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MICROBIOLOGICAL ACTIVITY OF SOIL AMENDED WITH GRANULATED FERTILIZER FROM SEWAGE SLUDGE

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Abstract

The study was conducted on a model of a pot experiment in which grey-brown podzolic soil developed from weakly loamy sand was amended with granulated dry organic-mineral fertilizer prepared from municipal sewage sludge. Three levels of pre-sowing fertilization with the granulate were applied: I – a dose which brought nitrogen in the amount of 0.35 g kg^{-1} d.m. of soil, II – a 50% higher dose, and III – a 100% higher dose. Additionally, a treatment with average mineral fertilization (NPK) was run. The control treatment was non-fertilized soil. Soil prepared as above was placed in pots and sown with white mustard (cv. Borowska) in spring in the 1st and 2nd year of the experiment. During the experiment, the soil moisture was maintained at the level of *ca* 60% t.w.c. Microbiological and biochemical soil assays were conducted three times in the first and second years of the experiment, i.e. in May and July and 41 days from harvesting the crop and mixing of its fragmented residues with the soil. The scope of the analyses included numbers of bacteria with low and high nutrition requirements, total numbers of filamentous fungi, cellulolytic, proteolytic and lipolytic bacteria and fungi. Other analyses included determinations of the respiratory activity in soil, the rate of cellulose mineralization and the activity of dehydrogenases, lipase, protease and acid phosphatase. The study showed a stimulating effect of the sludge granulate on the above groups of bacteria and fungi, both in the 1st and in the 2nd years of the experiment. The effect generally weakened with time. The fertilization also stimulated the respiratory rate and activity of phosphatase. However, it inhibited the mineralisation of cellulose as well as the activity of dehydrogenases, lipase and protease in soil. The application of mineral fertilization in the form of NPK did not cause any changes in numbers of the analyzed bacterial groups, except a certain decreasing tendency in the second year. In both years, mineral fertilization contributed to the stimulation of the growth of most fungi. It also enhanced the respiratory activity, rate of cellulose mineralisation, and the activity of dehydrogenase and acid phosphatase, albeit only in the first year of the experiment. Finally, in the soil with mineral fertilization the activity of protease and lipase was observed to have declined.

Keywords: grey-brown podzolic soil, mustard, sludge granulate, NPK, numbers of bacteria and fungi, biochemical activity.

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AKTYWNOŚĆ MIKROBIOLOGICZNA GLEBY NAWOŻONEJ GRANULATEM Z OSADU ŚCIEKOWEGO

Abstrakt

Badania przeprowadzono na modelu doświadczenia wazonowego, w którym glebę płową, wytworzoną z piasku słabo gliniastego, nawieziono granulowanym suchym nawozem organiczno-mineralnym, wykonanym na bazie osadu ścieków komunalnych. W doświadczeniu zastosowano przedsiewnie trzy poziomy nawożenia granulatem: I – dawka z którą wniesiono azot w ilości 0,35g kg^{-1} s.m. gleby; II – dawka zwiększona o 50%, III dawka zwiększona o 100%. Dodatkowo wprowadzono kombinację z przeciętnym nawożeniem mineralnym (NPK). Kontrolę doświadczenia stanowiła gleba nienawożona. Tak przygotowaną glebę umieszczono w wazonach i obsiewano wiosną w 1. i 2. roku badań gorczycą białą odmiany Borowska. Wilgotność gleby w czasie doświadczenia utrzymywano na poziomie ok. 60% c.p.w. Analizy mikrobiologiczne i biochemiczne gleby wykonano trzykrotnie w 1. i 2. roku doświadczenia, tj. w maju i lipcu oraz po 41 dniach od zbioru rośliny i wymieszaniu jej rozdrobnionych resztek z glebą. Określano liczebność: bakterii o małych i dużych wymaganiach pokarmowych, grzybów nitkowatych ogółem, bakterii i grzybów celulolitycznych, proteolitycznych oraz lipolitycznych. Ponadto w glebie analizowano aktywność oddechową, tempo mineralizacji celulozy oraz aktywność dehydrogenaz, lipazy, proteazy i kwaśnej fosfatazy.

