BABES-BOLYAI UNIVERSITY OF CLUJ-NAPOCA
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

DOCTORAL SCHOOL OF CHEMISTRY

PhD THESIS

ASSESSING OF ORGANIC COMPOUNDS PATH IN LIVING SYSTEMS BY EXPERIMENTAL AND NUMERICAL MODELLING

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Thesis content

Abstract content

Keywords

nsaid, lycopersicon esculentum, pathway, transformation products, attenuation, difussion, sem-pls-pm, dynamic boundary

Chapter 1. Introduction

Although, pharmaceutical industry has developed continuously and progressively since the last decade of the 19th century, with a significant peak in the immediate aftermath of World War II, inclusion it as a concern for environment is quite recent – only in the early 2000 [4]. Until that period, consideration of this branch of industry as an environmental issue was attributed to be insignificant and the only potential to pollute the environment was considered arise solely from the manufacturing facilities [5]. However, these were estimated to be relatively small with controlled emissions, while the probability for an accidentally spills was appreciated to be reduced. Concerns surrounding these chemicals appeared in 1994 when presence of clofibric acid was identified by Stan et al., [6] in surface waters from Germany. After that, once because of accorded interest and secondly due to registered developments in analytical instrumentation technologies, pharmaceuticals active ingredients started to be reported in multiple environmental matrices (surface water, groundwater, sediments, soil, etc.) all over the world [7-13]. Now, pharmaceuticals active ingredients belonging to analgesics, antifungals, antacids, antibiotics, anticancer drugs, anticonvulsants, antidepressants, antidiabetic drugs, anthelmintics, antihistamines, antihypertensive drugs, anti-inflammatories, antipsychotics, β-blockers, contraceptives, contrast media, lipid regulators, antivirals, diuretics, psychiatric drugs, antiallergics, stimulants, bronchodilators and other drugs are widely detected in different environmental matrices at global level [7; 9; 12; 14-21].

With the spread of reports about pharmaceuticals presence in environment, they have become a global issue of growing concern. Concerns became justified as studies showed that pharmaceutical presence in environment are persistent and pseudo-persistent [22-26] with a significant bioaccumulation potential in living organisms of different trophic level [27-31]. Their direct and indirect ways of entry into environment were well established. Metabolic excretion by humans, livestock and industrial activities are considered as direct ones, while improper disposal and sewage treatment are considered as representative indirect ones.

As many pharmaceuticals are partially metabolized by consuming organisms and wastewater treatment plant not entirely have the ability to remove these products causing their liberation into environment both in unchanged and transformed forms of them, questions have been raised relating to potential negative effects occurrence on the environment, biota and not least on any living organisms connected to the contaminated environment or biota.

Non-steroidal anti-inflammatory drugs (NSAIDs) are known to have the highest consumption rate worldwide because of their wide spectrum of clinical applications. Today NSAIDs are ranked as the second medical prescription class of drugs as follow antibiotics [3]. These are over the counter remedies available all over the world used mainly for their antipyretic (relieve fever), anti-inflammatory (reduce inflammation), and analgesic (relieve pain) properties.

Pharmaceuticals, as NSAIDs, reach soil and water environment through numerous ways of entry, and are redistributed through different environmental compartments. One of the reasons of that is because current WWTPs technologies failed in their complete removal. Resulted WWTP products as effluents and sludge reach either water and soil environment. Continuous use of these pharmaceuticals and their improper removal assures a continuous influx of them in environment. At moment, the advances in analytical technologies made possible the detection and quantification of NSAIDs as diclofenac, ibuprofen and ketoprofen active ingredients in environmental and biological samples. These NSAIDs active ingredients may be mobile and persistent in different environmental compartments and ecological receptors even at ppb levels. However, relevant data on their pathway and behavior in the environment, as well on threat to ecological and non-targeted living organism health are still lacking. Moreover, the ecotoxicological significance of NSAIDs related TPs remains largely unknown at moment. Such knowledge is imperatively needed to boost research and development for new and effective treatment technologies, in line with the mode of fate, uptake and consequences of each NSAIDs active ingredients. Addressing the topic of TPs formation, pathway in environment, bioaccumulation potential in non-targeted organisms is important as our previous experience with other organic chemical contaminants has proved that TPs often could possess more accentuated adverse effects and impact on organisms and environment than corresponding parent compounds. In case of NSAIDs, recently started to be published toxicological studies on ibuprofen hydroxylated TPs adverse effects on living organisms. These studies evidenced cyto-toxicological, teratogenic, and mutagenic effects. Although other organic contaminants, e.g., agrochemicals, are only periodically introduced into environment, NSAIDs – as popular, frequently used, over-to-counter pharmaceuticals, and who are inefficiently removed by WWTPs, are continuously introduced in environment. Therefore, the incidence of unwanted exposure to NSAIDs of non-targeted living organisms is obvious and real.

Potential impact of climate change on fate of organic contaminants in environment has reached a growing interest as frequency and amplitude of extreme events as heat waves, flood

or drought become more pronounced all over the world. The impact of warmer temperatures and meteorological anomalies on NSAIDs active ingredients are missing at moment, although there are a growing number of studies that are being to study and evaluate these impacts and changes in a quantitative way on regulated organic chemical contaminants. Based on available literature search, studies on environmental cycling of NSAIDs, and especially their TPs formation potential, are not existent at moment up to our knowledge. So, examination and evidence acquiring on how chemical and biological processes are changed for major environmental compartments (soil, water), and how these indirect effects of climate change impact NSAIDs behavior are a requirement to fulfil the knowledge gap about these chemical pathways in environment. Moreover, if significant changes to the biotic and abiotic components and properties of the environment are attributed due to climate change, these in turn may impact how we currently assess the environmental risk of organic chemical contaminants.

Chapter 2. Experimental concept description

2.1. Exposure scenario proposed for model plant

Generally, pharmaceuticals could reach the environment through multiple ways. Although improper disposal or accidental discharge contribute to pharmaceuticals entrance into the environment, this route is considered with minor contribution in case of NSAIDs if we take into account their large presence in different environmental compartments all over the world. Main way throughout the selected pharmaceuticals reaches the environment is human and animal excretion that enter into sewage systems and wastewater systems [7; 369-371]. Increased amounts of resulting sludge from wastewater treatment plants and sewage system plants, gathered a reuse potential given by their increased content of organic matter, minerals and other benefic fertilizing components [372; 373]. These made from their disposal on soils (agricultural, forest, etc.) a proper way to handle them. Also, increased water scarcity due to climate changes, made treated wastewater reuse to become a solution for agricultural lands irrigation [374-376]. A significant problem connected with sewage and wastewater systems current technologies are their reduced capacity to eliminate the new generation of contaminants like pharmaceuticals [377-381].

According to these, pharmaceuticals as NSAIDs could reach the environment through multiple ways. Therefore, tomato (*Lycopersicon esculentum*) could get contact with NSAIDs through multiple routes as soil, water, pulverized water (as precipitation), or combinations of one or more of these major routes. In this study each of these exposure routes were considered in order to evaluate their potential amplitude in contamination with NSAIDs of *Lycopersicon esculentum*, especially its edible part - the tomato fruit.

Tomato plants were grown in a controlled environment of a climate change chamber. Climate change chamber (1 m^3) was designed and constructed in the laboratory with plexiglass. This chamber allowed controlled light-dark cycle, humidity and temperature. Briefly, 14 days old tomato (*Lycopersicon esculentum*) seedlings were planted in approximately 2 L volume experimental pots. Germination of tomato seeds was performed at 25 °C at an incubator (LabCompanion, Billerica, MA, USA). After seedling transfer in experimental pots at the climate chamber the following conditions were kept for plant development: 14 h day light, 10 h darkness; day temperature 25 °C and night temperature 18 °C. Daylight blend matching the natural sunlight was done with Mammoth LED that enclose an unremitting range of wavelengths from blue and green to red. Soil humidity was adjusted at 58 % water holding

capacity. To ensure tomato development 5 g of fertilizer were added to each pot as described by Kovacs et al. [382].

First, uptake of diclofenac, ibuprofen and ketoprofen in the edible part of tomato was quantified. Secondly, translocation factors of these pharmaceuticals active ingredients form contaminated environmental compartment to plant as well translocation factors from one anatomical compartment to another of tomato was also estimated. And not at last, metabolites of studied NSAIDs were kept in attention for the whole tomato plant. Studies were performed involving all development stages of tomato (time vs. plant evolution). Therefore, tomato plant samples were collected at each four major stages of their development: *stage 1* - vegetative, *stage 2* - flowering; *stage 3* - fruit formation, and *stage 4* - mature fruit (harvesting).

Moreover, as pharmaceuticals active ingredients reach one or more environmental compartments [369], they undergo several biotic and abiotic transformations. These are complex processes that result in formation of new chemicals called TPs about whose persistence in the environment or potential bioavailability and toxicity are little knowledge at this moment. To fulfill these identified gaps of knowledge, the fate of each selected NSAIDs in soil and water was studied. In this case no plants were present in experiment boxes. Influence of biotic and abiotic processes were quantified through laboratory experiments under controlled conditions (Table 4). Influence of different physical, chemical and biological parameters was evaluated also as additional experimental variables for a better assessment of diclofenac, ibuprofen and ketoprofen fate in these environmental compartments.

Table 4. NSAIDs fate evaluation in main environmental compartments

Environmental matrices	Monitored processes	Main evaluated parameters
✓ Soil	✓ Biotic processes	✓ Active ingredients quantification in time
✓ Water	✓ Sorption processes	✓ GC-MS identification of potential transformation processes
	✓ Abiotic processes	

2.2. Modelling concept planned for NSAIDs pathway assessment in complex systems

Understanding NSAIDs pathway in environment is particularly important in relations with assessment of their potential impact on non-targeted living organisms. It is important to find out both the concentrations as well the formation potential of related TPs in different environmental matrices and living systems as well. This is an interest, since there are

assumptions based on previous evidence that organic chemical contaminants could have unwanted effects on environment and biota [383].

To properly estimate NSAIDs impact on a living organism it is necessary first to figure out as accurate as possible their pathway in individual environmental compartments. Thus, become possible to acquire information about their time spend in the specific environmental compartment, reactions in that could be involved (depletion and formation potential of new products – TPs) as well impact on the environmental matrix. If these are known, the assessment of the contaminant impact on any living organisms become more realistic as it quite possible that the organisms to be impacted not only by the initial contaminant but also by the resulted TPs in the environment.

Starting with these assumptions, in this thesis, inter- and intra-compartment mode of assessment of NSAIDs pathway was applied for experimental data acquisition (see Figure 6). These obtained experimental data were used further for numerical model built, model that allow the prediction of NSAIDs impact on a living organism – in this case on *Lycopersicon esculentum.*

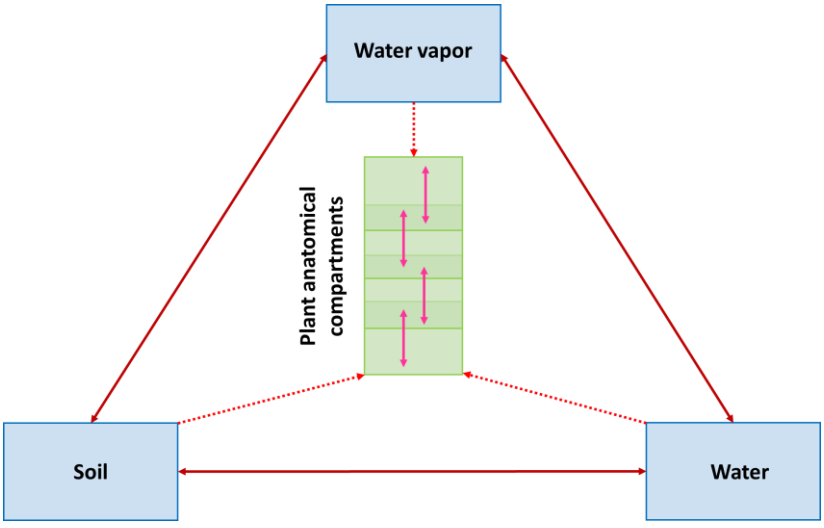


Figure 6. Schematic concept of experimental data collections for model formulation

For each major system specific model was applied in order to estimate the NSAIDs fate and not ultimately the impact rate of NSAIDs on *Lycopersicon esculentum.*

Structural Equation Modelling (SEM) was applied for NSAIDs fate assessment in surface water, as this environmental matrix is considered a potential major rout through that *Lycopersicon esculentum* could get contact with NSAIDs. This model combined with *Partial Least Squares Path modelling* (PLS-PM) allowed to quantitatively test the proposed theoretical

model of NSAIDs fate in surface water based on set of experimental variables that are hypothesized to influence their fate in this environmental system.

In soil system, NSAIDs fate as depletion kinetics (time-scale) and spatial distribution of parent and TPs through soil was modelled applying COMSOL solver with components from COMSOL Multiphysics and Subsurface Flow Model. The COMSOL proposed pollutant transport application, built on Richard's equation coupled with Fick convection-dispersion equation, was adapted to diclofenac, ibuprofen and ketoprofen case study under different environmental conditions (simulated drought condition and simulated flood conditions).

In both these environmental systems, NSAIDs pathway is influenced by non-random spatial and temporal variations of physical, chemical and biological components of the environmental matrix.

In case of tomato (*Lycopersicon esculentum*) uptake of NSAIDs from environment (soil, water, and pulverized water as precipitation) into different anatomical compartments (root, stem, leaf, fruit) was simulated applying Rein et al., [384] uptake model adapted to studied NSAIDs, which allow diclofenac, ibuprofen and ketoprofen continuous and/or periodic input simulation, degradation in soil of their active parent compounds, and diclofenac, ibuprofen and ketoprofen active ingredients dilution through plant growth and metabolites formation through plant development. For studied NSAIDs and corresponding representative TPs distribution through tomato fruit body, model proposed by Xiao et al., [385] was adapted. This model involved to predict the dynamic distribution (moving boundary) of diclofenac, ibuprofen and ketoprofen concentration in tomato fruit.

In each case, either environmental compartment or anatomical compartment of tomato, the obtained predicted data were compared with experimental ones in order to evaluate the goodness of fit of proposed model.

Chapter 3. NSAIDs (ibuprofen, diclofenac, ketoprofen) pathway assessment in water system

Environmental degradation of NSAIDs in surface water systems involve processes as sorption, abiotic degradation and biodegradation. However, it should be accentuated that these processes rather induce a transformation of parent active pharmaceutical ingredient than their elimination. This might lead in formation of a large number of TPs. Although there are studies that have been evidenced these processes impact on certain pharmaceuticals, few data are available for studied NSAIDs fate and degradability in natural surface waters. Moreover, after our knowledge no one quantified the individual contribution of these processes on diclofenac, ibuprofen and ketoprofen fate in surface water environment.

3.1. NSAIDs path modelling in surface water

Changes of NSAIDs amount during experiments were observed in all performed studies although the extent differed. This could be motivated through that the studied processes impact the active ingredient pathway was different. Because assessment of NSAIDs active ingredients through monitoring studies could be cost and time inefficient, identification and development of proper numerical approaches that allow their fate assessment become of interest both for environmental assessors as well for regulators. In that part of the thesis targeted NSAIDs active ingredients pathway in water environment was formulated numerically based on acquired experimental data.

3.2. Structural Equation Model involving Partial Least Squares Path Modelling (SEM-PLS-PM)

Multivariate data analysis is commonly applied to test empirically hypothesized correspondence between variables that could shape organic compounds pathway in water. Frequently used approaches are multiple regression, logistic regression or analysis of variance. However, over time, these approaches have been showed several limitations. Some of that are (***i.***) assumption of simple model structure with one layer of dependent and independent variables; (***ii.***) consideration only of the observable variables, neglecting the abstract properties or attributes; and (***iii.***) premise that all measured data are free of errors.

