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THE INFLUENCE OF A LONG-TERM RECLAMATION ON THE MICROBIOLOGICAL PROPERTIES OF SOILS FORMED FROM POST-MINING MATERIALS

Summary

The aim of the research was to determine an enzymatic activity and the number of bacteria, Actinomycatales and fungi in anthropogenic soils formed from post-mining materials. Experimental factors included two crop formations and three levels of mineral fertilization. The conducted research showed that higher microbiological activity occurred in the soils from the fodder- frumentaceous crop rotation, where lucerne had been cultivated for four years. Important influence of mineral fertilization on the microbiological activity of soils in frumentaceous-rapeseed crop rotation was found, whereas in soils where fodder- frumentaceous crop rotation was implemented, no major influence of mineral fertilization on soil's microbiological activity was found.
***Key words**: post-mining lands, reclamation, fertilization, enzymatic activity, microorganisms*

WPŁYW WIELOLETNIEJ REKULTYWACJI NA WŁAŚCIWOŚCI MIKROBIOLOGICZNE GLEB TWORZĄCYCH SIĘ Z GRUNTÓW POGÓRNICZYCH

Streszczenie

Celem przeprowadzonych badań było określenie aktywności enzymatycznej oraz liczebności bakterii, promieniowców i grzybów w glebach antropogenicznych wytworzonych z gruntów pogórniczych. Czynnikami doświadczenia były dwa płodozmiany oraz trzy poziomy dawki nawożenia mineralnego. Przeprowadzone badania wykazały, że większą aktywnością mikrobiologiczną charakteryzowały się gleby pobrane spod płodozmianu paszowo-zbożowego, w którym przez cztery lata uprawiana była lucerna. Stwierdzono istotny wpływ nawożenia mineralnego na aktywność mikrobiologiczną gleb w płodozmianie zbożowo-rzepakowym, natomiast w glebach, na których stosowany był płodozmian paszowo-zbożowy, nie stwierdzono istotnego wpływu nawożenia mineralnego na aktywność mikrobiologiczną gleby.
***Key words**: grunty pogórnicze, rekultywacja, nawożenie, aktywność enzymatyczna, właściwości mikrobiologiczne*

1. Introduction

Brown coal mining in Konin and Turek Basin (pl. *Konińsko-Tureckie Zagłębie Węglowe*) has been functioning for more than 60 years. It caused several geomechanical transformations of the landscape. Especially terrain morphology and hydrological conditions were changed. This changes influence on the local biodiversity (flora, fauna and microbial). Removed soil and rock material overlying the brown coal deposit are deposited as inner or outer spoil dumps. Artificially formed spoil dumps are reclaimed with the so called *PAN* method. Main idea of this model is to regulate the chemistry of the deposited material (rock, soil). Mineral fertilization and the implementation of agriculture plants are used to achieve this objective. According to the research conducted by various authors [7, 8, 9, 19] the reclaimed ground gains the traits of a soil after ten years of reclamation operations. The measurement of soils biological activity is one of the most important criteria which determines the progress of reclamation process. It depends on various elements such as the content of organic matter, flora, soil reaction [5]. Biological activity of a soil is marked with direct methods – by measuring the concentration of microbial activity products. This parameter is usually defined on the basis of enzyme activity, which may be also assessed with indirect methods, according to which the number of microorganisms that live in the soil is determined [12]. In fertile soils with proper hydrological conditions, high enzymatic activity is observed. It is the measure of soils fertility and productivity [11, 17].

2. Materials and methods

Analytical materials for laboratory research (soil samples) were collected from a long-term experiment conducted in the outer spoil dump in Pątnów post-mining area. This experiment was established in 1978 by professor Bender. Experimental factors included two crop rotations: rapeseed- frumentaceous crop rotation (the rotation of winter rapeseed and winter wheat) and fodder- frumentaceous one (four years of lucerne and orchard grass tillage in rotation with two years of winter wheat tillage), and three levels of mineral fertilization (0 NPK, 1 NPK and 2 NPK) (Tab. 1). Soil samples were collected in two vegetation seasons: during plants' intensive growth (May) and at the end of the vegetation season (September 2014). Medium-top samples from the arable-humus horizon Ap (0-20 cm) of the reclaimed outer spoil dump were collected with an automatic sampler from fifteen fields. In the collected soil material, the following chemical properties were marked: soil reaction was determined potentiometrically in potassium chloride (1 M KCl dm^{-3}) and water solutions, total organic carbon (TOC) content by elementary analysis on Vario Max analyzer with previously removal of carbonates by phosphoric acid, total nitrogen (TN) on a Vario Max analyzer.

