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# Efects of chemical and physical soil properties on activity phosphomonoesterase

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**Abstract:** In the present work, effects of hemical and phisical soil properties on activity phosphomonoesterase are presented. Chemical and physical properties of calcomelanosols, chernozems, humogleys, solonetzes and fluvisols have been determined by standard methods. The highest total activities of PME were found in brownized calcareous black soil and lowest in Bt,na in the solonetz horizon. PME activity is correlated to humus content. pH is negatively and significantly correlated with the activity of acid PME. The activity of PME has not been significantly influenced by inorganic P and K contents in the investigated soils. Activites of alkaline and acid PME are not in significant correlations with the contents of the investigated soil fractions,

**Key words:** phosphomonoesterases, microbiological biomass, microorganisms solubilizing phosphates, soil.

## Introduction

Phosphomonoesterases (alkaline, EC 3.1.3.1 and acid, EC 3.1.3.2) that catalyze hydrolysis of orthophosphorous acid esters are involved in processes of mineralization of organic P compounds. Considering that orthophosphate monoesters comprise 72-85% of extractable P (Markov et al. 1996) and phosphatase hydrolizable P 33-93% of the total organic P (Negrin et al. 1995), these enzymes have an important role in P cycling and supplying of plants with available P.

Functioning of soil enzymes is a complex process consisting of integrated ecologically connected synthetic processes, immobilization and enzyme stability (Khaziyev and Gulke 1991). Phosphomonoesterases from soil originate from plant roots and microorganisms (Dick and Tabatabai 1984; James et al. 1991) that produce extracellular phosphatase both as a constitutive and an inducible enzyme. Phosphatases are present in soil as short-living free proteins, associated with living, resting or dead cells (Burns 1986). The activity of free enzymes in soil solution is negligible because they are very quickly inactivated by denaturation, proteolysis or binding to soil particles and colloids. Phosphatases form stable complexes with or on clay minerals (Rao et al. 1996), polysaccharides (Mayandon and Sarcar 1974) and humus matter (Vuorinen and Saharinen 1996) by immobilization, obtaining resistance towards proteolysis and other inactivating factors (Garazilo et al. 1996). The type of immobilization and the degree of stabilization of the immobilized enzymes depend on enzyme molecular structure and the type of conformational changes of protein molecules that may induce a significant loss of the enzyme activity (Gienferda and Bollag 1994). The activity of immobilized enzymes depends on the concentration of enzymes bound to adsorbents, chemical acitivity and concentration of substrate, presence of cofactors, inhibitors and activators (Khaziyev and Gulke 1991), but also on chemical and physical soil properties (type of soil, organic matter content, total N content, C/N ratio, pH, total P content and its labile fractions contents, soil mechanical composition).

Every soil contains a certain supply of immobilized enzymes that determine soil metabolism processes (McLaren 1975), which depend on its chemical, physical, microbiological and biochemical properties. The present work includes investigations of influence of chemical, physical, soil properties on phosphatase activities.

## Materials and methods

The investigated soils belong to different types, subtypes, modes of utilization and sampling sites in Serbia (Table 1). Ten pedological profiles were opened per each type and subtype of soil, selecting representative ones, from which samples were collected along genetical horizons down to original substrate, except in the case of chernozem in Zemun Polje. In air-dried soil samples that were sieved through a 2 mm-pore size, chemical characteristics were determined by following methods: reaction (pH in $H_2O$) of soil - by electrochemical method, total organic carbon - by dichromate oxidation, total nitrogen - by Kjeldahl digestion and steam distillation, available P and K - by Egner-Riehm method. Sand, powder and clay contents were determined by pipette method (Loveland and Whalley 1991). Soil classification according to textural class has been performed on the basis of FAO soil texture classification (Table 1, 2). All the results are expressed per absolutely dry soil mass.

