Przegląd Naukowy – Inżynieria i Kształtowanie Środowiska (2016), 25 (3), 323–332
Prz. Nauk. Inż. Kszt. Środ. (2016), 25 (3)
Scientific Review – Engineering and Environmental Sciences (2016), 25 (3), 323–332
Sci. Rev. Eng. Env. Sci. (2016), 25 (3)
http://iks_pn.sggw.pl

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Activity of phosphomonoesterases and the content of phosphorus in the eroded Luvisols of orchard and arable soils
Aktywność fosformonoesteraz oraz zawartość fosforu w zerodowanej glebie płowej użytkowanej sadowniczo i rolniczo

Key words: alkaline and acid phosphatase, erosion, Luvisols
Słowa kluczowe: alkaliczna i kwaśna fosfataza, erozja, gleby płowe

Introduction

The effects of the intensive soil use are visible in the soil cover of most countries of the European Union, posing a serious threat to maintaining the balance in agroecosystems. An irrevocable effect of tillage erosion is a regular upper horizon truncation and the translocation and accumulation of the eroded soil material at local footslope position (De Alba et al., 2004). The erosion in the slope summit zone and the slope shoulder mostly concerns the losses of fine soil fractions and depletion in humus and nutrients (Wojtasik et al., 2008; Van Oost et al., 2009). De Gryze et al. (2008) found that the C, N, P content was lower in the erosion zone of the slope and the erosion effects are much more visible in the part of the field cultivated in the tillage system. The basic task of sustainable agriculture is maintaining the optimal content of nutrients in soil and minimising the losses caused by their migration to surface waters, which can cause eutrophization (Geisseler et al., 2011). Phosphorus is a nutrient indispensable for the adequate growth and development of all the living organisms. An inconsiderable part of phosphorus is available to plants in a form of inorganic orthophosphate. The element enhances the fruit fleshiness, the size and the colour as well as prevents diseases which occur during storage. The biogeochemical phosphorus cycle involves the participation of enzymes, e.g. phosphatases which catalyse the hydrolysis of organic phosphorus compounds to the inorganic forms which can be uptaken by the plant root system (Lemanowicz, 2013). The soil profile truncation as a result of erosion is reflected in decreasing the fertility and productivity of soils. The indicator of changes which occur in intensively used soils can be the measure of enzymatic activity (An et al., 2008). The use of enzymatic indices for the comprehensive evaluation of the ecochemical state of eroded soils can facilitate long-term monitoring and identifying the trends of changes (Fu et al., 2012).

The aim of this paper was to determine the effect of typical field operations in the area with a varied landscape on the content of available phosphorus for the plants as well as the activity of alkaline and acid phosphatase.

Material and methods

The soil profile pits were located on the hill slopes between the slope summit and its footslope in the zone of low-rolling moraine uplands found in the Vistula river glaciation. The paper describes six profiles of eroded Luvisols covered by research project 0700/P06/2003/25 co-financed by Polish Ministry of Science and Higher Education. A common feature of all the soils studied was a lack of horizon Et (*luvic*) in the soil profile. The arable soil was sampled from the soil test pits of arable Luvisols eroded in the vicinity of: Olszewka – P1; Strzelewo – P2; Trzeciewnica – P3 (Central Poland). The soil was sampled in the orchards from the soil test pits performed in the herbicide strips of three apple tree orchards in the vicinity of: Wtelno – S-1 (30-year old orchard); Tryszczyn – S-2 (27-year old orchard); Gościeradz – S-3 (30-year old orchard) (Central Poland). The fertilization with phosphorus treatments was equal to 25 $kg \cdot ha^{-1}$ in arable soils and 40 $kg \cdot ha^{-1}$ in orchard soils for a few years preceding the research.

