MIDDLE POMERANIAN SCIENTIFIC SOCIETY OF THE ENVIRONMENT PROTECTION
ŚRODKOWO-POMORSKIE TOWARZYSTWO NAUKOWE OCHRONY ŚRODOWISKA

Annual Set The Environment Protection
Rocznik Ochrona Środowiska

Volume/Tom 17. Year/Rok 2015 ISSN 1506-218X 125–142

Drug-Resistant Bacteria in Soils Fertilized with Sewage Sludge

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Introduction

Apart from chemical contaminants such as heavy metals or organic compounds, sewage sludge might also contain substantial amounts of bacteria, fungi, viruses and helminth eggs. The scientific literature has reported that sewage sludge is also characterized by high contamination with pathogenic microorganisms [6,7]. Microorganisms which are particularly dangerous to humans are often found in sewage sludge, including bacilli of typhoid fever (*Salmonella*) dysentery (*Shigella*), tetanus (*Clostridium tetani*) gas gangrene (*Clostridium perfringens*) and bacilli that cause foodborne disease (*Clostridium botulinum*). Examinations of microbiological composition of sewage sludge reveal also tuberculosis bacterial species (*Mycobacterium tuberculosis*) and anthrax bacilli (*Bacillus anthracis*). These microorganisms cause serious diseases in both cattle and in humans [4]. The properties of sewage sludge (physical, chemical and biological) are significantly affected by the way it is processed [8,19,20,21]. The uncontrolled use of antibiotics in medical care of humans and animals also contributes to the threat connected with development of new drug-resistant forms of microorganisms in sewage and sewage sludge. Despite numerous studies that have investigated the problems of the use of sewage sludge in nature and agriculture, no sufficient information has been provided to date about certain parasitic and pathogenic forms that are resistant to the drugs used which can survive in sludge and soils fertilized with sludge and in the plants grown in these soils. The effects of introduction of sewage sludge to the soil might be observed even many years after fertilization [14,15]. There are few reports on the presence of drug-resistant bacteria in sludge in the literature concerning sludge fertilization [9,10]. In both sludge and a conventional organic fertilizer provided by manure, numerous pathogenic forms have been found while they acquired or developed resistance patterns with respect to a number of antibiotic available in health care [2]. This problem is connected with excessive and often needless overuse of antibiotics in health prevention and health care. Antibiotics have also been popular in animal farming, not only for medical purposes, but often used with small doses for a longer period of time in order to increase animals' weight. One example is the United States of America, where some 40% of the antibiotics production is used for such purposes. In some countries, antibacterial drugs are used in farming and horticulture. It should also be noted that consumption of food that contains antibiotics with small contents prepares bacteria (e.g. those contained in the intestinal flora) for development of resistance patterns if the appropriate conditions occur [5]. These practices lead to selection of microflora that is resistant to antibiotics. Drug-resistant bacteria present in sludge accumulate in sewage sludge during processing and become a real threat to the environment. Few studies on the problems of drug-resistant bacteria in sewage and sewage sludge and the results obtained by the authors of the present paper point to the probability of a substantial survival of these particularly dangerous microorganisms in sewage sludge and in the soils fertilized with the sludge [10,14].

Experimental

Sewage sludge from the wastewater treatment plant in Myszków, Poland, was used in the experiments. The sludge was sampled after biological processes of sewage treatment, without fermentation. Stabilization occurred through anaerobic pathways. After dehydration (with addition of polyelectrolytes) in presses, part of the samples were subjected to liming and stored in a storage area for 6 months. Other samples were dried after liming in a solar dryer. After this process, sludge was also stored for the period of six months. One type of soil was fertilized. This was sandy soil and its characterization (similar to sewage sludge and manure) is provided in Tables 1 and 2. Properties of the fertilized soil were consistent with the recommendations for the use of sewage sludge [17,18]. For comparison purposes, the authors used straw-based cattle manure, which was also stored before the evaluation. The examinations were carried out under field experiment conditions (small beds with the surface area of 10 m^2). The doses of fertilizers used were 10, 20, 30 and 40 Mg d.m.·ha^{-1}, added in spring to arable layer at the thickness of 30 cm. The duration of the experiment was one vegetation season. White mustard (*Sinapis alba*) was planted in the fertilized soils. In autumn, after harvest of the plants, biological analyses of the fertilized soils were carried out. The experiments were carried out with 3 repetitions. The results obtained are represented by the means from these repetitions.

