UNIVERSITY OF PARDUBICE

FACULTY OF CHEMICAL TECHNOLOGY

Nikola Roulová

PROBLEMS OF THE ANALYTICAL AND MICROBIOLOGICAL QUALITY OF WASTEWATER

Theses of the Doctoral Dissertation

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Author: Ing. Nikola Roulová

Supervisor: doc. Ing. Marcela Pejchalová, Ph.D.

Expert supervisor: Ing. Petra Moťková, Ph.D.

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Abstract

The common feature of the three parts of this dissertation was wastewater, but it was viewed from different perspectives. First, as a specific matrix for microbiological analysis, but also as a source of o contaminants for the environment. In the first part of the dissertation, culture protocols for the detection of pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, and *Salmonella* spp. from wastewater were optimised. In the second part of the dissertation, the uptake of the polyfluorinated pharmaceuticals efavirenz and flufenamic acid was confirmed under hydroponic conditions by cress (*Lepidium sativum*), rocket (*Eruca sativa*), radish (*Raphanus sativus),* and pea (*Pisum sativum).* In this part of the dissertation, the extraction procedure of efavirenz and flufenamic acid from plants was optimised and an HPLC/MS method for their detection in plant extracts was designed. For the third part of the dissertation, the conditions of the freshwater algae growth inhibition test were modified with respect to the use of *Chlorella kessleri* species. The test was used to evaluate changes in the acute toxicity of reaction solutions during photocatalytic degradation of the chloroacetanilide herbicides alachlor, metolachlor, and acetochlor.

Abstrakt

Společným znakem všech tří částí této disertační práce byla odpadní voda, na kterou však bylo nahlíženo z různých úhlů pohledu. Jednak jako na specifickou matrici pro mikrobiologický rozbor, ale také jako na zdroj znečišťujících látek pro životní prostředí. V první části disertační práce byly optimalizovány kultivační protokoly pro detekci patogenních bakterií *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica* a *Salmonella* spp. z odpadních vod. V druhé části disertační práce byl potvrzen příjem polyfluorovaných léčiv efavirenzu a kyseliny flufenamové za hydroponických podmínek řeřichou setou (*Lepidium sativum*), roketou setou (*Eruca sativa*), ředkvičkou setou (*Raphanus sativus*) a hrachem setým (*Pisum sativum*). V této části práce byla optimalizována metoda extrakce efavirenzu a kyseliny flufenamové z rostlinné matrice a dále navržena HPLC/MS metoda pro jejich detekci v rostlinných extraktech. Pro třetí část disertační práce byly upraveny podmínky zkoušky inhibice růstu sladkovodních řas s ohledem na použití

druhu *Chlorella kessleri*. Zkouška byla použita pro hodnocení změn akutní toxicity reakčních roztoků v průběhu fotokatalytické degradace chloracetanilidových herbicidů alachloru, metolachloru a acetochloru.

Keywords

wastewater, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica, Salmonella,* plant uptake, polyfluorinated pharmaceuticals, hydroponic cultivation, acute toxicity, chloroacetanilide herbicides, *Chlorella kessleri*

Klíčová slova

odpadní voda, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Salmonella*, příjem léčiv rostlinami, polyfluorovaná léčiva, hydroponické pěstování, akutní toxicita, chloracetanilidové herbicidy, *Chlorella kessleri*

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1 Detection, occurrence, and antimicrobial susceptibility of selected pathogenic bacteria in wastewater

The first part of this dissertation focused on the detection, occurrence, and antimicrobial susceptibility of the pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica,* and *Salmonella* spp. in wastewater. The main objective of this part of the dissertation was to determine optimal culture protocols for the isolation of target pathogenic bacteria from wastewater. The essence was to compare different culture protocols that were either newly designed or resulted from modifications of existing standardised culture methods. In both cases, emphasis was placed on considering the specific characteristics of the wastewater to ensure that the culture protocols were more applicable to this complex matrix. For this reason, only naturally contaminated wastewater samples were used. The samples used for this dissertation were raw and biologically treated municipal wastewater, raw infectious hospital wastewater, and raw hospital wastewater.

1.1 Introduction

Culture methods can detect microorganisms that are viable at a given time and for which the growth conditions are known. However, depending on the type of environment, less than 1–10% of bacteria can be cultured. Despite the skipping of non-culturable bacteria, the value of culture methods cannot be underestimated, as they are crucial to understanding the phenotypic characteristics of isolates (Manaia *et al.* 2018; Pazda *et al.* 2019). In addition, culture methods allow the enumeration of viable cells and the possibility of assessing antibiotic resistance profiles of isolates, being the most used method to determine multidrug resistance phenotypes (Manaia *et al.* 2018).

Microbiological control of water quality has historically been associated with culture methods (Helmi *et al.* 2015). Methods for the detection of selected pathogenic bacteria in water are established by standards. These are mainly indicator-value pathogenic bacteria or important agents of waterborne diseases whose detection is important from an epidemiological point of view. There are no specific methods defined for the detection of pathogenic bacteria in wastewater, but some of the standards in the field of water microbiology specify methods for the detection of pathogenic bacteria applicable to all types of water, or it is possible to rely on the principles of standardised methods that are intended only for certain types of water or even for the food chain. However, in any case, the culture protocol used must be adapted to the specific characteristics of the wastewater.

1.2 Wastewater samples

Raw and treated municipal wastewater were obtained from a municipal WWTP with conventional biological wastewater treatment based on activated sludge. The WWTP serves a population equivalent of 40,000 and receives household, industrial, and agricultural wastewater. Samples were taken from the influent and the final effluent of the municipal WWTP.

The raw hospital wastewater was acquired from a hospital with a capacity of 1,300 beds. Wastewater samples were taken at two sampling sites and differed in composition. The first sampling site was the influent of the hospital's internal WWTP. The samples collected at this sampling site represented infectious wastewater that contained discharges generated purely by medical activities. The second sampling site was located in the central inspection shaft of the hospital's sewer system. The samples collected at this sampling site were hybrid wastewater composed of discharges generated by medical and nonmedical activities.

1.3 Sample collection

Wastewater sampling was performed between July 2020 and May 2022. In total, 56 wastewater samples were collected, corresponding to 14 samples at each sampling point. The samples were collected during all seasons of the year to cover all weather conditions. The time interval between sampling was kept between four and six weeks. Wastewater (500 mL) was taken as a grab sample. Samples were transported to the laboratory in a cooling box and analysed within two hours after collection.

1.4 Antimicrobial susceptibility testing

The antimicrobial susceptibility of wastewater isolates was tested using the disc diffusion method, according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2023). Mueller-Hinton agar and commercial antimicrobial test discs were used. The incubation was carried out at 35 ± 2 °C for 18 ± 2 h. A panel of six antimicrobial agents was selected for each bacterial species. The zones of inhibition were interpreted according to the current EUCAST Breakpoint Tables (EUCAST 2020, 2021, 2022). Susceptibility results were classified as susceptible, standard dosing regimen (S), susceptible, increased exposure (I), or resistant (R) according to EUCAST recommendations (EUCAST 2019). When an isolate was resistant to at least one antimicrobial agent in three or more different classes of antibiotics, it was considered multidrug-resistant (MDR).

