PHOSPHATASE ACTIVITY OF TYPICAL CHERNOZEM SOIL IN FIELD CROP ROTATION

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Abstract

Soils of the Republic of Moldova are characterized mostly by a low content of mobile phosphorus. In the context of sustainable agriculture restoration and maintenance of soil fertility has become a key problem. The main role in the process of phosphorus mineralization in the soil, from organic compounds, belongs to orthophosphoric monoester phosphohydrolases (phosphatases). The aim of this research was the assessment of phosphatasic activity in soil, depending on the system of fertilization and crop type. The long-term field experiment (since 1972) is located in Balti steppe, RM, on the typical chernozem soil. The results of comparative analysis of phosphatasic activity (acid – pH 5.0, natural – un-buffered conditions and alkaline – pH 10.2) are presented. The extracellular activity was determined by soil incubation with toluene, and with disodium p-nitrophenyl phosphate hexahydrate salt as the substrate. The soil samples were collected under the following crops: vetch + oats, winter wheat and sugar beet, which are a part of a field crop rotation. Three fertilization systems were analyzed: mineral, mineral + organic, organic and control – un-fertilized soil. The investigation results revealed that in all examined treatments, the phosphatasic activity determined under conditions of natural soil pH had higher values than those of acid and alkaline ones. Phosphorus mineralization process went more intense in variants with organic fertilization. The soil enzymatic activity under the sugar beet was lower than in the soil collected under the vetch + oats and winter wheat.

Key words: typical chernozem, field crop rotation, organic fertilization, soil phosphatasic activity

Currently, the need to develop scientifically grounded principles of soil management, based on restoring and maintaining of natural soil fertility becomes more important.

Phosphorus is highly essential in formation of soil fertility and plant nutrition, and thus helping to achieve a high-quality yield. Crops, extract annually considerable amounts of nutrients from the soil. Long plant cultivation without soil application of fertilizers causes phosphorus exhaustion of the soil and yields decreasing. Moldavian soils are relatively rich in nutrient elements but in most cases, the content of plant available, mobile phosphorus is very low (Andrieş S., 2007).

Many recent studies from around the world show that organic farming system contributes to increase biological activity and respectively the soil quality (Herrick J., 2000; Kumar, 2010). Determination of hydrolytic enzymatic activity in the rhizosphere is widely used to study the effects of agricultural practices on soil quality (Khaziev F., 1990).

Soil organic phosphorus compounds are divided into two groups: specific humus substances (humic, fulvic and huminic acids) and nonspecific, including: nucleic acids, phospho-lipids' and phospho-proteins' derivatives, metabolic phosphates (sugar phosphates, glicero-phosphates). Organic phosphorus is assimilated by plants and microorganisms after its mineralization by extracellular phospho-hydrolases issued by plant roots, microorganisms and those accumulated on the soil particles (Khaziev F., 1982).

Among the enzymes involved in phosphorus cycle in the soil, ortho-phosphoric monoester phosphohydrolases (phosphatases) have been extensively studied in soil because they catalyze the hydrolysis of labile organic compounds (phospho-monoesters) to inorganic phosphorus which can be taken up by plants. The soil acidity is most important chemical property for phosphatase activity. According to their optimum pH, they are classified as acid, neutral and alkaline phosphatases (Ph) (Alef K., 1995, Shukla G., 2010). Phosphatasic activity determined at the natural soil pH is also used (Taylor M., 2002).

Important alkaline phosphatase providers are soil microorganisms and fauna, as opposed to acidic, produced, in particular, by plant roots, and certain by rhizodermal and root caps cells (Alef K., 1995, Gould W., 1979). Both acid and alkaline

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phosphatases are supposed to play an important role in plant nutrition because their activity in rhizosphere is higher than in the bulk soil (Alef K., 1995, Shukla G, 2010). Phosphatases are concentrated in the rhizosphere and in the surface layer of soil, where less humified organic matter prevail (Rojo M., 1990).

Phosphatasic activity reflects the level biochemical process' activity of phosphorus mobilization in the soil. The functional relationship between phosphatase activity and phosphorus uptake by plants was experimentally confirmed (Khaziev F., 1982).

The aim of the present study was to assess soil acid, alkaline and natural (summary) phosphatasic activity, as influenced by the system of fertilization and crop type.

