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# Changes of biological and chemical indicators in soil after dehydrated sewage sludge application

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ABSTRACT

The aim of the study was to determine how fast the hygienization process of sewage sludge used for lawns in Bialystok takes place. The study also included the determination of concentrations of Cd, Cu, Cr, Ni, Pb, and Zn and their relations with biological factors in studied soil samples. In the tested samples, neither *Salmonella* sp., nor invasive nematode eggs belonging to the genera *Ascaris*, *Trichuris,* and *Toxocara* were found. Moreover, a significant reduction in microbiological indicators was determined, that is psychrophilic and *mesophilic* plate count bacteria, total coliforms and fecal coliforms bacteria as well as *Clostridium* sp. and *Enterococcus* sp., to the level corresponding with control sectors. On the basis of the studies, one could observe a lower content of certain heavy metals such as Cd, Ni, Zn, and Pb in 2012 compared with 2011, while the content of chromium and copper in the soils increased significantly in 2012. All studied heavy metal contents in soil in 2011 and 2012 were in the acceptable level for urban soil defined in the regulation of the Polish Ministry of the Environment.

*Keywords:* Sewage sludge; Soil; Heavy metals; Sanitary indicators; Sediment management

## 1. Introduction

Urban soils are vulnerable to contamination due to continuous development of industry and communication transport. Chemical contamination of soils, including heavy metals which adversely affect living organisms occurring there, results in the elimination of sensitive species and, consequently, lead to disturbances in the circulation of matter and energy, preventing its proper functioning [1]. Heavy metals accumulate in the soil and then get into the food chain, which may lead to erosion of proper development of all organisms [2]. The consequences of soil pollution with heavy metals are rarely seen in the short term, and they can lead to dangerous ecological changes which are delayed in time. An important feature which distinguishes heavy metals and other undesirable substances is that they are not biodegradable, but only undergo biotransformation, as a result, of complex physico-chemical processes and biological processes in soil. These processes determine the

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mobility and bioavailability of heavy metals in the soil–plant system. An important role in the binding of metals in the soil plays: pH, hydroxides of iron, aluminum and manganese, organic matter, a soil clay fraction, and moisture sorption capacity [3].

As Adriano et al. pointed out [4], the dynamics of metal forms is most intense in the top layer of soil and depends on a diverse population of microorganisms, organic matter, sorption capacity, and the biological interactions associated with rhizosphere microorganisms and also plant. All these factors cause that urban soil requires continuous remediation treatment because of the progressive chemical degradation that leads to the permanent deterioration of their properties [5]. On the other hand, due to a limited amount of the natural fertilizer, which could enrich the urban soil, the nutrition of the top layer of soil with sewage sludge seems to be a reasonable solution.

Sewage sludge production has been an important issue in the protection of the environment for many years. The progress that has been made recently in terms of new or improved sewage treatment technology influenced the amount of sludge production. In 2011, municipal wastewater treatment plants in Poland produced 519,000 tons of dry matter (DM), while industry produced 397.6,000 tons of DM [6]. A systematic increase in the amount of sediment and the prohibition of its storage since 1 January, 2013 made the management of sewage sludge an important environmental, technical, and technological problem [7]. More than half of the load of pollutants in wastewater is deposited in sludge [8–10].

Pollutants that are found in sewage sludge can be divided into the following categories [11]:

(1) inorganic contaminations (e.g. metals and other trace elements);
(2) organic contaminants (e.g. polychlorinated biphenyls dioxins, pharmaceuticals, and surfactants); and
(3) pathogens (e.g. bacteria, viruses, parasites, and protozoans pathogens).

The appropriate provisions, which define the limits of chemical and biological contaminants, decide whether the use of sewage sludge is possible. In Poland, among the most important acts are as follows: the Waste Act of December 14, 2012 [12], which clarifies the concept of municipal sewage sludge, and the regulation of the Minister of Environment of July 13, 2010 on municipal sewage sludge [13]. The regulation quoted above defines the conditions which must be met by the use of municipal sewage sludge, among others, in agriculture, for land reclamation, for growing plants in order to produce compost or crops not intended neither for human consumption nor for animal fodder. The regulation also contains some guidance on the scope, frequency, and methods used in the study of municipal sewage sludge and the land on which settlements are to be used.

With regard to Polish regulations, in order to classify sewage sludge, the content of heavy metals and selected biological indicators, which include bacteria of the genus *Salmonella* and invasive nematode eggs belonging to the genera *Ascaris, Trichuris,* and *Toxocara* (ATT indicator), are taken into account. But there is no appropriate legislation relating to the industrial sludge.

A large amount of valuable organic matter, nitrogen, phosphorus, and other nutrients in sewage sludge justify their use as good fertilizers [14,15]. In Belgium, 57% of the sludge is applied to land, while in France even 60%. The soil, fertilized with stabilized sewage sludge or compost made on the basis of sewage sludge, creates proper conditions for the rapid development of plants [16]. Landfilling and land application of the sewage sludge are suggested to be the most economical sludge disposal methods [17,18].

Too high content of toxic heavy metals or biological factors may be the barrier, which limits sewage sludge usage [19–22]. Biological factors are of major concern in the use of sludge for agricultural or environmental purposes. The use of sewage sludge from municipal wastewater treatment plants is justified especially in the regions with a low degree of industrialization. Podlaskie Region is a typical agricultural area, where the agri-food industry is the dominant branch of production. Agri-food industry does not cause the generation of abnormal quantities of heavy metals in sludge. Much greater obstacle to the non-industrial usage of sludge constitutes pathogenic organisms. The aim of the study was to determine how fast the hygienization process of sewage sludge used for lawns in Bialystok follows. The study also included the determination of concentrations of Cd, Cu, Cr, Ni, Pb, and Zn and its relations with biological factors in studied soil samples.

