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EVALUATION OF SOIL ENZYME ACTIVITIES UNDER DIFFERENT CROPLANDS

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ABSTRACT

Soil enzyme activities play key roles in the biochemical functioning of soils, nutrient cycling, and in the degradation of litter and artificial substances. Knowledge of soil enzyme activities can provide information on changes in soil quality due to land use management. In this study, we report the actual and potential dehydrogenase and catalase activities involved in intracellular metabolism and phosphatases (phosphomonoesterases) activities involved in phosphorus metabolism under different croplands. Soil was sampled from the 0-10-, 10-20- and 20-30-cm depths from typical clay soil at Livada (Satu Mare County), and from typical chernozem and histosol at Berveni (Satu Mare County). Significant and unsignificant differences were registered in the soil enzymatic activities depending on the kind of enzymatic activity and the type of soil. All enzymatic activities studied were greater in typical chernozem compared to the typical clay soil and histosol. Based on the absolute values of the enzymatic activities, the enzymatic indicator of soil quality (EISQ) was calculated. The EISQ values ranged between 0.605 and 0.989 indicating an appreciable intensity of the enzymatic activities. These values mean that by determination of soil enzyme activities valuable information can be obtained regarding fertility status of soils. The enzyme activities were significantly intercorrelated with *r* values from 0.814 to 0.989 ($p<0.05$). A perfect positive correlation between enzymatic indicator of soil quality and grain yield was establish.

Keywords: cropland, soil enzyme activities, enzymatic indicators of soil quality.

INTRODUCTION

Soil is a complex and dynamic biological system where all biochemical processes are taking place through enzymatic activities produced by microorganisms (Dick, 1992). The microbial activity of a soil directly influence ecosystem stability and fertility and it is widely accepted that a good level of microbiological activity is essential for maintaining soil quality (Moore et al., 2000).

Many investigators often concentrate on finding out which soil properties best reflect the change of soil quality. According to Gil-Strotes et al. (2005) for estimation of soil quality, 40% of the consulted published papers used a general biochemical parameter such as microbial biomass, dehydrogenase activity, soil respiration, nitrogen mineralization capacity, while the remaining 60% considered a specific biochemical parameter such as urease or phosphatase activities. Amongst the general parameters, the dehydrogenase activity is the second most reliable (28% of authors) while phosphatase (28%) is the most frequently used among the specific biochemical parameters.

The objective of this study was to investigate the status of several enzyme activities involved in metabolism intracellular and in P-cycling in soil under different cropland use. Enzyme activities have the potential to anticipate changes in soils before they are detected by other soil properties (Ndiaye et al., 2000). The dehydrogenases were studied because these represent enzymes which indicate the status of environment and can be taken as a measure for the intensity of microbial metabolism in soil. Dehydrogenase activity assessments can

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be used in the evaluation of soil quality (Blonska et al., 2017). Dehydrogenase activity has also been used to evaluate the degree of recovery of degraded soils, being considered as a good indicator, even in soils that have been contaminated by petroleum spillage (Margesin et al., 2000).

The catalase activity was studied because this enzyme is used to characterize soil microbial activities. The catalase of aerobic organisms splits the toxic H_2O_2 produced from the mitochondrial electron transport and from various hydroxylation and oxygenation reactions into water and oxygen. Dehydrogenase and catalase are considered as indicators of the global and respiratory activity of soil and are used by microorganisms in the soil to break down organic matter (Utobo and Tewari, 2015). Soil organic matter is a frequently recalcitrant complex that is both synthesized and degraded by microbial enzyme activities. The balance between these two competing processes determines how much carbon is sequestered as well as contributing to soil aggregate structure and stability, plant nutrient availability, microbial diversity and activity and a host of enzymatic properties that determine soil fertility and plant productivity (Burns et al., 2013).

The phosphatases (acid and alkaline phosphatase) were studied because they are significantly affected by soil pH, which controls phosphorus availability, despite of organic matter content or levels of disturbance. Phosphatases have been correlated to P stress and plant growth, showing that they are good indicators of the P supply in a soil system (Dodor and Tabatabai, 2003).

