Monika Harnisz , PhD **Appendix 3**

SUMMARY

of Scientific, Research, Organizational and Academic Accomplishments

Olsztyn, 2015

1. Name

Monika Diana Harnisz

2. Held diplomas, degrees / art - with the name, location and the year of their acquisition

1998 M.Sc. – at Faculty of Environmental Protection and Fisheries Academy of Agriculture and Technology, Olsztyn, Poland, title: „Chemistry of modern bottom sediments of meromictic Zapadłe Lake" Promoter: professor assistant Czesław Mientki

2002 Ph.D. in agriculture science – at the Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, Poland, title: „Microorganisms during cage culture of European catfish (*Siluris glanis* L.) in cooling water*",* Promoter: professor Izabella Zmysłowska

3. Information on previous employment in scientific/artistic

1998 – 2002 Ph.D. study at University of Warmia and Mazury – Ph.D. student

2002–2004 Department of Environmental Microbiology, University of Warmia and Mazury in Olsztyn – Assistant,

since 2004 Department of Environmental Microbiology, University of Warmia and Mazury in Olsztyn – Assistant Professor.

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

a) the title of the scientific achievement:

Treated municipal wastewater and production waters from fish farms as a vector of antimicrobial resistance

b) Publications included in the scientific achievements:

1) **Harnisz M.,** Tucholski S., 2010. Microbial quality of common carp and pikeperch fingerlings cultured in a pond fed with treated wastewater. Ecological Engineering 36 (4): 466-470. **IF_{2014}=3,041; MNiSW=35 pkt.**

   I contributed to this paper by developing the concept of the research, conducting the experiment as well as performing the analysis and presentation of the results. I estimate my contribution at 90%.

2) **Harnisz M.,** Gołaś I., Pietruk M., 2011. Tetracycline-resistant bacteria as indicators of antimicrobial resistance in protected waters - The example of the Drwęca River Nature Reserve (Poland). Ecological Indicators 11: 663-668. **IF_{2014}=3,230; MNiSW=35 pkt.**

   I contributed to this paper by developing the concept of the research, conducting the experiment as well as performing the analysis and presentation of the results. I estimate my contribution at 90%.

3) **Harnisz M.,** 2013. Total resistance of native bacteria as an indicator of changes in the water environment. Environmental Pollution 174: 85–92. **IF_{2014}=3,902; MNiSW=40 pkt.**

   I contributed to this paper by developing the concept of the research, conducting the experiment as well as performing the analysis and presentation of the results. I estimate my contribution at 100%.

4) **Harnisz M.,** Korzeniewska E., Ciesielski S., Gołaś I., 2015. *tet* genes as indicators of changes in the water environment: relationships between culture-dependent and culture-independent approaches. Science of the Total Environment 505: **704-711. IF_{2014}=3,163; MNiSW=35 pkt.**

I contributed to this paper by developing the concept of the research, conducting the experiment as well as performing the analysis and presentation of the results. I estimate my contribution at 70%.

5) **Harnisz M.,** Korzeniewska E., Gołaś I., 2015. Impact of freshwater fish farm on the community of tetracycline resistant bacteria and structure of tetracycline resistance genes in river water. Chemosphere, 128: 134-141. **IF_{2014}=3,499; MNiSW=35 pkt.**

I contributed to this paper by developing the concept of the research, conducting the experiment as well as performing the analysis and presentation of the results. I estimate my contribution at 90%.

** Statements contributors work together with determination of the individual contribution of each of them in the creation of individual manuscript can be found in Appendix 7*

c) description of research objective and results of the above study/studies with an indication of practical applications

Surface water is one of the key elements of the natural environment which must be protected against the negative effects of human activity. Main sources of micro-pollutants penetrating into surface water are wastewater treatment plants and centres for farming and breeding livestock.

Municipal wastewater and wastewater from livestock breeding facilities may contain residues of antibiotics due to their wide use both in human and veterinary treatment. This occurs because antibiotics are only partially metabolized in the body, and therefore are excreted with faeces, e.g. to wastewater[1,2,3] or post-production water from fish farms[4,5,6], as parent drugs or their metabolites. These soluble components are only slightly removed in the wastewater treatment process, and they can penetrate into surface water being a collector for treated wastewater. Their concentration in water is usually low, but in the longer term, even subliminal doses of drugs can cause increased resistance in microorganisms that inhabit these ecosystems. Moreover, antibiotics released to the environment may change the structure of bacterial communities.

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[1] Hirsch R, Ternes T, Haberer K, Kratz KL, 1999. Sci Total Env 225: 109-118.
[2] Brown KD, Kulis J, Thomson B, Chapman,TH, Mawhinney DB, 2006. Sci Total Env 366: 772-783.
[3] Gulkowska A, Leung HW, So MK, Taniyasu S, Yamashita N, Yeunq LWY, Richardson BJ, Lei AP, Giesy JP, Lam PKS, 2008. Water Res 42: 395-403.
[4] Zou S, Xu W, Zhang, R, Tang J, Chen Y, Zhang G, 2011. Environ Pollut 159: 2913-2920.
[5] Buschmann AH, Tomova A, Lopez A, Maldonado MA, Henriquez LA, Ivanova L, Moy F, Godfrey HP, Cabello, FC, 2012. Plos One 7.
[6] Gao P, Mao D, Luo Y, Wang L, Xu B, Xu L, 2012. Water Res 46: 2355-2364.

