Phosphatase activities (ACP, ALP) in agroecosystem soils

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ABSTRACT

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Soil phosphatases play a major role in the mineralization processes of organic phosphorus substrates. Enzymes in soils originate from animal, plant and microbial sources and the resulting soil biological activity includes the metabolic processes of all these organisms. The activity of soil phosphatases can be influenced by numerous factors and soil properties and farming systems play a key role among them.

Our research adds to the growing knowledge on soil phosphatases in their interactions with specific soil characteristics at nine sites in the Czech Republic with common soil types. The results show correlations and linear equations between phosphatase activity and soil characteristics. Positive correlations were found between enzymatic activity and organic carbon, and with nitrogen; and between acid phosphatase activity and total phosphorus. Negative correlations were with the quality of humus (humic:fulvic acids ratio) and available phosphorus, and between acid phosphatase activity and clay content and pH.

The trials were also conducted at experimental locations and farms in Uppland (Sweden) with eutric cambisol soil type. The results demonstrate the correlation between acid and/or alkaline phosphatase and several soil characteristics (clay content, C_{org}, N_{tot}, pH, humic:fulvic acids ratio, Pavail, basal soil respiration). At the experimental plots were also studied the effect of different farming systems (conventional farming without animal husbandry /A/, organic farming with animal husbandry /B/, organic without animal husbandry with standard soil cultivation /C/, and organic agricultural system without animal husbandry with minimum soil management /D/). The data show that the highest acid and alkaline phosphatase activity was found in system D followed by system B. The lowest means were recorded in system A and C.

Enzymes in soils originate not only from microbial sources but also from animals and plants and the resulting soil biological activity includes the metabolic processes of all these organisms. In our research we also concentrated toward acid phosphatase activity linked to cultivated plants. Our evaluation was carried out on the root systems of both the chosen species and cereal varieties and also in nutrient medium on which crops were planted under conditions of changing pH and phosphorus supply. The results show that the acid phosphatase activity in the root system of various species and cereal cultivars is negatively correlated with increasing pH and available phosphorus level in the nutrient medium.

Attention was also paid to acid phosphatase and alkaline phosphatase active colonies of bacteria, isolated from soils. The activity of acid phosphatase and other soil properties (the number of aerobic bacteria, basal respiration, the level of ammonification, the number of bacteria active in ammonification, the level of nitrification, the number of micromycetes) were compared with the number of bacteria belonging to the genus *Micrococcus*. Numerous correlations were described but our results didn't show any correlation between phosphatase active colonies of bacteria and acid and alkaline phosphatase activity in the investigated soils. This is probably due to the production of phosphatates by organisms other than bacteria, for example, soil protozoa or other groups. Enzymes in soils do not only originate from microbial sources, but also from animals and plant roots.

Key words: soil, activity, enzymes, phosphatases, ACP, ALP, soil properties, farming systems, plants

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Papers I – IV

The present thesis is based on the following papers and will be referred to by their Roman numerals.

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INTERACTIONS BETWEEN PHOSPHATASE ACTIVITY AND SOIL CHARACTERISTICS AT SOME LOCATIONS IN THE CZECH REPUBLIC

II.
PHOSPHATASE ACTIVITY OF EUTRIC CAMBISOLS (UPPLAND, SWEDEN) IN RELATION TO SOIL PROPERTIES AND FARMING SYSTEMS

III.
EFFECT OF pH AND PHOSPHATE SUPPLY ON ACID PHOSPHATASE ACTIVITY IN CEREAL ROOTS

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RELATIONSHIP BETWEEN PHOSPHATASE ACTIVE BACTERIA AND PHOSPHATASE ACTIVITIES IN FOREST SOILS

1 INTRODUCTION

Agriculture is an integral part of our environment because it is practiced on about 30 percent of the earth's land. Ecosystems managed by man – e.g. agroecosystems, are often different from natural ecosystems. Odum (1982) defined agroecosystems as domesticated ecosystems that are in many basic ways intermediate between natural ecosystems, such as grasslands and forests on the one hand, and fabricated ecosystems, such as cities, on the other hand, or between solar powered natural ecosystems and fuel powered urban – industrial systems. The differences between an agroecosystem and a natural ecosystem have been described by Altieri (1987). One of the most ecologically significant contrasts between ecosystems is that the agroecosystem is a much more open system with a greater number and a larger volume of inputs and outputs than that of unmodified ecosystems. The agroecosystem is frequently subsidized by the addition of direct or indirect energy which is often seen as necessary for cultivation and increased yields. This more highly concentrated energy flow in the agroecosystem is accompanied by a disruption of the natural nutrient cycle.

The mineral and organic matter of the soil is part of the environment of most terrestrial ecosystems including the agroecosystem. According to USDA Soil Survey "soils are considered natural bodies, covering parts of the earth surface that support plant growth, and that have properties due to the integral effect of climate and organisms acting upon the parent material, as conditioned by relief, over period and time" (Tan 1994). Soil constitutes a subsystem of a larger terrestrial system wherein it is possible to study the complex relationship between its structure and function, including its various cycles. An understanding of these cycles and their interactions is essential for the use of soil as a medium for plant growth and the life and functions of the organisms.

Soil consists of mineral material, the roots of plants, microbial and animal biomass, organic matter in various states of decay, as well as water and gaseous atmosphere. The distribution of these components, their composition, function are studied by soil scientists, soil biologists and ecologists and are described in many papers and monographs. Soil harbours a diverse population of living organisms, both animals and plants. The soil subsystem does not have the capacity to capture a substantial amount of solar energy as do the other subsystems and depends instead on substances from outside. These materials form plant residues which are used in the soil by other organisms and form soil organic matter including humic substances. Where plant residues enter the soil, there is an initial flush of decomposition (two thirds of most plant residues generally decompose in one year), followed by a much slower steady breakdown (Killham 1994). Organic molecules such as cellulose, hemicellulose and lignin are associated with three elements – carbon, hydrogen and oxygen. Many other organic molecules contain other important plant nutrients, including phosphorus (nucleic acids, phospholipids, phytin, etc.). The release of organically bound nutrients into plant available, in inorganic forms, is termed mineralization which is of critical

importance to the productivity of ecosystems. All available nutrients are not only required by plants, but decomposer soil organisms also need these nutrients. Because of the dependence of decomposers on an adequate nutrient flow in the soil, the decomposition of organic molecules must not be considered as a series of independent processes.

The bulk of most nutrient elements is held in the molecular or structural framework of primary and secondary minerals and organic matter. Over time, these elements may be released for plant use and the structural framework is a significant source of these essential elements (Brady, Weil 2002). This is also the problem of phosphorus and the maintenance of this nutrient in an available form is the object of this thesis. It may have significance at this time mainly in organic or sustainable farming systems with limited inputs of nutrients. This importance can also be seen in conventional agriculture with a high level of human intervention (mechanization, chemicals, genetic manipulations, etc.), where the decomposition process is altered and soil fertility maintained, not through nutrient recycling, but with fertilizers. The intensive use of inorganic fertilizers is a key factor in the high yields associated with "modern" agriculture. Intensive farming in this sense needs bigger capital resources which influence the whole agroecosystem, the environment and natural resources. Along with this serious problem is that of the limited reserves of rock phosphates. That is why it is necessary to study and optimize agroecosystems to be less dependent on high inputs, to maintain productivity and soil fertility, and minimize negative effects on the environment. The study of biogeochemical cycles including phosphorus, which consists of an organic phase of the agroecosystem and involving living organisms, as well as an abiotic phase, must also be taken into consideration. The aim of this work is to at least help in this way.

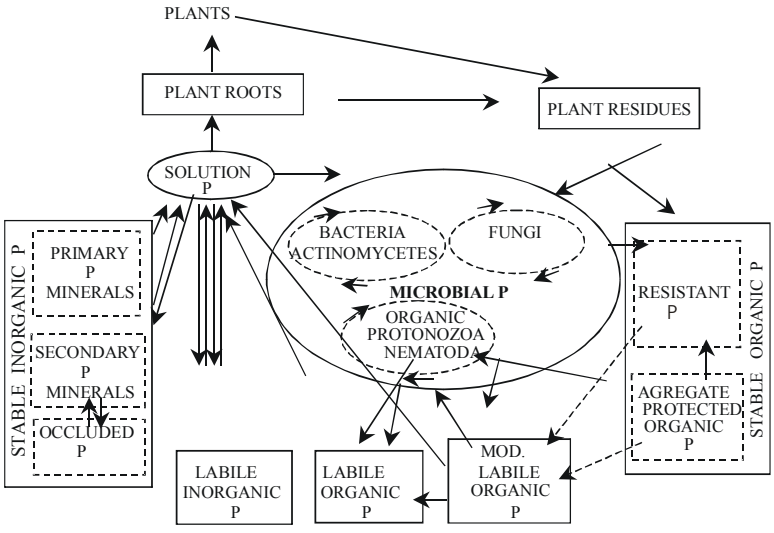
2 PHOSPHORUS IN AGROECOSYSTEM SOILS

In agricultural ecosystems, phosphorus constraints are much more critical because phosphorus in the harvested crops is removed from the system, with only limited quantities being returned in crop residues and animal manures. As a result, extreme phosphorus deficiencies are quite common where no supplementary sources of this element are applied to soils. Such conditions are widespread today, for example, in most countries of sub-Saharan Africa, where phosphorus-bearing fertilizers are either not available or where the cost of their being transported and applied is prohibitive (Brady, Weil 2002). Phosphorus as a plant nutrient can also have impacts on the environment. Too much or too little phosphorus can have severe and widespread negative impacts on environmental quality. The principal environmental problems related to soil phosphorus are land degradation caused by too little available phosphorus and accelerated eutrophication caused by too much. Both problems are related to the role of phosphorus as a plant nutrient.

The phosphorus cycle in soil is a system which involves soils, land and microorganisms. Major processes include the uptake of soil phosphorus by plants, recycling (the return of plant and animal residues), biological turnover (mineralization and immobilization), fixation to clay, solubilization (Stevenson 1986). Phosphorus is not supplied through biochemical fixation but, must come from other sources to meet plant requirement. These sources include commercial fertilizers, animal manures, plant residues, wastes and native compounds of phosphorus, both inorganic and organic already present in the soil.

The phosphorus cycle has been described in both the ecological and the pedological literature. There, the differences between an unmanaged ecosystem and an agroecosystem (inputs, outputs, etc.) have been described, for example by Tivy (1990).

The next figure shows the general cycle of phosphorus in soils (portion of organic and inorganic forms of phosphorus) into pools based on its availability to plants (Stewart, McKercher 1982):



2.1 Phosphorus as a plant nutrient

The phosphorus requirement for optimal growth falls within the range of 0.3 to 0.5 percent of plant dry weight. Phosphorus deficiency is exhibited by retarded growth and often by reddish coloration. The plants often have a darker green color. These plants have a much lower photosynthetic efficiency per unit of chlorophyll and this deficiency leads to a reduction in most metabolic processes.

Phosphate in plants remains in the the highest oxidized form. Only a small proportion of organic phosphorus is directly obtained by plants. Generally, plants acquire this element through the uptake of soluble inorganic forms (Loughman 1978, Hedley et al. 1982). After uptake as $H_2PO_4^-$ it remains as inorganic phosphate (Pi) or is esterified to a carbon chain as a phosphate ester or attached to another phosphate by the energy rich pyrophosphate bond (e.g. ATP). Exchanges between Pi and the P in the ester or pyrophosphate bond are very high.

This phosphorus is a constituent of macromolecular structures in nucleic acids. As units of the DNA molecule, they are carriers of genetic information and, as units of the RNA molecule, they are the structures responsible for translating genetic information. In both DNA and RNA, phosphate forms a bridge between ribonucleoside to form macromolecules.

The bridging form of phosphorus in diester is also abundant in the phospholipids of biomembranes (bridge between a diglyceride and another molecule – amino acid, amine, alcohol).

Most phosphate esters are intermediates in the metabolic pathway of biosynthesis and degradation. Their functions and formation are directly related to the energy metabolisms of the cells and to energy rich phosphates. ATP is the principal energy rich phosphate required for starch synthesis. The energy rich phosphate bonds of ATP can be transmitted to other coenzymes (UTP, GTP).

Inorganic phosphate has essential functions in the metabolic pool. In many enzyme reactions Pi is either a substrate or an end product. Pi controls some key enzyme reactions. Therefore, compartmentation of Pi is essential for the regulation of metabolic pathways in the cytoplasm and in the chloroplasts. A fascinating control function of Pi in photosynthesis and carbohydrate metabolism has been discovered.

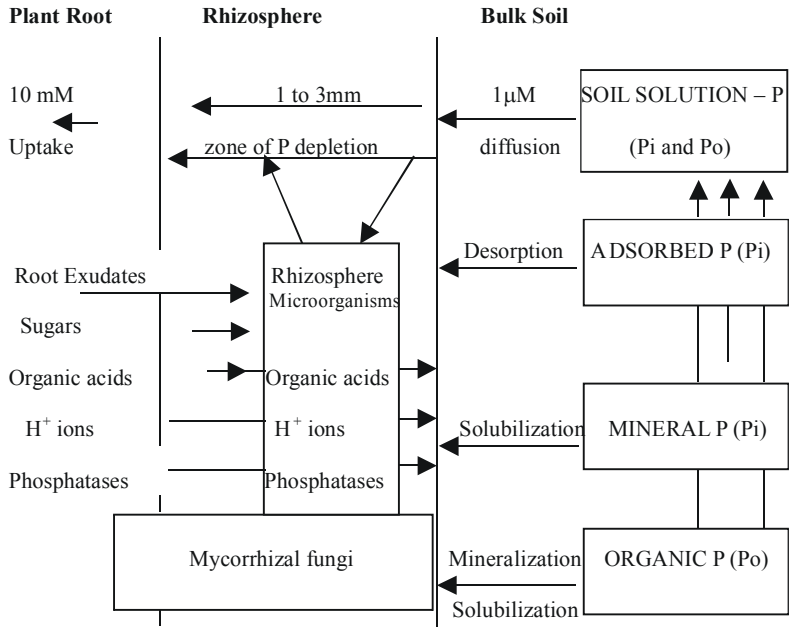
The reaction of Pi to triosephosphates determines the rate of starch synthesis in chloroplasts (Portis 1982 in Marschner 1990). The other mechanisms regulated by Pi is the relase of triosephosphates, the main products of the CO_2 acceptor, ribulose biphosphate. A high external Pi concentration also inhibits total CO_2 fixation.

Carbohydrate metabolism in leaves and sucrose translocation is also affected by Pi. The storage of phosphate in cells as inorganic phosphates is widespread among lower plants. It also been found in higher plants – for example in apple leaves (Smith, Buban 1971, in Marschner 1990).

With an increase in the supply of P (from the suboptimal to the optima level) all the phosphorus fractions in the leaves increase (Kakie 1969, in Marschner 1990). In seeds, the level of the Pi at maturity is very low and most of the phosphate is present as phytate (salt of phytic acid). Phytate phosphorus makes up to 50 percent of the total phosphorus in legume seeds, 60 – 70 percent in cereal grains (Lolas et al. 1976). The level of phytate rises during the period of rapid starch synthesis.

During this period the Pi level declines (Ogawa et al. 1979 , in Marschner 1990). Some phosphorus is incorporated into the starch grains (in potato tubers up to 40 percent of total phosphorus).
During seedling growth the embryo requires mineral nutrients. The degradation of phytates, catalyzed by phytases, leads to a rapid decline in phytate bound phosphorus. The degradation of phytate continues and finally the levels of DNA and RNA phosphorus increase.

Plants essentially derive their P requirements from phosphate anions ($H_2PO_4^-$, HPO_4^{2-}) in the soil. On the other hand, this pool of available phosphorus is small. Soil solution P must, therefore, be replenished continually in order to maintain optimal plant growth. The schema of the major physiological and chemical processes that influence phosphorus availability is shown in the following figure (Richardson 1994):



2.2 Reserves of phosphorus in the Earth

Major reserves of phosphorus in the earth are marine sediments, terrestrial soils, dissolved inorganic phosphorus in the ocean, mineral rocks and biota. Total global reserves of phosphorus (in 10^{12} kg) are shown in the next table (Rochet 1983, in Stevenson 1986):

reservoir	total P x 10^{12} kg
land	
soil	96 – 160
mineral rock	19
biota	2.6
fresh water	0.090
ocean	
sediments	840,000
dissolved	80
detritus	0.65
biota	0.050-0.12

Stevenson estimated that the annual uptake of phosphorus by terrestrial plants is 200 x 10^{19} kg. Losses of phosphorus due to erosion are judged to be 17 x 10^{19} kg.

The phosphorus cycle is influenced by man through the mining and processing of phosphates for fertilizing. Known reserves of rock phosphates representing about 5 x 10^{12} kg of phosphorus (when P content in rock is calculated at 10 percent). Cosgrove (1977) attributed the phosphate problem to the mining rate (about 7 x 10^{10} of rock phosphate annually). The reserves are judged to be about 700 years. Higher rates of use will reduce reserve time.

Large reserves of phosphorus are found in soils. The phosphorus content of common soils varies from 0.01% to 0.2%. the average content is about one half of N and one twentieth of K. Agricultural soils generally contain large amounts of total phosphorus, although only a small proportion (less than 1%) of it is immediately available for plant uptake (Russel 1973). Phosphate in soil can be absorbed on colloidal surfaces and form insoluble complexes with di-and trivalent cations. Therefore, leaching of P from soils is generally small. The main mechanism of P loss from agricultural soils is through erosion. Each metric ton of soil lost by erosion contains 0.2 – 0.8 kg of P (Stevenson 1986).

2.3 Chemistry of phosphorus in soil

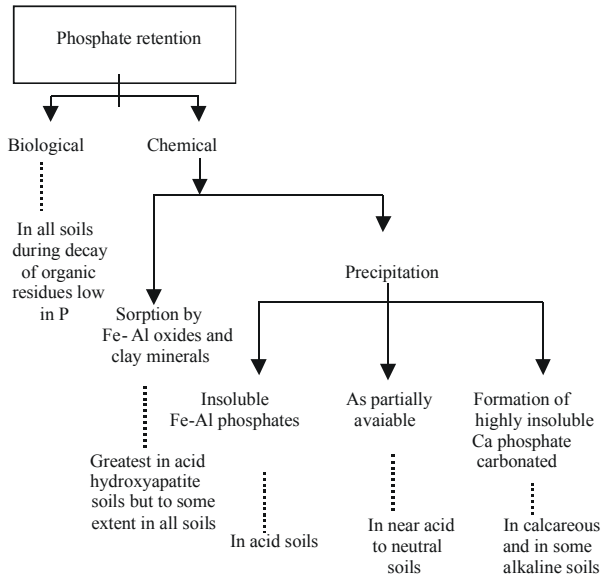
The phosphorus compounds in soil can be placed into (Stevenson 1986):
- soluble inorganic and organic compounds in the soil solution,
- weakly adsorbed (labile) inorganic phosphate,
- insoluble phosphate (Ca in alkaline and Fe and Al in acid soils),
- phosphates strongly adsorbed and/or occluded hydrous oxides of Fe and Al,
- fixed phosphates of silicate minerals, and
- insoluble organic forms such as that:
  - of the soil biomass
  - of undecomposed plant and animal residues
  - part of the soil organic matter (humus).

The native phosphorus in soils was derived from the apatite (complex compound of tricalcium phosphate). The most common minerals are the chloro-, fluor, hydroxy- and carbonate apatites. During their weathering and soil development phosphorus is liberated and is:
- absorbed by plants and recycled,
- incorporated into the organic matter of soils and sediments, and
- redeposited as either insoluble or slowly soluble mineral forms.

Only a small fraction of phosphorus occurs in water soluble forms. Approximately 90 percent of the soil phosphorus occurs in insoluble or fixed forms (primary phosphate minerals, humus P, phosphates of Ca, Fe, Al, phosphates fixed by colloidal oxides and silicate minerals).

Phosphorus is absorbed by plants largely as the negative charged primary and secondary orthophosphate. Thus, water soluble pools have a direct effect on plant growth.

The phosphorus applied to soil in water soluble forms can be converted by both biological and chemical processes to one of many insoluble forms as described by Sauchelli (1951, in Stevenson 1986):



According to Brady (1990) the availability of inorganic phosphorus is determined by:

- soil pH,
- soluble iron, aluminium and manganese,
- presence of iron-, aluminium, and manganese-containing minerals,
- available calcium and calcium minerals,
- amount of decomposition of organic matter, and
- activities of microorganisms.

Most factors are interrelated with pH. In highly acid solutions only $H_2PO_4^-$ ions are present. If the pH is increased, HPO_4^{2-} and finally PO_4^{3-} ions dominate. At intermediate pH levels both $H_2PO_4^-$ and HPO_4^{2-} are found. $H_2PO_4^-$ ions are more available than HPO_4^{2-}, but in acid soils where is dominated could with some soluble iron, aluminium or manganese to insoluble hydroxy phosphates.

The $H_2PO_4^-$ ion can react with insoluble hydrous oxides or elements. The other fixation of phosphorus involves silicate minerals. There are some ideas that the fixation could be a surface reaction between OH groups and $H_2PO_4^-$ ions or that aluminium and iron ions are removed from the silicate crystal and then form hydroxy phosphates.

Phosphorus can also participate in simple anion exchange reactions. The fixation is than influenced by clay mineralogy and decreases in order: amorphous hydrous oxides - goethite - gibbsite - kaolinite - montmorillonite.

In calcareous soils the less soluble di-and tricalcium phosphate are formed by conversion to carbonate apatite. This compound is highly insoluble and its availability may be enhanced by the presence of growing plants (action of organic acid secreted from plant roots).

For most soils, maximum availability is in the slightly acid to neutral pH range. The pH of the rhizosphere changes locally and alters the availability of different phosphorus sources via its influence on the mobilization of P (Marschner 1995). Detailed information about phosphate fixation and availability is in pedological or soil ecological literature (for example Stevenson 1986, Brady 1990).

