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ÚSTAV CHEMIE A TECHNOLOGIE OCHRANY ŽIVOTNÍHO PROSTŘEDÍ

EVALUATION OF THE OCCURRENCE OF SELECTED PHARMACEUTICALS IN THE SOIL ECOSYSTEM

HODNOCENÍ VÝSKYTU VYBRANÝCH LÉČIVÝCH LÁTEK V PŮDNÍM EKOSYSTÉMU

DOCTORAL THESIS

DIZERTAČNÍ PRÁCE

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ABSTRACT

Pharmaceuticals, including antibiotics, are essential for treating infectious diseases in humans, livestock, and aquaculture. However, up to 90% of the administered dose may be excreted unmetabolised as the parent compound in faeces or urine. Consequently, these pharmaceuticals, along with their degradation products and metabolites, can enter the terrestrial environment through various pathways, such as irrigation with contaminated wastewater, incorporation of biosolids, or application of animal manure. Pharmaceutical residues in soil pose ecotoxicological risks and can be taken up by plants and soil organisms, potentially disrupting soil ecosystems and amplifying their environmental impact. Additionally, there are growing concerns about the risks to human health associated with the long-term intake of these micropollutants through food, including their potential contribution to the development of antimicrobial resistance. Recognizing these threats, the European Union and international organizations such as World Health Organization, United Nations Environment Programme, and Organisation for Economic Co-operation and Development have highlighted pharmaceutical residues in the environment as a major concern, though current scientific knowledge remains inadequate to constructing relevant policy and management recommendations.

This doctoral thesis presents multiresidue methods for quantifying up to 42 pharmaceutical residues in soil using SPE, and in lettuce and earthworms using QuEChERS, followed by UHPLC-MS analysis. A novel analytical workflow for pharmaceutical metabolite annotation, utilising *in silico* spectral libraries coupled with LC-HRMS, was introduced for lettuce and earthworms, leading to the annotation of 26 compounds. Furthermore, uptake experiments with *Eisenia fetida* in soil and *Lactuca sativa* under both soil and hydroponic conditions, exposed to varying pharmaceutical concentrations, were conducted to assess the fate of pharmaceuticals (including bioconcentration and translocation factors, as well as pharmaceutical degradation kinetics) and ecotoxicological endpoints (e.g., mortality rates and biomass weight). The data from these experiments were statistically analysed to enable meaningful and objective evaluation of the conclusions, trends, and effects of pharmaceutical residues on these organisms. In addition, risk assessments were performed to evaluate the potential for antimicrobial resistance emergence in the environment and to estimate potential health risks associated with lettuce consumption due to pharmaceutical residues. Finally, various advanced oxidation processes for wastewater treatment, fermentation of animal manure and the effect of biochar in soil were assessed as potential solutions for mitigating pharmaceutical residues in the environment. In particular, the impact of biochar on the bioavailability of these compounds to organisms, including lettuce and earthworms, was evaluated.

KEYWORDS

pharmaceutical residues, solid-phase extraction, QuEChERS, liquid chromatography, mass spectrometry, pharmaceutical uptake, ecotoxicology, risk assessment

OBJECTIVES OF THE DISSERTATION

Overall, this doctoral thesis aimed to contribute to filling the following research gaps:

1) Development and optimisation of multiresidue analytical methods for pharmaceutical extraction from various matrices,

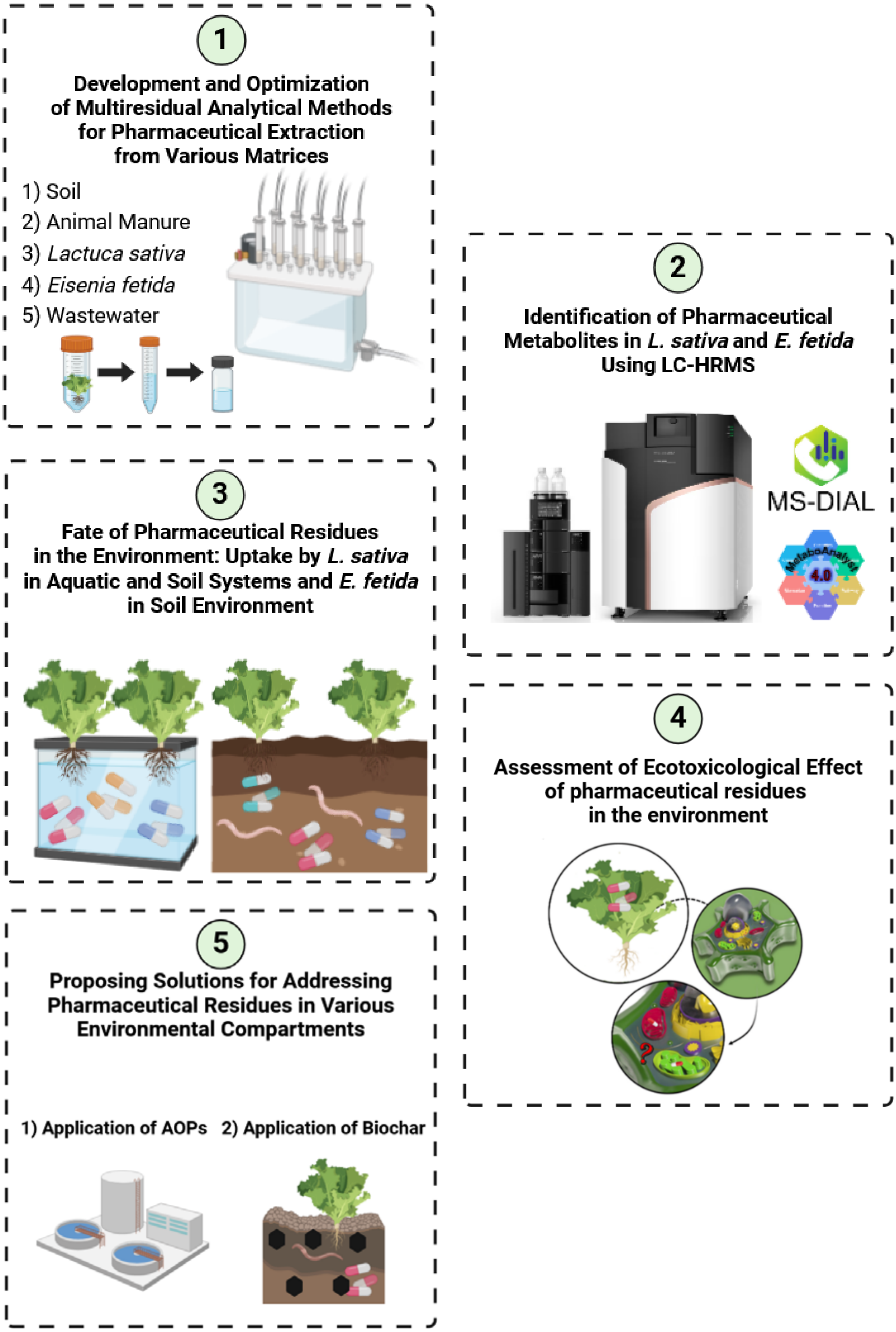
2) Identification of pharmaceutical metabolites in *L. sativa* and *E. fetida* using LC-HRMS,

3) Fate of pharmaceutical residues in the environmental: uptake by *L. sativa* in aquatic and soil systems and *E. fetida* in soil environment,

4) Assessment of the ecotoxicological effect of pharmaceutical residues in the environment, and

5) Proposing solutions for addressing pharmaceutical residues in various environmental compartments

GRAPHICAL ABSTRACT



1

Development and Optimization of Multiresidual Analytical Methods for Pharmaceutical Extraction from Various Matrices

1) Soil
2) Animal Manure
3) *Lactuca sativa*
4) *Eisenia fetida*
5) Wastewater

2

Identification of Pharmaceutical Metabolites in *L. sativa* and *E. fetida* Using LC-HRMS

3

Fate of Pharmaceutical Residues in the Environment: Uptake by *L. sativa* in Aquatic and Soil Systems and *E. fetida* in Soil Environment

4

Assessment of Ecotoxicological Effect of pharmaceutical residues in the environment

5

Proposing Solutions for Addressing Pharmaceutical Residues in Various Environmental Compartments

1) Application of AOPs
2) Application of Biochar

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CHAPTER 1 INTRODUCTION

The use of antibiotics (and pharmaceuticals in general) plays a crucial role in the treatment of infectious diseases in humans, livestock, and aquaculture [1, 2]. Statistics on pharmaceutical consumption indicate that in 2022, 4,458 tonnes of veterinary antibiotics were used for livestock within the European Union [3]. In some countries, veterinary antibiotics are used not only for therapeutic purposes but also to increase production in animal farming (prohibited in the European Union since 2006) [4]. Outside the European Union, countries such as Mexico, South Korea, and New Zealand have also banned the use of antibiotics as growth promoters; however, there are still many countries where this practice is not prohibited (e.g., Argentina, China, India, Russia, South Africa) [5]. Depending on pharmacokinetics, specific transformation processes, the route of drug administration, and the class of antibiotics, however, 30% to 90% of the administered drug dose may be excreted unmetabolised in faeces or urine [6–8].

As a result, these pharmaceuticals (including their degradation products and metabolites) can enter the environment (particularly soil or water environment) through several different routes. Some pharmaceutical residues are not entirely removed by conventional methods used in wastewater treatment plants and, therefore, may be present in the effluent from wastewater treatment plants as well as in the sewage sludge. These residues can subsequently enter the soil, for example, through the irrigation of wastewater (wastewater recycling), the application of animal manures (e.g., pig manure, poultry litter, cattle manure or slurry), or the application of treated sewage sludge (biosolids) to agricultural land [9]. A less significant route of dissemination for these micropollutants into the environment can be atmospheric transport, where contaminated dust particles from animal farming are carried by the wind [5]. Contrary to expectations, the pharmaceutical industry can also be a significant source of contamination, especially when waste, such as penicillin fermentation remains, is not properly treated [10, 11].

Concentrations of pharmaceutical residues in wastewater typically range from tenths of $ng \cdot L^{-1}$ to several $mg \cdot L^{-1}$ [11–20], in river and pond sediments from hundredths of $ng \cdot g^{-1}$ to hundreds of $\mu g \cdot g^{-1}$ [20–22], in soil ecosystems from hundredths of $ng \cdot g^{-1}$ to hundreds of $\mu g \cdot g^{-1}$ [18, 20, 23–25]. In organic manures, concentrations range from units of $ng \cdot g^{-1}$ to thousands of $\mu g \cdot g^{-1}$ [18, 24], in biosolids from units to tens of thousands of $\mu g \cdot kg^{-1}$ [19, 26] and in plants from tenths of $ng \cdot g^{-1}$ to hundreds of $\mu g \cdot g^{-1}$, depending on the level of soil contamination [20, 27, 28]. In soil organisms, concentrations are on the order of $ng \cdot g^{-1}$, depending on the level of terrestrial contamination [29].

