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TOXICITY ASSESSMENT OF SEWAGE SLUDGE FROM MUNICIPAL SEWAGE TREATMENT PLANTS

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ABSTRACT: The aim of this study was to assess the toxicity of sewage sludge from the municipal sewage treatment plant in Bialystok. Raw sewage, sewage sludge from the primary settling tank, activated sludge, sludge before the fermentation chamber, recirculated sludge and sewage sludge after fermentation and dehydration on the press were tested. The Microtox Model 500 kit using *Aliivibrio fischeri* luminescent bacteria was used for the toxicity analysis. The EC50 index (indicating the concentration of toxic substances resulting in a 50% reduction in the intensity of luminescence of the bacterial strains used) converted into the TUa toxicity units was adopted as the value describing the toxicity of the tested media. The obtained results showed high and very high ecotoxicity of raw sewage and sewage sludge from the primary settling tank, sludge in front of the fermentation chamber and after fermentation and dehydration in the press. The use of tests determining the toxicity of sewage flowing into the sewage treatment plant and sewage sludge generated at various stages of the treatment process allows for the detection of the danger associated with the uncontrolled discharge of toxic substances into the sewage system.

KEYWORDS: toxicity, *Aliivibrio fischeri*, sewage sludge

Introduction

The development of new industrial technologies, agriculture, and the increase in consumption, contribute to the rise in the amount and variety of chemical compounds and their mixtures getting into the environment. A huge number of chemical compounds and their combinations are commercially available. Placing chemicals (substances and/or chemical mixtures) on the market in the European Union is currently subject to numerous formal requirements. Their type and nature are related to the type of the product being introduced, the potential threat to human health or the environment, and its use. The development of new methods of chemical analysis enables the detection and determination of most compounds, but on the one hand, it is costly, requires specialised equipment and the use of a complicated procedure for their analysis; on the other – it does not answer two fundamental questions: how a given sample can act on living organisms living in the environment and how it can, directly and indirectly, affect the human body. Bioindication is the method that allows us to find out the total toxicity of all harmful substances, in many cases acting synergistically. It uses the so-called living organism as an indicator, a bioindicator, the reaction of which may be the basis for assessing the overall biological activity of the system under study. The quantitative and qualitative composition of the impurities contained in the tested substance does not fully reflect its harmfulness. This makes it difficult to assess the existing state of emergency unequivocally. For this reason, an objective assessment of the degree of risk is provided by toxicological tests, including bioindication methods.

Currently, several dozen bioindication methods are used worldwide, some only for scientific purposes, others routinely to control the toxicity of newly introduced chemical compounds. Tests using the Microtox kit and *Vibrio fischeri* luminescent bacteria as bioindicators are widely recognised and applied. According to the current nomenclature, these bacteria were included in the new genus *Aliivibrio* (Urbanczyk, 2007). All bacteria belonging to this genus have the characteristic shape of curved sticks, i.e. commas. *Aliivibrio fischeri* are heterotrophic Gram-negative facultatively anaerobic bacteria, 0.5 μm wide and 3 μm long.

A characteristic feature of these bacteria is their ability to luminescence, i.e. to produce light under the influence of enzymatic reactions in cells. They occur in the environment of the seas and oceans (Widder, 2010, Włodarczyk et al., 2017), however, the abundance of these bacteria in marine waters is not high. For this reason, they cannot glow directly in sea waters, but they can be seen in some marine organisms where the concentration of these bacteria is around 1010 cells/cm^3. *Aliivibrio fischeri* are a large group of organisms

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that live in symbiosis with other aquatic organisms, e.g. squids (Widder, 2010). The squid of the species *Euprymnascolopes* provide food for *A. fischeri*, and in return, the bacteria produce light in the squid's light organ (Nałęcz et al., 2010; Salyers & Whitt, 2010). Free-living bacteria survive on decaying organic matter. They are polar ciliated and, therefore, can move. The phenomenon of bioluminescence can be linked to the chemiluminescence occurring in some bacterial cells. Luminescence occurs under the influence of the luciferase enzyme, catalysing oxidation with molecular oxygen, reduced nucleotide $FMNH_2$ and aliphatic long-chain aldehyde (R-CHO) to the unstable complex ($LFMNH_2O_2R$-CHO), which after decay, emits photons, i.e. quanta of light energy with a wavelength of 490 nm.

$$FMNH_2 + O_2 + R — CHO \rightarrow FMN + R — COOH + H_2O + light. \quad (1)$$

The light intensity changes, or decreases, along with the increase in the concentration of the toxic substance. All factors disturb cell metabolism and significantly influence the functioning of enzymes and enzyme systems to reduce the level of bacterial glow. And it is this property that was used in the development of the Microtox luminometer.

