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## MICROBIOLOGICAL TRANSFORMATIONS OF PHOSPHORUS AND SULPHUR COMPOUNDS IN ACID SOILS

**ABSTRACT:** The dynamics of phosphorus and sulphur in soil is closely related to the dynamics of the biological cycle in which microorganisms play a central role. There is not much microbiological activity in acid soils because aerobes are scarce, rhizosphere is restricted to the shallow surface layer, and the biomass of microorganisms decreases with higher acidity. The aim of the research was to investigate the number of microorganisms, which decompose organic and inorganic phosphorus compounds and organic sulphur compounds in calcocambisol, luvisol, and pseudogley.

The following parameters were determined in the soil samples: pH in H<sub>2</sub>O and in 1MKCl; the content of CaCO<sub>3</sub> (%); humus content (%), nitrogen content (%); the content of physiologically active phosphorus and potassium (mg P<sub>2</sub>O<sub>5</sub>/100g of soil; mg K<sub>2</sub>O/100g of soil). The number of microorganisms was determined by the method of agar plates on appropriate nutrient media: the number of microorganisms solubilizing phosphates on a medium by Muramcov; the number of microorganisms that decompose organic phosphorus compounds on a medium with lecithin; and the number of microorganisms that transform organic sulphur compounds on a medium by Baar.

All three types of soil are acid non-carbonate soils with a low level of available phosphorus and a more favorable amount of potassium, nitrogen, and humus. The largest number of bacteria, which transform organic phosphorus compounds, was found in calcocambisol. The largest number of phosphate solubilizing bacteria was recorded in pseudogley, whereas the largest number of phosphate solubilizing fungi was recorded in calcocambisol. The largest number of bacteria, which transform organic sulphur compounds, was recorded in pseudogley.

**KEY WORDS:** acid soil, microorganisms, phosphorus, sulphur

## INTRODUCTION

Soil microorganisms play a major role in the decomposition of plant residues, creation of humus and maintenance of stable soil structure. They also participate in the cycles of the most important macro and microelements such as phosphorus and sulphur (C a i r n e y, 2000; K l i r o n o m o s et al., 2000).

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The dynamics of phosphorus in soil is closely related to the dynamics of the biological cycle in which microorganisms play a central role (Wakelin et al., 2004; Vassilev et al., 2006). Microorganisms affect the amount of phosphorus accessible to plants by means of mineralization of organic phosphorus compounds, immobilization of available phosphorus and solubilization of non-soluble phosphorus minerals such as tricalcium phosphate (Chen et al., 2006; Kang et al., 2002; Pradhan and Sukla, 2005). Organic phosphorus compounds in soil are an important supply of this plant nutrient but only after they have been mineralized (Gyaneshwar et al., 2002). Mineralization is catalyzed by microbiological enzymes phosphatases (Oberson et al., 2001). Organic phosphorus mineralization in soil depends on temperature, pH, soil moisture, degree of aeration (Dalla, 1977). According to Wittmann et al. (2003a), optimum pH value for phosphatase activity is 4-5 and optimum temperature 35° C.

Sulphur is one of the essential plant nutrients contributing to yield and quality of crops. There are two main forms of sulphur in soil, inorganic and organic sulphur (Landers et al., 1983). In soil, sulphur is mainly found in organic form (90%). Organic sulphur is present in three forms, ester sulfate-S, C-bonded S and non-reducible organic sulphur (Freny et al., 1975). Sulphur transformations in soil are considered to result primarily from microbial activity, which involves processes of mineralization, immobilization, oxidation, and reduction. Mineralization of organic sulphur compounds and transformation into forms accessible to plants is catalyzed by enzyme sulphatase (Eivazi and Bayan, 1996; Hayes et al., 2000). Sulphatase activity has been noticed even in soils with extremely low values of pH (pH 3-5), but its effects in such extreme conditions are unknown (Kahkonen et al., 2002).

