

Appendix 4

Białystok, 11 September 2013

Andrzej Butarewicz Ph.D.
Faculty of Civil and Environmental Engineering
Białystok University of Technology

Summary of Professional Accomplishments

1. Name and Family name: Andrzej Butarewicz

2. Held diplomas, degrees including the name, location and the year of acquisition of these diplomas/degrees:

- Master of Science in Civil Engineering, Specialization: Sanitary Facilities, Institute of Civil Engineering, Białystok University of Technology, 1982

- Doctor of Engineering Sciences in the field of Environmental Engineering, Faculty of Civil and Environmental Engineering, 1992. PhD dissertation titled: “*Aeromonas sp.* and their use to estimate sanitary state of surface water”, thesis supervisor: professor, habilitated Doctor Zofia Kańska, Warsaw University of Technology.

3. Information on previous employment in scientific/artistic institutions

1982-1992 - research assistant, Institute of Civil and Environmental Engineering

1993 - up to now- assistant professor at The Faculty of Civil and Environmental Engineering

4. Indication of achievement according to the article 16, paragraph 2 of the Act from 14 March 2003 on academic degrees and titles in the arts (Journal of Laws No. 65, item. 595, as amended.)

A. Author/publication, title/ the year of publication, the name of publisher

According to the Act on academic degrees, Article 16, paragraph 2, for the habilitation achievement to be defined as the habilitation achievement, it should be a work published in the form of the habilitation monograph.

Andrzej Butarewicz, “Pathogenic organisms in sewage sludge – the detection and neutralization”, 2013, The Publishing house of Białystok University of Technology.

B. Description of research objective and results of the above study with an indication of applications

Systematic growth of the amount of sediments and the storage prohibition after January 1, 2013 has led to the fact that sewage sludge management has become a serious

ecological, technical and technological problem. While making a decision about sewage sludge management, one should bear in mind the chemical and biological aspects, especially those concerned with the threat of pathogenic organisms which can live in the sediments. Both municipal and industrial sewage sludge can be contaminated with heavy metals and other toxic substances and numerous pathogenic organisms.

While the chemical aspect of the sewage sludge has been widely recognized, the clarification of the aspects connected with biological contamination requires more research and analysis. The presented habilitation dissertation constitutes a summary of multiannual experiences of the author himself. The dissertation refers to microbiological and parasitological research of sewage sludge from wastewater treatment plants based in Podlaskie Voivodeship.

The aim of this dissertation was to identify the main microbiological and parasitological threats of sewage sludge and defining the aspects which have impact on the reduction of pathogens. Last but not least important aim of this dissertation was the recognition and verification of different research procedure used to detect indicator organisms in sewage sludge samples. The dissertation presents author's own diagnostic methods which can be introduced by reference laboratories and small laboratories located next to biological wastewater treatment plants.

Presented monograph provides comprehensive coverage of issues related to sewage sludge. On the basis of foreign literature, as well as national publications, pathogenic organisms detected in sewage sludge, with particular emphasis on indicator organisms, have been presented. A lot of pieces of information about the influence of chemical and physical factors on the neutralization or destruction of pathogenic organisms have been collected, and the most effective biological methods of sewage sludge treatment have been submitted. Indicator organisms diagnostics constitutes an important element of the presented monograph. Until the end of 1990s, the author's interest in biological aspects associated with the sewage sludge had been mainly connected with the detection of various indicator organisms such as bacteria and fungus. The first helminthological tests were conducted with the use of the flotation method- the Spindler's method modified by Vasilkova and Gefter as well as by Quinn and his partners. At this time, there were no precise microbiological and parasitological assessment criteria of the sewage sludge in Poland and greater attention was paid to chemical testing. Finally, the Regulation of 11 August 1999, passed by the Polish Minister for the Environment, Natural Resources and Forestry set the biological criteria. The currently valid Act of Minister of Environment of 13 July 2010 on the municipal sewage sludge (Journal of

Laws of 2010 No. 137, item. 924), although sets out the conditions that must be met while using municipal sewage sludge and recommends reference methods for the study, but does not indicate any standard, which specifies in details the conduct of such research.

