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DRUG-RESISTANT MICROORGANISMS IN SOILS FERTILIZED WITH SEWAGE SLUDGE

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Abstract: One of the methods of sewage sludge disposal, which is based on its fertilizing properties, is its use in nature, e.g. in farming (if all the permissible standards are met). However, the sludge used for soil fertilization might also contain heavy metals, pathogenic microorganisms, thus causing contamination in soil foundation and deterioration of the conditions for development of indigenous organisms. Particular threat is posed by the existence of drug-resistant microorganisms in sewage sludge. This problem has not been researched in detail yet.

The authors of the present study aimed to determine qualitative changes in drug-resistant microorganisms in sandy soil fertilized with selected sewage sludge. Sewage sludge after different types of drying process (natural and solar) was added to the degraded sandy soil. The effect of the methods of sewage sludge drying on concentration of drug-resistant microorganisms in soil fertilized with the sludge was analysed.

The study demonstrated that sewage sludge dried naturally in drying beds pose threat to soil environment and, potentially, to people and animals which have contact with fertilized soils. In sandy soils fertilized with these types of sewage sludge, pathogenic forms which exhibit resistance to first-line antibiotics can be found.

INTRODUCTION

Waste treatment is always accompanied by generation of sewage sludge. One of the methods of sewage sludge disposal, which is based on its fertilizing properties, is its use in nature, e.g. in farming (if all the permissible standards are met) [9, 10, 16]. However, the sludge used for soil fertilization might also contain heavy metals, pathogenic microorganisms, thus causing contamination in soil foundation and deterioration of the conditions for development of indigenous organisms [14, 15]. Particular threat is posed by the existence of drug-resistant microorganisms [2, 11, 12]. This problem has not been researched in detail yet.

The authors of the present study aimed to determine qualitative changes in drug-resistant microorganisms in sandy soil fertilized with selected sewage sludge and manure. The investigations were carried out under conditions of pot experiment. The following doses of organic fertilizers were used: 0, 10, 50, 100 and 200 Mg/ha (maximal reclamation dose). Sewage sludge after different types of drying process (natural and solar) was added to the

degraded sandy soil. The effect of the methods of sewage sludge drying on concentration of drug-resistant microorganisms in soil fertilized with the sludge was analysed.

EXPERIMENTAL PROCEDURES

The experiments were carried out using light loamy soil. The soil pH was 6.5. The soil was sampled in the Częstochowa region in Poland. Two types of sludge, which differed in treatment process, were used for comparison. The sludge was dried by means of natural and solar drying methods. The sludge introduced to the soil according to the standards was suitable for use in nature or for agricultural applications. The experiment was carried out under laboratory conditions of pot experiment. The soil was fertilized with increasing doses of sewage sludge, which were calculated per pot so that they corresponded to the amount of 10, 50, 100 and 200 Mg of fertilizer per hectare (10 = dose for agricultural application, 50-200 = reclamation doses according to the ordinance which was in force at the time of experiment [13]). The experiment was carried out during the period of one vegetation season. White mustard (*Sinapis alba*) was planted in the fertilized soils. Humidity in each pot during the experiment was maintained at the level of 60%. After the vegetation period, which took 6 months, the soil samples were taken for microbiological investigations.

The samples were taken from each pot to sterile containers. The procedures identical to sampling of infectious substances were used. Under laboratory conditions, the samples of soil of 10 g were taken and placed in sterile Petri dishes. Each sample was submerged with 2.5 ml of distilled water in order to ensure appropriate humidity. The material was inoculated with bacteriological loop on blood agar plates and MacConkey agar plates.

After 18-hour incubation at 37°C, the assessment of growth and isolation of individual strains was done. The isolates were left for the next 18 hours at 37 degrees Celsius, whereas biochemical tests were carried out in order to identify individual species. At the same time, drug-resistance tests were carried out according to national guidelines [3]. Mueller-Hinton plates were employed during drug-resistance testing, used in bacteriological laboratories for determination of antibiotic resistance. Then, the plates with the following antibiotics were added:

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

After 15-minute pre-incubation, the plates were placed in a thermostat at the temperature of 37°C for 18 hours. After that time, the results were read.