In Europe, the practice of recycling organic manures on arable land is widely supported within the framework of the circular economy as a potential substitute or supplement to mineral fertilisers [30]. Worldwide, approximately 5.6 billion cubic meters of wastewater are used for agricultural irrigation, which represents less than 1% of the water consumed in agriculture. However, for some countries, wastewater is an essential irrigation resource; for example, in Israel, more than 85% of the wastewater produced is used for irrigation, in Jordan 38%, in California approximately 46%, and globally, the use of wastewater in agriculture is expected to continue increasing due to drought and limited freshwater resources. Additionally, in the USA and Europe, a total of 7.2 and 4.7 million tonnes (dry weight) of sewage sludge are produced each year, respectively (with this quantity continually rising due to increasing population and urbanisation), and approximately 50% of this amount is applied to agricultural land, which has a positive impact on soil properties [31, 32]. However, this circular economy may indirectly contribute to the spread of pharmaceutical residues into various environmental compartments [30]. These pharmaceutical residues can be retained in the specific environmental compartment, and depending on their properties and the surrounding matrix, some of these substances may be further transported through the environment. For instance, in the case of soil, pharmaceutical residues can be washed out into surface- and groundwater through surface runoff and infiltration [33].

Several studies have shown that crops grown in contaminated soil, following the application of manure, wastewater, or sewage sludge, may contain trace amounts of pharmaceutical residues (including their degradation products and metabolites). This raises concerns about potential risks to human health associated with the long-term intake of these micropollutants through food. Additionally, there are concerns about the impact of these substances on human microbiota and the development of antimicrobial resistance [5, 34, 35].

Soil contamination with these substances can also inhibit seed germination and crop growth [5]. Studies [29, 36] have shown that pharmaceuticals can be taken up and bioaccumulated by soil organisms, particularly invertebrates such as earthworms. These organisms are vital for maintaining soil fertility and form the basis of many food chains, potentially serving as prey for predators and thus can lead to secondary poisoning due to the bioaccumulation of substances.

Increasing concentrations of antimicrobial substances (including their metabolites and degradation products) in the environment pose a potential threat to all organisms [1, 20]. Even concentrations of antibiotics lower than the minimum inhibitory concentration can foster genetic changes in bacterial genomes and the transfer of antibiotic resistance genes and related mobile genetic elements. Contaminated aquatic and soil ecosystems create conditions that promote the selective growth of resistant bacteria, altering the sensitivity of entire microbial communities to antibiotics. Unfortunately, these resistance genes can also be transferred to bacteria that colonise the human body [1].

Antimicrobial resistance can be either intrinsic or acquired through mutations (vertical evolution) or through transfer from other microorganisms (horizontal evolution) that already possess resistance, via processes such as conjugation, transformation, or transduction. Most bacterial strains present in aquatic environments carry antibiotic resistance genes that can be incorporated into mobile genetic elements like integrons, transposons, and plasmids. These elements can easily transfer these genes and result in the mutation of naturally occurring bacterial communities. Several effective mechanisms by which microorganisms acquire antimicrobial resistance have been identified, including efflux pumps, horizontal gene transfer, plasmids, mutations, and enzymatic modification (Figure 1.1) [37, 38]. Ultimately, antimicrobial resistance can severely diminish our chances of effective treatment for diseases, as no new class of antibiotics has been discovered since 1987 [7].

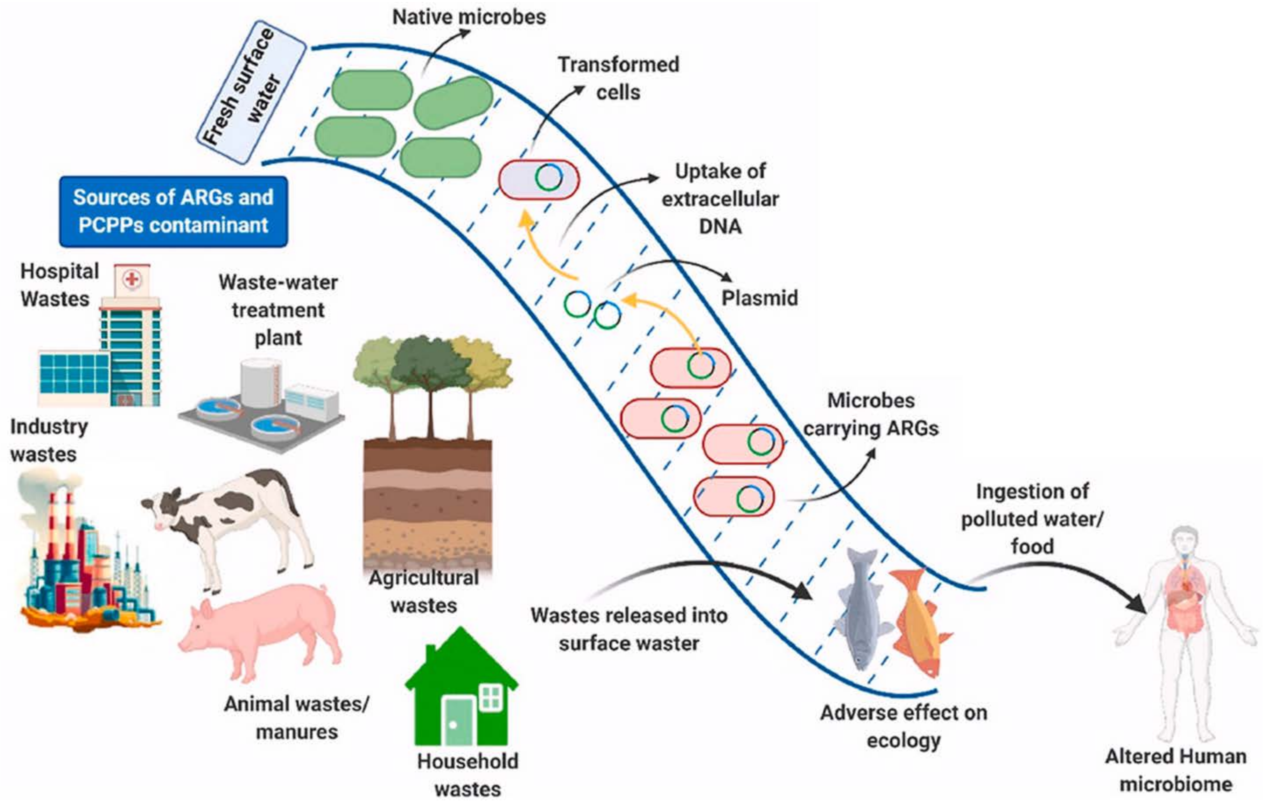

Figure 1.1 *The Origin of Antimicrobials in the Environment and their Impact [38]*

A 2016 review [39] estimates that antimicrobial resistance currently causes approximately 700,000 deaths annually, with projections indicating that this number could rise to 10 million per year by 2050. A 2019 study [40] conducted a statistical analysis of the impact of antimicrobial resistance on incidence rates, mortality, hospitalisation duration, and healthcare costs for specific pathogen-drug combinations in selected locations. The results are highly concerning, as statistical models estimate that globally, antimicrobial resistance causes between 0.91 and 1.17 million deaths, with an additional 2.62 to 4.78 million cases associated with antimicrobial resistance (particularly with bacteria resistant to third-generation cephalosporins, carbapenems, or fluoroquinolones) [40].

One of the greatest challenges of our time is maintaining the quality of life with respect to environment. According to the EU, one of the most pressing issues is pharmaceuticals in the environment. Although the occurrence, fate (including transformation), and effects of antibiotics in agricultural soil have been studied, our understanding of this area remains incomplete. Knowledge about the potential risks to both the environment (soil ecology, terrestrial and aquatic organisms/plants) and human health is still limited [41, 42].

The European Union and international organisations (such as World Health Organization, United Nations Environment Programme, and Organisation for Economic Co-operation and Development) have recognised that pharmaceutical residues in the environment pose a potential threat. Numerous initiatives have been adopted or are in progress, including the European Green Deal, the Farm to Fork Strategy, the Eighth Environmental Action Programme, and the Circular Economy Action Plan [43, 44]. The Antimicrobial Expert Group within the European Union and World Health Organization have identified several classes of antibiotics as critically important for human medicine. These include cephalosporins, polymyxins, fluoroquinolones, quinolones, and macrolides, which are still authorised for use in veterinary medicines within the EU [45]. Additionally, the EU implemented new regulations on January 28, 2022, introducing strict limits on antibiotic use in farm animals. Antibiotics cannot be used routinely or as a substitute for poor hygiene, inadequate animal care, or poor farm management. Their use for disease prevention in groups of animals (metaphylaxis) is permitted only when there is a high risk of disease spreading and no suitable alternatives are available. These measures aim to combat antibiotic resistance and encourage responsible antibiotic use in agriculture [46].

Furthermore, EU legally mandates that foodstuffs such as meat, milk, and eggs must not contain residue levels of veterinary medicines or biocidal products that could pose a risk to consumer health. The maximum residue limits vary depending on the specific pharmaceutical residue and animal tissue, ranging from cases where no maximum residue limit is required to several hundred $\mu g \cdot kg^{-1}$ [47]. Additionaly, World Health Organization has established an acceptable daily intake (ADI) of less than 50 $\mu g \cdot kg^{-1}$ body weight per day for most pharmaceutical residues. Specifically, the ADI for tetracycline is $< 30\ \mu g \cdot kg^{-1}$, for enrofloxacin $< 2\ \mu g \cdot kg^{-1}$ and for sulfamethazine $< 50\ \mu g \cdot kg^{-1}$ [48].

Regarding pharmaceuticals in the aquatic environment, EU updates the Watch List every two years to collect data and assess whether certain substances pose a risk at the EU level. Based on this assessment, Environmental Quality Standards may be established if necessary. The current 5th Watch List includes various organic micropollutants, including pharmaceutical residues such as fluoxetine, propranolol, oxytetracycline, tetracycline, norfloxacin, and tylosin [49, 50]. Additionally, in 2024, the European Union published the Urban Wastewater Treatment Directive to mitigate the environmental impact of wastewater discharges, including adverse effect of micropollutants. Growing concerns over pharmaceutical pollution have driven recent policy updates, mandating the gradual implementation of tertiary treatment technologies (e.g., activated carbon, ozonation) based on the size of wastewater treatment plants. Furthermore, the removal efficiency of specific organic substances, such as amisulpride, carbamazepine, citalopram, clarithromycin, diclofenac, hydrochlorothiazide, metoprolol, and venlafaxine, must be monitored to ensure compliance with the required minimum removal efficiency of 80%. In addition, the EU promotes the reuse of treated wastewater and sewage sludge in agriculture, provided adequate treatment standards are met [51].