MATERIAL AND METHODS

The experimental fields were located at the Agricultural Research and Development Station in Livada (Satu Mare County) on a typical clay soil with a pH of 5.6, a clay content of 22.4% and a humus content of 1.8 and at Berveni (Satu Mare County) on two soil types: typical chernozem with a pH of 7.9, a clay content of 27.5% and a humus content of 3.4 and histosol with a pH of 5.1 and the organic content of 86%, being a nutrient-rich soil.

The experiments were conducted using the Latin rectangle method in three blocks, the plot area being 21 square meters.

In October 2018, soil samples were taken from all soils under wheat and maize crops. Sampling depths were 0-10-, 10-20- and 20-30-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 Casida et al. (1964). 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 solution, respectively, for potential dehydrogenase. All reaction mixtures were incubated at 37^{o}C for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm. 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 (Drăgan-Bularda, 2000). The reaction mixtures consisted of 3.0 g soil, 2 ml H_2O_2 3%, and 10 ml phosphate buffer. It suffered incubation at 37^{o}C for 1 hour. Catalase activity was recorded as mg H_2O_2 decomposed by 1 g of soil in 1 hour.

Disodium phenylphosphate served as phosphate 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 Őhlinger (1996). The reaction mixtures consisted of 2.5 g soil, 2 ml toluene (antiseptic), 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 37°C 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. Phosphatase activities were expressed in mg phenol/g soil/ 2 hours.

The activity values were submitted to statistical evaluation by the t-test (Sachs, 2002).

RESULTS AND DISCUSSION

Results of the enzymological analyses are presented in Tables 1 and 2, and those of the statistical evaluation are summarised in Table 3.

Table 1. Enzymatic indicators of soil quality (EISQ) in lands under maize crop

Type of land	Soil depth (cm)	Soil enzymatic activity*					EISQ**
		Dehydrogenase		Catalase	Phosphatase		
		Actual	Potential		Acid	Alkaline	
Typical clay soil	0-10	6.981	7.224	3.50	0.219	0.144	0.605
	10-20	7.392	7.448	5.80	0.223	0.140	0.665
	20-30	7.952	8.232	5.90	0.222	0.162	0.707
Typical chernozem	0-10	7.672	9.072	8.80	0.262	0.366	0.916
	10-20	8.176	9.296	8.50	0.303	0.373	0.958
	20-30	8.600	9.876	9.50	0.220	0.367	0.941
Histosol	0-10	7.176	8.456	5.50	0.224	0.169	0.692
	10-20	7.840	8.240	5.70	0.243	0.170	0.724
	20-30	7.896	8.296	5.80	0.238	0.158	0.714

*Dehydrogenase activity (mg TPF/10 g soil/24h). Catalase activity (mg H_2O_2/g soil/h). Phosphatase activity (mg phenol/g soil/2h).
**EISQ - Enzymatic indicators of soil quality.

Table 2. Enzymatic indicators of soil quality (EISQ) in lands under wheat crop

Type of land	Soil depth (cm)	Soil enzymatic activity*					EISQ**
		Dehydrogenase		Catalase	Phosphatase		
		Actual	Potential		Acid	Alkaline	
Typical clay soil	0-10	5.708	6.650	2.90	0.241	0.192	0.630
	10-20	6.060	7.350	4.50	0.244	0.199	0.695
	20-30	5.640	7.943	4.80	0.207	0.143	0.649
Typical chernozem	0-10	6.772	7,779	6.60	0.268	0.316	0.844
	10-20	7.412	8.093	10.0	0.293	0.386	0.989
	20-30	6.844	8.552	9.40	0.250	0.375	0.938
Histosol	0-10	5.044	7.431	3.20	0.217	0.162	0.606
	10-20	6.523	7.554	4.70	0.245	0.177	0.705
	20-30	5.898	7.743	4.00	0.209	0.162	0.646

*Dehydrogenase activity (mg TPF/10 g soil/24h). Catalase activity (mg H_2O_2/g soil/h). Phosphatase activity (mg phenol/g soil/2h).
**EISQ - Enzymatic indicators of soil quality.

Variation of the five enzymatic activities in dependence of sampling depth

It is evident from Tables 1 and 2 that each enzymatic activity under both crops in the 10-20-cm were more than 1.2-fold higher than in the 0-10- and 20-30-cm depth. Previous studies with soils from various regions have shown that enzyme activities are sensitive to soil changes due to depth (Samuel et al., 2008; 2011), cropping systems (Saha et al., 2008) and land use (Acosta-Martinez et al., 2007).

