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Assessment of soil phosphatase activity, phosphorus and heavy metals content depending on the mineral fertilization
Ocena aktywności fosfataz, zawartości fosforu i metali ciężkich w zależności od nawożenia mineralnego

Key words: alkaline and acid phosphatase, heavy metals, mineral fertilization, phosphorus, total organic carbon
Słowa kluczowe: alkaliczna i kwaśna fosfataza, metale ciężkie, nawożenie mineralne, fosfor, całkowity węgiel organiczny

Introduction

Brassica napus L. is one of the key oilseed crops, however its nutrition requirements, e.g. as compared with winter wheat, are twice as high as in the case of nitrogen, phosphorus or potassium. In the system of balanced fertilization, satisfying the nutrition requirements of plants at the level adjusted to the expected yields follows the principle of the right nutrients management in the soil–plant system (Lemanowicz and Bartkowiak, 2013a). Those functions can be played in terms of ensuring the adequate fertilization with both macro- and microelements (Zakarauskaitë et al., 2008). Unfortunately, recently it has been observed that to increase the yields, excessive amounts of nitrogen fertilizers are applied without maintaining the right proportions with the other nutrients (Lemanowicz, 2013; Bartkowiak and Lemanowicz, 2014). Unfavorable changes can occur as a result of many-year application of unbalanced mineral fertilization (He et al., 2005). It can lead to disturbed physiological functions of the plants and, as a result, to reduced yields and a decrease in soil fertility (Guala et al., 2010). They are seen first as a change in the soil acidity and then as changes in the content of available forms of nutrients, including heavy metals. The Regulation of Minister of the Environment (2002), based on the Nitrate Directive (European Council, 1991), was the first step to reduce the negative impact of agriculture on the environment. Fertilizers usually contain some amounts of heavy metals which, having been introduced into soil, increase its concentration of available forms. Mineral fertilizers most contaminated with heavy metals are as follows: phosphorus > calcium > potassium > nitrogen fertilizers (Conceição et al., 2013) at the same time heavy metals are components of some enzymes; they are also indispensable for the right pattern of biochemical processes, they affect the metabolism of soil microorganisms causing the denaturation of proteins as well as the destruction of cell membranes (Kandeler et al., 2000). To evaluate the state of soil contamination, biological methods are used; hence the use of the measurements of the enzymatic activity, mostly dehydrogenases, phosphatases, ureases and proteases (Hinojosa et al., 2008; Lemanowicz and Bartkowiak, 2013b). The enzymes reacting fastest to the increase in the content of heavy metals in soil include alkaline phosphatase and acid phosphatase, catalyzing the hydrolysis of organic phosphorus bonds. The activity of phosphomonoesterases is used to evaluate the potential rate of mineralization of those compounds in soil. Their activity depends on various factors as the soil type and its fertility, type of fertilization and nutrient management, organic matter, soil pH and varieties of higher plant species.

This research was undertaken to ascertain changes in soil: the content of total organic carbon, available phosphorus and available forms of selected heavy metals (zinc, copper, lead and cadmium) against selected chemical properties, the acid and alkaline phosphatase activity under the effect of application of mineral fertilization under *Brassica napus*.