Polish reference laboratories base their standards on PN-Z-19000-4 norm, which deals with the method of detecting intestinal parasites *Ascaris lumbricoides* and *Trichuris trichiura* in soil.

According to the research conducted by the author, in case of certain deposits, especially these, in which chemical preparations assisting dehydration were utilized, the use of the above-mentioned standard is difficult or even impossible.

Besides, the necessity of detecting *Toxocara cati* and *Toxocara canis* was disregarded in the above mentioned norm, which is also a huge drawback not only in studying the sewage sludge but also the soil. Moreover, this standard does not include any reference to the eggs of the parasitic nematode *Ascaris suum*.

It should be stated that there is no such a thing as one universal research method which would be effective in detecting the parasitic organisms in sewage or sewage sludge and that the effectiveness of such methods is restricted. The choice of the suitable method should be dependent on the kind of the sediment sample. The value of detection of parasitic eggs in sewage sludge is conditional on the kind of the method used in research as well as the efficiency of the operator.

Independently from the kind of sediment, all the available methods for the detection of the eggs of parasites are based on two fundamental rules: either the eggs of parasites float in high – density solutions in contrast to other particulates; fats, and other components are separated by means of solutions such as ether or ethyl acetate. Or the eggs of parasites settle at the bottom of a not-miscible layer of solution.

Presented monograph includes a critical review of the world's most widely used research procedures for the detection of parasite eggs. In Annex 1, in addition to Polish test procedure, different diagnostic solution advised by WHO, U.S. EPA and other laboratories in the United States and Mexico have been presented. Moreover, the most commonly used solutions for flotation with their preparation procedures have been described. In addition to the above-mentioned research procedures used worldwide to detect nematode eggs, author's own solutions resulting from experience have been proposed. The monograph presents also, an original methodology, modified by the author, for testing indicator nematode eggs. The presented methodology outlines the stages (one after another) to detect invasive nematode eggs paying attention to various diagnostic problems which may arise in their detection.

The proposed research procedure allows the detection of parasite eggs with better results than the methodology applicable to the study of soil samples, and also allows adjusting it to the type of sludge in the course of diagnostic tests.

The CD atlas with images of nematode eggs detected in sewage sludge from wastewater treatment plants in Podlaskie Voivodeship was attached as a supplement to the monograph. The pictures of nematode eggs were made with the use of an optical microscope equipped with a CCD camera. The detected eggs of *Ascaris sp.*, *Trichuris sp.* and *Toxocara sp.* were in various stages of development from unfertilized eggs to eggs with visible invasive larvae. During the 18-month test cycle of sewage and sewage sludge from the selected wastewater treatment plant, the fluorescence microscope with Nomarski Interference Contrast (NIC) was used to analyze the sediments. The obtained pictures have been presented in the attached atlas, which constitutes an original elaboration on a national scale, to be used by diagnostic laboratories.

The second, not less important indicator of sewage sludge is *Salmonella*. Under the Polish law, the detection of *Salmonella sp.* in sewage sludge is an extremely important issue, since the presence of this bacteria eliminates any possibility of the use of sewage. The reference methods defined in Regulation and used to detect the presence of pathogenic bacteria of *Salmonella* species in a sample of sludge depend on the conduct of two types of culture media: enrichment and selective-differential media. The methods also require biochemical and 'serovar' confirmation. Similarly, like in the case of the nematode belonging to ATT, there are no special methods for the determination of *Salmonella sp.* in sewage sludge in Poland. There is only a PN-Z-19000-1:(2001) norm for detecting *Salmonella sp.* in the soil which is used for testing sewage sludge. The use of '1682' research methodology proposed by the Environmental Protection Agency specifically for the study of sewage sludge is an alternative to the proposed procedures for the detection of *Salmonella* in surface waters, wastewater or soil in Poland. The methodology was not only tested on samples of sludge in this study, but also on raw and treated wastewater. It was proposed on the basis of the conducted research that the research methods need to be simplified via the introduction of genetic tests at the stage of isolation of colony forming units on selective-differential media.