Table 1. Fertilization combinations and doses of mineral ingredients
Tab. 1. Kombinacje nawozowe i dawki składników mineralnych.

Fertilization treatment	Rapeseed			Wheat			Lucerne		
	N	P	K	N	P	K	N	P	K
0 NPK	Without fertilization								
1 NPK	200	70	90	160	40	80	130	60	100
2 NPK	400	140	180	320	80	160	260	120	200

Source: own studies / Źródło: badania własne

The following microbiological analysis were done:

- Number of bacteria
- Number of fungi
- Number of *Actinomycatales*
- Number of *Aotobacter sp.*
- Dehydrogenases' activity with Thalmanmn's method [22]
- Protease's activity with Ladd's and Butler's method [15]
- Alkaline phosphatase's activity with Tabatabia's and Bremner's method [21] with the implementation of a substrate of 0,8% sodium nitrophenylphosphat solution, expressed in millimoles of p-nitrophenol kilogram per hour
- Ureases' activity with Hoffmann's and Teicher's method [10].

The obtained results were statistically processed with *Statistica 12* program.

3. Results and discussion

An over-30-year-long period of the implementation of various fertilization and crop rotations have influenced the chemical properties of forming soils. The content of organic carbon oscillated between 0,36% (rapeseed- frumentaceous crop rotation - control 0NPK) and 1,06% (fodder- frumentaceous crop rotation, 2NPK). Mineral fertilization which was used in the experiment both in the dose of 1NPK and 2NPK has led to statistically significant increase of the content of total organic carbon in the soils of frumentaceous-rapeseed crop rotation when compared to control (0NPK). In the fields of this crop rotation when 1NPK dose was implemented, it was found that the content of total organic carbon doubled when compared to control 0NPK. Further advance of mineral fertilization to the so called dose of 2NPK caused some increase of this ingredient, yet not statistically important (Tab. 2). Varied doses of mineral fertilization used in the soils of fodder- frumentaceous crop rotation did not lead to statistically significant growth of the content of organic carbon. Similar, not majorly different values were obtained in the combinations: 0NPK - 0,84%, 1NPK- 0,88% and 2NPK - 1,06%. When comparing the content of total organic carbon in the soils of both crop rotations, its higher content was found in the soils of the fodder- frumentaceous crop rotation. When analysing the values of humus and total nitrogen, similar values were found as in TOC. The research conducted by Spychalski et al. [19, 20] also showed positive influence of mineral fertilization on the growth of organic carbon and total nitrogen in the reclaimed post-mining lands. Soils reaction – the concentration of hydrogenous ions, has an impact on the functioning of numerous enzymes, and especially on acid phosphatase. The enzyme activity grows when pH is close to neutral, and a small number of microorganisms and low enzymatic activity in acid soils are observed [12]. The analysed soils were characterised by an alkaline reaction both in the marked water solution and in the solution of potassium chloride (Tab. 2). Its values expressed in pH units oscillated from 8,00 to 8,29 when marked in water, and from 7,35 to 7,55 when marked in potassium chloride. Long-term implementation of mineral fertilization in the dose of 1NPK in the soils of frumentaceous-rapeseed crop rotation did not result in statistically significant changes of the reaction; similar and not statistically varied values of pH were obtained, from 8,29 to 8,14, respectively. Further advance of mineral fertilization to the dose of 2NPK resulted in the statistically significant decrease of the values of this parameter when compared to control. Similar regularities were observed in the case of potassium chloride. Long-term implementation of mineral fertilization in the fodder- frumentaceous crop rotation did not lead to any important changes of the soil reaction determined both in H_2O and KCl solutions.

Table 2. Selected properties of soils forming from post-mining materials - average values for combinations
Tab. 2. Wybrane właściwości gleb rozwijających się z gruntów pogórniczych. - wartości średnie dla kombinacji

Fertilization treatment	Plant	pH		TOC [%]	OM [%]	TN [%]	C/N
		H_2O	KCl				
Rapeseed – frumentaceous rotation							
0 NPK	rapeseed wheat	8,29b	7,55b	0,354a	0,611a	0,036a	9,8a
1 NPK	rapeseed wheat	8,14a	7,43a	0,607b	1,047b	0,064b	9,5a
2 NPK	rapeseed wheat	8,11a	7,40a	0,615c	1,060c	0,067c	9,1a
Fodder – frumentaceous rotation							
0 NPK	lucerne wheat	8,16a	7,39a	0,83a	1,43a	0,084a	9,8a
1 NPK	lucernę wheat	8,00a	7,42a	0,85a	1,46a	0,088a	9,6a
2 NPK	lucerne wheat	8,02a	7,35a	1,02a	1,76a	0,106a	9,7a