Topsoil P content is usually greater than in the subsoil due to the sorption of added P and greater biological activity and the accumulation of organic material. However, soil P content varies with parent material, extent of pedogenesis, soil texture, and management factors, such as rate and type of P applied and soil cultivation. These factors also influence the relative amounts of Pi and Po (Sharpley 2000).

2.4 Organic forms of phosphorus

Some part of the phosphorus in soils is bound organically. Organic P is often the dominant form in topsoil, the main rooting compartment, though largely unavailable for plants. The exact amount depends to a large extent on the organic matter content of the soil (Barber 1995, Jennings 1995). When inorganic phosphorus (fertilizers, component of crop and animal residues) is applied to soil, a large proportion of the P is transformed into organic forms as a direct result of microbial activity (Cosgrove 1976). Most soils contain between 50 - 500 mg org. P kg^{-1} soil. The average content of organic phosphorus in cultivated soils ranges from 5 - 50 percent of total P. Detailed data are described by Harrison (1987). Brady and Weil (2002) summarized data of other researchers concerning the percentage of total phosphorus in organic forms ranging between 19 - 75 percent. Halstead and McKercher (1975) state that as much as 5 -10 percent of the organic phosphorus is associated with living microbial tissue. The phosphorus content of microbial tissue is reported to be between 1.5 - 5 percent (White 1987, Úlehlová 1988 and others) (detailed information about P biomass is given in the next chapter).

The total organic phosphorus content of a soil is related to some soil properties, land use, soil order, parent material, climatic zone, etc.

A significant relationship between the organic phosphorus content and the total phosphorus content of soils has been found by a number of researchers (e.g. Harrison 1987).

Several researchers summarized by Harrison (1987) found a relationship between:

- the total organic P content of soils and their organic matter content,
- pH and organic P when acid soils tend to accumulate more total organic P than alkaline soils, and
- nitrogen and organic phosphorus.

The C/P_{org} and N/P_{org} are more variable than the C/N ratio in soils (Neptune, Tabatabai and Hanway 1975, Somani and Sarena 1978, in Stevenson 1986). Uriyo and Kesseba (1975) derived the relationship between organic P and organic C in the equation:

Organic C ($mg.g^{-1}$ soil) = 4.9 + 0.059 Organic P ($\mu g.g^{-1}$ soil), which produces an organic C/P ratio of 115.

Stevenson (1986) summarizing the work of others, published data about the organic C, total A, organic P and organic S relationship in soils:

Location	Number of soil	C/N/P/S
Iowa	6	110:10:1.4:1.3
Brazil	6	194:10:1.2:1.6
Scotland		
calcareous	10	113:10:1.3:1.3 ($S=S_{tot}$)
noncalcareous	40	147:10:2.5:1 ($S=S_{tot}$)
New Zealand	22	140:10:2.1:2.1
India	9	144:10:1.9:1.8

The total organic phosphorus in soils tends to be absorbed onto the clay and silt. Analysis shows a highly significant difference in the organic P content of different soil texture classes.

It´s not easy to determinate the relations between individual climatic factors with total organic phosphorus. However, some date from South Dakota exists where the organic P content of soils increases with:

- increasing temperature and rainfall,
- higher organic P being associated with sites of crop productivity (Westin, Buntley 1967).

Some researchers have described the lesser effects of climate on soil organic P content. For an analysis of data of climate on organic phosphorus, the world was divided into two zones:

- warmer - between the 40^0 parallels,
- cooler - areas influenced by polar air masses (at least in winter).

The analysis of data from the two zones shows significant differences with a higher level in winter in the cold regions (Harrison 1987).

In the temperate or in the humic tropical zones organic P content increases with increased rainfall intensity (Uriyo, Kesseba 1975). In some reports higher total and organic phosphorus in poorly drained soils is described in relation to better drained soils (restricted mineralization) (Jencks et al. 1964).

Forest soils have a higher organic P content than arable or cultivated soils (Brogowski 1966).

An analysis of the published data (Harrison 1987) shows that the total organic P content of soils does vary with land use. Soils of temperate grassland have the highest amount of organic phosphorus whilst those of the warmer zones have the least.

The organic phosphorus content varies with soil type (Kalpage, Wong 1977), and soil parent material (Udo, Ogunwale 1977). The distribution of organic

phosphorus is related to the distribution of organic matter in the soil profile and its content in mineral soils generally decreases with depth (Smeck, Runge 1971).

The organic P content can be affected by human activities. For example, the addition of P fertilizer could result in an increase in the organic P content of surface soils (Batten et al. 1979). In other studies an increase has yet to be found.

The level of organic P could be affected by cultivation, crop type (effect of legumes - ability to fix nitrogen and the increase in organic P, quality and quantity of organic matter returned to the soil) grazing, etc. (Harrison 1987).

A large reservoir of organic phosphorus exists which is unavailable to plants. Here, the microbial oxidation of organic substrates is an important supplementary source of inorganic phosphate.

Nucleic acids and phospholipids are phosphodiesters whose contents are high in newly deposited organic matter. The esters of orthophosphoric acid, and especially inositol phosphates, tend to accumulate in the soil (Stevenson 1994, Jennings 1995).

Soil organic matter contains:

1. Inositol phosphates (from mono to hexa) - The mixed inositol polyphosphates are known as soil phytate (soil phytin). Phytic acid is myositol hexaphosphate and phytin or phytate is calcium/magnesium salt (Cosgrove 1977).
The amount of soil phytate is generally between 15 -30 percent of the total organic P. Myositol hexaphosphate (phytin) is found in plants and animals and a variety of other forms is synthesized by microorganisms (Richards 1987). For many years the inositol hexaphosphates in soil were thought to be derived from the phytin of higher plants, but recent work indicates that inositol hexaphosphates in soil are probably of microbial origin (Stevenson 1986). Jennings (1995) described that up to 60 % of the organic phosphorus content in soils may consist of phytate (hexa-phosphate ester), and it often forms salts with different ions. The calcium salt of phytic acid is a soluble salt, while the other salts, mainly iron and aluminium salts, only have a low solubility.

2. Smaller quantities of organic phosphorus are in nucleic acids and in phospholipids. Nucleic acids (DNA, RNA) are found in all living cells and they are synthesized by soil microorganisms during the decomposition of residues. The amount is 2 - 5 percent of the total organic P.
Phospholipids are biologically important compounds that are soluble in fat solvents and the amount is 1 - 2%.

3. Traces of P come from other sources (phosphoproteins, metabolic phosphates, etc.)

4. Many of the organic phosphorus sources are not identified (Richards 1987).

2.5 Phosphorus of soil microbial biomass

It has been shown that the P contained within microbial biomass can constitute a significant component of total soil P, with values in the range of 1 - 10% being common for agricultural soils (Richardson 1994). These values correspond with 10 - 100 kg $P.ha^{-1}$ in the top 10 cm of soil.

Microbial P is influenced by numerous factors (soil moisture and temperature, management practices, etc.). Microbial P is generally higher in permanent grassland compared to arable soil. The soil biomass P content can be increased by the addition of C (Thien, Myers 1992) or lime (Condron, Goh 1990).

Soil microorganisms can derive a significant proportion of their requirement through the mineralization of P_o (Thien and Myers 1992). A significant component of soil P is contained within and cycled through the microbial biomass (Richardson 1994). The P cycled through soil microorganisms is not necessarily directly available to plants, but is also (like fertilizer P) subject to immobilization and the physical and chemical reactions taking place in the soil.

The concepts of using specific soil microorganisms to increase the availability of P to plants is described by Richardson (1994). Two major strategies exist for the manipulation of soil microorganisms to increase the availability of soil P:

a) the management of existing populations of microorganisms in soil so as to optimize their capacity for P transformation,

b)the introduction of specific bacteria or fungi as inoculants.

Inoculation of soil with *Bacillus megatherium* var. *phosphaticum* (phosphobacterin) was used in Russia to increase crop yield (Cooper 1959). Legget et al (1993) described the using of *Penicillium bilaii* which solubilized Pi through the production of organic acids. Few efforts have attempted to directly manipulate microbial populations which are able to increase P_o mineralization and this problem is being solved by researchers.

The symbiotic interaction between plant roots and mycorrhizal fungi is studied mainly in forestry where the introduction of ectomycorrhizal inoculants has been successful. The vesicular - arbuscular (VA mycorrhizas form associations with agricultural plant species. Their widespread utilization is limited by restricted routine cultivation (Gianinazzi, Gianinazzi - Pearson 1986).

The P biomass in the soil can be estimated from cell weights based on microbial counts, from inorganic P released during the incubation of partially sterilized soil or from ATP measurements as described by Stevenson (1986).

2.6 Mineralization of organic compounds

Soil microorganisms are integral components of the soil - plant system and of particular importance is their influence on plant nutrients such as phosphorus. Microorganisms can affect the P supply to higher plants through the decomposition of organic P compounds, through the immobilization of available phosphates and by promoting the solubilization of fixed or insoluble mineral forms (chelating agents). The microbial biomass itself contains a large pool of nutrients that are potentially available to plants.

The level of soluble phosphate in the soil depends on two opposing processes:

The mineralization of phytates is relatively slow, but these are an important source of PO_4^{3-} for plants, since phytase activity is common among soil and rhizosphere microorganisms and mycorrhizal fungi (Greaves, Webley 1965, Moser, Haselwandter 1983).
Such compounds as phospho-sugars, nucleic acids, and phospholipids which form only a small part of the organic phosphorus content are mineralized rapidly. The labile part of organic P is defined as "the organic pool undergoing rapid transformation and contributing to P available to plants through mineralization" (Harrison 1987). The labile P pool is determined either as that part of the organic P soluble in sodium bicarbonate solution or in potassium carbonate solution and hydrolysed by hypobromide (Olsen et al. 1954, Harrion 1981).
The P content of decomposing organic residues plays a key role in regulating the quantity of soluble phosphorus. Net immobilization will occur when the C/P_{org} is 300 or more, net mineralization when the ratio is 200 or less (Stevenson 1986).

The mineralization and immobilization of organic phosphorus as a biological process is strongly influenced by various physical and chemical properties of soil and by human induced.
For example, Harrison (1987) and Stevenson (1986) describe the factors which affect the rate of mineralization of organic phosphorus:

This mineralization is related to:
1. Temperature - the rate of mineralization generally increases with temperature. It is quite rapid at temperatures above 30^0 C (Martin, Cunningham 1973).

2. Moisture - some researchers have suggested that the greatest mineralization occurs under waterlogged conditions (Racz 1979) while others indicate that most mineralization exists under drier conditions (Biddappa, Perur 1978). An important aspect is the alteration of dry with wet conditions.

3. pH - some studies show that the rate of mineralization increases with the increase in soil pH and declines when pH is above 7. It could be associated with changes in the solubility of organic phosphorus. Organic P is most stable below pH 5 and higher than 7.5 and liable to mineralization between 5 -7 (Envezor 1967).

4. Microbial activity - it has been demonstrated that soil bacteria, actinomyces, fungi and protozoa can hydrolyze organic P compounds (Harrison 1987). Mineralization processes are indicated indirectly by correlations between mineralization rates of P and organic C or soil respiration.

5. Varying soil conditions (aerobic and anaerobic conditions) in which microorganisms vary in their activity to hydrolyze organic phosphates.

6. Inorganic phosphate - some studies have shown that increasing the rate of mineralization after the addition of inorganic P (Pi displaced some P_{org} bound to Fe, Al, Ca) leads to organic P being more readily dephosphorylated by enzyme action. Many studies have demonstrated no effect of Pi on mineralization.

7. Plants are the source of organic matter in soil, but their presence should increase the rate of P_{org} mineralization (for example by phosphatase activity).

8. enzymatic activity - phosphatase activity - the mineralization of organic phosphorus is effected by the action of phosphatases. Details of enzyme activity in the soil, mainly phosphatases activity are described in the following text.

9. Cultivation - some studies have suggested that cultivation stimulates the mineralization of organic phosphorus.

The mineralization of labile P_o has been shown to be important in both low and high fertility soils (Stewart, Tiessen 1987, Tate et al. 1991). Amounts of P_o mineralized in temperate dry land soils range from 5 – 20 kg P ha^{-1} yr^{-1} (Stewart, Sharpley 1987) and tends to be higher in the tropics (67 – 157 kg P ha^{-1} yr^{-1}). Brady and Weil (2002) described mineralization of organic phosphorus in temperate regions ranged between 5 to 20 kg P ha^{-1} yr^{-1} , most of which is readily adsorbed by growing plants. These values can be compared to the annual uptake of phosphorus by most crops, trees, and grasses, which generally ranges from 5 to 30 kgP ha^{-1}.
P_o may also be resistant to hydrolysis by phosphatase through complexation with Al and Fe (Tate 1984).
A number of approaches can be suggested to amelioration deficiencies and excesses of phosphorus in soils including also the cycling of organic matter and the choice of phosphorus-efficient plants.

3 SOIL ENZYMES

Biochemical actions are dependent on or related to the presence of enzymes. Many reactions involving soil organic matter transformations may be catalysed by enzymes existing outside the microorganisms and plant root system. Each soil may have a characteristic pattern of specific enzymes as described by Kuprevich and Scherbakova (1971). The differences in the level of enzymatic activity are caused primarily by the fact that every soil type, depending on its origin and developmental conditions, is distinct from every other in its content of organic matter, in the composition and activity of living organisms inhabiting it, and consequently, in the intensity of biological processes. Obviously, it is probable that each type of soil has its own inherent level of enzymatic activity.

Oxidoreductases (catalyzing electron transfer reactions), transferases (catalyzing reactions with transfer of molecular groups, such as – NH^2, R-CO-, etc.) and hydrolases (catalyzing bond hydrolysis) are the most studied enzyme systems in soil because of their role in the oxidation and release of inorganic nutrients from soil organic matter (Dick 1994, Nannipieri 1994).

A list of enzymes in soils is given in the following table (Stevenson 1986):

Enzyme	Reaction catalysed
α and β amylase	hydrolysis of 1,4 - glucosidic bonds
arylsulfatases	$R\text{-}SO_3^- + H_2O = ROH + H^+ SO_4^{2-}$
asparaginase	asparagine + H_2O = aspartate + NH_3
cellulase	hydrolysis of -1,4 - glucan bonds
deamidase	carboxylic acid amide + H_2O = carboxylic acid + NH_3
dehydrogenases	XH_2 + acceptor = X + acceptor H_2
α and βgalactosidase	galactoside + H_2O = ROH + galactose
α and β glucosidase	glucoside + H_2O = ROH + glucose
lichenase	hydrolysis of 3 - 1,3 cellotriose bonds
lipase	triglyceride + $3H_2O$ = glycerol + 3 fatty acids
nucleotidases	dephosphorylation of nucleotides
phenoloxidases	diphenol + $1/20_2$ = guionone + H_2O
phosphatase	phosphate ester + H_2O = ROH + PO_4^{3-}
phytases	inositol hexaphosphate + $6H_2O$ = inositol + 6 PO_4^{3-}
proteases	proteins = peptides and aminoacids
pyrophosphatase	pyrophosphate + H_2O = 2 PO_4^{3-}
urease	urea = $2NH_3 + CO_2$

During the study of soil enzymes the question of their origin was solved. The first report of extracellular enzymes (Woods 1899) showed that catalase was

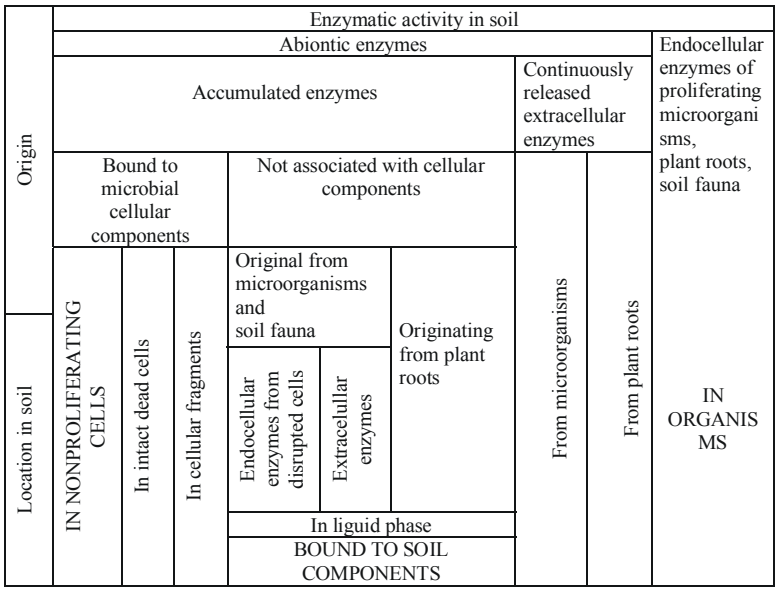
excreted by plant roots. Activity in the rhizosphere was made higher not only by the plant root contribution, but by a higher density of microbial microflora. In the 1960´s the rhizosphere effect on soil enzymatic activities was studied by a number of researchers. For example, Voets and Dedeken (1966) describe the increase of invertase, urease, glucosidase and phosphatase in the rhizosphere of barley, rye and wheat. Some research assumes that the source of most soil enzymes is the soil microflora. Hoffmann (1963) demonstrated that microorganisms were the sole sources of enzymes in soils. Kiss (1957), Kozlov (1965) published reports on the influence of soil fauna on soil enzymes. The fauna contribute to enzyme content but this contribution is limited. De Jorge and Sawaya described earthworm production of acid and alkaline phosphatase. The contribution of enzymes to soil from soil fauna has been poorly investigated (Dick 1994).

Enzymes in soils originate from animal, plant and microbial sources. Speir and Ross (1978) demonstrated that microorganisms supply most of the soil enzyme activity, with their large biomass, high metabolic activity and short lifetime under favourable conditions. The microbial populations are influenced indirectly by plants (rhizosphere, supply of carbon essential for microbial growth). Ladd (1985) also described that microorganisms are the most important sources.

Many organisms, including soil fungi, release phosphatases into their environment (Haas et al. 1992). These phosphatases are introduced into the soil by active exudation, leakage or cell lyses (Tadano et al. 1993). P deficiency often enhances extracellular phosphatase activity from plant roots, fungi and other microorganisms (Tadano et al. 1993, Li et al. 1997).

Ultracytochemical and ultrahistochemical methods applied to soil with the examination of preparations by electron microscope have allowed the detection of enzymes, such as acid phosphatases in root fragments, mycorhizae, soil microorganisms and fragments of microbial membranes as small as 7 – 20 nm (Ladd et al. 1996). Unfortunately, these ultracytochemical tests used to locate enzymes have limits in electrontransparent materials, such as microbial and root cells or cell fragments but not in naturally electrondense components, such as soil minerals (Nannipieri, Landi 2000).

The schema of the components of soil enzyme activities described by Skujins (1978) is as follows:



Ladd (1978) drawing upon Kiss et al. (1975) says that accumulated enzymes are those "present and active in a soil in which no microbial proliferation takes place". They include enzymes which are:

- extracellular - i.e., free in the soil solution or bound to inorganic and organic soil constituents,
- present in cell debris, and
- present in dead cells or in viable but non-proliferating cells.

The enzymes present in soil are associated with various biotic and abiotic components. They may be associated with proliferating and non-proliferating cells or with dead cells and cell debris. They may have leaked from cells or lysed cells, and they may exist temporarily in enzyme - substrate complexes or may be adsorbed to clay minerals or be associated with humic colloids (Nannipieri 1994). Detailed information on soil enzymes may be found, for example in the monograph edited by Burns (1978).

3.1 Activity of soil enzymes as an indicator of soil quality

Soil microorganisms as one part can be sensitive biological markers and can be used to assess soil quality or degradation. Microbiological properties can serve as soil quality indicators because after plants soil microbes are the second most important biological agent of the agricultural ecosystem. There are many indicators of soil microbiological properties including microbial biomass content, microbial diversity and activity, and enzyme activity. Following text (published in Environmental research – 99, Lithuanian University of Agriculture, Kaunas, 30-35) shortly demonstrates the problem of soil quality evaluation and possible use of enzymes activity:

The pressure of humans on soils during the last decades begs many questions about its quality and productivity. People are dependent on soil and vice versa good soils are dependent on people. Unfortunately, soil resources have been degraded through erosion, the loss of tilth and biological activity, salinization and the build up of toxic compounds, etc. Many discussions center on the effect of farming practices, such as chemicals and fertilizers on soil microorganisms, soil tilth and environmental quality.

Soil quality

Doran and Parkin (1996) have defined soil quality as the capacity of the soil to function within ecosystem boundaries to sustain biological productivity, to maintain environmental quality, and to promote plant and animal health. The relation of soil quality to crop productivity is probably best understood from soil quality components. The soil is the primary supplier of water and nutrients for plants, which, in turn provide nutrients to animals and humans. Maintaining soil quality for plants will help to ensure normal growth and development for plants, animals and humans. Doran et al. (1996) outlined three avenues where soils interact with and affect the health of animals (direct poisoning by chemicals in the soils, the ability of soils to filter contaminants from water, the provision of a medium that contains essential and/or nonessential nutrients to plants that can alter the quality and quantity of plant growth).

Assessing soil quality

Problems exist with measuring and evaluating soil quality. We have acceptable measurements for water and air quality, but not for soil, which is a key issue relating to agricultural sustainability. Researchers in many laboratories are interested in an integrative soil quality index to monitor changes in soil over time. Ideally, a soil quality index would be formulated by an integration of parameters that account for the soil´s capacity to perform productivity, environmental and health functions. There are numerous soil properties that change in response to changes in management practices and land use, some of which are highly sensitive, whereas other are more subtle. These properties will also vary spatially and temporary.