The effect of cropland on the enzymatic activities in soil

The enzyme activities were generally lowest in the typical clay soil because it contained the lowest clay content (22.4%) and the lowest humus content (1.8%) among the soils. In addition, this soil contains the lowest total C (6%) and N (0.16%) contents among the soils. Actual and potential dehydrogenase and catalase activities were higher than acid and alkaline phosphatase activities in the soil studied under both crops

(Table 3). The different values of the soils studied may be related to the different of the enzymes: dehydrogenase and catalase activities are considered as indicators of the global and respiratory activity of soil, whereas phosphatase activities are related to the P-cycling in soil.

The predominance of soil enzyme activities is more related to the ecological role and kinetic characteristics of the enzymes studied despite the effects of chemical and physical properties, geology, and land use of the soils studied (Landgraf and Klose, 2002).

For evaluation of this effect, the results obtained in the three soil layers analyzed were considered together. In the soil under maize, actual dehydrogenase and catalase activities were significantly higher (at least at $p<0.05$) in typical chernozem than in the typical clay soil and histosol, respectively. Similar to potential dehydrogenase activity, alkaline phosphatase activity was significantly higher ($p<0.01$) only in typical chernozem than in typical clay soil while acid phosphatase activity was unsignificantly higher ($p>0.05$) in typical chernozem than in the other soils. It is evident from Table 3, that each enzymatic activity was unsignificantly higher ($p>0.05$), excepting potential dehydrogenase which was significantly higher ($0.05>p>0.02$) in histosol than in typical clay soil.

In the soil under wheat, dehydrogenase and catalase activities were significantly higher (at least at $p<0.05$) in the typical chernozem than in the other soils studied. Each of the two phosphatase activities determined was unsignificantly higher ($p>0.05$) in typical chernozem than in the other soils, excepting alkaline phosphatase activity which was significantly higher ($0.02>p>0.01$) in typical chernozem than in histosol. In addition, Table 3 shows that dehydrogenase activities were unsignificantly higher ($0.10>p>0.05$) in histosol than in typical clay soil whereas catalase and phophatase activities were unsignificantly higher ($0.10>p>0.05$) in typical clay soil than in histosol.

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 kind of enzymatic activity, the nature of crop and the type of soil. Based on the differences the following decreasing orders of the activities could be established in the soils:

- actual dehydrogenase activity:
  - typical chernozem>histosol>typical clay soil, under maize;
  - typical chernozem>histosol>typical clay soil, under wheat;
- potential dehydrogenase activity:
  - typical chernozem>histosol>typical clay soil, under maize;
  - typical chernozem>histosol>typical clay soil, under wheat;
- catalase activity:
  - typical chernozem>histosol>typical clay soil, under maize;
  - typical chernozem>typical clay soil> histosol, under wheat;
- acid phosphatase activity:
  - typical chernozem>histosol>typical clay soil, under maize;
  - typical chernozem>typical clay soil> histosol, under wheat;
- alkaline phosphatase activity:
  - typical chernozem>histosol>typical clay soil, under maize;
  - typical chernozem>typical clay soil> histosol, under wheat.

For establishing a hierarchy of the soils under both crops admitting equal importance for the five enzymatic activities, we have used the formula, referred to in Muntean et al. (1996), 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; Vmax(i) = maximum theoretical individual value.

The maximum theoretical individual value calculated from the composition of the reaction mixtures are: 13.45 mg formazan (dehydrogenase activities), 60 mg H_2O_2 (catalase activity) and 21.56 mg phenol (phosphatase activities). We mentioned that the enzymatic indicator may have values ranging 0 (when no real activity of any of the studied enzymes detected) and 1 (when all the activities have real individual values equal to the maximum theoretic values).

Tables 1 and 2 illustrate that all plots exceed the 0.5 value of the EISQ. Based on the results we may consider that the analyzed soils have an appreciable biological potential.