To overcome these limitations, second generation multivariate data analysis approaches should be applied. *Structural equation modelling* (*SEM*) could be a proper approach to simulate NSAIDs active ingredients pathway in water environment. This approach is suited for estimate a network of causal relationship of abiotic, sorption and biotic processes defined according to a theoretical model linked to several latent complex concepts as NSAIDs active ingredients potential attenuation due to photolysis, sorption to water sediments or biodegradation performed by bacterial and fungal communities – called as *latent variables* (*LV*), measured through a number of observable indicators (eq. water related physiochemical properties, microbiological characteristics, and active ingredient amount variation) – called as *manifest variables* (*MV*).

SEM approach represent a connecting point between path analysis (PA) and confirmatory factor analysis (CFA). Further, *partial least squares* (PLS) method of SEM named also as *PLS path modelling* (PLS-PM) was used in further to assess the representative processes impact on studied NSAIDs active ingredients pathway in water system, as this approach is a component-based estimation procedure compared with LISREL method which is built on covariances. As iterative algorithm, this approach resolves the problem of measurement model and then it assumes the path coefficients in SEM. PLS-PM well describe the residual variance of LV and sometime of MV in any regression run of proposed model. Therefore, this approach is mainly dedicated to improve predictions (explained variance) than estimates which could present less statical accuracy.

PLS-SEM is construed by two set of linear equations of that the first represent the *structural model* (inner model) and the second one represents the *measurement model* (outer model). The structural model designates the relation between LV while the measurement model expresses the relation between LV and corresponding MV (indicator variable). LV that are not dependent are also called as *exogenous variable* (ξ). If it is dependent, it is called as *endogenous variable* (η). These variables are operational through measurement indicator variables (x, y). The combination of these models (structural model and measurement model) results in PLS model.

The structural model for exogenous LV and endogenous LV could be mathematically expressed through (Eq.48.):

$$\eta_j = \gamma_{ji} \cdot \xi_i + \varsigma_j \qquad \text{(Eq.48.)}$$

where ξ and η are vectors of exogenous and endogenous LV, respectively; LV_i explain LV_j; γ represent the matrix of coefficients of corresponding relationships; ξ represent the structural model residuals.

In case of a relationship between two endogenous variables the structural model is defined according with (Eq.49.):

$$\eta_j = \beta_{ji} \cdot \eta_i + \varsigma_j \qquad \text{(Eq.49.)}$$

where η is the vectors of endogenous LV, respectively; LV_i explain LV_j; β represent the matrix of coefficients of corresponding relationships; ξ represent the structural model residuals. Reflective and formative measurement models are common outer models in PLS-SEM.

Assumed hypothesis, variables and data entries

Hypothesis: The main hypothesis assumed was that abiotic, biotic or sorption processes influence NSAIDs active ingredients pathway in surface water, although the extent of corresponding consequences on active ingredients are not well known at moment. Hypothesized and tested relations between defined processes and targeted NSAID active ingredients amount listed in Table 20:

Table 20. Primary and secondary assumption corresponding SEM-PLS-PM segments

Assumption	Segment	MV (manifest variables)
Primary assumption	Abiotic process → NSAID attenuation	pH → Abiotic process
		Temperature → Abiotic process
		Photolysis → Abiotic process
		Non-photolysis → Abiotic process
	Sorption process → NSAID attenuation	pH → Sorption process
		Temperature → Sorption process
		Sediment organic matter → Sorption process
		Attenuation → Sorption process
	Biotic process → NSAID attenuation	Total microbiota community → Biodegradation process
		Bacteria community → Biodegradation process
		Fungal community → Biodegradation process
		Microeukaryotes community → Biodegradation process
		Aerobic bacteria → Biodegradation process

Assumption	Segment	MV (manifest variables)
		Anaerobic bacteria → Biodegradation process Gram positive bacteria → Biodegradation process Gram negative bacteria → Biodegradation process Methanotroph bacteria → Biodegradation process Attenuation → Biodegradation process
Secondary assumption	Abiotic process → Biotic process	Abiotic process MV → Biotic process MV → NSAID attenuation Abiotic process MV → Biotic process MV → Sorption process MV → NSAID attenuation
	Abiotic process → Sorption process	Abiotic process MV → Sorption process MV→ NSAID attenuation
	Biotic process → Sorption process	Biotic process MV → Sorption process MV → NSAID attenuation

Variables and data entries: Studied NSAIDs active ingredients non-standardized MV main characteristics are listed in Table 21. Collected experimental data were the support the NSAIDs active ingredient SEM-PLS-PM model.

Table 21. Manifest variables (MV) main characteristics

NSAID	MV	Mean	Median	Standard deviation	Excess kurtosis	Skewness
Diclofenac	*AeroB*	3.3	3.6	0.693	-1.643	-0.409
	AnaeroB	2.9	3.3	0.601	-1.906	-0.524
	Bact	32.8	33.2	4.786	-2.052	-0.174
	Bioat	23.2	24.3	7.849	-0.587	-0.631
	Fungi	2.3	2.4	0.526	-1.753	0.271
	G+B	3.6	3.6	0.393	-1.524	-0.031
	G-B	21.5	21.2	6.485	-2.073	-0.098
	Methano	1.4	1.4	0.128	-1.111	-0.304
	Meuk	9.7	9.7	0.515	-1.657	-0.223
	NonPhot	5.4	4.6	3.654	-1.047	0.699
	pH	6.5	6.5	0.057	0.413	0.028
	Phot	15.8	16.7	7.277	-1.546	-0.218
	Som	6.1	6.2	0.077	2.037	-1.923
	Sorbat	11.7	12.3	3.861	-0.519	-0.701
	Temp	12.4	12.5	0.18	0.167	-1.317
	Total M	77.5	78.4	9.589	-2.036	-0.181
Ibuprofen	*AeroB*	6.2	6	1.778	-1.403	0.246

NSAID	MV	Mean	Median	Standard deviation	Excess kurtosis	Skewness
	AnaeroB	1.6	1.5	0.302	-1.189	0.526
	Bact	29.7	28.7	5.978	-1.195	0.378
	Bioat	42.8	51.1	17.4	-0.909	-0.708
	Fungi	1.5	1.6	0.207	-0.634	-0.912
	G+B	2.9	2.1	1.077	-0.429	0.959
	G-B	17.9	18.1	5.678	-1.179	-0.020
	Methano	1.6	0.9	0.401	4.470	2.111
	Meuk	14.7	17	4.283	-0.052	-1.294
	NonPhot	11.1	11.3	6.539	-1.669	-0.279
	pH	6.5	6.5	0.045	-0.431	-0.036
	Phot	24.3	22.5	10.469	-11.036	0.118
	Som	6.2	6.2	0.097	1.919	-1.858
	Sorbat	19.2	20.1	10.881	-0.736	-0.624
	Temp	12.4	12.5	0.243	2.475	0.732
	Total M	75.7	76.0	15.766	-1.129	-0.125
Ketoprofen	*AeroB*	4.8	4.8	0.567	-1.074	0.23
	AnaeroB	2.1	2.1	0.273	-1.364	0.329
	Bact	31.1	30.6	5.396	-1.740	0.036
	Bioat	35.4	39.5	9.596	1.417	-1.355
	Fungi	1.9	2.0	0.165	-1.770	-0.534
	G+B	3.2	2.9	0.729	-1.003	0.729
	G-B	19.7	19.6	6.022	-1.696	-0.104
	Methano	1.3	1.2	0.248	2.189	1.469
	Meuk	12.2	13.3	2.372	-0.182	-1.219
	NonPhot	8.8	12.7	6.084	-2.231	-0.239
	pH	6.5	6.5	0.045	-0.431	-0.036
	Phot	37	42.6	19.909	-1.555	-0.492
	Som	6.2	6.2	0.097	1.919	-1.858
	Sorbat	20/5	24.7	9.395	-1.018	-0.781
	Temp	12.4	12.4	0.243	2.475	0.732
	Total M	76.3	76.5	12.710	-1.569	-0.193

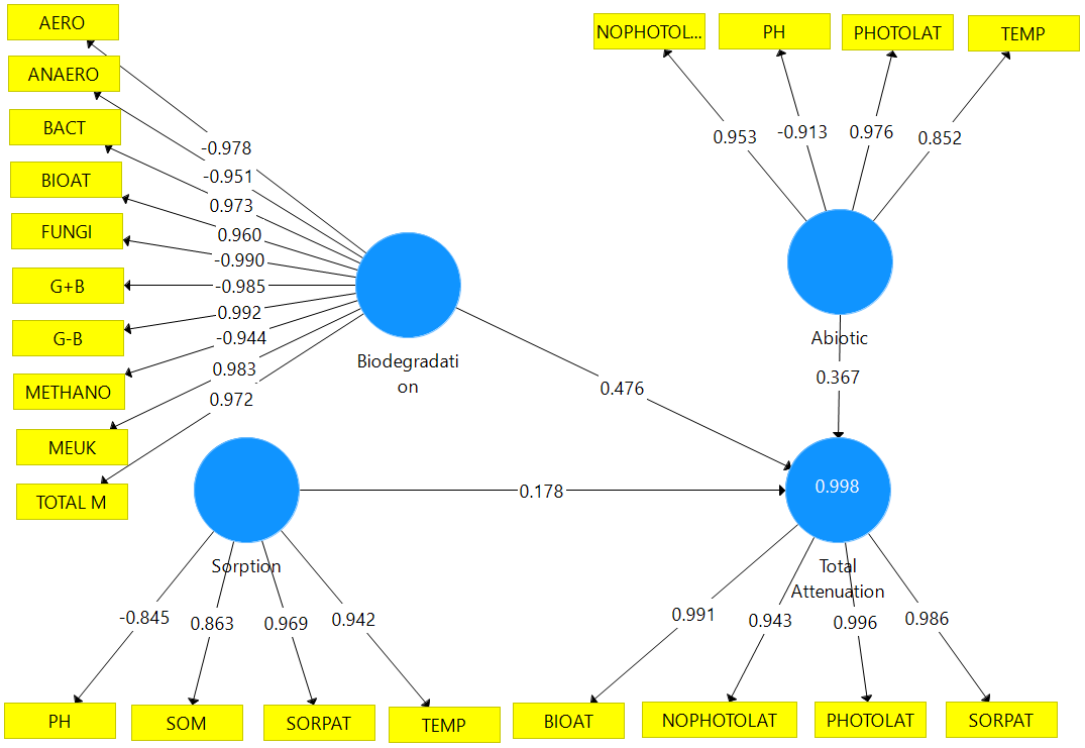
AeroB – aerobe bacteria; *AnaeroB* – anaerobe bacteria; *Bact* – bacteria; *Bioat* – biotic process induced attenuation; *G+B* – gram positive bacteria; *G-B* – gram negative bacteria; *Methano* – methanotroph bacteria; *Meuk* – microeukaryotes, *NonPhot* – non photolysis; *Phot* – photolysis; *Som* – sediment organic matter; *Sorbat* – sorption process induced attenuation; *Total M* – total microbiota;

3.3. SEM-PLS-PM path diagram for NSAIDs fate in surface water

The SEM-PLS-PM was solved using *SmartPLS* software. The algorithm, through the preparatory phase normalize the MV followed by outside approximation and inside approximation processes, respectively. Through outside approximation the LV are estimated as weighted aggregates of MV applying ordinary least squares regression. Through inside

approximation endogenous LV are associated with adjacent LV and proxies are established applying once again ordinary least squares regression. Performing the algorithm, the path diagram, weight of formative indicators, reflective indicators and coefficients of each hypothesized paths between LV are generated.

Using previously obtained experimental data, the path diagram for primary assumption and secondary assumptions are presented for each studied NSAIDs active ingredient. In Figure 28 are presented the path diagrams for diclofenac, in Figure 29 are presented the path diagrams for ibuprofen, and in Figure 30 are presented the path diagrams for ketoprofen.



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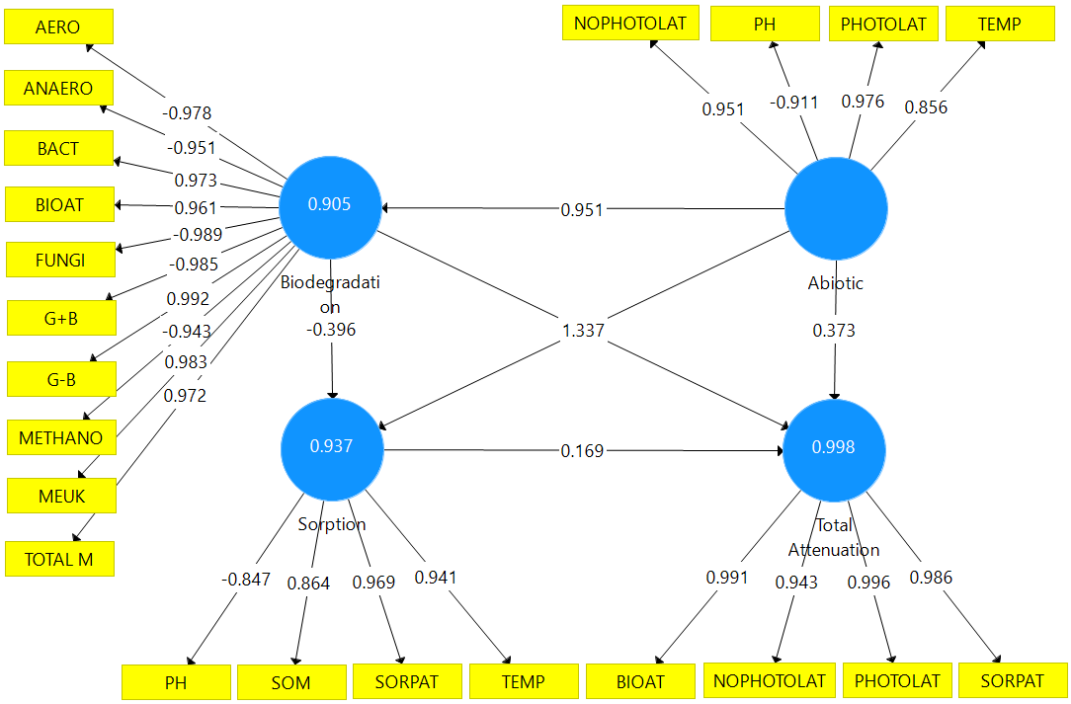
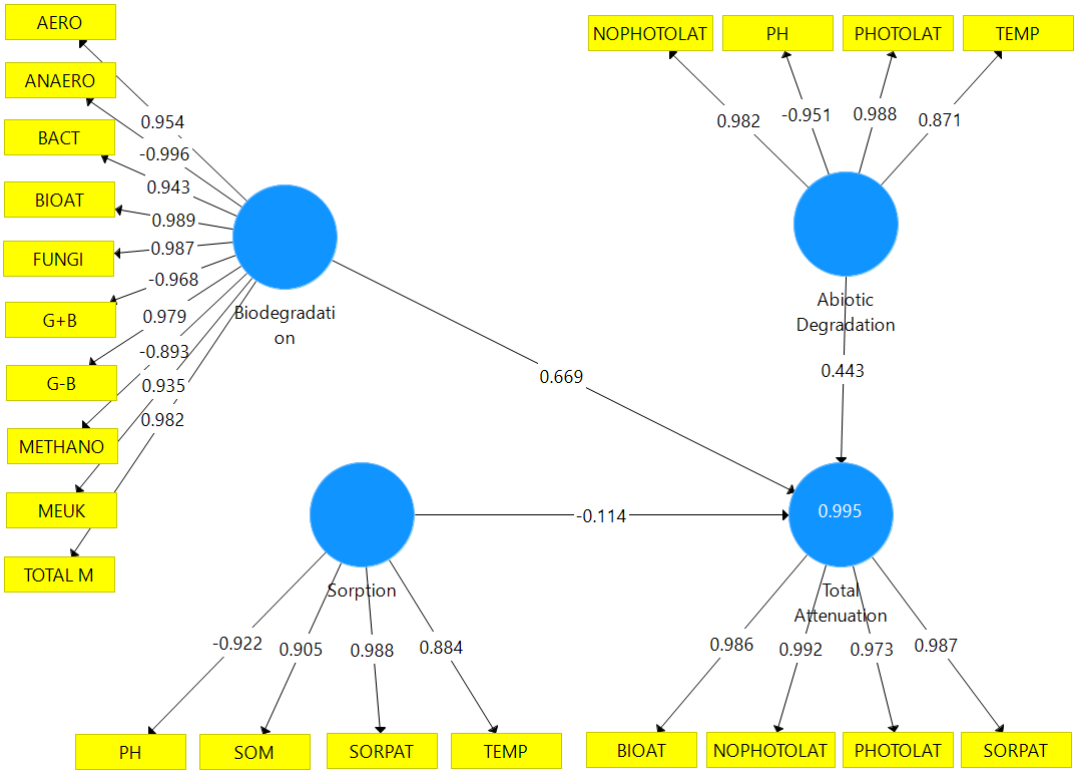


(b.)