Doran and Parkin (1996) state that soil quality - health indicators should: correlate well with ecosystem processes; integrate soil physical, chemical and biological properties and processes, and serve as the basic inputs needed for the estimation of soil properties or functions which are more difficult to measure directly; be relatively easy to use under field conditions and be assessable by both specialists and producers; and be sensitive to variations in management and climate. The indicators should be sensitive enough to reflect the influence of management and climate on long term changes in soil quality but not be so sensitive as to be influenced by short-term weather patterns, and where possible, should be a component of existing soil data bases.
According to these authors the indicators should be:

- physical - texture, depth of soil, infiltration and soil bulk density, water holding capacity,
- chemical - soil organic matter, pH, electrical conductivity, extractable N, P and K,
- biological - microbial biomass C and N, potentially mineralizable N, soil respiration.

Sarrantonio et al. (1996) described recommendations for on-farm assessment of soil quality and health. The tested parameters are soil pH, electrical conductivity, bulk density, infiltration rate, water holding capacity, soil respiration rate, and soil nitrates.
Oades and Walters (1994) described indicators of soil quality for cropping systems. They divided these into: water use efficiency and physical indicators, chemical indicators, predictive models (organic matter), plant indicators and biological indicators. The last one includes biomass, enzyme activity, respiration, cellulose decomposition, nitrogen fixation, *Rhizobium*, earthworms, and soil fauna.

Soil enzymes activity

Soil microorganisms as one part can be sensitive biological markers and can be used to assess disturbed or contaminated soils. Microbiological properties can also serve as soil quality indicators because after plants soil microbes are the second most important biological agent of the agricultural ecosystem. There are many indicators of soil microbiological properties including microbial biomass content, microbial diversity and activity, and enzyme activity (Sikora et al, 1995) Each soil has a characteristic pattern of enzymes because all biochemical actions are dependent on or related to their presence. Soil enzyme assays are process level indicators and are presented as a means of determining the potential of a soil to degrade or to transform substrates. This can be useful as an indicator of how well a soil carries out important steps in different processes. Oxidoreductases, transferases and hydrolases, have been the most studied enzyme activities of soil because of their role in the oxidation and release of inorganic nutrients from organic matter. Ideally the activity of a soil should be attributable to enzymes of different origins, functioning according to their concentration and catalytic properties as expressed under the conditions of the soil micro-environment in which they are located (Ladd, 1978). Enzymes in soils originate from animal, plant and microbial sources. Speir and Ross (1978) have demonstrated that

microorganisms supply most of the soil enzyme activity, with their large biomass, high metabolic activity and short lifetime under favourable conditions.

Effect of the agroecosystem on activity of soil enzymes

Soil enzyme activities are influenced by management practices because they are also related to microbial biomass which is sensitive to different treatments. Soil enzymes activities influence the following factors:

- soil moisture, temperature, aeration and structure, pH, content of inorganic and organic colloids,
- the presence of important trophic substances supplying soil organisms with nutrients,
- soil biocenoses, vegetation cover,
- organic matter quality and quantity, and
- the presence of inhibitors and activators.

In the literature there are many references about the relationship of enzyme activities and various soil properties. McLaren (1975) referred to soil as a system of humus- and clay- immobilized enzymes with the persistence and stability of these enzymes in soils being generally attributed to their association with both clay and humus. Even more research exists on how the physicochemical properties of soil can influence the activity of soil enzymes.

In our research of soil phosphatase activity at locations in the Czech Republic with different soil types (Šarapatka and Kršková, 1997) we described the correlations of this activity with clay content, C_{org}, N_{tot}, humic and fulvic acids ratio, available and total phosphorus, and pH.

From research it is evident that soil enzyme activities are influenced by the system of agriculture, soil tillage, inputs of fertilizers and pesticides, inhibitors, etc. Microbial biomass and enzyme activities could increase after the addition of an energy source. The stimulation of soil enzyme activity by organic amendments is higher than that induced by organic fertilizers. Other investigations have evaluated the effects of tillage and farming systems on enzyme activities. The results observed for the effect of tillage and rotation on soil enzyme activities were strongly correlated with organic C concentration. The results indicate, for example, higher activities in the top of the profile where there was no-tillage compared to conventional tillage practices. The effect of cultivation (for example of grassland) induced significant changes in the quality, chemical composition and molecular size of organic matter which in turn influenced the activities of enzymes involved in the C, N and P cycles. There have been fewer investigations concerning ecological agriculture and enzyme activity. One example could be from the long term field trials in Oberwil where enzyme activities were higher in biological production systems than in conventional systems. Enzyme activity is also influenced by plants because they have been shown to stimulate the activity of enzymes in the rhizosphere and can be higher in planted than in unplanted soils.

On the other hand, there, are also some inhibitors of enzyme activity. Phosphatase activity can be depressed by phosphate fertilizers, N fertilizers can have a similar effect on enzymes involved in the nitrogen cycle. Various pesticides have been evaluated to determine their effect on the activity of soil enzymes. It is not possible to cover all the problems here. Detailed information has been

provided, for example, by Schäffer (1993), who summarized world research into pesticides and soil enzymes. There are also many investigations on the anthropogenic effects of heavy metals where a negative correlation generally exists between the enzymatic activities of soils and the heavy metal.

Evaluation of soil enzyme activity

In general, enzyme activity is a good indices of soil quality because enzymes play an important role in nutrient cycles. The rationale for soil enzyme activity as a soil quality indicator is after Dick et al. (1996). Enzyme activities:

- are closely related to important soil quality parameters,
- can begin to change much sooner than other properties,
- can be an integrative soil biological index of past soil management, and
- involve procedures that are relatively simple compared to other important soil quality properties.

At this time data are limited and incomplete drawn from diverse environments (across soil types, environments, soil management systems) to determine the potential of soil enzyme activity to characterize soil quality including the development of calibration data to interpret enzyme activities.

From the above text it is also perceptible that in the soil there exist many complex processes and it´s unrealistic to expect to find a simple relationship between a single enzyme activity and the fertility or quality of soil. It is the endeavour of many laboratories to describe some indices for the evaluation of soil fertility. Stefanic et al. (In: Nannipieri, 1994) described the "Biological Fertility Index" which is calculated using dehydrogenase and catalase activity. In 1984, Beck (In: Nannipieri, 1994) developed a more comprehensive parameter "Enzyme Activity Number" which is calculated with dehydrogenase, alkaline phosphatase, protease, catalase and amylase activities. This complex view, which is being developed by specialized laboratories, can also help to evaluate changes in agricultural soil fertility in the modern sense of reduced chemical and energy inputs. This evaluation could help in assessing the sustainability of farming systems apart from profitability and an absence of adverse impacts on the environment, etc. Soil stability and quality play a key role.

4 SOIL PHOSPHATASES

Large proportions of the phosphorus in many soils is organically bound and the mineralization of these portions is of agricultural and economic importance. Organic phosphorus compounds in soil can constitute 5 – 50 percent of total phosphorus and the assimilation of this phosphorus by plants and microorganisms is preceded by soil enzymes. Several enzymes are involved in the decomposition of organic phosphorus compounds (Jennings 1995). Those enzymes that hydrolyse P esters are commonly called phosphatases. Soil phosphatases then play a major role in the mineralization processes (dephosphorylation) of organic P substrates. Appiah and Thompson (1974) proposed that the mineralization of organic phosphorus is principally a microbial phenomena and they suggest further that phosphatase activity becomes important after the initial breakdown of soil organic matter once catalysed by a host of microbial enzymes. The initial breakdown could be the rate – limiting step of organic phosphorus mineralization. The organic phosphorus mineralization in soil correlated with the nitrogen and carbon mineralized (Thompson et al. 1954). Ridge and Rovira (1971) describe the important role of root surface enzymes in phosphorus mineralization compared to the larger amounts of enzymes more distant from the roots and bound to clay and organic matter. Some results indicate a more important role might be played by microbial phosphatase than cell-free phosphatase. However, under conditions in soils cell-free phosphatase constitutes the major proportion of these enzymes and should play a considerable role in the constant mineralization of organic matter (Speir, Ross 1978).

The enzymes have trivial names according to their substrates, but are either phosphoric monoester hydrolases (phosphoric monoester hydrolases) (EC 3.1.3) or phosphoric diester hydrolases (phosphoric diester hydrolases) (EC 3.1.4).
To the first group belong:
- phytase
- nucleotidases
- sugar phosphatases
- glycerophosphatase

To the second group belong:
- nucleases
- phospholipases (Speir, Ross 1978).

These enzymes are often referred to as phosphatases. Hoffmann (1968) suggested three types of phosphatases – acid, neutral and alkaline (different pH optima).

4.1 Determination of soil phosphatases activity

The name phosphatases denotes a group of enzymes that are able to hydrolyze esters and phosphoric acid anhydrides.
Soils contain:
- phosphomonoesterases (e.g. phytases, glycerophosphatase, nucleotidases),
- phosphotriesterases,
- polyphosphatases (e.g. ATPases, pyrophosphatases), and
- P-N hydrolases (e.g. phosphoamidases) (Schinner et al. 1993).

The activity of phosphatases can be determined by means of a phosphate originating in the process of mineralization of natural phosphoesters, e.g. phytine, or using organic fractions following the mineralization of "artificial" organic substrates (3-naphthylphosphate, p-nitrophenylphosphate). In most instances p-nitrophenylphosphate (Tabatabai, Bremner 1969) or phenylphosphate dinatriumsalz (Hoffmann 1968) are used as substrates being split up by phosphomonoesters to get ROH and HPO_4^{2-}. The determination of the individual phosphatases has been described by Őhlinger, Margesin, Kandeler (in Schinner et al. 1993).

Phosphomonoesterases are characterized by substrate specificity and optimum pH. Consequently, it is easy to distinguish between acid and alkaline phosphatases.

Two methods to be described here:

1. The method according to Hoffmann (1968) a mixed soil samples with phenylphosphate dinatriumsalz solution are incubated at 37^0C for 3 hrs. The removed phenol is dyed with 2,6-dibromchinonchlorimide, and measured by means of spectrophotometry at 614 nm.

2. The slightly modified method of Tabatabai and Bremner (1969), used in our laboratory, is based on the incubation of soil samples mixed with a buffer solution of p-nitrophenylphosphate at 37^0C for 1 hr. The released p-nitrophenol is stained, and measured spectrophotometrically at 400 nm.

It is not very common to assess phosphodiesterase, phosphotriesterase and polyphosphatase activity as a part of soil analysis. Nevertheless, there are some basic data to be presented here.

According to Browman and Tabatabai (1978 in Margesin 1993) phosphodiesterases are determined following the incubation of soil samples supplemented with bis(p-nitrophenyl)phosphate solution at 37^0C. The enzyme-released amount of p-nitrophenol is stained with an alkali solution, TRIS, and measured spectrophotometrically at 400nm.

Pyrophosphatase activity is estimated in the course of soil sample incubation together with a pyrophosphate solution. Enzyme-released orthophosphate is extracted using sulphurous acid, and is stained with ammoniummolubdate where

upon it is then measured photometrically at 700 nm (Dick, Tabatabai 1978, in Margesin 1993).

Kandeler (1993) described the determination of phospholipase C activity using the original method of Kuroshima and Hayan. Soil samples were incubated with the substrate p-nitrophenylphosphorylcholine at 30^0C for 2 hrs, and the formed p-nitrophenol was measured photometrically.

All the above procedures are based on the use of a reaction mixture containing substrates together with the soil to bring about the release and storage of substrate hydrolysis products. Soil phosphatases can promote hydrolysis at a certain optimum pH level. It is essential then to maintain the required pH value of the reaction mixture during the course of incubation.

For example, Tabatabai and Bremner (1969) used MUB (modified universal buffer). Nevertheless, there are other types of buffers reported by various authors. Zvjagincev et al. (1980) described the application of acetin buffers whilst Szegi (1983) used a borate buffer.

There are some references on the use of bacterostatics, especially in those environments where soil organisms are ready to consume the products of substrate disintegration immediately, and consequently, the enzyme activity recorded is somewhat lower compared to the original test source (cf. Juma, Tabatabai 1978, Pang, Kolenko 1986).

Enzymic reaction is most often ceased by NaOH. Numerous authors, e.g. Tabatabai and Bremner, reported the point use of NaOH and $CaCl_2$ which was tried in our study too. Other authors applied other hydroxides (e.g. KOH, NH_4OH) or trichloroacetic acid to stop the reaction.

In research is also effort to develop new methods in determination of soil phosphatases activity. Extracellular enzymes in soil often occur in immobilised forms, a state that may alter their interactions with substrates in comparison with enzymes in the solution phase. De Cesare et al. (2000) evaluated sonification for its usefulness in studying immobilised acid phosphatase by dispersing soil aggregates. An increase in activity with sonication, which coincided with a release of soil chromophores, might be related to the exposure or release from aggregates of the extracellular enzyme fraction immobilised on colloids.

Marx et al. (2001) described a microplate fluorimetric assay which has been developed to study enzyme diversity in soil as an approach to understanding functional diversity. They also carried out a comparative study between the new microplate fluorimetric assay and a standard colorimetric enzyme assay based on p-nitrophenyl substrates. Kremer (1994) described the microplate method as similar to the standard method in accuracy of determination, required fewer chemical reagents, and considerably reduced the time required for analysis.

Mimmo et al. (2002) determined the effects of sample diversity on mid-infrared diffuse reflectance spectroscopic calibrations for biological measures in soils. The results of this research show that diffuse reflectance infrared Fournier transform spectroscopy was capable of determining, to some degree, compositional parameters (total C and N) and biological activity as reflected by three enzymes. The results using mid-infrared spectra were more accurate than those using mid-infrared spectra for the same samples.

Knowledge of microbial numbers and activity could be essentials for understanding the transformations in soil. In our research (Hýsek, Šarapatka 1998)

we compared the activity of acid phosphatase and other soil properties with the number of bacteria belonging to the genus *Micrococcus*. Taylor et al. (2002) described strong positive correlations between all enzyme activities and between all methods of estimating bacterial abundance. Positive correlations were also found between bacterial abundance and enzyme activities and between enzyme activities and organic matter content.

In root system and in external hyphae is possible to investigate fungal phosphatase activity using fluorogenic substrate that forms a fluorescent crystaline precipitate at the site of the phosphatase activity. Van Aarle et al. (2001) compared this method with others, for example, with using of p-nitrophenylphosphate. The result is that microscopic detection using fluorogenic substrate can be used to visualize hyphal phosphatase activity in both roots and external hyphae.

4.2 Factors influencing soil phosphatase activity

The basic ecological factors influencing soil enzyme activities have been studied by numerous authors. Rejšek (1988) stressed the following ones:

- soil moisture, temperature, aeration and structure, pH, content of inorganic and organic colloids,
- the presence of important trophic substance supplying soil organisms with nutrients,
- soil biocenose, vegetation cover,
- organic matter quality and quantitiy, and
- the presence of inhibitors and activators.

The above factors and anthropogenic effects will be described in detail in the following chapters:

- soil properties and soil phosphatase activity,
- soil tillage, fertilization, farming system as compared to soil phosphatase activity
- management of the agroecosystem and soil phosphatase activity, and
- effect of plants, rhizosphere effect, mycorrhiza and animals on soil phosphatase activity.

4.2.1 Soil properties and soil phosphatase activities

According to Gispert and Arcara (1988) the physicochemical properties of soil can influence the respiration rate and saccharase activity. Urease, phosphatase and protease activity were well correlated with them. Beyer et al. (1992) studied microbial dehydrogenase and alkaline phosphatase activities. In loamy Luvisols and Gleysols, biological activity was greater under organic supply despite poorer nutrient supply.

Pagliai et al. (1993) showed that the relationship of enzyme activities (urease and phosphatase) and the various pore size classes in each type of soil showed a positive common trend between the two enzyme activities and the percentage area

of pores ranging from 30 – 200 mum in equivalent pore diameter. Appreciable quantities (more than 48 percent) of commercially active phosphatase were immobilized by soil colloidal particles in rendzina soil in the research of Perez – Matos et al. (1991).

In some research soil phosphatase correlated with other chemical and biological properties of the soils. Bonmati et al. (1991) assert that phosphatase activity in soils from experimental plots, after storage at room temperature for 1 year. before being analysed, was correlated with protease, total N and organic C. Zehlen and Schroder (1990) measured the biological activity in crop rotation using microbial biomass and enzyme activity as parameters. Microbial biomass and alkaline phosphatase in barley fields were correlated to pH values and the organic C content of the soil. The influence of pH on the rate of phosphatase activity has also been described by Herbien and Neal (1990). Acosta-Martinez and Tabatabai (2000) described the correlations between phosphatases activity and pH as the effect of eight lime application rates. Research done in the 1980s by Chhonkar and Tarafdar (1984) described positive correlations of phosphatase activity with organic carbon, organic phosphorus and bacterial populations, and a negative relationship with soil pH. In this research no correlation was found between phosphatase activity and clay content or soil actionomyces. In the same year Beck described significant correlations between all individual microbial soil properties. This close relationship between the tested biomass and enzyme activities was used for calculating an overall index of soil microbial activity.

In a investigation by Nahas et al. (1994) the activity of acid and alkaline phosphatase correlated with organic matter and total phosphorus content, but not with available phosphorus and organic phosphorus content. The phosphatase producing bacterial population was favoured by the level of available phosphorus.

After studying the literature, soil phosphatase activity can be related to soil organic matter (Kiss et al. 1974, Nannipieri et al. 1973, Jordan and Kremer 1994, Aon and Colaneri 2001 and others), total nitrogen (Speir 1978, Aon and Colaneri 2001 and others), soil organic phosphorus content (Gavrilova et al. 1973 and others).

Soil phosphatase activity is also affected by soil moisture and soil depth. Many authors, including Harrison (1983, 1987), Herbien and Neal (1990) have emphasized the significance of soil moisture. It affects phosphomonoesterases by promoting the development of microbial communities and plant roots. There is a balance between soil moisture and soil biocenose. A soil with a certain moisture level corresponds to the density of edaphon. As a result, temperature alterations of the soil are only significant when concerned with the speed at which this stage is attained. There are certain temperature thresholds which control phosphomonoesterase activity. The optimum temperature for activity is within the range 45 – 60^0C (Harrison 1987).

Several reports have compared phosphatase activity with soil depth (Kiss et al. 1974, Arutyunyan, Galstyan 1974). Phosphatase activity decreases with soil depth and corresponds to the distribution of microorganisms in the soil profiles (Khazirev, Burangulova 1965) and organic matter content (Arutyunyan, Galstyan 1974).

McLaren (1975) referred to soil as a system of humus- and clay-immobilized enzymes with the persistence and stability of these enzymes in soils being

generally attributed to their association with both clays and humus. The enzymes may become electrostatically bound to clays via cation exchange reactions (Boyd, Mortland 1990). Other forms of binding include van der Wals interactions, hydrogen bonding, and ion-dipole interactions with metal exchange ions on clay surfaces (Mortland 1970). The clay adsorbed enzymes can retain their catalytic activities. The adsorption does not necessarily result in denaturation through conformation changes. The resistance of clay-adsorbed enzymes to enzymatic hydrolysis were also studied. The biological stability of enzymes may be affected by adsorption onto clay. The microbial degradation of acid and alkaline phosphatases and urease adsorbed on homoionic clays was greatest with Ca-clays, intermediate with Na-clays, and lowest with Al-clays (Chhonkar, Tarafdar 1985). The most protective effects provided by clay to enzymes are most prominent when they are adsorbed into the interlamellar regions of swelling clays.

The activities of clay-adsorbed enzymes are often lower than those of free enzymes in solution. Mortland and Gieseking (1952) show that the presence of clay reduced the hydrolytic activity of phosphatases. This reduction was in relation to the total surface area of clay mineral and it decreased in the order smectite – illite and kaolinite. The general observation is that swelling clays with high surface areas produce higher effects than nonswelling clays with low surface areas (Boyd, Mortland 1990). There are exceptions, for example Makboul and Ottow (1979), reported increased activity of alkaline phosphatase in the presence of Ca-smectite. Detailed information on enzyme interaction with clay has been published by Boyd and Mortland (1990).

Soil enzymes may also be associated also other soil constituents (clay- organic matter complexes and soil humus). The possible mechanisms of the formation of humus – enzyme complexes may include their physical entrapment in humus or clay – humus particles, H – bonding, ionic bonding and covalent attachment of the enzyme to soil humic substance (Burns 1978). Boyd and Mortland (1990) presented the hypothesis that enzymes may be associated with organic matter through nonpolar interactions.

Studies show that the enzymatic activity is dispersed throughout a range of molecular weight fractions and that most persistent enzymes are likely to be associated with high molecular weight polycondensates (Nannipieri et al. 1996). The association of the enzyme with the humic moiety probably stabilizes the tertiary structure of the protein thus improving the resistance of the enzyme to thermal denaturation. Bonding and entrapment of these enzymes in humic complexes may protect the enzyme protein but may render it inaccessible and inactive toward high molecular weight substrates (Ladd 1985). Burns (1982) suggested that enzymes immobilized on organic colloids or occluded by humic molecules play an important role in soil microbial ecology. Even if the enzyme survives long enough to detect the exogenous polymer and the microenvironment surrounding the substrate is suitable for catalysis, the product may not be taken up by the enzyme-producing microorganisms due to competitive factors such as adsorption by soil particles, nonbiological degradation and uptake by opportunistic microorganisms (Burns 1982).

Soil physical properties are important for biological activity, for example porosity. Marinari et al. (2000) described positive correlations between soil soil porosity, enzymatic activity and CO_2 production in organic and mineral treatment.

Organic treatment stimulated soil biological activity probably due to an enrichment of soil organic matter, mineral fertilizer enhanced soil porosity by increasing regular and irregular pores and caused a priming effect of native soil organic matter.