Table 1. Physical and chemical properties of soil, sewage sludge and manure used in the experiments
Tabela 1. Właściwości fizyczne i chemiczne gleby, osadów ściekowych i obornika stosowanych w badaniach

Parameter	Unit	Soil	Sewage Sludge		Manure
			Myszków (after natural drying)	Myszków (after solar drying)	
		Contents			
Organic substance	[% d.m.]	0.29	42.2	39.0	61.0
Reaction (pH_{H2O})	-	6.87	7.01	7.2	8.01
Organic carbon	[g·kg^{-1} d.m.]	13.40	218.4	215.0	246.6
Total nitrogen		0.64	39.1	35.0	39.8
Available P	[mg·kg^{-1} d.m.]	34.0	616.70	601.0	470.0
Available K		21.8	238.6	219.6	2260.0
Available Mg		55.6	893.6	901.0	994.3
Cr		10.4	17	19	2.1
Zn		75.0	908	913	132
Pb		17.8	21	23	7.3
Cu		12.6	139	142	21
Cd		0.4	2.1	2.3	1.2
Ni		10.3	102.5	120.0	2.2
Hg		0.003	1.6	1.82	0.11

The contents of 7 standard heavy metals (Cd, Cu, Ni, Pb, Zn, Hg, Cr) in the sewage sludge used for the experiments and in the manure did not exceed the permissible values that are a prerequisite for their use in nature. The contents of heavy metals found in the sewage sludge allowed even for their agricultural use and soil reclamation for agricultural purposes. Furthermore, the contents of heavy metals in the soils used in the experiments did not exceed permissible values for the use in agriculture and for reclamation of soil [17,18].

Biological conditions of research materials used in the field experiments are presented in Table 2.

Table 2. Biological properties of soil, sewage sludge and manure used in the experiments
Tabela 2. Własności biologiczne gleby, osadów ściekowych i obornika stosowanych w badaniach

Parameter	Unit	Soil	Sewage Sludge		Manure
			Myszków (after natural drying)	Myszków (after solar drying)	
		Contents			
Escherichia coli	Coliform index	10^{-1}	10^{-5}	10^{-5}	10^{-5}
Proteus vulgaris	Coliform index	10^{-2}	10^{-5}	10^{-4}	10^{-5}
Clostridium perfringens	Coliform index	n.s.	10^{-5}	10^{-4}	10^{-4}
Salmonella species bacteria	[JTK/g d.m.]	n.s.	n.s.	n.s.	n.s.
Total bacterial count	[JTK/g d.m.]	$5.2*10^{5}$	$21*10^{9}$	$31*10^{8}$	$29*10^{10}$
Total fungi count	[JTK/g d.m.]	$2.5*10^{4}$	$25*10^{6}$	$31*10^{3}$	$51*10^{6}$
Total actinobacteria count	[JTK/g d.m.]	$1.4*10^{3}$	$43*10^{6}$	$49*10^{6}$	$55*10^{6}$
Total count of *Fusarium* fungi species	[JTK/g d.m.]	n.s.	$21*10^{3}$	$21*10^{1}$	$23*10^{10}$
ATT index		n.s.	n.s.	n.s.	n.s.

n.s. – no presence was found

The sanitary state of the sewage sludge determined before the experiments (Tab. 2) allowed for its use in agriculture. No *Salmonella* bacteria (detected on the medium SS) were isolated and no helminths' eggs (detected by Wasilkowa flotation method), which are the main sanitary indicator that determines possibility of the use of sewage sludge in nature, were found in the sludge.

Laboratory tests methodology

The fertilized soils were evaluated with respect to bacterial count concerning the bacteria that exhibited resistance to first-line antibiotics. After obtaining the positive results of the first determinations, the authors analysed the resistance of conditional pathogens to the final antibiotic (vancomycin).