Explanation: TOC – Total organic carbon, OM – organic matter, TN- total nitrogen

Source: own studies / Źródło: badania własne

Long-term implementation of varied fertilization in the two crop rotations influenced the number of microorganisms in the analysed anthropogenic soils. Only in the case of the total number of bacteria it was observed that the implementation of mineral fertilization in the dose of 1NPK led to statistically important decrease of their number from 35,70 (0NPK) to 17,10 (1NPK) (Rapeseed – frumentaceous rotation). Further advance of the fertilization to the dose of 2NPK resulted in the growth of their number; similar values were obtained in 0NPK and 2NP combinations. A total number of Actinomycatales, fungi and bacteria from Azotobacter sp. was presented in tables 3-5. Statistically significant influence of varied fertilization on the numeral changes of microorganisms was observed neither in the soils of frumentaceous-rapeseed crop rotation (Tab. 3), nor in the ones of fodder- frumentaceous crop rotation (Tab. 4). When comparing the total number of bacteria, their statistically larger number was found in soil samples collected from the fodder- frumentaceous crop rotation, whereas there were much more bacteria from Azotobacter sp. in the soils of frumentaceous-rapeseed crop rotation.

According to numerous authors, the level of enzymatic activity may be a reliable indicator of soils' fertility [14, 16, 18]. What is more, the indicator informs about the changes taking place in the soil environment. The authors pay special attention to the beneficial role of Fabaceae in the formation of soils' fertility enzymatic indicator. Factors used in the experiment such as fertilization and crop rotation has influenced not only the chemical properties but also soils' different enzymatic activity in various ways.

Phosphates are considered an indicator of the intensity of mineralization of phosphorus' organic combinations. Soil microorganisms and plants' roots are the source of phosphate in the soil environment.

Table 3. Selected chemical properties of soils forming from post-mining materials – average values for crop rotations
Tab. 3. Wybrane właściwości chemiczne gleb rozwijających się z gruntów pogórniczych - wartości średnie dla płodozmianów

Crop rotation	pH		TOC	OM	TN	C/N
	H_2O	KCl	[%]	[%]	[%]	
Rapeseed – frumentaceous rotation	8,06a	7,39a	0,525a	0,907a	0,056a	9,5a
Fodder – frumentaceous rotation	8,18a	7,46a	0,899b	1,551b	0,092b	9,7a

Explanation: TOC – Total organic carbon, OM – organic matter, TN- total nitrogen

Source: own studies / Źródło: badania własne

Table 4. Average number of selected microorganisms in the top horizon of soil collected from frumentaceous-rapeseed crop rotation
Tab. 4. Średnia liczebność wybranych mikroorganizmów w warstwie wierzchniej gleby pobranej z płodozmianu zbożowo-rzepakowego

Crop rotation	Fertilization treatment	Bacteria	Actinomycatales	Fungi	***Azotobacter sp.***
		10^5 cfu ·g^{-1}	**10^4 cfu ·g^{-1}**	**10^3 cfu ·g^{-1}**	**cfu ·g^{-1}**
Rapeseed – frumentaceous rotation	0NPK	35,70a	43,96a	32,39a	10,18a
	1NPK	17,10b	23,25a	18,90a	16,79a
	2NPK	32,71a	28,45a	61,75a	10,00a

CFU colony forming units. Explanations: means marked with the same letters did not differ significantly at α=0,05.

Source: own studies / Źródło: badania własne

Table 5. Average number of selected microorganisms in the top horizon of soil collected from fodder-frumentaceous crop rotation
Tab. 5. Średnia liczebność wybranych mikroorganizmów w warstwie wierzchniej gleby pobranej z płodozmianu paszowo-zbożowego

Crop rotation	Fertilization treatment	Bacteria	Actinomycatales	Fungi	***Azotobacter sp.***
		10^5 cfu k·g^{-1}	**10^4 cfu ·g^{-1}**	**10^3 cfu ·g^{-1}**	**cfu ·g^{-1}**
Fodder – frumentaceous rotation	0NPK	63,83a	95,54a	47,03a	20,28a
	1NPK	60,55a	56,25a	47,88a	18,16a
	2NPK	56,15a	51,63a	46,61a	18,66a