As acid soils are found in many areas in our country, the aim of the research was to investigate the number of microorganisms that transform inaccessible phosphorus and sulphur compounds into forms accessible to plants.

## MATERIAL AND METHODS

Acid soil samples were taken from the slopes of Golija mountain (calco-cambisol), Radočevo (luvisol), and Kraljevačka valley (pseudogley).

The following chemical properties of soil were determined: pH in H<sub>2</sub>O and in 1MKCl; the content of CaCO<sub>3</sub> (%); humus content (%); total nitrogen content (%); the content of physiologically active phosphorus and potassium (mg P<sub>2</sub>O<sub>5</sub>/100g of soil; mg K<sub>2</sub>O/100g of soil).

The number of microorganisms was determined by the method of agar plates (Trollenier, 1996) on appropriate nutrient media (Hi Media Laboratories Pvt. Limited Mumbai, India): the number of bacteria decomposing organic phosphorus compounds on a medium with lecithin, the number of phosphate solubilizing bacteria and fungi on a medium with aluminum phosphate, and the number of microorganisms transforming organic sulphur compounds on a medium by Baar. All groups of microorganisms were introduced into Petri

dishes with 0.5 ml soil suspension from  $10^{-4}$  dilution. The microorganisms were incubated at the constant temperature of 28° C. The incubation of microorganisms, which transform phosphorus compounds, lasted 2-10 days, whereas the incubation of microorganisms, which transform sulphur compounds, lasted 7-10 days.

Statistical data analysis was performed using STATISTICA 10 software.

The significance of differences in the number of microorganisms between different soil types was determined upon the least significant difference (LSD).

## RESULTS AND DISCUSSION

The investigated soil types are characterized by the chemical properties shown in Table 1.

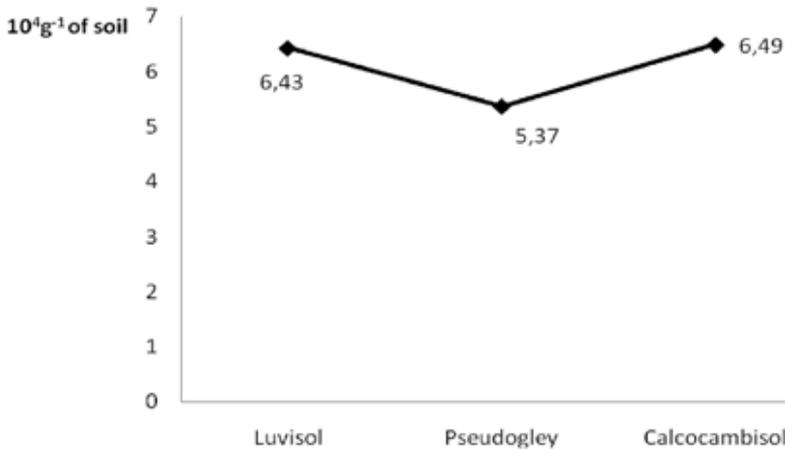
Tab. 1. – Chemical properties of the investigated soil types

Soil	pH		Humus %	N %	CaCO <sub>3</sub> %	mgP <sub>2</sub> O <sub>5</sub> u 100 g	mgK <sub>2</sub> O u 100 g
	In H <sub>2</sub> O	In 1M KCl					
Calcocambisol	6.2	4.8	3.2	0.26	0.26	4.9	12.25
Pseudogley	5.1	3.7	2.4	0.18	0.46	4.3	14.3
Luvisol	4.0	3.6	3.7	0.43	0	2.1	15.7

Calcocambisol is a low carbonate soil. It abounds in nitrogen and has high humus content. The amount physiologically active phosphorus in it is minimal whereas the amount of potassium is greater. Pseudogley is an extremely acid type of soil. It has a medium amount of nitrogen and humus. It is characterized by a very low amount of phosphorus and calcium carbonate and a medium amount of potassium. Luvisol is an extremely acid non-carbonate soil type. It is well- provided with nitrogen and humus. The amount of phosphorus in it is low whereas the amount of potassium is somewhat greater.

