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Enzymatic activity in soil samples from Gilău and Florești transect, Cluj, Romania

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Abstract. The aim of this paper was to assess the activity of some enzymes from soils of polluted zones in order to understand the complex processes that happen in these habitats of special significance. The sampling sites were 5 zones from Gilău and Florești transect. There were established the types of the soil samples collected from different places. In the soil samples the following enzymatic activities have been quantitatively determined: actual and potential dehydrogenase, phosphatase and catalase. The studied activities were detected in all five soil samples, with differences noticed only as regards the intensity of the processes. Based on the absolute values on enzymatic activities, the enzymatic indicator of soil quality (EISQ) was calculated. The EISQ values ranged between 0.05 and 0.27, indicating a low intensity of the enzymatic activities.

Key Words: soil type, enzymatic activity, enzymatic indicator of soil quality.

Rezumat. Scopul acestei lucrări a constat în determinarea activității unor enzime din solurile unei zone poluate cu scopul de a înțelege procesele complexe care au loc în aceste habitate cu importanță specială. Au fost prelevate probe din 5 zone cuprinse pe transectul dintre localitățile Gilău şi Florești. A fost stabilit tipul de sol al fiecărei probe prelevate. În probele de sol au fost determinate cantitativ următoarele activități enzimatice: activitatea dehidrogenazică actuală și potențială, activitatea fosfatazică și activitatea catalazică. Activitățile enzimatice au fost detectate în toate cele 5 probe de sol, cu diferențe între ele în ceea ce privește intensitatea proceselor. Pe baza valorilor absolute a activităților enzimatice s-a calculat indicatorul enzimatic de calitate al solurilor (IECS). Valorile IECS au fost cuprinse între 0.05 și 0.27, indicând o intensitate scăzută a activităților enzimatice.

Cuvinte cheie: tip de sol, activitate enzimatică, indicatorul enzimatic al calității solului.

Introduction. Soil enzymes are a group of enzymes found in soil and they are constantly playing an important role in maintaining soil ecology, physical and chemical properties, fertility of soils and contribute to the health of soils. Soil enzyme activities are often closely related to soil organic matter, soil physical properties and microbial activity or biomass, changes quicker than other parameters, thus providing early indications of changes in soil health, and involve simple procedures (Dick et al 2000). In addition, soil enzyme activities can be used as measures of microbial activity, soil productivity, and inhibiting effects of pollutants.

Soil enzyme data have constituted the foundation for the development of conceptual models that provide a more comprehensive understanding of those key processes linking microbial populations and nutrient dynamics (Schimel & Weintraub 2003).

Soil contamination with heavy metals has become a serious issue all over the world, as a result of wastewater irrigation, sludge application, solid waste disposal, automobile exhaust and industrial activities (Cui et al 2005).

Soil enzymes are synthesized by microorganisms and act as biological catalysts to facilitate different reactions and metabolic processes, to decompose organic pollutants and produce essential compounds for both microorganisms and plants (Moreno et al 2003). Soil enzymatic activities are recognized as a more sensitive bio-indicator than plants and animals of any natural and anthropogenic disturbance (Hinojosa et al 2004).

Soil enzyme assays have been viewed as one of the cheapest and easiest techniques and have been used before as sensors for measuring the soil pollution and also to integrate information about microbial community status (Baum et al 2003). Enzymatic activities are frequently used for determining the influence of various pollutants, including heavy metals, on soil microbiological quality (Khan et al 2007).

The analysis of the enzymatic activity in soils from different ecosystems is a research method in evaluation of the functional diversity of the microbiota involved in the biogeochemical cycles. The enzymatic activity in the soil is mainly of microbial origin, being based on intracellular, cell-associated and free enzymes. A unique balance of chemical, physical and biological (including microbial enzyme activities) components contribute to maintaining soil health. Thus, evaluation of soil health requires indicators of all these components.

The enzymatic activity assessing offers, faster than the microbiological analysis, the suggestive data regarding the processes that are taking place in soils or in other natural habitats (Carpa 2011).

The aims of the present paper are: to establish the types of the soil samples collected from different environments; to investigate enzymatic activities on two oxidoreductases (dehydrogenase and catalase) and one hydrolase (involved in the cycle of P-phosphatase) and to calculate the enzymatic indicator of soil quality. This approach may permit an evaluation of the status of ecosystems.

Materials and Methods

Site characteristics and soil sampling. The soil samples were taken from the Gilău and Florești transect. Soil samples were collected using an auger (5 cm diameter) at 0-5, 10 and 15 cm soil depths. The samples were kept at 4 °C until soil enzymatic analysis was performed.

Chemical and physical analyses. Physico-chemical analyses were performed on the soils. The pH of soil samples was measured with Mettler Toledo electrode. Soil texture and humus of soil samples were determined in Laboratory of Pedology, USAMV Cluj. The type of soil specific to each collected sample was determined (Blaga et al 2005).

