SILESIAN UNIVERSITY OF TECHNOLOGY

FACULTY OF CHEMISTRY

DEPARTMENT OF INORGANIC ANALYTICAL CHEMISTRY AND ELECTROCHEMISTRY

MSc Eng. Klaudia Stando

SUMMARY OF THE DOCTORAL DISSERTATION

Opracowanie i zastosowanie nowych procedur analitycznych do oznaczania wybranych zanieczyszczeń oraz produktów ich transformacji w środowisku

Przewodnik po monotematycznym cyklu publikacji

Development and application of new analytical procedures for determining selected pollutants and their transformation products in the environment

Guide to a Thematic Series of Publications

Supervisor: Dr hab. Eng. Sylwia Bajkacz, prof. SUT

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The doctoral dissertation was prepared based on nine thematically related articles with a total Impact Factor (IF) of 52.960 and a total sum of MNiSW points of 1170:

[P1]   **Kokoszka (Stando), K.;** Wilk, J.; Felis, E.; Bajkacz, S.* Application of UHPLC-MS/MS method to study occurrence and fate of sulfonamide antibiotics and their transformation products in surface water in highly urbanized areas. *Chemosphere* **2021**, 283, 131189, doi:10.1016/j.chemosphere.2021.131189. **(IF=8,943; 140 pkt)**

[P2]   **Kokoszka (Stando), K.;** Zieliński. W.; Korzeniewska, E.; Felis, E.; Harnisz, M.; Bajkacz, S.* Suspect screening of antimicrobial agents transformation products in environmental samples development of LC-QTrap method running in pseudo MRM transitions. *Sci. Total. Environ.* **2022**, 808, 152114, doi:10.1016/j.scitotenv.2021.152114. **(IF=10,753; 200 pkt)**

[P3]   **Stando, K.*;** Kasprzyk, P.; Felis, E.; Bajkacz, S. Heterogeneous Photocatalysis of Metronidazole in Aquatic Samples. *Molecules* **2021**, 26(24), 1-16, doi: 10.3390/molecules26247612. **(IF=4,927, 140 pkt)**

[P4]   **Stando, K.**; Korzeniewska, E.; Felis, E.; Harnisz, M.; Buta M.; Bajkacz, S.* Determination of antimicrobial agents and their transformation products in an agricultural water-soil system modified with manure. *Sci. Rep.* 2022, 12, 17529, doi: 10.1038/s41598-022-22440-5. **(IF=4,996; 140 pkt)**

[P5]   **Stando, K.*;** Korzeniewska, E.; Felis, E.; Harnisz, M.; Bajkacz, S.* Uptake of pharmaceutical pollutants and their metabolites from soil fertilized with manure to parsley tissues. *Molecules* **2022**, 27(14), 1–20, doi: 10.3390/molecules27144378. **(IF=4,927; 140 pkt)**

[P6]   **Stando, K.*;** Czyż, A.; Gajda, M.; Felis, E.; Bajkacz, S.* Study of the Phytoextraction and Phytodegradation of Sulfamethoxazole and Trimethoprim from Water by *Limnobium laevigatum*. *Int. J. Environ. Res. Public Health* **2022**, 19, 1-18, doi:10.3390/ijerph192416994. **(IF=4,614; 140 pkt)**

[P7]   **Stando, K.**; Wilk, J.; Jakóbik-Kolon, A.; Felis, E.; Bajkacz, S.* Application of UHPLC-MS/MS method to monitor the occurrence of sulfonamides and their transformation products in soil in Silesia, Poland. *Enviro. Sci. & Pollution Res.* **2023**, 30, 112922–112942, doi: 10.1007/s11356-023-30146-y. **(IF=5,800; 100 pkt)**

[P8]   **Stando, K.**; Grzybowski, M.; Byczek-Wyrostek, A.; Bajkacz, S.* Efficiency of phytoremediation and identification of biotransformation pathways of fluoroquinolones in the aquatic environment. *Int. J. Phytoremediation* **2023**, 26(7), 1027–1037, doi: 10.1080/15226514.2023.2288898. **(IF= 3,700; 100 pkt)**

[P9]   **Stando, K.*;** Kopiec, M.; Pieszczek, M.; Zogornik, P.; Kowalska, K.; Felis. E.; Bajkacz, S.* Degradation of fluoroquinolones and macrolides by solar light-driven heterogeneous photocatalysis – Proposed drug transformation pathways. *J. Photochem. Photobiol. A* **2024**, 453, 115651, doi: 10.1016/j.jphotochem.2024.115651. **(IF=4,300; 70 pkt)**

$\sum_{IF\ (P1-P9)} = 52,960$ $\sum_{MNiSW\ (P1-P9)} = 1170$ * – corresponding author

Purpose of the doctoral dissertation

The aim of the doctoral dissertation was to develop **new analytical methods useful for analyzing the presence and stability of a wide range of antimicrobial agents (AMs) and their degradation products in environmental samples. The research also addressed a gap in understanding the fate and transformation pathways of AMs after their introduction into the environment.** As part of the doctoral dissertation:

I. Conditions for the determination of selected AMs in four types of environmental samples (water, wastewater, soil, plant tissues) using LC-ESI-MS/MS were developed **[P1–P9]**.

II. Procedures for extraction of selected AMs from four types of environmental samples were developed **[P1,P2,P4–P8]**.

III. The physicochemical properties of selected environmental samples were characterized, and which of them may affect the reproducibility of the method were determined **[P2,P7]**.

IV. The developed analytical procedures were validated **[P1,P2,P4–P8]**.

V. Monitoring studies of the aquatic environment were conducted to determine the sources of AMs introduction into the environment and to assess their prevalence **[P1,P2]**.

VI. The mobility and bioavailability of selected veterinary AMs were investigated after their introduction with manure to the soil under field conditions **[P4,P5,P7]**.

VII. The effectiveness of AMs removal from the aquatic environment was investigated using two purification methods – photocatalysis and phytoremediation – as an alternative to the traditional method using activated sludge **[P3,P6,P8,P9]**.

VIII. The TPs of AMs present in each of the environmental samples tested were identified, and, if possible, their transformation path was proposed **[P1–P9]**.

Doctoral dissertation thesis

Three main hypotheses were defined and then they were verificated and this verification was the aim of the doctoral dissertation:

(I) Antimicrobial agents are common environmental pollutants that, depending on their physicochemical properties, exhibit the ability to **migrate or accumulate** in surface water, groundwater, and soil.

(II) **Once introduced into the environment, antimicrobial agents transform** under the influence of biotic and abiotic factors. The resulting transformation products **are not detected in traditional screening tests** due to the lack of information on their structure and physicochemical properties.

(III) The wastewater treatment methods commonly used in Poland, such as activated sludge, are insufficient to remove the remains of antimicrobial agents from wastewater entirely. Treated wastewater discharged into surface waters is a source of pharmaceutical contamination and their transformation products.

1. Theoretical Framework

The treatment of infectious diseases of bacterial origin is based on antimicrobial agents (AMs), which eliminate bacteria or inhibit their growth. Medical preparations containing AMs are used to combat and prevent diseases in medicine and veterinary medicine, and the main difference between them is the dose of active substances and the duration of treatment. AMs have been used for years in livestock farming and aquaculture to increase the production efficiency of animal products (meat, milk, eggs) [1]. The primary sources of AMs introduction into the environment are wastewater treatment plants (WWTPs) and run off from agricultural fields fertilized with animal fertilizers. AMs are not fully metabolized after consumption, and their residues are determined in a wide range of concentrations (ng/g – μg/g) in human and animal feces [2]. Human feces are fed to WWTPs with domestic wastewater, while animal feces are used as a natural fertilizer due to the high content of organic carbon (OC) and nitrogen [3].

AMs introduced into the soil with animal excrements, depending on their physicochemical properties (solubility, volatility, pK_a, logP), soil properties (OC content, pH, sorption properties), and environmental conditions (season, temperature, rainfall), can migrate in the soil or accumulate in the environment. Moreover, AMs are also transformed by biotic and abiotic environmental factors to form transformation products (TPs) [4,5]. The migration of AMs in soil leads to surface and groundwater contamination. After the dispersion of AMs in the environment, depending on their bioavailability, AMs can affect the growth and condition of plants, cause oxidative stress, inhibit photosynthesis, and accumulate in their tissues [6]. The stability of AMs in the environment can range from several hours to many months, depending on their physicochemical properties and environmental factors [7].

Discharges of treated wastewater (TWW) into rivers are the primary source of AMs being introduced into the aquatic environment. Depending on the wastewater treatment technology used in WWTPs and the physicochemical properties of AMs, they can (I) be removed partially or entirely, (II) remain unchanged, and (III) be transformed to form TPs [8,9]. The type, amount, and concentration of AMs in untreated wastewater (UWW) supplied to WWTPs depend on the season, its location, climate, and the sources from which they originate (urban, hospital, industrial) [10,11]. After the introduction of AMs into the aquatic environment, despite their low concentration (ng/l – mg/l) and large dispersion in water, they negatively affect the condition of aquatic organisms (their behavior, development, and fertility) [12].