Material and methods

The soil for laboratory analyses sampled from static field experiment set up by the Department of Plant Nutrition and Fertilisation of the Institute of Soil Science and Plant Cultivation in Puławy in the area of the Experiment Station in Grabowo upon Vistula in the Mazowieckie province, Zwoleński county, Przyłęk commune (Poland). The location of the experiment station is determined by altitude 51°21′8″N and longitude 21°40′8″E; the lowland climate of moderate altitudes. The experiment (in years 2005–2008) was performed on the soil typical of Poland, classified as light loamy and sand texture (haplic luvisol, LVha) according to FAO soil classification (Rutkowska and Pikuła, 2013). The studied soils were very similar in granulometric composition with the clay fraction in the range of 4.34 to 5.26%, and have been classified as sandy loam texture according to USDA (United States Department of Agriculture) soil classification. Soil exchangeable acidity ranged from 5.1 to 5.8, which classifies the soils as slightly acid and acid. The soil was sampled from horizon Ap (0–20 cm) after *Brassica napus* harvest. Four replications of the cultivated plots of 40 m^2 acreage were created. The experiment was carried out as a two-factor experiment, in randomized block design in three reps. The first factor was fertilization with phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulphur (S) in six fertilizer combinations: 1 – PKMgCaS, 2 – KMgCaS, 3 – PMgCaS, 4 – PKCaS, 5 – PKMgS, 6 – PKMgCa. The second factor was nitrogen fertilization in the form of ammonium nitrate (34% N) at the rates of: N0 – 0, N1 – 50, N2 – 100, N3 – 150, N4 – 200, N5 – 250 kg·ha^{-1} of N. The following fertilizer forms were used: for the treatments with sulphur there were applied phosphorus and potassium fertilizers containing sulphur – single superphosphate and potassium sulfate(IV), for the treatment without sulphur there were used phosphorus and potassium fertilizers which do not contain sulphur: triple superphosphate and high-percentage potassium salt, for the treatment with calcium and magnesium there was applied dolomite containing 21.3% Ca and 6% Mg, in the plots without magnesium, lime was used at the amount of 142 kg·ha^{-1} of Ca, while in the case of calcium deficit, magnesium sulfate was supplied at the rate of 42 kg·ha^{-1} of Mg. The rates of minerals applied in the experiment were as follows: 39.2 kg·ha^{-1} of P, 107 kg·ha^{-1} of K, 42.2 kg·ha^{-1} of Mg, 143 kg·ha^{-1} of Ca and 20 kg·ha^{-1} of S. The experiment was performed in a four-year crop rotation: winter wheat + intercrop, maize grown for grain, spring barley, winter rape (Table 1).

Field-moist samples were sieved (2-mm mesh size) and stored in a plastic box at 4°C for not less than 2 days in order to stabilize the microbial activity and then were analyzed for phosphomonoesterases activity within one week. Determined the activity of selected enzymes representing the class of hydrolases: alkaline phosphatase [E.C. 3.1.3.1] (AlP) and acid phosphatase [E.C. 3.1.3.2] (AcP) with the method of Tabatabai and Bremner (1969), based on the colorimetric determination of freed substrate: p-nitrophenol (pNP) after the incubation of soil with of MUB (modified universal buffer) at pH 6.5 for acid phosphatase and pH 11.0 for alkaline phosphatase samples for 1 h at the temperature of 37°C. A subset was air dried in the laboratory and stored in closed polyethylene bags for physicochemical analysis. In the soil material the following were assayed: pH in 1M KCl measured potentiometrically (PN-ISO 10390:1997) the content of available phosphorus (AP) according to the Egner-Riehm method (DL) accord-

TABLE 1. Rates and the fertilizers applied in a four-year crop-rotation
TABELA 1. Dawki i nawożenie stosowane w czteroletnim zmianowaniu

Year Rok	Rates of mineral fertilization/Dawki nawozów mineralnych [kg·ha^{-1}]										
	P	K	Mg	Ca	S	N0	N1	N2	N3	N4	N5
Winter wheat/ /Pszenica ozima (2005)	30.5	74.7	42.2	143	20	0	40	80	120	160	200
Maize/Kukurydza (2006)	34.9	116	42.2	143	20	0	50	100	150	200	250
Spring barley/ /Jęczmień jary (2007)	30.5	66.4	42.2	143	20	0	30	60	90	120	150
Winter rape/Rzepak ozimy (2008)	39.2	107	42.2	143	20	0	50	100	150	200	250

N0, N1, N2, N3, N4, N5 – rates of the nitrogen.

ing to PN-R-04023:1996 which involves the spectrophotometric measurement of the intensity of the color of phosphorus-molybdenum blue created by orthophosphoric ions with molybdenum ions in the acid environment in the presence of tin(II) chloride. Total organic carbon with the use of the TOCN FORMACTS™ analyzer provided by SKALAR. Besides, there was assayed the content of available forms of zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) extracted with the DTPA (1M diethylenetriaminepentaacetic acid), solution according to PN-ISO 14870:2007 The content of available forms was determined using the atomic absorption spectrophotometry method on PU 9100X spectrometer (Philips). All the assays were made in three reps; the paper demonstrates the arithmetic means of the results.