Biological tests methodology

Determination of total count of pathogenic bacteria resistant to first-line antibiotics

Quantitative and qualitative analysis of individual groups of microorganisms was carried out using the Koch's dish method in agar plates. In order to determine drug resistance of the microorganisms, the authors used blood agar plates and MacConkey agar plates. After 18-hour incubation at 37 degrees Celsius, the authors evaluated growth and isolation of individual strains. Biochemical examinations aimed at identification of individual bacteria species from the family *Enterobacteriaceae,* employed Enterotest and non-fermenting gram-negative bacilli test (Nefermtest, Lachema). Simultaneous tests of drug resistance were also carried out. Drug resistance tests used Mueller-Hinton plates.

Furthermore, the plates with the following antibiotics were added:

- ampicillin 10 mg,
- gentamicin 10 mg,
- amikacin 10 mg,
- ceftazidime 30 mg,
- amoxicillin with clavulanic acid 20/10 mg,
- cefotaxime 30 mg.

The tests were carried out at the temperature of 37 degree Celsius for 18 hours. Antibiotics were chosen so that they were consistent with the basic antibiogram (first-line antibiotics) according to the recommendations for test selection of the National Reference Centre for Microbial Drug Sensitivity [3].

Determination of total vancomycin-resistant mesophilic bacteria count

Determination was carried out using Koch's dish method with agar plate which was added the appropriate dose of the antibiotic (6mg/l according to the guidelines of KORLD) [3]. Then, the plates were inoculated with the suspension of bacteria from dilutions while using spread plate method. Incubations were carried out for 24 hours at 37˚C. After 24 hours, the authors enumerated the colonies that had grown and the results of the determination were given as vancomycin-resistant mesophilic (conditional pathogen) bacteria count per 1g of soil, mixtures of soil with sewage sludge and those with manure. The experiments were carried out with 3 repetitions. The results obtained are represented by the means from these repetitions. The results obtained were analysed statistically using the Kruskal-Wallis test.

Results

The results obtained for qualitative determinations and drug-resistance (compared to first-line antibiotics) of conditionally pathogenic microorganisms in the sandy soils fertilized with sewage sludge and manure after a year from fertilizing under conditions of field experiment are presented in Tables 3–5. Figure 1–4 presents a comparison of mean susceptibility or resistance of pathogenic microorganisms to these drugs in the fertilized soils (collectively for all the doses).

Figure 5 presents mean bacteria count for conditionally-pathogenic vancomycin-resistant bacteria for all the doses determined in the soils fertilized with sewage sludge and manure.

Table 3. Results obtained for determination of drug resistance of conditionally pathogenic microorganisms in sandy soils fertilizedwith sewage sludge from Myszkow wastewater treatment plant after natural drying
Tabela 3. Wyniki lekooporności drobnoustrojów warunkowo chorobotwórczych w glebie piaszczystej nawożonej osadami ściekowymi z oczyszczalni ścieków w Myszkowie po naturalnym suszeniu

Fertilizer dose [Mg d.m.·ha^{-1}]	Type of isolated microorganisms	Susceptibility of the microorganisms to the antibiotic used					
		Amikacin	Amoxicillin with clavulanic acid	Ceftazidime	Cefotaxime	Gentamicin	Ampicillin
10	*Enterobacter sp*	s	r	s	s	s	r
	Photorhabdus luminescens	s	s	s	s	s	r
	Escherichia coli	s	s	S	s	s	r
	Proteus vulgaris	s	s	S	s	s	ms
20	*Enterobacter sp*	s	r	S	s	s	r
	Photorhabdus luminescens	s	s	S	s	s	r
	Escherichia coli	s	s	S	s	s	r
	Proteus vulgaris	s	s	S	s	s	ms
	Klebsiella oxytoca	ms	r	S	r	s	r
30	*Enterobacter sp*	ms	r	S	s	s	r
	Photorhabdus luminescens	s	s	S	s	s	r
	Escherichia coli	s	ms	S	s	s	r
	Proteus vulgaris	s	s	S	s	s	r
	Klebsiella oxytoca	ms	r	S	r	s	r
	Enterobacter kobei	ms	s	S	s	s	r
40	*Enterobacter sp*	r	r	S	s	s	r
	Photorhabdus luminescens	s	s	S	s	s	r
	Escherichia coli	s	r	S	s	s	r
	Proteus vulgaris	s	s	S	s	s	r
	Klebsiella oxytoca	r	r	S	r	s	r
	Enterobacter kobei	r	s	S	s	s	r

