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Dynamics of Changes in Microbiological Composition of Stored Sediments

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ABSTRACT

The composition of the sediments formed in the process of stormwater pretreatment is diversified and depends on many parameters, mainly on the quality of stormwater and the land use of the catchment. The stormwater sediments are characterized by heterogeneous chemical and microbiological composition. The aim of this paper was the microbiological evaluation of the sediments from four stormwater catchments in terms of hazard to humans and the environment. The pH, and the content of organic and mineral matter were determined for the examined sediments. The microbiological analysis included the determination of the total number of psychrophilic, mesophilic, and coliform bacteria, including *E. coli*, and also the number of faecal *Enterococc*i, *Salmonella* and *Shigella*. The study was conducted for fresh deposits and those stored for one year in order to determine the dynamics of changes in their biological activity.

Keywords: sediments, stormwater treatment plant, microbiological analysis

INTRODUCTION

In general, both naturally formed bottom sediments and also sewage sediments, play various roles in the environment. They perform environmental, geochemical and economic functions. As regards the environmental function, the sediments formed in the stormwater system, also categorised as the aquatic environment, provide a habitat to an enormous number of plant and animal organisms. The geochemical function of sediments is related to the fact they are thought to operate as the sites of deposition of pollutants that enter the water environment. Then, due to their properties and composition, sediments can be treated as natural geosorbents. As regards their economic value, sediments are considered a natural resource, widely used in agriculture to supply organic matter, in construction industry as a building material, and also to support the ecosystems of swamps and beaches [Królikowska 2012].

Sediments are formed at different points of the stormwater system, including catchments that are diversified in character. As a result, sediments differ in the physicochemical properties, particle size distribution, and primarily, in the content of various kinds of pollutants [Marsalek 1997, Bojakowska 2006]. When the stormwater system is in operation, sediments come as a by-product of the system function; they should be removed from it and managed. However, in order to correctly evaluate the means of rendering the wastes harmless, it is necessary to determine the contents of substances that are hazardous to the natural environment. They include petroleum substances, heavy metals, and polycyclic aromatic hydrocarbons. The presence of organisms that may pose a sanitary threat is another issue. It is difficult to conduct a risk analysis, considering that no regulations to control the management of sediments derived from stormwater system facilities are currently in force. That refers to both the chemical pollution and biological hazard [Hvitved – Jacobsen 1991, Samara 2016]. The Regulation of the Minister of Environment on conditions to be met for the introduction of wastewater into the water or the ground, and the substances particularly harmful to the aquatic environment refers only to the stormwater and does not account for the sediments formed in it. On the other hand, the Regulation of the Minister of Environment of 06 February 2015 on municipal sewage sediments could be applicable, but only to the situations in which sediments are intended for non-industrial use, namely they are to be used for soils [Regulation of the Minister of Environment… 2002]. In this document, § 2.1. states that *"Municipal sewage sediments can be applied to soils if the following conditions are met:*

1. *the content of heavy metals in these sediments does not exceed the amount specified in Annex 1 to the regulation;*
2. *in the case these sediments are used in agriculture, and for soil reclamation for agricultural purposes, when Salmonella bacteria were not isolated from the representative sample, 100 g in weight, obtained in accordance with § 5 subparagraph 3;"*

The microbiological tests conducted for the study aimed at specifying the hazards potentially posed by the sediments obtained from the stormwater system, both in real time when the material was collected, and after one-year storage under variable temperature conditions.

MATERIALS AND METHODS

The investigations concerned the sediments collected from the storage tanks of stormwater treatment plants located in the city of Kielce. Four facilities were selected and named after the street they are situated: Piekoszowska (P), Witosa (W), Jesionowa (J)) and Kaczmarka (K). They differ in terms of the time they have been in operation, the type of the catchment management, and also the type of the tank for sediment storage [Statement of water management…2007, State of the environment 2011]. The parameters of all facilities are shown in Table 1. The samples of sediments were collected from the sedimentation tanks of the treatment facilities in the spring of 2015. The samples were taken in accordance with the recommended methodology [PN-EN ISO 5667–15:2009] using an Eijkelkamp sampler. Directly after collection, pH, dry residue, the content of organic components, and grain size composition of the samples were analysed. Additionally, the sediments were subjected to the microbiological analysis. In May 2016, the same tests were carried out on the same samples of sediments stored under variable temperature conditions (+4°C ÷ +25°C) to identify the changes in microbiological composition. The parameters were determined in accordance with the current standards [PN-EN 12176:2004, PN-78/C-04541, Regulation of the Minister of Environment…2002].

