Appendix no 3

**SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS**

Discussion of the publication cycle entitled:

# *HUMAN-ASSOCIATED BACTERIA WITH ANTIMICROBIAL-RESISTANCE PATTERNS IMPORTANT MINORITY IN WASTEWATER AND WASTEWATER IMPACTED ECOSYSTEMS*

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Department of Water and Wastewater Technology

***ANETA ŁUCZKIEWICZ***

Gdańsk, 2014

# Table of Contents

**1. NAME**

Aneta Łuczkiewicz

**2. DIPLOMAS AND DEGREES:**

**2003** - Ph.D. in technical science at the Faculty of Hydraulic and Environmental Engineering (now Faculty of Civil and Environmental Engineering), Gdansk University of Technology. The dissertation title: "Soil and water contamination as a result of sewage sludge application" (*awarded*). Supervisor: dr hab. inż. Bernard Quant, prof. PG.

**1997**- M.Sc. in technical science at the Faculty of Environmental Engineering (now Faculty of Civil and Environmental Engineering), Gdansk University of Technology. The dissertation title: "Microbiological risk assessment at water treatment plants". Supervisor: prof. dr hab. inż. Krystyna Olańczuk Neyman.

**3. EMPLOYMENT IN SCIENTIFIC INSTITUTIONS**

**11.2003 - present** – adiunkt at the Department of Water and Wastewater Technology, Faculty of Hydraulic and Environmental Engineering (now Faculty of Civil and Environmental Engineering), Gdansk University of Technology,

**10.1997 - 10.2003** – teaching and research assistant at the Department of Water and Wastewater Technology, Faculty of Environmental Engineering (now Faculty of Civil and Environmental Engineering), Gdansk University of Technology; from 1996 to 2003 Ph.D. studies at the Faculty of Environmental Engineering, Gdansk University of Technology.

**4. ACHIEVEMENTS UNDER ART. 16 PARAGRAPH 2 OF THE ACT OF 14 MARCH 2003 ON ACADEMIC DEGREES AND TITLE**

**4a)** ***Title of scientific achievement***

The results of my scientific achievement, entitled: **Human-associated bacteria with antimicrobial-resistance patterns - important minority in wastewater and wastewater impacted ecosystems,** are presented in the following monothematic publications published between 2006-2014 with a total impact factor *IF = 12,249* and the value of PM = *101,5* Ministry of Science and Higher Education scoring.

**4b)** ***Series of monothematic publications included in scientific achievement***

1. **Łuczkiewicz A.**, Jankowska K., Olańczuk-Neyman K., 2007. Presence and distribution of antibiotic-resistant bacteria in multiphase activated sludge system. Polish Journal of Environmental Studies. nr 2A, pt. 3 suplement 546-551 **(PM - 5)**

2. **Łuczkiewicz A.**, Fudala-Książek S., Jankowska K., Quant B., Olańczuk-Neyman K., 2010. Diversity of fecal coliforms and their antimicrobial resistance patterns in wastewater treatment model plant. Water Science and Technology, 61 (6), 1383-92 (IF – 1.146, PM - 20)

3. **Łuczkiewicz A.**, Fudala-Książek S., Jankowska K., Olańczuk-Neyman K., 2010. Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. Water Research, 44 (17), 5089-5097 **(IF – 5.315, PM - 45)**

4. **Łuczkiewicz A.**, Fudala-Książek S., Jankowska K., Quant B., Olańczuk-Neyman K., 2010. Antimicrobial resistance of *Enterococcus* spp. in municipal wastewater treatment plant – model study, Polish Journal of Environmental Studies, vol. 2, Series of Monographs, HARD, 146-152 **(PM - 5)**

5. **Łuczkiewicz A.**, Jankowska K, Bray R., Kulbat E., Quant B., Sokołowska A., Olańczuk-Neyman K., 2011. Antimicrobial resistance of fecal indicators in disinfected wastewater. Water Science and Technology, 64 (12), 2352-61 **(IF – 1.146, PM - 20)**

6. **Łuczkiewicz, A.**, Felis, E., Ziembinska, A., Gnida, A., Kotlarska, E., Olańczuk-Neyman, K., Surmacz-Gorska, J., 2013. Resistance of *Escherichia coli* and *Enterococcus* spp. to selected antimicrobial agents present in municipal wastewater. Journal of Water and Heath, 11(4), 600-12 **(IF – 1.542, PM - 25)**

   The results were also partly presented by: **Łuczkiewicz, A.**, Olańczuk-Neyman, K. Felis, E., Ziembińska, A., Gnida, A., Surmacz-Górska, J., 2013. Fecal indicators resistance to antimicrobial agents present in municipal wastewater. In: Proceedings of the Conference on Environmental Engineering IV Lublin, Poland; 3 - 5 September 2012; Eds. A. Pawłowski, M.R. Dudzińska, L. Pawłowski. 2013 Taylor & Francis Group, 151-159

7. Sadowy E. & **Łuczkiewicz A.**, 2014. Drug-resistant and hospital-associated *Enterococcus faecium* from wastewater, riverine estuary and anthropogenically impacted marine catchment basin. BMC Microbiology, DOI:10.1186/1471-2180-14-66 **(IF – 3.10, PM - 25)**

## 4c) *Discussion of the Scientific Achievements*

### Introduction

In 1997 the World Health Organization (WHO) reported antibiotic resistance infections as a major threat to public health, while a year later European Parliament and the Council (Decision No 2119/98/EC) requested an endeavor effort and multidisciplinary strategy to prevent dissemination of antibiotic resistance.

Several studies have been already undertaken among healthcare facilities. However, there is limited information about the release and subsequent survival of drug-resistant bacteria in the environment. In my opinion, the potential role of wastewater treatment plant (WWTP) effluents in dissemination of clinically relevant bacteria and genes needs more attention. As a sanitary engineer, who provides adequate water supply and effective sanitation of wastewater, I aimed to find out if antimicrobial-resistance patterns detected in hospital settings could be also beneficial for survival in environmental compartments. I focused on Gulf of Gdansk and its shallow western part, the Puck Bay, important ecosystem subjected to strong anthropogenic impact. Significant pollution load is discharged to this coastal area through the numerous local rivers and marine outfalls. Due to limited water exchange in the Gulf of Gdansk, especially safe wastewater disposal is essential to prevent the environmental degradation and to preserve the public health. However, no microbiological standards have been set on WWTP effluents in the majority of European countries, it is required instead to monitor the quality of recreational water by detection of fecal enterococci, together with *Escherichia coli* as indicators of fecal contamination of bathing water [1].

Thus primary objective of my scientific achievement was to analyze the occurrence and spread of antimicrobial resistance among fecal indicators in wastewater treatment processes. In the study particular attention was given to:

1. survival of indicator bacteria with antimicrobial-resistance patterns in wastewater processes based on activated sludge system (laboratory and full-scale study),

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[1] DIRECTIVE 2006/7/EC of the European Parliament and of the Council, 15 February 2006, concerning the management of bathing water quality and repealing Directive 76/160/EEC. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?-uri=OJ:L:2006:064:0037:0051:EN:PDF

2. incidence of antimicrobial resistance among indicator bacteria isolated from direct wastewater receivers,
3. effectiveness of wastewater disinfection (ozonation, UV irradiation and micro/ultrafiltration) in removal of indicator bacteria with antimicrobial-resistance patterns (pilot-scale study).

In line with objectives of this study I collected and tested raw and treated wastewater as well as an activated sludge from two main local WWTPs: Gdansk-Wschod and Gdynia - Debogorze. Since both WWTPs discharge the treated wastewater via marine outfalls, I also sampled the costal water of Gulf of Gdansk, about 2.5 km from the shoreline, where the submarine collectors end with diffuser systems. Additionally, Vistula river mouth was sampled (Fig. 1). I analyzed the antimicrobial resistance and selected resistance determinants among *Escherichia coli* and *Enterococcus* spp. Both bacteria are commensals of human intestinal tract and are regarded as the indicators of fecal contamination in environmental studies. Even more important is the fact that both *E. coli* and enterococci are also responsible for hospital-acquired infections in Europe[2]. Enterococci cause a range of illnesses including bloodstream infections, surgical site infections, and together with *E. coli* urinary tract infections.

Among enterococci in the human intestine *E. faecalis* and *E. faecium* are widely spread (90% and 5%, respectively)[3], while species such as *E. avium*, *E. casseliflavus*, *E. durans*, *E. gallinarum*, and *E. raffinosus* are occasionally detected. The last two species are also most commonly isolated from human enterococcal infections, however, in the recent years, the relative proportion of *E. faecium* to *E. faecalis* is increasing in the US and Europe. It is connected with the spread of a particular hospital-adapted polyclonal high-risk enterococcal complex (HiRECC) of *E. faecium*, initially described as clonal complex 17, CC17[4]. An important feature of HiRECC is the acquisition of resistance to antimicrobials of several classes (multi-drug resistance, MDR), leaving few or no treatment options. Among enterococci, acquired resistance of clinical relevance includes resistance to glycopeptides (vancomycin-resistant enterococci, VRE) and high-level resistance to aminoglycosides

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[2] ECDC. Annual Epidemiological Report 2012. Stockholm, 2013

[3] Tendolkar PM, Baghdayan AS, Shankar N, Cell Mol Life Sci, 2003, 60:2622-2636

[4] Leavis HL, Bonten MJ, Willems RJ: Identification of high-risk enterococcal clonal complexes: global dispersion and antibiotic resistance. Curr Opin Microbiol 2006, 9:454-460

(HLAR). Among *E. coli* (and other *Enterobacteriacaea*) extended spectrum β-lactamase producing (ESBLs) strains, resistant to a wide variety of penicillins and cephalosporins are of great concern.