In Poland, the most commonly used wastewater treatment technology is activated sludge (AS). Treatment of wastewater using AS from AMs micropollutants is not effective because it depends on the composition of the activated sludge microorganism population, process parameters (temperature, sludge retention time, type of bioreactor), and the composition of the supplied UWW [13]. AMs can significantly affect the composition of bacterial communities in AS, promoting species growth with higher tolerance to pharmaceutical contaminants and limiting the growth of more sensitive organisms [14]. The efficiency of wastewater treatment using AS strongly depends on the ambient temperature. A decrease in ambient temperature

slows down the metabolic processes of microorganisms and reduces their growth rate, which reduces the efficiency of AMs removal [14,15]. The main mechanisms regulating the removal of AMs by AS are adsorption and biodegradation [13]. The removal efficiency depends on the physicochemical properties of AMs (water solubility, logP, pKa), species of microorganisms used, and the operating conditions of the bioreactor (temperature, presence of metal ions, pH) [13].

Due to the insufficient effectiveness of currently used biological wastewater treatment methods, the priority challenge is developing new solutions. The doctoral dissertation focused on two wastewater treatment methods that were considered environmentally friendly and economical – phytoremediation and heterogeneous photocatalysis. Phytoremediation uses natural plant mechanisms (phytoextraction, phytoaccumulation, phytostabilization, phytodegradation, phytovolatilization) to collect and accumulate organic and inorganic pollutants in plant tissues [16]. All plants can uptake pollutants along with nutrients from soil and water, but not every species is suitable for phytoremediation. Plants characterized by a fast growth rate, a wide range of tolerance to changing environmental conditions, resistance to biotic and abiotic stress, the ability to collect pollutants in a wide range of concentrations, ease of cultivation and harvesting, and high biomass are used for environmental purification by phytoremediation [17,18]. After the contaminants are up taken by the plant, they are deposited in vacuoles or apoplast in the original form or as metabolites [19]. Free-floating plants or plants from wetland ecosystems are often used for phytoremediation of AMs from water and wastewater. The effectiveness of AMs removal from water by phytoremediation depends mainly on their type, concentration, bioavailability, and plant species [20].

Heterogeneous photocatalysis is one of the methods of advanced oxidation processes (AOPs), characterized by high efficiency in removing organic pollutants from water and wastewater. This process involves the use of chemical reactions occurring on the surface of the photocatalyst, as a result of which highly reactive hydroxyl radicals are generated. Hydroxyl radicals initiate and accelerate the removal of organic pollutants, which leads to their partial or complete mineralization. The efficiency of pollutant removal in heterogeneous photocatalysis depends on the type and concentration of the catalyst, pH, process temperature, type and concentration of pollutant, amount of dissolved oxygen, and light source parameters [21]. Semiconducting metal oxides (such as TiO_2, ZnO, WO_3, and ZrO_2) are often used as photocatalysts in heterogeneous photocatalysis, individually, as mixtures or composites [22]. Despite the large number of AMs photodegradation procedures described in the literature, the comparison of their removal efficiency by different photocatalysts is difficult due to the various process conditions [23–25]. The efficiency of AMs removal from other groups of drugs (beta-lactams (β-Ls), fluoroquinolones (FQs), tetracyclines (TCs), sulfonamides (SAs)) can be close to 100% with appropriately selected process parameters [23,26]. However, the complete removal of AMs from water does not equal their complete mineralization. It is also necessary to determine the degree of sample mineralization because it considers the possibility of the formation of transformation products (TPs) in the purification process.

In 2015, the World Health Organization (WHO) developed a global action plan to stop bacterial drug resistance, which called for limiting the inappropriate use of AMs in humans and

animals [27]. However, the applicable Polish and European law does not require screening of water and soil for AMs contamination to date. The watch lists of substances prepared by the EU Commission since 2015 suggest monitoring of selected AMs (amoxicillin (AMOX), ciprofloxacin (CIP), sulfamethoxazole (SMX), trimethoprim (TRI), clindamycin (CLD), ofloxacin, cephalexin). However, they do not constitute formal legal documents that would impose a direct order to monitor the compounds listed therein. This is insufficient in the context of sustainable socio-economic development of countries, which assumes minimizing the impact of human activity on the environment and caring for the availability of natural resources for future generations. The type and concentration of AMs in liquid environmental samples depend on the geographical location of the sampling point and its demographic characteristics, access to municipal wastewater systems, and the frequency of drug use in a given region [28,29]. AMs are also present in solid environmental samples (soils, sediments, manure, plant tissues) in a wide range of concentrations (ng/kg – μg/kg) [30–32]. The main factors influencing the dispersion of AMs in soil are their sorption capacity, stability, and bioavailability for plants [33].

Liquid chromatography (LC) coupled with tandem (MS/MS) or high-resolution mass spectrometer (HRMS) is commonly used to determine trace amounts of AMs in environmental samples. Using a mass spectrometer as a detector allows for achieving low detection limits (ng/l or ng/g) and high sensitivity and selectivity of the method. The most commonly used method for extracting AMs from liquid samples is solid-phase extraction (SPE) [34–36]. The most important parameters influencing the efficiency of SPE are the sorbent's type and mass, the sample's pH, and the amount of solvent used for conditioning and elution of the sorbent. Extraction of AMs from solid environmental samples (soil, manure, plant tissues) requires more complex procedures due to (I) their large dispersion in the environment, (II) low concentrations (ng/kg – μg/kg), and (III) interaction of AMs with organic matter contained in the samples [37]. In the first stage, techniques such as solid-liquid extraction (SLE), pressurized liquid extraction, or QuEChERS extraction are used to extract AMs from solid samples. Then, the obtained extract is purified and enriched in AMs, most often using SPE [38,39]. However, despite the large number of procedures described in the literature, their reproduction in laboratory conditions is complicated and sometimes impossible. The lack of complete information on the characteristics of the sample used to develop the analytical method, the lack of crucial details in the methodological description, and the use of other laboratory equipment or reagents than those indicated in the method significantly limit the scope of applicability of the analytical method. Moreover, the development of a procedure for the simultaneous extraction of AMs with different physicochemical properties (polarity, solubility, logP, pK$_a$) requires a compromise in the selection of experimental conditions that will allow for obtaining a large amount of information on the qualitative and quantitative composition of the sample. For this reason, the recovery of individual AMs may differ by up to 40% [37,38].

Most of the available literature reviews on the fate of AMs in the environment do not take into account the issue of the formation of their TPs. After being introduced into the environment, AMs are transformed by biotic factors (microbial decomposition) and abiotic factors (hydrolysis, photolysis) [40]. The determination of TPs AMs using the commonly used targeted analysis (TA) is only possible if they have been included in the scope of the analytical method. To identify

TPs of unknown structure and physicochemical properties, non-targeted searching is necessary, which makes it possible to collect information about all contaminants present in the sample within a single analysis without needing information about their composition and origin. Non-targeted analysis (NTA) is mainly performed using liquid or gas chromatography coupled with MS/MS or HRMS. The results obtained in NTA are qualitative, and their interpretation compares the obtained mass spectrum with reference spectra available in chemical databases and scientific literature [41]. The number of compounds detected in NTA is influenced by the type of sample, extraction conditions, pH, chromatographic conditions, type of mass spectrometer, and the ionization mode [42]. For this reason, to use the full potential of NTA in the identification of TPs AMs, it is necessary to know the limitations of the method and adopt appropriate assumptions and criteria enabling the selection of the obtained data.

For the research conducted as part of the doctoral dissertation, 22 AMs were selected (amoxicillin (AMOX), ampicillin (AMP), ciprofloxacin (CIP), doxycycline (DOX), enrofloxacin (ENF), clarithromycin (CLR), clindamycin (CLD), levofloxacin (LVF), metronidazole (MET), oxytetracycline (OTC), sulfadiazine (SFD), sulfamerazine (SFR), sulfamethazine (SFM), sulfamethizole (SFH), sulfamethoxazole (SMX), sulfapyridine (SFP), sulfathiazole (SFT), sulfisoxazole (SFX), tetracycline (TC), trimethoprim (TRI), tylosin (TYL), vancomycin (VAN)) from 7 drug groups. The selection of AMs was based on the WHO report on the surveillance of antibiotic consumption in 2016–2018 [43], the results of environmental screening studies [44–47], and the analysis of drug consumption [48,49] in Poland.

2. Discussion of the results

2.1 Isolation and identification of selected antimicrobial agents and their transformation products in environmental samples

As part of the research conducted for the needs of the doctoral dissertation, 4 methods of extraction of selected AMs from liquid environmental samples (groundwater, surface water, wastewaters) using SPE **[P1,P2,P6,P8]** and 7 methods of extraction of AMs from solid environmental samples (soil, plant tissues) using two-stage SLE-SPE **extraction [P4–P8]** were developed. The selection of SPE parameters included the type of sorbent and its mass, sample pH, type and volume of solvents used for elution and dissolution of sample residues after the evaporation stage, and, if necessary, the material and size of the syringe filter.

All procedures for extraction of AMs from liquid samples were developed using the SPE method. OASIS HLB sorbent (500 mg, 6 ml) was used to conduct experiments **[P1,P2,P6,P8]**. The sample pH was an important parameter influencing the retention of AMs from TCs, FQs, SAs and MQs groups on the sorbent. Adjusting the sample pH to 3 – 4 minimized the probability of changing the ionic form by AMs and reduced the potential losses of analytes. The elution efficiency of AMs from the sorbent depends mainly on their ionic form and the interaction of functional groups with the sorbent. The highest elution efficiency of SAs, MQs, and TCs from the sorbent was obtained using MeOH. It was different in the case of FQs, whose elution efficiency from the sorbent was the highest after using 0.1% acetic acid in MeOH (62 –

97%) **[P8]** in comparison to MeOH (53 – 71%) **[P2]**. Finally, in the extraction methods of 14 AMs from various groups of drugs from wastewater and surface water samples, MeOH elution was performed due to the necessity of obtaining a high recovery of all analytes (51 – 102%), not only FQs **[P2]**. If necessary, extracts from water and wastewater samples were filtered immediately before LC-MS/MS analysis to obtain uniform, clear solutions **[P1,P2]**.