s – susceptible, ms – medium susceptible, r – resistant

Table 4. Results obtained for determination of drug resistance of conditionally-pathogenic microorganisms present in sandy soil fertilized with sewage sludge from Myszków wastewater treatment plant after solar drying
Tabela 4. Wyniki lekooporności warunkowo chorobotwórczych mikroorganizmów obecnych w glebie piaszczystej nawożonej osadami ściekowymi z oczyszczalni ścieków w Myszkowie po solarnym suszeniu

Fertilizer dose [Mg d.m.·ha^{-1}]	Type of isolated microorganisms	Susceptibility of the microorganisms to the antibiotic used					
		Amikacin	Amoxicillin with clavulanic acid	Ceftazidime	Cefotaxime	Gentamicin	Ampicillin
10	*Alcaligenes faecalis*	s	s	S	s	s	s
20	*Alcaligenes faecalis*	s	s	S	s	s	s
	Achromobacer piechandii	s	s	S	s	s	s
30	*Alcaligenes faecalis*	s	s	S	s	s	s
	Achromobacer piechandii	s	s	S	s	s	s
	Pseudomonas alcaligenes	s	s	S	s	s	r
40	*Alcaligenes faecalis*	s	s	S	s	s	s
	Achromobacer piechandii	s	s	S	s	s	ms
	Pseudomonas alcaligenes	s	s	S	s	s	r

s – susceptible, ms – medium susceptible, r – resistant

Table 5. Results obtained for determination of drug resistance of conditionally-pathogenic microorganisms in the sandy soil fertilized with manure
Tabela 5. Wyniki lekooporności warunkowo-patogennych drobnoustrojów w piaszczystej glebie nawożonej obornikiem

Fertilizer dose [Mg d.m.·ha^{-1}]	Type of isolated microorganisms	Susceptibility of the microorganisms to the antibiotic used					
		Amikacin	Amoxicillin with clavulanic acid	Ceftazidime	Cefotaxime	Gentamicin	Ampicillin
10	*Enterobacter sp*	s	s	S	s	s	r
	Serratia fonticola	s	ms	S	ms	s	r
	Yersinia rodhei	s	r	S	ms	s	r
20	*Enterobacter sp*	s	s	S	s	s	r
	Serratia fonticola	s	ms	S	ms	s	r
	Yersinia rodhei	s	ms	S	ms	s	r
	Photorhabdus luminescens	s	r	S	r	s	r
	Enterobacter kobei	s	ms	S	s	s	r
30	*Enterobacter sp*	s	s	S	s	s	r
	Serratia fonticola	s	r	S	r	s	r
	Yersinia rodhei	s	r	S	r	s	r
	Photorhabdus luminescens	s	r	S	r	s	r
	Enterobacter kobei	s	ms	S	s	s	r
	Citrobacter brakii	s	r	S	ms	s	r
40	*Enterobacter sp*	s	r	S	s	s	r
	Serratia fonticola	s	r	S	r	s	r
	Yersinia rodhei	s	r	S	ms	s	r
	Photorhabdus luminescens	s	r	S	r	s	r
	Enterobacter kobei	s	r	S	r	s	r
	Citrobacter brakii	s	r	S	s	s	r