## 2. Materials and methods

### 2.1. The experiment area and sewage sludge applied

The experimental was set up on the lawns located in the main traffic routes of Bialystok. Designated lawns were reclaimed in a way that the soil could be mixed with sewage sludge. Prior to application of sludge to soil, chemical and microbiological–parasitological tests were performed in order to determine the possibility of its usage. To enrich the ground for lawns, stabilized and dehydrated sewage sludge from wastewater treatment plant in Sokółka was used, with various content of macro- and micronutrients essential for plant growth. Each plot was divided into 18 sectors with 5 $m^2$ area for each sector (Fig. 1). Location sectors were selected randomly. Six of them were left without fertilization, six were fertilized with sewage sludge in the amount of 7.5 kg/$m^2$ (14.5 t/ha of DM) per each sector, the rest was fertilized in doses of 15 kg/$m^2$ (29 t/ha of DM) of sludge per sector.

The experiment was set on four lawns located in the main traffic routes: Popieluszki Street, Hetmanska Street, Piastowska Street, and Raginisa Street. The volume of vehicle traffic of a road at each experimental plots measured at peak hours in 2011 was respectively: 2,320, 1,169, 1,407, 538 vehicles per hour. The plots were fertilized with sewage sludge in autumn 2010, and the grass was sown in spring 2011.

Physico-chemical analyses of sewage sludge and soil samples before foundation of experiment were done by the reference laboratory of the Regional Chemical and Agriculture Station in Bialystok. Biological studies of sewage sludge samples and soil samples before and after the application of sewage sludge into the soil were carried out in the laboratory of the Division of Sanitary Biology and Biotechnology, Bialystok University of Technology.

### 2.2. Determination of chemical and physical properties of samples

Three samples of soil were taken from each plot. They were mixed and sieved through a sieve with a mesh diameter of 0.25 mm, and the sample averages for each plot were taken. The determination of particle size was done by the Casagrande areometric method modified by Prószyński, which consists in measuring the density of soil suspension during progressive sedimentation of soil particles at a constant temperature. Density measurements were made with the Prószyński hydrometer, and the density of the soil suspension was read in the periods indicated in the tables developed by Prószyński. This procedure is regulated by the PN-R-04032 [23] standard published mostly for agricultural soil analyses. Additionally, two times in season pH of the soil were measured in water.

### 2.3. Determination of total heavy metals' concentrations in soils

In October 2011 and 2012, samples of soil (0–20 cm) were collected. Heavy metals' concentrations in soils with sewage sludge were determined using atomic absorption spectrometry. The samples of soil were mineralized in temperature at about 450˚C, and remains were dissolved in aqua regia (3:1 mixture HCl and $HNO_3$) at 80˚C (PN-ISO 11047:2001) [24].

### 2.4. Determination of microbiological and parasitological tests

The parasitological tests were carried out in accordance with Polish Standard PN-Z-19000-4 [25] and recommended by US EPA [14]. The analyses of *Salmonella* sp. were carried out using EPA Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis Medium [26]. The total viable count of heterotrofic psychrophilic bacteria incubated in 22˚C was carried out in accordance with Polish Standards PN-EN ISO 6222:2002 [27]. Most probable number of total coliforms (TC) was performed according to PN-EN ISO 9308-3:2002 [28], and *fecal* coliforms (FC) were performed according to US

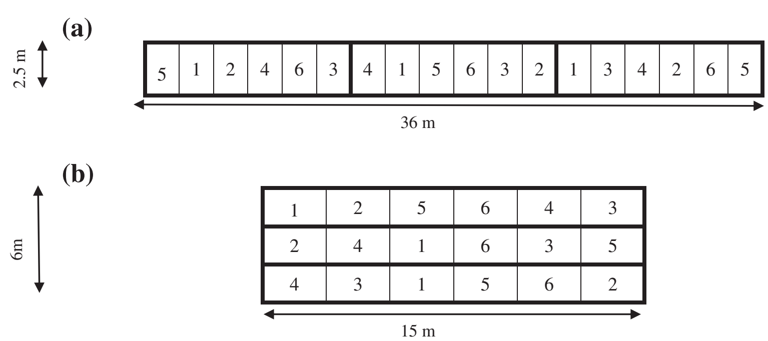


Fig. 1. The system of experimental plots (a) at Hetmanska, Piastowska, and Raginisa Streets; (b) at Popieluszki Street. 1–0 kg/$m^2$ sewage sludge, 2–7,5 kg/$m^2$ sewage sludge, 3–15 kg/$m^2$ sewage sludge, 4–0 kg/$m^2$ sewage sludge, 5–7.5 kg/$m^2$ sewage sludge, 6–15 kg/$m^2$ sewage sludge.

EPA Method 1681: fecal coliforms in sewage sludge (biosolids) by Multiple-Tube Fermentation using A-1 Medium [29]. *Enterococci* were detected according to Polish Standard PN-EN ISO 7899-2:2004 [30], and *Clostridium* was detected according to Polish Standard PN- PN-EN 26461-2:2001 [31].

Dry matter was determined according to Polish Standard PN-EN 12880:2004 [32].

### 2.5. Data analysis

The correlation between biological factors, soil properties, and heavy metals' concentration in soil fertilized with different doses of sewage sludge was calculated using Spearman's correlation factor $r$ for $p \le 0.05$ with Statistica 10.0. The impact of experimental factors on the content of heavy metals in soils was analyzed using a multivariate analysis of variance at a significance level for $p < 0.05$ and for $p < 0.01$, and the significant differences rated by Tukey's test.

## 3. Results and discussion

According to the regulation of the Minister of the Environment of July 13, 2010 on municipal sewage sludge [13], the tested sludge from sewage treatment plant in Sokółka and averaged soil samples taken from the top layer of plots met all the criteria set out in the regulation (Tables 1 and 2).

Sewage sludge did not contain *Salmonella* or invasive nematode eggs belonging to ATT, and heavy metals did not exceed permitted limits. Soil samples also met the guidelines set out in the regulation. Therefore, the sludge could be applied to the soil for lawns in the city.