4.2.2 Soil tillage, fertilization, system of agriculture as compared to soil phosphatase activity

Phosphatase activity can significantly increase after the application of organic manures (Guan 1989). Microbial biomass and enzyme activities could increase after the addition of energy sources. This increase took place, for example, after the addition of glucose or ryegrass with NO_3^- to clay loamy soil (Nannipieri et al. 1983). Increases in phosphatase and urease activities coincided mainly with increases in bacterial biomass and with the rapid immobilization of labeled N. The effect of long term cattle manure application on soil phosphorus levels and phosphatase activities was studied by Parham et al. (2002). Microbial biomass C and alkaline phosphatase activity were significantly higher in the soil treated with cattle manure and acid phosphatase activity, however, was significantly higher in soils treated with chemical fertilizers. Results from this study suggested that manure-P is relatively more mobile than inorganic fertilizer-P. Soil organic matter content and soil microbial activities, vital for the nutrient turnover and long term productivity of soil, are enhanced by the balanced application of nutrient and manure (Kanchikerimath, Singh 2001). The results of Kandeler and Eder (1993) from experimental grassland fields showed that spurty treatment influenced enzyme production by microbial biomass. The application of municipal waste compost stimulated the phosphatase producing microorganisms (Perucci, Giusquiani 1991). Pig slurry can stimulate phosphatase and urease activity (Grejtovský 1991). Some research investigated the quality of manures on the enzymatic activity of soils. Neweigy et al. (1987) studied the effect of „biomass“ manure and two traditional organic manures. Digested sewage sludge treatment produced the highest activity in sandy soil. The phosphatase in sandy soil showed lower activity than that shown in alluvial soil in all treatments. Significant increases of phosphodiesterase activity were produced by the high rate application of aerobic sludge and the mid- and high-rates of anaerobic sludge (Albiach et al. 2001). The activity of phosphatase in soil first increased, then decreased with an increasing rate of sewage sludge application (Tan et al. 2002). Therefore, phosphatase might be used to reflect the effects of sewage sludge application on soil environment and quality.

Phosphatase activity is also influenced by mineral fertilizers. Katai et al. (1986) found that a large herbicide of fertilizer application before germination reduced phosphatase activity. A medium dose of fertilizer stimulated phosphatase activity. According to Haynes and Swift (1988) additions of lime generally increased protease and sulfatase activity, but decreased phosphatase activity. Additions of phosphatase decreased the activities of all three enzymes. The influences of fertilization and crop rotation on enzymatic activity in four different field trials were studied by Kandeler (1988). For alkaline phosphatase, P fertilization

increased Km of a grassland soil. The Km values of soil samples receiving different fertilization (mineral or organic) also increased.

Kirchner et al. (1993) described the effect of reduced N fertilization and green manuring on microbial biomass and enzyme activity of four treatments (continuous maize, no-till, conventional + N, conventional + green manuring). The results show that acid phosphatase was greater in the fertilized than the unfertilized soil, and that alkaline phosphatases under green manuring compared to N-fertilized conventionally tilled soil.

4.2.3 Management of agroecosystem and soil phosphatase activity

It has been demonstrated after many investigations that the enzymatic activity of soil is significantly influenced by tillage, crop rotation and fertilization. Tillage causes soil disturbance, altering the vertical distribution of soil organic matter and plant nutrient supplies in the soil surface, and it may affect both enzyme activity and microbial biomass which are responsible for the transformation and cycling of organic matter and plant nutrients (Curci et al. 1997). Microbial biomass and soil enzyme activities in particle-size fractions were researched by Kandeler et al. (1999a). These were affected mostly by the type of tillage and to a lesser extent by the date of soil sampling. Grocholl and Ahrens (1990) studied the effect of four long term tillage regimes on the microbial activities of soils. Ploughing led to less biological activity in both soils under investigation than non – inversion tillage treatments. A similar investigation published in the same year by Haynes and Knight investigated enzyme activity an other soil properties in conventional tillage and no-tillage treatments. Phosphatase activity was higher under no-tillage to a depth of 0.1 – 0.2 m. The higher accumulation of C and N was in this system in surface to the 0.05 m depth. A zero-till continuous wheat system has increased total organic matter, microbial biomass N, C and N mineralization and phosphatase activity in the top 0.075 m of the soil in the research done by Campbell et al. (1989). The effect of long term no-tillage practices on the activity of enzymes in soil was studied by Dick (1984). The results indicate higher activities of acid phosphatase and alkaline phosphatase in 0.075 m profile where no-tillage was compared to conventional tillage practices. The results observed for the effect of tillage and rotation on soil enzyme activities were strongly correlated with organic C concentrations. Similar results concerning no-tillage treatment were published by Angers et al. (1993). They measured alkaline phosphatase and other soil properties after 4 years of different combinations of crop rotation and soil tillage. Alkaline phosphatase in the experiment was greater under no-tillage and chisel ploughing than under mouldboar ploughing and 15% greater in the rotation than in the continuous barely. The management induced differences were greater in the top layer (0 – 0.075 m) than in the lower layer of the Ap horizon (0.075 – 0.15 m). The differences between activities in different soil depths are also described in other articles, e.g. Samuel et al. (2000).

In the general context of the search of quality index for soil as an indicator of sustainable management, Aon et al. (2001) analysed a soil (incl. phosphatase activity) under no-till or conventional tillage at different times in a season. The fact that several of the variables measured were strongly linked despite season and

crop presence points to the existence of a core highly interrelated process in the soil.

The effect of temporary grassland and conventional tillage on aggregate stability and soil microbial processes was studied by Kandeler and Murer (1993). Temporary grassland increases microbial biomass and secondly, microorganisms produce enzymes which mineralize organic compounds. After ploughing grassland, soil microbial processes decreased rapidly. The effect of cultivation on soil organic matter quality and the mechanism of soil organic matter stabilization has been described by Schulte et al. (1995). Cultivation induced significant changes in the quality, chemical composition and molecular size of soil organic matter. Increases in molecular size and bonding complexity in the cultivated samples were accompanied by decreases in activities of enzymes involved in C, N and P cycle. Decreases in dehydrogenase, urease and acid phosphatase activity ranged from 60 to 80%. Haynes (1999) described changes in soil properties under grass and arable management with the result that acid phosphatase activity (and other soil properties) increase caused 5 years of continuous pasture (in comparison with arable land with barley) was 100 – 180 %, which was considerably greater than that for organic C (i.e. 60 %). After Masciandaro and Ceccanti (1999), going from native to set-aside to intensively cultivated soils, the sodium-pyrophosphate extracted humic substances and the absolute enzymatic activity decreased (phosphatase activity was also measured), while the specific enzyme activities (activity per unit of extracted carbon) showed similar values.

There have been fewer investigations concerning ecological agriculture and the phosphatase activity of soils. According to Engels et al. (1993) who studied an agroecosystem cultivated according to IFOAM guidelines, exo-enzymes are useful in the monitoring of specific nutrient cycles. The soil phosphorus dynamic in biodynamic, bio-organic and conventional plots was investigated by Oberson et al. (1993) and Mäder et al. (2002). The results show that the soil microbial biomass and the activity of acid phosphatase were higher in both biologically managed systems. These results were attributed to the higher quantity of organic C and organic P applied to these systems, but also to the absence of, or severe reduction in, chemical plant protection. The higher activity of acid phosphatase in biologically cultivated plots indicate an increased potential to mineralize organically bound P in these soils (Oberson et al. 1995). In ecologically farmed experimental plots (Beyer et al. 1992) there was a strong correlation between the microbial biomass and dehydrogenase and the microbial biomass and alkaline phosphatase activity.

Phosphatase activity can also be influenced by soil erosion. Arutyunyan and Simonyan (1975) have shown that erosion results in decreased soil phosphatase and the loss of the enzyme in the soil surface layer.

4.2.4 Effect of plants, rhizosphere effect, mycorrhiza and animals on soil phosphatase activity

There is no doubt that the presence of plants has an effect on enzyme activities, including phosphatase (Gavrilova et al. 1973, Kiss et al. 1974, McLachlan 1980, Juma, Tabatabai 1988, Tadano et al. 1993). The effect of plants can be direct when

the roots secrete acid phosphatase and indirect when related to changes in soil organic matter content and microbial populations (rhizosphere effect).

A greenhouse study by Raddy et al. (1987) proved that enzyme activity was higher in rhizosphere soils than in before planting and non – rhizosphere soils. According to Hedley et al. (1983) in unamended soil, rhizosphere phosphatase activity increased with an increase in P deficiency caused by increased root density and decreased soluble inorganic P levels. Plant P uptake and yield will correlate with the phosphatase activity in the rhizosphere (Tarafdar, Rao 1990).

Most plant families are able to form mycorrhiza and the arbuscular mycorrhiza association is the most common mycorrhiza type involved in agricultural systems. Given the effects of arbuscular mycorhizal fungi innoculation on plant growth and health, it is generally accepted that appropriate management of this symbiosis should permit reduction of agrochemical inputs, and thus provide for sustainable and low-input plant productivity (Gianinazzi et al. 2002).
The positive effect for using the innoculum of mycorrhiza can increase:
- plant nutrient, eg. P,
- tolerance of root pathogens by the plant system,
- tolerance to water stress and adverse environmental condition,
- efficacy of N-fixation by *Rhizobium*,
- plant biodiversity in restored ecosystems,
- stability of soil (Vosátka, Dodd 2002).

Arbuscular mycorrhiza can enhance the plant uptake of inorganic phosphorus from soil through hyphal scavenging of soil volumes that are not accessed by roots. Plants also obtain considerable amounts of P from pools of organic phosphorus in soil following mineralization (Dalal 1977). A central role in this process is played by phosphatases. The role of arbuscular mycorrhiza fungal phosphatases in the mineralization of organic phosphorus in soil, and thus plant nutrition, is unclear (Joner et al. 2000). These authors described intracellular and extracellular arbuscular mycorrhiza phosphatases and the effect of phosphatases on P mineralization. In 1994 Tarafdar and Marschner first measured phosphatase in root free soil using compartment pots. They published a significant increase in both acid and alkaline phosphatase in hyphal compartments. Similar research was done by Joner and Jakobsen (1995). Calculations of the hyphal contribution to total phosphatase activity in soil are in the range of 2 – 4 % before any adverse effects on activity has been taken into account (Joner, Johansen 2000). The quantitative importance of extracellular phosphatase of arbuscular mycorrhiza (AM) hyphae for the P nutrition of AM plants thus seems to be low. The discussion of mycorrhizal phosphatases in the paper by Joner et al. (2000) supports the view that the extracellular phosphatases of roots and micro-organisms are to a large extent released incidentally into soil and that the source has limited benefit from its activity.

Phosphatase activity can be affected by earthworms and other soil animals. In research provided by Satchel and Martin (1984) phosphatase activity was greater in the presence of earthworms than in a control substrate without earthworms. The presence of earthworms also resulted in increased enzyme activities in some

sampling done by Ross and Cairus (1982). Weiss and Trespendorfer (1993) have described a similar influence of earthworms on the enzyme activity of the surrounding soil. Earthworms occurred in greater numbers in the outdoor experiment compared with vessel investigation and showed a short term increase in phosphatase, dehydrogenase, protease and nitrogenase activity. The contribution of the earthworm *Pontoscolex corethrurus* to soil properties was studied by Mba (1999) with the results that earthworms enhance soil acid phosphatase, and dehydrogenase activities, increased soil total and macro-porosity, soil saturated hydraulic conductivity, and CEC. Significantly increase phosphatase activity by earthworms was described by Cepeda et al. (1998). Zhang et al. (2000) studied changes in organic matter fractions after passage through earthworm guts and they also measured enzyme activities. Phosphatase activities were significantly higher in the gut of the anecic species as opposed to the epigeic species. They concluded that microorganisms are used by earthworms as a secondary food resource, and that passage through the earthworm gut decreases the total soil MB and increases the active components of MB.

4.2.5 Inhibitors of phosphatase activity

Various pesticides have been evaluated to determine their effect on soil phosphatase activity. Some of the pesticides increased activity, some had no effect, but many, in fact most reduced the activity of phosphatases. For example Tarafdar (1986) applied fluchloralin, methabenzthiazuron, metoxuron, 2,4 – D and isoproturon to a sandy loam soil at the usual rates. After application the activity of phosphatase was depressed by 60 to 74%. By harvest time (110 days after application) activities had almost recovered. Perucci et al. (1988) described how alachlor, metolachlor and atrazine increased in the first stage activities of acid and alkaline phosphatases. Then the activity decreased to the level of the control after about 60 days. Greaves et al. (1981) published that dalapon inhibited phosphatase activity for 2 weeks but that the activity recovered afterward. Davies and Greaves (1981) found that glyphosate, paraquat, trifluralin and atrazine did not change the phosphatase activity. On the other hand in 1974 Voets et al. asserted that atrazine had significantly reduced phosphatase and other enzyme activities. Burrows and Edwards (2000) published results from integrated terrestrial microcosm where effects of pesticide on representative soil organisms and ecosystem processes were studied. From these processes attention was paid also to phosphatase activity which was one from most affected endpoints, usually at the higher doses of carbendazim. Singh et al. (2001) described a significant reduction in soil microbial biomass and enzyme activities (incl. phosphatase) after the use of the pesticide chlorothalonil. A general inhibitory effect of glyphosate for phosphatase (5-98 %) was in the evaluation of Sannino and Gianfreda (2001).

In some cases the results of the effects of agrochemicals on enzyme activities in soils have differed depending on author and type of research. Detailed information is provided by Schäffer (1993) who summarized world research into pesticides and soil enzyme – phosphatase activity.

In literature, for example Pozo et al. (1994), this problem has been mentioned. In their investigation phosphatase activity decreased significantly, initially at

concentration of 5 – 10 kg ha^{-1} of alachlor, but recovered to levels similar to the control. In all studied herbicides (atrazine, alachlor and metolachlor) Perucci and Scarponi (1990) described increases in the soil alkaline phosphomonoesterase whereas phosphodiesterase activity was stimulated by acetanilide herbicides and inhibited by atrazine. Tu (1992) observed no inhibitory effect of pesticides (haloxyfop, tridiphane, pyroxyfur) on the activity of phosphatase.

It has been found that several cations inhibit soil phosphatase activity. Tyler (1976) has shown that Cu and Zn ions have a marked inhibitory effect on phosphatase activity. This activity decreased most markedly at the highest Zn rate in the investigation of Kucharski and Niklewska (1992). Sjöqvist (1995) showed after investigation in a highly polluted area that acid phosphatase activity and dehydrogenase activity are sensitive indicators of Cu and Zn pollution. Detailed results were obtained by Shindo and Huang (2001) who evaluated the influence of Cu, Zn and Cd on the activity and kinetics of acid phosphatase immobilized by two soil clays. The ability of Cu to inhibit the enzyme activity was higher that of Zn, the ability of Cd was negligible. Johansson et al. (1999) showed with use of principal component analysis and discriminant function analysis that the sewage sludge affected several of the biological and chemical soil parameters investigated. Tyler (1976) also studied the effect of vanadium on soil phosphatase. The vanadium when added to spruce needle inhibited this activity. The effect of lead pollution on soil enzyme activities (Marzadori et al. 1996) was found in the experiment only at the highest Pb concentration. Stott et al. (1985) studied the effect of 21 trace elements on the activity of phosphatase in soils. Results showed that 19 of these elements inhibited pyrophosphatase activity. The short- and long-term effects of heavy metal pollution on enzymatic activity has been studied by Doelman and Haanstra (1989). They present the results graphically in logistic dose – response curves, and the data could be very useful in limiting soil pollution by heavy metals.

Some investigations fail to confirm a decrease in this activity. Bargett et al. (1994) observed the effects of soil contamination by Cr, Cu and As which did not affect basal respiration, phosphatase urease and invertase activities, but did decrease other soil biological parameters. The effect of heavy metals from composted municipal waste on enzyme activities was studied by Giusguiani et al. (1994). The results show that heavy metals did not affect soil enzyme activities up to additional rates of 3 x the limits specified by Italian law. In some investigations it is difficult to say that heavy metals decrease phosphatase activity. The presence of available soil heavy metals due to the addition of the heavy metal rich organic materials did not negatively affect dehydrogenase, beta glucosidase and urease activity, but there were negative correlations between heavy metals and phosphatase activity in the beginning of the experiment (Madejon et al. 2001). But this negative correlation was probably due to high levels of available P which were also found.

The effect of miscellaneous compounds added to the soil on phosphatase activity was studied by Ambrož (1973). The results show that penicilin and streptomycin inhibit it markedly, while benzene, xylene, chloroform, hydrogen peroxide and ether had no effect at all. The same author (1973) states that the effluent from a starch production plant decreased soil phosphatase activity.

Large areas in the world are also influenced by salinization. The detrimental effects of salts on microbial activity may be due to the toxicity of specific ions, the elevation of osmotic pressure or the increase in alkalinity which may restrict the availability of water. The increase in soil salinity had a negative effect on a soil´s microbiological activity. In the research of Okur et al. (2002) in the increasing salinity of the irrigation water there was also a reduction in soil respiration and phosphatase activity.

5 OBJECTIVES, GENERAL DISCUSSION AND CONCLUSIONS

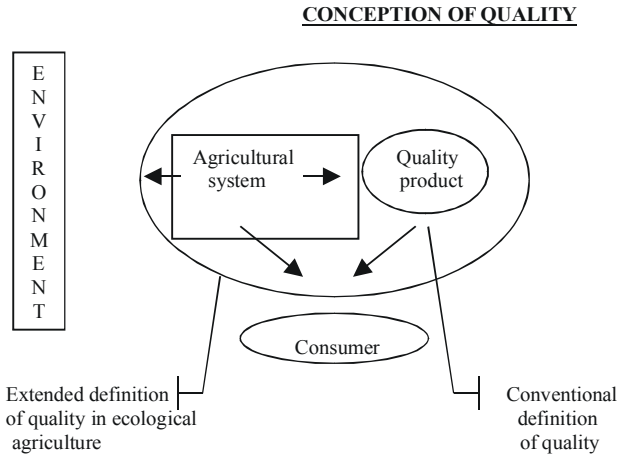
Contemporary production farming methods have a negative impact both on live organisms and their environment. Industrial agriculture is dependent on external inputs, and ecological as well as biological imbalances are mainly managed by using synthetic pesticides and chemical fertilizers. It is now quite obvious that this system cannot meet the requirements of sustainable development. The research described in this thesis covers only a small part of the difficulties associated with agroecosystems. In the future, science and research should focus on developing new methods and production processes throughout society to satisfy such demands. It is necessary to reduce the depletion of raw material resources and minimize the change-over of this valuable wealth to dispersed pollutants. A major effort has to be directed at reducing environmental contamination by alien substances. All human activities in the landscape should respect both its capacity and natural potential in the context of sustainable development.

To tackle the above problems will require new priorities in agricultural research focusing on:
- multidisciplinary and interinstitutional approaches,
- definition of quality parameters and indicators of whole systems and their effects on the environment, product quality, consumers and efficiency,
- developing new farming systems that would have a positive impact on consumers and meet all the requirements of sustainable development.

Future farming systems should head toward a better buffering capacity, lower vulnerability and higher tolerance to stress and variation of external factors, maximum biological diversity, capacity for maximum exploitation of biological processes, maximum recirculation ability within closed systems, etc.

This thesis concentrates only on one part of the soil system connected with phosphorus supply. Described phosphatases could also be used in the evaluation of arable soil quality. This quality encompasses not only the capacity of soil for crop productivity, but also food safety and animal and human health and is, therefore, a concept that has proven difficult to define and quantify (Torstensson et al. 1998). Qestions of quality are a crucial aspect of ecological farming closely related to the quality of the system itself.

Much attention is being paid to impacts on the environment, properties of products as well as sociopsychological and ethical aspects (Dlouhý, Šarapatka 2003).



The research work published in this thesis focuses on four main areas:

- interactions among soil phosphatases activity and specific physical and chemical and biological soil properties (paper 1 and 2),
- the effect of different systems (i.e. organic farming and conventional agriculture on the activity of soil phosphatases with the aim of optimizing farming systems (paper 2),
- the evaluation of the activity of acid phosphatase in the root systems of both the chosen cereal species and varieties planted in a nutrient medium under conditions of changing pH and phosphorus supply which can reflect changing soil conditions (paper 3),
- investigation of the number of phosphatase active colonies and discovery of correlations between these and chemical and biological soil properties (paper 4).

The first paper (*Interactions between phosphatase activity and soil characteristics at some locations in the Czech Republic*) describes a major role of soil phosphatases in the mineralization processes of organic phosphorus substrates. Their activity can be influenced by numerous factors and soil properties play a key role among them. Our research adds to the growing knowledge on soil phosphatases in their interactions with specific soil characteristics at nine sites in the Czech Republic with common soil types.

The results show correlations and linear equations between phosphatase activity and soil characteristics. The results clearly state that in the soil samples taken from the locations in the Czech Republic positive correlations exist between acid and alkaline phosphatase and organic carbon content. This finding is in agreement with the results of other authors, e.g. Nannipieri et al. (1973), Gehlen and Schroder (1990), Nahas et al. (1994), Kandeler et al. (1999) and others. Enzyme activities decrease after decreasing total organic carbon (Beyer et al. 1999) as can

be seen in different intensively cultivated agricultural soils. Organic carbon content was found to correlate with total nitrogen content. There was a positive correlation between this element and phosphatase activity, which corresponds with the data reported by Speir (1978), Bonmati et al. (1991), and others. Aon and Colaneri (2001) described strong relationships between organic carbon and total nitrogen with enzymatic activities including acid and alkaline phosphatases.

The humus quality was expressed by the humic : fulvic acid ratio. A negative correlation was determined between the above ratio and the activity of acid and alkaline phosphatases. The increasing humic : fulvic acid ratio brought about a decrease in the activity of the enzymes which may be due to the fact that those substances with a lower molecular weight decompose more easily. Increases in molecular size and bonding complexity in the cultivated soil sample were, in the evaluation of Schulten et al. (1995), accompanied by decreases in the activities of the enzymes involved in C, N and P cycles. Decreases in dehydrogenase, urease and acid phosphatase activity ranged from 60 to 83 %.