MATERIAL AND METHOD

As the object of study served samples of typical chernozem soil, taken from the long-term (since 1972 year) experimental fields of the Research Institute for Field Crops "Selectia". The territory is located in Balti steppe, in the northern region of Republic of Moldova.

The soil sampling was conducted in September 2010, under the following crops: vetch + oats, winter wheat and sugar beet, which are a part of a field crop rotation of 6 crops.

It was chosen three fertilization systems:

FS1 – mineral fertilization (130 kg NPK / ha rotation surface),

FS2 – mineral + organic fertilization (130 kg NPK + 15 t manure / ha rotation surface),

FS3 – organic fertilization (15 t manure / ha rotation surface) and

Control – un-fertilized soil.

Soil samples were taken from the arable layer, at a depth of 0-20 cm, sieved through a 2 mm aperture grid, air-dried and stored at room temperature conditions. All visible root, litter material and fauna debris were removed prior to sieving.

When determining the enzymatic activity (6 months later), soil biological activity required rehabilitation. For this purpose the soil was pre-conditioned: moistened to 50% water holding capacity and incubated at +27°C for 7 days, with regular addition of water.

Soil moisture was measured by gravimetric method. Soil samples were dried in the oven for 8 hours at +105°C. Soil pH values were 6.4 (20 g soil: 50 ml bidistilated water) and 5.8 (20 g soil: 50 ml 1N KCl).

Soil was analysed for the potential, extracellular phosphatasic activity (Ph), using the method proposed by Tabatabai and Bremner (1969) (Khaziev F., 1990). Thus, soil (1 g) was incubated 1 hour at +37°C in the presence of toluene (0.5 ml) as antiseptic, 5 ml buffer solution and 1 ml 0.025 M solution of disodium p-nitrophenyl phosphate hexa hydrate salt, as the substrate. The acetate buffer (pH 5.0) was used for acid phosphatase (AcPh, EC 3.1.3.2) analysis, the monoetanol-amine buffer (pH 10.2) – for alkaline phosphatase (AlkPh, EC 3.1.3.1) determination. Besides that, the assay was conducted at the natural pH of the soil (un-buffered soil, the buffer was replaced by distilled water) and was considered as soil natural phosphatase activity (NatPh, EC 3.1.3). The reaction was stopped with 1 ml 0.5 M $CaCl_2$, and 4 ml 0.5 M NaOH.

The standard curve was plotted using a range of p-nitrophenol concentrations. Extinction value of the solution was measured at λ = 440 nm. Phosphatase activity was expressed in µg p-nitrophenol g^{-1} soil 1 $hour^{-1}$.

All mathematical and statistical analyses of the results were performed using Microsoft Office Excel for Windows XP. Reviewed parameters included: arithmetic mean, the standard error, level of significance (p) of the Student's t-test and Pearson correlation coefficient (r).

RESULTS AND DISCUSSIONS

Comparative study of phosphatase activity (Ph) in soil from all investigated treatments, revealed a significant difference ($p < 0.001$) of this activity *(*Table 1, Fig. 1*)* determined in different pH conditions (5.0, 6.4, 10.2). Thus, natural Ph activity measured in un-buffered conditions of the soil indicated maximum values and acidic Ph, higher values than the alkaline Ph. Schematically the intensity of enzymatic activity, depending on the pH of the buffer, of typical chernozem, can be represented as follows: natural Ph activity > acid Ph activity ≥ alkaline Ph activity.

Statistical analysis showed a positive correlation between all three types of soil phosphatasic activity (Table 1). Between acidic and natural Ph activity there is a correlation of high level ($r = 0.88$).

The literature indicates that the intensity of Ph activity, depending on the pH of the environment differ from one soil type to another (Alef K., 1995). Similar results, when soil natural Ph activity was significantly higher than those of acidic and alkaline ones have been obtained for the clay and sandy soils (Taylor M., 2002). Acid phosphatasic activity in preluvosoil had greater values than alkaline Ph (Samuel A., 2010).

Our results indicate that the phosphatasic activity varies significantly depending on fertilization system.