The results were exposed to the analysis of variance and the significance of differences across the means was verified with the Tukey test at the confidence level of $p = 0.05$. For the purpose of the calculations, there was applied Microsoft Excel-based ANOVA (Statistica 7.0 StatSoft Inc, Tulsa, USA). At the same time the results of the analyses of the properties investigated were exposed to the analysis of simple correlation ($p < 0.05$), to define the level of dependence between the respective properties. The analysis of the correlation was performed with Statistica 8.1 for Windows PL software. The coefficient of variation (CV) of the parameters of mineral nitrogen was calculated from the formula: $CV = (SD / X) \cdot 100$, where: CV – coefficient of variation (%), SD – standard deviation, X – arithmetic mean. The values of 0–15, 16–35 and above 36% indicate low, moderate, or high variability, respectively.

Results and discussion

The exchangeable acidity (pH_{KCl}) measured in the plough horizon of the soil investigated ranged from 5.1 to 5.8 (Table 2). Based on those values the soil has been classified to represent acid and slightly acid soils. The mineral fertilisation slightly differentiated the soil reaction. Nitrogen in ammonium form contributes to the soil solution acidification both as a result of the nitrification process and the uptake of cation NH_4^+ by the root system of the plants.

The amount of the organic substance is mostly connected with the type and the soil type. The variation in the content

TABLE 2. Values of pH_{KCl} in soil samples
TABELA 2. Wartości pH_{KCl} próbek glebowych

Treatments Zabiegi	Nitrogen fertilization Nawożenie azotowe [kg·ha^{-1}]					
	N0	N1	N2	N3	N4	N5
1	5.8	5.6	5.6	5.5	5.3	5.2
2	5.8	5.7	5.7	5.5	5.4	5.3
3	5.8	5.8	5.6	5.6	5.5	5.5
4	5.7	5.7	5.6	5.7	5.5	5.4
5	5.5	5.6	5.5	5.3	5.2	5.1
6	5.7	5.8	5.7	5.8	5.7	5.6

of that parameter also depends on their different use and fertilization (Kong et al., 2005; Bęś and Warmiński 2015). The content of TOC in the soil investigated due to the factors applied was low (7.47 g·kg^{-1} mean for the entire experiment) defined for soil, which was due to a lack of natural or organic fertilization, which usually increases the content of TOC in soil even by 27% (Steiner et al., 2007; Lemanowicz et al., 2014a). There was reported a significant effect of experimental factors on the content of total organic carbon. Increasing nitrogen rates increased the content of total organic carbon. The highest content of TOC was recorded in the soil fertilized with nitrogen at the rate of 250 kg·ha^{-1} (7.59 g·kg^{-1}). Fertilization with the combination without sulphur (PKMgCa) also increased the content of total organic carbon (7.97 g·kg^{-1}) (Table 3).

