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CHANGES IN NUMBERS OF MICROORGANISMS PARTICIPATING IN NITROGEN METABOLISM IN SOIL UNDER HORSE BEAN CULTIVATION AROUND A MUNICIPAL WASTE LANDFILL SITE IN TARNOW

ZMIANY LICZEBNOŚCI DROBNOUSTROJÓW BIORĄCYCH UDZIAŁ W METABOLIZMIE AZOTOWYM W GLEBIE POD UPRAWĄ BOBIKU WOKÓŁ SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE

Abstract: Field research connected with the subject of the paper was conducted from March 2006 to September 2007. For the sake of the experiment 8 experimental points were established on each side of the municipal waste landfill in Tarnow in two zones: 50–200 m and 250–500 m from its boundaries and samples were collected from the soil in which horse bean, Nadwiślański c.v. was cultivated. An additional ninth point was located in the landfill area, in the inactive, reclaimed landfill sector. The results of microbiological tests show obvious differences in the quantitative composition of microflora participating in nitrogen metabolism depending on the distance from the active landfill sector. Comparison of all results revealed an apparent increase in the number of microorganisms participating in nitrogen metabolism during the horse bean vegetation period in comparison with their occurrence before and after the completed vegetation season.

Keywords: soil, microflora, municipal waste landfill site

Soil, as the natural living environment of various microorganism taxonomic groups, for many of them is a suitable ecological niche where numerous abiotic and biotic factors affect one another. Soil quality depends not only on its physical and chemical properties but is also strictly connected with its biological properties, and in the first place with microbiological processes [1, 2].

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Soil microflora is the component of environmental biocenosis which is the fastest growing and responding to changes in environmental parameters. It is conditioned by diversity of biochemical functions, specific for microorganisms and their very high physiological activity. There is also a distinct interrelation among soil environment parameters, microorganisms settling soil and plants [3, 4].

Therefore, soil microorganisms are the factor which together with the vegetal cover determines both the direction and character of biochemical processes and all basic biological transformations connected with biological activity and physicochemical properties of arable soils. The results of the activity comprise not only mineralization and humification of various organic compounds (including humus synthesis) but also mobilization of many mineral compounds which are of primary importance for the life of plants and soil animals [2, 3]. Therefore, it is most important to identify precisely and analyze biocenotic relationships formed in the soil environment, which allow for observation of the scale of changes in soil microorganism biodiversity [5]. The present paper aimed to determine the effect of an active municipal waste landfill on microorganisms participating in nitrogen transformation in the soil environment.

Material and methods

Field research connected with the subject matter was conducted from March 2006 until September 2007 as a model field experiment set up in the vicinity of a municipal waste landfill site in Tarnow. Eight experimental points for soil sampling were established on each side of the investigated object in two zones: below 250 m and 250–500 m from its boundaries and experimental plots were designated in them. Horse bean, Nadwiślański c.v. was cultivated on the plots. An additional ninth point was localized in the landfill area, on an inactive sector, which was formerly reclaimed. Labelling of the points has been presented in Table 1.

Table 1

Plots situated in the vicinity of the municipal waste landfill site in Tarnow 2006–2007

No.	Point	Location of plots with respect to landfill site	
		Direction	Zone [m]
1	W I	West	50–200
2	W II – control	West	250–500
3	N I	North	50–200
4	N II	North	250–500
5	E I	East	50–200
6	E II	East	250–500
7	S I	South	50–200
8	S II	South	250–500
9	Z	Landfill site – reclaimed sector	

Soil samples for microbiological analyses were collected four times in 2006 and 2007 (from March till September) from the root zone at various periods of horse bean vegetation. Collected soil samples were brought to the microbiological laboratory of the Agricultural University in Krakow, where soil moisture and pH were measured and microbiological analyses were conducted. These comprised determination of the numbers of ammonificators, proteolytic microorganisms and aerobic atmospheric nitrogen assimilators of the genus *Azotobacter*. Moreover the course of nitrification and denitrification processes was determined. The number of microbial colony forming units (CFU) was assessed using the method of dilution inoculation and converting the assessment result into 1 gram of soil dry matter or the count was assayed in diluted soil starting from 10–1, ie in 0.1 g.

An important agent contributing to pollutant spread from a landfill are winds. Characteristics of wind directions in the region of the municipal landfill site in Tarnow was presented in Table 2.