s – susceptible, ms – medium susceptible, r – resistant

Statistical analysis of the results obtained in the field experiment based on Kruskal-Wallis test revealed that the resistance to the antibiotics used differed between the samples. In the case of fertilization with the sludge after natural drying, amikacin-resistance bacteria were found. They were not present after fertilization with manure and the sludge after solar drying. With regard to amoxicillin with clavulanic acid in the soil after the use of the solar-dried sludge, all the bacteria exhibited susceptibility. After fertilization with manure and the sludge after natural drying, the cases of medium susceptibility and resistance to the drug were observed. Susceptibility to cefotaxime was found in all the potentially pathogenic bacteria isolated from sandy soil fertilized with the sludge after solar drying. In the soils after fertilization with manure and the sludge after natural drying, the cases of medium susceptibility and resistance to the drug were observed. With regard to amipicilin, the most of the bacteria in the soils fertilized with the sludge after solar drying were susceptible. Furthermore, fertilization of the soil with manure and the sludge after natural drying caused, in the most of the cases, the development of pathogenic forms of bacteria which were resistant to this antibiotic. In the case of ceftazidime and gentamicin, all the analyses revealed the sensibility of the isolated conditionally pathogen forms to these antibiotics. Furthermore, it was found in the field experiment that the increase in the fertilizer dose contributed significantly to the increase in resistance of pathogenic forms in the soils studied.

All the sludge and manure samples and the samples of soils fertilized with them contained vancomycin-resistant microorganisms. Presence of insignificant number of bacteria that exhibited resistance to vancomycin was observed in the non-fertilized control soil. The most of these forms ($22*10^4$) were found in manure and soils fertilized with manure. Sewage sludge after solar drying contained the more of vancomycin-resistant bacteria compared to the sludge after natural drying.

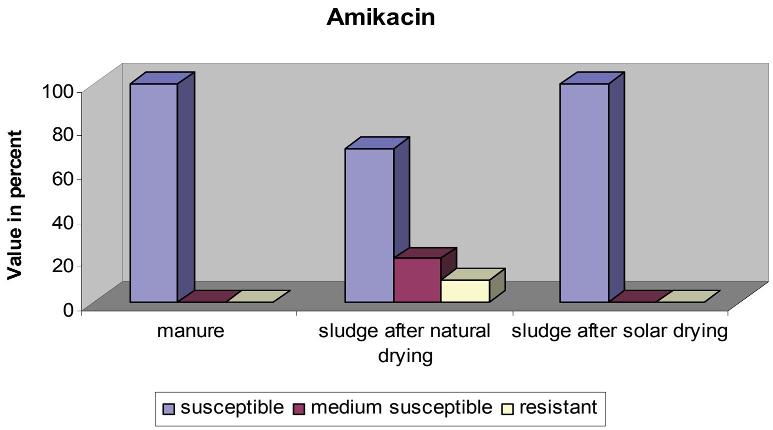

Fig. 1. Mean overall sensitivity to selected first-line antibiotics in drug-resistant conditionally-pathogenic to Amikacin microorganisms after fertilization of sandy soil with sewage sludge and manure [%]

Rys. 1. Średnia wrażliwość na wybrane antybiotyki pierwszego wyboru wśród lekoopornych warunkowo chorobotwórczych mikroorganizmów na Amikacynę po nawożeniu gleby piaszczystej osadami ściekowymi i obornikiem [%]

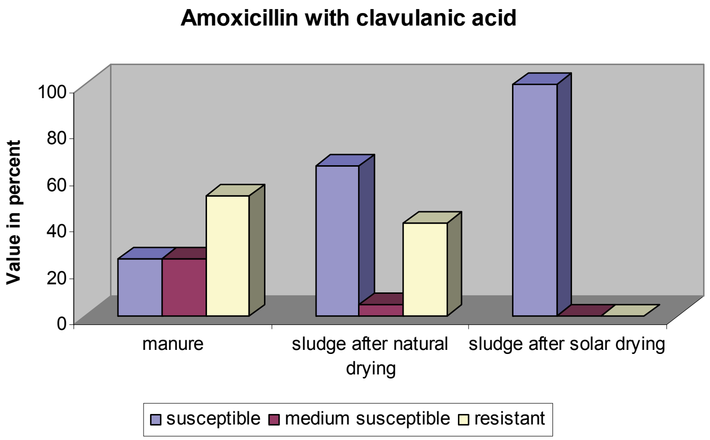

Fig. 2. Mean overall sensitivity to selected first-line antibiotics in drug-resistant conditionally-pathogenic to Amoxicilin with clavulanic acid microorganisms after fertilization of sandy soil with sewage sludge and manure [%]