1.5 *Staphylococcus aureus*

1.5.1 Experimental Part

Two culture methods were used for the detection of *Staphylococcus (St.) aureus* in wastewater, which differed in the use of an enrichment step prior to plating on solid media. Whereas one method involved the enrichment of wastewater samples in liquid media, the other method used the direct spread of wastewater samples onto solid media. A total of three liquid media were used, which varied in selectivity and composition. Then, two solid media were used in both methods. Depending on the liquid media or solid media used, the methods were further divided into individual protocols. Considering all combinations, each wastewater sample was examined in parallel using eight culture protocols for the detection of *St. aureus* (Table 1).

Table 1 *Scheme of culture protocols used for the isolation of Staphylococcus aureus from wastewater*

Culture method/protocol	Enrichment in liquid medium	Plating out	Solid medium
Method with wastewater enrichment in liquid medium			
BHI 6.5% NaCl/ B-P agar (A)	90 mL of BHI broth with 6.5% NaCl + 10 mL of wastewater 36 ± 1 °C, 21–24 h	streaking by loop	B-P agar 36 ± 1 °C, 24 ± 3 h
BHI 6.5% NaCl/ MS agar (B)			MS agar 36 ± 1 °C, 24 ± 3 h
BHI/B-P agar (C)	90 mL of BHI broth + 10 mL of wastewater 36 ± 1 °C, 21–24 h	spreading of 100 µl of the respective dilutions of the enriched culture	B-P agar 36 ± 1 °C, 24 ± 3 h
BHI/MS agar (D)			MS agar 36 ± 1 °C, 24 ± 3 h
TSB/B-P agar (E)	90 mL of TSB broth + 10 mL of wastewater 36 ± 1 °C, 21–24 h.	spreading of 100 µl of the respective dilutions of the enriched culture	B-P agar 36 ± 1 °C, 24 ± 3 h
TSB/MS agar (F)			MS agar 36 ± 1 °C, 24 ± 3 h
Spread plate method			
spread/B-P agar (G)	---	spreading of 100 µl of wastewater and other dilutions	B-P agar 36 ± 1 °C, 24–48 h
spread/MS agar (H)			MS agar 36 ± 1 °C, 24–48 h

BHI broth – Brain Heart Infusion broth; TSB broth – Tryptone Soya broth; B-P agar – Baird-Parker agar; MS agar – Mannitol Salt agar

1.5.2 Results

Of the 48 wastewater samples examined, 34 (70.8%) were found to be positive for *St. aureus* by at least one of the culture protocols used. *St. aureus* was detected in all types of wastewater. However, the prevalence varied according to the origin of the wastewater, between 50.0% and 100%. The main sources of isolates were raw and treated municipal wastewater.

Culture protocols with selective enrichment in BHI broth with 6.5% NaCl (protocols A and B) provided the highest *St. aureus* detection rate (97.1% and 85.3%, respectively) and were able to detect almost all positive samples. The culture protocol with enrichment in non-selective liquid medium (protocols C to F) yielded significantly fewer positive samples compared to the culture protocols with enrichment in selective liquid medium (protocols A and B). Regarding the non-selective liquid media used, better results were obtained using BHI broth (protocols C and D) compared to TSB broth (protocols E and F). Culture protocols with the spread of wastewater onto solid media (protocols G and H) resulted in the lowest detection rate of *St. aureus* (20.6% and 5.9%, respectively) and performed poorly compared to culture protocols with enrichment step. For culture protocols with enrichment step, comparable *St. aureus* isolation rates were observed from both solid media used. On the contrary, the superiority of B-P agar in terms of the number of positive samples detected was evident in the protocols with the spread of wastewater onto solid media.

In general, the morphology of presumptive *St. aureus* colonies on MS agar was ambiguous. In contrast, B-P agar provided presumptive *St. aureus* colonies that were very easily visually identifiable, especially based on the presence of a clear zone around the colonies.

A panel of six antibiotics for the *St. aureus* strains included cefoxitin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), clindamycin (2 µg), erythromycin (15 µg), and trimethoprim-sulfamethoxazole (1.25-23.75 µg). A total of 76 *St. aureus* isolates were selected for antimicrobial susceptibility testing. Of these, 89.5% (68/76) were susceptible to all antibiotics tested. Three methicillin-resistant *St. aureus* (MRSA) (3.9%, 3/76) were detected based on resistance to cefoxitin. Two MRSA isolates were detected in treated municipal wastewater, while one MRSA isolate was observed in hospital wastewater.

1.6 *Pseudomonas aeruginosa*

1.6.1 Experimental Part

Two culture methods were used for the detection of *Pseudomonas (P.) aeruginosa* in wastewater, which differed in the use of an enrichment step prior to plating on solid media. Whereas one method involved the enrichment of wastewater samples in liquid media, the other method used the direct spread of wastewater onto solid media. In total, two non-selective liquid media and one solid

medium were used. Considering all combinations, each wastewater sample was examined in parallel using three culture protocols for the detection of *P. aeruginosa* (Table 2).

Table 2 *Scheme of culture protocols used for the isolation of Pseudomonas aeruginosa from wastewater*

Culture method/protocol	Enrichment in liquid medium	Plating out	Solid medium
Method with wastewater enrichment in liquid medium			
BHI/CET agar (A)	90 mL of BHI broth + 10 mL of wastewater 36 ± 1 °C, 21–24 h	spreading of 100 µl of the respective dilutions of the enriched culture	CET agar 36 ± 1 °C, 24–48 h
TSB/CET agar (B)	90 mL of TSB broth + 10 mL of wastewater 36 ± 1 °C, 21–24 h	spreading of 100 µl of the respective dilutions of the enriched culture	CET agar 36 ± 1 °C, 24–48 h
Spread plate method			
spread/CET agar (C)	---	spreading of 100 µl of wastewater and other dilutions	CET agar 36 ± 1 °C, 24–48 h

BHI broth – Brain Heart Infusion broth; TSB broth – Tryptone Soya broth; CET agar – Cetrimide agar

1.6.2 Results

P. aeruginosa was detected by at least one of the culture protocols used in 87.5% (42/48) of the wastewater samples examined. High prevalence rates were observed for all types of wastewater, ranging from 75.0% to 100%.

The culture protocol with non-selective enrichment in TSB broth (protocol B) provided the highest detection rate of *P. aeruginosa* in wastewater (92.9%, 39/42). In contrast, the culture protocol with non-selective enrichment in BHI broth (protocol A) gave the lowest detection rate of *P. aeruginosa* (7.1%, 3/42) and this enrichment step did not work adequately and led to an unacceptably high number of false negative results. The efficiency (76.2%, 32/42) of the culture protocol with the spread of wastewater onto solid media (protocol C) was similar to the protocol with non-selective enrichment in TSB broth (protocol B). Cetrimide agar did not give false positive

colonies. However, the growth of the accompanying microflora was not completely suppressed, and the presence of *Klebsiella* spp. and *Proteus* spp. was observed on cetrimide agar.

A panel of six antibiotics for the *P. aeruginosa* strains included piperacillin-tazobactam (30-6 µg), cefotaxime (5 µg), meropenem (10 µg), ciprofloxacin (5 µg), aztreonam (30 µg), and amikacin (30 µg). A total of 89 *P. aeruginosa* isolates were selected for antimicrobial susceptibility testing. The results showed that 97.8% isolates (87/89) had no resistance to any of the six antibiotics tested. On the contrary, two isolates were resistant to more than three antibiotics (2.2%, 2/89) and were classified as multidrug-resistant (MDR). MDR isolates was detected in hospital wastewater.

