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PSEUDOMONAS FLUORESCES OCCURRENCE IN SOIL AFTER FERTILIZATION WITH SEWAGE SLUDGE

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ABSTRACT: The aim of the study was to analyze the occurrence of *Pseudomonas fluorescens* in urban soil in the second year after fertilization with unprocessed sewage sludge from the wastewater treatment plant in Sokółka and processed sludge from wastewater treatment plant in Bialystok. The study was conducted on experimental plots located in the green belts along the main roads in Bialystok (Piastowska and Hetmańska streets). For the studied soil, two different types of sewage sludge were used: after-press dewatered sludge from the treatment plant in Sokółka and dry sludge in the form of pellets from the treatment plant in Bialystok. The experiment plots were fertilized with three doses of sewage sludge: 0-control, 14.5 and 29 Mg D.M./ha. In the second year after application of sewage sludge, microbiological tests of the rhizosphere area showed seasonal fluctuations in the number of *Pseudomonas fluorescens* bacteria. The highest number of *Pseudomonas fluorescens* bacteria was observed in April and in October, while the lowest number of bacteria occurred in July, which could have been conditioned by atmospheric factors. The analysis of the correlation indicates that in the urban soil mixed with sewage sludge the number of *Pseudomonas fluorescens* was significantly positively correlated with C:N ratio, organic carbonand content of phosphorus.

KEY WORDS: *Pseudomonas fluorescens*, urban soil, sewage sludge

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Introduction

One of the forms of sewage sludge management is the environment use which is conditioned by its mineral and organic composition, similar to that of soil humus. Siuta (2003) reports that in sewage sludge organic fraction constitutes 35-40%. The organic matter and nutrients contained in the waste constitute a potential that should return to the natural cycle and should be used by plants. In addition, an important purpose of the use of sediments is to inhibit water or wind erosion of land by planting vegetation on the surface (Bień, Wystalska, 2011). Moreover, apart from the positive aspect of the use of sludge, there is also a negative aspect. As reported by Merrington and Smernik (2004), one of important threats related to sewage sludge use may occur when various xenobiotics are introduced into the soil along with sewage sludge. According to Singh and Agrawal (2008), the concentration of xenobiotic compounds in sewage sludge depends on such factors as sludge origin, sewage treatment and processing, the bioavailability of xenobiotics brought with sludge into the soil which are determined by soil properties such as pH, redox potential, content of organic matter as well as the size of applied sewage sludge dose (Wilk, Gawronek, 2009).

The biomass of microorganisms in soils constitutes approximately 85% of the total biomass of all organisms living in this environment. Approximately 90% of resulting carbon dioxide in soil is produced in microbes life, which indicates the high metabolic activity and the great importance of microorganisms for most processes occurring in the soil environment (Dahm et al., 2010). As a result of microbial processes of organic matter transformation, humus is formed, the quantity of which is one of the most important factors determining the soil ability to store water and nutrients. Microorganisms also contribute to the degradation and detoxification of various soil pollutants, reduce the development of pests and pathogens of plants and directly (symbiosis, mycorrhiza) or indirectly influence the growth of plants (Martyniuk, Księżak, 2011). Among many microorganisms found in soil, the numerous Pseudomonas bacteria present in the rhizosphere of plants, deserve scholarly attention.

Pseudomonas fluorescens belong to the most widespread groups of microorganisms in the environment which can occur in soil, on plants, in fresh water and in deep sea. These gram-negative sticks produce fluorescein – a dye that causes them to glow in UV light. The major source of their energy is hydrocarbons (Nagarajkumaret et al., 2004).

Bacteria of the genus Pseudomonas are representatives of the so-called rhizobacteria, which are characterized by forming associations with plant roots. These bacteria degrade many different sugars, amino acids, alcohols, as well as highly molecular compounds, e.g. humic acids or pesticides (Nagarajkumaret et al., 2004). Some bacteria of the genus Pseudomonas produce diffusing, fluorescing compounds called siderophores which have a high affinity with iron compounds. These species are used for biological control of phytopathogens found in soil. These bacteria that produce siderophores trap iron and prevent pathogens from obtaining this compound from the environment (Mercado-Blanco et al., 2001).

The aim of the study was to analyze the occurrence of *Pseudomonas fluorescens* in urban soil in the second year after fertilization with two kind of sewage sludge obtainedfrom the treatment plant in Bialystok and the treatment plant in Sokółka.

Research methods

Experimental design

The study was conducted on experimental plots located in the green belts along the main roads in Bialystok (Piastowska and Hetmańska streets). Each of the test points of an area of 90 m^2 was divided into three blocksof an area of 30 m^2 each, which constituted further repetitions. For the studied soil, two different types of sewage sludge were used: after-press dewatered sludge from the treatment plant in Sokółka and dry sludge in the form of pellets from the treatment plant in Bialystok. The experiment plots were fertilized with three doses of sewage sludge: 0-control, 14.5 and 29 Mg D.M./ha.