Apart from antibiotics, antibiotic resistance genes (ARG) and drug resistant bacteria (antibiotic resistant bacteria – ARB)[7,8,9] can also be classified as dangerous micropollutants which penetrate into the environment together with treated wastewater and wastewater from fish farms. In anthropogenic ecosystems, the occurrence of microorganisms resistant to almost all drugs has been reported[9]. It creates a potential risk for human and animal health, because ARG and ARB introduced into the environment may re-enter humans and animals.

According to the World Health Organization (WHO) and its World Health Report, microbial drug resistance constitutes a global public health threat. The World Health Assembly, during its 58th session in Geneva, held on 16-25 May 2005, adopted a Resolution[10] concerning the problem of antibiotic resistance. It proposes a quantitative and qualitative monitoring of antibiotic consumption patterns, as well as of propagation of antibiotic-resistant microorganisms in different ecosystems.

Therefore, research studies on the determination of whether treated municipal wastewater and production water from fish farms are carriers of drug-resistant bacteria have been undertaken. The study also comprised the impact of the discharge of treated municipal wastewater and production water on antibiotic resistance of bacteria inhabiting rivers which serve as their collectors.

The first thematic area (A), concerning issues related to drug resistance of bacteria in treated wastewater, included studies conducted in the Olsztyn Wastewater Treatment Plant "Łyna". The activity of the treatment plants is based on multiphase activated sludge with nitrification and simultaneous denitrification, along with an anaerobic chamber for dephosphatation and a chamber for predenitrification of returned sludge. A collector of treated wastewater is the Łyna River. The treatment plant highly reduces organic pollution, and its activity enables limiting the amount of nutrients discharged into the Łyna River. According to the author's own research (unpublished data), this treatment plant is also characterized by a very high (99.7%) removal rate of bacteria. However, despite this high efficiency, during one day, 10^{14} coliform bacteria and faecal streptococci and 10^{12} faecal coliform bacteria (MPN)

[7] Kuemmerer K, 2004. J Antimicrob Chemoth 54: 311-320.
[8] LaPara TM, Burch TR, McNamara PJ, Ta, DT, Yan M, Eichmiller JJ, 2011. Environ Sci Technol 45: 9543–9549.
[9] Pruden A, Pei R, Storteboom H, Carlson KH, 2006. Environ Sci Technol 40: 7445-7450.
[10] World Health Organization 2005. Fifty-Eighth World Health Assembly, Geneva, 16-25 May 2005, Resolutions and Decisions Annex, Geneva 2005.

are released from the treatment plant. In this study, the author attempted to demonstrate that microorganisms occurring in treated wastewater also include many drug-resistant bacteria.

The second research area (B) concerning the occurrence of drug-resistance bacteria in post-production water from fish farms comprised:

1. A pond located within the wastewater treatment plant in Olsztynek. Wastewater treatment in this treatment plant is performed using the activated sludge method with enhanced capacity for removing phosphorus and nitrogen in CBR-FOS reactors. A batch reactor with five cycles is used. By adjusting the length of individual phases and technological parameters, it is possible to achieve high stability of the process and increase its efficiency. Treated wastewater is discharged into the Jemiołówka River.

The investigated pond was filled with water coming from the sources and periodically fed with treated wastewater comprising a mixture of domestic and municipal wastewater and wastewater from the fruit and vegetable processing industry. In June 2007, the pond was stocked with juvenile carps and zanders. No additional fish feeding or pharmaceuticals were applied.

2. Fish farms located on the Drwęca River being a part of the Natura 2000 network. The main objective of the network is to preserve certain types of natural habitats and species that are considered rare and endangered across Europe, as well as the protection of biodiversity. Fish breeding takes place in ponds supplied with water from the Drwęca River, and post-production water is discharged back into the river. In the years 2006-2007, the study was conducted in three farms where no treatment of post-production water was performed. In the years 2010-2011, one of three of the above farms, which was characterized as the main environmentally troublesome source of micropollutants, was selected for further investigation. To prevent the river from contamination, a post-production water sedimentation tank was applied within the farm as a method for post-production water treatment.

A)

Treated wastestewater as a reservoir of resistant bacteria.

Antibiotic resistance can be divided into natural, or constant and encoded in the bacterial genome for a very long time, and acquired, or appearing under the influence of changes in the genome. An example of the former can be ampicillin resistance of *Aeromonas hydrophila*, which is used for its culture on selective media. Examples of acquired resistance include methicillin-resistant *Staphylococcus aureus* (MRSA) or increasingly occurring isolates of *Klebsiella pneumoniae* producing carbepenemases (KPC).

The study on the occurrence of drug resistance in treated wastewater, and water in the collector conducted by the author, was based on functional indicators, or non-uniform groups of bacteria, which are all insensitive to particular antibiotic activity. Such groups may comprise bacteria characterized by both natural and acquired resistance: pathogens, potential pathogens and saprophytes.

In order to study the count of drug-resistant bacteria, the author selected microorganisms insensitive to the activity of eight antibiotics belonging to four classes of antibiotics (tetracyclines: oxytetracycline OTC and doxycycline DOX, β-lactams: amoxicillin AMO and cefuroxime sodium CEF, fluoroquinolones: norfloxacin NOR and enrofloxacin ENR, macrolides: roxithromycin ROX and erythromycin ERY). The selected drugs are widely used in Poland and throughout the world, both in human and veterinary medicine[11].