Table 1. Types of the sampled soils

| **No** | **Soil type (soil subtype)** | **Depth (cm)** | **Site** | **Vegetation type** | **Texture class** |
|---|---|---|---|---|---|
| 1. | CALCOMELANOSOL<br>(Organo-mineral black soil) | 0 - 20 | RAJAC | Pasture | Silty clay |
| 2. | CALCOMELANOSOL<br>(Brownized calcareous black soil) | 0 - 20<br>20 - 40<br>40 - 60 | RAJAC | Pasture | Heavy clay |
| 3. | KALKOMELANOSOL<br>(Lessive calcareous black soil) | 0 - 15<br>30 - 50<br>50 - 70 | RAJAC | Pasture | Silty clay |
| 4 | SOLONETZ | 0 - 15<br>30 - 50<br>50 - 70 | KUMANE | Uncultivated land | Silty clay |
| 5. | CHERNOZEM | 0 - 20<br>20 - 40 | ZEMUN POLJE | Maize | Silty clay |
| 6. | CHERNOZEM | 0 - 20<br>20 - 40 | RIMSKI [AN^EVI | Soybean | Light clay |
| 7. | HUMOGLEY | 0 – 31<br>31 - 93 | BE^EJ | Winter wheat | Heavy clay |
| 8. | FLUVISOL | 0 - 31<br>31 - 68 | KA]KA [UMA | Poplar plantation | Sand |

Acid and alkaline phosphomonoesterase (PME) activities were determined utilizing a modified universal buffers pH 6.5 and 11 according to Tabatabai (1982). Phosphomonoesterase activity was determined in four replications and was expressed in pmol p-nitrophenole $g^{-1}$ $s^{-1}$.

As a mathematical model for the interrelation of the investigated parameters, correlation coefficient was calculated, expressing the strenght of relationship between the investigated components.

## Results and discussion

Soil function mainly involves recycling of organic inputs and maintenance of physical strucure. These processes are regulated by a suite of hierarchically organized factors (Beare et al. 1995) operated at nested scales of space and time in other: climate, soil (clay and nutrient status) properties, organic matter, macro- and microorganisms. PME activity, microorganism abundance and microbiological biomass have been determined in soils formed under the influence of various pedogenetic factors and which differ from one another by chemical and physical properties (Djordjevi} 1993). Active acidity of the investigated soils ranges from 4.94 (calcomelanosol, humous horizon) to 8.70 (fluvisol, subhumous horizon), (Table 2). Humus content varies both between different types and along profile depth. The highest humus content (13.66%) is in the Ah horizon of calcomelanosol, while the lowest is in Bt, in the solonetz horizon. Total nitrogen content varies between 1.26% and 0.02%. The soils are poor (to moderately supplied) with highly mobile $P_2O_5$ forms, while they are predominantely moderately supplied with highly mobile $K_2O$.