1.7 *Yersinia enterocolitica*

1.7.1 Experimental Part

Two culture methods were used for the detection of *Yersinia (Y.) enterocolitica* in wastewater, which differed mainly in wastewater sample processing prior to enrichment in selective liquid medium. One method included direct inoculation of wastewater into selective liquid medium, while the second method used the initial concentration of wastewater by centrifugation prior to selective enrichment in liquid medium. Two selective liquid media and one solid medium were used in both culture methods. With respect to the selective liquid medium used and the alkaline treatment of the enrichment cultures, the culture methods were further divided into individual culture protocols. Considering all combinations, each wastewater sample was examined in parallel using six culture protocols for the detection of *Y. enterocolitica* (Table 3).

1.7.2 Results

Of the 56 wastewater samples examined, 39 (69.6%) were positive for *Y. enterocolitica* by at least one of the culture protocols used. The prevalence of *Y. enterocolitica* varied according to the origin of the wastewater, between 21.4% and 100%. The lowest prevalence (21.4%, 3/14) of *Y. enterocolitica* was observed in infectious hospital wastewater. All *Y. enterocolitica* wastewater isolates belonged to biotype 1A.

In general, enrichment in PSB broth was more efficient for the isolation of *Y. enterocolitica* from wastewater than enrichment in ITC broth. In detail, culture protocols C (PSB/ALK/CIN agar) and F (CENT/PSB/ALK/CIN agar) provided the highest detection rate of *Y. enterocolitica* (89.7% and 76.9%, respectively). Additionally, culture protocol C (PSB/ALK/CIN agar) was the only protocol by which *Y. enterocolitica* was isolated from infectious hospital wastewater samples. Regarding the processing of the sample, direct inoculation of wastewater in a selective liquid medium provided substantially more positive samples than the initial concentration of wastewater by centrifugation.

Table 3 *Scheme of culture protocols used for the isolation of Yersinia enterocolitica from wastewater*

Culture method/protocol	Selective enrichment	Alkaline treatment	Plating out	Solid medium
Method with direct inoculation of wastewater				
ITC/ALK/ CIN agar (A)	90 mL of ITC broth + 10 mL of wastewater 25 ± 1 °C, 44 ± 4 h	0.5 mL of enriched culture + 4.5 mL of KOH solution 20 ± 5 s	streaking by loop ― spreading of 100 µl of alkalized culture	CIN agar 30 ± 1 °C 24 ± 3 h
ITC/CIN agar (B)	90 mL of ITC broth + 10 mL of wastewater 25 ± 1 °C, 44 ± 4 h	without alkaline treatment	streaking by loop	CIN agar 30 ± 1 °C 24 ± 3 h
PSB/ALK/ CIN agar (C)	90 mL of PSB broth + 10 mL of wastewater 25 ± 1 °C, 44 ± 4 h	0.5 mL of enriched culture + 4.5 mL of KOH solution 20 ± 5 s	streaking by loop ― spreading of 100 µl of alkalized culture	CIN agar 30 ± 1 °C 24 ± 3 h
PSB/CIN agar (D)	90 mL of PSB broth + 10 mL of wastewater 25 ± 1 °C, 44 ± 4 h	without alkaline treatment	streaking by loop	CIN agar 30 ± 1 °C 24 ± 3 h
Method with initial concentration of wastewater by centrifugation				
CENT/ITC/ ALK/CIN agar (E)	10 mL of ITC broth + 50 mL of wastewater/ centrifugation 25 ± 1 °C, 44 ± 4 h	0.5 mL of enriched culture + 4.5 mL of KOH solution 20 ± 5 s	streaking by loop ― spreading of 100 µl of alkalized culture	CIN agar 30 ± 1 °C 24 ± 3 h
CENT/PSB/ ALK/CIN agar (F)	10 mL of PSB broth + 50 mL of wastewater/ centrifugation 25 ± 1 °C, 44 ± 4 h	0.5 mL of enriched culture + 4.5 mL of KOH solution 20 ± 5 s	streaking by loop ― spreading of 100 µl of alkalized culture	CIN agar 30 ± 1 °C 24 ± 3 h

ITC broth – Irgasan Ticarcillin Chlorate broth; PSB broth – Peptone Sorbitol Bile broth; CIN agar – Cefsulodin Irgasan Novobiocin agar; ALK – alkaline treatment; CENT – centrifugation

Alkaline treatment was crucial for the successful recovery of *Y. enterocolitica* from wastewater, and its application in the isolation procedure was necessary, as it significantly reduced the levels of the accompanying microflora. The importance of alkaline treatment was reflected in the zero-isolation rate of *Y. enterocolitica* by culture protocols B (ITC/CIN agar) and D (PSB/CIN agar), in which the enriched cultures were not treated with a KOH solution. However, adjustment of the concentration of the KOH solution was essential to prevent the reduction of *Y. enterocolitica* itself. The best results were achieved with 0.4% KOH solution for 20 s.

On CIN agar the growth of colonies quite similar in appearance to *Y. enterocolitica* was observed. However, although these colonies were characterised by the typical colouration of the presumptive colonies of *Y. enterocolitica*, they were relatively visually distinguishable from them. The most noticeable difference was the wider, most often light pink, centre, which made it relatively easy to identify the presumptive colonies.

A panel of six antibiotics for the *Y. enterocolitica* strains included ampicillin (10 μg), cefotaxime (5 μg), ciprofloxacin (5 μg), gentamicin (10 μg), tetracycline (30 μg), and trimethoprim-sulfamethoxazole (1.25-23.75 μg). A total of 98 *Y. enterocolitica* isolates were selected for antimicrobial susceptibility testing. The highest resistance rate was observed for ampicillin (92.9%, 91/98). Furthermore, six isolates were resistant to ciprofloxacin (6.1%, 6/98). All 98 strains were susceptible to cefotaxime, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole.

1.8 *Salmonella spp.*

1.8.1 Experimental Part

Three culture methods were used for the detection of *Salmonella* in wastewater, which differed mainly in the number of enrichment passages and in the processing of the wastewater sample. One method included both the non-selective and selective enrichment steps, whereas the other two methods included only the selective enrichment step and differed in the wastewater sample processing, as one method included direct inoculation of wastewater into selective liquid medium and the second method used the initial concentration of wastewater by centrifugation prior to selective enrichment in liquid medium. All three culture methods used two selective liquid media and four solid media. Depending on the liquid medium and the incubation temperature used for the selective enrichment step, the culture methods were further divided into individual protocols. When considering all combinations, each wastewater sample was examined in parallel using 10 culture protocols for the detection of *Salmonella* (Table 4).

Table 4 Scheme of culture protocols used for the isolation of Salmonella from wastewater

Culture method/protocol	Non-selective pre-enrichment	Selective enrichment	Solid medium
Method with non-selective and selective enrichment steps			
BPW/MKTTn 37 °C (A)	10 mL of WW + 90 mL of BPW 37 °C, 16 h	1 mL of BPW culture 10 mL of MKTTn broth 37 °C, 21–24 h	XLD agar DC agar BG agar Rambach agar 36 ± 1 °C 24 ± 3 h
BPW/MKTTn 41.5 °C (B)		1 mL of BPW culture 10 mL of MKTTn broth 41.5 °C, 21–24 h	
BPW/RVS 37 °C (C)		0.1 mL of BPW culture 10 mL of RVS broth 37 °C, 21–24 h	
BPW/RVS 41.5 °C (D)		0.1 mL of BPW culture 10 mL of RVS broth 41.5 °C, 21–24 h	
Method with selective enrichment step with direct inoculation of wastewater			
MKTTn 37 °C (E)	----	1 mL of WW 10 mL of MKTTn broth 37 °C, 21–24 h	XLD agar DC agar BG agar Rambach agar 36 ± 1 °C 24 ± 3 h
MKTTn 41.5 °C (F)		1 mL of WW 10 mL of MKTTn broth 41.5 °C, 21–24 h	
RVS 37 °C (G)		1 mL of WW 10 mL of RVS broth 37 °C, 21–24 h	
RVS 41.5 °C (H)		1 mL of WW 10 mL of RVS broth 41.5 °C, 21–24 h	
Method with selective enrichment step with initial concentration of wastewater by centrifugation			
CENT/MKTTn 41.5 °C (I)	----	50 mL of WW/centrifugation 10 mL of MKTTn broth 41.5 °C, 21–24 h	XLD agar DC agar BG agar Rambach agar 36 ± 1 °C 24 ± 3 h
CENT/RVS 41.5 °C (J)		50 mL of WW/centrifugation 10 mL of RVS broth 41.5 °C, 21–24 h	