The study of selected physico-chemical parameters of soils without and with applied doses of sludge was conducted in 2011 and 2012. Table 3 shows the results of the determination of pH and organic carbon. The pH of the soil is the major factor determining solubility of heavy metals in soil. Solubility of heavy metals determined by exchangeable sorption processes is low

Table 1
Selected chemical and biological indicators in sewage sludge

| Indicators | Heavy metals' content in mg/kg of DM | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cd | Cu | Ni | Pb | Zn | Hg | Cr |
| Metal content in sludge form wastewater treatment plant in Sokółka | <0.5 | 194 | 22.0 | 23.5 | 1,459 | 1.04 | 58 |
| Limit value in the application of sludge to land reclamation for non-agricultural purposes [13] | 25 | 1,200 | 400 | 1,000 | 3,500 | 20 | 1,000 |
| Viable helminth ova of *Ascaris* sp., *Trichuris* sp., *Toxocara* sp. (ATT) | n.d. | | | | | | |
| Bacteria of the genus *Salmonella* in 100 g of sludge | n.d. | | | | | | |
| *Other indicators determined in sludge* | | | | | | | |
| pH | 6.7 | Dry matter (%) | | | 19.3 | | |
| Total nitrogen (% DM) | 4.0 | Calcium (% DM) | | | 5.5 | | |
| Ammonium nitrogen (% DM) | 0.1 | Magnesium (% DM) | | | 0.7 | | |

Table 2
Selected chemical indicators in the soil samples before application of sludge

| Soil samples | Heavy metals' content in mg/kg of DM | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cd | Cu | Ni | Pb | Zn | Hg | Cr |
| Popiełuszki Street | 1.65 | 17.9 | 5.89 | 26.5 | 82.4 | 0.14 | 13.1 |
| Hetmanska Street | <0.5 | 9.46 | 4.48 | 12.4 | 40.9 | 0.06 | 10.9 |
| Piastowska Street | <0.5 | 16.8 | 10.8 | 23.9 | 195 | 0.02 | 13.6 |
| Raginisa Street | <0.5 | 8.8 | 4.6 | 12.1 | 36.6 | 0.05 | 8.3 |
| The allowed amount of heavy metals in the surface soil layer (0–25 cm soil light) in the application of municipal sewage sludge for land reclamation for non-agricultural purposes [13] | 3 | 50 | 30 | 50 | 150 | 1 | 100 |

Table 3
The content of organic carbon and pH of the soil samples fertilized with different doses of sludge

| | | pH | | $C_{org}$ (%) | |
|---|---|---|---|---|---|
| Experiment | Dose of sewage sludge (kg/m$^2$) | 2011 | 2012 | 2011 | 2012 |
| Hetmańska Street | 0 | 7.66 | 7.82 | 1.96 | 1.82 |
| | 7.5 | 7.76 | 7.84 | 1.99 | 1.68 |
| | 15 | 7.58 | 7.97 | 2.19 | 1.99 |
| Piastowska Street | 0 | 7.53 | 7.79 | 1.52 | 1.68 |
| | 7.5 | 7.42 | 7.76 | 1.77 | 1.75 |
| | 15 | 7.34 | 7.73 | 1.93 | 1.98 |
| Popieluszki Street | 0 | 7.62 | 7.80 | 1.18 | 1.68 |
| | 7.5 | 7.43 | 7.87 | 2.03 | 1.60 |
| | 15 | 7.35 | 7.68 | 1.47 | 1.86 |
| Raginisa Street | 0 | 7.83 | 7.74 | 1.77 | 1.64 |
| | 7.5 | 7.61 | 7.82 | 1.83 | 1.58 |
| | 15 | 7.27 | 7.99 | 1.90 | 1.65 |
| Min | | 7.27 | 7.68 | 1.18 | 1.58 |
| Max | | 7.83 | 7.99 | 2.19 | 1.99 |
| Mean | | – | – | 1.78 | 1.75 |
| Median | | – | – | 1.83 | 1.68 |

in the range of neutral and alkaline reactions and increases with decreasing pH [33]. The sludge added to the soil, both in a single as well as double dose, contributed to a slight decrease in pH in the first year of the study. In our study, active acidity for all tested samples ranged from 7.27–7.83 in 2011 to 7.68–7.99 in 2012 (Table 3), which means that at any point, the alkaline reaction in soil occurred. Thus, soil and heavy metals contained in it should not pose a significant threat to the environment.

According to Lal [34], organic matter in soil is the main element having an impact on its quality and, as a result, on the creation and sustainability of aggregates in soil. Additionally, soil water retention, soil biodiversity, and the density depend on the content of organic matter. It was shown that soil in urban areas is characterized by a very low content of C-organic. Even sludge doses used in our experiment did not cause any significant increase in carbon content. The highest value of organic C was observed at the highest fertilization with sewage sludge applied (15 kg/m$^2$) in 2011 and 2012 (respectively, 2.19 and 1.99%) of the plots at Hetmańska Street (Table 3).

Fig. 2 shows concentrations of heavy metals in the urban soil fertilized with different doses of sewage sludge in 2011 and 2012. On the basis of the studies, one observed a lower content of certain heavy metals such as Cd, Ni, Zn, and Pb in 2012 compared with 2011, while the content of chromium and copper in the soils increased significantly in 2012.

The analysis of the concentration of heavy metals in the soils showed that the contents of Cd, Cr, Cu, Ni, Pb, and Zn were within the limit values for soils of the urban areas defined in the regulation of the Ministry of Environment, September 9, 2002 on the standards of soil quality and standards of land quality. According to Lis and Pasieczna [35], the natural content of heavy metals in soil in relation to the geochemical background was in 2011 and 2012, respectively, Cd < 0.5 mg/kg (0.70 and 0.49), Cr 5–10 mg/kg (5.5 and 15.76), Cu 20–40 mg/kg (16.24 and 33.35), Ni < 5 mg/kg (44.76 and 11.12), Pb 12.5–100 mg/kg (37.08 and 11.24), Zn 25–400 mg/kg (88.6 and 62.76) (in parentheses are given the average values of the individual accumulation of metals in the soil). The plots located at Raginisa Street showed the lowest content of metals in the soil, for example, Cu, Zn, and Pb, while at Piastowska Street, the plot was characterized by the highest content of metals such as Cd, Zn, and Pb (Fig. 2).