Also the addition of a carbon source to the soil could affect soil properties. Falih and Wainwirght (1996) found that the addition of sugarbeet to soils as a source of C led to an increase in the availability of easily utilizable C, which in turn markedly increased the number of soil bacteria and soil activity including phosphatase activity. Also the long term application of cattle manure promoted microbiological activities and P cycling and can have positive effect on soil phosphatase activity (Parham et al. 2002). Positive correlations can also be found between phosphatase activity and biomass C determined, for example, by the fumigation – extraction method (Tarafdar 1998).

The negative correlation was also found between the available phosphorus content and both acid and alkaline phosphatase activity. This result agrees with the results published by Haynes and Swift (1988). Our study reveals a positive correlation between acid phosphatase activity and total inorganic phosphorus content, but no correlation with organic phosphorus was found. This has been described previously in the literature, for example, by Chhonkar and Tarafdar (1984). Similar to other experiments (cf. Gehlen, Schroder 1990), our trials have also confirmed a negative correlation between acid phosphatase activity and pH. At our locations, phosphatase activity of soil decreases with increasing clay content. This is related to the fact that soil enzymes are associated with clays. The activities of clay adsorbed enzymes are often lower than those of free enzymes, as previously described by Boyd and Mortland (1990).

In the second paper (*Phosphatase activity of eutric cambisols (Uppland, Sweden) in relation to soil properties and farming systems*) the aim of the study was to assess the activity of soil phosphatases in agricultural soils in relation to some physical, chemical and biological properties. The trials were conducted at experimental locations and farms in Uppland (Sweden) with eutric cambisol soil type.

The results demonstrate the correlation between acid and/or alkaline phosphatase and several soil characteristics (clay content, C_{org}, N_{tot}, pH, humic:fulvic acids ratio, P_{avail}, basal soil respiration). It is in agreement with our data from the first paper and also with data from the papers of other scientists. Concerning organic matter content, in the results published by Bergstrom et al.

(2000), phosphatase and arylsulfatase activity in the Ap horizon behaved as indices of soil organic matter content along the topographic gradient.

At the experimental plots the effect of different farming systems were also studied: (conventional farming without animal husbandry /A/, organic farming with animal husbandry /B/, organic without animal husbandry with standard soil cultivation /C/, and organic agricultural system without animal husbandry with minimum soil management /D/).

Organic farming system B was the only one to comprise animal husbandry where the farmyard manure was applied (25 t/hectare). Another crop was clover grass which leaves large amount of post harvest residue. The organic farming system with minimum soil cultivation (B) was similar to that with animal husbandry. On the one hand, the crop rotation involved biennial grass, and on the other hand no farmyard manure was applied. Moreover, the organic matter supply was supplemented with cut straw scattered over the soil surface. These farming systems show a higher activity of phosphatases than systems A and C.

Conventional farming system (A) and organic farming system with standard soil management (C) were characterized by similar organic matter supply as well as comparable soil phosphatase activities. Conventional system A did not comprise any animal husbandry and organic matter supplies was guaranteed by straw incorporation. Organic farming system C was given organic matter through straw and green manure incorporation.

The above results are in close agreement with the fact that enzyme activities can be increased after the addition of energy sources (Nannipieri et al. 1983). The results of the effects of organic farming systems on higher enzyme activity are in agreement with data published (e.g. Oberson et al. 1993; Mäder et al. 1993). Similar results are from our investigations done at experimental plots at the Agricultural Research Institute of Kroměříž where comparisons were carried out on different farming systems (conventional, Norfolk, organic) including monocultures. It was surprising to find the highest acid phosphatase activity in barley monocultures following straw incorporation and green manuring, which was one of the inter-crops in the crop rotation each year. In recent years, large amounts of organic matter had been incorporated into the soils under study which could have influenced their microbial activity, and consequently their final enzyme activity. In this research variant there was a strong prevalence for acid phosphatase activity due to the annual application of ammonium sulphate whose action is physiologically acid having decreased the pH of the plots by ca. 1 (from an average 6.6 to 5.6). The higher enzyme activity found in monocultures in comparison to that found in crop rotations was also reported by Anwarzay et al. (1990) where the increase was related to a larger organic matter supply. In our research, higher values of phosphatase activity were also recorded in conventional crop rotation when biennial alfalfa was followed by wheat. Alfalfa could have influenced the biological activity of the soil by its rhizosphere effect and large amounts of post-harvest residues. An increase in phosphatase activity was also found in barley grown after sugar-beet where the amount of post-harvest residue was not as ample as with alfalfa, but in which high rates of farmyard manure had been applied (i.e. 40 t per hectare).

The enzyme activity could also be higher in the systems with minimum or no-tillage because of higher amounts of organic matter in the topsoil layer (e.g.

Angers et al. 1993). In minimum tillage and reduced tillage the phosphatase activity was higher compared with other tillage systems in other research projects as well, e.g. Piovanelli et al. (1998), and these systems can induce positive effects on soil biochemical and chemical properties (Canarutto et al. 1995). Increasing the carbon, total nitrogen and phosphorus content could serve as a basis for increasing both biological and enzymatic soil activities.

In conclusion, the results indicate that enzyme activities are directly dependent on the content of organic substances in the soil (C, N forms) which may be influenced by farming activities (e.g., integrated and ecological agriculture). Our research provides results which can help to optimize inputs to the agroecosystem with respect to the protection of the environment.

Phosphate from organic phosphorus substrates, to be available to plants, must be hydrolysed by phosphatases. Enzymes in soils originate from animal, plant and microbial sources and the resulting soil biological activity includes the metabolic processes of all these organisms. The literature shows that under favourable conditions microorganisms supply most of the enzyme activity. The effect of plants on soil enzymatic activity is due to changes in organic matter content and microbial populations, but is also formed by accumulated enzymes and by continuously released extracellular and endocellular enzymes; all of which originate in the plant roots. In our research, published in paper three (*Effect of pH and phosphate supply on acid phosphatase activity in cereal roots*), we concentrated on acid phosphatase activity linked to the previous source, by which we mean cultivated plants. Our evaluation was carried out on the root systems of both the chosen species and cereal varieties and also in nutrient medium on which crops were planted under conditions of changing pH and phosphorus supply.

Different varieties of winter wheat, spelt, barley and rye were used and after sterilization the seeds were sown on Murashige – Skoog nutrient medium with pH 5.6, 6.2 and 6.8 and a phosphorus supply between 30 – 160 mg P_2O_5/l of medium. After 10 days of cultivation the plant roots were harvested, homogenized and the acid phosphatase activity was measured.

The results show that the acid phosphatase activity in the root system of various species and cereal cultivars is negatively correlated with increasing pH and available phosphorus level in the nutrient medium.

The results of our research correspond with the results of Beissner and Römer (1999) who found the effect of P deficiency on phosphatase activity in sugarbeet roots from the acid to the neutral pH range. McLachlan (1980) stated that P deficient plants had greater activities than those with sufficient levels. Similar conclusions were found by Gilbert et al. (1999) for white lupin roots and Richardson et al. (2000) for wheat. Richardson et al. (2000) also described a limited ability to obtain P from inositol hexaphosphate, whereas other monoester substrates, such as glucose 1- phosphate, were equivalent sources of P for plant growth when compared with inorganic phosphate. Gilbert et al. (1999) describe the development of proteoid roots when grown in phosphorus deficient conditions and these roots are adapted to increased P availability. White lupin roots from P deficient plants had significantly greater acid phosphatase activity in both the root extracts and the root exudates than comparable samples from P – sufficient plants.

The second factor in our research was pH of the medium. In some papers (e.g. McLachlan 1980; Beissner, Römer 1999) published results show optimum pH for acid phosphatase activity to be in an acid environment. McLachlan (1980) described that pH optima for the activity peaks of phosphorus-sufficient and phosphorus deficient plants were similar and phosphatase activity for all species was greater in pH 5 – 6. For our research we used the pH range typical for soils in the Czech Republic. The results then show higher acid phosphatase activity in pH 5.2 which is more frequent for example in Cambisols than in 6.2 or 6.8.

In the rhizosphere of a plant there may be a greater concentration of water-soluble organic C, organic phosphorus and microbial biomass, and also higher alkaline phosphatase and phosphodiesterase enzyme activities as described by Chen et al. (2002) from their research with radiata pine and perennial ryegrass.
Root surface and secreted phosphatases may be involved in the release of phosphate from exogenous organic phosphate compounds (Bieleski 1973) or be part of a salvage system by releasing phosphate from organic phosphates that have been leaked out of the root cells (Barrett – Lennard et al. 1993). In research of Asmar and Gissel – Nielsen (1997) the activity of the enzymes associated with the roots was 20 – 80 times higher than the activity of those released by the roots to the surrounding nutrient solution. This and our results also indicates that extracellular phosphomono- and phosphodiesterase is mostly attached to the roots and not released to the surroundings in larger amount. This is also in agreement with McLachlan´s results (1980) and extracellular phosphatase on plant roots may be associated with rhizodermal cell walls (Bieleski, Johnson 1972). The secretion of acid phosphatase is at the same time higher under P deficient conditions (Tadano et al. 1993) which was confirmed in our research in case of Triticum aestivum.

Our current and future research may help to indicate varieties with differing potential for obtaining phosphorus from soil reserves. The study of phosphatase activity in different species and varieties can be used in practice as an indicator of these plants´ potential to exploit different phosphorus conditions. Asmar and Gissel – Nielsen (1997) show that genotypes have significantly higher activity of extracellular phosphomonoesterase than that of phosphodiesterase both associated with and released by their roots. Practical aspects from the evaluation of varieties are useful, for example, in the breeding of varieties for organic or low-input farming. In plants, reduced phosphatase activity could indicate the potential of varieties for greater efficiency in obtaining phosphorus. From our research, in keeping with the results of other scientists, we found different reactions of plant species to different supplies of phosphorus. In future research, for the practical purposes of plant breeding, we would like to describe the reaction of particular cereal varieties to different pH and especially in relation to phosphorus.

Acid phosphatase and alkaline phosphatase active colonies of bacteria, isolated from soils, were stained and published in paper four (*Relationship between phosphatase active bacteria and phosphatase activities in forest soils*). The activity of acid phosphatase and other soil properties (the number of aerobic bacteria, basal respiration, the level of ammonification, the number of bacteria active in ammonification, the level of nitrification, the number of micromycetes) were compared with the number of bacteria belonging to the genus *Micrococcus*.

Soil samples were taken from the flowing horizons: F-AO1 (fermentative), H-AO2 (humic), and A (basic). the soil samples were taken from beneath forest stands in the Izera Mountains (North Bohemia, Czech Republic).

The number of acid phosphatase active colonies correlated positively with the number of alkaline phosphatase active colonies in the F-AO1 horizon, and there was a high, positive correlation between the former and the level of ammonification in the H- AO2 horizon. The number of alkaline phosphatase active colonies correlated positively with organic carbon, the number of ammonification bacteria, and the number of micromycetes in the H-AO2 horizon. The A horizon was almost biologically inactive.

Our results didn't show any correlation between phosphatase active colonies of bacteria and acid and alkaline phosphatase activity in the investigated soils. This is probably due to the production of phosphatates by organisms other than bacteria, for example, soil protozoa (Hattori 1993) or other groups. Enzymes in soils do not only originate from microbial sources, but also from animals and plant roots. The presence of plants positively affects enzyme activities, including that of phosphatase (Juma, Tabatabai 1988, Tadano et al. 1993). Phosphatase activity can also be affected by earthworms and other soil animals (Satchel, Martin 1984, Weiss, Trespendorfer 1993), and Gerretsen (1948) argued almost half a century ago that micro-organisms in the soil may bring P into solution and thereby improve the growth and P nutrition of plants. Brown (1974) discussed the stimulatory effects of microorganisms on plant growth. According to Greaves and Wembley (1965), up to 90% of the macroflora in the rhizosphere are capable of producing phosphatases. The lack of significant correlation between the number of *Micrococcus* colonies with either acid or alkaline phosphatase activities shows that other factors affect the level of enzyme activity in forest soil. Bacterial counts in our search showed that only those micro-organisms which can be cultured, i.e., a low percentage of bacteria inhabiting soils, could have influenced the characteristics of soil in terms of enzyme activity. In forest soils there are other organisms, such as micromycetes, which can increase total soil enzyme activity.

Agroecosystems are more open systems when compared with unmodified ecosystems and have many problems including phosphorus nutrition. This is also a problem discussed at many scientific conferences and meetings of farmers oriented toward organic farming. Our research provides results which can help in agroecosystem optimization with respect to the minimization of inputs and protection of the environment. This trend in agricultural production is preferred both in EU countries and in Associated Central European States and is also supported by subsidies (Šarapatka, Dlouhý 1995). These systems are friendly to the environment and present a sustainable future. In the future research of soil enzyme activities, including phosphatases, we would like to concentrate on:

- to research relations with other soil properties, mainly with the quality of soil organic matter which is influenced by farming activities. According to data published recently (e.g. Šarapatka 1995) the soil carbon content has fallen by up to 30 % in some regions of the Czech Republic and changes have also been observed in humus quality. One example could be our research of changes in organic matter decomposition and humification, and soil biological activity after using biological preparates.

- to evaluate changes in soil biological and biochemical properties during the transition period to low-input and organic farming because of slow changes of some soil characteristics (e.g. research done in Oberwil). The results could help in the selection of soil indicators used in the evaluation of soil quality and soil degradation. Suitable characteristics will be proposed to the Central Institute for Supervising and Testing in Agriculture, Brno to enrich the monitored parameters of soils in the Czech Republic.
- to determine the activity of soil phosphatases to introduce or to optimize the method convenient for square soil monitoring in the Czech Republic (e.g. microplate method).
- to give detailed information about phosphatase activity of different sorts to plant breeders interested in the selection and breeding of varieties suitable for low-input and mainly for organic farming. Attention will also be concentrated on extracellular phosphatases and in co-operation with specialized institutions (e.g. Institute of Botany of the Czech Academy of Science) to the effect of arbuscular mycorrhiza to extracellular soil phosphatase activity. The arbuscular mycorrhiza association is the most common mycorrhiza type involved in agricultural systems and can also increase plant nutrients, eg. P.

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I

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INTERACTIONS BETWEEN PHOSPHATASE ACTIVITY AND SOIL CHARACTERISTICS AT SOME LOCATIONS IN THE CZECH REPUBLIC

INTERAKCE MEZI AKTIVITOU FOSFATÁZ A PŮDNÍMI CHARAKTERISTIKAMI VE VYBRANÝCH LOKALITÁCH ČR

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ABSTRACT: Soil phosphatases play a major role in the mineralization processes of organic phosphorus substrates. Their activity can be influenced by numerous factors and soil properties play a key role among them. This research adds to the growing knowledge on soil phosphatases in their interactions with specific soil characteristics of nine sites in the Czech Republic with common soil types. The results show correlations and linear equations between phosphatase activity and soil characteristics. Positive correlations were found between enzymatic activity and organic carbon, and with nitrogen; and between acid phosphatase activity and total phosphorus. Negative correlations were with the quality of humus (humic:fulvic acids ratio) and available phosphorus, and between acid phosphatase activity and clay content and pH.

soil; enzymes; phosphatase activity; soil characteristics

ABSTRAKT: Část půdního fosforu se nachází v organických vazbách. Při jeho zpřístupňování mají velký význam půdní fosfatázy, jejichž aktivita je ovlivňována řadou faktorů, včetně půdních podmínek. Práce se zabývá vztahy mezi fyzikálními a chemickými charakteristikami půd a aktivitou zkoumaných půdních enzymů. Sledování probíhalo v letech 1993 až 1995 na území devíti zemědělských podniků a výzkumných ploch s půdními typy běžnými v ČR. Ve vegetační sezóně byly v rámci každého podniku odebrány vzorky půdy průměrně z osmi ploch (pouze Štěpánov a Olomouc – dvě plochy) z horizontu 0 až 20 cm. Pro zkoumání aktivity půdních fosfatáz byla použita publikovaná metodika (Tabatabai, Bremner, 1969); fyzikální a chemické charakteristiky byly zjišťovány podle metodik běžně používaných v pedologických laboratořích. Výzkumem byly zjištěny korelace na hladinách významnosti 95, resp. 99 % mezi enzymatickou aktivitou a dalšími

proměnnými a následně pak závislosti, které jsme vyjádřili lineárními regresními rovnicemi. Z výsledků byly rovněž krokovou analýzou vypočteny vícenásobné lineární rovnice pro aktivitu kyselé a alkalické fosfatázy. Z těchto zjištění je zřejmá kladná závislost mezi aktivitou fosfatáz a obsahem organického uhlíku i celkového dusíku v půdě. Se zvyšujícím se množstvím organických látek vzrůstala i aktivita fosfatáz. U organické hmoty byla stanovována i kvalita humusových látek vyjádřená kvocientem Q4/6. U této veličiny jsme zaznamenali korelaci zápornou, stejně jako mezi enzymatickou aktivitou a obsahem jílnatých částic a mezi aktivitou kyselé fosfatázy a pH půdy. U fosforu nebyly zaznamenány závislosti mezi obsahem organického fosforu a aktivitou fosfatáz, byl však zjištěn kladný vztah mezi aktivitou kyselé fosfatázy a obsahem celkového fosforu a záporný vztah mezi aktivitou fosfatáz a obsahem přijatelného fosforu. Získané výsledky mohou být využity při optimalizaci osevních postupů s cílem posílit půdní úrodnost i biologické a biochemické procesy probíhající v půdním prostředí.

půda; enzymy; aktivita fosfatáz; půdní charakteristiky

INTRODUCTION

The phosphorus cycle in agroecosystems is influenced by man through the mining and processing of phosphates for fertilizing. Known reserves of rock phosphates are very limited and are only a part of the phosphorus found in soils (Stevenson, 1986). The activity of soil enzymes is important in ensuring the availability for plants. Such is the case of phosphorus, in which a portion of this element within the soil is bound organically and soil phosphatases play a major role in the mineralization processes of organic phosphorus substrates. The mineralization of such organic fractions is of great agricultural and economic importance.

Enzyme reactions, which take place in the cells of soil organisms, plant roots, and directly in the soil due to enzyme accumulation, form the basis of soil metabolism (Chaziev, 1972). Phosphatase activity can be influenced by numerous factors and soil properties play a key role among them. In some research phosphatase activity has correlated with other physical, chemical and biological soil properties. Relationships between phosphatase activity and organic carbon have been described by Gehlen and Schroder (1990), Bonmati et al. (1991) and Nahas et al. (1994). The correlation with total nitrogen has been described by Speir (1977), Bonmati et al. (1991), with pH by Chhonkar and Tarafdar (1984), Herbien and Neal (1990). A study of the literature provides information that soil phosphatase activity can also be related to organic and inorganic phosphorus content, clay content, and soil moisture.

The aim of our research was to determine the activity of acid and alkaline phosphatase in the agroecosystem and to increase our understanding of the interactions among soil phosphatases and some specific physical and chemical soil characteristics.

MATERIAL AND METHODS

The research was conducted at experimental locations and farms in the Czech Republic during the years 1993 to 1995. The experimental locations comprised some plots at Uhříněves near Prague belonging to the Czech Agricultural University (luvisols, 295 m above sea level) and the Agricultural Research Institute in Kroměříž (chernozems, 220 m above sea level). Parallel sampling was carried out on farms as follows:

Location (District)	Major soil group	Height above sea level (m)
Medlov (Olomouc)	luvisol	260
Staré Město pod Sn. (Šumperk)	cambisol	560
Pitín (Uherské Hradiště)	cambisol	390
Králíky (Ústí nad Orlicí)	cambisol	610
Rovečné (Žďár nad Sázavou)	cambisol	510
Štěpánov and Olomouc (Olomouc)	fluvisol	230

At the nine research sites soil samples were taken in the vegetation period from an average of eight fields at each site, except Štěpánov and Olomouc where two research fields were sampled. Soil samples were taken from the 0 to 20 cm horizon.

Most of the analyses were performed in the Department of Ecology laboratory, Palacký University, Olomouc. The main goal of the study was to determine soil phosphatase activity using the method according Tabatabai and Bremner (1969). During the analyses soil was incubated in a solution with p-nitrophenyl phosphate (PNP-P), and the p-nitrophenol formed was determined spectrophotometrically.

Granulometric analyses of the soils were carried out by means of the pipette method. Particle size, used for further trials, was below 0.01 mm and for clay was smaller than 0.002 mm. The soil samples were tested for exchangeable pH in soil extract 0.01 M $CaCl_2$ solution. Chemical analyses comprised the assessment of organic carbon, humus quality, total nitrogen, available phosphorus, total phosphorus, inorganic phosphorus and organic phosphorus. Most of the analyses were performed according to the method of Javorský et al. (1987) and Králová et al. (1990). Soil carbon was determined by its oxidation with $K_2Cr_2O_7$ and titration with diammonium iron(II) bissulfate hexahydrate. Humus quality, expressed as a humic acids:fulvic acids ratio, was determined by means of colour quotient Q4/6. Soil samples were extracted using an alkaline solution of sodium diphosphate, and the clear extract was measured spectrophotometrically at 465 nm and 665 nm, respectively. Soil nitrogen was determined after mineralization and distillation into boric acid. Available phosphorus was assessed following soil extraction with a calcium lactate solution. Phosphorus content was determined spectrophotometrically. The determination of total, mineral and organic phosphorus in soils was accomplished according to Bowman (1989), which is based on a high efficiency of organic phosphorus being extracted from the soil samples using the heat of the solution created by the addition of water to concentrated H_2SO_4.

Analyses of clay content, organic carbon, total nitrogen, pH and available phosphorus were performed on soil samples from all localities; total phosphorus, inorganic phosphorus and organic phosphorus in samples from Staré Město, Pitín, Medlov, Štěpánov, Olomouc; and the results of humic : fulvic acid ratio are from Staré Město, Pitín, Medlov, Kroměříž, Štěpánov and Olomouc.