The content of available P in soil ranged from 38.55 to 95.03 mg·kg^{-1} (mean for the experiment 57.32 mg·kg^{-1} of AP) (Table 3), which according to PN-R-04023:1996 classifies it as class III with an average content of that nutrient. There was found a significant effect of the experimental factors applied on the changes in the content of P available to plants. The lowest (45.85 mg·kg^{-1} of AP on average) content of AP was recorded in the soil fertilized without P (KMgCaS). Complete mineral fertilization (PKMgCaS) increased AP by 27% (62.53 mg·kg^{-1} of AP on average). Increasing nitrogen rates resulted in a regular decrease in the content of P available in soil. The lowest content of AP was reported in the soil sampled from the treatments with nitrogen fertilization at the rate of 250 kg·ha^{-1} of N (43.27 mg·kg^{-1} of AP on average), which changed the soil classification in terms of the richness in P from average to low. In that case one should apply increased phosphorus fertilization. Application of increasing N rates results in a decrease in soil fertility, an increase in its acidity, a decrease in the share of alkaline cations in the sorption capacity and in a decrease in the content of mobile forms of nutrients, including P. Most P is released when pH of soil ranges from 6 to 7 (Lemanowicz et al., 2014b). The content of the available phosphorus in soil showed a moderate variability (CV = 23.75%) (Table 3). According Lemanowicz et al. (2013), a lack of organic fertilization and unbalanced mineral fertilization decreased the contents of the P form available to plants. Long term field experiments, such as those carried out at the Rothamsted's research, have shown that the use of farm yard manure (FYM) has positive effects on the P content of agricultural soils as compared to chemical fertilizers, which contain highly soluble P. Application NPK on the long term causes high P losses from soil due to the relatively P retention capacity of soils, with detrimental effects on the surface and groundwater (Fortune et al., 2005).

The activity of alkaline and acid phosphatase in soil depended on the mineral fertilization applied. A lack of phosphorus fertilization (KMgCaS) increased the activity of the phosphatases (alkaline – 1.588 mM pNP·kg^{-1}·h^{-1}, acid – 3.297 mM pNP·kg^{-1}·h^{-1} – Table 4). It is connected with the fact that an available P deficit in the soil sampled from the plots increases the production and the secretion of acid extracellular phosphatases to the subsoil, and so the activity of phosphatases is, to a large extent, conditioned

TABLE 3. The content of total organic carbon (TOC) and available phosphorus (AP) in soil samples
TABELA 3. Zawartość węgla organicznego (TOC) i fosforu przyswajalnego (AP) w próbkach glebowych

Treat-ments Zabiegi	Total organic carbon/Węgiel organiczny [g·kg^{-1}]							Available phosphorus/Fosfor przyswajalny [mg·kg^{-1}]						
	nitrogen fertilization/nawożenie azotowe [kg·ha^{-1}]													
	N0	N1	N2	N3	N4	N5	mean	N0	N1	N2	N3	N4	N5	mean
1	7.28	7.28	7.15	7.02	7.87	7.28	7.31	75.73	71.33	68.30	61.38	51.93	46.53	62.53
2	6.96	7.15	5.98	7.28	7.67	7.15	7.03	51.99	49.21	48.21	44.04	42.69	38.55	45.86
3	6.57	5.53	7.22	7.41	8.78	7.41	7.12	85.08	65.89	60.75	55.60	50.45	41.66	59.91
4	5.66	8.39	7.54	7.80	8.78	7.41	7.59	95.02	68.41	62.70	59.75	48.78	46.98	63.61
5	7.35	7.93	7.74	8.06	8.19	7.48	7.79	76.66	60.43	56.87	54.59	49.48	46.66	57.45
6	7.41	8.19	7.93	7.02	8.45	8.84	7.97	78.74	69.14	49.60	47.17	43.31	39.27	54.54
Mean	6.87	7.38	7.26	7.43	8.29	7.59	7.47	77.20	64.07	57.82	53.76	47.77	43.27	57.32
$LSD_{0.05}$ I 0.64 II 0.64 II/I 1.56 I/II 1.56								$LSD_{0.05}$ I 2.068 II 2.068 II/I 5.066 I/II 5.066						
SD 8.35 *CV* 11.20								*SD* 13.61 *CV* 23.75						

1 – PKMgCaS, 2 – KMgCaS, 3 – PMgCaS, 4 – PKCaS, 5 – PKMgS, 6 – PKMgCa, *SD* – standard deviation, *CV* [%] – coefficient of variation.