Table 2

Wind directions in Tarnow

Wind direction	Proportion
North wind	6.0
North-eastern	7.1
Eastern wind	16.7
South-eastern wind	4.8
South wind	14.8
South-western wind	7.4
Western wind	22.6
North-western wind	8.8
Silences	11.8

Results and discussion

The numbers of soil microorganisms is one of the parameters allowing for an assessment of processes occurring in the polluted soils and their potential ability for pollutant biodegradation. Mineralization of organic compounds containing nitrogen is the basic microbiological process supplying nitrogen for plants and microorganisms in an easily available mineral form. Proteins and other nitrogen containing substances occurring in the soil environment undergo gradual microbiological decomposition by proteolytic, ammonification and denitrifying bacteria [1, 6, 7].

Results of microbiological analyses show apparent differences in the quantitative composition of the microflora participating in nitrogen metabolism depending on the distance from the active landfill. Analytical data presented in Figure 1 show that between 3000 and 172000 CFU proteolytic bacteria occur in 1 g of soil dry matter in the municipal waste landfill in Tarnow and in the surrounding area, in soil under horse bean, Nadwiślański c.v. cultivation. The maximum value for this group of bacteria was assessed in September 2006 on an experimental plot situated in the 50–250 m zone

south from the landfill area (Fig. 1, plot S I). The minimum numbers of proteolytic bacteria were registered in September 2007 on the control plot – W II, which was located in 250–500 m zone in front of the landfill entrance gate (to the west). A slightly higher number of these bacteria was also observed on S II point (Fig. 1) in September 2006. Quantitative analyses of proteolytic bacteria at 9 experimental points shows their different numbers, which were larger in soils on the plots situated to the north and south, at the distance below 250 m from the landfill (plots S I and N I). Attention should be paid to an apparent decrease in proteolytic bacteria numbers in the initial and final period of the experiment, ie in March 2006 and September 2007 (Fig. 1).

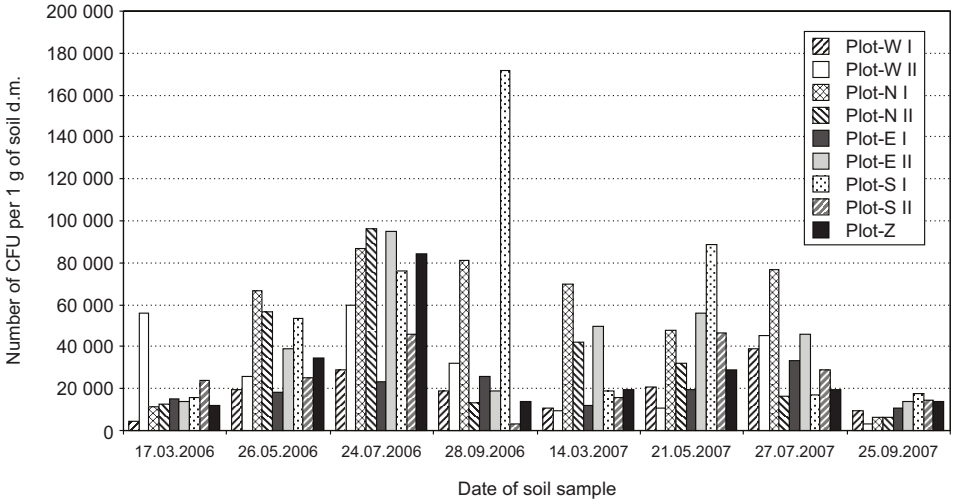

Fig. 1. Changes in proteolytic bacteria numbers in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow (in colony forming units (CFU) per 1 g of soil d.m.)

The analysis of the changes in ammonification bacteria numbers according to the date and place revealed considerable changes in their numbers, fluctuating depending on the research point from 113500 (March 2007) on E I plot to 7881066 CFU I 1 g soil d.m. (March 2006) at the research point located 250 m north of the landfill (N I plot) (Fig. 2). Definitely the lowest values for ammonification bacteria were registered on the experimental plot E II located in the 250–500 m zone east of the landfill. A comparison of these bacteria numbers in the experiment on all plots shows an apparent increase in their numbers during horse bean growing season from May till July 2006–2007, which was most probably caused by intensive mineralization of the soil organic matter due to the thermal and moisture condition in this period prevailingly advantageous for the microflora development [8].

The obtained results evidence that the course of ammonification process depends on various environmental factors, among others the soil type, total carbon and organic nitrogen concentrations, mineral and organic fertilization [9].