Current policies focus solely on monitoring, research, and improving wastewater and agricultural practices to limit environmental contamination. However, there are currently no EU regulations establishing maximum residue limits for pharmaceutical residues in soil, animal manure (with policies regulating only organic matter and nitrogen, not pharmaceuticals), sewage sludge (where policies primarily focus on heavy metal concentrations), or vegetables for human consumption (with policies focusing on pesticide residue levels) [52–54].

Several federal agencies recognise that further research is necessary before deciding on regulations or restrictions regarding pharmaceuticals (including veterinary antibiotics) to achieve sustainability in agriculture, environmental protection, and most importantly, human health. Given the need for additional research in these areas, the following objectives (mentioned on Page 4 as Objectives of the Dissertation) have been set for the experimental part of this doctoral thesis [5, 55]:

1. Development and optimisation of multiresidue analytical methods for pharmaceutical extraction from various matrices (Chapter 2 on Page 13)
2. Identification of pharmaceutical metabolites in *L. sativa* and *E. fetida* using LC-HRMS (Chapter 3 on Page 14)
3. Fate of pharmaceutical residues in the environment: uptake by *L. sativa* in aquatic and soil systems and *E. fetida* in soil environment (Chapters 5–7 on Pages 16–18)
4. Assessment of the ecotoxicological effect of pharmaceutical residues in the environment (Chapters 5–7 on Pages 16–18)
5. Proposing solutions for addressing pharmaceutical residues in various environmental compartments (Chapters 4–8 on Pages 15–19)

CHAPTER 2 OPTIMISATION AND VALIDATION OF MULTIRESIDUE EXTRACTION METHODS FOR PHARMACEUTICALS IN SOIL, LETTUCE AND EARTHWORMS

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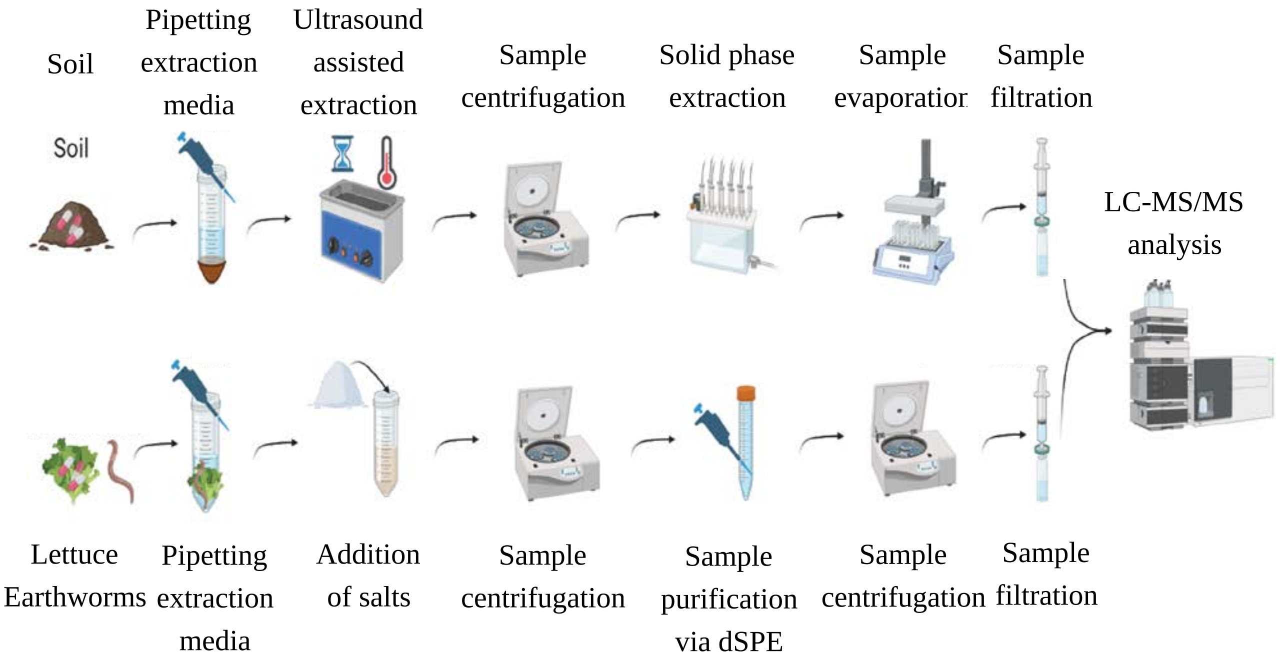
GRAPHICAL ABSTRACT



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CHAPTER 3 PHARMACEUTICAL METABOLITE IDENTIFICATION IN LETTUCE (*LACTUCA SATIVA*) AND EARTHWORMS (*EISENIA FETIDA*) USING LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY AND IN SILICO SPECTRAL LIBRARY

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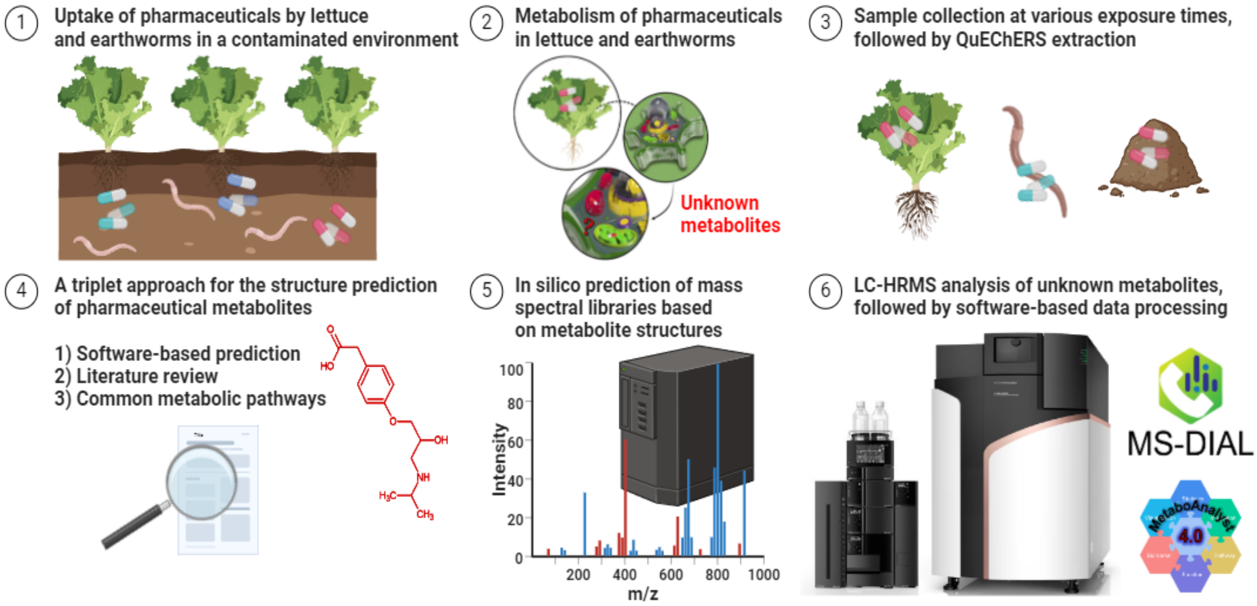

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CHAPTER 4 FATE OF FLUOROQUINOLONES IN FIELD SOIL ENVIRONMENT AFTER INCORPORATION OF POULTRY LITTER FROM A FARM WITH ENROFLOXACIN ADMINISTRATION VIA DRINKING WATER

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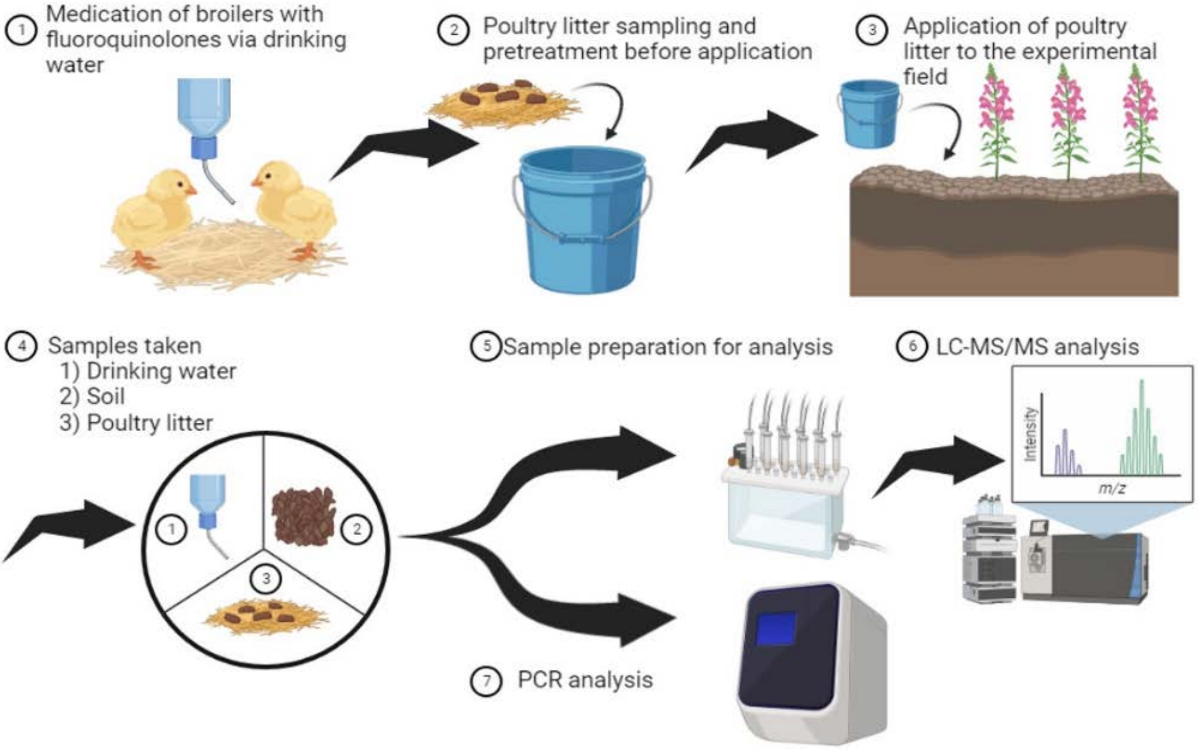

