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IMPACT OF THE MUNICIPAL LANDFILL SITE ON BACTERIA PARTICIPATING IN TRANSFORMATION OF SOIL NITROGEN

WPŁYW SKŁADOWISKA KOMUNALNEGO NA BAKTERIE BIORĄCE UDZIAŁ W PRZEMIANACH AZOTU GLEBOWEGO

Abstract: Field research on the subject presented in the paper were conducted from March 2006 until September 2007. For the experimental reasons 8 research plots for soil sampling were established on each side of the municipal waste landfill site in Tarnow in two zones: 50–200 and 250–500 m from its boundaries. Spring wheat, Zura c.v. was cultivated on the plots. An additional experimental plot was set up in the reclaimed part of the landfill site. Analysis of the obtained results points to differences in the quantitative composition of microflora participating in nitrogen metabolism in the analyzed soils. It was found that on the experimental plots under wheat the number of proteolytic bacteria in 1 g of the soil dry mass ranged from 2 300 to 96 700 cfu, ammonifying bacteria from 122 000 to 4 860 000 and bacteria from Azotobacter genus from 0 to 330 cfu. Over the whole period of investigations also *Clostridium pasteurianum* bacteria counts were determined within the range of 0.01 to 0.00001, the values of nitrification process index from 0.01 to 0.000001 and denitrification index from 0.001 to 0.00001 were assessed in the whole analyzed soil environment.

Keywords: municipal landfill sites, soil, bacteria

Soil microflora is the component of its biocenosis, which most rapidly grows and responds to changes of environmental parameters. Microorganisms change their biomass, metabolic activity and microbiocenotic composition in response to numerous stressors and stimulants which may occur in the soil environment [1, 2]. Even non-farmed soil is not a neutral environment without any interactions, either. It is the area where the conditions, biotic and abiotic factors often determine change in the number of the organisms living in it [3]. It should be remembered that soils belong to

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non-renewable or hardly renewable resources [4]. Therefore, a thorough identification of biocenotic systems formed in the soil environment and allowing for observation of the scale of changes in soil microorganism biodiversity gains a crucial importance [5]. Microorganisms are an important element, necessary for the proper functioning of biological systems, because any damage to microorganisms in soils, whatever its cause, would always lead to recession of individual either eco- or agrosystem [6]. Microorganisms which mobilize unavailable biogen forms enable the growth and development of higher plants conditioning the functioning of whole terrestrial ecosystems [7].

Therefore, the present work aimed at an assessment of the impact of municipal waste landfill site on the occurrence of bacteria participating in nitrogen transformation process.

Material and methods

The research presented in the paper was conducted in the area and in the neighbourhood of municipal waste landfill site in Tarnow during the period from March 2006 until September 2007. The research was conducted on a field experiment model. Therefore, two zones were marked out: I – 250 m and II – between 250 and 500 m from its boundaries. A total of 8 experimental plots, on which spring wheat, Zura c.v. was cultivated, were set up in the established zones. An additional ninth plot was situated in the landfill site area, in the reclaimed sector. The marking of sites was presented in Table 1.

Table 1

Plots situated in the vicinity of municipal waste landfill site in Tarnow

Plot	Plot localization zone [m]	Soil pH	Range [%]
WI	50–200	5.1	6.7–19.4
WII	250–500	5.1	6.1–17.7
NI	50–200	5.6	9.2–23.7
NII	250–500	4.8	7.5–25.8
EI	50–200	4.8	8.9–27.4
EII	250–500	4.9	7.3–24.3
SI	50–200	7.5	9.8–31.3
SII	250–500	4.7	9.7–29.1
Z	Reclaimed sector area	4.7	12.4–39.6