Figure 28. Diclofenac path diagram in surface water. **a.)** primary assumption; **b.)** secondary assumption.



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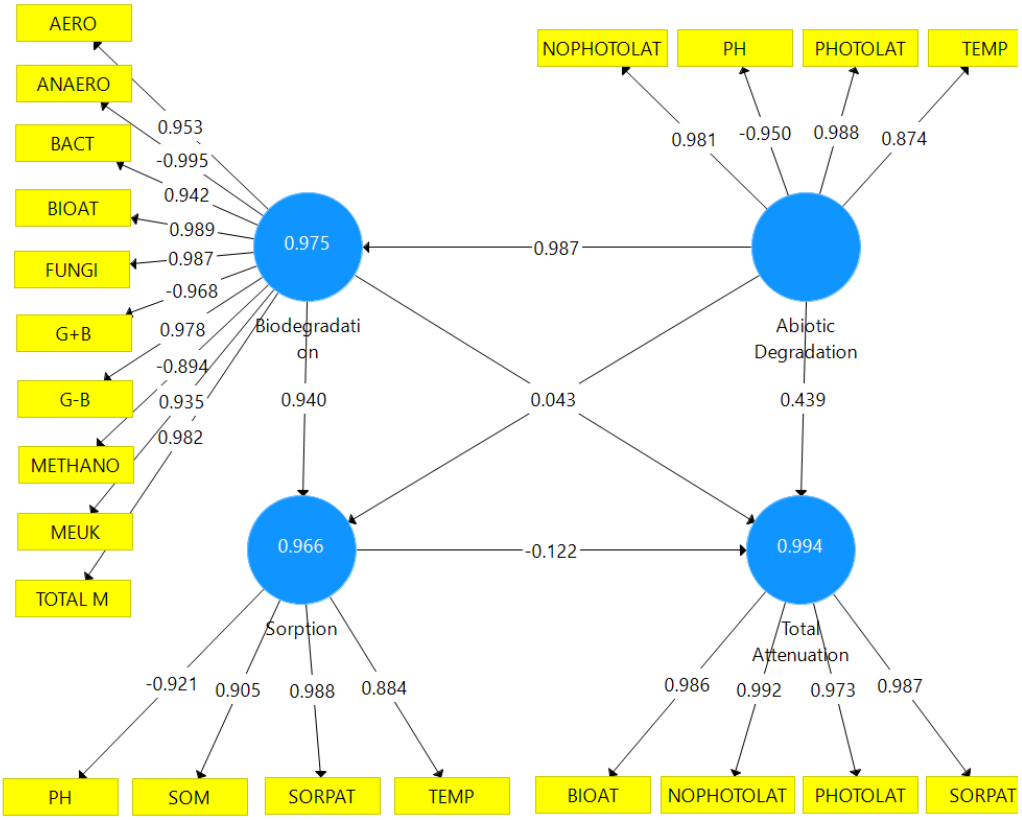
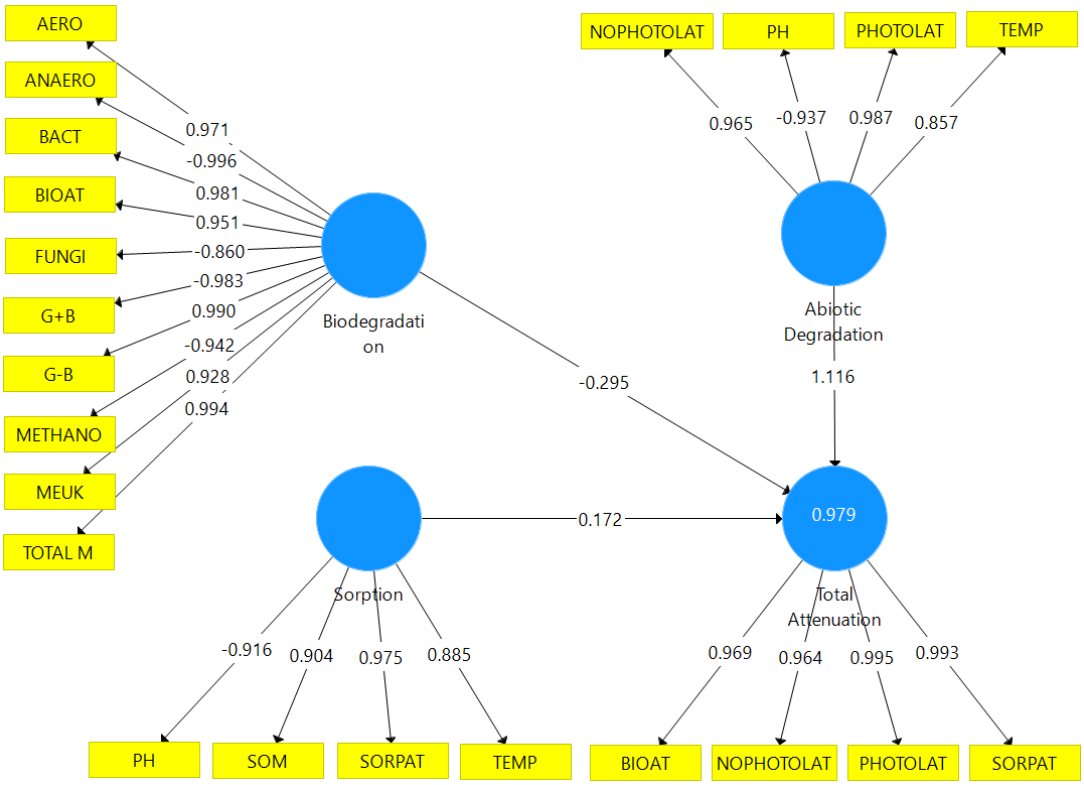


(b.)

Figure 29. Ibuprofen path diagram in surface water. **a.)** primary assumption; **b.)** secondary assumption.



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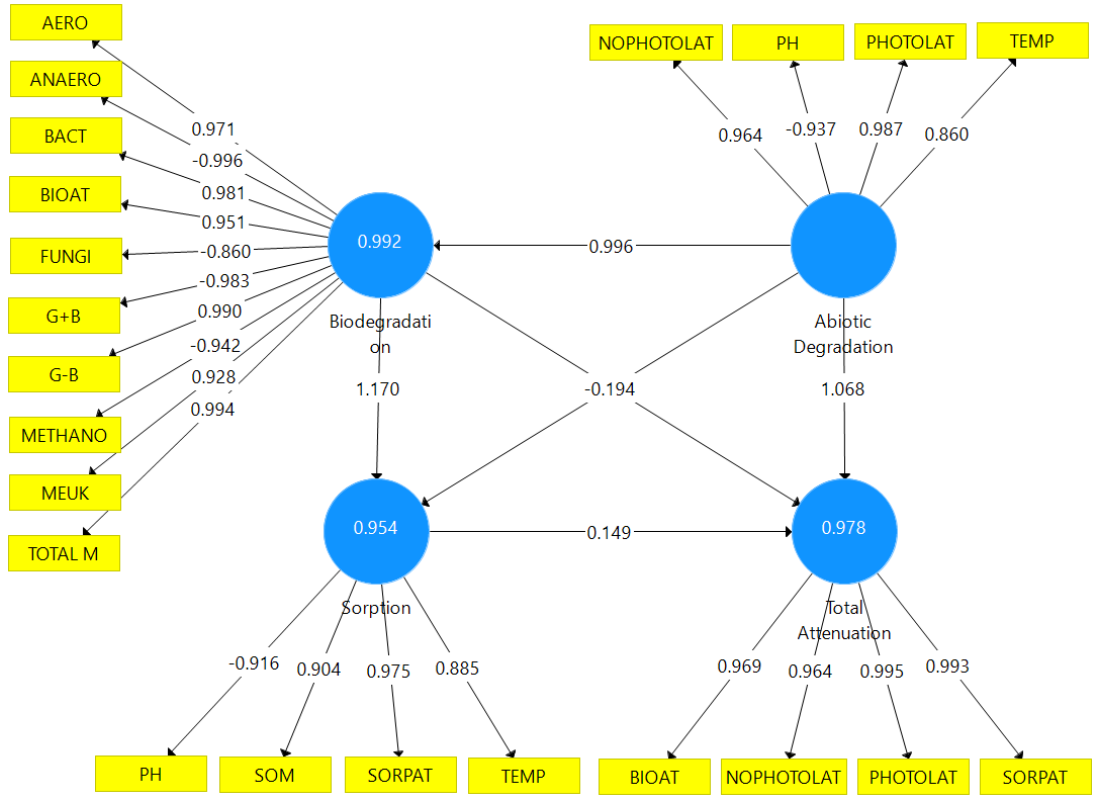


(b.)

Figure 30. Ketoprofen path diagram in surface water. **a.)** primary assumption; **b.)** secondary assumption.

Proposed model veracity test: quality index evaluation

In Table 25 – 27 are listed the representative qualitative indexes obtained for the proposed path models. Analyzing the obtained data for tested quality index, the proposed path model for each studied NSAID active ingredient in water environment was accepted.

Table 25. Proposed path model quality indexes: diclofenac case study

Assumption	Parameters
Primary assumption	***Construct reliability and validity* (ρ)** *Abiotic process*: 0.949 *Biotic process*: 0.994 *Sorption process*: 0.942 ***Discriminated validity***

	Abiotic	Biotic	Sorption	Total attenuation
Abiotic	0.925			
Biotic	0.951	0.973		
Sorption	0.959	0.877	0.907	
Total attenuation	0.99	0.98	0.947	0.979

Assumption	Parameters		
Secondary assumption	***Construct reliability and validity* (ρ)**		
	Abiotic process: 0.947 Biotic process: 0.994 Sorption process: 0.938		
	LV characteristics		
		Excess kurtosis	*Skewness*
	Abiotic process	-0.62	-0.252
	Biotic process	-1.898	0.011
	Sorption process	1.067	-1.206
	Total attenuation diclofenac	-1.174	-0.237
Model performance	***Reliability for diclofenac attenuation***		
	α (Cronbach alpha): 0.985 *ρ:* 0.986 *Composite reliability:* 0.989 *Average variance extracted:* 0.958 ***r^2:*** 0.998 ***Model RMSEA:*** 0.082 (model fit)		

Table 26. Proposed path model quality indexes: ibuprofen case study

Assumption	Parameters			
Primary assumption	***Construct reliability and validity* (ρ)**			
	Abiotic process: 0.968 *Biotic process*: 0.992 *Sorption process*: 0.952			
	Discriminated validity			
		Abiotic	Biotic	Sorption	Total attenuation
	Abiotic	0.949			
	Biotic	0.987	0.963		
	Sorption	0.972	0.983	0.926	
	Total attenuation	0.993	0.995	0.974	0.985
Secondary assumption	***Construct reliability and validity* (ρ)**			
	Abiotic process: 966 Biotic process: 0.992 Sorption process: 0.951			
	LV characteristics			
		Excess kurtosis		*Skewness*
	Abiotic process	-0.985		-0.202
	Biotic process	-0.812		-0.613
	Sorption process	0.021		-0.845
	Total attenuation diclofenac	-1.183		-0.388
Model performance	***Reliability for diclofenac attenuation***			

Assumption	Parameters
	α (Cronbach alpha): 0.990 *ρ:* 0.990 *Composite reliability:* 0.992 *Average variance extracted:* 0.970 ***r²:*** 0.972 ***Model RMSEA:*** 0.05 (model fit)

Table 27. Proposed path model quality indexes: ketoprofen case study

Assumption	Parameters
Primary assumption	***Construct reliability and validity* (ρ)** *Abiotic process*: 0.961 *Biotic process*: 0.992 *Sorption process*: 0.949 ***Discriminated validity***

	Abiotic	Biotic	Sorption	Total attenuation
Abiotic	0.938			
Biotic	0.996	0.960		
Sorption	0.970	0.977	0.921	
Total attenuation	0.989	0.984	0.966	0.981

Secondary assumption	***Construct reliability and validity* (ρ)** Abiotic process: 0.958 Biotic process: 0.992 Sorption process: 0.948 ***LV characteristics***

	Excess kurtosis	*Skewness*
Abiotic process	-1.284	-0.372
Biotic process	-1.176	-0.397
Sorption process	-0.008	-0.908
Total attenuation diclofenac	-1.163	-0.641

Model performance	***Reliability for diclofenac attenuation*** *α (Cronbach alpha):* 0.987 *ρ:* 0.987 *Composite reliability:* 0.990 *Average variance extracted:* 0.962 ***r²:*** 0.991 ***Model RMSEA:*** 0.057 (model fit)

Chapter 4. NSAIDs (ibuprofen, diclofenac, ketoprofen) pathway assessment in *Lycopersicon esculentum*

Three major processes might influence NSAIDs fate in soil environment. One of those processes is that of pharmaceuticals active ingredients adsorption onto active surface of soil environment, the second one is that of biodegradation by soil inhabiting microorganisms, and the third is the translocation potential by higher trophic level organisms. These processes define potential persistence as well transformation rate of these compounds in the soil environment.

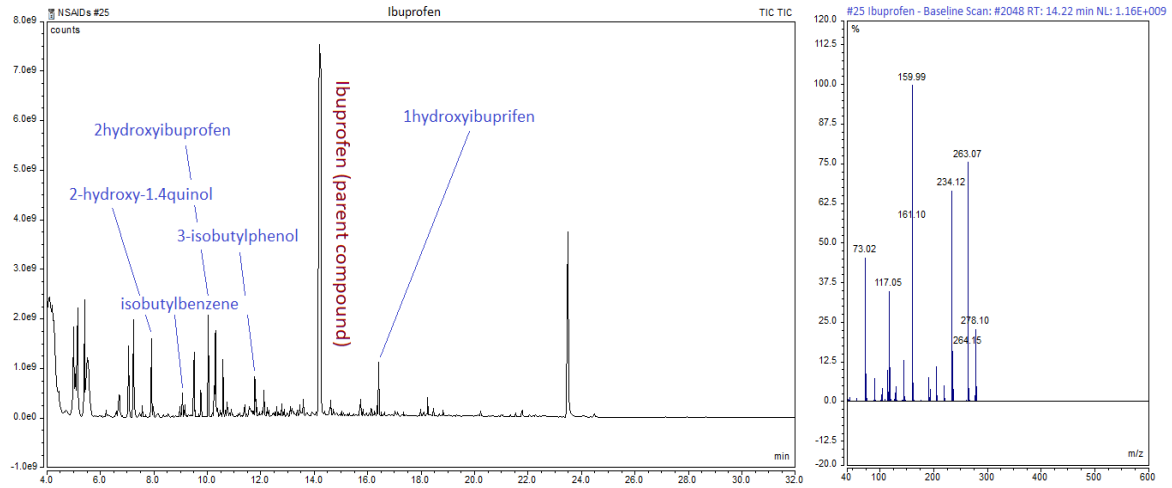
According with literature, two major challenges have been identified in assessment of NSAIDs pathway in soil environment. One of this refer at these pharmaceutical active ingredients fate under current challenges of climate change, namely under precipitation and temperature anomalies [423-426] while the second one is about the formation potential of TPs that could possess more improper environmental and toxicological properties than the parent compound [363; 369; 402; 424; 427].

Based on these two major identified challenges, in this part of thesis diclofenac, ibuprofen and ketoprofen fate and transformation in soil under different environmental conditions have been studied.