Although un-fertilized soil was expected to have a lower biological activity in the Control variant, natural and acid Ph activity showed the lowest values, only the soil under winter wheat,

Table 1

The significance of phosphatasic activities (acid, natural and alkaline) differences of typical chernozem, as influenced by the system of fertilization and crop type

Fertilization system	Acid phosphatase, p-nitrofenol, µg g^{-1} soil 1 $hour^{-1}$			Natural phosphatase p-nitrofenol, µg g^{-1} soil 1 $hour^{-1}$			Alkaline phosphatase p-nitrofenol, µg g^{-1} soil 1 $hour^{-1}$			Significance, p		
	Mean ± SE	%	p	Mean ± SE	%	p	Mean ± SE	%	p	Ac/ Nat	Nat/ Alk	Ac/ Alk
	Vetch + oats											
Control	720.8 ± 3.4	100		758.9 ± 10.4	100		446.9 ± 13.8	100		*	***	***
FS 1	658.6 ± 6.1	91.4	***	778.1 ± 4.6	102.5	–	434.4 ± 15.7	97.2	–	***	***	***
FS 2	488.3 ± 6.7	67.7	***	649.9 ± 4.7	85.6	***	445.8 ± 39.5	99.8	–	***	*	–
FS 3	613.1 ± 5.4	85.1	***	763.6 ± 4.2	100.6	–	517.0 ± 24.0	115.7	–	***	**	*
	Winter wheat											
Control	447.8 ± 3.3	100		670.0 ± 4.8	100		434.1 ± 24.3	100		***	**	–
FS 1	511.2 ± 5.0	114.1	***	706.5 ± 7.9	105.4	*	373.5 ± 11.3	86.0	–	***	***	***
FS 2	661.5 ± 1.5	147.7	***	799.1 ± 1.6	119.3	***	447.5 ± 23.3	103.1	–	***	***	*
FS 3	600.6 ± 6.3	134.1	***	804.8 ± 2.9	120.1	***	452.4 ± 6.8	104.2	–	***	***	***
	Sugar beet											
Control	485.9 ± 2.6	100		681.8 ± 3.4	100		425.1 ± 8.5	100		***	***	**
FS 1	504.7 ± 2.3	103.9	**	710.0 ± 9.7	104.1	–	400.1 ± 15.0	94.1	–	***	***	**
FS 2	454.0 ± 1.8	93.4	***	646.6 ± 11.0	94.8	*	414.6 ± 27.0	97.5	–	***	**	–
FS 3	438.0 ± 2.4	90.1	***	616.7 ± 2.3	90.5	***	406.1 ± 21.2	95.5	–	***	**	–
AcPh	–			r=0.88			r=0.49					
NatPh	r=0.88			–			r=0.47					
AlkPh	r=0.49			r=0.47			–					

Note: SE - standard error of mean,**%** - percentage as compared to the Control (un-fertilized soil), **p** - significance of the differences as compared to the Control (un-fertilized soil), ***** - p <0.05, ****** - p <0.01, ******* - p <0.001, **Ac/Nat** - significance of the differences between acid and natural phosphatasic activity, **Nat/Alk** – significance of the differences between natural and alkaline phosphatasic activity, **Ac/Alk** - significance of the differences between acid and alkaline phosphatasic activity, **r** - Pearson coefficient, **AcPh** - acid phosphatase activity, **NatPh** - natural phosphatasic activity, **AlkPh** - alkaline phosphatasic activity.