The study confirmed that treated wastewater constitutes a reservoir of drug-resistant bacteria, because the highest concentrations of the investigated bacterial groups were found in the samples of treated wastewater, while the lowest were in the water of the Łyna River collected before the discharge of wastewater. The count of individual investigated groups of microorganisms was associated with the spectrum of antibiotics and their generation. The most numerous were bacteria resistant to macrolides, antibiotics that have been widely used for 40 years, especially active against bacteria other than *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Treated wastewater containing significant amounts of enterobacteria may be characterized by a high percentage of bacteria resistant to macrolides. In groups of bacteria resistant to β-lactame and tetracycline activity, much greater counts of bacteria resistant to older-generation antibiotics, namely amoxicillin and oxytetracycline, were observed.

Among all studies of groups of drug-resistant microorganisms, only a group of tetracycline-resistant bacteria met the conditions established for a functional bioindicator. Their share in the total count of bacteria was ten times higher in treated wastewater samples and about seven times higher at the site of discharge of pollutants into the Łyna River in

[11] www.esac.ua.ac.be www.antybiotyki.edu.pl www.wetgiw.gov.pl

comparison to the control station. Accordingly, tetracycline-resistant isolates (252 OTC^R isolates and 163 DOX^R isolates) were qualified for a detailed study on bacterial resistance to drugs.

In this group, 218 OTC^R isolates and 105 DOX^R isolates were identified using sequencing of the 16S rRNA gene. In treated wastewater, a clear dominance of microorganisms of the *Aeromoas* sp. and *Acinetobacter* sp. was found for the first bacterial group, while the second bacterial group was dominated by *E. coli*, *Shewanella putrefaciens* and *Providencia* sp. This shows that their only source is treated wastewater. Other genera of bacteria occurred both in the treated wastewater samples and in river water collected before and after the discharge of treated municipal wastewater.

It was found that tetracycline-resistant isolates from treated wastewater are characterized by higher minimal inhibitory concentrations (MIC) of doxycycline, as compared to isolates from the Łyna River. Antibiotic resistance is determined by the occurrence of resistance genes located in the bacterial chromosome or mobile elements such as plasmids or transposons[12,13]. Resistance to tetracyclines is determines by the presence of *tet* genes in the bacterial genome[14]. In OTC^R isolates, a dominance of *tet(A)*, *tet(O)* and *tet(L)* genes was found. Doxycycline resistance was determined primarily by *tet(B)* and *tet(L)*. The presence of the *tet(B)* gene was observed in all doxycycline resistant *Escherichia coli* isolated from treated wastewater. According to the studies of other authors[15,16], *tet(B)* is often associated with a particularly virulent enterohemorrhagic O157:H7 strain of *E. coli* and is responsible for its resistance to tigecycline. This antibiotic is a new generation drug from tetracycline group and a very promising therapeutic agent. In this study, more than 50% of isolates of *E. coli* were resistant to tigecycline, which confirms that treated wastewater is also a source of clinical isolates resistant to tetracyclines.

Wastewater treatment plants, and especially aeration tanks and trickling filters, are a particularly predisposed environment for the transfer of genes, including resistance genes. The exchange of genetic information between bacteria in these environments is often a result of nutrients availability, suitable temperature for the growth of bacteria, very high density of

[12] Marti E, Jofre J, Balcazar LJ, 2013. Plos One 8.
[13] Mokracka J, Koczura R, Kaznowski A, 2012. Water Res 46: 3353–3363
[14] Chopra I, Roberts M., 2001. Microbiol Mol Biol R 65: 232-260
[15] Wilkerson C, Samadpour M, van Kirk N, Roberts MC., 2004. Antimicrob Agents Ch 48: 1066-1067.
[16] Tuckman M, Petersen PJ, Howe AYM, Orlowski M, Mullen S, Chan K, *et al,*. 2007. Antimicrob Agents Ch 51: 3205-3211.

bacterial cells, the presence of both donor and recipient cell and the presence of selective pressure[17].

Tetracycline resistance genes are very often located on mobile elements, so that they can easily change the host. The conducted conjugation experiment demonstrated a possibility to transfer almost all of the investigates *tet* genes (*(A), (B), (C), (D), (E), (K), (L), (M), (O), (S), (Q)* and *(X)*) under laboratory conditions. At the same time, it means that a transfer of drug resistance genes may probably also occur in the environments characterized by favourable conditions for the exchange of genes between bacteria.

Thanks to the application of a very modern diagnostic tool, which is a real-time PCR (quantitative PCR), it was proven that treated wastewater is a source of tetracycline resistance genes and that they affect the number of genes associated with drug insensitivity in treated wastewater collectors. Relative concentrations (normalized according to the number of copies of 16S rRNA gene) of *tet* genes (*tet(A), tet(B), tet(M), tet(O), tet(Q)* and *tet(X)*) were the highest in treated wastewater samples, as compared to water samples from the Łyna River collected before and after the discharge of treated wastewater. Statistical analysis confirmed the existence of significant differences between relative concentrations of these genes in water samples collected from the Łyna River before the discharge of wastewater and treated wastewater and between samples of treated wastewater and samples of water collected from the Łyna River after the discharge of wastewater. Only in the case of *tet(B)* concentration were there statistically significant differences between samples of water collected from the Łyna River before wastewater discharge and samples of water collected from the Łyna River after wastewater discharge, which indicates that these genes are introduced into collector water mainly along with treated wastewater.