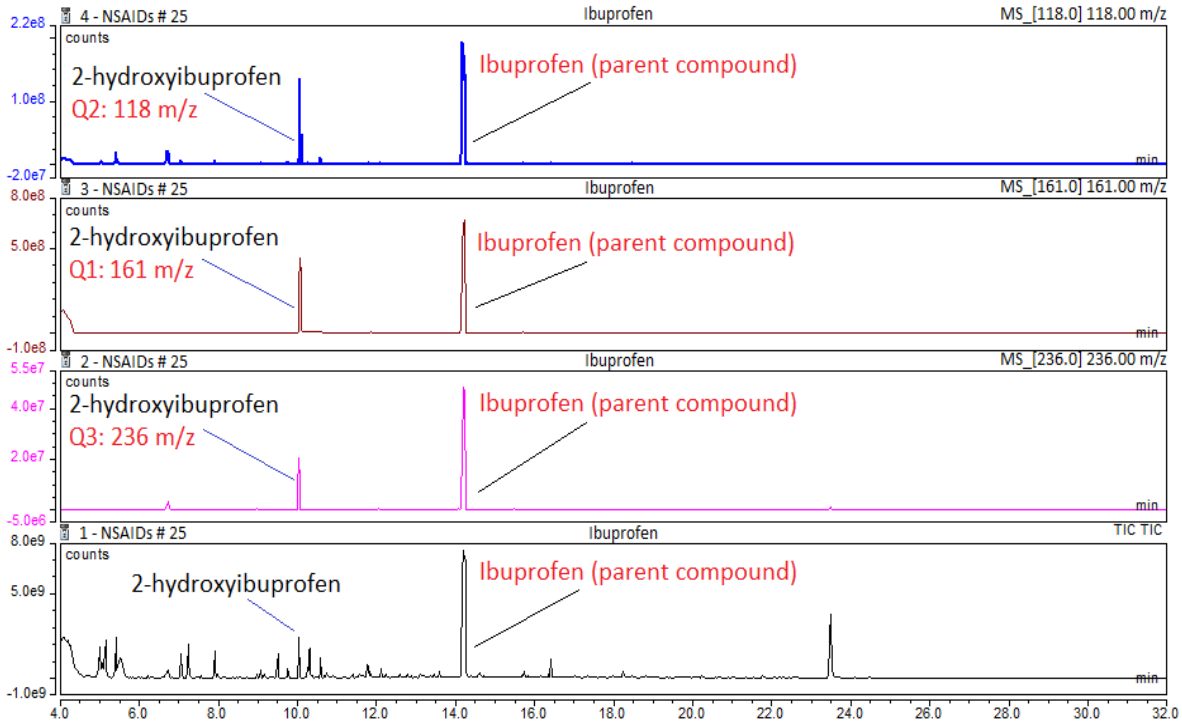
4.1. Identified NSAIDs related TPs formation potential through assay

According with obtained experimental data (both from performed pot and column experiments) positive correlation was identified between specific soil microbiota community and NSAIDs depletion in soil based on Monod equation kinetic parameters when formation potential of transformation products for studied NSAIDs was questioned. Analyzing the available literature, transformation products could be formed when microorganisms use these compounds as carbon source through their metabolic functions [250; 442- 445]. Therefore, through experiment GC-MS analysis in full scan mode were performed to identify potential transformation products of these studied NSAIDs targeting corresponding qualifier and quantifiers ions (m/z) suggested by literature [446- 450].

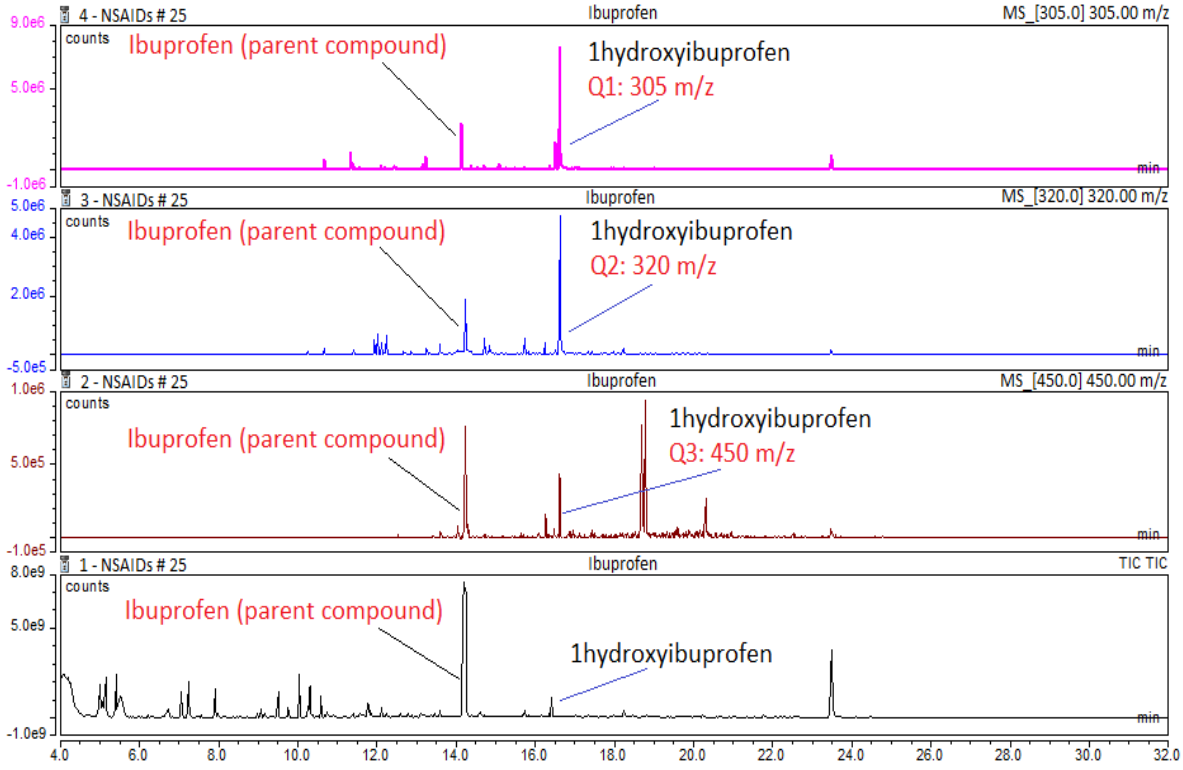
During the thirty days of our soil pot experiments we identified the following seven TPs in soils: 1-hydroxyibuprofen, 2-hydroxyibuprofen, 1,2-dihydroxyibuprofen, 2-hydroxy-1,4-quinol, isobutyl benzene and 3-isobutyl phenol. Their mass spectra are presented in Figure 41.



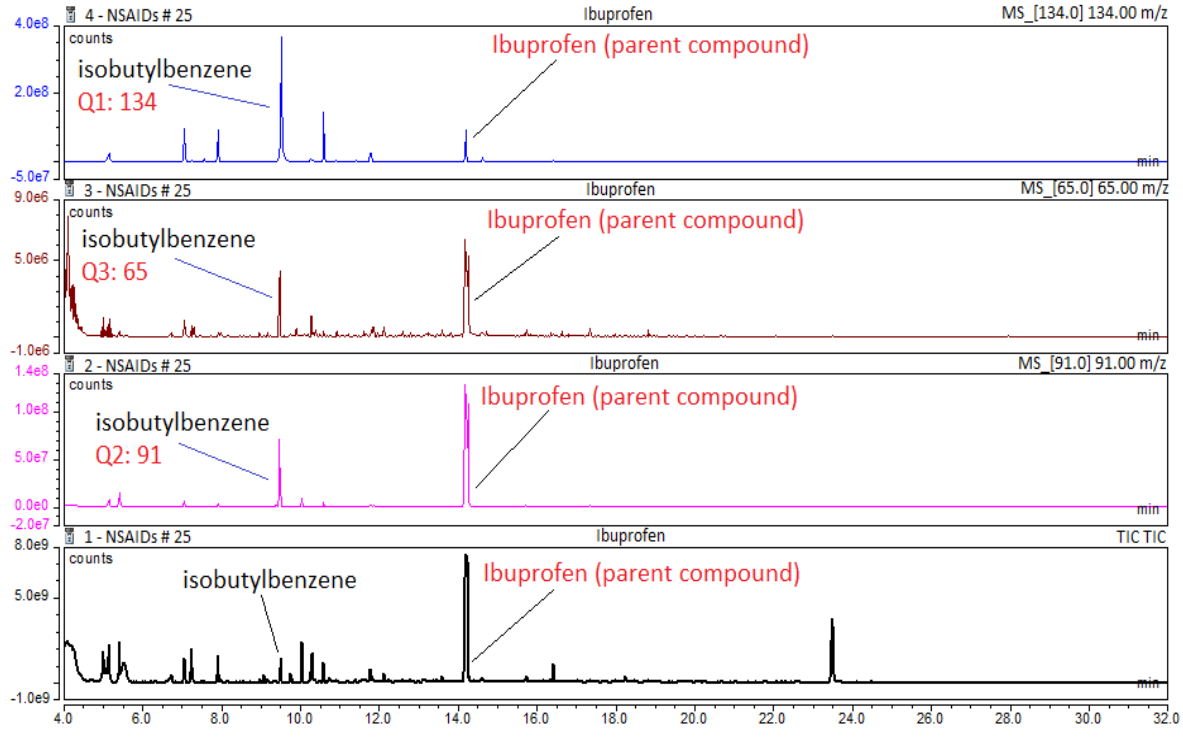
(a.)



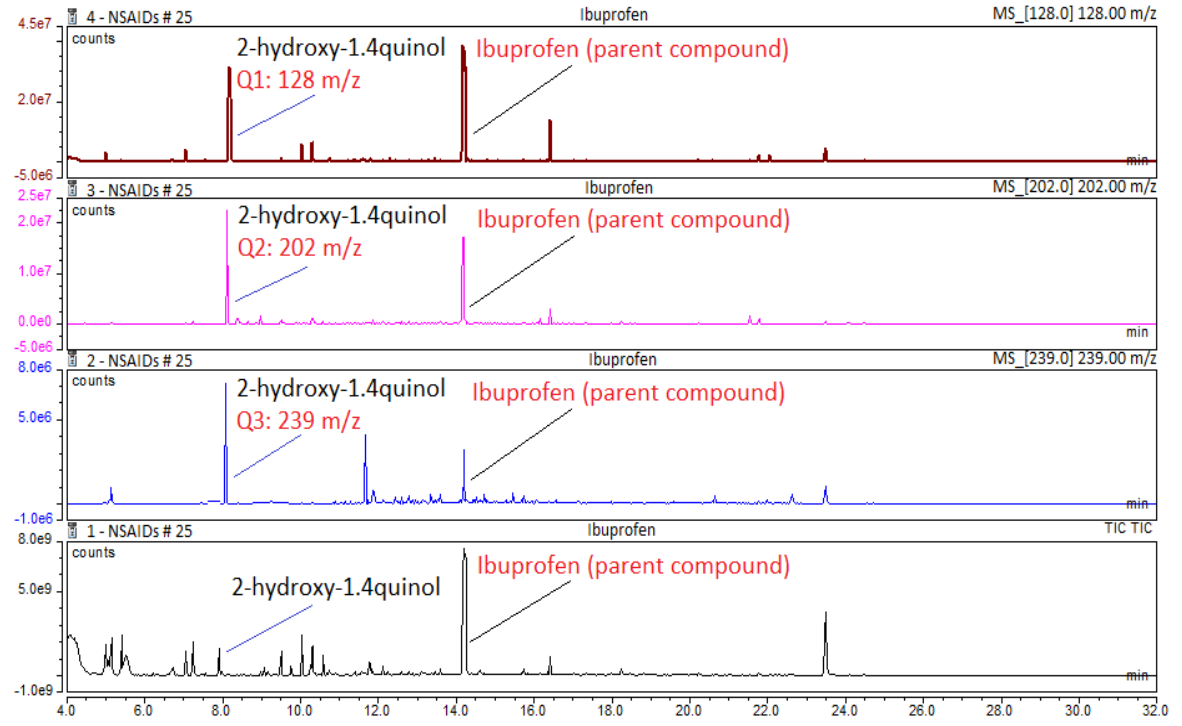
(b.)


4 - NSAIDs # 25
Ibuprofen
MS_[305.0] 305.00 m/z
counts
Ibuprofen (parent compound)
1hydroxyibuprofen
Q1: 305 m/z
min
3 - NSAIDs # 25
Ibuprofen
MS_[320.0] 320.00 m/z
counts
Ibuprofen (parent compound)
1hydroxyibuprofen
Q2: 320 m/z
min
2 - NSAIDs # 25
Ibuprofen
MS_[450.0] 450.00 m/z
counts
Ibuprofen (parent compound)
1hydroxyibuprofen
Q3: 450 m/z
min
1 - NSAIDs # 25
Ibuprofen
TIC TIC
counts
Ibuprofen (parent compound)
1hydroxyibuprofen
min

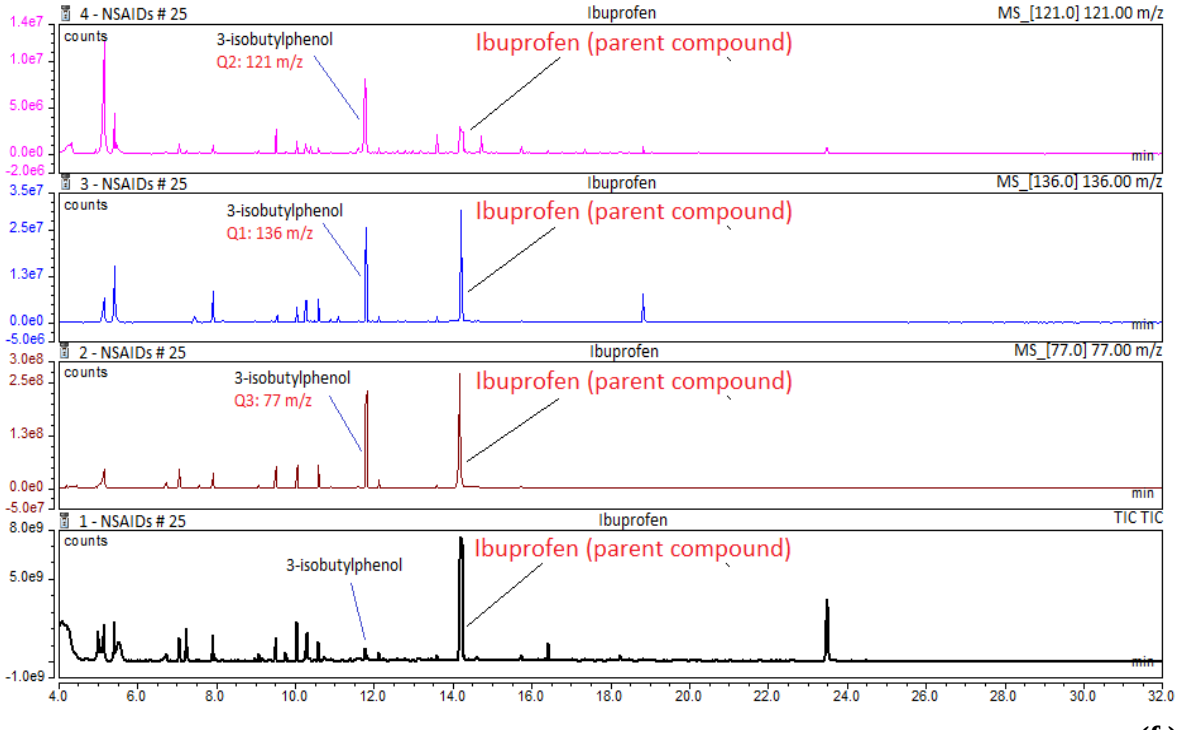
(c.)