The direct isolation of *Salmonella* DNA from the sediment samples or sewage sediment may be extremely difficult, because the number of bacilli belonging to the genus *Salmonella* in the samples is very low. Genomic Mini by DNA Gdansk (nowadays, the company is called Blirt) was used to isolate the genomic DNA. The amplification of the characteristic DNA fragment was performed using the PCR technique and the kit of Blirt was

used to detect the rods belonging to the genus *Salmonella* in food. The conducted genetic testing confirmed that all isolated by conventional methods strains belong to *Salmonella* sp. In two doubtful cases, in which the negative results in the latex test were obtained, the genetic research proved the presence of *Salmonella* rods in sludge. Genetic testing should be soon used by reference laboratories testing sludge. In large wastewater treatment plants with appropriate research facilities, genetic testing should replace the biochemical tests, which would shorten the test procedure for a day.

On the basis of the research, a scheme to detect *Salmonella* rods in sewage and sewage sludge has been proposed, using conventional methodology, but also genetic testing.

A review of available publications and studies has led to the following conclusions:

1. Based on the review of the sanitary regulations in force in many countries, it has been concluded that there are no uniform biological criteria used to determine the quality of sanitary sewage. This applies to the types of the used indicator organisms and determination of their permissible size as well as the weight of the sediment, which is converted into the final result. This creates a lot of problems in the interpretation of test results.
2. The standards to be used in detecting parasite eggs in wastewater and sewage sludge from municipal and industrial wastewater treatment plants should be developed in Poland. The standard should include the appropriate research procedure, such as the one proposed by the author, for the detection of three species of nematodes: *Ascaris*, *Trichuris* and *Toxocara* specified in the Regulation of the Minister of the Environment from 2010 on municipal sewage sludge. The proposed and presented in detail in the monograph research procedure allows the detection of parasite eggs with better results than the methodology applicable to the study of soil samples, and also allows adjusting it to the type of sludge in the course of diagnostic tests
3. One should develop the standards for the detection of *Salmonella* rods in sewage and sewage sludge which would be adapted for the development of research techniques. Molecular studies should be more widely used to detect the bacteria by reference laboratories. The kits for the detection of rods belonging to the genus *Salmonella* in food can be successfully used in the study of sediments. Due to the specific nature of the sludge, the applied molecular studies will be possible after having isolated the characteristic colony

forming units on selective-differential media. This will shorten the test time, while eliminating the need for biochemical tests and serological confirmation test.

4. It was found on the basis of tests that the sediment sample had been dominated by the eggs of *Ascaris sp.* There were less eggs of *Toxocara sp.* whereas invasive eggs of *Trichuris sp.* were detected only once out of 18 analyzed sediment samples. Epidemiological status of the Podlaskie Voivodeship confirms drop in new cases of trichuriasis. The detection of a large number of indicator nematodes in sediments should be a signal to carry out comprehensive research in the community, as well as livestock and wild animals. Limiting the number of cases is only possible when using a wide community deworming campaigns and animals.

5. Laboratories for sludge research should be equipped with the appropriate test equipment to document the results of the research on nematodes detected in sewage sludge. The attached to the monograph atlas, includes the photos of indicator nematode eggs, taken with a classic optical microscope and a fluorescence microscope equipped with Nomarski Interference Contrast (NIC). The atlas is of practical importance and can be used by laboratories for diagnostics of indicator nematodes.

6. The periodic occurrence of *Salmonella* in treated wastewater should be a signal to enter the disinfection process.

5. Other scientific accomplishments

Before receiving a doctoral degree, in the years 1982 - 1985 I was involved in the research on the occurrence of the gram-negative bacteria in surface waters characterized by extrachromosomal resistance to heavy metal compounds as well as antibiotics in the problems of The Inter MR.II.17 topic 5.4.6. From years 1987-1988 I took an active part in the research on microbial activity, microbial use and their suppression in the framework of CPBP 04.02. 1987/88 program. The head of the two above-mentioned work was Doctor of Engineering Miroslaw M. Bobrowski.