The phenomenon of antibiotic resistance is closely related to the occurrence of antibiotic selective pressure, which makes microorganisms acquire new features allowing them to survive in a changed environment[18,19,20]. In the study, thanks to determination of a concentration of tetracycline group antibiotics (tetracycline, oxytetracycline and doxycycline) in samples of treated wastewater and water from the Łyna River, a selective pressure of

[17] Seveno NA, Kallifidas D, Smalla K, van Elsas JD, Collard JM, Karagouni AD, Wellington EM, 2002. Rev Med Microbiol 13, 1-13

[18] Aminov RI, Mackie RI., 2007. FEMS Microbiol Lett 271: 147-161.

[19] Baran W, Adamek E, Ziemiańska J, Sobczak A., 2011. J Hazard Mater 196: 1-15.

[20] Tello A, Austin B, Telfer TC., 2012. Environ Health Persp 120: 1100-1106.

doxycycline on communities of bacteria isolated from treated wastewater and collector water was demonstrated. Moreover, a statistically significant positive correlation between doxycycline concentration and the *tet(B)* gene concentration was found. Such correlations were not found in the case of older generation drugs (tetracycline and oxytetracyline), which probably results from the fact that resistance to these drugs has spread among bacteria due to their long-term use.

The study confirmed that despite the fact that wastewater treatment plants are characterized by a high efficiency in pollutants removal, a large pool of drug-resistant bacteria and drug resistance genes is introduced into collector water along with treated wastewater. The study helped to identify a group of tetracycline-resistant bacteria, as the one which is particularly associated with anthropopressure, and the direct influence of treated wastewater on the bacterial pattern in the collector.

The above-mentioned results I presented in the following articles:

- Harnisz M., 2013. Total Resistance of Native Bacteria As an Indicator of Changes in the Water Environment. Environmental Pollution 174: 85–92 IF_{2014}=3,902; MNiSW=40 pkt.

- Harnisz M., Korzeniewska E., Ciesielski S., Gołaś I., 2014. *tet* Genes as Indicators of Changes in the Water Environment: Relationships Between Culture-Dependent and Culture-Independent Approaches. Science of Total Environment 505: 704-711, IF_{2014}=3,163; MNiSW=35 pkt.

Studies to these articles were made as part of a research project NCN No. N N305 164339 "Tetracycline resistant bacteria as indicators of drug resistance in surface waters receiving treated municipal wastewater and discharges from fish farms", in which I was the project manager.

B)

Fish farming water as a carrier for drug resistance

B1)

Nowadays, environmental engineering aims to create low-cost and user-friendly forms of wastewater treatment. Issues of aquaculture and wastewater treatment seem to be unrelated, but the use of wastewater to fertilize production water for aquaculture is very popular all over the world. Thanks to this engineering solution, it is possible to create a stable ecosystem, which has a positive impact on local economic development[21] and reduces the amount of pollutants which are released to collector water.

In fish ponds, various types of wastewater are employed, including raw sewage stored in containers suspended inside ponds[22], raw sewage after sedimentation[23], sewage treated with the activated sludge method[24] and contaminated river water[25]. The main aim of this practice is to fertilize the ponds and to enhance their natural, primary and secondary productivity.

The microbiological quality of water in ponds fertilized with wastewater is very important as it reflects microorganisms inhabiting fish[26]. It is believed that the total number of heterotrophic bacteria exceeding 10^4 CFU/ml creates a risk for fish to be penetrated by human pathogens[27]. To determine the quality of such water, standard bacterial indicators such as coliforms and faecal streptococci are typically used[28]. According to the guidelines of the World Health Organization[29], the count of coliform bacteria in fish ponds should not exceed 10^3 CFU per 100 ml.

The contamination of fish farming water with wastewater affects the development of multiple drug-resistant bacilli of the *Aeromonas*[30] genus, considered as potential pathogens of

[21] Yan J, Wang R, Wang M., 1998. Ecol Eng 10: 191–208.
[22] Edwards P., 2005. Urban Aquaculture, CABI Publishing 45–59.
[23] Olah J, Sharangi N, Datta NC., 1986. Aquaculture 54: 129–134.
[24] Tucholski S., 1994. Fish Rearing in Ponds Fed with Treated Wastewater. Wydawnictwo IRŚ, Olsztyn.
[25] Liang Y, Cheung RYH, Everitt S, Wong MH, 1999. Water Res 33: 2099–2109.
[26] Guzmán MC, Bistoni M, Tamagnini LM, González RD., 2004. Water Res 38: 2368–2374.
[27] Mara D., Cairncross S., 1989. Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture: Measures for Public Health Protection. World Health Organization, Geneva
[28] Molleda P., Blanco I., Ansola G., de Luis E., 2008. Ecol. Eng 33: 252–257.
[29] World Health Organization (WHO), 2006. Wastewater and Excreta Use in Aquaculture. Geneva, Switzerland
[30] Thayumanavan T, Vivekanandhan G, Savithamani K, Subashkumar R, Lakshmanaperumalsamy P, 2003. FEMS Immunol Med Microbiol 36: 41–45

fish. These bacteria are known for fast acquisition of new drug resistance features[31], which makes them a suitable tool to study the spread of this phenomenon.