Enzymatic analyses. The enzymological analyses consisted in assessing the following enzymatic activities: actual and potential dehydrogenase (ADA and PDA) – activities expressed in mg formazan/g soil dry matter (Casida 1977); phosphatase activity (PA) expressed in mg phenol/g soil dry matter; catalase (CA) – activity expressed in mg splitted H_2O_2/g soil dry matter (Carpa et al 2014). Based on theoretical values of enzymatic activity, enzymatic indicator of soil quality (EISQ) was calculated.

Results and Discussion

Chemical and physical analyses. Soil samples from different area were characterized and classified according to the Romanian Soil Classification System (2003) using the criteria established by the Pedology Lab from USAMV, Cluj (Table 1).

All the soil samples have presented a pH around the neutral value and the humus quantity varied from 1.2 to 3.8 at samples from zone 5. The texture of soil samples was medium (for P1, P2 and P5) to coarse (for P3 and P4). It was established that all the 15 soil samples belong to Alluvial type from typical (for P1, P2 and P5) to arenic (for P3 and P4) (Table 1).

Soil enzyme activities have been related to soil physio-chemical characters (Amador et al 1997), microbial community structure (Caldwell 2005), vegetation (Sinsabaugh et al 2002), disturbance (Boerner et al 2000), and succession (Tscherko et al 2003; Caldwell 2005).

Table 1

Samples	рН	Texture	Humus	Soil type
P1	7.1	Medium, sandy SM	2.11	Alluvial typical
	7 neutral	Medium, medium sandy clay SM	2.91	Alluvial typical
	7.2	Medium, sandy loamy SM	2.98	Alluvial typical
P2	7.2	Medium, medium sandy clay SM	2.43	Alluvial typical
	7	Medium, medium sandy clay SM	2.60	Alluvial typical
	7.5	Medium, medium sandy clay SM	2.92	Alluvial typical
Р3	7 neutral	Coarse, fine loamy sand UF	1.21	Alluvial arenic
	7	Coarse, fine loamy sand UF	1.32	Alluvial arenic
	7.3	Coarse, fine loamy sand UF	1.67	Alluvial arenic
P4	7.4	Coarse, fine loamy sand UF	1.27	Alluvial arenic
	7.6	Coarse, fine loamy sand UF	1.42	Alluvial arenic
	7.7	Coarse, fine loamy sand UF	1,85	Alluvial arenic
Р5	7	Medium, medium clay LL	2.91	Alluvial typical
	7.2 neutral	Medium, medium clay LL	3.2	Alluvial typical
	7.6	Medium, loamy LL	3.8	Alluvial typical

Soil types on Gilău and Florești transect

Enzymological analyses. In the soil environment, almost all reactions are catalysed by enzymes that are largely of microbial origin and associated with viable cells (Dick 1997).

Several environmental factors, including soil moisture, oxygen availability, oxidation-reduction potential, pH, organic matter content, depth of the soil profile, temperature, season, heavy metal contamination and soil fertilization or pesticide use can significantly affect DHA in the soil environment.

The faintest actual dehidrogenases activity was recorded at the samples from P2 zone, presumable due to the environmental conditions with neutre pH influencing the developing of microorganisms. Overall, PDA is much more intense than ADA (Table 2). This shows the stimulating action of the carbon source (added glucose) on the enzymes synthesis by microorganisms.

Soil dehydrogenases (EC 1.1.1.) are the major representatives of oxidoreductases enzymes class (Gu et al 2009). Among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity (Gu et al 2009; Salazar et al 2011), because they are found intracellularly in all living microbial cells (Moeskops et al 2010; Yuan & Yue 2012). Moreover, they are tightly linked with microbial oxidation-reduction processes (Moeskops et al 2010). An important aspect is that dehydrogenases do not accumulate extracellular in the soils. Dehydrogenase activity plays an important role in the oxidation of organic matters (Dick et al 2000).

Dehydrogenase activity appeared to be one of the most sensitive parameters, among the selected enzyme activities. It could be used as a good indicator in a soil ecotoxicological test regarding heavy metals (Maliszewsk-Kordybach & Smeeczak 2003; Hinojosa et al 2004).

The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are the part of respiration pathways of soil microorganisms and are closely related to the type of soil and soil air-water conditions.

Table 2

Samples	Depth	Enzymatic Activities					
	(cm)	Phosphatase	Catalase	Dehydrogenase		_	
		mg phenol	mg H_2O_2	Actual	Detential		
		/2.5 y	spiit/1.5g	ACLUAI	Polenila		
				formazan/2.5q	formazan/2.5 g		
				,	, J		
P1	5	6.794	12.350	0.785	1.014	0.164	
	10	2.904	24.770	0.318	0.933	0.160	
	15	3.545	26.755	0.486	1.091	0.182	
P2	5	5.852	34,352	0.963	1.554	0.258	
	10	5.680	39.536	0.397	1.012	0.257	
	15	7.227	39.428	0.235	1.143	0.274	
22	5	4 802	32 978	0 489	1 080	0 222	
15	10	4 744	35 249	0.334	0.949	0.222	
	15	5.766	38.489	0.345	0.950	0.251	
P4	5	1.876	10.229	0.245	0.433	0.077	
	10	1.498	8.257	0.079	0.108	0.055	
	15	1.743	8.918	0.720	0.978	0.089	
P5	5	1.941	11.451	0.076	0.104	0.074	
	10	1.602	13.179	0.493	0.634	0.094	
	15	1.474	13.143	0.133	0.181	0.078	

Enzymatic activities in soil samples from Gilău-Florești transect

Soil phosphatases are important in soil P cycling, involving in mineralization of organic P and releasing phosphate for plants (Pant & Warman 2000; Khan et al 2007).