Microbial communities are important in soils because of their key function in ecosystems processes such as decomposition, nutrient cycling, and plant symbioses (N a n n i p i e r i et al., 2003). All these processes enable mutual relations of microorganisms, soil, and plants. Soil pH is one of the most important soil properties related to the composition of microbial communities (B a t h and A n d e r s o n, 2003; N i l s s o n et al., 2007; W u et al., 2009; B r a d y and W e i l, 2002).

Three types of acid soils were used in this research, which certainly affected the number of microorganisms. The number of bacteria decomposing organic phosphorus compounds ranged from 5.37 to 6.49 x 10<sup>4</sup> g<sup>-1</sup> of absolutely dry soil (Graph 1). The differences were not statistically significant (LSD for 5% =5.42).



Graph 1. – The number of bacteria transforming organic phosphorus compounds ( $10^4 \text{ g}^{-1}$  of soil)

Tarafdara and Claasen (1988) documented that almost half of the microorganisms in soils and on plant roots were able to mineralize organic P through the phosphatase action. Organic phosphorus compounds are mainly phospholipids, phytin, and nucleoproteins. A large number of bacteria are able to use phospholipids and nucleoproteins transforming them into phosphate anions. In this research, a large number of bacteria decomposing organic phosphorus compounds could provide plants with a significant amount of this nutrient. However, in acid soils, phosphate anions form bonds aluminum and iron cations and become available to plants only after the activity of microorganisms. In acid soils, fungi are more active solubilizers of insoluble phosphates, whereas in neutral soils, bacteria are more active. In this research, the number of fungi solubilizing phosphates inaccessible to plants was similar in all three types of soil, ranging from  $1.41$  to  $1.73 \times 10^4 \text{ g}^{-1}$  of absolutely dry soil (Graph 2). The differences in the number of this group of microorganisms in the investigated types of soil were not statistically significant (LSD for 5% = 1.21).

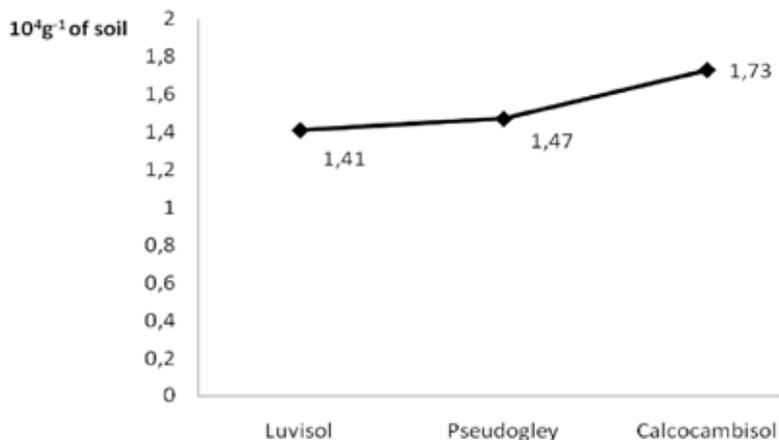
Phosphate solubilizing bacteria were not found in calcocambisol, whereas in the other two types of soil their number ranged from  $0.49 \times 10^4 \text{ g}^{-1}$  to  $8.81 \times 10^4 \text{ g}^{-1}$  of absolutely dry soil (Graph 3). A statistically significant difference in the number of bacteria was recorded between pseudogley and the other two soil types and it was on the level  $P < 0.05$  (LSD for 5% = 7.09).

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Goldstein, 1986). It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms (Halderson, 1990; Leyval, 1989; Salih, 1989). Among them, gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. Another organic acid identified in strains with phosphate-solubilizing ability is 2-ketogluconic acid (Halderson, 1990, 1993). Bacteria are more effective in

phosphorus solubilization than fungi (Alam et al., 2002). Among the whole microbial population in soil, phosphorus-solubilizing bacteria constitute 1 to 50 %, while phosphorus-solubilizing fungi are only 0.1 to 0.5 % in P solubilization potential (Chen et al., 2006).