TABLE 4. The activity of alkaline (AlP) and acid phosphatase (AcP) in soil samples
TABELA 4. Aktywność fosfatazy alkalicznej (AlP) i fosfatazy kwaśnej (AcP) w próbkach glebowych

Treat-ments Zabiegi	Alkaline phosphatase/Fosfataza alkaliczna [mM pNP·kg^{-1}·h^{-1}]							Acid phosphatase/Fosfataza kwaśna [mM pNP·kg^{-1}·h^{-1}]						
	nitrogen fertilization/nawożenie azotowe [kg·ha^{-1}]													
	N0	N1	N2	N3	N4	N5	mean	N0	N1	N2	N3	N4	N5	mean
1	1.526	1.466	1.408	1.366	1.335	1.298	1.400	1.866	1.972	2.070	2.138	2.237	2.304	2.098
2	1.865	1.723	1.615	1.534	1.434	1.255	1.588	3.970	3.144	3.261	3.375	3.414	3.495	3.297
3	1.482	1.425	1.370	1.298	1.236	1.209	1.337	1.710	1.792	1.835	1.935	2.004	2.072	1.891
4	1.432	1.401	1.358	1.296	1.258	1.203	1.325	2.598	2.674	2.744	2.836	2.931	2.996	2.796
5	1.418	1.372	1.328	1.260	1.224	1.163	1.294	2.957	3.041	3.170	3.322	3.372	3.464	3.221
6	1.450	1.399	1.357	1.311	1.249	1.231	1.333	2.508	2.572	2.633	2.718	2.872	3.011	2.719
Mean	1.529	1.464	1.406	1.344	1.289	1.243	1.379	2.456	2.533	2.619	2.721	2.805	2.890	2.670
$LSD_{0.5}$ I 0.011 II 0.011 II/I 0.027 I/II 0.027 *SD* 0.145 *CV* 10.53									*LSD* I 0.018 II 0.018 II/I 0.044 I/II 0.044 *SD* 0.554 *CV* 20.74					

1 – PKMgCaS, 2 – KMgCaS, 3 – PMgCaS, 4 – PKCaS, 5 – PKMgS, 6 – PKMgCa, *SD* – standard deviation, *CV* [%] – coefficient of variation.

TABLE 5. The content of available zinc (Zn) and cooper (Cu) in soil samples
TABELA 5. Zawartość przyswajalnego cynku (Zn) i miedzi (Cu) w próbkach glebowych

Treatments Zabiegi	Zinc/Cynk [mg·kg^{-1}]							Copper/Miedź [mg·kg^{-1}]						
	nitrogen fertilization/nawożenie azotowe [kg·ha^{-1}]													
	N0	N1	N2	N3	N4	N5	mean	N0	N1	N2	N3	N4	N5	mean
1	1.511	1.295	1.409	1.293	1.387	1.439	1.389	0.369	0.289	0.341	0.331	0.357	0.339	0.338
2	1.351	1.155	1.099	1.147	1.167	1.375	1.216	0.389	0.331	0.263	0.353	0.355	0.409	0.349
3	1.422	1.411	1.435	1.315	1.429	1.317	1.388	0.389	0.428	0.438	0.411	0.359	0.459	0.414
4	1.109	1.517	1.093	1.183	1.479	1.117	1.250	0.353	0.381	0.359	0.385	0.369	0.383	0.372
5	1.469	1.389	1.655	1.663	1.447	1.679	1.550	0.497	0.469	0.633	0.427	0.459	0.475	0.493
6	1.353	1.241	1.195	1.119	1.265	1.351	1.254	0.395	0.417	0.379	0.381	0.409	0.449	0.405
Mean	1.369	1.335	1.287	1.287	1.362	1.380	1.341	0.399	0.386	0.402	0.381	0.385	0.418	0.395
LSD I 0.001 II 0.001 II/I 0.002 I/II 0.002 *SD* 0.161 *CV* 12.05								*LSD* I 0.001 II 0.001 II/I 0.002 I/II 0.002 *SD* 0.065 *CV* 16.53						