BPW – Buffered Peptone Water; MKTTn broth – Muller Kauffmann Tetrathionate Novobiocin broth; RVS broth – Rappaport Vassiliadis Soya Peptone broth; XLD agar – Xylose Lysine Deoxycholate agar; DC agar – Deoxycholate Citrate agar; BG agar – Brilliant Green agar; WW – wastewater; CENT – centrifugation

1.8.2 Results

In total, 40 of the 56 wastewater samples (71.4%) examined were found to be positive for *Salmonella* by at least one of the culture protocols used. However, the prevalence varied according to the origin of the wastewater, from 85.7% to 35.7% The lowest prevalence (35.7%, 5/14) of *Salmonella* was observed in infectious hospital wastewater.

The results showed that the crucial factor for the detection of *Salmonella* in wastewater was the enrichment step. The conditions of selective enrichment (that is, the liquid medium and incubation temperature used) had a significant effect on the multiplication of *Salmonella* to detectable levels, suppressing competing bacteria, and on the efficiency of solid media, thus significantly influencing the isolation rates of *Salmonella* from wastewater. In contrast, the pre-enrichment in non-selective liquid medium, as well as the method of processing of the wastewater sample prior to selective enrichment step, had only a minimal effect on the efficacy of individual culture protocols.

In general, enrichment in MKTTn broth was more effective than enrichment in RVS broth for the isolation of *Salmonella* from wastewater. Regarding the temperature of selective enrichment, *Salmonella* was isolated more frequently after incubation at an elevated temperature of 41.5 °C, regardless of the selective liquid medium used. In detail, the culture protocols B (BPW/MKTTn 41.5 °C), F (MKTTn 41.5 °C), and I (CENT/MKTTn 41.5 °C) gave the highest *Salmonella* detection rate (65.0%, 60.0%, and 57.5%, respectively). However, they were unable to isolate *Salmonella* from all positive samples. On the contrary, protocols A (BPW/MKTTn 37 °C), and J (CENT/RVS 41.5 °C) resulted in the lowest *Salmonella* detection rate (15.0% and 20.0 %, respectively) and performed poorly compared to other culture protocols. The culture protocols with selective enrichment in RVS broth at 37 °C (protocols C and G) provided zero detection rate of *Salmonella*. Thus, these selective enrichment conditions did not perform adequately.

Regarding the solid media used, the most effective for the isolation of *Salmonella* from wastewater was XLD agar, followed by DC agar and Rambach agar. All the solid media used gave false positive colonies. *Proteus* spp. were the bacteria most frequently identified that gave false positive reactions on XLD agar and DC agar.

A panel of six antibiotics for *Salmonella* strains included ampicillin (10 μg), pefloxacin (5 μg), cefotaxime (5 μg), gentamicin (10 μg), chloramphenicol (30 μg), and trimethoprim-sulfamethoxazole (1.25-23.75 μg). A total of 93 *Salmonella* isolates were selected for antimicrobial susceptibility testing. Of these, 71,0 % (66/93) were susceptible to all antibiotics tested. The highest resistance rate was observed for pefloxacin (18.3%, 17/93). Resistance to gentamicin was found in six isolates (6.5%, 6/93). Three isolates showed resistance to ampicillin and trimethoprim-sulfamethoxazole (3.2%, 3/93). All 93 strains were susceptible to cefotaxime and chloramphenicol.

2 Uptake of polyfluorinated pharmaceuticals efavirenz and flufenamic acid by plants under hydroponic conditions and their detection in plant extracts

The purpose of the second part of the dissertation was to evaluate the uptake of polyfluorinated pharmaceuticals efavirenz and flufenamic acid from contaminated water by different plant species, namely cress (*Lepidium sativum*), rocket (*Eruca sativa*), radish (*Raphanus sativus)*, and pea (*Pisum sativum*). The ability to naturally absorb target pharmaceuticals by the selected model plants was evaluated under hydroponic conditions using aqueous solutions of each pharmaceutical as the growing medium. In this part of the dissertation, conditions for the hydroponic cultivation of plants and the method for the processing of plant material before extraction of the target pharmaceuticals were designed. Furthermore, the extraction procedure of efavirenz and flufenamic acid from plants was optimised. Finally, an analytic method based on HPLC/MS analysis for the detection of efavirenz and flufenamic acid in plant extracts was proposed.

2.1 Introduction

Reuse of treated wastewater for irrigation leads to the introduction of contaminants of emerging concern into the soil environment (Kampouris *et al.* 2021). A major concern regarding agricultural applications of treated wastewater is the introduction of contaminants into crops through plant uptake. Contamination of crops by these chemicals may pose potential health risks to humans (Wu *et al.* 2015).

Hydroponic experiments in which plants are exposed to target pharmaceuticals through a growing medium can be used to quickly screen and identify pharmaceuticals with high potential for uptake by plants (Wu *et al.* 2015; Madikizela *et al.* 2018; Klampfl 2019). Hydroponic experiments are simple to perform and reduce workload. Moreover, hydroponic cultivation allows minimising the influence of bacterial activity on the experiments. However, because of the complex processes of pharmaceuticals in the soil, the uptake of target pharmaceuticals from the growing medium under hydroponic conditions and from the soil can be considerably different. Therefore, it should be cautious to make predictions about the uptake of pharmaceuticals by plants in real environments based on hydroponic experiments (Wu *et al.* 2015).

2.2 Experimental Part

2.2.1 Model plants and hydroponic experiments

Cress (*Lepidium sativum*), rocket (*Eruca sativa*), radish (*Raphanus sativus)*, and pea (*Pisum sativum*) were used as model plants. Solutions of efavirenz and flufenamic acid in distilled water at concentrations of 1.00, 3.75, and 7.50 mg/L were used as a growing medium. The plants were cultivated under hydroponic conditions in Petri dishes covered with synthetic polyester wool for seven days in total. A gram of cress, rocket, and radish seeds or 20 seeds of pea were sown. The plants were exposed to pharmaceuticals throughout the 7-day cultivation period, that is, even during the germination period. The total volume of watering during the 7-day cultivation period was 150 mL; however, the daily volume of watering was not identical and varied depending on the growth stage of the plants. Plant samples were taken once at the end of the 7-day cultivation period or daily throughout the 7-day cultivation period. The cultivation conditions and the watering system were identical for both sampling methodologies (Table 5). Before harvesting, the plants were rinsed with distilled water and dried with a paper towel. All plants grown in the Petri dish were collected for analysis. The plants were freeze-dried and then pulverised to a fine powder.