Table 3. Significance of the differences between enzymatic activities in the three types of soil

Type of land	Soil enzymatic activity*	Soil depth (cm)	Mean activity values			Significance of the differences
			a	b	a-b	
Maize crop Typical clay soil (a) vs. typical chernozem (b)	ADA PDA CA AcPA AlkPA	0-30	7.441 7.634 5.066 0.221 0.148	8.149 9.414 8.933 0.261 0.368	-0.708 -0.178 -3.867 -0.040 -0.220	0.01>p>0.001 0.01>p>0.001 0.05>p>0.02 0.10>p>0.05 0.01>p>0.001
Maize crop Typical clay soil (a) vs. histosol (b)	ADA PDA CA AcPA AlkPA	0-30	7.441 7.634 5.066 0.221 0.148	7.637 8.330 5.666 0.235 0.165	-0.196 -0.696 -0.600 -0.014 -0.017	p>0.10 0.05>p>0.02 p>0.10 0.10>p>0.05 0.10>p>0.05
Maize crop Typical chernozem (a) vs. histosol (b)	ADA PDA CA AcPA AlkPA	0-30	8.149 9.414 8.933 0.261 0.368	7.637 8.330 5.666 0.235 0.165	0.512 1.084 3.267 0.026 0.203	0.05>p>0.02 0.10>p>0.05 0.05>p>0.02 0.10>p>0.05 p>0.10
Wheat crop Typical clay soil (a) vs. typical chernozem (b)	ADA PDA CA AcPA AlkPA	0-30	5.802 7.314 4.066 0.230 0.178	7.009 8.141 8.666 0.270 0.359	-1.207 -0.827 -4.600 -0.040 -0.181	0.01>p>0.001 0.05>p>0.02 0.02>p>0.01 0.10>p>0.05 0.10>p>0.05
Wheat crop Typical clay soil (a) vs. histosol (b)	ADA PDA CA AcPA AlkPA	0-30	5.802 7.314 4.066 0.230 0.178	5.821 7.576 3.966 0.223 0.167	-0.019 -0.262 0.100 0.007 0.011	0.10>p>0.05 0.10>p>0.05 0.10>p>0.05 0.10>p>0.05 0.10>p>0.05
Wheat crop Typical chernozem (a) vs. histosol (b)	ADA PDA CA AcPA AlkPA	0-30	7.009 8.141 8.666 0.270 0.359	5.821 7.576 3.966 0.223 0.167	0.396 0.565 4.700 0.047 0.192	0.05>p>0.02 0.05>p>0.02 0.02>p>0.01 0.10>p>0.05 0.02>p>0.01

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

The variation in enzyme activities as affected by the soil orders is associated in part with the geology of the parent material. In addition to the geology and soil texture, the variation of the enzyme activities is due to the differences in organic C content among soil orders. The trends found in the enzyme activities as affected by land use are in agree with previous studies. According to Acosta-Martinez et al. (2007) the soils under pasture sustained higher enzyme activities, compared to the correspondence agricultural soil, are due to the positive impacts of the surface cover, vegetation, and lack of tillage of pasture on soil properties, including the microbial populations and activities.

Previous studies have shown that changes in the microbial communities may influence the potential of soils for enzyme mediated substrate catalysis (Kandeler et al., 1999). Acosta-Martinez et al. (2003) reported that the higher enzyme activities under pasture than soils under agriculture were correlated to higher microbial biomass and fatty acid indicators of protozoa and fungal populations.

Another study found significant differences in enzyme activities between forest and agricultural soil at 0-15-cm depth. The lowest activities of dehydrogenase and urease were recorded in the soils where crops were cultivated thus demonstrating that enzyme activity is influenced by the organic matter content of the soil (Blonska et al., 2017).

Relationships of enzyme activities.

The relationships among enzyme activities were investigated. Linear regression analysis showed a trend of a negative relationship (Table 4) between enzyme activities involved in intracellular metabolism (dehydrogenase and catalase) and enzymes involved in phosphorus metabolism (phosphatase). These findings will apply for the typical clay soil and for the typical chernozem. Enzyme activities in a histosol were positive intercorrelations probably due to higher organic matter levels (86%) which support greater microbial activity because of greater supplies of energy and nutrients. The enzyme activities were significantly intercorrelated with *r* values from 0.814 to 0.989 ($p<0.05$). The intercorrelations between the enzyme activities studied indicate the enzymes responded similarly to the land use systems. This is consistent with other studies that have shown this relationship.