Unlike these results, in this research the number of phosphate solubilizing fungi was larger than the number of bacteria apart from pseudogley where the number of bacteria was larger. This could be the result of unfavorable conditions for microorganism activity in the investigated soil types. The investigated soil types are characterized by heavy soil texture, unfavorable water-air ratio, and acidity (Milković, 1996). Our results confirmed the results of Yahya and Azawai (1998) who concluded that phosphate solubilizing fungi in infertile soils were more numerous than bacteria.

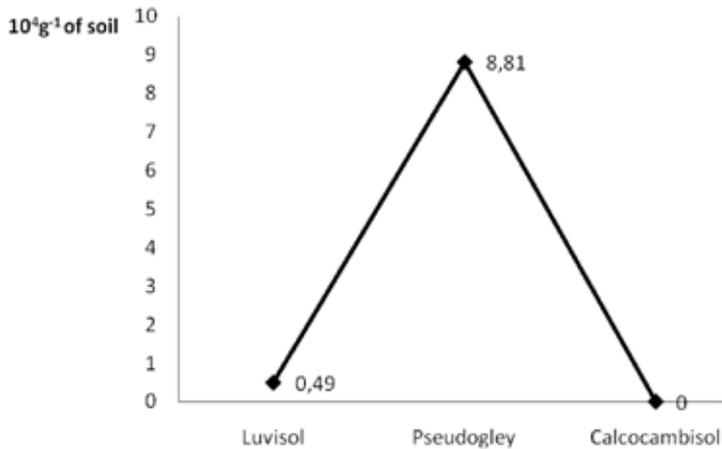
The number of bacteria, which transform organic sulphur compounds, amounted to thousands (Graph 4). Their smallest number was recorded in luvisol ( $12.16 \times 10^4 \text{ g}^{-1}$ ), whereas their largest number was recorded in pseudogley ( $28.01 \times 10^4 \text{ g}^{-1}$ ). The differences in the number between the investigated soil types were not statistically significant (LSD for 5% = 20.45).



Graph 2. – The number of phosphate solubilizing fungi ( $10^4 \text{ g}^{-1}$ )

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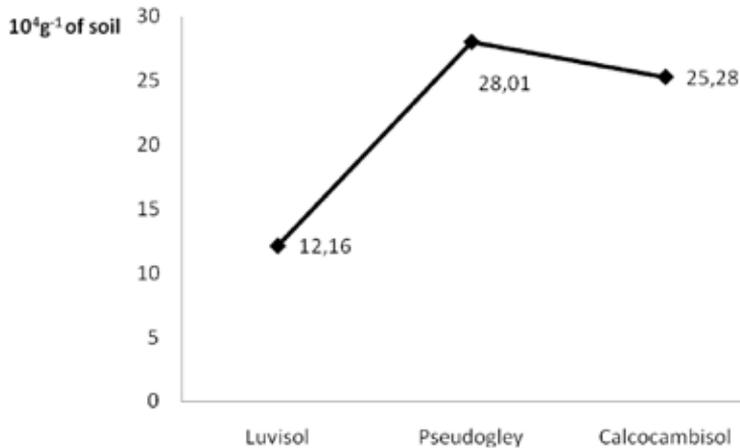
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acid seems to be the most frequent agent of mineral phosphate solubilization. Another organic acid identified in strains with phosphate-solubilizing ability is 2-ketogluconic acid (Haldler, 1990, 1993). Bacteria are more effective in phosphorus solubilization than fungi (Alam et al., 2002). Among the whole microbial population in soil, phosphorus-solubilizing bacteria constitute 1 to 50 %, while phosphorus-solubilizing fungi are only 0.1 to 0.5 % in P solubilization potential (Chen et al., 2006).