Table 5 *Plant watering system and sampling methodology*

Day of cultivation	Daily volume of watering	One-time sampling	Daily sampling
Sowing of plants (Monday)	40 mL	---	---
Day 1 (Tuesday)	30 mL	---	✓
Day 2 (Wednesday)	20 mL	---	✓
Day 3 (Thursday)	20 mL	---	✓
Day 4 (Friday)	40 mL	---	✓
Day 5 (Saturday)	without watering	---	✓
Day 6 (Sunday)	without watering	---	✓
Day 7 (Monday)	without watering	✓	✓

2.2.2 Extraction of efavirenz and flufenamic acid and HPLC/MS analysis

The extraction of efaverine and flufenamic acid was carried out using 20 mL of methanol/1M NaOH (10:1, v/v) mixture in an ultrasonic bath for 60 minutes. The plants were separated from the extraction solvent by centrifugation at 4688 x *g* for 30 min at 4 °C. The plant extracts were stored frozen at -20 °C until HPLC/MS analysis. Analysis of efavirenz and flufenamic acid in plant extracts was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with mass spectrometric (MS) detection. The quantitative analysis of the target pharmaceuticals was performed by the calibration curve method. Optimal separation was achieved using an Ascentis Express C18 column (150 × 3 mm, particle size 2.7 µm) in combination with a mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) operating in the gradient mode as follows: 0 to 2 min: 20% B, 2.5 to 3 min: 90% B, and 6 min: 20% B.

2.3 Results

2.3.1 Optimization of extraction of efavirenz and flufenamic acid from plants

The extraction procedure of efavirenz and flufenamic acid from plants was optimized with respect to the type of extraction solvents used, the extraction time in the ultrasonic bath, and the overall time and manual effort of the extraction, including the drying process. For the extraction of the target pharmaceuticals, three extraction solvents were tested, methanol/HCl (10:1, v/v), acetonitrile/HCl (125:1, v/v) and methanol/1M NaOH (10:1, v/v). The extraction times tested were 15, 30, 45 and 60 minutes. The best extraction yield for both pharmaceuticals was achieved using the methanol/1M NaOH (10:1, v/v) mixture, during extraction in an ultrasonic bath for a period of 60 minutes. Various forms of processing of the acquired extract were tried before HPLC/MS analysis. The extract was evaporated under a nitrogen stream and redissolved in 1 mL of mixture corresponding to the mobile phase at the beginning of the gradient (20% methanol). Furthermore, 90% methanol and 20% acetonitrile were tested to redissolve the evaporated extract. The results obtained showed that evaporation and redissolution of the dried extract reduced the yield compared to the analysis of the untreated extract obtained directly after extraction by 20 mL of mixture of methanol/1M NaOH (10:1, v/v). Therefore, evaporation and reconstitution steps were not used in the proposed extraction procedure. Furthermore, SPE extraction was tested on columns with different types of stationary phases (Strata C18 and Oasis HBL). Methanol was used as elution solvent. However, the SPE step did not provide sufficient results since it not only prolonged the sample processing time, but also contributed to the loss of target pharmaceuticals. Therefore, the SPE step was not included in the proposed extraction procedure.

2.3.2 Uptake of efavirenz and flufenamic acid by model plants

Both target pharmaceuticals were detected in all plant species at all three levels of pharmaceutical concentration in the growing medium. As expected, the highest concentration of flufenamic acid and efavirenz was detected in plants at the highest concentration of pharmaceuticals in the growing medium (7.50 mg/L).

In terms of plant species used, the radish exhibited the highest ability to absorb flufenamic acid and efavirenz. On the contrary, the rocket showed the lowest uptake of both target polyfluorinated pharmaceuticals. Regarding target pharmaceuticals, higher uptake by plants was found for efavirenz. At identical concentration levels of both target pharmaceuticals in the growing medium, up to an order of magnitude higher concentrations in µg/g were determined in plants in the case of efavirenz (Table 6).

Table 6 *Flufenamic acid and efavirenz concentrations in plants at the end of the 7-day cultivation period (µg/g fresh weight)*

Pharmaceutical	**Concentration of the pharmaceutical in the growing medium**	**Model plant**			
		Cress	**Rocket**	**Radish**	**Pea**
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Flufenamic acid	7.50 mg/L	2.33 ± 0.17	0.85 ± 0.08	6.80 ± 0.24	1.80 ± 0.17
	3.75 mg/L	0.89 ± 0.20	0.69 ± 0.19	2.09 ± 0.15	1.59 ± 0.15
	1.00 mg/L	0.37 ± 0.06	0.45 ± 0.18	1.41 ± 0.22	0.77 ± 0.10
Pharmaceutical	**Concentration of the pharmaceutical in the growing medium**	**Model plant**			
		Cress	**Rocket**	**Radish**	**Pea**
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Efavirenz	7.50 mg/L	7.46 ± 0.82	2.20 ± 0.26	23.60 ± 5.11	9.92 ± 1.13
	3.75 mg/L	6.15 ± 0.99	1.94 ± 0.28	17.46 ± 1.43	4.68 ± 0.62
	1.00 mg/L	0.75 ± 0.10	1.04 ± 0.30	4.68 ± 0.51	2.17 ± 0.20

[a] Average concentration (n=6).

A significant decrease in the detected amount of flufenamic acid in radish and cress was observed when the concentration of this pharmaceutical in the growing medium was reduced to 3.75 mg/L and 1.00 mg/L, respectively. In contrast, for rocket and pea, the difference between the amount of flufenamic acid detected in plants at different concentration levels of this pharmaceutical in the growing medium was significantly lower. This phenomenon was especially evident for rocket, in which the concentration of flufenamic acid at all concentration levels of this pharmaceutical in the growing medium ranged from 0.45 to 0.85 µg/g (Figure 1).

In radish and cress, a significant decrease in the detected amount of efavirenz was observed only at the lowest concentration of this pharmaceutical in the growing medium (1.00 mg/L). In contrast, at concentrations of 3.75 mg/L and 7.50 mg/L of efavirenz in the growing medium, the amount of this pharmaceutical detected was comparable. In the rocket there was again only a slight difference evident between the concentrations of efavirenz detected in plants at different concentration levels of this pharmaceutical in the growing medium. In pea, the trend of decreasing the amount of efavirenz detected in plants corresponded to a gradual decrease in the concentration of this pharmaceutical in the growing medium (Figure 2).