1 – PKMgCaS, 2 – KMgCaS, 3 – PMgCaS, 4 – PKCaS, 5 – PKMgS, 6 – PKMgCa, *SD* – standard deviation, *CV* [%] – coefficient of variation.

by the concentration of P in soil (Lemanowicz, 2013). The increasing nitrogen rates stimulated the activity of acid phosphatase in soil (2.890 mM pNP·kg^{-1}·h^{-1} at the rate of 250 kg·ha^{-1} of N), while the activity of alkaline phosphatase got inhibited (1.243 mM pNP·kg^{-1}·h^{-1} at the rate of 250 kg·ha^{-1} of N), which was related with the slightly acid and acid soil reaction, and phosphatases are enzymes sensitive to its changes, which must have been due to the process of nitrification of the ammonium fertilizer applied. Coefficient of variation is a useful statistical tool for measuring the variability of soil properties. Differentiation of the alkaline and acid phosphatases activity was low, which was confirmed by coefficients of variation values (*CV%*) amounting for 10.53% (AlP) and 20.74% (AcP).

The content of the available forms of the metals analyzed in soil was modified by both mineral fertilization and the fertilization with increasing nitrogen rates (Tables 4–5). There was found a significantly highest content of Zn (1.550 mg·kg^{-1}), Cu (0.493 mg·kg^{-1}) and Cd (0.249 mg·kg^{-1}) available in soil exposed to mineral fertilization without Ca (PKMgS). The mineral fertilization applied without P (KMgCaS) applied decreased the content of Zn (1.216 mg·kg^{-1}), Cu (0.349 mg·kg^{-1}) and Cd (0.100 mg·kg^{-1}) (Tables 4 and 5). According to Thomas et al. (2012), phosphorus fertilizers can be the source of soil contamination with heavy metals, especially with Cd. Based on the earlier research performed by Berbecea et al. (2011), it was found that high nitrogen rates (100, 200 kg·ha^{-1} of N), when exposed to a lack of phosphorus and potassium fertilization, increased the content of available Zn, Mn, Cu and Ni, which the authors relate to a decreased soil acidity. In the soil from the treatments without magnesium fertilization (PK-CaS) there was identified a significantly higher content of Cu and Cd, as compared with the control samples (PKMg-CaS). Magnesium fertilization can limit the unfavorable effect of heavy metals on the soil and thus on the crops. It is connected with an improved soil reaction. Fertilization without S (PKMgCa) showed a significant effect on a decrease in the content of Zn (1.254 mg·kg^{-1}) and Pb (0.348 mg·kg^{-1}). There was noted a significant increase in the content of Cd (0.245 mg·kg^{-1}) and Cu (0.405 mg·kg^{-1}), as compared with the content of those elements in soil with complete fertilization (PKMgCaS). According to Skwierawska et al. (2012), soil fertilization with sulphate sulphur (S-SO_4^{-2}) and elemental sulphur (S-SO) did not change the Cu content; it decreased the Zn content and slightly affected the Pb content. In the soil from the treatments without Ca (PKMgS) fertilization there was identified a significant increase in the content of Zn (1.150 mg·kg^{-1}), Cu (0.493 mg·kg^{-1}) and Cd (0.246 mg·kg^{-1}).

The increasing nitrogen rates resulted in a significant increase in the content of Zn, Cu and Cd in soil. The highest accumulation of Zn (1.380 mg·kg^{-1}), Cu (0.418 mg·kg^{-1}) and Pb (0.482 mg·kg^{-1}) was received in the soil sampled from the treatments fertilized with N at the rate of 250 kg·ha^{-1}. The ammonium nitrate applied, representing physiologically acid fertilizers, decreased the soil pH (Li et al., 2009). The change in the soil reaction considerably affects the concentration and the mobility of Zn. Due to the