Sludge generated at various stages of wastewater treatment in municipal treatment plants can be contaminated to varying degrees. From the point of view of the operation of a municipal wastewater treatment plant, the most relevant is stabilised and dewatered sludge, which is subject to joint control. Still, this control in Poland is limited to only a few chemical factors, including heavy metals. It does not consider the problem of overall toxicity, which can result from the synergistic interaction of various chemical compounds contained in wastewater and deposited at different stages of its treatment. For this reason, a study was undertaken to determine whether the wastewater flowing into the wastewater treatment plant and the sludge generated at the various stages of its treatment exhibit toxicity and what effect the toxic substances have on the natural part of wastewater treatment.

Research methods

Raw sewage samples and sewage sludge from the Bialystok Sewage Treatment Plant were used in the research: sludge from the primary sedimentation tank, activated sludge, recirculated sludge, mixed sludge subjected to the fermentation process in separate fermentation chambers, and sludge dewatering in the press. The first series of measurements was

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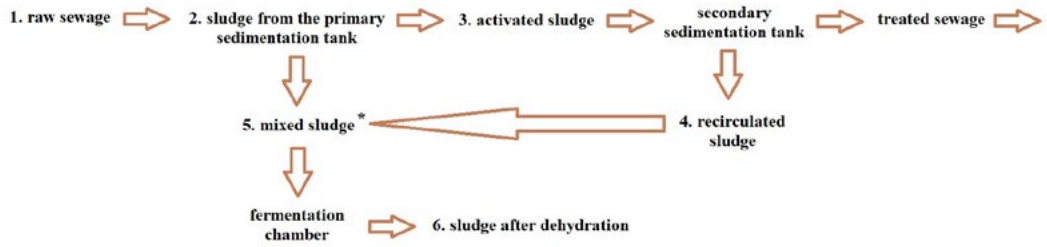
made in October and November 2021, and the second series in March and April 2022. Figure 1. presents a simplified diagram of the sewage treatment plant and points from which samples were collected for testing.



*mixed sludge – 50% recirculated sludge and 50% sludge from the primary sedimentation tank

Figure 1. Simplified diagram of the sewage treatment plant and points from which samples were collected for testing

Source: author's work.

Toxicity testing of the samples mentioned above was performed on a Microtox Model 500 luminometer. During the determinations, the reagents of the ModernWater company and the lyophilised *Aliivibrio fischeri* bacteria designated by the manufacturer as *Vibrio fischeri* NRRL B-11177 were used, as well as the following solutions:

- bacterial regeneration solution – Reconstitution Solution,
- osmotic solution – Osmotic Adjusting Solution,
- dilution water – Diluent for the liquid and solid phases.

As a bioindicator, luminescent bacteria of the species *Aliivibrio fischeri* were used. In lyophilised form, these bacteria can be stored for more than one year at -20°C and used for testing according to the procedure used. The test reaction was to reduce the luminescence of bacteria after a specified incubation time of the tested raw sewage samples and sewage sludge. The Microtox analyser was connected to a computer with appropriate software (Microtox Omni 4.1). That controlled the test execution and enabled the calculation of the results and the subsequent generation of statistical reports.

In the first series of tests, samples of highly hydrated sludge included sediment from the primary settling tank, activated sludge and recirculated sludge. Additionally, raw sewage flowing into the wastewater treatment plant was subjected to a liquid phase test with dilutions. It should be mentioned that sewage sludge requires a customised approach to sample preparation for testing. In many cases of sediment testing, the biggest problem is the filtration of the sample, which is necessary to make a toxicity determination.

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The sludge above samples was sedimented, and the resulting supernatant was filtered until a clear solution was obtained. Samples prepared this way were used for toxicity determination using the liquid phase test according to the software recommendation. In the case of press-dewatered sludge having a solid consistency (about 80% hydration), a solid phase test – the so-called Basic Solid Phase Test – was applied according to the software's recommendation. Preparation of the sample for testing consisted of the following steps recommended by the test procedure. The corresponding mass of precipitate (7.00 g) was placed in a beaker with diluent for the solid phase (35mL) and then, after mixing, was subjected to the procedure of centrifugation on a magnetic stirrer. After phase separation during centrifugation, the supernatant portion was collected, which was then subjected to filtration through unique filter columns, which were an integral part of the Microtox solid phase test kit equipment. The subsequent steps followed the measurement procedure recommended by the solid phase test software. The samples were serially diluted. In further action, the initial measurement of the luminescence of the bacteria was performed, and then the dilutions made were transferred to tubes with *Aliivibrio fischeri* bacteria. After a specific contact time between the test sample and the bacteria, the luminescence of the bacteria was measured again. Based on the results, a final report was generated.

In the second series, three sewage sludges, i.e., pretreatment sludge, mixed sludge and sludge after digestion and dewatering, were tested using the solid phase test (BSPT). The tests were strictly performed with the necessary equipment for the solid phase test according to the recommended procedure.