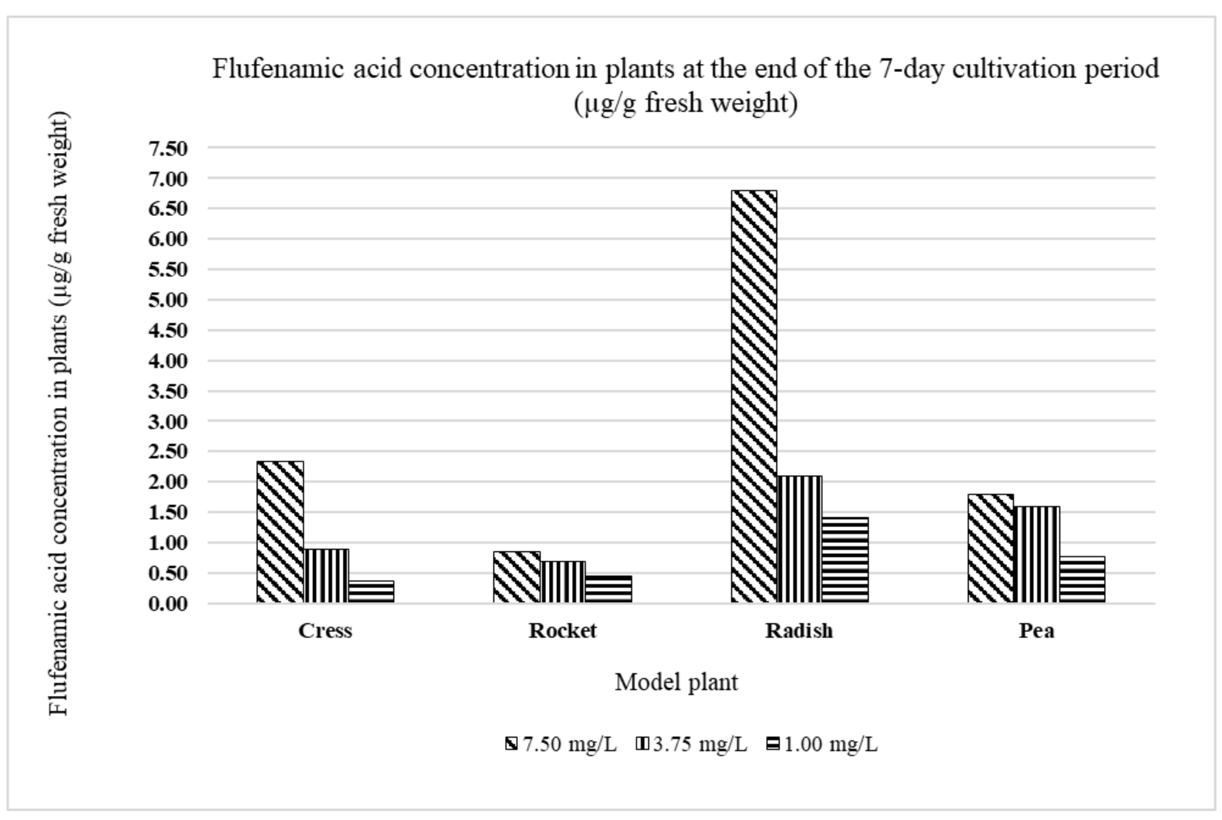

Figure 1 *Flufenamic acid concentration in plants at the end of the 7-day cultivation period (µg/g fresh weight)*

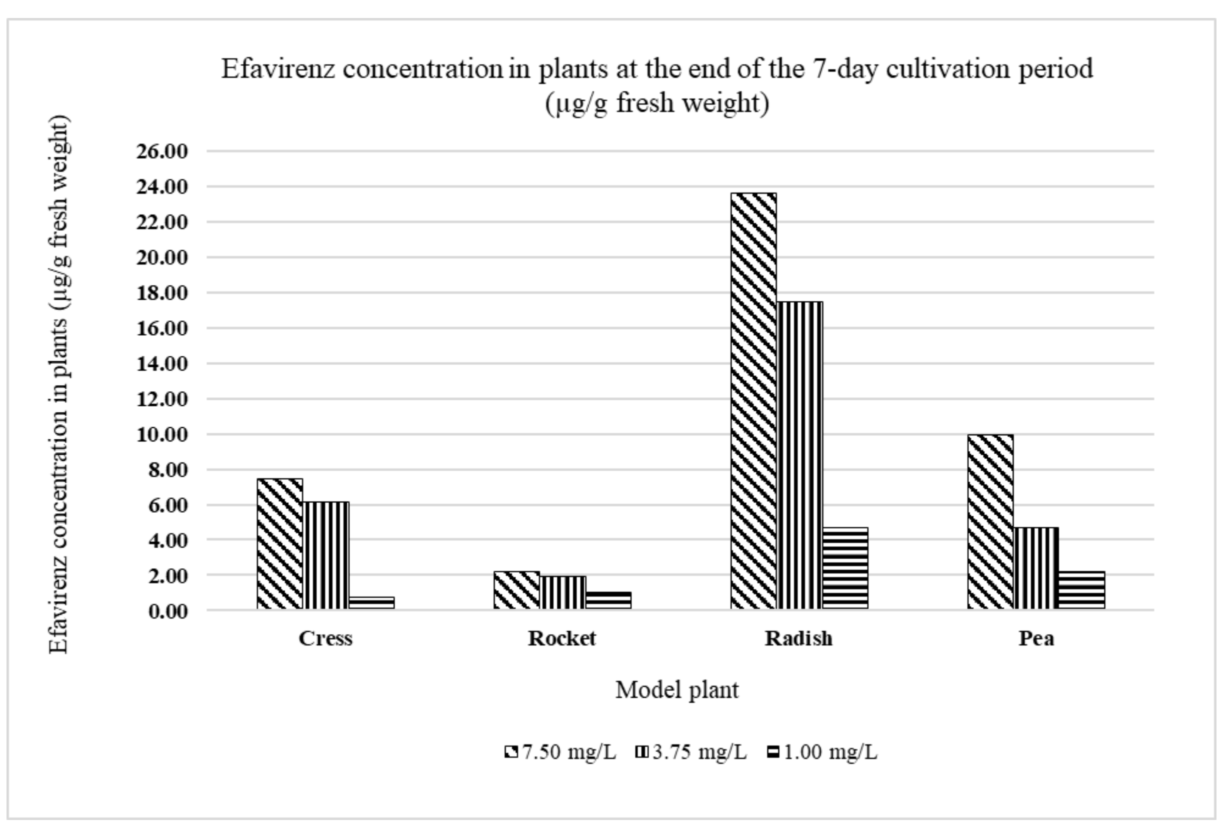

***Figure 2** Efavirenz concentration in plants at the end of the 7-day cultivation period (µg/g fresh weight)*

The trends in the uptake of pharmaceuticals by plants did not depend on the type of pharmaceutical, but on the type of plant. Furthermore, the trends did not depend on the concentration of pharmaceuticals in the growing medium, as showed quite the same behaviour for the three concentrations studied. The results are detailed in Tables 7, 8, and 9.

In cress and rocket, the uptake of flufenamic acid and efavirenz showed a very similar trend. This consisted of a very rapid increase in the concentration of pharmaceuticals in the plant, with the highest concentrations detected reached on the third or fourth day of cultivation. This was followed by a phase characterised by a steep decrease in the concentration of pharmaceuticals in the plant with time of exposure. In radish and pea, the uptake of both flufenamic acid and efavirenz was characterised by a continuous increase in the concentration of pharmaceuticals in the plant with exposure time. The highest concentration of target pharmaceuticals was detected in the plants on the last day of cultivation.

