vital natural equilibrium in soils, we are giving more attention to those.

Analysis of results from Table 2 makes evident the benefical effect of soil supplying with organo-mineral manures for a long time on the potential of hydrolase pedoenzymes in all the soils researched, indifferently of their nitrogen and phosphorous content and saturation degree in bases (V%), as Table 2 attests. Generally, the high content in humus and organo-mineral manuring must be stimulative of soil life and as consequence, the pedoenzyme accumulation to be higher, lest to speak still of periodical addition of enzymes by farmyard manure.

Catalasic potential was depreciated as consequence of organo-mineral manuring. This effect can't be explained by agrochemical indicators. Catalase being an enzyme that governs the slow oxydation of organic material in soil, and originating in aerobic microorganisms of soil, might to be stimulated by farmyard manure (Pavlovschi and Groza, 1947). It might be possible to give an explanation: the high consume of atmospheric oxygen by microorganisms, for transforming the organic material added in soil, by mineralization and humification, produces an oxygen lack, and it makes difficult the formation of peroxydes adsorbed in soil and also of the catalase.

The data of Table 3 sustain the increase of humus content and of humic fractions determined by organic manuring of long time. Total nitrogen (kjeldahl) and phosphorus – organical combined, which are, in the best part, humic components and of soil biomass, put in evidence the role of organo-mineral consistent manuring.

The saturation degree in bases (V%) increased, in all analysed soils, indifferently of soil type, what is very favorable, particularly for acid soils stimulating on this way, the soil vital activity, in the limits of the equilibrium mineralization/ humification, in benefit of soil entropy increase.

Indicator of Enzymic Activity Potential (IEAP%) realized by role of sinthetical estimation of soil pedoenzymic potential (Table 2) generalizes the fact that organic manuring is favorable to biochimic processes in soil. It is possible as analytical results, which make negative exception to be due both of some analytical mistakes and / or it may be to uncorresponding quality of farmyard manure in some agricultural stations.

Table 3

Modification of some agrochemical parameters under the influence of organo-mineral fertilization for a long time in several agricultural soils from Romania

Soil enzyme potential		a_1-Chernozem	a_2-Reddish preluvosol	a_3- Albic luvosol
Humus – $C_{t\%}$	b_1	b 1.75 a	b 0.78 c	b 1.32 b
	b_2	a 2.09 a	a 1.08 c	a 1.43 b
	LD P0.1% Factor A =0.04 Factor B = 0.01			
Extractable carbon – $C_{e\%}$	b_1	b 0.75 b	b 0.38 c	b 0.80 a
	b_2	a 0.86 a	a 0.46 b	a 0.87 a
	LD P0.1% Factor A =0. 03 Factor B = 0.01			
Carbon from huminic acides – $C_{ah\%}$	b_1	b 0.49 a	a 0.26 b	b 0.28 b
	b_2	a 0.58 a	a 0.26 c	a 0.30 b
	LD P0.1% Factor A =0.02 Factor B = 0.01			
Total nitrogen $N_{t\%}$	b_1	b 0.17 a	b 0.11 b	b 0.11 b
	b_2	a 0.21 a	a 0.14 b	a 0.13 b
	LD P0.1% Factor A = 0.01 Factor B = 0.002			
Organic phosphorus mg P/ 100g soil	b_1	b 10.7 a	b 3.6 c	b 4.1 b
	b_2	a 17.5 a	a 5.4 c	a 6.3 b
	LD P0.1% Factor A = 1.0 Factor B = 0.5			
Saturation degree in bases V%	b_1	b 94.4 a	b 81.1 b	b 80.7 b
	b_2	a 95.5 a	a 87.3 a	a 93.0 a
	LD P0.1% Factor A = 10.9 Factor B = 0.4			
Chemical Synthetic Indicator %	b_1	a 66.6 a	b 46.3 c	b 49.2 b
	b_2	a 74.6 a	a 52.1 c	a 61.7 b
	LD P0.1% Factor A =1.7 Factor B = 0.9			

*b_1 – unfertilized; b_2 – organo-mineral fertilized and limed of 3 folds at 10 years;
**the letters placed anterior of numbers marked the same group of values statistical significative of factor A;
*** the letters placed after the numbers marked the same group of values statistical significative of factor B;

The data of Tables 2 and 3 permit the observation that fertile soils (chernozems) present higher values than those with smaller fertility (reddish-brown = reddish preluvosol and albic luvisol = albic luvosol = podzole). The most easy one observes that, from comparison of pedoenzymic synthetic indicators with those agrochemicals (ISC%), both proposed by Ştefanic (1994 a and b; Ştefanic, 1999 and 2006; Ştefanic *et al.*, 1997; 1998 and 2001), for forming the Synthetic Indicator of Soil Fertility (SISF%).

Figure 1 attests the positive and linear – significant correlation between ISC% and IEAP%, both for unfertilized soils and for those fertilized organo-mineral. Positive and significant liniar correlations were put in evidence between other parameters also, as examples: humus (Ct%) with saccharase (Fig. 2), with urease (Fig. 3) or with total phosphatase potentials (Fig. 4) and also, between organic phosphorus (nativ in soil) and total phosphatase potential (Fig. 5) in organo-mineral fertilized soils.

