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THE ESTIMATION OF PHOSPHATASE ACTIVITY IN SOIL

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Abstract: *The importance of phosphatase for plant nutrition has repeatedly been pointed out. In most soils, the organically bound P- fraction is higher than the inorganic. Phosphorus uptake by plants requires mineralization of the organic P-component by phosphatases to orthophosphate. Phosphatases are inducible enzymes that are produced predominantly under conditions of low phosphorus availability. Phosphatases are excreted by plant roots and by microorganisms. Microbial phosphatases dominate in soils. The phosphomonoesterases, so-called phosphatases differ in their substrate specificity and their pH optimum. One can thus diferentiate between acid and alkaline phosphatases in the soil. Phosphatase activities were determined in the 0-20-, 20-40- and 40-60-cm layers of a preluvosoil submitted to a complex tillage (no-till and conventional tillage), crop rotation (2- and 3-crop rotations) and fertilisation [mineral(NP) fertilisation and farmyard-manuring] experiment. It was found that the activities decreased in the order: acid phosphatase activity > alkaline phosphatase activity. Each activity decreased with increasing sampling depth. No-till –in comparison with conventional tillage – resulted in significantly higher soil phosphatase activities in the 0-20-cm layer and in significantly lower activities in the deeper layers. The soil under maize or wheat was more phosphatase-active in the 3-than in the 2-crop rotation.In the 2-crop rotation higher soil phosphatase activities were recorded under wheat than under maize. Farmyard-manuring of maize – in comparison with its mineral fertilisation – led to a significant increase in each activity.*

Key words: *crop rotation, farmyard-manured, phosphatase, preluvosoil, tillage*

INTRODUCTION

The degradation of plant and animal matter, the release and binding of nutrients and trace elements, is one of the most important functions of soil organisms (BANDICK, 1999). The microorganisms are important for the enzymatic degradation of the complex organic substances to nutrients and for the release of nutrients and trace elements from the mineral soil fraction (DICK, 1992; DICK et al., 1988). The name phosphatase describes a group of enzymes that hydrolyzes esters as well as anhydrides of phosphoric acid (DICK et al., 1994). To determine phosphatase activity, one can use either phosphate, which is produced through the mineralization of natural organic phosphate esters, or organic components after mineralization of artificial organic substrates (BALOTA et al., 2003; CANARUTTO et al., 1995).

The phosphomonoesterases, so-called phosphatases differ in their substrate specificity and their pH optimum (KANDELER and MURER, 1993; KIRCHNER et al., 1993). One can thus diferentiate between acid and alkaline phosphatases in the soil (CLARHOLM and ROSENGREN-BRINK, 1995; DENG and TABATABAI, 1997).

In this aim, we determined acid and alkaline phosphatase activities in a preluvosoil submitted to a complex tillage, crop rotation and fertilisation experiment at the Agricultural Research and Development Station in Oradea (Bihor county).

MATERIALS AND METHODS

The ploughed layer of the studied soil is of mellow loam texture, it has a pH value of 5.5, medium humus(2.32%)and P(22 ppm) contents, but it is rich in K (83 ppm).

The experiment started in 1992. The experimental field occupying 2.84 ha was divided into plots and subplots for comparative study of no-till and conventional tillage, rotations of 2 and 3 crops, and mineral (NP) fertilisation and farmyard-manuring. The plots (and subplots) were installed in three repetitions.

In October 2009, soil was sampled from all subplots. Sampling depths were 0-20, 20-40 and 40-60 cm. The soil samples were allowed to air-dry, then ground and passed through a 2 mm sieve and, finally, used for determination of phosphatase activities. Disodium phenylphosphosphate served as enzyme substrate (ŐHLINGER, 1996). 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 (SAMUEL and KISS, 1999).

The reaction mixtures consisted of 2.5g soil, 2 ml toluene (antiseptic), 10 ml buffer solution and 10 ml 0.5% substrate solution. Reaction mixtures without soil or without substrate solution were the control. All reaction mixtures were incubated at 37^0C 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 are expressed in mg phenol/g soil/2 hours. The activity values were submitted to statistical evaluation by the two –way t-test (SACHS, 2002).

RESULTS AND DISCUSSION

Results of the determination of phosphatase activities are presented in Table 1, and those of the statistical evaluation are summarised in Table 2.

Comparison of the two phosphatase activities measured. At the same soil depth (0-20-, 20-40-, or 40-60-cm) in both subplots under wheat and maize crop of both 2- and 3- crop rotations, the activities decreased in the order: acid phosphatase activity > alkaline phosphatase activity (Table 1). This decreasing order is also valid for the mean values of the two activities (Table 2).

Table 1

The effects of soil management practices on phosphatase activities in a preluvosoil

Soil phosphatase activity*	Soil depth (cm)	Rotation of 2 crops**				Rotation of 3 crops**					
		Wheat		Maize		Maize		Wheat		Maize (FYM)	
		N.t.	C.t.	N.t	C.t.	N.t	C.t.	N.t	C.t.	N.t	C.t.
Acid	0-20	0.26	0.20	0.22	0.20	0.28	0.24	0.33	0.31	0.30	0.29
	20-40	0.16	0.23	0.19	0.19	0.15	0.16	0.20	0.22	0.17	0.20
	40-60	0.12	0.16	0.11	0.13	0.12	0.14	0.12	0.15	0.16	0.16
Alkaline	0-20	0.20	0.19	0.25	0.17	0.24	0.19	0.26	0.24	0.31	0.25
	20-40	0.13	0.16	0.11	0.15	0.14	0.16	0.17	0.20	0.20	0.20
	40-60	0.05	0.08	0.04	0.07	0.06	0.09	0.08	0.09	0.05	0.06

Variation of the two soil phosphatase activities in dependence of sampling depth. It is evident from Table 1 that each phosphatase activity decreased with sampling depth in both subplots under wheat and maize crops. In addition, Table 2 shows that the mean values of each of the two activities in both non-tilled and conventionally tilled subplots also decreased with increasing soil depth.