Examinations of all materials were performed in triplicate.

RESULTS AND DISCUSSION

Non-pathogenic aerobic bacilli were grown in control material (sandy non-fertilized soil). Conditional pathogens were found in the soils fertilized with sewage sludge. The results of determination of drug resistance for microorganisms in the plates included in the study are compared in Tables 1 and 2.

Table 1. Results of drug-resistance readings for pathogenic strains grown in sandy soil samples fertilized with sewage sludge after natural drying process.

Type of isolated microorganism	Sewage sludge dose [t/h]	Susceptibility of microorganisms to the type of antibiotic					
		Amikacin	Amoxicillin with clavulanic acid	Ceftazidime	Cefotaxime	Gentamicin	Ampicillin
Alcaligenes faecalis	10	s	s	s	s	s	s
Achromobacter denitryficans	10	s	s	s	s	s	r
Enterobacter sp.	10	ms	r	s	s	s	r
Photorhabdus luminescens	10	s	s	s	s	s	r
Alcaligenes faecalis	50	s	r	s	r	s	r
Achromobacter denitryficans.	50	s	s	s	s	s	r
Enterobacter sp.	50	ms	r	s	s	s	r
Alcaligenes faecalis	100	s	r	s	r	s	r
Klebsiella oxytoca	100	r	r	s	r	s	r
Escherichia coli	200	s	r	s	s	s	r
Moraxella caprae	200	s	s	s	s	s	r
Klebsiella oxytoca	200	r	r	s	r	s	r
Enterobacter kobei	200	r	s	s	s	s	r
Citrobacter freundii	200	ms	r	r	r	s	r

s – susceptible; **ms** – medium susceptible; **r** – resistant

Table 2. Results of drug-resistance readings for pathogenic strains grown in sandy soil samples fertilized with sewage sludge after solar drying process

Type of isolated microorganism	Sewage sludge dose [t/h]	Susceptibility of microorganisms to the type of antibiotic					
		Amikacin	Amoxicillin with clavulanic acid	Ceftazidime	Cefotaxime	Gentamicin	Ampicillin
Alcaligenes faecalis	10	s	s	s	s	s	s
Achromobacter piechandii	10	s	s	s	s	s	s
Alcaligenes faecalis	50	s	s	s	s	s	s
Alcaligenes faecalis	100	s	s	s	s	s	s
Pseudomonas alcaligenes	200	s	s	s	s	s	r

s - susceptible; **ms** – medium susceptible; **r** – resistant

Municipal waste contains pathogenic microorganisms which originate from sick people and healthy carriers. It is also known that the processes of waste treatment do not eliminate them entirely. The most frequent bacteria present in sewage and sewage sludge include *Escherichia coli*, *Salmonella typhi*, *Clostridium botulinum*, *Vibrio cholerae*, *Mycobacterium tuberculosis* [1, 5]. Manure is a reservoir of faecal bacteria, i.e. *Escherichia coli*, *Streptococcus faecalis* and coccobacillus from the family of *Enterobacteriaceae* [17].

The most dangerous strains are those characterized by drug resistance to all popular antibiotics, because they might cause a serious threat to people and animals. Overuse and irrational use of antibiotics might cause development of mutations which lead to acquired resistance to antibiotics. This has made many Polish doctors experience the challenge of lack of appropriate drugs to treat patients with severe infections.

The present study determined resistance of pathogenic forms, which can be found in soils after introduction of selected types of sewage sludge, to antibiotics which are most frequently used in medicine. The lowest efficiency in relation to these microorganisms was demonstrated for ampicillin, amikacin and amoxicillin with clavulanic acid. The literature reports support the results obtained by the authors of the present paper. *Coccobacilli* from the family of *Enterobacteriaceae*, represented by *Escherichia coli*, which were isolated from sewage and sewage sludge exhibited resistance to ampicillin and penicillin in the study by Junko-Tejedor [4]. In the study by Yang, strains from hospital sewage were analysed. The study demonstrated the existence of Gram-negative bacteria which exhibited resistance of 85.6 to 94.1% in relation to ampicillin [19].