Frankenberger and Dick (1983) observed a similar relationship that exist between the various soil enzyme activities and microbial numbers and biomass. Of the 11 enzymes evaluated, alkaline phosphatase, amidase, and catalase were concluded to be the most satisfactory choices in determining the relative activity and mass of the microbial population in soils.

According to Colvan et al. (2001) hay meadow soils treated with farmyard manure for about 100 years had higher acid and alkaline phosphomonoesterase and higher extractable P than those receiving mineral P. Alkaline phosphomonoesterase activity was positively correlated with extractable P in soils treated with farmyard manure or with phosphate, whereas acid phosphomonoesterase activity was negatively correlated with extractable P. In addition, acid phosphomonoesterase activity was negatively correlated with alkaline phosphomonoesterase when considering all treatments but was always higher than the alkaline phosphomonoesterase because all soils were acidic.

In a study on a semiarid soil, linear regression analyses indicated that enzyme activities were significantly intercorrelated with *r* values up to 0.98 ($P<0.001$) and a positive correlations between enzyme activities and total C, *r* values up to 0.96, $P<0.01$ (Acosta-Martinez et al., 2003).

Dehydrogenase, catalase and acid phosphatase were determined in the 0-20-, 20-40- and 40-60-cm layers of a typical clay soil submitted to a complex tillage and crop rotation experiment were significantly intercorrelated with *r* values from 0.912 to 0.998, which suggest that tillage and crop rotations systems have similar effects on these activities (Samuel et al., 2017).

Table 4. Correlation matrix (*r* values) between soil enzyme activities

Variables	Actual dehydrogenase	Potential dehydrogenase	Catalase	Acid phosphatase	Alkaline phosphatase
Typical clay soil					
Actual dehydrogenase	-	0.471	0.634	-0.227	-0.421
Potential dehydrogenase	-	-	0.814*	-0.632	-0.454
Catalase	-	-	-	-0.380	-0.410
Acid phosphatase	-	-	-	-	0.908*
Alkaline phosphatase	-	-	-	-	-
Typical chernozem					
Actual dehydrogenase	-	0.894*	0.383	-0.180	0.400
Potential dehydrogenase	-	-	0.393	-0.420	0.404
Catalase	-	-	-	-0.138	0.937*
Acid phosphatase	-	-	-	-	0.138
Alkaline phosphatase	-	-	-	-	-
Histosol					
Actual dehydrogenase	-	0.855*	0.989*	0.684	0.108
Potential dehydrogenase	-	-	0.822*	0.271	-0.103
Catalase	-	-	-	0.663	0.169
Acid phosphatase	-	-	-	-	0.528
Alkaline phosphatase	-	-	-	-	-

*Significant at 0.05 level.

The effect of cropland on the grain yield

Maize yield varied from 81.10 q/ha for histosol to 95.20 q/ha for typical chernozem. In the case of wheat, it ranged from 63.79 q/ha to 95.20 q/ha, highest for typical chernozem and lowest for typical clay soil. Grain yields of maize and wheat were significantly influenced by types of soils (Figures 1 and 2). One can see from figures a perfect positive correlation (r=1 and r=0.967) between enzymatic indicator of soil quality and maize, respectively, wheat yield.

The first data on the grain yield in the three soil types (typical clay soil, typical chernozem and histosol) were published by Mondici et al. (2019). They studied the influence of the herbicide treatments at wheat crops in the three types of soil and found that yield increases were obtained on each type of soil, but on typical clay soil not all variants produced statistically significant increase as on typical chernozem and histosol. In addition, the costs of the herbicide treatments are different, but the yields were also different due to different efficacy on the floral composition of the weeds on the three soil types.