As Chan and Lam [36] point out, while mixing sludge with soil, heavy metal forms undergo transformation, which alters their bioavailability due to changes in their equilibrium and kinetic reactions in the soil. Some metals are absorbed by the organic matter, clay minerals, oxidized forms of iron, manganese, and calcium carbonate contained in the soil. The result is a reduction in the concentration of metals in a dissolved form.

Based on these results, it was found that the average cadmium content in the soils differed significantly depending on the location of the plots (Fig. 2). The highest average cadmium content was recorded in soil samples collected at Popiełuszki (0.90–0.41 mg/kg

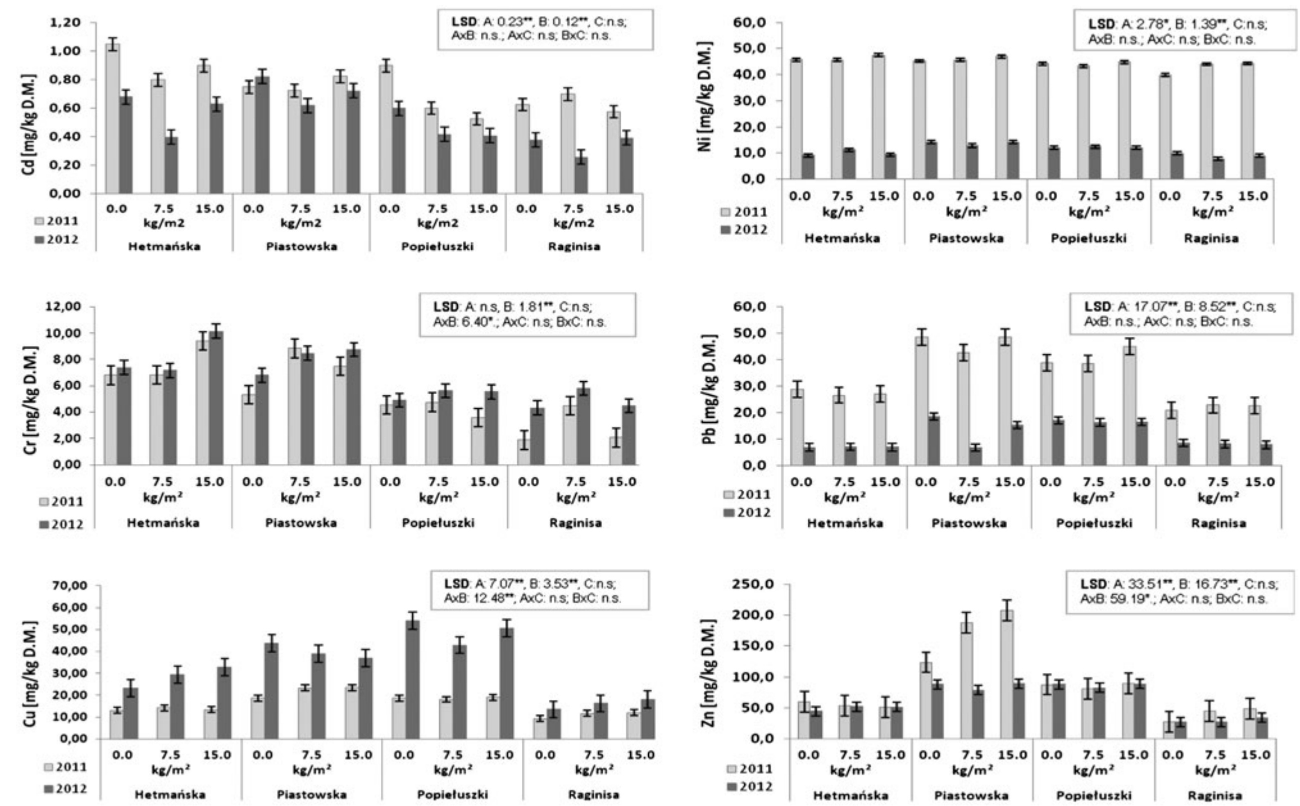


Fig. 2. Concentrations of heavy metals in urban soil fertilized with different doses of sewage sludge at Hetmańska, Piastowska, Popiełuszki, and Raginisa Street (A: location, B: year, C: dose of sewage sludge; *Significant differences for $p < 0.05$, **Significant differences for $p < 0.01$; n.s.—no significant differences).

DM) and Hetmańska Streets (1.05–0.39 mg/kg DM) and the lowest at Raginisa Street (0.70–0.25 mg/kg DM). Before the experiment was started in 2010, the content of cadmium had been too high at Popiełuszki Street where it was 1.65 mg/kg DM. In the following years, that is, 2011 and 2012, with the applied mixtures of grasses and at different fertilization with sewage sludge, cadmium significantly decreased in the control plots to the value of approx 0.9–0.6 mg/kg: at 7.5 kg/m$^2$, it was 0.6–0.4 mg/kg of DM and at 15 kg/m$^2$—0.5–0.4 mg/kg DM, respectively. Such a decrease in the amount of cadmium in the soil in the first year of the experiment could be due to the fact that the element was cumulated in large quantities by the grass, which is confirmed by the research study conducted by Wołejko et al. [22].