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The number of bacteria, which transform organic sulphur compounds, amounted to thousands (Graph 4). Their smallest number was recorded in luvisol (12.16 x 10<sup>4</sup> g<sup>-1</sup>), whereas their largest number was recorded in pseudogley (28.01 x 10<sup>4</sup> g<sup>-1</sup>). The differences in the number between the investigated soil types were not statistically significant (LSD for 5% = 20.45).

Sulphur is one of the essential plant nutrients classified as secondary nutrient. Sulphur transformations in soil are considered to result primarily from microbial activity which involves processes of mineralization, immobilization, oxidation and reduction (Vidyalakshmi, 2009). So far, a great deal of attention has been paid to the isolation and identification of Sulphur Oxidizing Bacteria (Chapman, 1990; Wood, 1991; Johnson, 1992), whereas very little attention has been devoted to investigating their number and activity in soil. A relatively large number of bacteria, which transform



Graph 4. – The number of bacteria that transform organic sulphur compounds ( $10^4 \text{ g}^{-1}$  of soil)

organic sulphur compounds in the investigated soils, confirms the results of Wittman et al. (2003b) who stated that the optimum pH value for sulphatase is 4-5, which indicates that the hydrolytic activity of the enzyme is adapted to acid soils.

## CONCLUSION

All three types of soil belong to the group of acid, non-carbonate soils with a low level of accessible phosphorus and a more favorable content of potassium, nitrogen, and humus.

The number of bacteria decomposing organic phosphorus compounds ranged from  $5.37 \times 10^4 \text{ g}^{-1}$  to  $6.49 \times 10^4 \text{ g}^{-1}$  of absolutely dry soil.

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## МИКРОБИОЛОШКЕ ТРАНСФОРМАЦИЈЕ ЈЕДИЊЕЊА ФОСФОРА И СУМПОРА У КИСЕЛИМ ЗЕМЉИШТИМА

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### Резиме

Динамика фосфора и сумпора у земљишту тесно је повезана са динамиком биолошког циклуса у којем микроорганизми имају централну улогу. Микробиолошка активност у киселим земљиштима није велика, јер су потиснути аероби, ризосфера је ограничена на плитку површинску зону, а са повећањем киселости смањује се и биомаса микроорганизама. Циљ ових истраживања је био да се испита заступљеност микроорганизама који разлажу органска и неорганска једињења фосфора и органска једињења сумпора у калкокамбисолу, лувисолу и псеудоглеју.

У узорцима земљишта одређени су следећи параметри: реакција земљишта (pH) у H<sub>2</sub>O и у 1M KCl; садржај CaCO<sub>3</sub> (%); садржај хумуса (%); садржај азота (%); садржај физиолошки активног фосфора и калијума (mg P<sub>2</sub>O<sub>5</sub>/100g земљишта; mg K<sub>2</sub>O/100g земљишта). Број микроорганизама одређиван је методом агарних плоча на одговарајућим селективним хранљивим подлогама: број микроорганизама који разлажу фосфате на подлози по: Мурамцов, број микроорганизама који разлажу органска фосфорна једињења на подлози са лецитином, а број микроорганизама који трансформишу органска једињења сумпора на подлози по Вааг-у.

Сва три типа земљишта спадају у групу киселих бескарбонатних земљишта, са ниским садржајем приступачног фосфора и повољнијим садржајем калијума, азота и хумуса. Највећи број бактерија које трансформишу органска једињења фосфора утврђен је у калкокамбисолу. Највећи број бактерија које разлажу фосфате утврђен је у псеудоглеју, а највећи број гљива које разлажу фосфате у калкокамбисолу. Највећи број бактерија које трансформишу органска једињења сумпора утврђен је у псеудоглеју.

КЉУЧНЕ РЕЧИ: кисело земљиште, микроорганизми, сумпор, фосфор