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CHAPTER 5 ASSESING EARTHWORM EXPOSURE TO MULTI-PHARMACEUTICAL MIXTURE IN SOIL: UNVEILING INSIGHTS THROUGH LC-MS AND MALDI-MS ANALYSES, AND IMPACT OF BIOCHAR ON PHARMACEUTICAL BIOAVAILABILITY

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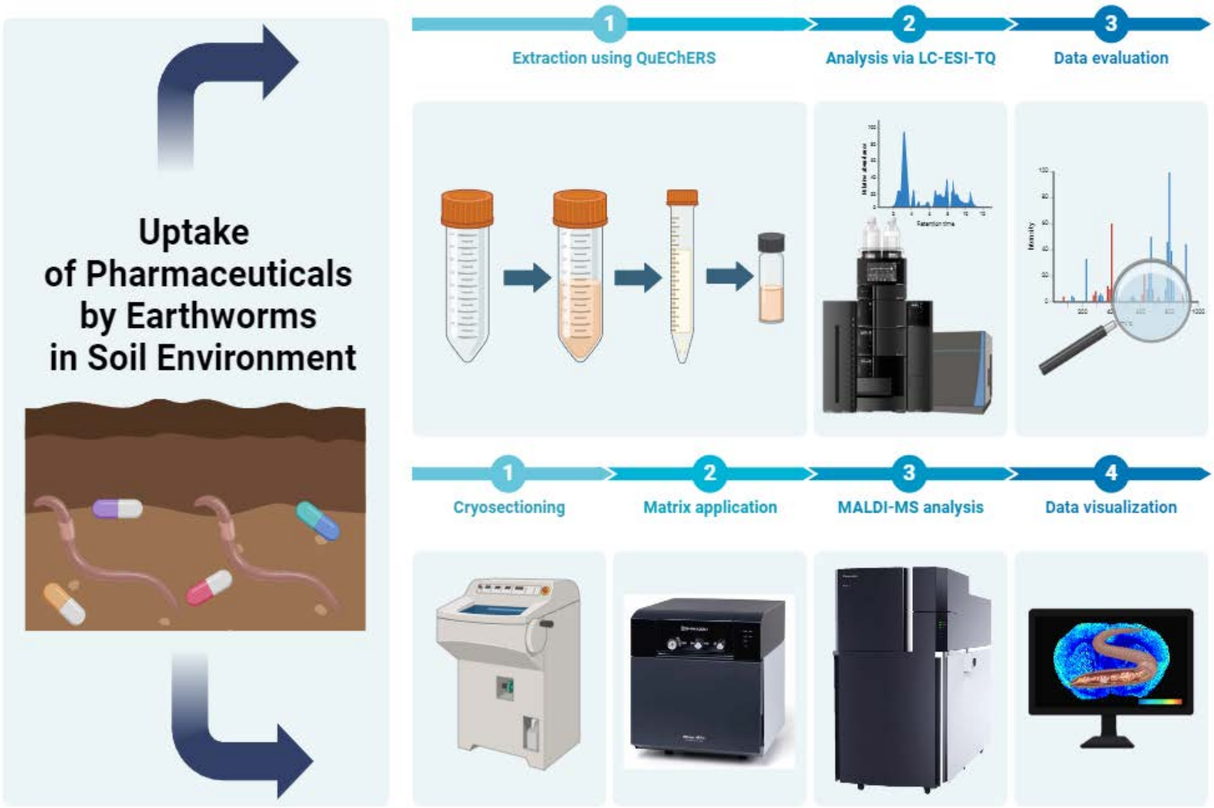

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CHAPTER 6 ASSESSING LETTUCE EXPOSURE TO A MULTI-PHARMACEUTICAL MIXTURE IN SOIL: INSIGHTS FROM LC-ESI-TQ ANALYSIS AND THE IMPACT OF BIOCHAR ON PHARMACEUTICAL BIOAVAILABILITY

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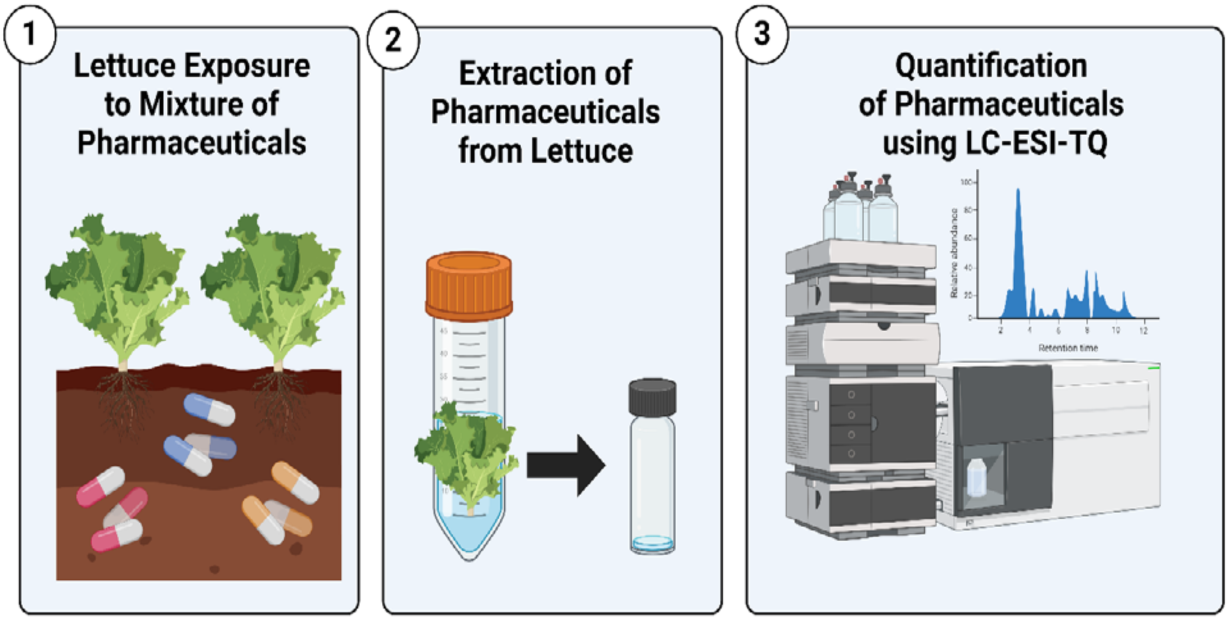

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CHAPTER 7 ASSESSING LETTUCE EXPOSURE TO A MULTI-PHARMACEUTICAL MIXTURE UNDER HYDROPONIC CONDITIONS: FINDINGS THROUGH LC-ESI-TQ ANALYSIS AND ECOTOXICOLOGICAL ASSESSMENTS

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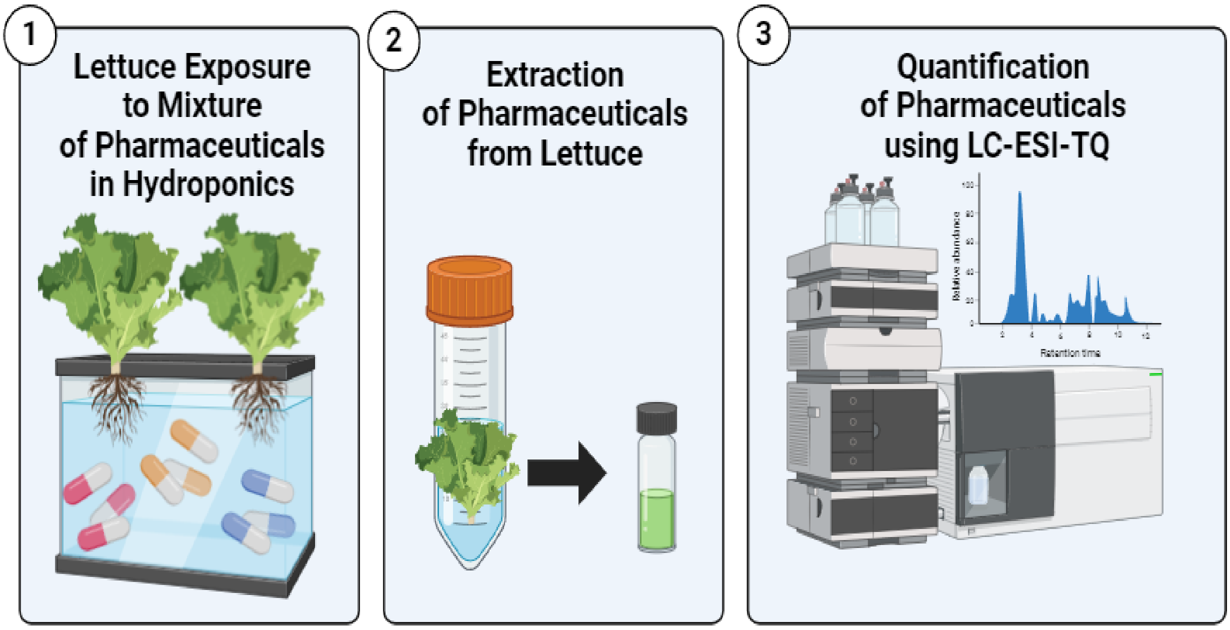

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CHAPTER 8 IMPACT OF VARIOUS OXIDATION PROCESSES USED FOR REMOVAL OF SULFAMETHOXAZOLE ON THE QUALITY OF TREATED WASTEWATER