Table 2. Chemical and physical soil properties

| Soil type | pH in $H_20$ | Humus % | Total N % | C/N | $P_20_5$ | $K_20$ | <0.02 mm (%) | <0.02-0.002mm (%) | <0.002 mm (%) | <0.01 mm (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | mg/100g | | | | | |
| 1 | 5.82 | 12.37 | 0.81 | 8.87 | 1.5 | 14.1 | 10.37 | 54.13 | 35.50 | 63.19 |
| 2 | 5.33 | 13.66 | 1.26 | 6.30 | 1.9 | 14.1 | 4.31 | 35.95 | 59.74 | 78.28 |
| | 5.46 | 12.10 | 1.07 | 6.57 | 1.2 | 21.2 | 4.31 | 32.78 | 62.91 | 79.86 |
| | 5.99 | 4.58 | 0.35 | 7.60 | 0.8 | 13.3 | 2.95 | 26.63 | 70.42 | 80.56 |
| 3 | 4.94 | 11.05 | 0.95 | 6.76 | 2.6 | 23.6 | 8.40 | 47.27 | 44.35 | 66.09 |
| | 5.07 | 2.97 | 0.22 | 7.82 | 1.0 | 10.4 | 9.38 | 40.87 | 49.75 | 68.31 |
| | 5.32 | 2.77 | 0.19 | 8.47 | 2.3 | 8.0 | 7.53 | 15.38 | 77.09 | 85.31 |
| 4 | 5.90 | 6.05 | 0.40 | 8.77 | 7.3 | 18.8 | 41.84 | 37.76 | 20.40 | 58.16 |
| | 7.38 | 1.69 | 0.10 | 9.80 | 1.5 | 12.5 | 26.32 | 34.68 | 39.00 | 73.68 |
| | 9.38 | 0.29 | 0.07 | 2.42 | 1.3 | 36.0 | 19.56 | 32.92 | 47.52 | 80.44 |
| 5 | 8.02 | 2.64 | 0.18 | 8.40 | 11.0 | 18.4 | 4.38 | 61.15 | 34.74 | 95.89 |
| | 8.25 | 2.48 | 0.16 | 8.88 | 10.0 | 19.2 | 4.24 | 62.21 | 33.55 | 96.76 |
| 6 | 8.05 | 2.63 | 0.17 | 8.84 | 13.4 | 18.0 | 33.84 | 29.76 | 36.40 | 66.16 |
| | 8.25 | 2.03 | 0.13 | 8.80 | 3.0 | 10.8 | 39.64 | 27.76 | 33.60 | 61.36 |
| 7 | 7.00 | 9.00 | 0.15 | 34.8 | 3.1 | 24.6 | 21.40 | 30.80 | 78.60 | 47.80 |
| | 5.65 | 2.27 | 1.15 | 1.14 | 3.1 | 9.2 | 26.16 | 22.68 | 73.12 | 50.44 |
| 8 | 8.53 | 1.59 | 0.06 | 15.3 | 3.1 | 9.2 | 96.40 | 7.60 | 3.20 | 10.80 |
| | 8.70 | 0.46 | 0.02 | 13.0 | 1.9 | 5.6 | 86.00 | 2.00 | 1.60 | 3.60 |

Acid and alkaline PMEdecrease with depth in all investigated soil types (Table 3). The highest total activities of these enzymes were found in brownized calcareous black soil and lowest in Bt,na in the solonetz horizon. Differences of PME activities between chernozem in Zemun Polje and chernozem in Rimski Šančevi are significant, as well as those between solonetz and humogley.

Coefficients of correlation between PME activities and soil properties are shown in Table 4. Several authors state that PME activity is correlated to humus content (Juma and Tabatabai 1978; Speir et al. 1984) and total nitrogen in soil (Bonmati et al. 1991; Lerios et al. 1998). Phosphatases are immobilized on humic acids into stabile humo-protein complexes, which prolongs enzyme half-life. Phosphatases immobilized on resorcinol copolymer show higher stability towards pH and temperature increases and proteolysis compared to free enzymes (Garzollo et al. 1996).

The results of Eivazi and Tabatabai (1977) indicate that the activity of acid PME is predominant in acid soils, and of alkaline PME in those with alkaline reaction. pH is negatively and significantly correlated with the activity of acid PME (Table 4). Juma and Tabatabai (1978) also found a negative and significant interdependence between the activity of acid PME and soil pH. The activity of PME has not been significantly influenced by inorganic P and K contents in the investigated soils (Table 4). Between the acivity of acid PME and inorganic P content a negative correlation has been found (r =-0.29). Šarapatka (1998) also mentiones a negative correlation between acid PME activity and available P content. Orthophosphate inhibits PME activity following the competitive inhibition pattern where it represents the actual inhibitor and regulator of enzyme synthesis (Pang and Kolenko 1986).