The development of the AM extraction method from solid samples began with the replication and modification of procedures described in the scientific literature. Although the AM recovery from solid environmental samples described in the literature was acceptable (>80%), none of them could be adapted to soil samples, for which the recovery of 11 out of 14 AMs was below 54%. A hypothesis was put forward stating that the problems with the reproducibility of AM extraction methods from soil samples result from differences in the physicochemical properties of soil samples (OC content, mineral content, pH). In order to determine the factors causing significant AM losses when using the SLE-SPE method for soil samples, the AM recovery was determined at individual stages of the sample preparation procedure: (1) SLE, (2) dissolution of SLE residues, (3) SPE, (4) sample evaporation **[P4,P7]**. The key stage in the AM extraction procedure from soil samples was SPE, where the highest analyte losses were observed. The presence of soil matrix components (humic and fulvic acids, organic matter) significantly reduces the recovery of AMs at the SPE stage, which is most likely related to the deactivation of the OASIS HLB sorbent **[P4]**. This problem was solved using an additional anion-exchange sorbent (OASIS WAX), which retained soil matrix components. The final recovery of the 14 AMs extraction procedure from soil samples enriched with manure was 57 – 95% **[P4]**.

The observed relationship between soil matrix components and the recovery of AMs in the extraction procedure using SLE-SPE led to the formulation of the following research hypothesis: issues with the reproducibility of AM extraction procedures arise from the incorrect assumption that they can be considered universal for different types of soil samples without taking their characteristics into account **[P7]**. The main objective of the studies described in **P7** was to determine how the soil matrix components (organic carbon, pH, minerals contained in the soil) affect the recovery of SAs at the extraction stage using the SLE-SPE method. Inorganic components included in the soil (Al^{3+}, $Ca^{2+,}$ and Mg^{2+}) and its pH did not significantly affect the efficiency of SAs extraction. It was observed that the presence of Na^+ and K^+ ions have a negative effect on the efficiency of SAs extraction because they increase the strength of their binding to the soil **[P7]**. The OC content in the soil exerted the most significant effect on SAs recovery. It was observed that increasing the OC content in the soil caused a significant decrease in SAs recovery **[P7]**. SAs extraction from soil was also carried out using the SLE-SPE method. However, the use of an additional OASIS WAX sorbent was omitted to precisely determine the effect of OC on the deactivation of the OASIS HLB sorbent. The highest SAs recovery was obtained when the OC content in the sample was <1% (81 – 110%). With an increase in OC by 1%, the SAs recovery systematically decreased: 42–80% (1≤OC≤2%), 31 – 69% (2≤OC≤3%), and 20 – 50% (3%≤OC) **[P7]**.

Depending on the plant species, its morphology, and the nature of the conducted research, three types of plant matrices were distinguished (leaves, roots, whole plant), for which

extraction methods of selected AMs were developed **[P5,P6,P8]**. Extraction of selected AMs from each of the above-mentioned matrices was carried out using SLE and SLE-SPE methods. It was noted that the efficiency of AMs extraction from leaf samples may be negatively affected by matrix components (lignin, cellulose, proteins, flavonoids, tannins, pigments), causing sample gelation. This effect was not observed in the case of plant root samples. As part of the doctoral dissertation, methods were developed for the extraction of selected AMs from four plant species –parsley (*Petroselinum crispum*) **[P5]**, *Limnobium laevigatum* **[P6]**, Japanese radish (*Raphanus sativus var. longipinnatus*) and duckweed (*Lemna minor L.*) **[P8]** – whose parameters were selected to ensure the highest possible recovery of selected analytes.

The determination of selected 22 AMs in all types of environmental samples (water, wastewater, soil, plant tissue) was performed using LC-MS/MS operating in multiple reactions monitoring mode (MRM) **[P1–P9]**. Four methods for determining selected AMs in environmental samples were developed as part of the doctoral dissertation. The selection of characteristic parameters for the compound (two precursor ion – product ion transitions, cluster disassembly potential (DP), collision energy (CE), collision chamber exit potential (CXP)) was performed using the syringe method, individually for each compound (Figure 1). Zorbax SB-C3 (150 mm × 3.0 mm, 5 µm) and Kinetex F5 (100 × 2.1 mm, 1.7 µm) columns were selected for the separation of AMs mixtures, which provided good resolution of compounds with similar structure and physicochemical properties. In all developed methods, the mobile phase consisted of two solvents – 0.1% formic acid in H_2O and ACN. The optimization of ion source parameters (curtain gas pressure (CUR), temperature (TEM), dispersion gas pressure (GS1), drying gas pressure (GS2), collision chamber gas pressure (CAD), and ion source voltage (IS)) was performed using flow injection analysis (FIA).

Each of the developed measurement methods was validated. Validation tests determined the accuracy, precision, selectivity, linearity, matrix effect (ME), limit of detection (LOD), limit of quantification (LOQ), and recovery (R) of the analytical method. Table 1 summarizes the validation parameters of the developed analytical methods. The obtained validation parameters were comparable with the values given in the literature. Therefore, they were found suitable for the analysis of environmental samples.
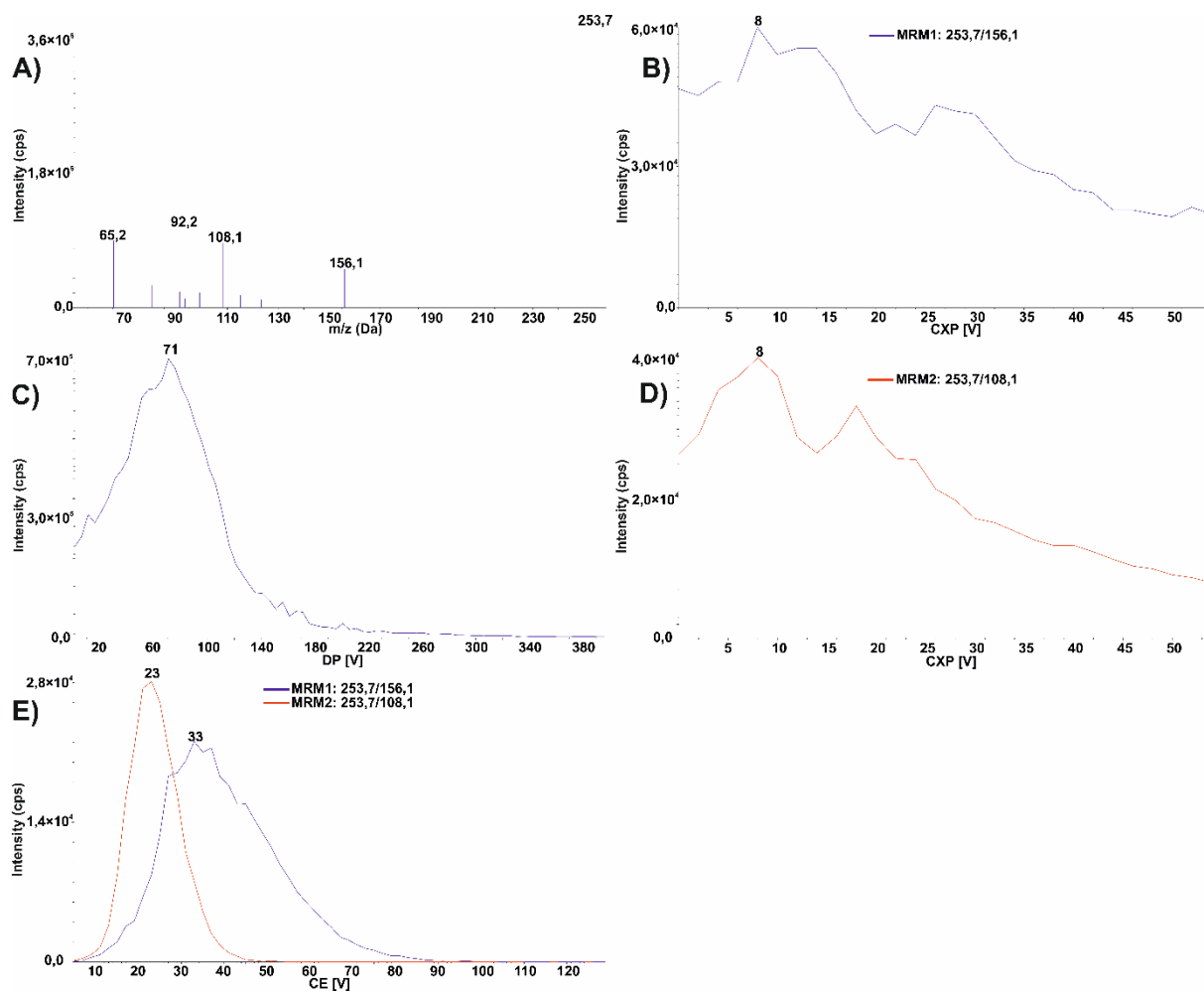
Figure 1. Selection of characteristic parameters for the analyte using the syringe method, based on the example of SMX; (A) fragmentation spectrum of SMX, plots of intensity versus (B, D) CXP, (C) DP and (E) CE for two transitions: precursor ion (m/z 253.7) – product ion (m/z 156.1 and m/z 108.1).