Grain size distribution was measured with the *wet laser diffraction method* (Mastersizer 3000, Malvern Instruments). Microbiological analyses involved specifying the total number of psychrophilic and mesophilic bacteria, number of coliforms, *E. coli*, faecal *Enterococci*, *Salmonella* and *Shigella*. The samples of sediments intended for microbiological determinations were stored in sterile containers. The determinations were performed immediately after the sediments were transported to the Laboratory of Microbiological Analysis. The samples of sediments underwent appropriate preparations prior to performing determinations. For that purpose, samples were homogenized. A 1 g sample was collected and placed in the centrifugal test tube that contained 10 cm^3 of sterile water. Then, the samples of sediments were subjected to subsequent 9-fold dilutions (the serial dilution method). The quantitative analysis of the microorganism was

Table 1. Characteristics of catchments and tanks selected for research

Specification	Tanks selected for analyses			
	Piekoszowska	Witosa	Jesionowa	Kaczmarka
Put in operation (year)	1992	2003	2001	2002
Type of tank	open	open	underground	underground
Total catchment area (ha)	804.6	82.0	355.0	224.0
Residential built environment (%)	63	89	33	78
Industrial areas (%)	9	0	65	14
Meadows, arable land (%)	27	0	0	0
Forests (%)	1	0	1	0
Green areas (%)	0	11	1	8

Table 2. Methodology of microbiological analysis

Type of analysis	Standard	Substrate	Temperature and incubation time
Mesophilic bacteria	PN-A-75052–05:19902	Nutrient agar	36°±2C, 24–48h
Psychrophilic bacteria	PN-A-75052–05:19902	Nutrient agar	20°±2C , 48–72h
Salmonella and *Shigella*	PN-Z-19000–1:2001	SS substrate; propagating media, API 20E tests	36°±2C, 24h
Faecal *Enterococci*	PN-EN ISO 7899–1:2002	Slanetz-Bartley agar	36°±2C, 24–48h
Coliforms and *E.coli*	PN-EN ISO 9308–1:2014–12	Lactose-TTC-Agar-with-Tergitol-7; test for cytochrome oxidase; test for indole detection	36°±2C, 24–48h

carried out using the surface plate method and dilutions from 10^{-1} to 10^{-5}. The cultures were inoculated into Petri dishes solidified with a suitable medium (Table 2) and incubated in the laboratory POL ECO's incubator. The number of microorganisms was determined in colony forming units / gram of dry weight [cfu·g^{-1} d.w.] according to the PN-78/C-04541 standard [Water and wastewater…2001]. The standard used for the soil analysis, PN-Z-19000–1:2001, was used for the determination of *Salmonella* bacteria, because there is no uniform methodology for the sediments from stormwater drainage system. This standard is based on the detection of the presence of *Salmonella* bacteria by culturing on propagating and differential-selective media and confirming the results by biochemical or serological identification. The sediments from the stormwater drainage system can be also compared to both bottom sediments and soil. The confirmation tests were performed using ready-made API 20E tests.

The methodology of the microbiological tests of sediments from stormwater system is not standardised; therefore, the procedures in the determinations of microorganisms were based on the standards applied to the microorganism identification in water, wastewater, soil, and municipal solid wastes samples (Table 2).

RESULTS AND DISCUSSION

The materials collected from four stormwater treatment facilities showed differences already at the stage of initial visual and organoleptic examination. The results of the analysis pertaining to the basic physicochemical parameters of sediments are summarised in Table 3.

All examined samples had alkaline pH values ranging from 7.11 to 7.56; in stored sediments the pH values were slightly reduced. The sediments collected from two open tanks, namely "P" and "W", were dark grey to black in colour and had rather loose, sticky texture. High hydration of samples (61% ÷ 75%) resulted in strong cementation when they were dried. With respect to this parameter, no significant changes were observed between the fresh and stored samples. The colour of the samples from underground tanks, "J" and "K", ranged from light yellow to dark grey. The share of sand/gravel fraction was predominant (63%÷80%), by contrast, the sediments from open tanks had a substantial, over 90%, content of dust/clay fraction, d<0.063 mm in diameter. Additionally, the hydration of sediments from those facilities was much lower than from the two others, and ranged from 28% to 37%. The differences in hydration and grain size composition between the fresh and stored samples were relative-