Before the beginning of the experiment, the sewage sludge and soil collected from all combinations were analysed according to the Directive of Environmental Minister of February 6th, 2015 concerning municipal sewage sludge (tables 1 and 2).

Table 1. Chemical-physic properties of the soil before sludge application

Soil samples	pH	Heavy metals content in mg/kg of DM of sediment							[%]		
		Cd	Cu	Ni	Pb	Zn	Hg	Cr	Sand	Silt	Clay
Hetmańska street	8.1	0.5	16.7	6.3	7.5	50.8	0.06	12.1	75.9	22.0	2.1
Popiełuszki street	7.7	0.6	26.3	8.5	5.6	74.4	0.14	11.5	75.7	22.3	2.0

Source: author's own work.

Sewage sludge from the treatment plant in Sokółka and the treatment plant in Bialystok used in the experiment contained: dry matter 19.3% and 81.7%, organic matter 58.4 and 56.9% D.M., N – 3.99 and 4.6% D.M., P – 2.73

and 3.26% D.M., Ca 5.51 and 3.79% D.M., Mg 0.66 and 0.57% D.M. and its pH was 6.7 and 8.2, respectively.

Table 2. Selected chemical and biological properties of sewage sludge

Indicators	Heavy metals content in mg/kg of dry matter (D.M.)					
	Cd	Cu	Ni	Pb	Zn	Cr
after-press dewatered sludge	<0.5	194	22	23,5	1459	58
dry sludge in the form of pellets	<1.25	198	30.1	26.0	1045	76.6
limit value in the application of sludge to land reclamation for non-agricultural purposes	25	1200	400	1000	3500	1000
viable helminth ova of Ascaris sp., Trichris sp., Toxocara sp.	not detected					
bacteria of the genus Salmonella in 100 g of sludge	not detected					

Source: author's own work.

Physico-chemical parameters of soil samples

The particle size was determined using the Casagrande areometric method modified by Prószyński according to PN-R-04032 standardwhich is dedicated to the analysis of agricultural soils. In spring and autumn, the pH of the soil was evaluated in distilled water in the ratio 1:2.5 (m:v) using a HACH Lange pH-meter.

The organic matter content was determined by drying the soil samples at 105°C (removing hygroscopic water) and then burnt in a muffle furnace at 500ºC. The total organic carbon (TOC) was measured by Tiurin method described by Ostrowska et al. (1991). The total nitrogen content was analysed by Kjeldahl method using Gerhardt's Vapodest 50s after mineralization using Kjeldatherm (Gerhardt) block digestion.

Microorganisms analyses

The rhizosphere soil samples for microbiological analyses were collected at three times: in April, July and October in the second year after the application of sludge. The grass roots were taken with soil adhering to them from each test plot to specially marked containers inserted into the fridge and transported to the laboratory for a further analysis. Then, 5 g of roots with the soil was suspended in 45 ml of sterile saline solution (solution of 0.85% NaCl) shaken for 10 min and then subjected to dispersion. A serial ten-fold dilution (10-1–10-6) was prepared for each of the samples and each dilution was used to inoculate Petri dishes. The number of bacteria of the species *Pseudomonas fluorescens* cultured in a mineral medium containing Bacto-

-Pepton (20.0 g); KH_2PO_4 (1.5 g); $MgSO_4 \times 7H_2O$ (1.5 g) and agar (15.0 g) per liter of deionized water. The pH was adjusted to 7.2 with 1MNaOH and glycerol (10%; v/v; 1.37 M) was added as a sole source of carbon (Galimska-Stypa et al., 1999). The bacteria were incubated at 28°C for 72 h and then the fluorescence was detected in UV light on the transluminator (UV Transilluminator (MD-20/HD-20) from Wealtec), then selected colonies were checked using API 20E tests.

The mean number of colonies in triplicate was presented as a colony-forming unit (cfu) in grams of roots and soil dry matter (DM).

Statistical analysis

The effect of selected soil properties on the number of *Pseudomonas fluorescens* was assessed using Spearman's correlation analysis at the significance level of $p < 0.05$. The effect of sewage sludge fertilization and the date of sampling were determined using the ANOVA variance analysis. Material differences were statistically evaluated using the Tukey test at the significance level of $p \leq 0.05$. The Statistica 13 was used.