Table 1. Developed and validated procedures for the determination of AMs in liquid and solid samples using LC-MS/MS

AMs	Matrix	Stationary phase	Mobile phase	LOD/LOQ	Linearity (R²)	CV [%]	RE [%]	ME [%]	Recovery [%]	Publication
8 SAs: SMX, SFD, SFP, SFM, SFT, SFR, SFH, SFX	surface water	Kinetex F5 (100 × 2,1 mm; 1,7 µm)	Gradient elution: A: 0.1% FA w H₂O B: ACN Flow rate: 0.3 ml/min	LOD: 0.03 – 0.27 ng/l LOQ: 0.1– 0.8 ng/l	0.9953 – 0.9984	1.4 – 7.3	-9.1 – 5.8	0.5 – 12.4	43 – 108	[P1]
	soil			LOD: 0.3 ng/g LOQ: 1.0 ng/g	0.9890 – 0.9959	0.3 – 15.0	-31.0 – 30.0	-6.4 – 36.0	20 – 110	[P7]
16 AMs: AMOX, AMP, TC, OTC, DOX, SMX, SFD, CIP, LVF, ENF, MET, TRI, VAN, TYL, CLR, CLD	wastewater, surface water, groundwater	Zorbax SB-C3 (150 × 3,0 mm, 5 µm)	Gradient elution: A: 0.1% FA w H₂O B: ACN Flow rate: 1.0 ml/min	LOD: 0.1 – 0.3 ng/l LOQ: 0.2 – 1.0 ng/l	0.9946 – 0.9998	1.5 – 7.5	-7.8 – 7.3	1.5 – 11.2	51 – 102	[P2,P3]
	soil			LOD: 0.2 ng/g LOQ: 0.5 ng/g	0.9946 – 0.9997	1.1 – 7.8	-6.3 – 6.3	2.9 – 10.3	57 – 95	[P4]
	plant tissues			LOD: 0.3 – 1.6 ng/g LOD: 0.3 – 1.6 ng/g	0.9858 – 0.9988	1.2 – 9.6	0.4 – 11.4	-11.3 – 11.3	46 – 97 (leaf) 45 – 95 (root)	[P5]
SMX, TRI	water	Kinetex Core-Shell C18 (75 × 2,1 mm, 2,6 µm)	Isocratic elution: A: 0.1% FA w H₂O B: ACN A:B (80:20; v/v) Flow rate: 0.6 ml/min	LOD: 0.4 – 7.0 ng/l LOQ: 1.3 – 10 ng/l	0.9992 – 0.9998	3.5 – 7.4	-6.5 – 6.4	1.5 – 7.8	74 (SMX) 70 (TRI)	[P6]
	plant tissues			LOD: 0.3 ng/g LOQ: 1.0 ng/g	0.9953 – 0.9970	4.30 – 7.04	1.1 – 3.6	-4.0 – 5.3	63 (SMX) 60 (TRI)	
CIP, ENF, LVF	water	Kinetex F5 (75 × 2,1 mm; 2,6 µm)	Isocratic elution: A: 0.1% FA w H₂O B: ACN A:B (85:15; v/v) Flow rate: 0.6 ml/min	LOD: 0.3 ng/l LOQ: 1.0 ng/l	0.9993 – 0.9998	1.2 – 8.0	1.3 – 6.5	-4.3 – 1.6	62 – 97	[P8,P9]
	plant tissues			LOD_leaf: 0.6 ng/g LOQ_root: 1.6 ng/g LOD_leaf: 2.0 ng/g LOQ_root: 5.0 ng/g	0.9985 – 0.9998	0.1 – 6.2	1.1 – 10.1	-11.7 – 10.5	78 – 113 (root) 52 – 99 (leaf)	

ACN – acetonitrile, AMOX – amoxicillin, AMP – ampicillin, CIP – ciprofloxacin, CLD – clindamycin, CLR – clarithromycin, CV – coefficient of variation, DOX – doxycycline, ENF – enrofloxacin, LOD – limit of detection, LOQ – limit of quantification, LVF – levofloxacin, ME – matrix effect, MeOH – methanol, MET – metronidazole, OTC – oxytetracycline, RE – relative error, SFD – sulfadiazine, SFH – sulfamethizole, SFM – sulfamethazine, SFP – sulphapyridine, SFR – sulfamerazine, SFT – sulfathiazole, SFX – sulfisoxazole, SMX – sulfamethoxazole, TC – tetracycline, TRI – trimethoprim, TYL – tylosin, VAN – vancomycin.

2.2 Antimicrobial agents in the aquatic environment: analytical methods for identifying sources of introduction and dissemination

Identifying sources introducing AMs into the aquatic environment and their distribution within the environment was the subject of two studies conducted as part of the doctoral dissertation. **The aim of the first study was to evaluate the efficiency of removing 14 selected AMs in the wastewater treatment process using the activated sludge method and to identify their TPs [P2].** Samples of untreated wastewater (UWW) from the bioreactor (AS), treated wastewater (TWW), and water from the river adjacent to the treatment plant, collected from a point before (URW) and after (DRW) the TWW discharge were taken between February and September 2019. Figure 2 presents the changes in the concentration of selected AMs in wastewater samples at various stages of treatment.
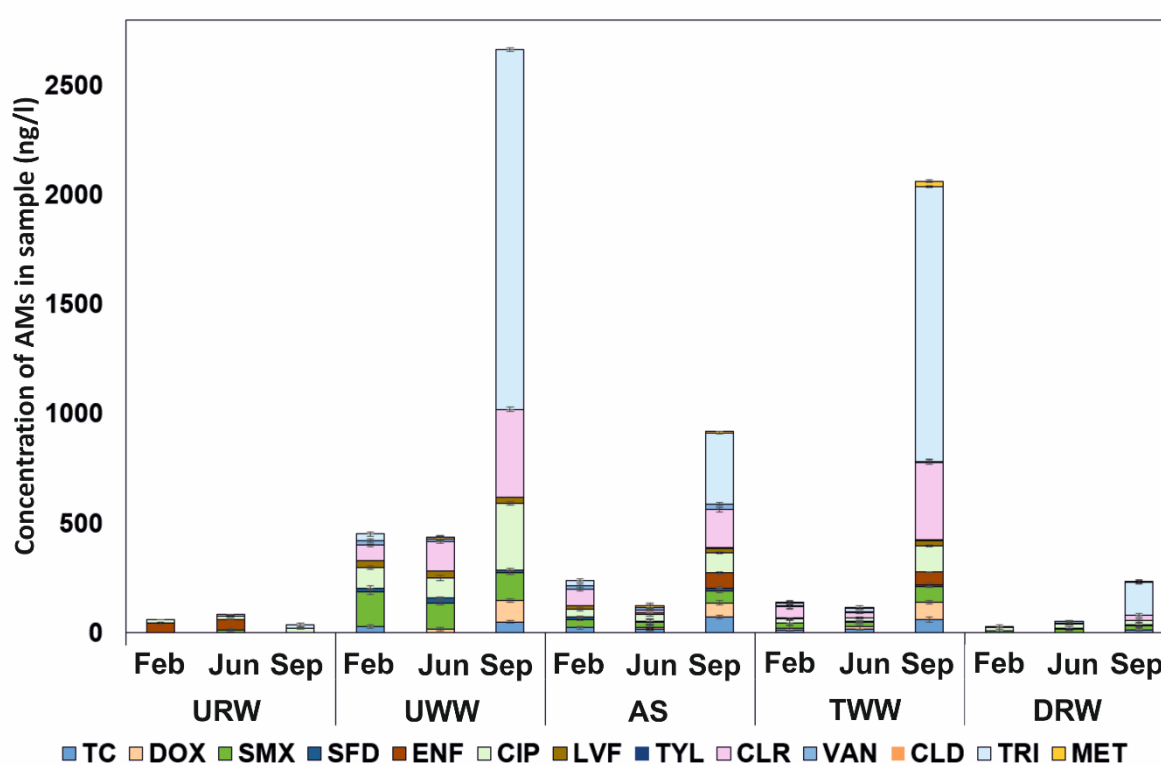

Figure 2. The concentration of selected AMs in wastewater samples collected at different treatment stages and river water (URW – river water before the wastewater discharge point, UWW – untreated wastewater, AS – wastewater from the bioreactor, TWW – treated wastewater, DRW – river water after the wastewater discharge point) [P2]. © 2021 Elsevier B.V. All rights reserved.