Table 3. Selected physical and chemical parameters of fresh and stored sediments

Facilities selected for testing	Samples sediments	Physicochemical parameters of fresh and stored sediments		
		pH	Dry residue [%]	Organic matter [%]
Piekoszowska	fresh	7.45	25.10±0.93	9.89±0.10
	stored	7.12	25.40±0.44	8.83±0.25
Witosa	fresh	7.23	39.32±1.57	9.30±0.21
	stored	7.30	33.55±0.64	6.80±0.40
Jesionowa	fresh	7.56	72.23±4.86	3.92±0.56
	stored	7.48	67.16±2.60	2.21±1.07
Kaczmarka	fresh	7.11	73.44±3.44	5.96±1.71
	stored	7.16	63.32±6.69	1.71±1.17

ly small. Both hydration and grain size composition of sediments is primarily affected by the type of catchment and its management. Piekoszowska and Witosa stormwater treatment plants serve the catchments with prevalent residential built environment, 63% and 89% respectively, but green areas and meadows are also found there, despite the fact that the catchments are located within the city's administrative borders. The prevalence of the finest fraction in the samples collected from those tanks is mainly attributable to the products of road surface and pavement abrasion. Another contributing factor is the runoff from the areas with typically agricultural land use. As regards Jesionowa and Kaczmarka stormwater treatment plants, hydration and grain size composition of the sediments were largely diverse. That is also closely related to the character of the catchments, as in addition to residential buildings, their built environment includes industrial facilities, and the share of green areas is low.

Microbiological analyses were conducted for all samples of sediments. After storage under different temperature conditions, the biological activity of selected organisms was examined as well. Table 4 shows the averaged results of the microbiological analyses for fresh and stored sediments collected from four treatment facilities.

As regards fresh sediments, the highest number of psychrophilic bacteria, namely 1.4×10^6 and 1.7×10^6 cfu·g^{-1}, was found at Piekoszowska and Witosa facilities. For the two other tanks, the values were a hundred times lower. More diverse results were obtained for the mesophilic bacteria count analysis. As a result, the tanks could be arranged in the decreasing order of the number of bacteria, namely Witosa > Jesionowa > Kaczmarka > Piekoszowska. Coliforms and *Salmonella* were present in two tanks, i.e. in the sediments collected from Witosa and Jesionowa, additionally, *E.coli* was also found in both of those tanks. Faecal *Enterococci* were observed exclusively in the Jesionowa tank. As regards *Shigella*, the Piekoszowska tank did not show any colonies, whereas in the other tanks small quantities were found (from 1.3×10^2 in the sediments from the Kaczmarka tank, to 1.0×10^4 cfu·g^{-1} dry weight in the Witosa tank sediments).

The physicochemical and microbiological analyses were repeated on the same sediments after they were stored for a year. The sediments were kept in closed polyethylene containers at room temperature. A change in the temperature conditions meant that the containers with sediments were periodically placed in the laboratory refrigerator, in which the temperature was in the range of 4°C÷8°C. It turned out that the biological activity of the selected living organisms did not cease, and in some cases, on the contrary, it increased. Definitely, coliforms, and thus *E.coli*, faecal *Enterococci*, *Salmonella* or *Shigella* were not found. A tenfold decrease in the psychrophilic bacteria count was recorded in Piekoszowska, Witosa and Jesionowa tanks, whereas in the Kaczmarka tank, the number almost did not change. The most spectacular changes in the bacterial count, however, were observed for the mesophilic bacteria. In the sediments from the Piekoszowska tank, the count in these bacteria increased a thousand times. For all other cases, the number of bacteria decreased tenfold or a hundred times. In order to explain this phenomenon, it is necessary to consider the content of the organic matter in the sediments, and also the occurrence of a num-