The results obtained were subjected to statistical evaluation. Attention was paid to both correlations and regression models. The statistical evaluations were performed using StatGraphics, HarvardGraphics and SPSS software.

RESULTS

The results of our research show the correlations among acid and alkaline phosphatase activity and numerous soil characteristics. The values of enzymatic activity and soil properties are in Tab. I.

The correlations of enzymatic activity and soil properties are given in Tab. II along with the data calculated to acquire the interactions between different soil characteristics and phosphatase activity at the significance levels of 95 and 99 %, respectively.

The associated Figs. 1 to 9 are supplemented with linear equations obtained by means of a single linear regression to confirm the above interactions between the variables (Y=a+b.x).

The single regression analysis of all values was followed by data estimation using multiple regression analysis where the variations of dependent variables could be explained by using numerous independent ones. The interactions between acid and alkaline phosphatase activity, clay, available phosphorus, organic carbon, total nitrogen contents, and pH/$CaCl_2$ were investigated using the step-by-step analysis with the following results:

- regression equation for acid phosphatase activity:

$$AC.P. = 7447.82 - 36.47 \times clay - 6.34 \times P_{avail.} + 984 \times C_{org.} - 638.12 \times pH/CaCl_2$$

(R2 = 53.9 %)

- regression equation for alkaline phosphatase:

$$Al.P. = -83.26 + 6.70 \times P_{avail.} + 0.84 \times N_{tot.} + 426.19 \times pH/CaCl_2$$

(R2 = 22.3 %)

I. The values of enzymatic activity and soil characteristics

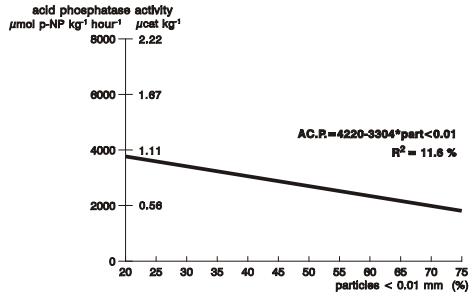
	Average	Median	Lower guartile	Upper guartile	Minimum	Maximum
Acid phophatase activity (µmol/kg/hour)	3 168.21	2 992.00	2 156.00	3 740.00	1 430.00	7 260.00
Alkaline phosphatase activity (µmol/kg/hour)	3 049.69	2 893.00	2 200.00	3 740.00	1 210.00	5 940.00
Particles <0.01mm (%)	36.79	36.00	25.60	41.46	20.50	74.60
Clay <0.002mm (%)	19.78	16.83	9.40	30.29	6.00	53.40
C_{org} (%)	1.46	1.47	1.05	1.71	0.59	2.68
N_{tot} (mg/kg)	1 764.19	1 788.50	1 296	2 056	957.00	3 407.00
Humic: fulvic acids (ratio)	0.55	0.41	0.33	0.76	0.24	0.92
P_{avail} (mg P_2O_5/kg)	126.88	117.00	99.50	148.50	50.00	263.00
P_{tot} (mg/kg)	922.80	710.00	625.00	1 250.00	520.00	1 775.00
P_{inorg} (mg/kg)	812.00	590.00	530.00	1 120.00	440.00	1 530.00
pH/$CaCl_2$	6.43	6.60	5.96	6.79	4.76	7.40

II. Correlations among phosphatase activity and selected soil properties

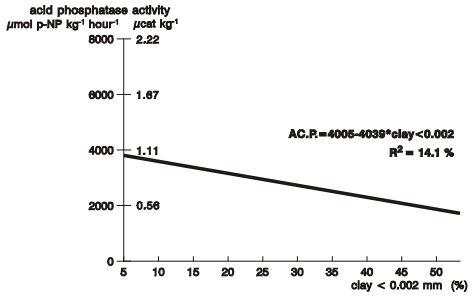
	ACID PHOSPHATASE ACTIVITY	ALKALINE PHOSPHATASE ACTIVITY
ACID PHOSPHATASE ACTIVITY		**
ALKALINE PHOSPHATASE ACTIVITY	**	
PARTICLES < 0.01 mm	**	-
CLAY < 0.002 mm	**	-
C_{org}	**	**
N_{tot}	**	**
HUMIC ACIDS : FLUVIC ACIDS	**	**
P_{avail}	*	*
P_{tot}	**	-
P_{inorg}	**	-
P_{org}	-	-
pH	**	-

** existing correlations (99 %), * existing correlations (95 %), - no correlations

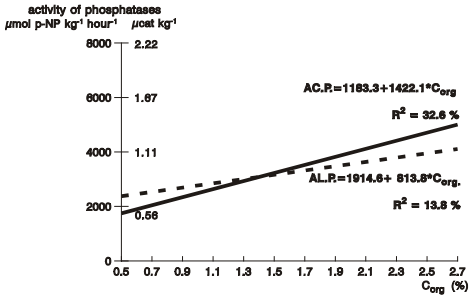
1. Relation of acid phosphatase activity to particles < 0.01 mml



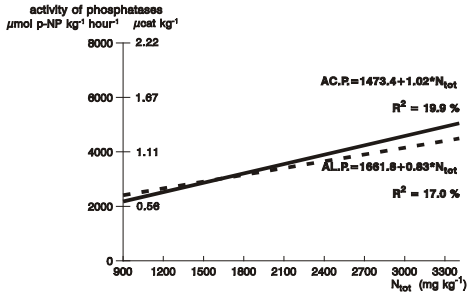
2. Relation of acid phosphatase activity to clay < 0.002 mm



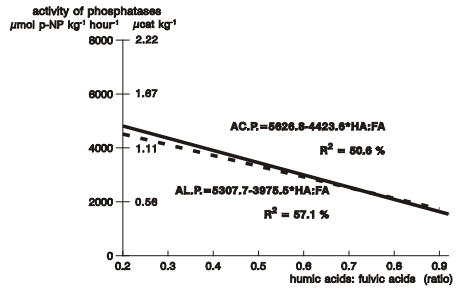
3. Relation of phosphatases activity to C_{org}



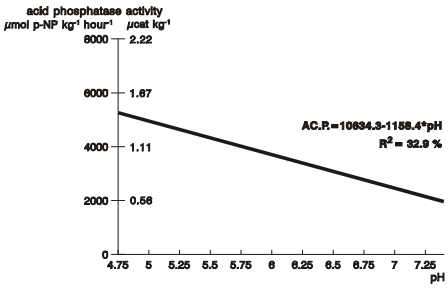
4. Relation of phosphatases activity to N_{tot}



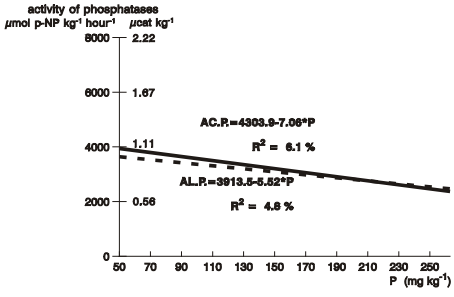
5. Relation of phosphatases activity to humic: fulvic acids ratio



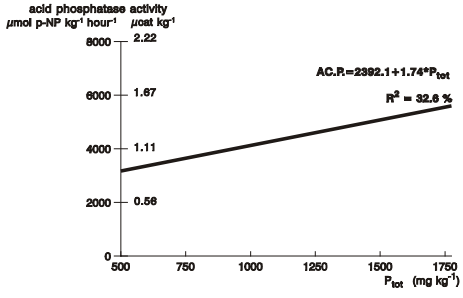
6. Relation of acid phosphatase activity to pH



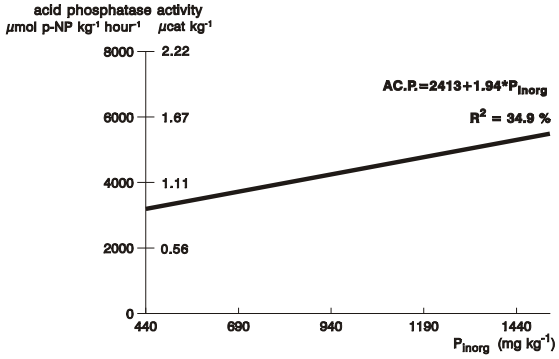
7. Relation of phosphatases activity to avallable P



8. Relation of acid activity to avallable P_{tot}



9. Relation of acid phosphatase activity to P_{Inorg}



Legend: ——— **acid phosphatase activity (AC.P.)**
- - - - - - - - - **alkaline phosphatase activity (AL.P.)**

DISCUSSION AND CONCLUSIONS

The results clearly state that in the soil samples taken from the locations in the Czech Republic positive correlations exist between acid and alkaline phosphatase and organic carbon content. This finding is in agreement with the results of other authors, e.g. Nannipieri et al. (1973), Gehlen and Schroder (1990), Nahas et al. (1994). Organic carbon content was found to correlate with total nitrogen content. There was a positive correlation between this element and phosphatase activity, which corresponds with the data reported by Speir (1977), Bonmati et al. (1991), and others. The humus quality was expressed by the humic : fulvic acid ratio. A negative correlation was determined between the above ratio and the activity of acid and alkaline phosphatases. The increasing humic : fulvic acid ratio brought about a decrease in the activity of the enzymes which may be due to the fact that those substances with a lower molecular weight decompose more easily. The negative correlation was also found between the available phosphorus content and both acid and alkaline phosphatase activity. This results agrees with the results published by Haynes and Swift (1988). Our study reveals a positive correlation between acid phosphatase activity and total inorganic phosphorus content, but no correlation with organic phosphorus was found. This has been described previously in the literature, for example, by Chhonkar and Tarafdar (1984). Similar to other experiments (cf. Gehlen and Schroder, 1990), our trials have also confirmed a negative correlation between acid phosphatase activity and pH. At our locations, phosphatase activity of soil decreases with increasing clay content. This is related to the fact that soil enzymes are associated with clays. The activities of clay adsorbed enzymes are often lower than those of free enzymes, as previously described by Boyd and Mortland (1990).

In conclusion, soil enzyme activities are believed to be able to discriminate between soil management treatments (Dick, 1993; Anwarzay et al., 1990; Mäder et al., 1993). The results of both our research and that of other research projects (Nannipieri et al., 1973; Nahas et al., 1994) show that soil enzyme activities are directly dependent on the content of organic substances (carbon, nitrogen forms). It is possible to say that unsuitable farming systems with a low input of organic substances have had a negative effect on the biological activity of the soils, including soil phosphatases. The application of soil conservation technologies, low-input farming systems, and well balanced carbon level could preserve soil fertility in favour of plant production and taken together are desirable factors for increasing the activity of soil enzymes in agroecosystems.

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II

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PHOSPHATASE ACTIVITY OF EUTRIC CAMBISOLS (UPPLAND, SWEDEN) IN RELATION TO SOIL PROPERTIES AND FARMING SYSTEMS

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The aim of the study was to assess the activity of soil phosphatases in agricultural soils in relation to some physical, chemical and biological properties. The trials were conducted at experimental locations and farms in Uppland (Sweden) with eutric cambisol soil type. The results demonstrate the correlation between acid and/or alkaline phosphatase and several soil characteristics (clay content, C_{org}, N_{tot}, pH, humic:fulvic acids ratio, P_{avail}, basal soil respiration). At the experimental plots the effect of different farming systems (conventional farming without animal husbandry /A/, organic farming with animal husbandry /B/, organic without animal husbandry with standard soil cultivation /C/, and organic agricultural system without animal husbandry with minimum soil management /D/) was also studied. The data show that the highest acid and alkaline phosphatase activity was found in system D followed by system B. The lowest means were recorded in system A and C. The research suggests that soil phosphatase activity was directly dependent on the content of organic substances in the soils (C,N forms) which may be influenced by farming activities. Our research provides results which can help to optimize the agroecosystem with respect to both minimizing inputs and protecting the environment.

phosphatase activity; physical, chemical and biological soil characteristics; farming systems

INTRODUCTION

All biochemical reactions are dependent on, and/or related to enzymes present in the environment. The same applies for those processes which take place in soils. Based on their origin and development, soil types differ in organic matter content, soil organism composition and activity, and consequently, in the intensity of the biological processes and soil enzymes (Kuprevich and Scherbakova, 1971).

The activity of soil enzymes is important for nutrient availability for plants. Our research focused on phosphorus - a major biogenic element that is made available through phosphatase.

Plants are able to take up and utilize inorganic phosphorus only, particularly the orthophosphate ion (anions $H_2PO_4^-$ and HPO_4^{2-}). In general, phosphorus occurs in soils in its inorganic form (both available and unavailable) as well as in organic bonds. Mineralization of such organic fractions, where a key role is played by phosphatases, is of great agricultural and economic importance. Enzyme reactions which take place in the cells of soil organisms, plant roots, and directly in the soil due to enzyme accumulation form the basis of soil metabolism (Chaziev, 1972; Kaprálek, 1986; Speir and Ross, 1978). A major step in organic matter conversion is its hydrolytic decomposition catalyzed by enzymes - hydrolases. A key role is played by esterase that cleaves ester bonds. Orthophosphates are released from the organic bonds by means of phosphoesterases that have their optimum pH either in acid or alkaline environments (acid and alkaline phosphatases, resp.).

In agricultural soils phosphatase activity is affected by physical, chemical, and biological soil properties, crop plants and cultural practices. As far as chemical characteristics are concerned, numerous studies have focused on carbon content and its positive impact on phosphatase activity (e.g. Nannipieri et al., 1973; Bonmati et al., 1991; Nahas et al., 1994; Šarapatka and Kršková, 1997), relationships between organic matter content (C_{org}) and other elements in the organic bonds (e.g. N and P), pH, and available phosphorus content. The amount of microbes is generally rendered a significant biological property of the soil. Various studies have shown either positive or negative correlations between the above soil properties and enzyme activity; others have reported none.

The aim of our study was to assess the activity of soil phosphatases in agricultural soils as it relates to the physical, chemical, and biological properties of soils with the intention of further applying the results obtained to the optimization of nutrient supply, particularly phosphorus within the scope of low input farming systems (e.g. ecological farming, low input agriculture).

MATERIALS AND METHODS

The trials were conducted at the experimental locations of Ultuna and Säby, both run by the Swedish University of Agricultural Sciences and on private farms (latitude 59 - 60 degrees north) with eutric cambisol soil type. Soil samples were taken after sowing winter wheat (September), with the forecrop being either clover-grass, on animal husbandry farms, or winter rape on the others. Afterward, soil samples were removed from the same plots planted with winter wheat (June). In total the sampling was carried out at 29 locations. From all localities 2 samples were taken each term from 0 - 20 cm horizon.

At the experimental locations at Ultuna and Säby, soil samples were also taken for evaluation of different farming systems on soil phosphatase activity. The research were focused on the following farming systems:

A. conventional farming without animal husbandry (barley, oats, w. wheat, spring rape, w. wheat),
B. organic farming with animal husbandry (oats + peas, barley + undersown, clover-grass, w. wheat),
C. organic farming without animal husbandry and standard soil management (peas, w. wheat (potatoes), oats + undersown, green manuring, w. wheat),
D. organic farming without animal husbandry and minimalized soil management (oats + peas, w. wheat + undersown, clover-grass, clover-grass, w. wheat).

Most of the analyses were conducted at the Department of Ecology and Environmental Sciences (Faculty of Science, Palacký University of Olomouc, Czech Republic) except for the biological soil activity - soil respiration which was determined at the Department of Microbiology (Swedish University of Agricultural Sciences in Uppsala, Sweden).

METHODS:

The soil samples were subjected to granulometric analysis by means of a pipetting method, and to soil chemical analysis. The parameters obtained were used to determine organic carbon by oxidation with a chromium-sulphur mixture followed by back-titration with diammonium. Total nitrogen was assessed by means of Kjeldahl´s method with nitrogen being mineralized by means of sulfuric acid. Dregs were determined by back titration with HCl. Moreover, available phosphorus was estimated after soil extraction with calcium lactate followed by spectrophotometric measurements of colour reaction, and exchangeable pH was determined in $CaCl_2$ extract (Králová, 1991; Javorský, 1987). The soil organic phosphorus was estimated by a method which is based on a high efficiency of $P_{org.}$ extracted from soils by the solution´s heat created by the addition of water to concentrated H_2SO_4. The acidic soil residue was next treated with NaOH to complete the extraction of $P_{org.}$ (Bowman, 1989).

The assessment of humic : fulvic acids ratio was made using the colour quotient Q 4/6 for humus substances. The visible part of the spectrum shows the highest and lowest absorbances of clear solutions of humus substances at 400 and 600 nm,

respectively. This quotient, dependent on the content of humic and fulvic acids, was introduced as a tool for humus determination by Welte (Králová, 1991).

Out of the soil-biological parameters of alkaline and acid phosphatase activity were assessed. To determine the activity of the above enzymes, soil was incubated using p-nitrophenylphosphate solution, and the resulting p-nitrophenol was estimated spectrophotometrically (Tabatabai and Bremner, 1969).

The basal respiration (hourly respiration rate over forty hours before the substrate addition) and SIR (substrate induced respiration rate a few hours after addition of glucose/ ammoniumsulphate/talcum mixture in 45/5/90 relation) were assessed. The CO_2 evolution, which is based on conductivity changes when CO_2 is trapped in KOH solution, was determined hourly at 20^{o}C with an improved version of the apparatus described by Nordgren (1988).

RESULTS

The results show the correlations among acid and/or alkaline phosphatase and numerous soil characteristics. The values of enzymatic activity and soil properties are in Table 1. The tests revealed correlations at significance levels of 95 and 99%, resp. Afterward, a method of simple linear regression was used to determine interactions between the variables, and to assess the formula $Y = a + b.x$ (see Table 2).

Analyses were carried out on the soils from experimental locations and from the farms. There was a positive correlation between acid phosphatase and organic matter content; and a negative correlation between acid phosphatase and pH. Our experiments demonstrated a positive correlation between alkaline phosphatase and basal soil respiration, organic matter content, and total nitrogen content, whilst the correlation between alkaline phosphatase and pH was negative.

Other interactions have been proven only in the soil samples from experimental locations, e.g. positive correlation between acid phosphatase and particles lower than 0.01 mm, and total nitrogen content; and between alkaline phosphatase and a content of particles lower than 0.01 mm and clay (lower than 0.002 mm).

Controversial results were obtained in the study on the interaction between acid phosphatase and clay content (0.002 mm); in this case the soil samples from experimental locations showed a positive correlation contrary to the farm soils which were characterized by a negative correlation. Similar results were recorded for alkaline and acid phosphatase in relation to humus quality expressed by a humic acids : fulvic acids ratio.

As far as the content of various phosphorus forms is concerned, the negative correlation between alkaline phosphatase and available phosphorus was found only at the location of Ultuna (Alkaline Phosphatase = 7835.52 - 20.74 P_{avail}). A positive correlation between alkaline phosphatase activity and SIR was observed at the same location (Alkaline Phosphatase = -1682.41+359.78 SIR).

PCA analysis (Ter Braak, 1993) was used to estimate the group of pedological characteristics and the results show similar correlations as those above (see Figs. 1 and 2).

Based on the data from research fields in Ultuna and Säby, mean values of acid and alkaline phosphatase activity were calculated in relation to the different

farming systems under study (Table 3). The data clearly show that the highest acid and alkaline phosphatase activity was found in system D (organic farming without animal husbandry with minimalized soil management, followed by system B (organic farming with animal husbandry). The lowest means were recorded in system A and C (conventional farming without animal husbandry, and organic farming without animal husbandry with standard soil management, respectively).

DISCUSSION AND CONCLUSIONS

The aim of the present paper was to undertake a comprehensive study on phosphatase activity in a widespread Swedish soil type, namely eutric cambisol dependent on soil properties. The results obtained can be successfully applied for farming system optimization, particularly within the scope of low input agriculture and ecological farming.

Several correlations found in our experiments correspond with the results of others. However, they were confined to few interrelationships occurring in the soil environment. The positive correlation between phosphatases and C and N is in good agreement with the results of Bonmati et al., (1991); Chhonkar and Tarafdar (1984); Šarapatka and Kršková (1997), the correlation between acid phosphatase and pH is similar to the observations of Gehlen and Schroder (1990); Herbien and Neal (1990); Chhonkar and Tarafdar (1984), and its relation to the microbial activity (referred to as respiration in our study) at some locations (i.e. farms, Ultuna + Säby) corresponds with the positive correlation demonstrated by Nannipieri et al. (1983); Chhonkar and Tarafdar (1984). It should be stressed that a negative correlation for the latter soil biological property was recorded at Säby where the phosphatase activity could have been affected by plant enzymes (Speir, 1976).

Increasing phosphatase activity with reduced mineral phosphorus content as recorded on the experimental plots of Ultuna resembles the results of Hedley et al. (1983) or Kandeler (1988). Nevertheless, our trials showed no positive correlation between phosphatases and organic phosphorus content as reported by e.g. Speir and Ross (1978) or Nahas et al. (1994). In addition, there was some correlation with the content of clay particles at the experimental locations.

Quality is considered an important parameter of organic matter. In our trials it was expressed as a humic acids:fulvic acids ratio. This parameter was characterized by a positive correlation in the experimental plots (Ultuna + Säby), whilst the correlation was negative on the farms. Having compared the experimental locations, the above ratio was higher at the Säby location and was accompanied by higher values of phosphatase activity. The situation on the farms was different due to the diversity of the respective locations. The increasing humic:fulvic acids ratio brought about a decrease in the activity of the enzymes which may be due to the fact that those substances with a lower molecular weight decompose more easily (Schulten et al., 1995; Šarapatka and Kršková, 1997). However, these findings contradict the results obtained from the sites at Ultuna and Säby, where there was a positive correlation between these variables. This is probably due to differences in organic matter and clay content.