Results of the research

Due to the high content of organic matter as well as its macro- and micro components sewage sludge is increasingly used in fertilizing agricultural and forestry areas and in recultivation of urban soil (Kaniuczak et al., 2009; Wołejko et al., 2015). Augustynowicz et al. (2010) draw attention to the beneficial properties of using sewage sludge as a fertilizer stimulating the number of soil bacteria. Based on the results of the present study, one observed that applied doses of sewage sludge from the two sewage treatment plants had an influence on the number of *Pseudomonas fluorescens* bacteria in the rhizosphere area (figures 1 and 2). The sewage sludge from the treatment plant in Sokółka applied on plots at Popiełuszki street significantly influenced the number of analyzed bacteria. In the growing season, the highest average number of *Pseudomonas fluorescens* bacteria was observed in the plots where the dose of sewage sludge was applied in the amount of 29 Mg D.M./ha (12.0 CFU*10^5 g^{-1}D.M.), and the lowest – on the control plots (7.6 CFU*10^5 g^{-1}D.M.). In turn, on the plots at Hetmańska street, it was observed that average number of *Pseudomonas fluorescens* bacteria was the highest on the control plots – 11.26 CFU*10^5 g^{-1}D.M., while for the plots fertilized with 29 Mg DM/ha, it was 5 CFU*10^5 g^{-1}D.M.

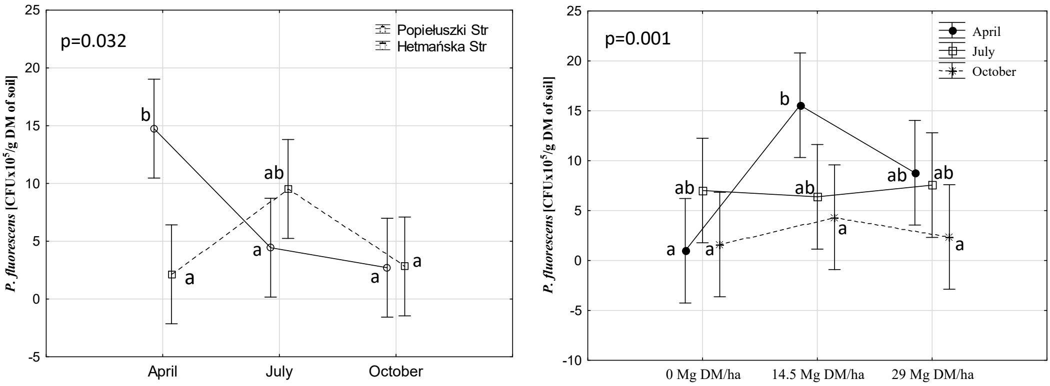

Figure 1. Occurrence of *Pseudomonas fluorescens* bacteria in the rhizosphere zone in the second year after application of sewage sludge from the treatment plant in Sokółka depending on the location and dose of sewage sludge. The same letters for average values mean no significant differences evaluated by Tukey test for p<0,05

Source: author's own work.

The present research study showed that in October there was the highest number of analyzed bacteria in the soil samples collected at Popiełuszki and Hetmańska streets, while in July the lowest was observed, which was probably caused by changes in environmental conditions (soil moisture and temperature) (figure 1). A similar relationship was also noticed in the research by Natywa et al. (2011) and Górska et al. (2007) who presented the dependence of the number of soil microorganisms on weather conditions in particular seasons. In the studied number of bacteria, periodic fluctuations may have been caused by the variability of atmospheric conditions, the length of precipitation, drought and changes in the microclimate.

On the plots where sewage sludge from the treatment plant in Bialystok was used, one observed the highest average number of *Pseudomonas fluorescens* at Popiełuszki street in April at the highest dose of sewage sludge of about 20.0 CFU*10^5 g^{-1}D.M., while the lowest was recorded on the control plots in September (3.5 CFU*10^5 g^{-1}D.M.) In turn, on the plots at Hetmańska street the highest number of *Pseudomonas fluorescens* bacteria was observed in July on the control plots (30.0 CFU*10^5 g^{-1}D.M.), while the lowest was recorded on the control plots in April (8.0 CFU*10^5 g^{-1}D.M.) (figure 2). The applied doses of sludge had a significant impact on the number of analyzed bacteria. On the plots fertilized with sewage sludge, the number of *Pseudomonas fluorescens* increased by approximately 15% compared with the control plots.As noted by Martin et al. (2004), after adding sewage sludge to the soil, it is very important to determine the (bio)chemical changes occurring in the vicinity of the roots, because they affect significantly the development of soil microorganisms and decomposition of xenobiotics present in soil.