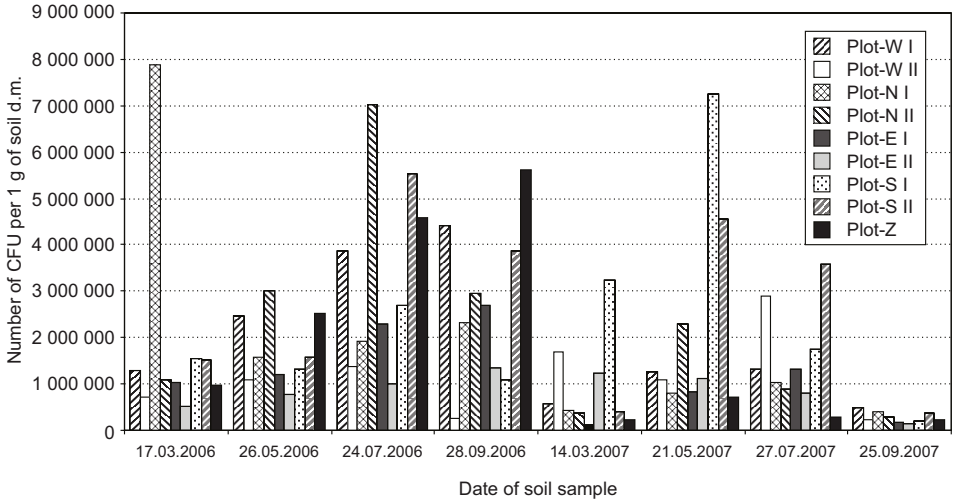

Fig. 2. Changes in ammonification bacteria numbers in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow (in colony forming units (CFU) per 1 g of soil d.m.)

Among bacteria participating in the atmospheric nitrogen assimilation are *Azotobacter* genus. The numbers of these free living soil assimilators under plots where horse bean was cultivated ranged between 0 at all experimental points to 460 colony forming units (CFU) per 1 g of soil d.m. at W II point (Fig. 3). Comparison of changes in *Azotobacter* numbers suggests the fact that in the presented experiment they were the

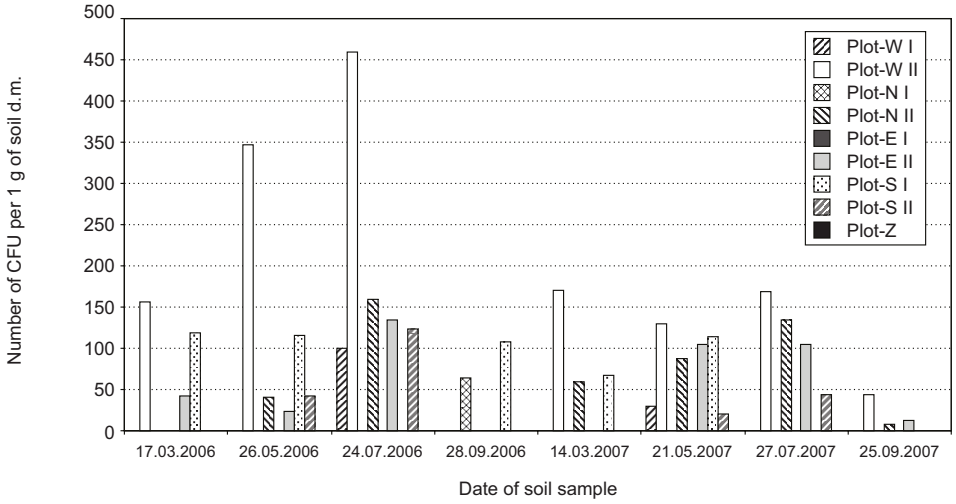

Fig. 3. Changes in *Azotobacter* bacteria numbers in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow (in colony forming units (CFU) per 1 g of soil d.m.)

most frequent and in greatest numbers present on the field located in front of the entrance to the landfill (to the west) at point W II and on the plots considerably distant from the landfill, in the 250–500 m zone – experimental points N II, E II and S II. No presence of *Azotobacter* was detected over the whole period of the experiment at the experimental point situated in the landfill area in the reclaimed sector (point Z) and in the point located east of the landfill in the 50–500 m zone.

It confirms that the numbers of *Azotobacter* depended on the soil pollution, which has a toxic effect visible either as a considerable decline in its numbers or leading to a total elimination of this microorganism group in the soil in the area of the municipal waste landfill or close to it [9].