Wykazano stymulujący wpływ zastosowanego granulatu osadowego na ww. grupy bakterii i grzybów, zarówno w 1., jak i w 2. roku doświadczenia. Obserwowane oddziaływanie na ogół słabło w miarę upływu czasu. Nawożenie to stymulowało również aktywność oddechową i aktywność fosfatazy. Natomiast hamowało tempo mineralizacji celulozy, a także aktywność dehydrogenaz, lipazy i proteazy w glebie. Zastosowanie nawozu mineralnego w postaci NPK w 1. roku badań nie spowodowało zmian w liczebności. badanych grup bakterii, a w kolejnym roku odnotowano nawet pewną tendencję spadkową w tym zakresie. W obu latach nawożenie mineralne przyczyniło się do pobudzenia rozwoju większości grup grzybów oraz tylko w 1. roku badań – aktywności oddechowej, tempa mineralizacji celulozy, aktywności dehydrogenaz i fosfatazy kwaśnej. W glebie z nawozem mineralnym odnotowano ponadto spadek aktywności proteazy i lipazy.

Słowa kluczowe: gleba płowa, gorczyca, granulat osadowy, NPK, liczebność bakterii i grzybów, aktywność biochemiczna.

INTRODUCTION

Owing its high fertilizing potential, sewage sludge is much more than just waste (Baran et al. 2002). When stored, often on sites inadequately prepared for the purpose, it constitutes a hazard to the environment. In contrast, after introduction to soil it has a beneficial effect on numerous physical, chemical, physicochemical and biological properties, as reported by Sastre et al. (1996), Księżopolska et al. (2002), Furczak and Joniec (2007), Pascual et al. (2007), Joniec and Furczak (2008), Ociepa (2011) or Scherer et al. (2011) and other researchers. Moreover, natural utilisation of sewage sludge means that large amounts of biogenic substances accumulated and immobilised in sewage sludge, primarily carbon, nitrogen and phosphorus, are returned into the cycling of elements. However, prior to using sewage sludge, a risk of its containing excessive levels of chemical pollutants should be considered, e.g. heavy metals, PAHs, pathogenic organisms, as well as of large amounts of organic matter and deficient levels of potassium (BARAN et al. 2002, OLESZCZUK 2007, WALCZAK, LALKE-PORCZYK 2009). Therefore, in order to improve the properties of sewage sludge, it is frequently subjected to various processes, e.g. composting (WOLNA-MARUWKA 2009, NICOLAS et al. 2012,) or phytoremediation with the use of alternative crop plants (LIPHADZI et al. 2006). One such plant is white mustard, characterised by phytosanitary and phytoremediation properties moreover; white mustard can also inhibit the migration of biogenic compounds to the environment and improve the soil structure (SAWICKA, KOTIUK 2007, VANEK et al. 2010). The production of granulated organic-mineral fertilizers from sewage sludge may turn to be another solution, ensuring better characteristics of sewage sludge and creating new possibilities of utilisation municipal sewage sludge (NOWAK et al. 2002).

In this study, an attempt was made to estimate the applicability of such fertilizer for the improvement of biological properties of grey-brown podzolic soil under cropped with white mustard. Additionally, the effect of the organic-mineral granulate was compared with conventional NPK mineral fertilization.