Rys. 2. Średnia wrażliwość na wybrane antybiotyki pierwszego wyboru wśród lekoopornych warunkowo chorobotwórczych mikroorganizmów na Amoksycylinę z kwasem klawulanowym po nawożeniu gleby piaszczystej osadami ściekowymi i obornikiem [%]

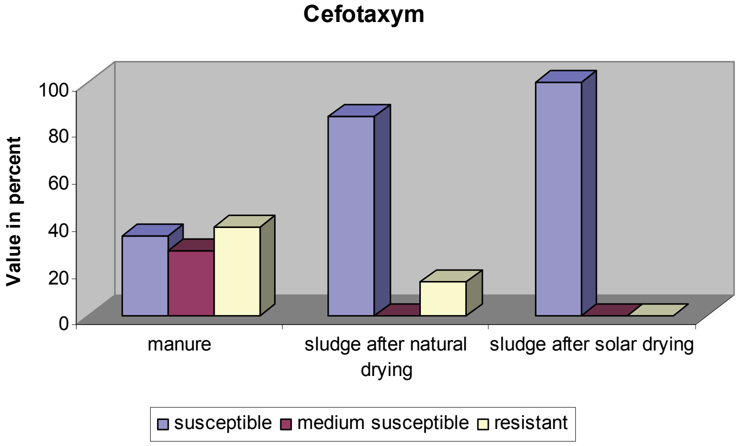

Fig. 3. Mean overall sensitivity to selected first-line antibiotics in drug-resistant conditionally-pathogenic to Cefotaxym microorganisms after fertilization of sandy soil with sewage sludge and manure [%]
Rys. 3. Średnia wrażliwość na wybrane antybiotyki pierwszego wyboru wśród lekoopornych warunkowo chorobotwórczych mikroorganizmów na Cefotaksym po nawożeniu gleby piaszczystej osadami ściekowymi i obornikiem [%]

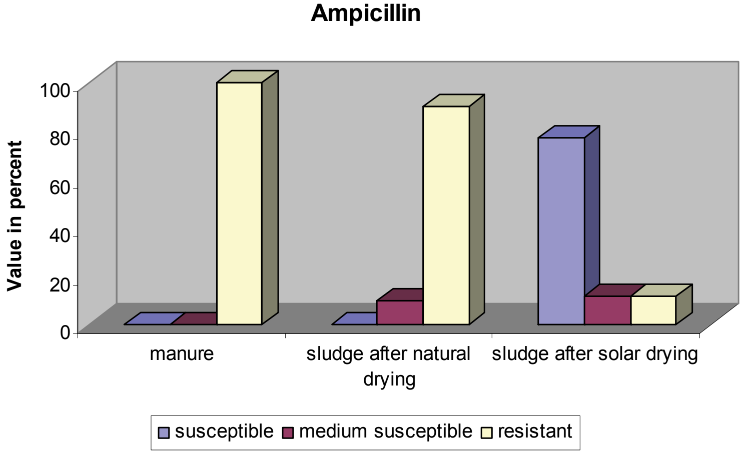

Fig. 4. Mean overall sensitivity to selected first-line antibiotics in drug-resistant conditionally-pathogenic to Ampicilin microorganisms after fertilization of sandy soil with sewage sludge and manure [%]
Rys. 4. Średnia wrażliwość na wybrane antybiotyki pierwszego wyboru wśród lekoopornych warunkowo chorobotwórczych mikroorganizmów na Ampicilinę po nawożeniu gleby piaszczystej osadami ściekowymi i obornikiem [%]