The soil was sampled for analyses from the arable layer (0–20 cm) from the experimental plots under spring wheat at different stages of its growth, from March 2006 until September 2007. Collected soil samples were brought to microbiological laboratory at the Microbiology Department, University of Agriculture in Krakow, where moisture and pH were measured and microbiological analyses were performed. These comprised determination of proteolytic microorganism count (medium acc. to Pochon), ammonifying bacteria count (medium acc. to Rougieux) and aerobic assimilators of

atmospheric nitrogen of *Azotobacter* genus (Ashby's medium) and well as bacteria assimilating atmospheric nitrogen in anaerobic conditions – *Clostridium pasteurianum* (medium acc. to Rougieux). Moreover, the course of nitrification processes (medium acc. to Winogradsky) and denitrification process was determined (medium acc. to Giltay). The number of cfu (*colony forming units*) of microorganisms was determined using dilution seeding method and converting the result into a gram of the soil dry matter, or determining the count in diluted soil starting from 10^{-1}, ie in 0.1 g.

Results and discussion

A current opinion states that "no healthy society is possible without a healthy soil". Healthy soil is connected with healthy food and to a great extent also with healthy water, air, microclimate and green areas surrounding our housing estates and work-places [7]. While seeking solutions to these problems, microbiological analyses were conducted on the soils under spring wheat cultivated on the experimental plots situated in the area and around the municipal waste landfill site in Tarnow. They revealed a diversified occurrence of bacteria participating in nitrogen metabolism. Mineralization of nitrogen containing organic compounds, occurring in the environment, carried on by proteolytic, ammonifying, nitrifying and denitrifying bacteria is the basic micro-biological process providing nitrogen in a mineral form easily available to plants [8–10].

Analytical data on the number of investigated soil microorganisms show apparent differences dependant on the plot location in relation to the landfill site. Considering the assessed number of proteolytic bacteria present in the experimental plots it may be seen that their number ranged from 2 300 top 96 700 cfu (Table 3). During the experiment their highest number was assessed in July 2007 in the soil from the experimental plot situated in the zone immediately adjoining the landfill site – SI plot, where also the highest average number was registered throughout the whole period of the experiment (Table 2). On the other hand, the lowest number of proteolytic bacteria was noted in September 2006 on the experimental plot situated in the zone located 250 m west of the landfill site – WI plot.

Considering the research results on the number of ammonifying bacteria in the soil of the established plots under spring wheat, which were given in Table 3, it was found that their number ranged from 122 000 to 4 860 000 cfu per 1 g of the soil dry mass. The highest number of ammonifying bacteria was noted in July 2006 in the soil of the experimental plot situated on the northern side of the landfill – NI plot, whereas the lowest number was revealed in March 2007 on the experimental plot located in the reclaimed sector, and slightly higher was registered on the WI plot located in the zone below 250 m in front of the entrance to the researched object. Much higher number of the analyzed bacteria was found in the investigated soil in the summer months (summer of 2006 and 2007) in comparison with the other periods. The average values show that the highest number of ammonifying bacteria occurred in the soil of the plots situated on the northern side of the landfill (plots NI and NII). The lowest average number (366 400 cfu) was registered in the soil of experimental plot on the western part of the landfill –

Table 2

Average number of bacteria participating in nitrogen metabolism in the soil under spring wheat in the vicinity of municipal waste landfill site in Tarnow

Analyzed microorganisms	Average number of cfu per 1 g of soil d.m.								
	Plots								
	W I	W II	N I	N II	E I	E II	S I	S II	Z
Proteolityc bacteria	9 566	18 902	15 790	32 391	28 980	40 209	40 960	20 176	34 949
Ammonifying bacteria	366 400	1 129 345	2 041 500	1 897 985	737 313	704 625	1 132 204	923 300	881 550
Bacteria of *Azotobacter* genus	14	123	72	64	0	16	124	28	0

Table 3

Number of bacteria participating in processes of nitrogen transformation in soils under Zura spring wheat in the vicinity of municipal waste landfill site in Tarnow (cfu per 1 g soil d.m/count)

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – WI								
Proteolytic bacteria	7 300	9 400	5 389	2 300	10 980	15 400	19 560	6 200
Ammonifying bacteria	420 300	478 300	678 000	415 500	127 800	155 000	372 100	284 200
Bacteria of *Azotobacter genus*	0	0	36	30	0	0	45	0
Nitrification	0.0001	0.001	0.00001	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
Clostridium pasteurianum	0.01	0.001	0.00001	0.001	0.001	0.001	0.001	0.001

Table 3 contd.