Studies by other authors have also confirmed relatively frequent resistance of *Escherichia coli* bacteria to ampicillin and amoxicillin with clavulanic acid. In the study by Nalewajek [8], drug-resistance of *Escherichia coli* strain originating from raw sewage amounted to nearly 14%, whereas this value in treated sewage was 6%. In relation to ampicillin, this value was 50%, whereas it reached 30% in the treated sewage [8].

According to literature data, the effectiveness of sewage treatment with respect to elimination of pathogenic bacteria ranges from 25 to 99% [18].

Analysis of the obtained results revealed that solar drying of sewage sludge considerably affected reduction in the count of conditional pathogens, including drug-resistant bacteria. In the soils fertilized with sewage sludge after solar drying process only one type of bacteria, *Pseudomonas alcaligenes*, was identified. It exhibited resistance to ampicillin, whereas it was susceptible to other types of antibiotics.

The seven conditional pathogens which exhibited resistance to the antibiotics used in the study were identified in the soils fertilized with sewage sludge dried naturally in the drying beds:

Achromobacter denitrificans − Gram-negative aerobic bacteria which are present in soil; they cause meningitis, endocarditis, peritonitis, ostitis, and urinary tract infections.

Enterobacter species − Gram-negative bacteria of this type are of faecal origin and cause, e.g. endocarditis and lower respiratory tract infections.

Photorhabdus luminescens − Gram-negative bacteria, which belong to the family of *Enterobacteriaceae*, frequently live in soils in symbiosis with nematodes; they are able to produce the compounds typical of urinary tract, gastrointestinal tract, bile duct, inflammations of paranasal sinuses, middle ear, meningitis, inflammations of soft tissue and osteomyelitis, they are pathogenic especially to insects, sporadically to humans.

Klebsiella oxytoca − belong to the family of intestinal coccobacilli (*Enterobacteriaceae*), and might cause sepsis, endotoxic shock, pneumonia, lung abscess or skin inflammations.

Moraxella caprae − Gram-negative bacteria with haemolytic properties, cause inflammations of respiratory tract in animals, sporadically in humans.

Enterobacter kobei − Gram-negative bacteria from *Enterobacteriaceae* family which cause nosocomial infections and urinary tract infections.

Citrobacter freundii − Gram-negative bacteria from *Enterobacteriaceae* family; they cause urinary tract infections and general infections, also in gastrointestinal and respiratory systems [18].

CONCLUSIONS

Overuse and irrational use of antibiotics increase the risk of development of bacterial mutations, which might acquire resistance to antibiotics [6, 7]. Particular threat is connected with the appearance of these forms of microorganisms in sewage sludge. Sewage and products of sewage treatment, i.e. sewage sludge, contain biological contaminants from the industry, hospitals and households.

The study demonstrated that sewage sludge dried naturally in drying beds pose threat to soil environment and, potentially, to people and animals which have contact with fertilized soils. In sandy soils fertilized with these types of sewage sludge, pathogenic forms which exhibit resistance to first-line antibiotics [3, 8] can be found. Solar drying caused essential decrease in drug-resistant bacteria count in sewage sludge.

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MIKROORGANIZMY LEKOOPORNE W GLEBACH NAWOŻONYCH OSADAMI ŚCIEKOWYMI

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 drobnoustrojów lekoopornych w glebie piaszczystej nawożonej wybranymi osadami ściekowymi. 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.

Badania wykazały, że osady ściekowe suszone naturalnie na poletkach osadowych stanowią zagrożenie dla środowiska glebowego i ewentualnie dla ludzi i zwierząt mających kontakt z nawożonym gruntem. W użyźnianych osadami ściekowymi glebach piaszczystych pojawiały się formy patogeniczne wykazujące odporność na tzw. antybiotyki pierwszego uderzenia.