Table 4. The results of microbiological analyses of fresh and stored sediments

Determination	Tank located at							
	Piekoszowska		Witosa		Jesionowa		Kaczmarka	
	[cfu·g^{-1} dry weight]							
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Total number of psychrophilic bacteria	1.4×10^6	1.4×10^5	1.7×10^6	1.0×10^5	5.5×10^4	7.8×10^3	6.2×10^4	7.8×10^4
Total number of mesophilic bacteria	7.2×10^5	1.2×10^8	2.0×10^8	2.0×10^6	2.1×10^7	1.4×10^4	2.2×10^6	6.0×10^5
Number of coliforms	0	0	1.0×10^5	0	7.6×10^4	0	0	0
Number of *E.coli*	0	0	2.5×10^4	0	1.6×10^4	0	0	0
Number of faecal *Enterococci*	0	0	0	0	2.7×10^2	0	0	0
Number of *Salmonella*	0	0	1.5×10^3	0	3.6×10^3	0	0	0
Number of *Shigella*	0	0	1.0×10^4	0	8.9×10^3	0	1.3×10^2	0

ber of other substances that facilitate or hamper the growth of bacterial flora. The living organisms that constitute the sediment can show very intense metabolic activity, closely related to the content of the organic matter [Bobrowski 2002, Kulikowska 2009]. The storage of the sediments leads to a clear decrement in the organic components in the samples collected from three tanks (Table 3). That is caused by the mineralisation processes and the fact that organic components are used by various microorganisms as a source of carbon [MacDonald 1994]. In the Piekoszowska tank, the degradation of the organic components was low and the organic matter content was reduced by 1% (9.89%→8.83%). That could create perfect conditions for the bacteria to multiply. Additionally, in the city of Kielce, the catchment of concern is the largest, the oldest and the most diversified one with respect to the built environment. As a result, the substances that contribute to the growth of microorganisms may have been delivered to the tanks together with stormwater. The presence of such a high number of mesophilic bacteria may indicate the occurrence of pathogens in the examined sediments. As regards the utilisation of stormwater system sediments in agriculture and land reclamation, the sediments from Witosa and Jesionowa tanks could not be employed for that purpose. In the other tanks, Salmonella was not found in the sediment samples. Thus, a problem arises how sediments could be evaluated with respect to the sanitary hazards. The issue has not been addressed by current legal regulations. In their study, Królikowska et al. [Królikowska 2012] proposed the limit values for the selected microbiological parameters that indicate the pollution of sediments. The parameters concern coliforms (limit value> 10^6), faecal coliforms (limit value> 10^5), *Enterococci* (limit value> 10^5) and *Clostridium* sp. (limit value> 10^6). The results of tests both on fresh and stored sediments do not show the exceedance of the limit values given above for the sediments of concern. Therefore, it is not necessary to disinfect sediments as they do not pose a hazard to the environment.

CONCLUSIONS

1. The sediments collected from four different tanks of the stormwater treatment facilities showed alkaline pH and substantial hydration. Another characteristic was the predominant dust-clay fraction. The physicochemical properties of sediments were closely related to the type of the catchment, its built environment and the length of time the facility had been in operation. The content of organic matter in sediment samples was reduced due to the mineralisation processes taking place in the material.
2. The microbiological analysis confirmed the presence of the microorganisms of concern, including, among others, psychrophilic and mesophilic bacteria, coliforms, *Escherichia coli* bacteria, faecal *Enterococci*, *Salmonella* and *Shigella*. The examined sediments, both fresh sediments and also stored ones, were characterised by high counts of psychrophilic (of the order of 10^3 to 10^6) and mesophilic bacteria (of the order of 10^4 do 10^8), which may indicate the presence of pathogenic organisms. The occurrence of coliforms and also of *Salmonella* was found in the sediments collected from two facilities, namely OWD_W and OWD_J. The occurrence of *E.coli* was also observed in the tanks of these two facilities. Faecal *Enterococci* occurred only in the tank of the Jesionowa OWD. As regards *Shigella, the* colonies of this type of bacteria were not found in the Piekoszowska tank, in the remaining tanks, only small quantities were present. In the stored sediments, coliforms, including *E.coli*, faecal Enterococci, *Salmonella* and *Shigella* were not found. Due to the presence of *Salmonella* (fresh samples from Witosa and Jesionowa tanks) sediments could not be used in agriculture or land reclamation. As regards all cases under analysis, the results of the tests conducted on both fresh and stored sediments do not indicate the exceedance of the limit values proposed by Królikowska et al. [Królikowska 2012]. Consequently, it is not necessary to disinfect sediments as they do not pose a hazard to the environment.
3. The lack of guidelines on the stormwater system sediments makes is necessary to produce and introduce the regulations on how sediments should be managed. The need is urgent due to the increasing quantities of sediments. As a result, it is also necessary to evaluate the stormwater system sediments with respect to chemical pollutants and microbiological hazard.

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