Acid Phosphomonoesterase Activity in Floodplain Forest Soils

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Abstract: The 4-nitrophenyl phosphate method for acid phosphomonoesterase (EC 3.1.3.2; acid orthophosphoric monoester phosphohydrolase) determination was slightly modified to increase the sensitivity and the stability of the 4-nitrophenol coloured complex in samples rich in humic substances and easily soluble organic compounds. Based on an approach used for mycorrhizal roots, the new analytical protocol was tested on samples taken from a single forest site with a large variation in soil types. Distinctive properties of forest soils and the accuracy/repeatability of the optimised technique were considered in the selection of the most appropriate analytical steps. Finally, the acid phosphomonoesterase activity was expressed in μg of 4-nitrophenol/g fresh soil recalculated dry matter/h released after hydrolysis of 4-nitrophenyl phosphate in sodium tetraborate/succinic acid buffer at pH 4.8. The modified method proposed was statistically compared with other procedures and the results obtained were found to give merit to these slight modifications. The rationale behind the slight modifications and their comparative significance was reported. Due to high soil complexity, it is suggested that a single laboratory measurement of chemical and biochemical properties is of limited application with forest soil and can be only one component of a broadly focused ecological analysis.

Keywords: soil acid phosphomonoesterases; forest soils

There were two aims of the work: (i) submitting the results of a comparative study where an optimised acid phospohomonoesterase (PME) activity assay was compared with the original method of Tabatabai and Bremner (1969) and four other traditional methods (Duxbury & Tate 1981; Clark & Clark 1981; Nannipieri *et al.* 1988; Trasar-Cepeda & Gil-Sotres 1988) for forest soils with high organic matter content, and (ii) trying to apply the procedure of Rejšek (1991) as proposed for mycorrhizal roots also to soil. The reason for such an application has come from the fact that if a method could be applied to both soil and mycorrhizal samples, it might lead to very general usage in ecologically-orientated studies

where similar comparative levels for both soil and mycorrhizal samples could be achieved. The procedure of Rejšek (1991) followed the method of Grunda and Rejšek (1990) using uranyl acetate as the inhibitor of enzymatic reactions. In addition, another objective of this assessing was to develop a quick, robust, and simple method that can be routinely used.

The problem of the study comes from the distinctive features of forest soils rich in extractable humus compounds: analysing such samples needs distinctive methodologies. Except for most Spodosols, Utisols and Oxysols, and some Alfisols, forest soils are typically characterised by prominent organic horizons and humic substances in differ-

ent quantities and qualities. The high content of these substances appeared to be the most serious analytical problem.

Despite long-term and extensive investigations of soil phosphorus (P) and its mobility, routine usage of techniques specific to forest soils is not common. In general, 70–90% of P in forest soils is often organically-bound (Firsching & Claasen 1996), and poorly soluble and insoluble phosphates prevail (McLaren & Cameron 2002). The decomposition of these organic compounds, followed by the release of orthophospatic anions, is catalysed by phosphatases. Acid orthophosphoric monoester phosphohydrolase, designated by Enzyme Nomenclature (1992) as EC 3.1.3.2., belongs among phosphohydrolytic enzymes (EC 3.1.3) with either an alkaline (EC 3.1.3.1) or an acidic (EC 3.1.3.2) pH-optimum. Esters of orthophosphoric acid, which enter the soil in the form of organic residues, immediately encounter both phosphomonoesterases adsorbed on colloids, phosphomonoesterases adaptively exuded by proliferating and nonproliferating cells of soil biota, and phosphomonoesterases associated with living plant roots and biological debris. Acidity of forest soils leads to a higher acid phosphomonoesterase acitivity – which is followed by the development of typical forest coenoses.