**Methods**

To analyze the phenomenon of antimicrobial susceptibility of indicator bacteria I applied standardized and conventional methods. Detection and enumeration of fecal indicators were carried out via membrane filtration, according to PN-ISO 9308-1:2000 and ISO 7899-2:2000. The species identification and drug susceptibility of presumptive *E. coli* and enterococci were determined by the Phoenix™ Automated Microbiology System (BD, New Jersey, USA) according to the manufacturer's instructions. Since it is not possible to distinguish *E. casseliflavus* and *E. gallinarum* using this system, the following classification was implemented: *E. casseliflavus/gallinarum*.

The susceptibility tests, based on microdilution, were carried out. Obtained minimal inhibitory concentrations (MIC) were evaluated according to the guidelines of Clinical and Laboratory Standards Institute(CLSI) [5] and European Committee for Antimicrobial Susceptibility Testing (EUCAST)[6]. Isolates were defined as multidrug-resistant (MDR) when they showed resistance to three or more compounds tested[7].

Differences in distributions were evaluated using the $\chi 2$ test, with the p values $< 0.05$ considered significant.

Scheme of sampling points location is given in Figure 1.

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[5] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 21st informational supplement 2011, Wayne, PA M100-S20

[6] European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs. 2013. http://www.eucast.org

[7] Magiorakos AP, Srinivasan A, Carey RB Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL: Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012, 18:268-281

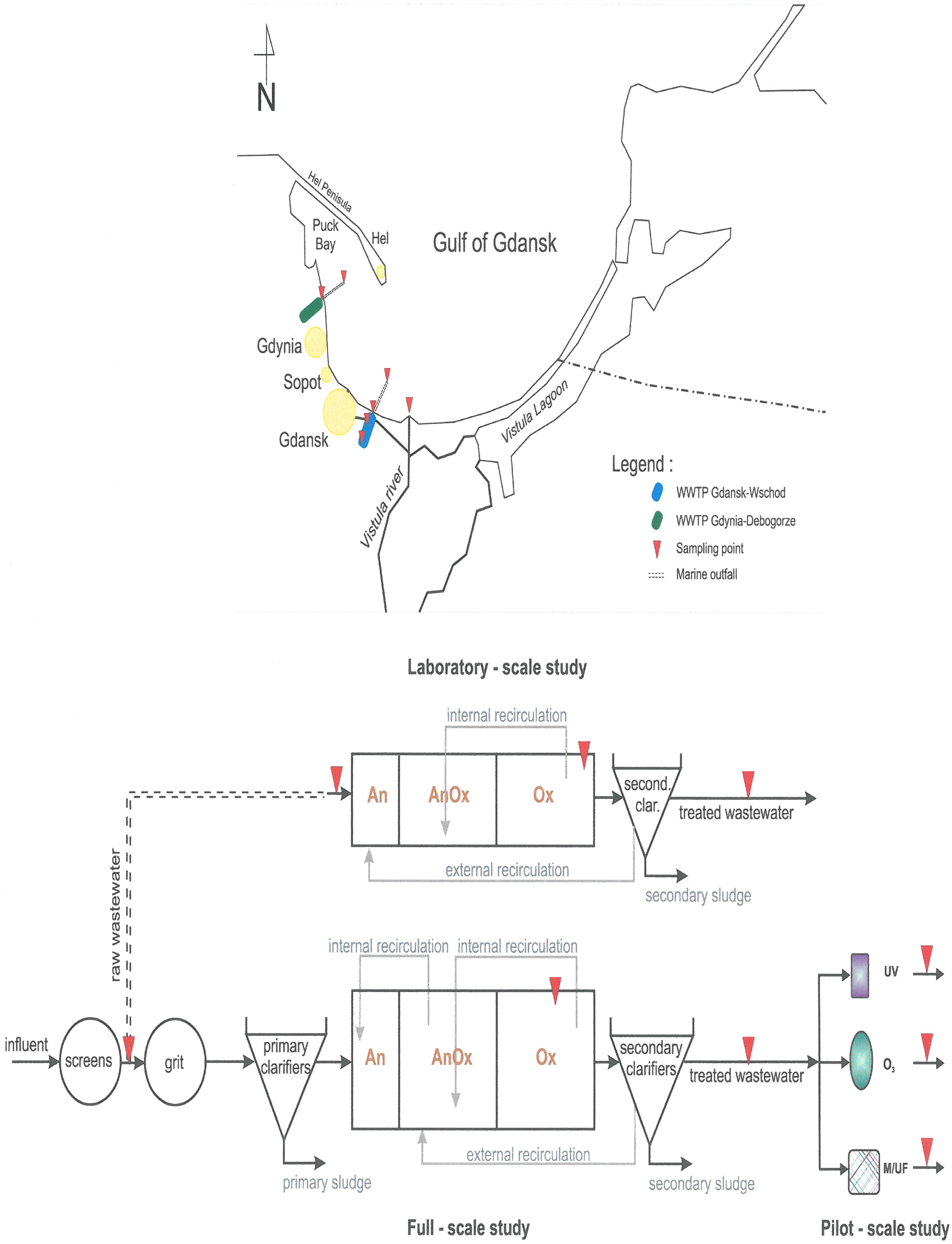


Figure 1. Scheme of sampling points location

*An - anaerobic conditions, AnOx - anoxic conditions, Ox - aerobic conditions, UV - UV module,* $O_3$ *- ozone module, M/ UF - micro / ultrafiltration*

**Ad topic no 1.**

***Survival of indicator bacteria with antimicrobial-resistance patterns in wastewater processes based on activated sludge system (laboratory and full-scale study)***

I started to analyze the occurrence and the antimicrobial susceptibility of fecal indicators in continuous-flow laboratory-scale wastewater treatment plant (M-WWTP), which is based on multiphase, activated sludge system and known as A2/O (anaerobic/anoxic/oxic). The M-WWTP (Qav. = 27 $m^3$/d) was inoculated with nitrifying activated sludge and fed continuously with the raw municipal wastewater (after screening) obtained from the Gdansk Wschod WWTP (Qav. = 96 000 $m^3$/d). During the experiment fecal indicators were isolated from the inflow and outflow as well as from mixed liquor of M-WWTP aerobic chamber. All together 222 isolates of *E. coli* and 152 of *Enterococcus* spp. were isolated.

According to the obtained results, 56.6% of enterococci were identified as *E. faecium*, 42.7% as *E. faecalis* and 0.7% as *E. hirae*. Most of them were resistant to erythromycin (up to 75% of isolates), up to 57% to ciprofloxacin and up to 46% tetracycline. With regards to *E. coli*, its resistance to ampicillin reached 41%, to tetracycline - 21%, to trimethoprim/sulfamethoxazole - 12% and to fluoroquinolones - 9%. Additionally, among tested isolates I detected the resistance patterns of clinical relevance: 2 extended-spectrum β-lactamase–producing *E. coli* (ESBL) as well as 11 *E. faecium* and 4 *E. faecalis* resistant to high level aminoglycosides (HLAR). It is important to outlined that both, HLAR and ESBL phenotype were always associated with resistance to other antimicrobials of at least two different chemical classes - multi-drug resistant (MDR) phenotypes. In general during this lab-scale experiment, I detected MDR patterns among 12% of *E. coli* and 27% of enterococci.

Detailed analyses of isolates distributions in raw and treated wastewater additionally showed statistically important selection of *E. coli* and *Enterococcus* spp. with MDR phenotypes. Such increase in number of antimicrobial-resistant strains to sensitive ones in treatment processes was also previously reported[8] and is explained by several factors, of which the most important are: selective pressure and/or transfer of resistance genes between bacteria.

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[8] Reinthaler et al., 2003 Antibiotic resistance of E. coli in sewage and sludge. Water Res. 37, 1685–1690.

The results obtained in laboratory scale led me to undertake further research in a technical scale. I continued my survey at the Gdansk-Wschod WWTP operating in modified University Cape Town (mUCT) system ($Q_{av.}$ = 96 000 $m^3/d$; PE = 675 000; 94.83% - municipal wastewater; 5% industrial and 0.2% not disinfected hospital wastewater). I isolated fecal indicators (*E. coli* and *Enterococcus* spp.) from raw wastewater, treated wastewater as well as from aerobic chamber.