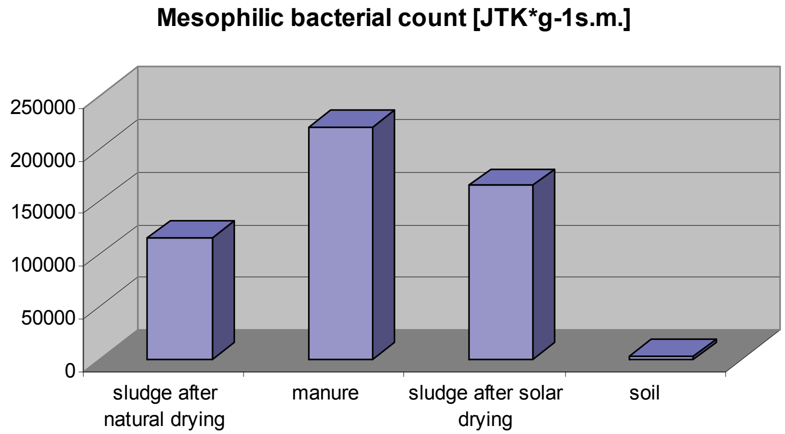

Fig. 5. Mean total bacterial count of conditionally pathogenic vancomycin-resistant bacteria in the soils fertilized with sewage sludge and manure
Rys. 5. Średnia całkowita liczba warunkowo patogennych bakterii odpornych na wankomycynę w glebach nawożonych osadami ściekowymi i obornikiem

Discussion

The attention should be paid to the risk of the use of sewage sludge in nature (and manure according to the available literature reports) which is caused by the presence of conditionally-pathogenic microorganisms which are dangerous to both people and animals. They often acquire resistance to popular drugs [2]. The sewage might contain a variety of residual contents of drugs, which then accumulate in the products of treatment i.e. sewage sludge, causing development of resistance patterns during the contact with microorganisms [11]. Substantial concentrations of Tamiflu (Oseltamiwir) [16] which is used for fighting viral infections were found in the sludge. The residual parts of drugs contained in sewage sludge used for fertilization might be transported to the soil profile. They might also cause resistance to drugs in soil autochthonous and allochthonous microorganisms. The study found that the use of sewage sludge and manure for fertilization of sandy soils caused development of conditionally-pathogenic microorganisms. A number of isolated microorganisms were characterized by drug resistance to first-line antibiotics. Some microorganism counts were similar to those isolated by Goyal and Adams [1]. The authors cited found in the sewage sludge stored in the Atlantic

Ocean, after 30 months from the drop to the water (which was one of the treatment methods): *Enterobacter cloacea, Klebsiella pneumoniae, Citrobacter freundii, Proteus vulgaris, Aeromonas hydrophila, Escherichia coli, Enterobacter agglomerans. Escherichia coli* turned out to be susceptible to all the antibiotics used, including ampicilin and gentamicin. *Klebsiella pneumoniae* exhibited resistance only with respect to ampicilin. *Citrobacter freundii* showed resistance to ampicilin and cephalosporins. *Klebsiella oxytoca*, apart from the resistance to the above two antibiotics, was also resistant to biseptol (sulfamethoxazol and trimethoprim). *Proteus vulgaris* was resistant to 6 of 13 antibiotics used, including ampicilin, cephalosporins and chloramphenicol. The authors found much higher resistance of the isolated forms of pathogenic forms with respect to the basic antibiotics compared to the findings published by Goyal and Adams [1]. A particularly worrying phenomenon is long survival rate of potentially pathogenic forms with resistance to basic antibiotics in the soils fertilized with sewage sludge and manure which was found by the authors of the study that was carried out for several years. One method of elimination or reduction in the content of these forms in organic fertilizers, mainly in the sewage sludge, might be provided by drying process, e.g. solar drying [12]. The field experiment demonstrated that this method of drying causes a significant decline in the content of drug-resistant pathogens in the sludge and the soil fertilized with the sludge. However, it is not always effective. This phenomenon was observed through determination of the vancomycin-resistant bacteria count (fig.5). The number of forms which were resistant to this antibiotic was significantly higher in the sludge after solar drying and the soils fertilized with this sludge compared to the use of the sludge after natural drying. The study found that the increase in the dose of sewage sludge and manure caused an increase in drug-resistant bacterial count. Similar findings were presented by Stańczyk-Mazanek et al. [13] and Stańczyk-Mazanek [14] in studies on the resistant pathogenic forms in the soils fertilized with sewage sludge. The increase in the dose of sewage sludge in the present study caused an increase in resistance of individual isolated pathogenic microorganisms. Higher resistance to the antibiotics used was found in allochthonous soil bacteria. It was found under conditions of field experiment that the manure used in the study also caused development of higher count of drug-resistant forms in the soils fertilized with manure. 100% of the bacteria from the soils fertilized with manure and ca. 90% of conditional pathogens from soils fertilized with sewage sludge were resistant to ampicilin (fig. 4). The study demonstrated that, similar to sewage sludge, manure can also be the source of drug-resistant bacteria in soil. These observations were also made by Holzel et al [2]. Solar drying of the sludge reduced the number of resistant forms to 10% in the soils where this type of sludge was introduced. The process of solar drying turned out to be an effective method that reduced the count of potential pathogens, including the forms resistant to first-line antibiotics in the sludge and then in the soil fertilized with them. However, this process was less effective with respect to vancomycin-resistant bacteria. This drug is used during serious infections with gram-positive bacteria which are resistant to other anti-bacteria drugs and in the patients with hypersensitivity to penicilin and cephalosporins. It is used for treatment of bacterial diseases such as infective endocarditis, sepsis, osteomyelitis, central nervous system infections, lower respiratory tract infections (pneumonia), skin infections and soft tissue infections.