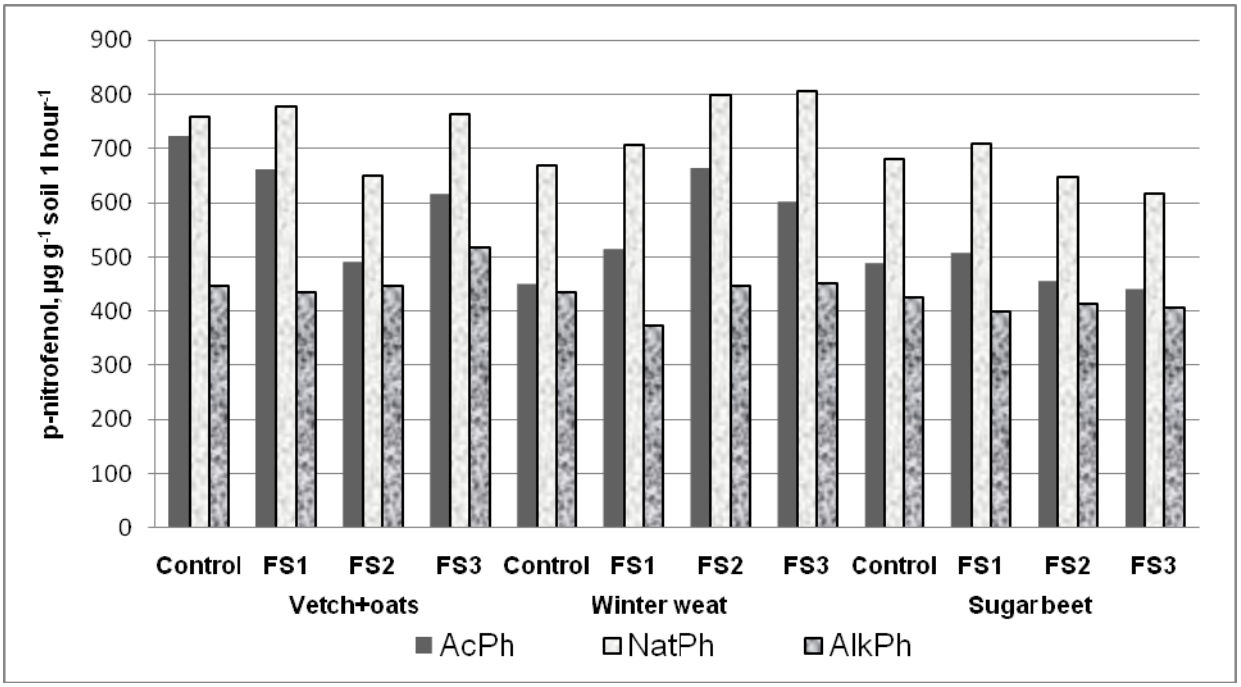

Figure 1 **Acid, natural and alkaline phosphatasic activities depending on crop type and fertilization system (FS): FS1 – mineral fertilization (130 kg NPK / ha rotation surface), FS2 – mineral + organic fertilization (130 kg NPK + 15 t manure / ha rotation surface), FS3 – organic fertilization (15 t manure / ha rotation surface) and Control – un-fertilized soil.**

reaching the values of 670.0 and 447.8 µg p-nitrophenol g^{-1} soil 1 $hour^{-1}$, respectively.

Alkaline phosphatase, considered of microbial origin, had a higher level, in the variant with organic fertilizers (**FS3**) administration only, in the soil under winter wheat and vetch + oats. The increased presence of organic matter fraction in the soil provides additional carbon source for edaphic microbes. Thus we can assume that in this treatment, microbial biomass has greater activity, and the processes of soil nutrients mobilization went more intense.

As noted in many papers, inorganic phosphorus introduced into the soil acts as inhibitor for this enzymatic activity (A. Wright, K. Reddy, 2001). Analyzing the data presented in *table 1* and *figure 1* we see that in the soil under all three crops, alkaline Ph activity was lower in treatment **FS1** (mineral fertilization).Thus the following values of alkaline Ph activity in the samples under the vetch+oats, winter wheat and sugar beet were recorded: 434.4, 373.5 and 400.1 µg p-nitrophenol g^{-1} soil 1 $hour^{-1}$, respectively.

The phosphatasic activity decreases in the soil under sugar beet, in the organic fertilization treatment: FS3 (organic fertilization) and FS2 (mineral + organic fertilization). Acid and natural Ph activity, in these fertilization systems, had lower values than in the FS1 (mineral fertilization), and significantly lower compared to the soil under winter wheat and vetch+oats.

Higher Ph activity in the samples collected under wheat and vetch+oats, in the FS2 and FS3, may be related to both increased number of rhizospheric microorganisms, and to the root enzymatic activity, which is of a major agronomic importance. It is known that with increasing the plant's root system the enzymatic flow to the soil is increased too.

Increased natural Ph activity in the soil under winter wheat, in the FS2 and FS3, shows the efficacy of organic fertilization of the soil and the location of this culture after vetch+oats.

CONCLUZIONS

The typical chernozem soil in all examined treatments had higher values of the phosphatase activity determined under natural soil pH than those of acid and alkaline ones.

There is a positive correlation between acid, natural and alkaline phosphatasic activities.

The alkaline phosphatase responsible for organic matter hydrolysis by soil microorganisms, revealed a very pronounced activity in the soil treated with organic fertilizers.

The presence of inorganic phosphorus introduced into the soil in the rate of 130 kg NPK/ha rotation surface, had reduced alkaline phosphatasic activity.

In the soil with organic fertilization with manure, under sugar beet, there was a reduced acid and natural phosphatasic activity.

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