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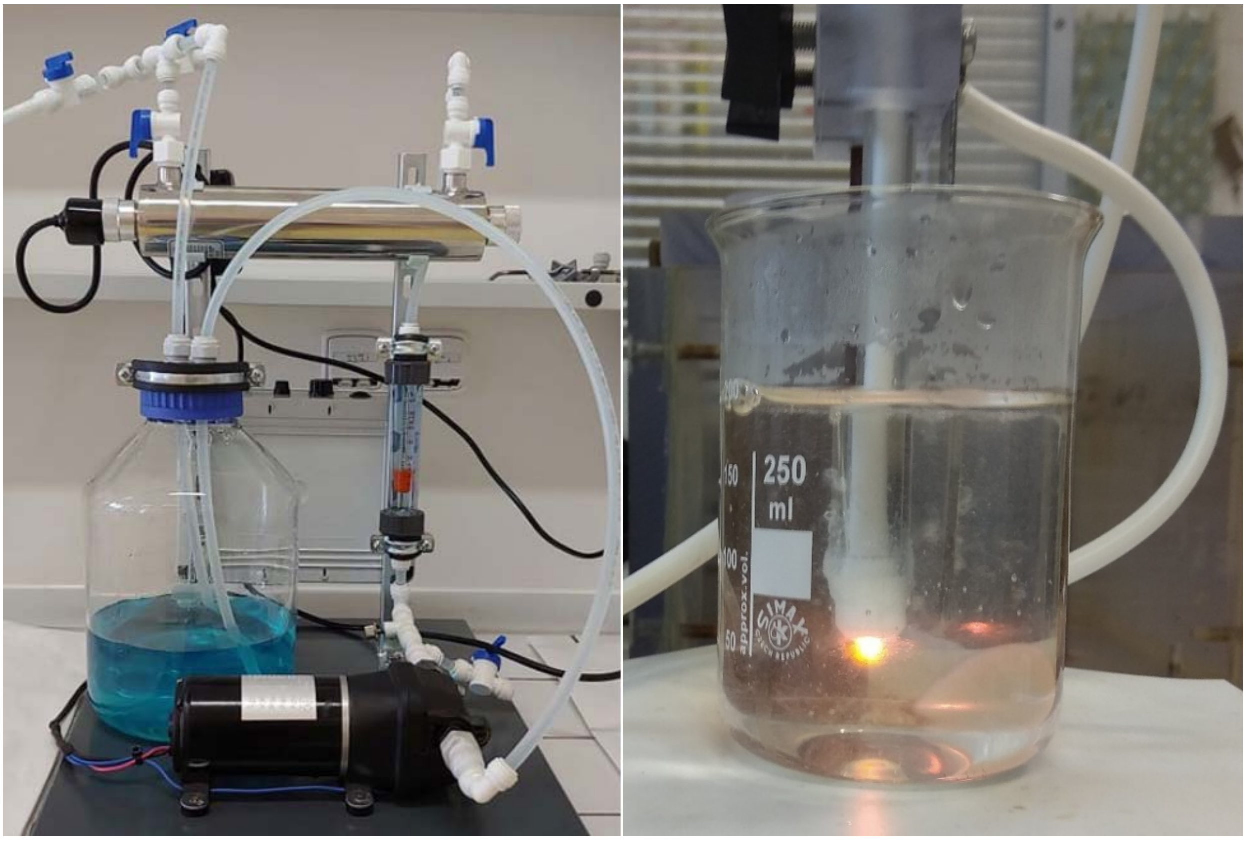

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CHAPTER 9 CONCLUSION

Before starting this doctoral research, several key objectives were defined to address existing research gaps and contribute to advancing the understanding of pharmaceutical residues in the environment. These research objectives include the following:

9.1 Development and Validation of Multiresidue Extraction Methods for Pharmaceutical Residues

Solid-phase extraction was applied to soil extracts, while the QuEChERS method was utilised for lettuce and earthworms. The extracted samples were then analysed using LC-MS, ensuring the accurate detection of pharmaceutical residues. The results of method validation demonstrated the robustness of these methods for a broad spectrum of PhACs. Specifically, 29 out of 42 PhACs were extracted with an average efficiency > 50% and RSD < 30% from the soil; 40 out of 42 PhACs exhibited an average efficiency > 50% and %RSD < 30% from the earthworms, while 39 out of 42 PhACs showed an average efficiency > 50% and RSD < 30% from the lettuce. This study presents three thoroughly validated methods for determining more than 40 PhACs in diverse matrices, enabling a comprehensive assessment of PhAC spread in the environment. These extractions methods were subsequently applied in the research conducted at the Faculty of Chemistry. Furthermore, they hold the potential for re-validation to target additional pharmaceuticals, provided appropriate pharmaceutical standards are available, as they have already been validated for a wide range of compounds across various therapeutic classes.

Moreover, these extraction methods have also been utilised in study focused on the identification of pharmaceutical metabolites. This suggests that these methods may also be suitable for the analysis of pharmaceutical metabolites and degradation products, further broadening their applicability. Additionally, the method developed for the extraction of pharmaceutical residues from lettuce could potentially be applied to other plant-based samples. It could also serve as a starting point for the optimisation of extraction protocols for different plant species, offering a flexible approach for broader environmental and agricultural studies.

9.2 Annotation of Pharmaceutical Metabolites in Lettuce and Earthworms

A novel analytical workflow for the annotation of pharmaceutical metabolites in lettuce and earthworm samples was introduced. This Metabolite identification workflow combined multiple strategies for pharmaceutical metabolite structure prediction, including literature searches, software predictions, and common metabolic pathways, followed by *in silico* prediction of MS^2 spectra. *L. sativa* and earthworms were exposed to a mixture of six pharmaceuticals from various therapeutic groups. Samples collected over several days were extracted using optimised QuEChERS methods and subsequently analysed using LC-qTOF in both positive and negative ionisation modes, employing both DDA and DIA approaches. This methodology led to the annotation of 26 statistically significant metabolites ($p < 0.05$), with DDA+ and DDA– outperforming DIA by successfully detecting 56/67 sample type:metabolite combinations. Lettuce roots had the highest metabolite count (26), followed by leaves (6) and earthworms (2). This study highlighted the importance of not only quantifying parent compounds in the environment, as this approach could lead to an underestimation of potential environmental and health risks. The data showed that pharmaceutical metabolites could occur at concentrations several times higher than their parent compounds in samples. Moreover, all software used for pharmaceutical metabolite structure prediction, *in silico* MS^2 spectral library prediction, LC-MS/MS data processing, and statistical analysis are open source and freely available to all scientists around the world. These tools are also independent of the instrument manufacturer, as LC-MS/MS data can be converted to the universal MS format (mzML) and used within this analytical workflow.

9.3 Fate of Antibiotics in Soil Following Animal Manure Application

The fate of fluoroquinolones in field soil environments after the incorporation of poultry litter from a poultry farm where enrofloxacin was administered via drinking water was investigated. Experiment was conducted under normal operational conditions that reflect common practices in poultry farming, the aim was to investigate the impact of incorporating poultry manure into soil, a practice supported by the European Circular Economy as a sustainable alternative to mineral fertilizers. While beneficial for agriculture, this practice may inadvertently contribute to the spread of antibiotic residues, resistant bacteria, and resistance genes, posing potential risks to both soil and public health. This study investigated the concentrations of enrofloxacin and its metabolite ciprofloxacin in medicated drinking water for broilers, poultry litter, and soil amended with poultry litter. PCR analyses were also employed to monitor the presence of Plasmid-Mediated Quinolone Resistance in poultry litter and soil samples. The results demonstrated the persistence of fluoroquinolones in the soil environment over 112 days, with enrofloxacin concentrations ranging from 36 $\mu g \cdot kg^{-1}$ to 9 $\mu g \cdot kg^{-1}$ after 100 days. The presence of resistance genes was detected in both poultry litter and soil samples, in agreement with the risk assessment indicating the potential for antimicrobial resistance selection in soil based on enrofloxacin concentrations. This study offers a comprehensive new perspective on the entry, long-term fate, and environmental consequences of antimicrobial residues following the incorporation of poultry litter into agricultural fields.

This study was part of a series of research projects commissioned by the Ministry of Agriculture of the Czech Republic, addressing the issue of antimicrobial resistance. Projects specifically focused on the persistence of these substances and their contribution to the development of antimicrobial resistance. The findings emphasized the critical need for the removal of pharmaceuticals from animal manure before its incorporation into soil. Subsequent projects [56–58] further support these conclusions, with additional investigations into the removal of pharmaceuticals from poultry litter (enrofloxacin contamination) and calf manure (tulathromycin contamination) through fermentation, prior to their incorporation into soil, where many antibiotics exhibit high persistence. Specifically, our 2023 research report [57] demonstrated that within one month, 78.3% of ciprofloxacin and 84.3% of enrofloxacin were degraded in poultry litter through anaerobic fermentation (initial concentrations were in units of $mg{\cdot}kg^{-1}$). However, concentrations plateaued in the following weeks. Additionally, our 2024 research report [58] revealed that 22% of tulathromycin degraded in calf manure over 42 days (initial concentration of tulathromycin 54 $mg{\cdot}kg^{-1}$), with its major metabolite remaining undetected.

9.4 Pharmaceutical Residues in Soil: Uptake and Ecotoxicological Effects on Earthworms

The effects of pharmaceutical residues on *E. fetida* in the soil environment was investigated, highlighting their crucial role in maintaining soil quality. The study provided valuable insights into the behaviour of 21 PhACs within the soil-earthworm system. Bioaccumulation factors for earthworms were determined using a novel time-weighted average approach, accounting for both initial soil contamination and pharmaceutical degradation. After 21 days of exposure, BAF values ranged from 0.0253 to 0.329, indicating low uptake by *E. fetida*. Biochar amendment had no significant impact on the bioavailability of pharmaceuticals to earthworms except on the first day ($p < 0.05$). However, biochar significantly prolonged the persistence of certain pharmaceuticals throughout the experiment ($p < 0.05$). Nevertheless, the available scientific literature presents conflicting results regarding the effectiveness of biochar amendment as a mitigation strategy for reducing the bioavailability of pharmaceutical residues and their ecotoxicological effects on soil organisms. This uncertainty underscores the critical need to prevent pharmaceutical residues from entering the soil environment as the most effective strategy.

No significant ecotoxicological effects on earthworm weight or mortality were observed ($p > 0.05$). However, the use of more sensitive biomarkers, such as proteome or lipidome analyses, would provide a more comprehensive assessment of potential ecotoxicological impacts. Additionally, mass spectrometry imaging revealed that pharmaceutical uptake by *E. fetida* occurs through both skin contact and ingestion, as pharmaceuticals were distributed uniformly throughout the entire earthworm tissue without specific localization. This analysis also served as a proof of concept, representing the first method published for spatially analysing small drug molecules within *E. fetida* following uptake from the terrestrial environment.

9.5 Pharmaceutical Residues in Soil: Uptake and Ecotoxicological Effects on Lettuce

The exposure of soil-grown lettuce to a multi-pharmaceutical mixture was conducted under two scenarios: growth in initially contaminated soil and irrigation with pharmaceutical-contaminated water across a wide range of concentrations to estimate bioconcentration factors and translocation factors. A novel approach utilising time-weighted average soil concentrations was employed, accounting for initial contamination and pharmaceutical degradation. Bioconcentration factors were significantly higher ($p < 0.05$) for irrigation with contaminated water compared to initially spiked soil for 20 of 25 PhACs, except for several soil-mobile sulfonamides. Conversely, translocation factors differed significantly ($p < 0.05$) for only 5 of 25 PhACs, suggesting that translocation is independent of the contamination route and more likely driven by pharmaceutical concentrations in plant roots. In contaminated soil, biochar amendment was evaluated. Similar to the findings in the earthworm-soil system, biochar had a negligible effect on the bioavailability of pharmaceuticals to lettuce roots and shoots ($p > 0.05$), while also decreasing the degradation rate of several pharmaceuticals in the soil. Furthermore, as anticipated, the ecotoxicological analysis showed increased mortality rate with higher pharmaceutical concentrations and a significant reduction in biomass weight ($p < 0.05$). This highlights the importance of preventing pharmaceuticals from entering the soil, as managing their sources, such as wastewater, animal manure, or biosolids, is more feasible. These matrices can be more effectively treated to remove pharmaceutical residues before they reach the soil.