Source: own studies / Źródło: badania własne

Table 6. Average number of selected microorganisms in the top horizon of soil collected from two crop rotations
Tab. 6. Średnia liczebność wybranych mikroorganizmów w wierzchniej warstwie gleby pobranej z dwóch płodozmianów

Crop rotation	Bacteria	Actinomycatales	Fungi	***Azotobacter sp.***
	10^5cfu·g^{-1}	**10^4 cfu ·g^{-1}**	**10^3 cfu ·g^{-1}**	**cfu ·g^{-1}**
Rapeseed – frumentaceous rotation	28,50a	31,89a	37,68a	12,32a
Fodder – frumentaceous rotation	60,18b	67,81a	47,18a	19,03b

Source: own studies / Źródło: badania własne

Phosphates are enzymes which catalyse an organic hydrolyse of organic compounds of phosphorus and are used for the assessment of the speed of compounds' mineralization [12]. Alkaline phosphate's activity's values in fertilization combinations oscillated from 24,19 to 0,54,72 mmole PNP·kg^{-1} s.m. soil·h^{-1}. Its especially low activity was observed in the soils of frumentaceous-rapeseed crop rotation in the control field – 0NPK. It was found that the implementation of mineral fertilization in the soils in this crop rotation in the dose of 1NPK had a statistically significant impact on the growth of these enzyme activity from the value of 24,19 mmole PNP·kg^{-1} s.m. soil·h^{-1} (control-0NPK) to 40,25 mmole PNP·kg^{-1} s.m. soil·h^{-1} (1NPK). The increase of fertilization to the dose of 2NPK had minor influence on the changes in phosphates' activity. Mineral fertilization implemented in the fodder- frumentaceous crop rotation did not significantly influence the activity of phosphates in the soil. Higher activity of an alkaline phosphate was observed in the soil with a fodder crop rotation, however, the difference was statistically unimportant when compared to the frumentaceous-rapeseed crop rotation.

Among the enzymes present in the soil, dehydrogenases are responsible for the oxidoreducing processes in the process of organic compounds' oxidation. Their appearance is a proof of the presence of physiologically active and alive microorganisms as they are active only inside living cells [12, 13]. Among all the dehydrogenases, the highest activity was observed in the soils from the fodder- frumentaceous fields crop rotation (Tab. 8). No statistically significant differences under the influence of varied mineral fertilization were observed in dehydrogenases' activity in this crop rotation. In the soils of the frumentaceous-rapeseed crop rotation, under the influence of mineral fertilization in the dose of 1NPK, a significant increase of dehydrogenases activity was observed when controlled to the control dose of 0NPK. The growth of mineralization to the so called dose of 2NPK did not cause any statistically significant increase of these enzymes' activity when compared to control. When comparing the activity of dehydrogenases in the soils of frumentaceous-rapeseed crop rotation and of fodder frumentaceous crop rotation, their statistically higher activity was observed in the soils from the fodder- frumentaceous crop rotation. In the analysed soil samples, the number of dehydrogenases is sometimes correlated with the content of nitrogen and organic carbon [2, 3, 4, 13]. In the soils of the discussed experiment, the correlation between the content of organic carbon and dehydrogenases' activity was very high – almost r=0,87.

Ureases catalyse the hydrolyse of urea to ammonia and carbon dioxide. Ureases' activity may be a bioindicative test in the recognition of the state of soil's quality. When analysing their activity in soil, samples collected from the experimental fields, their higher activity was found in the soils of the fodder crop rotation where lucerne was cultivated. No statistically significant influence of mineral fertilization on the activity of ureases in the soils of this crop rotation was observed (Tab. 8). Statistically similar values were obtained of 1,30 (0NPK0, 1,07 (1NPK), 1,14 (2NPK) µmole urea·ml^{-1}·18h^{-1}, respectively. The implementation of mineral fertilization in the soils of frumentaceous-rapeseed crop rotation led to the growth of ureases activity. A statistically important increase of ureases' activity was observed after the implementation of fertilization in the dose of 1NPK (Tab. 7). The increase of the fertilization dose to 2NPK did not cause any statistically significant growth of their activity and statistically similar values were obtained from 0,76 (1NPK) and 0,97 (2NPK) µmole urea·ml^{-1}·18h^{-1}. The activity of ureases [6] is strictly connected with the content of organic carbon and total nitrogen. In the analysed soils, the activity of ureases was additionally correlated with the content of organic carbon and total nitrogen r=0,88, which is confirmed by Bieleńska and Zukowska [6].