In UWW, 13 out of 16 selected AMs were detected, and their concentration was the highest in samples collected in September (11.4 – 1643.7 ng/l). In UWW samples collected in July and February, the concentration of AMs was significantly lower, in the 2.4 – 163.1 ng/l and 1.4 – 160.2 ng/l, respectively **[P2]**. Seasonal fluctuations in AMs concentration in UWW concerned drugs from the TCs, FQs, and MQs groups. The concentration of SAs (SMX and SFD) in UWW was at a similar level in all samples, which suggests a constant demand for these drugs throughout the year. The activated sludge wastewater treatment method was ineffective in

removing residual MQs (TYL, CLR). The concentration of TYL in UWW and TWW was similar throughout the year, while the removal efficiency of CLR ranged from 11% to 82% **[P2]**. SAs were detected in both DRW (2.5–19.2 ng/l), suggesting they are stable in the aquatic environment and may accumulate in it **[P2]**. **For this reason, the following study aimed to conduct screening studies of surface waters to determine the prevalence of 8 selected SAs (SMX, SFD, SFP, SFM, SFT, SFR, SFH, SFX) in the environment [P1].**

For the screening studies, artificial water reservoirs were selected in Sosnowiec (Stawiki Lake), Katowice (Borki Lake), and Tychy (Paprocany Lake), as well as the Jamna stream, which flows through the center and rural areas of Mikołów, and the drinking water intake in the Psary village . The selection of these sampling points was based on the reservoir's purpose (recreation, ecotourism, flood control), water retention time, and the location of point sources of pollution that increase the likelihood of SAs accumulation in the environment. In water samples from Mikołów and Tychy, 3 out of the 8 selected SAs (SMX, SFD, SFP) were detected. Depending on the location of the sampling point, SAs concentrations ranged from <LOQ (<0.1 ng/l) to a maximum of 75.8 ng/l **[P1]**. The most frequently detected compound was SMX, found in both Paprocany Lake (max. 75.8 ng/l) and the Jamna stream (max. 34.1 ng/l) **[P1]**. Water sampling points along the Jamna stream were arranged to reflect different liquid waste management profiles: forested, urban, and rural areas. It was observed that the highest concentrations of SAs were recorded at the sampling point located in the rural area, which can be attributed to the partial lack of access to municipal wastewater systems.

AMs transformation products were detected in all types of environmental samples **[P1,P2]**. TPs AMs were supplied to WWTPs together with UWW and were produced directly in wastewater treatment using activated sludge. TPs SMX and SFD were present in all samples of surface water collected in Silesia. Moreover, TPs of SAs drugs were also detected in water samples in which the parent compound was not detected. This suggests that long hydraulic retention time favors the accumulation of both SAs and their TPs in the aquatic environment.

2.3 The fate of antimicrobial agents in soil – analytical methods for assessing mobility and availability to plants

The pathways through which AMs are introduced into the soil have been thoroughly discussed in the literature, where animal manure has been identified as the primary source of contamination [3,50]. However, the fate of AMs after introduction into the environment, especially their ability to accumulate and bioavailability, remains largely unexplained. Field studies were conducted with the aim of assessing the mobility, stability, and bioavailability of four veterinary AMs (DOX, ENF, SMX, TYL) after their introduction into an agricultural hydrogeological ecosystem, which included groundwater, soils, and cultivated plants **[P4,P5]**. The experiment was conducted from May to September 2019 in environmental conditions on agricultural fields with an area of 4 m^2. The fields were fertilized with one of two types of animal manure (poultry or cattle) enriched with a mixture of four selected AMs, followed by the sowing of parsley seeds. **Simultaneously, monitoring studies of the soils were conducted to assess contamination by 10 of the most commonly used AMs (TC, OTC, CIP, LVF, MET,**

CLR, TRI, CLD, VAN, SFD), enabling an evaluation of their distribution and capacity for accumulation in the environment.

Although the fields used in the field studies were never fertilized with animal fertilizers, trace amounts of SMX (13.2–90.8 ng/l) were detected in groundwater. In soil samples collected before manure fertilization and in manure samples, none of the 4 selected AMs were detected, but they were contaminated with trace amounts of CIP **[P4]**. In groundwater collected on the last day of the experiment (day 133), DOX, TYL, and ENF were not detected despite their introduction into the soil with animal manure. The DOX, TYL, and SMX concentrations in the soil decreased over time (21–93%), and their loss depended on the type of animal manure used. The concentration of ENF in the soil remained constant throughout the experiment, regardless of the type of manure used. This suggests that the loss of DOX, TYL, and ENF was mainly related to the uptake by plants and their degradation processes in the soil, not to their leaching by rainfall. In both groundwater and soil samples, 7 additional AMs (OTC, TRI, SFD, CIP, CLR, CLD, MET) were detected but not introduced to the soil with manure. Their distribution in the soil profile was random, and their concentration was too low to draw deeper conclusions about their sources and fate in the environment **[P4]**. In parsley samples (leaf, root) collected after 133 days of vegetation, only 4 AMs were detected when manure was introduced **[P5]**. In freeze-dried plant tissues, the highest concentrations were determined for ENF (13.4–29.3 ng/g), followed by DOX (2.1–14.0 ng/g) and SMX (2.3–6.8 ng/g), while the concentration of TYL was lower than 1.0 ng/g **[P5]**. The tendency to accumulate AMs in selected plant tissues was observed only for ENF, which accumulated in leaves, and DOX, which was mainly collected in roots. Key conclusions from studies **P4** and **P5** regarding the effect of OC on the mobility of AMs in the environment and the stability of this group of compounds after introduction to the environment were the basis for formulating the concept of further studies.

As part of expanding the information on the fate of AMs in the environment, soil monitoring was conducted in the Silesian Voivodeship. The aim of the study was to determine the contamination of soils from urban and rural areas by 8 selected SAs (SMX, SFD, SFM, SFP, SFT, SFR, SFH, SFX) [P7]. A total of 27 sampling sites were selected, located in urban and rural areas in six localities: Tychy, Katowice, Sosnowiec, Dąbrowa Górnicza, Mikołów, and Przyłęk. The main criteria for selecting the soil sampling site were increased activity of domestic animals (dog runs, horse paddocks, city parks) or using organic fertilizers of animal origin (agricultural fields, pastures) in the indicated area. The most commonly detected compounds in urban areas and agricultural fields located on the outskirts of cities were SMX (23 out of 27 soil samples) and SFD (19 out of 27 soil samples) **[P6]**. In soils collected from dog runs and agricultural fields, each of the 8 selected SAs was determined, and their concentrations were in the ranges of 1.7–10.5 ng/g and 1.9–3.7 ng/g, respectively. Notably, the concentrations of SAs determined in samples collected from dog runs were higher than in samples collected from agricultural fields. It means that high activity and rotation of dogs and limited usable space (300–2000 m^2) contribute to increased accumulation of AMs in soils in places designated for them **[P6]**. In soil samples collected from city parks, only SMX and SFD were determined, and their concentrations were below 2.5 ng/g.

Despite similar dog rotation in city parks and dog runs, the area of parks is much larger, which may explain the smaller amount and lower concentrations of SAs in soils.

2.4 Phytoremediation and photocatalysis as alternative methods of purifying water from micropollutants with bactericidal agents

The low efficiency of purifying UWW using activated sludge **[P2]** from AMs was the basis for proposing two alternative methods of their removal – phytoremediation **[P6,P8]** and heterogeneous photocatalysis **[P3,P9]**. Both proposed methods are assumed to be ecological and economical, and it is possible to implement them on a larger scale in wastewater and surface water treatment processes. The efficiency of both methods was evaluated in laboratory conditions for selected AMs (MET, CIP, ENF, LVF, SMX, SFD, CLD, and TYL).

Effective removal of AMs residues from water using heterogeneous photocatalysis requires selecting an appropriate type of photocatalyst, its mass, and the intensity of electromagnetic radiation. The study's first aim was to evaluate the efficiency of MET removal from water samples using different heterogeneous photocatalysis parameters **[P3]**. The objective of the second study was to compare the efficiency of selected AMs removal (CIP, ENF, LVF, TYL, CLR) under the same photocatalytic conditions **[P9]**. In both studies, 5 commercially available semiconductor photocatalysts (TiO$_2$-anatase, TiO$_2$-P25, ZnO, ZrO$_2$, WO$_3$) were used. The highest efficiency of MET removal from water was achieved using TiO$_2$-P25 (90% after 60 min of the process). Promising photocatalytic properties were also shown by ZrO$_2$ and ZnO (80 – 90% after 90 min of the process) **[P3]**. The change in the photocatalyst mass had no significant effect on MET removal from water efficiency. It was found that, apart from the type of photocatalyst, the most critical parameter influencing the efficiency of AMs removal from water is the intensity of electromagnetic radiation. The change in irradiance in the range of 500 – 1000 W/m^2 shortened the time of complete MET removal from water from 90 to 30 minutes **[P3]**. Lower irradiance values (250 W/m^2) allowed only partial removal of MET from water (90% after 90 min of the process). Among the 5 selected photocatalysts, the FQs removal efficiency (CIP, ENF, LVF) was observed for TiO$_2$-P25, reaching over 90% after 3 – 5 min of the process. Only three photocatalysts (TiO$_2$-P25, TiO$_2$-anatase, and ZnO) indicated photocatalytic activity and allowed for the removal of 25 – 53% of CLR and 93 – 100% of TYL from water in a 120-minute photocatalytic process. Although the developed heterogeneous photocatalysis conditions (TiO$_2$-P25 (100 g/l), 500 W/m^2, 120 min) were suitable for removing 4 out of 5 selected AMs (MET, CIP, ENF, LVF, TYL) from water, they were not sufficient to completely remove CLR from water **[P3,P9]**. Moreover, a significant decrease in their removal efficiency was observed after using the developed photocatalytic method to remove selected FQs and MQs from TWW samples. After the photocatalysis process, analysis of the OC content in water samples showed that 120 min is insufficient to achieve total mineralization of AMs **[P3,P9]**. The decrease in the AMs content in the photocatalytic process was related to the formation of their TPs.