The remaining three sewage sludges were tested similarly to the first series using the liquid phase test. The highly hydrated sludges were initially subjected to sedimentation and filtration, followed by the appropriate test.

The test result was the EC50 value, expressed in%, which was converted into toxicity units. Only for the pressed sludge in the second step using the BSTP test the EC_{50} results are given in mg/L. In order to determine the toxicity of the sediment samples, the obtained values were converted into acute toxicity units.

Statistical analysis was performed by the Omni 4.1 software, which was an integral part of the Microtox system. In each report, the following are given: EC_{50} value expressed as % decrease in luminescence of *Alivibrio fischeri* bacteria, 95% confidence intervals; confidence coefficient, regression equation, correlation coefficient and coefficient of determination (R^2).

The obtained EC_{50} values were then converted into Acute Toxicity Units (TU_a).

The obtained EC_{50} values after 5 and 15 minutes were converted into toxicity units – *TU* according to the formula:

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$$TU_a = \frac{1}{EC_{50}} * 100, \quad (2)$$

where:
TU_a – Acute Toxicity Units,
EC_{50} – half maximal effective concentration.

In the case of determining the solid phase with the BSFT test, the obtained EC_{50} results were applied using the conversion system proposed by Falls et al. (2009).

$$TU_a = \frac{100}{EC_{50}} * 1000. \quad (3)$$

The system proposed by Persoone (Mantis et al., 2005) was used to determine the toxicity class of the tested samples of sewage and sewage sludge. The following toxicity classes have been identified:

- class 0 – TU_a=0 non-toxic sample,
- class 1 – 0.4<TU_a<1 no significant toxicity (low acute toxicity),
- class 2 – 1<TU_a<10 significant toxicity,
- class 3 – 10<TU_a<100 high acute toxicity,
- class 4 – TU_a>100 very high toxicity.

Additionally, the conductivity was determined with the HQ 40d meter, and the Hach Multi meter. Dry mass was also selected in the sewage sludge, which was determined in accordance with PN-EN 12880: 2004, with the use of a RADWAG moisture analyser.

Results of the research

The Municipal Sewage Treatment Plant in Bialystok accepts municipal sewage and sewage supplied from nearby towns, characterised by the following parameters shown in Table 1.

As a rule, they do not contain substances of toxic nature, especially their high concentrations, which is reflected in the correct operation of the treatment plant.

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Table 1. Quality of raw sewage from the Municipal Sewage Treatment Plant in Bialystok

Indicator	Unit	Value
pH	pH	7.75 ± 0.1
BOD5	mg/L	512.5 ± 86.17
COD		1310 ± 150.78
General suspension		757.5 ± 156.92
Total nitrogen		81.9 ± 4.82
Total phosphorus		9.42 ± 1.68
Wastewater temperature	°C	11.48 ± 0.73
Chlorides	mg/L	144
Sulfur		73.7
Volatile phenols		0.044
Zinc		0.299
Copper		0.081
Nickel		<0.010
Chrome		0.009
Lead		<0.010
Cadmium		<0.0005
Ether extract		252
Vanadium		<0.010
Arsenic		<0.010
Silver		<0.002
Quicksilver		<0.0005

Source: data provided by Wodociągi Białostockie Sp. z o.o., 2022.

Table 2. shows toxicity test results for different types of sewage sludge and raw sewage from the Bialystok Wastewater Treatment Plant. The EC_{50} values were converted into toxicity units, and the toxicity class of the tested sediments was determined on this basis.

In the second series, the toxicity of raw sewage flowing into the treatment plant and sewage sludge were determined, and their conductivity and hydration level were additionally determined. The obtained results are presented in Table 3.

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Table 2. Microtox test results of sewage and sewage sludge samples from the Bialystok Wastewater Treatment Plant – first series

Type of sewage sludge or sewage	Incubation time [min]				Toxicity class according to Persoone
	5		15		
	EC_{50} [%]	TUa	EC_{50} [%]	TUa	
The average value from three series of measurements					
raw sewage	7.855	13.19	5.408	18.49	3
sediment from the primary settling tank	2.525	39.60	2.095	47.73	3
recirculated sludge	824.125	0.12	152.250	0.66	1
activated sludge	5655.000	0.02	298.300	0.33	1
	Incubation time - 30 min		EC_{50} [mg/L]	TUa	
Sewage sludge after fermentation and dehydration on press			655,9 mg/L	152.46	4

Source: author's work according to Persoone et. al, 2003.