At the Swedish farms the phosphatase activity decreases with increasing clay content. This is related to the fact that soil enzymes are associated with clays and have lower activity (Boyd and Mortland, 1990).

Aditionally, differences between conventional and organic farming, as well as among different agricultural practices, were studied during the evaluation of phosphatases activity.

Organic farming system B were the only one to comprise animal husbandry, where the farmyard manure was applied (25 t/hectare). Another crop was clover grass which leaves large amount of postharvest residue. The organic farming system with minimum soil cultivation (B) was similar to that with animal husbandry. On the one hand, the crop rotation involved biennial grass, and on the other hand no farmyard manure was applied. Moreover, the organic matter supply was supplemented with cut straw scattered over the soil surface. These farming systems show a higher activity of phosphatases than systems A and C.

Conventional farming system (A) and organic farming system with standard soil management (C) were characterized by similar organic matter supplies as well as comparable soil phosphatase activities. Conventional system A did not comprise any animal husbandry and organic matter supplies was quaranteed by straw incorporation. Organic farming system C was given organic matter through straw and green manure incorporation.

The above results are in close agreement with the fact that enzyme activities can be increased after the addition of energy sources (Nannipieri et al., 1983). The results of the effects of organic farming systems on higher enzyme activity are in agreement with data published by e.g. Oberson et al., 1993; Mäder et al., 1993. The enzyme activity could also be higher in the systems with minimum or no-tillage because of higher amounts of organic matter in the topsoil layer (e.g. Angers et al., 1993). Increasing the carbon, total nitrogen and phosphorus content could serve as a basis for increasing both biological and enzymatic soil activities.

In conclusion, the results indicate that enzyme activities are directly dependent on the content of organic substances in the soil (C, N forms) which may be influenced by farming activities (e.g., integrated and ecological agriculture). Our research provides results which can help to optimize inputs to the agroecosystem with respect to the protection of the environment.

Acknowledgements - I am much indebted to the Swedish Institute and Ministry of Agriculture of the Czech Republic for their support of the research whose partial results are presented in this paper. My personal thanks to Dr. T. Sjöqvist, Dr. B. Stenberg and Dr. J. Dlouhý from SLU Uppsala for their help. Last but not least, I wish to express my thanks to my colleagues from Palacký University, Olomouc, namely Dr.M. Kršková and Mrs. S.Šlezarová for their assistance.

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Aktivita fosfatáz v kambizemích eutrofních (Uppland, Švédsko) ve vztahu k půdním vlastnostem a systému hospodaření

Cílem práce bylo stanovení aktivity fosfatáz v zemědělských půdách v závislosti na jejich fyzikálních, chemických a biologických vlastnostech. Výzkum byl prováděn na experimentálních lokalitách a farmách v Upplandu (Švédsko) s půdním typem kambizem eutrofní. Výsledky demonstrují korelace mezi aktivitou kyselé a/nebo alkalické fosfatázy a řadou půdních charakteristik (obsah jílu, C_{org}, N_{tot}, pH, poměr huminových kyselin a fulvokyselin, P, bazální respirace).
Na experimentálních lokalitách byl rovněž studován vliv zemědělských systémů [konvenční systém bez živočišné produkce /A/, ekologický zemědělský systém s živočišnou produkcí /B/, ekologický bez živočišné produkce s klasickým zpracováním půdy /C/ a ekologický systém bez živočišné produkce s minimalizovaným zpracováním půdy /D/). Získané výsledky ukazují nejvyšší aktivitu kyselé a alkalické fosfatázy u systému D následovaným systémem B. Nejnižší hodnoty byly zaznamenávány ve variantách A a C.
Výzkum potvrdil přímou závislost aktivity fosfatáz na obsahu organických látek v půdě s možným vlivem zvoleného způsobu hospodaření. Výsledky výzkumu jsou využitelné při optimalizaci agroekosystémů včetně minimalizace vstupů a tím i pro ochranu životního prostředí.

aktivita fosfatáz, fyzikální, chemické a biologické charakteristiky půdy, zemědělský systém

TABLE 1: The values of phosphatase activity and soil properties

Research fields

	Average	Medium	Mode	Stand. errors
Acid phosphatase(μmol.kg^{-1}.hour^{-1})	5 072.90	4 730.00	4 840.00	225.82
Alkaline phosphatase(μmol.kg^{-1}.hour^{-1})	4 458.79	3 740.00	5 940.00	258.08
particles < 0.01 mm (%)	54.34	60.40	60.10	2.83
clay < 0.002 mm (%)	33.19	37.80	41.60	1.86
C_{org} (%)	1.87	1.87	1.60	0.06
N_{tot} (mg . kg^{-1})	1 943.39	1 802.2	1 744.3	110.24
pH	5.80	5.75	5.48	0.06
Humic : fulvic acids (ratio)	0.60	0.64	0.64	0.04
Bas. respiration ($mgCO_2$.kg^{-1}.hour^{-1})	1.43	1.40	0.90	0.09
SIR ($mgCO_2$.kg^{-1}.hour^{-1})	16.25	15.24	14.73	1.27
P_{avail} (mg.kg^{-1})	99.43	102.00	59.00	6.43
$P_{tot.}$ (mg.kg^{-1})	1 068.00	1 020.00	966.00	41.83
$P_{org.}$ (mg.kg^{-1})	182.33	155.00	205.00	22.55

Uppland farms

	Average	Medium	Mode	Stand. errors
Acid phosphatase(μmol.kg^{-1}.hour^{-1})	4 855.44	4 741.00	5 610.00	290.04
Alkaline phosphatase(μmol.kg^{-1}.hour^{-1})	4 768.11	4 647.50	3 740.00	255.04
Particles < 0.01 mm (%)	55.46	55.25	52.10	2.36
Clay < 0.002 mm (%)	32.89	32.50	31.10	1.74
C_{org} (%)	1.78	1.62	1.35	0.08
N_{tot} (mg . kg^{-1})	1 855.26	1 760.25	1 696.6	99.51
pH	5.90	5.80	5.28	0.10
P_{avail} (mg . kg^{-1})	118.75	102.5	84.00	8.65
Humic : fulvic acids (ratio)	0.615	0.64	0.64	0.03
Bas. respiration ($mgCO_2$.kg^{-1}.hour^{-1})	1.43	1.28	1.05	0.10
SIR ($mgCO_2$.kg^{-1}.hour^{-1})	17.84	16.52	16.17	0.96

TABLE 2: Interactions between activity of phosphatases and soil properties

	Research fields	Farms
Acid phosphatase x particles < 0.01 mm	AC.P = 2622.1 + 45.50 x particles 0.01 mm R^2 = 33.96 %	-
Acid phosphatase x clay < 0.002 mm	AC.P = 2745.61 + 70.65 x clay <0.002 mm R^2 = 35.54 %	AC.P=7565-82.56xclay < 0.002mm R^2 = 26.474
Acid phosphatase x C_{org}	AC.P = 2415.97+1437.25 x C_{org} R^2 = 16.19 %	AC.P = 2300.41 + 1447.76 x C_{org} R^2 = 16.35 %
Acid phosphatase x N_{tot}	AC.P = 2182.81 + 1.50 x N_{tot} R^2 = 55.56 %	-
Acid phosphatase x humic:fulvic acids	AC.P = 3459.37 + 2712.60 x HA : FA R^2 = 28.93 %	AC.P=7200.24 – 3793.25 x HA : FA R^2 = 16.43 %
Acid phosphatase x pH	AC.P = 14903 - 1692.13 x pH R^2 = 21.47 %	AC.P = 16944.9 - 2041.89 x pH R^2 = 54.15 %

	Research fields	Farms
Alkaline phosphatase x particles < 0.01 mm	AL.P=952.26 + 64.87 x particles < 0.01 mm R^2 = 53.85 %	-
Alkaline phosphatase x clay < 0.002 mm	AL.P = 1150.52 + 100.18 x clay < 0.002 mm R^2 = 55.25 %	-
Alkaline phosphatase x C_{org}	AL.P = 1962.85 + 1580.11 x C_{org} R^2 = 23.82 %	AL.P = 677.30 + 2011.07 x C_{org} R^2 = 25.03 %
Alkaline phosphatase x N_{tot}	AL.P = 1031.98 + 1.76 x N_{tot} R^2 = 60.81 %	AL.P = 2385.18 + 1.28 x N_{tot} R^2 = 25.12 %
Alkaline phosphatase x humic:fulvic acids	AL.P = 2090.96 + 4029.77 x HA : FA R^2 = 46.06	AL.P=7051.83 – 3713.37xHA : FA R^2 = 19.12 %
Alkaline phosphatase x pH	AL.P = 11615.2 - 1160.94 x pH R^2 = 22.11 %	AL.P = 15735.7 - 1945.1 x pH R^2 = 22.35 %
Alkaline phosphatase x basal respiration	AL.P = 3058.12 + 1192.52 x BR R^2 = 23.64 %	AL.P = 2793.2 + 1163.07 x BR R^2 = 16.64 %

TABLE 3: Mean values of acid and alkaline phosphatase activity in research fields at Ultuna and Säby (in %)

SYSTÉM	ACID PHOSPHATASE	ALKALINE PHOSPHATASE
A. CONVENTIONAL WITHOUT ANIMALS	95.3	96.8
B. ORGANIC WITH ANIMALS	101.8	101.8
C. ORGANIC WITHOUT ANIMALS NORMAL TILLAGE	96.1	95.5
D. ORGANIC WITHOUT ANIMALS MINIMAL TILLAGE	106.8	105.9

FIG. 1 - Correlations among soil properties in research fields (PCA analysis)

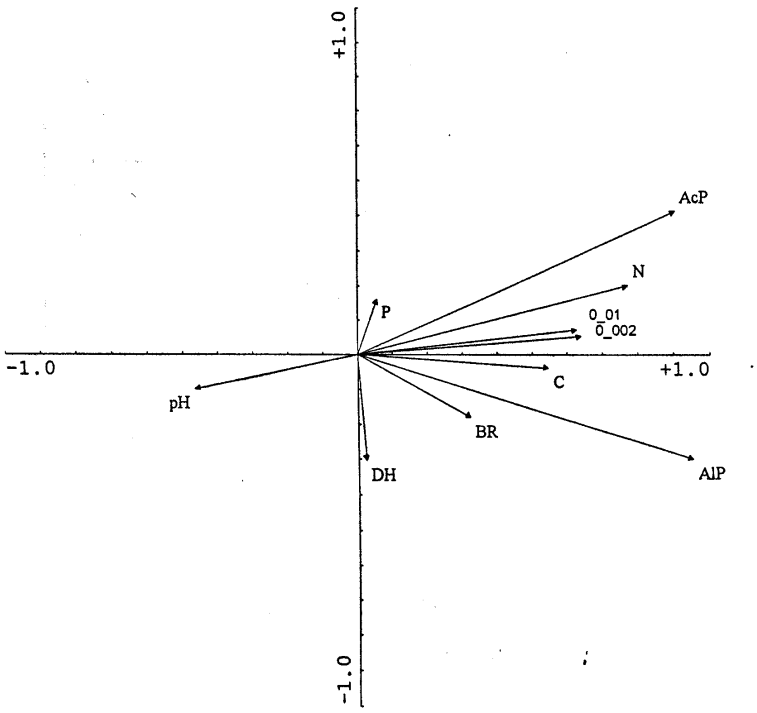
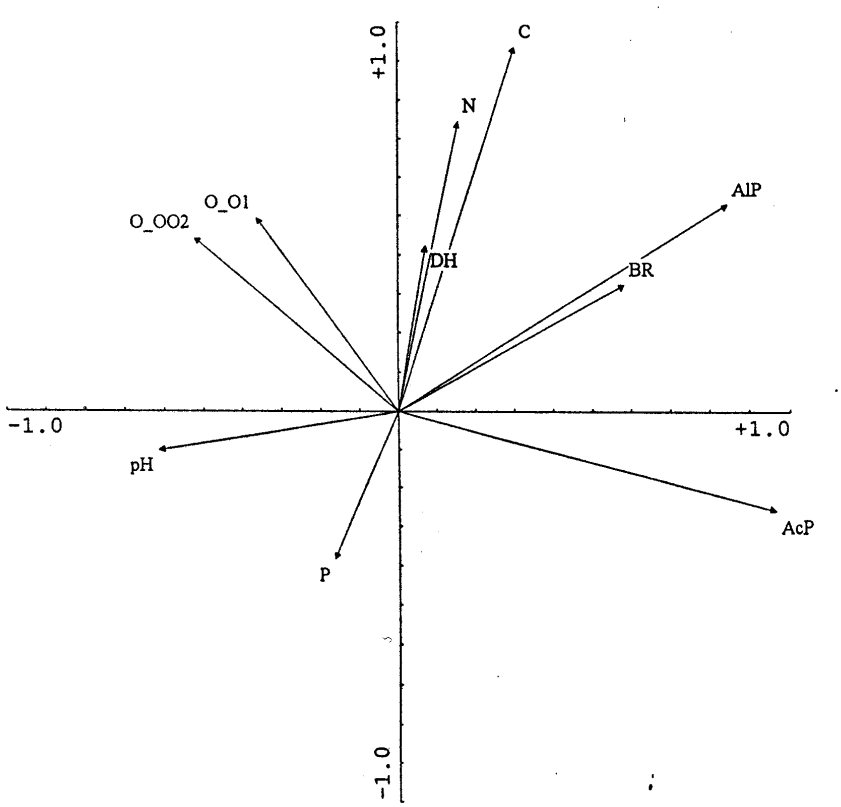


FIG. 2 - Correlations among soil properties in Uppland farms (PCA analysis)



Legend:

	AcP	acid phosphatase activity
	AlP	alkaline phosphatase activity
	DH	dehydrogenase activity
	BR	basal respiration
	0-01	particles lower than 0.01 mm
	0-002	clay lower than 0.002 mm
	C,N,P	symbols for C_{org}, N_{tot}, P_{avail}

III

Original paper, accepted for publication in Biologia

Effect of pH and phosphate supply on acid phosphatase activity in cereal roots

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Šarapatka, B., Dudová, L., Kršková, M., **Effect of pH and phosphate supply on acid phosphatase activity in cereal roots**.- *Biologia*

Enzymes in soils originate from animal, plant and microbial sources and the resulting soil biological activity includes the metabolic processes of all these organisms. The literature shows that under favourable conditions microorganisms supply most of the enzyme activity. The effect of plants on soil enzymatic activity is due to changes in organic matter content and microbial populations, but is also formed by accumulated enzymes and by continuously released extracellular and endocellular enzymes; all of which originate in the plant root.
Our research studies acid phosphatase activity linked to the previous source, by which we mean cultivated plants. Our evaluation was carried out on the root systems of both the chosen species and cereal varieties and also in nutrient medium on which crops were planted under conditions of changing pH and phosphorus supply.
Different varieties of winter wheat, spelt, barley and rye were used and after sterilization the seeds were sown on Murashige – Skoog nutrient medium with pH 5.6, 6.2 and 6.8 and a phosphorus supply between 30 – 160 mg P_2O_5/l of medium. After 10 days of cultivation the plant roots were harvested, homogenized and the acid phosphatase activity was measured.
The results show that the acid phosphatase activity in the root system of various species and cereal cultivars is negatively correlated with increasing pH and available phosphorus level in the nutrient medium.

Key words: phosphatase activity, soil, roots, cereals

Introduction

Plants meet their phosphorus requirement through the uptake of phosphate anions from the soil. To be available to plants, organic forms of soil phosphorus must be mineralized by those processes which are mediated by phosphatase enzymes (BIELESKI, FERGUSON, 1983). Some part of the total phosphorus in soil occurs in organic forms. The average content of organic phosphorus in soils ranges from 5 to 50 percent of total P (HARRISON, 1987) and forest soils have a higher organic P content than arable or cultivated soils. HALSTEAD and McKERCHER (1975) state that as much as 5 – 10 percent of the organic phosphorus is associated with living microbial tissue. Several researchers have described the relationship between total organic P content and other soil properties such as organic matter content, N and pH. The organic P content can also be affected by human activities. A large reservoir of organic phosphorus exists in forms which are unavailable to plants. The microbial oxidation of organic substrates is an important source of inorganic phosphate.

Soil phosphatases play a major role in the mineralization processes (dephosphorylation) of organic P substrates. In agricultural soils phosphatase activity is affected by soil properties, crop plants and farming systems. Relationships between phosphatase activity and soil properties have been described in numerous studies (e.g. SPEIR, ROSS, 1978; BONMATI et al, 1991; ŠARAPATKA, KRŠKOVÁ, 1997; MÄDER et al., 1993; OBERSON et al., 1993). These enzymes in soils originate from animal, plant and microbial sources and the resulting soil biological activity includes the metabolic processes of all these organisms. The literature shows that under favourable conditions microorganisms supply most of the soil enzyme activity (SPEIR, ROSS, 1978), with their large biomass, high metabolic activity and short lifespans. Plants have a marked effect on soil enzyme activity. This effect could be due to changes in organic matter content and the microbial population, but enzymatic activity in the soil is also formed by accumulated enzymes, continuously released extracellular enzymes and by endocellular enzymes; all of which originate in the plant roots. Rhizosphere phosphatase activity tends to be higher because of increased microbial numbers in the rhizosphere and the excretion of plant root enzymes.

The phosphatase activity associated with the roots of different plants has been studied by McLACHLAN (1980), BEISSNER and RÖMER (1999), GILBERT et al. (1999), RICHARDSON et al. (2000) and by other authors. Attention has also been dedicated to optimal pH, the effect of phosphorus on phosphatase activity, enzyme activities in root extracts or intact roots, activity in external root solutions, etc. BEISSNER and RÖMER (1999) described that sugar beet roots with P deficiency have high potential of phosphatase activity from the acid to the neutral pH range. Under these conditions they may effectively use dissolved organic phosphorus compounds. For *Triticum aestivum* McLACHLAN (1980) described increased activity of acid phosphomonoesterase under P-deficiency in intact roots and the optimum in the range pH 5-6. There was no evidence of alkaline phosphatase activity with phosphorus deficiency. GILBERT et al. (1999) found significantly greater acid phosphatase activity associated with white lupin roots in P-deficient plants.

The aim of this work is evaluate the activity of acid phosphatase in the root systems of both the chosen cereal species and varieties and also in a nutrient medium on which the crops were planted under conditions of changing pH and phosphorus supply which can reflect changing soil conditions.

Material and methods

Different varieties of winter wheat, spelt, barley and rye were used. *Triticum aestivum* L. represented cultivars Astella, Hana, Samanta, Siria and Trane, *Triticum spelta* L. - Ostro, Lueg, Oberkulmer Schwarzer, Altgold, Rouquin, *Hordeum sativum* Jessen - Norimberk, Rubín, Forum, Amulet and *Secale cereale* L. - Daňkovské nové and Rapid.

The seeds were sterilized using a solution of Savo preparation with chlorate and sown on the nutrient medium MS (MURASHIGE, SKOOG, 1962) without sacharose with a pH 5.6, 6.2 and 6.8 and phosphorus supply between 30 – 160 mg P_2O_5 / l of medium by KH_2PO_4.

After 10 days cultivation (2000 lx during 16 hrs/day, 26^o C) the plant roots were harvested and homogenized. After incubation at 30^o C, the acid phosphatase activity was measured using adjusting methods according to TABATABAI and JUMA (1988) with p-nitrophenyl phosphate as the substrate. After incubation the reaction was stopped by adding 0.5 M NaOH and the p-nitrophenol was then measured spectrophotometrically. The acid phosphatase activity was also set in the nutrient medium in which the plants were cultivated.

The results were statisticaly evaluated by means of linear regression analysis and analysis of variance using SPSS statistical system.

Results and discussion

The results show the effect of the concentration of phosphorus in the nutrient medium on the activity of acid phosphatases studied in the root system where the increasing amount of available P tends toward decreasing phosphatase activity, mainly between the first (30 mg P_2O_5 per l of medium) and second level (100 mg). These levels correspond approximately to the low and optimal supplies of phosphorus in the soil. Also, pH had a statistical effect on the activity of acid phosphatase where, in more acid media, a higher activity of acid phosphatase was evaluated. The correlations are possible to outline using linear regresion equations for:

P_2O_5: acid phosphatase activity = - 0.127 P2O5 + 135.5

pH: acid phosphatase activity = - 25.72 pH + 282.4

The basic statistical data (median, lower and upper quartile) of all cereals in different pH substrates and different phosphate supply, and trends we can see in Figures 1 and 2.