The effectiveness of phytoremediation of water containing residues of selected 5 AMs (SMX, TRI, CIP, ENF, LVF) was assessed in two studies using two species of free-floating

plants: creeping *Limnobium laevigatum* **[P6]** and duckweed (łac. *Lemma minor)* **[P8]**. The effectiveness of AMs removal depends on four processes: hydrolysis, photolysis, sorption of contaminants by the plant, and the activity of microorganisms present in the plant's root zone. Phytoremediation of AMs was carried out in laboratory conditions for 14 days. The removal efficiency of SMX and TRI at an initial concentration of 1.0 µg/l, after 14 days of phytoremediation using creeping limnobium, was 96% and 75%, respectively **[P6]**. SMX removal was primarily the result of hydrolysis and/or photolysis in the aquatic environment (83%), while the main mechanisms of TRI removal were uptake by the plant and microbiological decomposition in the root zone (51 – 58%) **[P6]**. The obtained results of phytoremediation of water from SMX and TRI residues were considered promising because the presence of AMs in water did not negatively affect the growth, development of the plant, and its ability to take up, accumulate, and metabolize them. However, the creeping *Limnobium laevigatum* did not show the ability to hyperaccumulate AMs, and therefore, in the next study, it was decided to replace it with duckweed. The phytoremediation efficiency of CIP, ENF, and LVF residue water was tested at two concentration levels, which corresponded to the average values of AMs concentrations in surface waters (1.0 µg/l; LCF) and TWW (10.0 µg/l; HCF) **[P8]**. The efficiency of FQs removal from water after 14 days of phytoremediation was the highest for ENF (96 (LCF) – 98% (HCF)), followed by LVF (86 (HCF) – 88% (LCF)), and the lowest for CIP (72 (HCF) – 80% (LCF)) **[P8]**. With the increase in FQs concentration in water, their concentration in plant tissues was also higher. Duckweed showed the ability to hyperaccumulate CIP, for which the bioaccumulation factor calculated for the dry weight of the plant (BAF$_{DW}$) was in the range of 11.5 – 18.2 **[P8]**. BAF$_{DW}$ for the other two FQs was lower (2.4 – 2.8 for ENF; 1.4 – 7.8 for LVF). However, it was still a satisfactory result compared to the literature values of BAF$_{DW}$ for other plant species [51,52].

Table 2 compares the effectiveness of the traditional wastewater treatment method using activated sludge, heterogeneous photocatalysis, and phytoremediation in the removal of FQs (CIP, ENF, LVF) from wastewater and water. The effectiveness of FQs removal from wastewater depended on the season and the concentration of these compounds in municipal domestic wastewater (UWW). With the increase in the concentration of FQs in UWW and the decrease in temperature characteristics for the autumn-winter period, their removal efficiency decreased **[P2]**. The use of heterogeneous photocatalysis with TiO$_2$-P25 as a photocatalyst enabled the almost complete removal of selected FQs from water and TWW. Heterogeneous photocatalysis using TiO$_2$-P25 as a photocatalyst and radiation intensity of 500 W/m^2 resulted in nearly complete (~99%) removal of all FQs from water and wastewater samples. However, depending on the matrix composition, the processing time was increased from 15 min to 120 min **[P6]**. The efficiency of phytoremediation of water from the residues of selected FQs, depending on their initial concentration, was in the range of 72 – 98% **[P8]**. However, phytoremediation is a long process (14 days) and requires favorable environmental conditions for plant growth and development, which is a serious limitation.

Table 2. Comparison of wastewater and water treatment methods from FQs residues

Purification method	Activated sludge method	Heterogeneous photocatalysis	Phytoremediation
Conditions	Flow rate: 60 000 m³/day BOD: 267 mg/l (UWW), 17 mg/l (TWW) COD: 382 mg/l (UWW), 173 mg/l (TWW) C_{FQs}: 9,5 – 306,7 ng/l	Catalyst: TiO_2-P25 (100 mg/l) Irradiance: 500 W/m² Time: 120 min C_{FQs}: 1 mg/l	Plant species: *Lemma minor* Time: 14 dni C_{FQs}: 1 – 10 µg/l
E_{CIP} [%]	Wastewater: 60 – 82	Water: 99 (15 min) Wastewater: 96(120 min)	Water: 72 – 80
E_{ENF} [%]	Wastewater: 15	Water: 99 (15 min) Wastewater: 99 (120 min)	Water: 96 – 98
E_{LVF} [%]	Wastewater: 8 – 100	Water: 99 (15 min) Wastewater: 99 (120 min)	Water: 86 – 88
TPs	+	+	+
Publication	**[P2]**	**[P9]**	**[P8]**

E – efficiency; TPs – transformation products; BOD – biochemical oxygen demand; COD – chemical oxygen demand; C – initial concentration of AMs.

2.5 Transformation pathways of antimicrobial agents in the environment

The identification of TPs for selected 22 AMs was performed using LC-ESI-MS/MS in various mass detector operating modes – MRM (multiple reaction monitoring), enhanced product ion (EPI), and enhanced MS scan (EMS) – as well as software enabling intelligent data acquisition (IDA). The identification of TPs in environmental samples was carried out in four steps: (I) screening of samples in pseudo-MRM mode, (II) non-targeted analysis of samples in EMS-IDA-EPI mode, (III) confirmation of the presence of TPs identified in the first step, and (IV) retrospective analysis of mass spectra to identify "new" TPs.

Transformation pathways were determined for 17 of the 22 selected AMs (MET, DOX, TC, OTC, TRI, CLR, TYL, LVF, ENF, CIP, SMX, SFR, SFD, SFM, SFP, SFT, SFX) in 9 types of environmental samples (UWW, TWW, AS, soils, plant tissues, surface waters, groundwaters and waters after photocatalysis and phytoremediation processes). A total of 119 TPs were identified in all environmental samples. The screening studies described in **P1 – P9** indicated that AMs can transform after their introduction into the environment and during the wastewater treatment process, regardless of the chosen method. Some TPs are more stable than their parent AMs because (I) they are resistant to the action of activated sludge **[P2]**, the action of strong oxidants **[P3,P8],** and (II) they are present in the aquatic environment, even when the parent drug is not detected in the sample **[P1]**. The lack of complete information on the sources of introduction of AMs into the environment also makes it difficult to determine their transformation paths because it is unknown whether the identified TPs were formed directly in the environment or were introduced into it. In addition, the chemical structure of SAs and FQs is similar within a given group of AMs, so they can create the same TPs. Hence, it is impossible to indicate the factor that caused the transformation of AMs and the place and time in which this transformation occurred solely on the basis of information on the presence or absence of

TPs in the environmental sample. An example of the MET transformation path is shown in Figure 3.
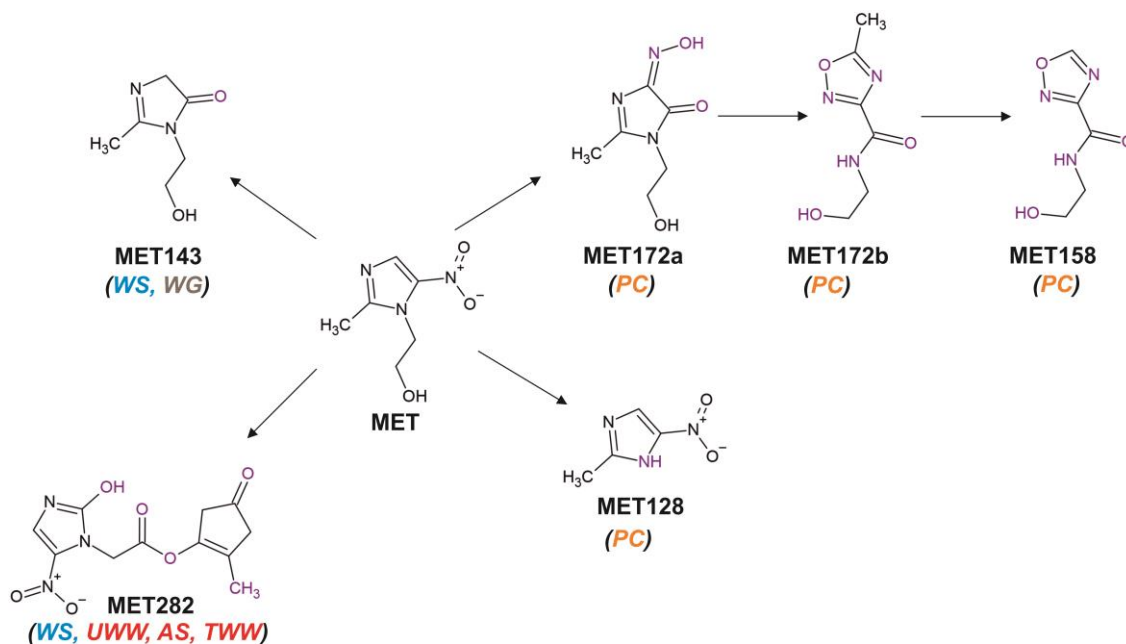

Figure 3. MET transformation pathway in the environment and the photodegradation process (WS – surface water, WG – groundwater, UWW – untreated wastewater, AS – bioreactor wastewater, TWW – treated wastewater, PC – photocatalysis).