There were significant differences in the content of cadmium in the soil in 2011 and 2012. There was a decrease in the average content of Cd in the soil in 2012 (from 0.26 mg/kg DM for Raginisa Street to 0.82 mg/kg DM for Piastowska Street) compared with 2011, when the average content of this element ranged from 0.33 mg/kg DM (Popiełuszki Street) to 1.05 mg/kg DM for Hetmańska Street. The application of sewage sludge did not significantly influence the content of cadmium in urban soils (Fig. 2). Differences in the content of cadmium in the soil depending on the position of research facilities may have resulted from the specific location, including, among others, the research points away from the edge of the road and traffic at various locations. According to Tchepel et al. [37], road transport is one of the main sources of emissions of air and soil pollutants in cities, strongly intercepting natural environment as a whole and having a negative influence on human beings. As a result of the combustion of fuels in engines and tear on tires and other parts of vehicles [38] into the air, heavy metals go and then go into the soil [37]. The correlation analysis showed positive correlation between the content of cadmium in the soil and the content of fine particles and $C_{org}$ (respectively, $r = 0.31$ and $r = 0.32$) (Table 4). Chan and Lam [36] reported that certain metals, including cadmium, can be absorbed by clay minerals in soil, which results in lowering the concentration of dissolved metals in the form of downloading and reduction of the element by the plants.

Table 4
Coefficients correlation between the total content of heavy metals in soil and pH, $C_{org}$, content of fine particles, and heavy metals in soil

| Chemical properties of the soil | Soil | | | | | |
|---|---|---|---|---|---|---|
| | Cd | Cr | Cu | Ni | Pb | Zn |
| pH | −0.25 | 0.48* | 0.19 | −0.51* | −0.57* | −0.29* |
| $C_{org}$ | 0.32* | 0.12 | 0.23 | 0.34* | 0.23 | 0.43* |
| Content of fine particles | 0.31* | 0.18 | 0.52* | 0.28 | 0.24 | 0.65* |
| Cd | 1.00 | −0.24 | −0.09 | 0.59* | 0.40* | 0.32* |
| Cr | −0.24 | 1.00 | 0.66* | −0.61* | −0.65* | 0.01 |
| Cu | −0.09 | 0.66* | 1.00 | −0.34* | −0.28 | 0.57* |
| Ni | 0.59* | −0.61* | −0.34* | 1.00 | 0.85* | 0.45* |
| Pb | 0.40* | −0.65* | −0.28 | 0.85* | 1.00 | 0.57* |
| Zn | 0.32* | 0.01 | 0.57* | 0.45* | 0.57* | 1.00 |

*Significant correlations for $p \leq 0.05$.

Chromium is one of the heavy metals, the concentration of which increases systematically in the environment [39]. The average content of chromium in this study ranged from 1.88 (Raginisa Street) to 9.43 mg/kg (Hetmańska Street) in 2011, and in the following year, the chromium content in the soil increased significantly and it was in the range from 4.32 for Raginisa Street to 10.15 mg/kg for Hetmańska Street (Fig. 2). The dose of sludge did not affect significantly the content of this element in the soil. Accumulation of chromium in all tested soil plots at various locations in relation to the geochemical background reveals contamination of the environment with this metal. Such an increase in the chromium content in the soil may be due to the fact that the sludge added to the soil contributed to a greater accumulation of this metal on loamy humus colloids and minerals in 2011, which could affect the accumulation of Cr in soil. The accumulation of heavy metals in the soil and their bioavailability for plants is determined by soil pH. The designated correlation coefficient ($r = 0.48$) indicates a lower solubility of chromium in the soil with the increase of the pH (Table 4). As the Seaman et al. [40] state, alkaline compounds can have varying effects on the mobility of chromates, depending on the soil pH changes. Increasing the pH above the neutral level can cause oxidation of $Cr^{3+}$ to more mobile and toxic $Cr^{6+}$ and increase its uptake by plants as well as accumulation in the soil.

Based on these results, it was found that the average copper content ranged from 9.25 (Raginisa Street) to 23.45 mg/kg (Piastowska Street) in 2011, and in the next year, the copper content in the soil increased significantly and ranged from 13.51 (Raginisa Street) to 54.03 mg/kg (Popiełuszki Street). The dose of the sludge did not affect the content of this element in the soil (Fig. 2). Of all the locations, only on plots located at Popiełuszki Street copper content in the soil was above the level of the geochemical background, which indicates that metal pollution. Copper is present in the soils in various forms, usually by creating a compounds in the form of low mobile carbonate and sulfate precipitates. While Cu's interaction with the environment is complex, research shows that most Cu introduced into the environment already is or rapidly becomes stable and results in a form which does not pose a risk to the environment. According to Wołejko et al. [22], fertilizing urban soil with sewage sludge resulted in greater accumulation of copper by the grass in proportion to the dose of sludge, which could affect the similar values of this element in the soil.

Copper is strongly sorbed by organic substance, which is confirmed by a statistically significant positive correlation coefficient ($r = 0.52$) (Table 4). In the first year of the experiment, the soil reaction was slightly lower at sites where sludge was applied when compared with the following year. Chan and Lam [36] reported that lowering the pH of the soil may cause the solubility of heavy metals and, as a result, increases their uptake by plants. In addition, the increased copper content in the surface soil levels is associated largely with the anthropogenic impact of public transport, as stated by Plesničar and Zupančič [41], in particular the use of the braking system and corrosion of car coolers.

Differences were found in the content of nickel in the soil, depending on the research location and vegetation season. In our study, the concentration of nickel in the soil decreased in 2012, almost four times compared with 2011. The average nickel content ranged from 39.85 (Raginisa Street) to 47.5 mg/kg DM

(Hetmańska Street) in 2011 and in 2012 ranged from 7.68 (Raginisa Street) to 14.18 mg/kg DM (Piastowska Street) (Fig. 2). The correlation analysis showed a positive correlation between the nickel and the content of organic matter in the soil ($r = 0.34$) what may indicate immobilization Ni in soil, which is enriched with organic matter in the form of sludge. This is confirmed by the results of Kabata-Pendias and Pendias [42], talking about a large accumulation of nickel in biolites, as well as the occurrence of mobile chelates bound to organic matter. At low pH, the binding strength of nickel by soil organic matter is small, but at neutral pH, its binding is very strong and it is important in respect to the bioavailability of the metal [43]. The solubility of nickel in the soil increases with increasing acidity. Non-ecological waste treatment, errors in fertilization, nickel metallurgy, and burning of coal and liquid fuels contribute to soil pollution with nickel [42]. The designated negative correlation coefficient ($r = -0.51$) indicates a higher solubility of nickel and the possibility of retrieving it by plants in alkaline medium (Table 4).