Conclusions

Fertilizing of sandy soils with municipal sewage sludge and with manure caused development of conditionally pathogenic microorganisms with acquired resistance to popular antibiotics. A number of isolated microorganisms (mostly from sewage sludge compared to manure) were characterized by considerable resistance to popular antibiotics i.e. first-line antibiotics. The highest resistance in bacteria isolated from soils fertilized with sewage sludge and manure was found for ampicilin (ca. 100% drug-resistant forms). The highest sensitivity of the most of conditional pathogens was found for ceftazidime. A very worrying finding is the presence of vancomycin-resistant microorganisms in both sewage sludge and manure (vancomycin is an antibiotic used for treatment of serious bacterial diseases. The contact with so contaminated soil after fertilization might represent the serious threat to the environment, including animals and humans. It seems essential to emphasize the effectiveness of stabilization and hygienization of sewage sludge in destruction of pathogenic microorganisms which might exhibit drug resistance. The process of solar drying of municipal sewage sludge was not always effective method of its processing in terms of reduction in the count of potential pathogens, including drug-resistant forms.

Acknowledgments

The research have been conducted at the Institute of Environmental Engineering, Czestochowa University of Technology.

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Obecność bakterii lekoopornych w glebach nawożonych osadami ściekowymi

Streszczenie

Oczyszczaniu ścieków zawsze towarzyszy wydzielanie osadów ściekowych. Jedną z metod utylizacji, wykorzystującą własności nawozowe osadów jest ich przyrodnicze m.in. rolnicze użytkowanie (jeśli tylko spełniają dopuszczalne normy). Wraz z wprowadzanymi osadami do gleb mogą się też przemieszczać metale ciężkie, mikroorganizmy patogenne, powodując skażenie podłoży i pogorszenie się warunków rozwojowych dla organizmów autochtonicznych. Szczególne niebezpieczeństwo związane jest z występowaniem w osadach ściekowych drobnoustrojów lekoopornych. Problematyka ta nie jest jeszcze szczegółowo zbadana.

Celem badań autorek pracy było określenie zmian jakościowych i ilościowych drobnoustrojów lekoopornych w glebie piaszczystej nawożonej wybranymi osadami ściekowymi i obornikiem. Badania prowadzono w warunkach doświadczenia polowego. Zastosowano następujące dawki nawozów organicznych: 0, 10, 20, 30 i 40 $Mg \cdot ha^{-1}$. Do zdegradowanej piaszczystej gleby wprowadzono osady po różnych procesach suszenia (naturalnie i solarnie). Badano wpływ metod suszenia osadów ściekowych na obecność mikroorganizmów lekoopornych w nawożonej nimi glebie. Porównywano również ilość bakterii lekoopornych po zastosowaniu osadów i obornika. Badania prowadzono przez jeden sezon wegetacyjny.

Słowa kluczowe:
osady ściekowe, obornik, nawożenie, gleba, mikroorganizmy lekooporne, przeżywalność

Keywords:
sewage sludge, manure, fertilization, soil, drug-resistant microorganisms, bacteria survival