In this paper, the acid PME activity assay for forest soils rich in humic substances was studied. The humic substances, which are easily alkali-soluble, form humic-phosphohydrolase complexes keeping a considerable part of the enzyme activity of topsoil. Because the floodplain forest soils are generally very rich in these compounds, these soils have been chosen for the testing. In relation to other works done in this field, alkalisation of the incubated soil samples by the addition of hydroxylic inhibitors to stop the enzyme reaction and efficiently extract phenol adsorbed by the soil is recommended. However, the addition of hydroxides leads to a massive release of the liquid organic compounds. The use of alkaline solutions produces a dark-brown soil extract due to a considerable solubilisation of humus. Consequently, the concentrations in the reaction mixture of chlorides and hydroxides used will be changed. For the forest soils that are extremely high in humic substances, the brown colouring of the soil suspension makes the final spectrophotometric assay difficult. Tabatabai and Bremner (1969), Cervelli et al. (1973) and Ho (1979) recommended the addition of calcium chloride to prevent both the non-desirable colouring of the soil suspension and the increased extraction of clay particles. In the humus-rich forest soils tested, however, the coagulating effect of CaCl$_2$ was not effective enough. Centrifugation (Brams & McLaren 1974; Gerritse & van Dijk 1978) was not successful either in preventing the non-acceptable discoloration. Further, the immediate discoloration of the incubation solutions could not be prevented by subsequent purification through activated charcoal. To exclude humic substances from the spectrophotometric assay and not to miss a marked part of actual PME activity, the only way is to add an inhibitor exclusively to the filtrate solution (Duxbury & Tate 1981; Clark & Clark 1981) with humus-rich forest soils. In addition, hydroxide-containing buffer solutions proved unacceptable because they contaminated the soil suspensions with extracted humic substances and could therefore not be utilised. However, the yellow colour of 4-nitrophenol ions will develop only in response to strong alkalisation-hydroxides that can be added after the filtrates are prepared (following separation of 4-nitrophenol from the soil samples). The approach came from Duxbury and Tate (1981) method without the centrifugation step. A clear yellow extract of 4-nitrophenol is obtained without the presence of CaCl$_2$. Dealing with the recovery of 4-nitrophenol, the absence of chlorides or chloride/hydroxide mixture in a ratio was successfully tested to make sure that it gives full recovery of the 4-nitrophenol released.

MATERIAL AND METHODS

Forest sites

The study forest area Ranspurk Virgin Forest (Ranspurk National Nature Reserve) is located in southern Czech Republic (48°37′ N, 16°56′ E). The region is near to the lower reaches of the March (Morava) and Thaya (Dyje) Rivers, having been developed on the deposits of Holocene sediments that filled widespread depressions in Neogene underlaying strata. The study plots are situated at 152 m above sea level, the mean annual temperature and the average annual precipitation being 9.1°C and 534 mm, respectively. The sites had not been used for intense agriculture or clear-cutting for centuries and since 1931 there has been no harvesting at all within the reserve. The study area with nearly half-millenary old oaks, a very rich species variety and

multiple canopy layers is dominated by English oak (*Quercus robur* L.) and narrow-leaved ash (*Fraxinus angustifolia* Vahl.). The second canopy layer comprised predominantly hedge maple (*Acer campestre* L.) and European hornbeam (*Carpinus betulus* L.). The understorey vegetation was dominated by smooth-leaved elm (*Ulmus carpinifolia* Gled.) and hawthorns (*Crataegus* sp.).

The studied fields manifesting different plant communities (Průša 1985) were situated in the same floodplain area, 200 m from each other. The sites were not fertilised, the peat formation did not occur (even not under *Ulmeto-Fraxinetum carpineum* – see Table 1).

Soils sampling

Cambisols, Regosols and Gleysols (ISSS-ISRIC-FAO 1998) were sampled in this correspondence to the plant communities: (*i*) *Ulmeto-Fraxinetum carpineum* (UFrc-I) on Eutric Cambisol, (*ii*) *Ulmeto-Fraxinetum carpineum*, type *Brachypodium sylvaticum* (Huds.) P. Beauv. (UFrc-II) on Haplic Fluvisol, (*iii*) *Querceto-Fraxinetum*, type *Deschampsia caespitosa* (L.) P. Beauv. (QFr-I) on Humic Regosol, (*iv*) *Querceto-Fraxinetum*, type *Ranunculus repens* L. (QFr-II) on Gleyic Regosol, (*v*) *Saliceto-Alnetum*, type *Baldingera arundinacea* (L.) Dumort (SAl-I) on Haplic Gleysol, and (*vi*) *Saliceto-Alnetum*, the initial stages (SAl-LL) on Histic Gleysol. Physical and chemical soil characteristics of the fields studied are given in Table 1.