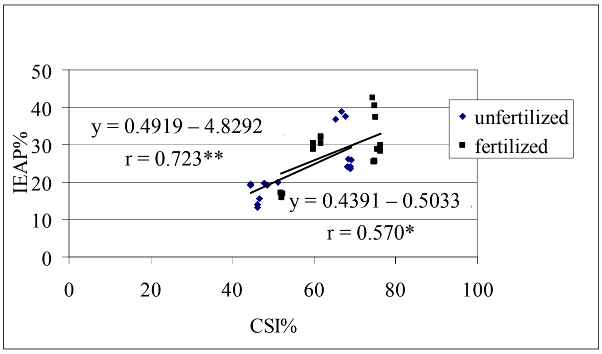

Fig. 1. Correlation between CSI and IEAP.

Positive correlations, above presented between humus content of soils and different pedoenzymic potentials, implicated in biotransformation of organic material (mineralization and humification) from different pedoclimatic zones of Romania are permitting us to give an *agronomical significance of pedoenzymic tests.*

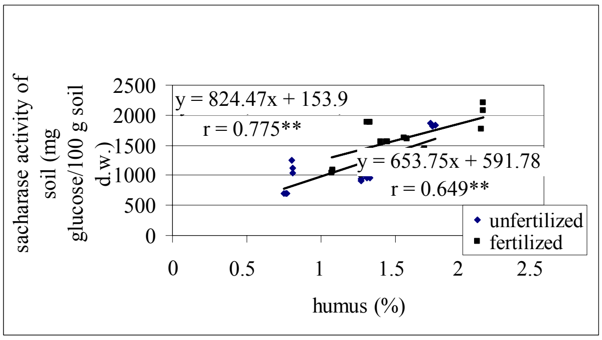

Fig. 2. Correlation between humus and saccharase activity of soil.

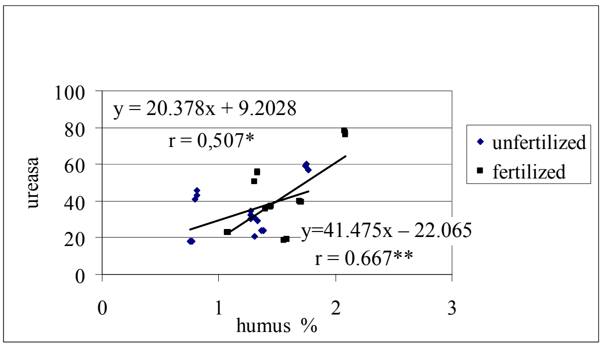

Fig. 3. Correlation between andurease activity of soil.

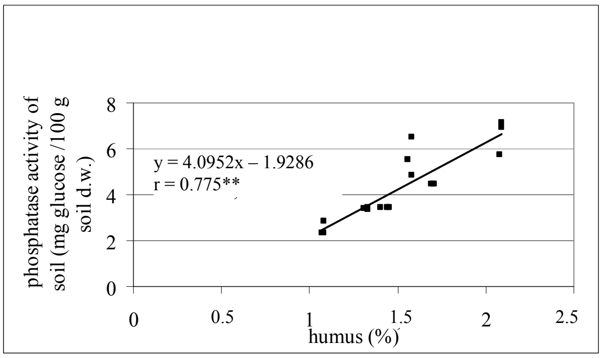

Fig. 4. Correlation between humus and phosphatase activity of soil.

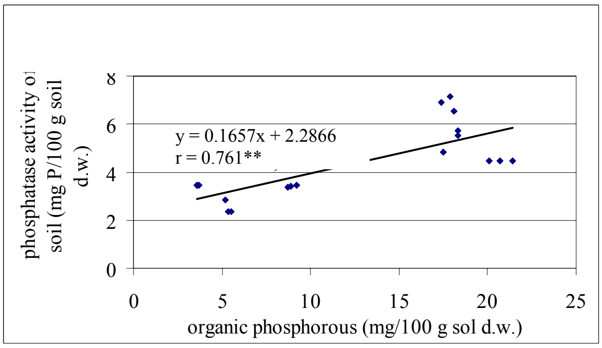

Fig. 5. Correlation between organic phospharous and total phosphatase of soil.

The worth of pedoenzymic tests consists in the fact that it estimates the soil potential for organic material transformation in closed dependence with the vital potential of soil and with agrotechnologies of: soil tillage, manuring, liming, irrigation, herbicide treatments, pests etc.

The pedoenzyme tests can surprise some rhythms of organic material biotransformations in soil under different agricultural interventions. Together with the tests of vital manifestations in soil (respiration, cellulolyse, free biologic fixation of nitrogen), reunited in Indicator of Vital Activity Potential (IVAP%), one obtains an agronomical significance regarding the evolution of land fertility status.

CONCLUSIONS

Agronomical signification of the pedoenzymic test by the fact that reflects the modification in pedoenzymic activity potential under the influence of different agricultural practices, depasses qualitatively the concept of *Biological significance of accumulate enzyme activity in soil.*

Between the results of agrochemical tests and those of pedoenzymic potentials exist some liniar and significant correlations which constitue a rational base for a complex control of fertility status of agricultural soils.

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