Proteases catalyse the hydrolyse of proteins in the soil to simpler compounds – polypeptides. During the process, they also dissemble peptide bindings (CO-NH) to amino acids [1]. The ability to protease formation is characteristic to proteolytic microorganisms such as oxygenic and anaerobic bacteria, some fungi and Actinomycatales. Proteases' activity oscillated within average values from 2,44-4,07 mg tyrosine·g-1 ·h-1 (Tab. 7) in the frumentaceous-rapeseed crop rotation and from 3,40-5,91 mg tyrosine·g^{-1} ·h^{-1} in the soils of the fodder- frumentaceous crop rotation (Tab. 8).

Table 7. Average enzymatic activity in the top horizon of soil collected from frumentaceous-rapeseed crop rotation
Tab. 7. Średnia aktywność enzymatyczna w warstwie wierzchniej gleby pobranej z płodozmianu zbożowo-rzepakowego

Crop rotation	Fertilization treatment	Alkaline phosphatase	Dehydrogenases	Urease	Protease
		mmol PNP·kg^{-1}·h^{-1}	mmol TPF·kg^{-1}·24h^{-1}	µmol urea·ml^{-1}·18 h^{-1}	mg tyrosine·g^{-1} ·h^{-1}
Rapeseed – frumentaceous rotation	0NPK	24,19a	0,33a	0,37a	2,44a
	1NPK	40,25b	1,36b	0,76b	4,07a
	2NPK	38,76b	0,68ab	0,97b	3,67a

Source: own studies / Źródło: badania własne

Table 8. Average enzymatic activity in the top horizon of soil collected from fodder-frumentaceous crop rotation
Tab. 8. Średnia aktywność enzymatyczna w warstwie wierzchniej gleby pobranej z płodozmianu paszowo-zbożowego

Crop rotation	Fertilization treatment	Alkaline phosphatase	Dehydrogenases	Urease	Protease
		mmol PNP·kg^{-1}·h^{-1}	mmol TPF·kg^{-1}·24h^{-1}	µmol urea·ml^{-1}·18 h^{-1}	mg tyrosine·g-1 ·h^{-1}
Fodder – frumentaceous rotation	0NPK	54,72a	1,95a	1,30a	3,74a
	1NPK	45,49a	2,51a	1,07a	3,40a
	2NPK	49,51a	2,13a	1,14a	5,91a

Source: own studies / Źródło: badania własne

Table 9. Average enzymatic activity in the top horizon of soil collected from two crop rotations
Tab. 9. Średnia aktywność enzymatyczna gleby pobranej z głębokości 0-20 cm z dwóch płodozmianów

Crop rotation	Alkaline phosphatase	Dehydrogenases	Urease	Protease
	mmol PNP·kg^{-1}·h^{-1}	mmol TPF·kg^{-1}·24h^{-1}	µmol urea ·ml^{-1}·18h^{-1}	mg tyrosine·g-1 s.m.gleby·h-1
Rapeseed – frumentaceous rotation	34,40a	0,79a	0,70a	3,39a
Fodder – frumentaceous rotation	49,91a	2,20b	1,17a	4,35a

Source: own studies / Źródło: badania własne

No statistically significant influence of the fertilization on their activity was observed. The observed differences in the activity of proteases between the analysed crop rotations turned out statistically unimportant (Tab. 9). According to numerous authors [4, 6], both proteases' and ureases' activity correlate with organic carbon and total nitrogen and in the analyse experiment was r = 0,81.

4. Conclusions

The obtained results allowed to define final conclusions:
1. The differentiation of mineral fertilization and crop rotation influenced the content of organic matter and total nitrogen positively.
2. The implementation of mineral fertilization in 1NPK dose in a crop rotation had a positive impact on the growth of microbiological activity of the forming soils.
3. Various mineral fertilization implemented in the soils of fodder- frumentaceous crop rotation did not have a major impact on the differentiation of microbiological parameters of the forming soils.
4. The fodder- frumentaceous crop rotation is more effective in the reclamation of post-mining materials. Soil tilled with this method had better biological and chemical properties, which was the result of lucerne's activity in this crop rotation.