4 - NSAIDs # 25
Ibuprofen
MS_[134.0] 134.00 m/z
counts
isobutylbenzene
Q1: 134
Ibuprofen (parent compound)
min
3 - NSAIDs # 25
Ibuprofen
MS_[65.0] 65.00 m/z
counts
isobutylbenzene
Q3: 65
Ibuprofen (parent compound)
min
2 - NSAIDs # 25
Ibuprofen
MS_[91.0] 91.00 m/z
counts
isobutylbenzene
Q2: 91
Ibuprofen (parent compound)
min
1 - NSAIDs # 25
Ibuprofen
TIC TIC
counts
isobutylbenzene
Ibuprofen (parent compound)
min

(d.)



(e.)



(f.)

Figure 41. Mass spectra of identified transformation products of diclofenac. **(a.)** total ion chromatogram of ibuprofen and transformation products; **(b.)** mass spectra of 2-hydroxyibuprofen qualifier and quantifier ions; **(c.)** mass spectra of 1-hydroxyibuprofen qualifier and quantifier ions; **(d.)** mass spectra of 1,2-dihydroxyibuprofen qualifier and quantifier ions; **(e.)** mass spectra of 2-hydroxy-1,4-quinol qualifier and quantifier ions; **(f.)** mass spectra of 3-isobutylphenol qualifier and quantifier ions;

Proposed degradation pathway considering their time in appearance through our experiment is presented in Figure 42.

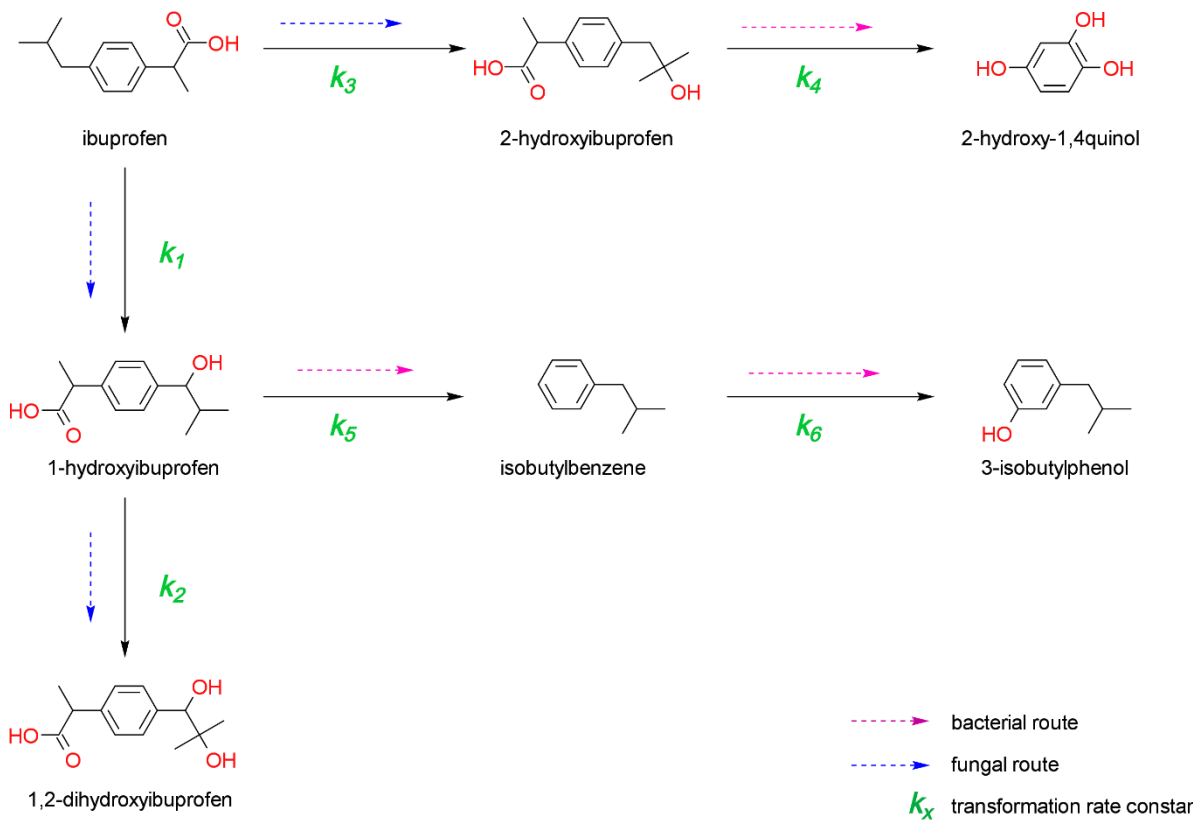
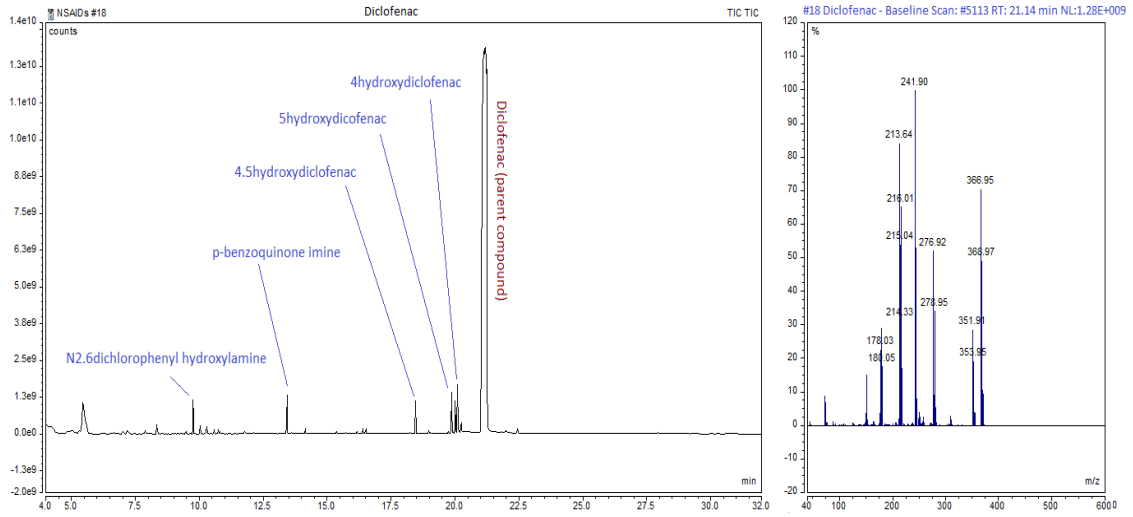
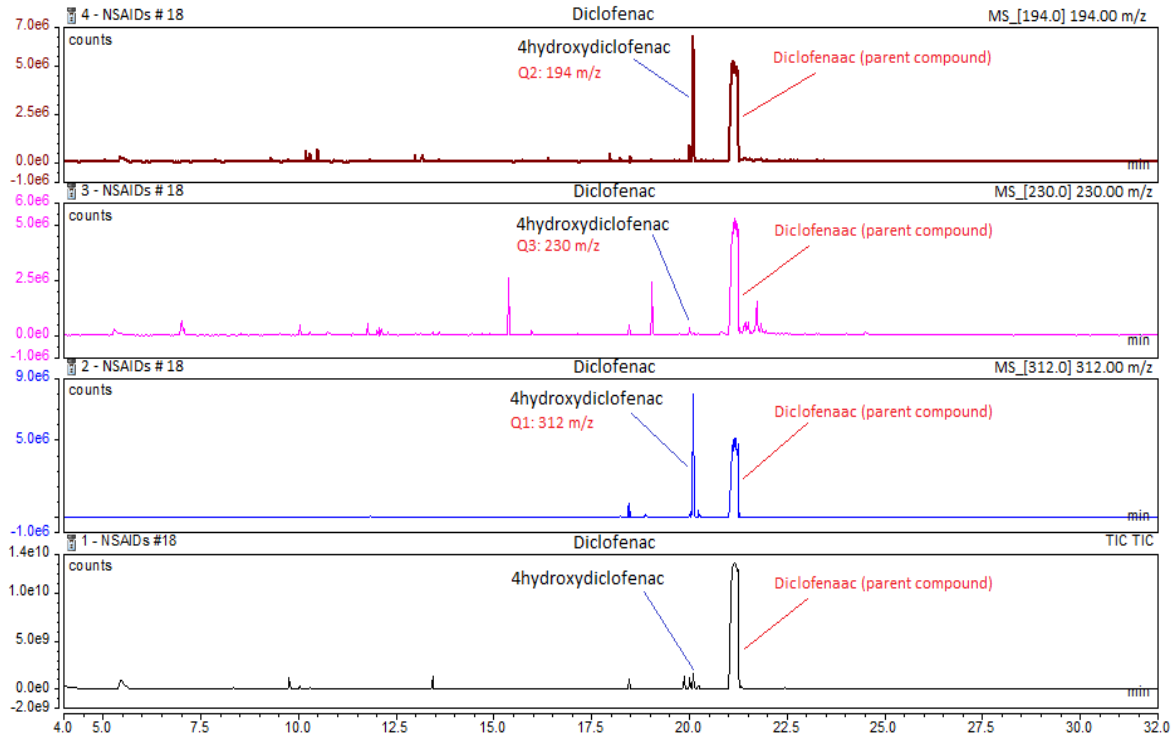


Figure 42. Established degradation pathway for ibuprofen in soil

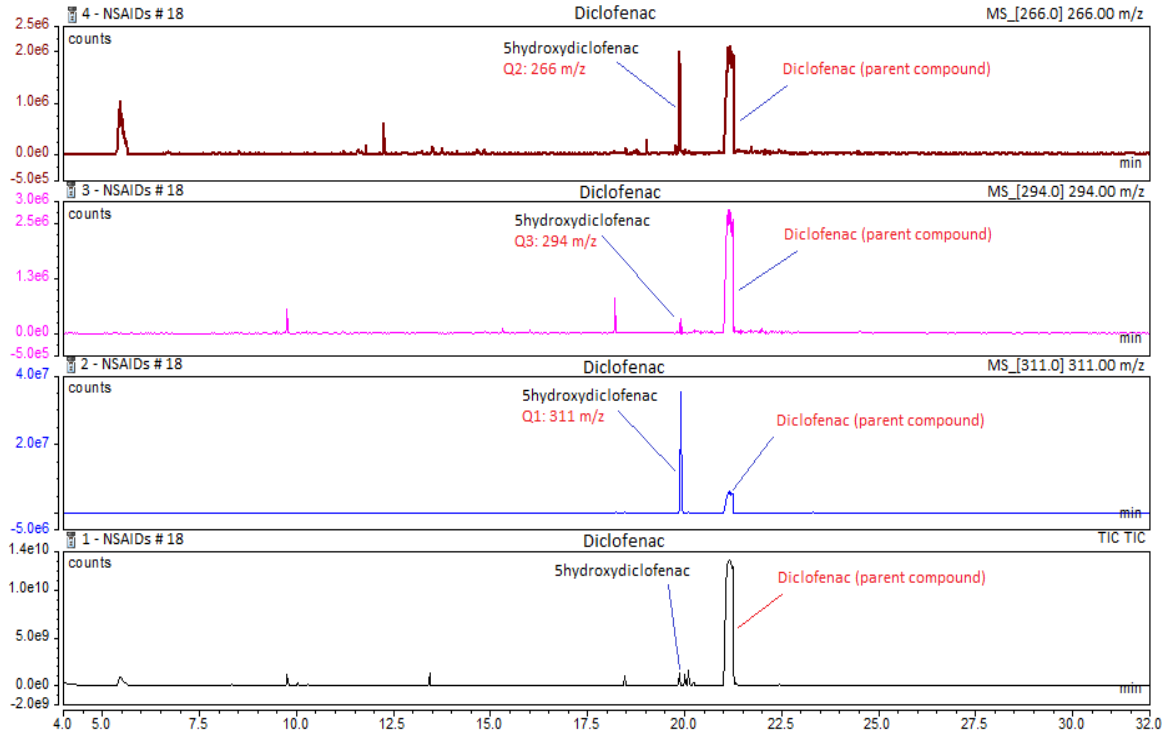
Identified TPs related to diclofenac: In case of diclofenac totally five TPs were identified during experiment. These were 4-hydroxydiclofenac, 5-hydroxydiclofenac, 4,5-hydroxydiclofenac, N-(2,6-dichlorophenyl)hydroxylamine and p-benzoquinone. Their mass spectra are presented in Figure 43. In view of experimental data, the proposed diclofenac pathway in soil is presented in Figure 44.


NSAIDs #18
Diclofenac
TIC TIC
counts
4hydroxydiclofenac
5hydroxydicofenac
4.5hydroxydiclofenac
p-benzoquinone imine
N2.6dichlorophenyl hydroxylamine
Diclofenac (parent compound)
min
#18 Diclofenac - Baseline Scan: #5113 RT: 21.14 min NL:1.28E+009
%
241.90
213.64
216.01
215.04
276.92
366.95
368.97
214.33
278.95
351.91
178.03
184.05
353.95
m/z

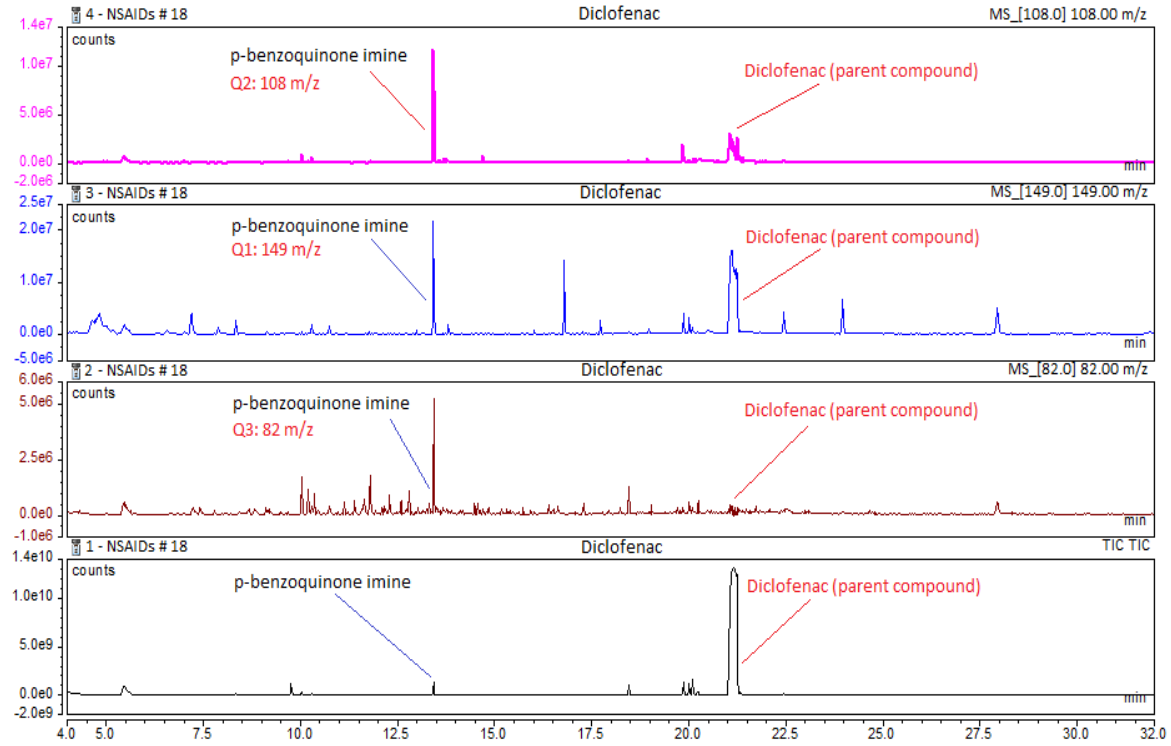
(a.)