Tabela 3. Biochemical soil properties

| **Soil type** | **Acid PME pmol NP $g^{-1}$ $s^{-1}$** | **Alkaline PME pmol NP $g^{-1}$ $s^{-1}$** |
|---|---|---|
| **1** | 958.4 | 489.2 |
| **2** | 1295.9 | 529.1 |
| | 718.8 | 510.2 |
| | 87.8 | 103.8 |
| **3** | 658.9 | 225.6 |
| | 74.38 | 37.9 |
| | 30.9 | 89.3 |
| **4** | 335.4 | 409.3 |
| | 159.7 | 120.8 |
| | 19.4 | 85.4 |
| **5** | 102.8 | 305.5 |
| | 96.8 | 244.6 |
| **6** | 119.8 | 347.4 |
| | 99.8 | 199.7 |
| **7** | 325 | 373.7 |
| | 303 | 131.8 |
| **8** | 91.6 | 155.3 |
| | 39.93 | 65.5 |

Textural soil composition influences the enzyme activity, because enzymes immobilize on different textural fractions of soil. The results of the present investigations show that the activites of alkaline and acid PME are not in significant correlations with the contents of the investigated soil fractions, (Table 4). Negative correlation with clay and physical clay contents, and a positive one with sand content. Harrison (1983) found a negative correlation between phosphatase activity and clay+powder content. Enzymes are immobilized with or on clay minerals forming enzyme-clay complex that reduces enzyme activity but does not eliminate it (Dick and Tabatabai 1987), which depends on mineralogical properties of the clay and enzyme molecular structure. Rojo et al. (1990) state that phosphatase activity is present on soil fractions between 100 and 2000 μm and that it is linked to plant remnants and less humified organic matter. Narco et al. (1996) also state that a higher content of hydrolizable organic matter can be found on bigger mechanical particles.

Table 4. Correlation coeffiients between chemical, physical and biochemical, microbiological soil properties

| | Humus | Total N | pH | C/N ratio | Available P | Available K | Sand | Powder | Clay | Fysical clay |
|---|---|---|---|---|---|---|---|---|---|---|
| Acid PME | 0.92 [a] | 0.85 [a] | -0.56 [b] | -0.09 | -0.24 | 0.10 | -0.34 | 0.29 | 0.23 | 0.12 |
| Alkaline PME | 0.80 [a] | 0.54[b] | -0.27 | 0.15 | 0.24 | 0.27 | -0.28 | 0.41 | 0.07 | 0.23 |

a - $P < 0.01$; b - $P < 0.05$

## Conclusion

On the basis of the results obtained it may be concluded:

- The highest total activities of PME were found in brownized calcareous black soil and lowest in Bt,na in the solonetz horizon;
- PME activity is correlated to humus content;
- pH is negatively and significantly correlated with the activity of acid PME;
- The activity of PME has not been significantly influenced by inorganic P and K contents in the investigated soils;
- Activites of alkaline and acid PME are not in significant correlations with the contents of the investigated soil fractions.

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## UTICAJ HEMIJSKIH I FIZIČKIH OSOBINA ZEMLJIŠTA NA AKTIVNOST FOSFOMONOESTERAZE

- originalni naučni rad -

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**Rezime**

U radu su ispitivani uticaji hemijskih i fizičkih osobina zemljišta na aktivnost fosfomonoesteraze. Hemijske i fizičke osobine kalkomelanazola, černozema, humogleja, soloneca i fluvizola su određene standardnim metodama. Najviša ukupna aktivnost fosfomonoesteraze (PME) ustanovljena je u smeđem kalceroznom humusu a najniza u B-t horizontu soloneca. PME aktivnost je u korelaciji sa sadrzajem humusa. pH je u negativnoj korelaciji sa aktivnošću kisele fosfomonoesteraze. PME aktivnost ispitivanih zemljišta nije bitno zavisila od sadržaja neorganskog fosfora i kalijuma. Uticaji alkalne i kisele PME nisu bili u znacajnoj korelaciji sa sadrzajima ispitivanih frakcija zemljišta.