Table 7 Flufenamic acid and efavirenz concentrations in plants during 7-day cultivation period (pharmaceuticals concentration in the growth medium 1.00 mg/L)

Pharmaceutical Concentration of the pharmaceutical in the growing medium	Day of cultivation	Model plant			
		Cress	Rocket	Radish	Pea
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Flufenamic acid 1.00 mg/L	1	0.39 ± 0.05	0.28 ± 0.09	0.40 ± 0.10	0.30 ± 0.06
	2	0.66 ± 0.11	0.55 ± 0.16	0.63 ± 0.13	0.36 ± 0.09
	3	1.29 ± 0.28	1.10 ± 0.30	2.41 ± 0.55	0.32 ± 0.11
	4	1.13 ± 0.12	0.67 ± 0.40	2.00 ± 0.18	0.29 ± 0.04
	5	0.74 ± 0.22	0.52 ± 0.12	1.49 ± 0.23	0.30 ± 0.06
	6	0.44 ± 0.12	0.46 ± 0.03	1.29 ± 0.43	0.50 ± 0.12
	7	0.38 ± 0.11	0.20 ± 0.12	1.30 ± 0.11	0.88 ± 0.22

Pharmaceutical Concentration of the pharmaceutical in the growing medium	Day of cultivation	Model plant			
		Cress	Rocket	Radish	Pea
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Efavirenz 1.00 mg/L	1	0.33 ± 0.04	0.39 ± 0.07	0.64 ± 0.03	0.28 ± 0.05
	2	0.91 ± 0.06	0.74 ± 0.08	1.55 ± 0.17	0.31 ± 0.05
	3	1.29 ± 0.11	1.61 ± 0.12	2.76 ± 0.20	0.34 ± 0.01
	4	1.14 ± 0.08	1.36 ± 0.06	4.29 ± 0.44	0.70 ± 0.09
	5	0.98 ± 0.16	1.19 ± 0.04	3.90 ± 0.27	1.19 ± 0.12
	6	0.74 ± 0.06	0.96 ± 0.16	3.02 ± 0.39	1.72 ± 0.04
	7	0.53 ± 0.07	0.41 ± 0.08	3.95 ± 0.52	2.05 ± 0.04

[a] Average concentration (n=3).

Table 8 *Flufenamic acid and efavirenz concentrations in plants during 7-day cultivation period (pharmaceuticals concentration in the growth medium 3.75 mg/L)*

Pharmaceutical Concentration of the pharmaceutical in the growing medium	Day of cultivation	Model plant			
		Cress	Rocket	Radish	Pea
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Flufenamic acid **3.75 mg/L**					
	1	0.27 ± 0.07	0.20 ± 0.05	0.91 ± 0.15	0.57 ± 0.05
	2	0.53 ± 0.09	1.24 ± 0.16	1.24 ± 0.10	0.72 ± 0.09
	3	1.55 ± 0.33	1.94 ± 0.24	4.43 ± 0.41	1.17 ± 0.13
	4	1.30 ± 0.20	2.30 ± 0.30	7.05 ± 0.98	1.29 ± 0.14
	5	0.99 ± 0.16	2.05 ± 0.20	7.50 ± 0.60	1.49 ± 0.20
	6	0.85 ± 0.05	0.35 ± 0.08	8.82 ± 1.01	1.60 ± 0.23
	7	0.96 ± 0.11	0.44 ± 0.10	11.38 ± 1.53	1.95 ± 0.19

Pharmaceutical Concentration of the pharmaceutical in the growing medium	Day of cultivation	Model plant			
		Cress	Rocket	Radish	Pea
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Efavirenz **3.75 mg/L**					
	1	1.22 ± 0.01	1.15 ± 0.18	1.46 ± 0.34	1.82 ± 0.19
	2	3.11 ± 0.21	3.12 ± 0.43	3.68 ± 0.11	1.97 ± 0.13
	3	5.44 ± 0.15	3.64 ± 0.39	5.96 ± 0.82	2.48 ± 0.35
	4	4.65 ± 0.15	3.74 ± 0.57	8.30 ± 0.89	3.01 ± 0.29
	5	0.74 ± 0.07	2.93 ± 0.29	9.35 ± 0.55	3.56 ± 0.47
	6	1.08 ± 0.12	2.43 ± 0.11	12.90 ± 1.48	3.68 ± 0.41
	7	3.11 ± 0.39	2.04 ± 0.37	16.03 ± 1.73	4.14 ± 0.21

[a] Average concentration (n=3).

Table 9 *Flufenamic acid and efavirenz concentrations in plants during 7-day cultivation period (pharmaceuticals concentration in the growth medium 7.50 mg/L)*

Pharmaceutical / Concentration of the pharmaceutical in the growing medium	Day of cultivation	Model plant: Cress	Rocket	Radish	Pea
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Flufenamic acid 7.50 mg/L	1	0.24 ± 0.07	0.87 ± 0.09	1.69 ± 0.15	0.99 ± 0.11
	2	0.38 ± 0.04	1.32 ± 0.12	2.91 ± 0.31	1.34 ± 0.18
	3	0.95 ± 0.09	1.92 ± 0.20	7.71 ± 0.89	1.44 ± 0.17
	4	2.16 ± 0.20	3.00 ± 0.39	13.71 ± 1.51	2.45 ± 0.19
	5	2.83 ± 0.21	2.30 ± 0.24	14.59 ± 1.99	3.50 ± 0.31
	6	3.04 ± 0.33	1.38 ± 0.17	16.08 ± 1.64	3.90 ± 0.32
	7	2.66 ± 0.31	0.71 ± 0.08	18.96 ± 2.01	4.37 ± 0.53
Pharmaceutical / Concentration of the pharmaceutical in the growing medium	**Day of cultivation**	**Model plant: Cress**	**Rocket**	**Radish**	**Pea**
		Concentration of the pharmaceutical in µg/g fresh weight[a]			
Efavirenz 7.50 mg/L	1	0.50 ± 0.01	2.32 ± 0.32	3.20 ± 0.25	1.53 ± 0.38
	2	3.38 ± 0.66	5.99 ± 1.40	7.53 ± 0.40	2.20 ± 0.77
	3	5.49 ± 0.99	6.20 ± 1.36	9.10 ± 1.36	2.50 ± 0.59
	4	5.98 ± 1.13	5.69 ± 0.43	11.52 ± 0.49	2.60 ± 0.25
	5	4.25 ± 0.75	4.26 ± 1.25	18.14 ± 2.65	3.14 ± 0.80
	6	6.48 ± 1.01	3.97 ± 1.46	23.52 ± 3.12	4.05 ± 0.42
	7	7.50 ± 1.60	3.84 ± 1.07	30.00 ± 2.99	6.50 ± 0.57

[a] Average concentration (n=3).

3 Evaluation of acute toxicity during photocatalytic degradation of chloroacetanilide herbicides alachlor, metolachlor, and acetochlor

The purpose of the third part of the dissertation was to evaluate the changes in acute toxicity of reaction solutions during the heterogeneous photocatalysis of the chloroacetanilide herbicides alachlor, metolachlor, and acetochlor via a growth inhibition test using the green alga *Chlorella kessleri* as a test organism. A toxicity assessment represented one of the approaches used to assess the suitability of various experimental setups of heterogeneous photocatalysis (TiO_2/UV-A) for the removal of selected chloroacetanilide herbicides from contaminated water. For this part of the thesis, the algae growth inhibition test was modified with respect to the use of the *Chlorella kessleri* strain.

3.1 Introduction

An important property of advanced oxidation processes (AOPs) is their ability to detoxify treated water through the decomposition of highly toxic organic compounds into less toxic or even nontoxic products (Babu *et al.* 2019). However, in some cases, the oxidation of the contaminants can lead to the creation of toxic degradation products, which are often more toxic than the parent compound itself. Similarly, even incomplete oxidation of contaminants leads to the creation of toxic intermediate products (Sharma *et al.* 2018; Babu *et al.* 2019). Therefore, acute toxicity tests are often employed during different stages of the AOP treatment of contaminated water. Additionally, toxicity testing also helps to demonstrate the utility of AOPs in reducing the toxicity of treated water for its safe disposal in the environment (Babu *et al*. 2019).

Biological toxicity tests use living organisms to determine the toxicity of a chemical (Ghosh *et al.* 2017). The resulting reaction of the test organism during the biological toxicity test is the consequence of the combined effects of the mixture of all the biologically available known and unknown compounds and metabolites present (Babu *et al.* 2019; De Baat *et al*. 2019). Algae are ideal organisms for toxicity testing since they are primary producers and the first level of the aquatic food chain. Therefore, any effect on algae will have an impact on higher trophic levels (Machado and Soares 2020).