4 - NSAIDs # 18
Diclofenac
MS_[194.0] 194.00 m/z
counts
4hydroxydiclofenac
Q2: 194 m/z
Diclofenaac (parent compound)
min
3 - NSAIDs # 18
Diclofenac
MS_[230.0] 230.00 m/z
counts
4hydroxydiclofenac
Q3: 230 m/z
Diclofenaac (parent compound)
min
2 - NSAIDs # 18
Diclofenac
MS_[312.0] 312.00 m/z
counts
4hydroxydiclofenac
Q1: 312 m/z
Diclofenaac (parent compound)
min
1 - NSAIDs #18
Diclofenac
TIC TIC
counts
4hydroxydiclofenac
Diclofenaac (parent compound)
min

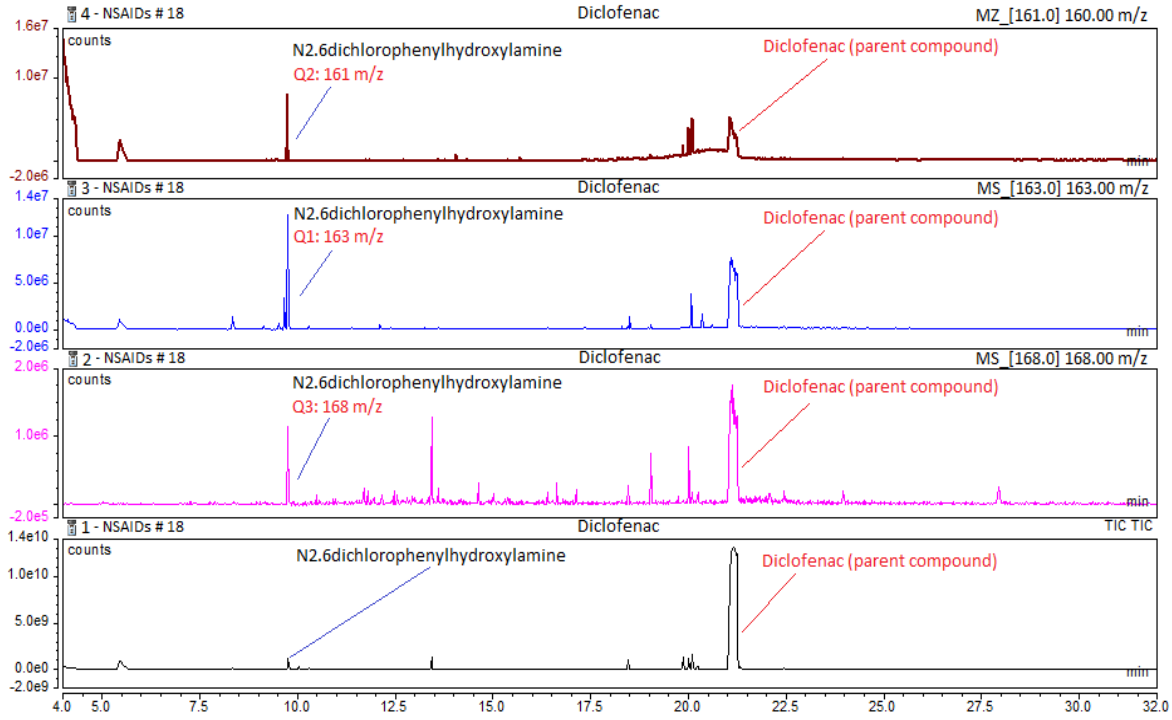
(b.)


4 - NSAIDs # 18
Diclofenac
MS_[266.0] 266.00 m/z
counts
5hydroxydiclofenac
Q2: 266 m/z
Diclofenac (parent compound)
min
3 - NSAIDs # 18
Diclofenac
MS_[294.0] 294.00 m/z
counts
5hydroxydiclofenac
Q3: 294 m/z
Diclofenac (parent compound)
min
2 - NSAIDs # 18
Diclofenac
MS_[311.0] 311.00 m/z
counts
5hydroxydiclofenac
Q1: 311 m/z
Diclofenac (parent compound)
min
1 - NSAIDs # 18
Diclofenac
TIC TIC
counts
5hydroxydiclofenac
Diclofenac (parent compound)
min
4.0 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 32.0

(c.)


4 - NSAIDs # 18
Diclofenac
MS_[108.0] 108.00 m/z
counts
p-benzoquinone imine
Q2: 108 m/z
Diclofenac (parent compound)
min
3 - NSAIDs # 18
Diclofenac
MS_[149.0] 149.00 m/z
counts
p-benzoquinone imine
Q1: 149 m/z
Diclofenac (parent compound)
min
2 - NSAIDs # 18
Diclofenac
MS_[82.0] 82.00 m/z
counts
p-benzoquinone imine
Q3: 82 m/z
Diclofenac (parent compound)
min
1 - NSAIDs # 18
Diclofenac
TIC TIC
counts
p-benzoquinone imine
Diclofenac (parent compound)
min
4.0 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 32.0

(d.)



(e.)

Figure 43. Mass spectra of identified transformation products of diclofenac. **(a.)** total ion chromatogram of diclofenac and transformation products; **(b.)** mass spectra of 4-hydroxydiclofenac qualifier and quantifier ions; **(c.)** mass spectra of 5-hydroxydiclofenac qualifier and quantifier ions; **(d.)** mass spectra of p-benzoquinone imine qualifier and quantifier ions; **(e.)** mass spectra of N-2,6-dichorophenyl hydroxylamine qualifier and quantifier ions

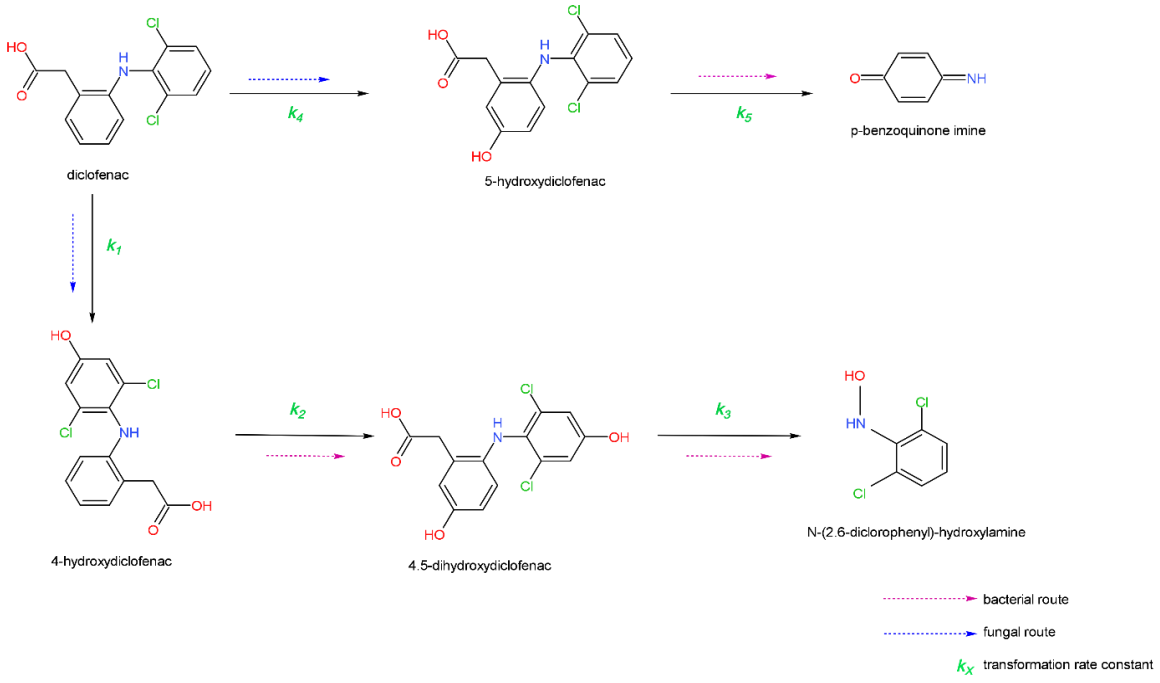
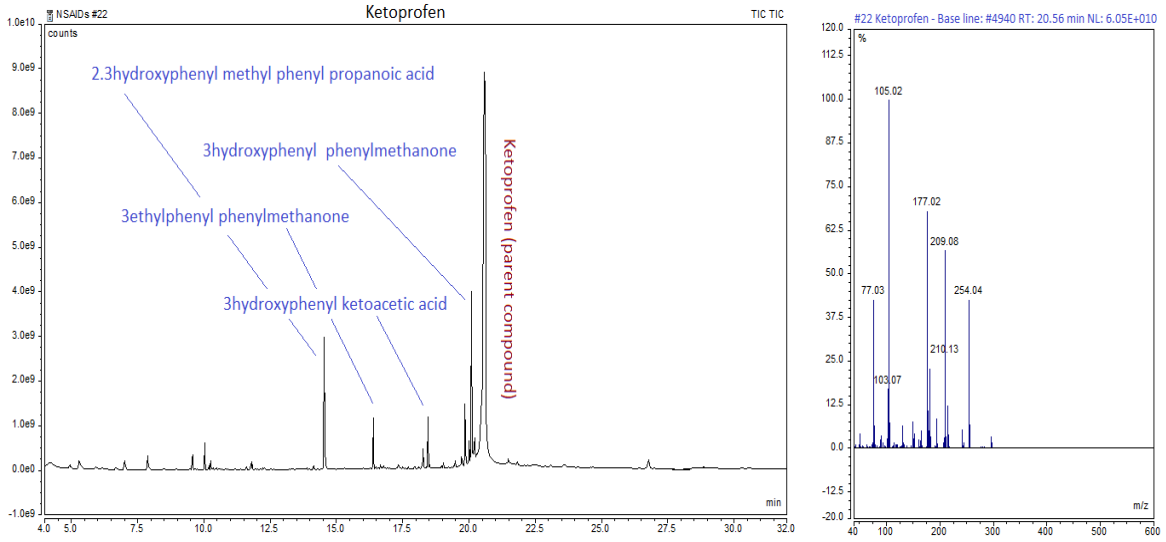
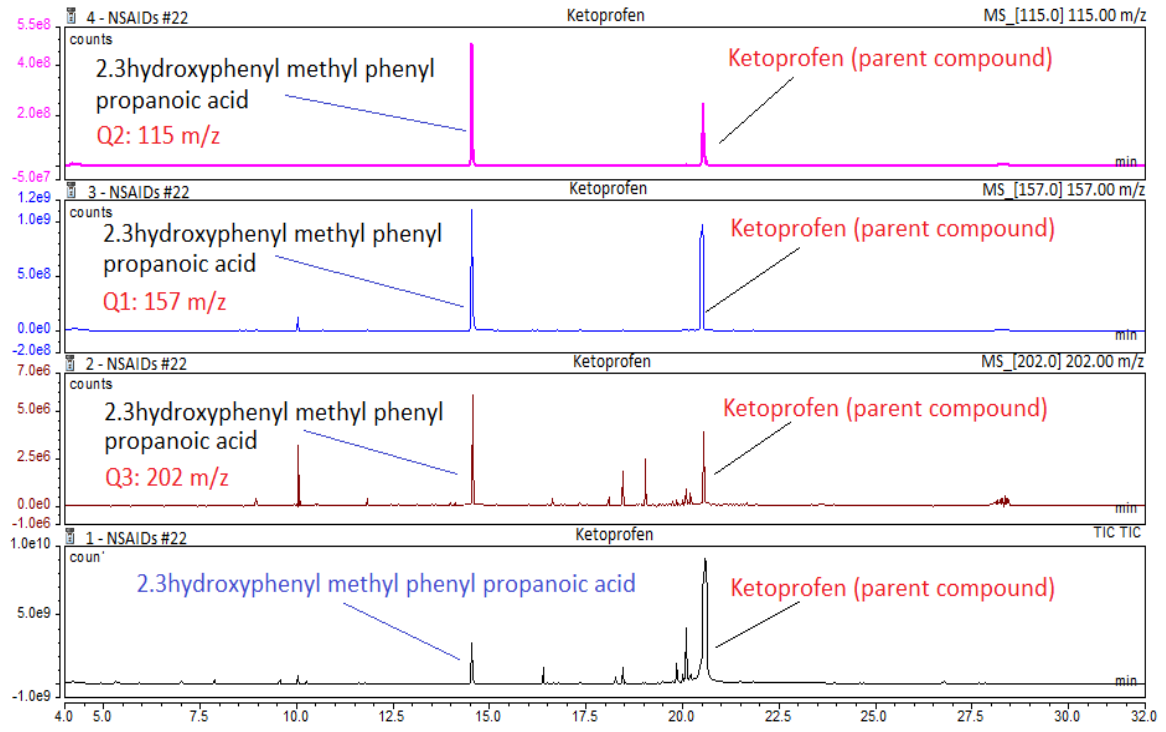


Figure 44. Established degradation pathway for diclofenac in soil

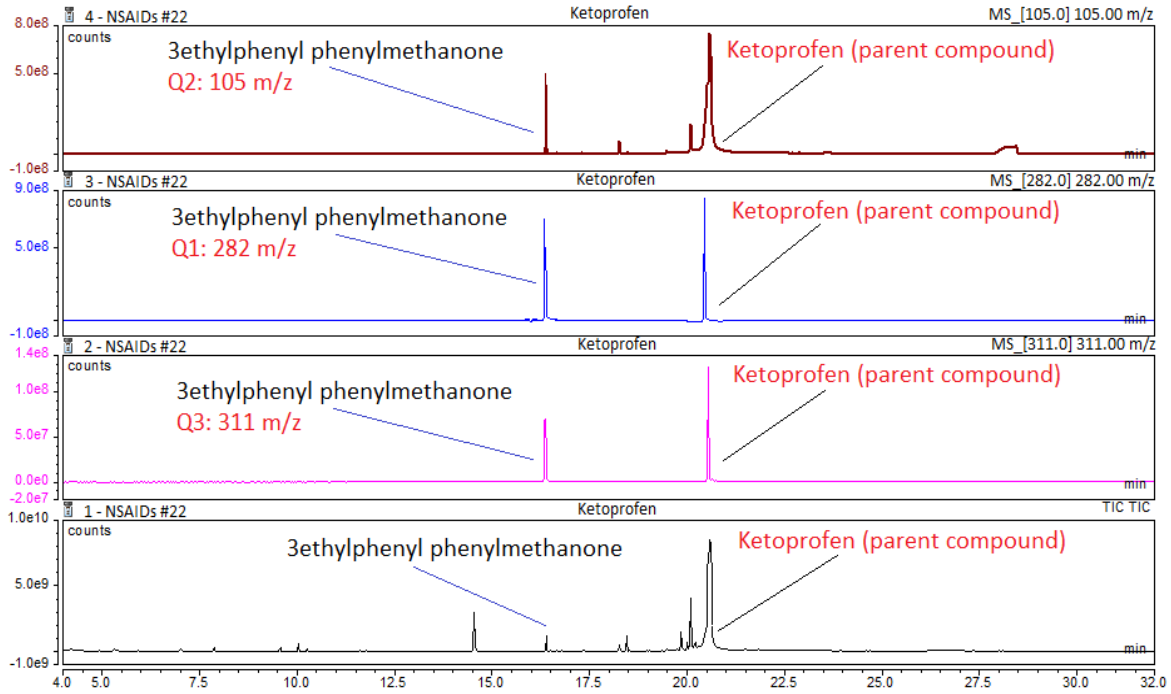
Identified TPs related to ketoprofen: In case studies on ketoprofen the identified TPs were 2-[3-(hydroxy-phenyl-methyl)phenyl]propanoic acid, (3-ethylphenyl)(phenyl)methanone, (3-hydroxyphenyl)(phenyl)methanone and (3-hydroxyphenyl)ketoacetic acid. Their mass spectra are presented in Figure 45. Ketoprofen transformation products amount (relative area) during soil pot experiments are presented in Table 40.