Table 3. Microtox test results of sewage and sewage sludge samples from the Bialystok Wastewater Treatment Plant – second series

Type of sewage sludge or sewage	Conductivity [µS/cm]	Hydration of the sludge [%]	$EC_{50/t}$ [mg/L]	TU_a	Toxicity class according to Persoone
sludge from primary settling tank	5780	95.011	4344	23.02	3
activated sludge	390	99.005	298.3% (t-15 min)	0.34	1
recirculated sludge	889	98.297	198.2% (t-15 min)	0.50	1
sludge before fermentation chamber	3800	96.866	22631	4.42	2
sewage sludge after fermentation and dehydration on press	36500	80.899	2396	41.74	3
raw sewage	-	-	1.794% (t-15 min)	55.74	3

Source: author's work according to Persoone et. al, 2003.

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Discussion

According to Park et al. (2005), single-substance chemical analyses do not determine the total toxic effect because we do not measure the levels of all chemicals in the sewage sludge or wastewater. We do not know the interactions between substances (additive, synergistic, antagonistic etc.) and have no insight into the effects on biota. These problems are solved by using Microtox for wastewater and sludge testing.

The sewage sludge test results confirmed the treatment plant's proper operation. The tested samples of municipal sewage flowing into the treatment plant in the first and second series were characterised by high acute toxicity from all hazardous substances, both organic and inorganic. This is evidenced by the toxicity of the sludge: the activated sludge and the recirculated sludge, which in both series of studies was at a low level defined by Persoone as having no significant acute toxicity. The rich microflora and microfauna of the activated sludge, especially bacteria and protozoa, conduct intensive enzymatic mineralisation of organic matter that contributes to low acute toxicity. Sludge toxicity increases slightly when sludge from the primary settling tank and recirculated sludge are mixed, and such sludge is stabilised in digesters. The sludge from the primary settling tank and the mixed sludge undergoing the digestion process show high specific conductivity in the eluates obtained, which indicates a high content of cations and anions mainly coming from inorganic compounds, e.g. salts. The sludge stabilised and dewatered on the press to approximately 19% dry weight and had the highest acute toxicity. This is related to the sludge dewatering process's chemical support and the belt presses' efficiency. Typically, mechanical compaction uses the addition of flocculants to increase compaction efficiency (Bien et al., 2015). The polymer consumption per kg d.m. of sewage sludge is in the range of 2-8g (Zielewicz-Madej & Fukas-Płonka, 1998). The Bialystok wastewater treatment plant uses Acefloc 80502 (cationic polyacrylamide) to assist with sludge dewatering. According to the literature, polyacrylamides are non-toxic substances, but their use in wastewater treatment processes may result in the release of acrylamides classified as toxic, carcinogenic and mutagenic substances (ICSC, 2006; Wodrow et al., 2008; Kanokhathai & Jittima, 2011; Włodarczyk et al., 2016).

In the case of the press-dewatered sludge study, very high or high toxicity was obtained, which is related to very high specific conductivity and thus to the content of inorganic compounds. In contrast, the organic matter content of the sludge decreases from about 72-76% to 55-60% after digestion (Butarewicz, 2016).

Testing sewage sludge and wastewater flowing into a municipal treatment plant by biological methods, especially with the Microtox kit, can be a

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valuable tool for determining their overall toxicity from all pollutants (Ricco et al., 2004). The results make it possible to decide whether substances of a toxic nature are deposited in sewage sludge at various stages of its generation. Examining the wastewater flowing into the treatment plant makes it easy to detect the danger of the uncontrolled discharge of toxic pollutants into the sewer system.

By performing toxicity testing of activated sludge, it is possible to determine whether the microorganisms conduct proper mineralisation of pollutants, including toxic substances. Therefore, ecotoxicological testing should be the standard used to assess the performance of a municipal wastewater treatment plant.

Conclusions

1. The tested samples of municipal wastewater flowing into the treatment plant and sludge from the primary settling tank in the first and second series were highly acute toxicity.
2. Activated sludge and recycled sludge had low toxicity. This testifies to the proper adaptation of activated sludge organisms to the pollutants present in the wastewater and the intensive biochemical-detoxification reactions they carry out.
3. Sewage sludge showed high toxicity after digestion and dewatering, primarily influenced by the agents used to dewater it. The Bialystok wastewater treatment plant uses Acefloc 80502 (cationic polyacrylamide) to assist with sludge dewatering.
4. Ecotoxicological testing should be the standard used to assess the performance of a municipal wastewater treatment plant. Economic considerations support the use of the Microtox test to determine the toxicity of raw wastewater and sewage sludge, as the cost of toxicity determinations of individual chemical parameters is much higher compared to the control of overall acute toxicity from all chemical pollutants.

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The contribution of the authors

Eliza Hawrylik – conception, literature review, laboratory tests, analysis and interpretation of data – 40%.

Andrzej Butarewicz – conception, literature review, laboratory tests, analysis and interpretation of data – 40%.

Marzanna Andraka – laboratory tests, analysis and interpretation of data – 20%.

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