MATERIAL AND METHODS

The study was conducted on a model of a pot experiment set up in a greenhouse of the Faculty of Environmental Microbiology, University of Life Sciences in Lublin. Each of the pots contained 5 kg of grey-brown podzolic soil developed from weakly loamy sand (pH_{KCl} – 5.3; $C_{org.}$ – 9.12 g kg^{-1}), fertilized once with dry granulated organic-mineral fertilizer from municipal sewage sludge, containing: N – 61 g kg^{-1}; P – 21.4 g kg^{-1}; K – 108 g kg^{-1}; Ca – 28.6 g kg^{-1} and Mg – 6.03 g kg^{-1}. The granulate was composed of 42.5% of organic matter and 57.5% of inorganic components. The fertilizer was synthesised at the Department of Coke Engineering and Environmental Protection, Institute of Chemical Processing of Coal in Zabrze, and consisted of 70% of sewage sludge and 30% of potassium nitrate (KNO_3). Detailed characteristics of the fertilizer are given by Wiater and Żebranowicz (2005). Three levels of pre-sowing fertilization with the granulate were applied in the experiment: I – dose which brought nitrogen in the amount of 0.35 g kg^{-1} d.m. of soil, II – a 50% higher dose, and III – a 100% higher dose. In another experimental object, an average mineral fertilization treatment was applied in the form of NPK. The control treatment consisted of soil without any fertilization. The experimental design included three replications for each of the treatments. The soil in all the pots was stabilised at a moisture level of 60% t.w.c. and sown with white mustard (cv. Borowska). That level of soil moisture was maintained fairly stable throughout the experiment. After harvesting aerial parts of the plants, the residues were fragmented, mixed with the soil and left till the next year. White mustard was sown again into the same pots in spring the following year to observe the residual effect of the fertilization on the biological parameters of the soil.

Microbiological and biochemical analyses were performed three times (in May and July, i.e. during the growth of the plant, and 41 days after harvesting the crop and mixing fragmented plant residues with the soil) in the first and second years of the experiment.

The scope of the analyses included assays of the total number of oligotrophic bacteria, on a medium with soil extract or sludge extract (350 cm^3 dm^{-3}) and K_2HPO_4; total number of macrotrophic bacteria on the Bunt-Rovira medium (1955); so-called total number of filamentous fungi on the Martin medium (1950); numbers of cellulolytic bacteria on liquid medium accoring to Pochon and Tardieux (1962), the most probable number of those bacteria was read from McCrady's Tables; numbers of cellulolytic fungi on mineral agar, with an addition of antibiotics in the amount recommended by Martin (1950), covered with Whatman paper discs; numbers of bacteria decomposing proteins, on the Frazier gelatine medium (Rodina 1968); numbers of fungi decomposing proteins, on the Frazier gelatine medium (Rodina 1968) with an addition of antibiotics in the amount recommended by (Martin 1950); numbers of lipolytic bacteria on agar medium with tributyrate (Burbianka et al. 1971) in which yeast extract was replaced with broth (Nowak et al. 1992); numbers of lipolytic fungi on the medium as above, with an addition of antibiotics in the amount recommended by (Martin 1950); respiratory activity with the method of Rühling and Tyler (1973); rate of cellulose mineralisation in 25-gram weighed portions of soil enriched with 0.5% of powdered Whatman cellulose, from the amount of CO_2 emitted from soil during 20 days assayed with the method of Rühling and Tyler (1973); activity of dehydrogenases with the method of Thalmann (1968); lipase activity with the method of Pokorna (1964) modified by Kuhnert-Finkernagel and Kandeler (1996); protease activity with the method of Ladd and Butler (1972); activity of acid phosphatase with the method of Tabatabai and Bremner (1969).

All analyses were made in three replications. The results of the microbiological and biochemical analyses were processed statistically by analysis of variance. The significance of differences was determined with the Tukey's test at $p = 0.05$.

RESULTS AND DISCUSSION

The study indicated that the applied doses of the granulate caused an increase in the numbers of almost all of the bacterial groups in the 1st year, i.e. oligotrophic, macrotrophic, proteolytic and lipolytic bacteria (Tables 1, 2 and 3). The effect was the strongest in the case of proteolytic bacteria and

Table 1

Total numbers of bacteria in the soil

Fertilization	Oligotrophic bacteria (cfu 10^9 kg^{-1} d.m.of soil)		Macrotrophic bacteria (cfu 10^9 kg^{-1} d.m.of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	5.078	12.456	3.622	22.611
Soil + NPK	1.633	7.400	3.889	7.111
Soil + I dose of granulate	6.678	9.267	8.378	16.311
Soil + II dose of granulate	5.133	36.956	8.889	122.200
Soil + III dose of granulate	6.889	8.900	9.433	17.467
Mean	5.082	14.996	6.842	37.140
LSD year LSD year x fertilization	0.416 1.530		0.600 2.203	