Additionally, the estimated daily intake of PhACs through the consumption of *L. sativa* indicated negligible health risks if lettuce were the sole contaminated vegetable consumed. However, the health risk would significantly increase if other vegetables were similarly contaminated with trace residues. It is important to note that bioconcentration factors were significantly higher when *L. sativa* was irrigated with contaminated water, and real wastewater typically contains a broader range of compounds, including metabolites and degradation products. This complexity could potentially amplify health risks several-fold, potentially reaching levels where the risk is no longer negligible. Furthermore, this study evaluated the environmental risk associated with the emergence of AMR in soil, which was classified as medium to high. These findings underscore the multifaceted challenges of pharmaceutical contamination in agricultural systems and emphasize the critical need for proactive measures, such as removing pharmaceutical residues from wastewater, animal manure, or biosolids before their application to agricultural soils.

9.6 Pharmaceutical Residues in Hydroponic System: Uptake and Ecotoxicological Effects on Lettuce

Obtained results highlighted the potential risks of hydroponically grown lettuce in pharmaceutical-contaminated water. Bioconcentration factors were determined using time-weighted average aquatic concentrations, accounting for initial contamination and pharmaceutical degradation. The findings revealed a wide range of bioconcentration factors (2.3 to 880 $L \cdot kg^{-1}$) and translocation factors (0.019–1.48), indicating a high potential for pharmaceutical uptake and translocation by *L. sativa*. The degradation of 20 pharmaceuticals within the water-lettuce system followed first-order degradation kinetics. Substantial ecotoxicological effects were observed, including increased mortality rate, alterations in root morphology and length, and changes in biomass weight ($p < 0.05$).

For health risk assessment, Monte Carlo simulations indicated that at a concentration of 10 $\mu g \cdot L^{-1}$, there is a 66.18% probability that the health hazard index will exceed the threshold of 0.01, signifying a considerable human health risk associated with long-term intake. At a concentration of 50 $\mu g \cdot L^{-1}$, the probability increases to 66.51% that the hazard index will exceed 0.05, suggesting a distinct health risk. Although risk quotients could not be determined for all 20 PhACs due to incomplete data on translocation factors, the assessment focused solely on the HI for PhAC intake through lettuce consumption. Since lettuce is just one of many commonly consumed vegetables, similar contamination levels could be present in other vegetables grown in contaminated water under hydroponic conditions.

Moreover, real-world scenarios often involve wastewater contamination with a broader spectrum of PhACs, including their metabolites and degradation products, many of which remain unidentified. These findings underscore a significant health risk posed by pharmaceutical contamination in commonly consumed vegetables, particularly hydroponically grown lettuce, through long-term exposure. While these results were obtained under controlled hydroponic conditions, they may not fully reflect real-world conditions, highlighting the need for further research into the broader impact of wastewater contamination on edible plants and the importance of monitoring PhACs and their metabolites in agricultural water sources.

Additionally, the consumption of fresh vegetables like lettuce may inadvertently contribute to the transfer of antibiotic-resistant genes in the human gastrointestinal tract, posing a significant threat to public health. To assess this risk, sum of risk quotients was determined, revealing a high potential for the emergence of antimicrobial resistance at both 10 and 50 $\mu g \cdot L^{-1}$ concentration. Although risk quotients showed a gradual decrease over time due to antibiotic degradation, the formation of degradation products or metabolites was not considered in the calculation of the risk quotients. This highlights the urgent need for appropriate legislation to regulate permissible PhAC residues in both vegetables and wastewater. Furthermore, it underscores the importance of exploring and implementing tertiary treatment processes, such as advanced oxidation processes, at wastewater treatment plants to mitigate pharmaceutical contamination and reduce public health risks.

9.7 Impact of Advanced Oxidation Processes on the Removal of Sulfamethoxazole from Wastewater

Efficiency of various advanced oxidation processes (AOPs) on the removal of sulfamethoxazole (SMX) from both model water and real wastewater was investigated. The processes studied included ozone, UV, a combination of ozone and UV, and plasma discharge. Removal efficiencies were assessed by calculating reaction rate coefficients using a pseudo-first-order kinetic model. For real wastewater, the removal efficiency of AOPs ranked as follows: 6.5 $g \cdot h^{-1}$ O_3 > plasma > 100 $mg \cdot h^{-1}$ O_3 + UV > 100 $mg \cdot h^{-1}$ O_3 > UV. Reaction rate coefficients were higher for model water due to the simpler matrix, highlighting the challenges of treating complex wastewater samples.

These AOPs have potential as tertiary treatments in conventional wastewater treatment plants. Chemical and microbiological analyses of treated wastewater showed no significant changes in inorganic composition. Although this study did not examine the SMX degradation products, their potential biological activity and toxicity warrant further investigation.

Ecotoxicological tests using *Daphnia magna* and *Lemna minor* were conducted on treated wastewater. Among the processes tested, only plasma treatment caused significant adverse effects on ecotoxicological endpoints, raising concerns about the byproducts generated during this process.

9.8 Key Findings and Implications: A Comprehensive Overview

In summary, this thesis has yielded several key results for both future research and potential policy-making.

1. **Development of Analytical Methods**: Robust and validated multiresidue extraction methods were successfully developed for pharmaceutical residues in diverse matrices, including soil, lettuce, and earthworms. These methods enable comprehensive environmental assessments of over 40 pharmaceutical compounds. Additionally, the number of compounds that can be analysed could easily be expanded, as the methods are optimised for various therapeutic classes.
2. **Mass Spectrometry Imaging**: Analysis revealed that pharmaceutical uptake by *Eisenia fetida* likely occurs through both skin contact and ingestion, as pharmaceuticals were distributed uniformly throughout the entire earthworm tissue without specific localization. This analysis also serves as a proof of concept, representing the first published method for spatially analysing small drug molecules within *E. fetida* following uptake from the terrestrial environment.
3. **Pharmaceutical Metabolites**: A novel workflow for pharmaceutical metabolite identification using open-source software was described. Additionally, the identification of pharmaceutical metabolites in lettuce and earthworms showed that their concentrations can exceed those of parent compounds. This highlights the importance of including metabolites and degradation products in both environmental and health risk assessments.
4. **Incorporation of Insufficiently Treated Animal Manure**: Incorporation of insufficiently treated animal manure, contaminated with antibiotic residues, can lead to soil contamination. Certain antibiotics, such as fluoroquinolones and macrolides, exhibit a high persistence in the soil. Moreover, the PCR results confirmed the presence of plasmid-mediated quinolone resistance genes in both animal manure and soil, underscoring the environmental and health risks associated with pharmaceutical residues in terrestrial environments.
5. **Pharmaceutical Uptake in Laboratory Experiments**: Laboratory experiments with *L. sativa* and *E. fetida* confirmed the uptake of pharmaceutical residues across a wide range of concentrations, allowing the estimation of bioconcentration factors and translocation factors. A novel approach using time-weighted average soil concentrations was applied, accounting for both initial contamination and pharmaceutical degradation.

6. **Biochar Amendments and Pharmaceutical Bioavailability**: Biochar amendments had limited effects on reducing pharmaceutical bioavailability, highlighting the importance of preventing soil contamination at the source (e.g., wastewater, animal manure, biosolids), as the soil matrix is considerably more complex. Contrary to our results, some studies report that biochar can either reduce the bioavailability of pharmaceutical residues or increase degradation rates, depending on biochar properties. Therefore, future research in this area is highly recommended.
7. **Ecotoxicological Risks**: Pharmaceutical contamination in soil and hydroponic systems caused significant ecotoxicological effects in *L. sativa*, while no adverse effects were observed in *E. fetida* at the whole-organism level based on our primary ecotoxicological endpoints. However, more sensitive ecotoxicological tests could potentially detect negative effects in earthworms. These findings underscore the risks associated with pharmaceutical residues in agricultural systems and highlight the need for effective strategies to mitigate their spread over agricultural land.
8. **Assessment of Environmental and Health Risk**: The health risk assessment for the consumption of contaminated lettuce showed a negligible health risk when grown in soil environments (100 and 1 000 $ng \cdot g^{-1}$ when the soil was initially contaminated or irrigated with contaminated water at concentrations of 5 and 50 $\mu g \cdot L^{-1}$). However, when grown under hydroponic conditions (with concentrations of 10 and 50 $\mu g \cdot L^{-1}$), the health risk was not negligible. It is important to note that the contamination included only up to 24 pharmaceuticals, while real wastewater may contain a significantly higher number of pharmaceuticals and other organic micropollutants, including their degradation products and metabolites. This suggests an alarming situation. Moreover, in agreement with the PCR analyses from the study under real-world conditions, the risk quotients for the rise of antimicrobial resistance in both soil and water indicated high risks under laboratory conditions. These findings underscore the need to prevent contamination of both agricultural and hydroponic systems for crop production.
9. **Recommendations for Future Practices**: The results of several projects/studies emphasize the critical need to remove pharmaceuticals from animal manure or wastewater before incorporating them into agricultural soils or using them in hydroponic systems. Potential solutions, such as fermentation processes and advanced oxidation processes, were investigated to remove pharmaceutical contamination and proved to be quite efficient, unlike biochar. However, further research is needed to address the potential formation of degradation products.