The soil samples were obtained from both surface mineral horizons (designated as Ah or Ae) and subsurface horizons (designated as Ahb, AC, Bm, Bg and Cg) of unfertilised pristine forest soils throughout the year – Table 1 lists the ranges of the individual depths sampled. The soil profiles were sampled from open soil pits in May, July, October, and January for three years. Based on the current approach for the soil sampling (Pansu *et al.* 2001), the procedure consisted of a random sampling of individual soil samples (per soil type and depth) followed by mixing them into representative samples and analysing in four replicates. The sampling was performed seasonally following the proportionate spatial sampling plan. For each season, each particular soil is characterised by the data measured in four replicates of the mixed samples obtained from nine individual soil samples taken from three independent sampling plots. Considering the possible microsampling

effect in such complex and variable materials as forest soils are, the nine particular soil samples for each horizon of each experimental plots for each season were taken separately, mixed together (Stein & Ettema 2003), placed in PE-bottles, immediately refrigerated at 4°C and analysed within one or two weeks.

Soil physical and chemical analysis

Particle-size classes were determined in U.S.D.A. system (diameters of 0.002–0.05–0.1–2 mm) by the pipet method (Kalra & Maynard 1991). The soil reaction was measured using air-dried soil-water suspension (1:5 v/v, shaken for 5 min) at room temperature (ISO/DIS 10390 1992). The content of organic matter was determined after sulfochromic oxidation followed by spectrophotometric measurement (ISO/DIS 14235 1995). The analyses of total/organic carbon and total nitrogen were carried out on Autoanalyser LECO FP-2000 Makro. The contents of humus substances were obtained by multiplying the percentage of organic carbon content with the conventional factor 1.724, C:N ratio was calculated.

Determination of acid phosphomonoesterase activity

Both surface and subsurface (zero to more than 50 cm) forest soil horizons were sampled to obtain (Table 1) samples with a wide range of the organic matter content (0.01%–29.5%), texture (sand: 9–87%, silt: 5–41%, clay: 3–48%), and pH/H_2O (4.9–6.0). The original procedure of Tabatabai and Bremner (1969) and its traditional modification by Nannipieri *et al.* (1988) were tested together with three methods focused on the exclusion of the interfering extractable organic compounds from the final spectrophotometric assay: the methods of Trasar-Cepeda and Gil-Sotres (1988), Clark and Clark (1981) method with the addition of inhibitors to the filtrates, and the measurement using no buffer by Duxbury and Tate (1981). The methods based on disodium phenyl phosphate (Kroll & Kramer 1955) or other phenolic esters of monophosphoric acid (Rejšek 1994) were not tested. In addition, the particular uses of maleate (Brams & McLaren 1974), borate (Kramer & Erdeli 1959), citrate (Hoffmann 1968) and ethanol-amine-acetate (Zvyagintsev *et al.* 1980) buffers, toluene (Rogers 1942), trichloracetic

Table 1. Selected physical and chemical properties of the six forest soils from Lanzhot Virgin Forest, south Moravia (Czech Republic)

Depth (mm)	Horizon	Sand (%)	Fine s. (%)	Silt (%)	Clay (%)	pH/H_2O	Humus (%)	C_{ox} (%)	N_t (%)	C/N
Eutric Cambisol										
10–130	Ah	77	7	11	5	5.7	29.48	17.10	0.705	24.3
130–370	Bm	87	5	5	3	5.5	2.29	1.33	0.137	9.7
370+	C	76	6	7	11	5.8	0.62	0.35	0.054	6.5
Haplic Fluvisol										
20–170	Ah	63	7	25	5	5.9	14.52	8.42	0.395	21.3
170–330	AC	51	16	29	4	5.9	2.03	1.17	0.105	11.1
330+	C	44	16	22	18	5.9	2.61	1.51	0.123	12.3
Humic Regosol										
10–170	Ah	45	14	27	14	4.9	8.47	4.91	0.360	13.6
170–350	Ahb	39	10	41	10	5.4	4.22	2.45	0.152	16.1
330+	C	41	19	10	20	6.0	0.51	0.29	0.105	2.8
Gleyic Regosol										
20–80	Ae	39	20	23	18	5.7	7.38	4.28	0.486	8.8
80–250	Cg1	24	33	18	25	5.2	0.96	0.56	0.367	1.5
250–500	Cg2	14	15	23	48	5.7	3.28	1.90	0.173	11.0
500+	Cg3	8	17	27	48	6.0	1.33	0.65	0.066	9.8
Haplic Gleysol										
20–130	Ah	39	9	29	23	4.9	2.27	1.58	0.142	11.1
130–400	Cg1	33	8	27	32	5.8	0.33	0.19	0.047	4.0
400+	Cg2	17	19	29	35	5.9	0.01	0.01	0.048	0.2
Histic Gleysol										
20–50	Ae	38	12	26	24	4.9	3.01	1.65	0.142	11.6
50–500	Bg	31	11	19	39	6.0	0.23	0.15	0.039	3.9
500+	Cg	9	22	28	41	5.9	0.78	0.45	0.100	4.0