In full-scale experiment as in laboratory-scale, among enterococci (n = 199) the predominant species were *E. faecium* (60.8%) and *E. faecalis* (22.1%). The remaining isolates were, however, up to 17.1% and belonged to *E. hirae* (12.1%), *E. casseliflavus/gallinarum* (4.5%), and *E. durans* (0.5%). In general among enterococci resistance to erythromycin was the most prevalent and followed by fluoroquinolones and tetracycline while among *E. coli* (n=153) resistance to ampicillin was followed by resistance to tetracycline, trimethoprim/sulfometoxazole, and fluoroquinolones. Although the general resistance tendency was similar in both: laboratory and full-scale studies, the resistance rates obtained for the WWTP Gdansk-Wschod were significantly lower. For example, in full-scale resistance to erythromycin among *E. faecium* and *E. faecalis* reached 45% and 64%, respectively, while in continuous-flow laboratory scale model (M-WWTP) up to 75%. In WWTP Gdansk-Wschod lower incidence of clinically-relevant resistance phenotypes was also noted, e.g.: resistance to high-level aminoglycosides reach 5.4% among enterococci while ESBL-producing *E. coli* was detected once, in the aeration chamber.

It is important to note, however, that both studies, carried out on a laboratory and full-scale, demonstrated selection of multi-drug resistant *E. coli* and *Enterococcus* spp. (MDR phenotypes). Additionally, during wastewater treatment processes significant increase in resistance prevalence ($p < 0.05$) was observed in both *E. faecalis* and *E. faecium* resistant to fluoroquinolones. In terms of *E. coli*, the resistance to penicillins, tetracycline fluoroquinolones as well as trimethoprim/sulfamethoxazole was also more prevalent in treated wastewater.

As mentioned above, several factors have been recognized to promote the resistant strains in wastewater processes. In general solids retention time as well as high density of bacterial cells in activated sludge are regarded as the most important. In the presented studies solids retention time in laboratory-scale M-WWTP, approximate three times higher (70 days) comparing to full-scale WWTP (20 days), may explain higher resistance rate obtained in laboratory-scale experiment. Except for retention time, distribution of resistance patterns in activated sludge processes can be enhanced by horizontal genes transfer, due to the high diversity and density of bacteria[9 10]. The diverse mobile genetic elements, such as: plasmids, transposable elements or integron-specific gene cassettes may play an important role in dissemination of resistance patterns by conjugation, transformation or transduction[11].

In my research work, the distribution of sulphonamide resistance genes *sul*1-3 (normally located on mobile genetic elements) was studied among *E. coli*. The molecular analysis showed the prevalence of *sul*2 (81%) and *sul*1 (50%) genes. Five isolates (31%) simultaneously carried both genes. Additionally, the recently discovered gene *sul*3 was also detected in this study, twice (6%) in the WWTP effluent. So far, the incidental presence of this new plasmid-borne sulfonamide resistance gene has been reported for *E. coli* isolated from urinary tract infection[12], from poultry meat[13], and wastewater origin[14 15], but to my knowledge it was first time detected in wastewater in Poland. It is important to note that association of *sul*3 genes with conjugative plasmids could facilitate (and explain) further spread of this gene to bacteria of different environmental compartments.

Recent reports in the literature suggest that resistance genes can be transferred not only among bacteria of the same species but also among unrelated genera[16], thus human-associated bacteria can be regarded as vectors in two-way gene transmission between the pathogenic and environmental populations. Additionally, mobile genetic elements may be important in adaptation of bacterial cells in adverse environmental conditions. Also in wastewater processes, due to presence of antimicrobial agents and their metabolites, even in sub-therapeutic concentration may be beneficial for resistant bacteria survival. Data

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[9] Schwartz et al., 2003 Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol. Ecol. 43(3), 325–335.

[10] Tennstedt et al., 2003 Occurrence of integron-associated resistance gene cassettes located on antibiotic resistance plasmids isolated from a wastewater treatment plant. FEMS Microbiol. Ecol. 45(3), 239–252.

[11] Schlüter et al., 2007 Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS Microbiol. Rev. 31(4), 449–477.

[12] Grape et al., 2003 Sulphonamide resistance gene sul3 found in *Escherichia coli* isolates from human sources. J. Antimicrob. Chemother. 52, 1022–1024.

[13] Soufi et al., 2011. *Escherichia coli* of poultry food origin as reservoir of sulphonamide resistance genes and integrons. Int. J. Food. Microbiol. 144, 497–502.

[14] Perreten et al., 2003. A new sulfonamide resistance gene (*sul*3) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob. Agents Chemother. 47:1169-1172.

[15] Szczepanowski et al., 2009. Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiology. 155, 2306–2319.

[16] Norberg P., Bergström M., Jethava V., Dubhashi D., Hermansson M.. The IncP-1 plasmid backbone adapts to different host bacterial species and evolves through homologous recombination. Nature Communications, 2011; 2: 272

obtained in laboratory and full-scale studies reflected the following: the resistance to antimicrobials observed among *E. coli* and enterococci of clinical origin[17] as well as the current trends in antimicrobial agents' consumption in Poland[18]. As an example: the increase in usage of fluoroquinolones has been followed by increasing resistance of targeted bacteria (including *E. coli* and enterococci) and MDR phenotype involving fluoroquinolones is regarded nowadays as an increasing problem among clinical isolates[19].

Based on the results presented above I have concluded that conventional wastewater processes do not prevent the receiver from dissemination of fecal indicators with clinically relevant resistance patterns. Furthermore, bacteria with resistance patterns were positively selected during the wastewater treatment processes based on activated sludge. Therefore, in the environmental risk assessment the continuous input of resistant bacteria should be monitored.

**The above-discussed results were summarized in the articles listed below:**

- Łuczkiewicz A., Jankowska K., Olańczuk-Neyman K., 2007, Presence and distribution of antibiotic-resistant bacteria in multiphase activated sludge system. Polish Journal of Environmental Studies. nr 2A, pt. 3 suplement 546-551 (Appx. no 5, I.B. item 1),
- Łuczkiewicz A., Fudala-Książek S., Jankowska K., Quant B., Olańczuk-Neyman K., 2010, Diversity of fecal coliforms and their antimicrobial resistance patterns in wastewater treatment model plant. Water Science and Technology, 61 (6),1383-92 (Appx. no 5, I.B. item 2),
- Łuczkiewicz A., Jankowska K., Fudala-Książek S., Olańczuk-Neyman K., 2010, Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. Water Research, 44 (17), 5089-5097 (Appx. no 5, I.B. item 3),
- Łuczkiewicz A., Fudala-Książek S., Jankowska K., Quant B., Olańczuk-Neyman K. 2010. Antimicrobial resistance of *Enterococcus* spp. in municipal wastewater treatment plant – model study, Polish Journal of Environmental Studies, vol. 2, Series of Monographs, HARD, 146-152 (Appx. no 5, I.B. item 4),
- **Łuczkiewicz, A.**, Felis, E., Ziembinska, A., Gnida, A., Kotlarska, E., Olańczuk-Neyman, K., Surmacz-Gorska, J. 2013. Resistance of *Escherichia coli* and *Enterococcus* spp. to

[17] European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2010. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2011.

[18] Elseviers et al., ESAC project group, 2007. Antibiotic use in ambulatory care in Europe: trends, regional differences and seasonal fluctuations. Pharmacoepidemiol. Drug Saf. 16 (1), 115-123.

[19] Lautenbach et al., 2009. Gastrointestinal tract colonization with fluoroquinolone resistant Escherichia coli in hospitalized patients: changes over time in risk factors for resistance. Infect. Control Hosp. Epidemiol. 30(1), 18–24.

selected antimicrobial agents present in municipal wastewater. J Water Health. 2013 11(4):600-12 (Appx. no 5, I.B. item 6)

**The studies were supported by following grants:**

- **N523 077 32/2900**, Polish Ministry of Science and Higher Education pt.: „Zmienność zagrożeń ekotoksykologicznych i mikrobiologicznych towarzysząca biologicznemu oczyszczaniu odcieków ze składowisk odpadów komunalnych", 2007 - 2009 (Appx. no 5, II.J.2 item 4),

- **WFOŚ/D/201/187/2010,** dotacja celowa Wojewódzkiego Funduszu Ochrony Środowiska i Gospodarki Wodnej w Gdańsku pt.: „Wspólne oczyszczanie odcieków ze składowisk odpadów wraz ze ściekami w komunalnych oczyszczalniach ścieków", 2010 – 2011 (Appx. no 4, II.J.2 item 5),

- **N N523 493134**, Polish Ministry of Science and Higher Education pt.: „Antybiotyki w środowisku wodnym a przenoszenie antybiotykooporności przez bakterie osadu czynnego", 2008 - 2011 (Appx. no 5, II.J.2. item 6),

- **N N305 461 139**, Polish Ministry of Science and Higher Education pt.: „Badania lekooporności, wirulencji i zróżnicowania genetycznego bakterii wskaźnikowych w odpływach z oczyszczalni ścieków i ich odbiorniku – morskich wodach przybrzeżnych", 2010-2013 (Appx. no 5, II.J.2 item 7).