Table 2

Numbers of proteolytic bacteria and fungi in the soil

Fertilization	Proteolytic bacteria (cfu 10^9 kg^{-1} d.m. of soil)		Proteolytic fungi (cfu 10^6 kg^{-1} d.m. of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	0.467	2.667	28.89	66.89
Soil + NPK	0.822	1.933	59.11	87.78
Soil + I dose of granulate	1.844	5.756	39.11	70.00
Soil + II dose of granulate	1.978	4.244	42.67	58.44
Soil + III dose of granulate	2.511	5.989	24.33	58.44
Mean	1.524	4.118	38.82	67.51
LSD year LSD year x fertilization	0.185 0.682		4.13 15.19	

Table 3

Numbers of lipolytic bacteria and fungi in the soil

Fertilization	Lipolytic bacteria (cfu 10^9 kg^{-1} d.m. of soil)		Lipolytic fungi (cfu 10^6 kg^{-1} d.m. of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	0.367	1.144	18.02	11.97
Soil + NPK	0.278	1.167	17.79	19.31
Soil + I dose of granulate	0.689	1.911	21.98	12.30
Soil + II dose of granulate	0.822	1.411	23.13	15.93
Soil + III dose of granulate	1.000	1.333	21.00	11.71
Mean	0.631	1.393	20.38	14.24
LSD year LSD year x fertilization	0.056 0.207		0.96 3.55	

the weakest regarding oligotrophic bacteria (Tables 1, 2 and 3). Moreover, the growth of proteolytic and lipolytic bacteria was more intensive under higher doses of the sludge granulate. It was only the number of cellulolytic bacteria that was slightly reduced in the first year of the experiment, and the decrease was the biggest under the effect of the largest dose of the granulate (Table 4).

Table 4

Numbers of cellulolytic bacteria and fungi in the soil

Fertilization	Cellulolytic bacteria (10^6 kg^{-1}· d.m. of soil)		Cellulolytic fungi (cfu·10^6 kg^{-1} d.m. of soil)	
	1st year	2nd year	1 st year	2 nd year
Non-fertilized soil	52.41	1.60	28.15	17.01
Soil + NPK	45.00	1.11	41.20	36.80
Soil + I dose of granulate	47.02	7.42	30.50	23.24
Soil + II dose of granulate	47.31	4.20	35.32	25.20
Soil + III dose of granulate	30.43	7.70	38.52	16.98
Mean	44.43	4.41	34.74	23.85
LSD year LSD year x fertilization			1.17 4.31	

In the second year, the growth of oligo- and macrotrophic bacteria was stimulated only by the middle dose of the granulate (Table 1) whereas the other two doses (I and III) caused a slight decreasing tendency in the numbers of those bacteria. On the other hand, the stimulated growth of proteolytic and lipolytic bacteria proceeded in the 2nd year of the experiment in all the treatments with the granulate (Tables 2, 3), although it was weaker than in the previous year. The growth of cellulolytic bacteria, as opposed to the first year, was distinctly stimulated by all doses of the granulate (Table 4).

In a comparison of the effects of fertilization with the granulate versus NPK, it was noted that the granulate had a much better effect on the growth of the analyzed bacterial groups (Tables 1-4). In the 1st year, numbers of bacteria from most of the groups in the soil amended with NPK alone were on levels similar to or below the control treatments. However, in the second year, a tendency for the inhibited growth of most of the bacterial groups was observed (except for lipolytic bacteria) – Tables 1-4.

As with bacteria, numbers of all analyzed fungi, i.e. total, cellulolytic, proteolytic and lipolytic fungi, increased in the first year in the soil amended with the granulate (Tables 2-5). This effect was the most distinct for the total number of fungi.

In the second year, a slight stimulation by the granulate was still observed in the growth of most of the groups of fungi (Tables 2-5). As for total fungi, however, the stimulating effect was weaker with time, disappearing completely in the case of proteolytic fungi.