The soil was sampled from a particular genetic horizon, air-dried and sieved through a 2-mm screen. The following physical and chemical soil properties were determined using standard methods: texture by areometric Casagrande method in Prószyński's modification (soil suspension density is measured at regular time intervals from the start of sedimentation at constant temperature), pH in 1 $mol \cdot l^{-1}$ of KCl – potentiometrically using pH Meter CP-551 – Elmetron (Zabrze, Poland), hydrolytic acidity (Hh) according to the Kappen method, organic carbon content – using Vario MAX CN – Elementar (Hanau, Germany). Cation exchange capacity (CEC) was calculated on the basis of hydrolytic acidity and the content of exchangeable cations (Ca, Mg, K, Na) following the barium chloride method (PN-EN ISO 11260:2011). The analyses of cation concentrations were conducted with the atomic absorption spectrometer (Philips 9100X, Cambridge, UK). The content of available phosphorus (AP) was assayed according to the Egner–Riehm method – DL (Egner et al., 1960). Phosphorus was determined with the spectrophotometer Genesis 6 (Madison, USA). The activity of alkaline (AlP) [EC 3.1.3.1] and acid phosphatase (AcP) [EC 3.1.3.2] according to Tabatabai and Bremner (1969) is based on the colorimetric determination of freed substrate: p-nitrophenol after the incubation and the soil samples for 1 h at the temperature of 37°C. All laboratory analyses were performed in three replications (arithmetic mean) of each soil sample. The statistical analysis for surface horizons A and Ap of the profiles investigated was made using the Anova (Statistica 7.0, StatSoft Inc, Tulsa, USA) software for the experiment with a single factor: the method of soil use (the first level of the factor: orchard soil, the second factor level: arable soil) in completely randomised design. The differences at *p* <0.05 are considered significant. The descriptive statistical analysis, such as Pearson's correlation coefficients and cluster analysis with Ward's method (1963), were calculated using Statistica 7.0 (StatSoft Inc, Tulsa, USA).

Results and discussion

In the surface horizons the soils under study demonstrated the grain size composition of sandy loam, while in the subsurface horizons, most often, loam (Tables 1, 2). The content of the clay

TABLE 1. Properties of arable soils
TABELA 1. Właściwości gleb ornych

Symbol	Profiles Profile	Fraction Frakcja <2.0 μm	TOC	pH_{KCl}	TEB	Hh	CEC	AP	AlP	AcP
		%	$g \cdot kg^{-1}$		$cmol_{+}kg^{-1}$			$mg \cdot kg^{-1}$	$mM\ pNP \cdot kg^{-1} \cdot h^{-1}$	
P1	Ap	17	11.5	7.15	19.6	0.4	20.0	63.6	0.457	1.002
	Bt1	22	3.6	6.68	20.4	0.6	21.0	17.9	0.242	0.411
	Bt2	19	1.3	6.38	16.5	1.1	17.6	20.4	0.060	0.162
	Ck1	15	0.4	7.01	19.8	0.3	20.1	1.4	0.029	0.030
	Ck2	15	0.3	7.00	19.2	0.2	19.4	1.3	0.022	0.029
P2	Ap	12	9.7	6.91	15.8	0.6	16.4	93.9	0.640	0.861
	Bt1	17	2.4	6.69	15.4	0.6	16.0	57.0	0.160	0.198
	Bt2	19	0.6	6.35	13.2	0.7	13.9	39.8	0.030	0.083
	Ck1	16	0.2	7.13	12.0	0.3	12.3	11.3	0.010	0.026
	Ck2	15	0.1	7.11	13.7	0.1	13.8	7.1	0.006	0.008
P3	Ap	12	9.9	5.65	11.3	1.5	12.8	42.3	0.601	0.840
	Bt1	19	3.4	6.00	14.2	0.9	15.1	18.5	0.245	0.284
	Bt2	18	0.7	6.88	16.9	0.6	17.5	26.3	0.075	0.188
	Ck1	15	0.3	7.21	13.9	0.3	14.2	1.5	0.009	0.038
	Ck2	14	0.2	7.20	12.1	0.2	12.3	3.9	0.003	0.018

TOC – total organic carbon, TEB – total exchangeable bases, Hh – hydrolytic acidity, CEC – cation exchange capacity, AP – phosphorus available to plants, AlP – alkaline phosphatase, AcP – acid phosphatase.
TOC – zawartość ogólnego węgla organicznego, TEB – suma zasadowych kationów wymiennych, Hh – kwasowość hydrolityczna, CEC – kationowa pojemność wymienna, AP – zawartość przyswajalnego fosforu, AlP – fosfataza alkaliczna, AcP – fosfataza kwaśna.

fraction ranged from 12 to 23% with the characteristic accumulation in illuvial horizon Bt. Soils in orchards contained more carbon of organic compounds (13.9–17.0 $g \cdot kg^{-1}$) as compared with their content in horizon Ap of arable soils (9.7–11.5 $g \cdot kg^{-1}$).