It was found that TPs AMs can be divided into two categories: universal – present in all types of environmental samples and specific – characteristic for specific types of samples or processes. This division seems crucial for effectively identifying and monitoring the fate of AMs in the environment. Traditional wastewater treatment methods based on activated sludge lead to the formation of TPs resistant to its action, which are then introduced into the environment together with TWW **[P2]**. The formation of TPs was also observed in the process of heterogeneous photocatalysis, and their amount and type depended on the type of photocatalyst used **[P9]**. The ability to bioaccumulate TPs in plants used for water phytoremediation is particularly promising because they can be relatively easily collected and subjected to processing, which prevents their re-introduction into the environment **[P6,P8]**.

Summary and conclusions

Based on the research results obtained during the implementation of this doctoral dissertation, it can be stated with full confidence that all the assumed research goals have been achieved, and it has been proven that it is possible to simultaneously identify AMs and their transformation products using LC-MS/MS operating in different modes of the mass spectrometer. The dissertation extends the current knowledge on the sources of release of AMs residues and their spread in the environment. As part of the doctoral dissertation:

1. Successful extraction methods were developed for 20 out of 22 selected AMs from liquid and solid environmental samples **[P1–P9]**.

2. The extent to which agricultural runoff and wastewater treatment plant discharges contribute to introducing and disseminating AMs residues in the aquatic and terrestrial environment was determined **[P2,P4,P5]**.

3. Monitoring studies of the aquatic environment and soil confirmed that AMs (and especially SAs) are stable and can accumulate and spread in the environment, both in urban and rural areas **[P1,P7]**.

4. It was confirmed that the fate of AMs after introduction into the soil is determined by their physicochemical properties. Depending on sorption properties, water solubility, stability to environmental factors, and bioavailability, AMs can (I) migrate into the soil profile with rainfall, (II) accumulate in the soil, or (III) degrade to form TPs **[P4,P5]**.

5. Heterogeneous photocatalysis was the most effective method for removing AMs from water and untreated wastewater. A serious limitation of photocatalysis is the possibility of deactivation of the catalyst by components of the wastewater matrix, which can adsorb on its surface and inhibit photocatalytic reactions by absorbing light radiation. Moreover, the use of mild photocatalytic conditions simulating the average solar irradiance in Poland does not lead to complete mineralization of AMs in the sample **[P3,P9]**.

6. In the phytoremediation process, pollutants are removed mainly as a result of the action of biotic factors (uptake by the plant, microbiological decomposition) and abiotic factors (photolysis, hydrolysis). Which of the factors will be dominant depends on the physicochemical properties of AMs. For phytoremediation to be effective, it is necessary to provide optimal conditions for the growth and development of the plant **[P6,P8]**.

7. A total of 119 TPs were identified in all environmental samples. Transformation products of AMs were present in all environmental samples where the parent drugs were detected. These products may be resistant to biological and photochemical wastewater treatment methods or may form during the treatment processes. Transformation of AMs also occurs after their introduction into soil or water **[P1–P9]**.

8. After being taken up by the plant, AMs undergo metabolic transformations to form TPs, which, like the parent compounds, can be distributed within plant tissues and accumulated by the plant **[P5,P6,P8].**

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Academic achievements

Publication list:

1. Płonka, J.; Górny, A.; **Kokoszka (Stando), K**.; Barchanska, H. Metabolic profiles in the course of the shikimic acid pathway of Raphanus sativus var. longipinnatus exposed to mesotrione and its degradation products. *Chemosphere* **2020**, *245*, 125616, doi:10.1016/j.chemosphere.2019.125616. **(IF=7,086; 140 pkt, TOP10)**
2. Bajkacz, S.; Adamczewska, P.; **Kokoszka (Stando), K.**; Kycia-Słocka, E.; Sochacki, A.; Felis, E. Supramolecular Solvent-Based Microextraction of Selected Anticonvulsant and Nonsteroidal Anti-Inflammatory Drugs from Sediment Samples. *Molecules* **2020**, *25*, 1–20, doi:10.3390/molecules25235671. **(IF=4,411; 140 pkt)**
3. **Kokoszka (Stando), K.;** Kobus, A.; Bajkacz, S. Optimization of a method for extraction and determination of residues of selected antimicrobials in soil and plant samples using HPLC-UV-MS/MS. *Int. J. Environ. Res. Public Health* 2021, 18, 1–14, doi:10.3390/ijerph18031159. **(IF=4,614; 140 pkt)**
4. Buta, M.; Korzeniewska, E.; Harnisz, M.; Hubeny, J.; Zieliński, W.; Rolbiecki, D.; Bajkacz, S.; Felis, E.; **Kokoszka (Stando), K**. *Sci. Total. Environ*. Microbial and chemical pollutants on the manure-crops pathway in the perspective of "One Health " holistic approach. *Sci. Total Environ.* 2021, 785, 147411, doi:10.1016/j.scitotenv.2021.147411. **(IF=10,753; 200 pkt, TOP10)**
5. **Kokoszka (Stando), K.;** Wilk, J.; Felis, E.; Bajkacz, S. Application of UHPLC-MS/MS method to study occurrence and fate of sulfonamide antibiotics and their transformation products in surface water in highly urbanized areas. *Chemosphere* **2021**, *283*, 131189, doi: 10.1016/j.chemosphere.2021.131189. **(IF=8,943; 140 pkt, TOP10)**
6. Płonka, J.; Barchanska, H.; **Kokoszka (Stando), K**.; Krzyżanowska, A. Effect of herbicide stress on the content of tyramine and its metabolites in Japanese radish sprouts (Raphanus sativus). *J. Food Compost. Anal. .* **2021,** 104301, doi:10.1016/j.jfca.2021.104301. **(IF=4,520; 100 pkt)**
7. **Kokoszka (Stando), K.;** Zieliński. W.; Korzeniewska, E.; Felis, E.; Harnisz, M.; Bajkacz, S. Suspect screening of antimicrobial agents transformation products in environmental samples development of LC-QTrap method running in pseudo MRM transitions. *Sci. Total. Environ.* **2022**, 808, 152114, doi:10.1016/j.scitotenv.2021.152114. **(IF=10,753; 200 pkt)**
8. **Stando, K**.; Kasprzyk, P.; Felis, E.; Bajkacz, S. Heterogeneous Photocatalysis of Metronidazole in Aquatic Samples. *Molecules* **2021,** 26(*24*), 1-16, doi: 10.3390/molecules26247612. **(IF=4,927, 140 pkt)**
9. **Stando, K.;** Korzeniewska, E.; Felis, E.; Harnisz, M.; Buta M.; Bajkacz, S. Determination of antimicrobial agents and their transformation products in an agricultural water-soil system modified with Manure. *Sci. Rep.* **2022,** *12,* 17529*,* doi: 10.1038/s41598-022-22440-5. **(IF=4,996; 140 pkt, TOP10)**
10. **Stando, K.;** Korzeniewska, E.; Felis, E.; Harnisz, M.; Bajkacz, S. Uptake of pharmaceutical pollutants and their metabolites from soil fertilized with manure to parsley tissues. *Molecules* **2022**, 27(14), 1–20, doi: 10.3390/molecules27144378. **(IF=4,600; 140 pkt)**
11. **Stando, K.;** Czyż, A.; Gajda, M.; Felis, E.; Bajkacz, S. Study of the Phytoextraction and Phytodegradation of Sulfamethoxazole and Trimethoprim from Water by *Limnobium laevigatum. Int. J. Environ. Res. Public Health* **2022***, 19,* 1-18, doi:10.3390/ijerph192416994. **(IF=4,614; 140 pkt)**
12. Hasija, V.; Singh, P.; Thakur, S.; **Stando, K.**; Nguyen, V.; Le, Q.; Alshehri, S; Ahamad, T.; Wu, K.; Raizada, P. Oxygen doping facilitated N-vacancies in g-C$_3$N$_4$ regulates electronic band gap structure for trimethoprim and Cr (VI) mitigation: Simulation studies and photocatalytic degradation pathways. *Appl. Mater. Today* **2022**, *29*, 101676, doi:10.1016/j.apmt.2022.101676. **(IF=8,300; 140 pkt)**
13. Wolak, I.; Bajkacz, S.; Harnisz, M.; **Stando, K.**; Męcik, M.; Korzeniewska, E. Digestate from Agricultural Biogas Plants as a Reservoir of Antimicrobials and Antibiotic Resistance Genes-Implications for the Environment. *Int. J. Environ. Res. Public Health* **2022***, 19,* 1–18, doi:10.3390/ijerph20032672. **(20 pkt)**

14. **Stando, K.**; Wilk, J.; Jakóbik-Kolon, A.; Felis, E.; Bajkacz, S. Application of UHPLC-MS/MS method to monitor the occurrence of sulfonamides and their transformation products in soil in Silesia, Poland. *Enviro. Sci. & Pollution Res.* **2023**, 30, 112922–112942, doi: 10.1007/s11356-023-30146-y. **(IF=5,800; 100 pkt)**

15. **Stando, K.**; Grzybowski, M.; Byczek-Wyrostek, A.; Bajkacz, S. Efficiency of phytoremediation and identification of biotransformation pathways of fluoroquinolones in the aquatic environment. *Int. J. Phytoremediation* **2023**, 26(7), 1027–1037, doi: 10.1080/15226514.2023.2288898. **(IF= 3,700; 100 pkt)**

16. **Stando, K.**; Kopiec, M.; Pieszczek, M.; Zogornik, P.; Kowalska, K.; Felis. E.; Bajkacz, S. Degradation of fluoroquinolones and macrolides by solar light-driven heterogeneous photocatalysis – Proposed drug transformation pathways; *J. Photochem. Photobiol. A;* **2024**; 453; 115651; doi: 10.1016/j.jphotochem.2024.115651. **(IF=4,300; 70 pkt)**

$$\sum\nolimits_{IF} = 92,317 \qquad \sum\nolimits_{MNiSW} = 2050$$

h-index = 7 (according to Google Scholar and Scopus database)

Monographs:

1. Bajkacz, S.; **Kokoszka (Stando), K**. Analiza niecelowana jako narzędzie do poszukiwania produktów przemian ksenobiotyków w środowisku. *Bioanalityka tom 2, nowe strategie analityczne i rozwiązania aparaturowe*; Staneczko-Baranowska, I., Buszewski, B., Eds.; PWN: Warszawa, **2020**; pp. 643–660, ISBN: 978-83-01-21282-7.