5. References

[1] Baran S.: Ocena stanu degradacji i rekultywacji gleb. Przewodnik do ćwiczeń. Ed. AR Lublin, 2000.
[2] Bielińska E.J.: Wpływ długoletniej emisji azotowej na aktywność enzymatyczną gleb leśnych. Rocz. Glebozn., 2006, 57, 1/2, 32-40.
[3] Bielińska E.J.: Aktywność enzymatyczna gleby w sadzie wiśniowym w zależności od metody jej pielęgnacji. Rozpr. Nauk. AR Lublin, 2001.
[4] Bielińska E.J.: Enzymatic activity as an indicator of soil transformations under the influence of orchard use. Pol. J. Soil Sci., 2001, 34, 2: 89-97.
[5] Bielińska E.J., Mocek-Płóciniak A.: Fosfatazy w środowisku glebowym. Monografia, Wydawnictwo Uniwersytetu Przyrodniczego w Poznaniu, 2009.
[6] Bielińska E.J., Żukowska G.: Aktywność proteazy i ureazy w glebie lekkiej użyźnionej osadem ściekowym. Agrophysica 2002, 70: 41-47.
[7] Gilewska M., Bender J.: Rekultywacja gruntów pogórniczych a wartość użytkowa. Materiały Sympozjum XI Warsztaty Górnicze. Zagrożenia naturalne w górnictwie. IGSMiE PAN, Kraków. 2007.
[8] Gilewska M.: Rekultywacja biologiczna gruntów pogórniczych na przykładzie KWB „Konin". Roczniki Akademii Rolniczej w Poznaniu. Rozprawy Naukowe, 1991, Vol. 211.
[9] Gilewska M., Otremba K.: Składniki pokarmowe w glebach powstających z gruntów pogórniczych Kopalni „Konin". Roczniki Gleboznawcze, 2006, T. LVII, Nr 1/2: 82-92.
[10] Hoffman G., Teicher K.: Ein kolorimetrisches Verfahren zur Bestimmung der Ureaseaktivitat in Boden. Zeit. Pflanzenernaehr. Dung. Bodenkunde, 1961, 95, 55-63.
[11] Hoffmann E.: Phosphatases in the enzyme system of cultivated soils (in Germany) and possibilities of determining their activity. Z. Pflanzenernähr. Bodenkd., 1968, 118: 153-160.
[12] Januszek K. Aktywność enzymatyczna wybranych gleb leśnych Polski południowej w świetle badań polowych i laboratoryjnych. Zesz. Nauk. AR w Krakowie, 1999, Rozprawy: 250.
[13] Kieliszewska-Rokicka B.: Enzymy glebowe i ich znaczenie w badaniach aktywności mikrobiologicznej gleby. W: Drobnoustroje środowiska glebowego – aspekty fizjologiczne, biochemiczne, genetyczne. Red. H. Dahm, A. Pokojska-Burdziej, Marszałek, Toruń: 2001, 37-47.
[14] Koper J., Piotrowska A., Siwik-Ziomek A.: Wartość Enzymatycznego Wskaźnika żyzności w zależności od zróżnicowanego zmianowania i nawożenia gleby. Zesz. Probl. Post. Nauk Roln., 2004, 501: 219-225.
[15] Ladd N., Butler J.H.A.: Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem., 1972, 4, 19- 30.
[16] Russel S.: Drobnoustroje a życie gleby. Biblioteka Problemów. T. 199. Warszawa: PWN, 1974.
[17] Russel S.: Znaczenie badań enzymów w środowisku glebowym. Acta Agrophys. Rozpr. Monogr., 2005, 3: 5-9.
[18] Smolik B., Nnowak J.: Próba wyznaczenia wskaźnika zanieczyszczenia środowiska glebowego produktami ropopochodnymi poprzez badanie aktywności niektórych enzymów glebowych. Zesz. Probl. Post. Nauk Roln., 2003, 492: 311-319.
[19] Spychalski W., Mocek A., Gilewska M.: Potassium forms in soils formed from postmining lands. Nawozy i nawożenie w świetle badań polowych i laboratoryjnychZesz. Nauk. AR Krak., 2005, Rozpr. 250, (VII) Nr 3(24), 124-132.
[20] Spychalski W., Gilewska M., Otremba K.: Uziarnienie i skład chemiczny gleby wytworzonej z gruntów pogórniczych KWB "Konin". Roczniki Gleboznawcze, 2008, T. LIX Nr 2, 201-206.
[21] Tabatabai M.A., Bremner J.M. : Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem., 1969, 1, 301-307.
[22] Thalmann A.: Zur Methodik der Bestimmung der Dehydrogenase Aktivität in Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtsch. Forsch., 1968, 21.