Cation exchange capacity in arable soils ranged from 12.3 to 21.0 $cmol_{+}kg^{-1}$ (Table 1), while in the soils of apple tree orchards it ranged from 12.8 to 16.2 $cmol_{+}kg^{-1}$ (Table 2). The soil material in the surface horizon of apple tree orchards showed acid and very acidic reaction (pH_{KCl}), whereas in arable soils – the slightly acid and neutral reaction (Kobierski, 2006).

A significantly higher mean content of clay fraction and Hh in the soil materials sampled from orchard soil profiles, as compared with arable soil profiles was recorded (Table 3). The mean contents of the TEB and CEC were significantly higher in profiles of arable soils as compared with orchard soils.

The content of phosphorus available to plants in the soil of the apple tree orchards herbicide strip fell within a wide range from 1.60 to 92.1 $g \cdot kg^{-1}$ and it was the highest in the surface layer. The content of AP was clearly decreasing deep down each of the soil profiles, which must be due to an inconsiderable phosphorus mobility in soil (Wright, 2009; Xu et al., 2012). In the

TABLE 2. Soil properties in orchards
TABELA 2. Właściwości gleb w sadach

Symbol	Profiles Profile	Fraction Frakcja <2.0 μm	TOC	pH_{KCl}	TEB	Hh	CEC	AP	AlP	AcP
		%	$g \cdot kg^{-1}$		$cmol_{+}kg^{-1}$			$mg \cdot kg^{-1}$	$mM\ pNP \cdot kg^{-1} \cdot h^{-1}$	
S1	A	17	13.9	5.13	13.5	1.8	15.3	92.1	1.719	2.839
	Bt1	19	3.5	4.90	11.4	1.6	13.0	18.2	0.515	1.304
	Bt2	21	1.9	5.14	11.6	1.2	12.8	16.5	0.329	1.238
	BCk	21	0.8	7.42	12.6	0.4	13.0	5.5	0.202	0.436
	Ck	19	0.2	7.63	12.5	0.3	12.8	3.0	0.094	0.194
S2	A	18	17.0	5.43	12.6	2.1	14.7	61.8	1.762	2.905
	Bt1	19	2.3	5.19	12.2	2.4	14.6	14.4	0.296	0.938
	Bt2	22	1.6	5.88	13.8	1.3	15.1	12.9	0.203	0.723
	BCk	19	1.0	7.24	13.2	0.4	13.6	3.4	0.137	0.286
	Ck	18	0.4	7.36	12.9	0.4	13.3	2.0	0.094	0.152
S3	A	17	14.3	4.17	13.6	2.6	16.2	69.5	1.896	2.663
	Bt1	18	2.3	4.76	12.5	1.8	14.3	17.8	0.327	0.891
	Bt2	23	1.8	4.77	11.9	1.6	13.5	4.4	0.268	1.094
	BC	20	0.8	5.51	13.5	1.2	14.6	3.7	0.168	0.607
	C	18	0.3	6.14	12.4	0.8	13.2	1.6	0.126	0.448

Symbols, see Table 1/ Objaśnienia symboli patrz tab. 1.

TABLE 3. Results of statistical analysis (for Anova, the Tukey test)
TABELA 3. Wyniki analizy statystycznej (Anova, test Tukeya)

Parameter Parametr	Arable soils Gleby orne $n = 15$	Orchard soils Gleby w sadach $n = 15$	Significant level Poziom istotności (p)
	mean content		
Clay fraction	16.3	19.3	<0.005
TEB	15.6	12.7	<0.005
Hh	0.56	1.33	<0.005
CEC	16.2	14.0	<0.05

Symbols, see Table 1 / Objaśnienie symboli patrz tab. 1.

arable soil profiles the content of available phosphorus fell within a wide range (1.3–93.9 g·kg^{-1}) and it was the highest in horizon Ap (42.3–93.9 g·kg^{-1}).