In subsequent years, my work focused on the problem of the sanitary condition of water. Numerous literature sources published in prestigious journals in the world indicated that the applied analysis of the sanitary condition of water is insufficient and there is a need for new solutions. Some authors proposed to include additional indicatory organisms to the assessment of the sanitary condition of water e.g. rod-shaped *Aeromonas hydrophila*. The

tests were carried out (in the framework of doctoral dissertation) to determine the usefulness of these rod-shaped bacteria as additional indicators of health status. The dissertation was called "Bacteria of the genus *Aeromonas* and its use to assess the health status of surface water". The results demonstrated the usefulness of *Aeromonas* rods as a supplementary sanitary indicator used for the assessment of the quality of surface water. The new modified PXA medium was prepared to isolate these bacteria.

After receiving the degree of Doctor of Technical Sciences, my professional interests focused on a few research problems. The first one was doing the research related to microbiological air quality. I conducted a series of tests in the wastewater treatment plants (e.g. in the Wastewater Treatment Plant in Białystok and in Bielsk Podlaski) determining its area of nuisance on the adjacent environment. For many years I have been researching the microbiological quality of the air in the municipal landfill, and recently in waste sorting. I also conducted a study on air quality in the workplace for example in a textile factory "Fasty" in Białystok, and at the Białystok University of Technology. All the above-mentioned studies have in mind not only the cognitive aspect, but also utilitarian.

The second, no less important direction of my research interests, relates to the ability of using a variety of indicator organisms in toxicological studies. For many years I have been interested in chronic and acute toxicity tests and their practical application. In 1996 I conducted ecotoxicological studies for the PhD dissertation. The study included determination of thiobarbituric acid, ethionamide and 6-isopurine toxicity, in terms of acute toxicity to the bacteria of *Pseudomonas fluorescens* species, *Chironomus larvae*, crustaceans of the genus *Daphnia magna*. Currently my research is focused on determining the toxicity of wastewater. With the acquisition of modern set of Microtox, I conducted the research on toxicity of municipal and industrial wastewater from wastewater treatment plants. The obtained results were partially published and presented in the form of papers.

The third line of my research interests is connected with testing the sanitary quality of water and using various indicatory organisms. I made a lot of expertises of microbiological quality of drinking water, surface water or groundwater. In the research, I employed the latest diagnostic methods used in the world and at the same time, I paid attention to the possibility of their implementation into the research conducted in Poland, provided that all the necessary adjustments would be made. From 1998 to 2000 I conducted the research determining the purity of Suwalskie and Augustowskie Lake-district. Currently I am doing the research on the sanitary condition of bathing sites across the region of Podlasie. I also conducted a study of water quality in the river Narew and wells in the region of Podlasie within the framework of

“The trophic and sanitary condition of surface water in the Narew National Park in relation to the quality of water and areas adjacent to the soil”.

The third line of research is the microbiological quality of surface water and groundwater. From 1998 to 2000 I conducted the research determining the purity of Suwalskie and Augustowskie Lake-district. Currently I am doing the research on the sanitary condition of bathing sites across the region of Podlasie. I also conducted a study of water quality in the river Narew and wells in the region of Podlasie within the framework of “The trophic and sanitary condition of surface water in the Narew National Park in relation to the quality of water and areas adjacent to the soil”.

All studies of microbiological quality of drinking water (which I conducted) are extremely important for the safety of water users. The work on the quality of water in emergency wells located in Bialystok highlighted the existence of health problems to the municipal services in the case of the use of such wells, which usually takes place when the public water supply fail. Current research microbiology is associated with microbial biofilms in underground water supplies and the methods of the cleaning of such installations. The research is conducted in collaboration with the staff of the Technical University in Minsk and will continue in the coming years.

At the same time, I was constantly gathering experience and improving the quality of research related to biological sludge. In 2005, I become the main contractor of studies in the research project KBN No 5 T07E 043 23, which was run by Professor, habilitated Doctor of Engineering Andrzej Królikowski. The study included determination of the basic indicators of health, including parasitological indicators in sediment samples from septic tanks, separators and inlets of storm drainage system. The monograph of joint-authorship titled “The sediments built up in drainage system” was the final result of the pioneering studies of sediments from storm water. The monograph was released in 2006 by Polish Academy of Sciences and submitted for awards of the Polish Minister of the Environment by the Department of Civil and Environmental Engineering. At the same time I was doing microbiological-parasitological research in the framework of research project KBN No. 4 T09D 024 22 which was run by the Doctor of Engineering Lech Magrel. The aim of this study was to determine the possibility of using sewage sludge from dairy industry for producing compost, which could be used as a valuable fertilizer. Experiments were carried out on the basis of the prism formed in various using the dairy industry sludge as well as wood chips, sawdust and straw for this purpose. The results allowed us to determine the optimum parameters of the composting process which was carried out in the forest, as well as the outdoor. The research had a utilitarian aspect since

the obtained compost was used for the seedlings in the superintendence of Rudka. The result of this research study was the second monograph of joint-authorship called “ The research on the ultimate use of dewatered sewage sludge for non-industrial use”.