**Ad topic no 2.**

***Incidence of antimicrobial resistance among indicator bacteria isolated from direct and indirect wastewater receivers***

Since human-associated bacteria are suspected to be vectors in two-way gene transmission between the pathogenic and environmental populations, the results of antimicrobial resistance obtained for the fecal indicator in laboratory- and full-scale WWTP have prompted me to analyze the incidence of antimicrobial resistance among strains isolated from direct and indirect wastewater receivers[20].

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[20] D'Costa et al., 2006. Sampling the antibiotic resistome. Science. 311:374-377.

Currently in Poland and in other EU countries the quality of WWTP effluents is regulated only in terms of nutrients, organic matter, and suspensions content. Therefore wastewater treatment processes are designed and operated to fulfill those requirements. Other important micropollutants, like human-related microorganisms, are removed only unintentionally.

In advanced, highly effective WWTP the reduction of fecal bacteria reaches up to 99.9%. The effluents, however, occasionally may contain up to 7 $x10^5$ CFU of *E. coli*/100$cm^3$. Therefore, safe wastewater disposal is essential to prevent the environmental degradation, particularly in ecosystems subjected to strong anthropogenic impacts, such as the Gulf of Gdansk and its shallow western part, the Puck Bay. The coastal water of that gulf are recreationally important, however, receives significant pollution load through the numerous local rivers and marine outfalls.

In this study, I isolated *Enterococcus* spp. from treated wastewater of two main local WWTPs: Gdansk-Wschod (n = 82) and Gdynia–Debogorze (n = 73) as well as from their marine outfalls (n = 45, n = 71), which are placed approximately 2.5 km from the shoreline (Fig. 1) and have been operating since 2001 and 2010, respectively. Additionally, I decided to sample treated wastewater (n = 33) and activated sludge (n = 55) of WWTP Gdansk-Wschod as well as the Vistula River mouth (n = 69), due to ecological importance of this river flows for the Gulf of Gdansk area.

According to the results I obtained, *E. faecium* was dominated species cultivated from all sampling points (from 56 to 65% of isolates) except Vistula river mouth, where represented only 38% of isolates. In terms of *E. faecalis*, the presence of the species in treated wastewater was between 23 to 26%, while in costal water (WWTPs' outfalls as well as Vistula river mouth) did not exceed 10%. Interestingly *E. hirae*, rarely detected in treated wastewater (from 8 to 16%), in marine outfalls reach up to 38%, while in Vistula River mouth was predominant (54% of isolates). Occasional occurrence of other enterococci, like *E. durans*, *E. gallinarum/casseliflavus* and *E. avium* was also noted.

As in my previous studies, among enterococci resistance to erythromycin was predominant, however, varied over a broad range depending on sampling point. In general 51% of *E. faecium* and 65% of *E. faecalis* were found to be resistant to this compound. Also a relatively high percentage of isolates resistant to tetracycline (18% and 29%, respectively) and to fluoroquinolones (up to 40%) was detected.

Among resistance phenotypes of clinical relevance, high-level streptomycin resistance (HLSR) was prevalent in enterococci and reached up to 20.7% for *E. faecium* isolates from marine outflow of the Gdansk-Wschod WWTP and up to 33.3% of *E. faecalis* in the Gdynia-Debogorze WWTP effluent. The HLSR pattern was also detected in Vistula river mouth, but interestingly, among enterococci identified as *E. hirae* (3/37).

Species distribution in wastewater and environmental samples is not well understood, and may be affected by many factors including differences in diet, climate, season and methods of detection. In the human intestine, the *E. faecalis* is the most abundant (90%), while in mammals and birds the species composition of enterococci is more diverse. Nevertheless, *E. feacalis* was found to be the most prevalent only in wastewater samples in Sweden[21], while *E. faecium* dominated in Spain, UK, Canada, France, Switzerland[22 23 24] and Poland (based on my studies results). In Portugal and United States E. hirae dominated in wastewater samples[25].

I would like to highlight the fact that in recent years, the relative proportion of *E. faecium* to *E. faecalis* is increasing in enterococcal infection in the US and Europe[26 27]. It is probably caused by spread of a particular hospital-adapted polyclonal high-risk enterococcal complex (HiRECC) of *E. faecium* [28]. An important feature of hospital-associated *E. faecium*, beneficial to survival, is multi-drug resistance (MDR). In terms of HiRECCs release and subsequent survival in the environment, in this study, the clonal structure of *E. faecium* was additionally determined among resistant isolates, using multilocus VNTR analysis (MLVA) and mutlilocus sequence typing (MLST).

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[21] Kühn et al., 2003. Comparison of enterococcal populations in animals, humans, and the environment – a European study. Int J Food Microbiol, 88: 133-145.

[22] Lanthier et al., 2010. Frequency of virulence genes and antibiotic resistances in *Enterococcus* spp. isolates from wastewater and feces of domesticated mammals and birds, and wildlife. Can J Microbiol, 56: 715-729.

[23] Leclercq et al., 2013. Changes in enterococcal populations and related antibiotic resistance along a medical center-wastewater treatment plant-river continuum. Appl Environ Microbiol, 79: 2428-2434.

[24] Thevenon et al., 2012. Characterization of fecal indicator bacteria in sediments cores from the largest freshwater lake of Western Europe (Lake Geneva, Switzerland). Ecotoxicol Environ Safety, 78: 50-56

[25] Bonilla et al., 2006. Species assemblages of *Enterococcus* indicate potential sources of fecal bacteria at a south Florida recreational beach. Mar Pollut Bull, 52: 807-810

[26] Hidron et al., 2008. National Healthcare Safety Network Team; Participating National Healthcare Safety Network Facilities: NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol. 29: 996–1011

[27] De Kraker et al., 2012. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. Clin Microbiol Infect. doi: 10.1111/1469-0691.

[28] Leavis et al., 2006. Identification of high-risk enterococcal clonal complexes: global dispersion and antibiotic resistance. Curr Opin Microbiol, 9:454-460.

According to the obtained results, isolates belonging to the nosocomial HiRECC constituted altogether 24.6% of all *E. faecium* isolates subjected to typing, and were present at all sampling points. These isolates were abundant in resistance determinants, mainly located on mobile genetic elements such as plasmids and in pathogenicity factors (Esp, pili). Moreover, using an additional typing method (MLVA) the isolates with the same characteristics were found in the effluent of WWTPs and their marine outfall. This suggests the ability of such clones to survive in the environment for at least some time.

To my knowledge, in Poland the wastewater was first time recognized as important source of HiRECCs in environments. The presence of HiRECCs in wastewaters and marine/river environment highlights the need for further detailed analyses to better understand the survival and spread of drug-resistant strains in water ecosystems, and to elucidate the ways to limit such a dissemination. In my opinion, data obtained in my study will provide an important contribution to the discussion on the treated wastewater disinfection prior to introduction into aquatic ecosystems.

**The above-discussed results were summarized in the article listed below:**

- Sadowy E. and **Łuczkiewicz A.** Drug-resistant and hospital-associated *Enterococcus faecium* from wastewater, riverine estuary and anthropogenically impacted marine catchment basin. BMC Microbiology, DOI: 10.1186/1471-2180-14-66, IF – 3,10, PM – 30 (Appx. no 5, I.B. item 7).

**The studies were supported by the following grant:**

- **N N305 461 139**, grant of Minister of Science and Higher Education: "Studies of drug resistance, virulence and genetic diversity indicator bacteria in drains, sewage treatment plant and the receiver - marine coastal waters" 2010-2013 (Appx. no 5, II.J.2 item 7)
- **N N305 461 139**, grant of Minister of Science and Higher Education: „Dug resistance, virulence and genetic diversity of indcator bacteria wastewater and wastewater impacted marine waters", 2010-2013 (Appx. no 5, II.J.2 item 7)

**Ad topic no 3.**

**Effectiveness of wastewater disinfection (ozonation, UV irradiation and micro/ultrafiltration) in removal of indicator bacteria with antimicrobial-resistance patterns (pilot-scale study).**

According to the results discussed above, it seems plausible that hospital resistant clones accumulate several determinants that may promote their survival not only in the hospital settings but also in the environment. Most of mobile genetic elements together with antimicrobial resistance can gain resistance to disinfectants and ultraviolet light as well as ability to form biofilms and additional metabolic pathways. To prevent the wastewater receivers from the dissemination of resistant strains, the disinfection of final effluent seems to be the most efficient method.