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CHAPTER 11 CURICULUM VITAE

Jan Fučík

Analytical and Bioanalytical chemist, LC-MS specialist & Ph.D. student at environmental

CONTACT

+420 728 726 691
jan-fucik@seznam.cz
Brno, Czech Republic
LinkedIn profile
Publication list

EDUCATION

Chemistry and Technology of Environmental Protection (Doctoral degree)
Brno University of Technology, Faculty of Chemistry
2021-present

Chemistry for Medical Application (Master's degree)
Brno University of Technology, Faculty of Chemistry
2016-2021

AWARDS

- 2019 BUT FCH Dean's award
- 2022 BUT FCH Talented Ph.D. students
- 2022 Shimadzu lab4you
- 2024 BUT FCH Dean's award

SKILLS

- Development, optimization and validation of LC-MS methods
- LC-MS troubleshooting
- Knowledge of various extraction methods (SPE and QuEChERS)
- Interpretation of MS spectra
- Experience in managing small teams
- Experience in ordering consumables and chemicals
- Presentation skills from conferences and teaching

PROFILE

Over 5 years of experience in LC-MS analyses, specializing in method development, method validation, and data evaluation. Proficient in a wide range of LC-MS techniques, including LC-ESI-3D-IT, LC-ESI-SQ, LC-ESI-TQ, LC-APCI-TQ, LC-ESI-qTOF and MALDI-TOF, working with instrumentation from Agilent, Bruker, Shimadzu, and Waters. Experienced in utilising both vendor-provided software and open-source platforms for data processing and statistical analysis. Expertise spans the analysis of small molecules (**pharmaceuticals**, pesticides, PFAS, and chemical monomers used in polymer production) as well as large biomolecules (**oligonucleotides**). Proficient in using statistical tools for data analysis, including Origin, Minitab, and MetaboAnalyst, as well as various open-source tools for mass spectrometry, such as MS-DIAL and CFM-ID.

Ph.D. Student

Brno University of Technology, Faculty of Chemistry
Brno | Czech Republic | June 2021 – present

Ph.D. Topic: Evaluation of occurrence of selected pharmaceuticals in the soil ecosystem:

- Development, optimization and validation of extraction methods for >40 pharmaceutical substances from environmental matrices (wastewater, soil) and biological matrices (vegetables, poultry litter, soil organisms, ...)
- Sample extraction: SPE and QuEChERS
- Analytical instruments: LC-UV-MS, MALDI-TOF, GC-FID, UV-VIS, AES
- Maintenance, troubleshooting and calibration of LC-MS instrumentation
- Managing a small team of people in a laboratory (3–6 students)
- Teaching laboratory classes, including creating new laboratory tasks
- Training of students, including the drafting of manuals for the operation of LC-MS instrumentation

Other side projects/collaborations during Ph.D.

- Preparation and characterization of chitosan wound covers with controlled drug release (LC-MS analysis of released drugs)
- Identification of newly synthesized molecules (direct infusion MS or LC-MS analysis)
- Quantitative and qualitative analysis of substances released from dental materials by LC-MS for company ADM, a.s.
- Analysis of aqueous biochar leachates for the AdMaS centre (UV-VIS, AES)
- Method development for analysis of pesticides in soil samples
- Method development for analysis of PFAS in aquatic samples

LANGUAGES

- Czech Native
- English B2/C1
- German A2

SOFTWARE SKILLS

- Laboratory software for operating various analytical instruments from Shimadzu, Agilent, Bruker, Waters
- Open-Source Software for Mass Spectrometry (MS-DIAL, CFM-ID, etc.)
- Statistical Software (Origin, MiniTab, MetaboAnalyst)
- Microsoft Office pack
- ChemSketch
- Zoner Photo Studio

PROFESSIONAL EXPERIENCE

Analytical Chemist for Oligonucleotides R&D
Synthon | Blansko, Czech Republic |
June 2024 – May 2025

Systematic IP-RPLC-UV-MS method development for the purity and impurity analysis of single-stranded and double-stranded oligonucleotides using Design of Experiments (DoE, QbD), utilising both LC-SQ and LC-qTOF instrumentation. Utilising novel approaches and various software solutions for untargeted impurity analysis in combination with LC-HRMS. Working in a multidisciplinary team, supporting both synthetic and purification teams. Responsible for communication with service engineers and for LC-UV-MS troubleshooting within the oligonucleotide analytical team.

Abroad internship within the Shimadzu lab4you project
Shimadzu Europa GmbH | Duisburg, Germany |
July 2023 – September 2023

Project 1: Identification of unknown drug metabolites in lettuce and earthworms
Software prediction of drug metabolites, creation of *in-silico* MS/MS library of predicted metabolites, analysis of samples by LC-qTOF (DDA, DIA modes), data were processed by software MS-DIAL and statistical analysis was performed in MetaboAnalyst.

Project 2: Spatial distribution of drug substances in earthworms
Optimization of sample preparation for MALDI, analysis of earthworm samples by MALDI-TOF and data processing (ImageReveal, MSI reader).

Techinal Worker
Brno University of Technology, Faculty of Chemistry |Czech Republic|
November 2022 – December 2023

Responsible for the maintenance of LC-MS instruments, research activities including publication activity, consultation of bachelor's and master's theses and teaching laboratory classes.

Technical Worker for Applied Research Project
Materials research centre | Brno, Czech Republic |
July 2019 – June 2022

Employed under several part-time contracts during my master’s and doctoral studies, performing fundamental laboratory procedures such as sample preparation, grinding, and extractions. Operated essential laboratory equipment, including pH and EC meters, UV-VIS, AES, and GC-FID.

Assistant Chemist in Laboratory (Inorganic Analysis)
Public Health Institute Ostrava (laboratory Jihlava) | Jihlava, Czech Republic | July 2019 – September 2019

Responsible for sampling (wastewater, pool water, ...), sample preparation (grinding, filtration, ...) and inorganic analysis (UV-VIS).

CHAPTER 12 PUBLICATIONS RELATED TO THE TOPIC OF THE PHD THESIS, IN THE FIELD OF ENVIRONMENTAL CHEMISTRY

12.1 Peer-Reviewed Journal Publications

TULKOVÁ, T.; **FUČÍK, J.**; KOZÁKOVÁ, Z.; PROCHÁZKOVÁ, P.; KRČMA, F.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L.; SOVOVÁ, K. Impact of various oxidation processes used for removal of sulfamethoxazole on the quality of treated wastewater. *Emerging Contaminants,* 2023, roč. 9, č. 3, s. 1-11. ISSN: 2405-6650.

MRAVCOVÁ, L.; JAŠEK, V.; HAMPLOVÁ, M.; NAVRKALOVÁ, J.; AMRICHOVÁ, A.; ZLÁMALOVÁ GARGOŠOVÁ, H.; **FUČÍK, J.** Assessing Lettuce Exposure to a Multipharmaceutical Mixture under Hydroponic Conditions: Findings through LC-ESI-TQ Analysis and Ecotoxicological Assessments. *ACS OMEGA,* 2024, roč. 9, č. 50, s. 49707-49718. ISSN: 2470-1343.

MRAVCOVÁ, L.; AMRICHOVÁ, A.; NAVRKALOVÁ, J.; HAMPLOVÁ, M.; SEDLÁŘ, M.; ZLÁMALOVÁ GARGOŠOVÁ, H.; **FUČÍK, J.** Optimization and validation of multiresidual extraction methods for pharmaceuticals in Soil, Lettuce, and Earthworms. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH,* 2024, roč. 31, č. 24, s. 33120-33140. ISSN: 0944-1344.

FUČÍK, J.; AMRICHOVÁ, A.; BRABCOVÁ, K.; KARPÍŠKOVÁ, R.; KOLÁČKOVÁ, I.; POKLUDOVÁ, L.; POLÁKOVÁ, Š.; MRAVCOVÁ, L. Fate of fluoroquinolones in field soil environment after incorporation of poultry litter from a farm with enrofloxacin administration via drinking water. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH,* 2024, roč. 31, č. 13, s. 20017-20032. ISSN: 0944-1344.

FUČÍK, J.; FUČÍK, S.; REXROTH, S.; SEDLÁŘ, M.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L. Pharmaceutical Metabolite Identification in Lettuce (Lactuca sativa) and Earthworms (Eisenia fetida) Using Liquid Chromatography Coupled to High-Resolution Mass Spectrometry and *In Silic*o Spectral Library. *Analytical and Bioanalytical Chemistry,* 2024, roč. 416, č. 28, s. 6291-6306. ISSN: 1618-2650.

FUČÍK, J.; JAŠEK, V.; HAMPLOVÁ, M.; NAVRKALOVÁ, J.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L. Assessing Lettuce Exposure to a Multi-Pharmaceutical Mixture in Soil: Insights from LC-ESI-TQ Analysis and the Impact of Biochar on Pharmaceutical Bioavailability. *ACS OMEGA,* 2024, roč. 9, č. 37, s. 39065-39081. ISSN: 2470-1343.

FUČÍK, J.; JAROŠOVÁ, J.; BAUMEISTER, A.; REXROTH, S.; NAVRKALOVÁ, J.; SEDLÁŘ, M.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L. Assessing Earthworm Exposure to a Multi-Pharmaceutical Mixture in Soil: Unveiling Insights through LC-MS and MALDI-MS Analyses, and Impact of Biochar on Pharmaceutical Bioavailability. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH*, 2024, roč. 31, č. 35, s. 48351-58368. ISSN: 0944-1344.

12.2 Conference Abstracts

ZÁVODSKÁ, P.; JANEBOVÁ, D.; **FUČÍK, J.**; KLUČÁKOVÁ, M.; JAQUES, V. *Mobility of Sulfonamide Antibiotic Sulfapyridine in Different Types of Soils.* Ostrava: TangerLtd., 2022. s. 80-80. ISBN: 978-80-88365-07-5.

FUČÍK, J.; KARPÍŠKOVÁ, R; GELBÍČOVÁ, T.; POKLUDOVÁ, L.; POLÁKOVÁ, Š.; MRAVCOVÁ, L. *Persistence of fluoroquinolones in soil after application of poultry litter.* Chem2change Book of Abstracts. University of Pardubice, 2022. ISBN: 9788075604064.

FUČÍK, J.; TULKOVÁ, T.; KOZÁKOVÁ, Z.; MRAVCOVÁ, L. *DEGRADATION OF SULFAMETHOXAZOLE BY NON-THERMAL PLASMA DISCHARGE.* 3rd ZORH CONFERENCE, SPLIT, APRIL, 28th-29th, 2022. Split, Croatia: University of Split, Faculty of Chemistry and Technology, Croatia, 2022. ISBN: 978-953-7803-16-2.

ZÁVODSKÁ, P.; JANEBOVÁ, D.; **FUČÍK, J.**; KLUČÁKOVÁ, M.; PEKAŘ, M.; JAQUES, V.; ZIKMUND, T.; KAISER, J. Mobility of sulfonamide antibiotic sulfapyridine in different types of soil. In *NANOCON Conference Proceedings - International Conference on Nanomaterials.* 2022. s. 137-141. ISBN: 978-8-0883-6509-9.