As a part of the study on fish and water from a fish pond supplied with biologically treated wastewater from the water treatment plant in Olsztynek, 92 strains of *Aeromonas hydrophila* were isolated from the selective medium. Species of these strains were determined based on API NE biochemical tests available from BioMérieux. These isolates were subjected to multiple drug resistance investigation, including antibiotics of the following classes: fluoroquinolones: enrofloxacin and flumechina, aminoglycosides: neomycin, macrolides: erythromycin, β-lactams: amoxicillin and tetracyclines: tetracycline.

Isolates of *Aeromonas hydrophila* were found to be the least sensitive to amoxicillin (100% of insensitive strains). This antibiotic belongs to the class of β-lactam drugs, similarly to ampicillin. These bacteria are naturally resistant to ampicillin, which may cause their high resistance to amoxicillin. Approximately 20% of *A. hydrophila* strains were resistant to enrofloxacin, while less than 15% of isolates were resistant to erythromycin, neomycin and tetracycline.

Based on the conducted investigations, it can be stated that fertilization of the fish pond with biologically treated wastewater did not affect the development of multiple drug resistance of the bacterium *Aeromonas hydrophila* or sanitary and bacteriological condition of the water and fish inhabiting the studied pond. This is probably caused by the fact that the supply of treated wastewater to the pond occurred only twice in the fish breeding season, and the pond was supplied with water from underground sources, typically characterized by very good microbiological quality.

The above-mentioned results I presented in the article:

- Harnisz M., Tucholski S., 2010. Microbial Quality of Common Carp and Pikeperch Fingerlings Cultured in a Pond Fed with Treated Wastewater. Ecological Engineering 36 (4):466-470. IF_{2014}=3,041; MNiSW=35 pkt.

The research for this article were made as part of a research project of the Ministry of Science and Higher Education, "The impact of power biologically purified sewage ponds on the environment and the production of stocking material" No. N N305 2777 33, in which I was a main contractor.

[31] Schmidt AS, Bruun MS, Dalsgaard I, Larsen JL., 2001. Appl Environ Microbiol 67: 5675-5682.

B2)

In Polish aquaculture, fresh water is largely used for production of carp (900 fish farms) and rainbow trout (190 fish farms)[32]. From the point of view of environmental protection, aquaculture facilities can be considered a source of pollution only when the substances introduced into and produced in these facilities penetrate into the environment. Despite the fact that the concentration of pollutants in post-production water from fish farms is low, the water discharged from such farms should be treated[32].

However, in practice, the use of intense treatment methods is often economically unjustified. The most common method for treatment of post-production water from fish farms is the use of sedimentation tanks with a depth not exceeding 1 metre and a retention time of at least 30 minutes, which enables a reduction of total suspended solid by 45% and BOD_5 by 19%[33]. By the end of the first decade of the 21st century, most of the fish breeding centres in Poland did not use any method of post-production water treatment.

The significant increase in global aquaculture production in the last decades caused an intensification of the use of antibiotics to treat bacterial infections[34]. Nearly 80% of antibiotics used in aquacultures are released into the aquatic environment[35]. Their concentration in water can be so high that it exerts selective pressure on bacteria by the complete or partial inhibition of the growth of native bacteria sensitive to antibiotics.

In areas of aquaculture facilities, elevated counts of bacteria resistant to antibiotics and genes related to drug resistance are very often reported. Knowledge on the sources and mechanisms of the spreading of drug resistance is essential, as it can result in the development of effective strategies to control this phenomenon and assess the related risks for human health.

Investigations of post-production water as a drug resistance carrier were conducted in trout farms located on the Drwęca River, and they covered two periods: the years 2006-2007, when the studied fish farms did not use any wastewater treatment method, and the years 2010-2011,

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[32] Teodorowicz M, 2013. Arch Pol Fish 21:65-111.
[33] Goryczko K, 2008. Pstrągi. Chów i hodowla. Poradnik hodowcy. Wydawnictwo IRS Olsztyn.
[34] Boxall ABA, 2010. Veterinary medicines and the environment. Handbook of experimental pharmacology, 291-314.
[35] Le TX, Munekage Y, 2004. Mar Pollut Bull 49: 922-929.

when post-production water sedimentation tanks were used as a method of wastewater treatment.

In the studies on drug resistance performed in the years 2006-2007, tetracycline-resistant bacteria were used as functional bioindicators, similarly to the studies on treated wastewater. The study covered two groups of microorganisms with different temperature preferences: bacteria growing at 14 and 28°C (Tet^R 14°C and Tet^R 28°C). The first group was selected based on the mountain nature of the upper Drwęca, which results in the domination of psychrophilic bacteria among the native river microbiota. The second group of bacteria was selected due to the fact that potentially pathogenic bacteria and strict pathogens are typically mesophiles.

During the study, it was found that post-production waters from fish farms were the source of drug-resistant bacteria. Higher counts of bacteria belonging to both investigated groups, in comparison to control stations, occurred in post-production water from all three tested breeding centres. Moreover, it has been shown that post-production water is the main source of tetracycline-resistant mesophiles, as confirmed by statistical analysis. The penetration of large amounts of drug-resistant mesophilic bacteria together with post-production water into the river may change the structure of its bacterial communities, as well as their functions.