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – WII								
Proteolytic bacteria	14 247	26 300	15 300	11 470	19 400	16 000	35 300	13 200
Ammonifying bacteria	923 000	1 126 000	1 510 000	1 684 660	805 780	405 000	1 865 320	715 000
Bacteria of *Azotobacter genus*	104	264	330	0	0	110	178	0
Nitrification	0.00001	0.00001	0.0001	0.000001	0.000001	0.00001	0.0001	0.0001
Denitrification	0.0001	0.0001	0.0001	0.001	0.0001	0.000001	0.00001	0.0001
Clostridium pasteurianum	0.001	0.0001	0.0001	0.001	0.001	0.0001	0.01	0.001
Experimental plot – NI								
Proteolytic bacteria	21 500	26 400	16 680	19 000	11 000	7 000	16 700	8 040
Ammonifying bacteria	1 241 000	2 312 000	4 860 000	1 734 000	1 004 000	1 200 000	2 837 000	1 144 000
Bacteria of *Azotobacter genus*	0	167	200	205	0	0	0	0
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
Clostridium pasteurianum	0.001	0.01	0.001	0.0001	0.001	0.001	0.001	0.001
Experimental plot – NII								
Proteolytic bacteria	12 200	46 390	13 670	24 100	36 800	53 000	46 400	26 570
Ammonifying bacteria	829 280	1 994 000	3 935 000	3 290 000	766 200	1 790 000	1 490 200	1 089 200
Bacteria of *Azotobacter genus*	0	40	0	0	48	140	224	58
Nitrification	0.0001	0.00001	0.0001	0.000001	0.000001	0.00001	0.000001	0.00001
Denitrification	0.00001	0.00001	0.000001	0.0001	0.0001	0.000001	0.000001	0.00001
Clostridium pasteurianum	0.001	0.001	0.001	0.001	0.01	0.0001	0.001	0.0001

Table 3 contd.

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – EI								
Proteolytic bacteria	24 800	68 300	73 000	15 600	8 790	12 602	18 070	10 680
Ammonifying bacteria	739 000	1 000 400	830 000	1 380 400	282 500	345 200	842 100	478 900
Bacteria of *Azotobacter genus*	0	0	0	0	0	0	0	0
Nitrification	0.00001	0.00001	0.000001	0.000001	0.0001	0.000001	0.000001	0.01
Denitrification	0.001	0.001	0.0001	0.0001	0.001	0.001	0.00001	0.0001
Clostridium pasteurianum	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Experimental plot – EII								
Proteolytic bacteria	33 800	69 000	91 000	11 500	15 090	25 980	55 400	19 900
Ammonifying bacteria	402 000	685 200	1 985 000	399 500	192 800	295 000	1 495 000	182 500
Bacteria of *Azotobacter genus*	12	22	0	0	20	75	0	0
Nitrification	0.00001	0.0001	0.000001	0.00001	0.001	0.00001	0.000001	0.00001
Denitrification	0.0001	0.00001	0.0001	0.0001	0.0001	0.000001	0.00001	0.0001
Clostridium pasteurianum	0.0001	0.001	0.001	0.001	0.0001	0.0001	0.0001	0.001
Experimental plot – SI								
Proteolytic bacteria	25 800	53 400	46 100	26 000	16 630	35 500	96 700	27 550
Ammonifying bacteria	937 800	1 192 300	1 805 600	1 627 000	592 000	194 333	1 488 600	1 220 000
Bacteria of *Azotobacter genus*	0	0	226	270	0	185	263	48
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
Clostridium pasteurianum	0.001	0.01	0.0001	0.0001	0.001	0.001	0.001	0.001

Table 3 contd.