Thus, in the next study I analyzed the potential of physical (UV light and filtration) as well as chemical (ozonation) methods due to their effectiveness in removing fecal bacteria with antimicrobial resistance patterns. The experimental trials were carried out again in pilot-scale at the Gdansk-Wschod and Gdynia-Debogorze WWTP. The pilot UV system consisted of irradiation chamber of useful volume 2.34 L equipped with a low pressure UV lamp LBX3 (Wedeco) with nominal output of 33W at 254 nm. Flow rate was maintained in the range from 0.6 to 4.7 $m^3/h$, while UV dosages ranged from 3.2 to 110 $mJ/cm^2$. In case of ozonation, the unit was equipped with oxygen generator (OCS Modular 4HC, Wedeco), producing from 0.4 to 4.0 g $O_3$ per h. Ozone concentration in the wastewater ranged from 0.5 to 11 mg $O_3$/ $dm^3$. In membrane pilot station, the main component was a membrane with the total surface equal 0.8 $m^2$, and molecular weight cut off 200 kD (classified to both micro- and ultrafiltration range). The unit operated in a dead-end or cross-flow mode. Three disinfection units were fed with treated wastewater using pressured polyethylene pipe. The disinfection was applied to reach the level below 1,000 fecal coliform in 100 mL of disinfected wastewater, according to WHO standards set on wastewater reused in irrigation of crops, sports fields, and public parks[29].

The number of fecal coliforms and fecal enterococci detected in the treated wastewater before disinfection reached up to 3.5 × 104 CFU/100 mL and up to 1.8 × 104 CFU/100 mL,

[29] World Health Organization 1989. Health Guidelines for the use of Wastewater in Agriculture and Aquaculture: Report of a WHO Scientific Group. WHO Technical Report Series 778. World Health Organization, Geneva, Switzerland.

respectively. According to the obtained results, micro/ultrafiltration caused stable reduction of fecal bacteria (above 99%) in all wastewater samples, while in case of ozonation and UV irradiation, the dosages required to obtain a sufficient bacterial reduction depended on wastewater composition and experimental conditions. To obtain a sufficient bacterial reduction (1,000 fecal coliform in 100 mL) recommended dosage should be above 4 mg $O_3$/L concerning ozonation and from 10 to 20 mJ/$cm^2$ for UV irradiation.

In the survey, I focused on disinfection as a potential factor for the selection of antimicrobial resistance. According to the obtained results, applied UV irradiation and micro/ultrafiltration caused evident selection of isolates with MAR patterns among both tested fecal indicators. Additionally UV irradiation increased the antimicrobial resistance rate among fluoroquinolones-resistant *E. coli* and enterococcus as well as among *E. coli* showing resistance patters against tetracycline and trimethoprim/sulfamethoxazole. In case of micro/ultra filtration, the positive selection was also observed, but mainly among *E. coli*. It is suggested that the presence of antimicrobial agents can increase cell-wall elasticity enabling gram-negative bacteria to penetrate through the pores[30 31]. The protective effect against UV irradiation, is probably connected with the presence of plasmids that together with antimicrobial resistance also confers partial tolerance to UV light [32]. The signification of this phenomenon needs, however, further study.

**The above-discussed results were summarized in the article listed below:**

- **Łuczkiewicz A.**, Jankowska K, Bray R., Kulbat E., Quant B., Sokołowska A., Olańczuk-Neyman K. 2011, Antimicrobial resistance of fecal indicators in disinfected wastewater. Water Science and Technology, 64 (12), 2352-61 (Appx. no 5, I.B. item 5)

**The studies were supported by the following grants:**

- **EEA Grants E007/P01/2007/01/85** „New methods of emission reduction of selected pollutants and application of by- products from sewage treatment plants" Task 2 „Disinfection of treated wastewater discharged to the receiving waters" (Appx. no 5, II.J.2 item 1)

[30] Lorian, 1975. Some effects of subinhibitory concentrations of antibiotics on bacteria. Bull. N. Y. Acad. Med. 51 (9):1046-1055.

[31] Lebleu et al., 2009. Role of the cell-wall structure in the retention of bacteria by microfiltration membranes. J. Membr. Sci 326 (1), 178-185.

[32] Drabblwe & Stockerb, 1968. R (transmissible drug resistance) factors in *Salmonella typhimurium*: pattern of transduction by phage P22 and ultraviolet-protection effect. J. Gen. Microbiol. 53, 109-123

- **WFOŚ/D/201/185/2007** Regional Fund for Environmental Protection and Water Management in Gdansk „New methods of emission reduction of selected pollutants and application of by- products from sewage treatment plants" (Appx. no 5, II.J.2 item 2)

**Conclusions**

The results of the research work confirm the hypothesis that bacteria with clinically important resistance phenotypes are isolated from wastewater (raw, treated and subjected to disinfection) as well as in wastewater impacted ecosystem.

The resistance rates obtained for *E. coli* and enterococci of wastewater and environmental origin reflect both: the antibiograms of clinical isolates as well as the current trends in antimicrobial agents' consumption in Poland.

It was confirmed that wastewater treatment processes cause a positive selection of indicator bacteria exhibiting resistance to antimicrobial agents and that activated sludge age is an important factor of this process.

The association of resistance genes with mobile genetic elements as plasmids could facilitate (and explain) further gene dissemination to bacteria of different environmental compartments.

The obtained results are of high epidemiological importance and contribute to a better understanding of the potential human impact on the quality of water resources. They provide an important contribution to the discussion on the treated wastewater disinfection prior to introduction into aquatic ecosystems.

**According to the best of my knowledge in this study first time in Poland:**

- species identification of *Enterococcus* spp. present in wastewater and wastewater receivers was undertaken,
- presence of clinically-relevant antimicrobial phenotypes among fecal indicators, including *E. faecium* linked to high-risk enterococcal clonal complexes (HiRECC) was confirmed in both: in wastewater and wastewater impacted environment,
- an new plasmid-borne sulfonamide resistance gene called *sul*3 was found in *E. coli* isolated from wastewater treatment plant effluent.

Taking into account the above conclusions the results of my study significantly contribute to the multi-drug resistance monitoring program, which according to the requirements of the European Commission considers the environmental sector.

Lack of monitoring data was recognized as the most important gap in the full implementation of the programs and directives concerning sustainable management of the man-made and natural environments. Thus, the sanitary and epidemiological aspects of the undertaken research are of interest to many international institutions: the European Commission, the World Health Organization, the European Parliament and the Centers for Diseases Control and Prevention.

I strongly believe that data obtained in my research have contributed in raising awareness on the occurrence and spread of the antimicrobial resistance in wastewater processes.

## 5. OTHER SCIENTIFIC ACHIVEMENTS

### *5a) Before obtaining the Ph.D. degree*

Over the period 1992 to 1997 I was a student at Faculty of Hydrotechnology (now Faculty of Civil and Environmental Engineering) at Gdansk University of Technology (GUT) in the field of Water Management. During my studies I was a member of the student scientific club and conducted two research fellowships. In 1996, after the fourth year of my study, I participated in AIESTE (International Association for the Exchange of Students for Technical Experience) students exchange program and spent two months training at the Technische Universität Hamburg - Harburg, Germany. In the Department of Bioprozess und Bioverfahrenstechnik under the supervision of Professor Herbert Märkl I conducted research on possibility of thermophilic bacteria (mainly of the genus *Bacillus thermoleovorans*) to degrade lipids and certain enzymes. I published the obtained results in the proceedings of First Academic Seminar of Student Scientific Clubs 'Ecology - Construction – Technology' 6-7 December 1996, GUT, Gdansk. In the following year in July within the CEEPUS Program I spent one month on traineeship at the University of Zagreb in the Faculty of

Geotechnics. In the same year, under the supervision of prof. Krystyna Olańczuk - Neyman I prepared and defended my master thesis entitled "Evaluation of microbial hazards at water treatment plants". The results of the thesis I presented and published in the proceedings of Second Academic Seminar of Student Scientific Clubs 'Technical Aspects of Environmental Protection' 16-18 November 1997 GUT, Gdansk as well as in proceedings of the Symposium held on the occasion of the 15th anniversary of the Department of Water and Wastewater Technology, GUT, Gdansk. During the studies I also completed a two-year Pedagogical Course at GUT to get fully-qualified teaching skills and a training course in "Monitoring of water and air," organized by the Department of Chemistry, GUT.

In October 1997 as an assistant I joined the Department of Water and Wastewater Technology at the Faculty of Environmental Engineering, GUT, and continued my education on doctoral studies at GUT. Since 1997 to 2003 I was involved in several research activities connected with manganese content in the groundwater of an infiltration intake in the vicinity of a dammed river as well as with estimation of pollutant loads discharged to surface waters in the municipality of Gdańsk. Obtained results were referred and published in conferences proceedings (Appx. no 5, II.L.1 item 3-4), and presented as reports (Appx. no 5, II.L.1 item 4-7).

In my scientific work also important was the cooperation with Laboratoire d'étude des Transferts en Hydrologie et Environnement (LTHE) at the University Joseph Fourier in Grenoble. During two of my research internships I worked on the Biodegradation of organic pollutants, mainly 2,4-dichlorophenol (2,4-D), in contaminated soil (in 1999). Microbial reduction of toxic selenium oxyanions by *Ralstonia metallidurans* (in 2000) (Appx. no 5, III.L.1 item 6-7). This study, implemented on a laboratory scale, were supervised by prof. Jean-Paul Gaudet and carried out in cooperation with the DBMS/CB-CNRS, Centre d'études Nucléaires de Grenoble, a leading research institute in France.