The risk posed by the discharge of post-production water was also confirmed by sequencing of 16S rRNA genes from Tet^R 28°C isolates. This group was dominated by *Pseudomonas fluorescens* and *Aeromonas hydrophila* bacteria. Both of these species are potential pathogens of fish, and at this point, it should be mentioned that the Drwęca River is a part of the Natura 2000 network, which aims to protect, among others, rare species of fish inhabiting the river. Tet^R 28°C isolates were also characterized by high minimal concentrations of tetracycline needed to inhibit their growth (50% of the strains with MIC > 256 μg/ml). Moreover, a very high multiple drug resistance of Tet^R 28°C isolates was found: 100% of the isolated strains were resistant to ampicillin and amoxicillin (β-lactams), 94% to enrofloxacin (fluoroquinolones) and erythromycin (macrolides) and 83% to sulfamethoxazole with trimethoprim.

A very high share of tetracycline-resistant bacteria in the total count of bacteria determined in post-production water from fish farms, their fast response to changing environmental conditions expressed by high values of MIC and multiple drug resistance confirm the practical application of Tet^R bacteria as bioindicators of drug resistance changes.

Studies on post-production water was continued in the years 2010-2011 in a fish breeding facility, where in 2007, two sedimentation tanks for post-production water with an area of 1500 m^2 and volume of 1350 m^3 were built. The water retention time in the sedimentation tanks exceeded one hour at an average water flow of 0.19 m^3/s.

The study was based on bacteria resistant to oxytetracycline (OTC^R) and doxytetracycline (DOX^R) belonging to the tetracycline antibiotics.

No significant differences in OTC^R and DOX^R count in post-production water before and after its treatment were found. It should be added that the count of both investigated bacteria groups was also similar for the sites localized in the Drwęca River before discharge and after discharge of treated post-production water.

The frequent occurrence of *Aeromonas* sp., *Acinetobacter* sp. and *Pseudomonas* sp. was determined among 113 OX^R strains and 44 DOX^R strains using a method of 16S rRNA gene sequencing. The results of bacteria identification coincide with the results obtained in previous studies, which indicates the dominance of γ-Proteobacteria among breeding bacteria inhabiting freshwater.

No significant differences in the levels of MIC values for oxytetracycline and doxytetracycline and multiple drug resistance of isolates between different test sites were reported.

The occurrence of *tet* genes in two studied bacterial populations was similar. Both in the OTC^R and DOX^R group, a dominance of *tet (A, E, L, O)* genes was reported. In the presented study, the only recorded result of the impact of fish farming on bacterial community was a wider variety of *tet* genes determined in production water as compared to the waters of the Drwęca River. However, no statistically significant differences in *tet* gene diversity between treated and untreated production water were found.

The conjugation experiment showed that *tet (A, E, K, L, M, O)* genes from *Acinetobacter* sp. and *Aeromonas* sp. can be transferred into *E. coli* J53 (Rif^R), which suggests their location on the plasmids. The frequency of conjugation equalled from 1.0×10^{-6} to 3.5×10^{-5} per donor strain, which signifies a strong possibility of horizontal transfer of these genes.

The studies on the drug resistance of bacteria in post-production water from fish farms are a pioneer study, as for the first time in Poland and in the world, the method of quantitative PCR was used for investigations conducted in inland farms. For quantitative studies *tet(A), tet(C), tet(O)* and *tet(L)* genes were selected. The relative concentration of *tet* genes (normalized according to the number of copies of the 16S rRNA gene) was increased in post-production water before and after treatment, as well as in river water after the discharge of treated post-production water, as compared to the control station, but statistical analysis did not confirm these quantitative differences. The sedimentation tank, used as a method for post-production water treatment, had no effect on reduction of the relative number of *tet* genes; their concentrations were similar in water both before and after treatment.

Moreover, no statistically significant differences in the concentration of tetracycline antibiotics (tetracycline, oxytetracycline and doxycycline) between the investigated groups were reported.

It can be concluded that the use of sedimentation tanks as a method of post-production water treatment did not produce the expected results in the removal of pollutants. Considering the fact that tetracycline resistance genes can be transferred between unrelated bacterial species, which was confirmed by the conjugation experiment, actions aimed at increasing the efficiency of post-production water treatment for pollutant removal should be undertaken.

The above-mentioned results I presented in the article:

- Harnisz M., Gołaś I., Pietruk M., 2011. Tetracycline-resistant bacteria as indicators of antimicrobial resistance in protected waters—The example of the Drwęca River Nature Reserve (Poland). Ecological Indicators 11: 663-668. IF_{2014}=3,230; MNiSW=35 pkt.

- Harnisz M., Korzeniewska E., Gołaś I., 2014. Impact of Freshwater Fish Farm on the Community of Tetracycline Resistant Bacteria and Structure of Tetracycline Resistance Genes in River Water. Chemosphere 128: 134-141. IF_{2014}=3,499; MNiSW=35 pkt.

Research into these articles have been made in research projects:

• "The use of classical and molecular research methods to assess the impact of anthropogenic factors on the microbiological quality of water of the upper part of the river Drwęca" No. N305 110 313 667, in which I was the main contractor

• "Tetracycline resistant bacteria as indicators of drug resistance in surface waters receiving treated municipal wastewater and runoff from fish farms " No. N N305 164339, in which I was the project manager.

It can be concluded that all the above experiments comprise the issues related to environmental engineering, chemistry, microbiology and ecotoxicology, which makes them interdisciplinary. The obtained results contribute to a better understanding of the ways of spreading drug-resistant bacteria and resistance genes. This knowledge can be used to develop effective strategies to control the drug resistance phenomenon and to assess the risks for human health. Moreover, the obtained information is in line with global trends of drug resistance monitoring designated by the World Health Organization and the European Commission.