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – SII								
Proteolytic bacteria	20 400	25 300	32 000	22 510	11 800	9 000	18 700	21 700
Ammonifying bacteria	1 102 300	1 664 800	1 466 900	1 167 000	358 000	395 000	765 100	467 300
Bacteria of *Azotobacter genus*	0	0	148	0	0	40	34	0
Nitrification	0.00001	0.0001	0.001	0.01	0.0001	0.000001	0.0001	0.0001
Denitrification	0.00001	0.00001	0.000001	0.000001	0.0001	0.000001	0.000001	0.0001
Clostridium pasteurianum	0.001	0.001	0.0001	0.0001	0.001	0.001	0.0001	0.0001
Experimental plot – Z								
Proteolytic bacteria	19 060	74 700	60 000	11 600	16 330	29 000	49 400	19 500
Ammonifying bacteria	830 000	1 230 000	2 630 000	1 232 000	122 000	200 800	423 800	383 800
Bacteria of *Azotobacter genus*	0	0	0	0	0	0	0	0
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
Clostridium pasteurianum	0.01	0.001	0.01	0.01	0.001	0.01	0.001	0.01

WI plot (Table 2). The obtained results confirm that the course of ammonification process depends on various environmental factors, among others soil type, total contents of carbon and organic nitrogen, mineral and organic fertilization [9].

Bacteria active in atmospheric nitrogen assimilation comprise among others those from *Azotobacter* genus. When their number in the soil of the experimental plots under spring wheat changed, the changes were considerable, fluctuating from zero presence in the soils of all plots to 330 cfu per 1 g d.m. of soil (summer 2006) on WII plot situated in front of the entrance gate (west side) in the zone between 250–500 from the landfill boundaries (Table 3). Considering their number in the soil of the individual experimental plots, no presence was found in the analyzed soil environment on the plots situated in the reclaimed sector and in the area immediately adjoining the landfill from the east (plot EI). It may be due to the fact that the soil microflora is the fastest growing and responding to the changes of environmental parameters element of biocenosis. It is conditioned by typical for microorganisms diversity of biochemical functions and exceptionally high physiological activity [2, 7]. The analysis of data based on the obtained average values for these bacteria revealed, that *Azotobacter* bacteria were the most numerous on the experimental plots considerably distanced from the active part of the landfill site, most frequently to the west and south – plots WII and SII (Table 2). A comparison of these bacteria number in the experimental model reveals an apparent increase in their number in summer months of 2006–2007. It confirms that number of *Azotobacter* bacteria depended on the soil pollution, which had a toxic impact on it by significantly decreasing or leading to a total lack of these microorganisms in the soil [11].

Beside atmospheric nitrogen fixing aerobic microorganisms the research comprised also atmospheric nitrogen assimilators living in anaerobic conditions. As results from the data presented in Table 3, during the entire period of the experiment, the value of *Clostridium pasteurianum* in the soil of the experimental plots under spring wheat ranged from 0.01 (all experimental plots except plots EI, EII and SII) to 0.00001 on the experimental plot localized in the area west of the landfill, in the zone 250 m from its boundaries – WI plot. An apparent prevalence of the analyzed bacteria was visible in the soil of the plots situated on the southern part of the landfill – plots SI and SII in comparison with the other plots localized on different sides of the landfill. The lowest bacteria count was the most frequently noted in the soil of the plot localized in the reclaimed sector.

Attention should be also paid to the course of nitrification and denitrification processes in the analyzed soil. As seen in Table 3, the nitrification index determined in the soil of experimental plots localized in the neighbourhood of the municipal waste landfill site in Tarnow was on the level between 0.01 (all cultivated plots except WI) and 0.000001 (all plots except SI and SII). A markedly lower occurrence of nitrification process is observable in the soil of the experimental plots situated on the southern side of the landfill – plots SI and SII and in the reclaimed sector – plot Z. A tendency of increasing nitrification index was noticed during the summer – July of 2006 and 2007 but its decline in the final period of the investigations in autumn 2007. The obtained research results demonstrate that nitrification process may be disturbed by chemical

compounds present in soil. The course of this process may be misshapen also when there are other conditions in soil, unfavourable for nitrificators. Nitrification process is determined by pH, organic matter content and heavy metal concentrations in soil [12].