Considering the dates of analyses, the increase in *Azotobacter* populations may be noticed in the soil under horse bean cultivation in the presented experiment in the summer period (from May till June 2006 and 2007) in comparison with the spring-autumn season (March, September 2006 and 2007).

The research testified that the nitrifying and denitrifying activity of the soil depended both on the location of the experimental plot and on the period of the experiment. As results from Table 2, the nitrification count at the experimental points was determined on the level from 0.01 to 0.000001. An apparent predominance of nitrification process is visible on the plots localized to the north and south of the landfill site (experimental points N I and S II) in comparison with the plot situated in the landfill area in the reclaimed sector. General seasonal changes of the nitrification process were regular and distinctly evidencing that the highest activity of the nitrification process occurred during horse bean vegetation in the summer and early autumn period, ie in July and September 2006 and in July 2007.

The obtained research results prove that nitrification process may be disturbed by chemical compounds present in soil. The course of this process may be disturbed also when other conditions, unfavourable for nitrifiers occur in soil since the nitrification process is determined by pH, organic matter content and heavy metal concentrations in soil [10].

Different denitrification counts were registered in soil samples collected from the experimental plots situated in the landfill and its neighbourhood, ranging from 0.001 at the points situated west of the landfill in the below 500 m zone from the landfill entrance – points W I and W II and at the point located to the north at the distance of 250 m – point N I (Table 3). An apparent prevalence of the denitrification process was visible in the soil on plots localized in the zone between 250 and 500 m from the landfill and in the area of the reclaimed landfill sector in comparison with the plots situated in its vicinity.

It should be emphasized that soil provides an excellent substratum for the growth and development of miocroorganisms because it is usually well supplied in organic and mineral nutrients, has suitable moisture, pH and favourable aerobic conditions. Owing to these physicochemical properties, it is the natural habitat for large numbers of various forms of microflora and microfauna [11].

Table 3

The course of the nitrification and denitrification process in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow

Count	Sampling dates							
	17 March 2006	26 May 2006	24 July 2006	28 Sept. 2006	14 March 2007	21 May 2007	27 July 2007	25 Sept. 2007
	Plot W I – Control							
Nitrification	0.0001	0.001	0.000001	0.00001	0.001	0.0001	0.001	0.01
Denitrification	0.00001	0.00001	0.00001	0.0001	0.0001	0.000001	0.00001	0.0001
	Plot – W II							
Nitrification	0.00001	0.00001	0.0001	0.000001	0.00001	0.00001	0.0001	0.0001
Denitrification	0.0001	0.0001	0.0001	0.001	0.0001	0.000001	0.00001	0.0001
	Plot – N I							
Nitrification	0.00001	0.001	0.001	0.00001	0.001	0.001	0.001	0.001
Denitrification	0.001	0.00001	0.000001	0.000001	0.00001	0.001	0.00001	0.0001
	Poletko – N II							
Nitrification	0.0001	0.0001	0.000001	0.01	0.00001	0.00001	0.000001	0.001
Denitrification	0.00001	0.00001	0.000001	0.0001	0.0001	0.000001	0.000001	0.00001
	Plot – E I							
Nitrification	0.00001	0.00001	0.001	0.001	0.0001	0.00001	0.001	0.01
Denitrification	0.001	0.001	0.0001	0.0001	0.001	0.001	0.00001	0.0001
	Plot – E II							
Nitrification	0.00001	0.0001	0.000001	0.00001	0.001	0.00001	0.001	0.001
Denitrification	0.0001	0.00001	0.0001	0.0001	0.0001	0.000001	0.00001	0.0001
	Plot – S I							
Nitrification	0.00001	0.0001	0.001	0.000001	0.001	0.00001	0.00001	0.001
Denitrification	0.00001	0.00001	0.00001	0.000001	0.00001	0.000001	0.00001	0.00001
	Poletko – S II							
Nitrification	0.00001	0.0001	0.000001	0.000001	0.0001	0.000001	0.000001	0.0001
Denitrification	0.00001	0.00001	0.000001	0.000001	0.0001	0.000001	0.000001	0.00001
	Plot – Z							
Nitrification	0.001	0.0001	0.00001	0.001	0.01	0.0001	0.0001	0.0001
Denitrification	0.0001	0.00001	0.00001	0.000001	0.00001	0.00001	0.00001	0.0001