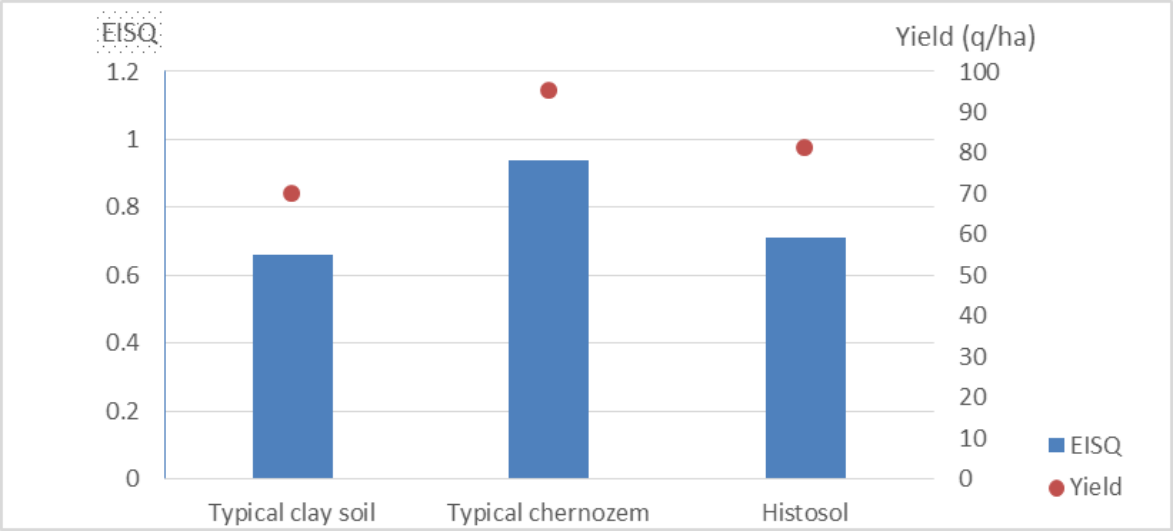

Figure 1. Correlation between enzymatic indicator of soil quality and maize yield

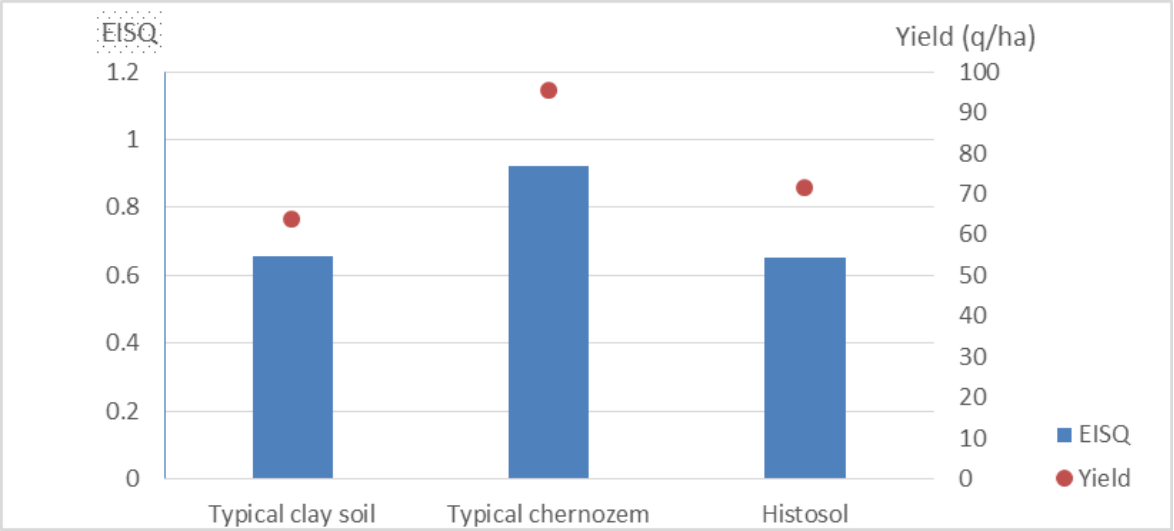

Figure 2. Correlation between enzymatic indicator of soil quality and wheat yield

CONCLUSIONS

The results provide information on important enzyme activities involved in intracellular metabolism and in phosphorus metabolism that should be taken into consideration under different land use and management decisions of the North-West region of Romania. The set of enzymes used in this study may have significant effects on soil biology, environmental management, growth, and nutrient uptake in plants growing in ecosystems.

The obtained results confirm the usefulness of assessment of enzyme activity to evaluate the differently managed soils. The results of the present study demonstrated that the soil enzyme activities have been reported as useful soil quality indicators due to their relationship to soil biology, being described as “biological fingerprints” of soil management and relate to soil structure.

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