In 2001 I received a positive opinion and my PhD grant No. 7 T09D 044 21 that was founded by Committee for Scientific Research (Appx. no 5, II.J.1 item 1). Additionally, my research was co-financed under the funds of the Voivodeship Fund for Environmental Protection and Water Management in Gdańsk WFOŚ/D/201/103/2001 (2001-2002) (Appx. no 5, II.J.1 item 1). I completed my doctoral studies in 2003 under supervision of dr hab. Bernard Quant prof. PG and successfully defended my doctoral desartation entitled: Soil and water contamination as a result of sewage sludge application. My dissertation received an award from Gdańsk Scientific Society for outstanding scientific achievements of young researchers in the field of technical sciences (Appx. no 5, II.K.1 item 1-2).

In the period 1997 - 2003 I participated in three courses on: modeling of wastewater treatment processes, microbiological and parasitological analysis of wastewater as well as migration of contaminants in porous media (Appx. no 5, III.L.1 item 5, 8-9).

From 2000 to 2003 I was also employed as an assistant environmental engineer in EcoTech Sp. z oo. This company is dedicated to many fields of public- and private-sector, such as water and sanitation, wastewater treatment, waste disposal, integrated permissions and environment protection programs. I was the author and co-author of the environmental impact reports (Appx. no 5, III.M.1 item 1-3) conceptions, projects and technical documentations for the planned water and wastewater systems (Appx. no 5, II.B.1 item 1, 4, 6-13) as well as industrial and urban wastewater treatment plants (Appx. no 5, II.B.1 item 2-3, 5, 14). The supervision of the construction and start-up of these installations has allowed me to gain the practical experience, so important for technical sciences, in both teaching and research areas.

To conclude, until 2003 the results of my research were published in chapter in a monograph (Appx. no 5, II.E.1 item 1), three full-text articles in the international conference proceedings (Appx. no 5, II.L.1 item 3-5) and three full-text articles in the national conference proceedings (Appx. no 5, II.L.1 item 1-2, 6). I was the main contractor of two grants (Appx. no 5, II.J.1 item 1-2), co-author of 14 projects and technical documentations in the field of sanitary engineering (Appx. no 5, II.B.1 item 1-14) as well as the co-author of three expertise and four reports (Appx. no 5, III.M.1 item 1-7).

### *5b) After obtaining the Ph.D. degree*

After completing my Ph.D., I continued my work as an adiunkt at the Department of Water and Wastewater Technology Department of Environmental Engineering, GUT.

Together with research related to my scientific achievements I was working on additional three main topics:

1. Prevalence of drug-resistant bacteria in anthropogenically impact aquatic ecosystems

2. Analysis of the taxonomic composition of bacteria of the genus Pseudomonas in municipal wastewater
3. The structure and stability of activated sludge bacterial community.

**Ad topic no 1.**

***Prevalence of drug-resistant bacteria in anthropogenically impact aquatic ecosystems***

Within this topic my interests were particularly focused on issues related to the occurrence of antimicrobial resistance patterns among fecal indicators (*Escherichia coli* and *Enterococcus* spp.) as well as *Pseudomonas* spp. of surface water origin. Thus the research can be divided into three main streams:

a) antimicrobial resistance, genomic diversity and phylogenetic affiliation of *Escherichia coli*
b) species distribution and antimicrobial resistance of *Enterococcus* spp.
c) species distribution and antimicrobial resistance of *Pseudomonas* spp.

Those research tasks were carried out under the grant entitled: The occurrence of drug-resistant bacteria in aquatic ecosystems subjected to anthropogenic pressure (N305 085 32/2959) (Appx. no 5, II.J.2 item 3). In the years 2007 - 2009, I isolated bacterial strains from representative points located on the Oliwski Stream and on the Reda river, two watercourses that significantly influence the microbiological quality of Gulf of Gdansk (Fig. 2). In the analyzed catchments the bacterial contaminations are suspected to be connected with the wild-life and livestock, in forestry and agriculture areas as well as with leaking septic tanks in the dispersed buildings regions with no central canalization system (points: O1, O2, R1, R2 and R5). In the remained sampling sites (points: O3, O4, O5; R3, R4), situated through the urbanized area fecal contaminants are discharged with the run off and with the cultivation of vegetables and fruits in the allotments. Not known wastewater treatment plant effluents are discharged to the studied watercourses.

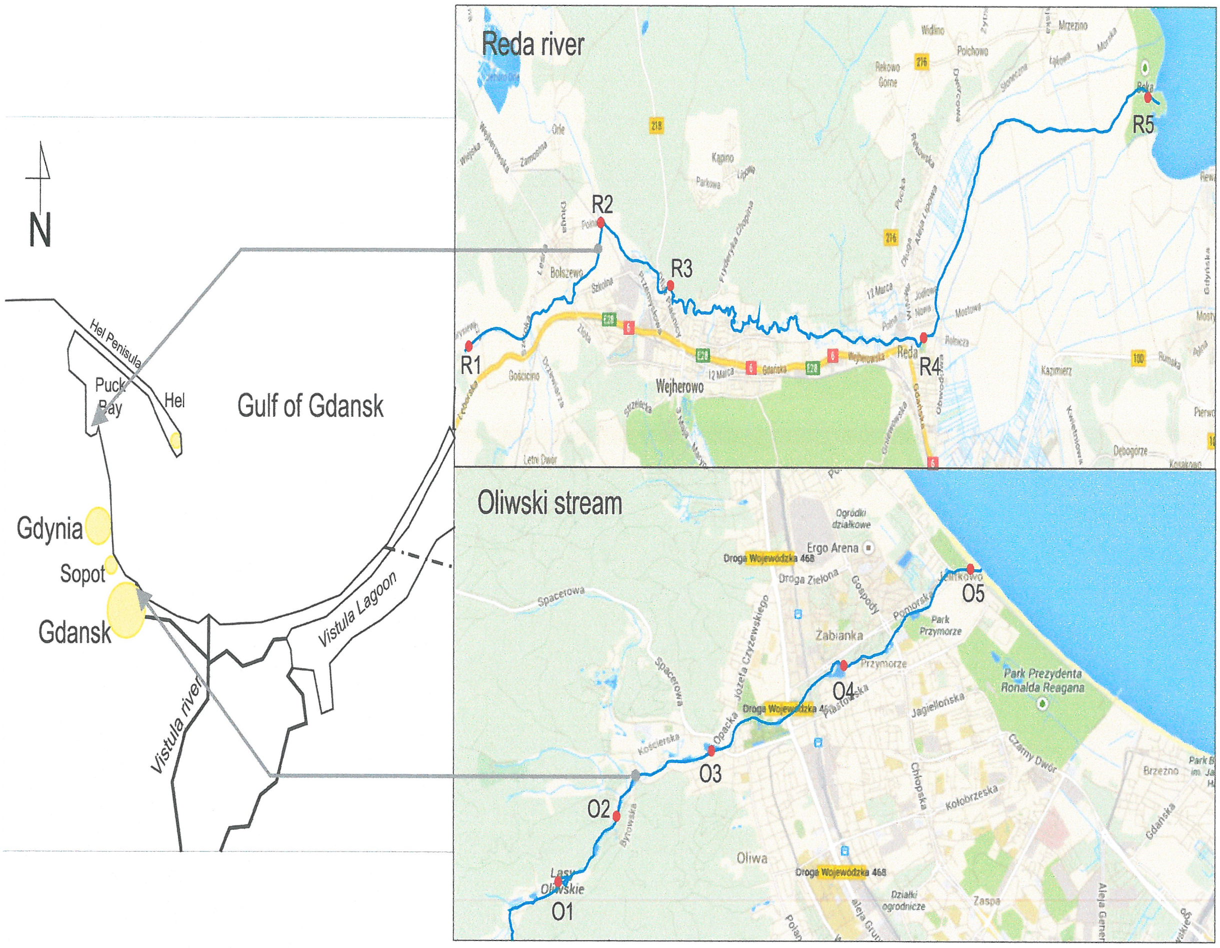


Fig. 2. Sampling points located on the Oliwski Stream (O1-5) and the Reda river (R1-5).

The physical, chemical and bacteriological quality of the analyzed watercourses was tested and compared with the legal requirements specified in the EU Bathing Water Directive 2006/7/EC (Appx. no 5, II.E.2 item 15). Simultaneously from the studied sampling points all together 222 strains of *E. coli*, 306 of *Enterococcus* spp. and 93 of *Pseudomonas* spp. were isolated.

Susceptibility testing conducted among *E. coli* of riverine origin showed that resistance to β-lactamases (ampicillin - 21%, piperacillin – 14%) was the most common and followed by resistance to tetracycline (16%); similar resistance tendency was observed among *E. coli* obtained from wastewater, what was discussed in scientific achievement). The multi-drug resistance phenotype (MDR) were observed among 9% of *E. coli* isolates (13 strains from the Oliwski Stream and 7 from the Reda river) (Appx. no 5, II.A.2 item 4).