2. Bajkacz, S.; **Stando, K.** Non-targeted analysis as a tool for serching transformation products*. Handbook of Bioanalytics;* Staneczko-Baranowska, I., Buszewski, B., Eds.; Springer Nature: Szwajcaria, **2022**; pp. 1–23, ISBN: 978-3-030-63957-0.

Oral presentations (personally delivered):

1. **K. Kokoszka (Stando),** S. Bajkacz, **oral presentation**: „New analytical tools for the determination of anthropogenic pollutants in the environmental samples". National Scientific Conference „Understand the Science" IV Edition, (26 September 2020), international conference (on-line).

2. **K. Kokoszka (Stando),** S. Bajkacz, **oral presentation**: „Analiza niecelowana jako narzędzie do identyfikacji produktów transformacji wybranych farmaceutyków w środowisku", e-Zjazd Wiosenny SSPTChem 2021, (27–29 May 2021), national conference (on-line).

3. **K. Kokoszka (Stando),** S. Bajkacz, **oral presentation**: „Oznaczanie pozostałości środków bakteriobójczych oraz identyfikacja ich produktów transformacji w środowisku wodnym". 63. Zjazd Naukowy Polskiego Towarzystwa Chemicznego (Łódź, 13–17 September 2021), national conference.

4. **K. Stando,** S. Bajkacz, E. Felis, M. Harnisz, E. Korzeniewska, **oral presentation**: „LC-MS/MS w badaniach akumulacji i degradacji wybranych leków weterynaryjnych w glebie oraz tkankach pietruszki". XI Polska Konferencja Chemii Analitycznej, PoKoChA 2022 (Łódź, 19–23 June 2022), national conference.

Poster presentations (personally presented):

1. J. Płonka, **K.Kokoszka (Stando), poster**: „Metabolity wtórne w procesie detoksyfikacji rośliny potraktowanej herbicydami". Aktualne problemy chemii analitycznej : XIII Seminarium naukowe, (Katowice, 17 May 2019), national conference.

2. **K. Kokoszka (Stando),** W. Zieliński, E. Korzeniewska, E. Felis, M. Harnisz, S. Bajkacz, **poster**: „Analysis of pharmaceutical residues and their transformation products in groundwater and municipal sewage samples". QUO VADIS Life Sciences (Opole, 23–27 June 2021), international conference (on-line). **(The poster was awarded in the category of Best Poster.)**

3. **K. Kokoszka (Stando),** S. Bajkacz, **poster**: „Development of a method for extraction and determination of residues of selected antimicrobials in soil and plant tissues". QUO VADIS Life Sciences (Opole, 23–27 June 2021), international conference (on-line), national conference.

4. **K. Stando,** J. Wilk, S. Bajkacz, **poster**: „Occurrence of transformation products of sulfonamides in environmental water samples from urbanized areas of Silesia, Poland". 5th Symposium on Biotransformations for Pharmaceutical and Cosmetic Industry (Kroczyce, 13–15 June, 2022), national conference.

5. **K. Stando,** S. Bajkacz, E. Felis, M. Harnisz, E. Korzeniewska, **poster**: „Distribution and degradation of antimicrobials in the soil-water system". 5th Symposium on Biotransformations for Pharmaceutical and Cosmetic Industry (Kroczyce, 13–15 June, 2022), national conference.

6. **K. Stando,** S. Bajkacz, P. Kasprzyk, E. Felis, **poster**: „Zastosowanie fotokatalizy heterogenicznej do oczyszczania wody z pozostałości metronidazolu". XI Polska Konferencja Chemii Analitycznej, PoKoChA 2022 (Łódź, 19–23 June 2022), national conference.

7. **K. Stando,** A. Czyż, M. Gajda, E. Felis, S. Bajkacz, **poster**: „Zastosowanie Limnobium Laevigatum do fitoremediacji wody zanieczyszczonej sulfametoksazolem oraz trimetoprimem". Zjazd Zimowy SMPTChem 2022 (Opole, 10 December 2022), national conference.

Co-authorship of lectures, oral presentations, and poster presentations:

1. N. Kuźnik, M. Wysocka-Gajda, M.M. Tomczyk, K. Bakalorz, A. Buchcik, M. Marczyk, **K. Kokoszka (Stando), oral presentation**: „Środki kontrastowe do obrazowania magnetyczno-rezonansowego". II Seminarium Ogólnoakademickie Metody fizykochemiczne w badaniach naukowych, (Sosnowiec, 23 April 2018), national conference.

2. J. Wilk, **K. Stando,** S. Bajkacz, **poster**: „Porównanie występowania wybranych sulfonamidów w środowisku wodnym i w glebie na terenach miejskich województwa śląskiego". Zjazd Zimowy SMPTChem 2022 (Opole, 10 December 2022), national conference.

3. A. Kicińska, **K. Stando,** S. Bajkacz, **poster**: „Rozkład tetracykliny w procesach inicjowanych światłem słonecznym". Zjazd Wiosenny SMPTChem 2023 (Chęciny, 3–7 May 2023), national conference.

4. J. Wilk, **K. Stando,** A. Grabowska, S. Bajkacz, **poster**: „Zastosowanie procesów inicjowanych światłem do usuwania deksametazonu z roztworów wodnych". XVI Konferencja Naukową „Mikrozanieczyszczenia w środowisku człowieka (Częstochowa, 18–20 September 2024), national conference.

5. J. Wilk, **K. Stando,** S. Bajkacz, **poster**: „Opracowanie metod ekstrakcji i oznaczania leków antybakteryjnych w próbkach środowiskowych". XIII Polska Konferencja Chromatograficzna (Katowice, 25–28 June 2023), national conference.

6. J. Wilk, **K. Stando,** A. Grabowska, S. Bajkacz, **poster**: „Zastosowanie LC-MS/MS w ocenie stopnia degradacji farmaceutyków w ciekłych próbkach modelowych". Zjazd Zimowy SMPTChem 2023 (Łódź, 09 December 2023), national conference.

7. A. Kicińska, **K. Stando,** S. Bajkacz, **poster**: „Rozkład mikrozaniczyszczeń w procesach inicjowanych światłem słonecznym – identyfikacja produktów degradacji". Zjazd Zimowy SMPTChem 2023 (Łódź, 9 December 2023), national conference.

8. A. Kicińska, **K. Stando,** E. Felis, S. Bajkacz, **oral presentation**: „Fotodegradacja tetracyklin w procesach inicjowanych światłem słonecznym". I Warszawskie Sympozjum Chemiczne „w ProbUWce" (Warszawa, 27–28 April 2024), national conference.

9. S. Bajkacz, **K. Stando**, J. Wilk, E. Korzeniewska, M. Harnisz, E. Felis, **oral presentation**: „Antibiotic residues in the environment". XIV Międzynarodowa Konferencja Naukowa „Chromatografia Jonowa i Techniki Pokrewne 2024 (Katowice, 9–10 April 2024), international conference.
10. S. Bajkacz, **K. Stando**, E. Felis, M. Harnisz, E. Korzeniewska, **oral presentation**: „Losy środków bakteriobójczych w ekosystemach rolniczych". 66. Zjazd Polskiego Towarzystwa Chemicznego, PTChem 2024 (Poznań, 15–20 September 2024), national conference.

Research projects:

1. Project leader (May 2020 – January 2021): „Oznaczanie antybiotyków sulfonamidowych w próbkach wód powierzchniowych", Badania statutowe, młodzi naukowcy (BKM-542/RCH-1/2020; 04/010/BKM20/0130).
2. Project leader (May 2021 – January 2022): „Badania wpływu procesów fotokatalitycznych na stabilność wybranych farmaceutyków", Badania statutowe, młodzi naukowcy (BKM-516/RCH1/2021; 04/010/BKM21/1023).
3. Project leader (May 2022 – January 2023): „Zastosowanie fitoremediacji do oczyszczania wód z pozostałości środków bakteriobójczych", Badania statutowe, młodzi naukowcy (BKM-593/RCH1/2022; 04/010/BKM22/1046).
4. Project leader (17 May 2022 – 16 November 2023): „Zastosowanie fitoremediacji do oczyszczania wód z pozostałości niesteroidowych leków przeciwzapalnych", Grant na rozpoczęcie działalności naukowej w nowej tematyce badawczej (IDUB: 32/014/SDU/10-22-38).
5. Project contributor (October 2019 – August 2022): „Lekooporność drobnoustrojów środowiskowych w perspektywie podejścia holistycznego "Jedno Zdrowie", Projekt OPUS 14 finansowany przez Narodowe Centrum Nauki (NCN) (2017/27/B/NZ9/00267).