Main achievements and conclusions from the research:

• Treated municipal wastewater are vector of residues of antibiotics, drug-resistant bacteria and genomic determinants of resistance to antibiotics.

• The presence of antibiotics in the environment exerts selective pressure on the bacteria, causing changes in their biodiversity, but it applies only to new generation antibiotics.

• Tetracycline-resistant bacteria are sensitive functional bioindicator which reactive quantitatively and qualitatively to changes in the environment.

• The treated municipal wastewater was dominated by doxycycline-resistant *Escherichia coli* with the determinant *tet (B)* and a very high resistance to tigecycline, which may be derived from hospital sources.

• Quantitative PCR confirmed that the treated wastewater are a source of resistance genes, especially gene *tet (B).*

• Under the influence of the discharge of treated sewage into the waters of the receiver followed the changes in the population of native bacteria.

• Production water from fish farms, are also a vector of drug resistance, but less than the treated wastewater, due to the high dilution of pollutants.

• The outflows from fish farms was dominated by antibiotic-resistant bacteria of the genera *Acinetobacter, Aeromonas* and *Pseudomonas*, which are potential pathogens of fish.

• Using claryfying ponds as a method of post-production water treatment did not produce expected results in the removal of the ARB and ARGs.

• To the best knowledge of the author, the quantitative PCR were used for the first time in Poland and in the world to the study of bacterial drug resistance in inland farms.

5. Other scientific accomplishments

I graduated the study in 1998 at the Faculty of Environmental Sciences and Fisheries, University of Agriculture - Technology in Olsztyn with the title of Master of Science in the field of environmental protection. Master's thesis have been performed at the Department of Environmental Engineering, supervised by prof. Czeslaw Mientki.

In the same year (1998) I began my Ph.D. studies at the University of Warmia and Mazury in Olsztyn. My promoter was prof. Izabella Zmysłowska. My thesis focused on the issues related to microbiological contamination of the environment due to human impact. I conducted research related primarily to assess the health of fish and microbiological status of the water in which they were fattening. The theme of my scientific interest was also the existence and role of microorganisms associated with intensive fish farming. The results of this work were used for the preparation of the doctoral thesis entitled: "Microorganisms during cage culture of European catfish (*Siluris glanis* L.) in the cooling-waters", defended in November 2002, at the Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn. The results indicated that the intensive farming of fish in the cooling waters did not significantly affect the sanitary-bacteriological quality of receiver. I found, however, that increasing density of fish in the cages was followed by an increase in the number of microbiota inhabiting the fish, which could lead to an increase in the incidence of disease. Furthermore, I noted that the quality of bacteria colonizing the fish, water and the fish feed were similar to each other. The results of study from this period I have been published in a number of articles (II.E.1, II.E.2, II.E.3, II.E.4, II.E.5, II.E.6, II.E .7, II.E.8, II.E.9, II.E.10, II.E.11). These observations and lessons learned indicated the desirability of further work on the changes in the anthropogenically altered environment (II.E.12, II.E.13, II.E.14, II.E.15, II.E.16). The subject of these works was also distributed at national and international scientific conferences (III.B.1, III.B.2, III.B.3, III.B.4, III.B.5, III.B.6, III .B.7, III.B.8, III.B.9, III.B.10, III.B.11, III.B.12, III.B.13, III.B.15).

Increasing knowledge of the changes in the human altered environment allowed for the preparation and implementation in 2006-2009 and 2007-2010 two research projects (projects: II.J.2, II.J.3). The aim of both of these projects was to assess the impact of fisheries on the state of the aquatic environment. Participation in them resulted in further scientific articles. It has been shown that the presence of the total number of psychro- and mesophilic bacteria, the nitrogen cycle bacteria and potentially pathogenic bacteria was closely associated with the intensification of fisheries management and physical-chemical parameters of water (II.A.2, II.A.3, II.E.18). These results were also presented in the form of reports at national and international scientific conferences (III.B.18, III.B.20, III.B.21, III.B.24, III.B.27).

At the same time I developed interest in the use of molecular biology methods in environmental research, which resulted in scientific articles (II.A.4, II.A.5, II.E.19) and speak at conferences (III.B.14, III. B.26).

Among the wide range of issues related to environmental microbiology I gave particular attention on drug-resistant bacteria. Research on this subject has focused initially on bacteria susceptible to antibiotics isolated from fish farms. The result of this work was the publication and a series of reports on the national and international scientific conferences (II.A.1, III.B.17, III.B.19, III.B.23, III.B.31). In subsequent years, the research also embraced the bacteria from sewage treatment plants resistant to β – lactams and tetracyclines, the most popular antibiotics in Poland. To address this issue meant that appeared more scientific work (II.A.6, II.A.7, II.A.8) and reports on national and international scientific conferences (III.B.22, III.B.25, III.B.28 , III.B.30).