The eroded surface genetic horizons of soils in the summit part of the slopes under cultivation make the depletion in nutrients available to plants and their accumulation in local toe slopes (De Gryze et al., 2008). The soil enzymatic activity reflects the course of biochemical processes as well as basic transformations connected with the soil biology against the present physical and chemical properties (Nannipieri et al., 2011). The activity of alkaline phosphatase in the profiles of eroded arable Luvisols ranged from 0.003 to 0.640 mM pNP·kg^{-1}·h^{-1} (Table 1). In the soil material sampled from the profiles located in herbicide strip of apple tree orchards, the activity of this enzyme was relatively higher and it was 0.094–1.896 mM pNP·kg^{-1}·h^{-1} (Table 2). The activity of acid phosphomonoesterase was similar. According to Zydlik et al., (2011), in the soil of herbicide strips in orchards a decrease (can be recorded) in the enzymatic activity as a result of triazine herbicides applied. Hence, a higher mortality rate of specialised soil microorganisms which, besides the plant roots, are a source of enzymes. In the arable field soils, the AcP activity ranged from 0.008 to 1.002 mM pNP·kg^{-1}·h^{-1} and it was lower, as compared with the herbicide belt soil (0.152–2.905 mM pNP··kg^{-1}·h^{-1}). According to An et al. (2008), a lower enzymatic activity of eroded soils is a result of depletion in nutrients, organic matter, soil microorganisms, as a result of including the eluvial horizon (Et) to the thickness of the plough horizon (Ap). The activity of alkaline and acid phosphatase was the highest in the surface horizons of all the soils under study and it was decreasing with depth. The decreasing enzymatic activity is connected with the spatial distribution of humus and soil microorganisms as well as a decreasing amount of carbon substrate available to microorganisms (Kizilkaya and Dengiz, 2010).

With the analysis of variance made for the surface horizons of the soils, there was found a significant effect of their method of use on the activity of phosphatases (Table 4). A higher activity of alkaline (1.789 mM pNP·kg^{-1}·h^{-1}) and acid phosphatase (2.803 mM pNP··kg^{-1}·h^{-1}) was noted in the soil of herbicide strips. However, there was found no significant effect of the soil use method on the content of available phosphorus. According to the criteria provided for in PN-R-04023:1996, the mean content of phosphorus available to plants in the surface layer of arable soils classifies the soils as demonstrating an average rich-

TABLE 4. Statistical analysis of results
TABELA 4. Analiza statystyczna wyników badań

Specification Wyszczególnienie	TOC [mg·kg^{-1}]	AP [mg·kg^{-1}]	AlP	AcP
			mM pNP·kg^{-1}·h^{-1}	
Orchard soils (horizon A)	15.06[a]	74.46	1.789[a]	2.803[a]
Arable soils (horizon Ap)	10.36[b]	66.00	0.566[b]	0.901[b]
$LSD_{0.05}$	3.132	n.s.	0.219	0.247
Mean	12.72	70.53	1.178	1.852
SD	2.855	19.66	0.675	1.046

[a, b] – Values with letters differ significantly at significance level *p*.
[a, b] – wartości opisane literami istotnie różnią się przy poziomie istotności *p*.
Significant at p <0.05, n.s. – non-significant difference, *SD* – standard deviation, Symbols, see Table 1/ /poziom istotności p <0,05, n.s. – nie istotne statystycznie, *SD* – odchylenie standardowe. Objaśnienie symboli patrz tab. 1.

ness class, while the soils under orchard use – a high content of AP (Table 4). In such a case preservative fertilisation with that nutrient is recommended. A significantly higher content of carbon of organic compounds was noted in horizon A of apple tree orchards.

The respective soils under orchard use or arable soils in their upper horizon showed slightly different physical and chemical properties, the content of available phosphorus and the activity phosphatase, which is confirmed by cluster analysis (Euclidean distance). A small distance between the variables is