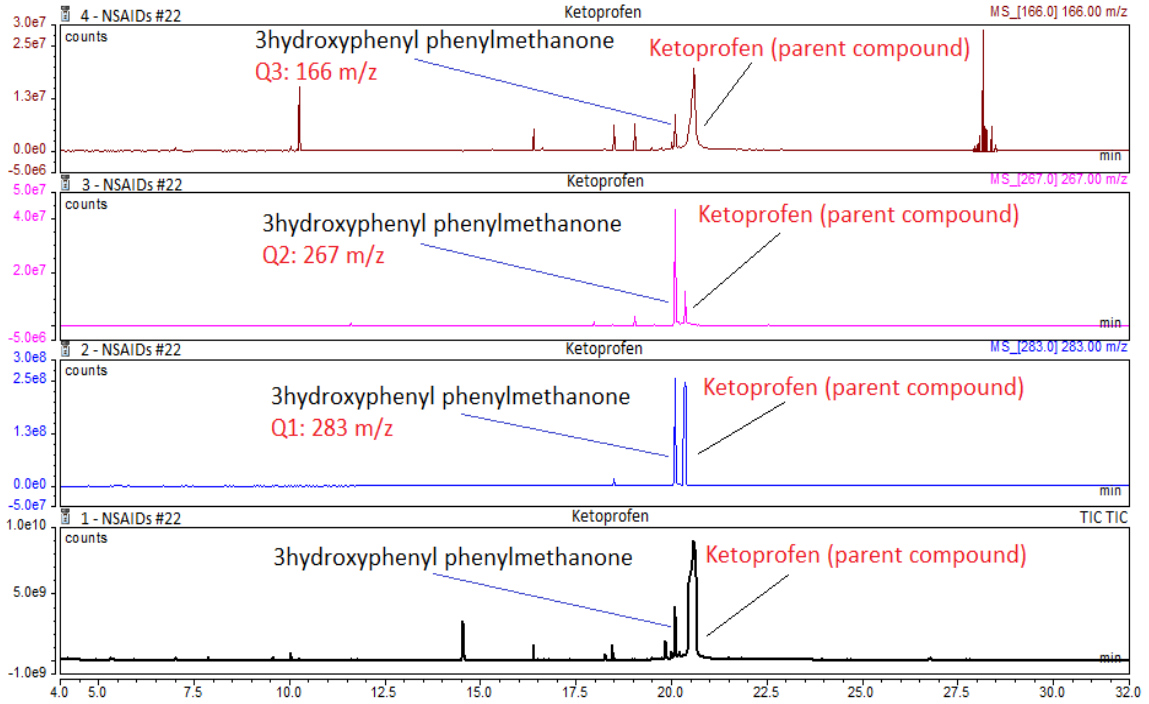
(a.)



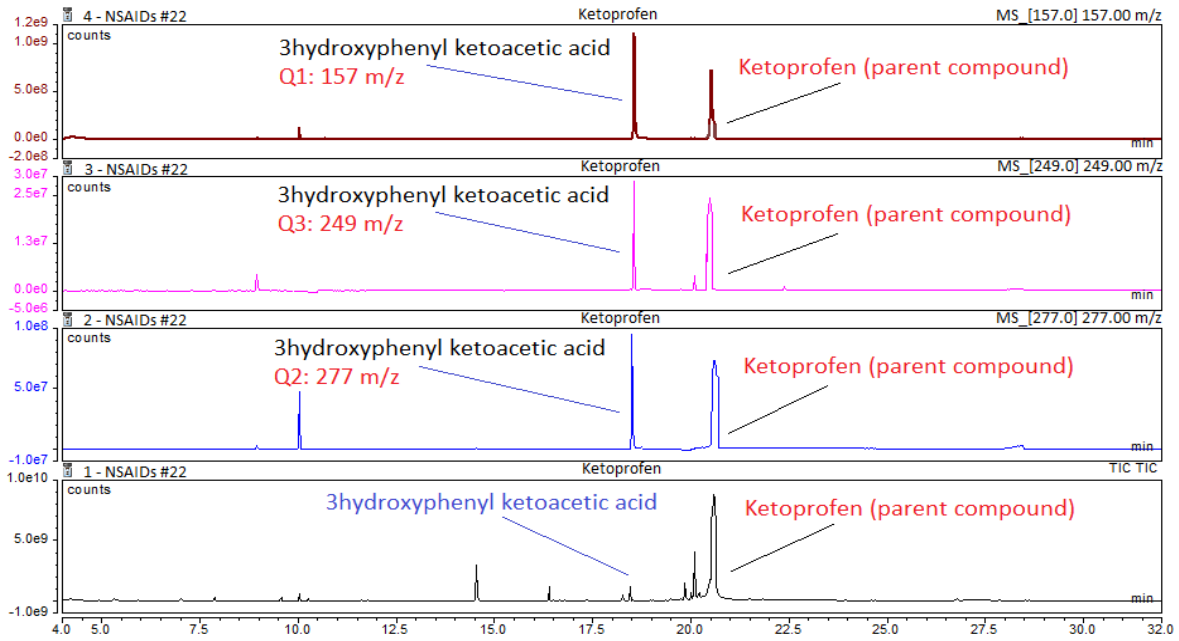
(b.)


4 - NSAIDs #22
Ketoprofen
MS_[105.0] 105.00 m/z
counts
3ethylphenyl phenylmethanone
Q2: 105 m/z
Ketoprofen (parent compound)
min
3 - NSAIDs #22
Ketoprofen
MS_[282.0] 282.00 m/z
counts
3ethylphenyl phenylmethanone
Q1: 282 m/z
Ketoprofen (parent compound)
min
2 - NSAIDs #22
Ketoprofen
MS_[311.0] 311.00 m/z
counts
3ethylphenyl phenylmethanone
Q3: 311 m/z
Ketoprofen (parent compound)
min
1 - NSAIDs #22
Ketoprofen
TIC TIC
counts
3ethylphenyl phenylmethanone
Ketoprofen (parent compound)
min
4.0 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 32.0

(c.)


4 - NSAIDs #22
Ketoprofen
MS_[166.0] 166.00 m/z
counts
3hydroxyphenyl phenylmethanone
Q3: 166 m/z
Ketoprofen (parent compound)
min
3 - NSAIDs #22
Ketoprofen
MS_[267.0] 267.00 m/z
counts
3hydroxyphenyl phenylmethanone
Q2: 267 m/z
Ketoprofen (parent compound)
min
2 - NSAIDs #22
Ketoprofen
MS_[283.0] 283.00 m/z
counts
3hydroxyphenyl phenylmethanone
Q1: 283 m/z
Ketoprofen (parent compound)
min
1 - NSAIDs #22
Ketoprofen
TIC TIC
counts
3hydroxyphenyl phenylmethanone
Ketoprofen (parent compound)
min
4.0 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 32.0

(d.)



(e.)

Figure 45. Mass spectra of identified transformation products of ketoprofen. **(a.)** total ion chromatogram of ketoprofen and transformation products; **(b.)** mass spectra of 2,3-hydroxyphenyl methyl phenyl propanoic acid qualifier and quantifier ions; **(c.)** mass spectra of 3-ethylphenyl phenyl methanone qualifier and quantifier ions; **(d.)** mass spectra of (3-hydroxyphenyl)(phenyl)methanone qualifier and quantifier ions; **(e.)** mass spectra of (3-hydroxyphenyl)ketoacetic acid qualifier and quantifier ions

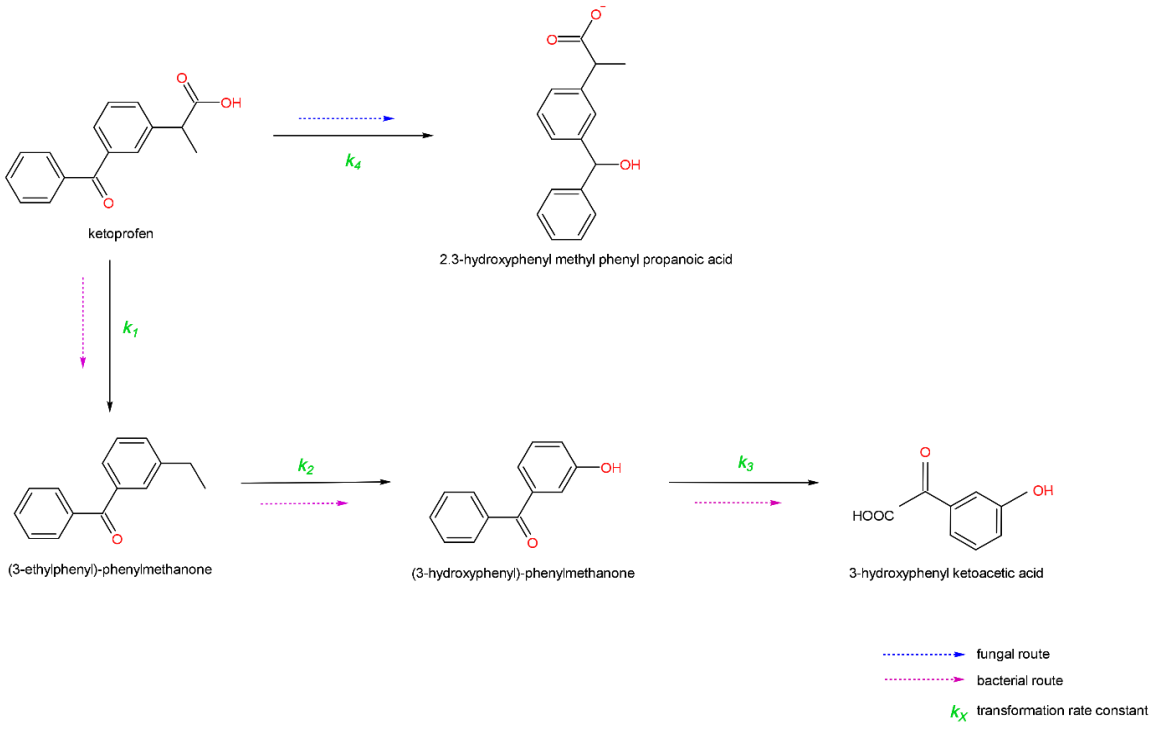


Figure 46. Established degradation pathway for ketoprofen in soil

4.2. NSAIDs path modelling in soil environment

In this study with aim to simulate studied pharmaceuticals fate in soil, a transport and reaction model was applied in COMSOL solver based on obtained experimental data. Model was defined according with: (*i*.) diclofenac, ibuprofen and ketoprofen depletion rate with time in soil; and (*ii*.) their TPs potential formation rate and stability in time.

Reaction and mass balance equation proposal for NSAIDs and related TPs formation

The mass balance equations for each NSAID and corresponding to each involved TP are presented in equations (Eq.58. – Eq.74):

Ibuprofen

$$\frac{\partial Ibuprofen}{\partial t} = -k_1 - k_3 \quad \text{(Eq.58.)}$$

$$\frac{\partial I.TP_1}{\partial t} = k_1 - k_2 - k_5 \quad \text{(Eq.59.)}$$

$$\frac{\partial I.TP_2}{\partial t} = k_2 \quad \text{(Eq.60.)}$$

$$\frac{\partial I.TP_3}{\partial t} = k_3 - k_4 \quad \text{(Eq.61.)}$$

$$\frac{\partial I.TP_4}{\partial t} = k_5 - k_6 \quad \text{(Eq.62.)}$$

$$\frac{\partial I.TP_5}{\partial t} = k_4 \quad \text{(Eq.63.)}$$

$$\frac{\partial I.TP_6}{\partial t} = k_6 \quad \text{(Eq.64.)}$$

where $I.TP_1$, $I.TP_2$, $I.TP_3$, $I.TP_4$, $I.TP_5$ and $I.TP_6$ represent ibuprofen transformation products as 1-hydroxyibuprofe, 1,2-dihydroxyibuprofen, 2-hydroxyibuprofen, 2-hydroxy-1,4-quinol, isobutyl benzene, and 3-isobutyl phenol, respectively; and *k* are the corresponding transformation rate constants.

Diclofenac

$$\frac{\partial Diclofenac}{\partial t} = k_1 - k_4 \quad \text{(Eq.65.)}$$

$$\frac{\partial D.TP_1}{\partial t} = k_1 - k_2 \quad \text{(Eq.66.)}$$

$$\frac{\partial D.TP_2}{\partial t} = k_2 - k_3 \quad \text{(Eq.67.)}$$

$$\frac{\partial D.TP_3}{\partial t} = k_3 \quad \text{(Eq.68.)}$$

$$\frac{\partial D.TP_4}{\partial t} = k_4 - k_5 \quad \text{(Eq.69.)}$$

$$\frac{\partial D.TP_5}{\partial t} = k_5 \quad \text{(Eq.70.)}$$

where in *D.TP₁*, *D.TP₂*, *D.TP₃*, *D.TP₄* and *D.TP₅* were symbolized diclofenac related TPs as 4-hydroxydiclofenac, 4.5-hydroxydiclofenac, N-(2,6-dichlorophenyl)hydroxylamine, 5-hydroxydiclofenac and p-benzoquinone imine, respectively.

Ketoprofen

$$\frac{\partial K.TP_1}{\partial t} = k_2 - k_3 \quad \text{(Eq.71.)}$$

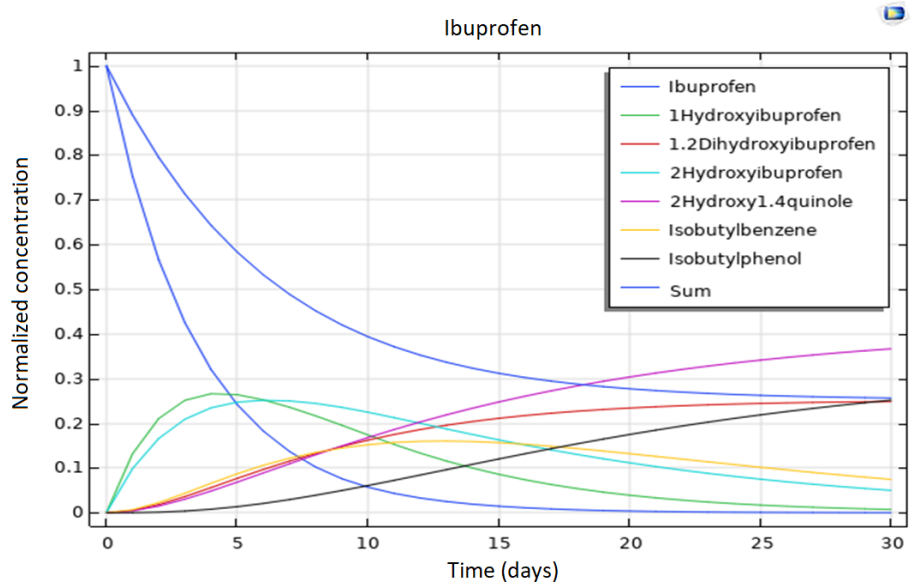
$$\frac{\partial K.TP_2}{\partial t} = r_3 \quad \text{(Eq.72.)}$$

$$\frac{\partial K.TP_3}{\partial t} = k_4 - k_5 \quad \text{(Eq.73.)}$$

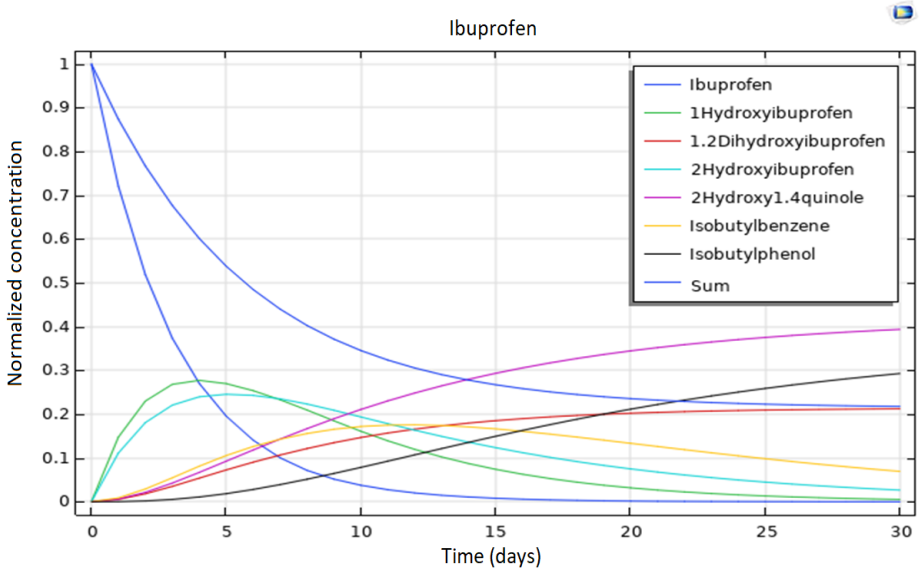
$$\frac{\partial K.TP_4}{\partial t} = k_5 \quad \text{(Eq.74.)}$$

where *K.TP₁*, *K.TP₂*, *K.TP₃* and *K.TP₄* were symbolized ketoprofen resulted TPs: (3-ethylphenyl)(phenyl)methanone, (3-hydroxyphenyl)(phenyl)methanone, (3-hydroxyphenyl)ketoaceticacid and 2-[3-(hydroxy-phenyl-methyl)phenyl]propanoic acid.