Table 5

Total number of filamentous fungi in the soil

Fertilization	Filamentous fungi ($cfu \cdot 10^6\ kg^{-1}$d.m. of soil)	
	1st year	2nd year
Non-fertilized soil	102.3	112.6
Soil + NPK	305.3	158.7
Soil + I dose of granulate	192.8	127.3
Soil + II dose of granulate	172.9	159.9
Soil + III dose of granulate	136.7	83.8
Mean	182.0	128.4
LSD year LSD year x fertilization	6.7 24.8	

The data in Tables 2, 4 and 5 show that the total number of filamentous, cellulolytic and proteolytic fungi increased also in the soil fertilized with NPK in the first year. As time passed, the positive effect on the total number of fungi and proteolytic fungi was weaker, although it continued also in the second year. Reversely, the stimulation of the growth of cellulolytic and lipolytic fungi intensified in later stages of the experiment (Tables 3 - 4). Populations of fungi in treatments with mineral fertilization were even larger than in treatments with the sewage sludge granulate. This indicates that mineral fertilization created more favourable conditions for fungi than sludge granulate. The application of mineral fertilization probably lowered the soil pH, an effect which is known to promote the growth of fungi. Lower soil reaction caused by mineral fertilizers is reported by Koper and Piotrowska (2003), among others. For soil quality, a more intensive growth of fungi is an adverse development. It may contribute to stronger acidification of soils and increased concentration of metabolites produced by fungi, including mycotoxins.

The stimulation of the growth of the analyzed bacterial and fungal groups by the sludge-mineral granulate was probably induced by the introduction of a certain amount of organic matter to soil because organic matter in sludge promotes the growth and activity of heterotrophic soil microorganisms (Sastre et al. 1996, Furczak, Joniec 2007, Pascual et al. 2007).

A review of the available literature shows absence of studies focused on the comparison of effects of sewage sludge granulate and mineral fertilization on soil microbiological parameters. On the other hand, there have been experiments on the effect of sewage sludge and mineral fertilization on microbial populations (Sastre et al. 1996). The cited authors demonstrated stimulated growth of various groups of bacteria and fungi, both by sewage sludge and by mineral fertilization.

In the first year of the current study, soil amendment with the experimental doses of the granulate stimulated the respiratory activity and the activity of acid phosphatase in soil (Tables 6-7). This effect was stronger in response to higher doses of the granulate. The positive effect of this fertilizer was also visible albeit more weakly in the second year of the experiment (Tables 6-7).

Both in the first and second year, the granulate doses most frequently caused a slight inhibition of the cellulose mineralization rate and the activity of dehydrogenases, lipase and protease (Tables 6-8).

In the first year of the experiment, mineral fertilization (NPK) stimulated the respiratory activity of soil and slightly improved the activity of dehydrogenases and acid phosphatase activity as well as the rate of cellulose mineralization (Tables 6-8). However, the positive effect of NPK was weaker, especially with respect to the respiratory and phosphatase activity, than the granulate. This was probably due to lower levels of organic carbon and, regarding phosphatase activity, to higher amounts of mineral phosphorus in soil fertilized with NPK, the reason also implied by MADEJÓN et al. (2001), GIANFREDA et al. (2005), and PASCUAL et. al. (2007) and others. With time, this effect was disappearing, and lasted only in the case of cellulose mineralisation, although it was much weaker than in the preceding year (Tables 6-8).

Table 6

Biochemical activity in the soil

Fertilization	Respiratory activity (mg C-CO_2 kg^{-1} d.m. of soil d^{-1})		Cellulose mineralization (mg C-CO_2 kg^{-1} d.m. of soil 20 d^{-1})	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	130.9	95.5	196.6	205.7
Soil + NPK	174.8	92.3	220.8	213.5
Soil + I dose of granulate	168.1	105.4	197.0	214.3
Soil + II dose of granulate	198.0	130.1	188.5	200.1
Soil + III dose of granulate	195.5	114.8	180.8	186.3
Mean	173.4	107.6	196.7	204.0
LSD year LSD year x fertilization	5.0 18.3			