In the course of my scientific work I published 13 works distinguished by the Journal Citation Reports database. I am also co-author of 19 publications published in other peer-reviewed journals and the distinguished (12 works) on the B list of MNiSW. I am also the author of 34 reports and papers presented at conferences and symposia, including 5 international. I participated and realized a total of 3 research projects (II.J.1, II.J.2, II.J.3), including one project that I was the project manager, and another two in which I was principal contractor. I made the 25 reviews of scientific publications (III.P.1, III.P.2, III.P.3, III.P.4, III.P.5, III.P.6), for such journals as Science of the Total Environment; World Journal of Microbiology and Biotechnology; CLEAN - Soil, Air, Water; Water Research and Aquaculture Research and one scientific review of the project proposed for the National Science Centre (III.O.1).

For achievements in the scientific field I received in 2005 Award from the Rector of the University of Warmia and Mazury and , in years 2006 and 2007, scholarships for special scientific activity (II.K.1, II.K.2, II.K.3). In 2014, I started working with the Netherlands Institute of Ecology. This cooperation concerns the study of changes in the biodiversity of bacteria involved in the turnover of methane, depending on the type of use of raised bogs.

Summary of quantitative and points of the most important achievements of scientific research

Specification				**Before PhD**	**After PhD**	**Total**
Originally published scientific creative work available in the social circuit, monographs and book publications (with an ISBN and EAN); A-publications included to scientific achievement, B-other publications						
Type of publication		**Score points of MNiSW**	**IF***			
Journals from JCR list	A	180	16,835	-	5	5
	B	190	13,657	-	8	8
Journals from Philadelphia list	A	-	-	-	-	-
	B	46	-	2	4	6
Other journals		26	-	2	11	13
Conference materials		-	-		8	24
TOTAL		**442**	**30,492**	**12**	**52**	**64**
Participation in projects						
Projects		MNiSW/NCN		-	3	3
TOTAL					3	3
Other achievements of scientific research						
Reviews of projects for NCN				-	1	1
Reviews of articles published in journals JCR				-	25	25
Indicators of scientific achievements						
Source of data				Web of Science	Scopus (Elsevier)	Google Scholar
The Hirsch index *h*				5	5	6
The number of citations				63	69	112
The number of publication in database				12	11	28

* ***Total impact factor*** *according to the Journal Citation Reports (JCR) in 2014*

6. Accomplishments in science promotion

My scientific work was complemented by active science promotion. Since 2010, I prepared and realized workshops for young people under the Olsztyn Days of Science and Art. I ran a four training courses for laboratory technicians "How to take samples for microbiological analysis in order to get the correct result of the test?".

In 2011, I participated in a program entitled "Commercialization of research findings and enterprise promotion at the University of Warmia and Mazury in Olsztyn through internships, training and academic enterprise initiatives" in PWiK Ostróda, a water supply and sewerage company in Tyrowo near Ostróda, as part of project No. POKL-08.02.01-28-001/08-00 co-financed by the European Social Fund.

In 2014 I founded a website dedicated to the issue of the prevalence of drug-resistant in the environment (http://uwm.edu.pl/antybiotykoopornoscwsrodowisku).

7. Organizational accomplishments

In the course of my work I carried a lot of organizational tasks for the benefit of the University. Since 2014 I am a member of the Faculty Council at the Faculty of Environmental Sciences as a representative of assistant professors. In the years 2009 - 2011 I was a coordinator of the schedule of teaching in the Department of Environmental Microbiology.

For my greatest success in organizational work for the benefit of UWM, I think being a part of teams working on the amendment of the training program for the Field Environmental Protection and the preparation of a training program for the Field Management of Water Resources. In 2012 I received a diploma from the Rector of the University of Warmia and Mazury in Olsztyn (III.D.1) for organizational achievements.

Since 1997 I am an active member of the Polish Society of Microbiologists (PTM).

8. Discussion of educational achievements

In the course of my work at the University of Warmia and Mazury in Olsztyn I prepared programs for the following subjects:

- Molecular microbiology (Field of Environmental Protection, Faculty of Environmental Sciences) - preparation and implementation of a program of lectures and classes,
- The techniques of microbiological sampling (Field of Environmental Protection, Faculty of Environmental Sciences) - preparation and implementation of a program of lectures and classes,
- The techniques of microbiological sampling (Field of Fisheries, Faculty of Environmental Sciences) - to prepare a program of lectures and classes,
- Diagnosis of microorganisms (Field of Microbiology, Faculty of Biology and Biotechnology) - preparation and implementation of a program of lectures and classes,
- Microbiological hazards of flood areas (Field of Environmental Engineering, Faculty of Environmental Sciences) - to prepare a program of lectures and classes,

In addition, I ran classes on the subject of Microbiology and Sanitary microbiology at the Faculty of Environmental Sciences, as well as the subject of Microbiology at the Faculty of Animal Bioengineering at the University of Warmia and Mazury in Olsztyn. I ran also seminars for students writing their master's theses and students performing their engineer's diploma work at the Faculty of Environmental Sciences.

I am scientific supervisor over PhD student Sebastian Wojciech Przemieniecki, as an auxiliary thesis supervisor. The working title of his doctoral dissertation is "Plant protection products and antagonistic bacteria in relation to the different genetic and phenotypic groups of *Pectobacterium* spp.". Doctoral cord was opened at the Faculty of Environmental Management and Agriculture in Olsztyn on 17.02.2015.

I am, since 2014, **s**cientific supervisor of Students' Research Association "Coccus," UWM in Olsztyn.

In the course of my work, I was scientific supervision over students writing their master's theses (16) and students performing their engineer's diploma work (22). I was also a reviewer of M.Sc. thesis and B.Sc. thesis (27).