3.2 Experimental Part

3.2.1 Test organism and algae growth inhibition test

The freshwater alga *Chlorella (Ch.) kessleri* Fott *et* Novak (strain LARG/1) obtained from the Culture Collection of Autotrophic Organisms (CCALA) of the Institute of Botany of the Czech Academy of Sciences was used as a test organism. The algae growth inhibition test was performed according to the recommendations described in Organisation for Economic Cooperation and Development Test No. 201 (OECD 201) but modified with respect to the use of the *Ch. kessleri* strain. OECD medium prepared according to OECD 201 was used as a growth medium. The test was carried out at 28 ± 2 °C for 72 ± 2 h under agitation on an orbital shaker with a frequency of 120 rpm in a culture box under white light in a light regime of 18 hours of light and 6 hours of darkness. The light intensity on the surface of the algal cultures was equivalent to 9000 lux. The cell concentration at the beginning of the test was $5 \cdot 10^4$ cells/mL.
At the end of the test, cell density was determined by measuring optical density at 684 nm (OD_{684}) using a spectrophotometer. Growth rates and percentage inhibition of growth rate were calculated according to OECD 201. The percentage inhibition of the growth rate was plotted against the time of photocatalysis to evaluate changes in the acute toxicity of the reaction solution during photocatalytic degradation.

3.3 Results

3.3.1 Modification of the algae growth inhibition test

The algae growth inhibition test conditions defined in OECD 201 are designed exclusively for the specific algal species recommended by the methodology, these being *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. Therefore, when *Ch. kessleri* was used, it was necessary to modify the test conditions to meet the required test validity criteria. To ensure sufficient biomass growth over 72 hours, it was necessary to use an order of magnitude higher initial cell concentration than that recommended by the OECD 201 methodology. To increase the growth rate, it was also necessary to increase the incubation temperature from the recommended range of 21 to 24 °C to 28 °C and use a light intensity corresponding to 9000 lux on the surface of the algal cultures. Nevertheless, despite these conditions under continuous lighting leading to a growth rate exceeding 1.4 d^{-1}, they also led to the rapid exhaustion of the algal cultures, which manifested as a transition from the exponential to the stationary growth phase as soon as 48 hours. Maintenance of algal cultures in an exponential growth phase for the entire test period was achieved by changing the light regime from continuous lighting to a regime of 18 hours of light and 6 hours of darkness. Despite this light regime leading to a mild reduction in growth rate, the average growth rate

in controls was still above 0.92 d^{-1} and, consequently, the test validity criterion was met. With the set light regime, the growth rates in the controls ranged from 1.2 d^{-1} to 1.3 d^{-1}. The coefficient of variation in the controls did not exceed 2.5% in any of the tests. For test validity, the coefficient of variation for controls must not exceed 5% (OECD 201).

3.3.2 Changes in acute toxicity of reaction solutions during photocatalytic degradation of alachlor, metolachlor, and acetochlor

Due to the concentration of herbicides in the initial reaction solution (10 mg/L), the samples were diluted (1000 times) with sterile deionized water to obtain concentrations approximately corresponding to the estimated values of EC_{50} of target chloroacetanilide herbicides.

Although the various experimental setups of heterogeneous photocatalysis resulted in the removal of 98.2% of the metolachlor, 97.5% of the alachlor, and 93.1% of the acetochlor from the reaction solution, this efficiency was not sufficient for a reduction in toxicity. Therefore, the initial hypothesis that with a decreasing concentration of herbicides in the reaction solution, its toxicity would also decline was not confirmed. During the degradation of metolachlor, a continuous increase in toxicity of the reaction solution was observed throughout photocatalysis. As a result, the toxicity of the reaction solution was highest at the end of photocatalysis at the 180th minute. In contrast, during the degradation of acetochlor, the toxicity of the reaction solution changed only minimally throughout photocatalysis, and the relation between the percentage inhibition of the growth rate of the green algae *Ch. kessleri* and the photocatalysis time was essentially parallel to the x axis. In the degradation of alachlor, a decrease in toxicity of the reaction solution was observed as the only herbicide during heterogeneous photocatalysis. However, the decrease in toxicity of the reaction solution occurred only during the first 15 minutes of the process, while from the 30th minute, the toxicity of the reaction solution gradually increased. At the 180th minute, the toxicity of the final reaction solution corresponded to that of the initial alachlor solution. Thus, for all herbicides, the acute toxicity of the final reaction solution after 180 minutes of heterogeneous photocatalysis was found to be comparable to or even greater than the acute toxicity of the initial reaction solution of 10 µg/L herbicides. Because the concentrations of all herbicides decreased during heterogeneous photocatalysis, it is likely that the increased acute toxicity of the reaction solutions was the result of the creation of photocatalytic degradation products that influenced algae growth to a greater degree than those of the original parent herbicides. However, neither ethanesulfonic acid nor oxanilic acid, considered the primary metabolites resulting from the degradation of chloroacetanilide herbicides, were detected in the reaction solutions.

4 Conclusions

The purpose of the first part of the dissertation was to determine the optimal culture protocol for the isolation of selected pathogenic bacteria from wastewater. In the case of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, this objective was met. For the isolation of *Staphylococcus aureus* from wastewater, a culture protocol that includes enrichment in BHI broth with 6.5% NaCl followed by plating on B-P agar can be considered optimal. The optimal culture protocol for the isolation of *Pseudomonas aeruginosa* combined enrichment in TSB broth followed by plating on cetrimide agar. However, in the case of *Yersinia enterocolitica* and *Salmonella*, it would be imprudent to recommend a single culture protocol, as it was evident that the performance of individual culture protocols varied between different types of wastewater. However, it can be stated that the culture protocol for the isolation of *Yersinia enterocolitica* from wastewater should include enrichment in PSB broth with alkaline treatment of the enriched culture before plating on CIN agar. For the isolation of *Salmonella* from wastewater, the combination of selective enrichment in MKTTn broth at an elevated incubation temperature of 41.5 °C followed by plating on XLD agar could constitute the best approach. However, it should be noted that the range of variables that can be evaluated in the optimisation of a culture protocol is considerable and not all of them were evaluated in this work.

In the second part of the dissertation, the uptake of the polyfluorinated pharmaceuticals efavirenz and flufenamic acid was confirmed under hydroponic conditions by all model plant species used. Although the growth medium contained the target pharmaceuticals in mg/L, in the plants, the unchanged pharmaceuticals were detected at a concentration level of µg/g. The radish exhibited the highest ability to absorb flufenamic acid and efavirenz. On the contrary, the rocket showed the lowest uptake of both target polyfluorinated pharmaceuticals. Regarding target pharmaceuticals, higher uptake by plants was found for efavirenz.

Heterogeneous photocatalysis using titanium dioxide as a photocatalyst proved to be a suitable technology for the removal of the chloroacetanilide herbicides alachlor, metolachlor, and acetochlor from aqueous solutions. However, even at the highest removal of target herbicides from the reaction solutions, there was no reduction in toxicity. Although the concentrations of all herbicides during photocatalysis decreased, the acute toxicity of the reaction solutions at the end of the process was comparable to or greater than the acute toxicity of the initial reaction solution of 10 µg/L herbicides. This phenomenon may be attributed to the formation of degradation photocatalytic products that influenced the growth of the green alga *Chlorella kessleri* to a greater extent than the original herbicides.

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6 List of Students' Published Works

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