acid (Kuprevitch & Stcherbakova 1966), centrifugation (Gerritse & van Dijk 1978), very high incubation temperature (Drobníková 1961) and very low incubation temperature (Harrison 1983) were tested as well.

Modified procedure

The modified method includes:
(*i*) the assay solution was not made alkaline before filtration,
(*ii*) reaching full recovery of 4-nitrophenol released,

(*iii*) no use of bacteriostatics,
(*iv*) no air-drying and crushing of the soil sample,
(*v*) incubation of the reaction mixture at 25°C,
(*vi*) solubilisation of the substrate in the buffer at pH 4.8.

Into each 50 ml-Erlenmayer flask, 1 g of fresh soil was put, followed by the addition of 12 ml of 500 µmol/l disodium 4-nitrophenyl phosphate $Na_2C_6H_4NO_2PO_4$ in the succinate-borate buffer of pH 4.8 (Nikolskij 1964) composed of 0.05 mol/l sodium tetraborate $Na_2B_4O_7 \cdot 10\ H_2O$ and 0.05 mol/l succinic acid $C_4H_6O_4$. The flasks were shaken shortly and let stand at 25°C for 60 min. The re-

action mixtures were filtered into test tubes and 8 ml of 1 mol/l KOH were immediately added (to stop the enzymatic reaction). The absorbances were measured at 410 nm and calibrated against standard solutions of 4-nitrophenol. To obtain standard solutions, 1 ml of 4-nitrophenol (initial dilution, 0.72 mmol/l $C_6H_4OH\ NO_2$) was diluted to 100 ml with the buffer. 12 ml of the buffer was mixed with 8 ml of 1 mol/l KOH and the mixture was used as the blank.

The results were given in µg of colorimetrically determined 4-NP released from 1 g of fresh soil during 1 h at 25°C. Considering the high content of organic matter, the gravimetric moisture contents were concurrently determined by drying the soil samples at 65°C for 24 h (Kutílek & Nielsen 1994) and the results obtained with 1 g of fresh soil were recalculated onto 1 g of dry matter.

Statistical methods

The results were analysed by standard methods of descriptive statistics (Kendall & Stuart 1979). In each soil horizon, the heterogeneity of variances of the datasets (Webster 2001) and the statistical suitability of the measured data according to normality by Kolmogorov-Smirnov-one-sample test (Masery 1951) were tested. Because the analytical data from the both modifications presented and also other techniques showed the normal distribution for all the soil horizons, the Anova test and the multiple comparison of Scheffe tests (Lee 1975) for the detail evaluation of variance was used to achieve the characteristics of the comparison (see Table 2) between six different analytical methods.

RESULTS AND DISCUSSION

The approach of Rejšek (1991) as proposed for mycorrhizal roots was used having in mind to reach similar comparative levels for both soil and mycorrhizal samples. The validation of a suitable application of the method optimised for soils rich in easily extractable organic compounds is presented in Table 2 where the descriptive statistics and the estimation of the data dispersion (standard error, standard deviation and variance) of it are compared with other methods. For the surface horizons, the coefficient of variance in the modification presented (CV = 2.54) is comparable only with CV of the method of Nannipieri *et al.* (1988) (CV = 1.28) and of Trasar-Cepeda and Gil-Sotres (1998) (CV = 2.53). For the subsurface horizons, CV in the modification presented is the lowest one

Table 2. Comparison of six methods for estimation of acid phosphomonoesterase activity; the activity is expressed as µg 4-nitrophenol h^{-1}/g dry soil weight (DM, dry matter); if a method uses fresh samples, the activity was measured from them and recalculated into dry soil weight