Zinc is an essential element in the regulation of metabolism of living organisms. In our study, the content of zinc in the soil was affected by the location of experimental fields. We also found out some significant differences in the content of this element in the soil in the subsequent years of the study. On the plots located at Piastowska Street, differently fertilized with sewage sludge, the excess of limit values of zinc in soils was observed in 2011 according to the regulation Directive of Environmental Minister of July 13th 2010 for land reclamation for non-agricultural to light soils. At the fertilization of 7.5 kg/m$^2$, it was 188.0 mg/kg of DM and at 15 kg/m$^2$, 207 mg/kg DM. In the following year, the zinc content on the analyzed plots was significantly lower by more than 50%, and at a dose of sludge fertilization of 7.5 kg/m$^2$, 78.0 mg/kg of DM and at 15 kg/m$^2$, 89 mg/kg DM at Piastowska Street. In turn, at other locations (Hetmańska, Popiełuszki, and Raginisa Street), the content of zinc in the soil was similar in both years (Fig. 2). Higher zinc content at the plots at Piastowska and Popiełuszki Streets may be associated with the development of communication routes in these areas, hence to an increase in traffic, which can affect the content of this element in the soil due to wear of tires, as stated by Plesničar and Zupančič [41]. The natural zinc content in soils varies depending on the type and kind of soil indicating close relationship between the mechanical composition and the content of organic substances [44]. With an increase in the organic substance, mobility of this metal decreases, whereby it is retained in the soil. This is confirmed by the correlation coefficients obtained between the zinc content and the content of organic carbon and clay minerals (respectively, $r = 0.43$ and $r = 0.65$). Chan and Lam [36] reported that zinc solubility decreases with increasing pH of the soil, however, the anionic complex organic-mineral compounds have a high mobility as an alkaline medium. Zinc can be accumulated in the plants even in large amounts (more than 1%) with no apparent toxicity symptoms. It is also worth noting that herbaceous grasses are the most sensitive to the deficiency of this nutrient [22,45]. The designated negative correlation coefficient ($r = -0.29$) indicates a higher solubility of zinc compounds and the possibility of retrieving it by plants in alkaline medium (Table 4).

In our study, Pb content reduced significantly in the soil at all locations in the second year of the study (2012). Its average content ranged from 20.75 (Raginisa Street) to 80.0 mg/kg DM (Popiełuszki Street) in 2011 and from 6.65 (Piastowska Street) to 18.46 mg/kg DM in 2012. For plots located at Hetmańska and Raginisa Streets, Pb content in the soil was below the level of the geochemical background (Fig. 2). Lead belongs to elements strongly bonded in soil and accumulated at the level of humus, and when mixed with soil and sludge, it is sorbed by the oxides and hydroxides of Mn and Fe as well as by organic matter. It migrates in the soil environment much less intensively than cadmium or zinc. The increased lead content in surface soil levels to a large extent is related to anthropogenic impact of industrial and communication emissions [36,44]. Based on the analysis of correlation, one found a negative correlation coefficient of lead from soil pH ($r = -0.57$), which may indicate a higher solubility of lead compounds and the ability to download it by the plants in alkaline medium (Table 4). Chan and Lam [36] add that the launch of lead can be caused by soil microorganisms, which in the process of microbial methylation of lead may increase its phyto availability.

The presence of other ions, including other heavy metals, may determine downloading metals in plants or soil or detaining them in soil. There was a significant relationship between the content of each metal in the soil. Nickel was positively correlated with Cd ($r = 0.59$), Pb ($r = 0.85$), and Zn ($r = 0.45$), while Ni was negatively correlated with Cr ($r = -0.61$) and Cu ($r = -0.34$). Total Pb content in soils was significantly positively correlated with the content of Cd ($r = 0.40$), Ni ($r = 0.85$), and Zn ($r = 0.57$), and for chromium ($r = -0.65$), a negative correlation was observed at $p \leq 0.05$. The correlation analysis also showed a

Table 5
Values of studied microbiological and parasitological indicators in soil samples before and after application of sewage sludge