For many years, my scientific work has been focused on environmental microbiology, especially on the diagnosis of microbial pathogens. I have been involved in the statutory work carried out in the Department of Sanitary Biology and Biotechnology, I performed the works financed from its budget on my own.

Recently I have been the main contractor for the research project with the registration number N N305 367438 titled “The Evaluation of the suitability and sustainability of lawn grass mixtures used on sidewalk lawns in ground fertilizing with sewage sludge”, which was run by Professor, habilitated Doctor of Engineering Tadeusz Łoboda. The project will be completed in 2014. Results to date have been published.

In addition to doing the scientific research financed from the state budget, I carried out a number of professional evaluations for businesses located in the region of Podlasie. I conducted a study of the quality of activated sludge and filamentous bacteria in wastewater treatment plants in Bielsk Podlaski and Sokółka. For many years, I have been working with sewage treatment plants situated mainly in the region of Podlasie. I made over 300 professional evaluations associated with the presence of pathogenic organisms in sewage sludge. The experience gained in this field become an excellent study material which was used in the habilitation monograph.

The list of the most important research works in which I participated in the years 1983 – 1992, which is before obtaining the degree of Doctor of Technical Sciences

1. The occurrence of the gram-negative bacteria in contaminated surface waters characterized by extrachromosomal resistance to heavy metal compounds as well as antibiotics in contaminated surface waters. The Problem of The Inter MR.II.17 topic 5.4.6 (1982-1985).
2. The Measurement of 30-minute concentrations of SO₂ in a random in selected cities in the region of Białystok.
3. The technique of milk processing for refined protein concentrates for the bakery. RNN-U-270/88.
4. Microbial activity, the use and control of bacteria in the framework of CPBP 04.02. 1987/88.

The list of the most important research works after obtaining the degree of Doctor of Technical Sciences

1. The Research Project No. S/IIŚ/23/95 titled “Microbiological processes occurring during the aerobic and anaerobic treatment of the sewage sludge and solid waste and the role of these processes in the transformation of heavy metals”.
2. The Research Project No. W/IIŚ/3 /98 titled: “The assessment of the sanitary-epidemiological risk of the sludge produced in the mechanical – biological wastewater treatment plants in the region of north-eastern Poland”.
3. The Research Project No. W/IIŚ/1/98 titled “The assessment of purity of Suwalskie and Augustowskie Lake-district”.
4. The Research Project No. S/IIŚ/26/01 titled: “The Microbiological contamination of the aquatic, soil and air environment in the region of north-eastern Poland”.
5. The Research Project W/WB/21/02 titled: "The trophic and sanitary condition of the surface water in the Narew National Park in relation to the quality of water and adjacent areas of the soil”.
6. The Research Project KBN No. 4 T09D 024 22 titled “ The Research on the ultimate use of dewatered sewage sludge for non-industrial use”.
7. The Research Project KBN No. T07E 043 23 titled „Qualitative and quantitative characterization of sediments from the drainage facilities in various urban catchments”.
8. The Research Project No. W/ WBiŚ/22/06 titled “The use of modern research techniques for the detection of pathogenic organisms in sewage sludge from municipal sewage treatment plants”.
9. The Research Project No. W/WBiŚ/3/2009 titled “Using conventional methods and modern molecular biology techniques for the detection of pathogenic organisms in treated wastewater and sewage sludge”.
10. The Research Project KBN (NCN) No N N305 367438 “The evaluation of the suitability and sustainability of lawn grass mixtures used on sidewalk lawns in ground fertilizing with sewage sludge”.
11. The Research Project W/WBiŚ „The assessment of purity of selected elements of the environment in Podlaskie”.