In the present study basic statistical methods were used to answer if antimicrobial resistance patterns are independent on the location of sampling point. To estimate the trend of antimicrobial resistance Chi square independence test and linear regression were used. According to the obtained data in the Oliwski Stream the ratio of *E. coli* with resistance patterns to all tested *E. coli* isolates against the monitoring points suggested positive correlation downstream. In case of Reda river similar tendency was observed in upper course – area with no central canalization system (Appx. no 5, II.F.2 item 8).

Since the result of antimicrobial susceptibility of *E. coli* showed resistance to ampicillin (AM) and tetracycline (TE) as the most frequent phenotype thus the occurrence and molecular diversity of tetracycline and ampicillin resistance genes was also determined in this study (in cooperation with the Department of Genetics and Marine Biotechnology Institute of Oceanology Polish Academy of Sciences). To identify the resistance determinants the primers specific for the *tet*A-C genes (3 primer pairs in total) and β-lactamase genes ($bla_{TEM}$, $bla_{OXA\text{-}B}$ and $bla_{SHV}$, 3 pairs in total) were used. The role of plasmids in the horizontal gene transfer of these resistance determinants was also attempted. Plasmid DNA was extracted from the bacteria using alkaline lysis method and the material was used for transformation of *Escherichia coli* DH5α cells. In the PCR assays 23 *E. coli* isolates were used (15 were TE-resistant and 20 were AM-resistant, some strains showed double resistance). $Tet^R$ genes were detected with predominance of *tet*B 47%, followed by *tet*A (13%). The amplification of *tet*C gene was not observed. Among tested β-lactamase genes only $bla_{TEM}$ was detected. Transformation of the *E. coli* DH5α recipient cells with plasmid DNA extracted from surface water isolates resulted in the transfer of resistance from 75% of AM-resistant and 20% of TE-resistant *E. coli*. This indicates that *E. coli* may be an important environmental source for horizontal transfer of resistance genes (Appx. no 5, II.F.2 item 7).

Together with resistance determinants the phylogenetic relationship of *E. coli* isolates was estimated by employing a BOX-PCR fingerprinting method. These studies were conducted in collaboration with the Department of Molecular Biology, Faculty of Biological Sciences, University of Zielona Gora. Among *E. coli* isolated from the Oliwski Stream phylogenetic analyses have indicated predominance of group A (A-90%, B1-10%), whereas the isolates from the Reda River represented all phylogenetic groups (A-40%, B1-20%, B2-20%, D-20%). It was also observed that isolates from phylogenetic group B2, frequently including extra-intestinal *E. coli* with virulence determinants, were less resistant to the tested antimicrobial agents than isolates from other groups. Thus this work, demonstrated that phylogenetic structure of riverine isolates may be influenced by catchments characteristic (Appx. no 5, II.F.2 item 9).

In this study the species distribution and antimicrobial resistance of *Enterococcus* spp. was tested. Among riverine enterococci, the identification data showed the predominance of *E. faecium* (68.6%) and *E. faecalis* (21.6%), with the remaining strains belonging to *E. casseliflavus/gallinarum* (5.2%), *E. hirae* (3.9%), and *E. durans* (0.7%). Resistance to erythromycin was common in enterococci (up to 66%). The isolates exhibited also resistance to fluoroquinolones (up to 28%) and to tetracycline (up to 26%). In this study resistance related to high-level aminoglycosides (HLAR) was reported for 5% of *E. faecium* (n=7) and for 11% of *E. faecalis* (n=5). Of analyzed *Enterococcus* strains, 73% (n=147) showed resistance patterns at least to one, while 9% (n=18) were regarded as multi-drug resistant (MDR) strains. MDR phenotypes were noted only among *E. faecium* (21%) and *E. faecalis* (6%) strains and detected in urbanized watershed of the Oliwski Stream (O4 and O5) and in upper course of the Reda river (R1-R3) (Appx. no 5, II.A.2 item 3; II.F.2 item 8).

Together with fecal indicators I analyzed the taxonomic composition and drug resistance of bacteria belonging to *Pseudomonas* spp.. My special attention was given to *P. aeruginosa*, that is commonly found in an aquatic environment and on the other hand is also an important etiologic factor in the bloodstream and respiratory tract infections. In studied watercourses, I occasionally noted *P. aeruginosa* (1%), while *P. putida* (65 %) was the most abundant and followed by *P. fluorescens* (16%), *P. oryzihabitans* (3%) and *P. mendocina* (1%). Out all tested *Pseudomonas*, applied biochemical methods failed to determine the species classification for 14%. *P. aeruginosa* was detected only in the Oliwski Stream estuary. Interestingly, in this sampling point, *P. aeruginosa* and other isolates identified as *P. putida* and *P. fluorescens* showed higher resistance rate than in other sampling points. Additionally their resistance patterns included third generation cephalosporins (cefotaxime and ceftazidime) and fluoroquinolones (ciprofloxacin and levofloxacin). Obtained results reveled that there was either periodical or continuous, illegal wastewater discharge in this sampling point. This is particularly important because the Oliwski Stream discharge influences significantly on coastal water use for recreational purposes. Although it should be underline that no multi-drug resistant (MDR) or extended spectrum β-lactamase (ESBL) producing isolates were detected neither in Oliwski Stream nor Reda river (Appx. no 5, II.F.2 item 2).

**Ad topic no 2.**

***Analysis of the taxonomic composition of bacteria of the genus Pseudomonas in municipal wastewater***

In the years 2007 - 2011 I was involved in the project "Antibiotics in the aquatic environment and the transfer of antibiotic resistance by activated sludge bacteria " (2008-2011, N N523 493134), conducted in collaboration with Environmental Biotechnology Department Faculty of Power and Environmental Engineering, Silesian University of Technology (Appx. no 5, II.J.2 item 6). My contribution to this grant (in addition to the work reported as my scientific achievement: AI, items 4, 8 - 10) was to determine the impact of wastewater treatment on species distribution among Pseudomonas spp. The experiments were carried out in wastewater treatment plant Gdansk-Wschod. The obtained results allowed to state, that in the wastewater, taxonomic composition of *Pseudomonas* spp. differed significantly from that observed in the watercourses (Oliwski Stream and Reda river). However in both cases, the dominant species was *P. putida* (53.5%), in the wastewater up to 21.7% of the isolates were identified as *P. aeruginosa*, and only 5.7% as *P. fluorescens*. The remain isolates, for which species identification was obtained, belonged to *P. pseudoalcaligenes* and *P. veronii*. In terms of the antibiotic resistance isolates of *Pseudomonas* (n = 125 ) were mainly resistant to betalactams. Importantly, among tested isolates resistance to cefepime (fourth-generation cephalosporin used only in the clinic) was also detected. Additionally *Pseudomonas* spp. isolated from activated sludge showed, comparing to raw and treated wastewater, statistically significant ($p <0.05$) increase of resistance to almost all of the tested antimicrobial agents. I hypothesized that *Pseudomonas* spp., able to carry out denitrification can also survive longer in activated sludge than, for example pathogenic or indicator bacteria. Density of bacteria in activated sludge may promote transfer and acquisition of resistance properties from other bacteria (by conjugation, transformation, or transduction). Additionally *Pseudomonas* spp. are longer exposed to antibiotics present in wastewater. Such prolonged exposure gives a selective advantage to drug resistant bacterial cells and on the other hand, may lead to stability of acquired resistance (Appx. no 5, II.F.2 item 4). Above hypothesis requires further study.

**Ad topic no 3.**

***The structure and stability of activated sludge bacterial community***

In addition to the sanitary aspects of environmental engineering as a environmnetal engineer, I have been interested in microbial community involved in wastewater treatment. Within the framework of the Innovative Economy Operational Programme 2007–2013, project no. UDA-POIG.01.03.01–22-140/09-04 (financially supported by European Regional Development Fund) I analyzed the structure and stability of activated sludge bacteria participating in denitrification supplemented with external carbon source: conventional (ethanol) and unconventional (fusel oils) (Appx. no 5, II.J.2 item 10). In this research study three methodological approaches were applied: a qualitative method (PCR-DGGE), quantitative method (FISH) and the analysis of the expression of *nir*K and *nir*S, genes that are involved in the denitrification (Real-Time PCR). Experiments were carried out in a laboratory-, pilot – and technical scale. The results indicate that, fusel oil like ethanol, do not constitute a strong selective agent to activated sludge bacteria. Increasing doses of fusel oils, however, influenced the structure of activated sludge flocs, contributing to flocs dispersion, but denitrification efficiency increased. Among the denitrifying bacteria, regardless of the type of external carbon source, representatives of α, γ-proteobacteria were predominat (especially *Acidovrax*, *Curvibacter*, *Azoarcus* and *Thauera*). *Acidobacteria*, *Verrucomicrobia* and *Firmicutes* were also identified. Between tested systems the similar numbars of clones belonging to the different phylogenetic groups were detected. The only difference was the increasing number of clones related to the genus *Acidovorax* detected in activated sludge fed with fusel oil. The results of expression of functional gene *nir*K and *nir*S have shown a significant number of copies of a nitrite reductase type transcripts *nir*S, while expression of *nir*K was low. The importance of undertaken research has been distinquist by TECHNICON-INNOVATION exhibition of Industrial Technology, Science and Innovation Fair in Gdansk

The results were presented at scientific conferences (Appx. no 5, II.L.2 item 6, III.B2 item 17, 29). An article “Acclimation of denitrifying activated sludge to a single vs. complex external carbon source during a start-up of sequencing batch reactors treating ammonium-rich anaerobic sludge digester liquors” (BIOD-D-13-00386) is under review. In the preparation is also a monograph on the subject.