Subject	Method	Mean		SD	Variance	CV	P-level (K-S)[1]	B[2]	ε[2]
		mean	SE						
Acid PME activity in the surface horizons Ah and Ae	modification presented	77.21	0.8	14.0	196.24	2.54	0.1	51.31	0.8
	Tabatabai & Bremner (1969)	276.5	1.4	41.8	1746.5	6.32	0.2	236.9	1.0
	Duxbury & Tate (1981)	100.1	0.9	20.4	414.35	4.14	1	72.41	0.9
	Clark and Clark (1981)	98.64	0.9	19.5	379.28	5.61	0	65.7	0.9
	Nannipieri *et al.* (1988)	106.9	1.2	11.7	137.67	1.28	0.4	93.26	1.0
	Trasar-Cepeda & Gil-Sotres (1988)	174.0	1.4	21.0	441.58	2.53	0.3	154.1	1.0
Acid PME activity in the subsurface horizons[3]	modification presented	58.5	0.6	12.2	149.1	1.49	0.2	38.44	0.8
	Tabatabai & Bremner (1969)	261.9	0.7	36.4	1323.4	5.05	0.7	211.5	1.0
	Duxbury & Tate (1981)	68.2	0.7	13.2	175.16	3.01	0.9	36.94	0.8
	Clark & Clark (1981)	67.76	0.7	13.1	179.94	3.14	0.2	37.69	0.8
	Nannipieri *et al.* (1988)	68.98	0.7	13.7	176.04	1.97	0.8	50.79	0.9
	Trasar-Cepeda & Gil-Sotres (1988)	152.7	0.6	17.2	294.73	1.93	0.8	127.8	1.0

[1]K-S test (test of normality); [2]Kolmogorov statistics; [3]Ahb in Humic Regosol, AC in Haplic Fluvisol, Bm in Eutric Cambisol, Bg Histic Gleysol and Cg in Gleyic Regosol, and Haplic Gleysol

SE – Standard error; SD – Standatd deviation; *CV* – Coefficient of variance

(*CV* = 1.49). Following the normality test, the whole dataset obtained from the surface horizons show such results on K-S test that it can be considered as normally distributed (*P*-level > 0.05). The whole dataset obtained from the subsurface horizons can be considered as normally distributed as well. In ANOVA test, factors *METHOD* and *SOIL* for the effect classification were considered. The effects from both factors and their interaction for both surface and subsurface horizons were proved to be significant. Although Standard Error Estimation values were not very different between the methods tested, the modification presented provided the smallest value of the standard error (SE) for the surface horizons and comparable values for the subsurface horizons. For the surface horizons, the size of effect belonging to each method was in lower border of error values and multiple comparison of each method by PostHoc Scheffe tests classified all methods as non-comparable. For the subsurface horizons, the size of effect belonging to each method was in the middle of error values and most of the methods were classified as non-comparable in multiple comparison of each method pair by PostHoc Scheffe tests, except for the interrelationship between the modification presented and method of Duxbury and Tate (1981). Methods of Clark and Clark (1981) and Nannipieri *et al.* (1988) provided comparable results of the descriptive statistics with the modification proposed but the modification showed a lower variability of measurements related to the standard error of the dataset.

The marked effect of the soil depth was demonstrated – the activities of the enzyme in the subsurface (mineral) horizons amounted usually to 60–70 % of the activities measured in the surface (organomineral) horizon. The spring activities are mostly quite high in comparison with the other while, the winter activities on contrary, formed the set of ones of the lowest values at all in both layers. In spring, the highest activities of the phosphohydrolases were measured in both layers of Rego Humic Gleysol, the lowest of Orthic Gleysol. The second highest activity was found in autumn (93.8 μg 4-nitrophenol released h^{-1}/g fresh soil, Rego Humic Gleysol in surface horizon), and the second lowest activity ever measured was found in summer (24.0 μg 4-nitrophenol released h^{-1}/g fresh soil, Orthic Gleysol in subsurface horizon).

Dealing with the methodology, it was widely expected that there could be particular analytical

steps unsuitable for soils rich in organic matter. An important fact was that it was not possible to analyse successfully all the humus-rich soil samples by the techniques tested that were principally designed for non-forest soils. The original method developed by Tatabatai and Bremner (1969) was tested on samples with organic carbon ranging from 1.44% to 2.95%, the slight modification presented here was tested on samples ranging in organic carbon from 0.01% to 17.1%.