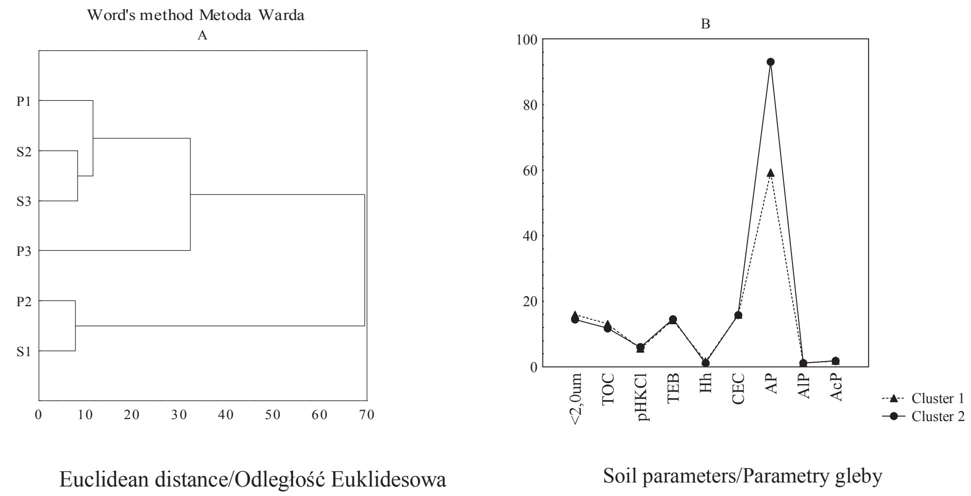

FIGURE. Cluster analysis (Euclidean distance) of soil parameters (A) (<2.0 μm, TOC, pH_{KCl}, TEB, Hh, CEC, AP, AlP, AcP) in the upper horizon arable soil profiles P1-P3 and orchard soil profiles S1-S3 and the graph of k-means clustering method (B)
RYSUNEK. Analiza skupień (odległość Euklidesowa) parametrów gleby (A) (<2,0 μm, TOC, pH_{KCl}, TEB, Hh, CEC, AP, AlP, AcP) w poziomie powierzchniowym profili gleb uprawnych P1-P3 i profili gleb w sadach S1-S3 oraz wykres metody k-średnich (B)

an indication of similarity and vice versa (Fig. A). Based on the conducted data clustering with Ward's method (1963), it was stated that cluster 1 includes four soil samples (cases S2, S3, P1, P3); while two soil samples (cases P2 and S1). Interpreting results of statistical analysis, it was used k-means clustering method. We found two clusters, where the cluster 2 (the case of P2 and S1) indicates the low accumulation of AP, while the other remaining parameters were in the same level (Fig. B).

With the statistical analysis of the results (Table 5), there was found a highly significant positive correlation between the content of available phosphorus and the activity of alkaline phosphatase (r = = 0.79, p <0.05) as well as acid phosphatase (r = 0.78, p <0.05) in arable soil profiles (Table 5). The practically complete dependence was noted in those soils between the content of carbon of organic compounds and the activity of alkaline phosphatase activity (r = 0.96 and acid r = 0.99, p <0.05).

Organic matter in soil plays a protective function for the enzymes prolonging the period of their activity, especially in unfavourable conditions of the soil environment (An et al., 2008). This is confirmed in the present study in soils covered by tillage erosion, where a lower content of available phosphorus (as well as carbon of organic compounds) decreased the activity of phosphatases. The practically complete dependence between the content of AP and the activity of AlP (r = = 0.96, p <0.05) and AcP (r = 0.94, p <0.05) as well as TOC (r = 0.95, p <0.05) was noted for the soils of the herbicide strip in apple tree orchards (Ta-

TABLE 5. Pearson's correlation coefficients
TABELA 5. Wartości współczynników korelacji Pearsona

Profiles Profile	pH_{KCl}	AlP	AcP	CEC	Hh	TOC
Arable soils / Gleby orne (n = 15)						
AP	n.s.	0.79	0.78	n.s.	n.s.	0.77
TOC	n.s.	0.96	0.99	n.s.	n.s.	–
TEB	n.s.	n.s.	n.s.	0.99	n.s.	–
Hh	0.84	0.53	n.s.	n.s.	–	–
AcP	n.s.	0.95	–	–	–	–
AlP	0.51	–	–	–	–	–
Orchard soils w sadach (n = 15)						
AP	n.s.	0.94	0.96	0.69	0.64	0.94
TOC	n.s.	0.99	0.96	0.67	0.66	–
TEB	n.s.	n.s.	n.s.	0.69	n.s.	–
Hh	0.63	0.67	0.76	0.72	–	–
AcP	0.57	0.96	–	–	–	–
AlP	0.52	–	–	–	–	–

Significant at p <0.05, n.s. – non-significant. Symbols, see Table 1 / Poziom istotności p <0,05, n.s. – nie istotne statystycznie. Objaśnienie symboli patrz tab. 1.

ble 5). One commonly observes a linear dependence between the activity of acid phosphatases and the amount of inorganic forms of phosphorus released to the soil solution (Nannipieri et al. 2011; Lemanowicz and Bartkowiak, 2015). The significant dependence noted between the activity of alkaline and acid phosphatase, and pH_{KCl} as well as a highly significant positive correlation between the hydrolytic acidity points to a high susceptibility of phosphomonoesterases to the changes in the soil reaction in orchards, which was also demonstrated in the research by Lemanowicz (2013).