From a technological point of view another interesting project I participated in was entitled "Ecotoxicological and microbiological hazards accompanying biological treatment of leachate from municipal landfills", (2007-2009) and implemented in collaboration with Environmental Biotechnology Department, Faculty of Power and Environmental Engineering, Silesian University of Technology in Gliwice. In this project I was particularly involved in the assessment of microbiological hazards associated with the treatment of leachate from municipal landfills as well as the co-treatmet of municipal wastewater with landfill leachate addition. In the research municipal wastewater was derived from wastewater treatment plant: "Gdańsk-Wschód", while the landfill leachates were obtained from landfills: 'Szadółki Landfill' in Gdansk ("old" leachates) and "Eco-Valley" in Łężyce near Gdynia ("young" leachates).

The laboratory-scale model known as A2O ( anaerobic - anoxic - oxic) was used in the experiment. Initially the A2O was inoculated with nitrifying activated sludge and during the start-up period was fed only with the raw municipal wastewater. Then during co-treatment period the mixture of raw municipal wastewater with increasing quantity (from 1 to 10% vol.) of landfill leachate was used.

Obtained results indicated that leachates did not substantially affect that efficiency. The removal of tested bacteria was high throughout the experiment: 99.9 % for fecal indicator (E. coli and *Enterococcuss* spp) and up to 99.8 % for saprophytic and mesophilic. Due to the potential toxicity of the leachates, in this project I additionally determined the metabolic activity of bacterial community using Live/Dead BacLight test. Analysis of number of bacterial cells with active membrane (MEM +) showed that increasing addition of tested leachates, however was noticeable, but did not influenced significantly bacterial activity. The results obtained during this study have been presented at scientific conferences (Appx. no 5, II.L.2 item 3, 5; III.B.2 item 2, 3, 11, 18, 24, 28).

Currently I have been continuing the cooperation with both the landfill "Eco-Valley " in Łężyce k/Gdyni and ZU "Szadółki" in Gdansk. It involved: monitoring of landfill leachate quality and advisory activities. The result of this cooperation, in addition to scientific papers (Appx. no 5, II.A.2 item 5) and reports (Appx. no 5, III.M.2 item 1-4) is also implementation (Appx. no 5, II.B.2 item 1).

In summary: after obtaining the Ph.D. degree (2004 – 2014), beyond the scientific achievement, I published in total 20 manuscripts, including five articles highlighted by the Journal Citation Reports (JRC) database (Appx. no 5, II.A.2 item 1-5), seven not distinguished by the JCR database (Appx. no 5, II.E.2 item 2-4, 6, 11, 12,15) and eight articles constituting chapters of monographs of Polish Academy of Science (Appx. no 5, II.E.2 item 1, 5, 7-10, 13-14).

In the years 2004 - 2014 I participated in six KBN research projects: two as manager (List.., II.J.2 item 3, 7) and four (Appx. no 5, II.J.2 item 4, 6-9) as a performer. Moreover, I participated in project financed by the EEA Grants (Appx. no 5, II.J.2 item 1) and Innovative Economy Operational Programme (Appx. no 5, II.J.2 item 10). Two above projects also received funding from the National Fund for Environmental Protection and Water Management (Appx. no 5, III.J.2 item 2, 5).

For industrial body I executed four expert opinions (Appx. no 5, III.M.2 item 1-4) as well as I am co-author of implementation (Appx. no 5, II.B.2 item 1).

Since 2007, I reviewed 17 publications in international journals highlighted by the Journal Citation Reports (JRC) database: Chemotherapy, Journal of Environmental Management, Ecotoxicology and Environmental Safety, Environmental Science and Pollution Research, Science of the Total Environment, Waste Management Research, Water Research, Water Science and Technology (Appx. no 5, III.P.2 item 1-17).

In 2004 and 2011, I received the third degree team scientific awards of the Rector of the Gdańsk University of Technology (Appx. no 5, II.K.2 item 1-2). In 2011 I also received a scholarships from the Funds of the Rector of the Gdansk University of Technology (Appx. no 5, III.K.2 item 4).

I participated in a number research internships (Appx. no 5, III.L.2 item 3, 4, 6) and specialized courses, especially connected with molecular techniques (Appx. no 5, III.L.2 item 5-7). I also delivered seven lectures papers (Appx. no 5, II.L.2 item 1-7) and presented 35 posters at international and national thematic conferences (Appx. no 5, III.B.2 item 1-35).

## 6. Summary of scientific achievements

The total impact factor of published papers is equal IF = 18,655 (scoring of the Ministry of Science and Higher Education PM = 187,25) (Appx. no 5, II.G).

After obtaining doctoral degree the impact factor of published papers equals above total impact factor IF = 18,655, but scoring of the Ministry of Science and Higher Education equals PM = 185,25.

The impact factor of scientific achievement equals IF= 12,249 (scoring of the Ministry of Science and Higher Education PM = 101,5) (Appx. no 5, II.G).

Number of citations by the Web of Science database is 42 (Appx. no 5, II.H).

Hirsch index published by the Web of Science database is h = 4 (Appx. no 5, II.I).

## 7. Teaching activities

Since 1997 I lectured in the framework of full-time and part-time studies, at bachelor and master degrees at the Faculty of Civil and Environmental Engineering. The scope of subjects have included: Chemistry, Environmental Biology, Water and Wastewater Technology, Environmental Law. Particularly important in my lecturer activity was formation of a new English Full-Time Msc. Studies in Environmental Engineering at Faculty of Civil and Environmental Engineering. Within these courses I have been responsible for three subjects: Environmental Impact Assessment, Environmental Microbiology and Environmental Chemistry (Appx. no 5, III.J.1 item 1, and III.J.2 item 1).

Within the framework of Erasmus Teaching Staff Mobility I also conducted series of lectures: in 2007 at Universidade de Beria Interior, Covilha, Portugal, in 2009 at Aalborg University, Denmark, in 2010 at Universidade Technica de Lisboa, Portugal (Appx. no 5, III.L.2 item 3, 4, 5). I also delivered lectures and presentations during meetings at the Polish Society for Microbiologist, the Baltic Festival of Science and during the Open Days at Gdansk University of Technology (Appx. no 5, III.I.2 item 1-4).

Since 2008, I supervised 4 and reviewed 10 engineer thesis as well as supervised 11 and reviewed 8 master thesis (Appx. no 5, III.J.2 item. 2, 3). Thanks to the cooperation with Instituto Superior Técnico, Universidade Técnica de Lisboa, Portugal three of thesis were conducted there and Mrs. Dorothy Cimochowska received a double degree (IST-UTL defense - in November 2011, the defense at GUT - March 2012).

I also try to actively cooperate with various student organizations. Since July 2013 I have been supporting and overseeing the academic supervisor the scientific circle "Microbiology in Environmental Engineering" at Faculty of Civil and Environmental Engineering (Appx. no 5, III.J item 4). Additionally, I also have been cooperating with the different student organizations which offer students exchange, like: Erasmus Student Network (ESN) and the International Association for the Exchange of Students for Technical Experience (IAESTE) (Appx. no 5, III.J item 5) .

**8. Organizational activities**

Since November 2004 till now, I have been the Dean's Representative for International Cooperation at the Faculty of Civil and Environmental Engineering, Technical University of Gdansk (Appx. no 5, III.Q.2 item 1).

In the years 2004 - 2006 I participated in the work of the Commission for University Education at the Faculty of Civil and Environmental Engineering (Appx. no 5, III.Q.2 item 2), then in years 2010 – 2012 I was a member of the Faculty Recruitment Committee (Appx. no 5, III.Q.2 item 2).

As part of the research projects I contributed to the development of laboratories by purchasing necessary equipment for specialized research as well as for routine laboratory work (Appx. no 5, III.Q.2 item 4). Currently, these items are used by students and Ph.D. students.

In 2010, I was a member of the Organizing Committee of the VI National Conference Hydromicrobial Conference "Micro-organisms in the environment - from Ecology to Technology" under the honorary auspicies of prof. Henryk Krawczyk, Rector of Gdansk University of Technology and prof. Bernard Lammek, Rector of the Gdańsk University (Appx. no, III.C.2 item 1). My organizational activity was awarded in 2011 by second degree team award of the Rector of the Gdansk University of Technology (Appx. no 5, II.K.2 item 3).

Aneta Łuczkiewicz