TABLE 6. The content of available cadmium (Cd) and lead (Pb) in soil samples
TABELA 6. Zawartość przyswajalnego kadmu (Cd) i ołowiu (Pb) w próbkach glebowych

Treatments Zabiegi	Cadmium/Kadm [mg·kg^{-1}]							Lead/Ołów [mg·kg^{-1}]						
	nitrogen fertilization/nawożenie azotowe [kg·ha^{-1}]													
	N0	N1	N2	N3	N4	N5	mean	N0	N1	N2	N3	N4	N5	mean
1	0.029	0.039	0.033	0.051	0.061	0.055	0.045	0.465	0.491	0.559	0.587	0.589	0.503	0.532
2	0.081	0.084	0.088	0.115	0.109	0.121	0.100	0.467	0.425	0.441	0.457	0.435	0.467	0.449
3	0.123	0.143	0.162	0.165	0.161	0.185	0.157	0.449	0.389	0.453	0.431	0.475	0.587	0.464
4	0.171	0.181	0.177	0.217	0.213	0.209	0.195	0.385	0.463	0.481	0.379	0.517	0.482	0.451
5	0.225	0.231	0.237	0.265	0.261	0.273	0.249	0.317	0.347	0.297	0.347	0.397	0.457	0.360
6	0.271	0.287	0.302	0.305	0.049	0.075	0.245	0.361	0.291	0.337	0.353	0.347	0.397	0.348
Mean	0.150	0.161	0.167	0.186	0.142	0.153	0.160	0.407	0.401	0.428	0.426	0.460	0.482	0.434
	LSD I 0.042 II 0.042 II/I 0.102 I/II 0.102 *SD* 0.0858 *CV* 53.29							*LSD* I 0.012 II 0.012 II/I 0.030 I/II 0.030 *SD* 0.079 *CV* 18.28						

1 – PKMgCaS, 2 – KMgCaS, 3 – PMgCaS, 4 – PKCaS, 5 – PKMgS, 6 – PKMgCa, *SD* – standard deviation, *CV* [%] – coefficient of variation.

experimental factors applied, the content of the heavy metals determined comes in the following order: Zn > Cu > Pb > Cd (Tables 5 and 6). Greater variation was observed for the content of Cd in soil (*CV* = 53.29%) in comparison to Zn (*CV* = 12.05%), Cu (*CV* = 16.53%) and Pb (*CV* = 18.28%) (Tables 5 and 6).

There was found a significant positive dependence between the content of TOC and the activity of alkaline phosphomonoesterase ($r = 0.462$, $p < 0.05$) in soil. Organic matter plays a key role as a precursor for enzyme synthesis, and in enzyme physical stabilization. Based on the values of the coefficients of correlation calculated, there was found a significantly negative relationship between the activity of alkaline phosphatase and the content of available Cu ($r = -0.368$, $p < 0.05$) and Cd ($r = -0.386$, $p < 0.05$) in soil as well as acid phosphatase and the content of Pb ($r = -0.345$, $p < 0.05$) (Table 7). According to Wang et al. (2007), the heavy metal contaminated soil had significant measurable effects on the P-transforming activity of this enzyme.

A significant value of the coefficient of correlation between the content of available phosphorus in soil and the activity of alkaline phosphatase ($r = 0.721$, $p < 0.05$) demonstrates that the enzyme is a good indicator of the rate of phosphorus transformations in soil and generally catalyze the P release by a wide range of orthophosphate esters and anhydrides, unlike the activity of acid phosphatase ($r = -0.478$, $p < 0.05$) (Table 7).