Table 7

Protease and acid phosphatase activity in the soil

Fertilization	Protease (mg tyrosine kg^{-1} d.m. of soil h^{-1})		Acid phosphatase (mg PNP kg^{-1} d.m. of soli h^{-1})	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	12.096	7.791	30.72	44.84
Soil + NPK	5.403	2.484	34.07	44.23
Soil + I dose of granulate	8.538	6.758	35.40	48.79
Soil + II dose of granulate	7.317	6.308	37.75	52.43
Soil + III dose of granulate	5.432	7.468	38.95	49.66
Mean	7.757	6.162	35.38	47.99
LSD year LSD year x fertilization	0.318 1.170		1.10 4.05	

Table 8

Dehydrogenases and lipase activity in the soil

Fertilization	Dehydrogenases (mg TPF kg^{-1} d.m. of soil d^{-1})		Lipase (units g^{-1} d.m. of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	0.909	0.876	0.633	1.068
Soil + NPK	1.050	0.886	0.516	0.930
Soil + I dose of granulate	0.708	0.897	0.550	1.038
Soil + II dose of granulate	1.132	0.686	0.644	0.834
Soil + III dose of granulate	0.962	0.813	0.580	0.867
Mean	0.952	0.831	0.585	0.947
LSD year	0.057		0.020	
LSD year x fertilization	0.208		0.073	

Analogously to our experiment, studies by some other authors demonstrated a better effect of sewage sludge on the respiratory and phosphatase activity in soil compared to NPK (SASTRE et al. 1996, PASCUAL et al. 2007, SCHERER et al. 2011).

Fertilization of soil with NPK caused a slight decrease of lipase activity, same as the sludge granuate, and a stronger decline of protease activity. These effects persisted throughout the whole experiment. Different results were obtained by PASCUAL et al. (2007), who only found a slight drop in protease activity in soil fertilized with NPK, and a significant increase of the same parameter under the effect of sewage sludge.

Based on the current results of the granulate affecting the microbiological and biochemical activity of soil, and also on the studies by WIATER and ŻEBRANOWICZ (2005) on its fertilizer value, it should be concluded that sludge-organic granulate can be an alternative to and possibly a better fertilizer than NPK.

CONCLUSIONS

1. In the first year of the experiment, the applied granulate doses caused stimulated almost all of the bacterial and fungal groups, and the effects persisted in the second year, although it was weaker. Initially, a slight inhibition affected only cellulolytic bacteria, but with time it changed into distinct stimulation.

2. Application of the experimental doses of the granulate resulted in an increase of respiration and acid phosphatase activity in the 1^{st} year. This effect continued also in the 2^{nd} year, but on a lower level. Throughout the experimental period, the doses of the granulate most frequently caused a slight decrease in the rate of dehydrogenases, lipase and protease activity.

3. In the 1st year, no response of most of the bacterial groups to mineral fertilization was observed. In the 2nd year, there was even a slight tendency towards their inhibited growth was noted (except for lipolytic bacteria). However, the mineral fertilization had a favourable effect on the growth of most of the fungal groups during the two-year period.

4. In the 1st year, mineral fertilization (NPK) stimulated respiration, cellulose mineralization and, to some degree, activity of dehydrogenases and acid phosphatase in soil. With time, this effect disappeared, remaining only in the case of cellulose mineralization, but on a notably lower level than in the preceding year. NPK fertilization of soil led to a decrease in the activity of protease and lipase, which lasted throughout the whole experiment. The decline was most pronounced in the activity of protease, especially in the second year.

5. The observations during the two years of the experiment proved that fertilization of soil under white mustard with granulated organic-mineral fertilizer had a more beneficial effect on the growth of the analyzed bacterial groups, and on the respiratory and phosphatase activity of the soil, than mineral NPK fertilization. In turn, mineral fertilisation was more favourable for the growth of the fungal groups, which is an adverse development from the viewpoint of soil quality. Both types of fertilizers had a negative effect on lipase and protease activity in soil, but that effect was generally more pronounced in soil fertilized with NPK.

6. The results of biological analyses indicate that sludge granulate has a better effect on most of the soil biological parameters than mineral fertilization. Thus, the tested sludge granulate can be a valuable fertiliser in agricultural practice.

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