On the basis of the investigated soil texture analysis they were classified to sandy, weakly loamy, light and strong loamy deposits (plots; W II, E I, E II, S I and S II), to light loams (plots W I, N I, N II, Z). Deposits with heavier (loamy) granulation occurred only on the plots established on the northern side of the landfill and on the plot Z situated on the already reclaimed landfill sector. The conducted measurements reveal considerable fluctuations of the soil pH from 4.8 to 7.1 (Fig. 4) in the investigated soil

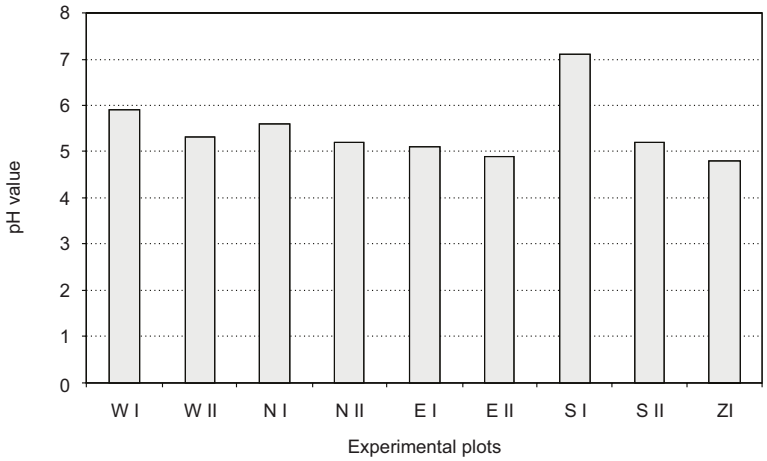

Fig. 4. Values of soil pH at experimental points localized around the municipal waste landfill site in Tarnow

environments. The reaction of all analyzed soils was acid at all experimental points except the soil taken from S I plot, which revealed alkaline pH. These conditions caused changes in the quantitative occurrence of microorganisms active in nitrogen transformation processes in the soil environment of the presented experiment.

On the basis of the obtained results it may be stated that a clear increase in the numbers of microorganisms active in nitrogen metabolism has been visible during the horse bean growing season. It confirms the fact that also various crop species affect the occurrence of various microorganisms in the soil environment, first during their growth in the vegetation season, through root system development and finally through the residue which remains after their die-back. In this way they also influence biological, chemical and physicochemical soil properties [12–14].

Conclusions

1. Numerous changes in the occurrence of microorganisms participating in nitrogen metabolism were found in the soil under horse bean cultivation in the vicinity of the active municipal waste landfill in Tarnow.

2. Microorganism numbers depended in the first place on the experimental plot localization and the period of the experiment.

3. A comparison of the obtained results allows for a conclusion that during the horse bean growing period an apparent increase in microorganism number participating in the nitrogen metabolism was noted in comparison with their presence after the season completion.

4. Analyses of the microbiocenotic composition of soil surrounding the landfill allow to improve knowledge about the dynamics but also on the range of pollutant spread in its vicinity and to decide upon the environment protection strategy.

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ZMIANY LICZEBNOŚCI DROBNOUSTROJÓW BIORĄCYCH UDZIAŁ W METABOLIZMIE AZOTOWYM W GLEBIE POD UPRAWĄ BOBIKU WOKÓŁ SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE

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Abstrakt: Badania terenowe związane z tematem pracy prowadzono w okresie od marca 2006 do września 2007 r. W tym celu z każdej strony od składowiska odpadów komunalnych w Tarnowie w dwóch strefach 50–200 i 250–500 m od jego granic wyznaczono w sumie 8 stanowisk badawczych pobierania próbek gleby, na których uprawiano bobik odmiany Nadwiślański. Dodatkowy punkt dziewiąty zlokalizowano na terenie składowiska odpadów na nieczynnym sektorze, który został wcześniej zrekultywowany. Wyniki przepro-

wadzonych badań mikrobiologicznych wskazują na wyraźne różnice w ilościowym składzie mikroflory biorącej udział w metabolizmie azotowym w zależności od odległości od czynnego składowiska. Porównując wszystkie wyniki, można wysunąć wniosek, że w okresie wegetacji bobiku widoczny jest wyraźny wzrost liczebności drobnoustrojów biorących udział w metabolizmie azotowym w porównaniu do ich występowania zarówno przed, jak i po zakończonym sezonie wegetacyjnym.

Słowa kluczowe: gleba, mikroflora, składowisko odpadów komunalnych