The phosphatase activity was detected in all the 15 soil samples from Gilau-Floresti transect, the distribution of the phosphatase activity values being shown in Tab. 2. It can be seen that FA was more intense in the samples from zone 2 and 1, compared with other zones. The faintest activity was recorded at the soil samples from zone 1. The phosphatase activity is limited by the availability of organic carbon in the soil.

Phosphatase plays a critical role in the hydrolysis of organic phosphorus (P) compounds in soil, and the ratio of alkaline phosphatase to acid phosphatase is usually treated as an indicator of susceptibility to soil pH (Dick et al 2000; Enowashu et al 2009).

In soil ecosystems, these enzymes are believed to play critical roles in P cycle as evidence shows that they are correlated to P stress and plant growth. Apart from being a good indicator of soil fertility, phosphatase enzyme plays a key role in the functioning of the soil system (Dick et al 2000).

The catalase activity was very intense in the soil samples from P2 zone and from P3 zone and less intense in the soil from P5 zone (Table 2). Catalase is an enzyme which accumulates in soil and thus it preserves for a long time its activity (Garcia-Gil et al 2000).

Catalase splits hydrogen peroxide into molecular oxygen and water. It is also correlated with the humus quantity in soil, the pH and the number of soil microorganisms. These enzymes also participate to degradation and/or incorporation of xenobiotic substances in the organic matter of the soil.

Enzymes are defined as biological catalysts for specific reactions, which depend on several biotic and abiotic factors such as: pH, temperature, presence or absence of inhibitors, soil organic matter composition, cultivation technique and other factors, which can directly influence the chemical reactions of these molecules within soil (Bowles et al 2014).

The origin of soil enzymes is mainly microbial. They can be found within microbial cells as well as extracellularly in the soil, bound to clay minerals and humic substances (Burns & Dick 2002).

Microbial enzymes can participate in adsorption, oxidation, reduction, hydrolysis, and complexation reactions, converting organic substances into other products to maintain the balance in the soil environment (Caldwell et al 2005; Cenciani et al 2011).

Enzymatic indicator of soil quality (EISQ). The analytical data serves as the base for calculating the enzymatic indicator of the soil quality (EISQ) (Muntean et al 1995-1996). Theoretically, the enzymatic indicator may exhibit values between 0 (where no activity exists in the studied samples) and 1 (where all the real individual values are equal to the maximal theoretical individual values of all activities).

Based on theoretical values of enzymatic activity, enzymatic indicator of soil quality (EISQ) was calculated, after formula:

$$EISQ = \frac{1}{n} \bullet \sum_{i=1}^{n} \frac{V_r(i)}{V_{\max}(i)}$$

Where, EISQ = the enzymatic indicator of soil quality;

n = number of activities;

V_r(i) = real individual value;

 $V_{max}(i)$ = maximum theoretical individual value.

In Fig. 1 there the variation of the enzymatic potential of analysed soils is depicted, as reflected by the values of the studied indicator. The studied soils have a relatively low enzymatic activity. The maximum value was recorded at the samples from zone 2 (0.27) and the minimum one, of 0.05, at the samples from zone 4. The quality of the soil is better as EISQ grows (Muntean et al 1995-1996; Carpa 2007). Considering EISQ, the soil from Gilău-Florești transect has a low enzymatic activity.



Figure 1. The enzymatic indicator of the soil quality (EISQ) in soil samples from Gilău-Florești transect.

Conclusions. It is quite essential to understand the possible roles that soil enzymes play in order to maintain soil health and apply a fertility management. The identified soil samples were of Alluvial type for all five zones and all have a neutral pH. All four studied quantitative enzymatic activities have shown variations of their intensities depending on the sampling place and on the necessary substrate from the enzymes synthesis by the microorganisms: a. In the soil samples PDA was higher than ADA, which shows the stimulating effect of the easy accesible carbon source (glucose), added to the samples, on enzyme synthesis by microorganisms.

b. Phosphatase activity (PA) presented slightly higher values. These values are due to accumulation of vegetal remains in soil.

c. Catalase activity (CA) was higher in the samples from zones 2 and 3.

Based on EISQ values, the soil from Gilău-Florești transect has a low enzymatic activity (between 0.05 and 0.27). The enzymatic indicators of soil quality reached the highest values (EISQ = 0.2) in P2 samples. These values indicate the presence of low enzymatic potential in these analyzed soils.

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