| Lp | Designation | 2011 | | | 2012 | | |
|---|---|---|---|---|---|---|---|
| | | The kind of tested sample per g of DM | | | | | |
| | | Soil | Soil + 7.5 kg/m$^2$ of sewage sludge | Soil + 15 kg/m$^2$ of sewage sludge | Soil | Soil + 7.5 kg/m$^2$ of sewage sludge | Soil + 15 kg/m$^2$ of sewage sludge |
| *Popieluszki Street* | | | | | | | |
| 1 | Total number of psychrophilic bacteria | 2.7 × E + 5 | 1.6 × E + 5 | 1.7 × E + 5 | 6.8 × E + 5 | 4.8 × E + 5 | 1.0 × E + 6 |
| 2 | Total coliforms | 2.8 × E + 1 | 2.8 × E + 2 | 8.3 × E + 3 | 1.4 × E + 2 | 2.1 × E + 2 | 1.5 × E + 3 |
| 3 | Fecal coliforms | n.d. | n.d. | n.d. | 1.0 × E + 0 | 1.0 × E + 0 | 3.8 × E + 1 |
| 4 | *Enterococcus* sp. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5 | *Clostridium* sp. | 1.2 × E + 1 | 2.0 × E + 2 | 4.8 × E + 2 | 5.6 × E + 1 | 4.6 × E + 3 | 1.2 × E + 3 |
| 6 | *Salmonella* sp. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 7 | ATT | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| *Hetmańska Street* | | | | | | | |
| 1 | Total number of psychrophilic bacteria | 2.0 × E + 5 | 2.6 × E + 5 | 1.3 × E + 5 | 1.6 × E + 6 | 6.3 × E + 5 | 2.0 × E + 6 |
| 2 | Total coliforms | 8.0 × E + 0 | 2.8 × E + 1 | 8.2 × E + 2 | 1.4 × E + 1 | 6.1 × E + 3 | 6.9 × E + 3 |
| 3 | Fecal coliforms | n.d. | n.d. | n.d. | 1.4 × E + 1 | 1.0 × E + 0 | 1.8 × E + 3 |
| 4 | *Enterococcus* sp. | n.d. | n.d. | n.d. | 1.1 × E + 1 | 1.0 × E + 2 | 2.0 × E + 2 |
| 5 | *Clostridium* sp. | 2.4 × E + 1 | 2.5 × E + 2 | 3.2 × E + 3 | n.d. | 1.1 × E + 2 | 1.1 × E + 2 |
| 6 | *Salmonella* sp. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 7 | ATT | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| *Piastowska Street* | | | | | | | |
| 1 | Total number of psychrophilic bacteria | 1.9 × E + 5 | 1.2 × E + 6 | 1.9 × E + 6 | 1.8 × E + 6 | 1.4 × E + 6 | 1.6 × E + 6 |
| 2 | Total coliforms | 2.8 × E + 1 | 2.9 × E + 2 | 3.5 × E + 4 | 6.7 × E + 1 | 7.0 × E + 1 | 6.9 × E + 2 |
| 3 | Fecal coliforms | n.d. | n.d. | n.d. | 9.0 × E + 0 | n.d. | 8.0 × E + 0 |
| 4 | *Enterococcus* sp. | n.d. | n.d. | n.d. | n.d. | 34 | n.d. |
| 5 | *Clostridium* sp. | 1.2 × E + 1 | 2.5 × E + 2 | 5.9 × E + 3 | 1.1 × E + 1 | 1.1 × E + 2 | 3.8 × E + 3 |
| 6 | *Salmonella* sp. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 7 | ATT | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| *Raginisa Street* | | | | | | | |
| 1 | Total number of psychrophilic bacteria | 3.7 × E + 6 | 1.6 × E + 6 | 3.8 × E + 6 | 4.9 × E + 5 | 1.0E + 5 | 2.6 × E + 5 |
| 2 | Total coliforms | 2.8 × E + 2 | 7.3 × E + 3 | 2.8 × E + 2 | 6.7 × E + 2 | 2.0×E + 1 | 7.0 × E + 1 |
| 3 | Fecal coliforms | n.d. | 3.9 × E + 1 | n.d. | 1.0 × E + 0 | n.d. | 2.7 × E + 1 |
| 4 | *Enterococcus* sp. | 1.2×E + 1 | 5.9 × E + 1 | 1.2 × E + 1 | n.d. | n.d. | n.d. |
| 5 | *Clostridium* sp. | 8.6 × E + 1 | 1.2 × E + 2 | 8.6 × E + 1 | n.d. | 1.8 × E + 1 | 2.2 × E + 2 |
| 6 | *Salmonella* sp. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 7 | ATT | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

significant correlation of Zn with Cd ($r = 0.32$) and Cu ($r = 0.57$), as well as chromium with Cu ($r = 0.66$) (Table 4).

Microbiological research of the averaged soil samples, taken from the tested plots, was carried out in 2011 and 2012. Table 5 summarizes the obtained test results.

As the studies revealed, 9 months after the application of sewage sludge, a significant reduction in all tested microbiological parameters was noticed. That decrease reached the level observed in the original soil samples without the addition of the precipitate. Survival time of microorganisms in soil is limited and depends on the type and species. According to the US Environmental Protection Agency [14], the average survival time of bacteria in soil is 2–3 months. In the case of invasive nematode eggs, that time reaches 2 years on average, and maximum up to 7 years. The risk connected with organisms' infection present in the sludge, dispensed into the ground, is not high and depends on the kind of organism, exposure time, and individual susceptibility to infection. According to Jenkins et al. [11], the infectious dose of *Cryptosporidium parvum* ranges from 1 to $10^3$ oocysts, whereas *Vibrio cholerae* and *Escherichia coli* require a dose $10^8$ to cause an infection. However, there are not enough documented outbreaks or disease cases that would be associated with microorganisms present in stabilized sludge applied to soil.

In the further part of the experiment, after the growth of grass mixtures, there was no significant difference between the sanitary indicators in the tested samples of the soil and those from the earlier tests.

The appearance of *fecal* coliforms in soil samples with sludge in 2012 (Popiełuszki Street and Hetmańska Street) could be caused by the phenomenon of their regrowth. However, these were not high figures per gram of dry weight of soil.

Sanitary evaluation of soil samples constitutes a significant problem. There is no regulation in Poland which would standardize the soil quality in terms of biology. In order to assess the contamination level of the soil fertilized with sewage sludge, the obtained results were compared to the sanitary state classification of soil by Gorbowa [46] (Table 6) and the classification by Parnakowa and Mayer [15] (Table 7).

According to these classifications, the tested soil after a year from sludge application was of good quality.

Sewage sludge is usually settled by microflora and microfauna, creating a biocoenosis, which can cause sanitary risks. It includes not only bacteria but also viruses, worms, parasites, fungi, protozoa, and other organisms. It is therefore assumed that the sludge must be perceived as potentially dangerous from the hygienic point of view [10]. For that reason, the development of appropriate provisions relating to the microbiological quality of the soil becomes a necessity.