Conclusion

The state of the soil environment determined the activity of the phosphomonoesterases studied, which is confirmed by the statistical analysis of the results between pH_{KCl}, hydrolytic acidity, cation exchange capacity, and the activity of alkaline and acid phosphatases. The analysis of variance found a significant effect of the soil use method on the activity of phosphatases in their surface horizons. A higher activity of phosphatases was noted in the soil surface horizon of the herbicide strip, rich in organic matter, as compared with the activity of those enzymes in arable soils, showing a similar structure of the soil profile (without Et horizon) and the parent material properties.

There was reported no significant effect of the field operations on the content of available phosphorus in the surface horizons of the profiles. The research of the activity of alkaline and acid phosphatase can be used to evaluate the changes in the content of phosphorus available to plants, which is seen from significant values of the coefficients of correlation between those parameters. The study of eroded soils should be continued since it can facilitate the ecological effects of tillage erosion on the soil environment.

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Summary

Activity of phosphomonoesterases and the content of phosphorus in the eroded Luvisols of orchard and arable soils. An irrevocable effect of the process of tillage erosion is truncation of the surface horizon and the translocation and accumulation of the eroded soil material at the foot of the slope. It concerns mostly fine soil fractions, humus and nutrients (C, N, P). The rate of that process depends on the original morphology of soil profiles and the susceptibility to erosion, the amount and intensity of precipitation, but mostly on the method and period of soil use.

The aim of the paper was to determine the effect of the use of eroded Luvisols on the content of available phosphorus and the activity of phosphomonoesterases against the physico-chemical properties selected. Based on the analysis of variance, there was found a significant effect of the use of soils on the activity of phosphatases in the surface horizons of the analysed soils. There was found a significantly higher activity of alkaline phosphatase (0.094–1.896 mM $pNP \cdot kg^{-1} \cdot h^{-1}$) and acid phosphatase (0.152–2.905 mM $pNP \cdot kg^{-1} \cdot h^{-1}$) in the soil material sampled from the herbicide strips of 30-year apple tree orchards, as compared with the activity of those enzymes in arable soils, with a similar soil profile morphology (without Et horizon), grain size composition and the properties of the parent material. Activity of phosphatases in the soil surface horizon of the herbicide strips was positively and significantly correlated with organic matter. There was reported no significant effect of the use of the eroded Luvisols on the content of available phosphorus in surface horizons.

Streszczenie

Aktywność fosformonoesteraz oraz zawartość fosforu w zerodowanej glebie płowej użytkowanej sadowniczo i rolniczo. Nieodwracalnym skutkiem procesu erozji uprawowej jest spłycanie poziomu powierzchniowego oraz translokacja i akumulacja zerodowanego materiału glebowego u podnóża stoku. Dotyczy to głównie drobnych frakcji glebowych, próchnicy oraz składników pokarmowych (C, N, P). Tempo tego procesu zależy od pierwotnej budowy profilu glebowego i podatności na erozję, ilości i natężenia opadów atmosferycznych, ale przede wszystkim od sposobu i okresu użytkowania gleb. Celem pracy było określenie wpływu sposobu użytkowania zerodowanych gleb płowych na zawartość fosforu przyswajalnego i aktywność fosfomonoesteraz na tle wybranych właściwości fizyko-chemicznych. Na podstawie analizy wariancji stwierdzono istotny wpływ sposobu użytkowania na aktywność fosfataz w poziomie powierzchniowym badanych gleb. Stwierdzono istotnie dużą aktywność fosfatazy alkalicznej (0,094–1,896 mM pNP$\cdot$kg$^{-1}\cdot$h^{-1}) i fosfatazy kwaśnej (0,152–2,905 mM pNP$\cdot$kg$^{-1}\cdot$h^{-1}) w profilach glebowych pobranych z pasów herbicydowych 30-letnich sadów jabłoniowych w porównaniu do aktywności tych enzymów w glebie użytkowanej rolniczo o podobnej budowie morfologicznej (bez poziomu Et), składzie granulometrycznym i właściwościach skały macierzystej. Aktywność fosfataz w glebie poziomów powierzchniowych pasów herbicydowych była istotnie dodatnio skorelowana z zawartością materii organicznej. Stwierdzono brak istotnego wpływu sposobu użytkowania zerodowanych gleb płowych gleb na zawartość fosforu dostępnego dla roślin w poziomach powierzchniowych.

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