Conclusion

Mineral fertilization accompanied by increasing nitrogen rates and a lack of organic fertilization resulted in an unfavorable decrease in the content of total organic carbon and available phosphorus in soil. There were found changes in the activity of alkaline and acid phosphatase as a result of increasing N rates. Increasing N rates and a lack of liming increased the soil acidity resulting in the inhibition of alkaline phosphatase, a decrease in the content of available P in soil and, at the same time, an increase in the content of phytoavailable forms of Cu and Zn. As a result of the analyses, there were found relatively low contents of the available forms of Zn, Cu, Cd and Pb, which points to their low mobility. The soil investigated can be classified as soils non-polluted with those metals. Mineral fertilization

TABLE 7. Relationship between activity phosphatase and selected soil properties in soil samples
TABELA 7. Zależność między aktywnością fosfataz i wybranych właściwości w próbkach glebowych

Variables/Zmienne		Equation/Równania	r	r^2
dependent/zależne	independent/niezależne			
total organic carbon	alkaline phosphatase	$y = 1.1132 - 0.2661x$	–0.462	0.214
acid phosphatase	available phosphorus	$y = 3.786 - 0.0195x$	–0.478	0.229
alkaline phosphatase	available phosphorus	$y = 1.2115 + 0.0029x$	0.724	0.519
alkaline phosphatase	copper	$y = -1.7031 - 0.819x$	–0.368	0.135
alkaline phosphatase	cadmium	$y = 1.4848 - 0.6003x$	–0.386	0.149
acid phosphatase	lead	$y = 3.7158 - 2.4083x$	–0.345	0.119
acid phosphatase	pH	$y = 8.7923 - 1.100x$	–0.373	0.142

with nitrogen fertilizer significantly influence soil properties, what may lead to the change of enzymatic activity of soil as well as the contents of the nutrients in soil. The research results allow for a closer look at the effect of mineral fertilization on the ecochemical state of soils and so it is necessary to continue the research further for the adequate farming system to be applied.

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Summary

Assessment of soil phosphatase activity, phosphorus and heavy metals content depending on the mineral fertilization. The paper presents the results of research into the activity of alkaline and acid phosphatase, the content of available phosphorus, heavy metals and total organic carbon, against in soil with mineral fertilization only. The first experimental factor was phosphorus, potassium, magnesium, calcium and sulphur fertilization in six fertilizer combinations: 1 – PKMgCaS, 2 – KMgCaS, 3 – PMgCaS, 4 – PKCaS, 5 – PKMgS, 6 – PKMgCa. The second factor was made up of nitrogen fertilization at the rates of: 0, 50, 100, 150, 200, 250 $kg \cdot ha^{-1}$ of N. Increasing nitrogen rates and a lack of liming increased the soil acidity inhibiting alkaline phosphatase, decreasing the content of available phosphorus in soil. A lack of phosphorus fertilization resulted in an intensive increase in the activity of both alkaline and acid phosphatase in soil. Due to the experimental factors applied, the content of the heavy metals assayed was as follows: zinc > copper > lead > cadmium.

Streszczenie

Ocena aktywności fosfataz, zawartości fosforu i metali ciężkich w zależności od nawożenia mineralnego. W pracy przedstawiono wyniki badań nad aktywnością fosfatazy zasadowej i kwasowej, zawartość przyswajalnego fosforu, metali ciężkich i węgla organicznego w glebie nawożonej wyłącznie mineralnie. Pierwszym czynnikiem doświadczenia było nawożenie fosforem, magnezem, wapniem i siarką w sześciu kombinacjach nawozowych: 1 – PKMgCaS, 2 – KMgCaS, 3 – PMgCaS, 4 – PKCaS, 5 – PKMgS, 6 – PKMgCa. Czynnikiem drugim było nawożenie azotem w dawkach: 0, 50, 100, 150, 200, 250 kg N·ha^{-1}. Wzrastające dawki azotu i brak wapnowania spowodowały wzrost kwasowości gleby, powodując inhibicje fosfatazy alkalicznej, zmniejszenie zawartości fosforu przyswajalnego w glebie. Brak nawożenia fosforem spowodował intensywny wzrost aktywności zarówno fosfatazy alkalicznej, jak i kwaśnej w glebie. Pod wpływem zastosowanych czynników doświadczalnych zawartość metali ciężkich kształtowała się w sposób następujący: cynk > miedź > ołów > kadm.

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