The effect of tillage practices on the phosphatase activities in soil. Each of the two phospatase activities determined was significantly higher (at least at $p< 0.01$) in the upper (0-20- cm) layer of the non-tilled subplots than in the same layer of the conventionally tilled

* Expressed in mg phenol/g soil/2 hours.
** N.t. – No-till. C.t. – Conventional tillage.
(FYM) – (farmyard –manured).

subplots. The reverse was true (at least at p< 0.01) in the deeper (20-40- and 40-60-cm) layers. These findings are valid for subplots under each crop of both rotations.

Table 2

Significance of the differences between phosphatase activities in a preluvosoil submitted to different management practices

Management practices	Soil enzymatic activity*	Soil depth (cm)	Mean activity values in management practices			Significance of the differences
			a	b	a-b	
No-till(a) versus conventional tillage(b)	AcPA	0-20	0.296	0.272	0.024	0.002>p>0.001
		20-40	0.178	0.202	-0.024	0.02>p>0.01
		40-60	0.128	0.148	-0.020	0.01>p>0.002
	AlkPA	0-20	0.256	0.218	0.038	0.01>p>0.002
		20-40	0.155	0.178	-0.023	0.001>p>0.0001
		40-60	0.060	0.080	-0.020	0.001>p>0.0001
The same crop in the two rotations						
Maize in 2-crop rotation (a)versus maize in 3-crop rotation (b)	AcPA	0-60	0.177	0.185	-0.008	0.01>p>0.002
	AlkPA	0-60	0.138	0.150	-0.012	0.0001>p
Wheat in 2-crop rotation(a) versus wheat in 3-crop rotation (b)	AcPA	0-60	0.194	0.227	-0.033	0.10>p>0.05
	AlkPA	0-60	0.138	0.179	-0.041	0.002>p>0.001
Different crops in the same rotation						
2-crop rotation						
Maize (a) versus wheat (b)	AcPA	0-60	0.177	0.194	-0.017	0.01>p>0.002
	AlkPA	0-60	0.138	0.138	0.000	-
3-crop rotation						
Maize (a) versus wheat (b)	AcPA	0-60	0.185	0.227	-0.042	0.02>p>0.01
	AlkPA	0-60	1.150	0.179	-0.029	0.01>p0.002
Maize (a) versus maize (FYM)**** (b)	AcPA	0-60	0.185	0.218	-0.033	0.001>p>0.0001
	AlkPA	0-60	0.150	0.181	-0.031	0.01>p>0.002
Wheat (a) versus maize (FYM) (b)	AcPA	0-60	0.227	0.218	0.009	0.01>p>0.002
	AlkPA	0-60	0.179	0.181	-0.002	0.02>p>0.01

The effect of crop rotations on the phosphatase activities in soil. For evaluation of this effect, the results obtained in the three soil layers analysed in the two subplots of each plot were considered together.

Soil phosphatase activities as affected by different crops in the same rotation

The 2-crop rotation. Acid phosphatase activity measured in the wheat soil exceeded significantly (p< 0.01) the coresponding activity recorded in the maize soil. Alkaline phosphatase activity is the same under wheat and maize crops.

The 3-crop rotation. Significant (p< 0.05 to p< 0.001) and unsignificant (p>0.05 to p >0.10) differences were registered in the soil phosphatase activities depending on the type of activity and the nature of crop. Based on these differences the following decreasing orders of the activities could be establised in the soil:

acid phosphatase activity: maize (FYM) > maize > wheat;

alkaline phosphatase activity: maize (FYM) > maize > wheat.

It is evident from these orders that position 1 is occupied by the farmyard-manured maize plot, followed by minerally fertilised cereal (maize and wheat) plots.

Soil phosphatase activities as affected by fertilisation. The two maize plots in the 3-crop rotation could serve for comparing the effect of mineral (NP) fertilisation (plot 1) and farmyard-manuring (plot 3) on the soil phosphatase activities. Each activity was higher in the farmyard-manured maize plot than in the minerally fertilised maize plot. The differences were significant (at least at p< 0.01).

* AcPA – Acid phosphatase activity.
AlkPA – Alkaline phosphatase activity.
** (FYM) – (farmyard-manured)

CONCLUSIONS

The soil phosphatase activities decreased in the order: acid phosphatase activity > alkaline phosphatase activity.

Each phosphatase activity decreased with increasing soil depth.

No-till in comparison with conventional tillage - resulted in higher phosphatase activities in the 0-20- cm soil layer and in lower activities in the 20-40- and 40-60- cm soil layers.

The 3-crop rotation – as compared to the 2-crop rotation – led, to higher phosphatase activities in the soil layers under maize or wheat.

In the 2-crop rotation, the soil layers under wheat were more phosphatase-active than those under maize.

Farmyard-manuring – in comparison with mineral (NP) fertilisation proved to be more efficient in increasing phosphatase activities in soil layers under maize in the 3-crop rotation.

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