FUČÍK, J.; JAROŠOVÁ, R.; BAUMEISTER, A.; REXROTH, S.; NAVRKALOVÁ, J.; AMRICHOVÁ, A.; HAMPLOVÁ, M.; SEDLÁŘ, M.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L. *Quantification and Spatial Distribution of Enrofloxacin in Earthworms after its Uptake from Contaminated Soil.* Sborník abstraktů - Studentská odborná konference Chemie je život 2023. Brno: Vysoké učení technické v Brně, Fakulta chemická, 2023. s. 50-51. ISBN: 978-80-214-6204-5.

FUČÍK, J.; AMRICHOVÁ, A.; HAMPLOVÁ, M.; NAVRKALOVÁ, J.; SEDLÁŘ, M.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L. *Uptake of pharmaceuticals by lettuce (Lactuca sativa) grown under hydroponic conditions and in a terrestrial environment and earthworms (Eisenia fetida) in the soil environment.* Dusseldorf, Germany: German Chemical Society, 2023. s. 104-104.

ZÁVODSKÁ, P.; SLANINOVÁ, K.; **FUČÍK, J.**; KLUČÁKOVÁ, M. *Interaction of sulfathiazole with soils differed by content of organic matter and calcium and its diffusion in real conditions.* 2023.

FUČÍK, J.; FUČÍK, S.; REXROTH, S.; HAMPLOVÁ, M.; NAVRKALOVÁ, J.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L. *Innovative Integration of High-Resolution Mass Spectrometry and In-Silico Libraries for Pharmacetical Metabolite Identification in Lettuce (Lactuca sativa).* Brno: Vysoké učení technické v Brně, Fakulta chemická, 2024. s. 65-65.

HAMPLOVÁ, M.; AMRICHOVÁ, A.; **FUČÍK, J.**; MRAVCOVÁ, L..; ZLÁMALOVÁ GARGOŠOVÁ H. *Assessing radish exposure to a multi-pharmaceutical mixture in soil.* Chémia a technológie pre život 26. celoslovenská študentská vedecká konferencia s medzinárodnou účasťou. Bratislava: Fakulta chemickej a potravinárskej technológie, 2024. ISBN: 978-80-8208-128-5.

12.3 Presentations

MRAVCOVÁ, L.; **FUČÍK, J.** *Occurrence of Pharmaceuticals in the Soil System.* 2022. Taiwan.

FUČÍK, J.; AMRICHOVÁ, A.; HAMPLOVÁ, M.; NAVRKALOVÁ, J.; SEDLÁŘ, M.; MRAVCOVÁ, L. *Identification of pharmaceuticals metabolites in lettuce (Lactuca sativa) and earthworms (Eisenia fetida).* 2022. Germany, Duisburg.

FUČÍK, J.; FUČÍK, S.; REXROTH, S.; HAMPLOVÁ, M.; NAVRKALOVÁ, J.; ZLÁMALOVÁ GARGOŠOVÁ, H.; MRAVCOVÁ, L. *Integration of High-Resolution Mass Spectrometry and In-Silico Libraries for Pharmaceutical Metabolite Annotation in Hydroponically Grown Lettuce (Lactuca sativa).* Wien: MassSpec Forum 2025 at the TU Vienna, 2025. p. 20-21.

12.4 Final Project Reports

MRAVCOVÁ, L.; **FUČÍK, J.**; BRABCOVÁ, K.; POKLUDOVÁ, L.; KARPÍŠKOVÁ, R; GELBÍČOVÁ, T.; POLÁKOVÁ, Š.; STOLÁŘ, P.; SLADEČEK, V.; ŠENK, D. *ANTIMIKROBIÁLNÍ REZISTENCE V PŮDĚ A PŘÍPADNĚ DALŠÍCH ČÁSTECH ŽIVOTNÍHO PROSTŘEDÍ.* 2021. s. 1-75.

KARPÍŠKOVÁ, R.; KOLÁČKOVÁ, I.; POKLUDOVÁ, L.; MRAVCOVÁ, L.; **FUČÍK, J.**; AMRICHOVÁ, A.; POLÁKOVÁ, Š. *Antimikrobiální rezistence v půdě a případně dalších částech životního prostředí.* 2023. s. 1-67.

KARPÍŠKOVÁ, R.; KOLÁČKOVÁ, I.; POKLUDOVÁ, L.; MRAVCOVÁ, L.; **FUČÍK, J.**; NAVRKALOVÁ, J.; POLÁKOVÁ, Š. *Antimikrobiální rezistence v půdě a případně dalších částech životního prostředí.* Brno: Ústřední kontrolní a zkušební ústav zemědělský v Brně, 2024. s. 1-78.

CHAPTER 13 PUBLICATIONS IN THE FIELD OF MEDICAL APPLICATIONS, MATERIALS AND PHYSICAL CHEMISTRY

13.1 Peer-Reviewed Journal Publications

JAŠEK, V.; **FUČÍK, J.**; IVANOVÁ, L.; VESELÝ, D.; FIGALLA, S.; MRAVCOVÁ, L.; SEDLÁČEK, P.; KRAJČOVIČ, J.; PŘIKRYL, R. High-Pressure Depolymerization of Poly(lactic acid) (PLA) and Poly(3-hydroxybutyrate) (PHB) Using Bio-Based Solvents: A Way to Produce Alkyl Esters Which Can Be Modified to Polymerizable Monomers. *Polymers,* 2022, roč. 14, č. 23, s. 1-18. ISSN: 2073-4360.

JAŠEK, V.; MELČOVÁ, V.; FIGALLA, S.; **FUČÍK, J.**; MENČÍK, P.; PŘIKRYL, R. Study of the Thermomechanical Properties of Photocured Resins Based on Curable Monomers from PLA and PHB for SLA 3D Printing. *ACS APPLIED POLYMER MATERIALS,* 2023, roč. 5, č. 12, s. 9909-9917. ISSN: 2637-6105.

JAŠEK, V.; **FUČÍK, J.**; MELČOVÁ, V.; FIGALLA, S.; MRAVCOVÁ, L.; KROBOT, Š.; PŘIKRYL, R. Synthesis of Bio-Based Thermoset Mixture Composed of Methacrylated Rapeseed Oil and Methacrylated Methyl Lactate: One-Pot Synthesis Using Formed Methacrylic Acid as a Continual Reactant. *Polymers,* 2023, roč. 15, č. 8, s. 1-21. ISSN: 2073-4360.

SZABOVÁ, J.; MIŠÍK, O.; **FUČÍK, J.**; MRÁZOVÁ, K.; MRAVCOVÁ, L.; ELCNER, J.; LÍZAL, F.; KRZYZANEK, V.; MRAVEC, F. Liposomal Form of Erlotinib for Local Inhalation Administration and Efficiency of Its Transport to the Lungs. *INTERNATIONAL JOURNAL OF PHARMACEUTICS,* 2023, roč. 634, č. march, s. 1-12. ISSN: 0378-5173.

JAŠEK, V.; **FUČÍK, J.**; KRHUT, J.; MRAVCOVÁ, L.; FIGALLA, S.; PŘIKRYL, R. A Study of Isosorbide Synthesis from Sorbitol for Material Applications Using Isosorbide Dimethacrylate for Enhancement of Bio-Based Resins. *Polymers,* 2023, roč. 15, č. 17, s. 1-18. ISSN: 2073-4360.

JAŠEK, V.; **FUČÍK, J.**; MELČOVÁ, V.; PŘIKRYL, R.; FIGALLA, S. Improvements in the Production of Isosorbide Monomethacrylate Using a Biobased Catalyst and Liquid-Liquid Extraction Isolation for Modifications of Oil-Based Resins. *ACS OMEGA,* 2024, roč. 9, č. 23, ISSN: 2470-1343.

FIGALLA, S.; JAŠEK, V.; **FUČÍK, J.**; MENČÍK, P.; PŘIKRYL, R. Poly(lactide) Upcycling Approach through Transesterification for Stereolithography 3D Printing. *BIOMACROMOLECULES,* 2024, roč. 25, č. 10, s. 6645-6655. ISSN: 1525-7797.

JAŠEK, V.; **FUČÍK, J.**; BARTOŠ, O.; FIGALLA, S.; PŘIKRYL, R. Photocurable Oil-Based Thermosets Containing Modifiers from Renewable Sources for Coating Applications. *ACS Polymers Au,* 2024, roč. December, č. 28.10., s. 60-73. ISSN: 2694-2453.

FUČÍK, J.; KEJÍK, P.; BYSTŘICKÝ, Z.; AMRICHOVÁ, A.; HAMPLOVÁ, M.; MRAVCOVÁ, L. Qualitative and quantitative analysis of leachables from dental composites under different extraction conditions using liquid chromatography coupled to mass spectrometry. *Journal of Applied Polymer Science,* 2024, roč. 141, č. 39, ISSN: 1097-4628.

13.2 Conference Abstracts

SZABOVÁ, J.; MIŠÍK, O.; **FUČÍK, J.**; MRÁZOVÁ, K.; MRAVCOVÁ, L.; KRZYZANEK, V.; LÍZAL, F.; MRAVEC, F. *Liposomal Form of Erlotinib for the Inhalation Drug Delivery.* Nanocon 2022 – Abstracts. Ostrava, CZ: Tanger Ltd., 2022. s. 89-89.

NEŠPOR, T.; **FUČÍK, J.**; SEDLÁŘ, M.; VOJTOVÁ, L.; SEDLÁČEK, P.; MRAVCOVÁ, L.; PEKAŘ, M. *Chitosan Hydrogels as Wound Dressings with Drug Release Potential.* Nanocon 2022 - Abstracts. Ostrava, CZ: Tanger Ltd., 2022. s. 86-87. ISBN: 978-80-88365-07-5.

FUČÍK, J.; NEŠPOR, T.; SEDLÁŘ, M.; PEKAŘ, M.; VOJTOVÁ, L.; SEDLÁČEK, P.; MRAVCOVÁ, L. *Preparation and characterization of chitosan films as wound covers with controlled drug release.* Nanocon 2022 – Abstracts. Ostrava, CZ: Tanger Ltd., 2022. s. 94-95. ISBN: 978-80-88365-07-5.

13.3 Final Project Reports

MRAVCOVÁ, L.; **FUČÍK, J.** *CHEMICAL EVALUATION OF MEDICAL DEVICES (DENTAL COMPOSITES) PART 1.* 2020. s. 1-16.

MRAVCOVÁ, L.; **FUČÍK, J.** *CHEMICAL EVALUATION OF MEDICAL DEVICES (DENTAL COMPOSITES) PART 2.* 2020. s. 1-16.

MRAVCOVÁ, L.; **FUČÍK, J.**; VAŠINOVÁ GALIOVÁ, M.; BILAVČÍKOVÁ, K. *CHEMICAL EVALUATION OF MEDICAL DEVICES (DENTAL MATERIALS).* 2023. s. 1-17.