While working on these regulations, at least two microbiological indicators should be taken into account: an indicator of fecal coliform and an indicator of species belonging to the *Clostridium perfringens*. Due to large differences in the hydration of the soil samples, identified indicators must be related to 1 g of dry weight of soil.

Heavy metals that leak into the soil, on the one hand, may inhibit the growth of microorganisms, and however, they can be bioaccumulated inside bacterial cells. On the basis of the correlation analysis, significant interconnections between the studied sanitary indicators and the content of individual metals in soil, pH and $C_{org}$ have been noticed. Statistical analyzes revealed a negative correlation coefficient between soil pH and the number of total coliforms or *Clostridium* sp. (respectively, $r = -0.30$ and $r = -0.31$), and however, there was a positive correlation between *Clostridium* sp. and $C_{org}$ ($r = 0.29$). Statistical analysis also showed a significant negative correlation with *Enterococcus* sp. with Pb ($r = -0.45$) and Zn ($r = -0.41$), while *Clostridium* sp. was significantly positively correlated with the content of Ni and Zn in soil (respectively, $r = 0.31$ and $r = 0.35$) at $p \leq 0.05$. The number of *fecal* coliforms showed a significant positive correlation with Cr and Cu (respectively, $r = 0.49$ and $r = 0.43$) and was negative correlation with Ni and Pb (respectively, $r = 0.59$ and $r = 0.58$) (Table 8).

Table 6
The sanitary state classification of soil by Gorbowa

| Contamination degree | MPN of total coliforms | MPN of *Clostridium perfringens* |
|---|---|---|
| Clean | $\leq 10^2$ | $\leq 10^3$ |
| Little contaminated | $10^2$–$10^4$ | $10^3$–$10^5$ |
| Contaminated | $10^4$–$10^5$ | $10^5$–$10^6$ |
| Heavily contaminated | $>10^5$ | $>10^6$ |

Source: Mrozowska [46].

Table 7
The classification of soil with varying degrees of contamination by Parnakowa and Mayer

| Contamination degree | MPN of total coliforms | MPN of *Clostridium perfringens* | Number of thermophilic bacteria on nutrient broth agar at 60°C in 1 g of soil |
|---|---|---|---|
| IV—not contaminated | $10^2$–$10^3$ | $10^2$–$10^3$ | 0–$5 \times 10^2$ |
| III—little contaminated | $10^3$–$10^4$ | $10^3$–$10^4$ | $5 \times 10^2$–$5 \times 10^4$ |
| II—medium contaminated | $10^4$–$10^6$ | $10^4$–$10^5$ | $5 \times 10^4$–$5 \times 10^5$ |
| I—heavily contaminated | $>10^6$ | $>10^5$ | $5 \times 10^5$–$5 \times 10^6$ |

Source: Zmysłowska [47].

Table 8
Correlation coefficients between studied sanitary bio-indicators in soil and pH, $C_{org}$ and heavy metals in soil

| | Sanitary indicators | | | | |
|---|---|---|---|---|---|
| Properties of the soil | Total number of psychrophilic bacteria | Total coliforms | *Fecal* coliforms | *Enterococcus* sp. | *Clostridium* sp. |
| pH | 0.07 | −0.30* | 0.28 | 0.02 | −0.31* |
| $C_{org}$ | 0.08 | 0.18 | −0.07 | −0.20 | 0.29* |
| Cd | 0.11 | 0.05 | −0.24 | −0.07 | 0.21 |
| Cr | −0.04 | −0.12 | 0.49* | −0.15 | 0.02 |
| Cu | 0.20 | 0.12 | 0.43* | −0.06 | 0.19 |
| Ni | −0.09 | 0.13 | −0.59* | −0.24 | 0.31* |
| Pb | −0.23 | 0.12 | 0.58* | −0.45* | 0.27 |
| Zn | 0.02 | 0.16 | −0.18 | −0.41* | 0.35* |

*Significant correlations for $p \leq 0.05$.

In Poland, stabilized sewage sludge coming from municipal wastewater treatment plants can and should be used more often as a valuable fertilizer for lawns in urban areas, provided that the appropriate guidelines from the relevant provisions are met.

On the basis of the obtained results, it was found that the application of sludge to soil should be accompanied by the biological monitoring for a period at least of 1 yr. The lack of provisions regulating microbiological quality of soil hinders the ability to evaluate and determine the safety threshold. It should be noted that despite the lack of invasive *Salmonella* and nematode eggs in applied sludge, it can contain other not identified pathogens and potentially pathogenic bacteria. Although their survival in the soil is limited, their short-term presence may cause a certain degree of risk. It is therefore necessary to develop and implement Polish sanitary rules on the quality of soil.

## 4. Conclusions

(1) Nine months after the application of sewage sludge in urban soils, a significant reduction in all tested microbiological parameters was noticed. It reached the level observed in the original soil samples without the addition of the precipitate.

(2) The application of different doses of sewage sludge from the municipal waste water treatment plant in Sokółka does not affect the content of heavy metals in urban soils. Average contents of studied heavy metals were significantly different depending on the year and the location of research areas. Differences in the content of cadmium in the soil depended on the location and could be due to the distance of the research points from the edge of the road and the intensity of traffic.

(3) The content of copper in the soil at Popiełuszki Street and the chromium content in the soil in the surveyed plots in all locations in 2012 were exceeded in relation to the geochemical background, which shows contamination of the urban soil with these metals.

(4) Accumulation of cadmium, nickel, copper, and zinc in urban soils mixed with sewage sludge could be caused by binding of these metals by organic matter and clay minerals, which is

confirmed by their positive correlation coefficients.

(5) The analysis of the correlation indicates that the contents of Ni and Zn in urban soil significantly influenced the growth and development of *Clostridium* sp., while the development of fecal coliforms was inhibited by Cr and Cu.

(6) The occurrence of Pb and Zn in urban soil suppressed the growth of *Enterococcus* sp., and the same influence of Ni and Pb on fecal coliforms was observed.

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