Fig. 1: Effect of phosphate level on acid phosphatase activity

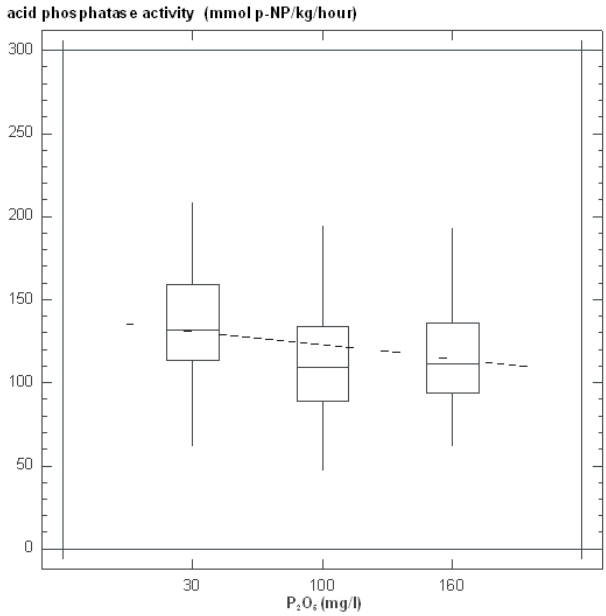
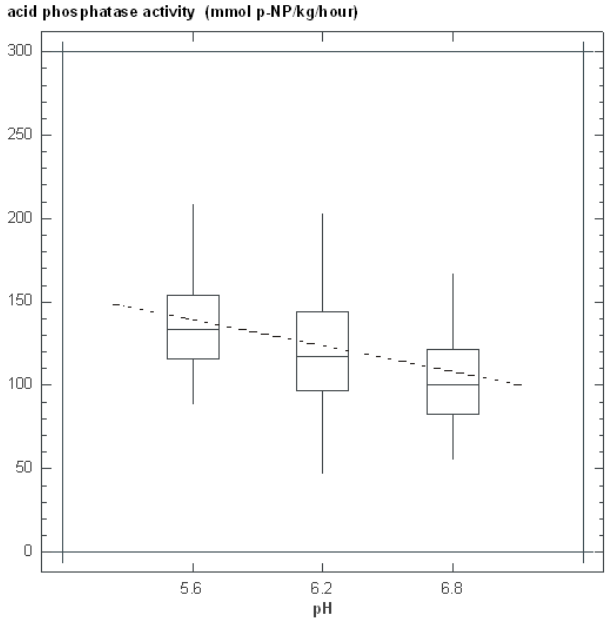
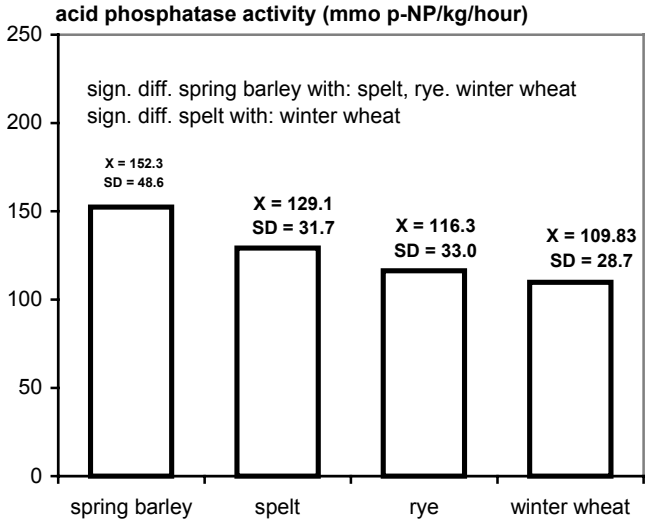


Fig. 2: Effect of pH on acid phosphatase activity



Differences were also found between the studied species: activity of the acid phosphatase in the root system of *Triticum spelta* differed from that of *Triticum aestivum* and *Hordeum sativum* differed from all other species. *Hordeum sativum* also had the highest activity of acid phosphatase which could be caused by the size of the root system and possibly by poorer utilization of nutrients.

Fig. 3: Acid phosphatase activity of cereal species



These differences were found not only between species. Statistical differences were found in the activity of the studied enzyme depending differing supplies of phosphorus and pH within an individual species:

- *Secale cereale* with pH levels of 5.6 and 6.8,
- *Hordeum sativum* with pH also with pH levels of 5.6 and 6.8,
- *Triticum aestivum* with pH at all levels, and with P_2O_5 - 30 and 100 mg/l,
- *Triticum spelta* with pH levels of 5.6 and 6.8; 6.2 and 6.8, and of all levels of P_2O_5.

Differences between acid phosphatase activity of single cereals in different pH and phosphorus supply are shown in Figs. 4 and 5.

Fig. 4: Effect of phosphate level on acid phosphatase activity of single cereal species

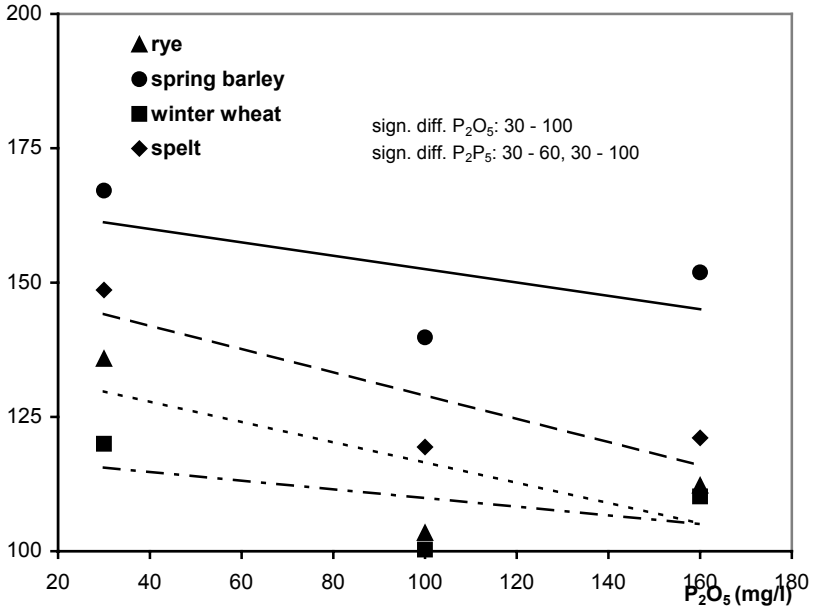
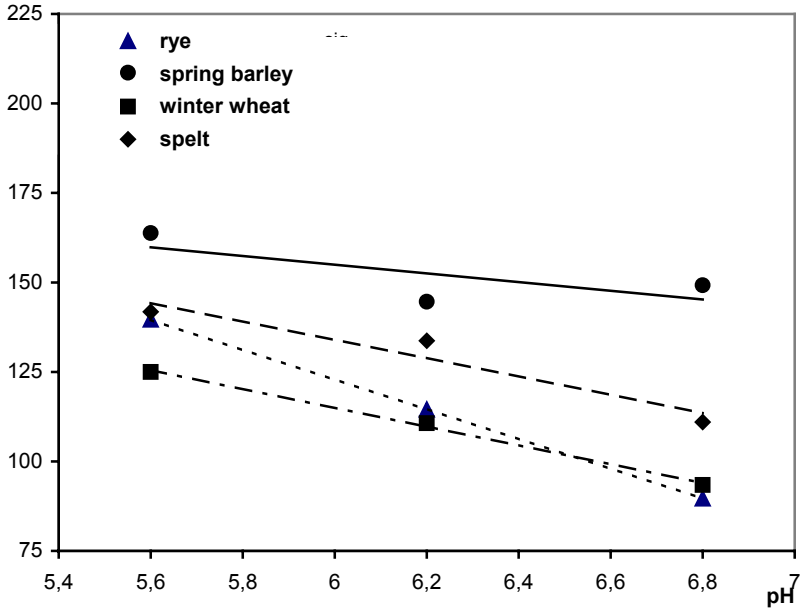


Fig. 5: Effect of pH on acid phosphatase activity of single cereal species



The results of our research correspond with the results of BEISSNER and RÖMER (1999) who found the effect of P deficiency on phosphatase activity in sugarbeet roots from the acid to the neutral pH range. McLACHLAN (1980) stated that P deficient plants had greater activities than those with sufficient levels. There was no evidence of alkaline phosphatase activity with phosphorus deficiency. Similar conclusions were found by GILBERT et al. (1999) for white lupin roots and RICHARDSON et al. (2000) for wheat. RICHARDSON et al. (2000) also described a limited ability to obtain P from inositol hexaphosphate, whereas other monoester substrates, such as glucose 1- phosphate, were equivalent sources of P for plant growth when compared with inorganic phosphate. GILBERT et al. (1999) describe the development of proteoid roots when grown in phosphorus deficient conditions and these roots are adapted to increased P availability. White lupin roots from P deficient plants had significantly greater acid phosphatase activity in both the root extracts and the root exudates than comparable samples from P – sufficient plants.
In some papers (e.g. McLACHLAN, 1980; BEISSNER and RÖMER, 1999), published results show optimum pH to be in an acid environment. For example, in McLACHLAN´s (1980) results phosphatase activity for all species was greatest in the acidic range – pH optima 5-6.
Our current and future research may help to indicate varieties used cereal breeding or in low input farming with differing potentials for obtaining phosphorus from soil reserves.

Conclusion

The study of phosphatase activity in different species and varieties can be used in practice as an indicator of these plants´ potential to exploit different phosphorus conditions. It´s useful, for example, in the breeding of varieties for organic or low input farming. In plants, reduced phosphatase activity could indicate the potential of varieties for greater efficiency in obtaining phosphorus. From our research, in keeping with the results of other scientists, we found different reactions of plant species to different supplies of phosphorus. In future research, for the practical purposes of plant breeding, we would like to describe the reaction of particular cereal varieties to different pH and especially in relation to phosphorus.

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We are grateful for the assistance and consultancy of scientists from the Department of Cell biology and Genetics, Palacký University Olomouc and laboratory work at the Department of Ecology and Environmental Sciences, Palacký University.

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IV

Original paper published in Biol Fertil Soils (1998) 26: 112 - 115

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Relationship between phosphatase active bacteria and phosphatase activities in forest soils

Abstract

Acid phosphatase and alkaline phosphatase active colonies of bacteria, isolated from forest soils, were stained. The activity of acid phosphatase and other soil properties (the number of aerobic bacteria, basal respiration, the level of ammonification, the number of bacteria active in ammonification, the level of nitrification, the number of micromycetes) were compared with the number of bacteria belonging to the genus *Micrococcus*. Soil samples were taken from the flowing horizons: F-AO1 (fermentative), H-AO2 (humic), and A (basic). the soil samples were taken from beneath forest stands in the Izera Mountains (North Bohemia, Czech Republic). The number of acid phosphatase active colonies correlated positively with the number of alkaline phosphatase active colonies in the F-AO1 horizon, and there was a high, positive correlation between the former and the level of ammonification in the H- AO2 horizon. The number of alkaline phosphatase active colonies correlated positively with organic carbon, the number of ammonification bacteria, and the number of micromycetes in the H-AO2 horizon. The A horizon was almost biologically inactive. Neither acid nor alkaline phosphatase activites correlated positively with the number of phosphatase active colonies of bacteria.

Key words
Phosphatase active colonies. Forest soil. *Micrococcus* spp. Ammonification. Micromycetes

Introduction

Soil enzyme activities have been studied in different soils and types of humus (mor, moder and mull). Extracellular enzymes, including phosphatases, are important for the degradation of organic substances in the soil, e.g., for organic phosphatase mineralization. The activity of phosphatates is influenced by various soil properties, soil biocoenoses, vegetation cover and the presence of inhibitors and activators. Wainwright (1980) found that the activity of soil phosphates decreased after their exposure to atmospheric deposition. In contrast, Falih and Wainwright (1996) found that the activity of these enzymes increased when a carbon source was added to the soil. The role of micro-organisms in the production of forms of phosphorus (P) which are available to plants, has been examined, for example, by Gerretson (1948), Greaves and Wembley (1965), and Brown (1974). Oberson et al. (1996) found a high, positive correlation between phosphatase activity and residual P, and correlations between phosphatase activity and other soil chemical characteristics have been described by Chhonkar and Tarafdar (1984), Bonmati et al. (1991), Gehlen and Schroder (1990) and others. Detailed information on the interaction between enzymes and clay has been published by Boyd and Mortland (1990). The effect of soil properties on the activity of phosphatates has been summarized by more authors e.g., Speir and Ross (1978) and Šarapatka (1997).

The results of enzyme activities, including soil phoshatase activity, could be compared not only with soil physical and chemical properties, but also with other biological factors (e.g. microbial biomass, the level of adenosine triphosphate, etc.) (Chhonkar and Tarafdar 1984). A global literature search indicated that, to date, there are no publications on the staining of phosphatates active colonies. In our research we investigated the number of phosphatase active colonies and tries to find correlations between these and chemical and biological soil properties.

Materials and methods

The soil samples were taken from five stands of 20-year-old spruce forest in the Izera Mountains (North Bohemia, Czech Republic). The altitude of all the stands was ca. 800m. The soil type was podzol and samples of approximately 1kg were taken from each horizon: F-AO1 (fermentative), H-AO2 (humic), and A (basic). Soil samples were not sieved but homogenized by an OMAS homogenizer TS-8. The samples were not stored at 5ºC under field conditions prior to cultivating the bacteria and evaluating the enzyme activity.

The evaluation of phosphatase active colonies

Acid and alkaline phosphatase active colonies of bacteria were evaluated using the following method (Koch`s plates). One millilitre of soil suspension (dilution 1:10000) was shaken and the pipetted onto the bottom of sterile Petri dishes. The agar, after cooling, was also poured onto the bottom of sterile Petri dishes. The agar contained p-nitrophenylphosphate (p-NPP) (0.1g l-1) and Thornton agar without p-NPP served as the control. After incubation for 48 h, 0.1 M HCl (ca. 2

ml) was poured onto one half of the Petri dishes and the same amount of 0.1 M NaOH was poured onto the second half. These solutions were washed out after 1h. Bacteria were cultivated for 48 h. Following the 0.5 h fixation with methylalcohol, the dead colonies were stained with basic fuchsin (hexazo-p-rosanilin). We mixed the same volume of two solutions: 4% solution of $NaNO_2$ and the solution of basic fuchsin (hexazo-p-rosanilin).
Basic fuchsin was preparedimmediately before staining as follows: 400 mg of basic fuchsin was dissolved 8 ml distilled water and 2 ml concentrated HCl was added. The mixture was mixed well and the cultures were stained for 5 h. The mixture lightened when the solution was mixed (this was the control for the use of fresh $NaNO_2$). The second staining solution (for the evaluation of basic phosphatase colonies) was prepared as follows: 50 mg Fast Blue BB was dissolved in 25 ml TRIS buffer (hydroxymethylaminomethane) pH 8.2-9.2. TRIS buffer was prepared as a mixture of 25 ml of 0.2 M hydroxymethylaminomethane with the prescribed amount of 0.1 M HCl. The staining mixture was filtered (Hayhoe and Quaglino 1994). The colonies from the Petri dishes containing p-NNP were not photographed because of the weak contrast. Following the diazo coupling reaction, the colonies were photographed and the Petri dishes served as the negative for black and white photography.

Determination of phosphatase activity in soil

The activity of soil phosphatases were determined using the method described by Tabatabai and Bremmer (1969). The soil was incubated in a solution with p-NPP and the formation of p-nitrophenol was measured using a spectrophotometer. The activities of acid and alkaline phosphatase were evaluated using a TRIS buffer at respectively, pH 6.5 (pH adjusted with HCl) and pH 11 (pH adjusted with NaOH).

Determination of soil respiration

The respiration of soil samples contained in 0.5 l bottles was evaluated using the classical method of absorption of CO_2 in 25 ml 0.1 M KOH and titration with a standard HCl solution, after precipitation of the carbonate with $BaCl_2$ (Dunger and Fiedler 1989).

Microbial counts

The number of micro-organism in the forest soils was evaluated using Koch`s plates with Thornton agar (aerobic bacteria), meat peptone agar (ammonification bacteria) and Czapek-Dox agar (micromycetes).

Determination of soil chemical properties

Soil organic carbon (C) was determined by $K_2Cr_2O_7$ oxidation and titration with diammonium iron(II)bisulphate hexahydrate (Javorský 1987). Total nitrogen (N) was determined using Kjeldahl`s method (Blum et al. 1989). The evaluation of N-NH_4^+ and N-NO_3^- followed extraction by $CaCl_2$ (ÖNORM L 1091 1989). NH_4^+ and NO_3^- (after reduction to NH_4^+) were evaluated after distillation with H_3BO_3

(Horáková et al.1989). Both the ammonification and nitrification levels were tested after 2 weeks of incubation at 25ºC (Frederic 1957). The available P in the soil was measured using a spectrophotometer, after its extraction by calcium lactate (Egner et al. 1960). We determined the quality of organic P by using Bowman`s (1989) method, which was based on the high efficiency of extracting organic P from soils by the heat of a solution created by the addition of water to concentrated H_2SO_4. The pH of soils samples extracted with KCl solution were also recorded.

Results and discussion

To our knowledge, this is the first study in soil biology to use hematological methods (Hayhoe and Quaglino 1994) to stain bacterial colonies. The resulting number of phosphatase active bacteria, the activity of soil phosphatases, and other and biological and chemical characteristics of the soil were analysed using StatGraphics. the results of the positive correlations between these characteristics are found in Tables 1-3. Figures 1 and 2 show phosphatase active colonies of bacteria which formed typical halo margins after staining. The staining was achieved by a diazo coupling reaction. The number of acid phosphatase active colonies of bacteria correlated positively with the number of alkaline phosphatase active colonies in the F-A01 horizon and with the level of ammonification in the H-A02 horizon.

The number of alkaline phosphatase active colonies correlated positively with organic C, the number of ammonification bacteria, and the number of ammonification bacteria, and the number of micromycetes in the H-A02 horizon. The A horizon was almost biologically inactive, although a positive correlation between acid phosphatase activity and organic matter content (organic C and total N) was found. All the colonies of phosphatase active bacteria were of grampositive cocci the genus *Micrococcus*.

Our results didn't show any correlation between phosphatase active colonies of bacteria and acid and alkaline phosphatase activity in the investigated soils. This is probably due to the production of phosphatates by organisms other than bacteria, for example, soil protozoa (Hattori 1993) or other groups. Enzymes in soils do not only originate from microbial sources, but also from animals and plant roots. The presence of plants positively affects enzyme activities, including that of phosphatase (Juma and Tabatabai 1988; Tadano et al. 1993). Phosphatase activity can also be affected by earthworms and other soil animals (Satchel and Martin 1984; Weiss and Trespendorfer 1993), and Gerretson (1948) argued almost half a century ago that micro-organisms in the soil may bring P into solution and thereby improve the growth and P nutrition of plants. Brown (1974) discussed the stimulatory effects of microorganisms on plant growth. According to Greaves and Wembley (1965), up to 90% of the macroflora in the rhizosphere are capable of producing phosphatases. The lack of significant correlation between the number of Micrococcus colonies with either acid or alkaline phosphatase actives shows that other factors affect the level of enzyme activity in forest soil. Bacterial counts in our search showed that only those micro-organisms which can be cultured, i.e., a

low percentage of bacteria inhabiting soils, could have influenced the characteristics of soil in terms of enzyme activity. In forest soils there are other organisms, such as micromycetes, which can increase total soil enzyme activity.

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Fig. 1 Acid phosphatase active colonies of bacteria

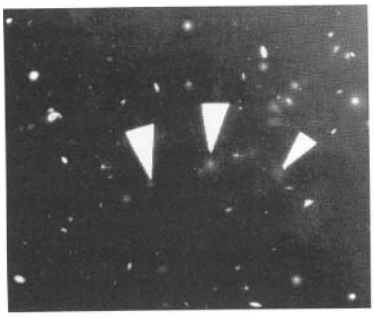

Fig. 2 Alkaline phosphatase active colonies of bacteria

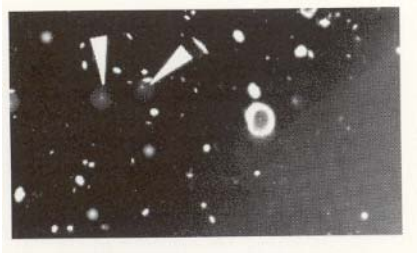

Table 1 Correlations between characteristics of the F-A01 horizon (fermentative)

(AB aerobic bacteria, AcPB acid phosphatase active bacteria, AlPB alkaline phosphatase active bacteria, AcP acid phosphatase activity, AlP alkaline phosphatase activity, C organic carbon, P organic phosphorus, N total organic nitrogen, C/N carbon/nitrogen ratio, BR basal respiration, N-NH_4^+ ammonium nitrogen, Am ammonification, N-NO_3^- nitrate nitrogen, Ni nitrification, AmB ammonifying bacteria, Microm micromycetes).

r values are given for P< 0.05

	AB	AcPB	AlPB	AcP	AlP	C	P	P	pH	N	C/N	BR	N-NH^+_4	Am	N-NO_3^-	Ni	AmB	Microm
AB																		
AcPB			0.98															
AlPB		0.98																
AcP																		
AlP																		
C									-0.96		0.97							
P																		
P															-0.74			-0.76
pH						-0.96				0.97	-0.99		0.95					
N									0.97		-0.99		1.00					
C/N						0.97			-0.99	-0.99			-0.98					
BR													-0.95					
N-NH^+_4									0.95	1.00	-0.98	-0.95						
Am																-1.00		
N-NO_3^-								-1.00										1.00
Ni														-1.00				
AmB																		
Microm								-1.00							1.00			

Table 2 Correlations between chararcteristics of the H-A02 horizon (humic).
For abbreviations see Table 1.

r values are given for $P< 0.05$

	AB	AcPB	AlPB	AcP	AlP	C	P	P	pH	N	C/N	BR	$N\text{-}NH^{+}_{4}$	Am	$N\text{-}NO_{3}^{-}$	Ni	AmB	Microm
AB							0.93	0.89										
AcPB														0.90				
AlPB						-0.76											0.90	0.66
AcP					0.97													
AlP				0.97														
C			-0.76									0.80					-0.84	
P	0.93							0.81										
P	0.89						0.81											
pH																		
N																		
C/N															-0.89			
BR						0.80												
$N\text{-}NH^{+}_{4}$																		
Am		0.90																
$N\text{-}NO_{3}^{-}$											-0.89							
Ni																		
AmB			0.90			-0.84												
Microm			0.66															

Table 3 Correlations between chararcteristics of the A horizon (basic).
For abbreviations see Table 1.

r values are given for P< 0.05

	AB	AcPB	AlPB	AcP	AlP	C	P	P	pH	N	C/N	BR	N-NH^{+}_{4}	Am	N-NO_3^-	Ni	AmB	Microm
AB		1.00	1.00															
AcPB	1.00		1.00															
AlPB	1.00	1.00																
AcP						0.97				0.97					0.96			
AlP																		
C				0.97						1.00								
P								1.00										
P							1.00											
pH																		
N				0.97		1.00												
C/N																		
BR													-0.98					
N-NH^{+}_{4}												-0.98						
Am																		
N-NO_3^-				0.96														
Ni																		
AmB																		
Microm																		