Concerning the value of denitrification index, it was found that it ranged from 0.001 (plots NI and EI) to 0.00001 on all experimental plots except the one situated on the eastern side of the landfill – plot E I (Table 3). General seasonal changes of the nitrification process in soil under spring wheat were little regular. The analyzed soil of the experimental model shows that the lowest soil denitrifying activity occurred on the experimental plot localized in the area immediately adjoining the landfill on its eastern side, plot EI. The analyses proved that nitrifying and denitrifying soil activity depended both on the localization of the experimental plot and the period of plant growth. According to the literature data, the age and development of plants affect the character of secretions, which in consequence has a considerable effect on microorganism populations. Soil is not only a substratum, the place of plant planting or a reservoir of biogenic elements, but also the habitat of numerous coexisting and mutually protective organisms [13, 14].

In the analyzed soil environments pH was assessed from 4.7 on SII and Z plots to 7.5 on the experimental plot SI. Considerable moisture fluctuations were registered from 6.1 % to 39.6 %, particularly in the reclaimed landfill sector, where between 12.4 % and 39.6 % were noted (Table 1). While comparing the number of bacteria participating in nitrogen metabolism on all plots, one may observe an apparent increase in their number during the plant growth period in May, July 2006–2007, which might have been caused by an intensive mineralization of soil organic matter in result of thermal and moisture conditions in this period usually favouring the microflora development [15]. The presented experiments corroborate also reports of other researchers who revealed the presence of microorganisms in soil in the area and in the neighbourhood of public utility facilities and different level of environmental contamination [1, 13, 16, 17].

Conclusions

1. The factor diversifying the presence of bacteria participating in nitrogen metabolism in the soil was the localization of the experimental plot.

2. Comparing the number of the analyzed bacteria, their successively increasing number was observed during the plant growth period, ie in the summer months, which might have been the result of favourable thermal and moisture conditions for microflora development in this period.

3. On the basis of conducted experiments it may be stated that the areas immediately adjoining the municipal waste landfills site (in the zone below 250 m) should be excluded from agricultural use.

4. The obtained results demonstrate that further extensive research should be continued in the vicinity of municipal waste landfill sites to select the plant positively affecting the beneficial soil microorganism groups which may contribute to the improvement of biological activity of the soils surrounding the operating municipal facilities.

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WPŁYW SKŁADOWISKA KOMUNALNEGO NA BAKTERIE BIORĄCE UDZIAŁ W PRZEMIANACH AZOTU GLEBOWEGO

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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 składowiska odpadów komunalnych w Tarnowie w dwóch strefach

50–200 i 250–500 metrów od jego granic wyznaczono 8 stanowisk badawczych (poletek) do pobrania próbek gleby, na których uprawiano pszenicę jarą odmiany Żura. Dodatkowe poletko doświadczalne założono na terenie zrekultywowanej części składowiska. Analiza otrzymanych wyników wskazuje na występowanie w badanych glebach różnic w ilościowym składzie mikroflory biorącej udział w metabolizmie azotowym. Na poletkach doświadczalnych pod uprawą pszenicy stwierdzono, że w 1 g suchej masy gleby liczba bakterii proteolitycznych kształtowała się w granicach od 2 300 do 96 700 jtk, bakterii amonifikacyjnych od 122 000 do 4 860 000 jtk, oraz bakterii z rodzaju *Azotobacter* od 0 do 330 jtk. Określano również w ciągu całego okresu badawczego w badanym środowisku glebowym wartości miana bakterii *Clostridium pasteurianum* w przedziale od 0,01 do 0,00001, wartości miana procesu nitryfikacji od 0,01 do 0,000001 oraz denitryfikacji od 0,001 do 0,00001.

Słowa kluczowe: składowiska odpadów komunalnych, gleba, bakterie