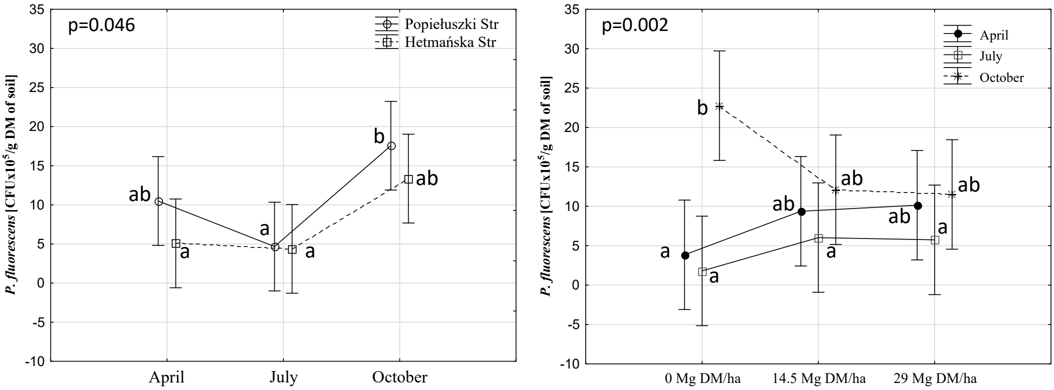

Figure 2. Occurrence of *Pseudomonas fluorescens* bacteria in the rhizosphere zone in the second year after application of sewage sludge from the Treatment Plant in Bialystok depending on the location and dose of sewage sludge. The same letters for average values mean no significant differences evaluated by Tukey test for p<0,05

Source: author's own work.

According to the research conducted by Siuta (2003), sludge produced as a by-product of wastewater treatment should be used to increase the biological activity in the soil. The development of microorganisms in the soil is influenced by many factors such as: physical and chemical properties of the soil, organic matter, applied fertilization as well as climatic and environmental conditions (Jezierska-Tys, Frąc, 2008). Based on our results, it was found that the ratio of C:N for the control plots ranged from 3.7 to 14.5, but for the plots where one applied after-press sludge it was 3.9 to 9.2, while for the plots where dry sludge, it was from 6.2 to 11.8. Moreover, on all the test plots soil pH was alkaline and ranged from 7.3 to 8.3 for the plots fertilized with after-press sludge and from 6.9 to 8.1 for the plots where dry sludge was used.

The statistical analysis revealed a significant correlation between the number of *Pseudomonas fluorescens* in the soil and the C:N ratio, silt and organic carbon (r=0.43, r=0.49 and r=0.64, respectively) at p ≤0.05. The normal development of soil bacteria will also depend on the availability of phosphorus in the soil. In this study, the content of phosphorus ranged from 15.0 to 72.0 mg P_2O_5/100 g D.M. for the plots where dry sludge was usedand for the plots where one applied after-press sludge it was 5.0 to 19.0 mg P_2O_5/100 g D.M. The content of this element in the soil influences the increase of biochemical and microbiological activity of the soil, which results in the transformation and availability of other nutrients (Bünemann et al., 2013).

In this study, one observed a positive correlation between the number of *Pseudomonas fluorescens* in the soil and content of phosphorus (r=0.63), which suggests that increases of the number bacteria may cause the increases of the content of phosphorus in the soil thus it can be more accessible to plants. Jezierska-Tys and Frąc, (2008) indicate that *Pseudomonas fluorescens* belong to the group of microorganisms that dissolve phosphates. This process consists in the secretion of phosphorus-releasing organic acids in an inorganic form. Moreover, in our study, one observed a negative correlation between the number of *Pseudomonas fluorescens* with a pH, sand and clay (r=-0.62, r=-0.45 and r=-0.55, respectively) at $p \leq 0.05$.

Conclusions

1. In the second year after the application of sewage sludge, microbiological tests of the rhizosphere area showed seasonal fluctuations in the number of *Pseudomonas fluorescens* bacteria.
2. The highest number of *Pseudomonas fluorescens* bacteria was observed in April and in October, while the lowest number of bacteria occurred in July, which could have been conditioned by atmospheric factors.
3. On the plots where dry sludge pellets were used, one observed an increase in the number of *Pseudomonas fluorescens* bacteria by approximately 15% compared with the control plots.
4. The analysis of the correlation indicates that in the urban soil mixed with sewage sludge the number of *Pseudomonas fluorescens* was significantly positively correlated with C:N ratio, organic carbonand content of phosphorus.

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The contribution of the authors

Elżbieta Wołejko – formulation of research concept and objectives, literature review, statistical analysis – 55%
Urszula Wydro – participation in the samples preparation for microbiological analysis and literature review – 30%
Agata Jabłońska-Trypuć – improving the manuscript after the reviews – 5%

Andrzej Butarewicz – participation in the literature review – 5%
Tadeusz Łoboda – participation in the literature review – 5%

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