The modification proposed showed the normal distribution. The coefficients of variance were very low in both horizons. Marginal mean estimations were either the lowest (in the enzyme in surface horizon) or one of the lowest (in the enzyme in subsurface horizon) with all the methods. ANOVA parameter estimations for the enzyme in both horizons reached the lowest values. The modification also had the lowest values of error influence. In comparison with the other basic methods tested, the modification provided the most stable measurements in terms of error influence. The exact data should be discussed in the viewpoint of the inherent heterogeneity of most forest soils where any very prominent decrease in variance could be followed by an increase in the probability of Type I errors. Therefore, the merit of the modification proposed producing lower means and variance compared to those generated by the other methods cannot be derived from the absolutely low data of these statistical parameters.

In order to allow assessing the actual phosphohydrolase activity, the modified technique was begun by analysing fresh soil samples: the approach led to the avoidance of the loss of enzymatic activity (Baligar *et al.* 1988) caused by air-drying the soils. The substrate used, 4-nitrophenyl phosphate, was both rapidly hydrolysed (Bolton *et al.* 1985) and highly sensitive to phosphomonoesterase action (Rojo *et al.* 1990). The substrate concentration was acceptable: no more than 10% of the 4-nitrophenyl phosphate was utilised. Toluene used as a bacteriostatic by Tabatabai and Bremner (1969) was not included in the modification presented because it may exhibit a direct effect on both free enzymes and the substrates of the enzymatic reaction (Scow *et al.* 1989), and may also be used as a substrate by some microbial coenoses (Nannipieri *et al.* 1990). In the testing, furthermore, an increased extraction of humus substances was regularly recorded after adding toluene to the soil suspension. The temperature of 37°C, which was

used by Tabatabai and Bremner (1969) as the incubation temperature for the reaction mixtures, was altered to 25°C (Brams & McLaren 1974). The combination of 60 min and 25°C appeared to be suitable for the field monitoring laboratories. The high pH of the reaction mixture (Tabatabai & Bremner 1969) was altered: using a lower pH for the reaction mixture for acid phosphohydrolase activity had been proposed previously (Trasar-Cepeda & Gil-Sotres 1988). The pH chosen provides a uniform comparative basis for ecological monitoring studies in different habitats. The utilisation of KOH according to its concentration and amount resulted in both immediate and permanent inhibition of further substrate hydrolysis. Following spectrophotometric determinations, the absorbance of 4-nitrophenol at 410 nm is given in weight units (Harrison 1983). The modification assumes no correction for the sorption of 4-nitrophenol by the soil (Malcolm 1983). The amount of 4-nitrophenol produced was determined using the Bouguer-Lambert-Beer Law; it came from the catalytic transformation of the substrate by free and bound enzymes.

In summary, a slightly modified method of Tabatabai and Bremner (1969) was proposed using the knowledge and the combinations of particular analytical steps by Rogers (1942), Kramer and Erdeli (1959), Drobníková (1961), Kuprevitch and Stcherbakova (1966), Hoffmann (1968), Brams and McLaren (1974), Gerritse and van Dijk (1978), Zvyagintsev *et al.* (1980) and Harrison (1983). The standard laboratory approaches (mixing a soil sample with the substrate of enzymatic reaction at a fixed pH level, incubation of the reaction mixture at a fixed temperature, termination of the enzymatic reaction by inhibition, followed by spectrophotometric measurement of the absorbance of the substrate decomposition products at a fixed wavelength) were modified for the determination of acid PME activity in forest soils that are extremely high in humic substances.

Forest soil is characterised by an extremely high number of linkages among the biotic and abiotic compartments. The sensitivity of phosphohydrolases and their linkages to the soil buffering capacity and the adsorption surface of the organo-nomineral complex were universally confirmed (Vuorinen & Saharinen 1996). Due to its high complexity, it was suggested (Klein & Paschke 2000; Griffiths *et al.* 2001) that neither a single

enzyme assay nor the knowledge of the activities of the pool of enzymes could be considered as a good indicator of the soil biological quality. Forest tree species grow for an extremely long time: therefore, the soil components, unavailable for agricultural plant species, can be split into active/bioavailable forms. By transforming organically-bound phosphorus into bioavailable forms in active cycles, phosphohydrolases carry out an essential biochemical function in ecosystems. However, phoshohydrolase activity must not be overestimated: the information about the activity of any particular enzyme is itself inadequate for characterising the biological activity of soil as a whole. On the contrary, the results obtained may reflect actual processes in the metabolic activity of soils. Using such enzymological techniques together with other monitoring methods, valuable results may be obtained.

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