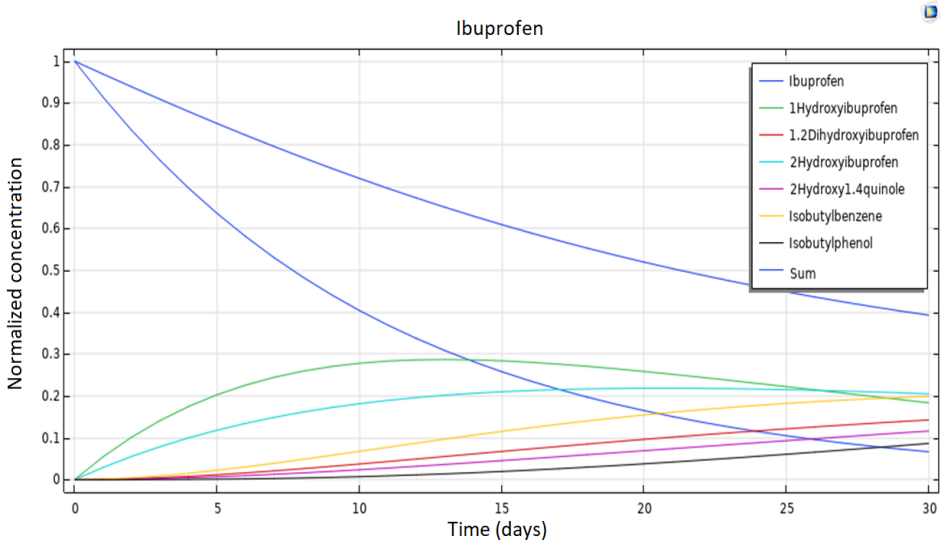
In Figure 47 are presented the fate of ibuprofen and its transformation products stability in time for all three tested experimental conditions.



(a.)

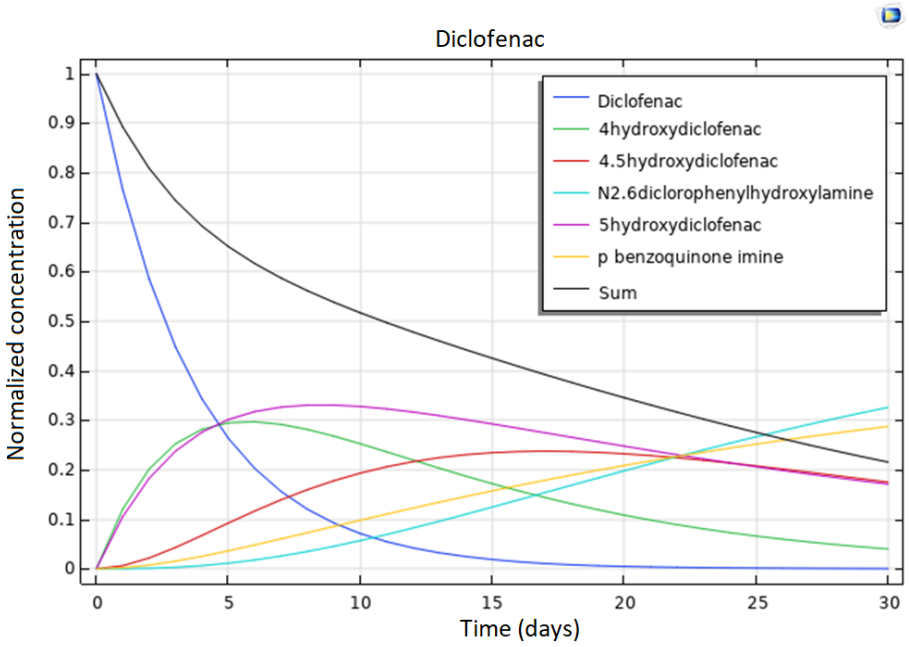


(b.)

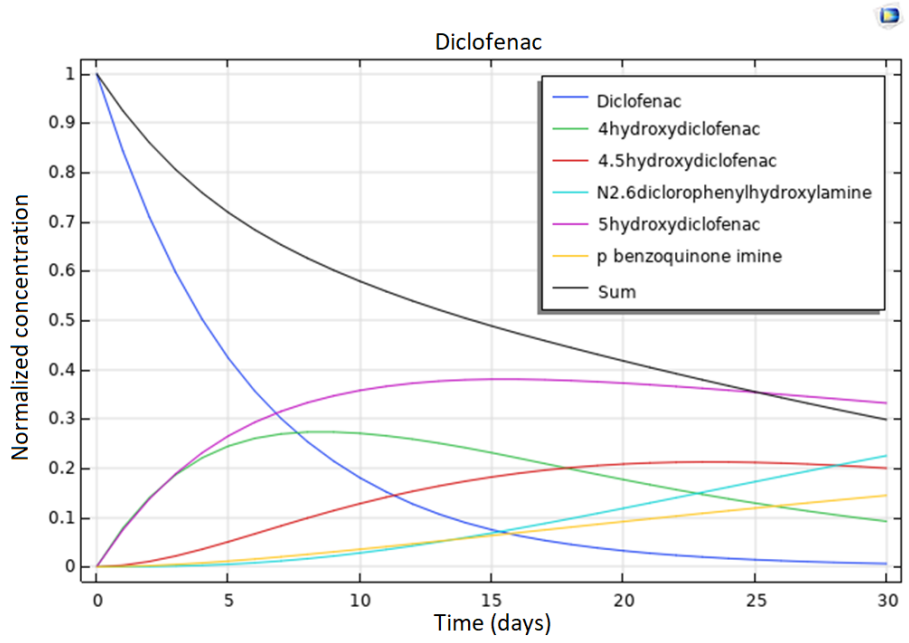


(c.)

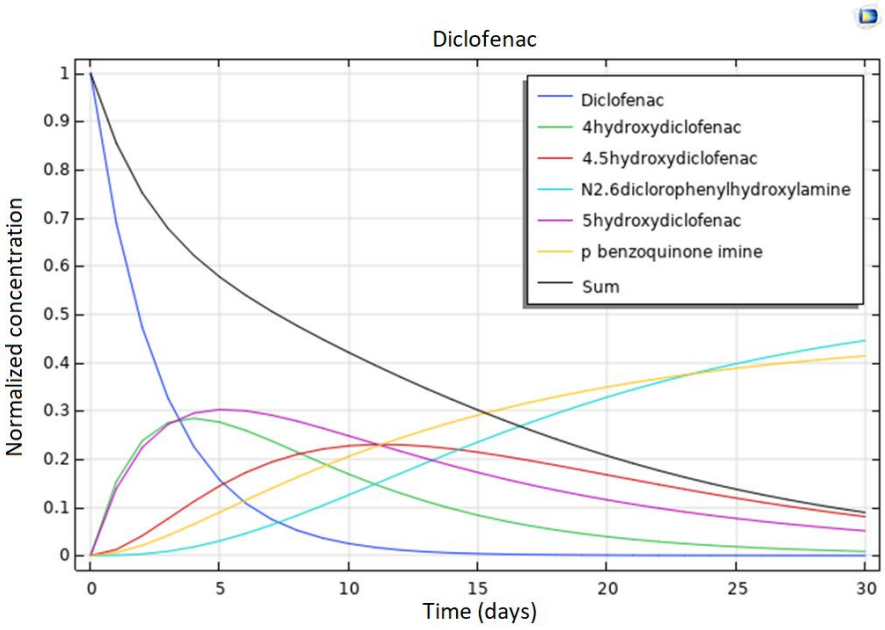
Figure 47. Ibuprofen and corresponding TPs fate simulation through COMSOL. **a.)** normal condition; **(b.)** simulated flood condition; **(c.)** simulated drought condition


Diclofenac
Normalized concentration
Time (days)
Diclofenac
4hydroxydiclofenac
4.5hydroxydiclofenac
N2.6dicloropheny lhydroxylamine
5hydroxydiclofenac
p benzoquinone imine
Sum

(a.)

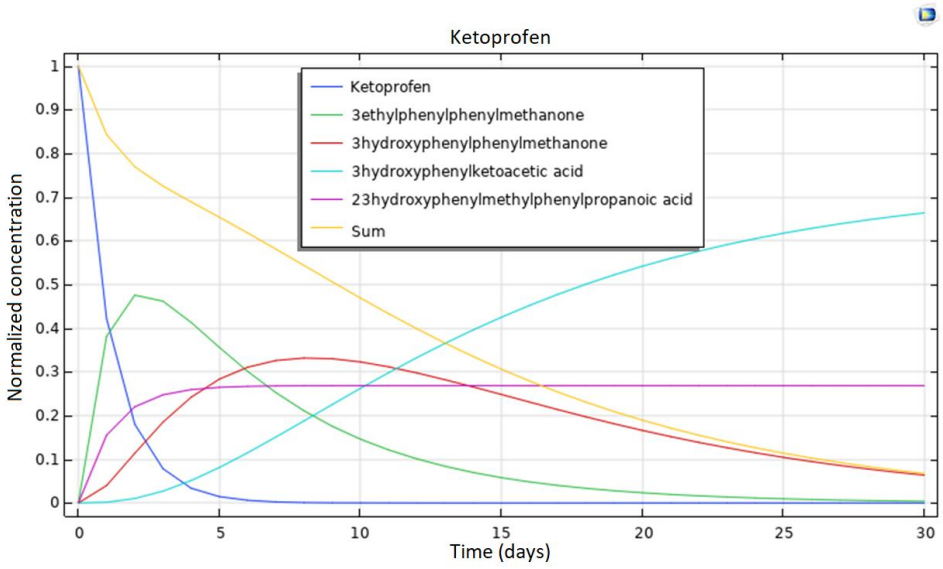

Diclofenac
Normalized concentration
Time (days)
Diclofenac
4hydroxydiclofenac
4.5hydroxydiclofenac
N2.6dicloropheny lhydroxylamine
5hydroxydiclofenac
p benzoquinone imine
Sum

(b.)

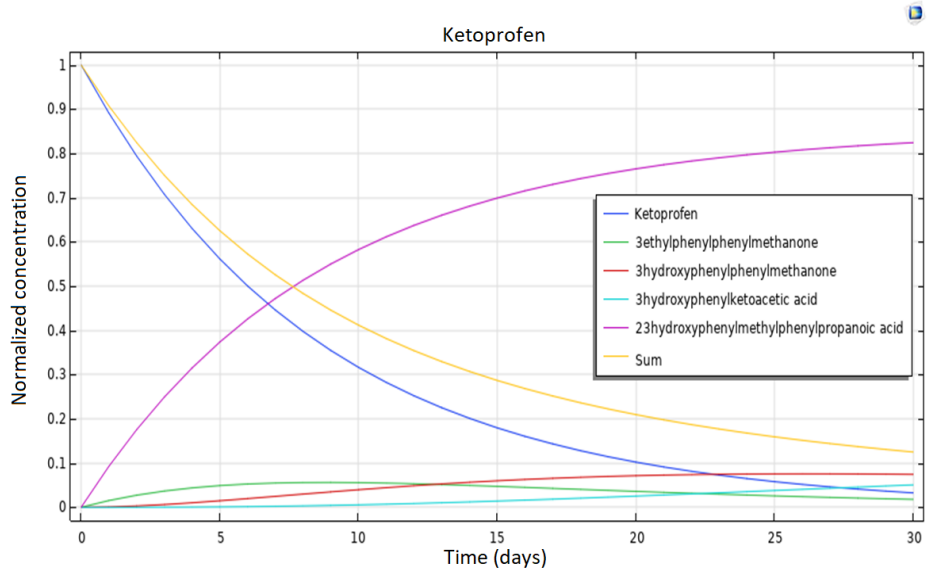


(c.)

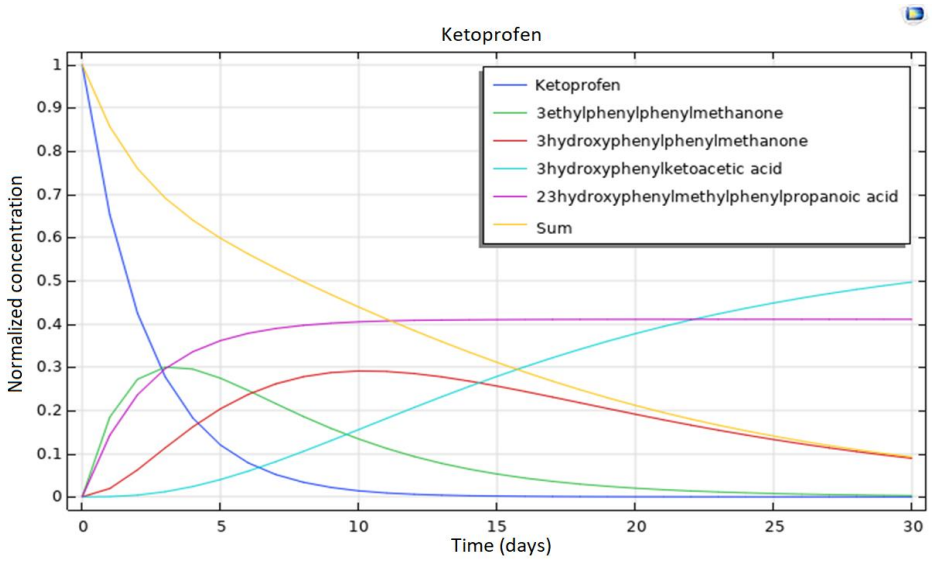
Figure 48. Diclofenac and corresponding TPs fate simulation through COMSOL. **a.)** normal condition; **b.)** simulated flood condition; **c.)** simulated drought condition



(a.)



(b.)



(c.)

Figure 49. Ketoprofen and corresponding TPs fate simulation through COMSOL. **a.)** normal condition; **b.)** simulated flood condition; **c.)** simulated drought condition

Chapter 5. NSAIDs (ibuprofen, diclofenac, ketoprofen) pathway assessment in *Lycopersicon esculentum*

The risk that human health will be compromised is increasing in our days. One significant way that could contribute at health deterioration is nutrition. Consumption of potential contaminated food as consequence of their growth and development in improper environment could be a cause. However, contaminants translocation from environment to subjects through food chain is a dynamic process whose amplitude and consequences are difficult to estimate at this moment.

Plants are important component of both human and animal diet. Although there are critical regulations regarding their quality and safety assurance, these didn't refer at the new generation of contaminants as pharmaceuticals. As plant could be exposed at organic contaminants through three major routes – soil, water and air – all these will be considered in this thesis. Also, because our experimental data about studied NSAIDs pathway in major environmental compartments clearly revealed their potential transformation, in experiments on plant exposed to NSAIDs also the identified TPs are targeted.

5.1. NSAIDs impact on *Lycopersicon esculentum* fruit quality

Lycopersicon esculentum fruit quality was evaluated in terms of volatile organic compounds content responsible for fruit aroma, carbohydrates, amino acids and fatty acids methyl esters.

Volatile organic compounds content variation in tomato fruit after multi-route exposure experiments: Among volatile organic compounds alcohols, ketones, aldehydes, esters, terpenes, phenols and others were identified. In control samples the alcohol compounds content was 26.4 %, followed by ketones (19.2 %), terpenes (17.9 %), aldehydes (15.9 %), phenols (3.8 %) and esters (2.1 %). Other compounds represented 14.7 %.

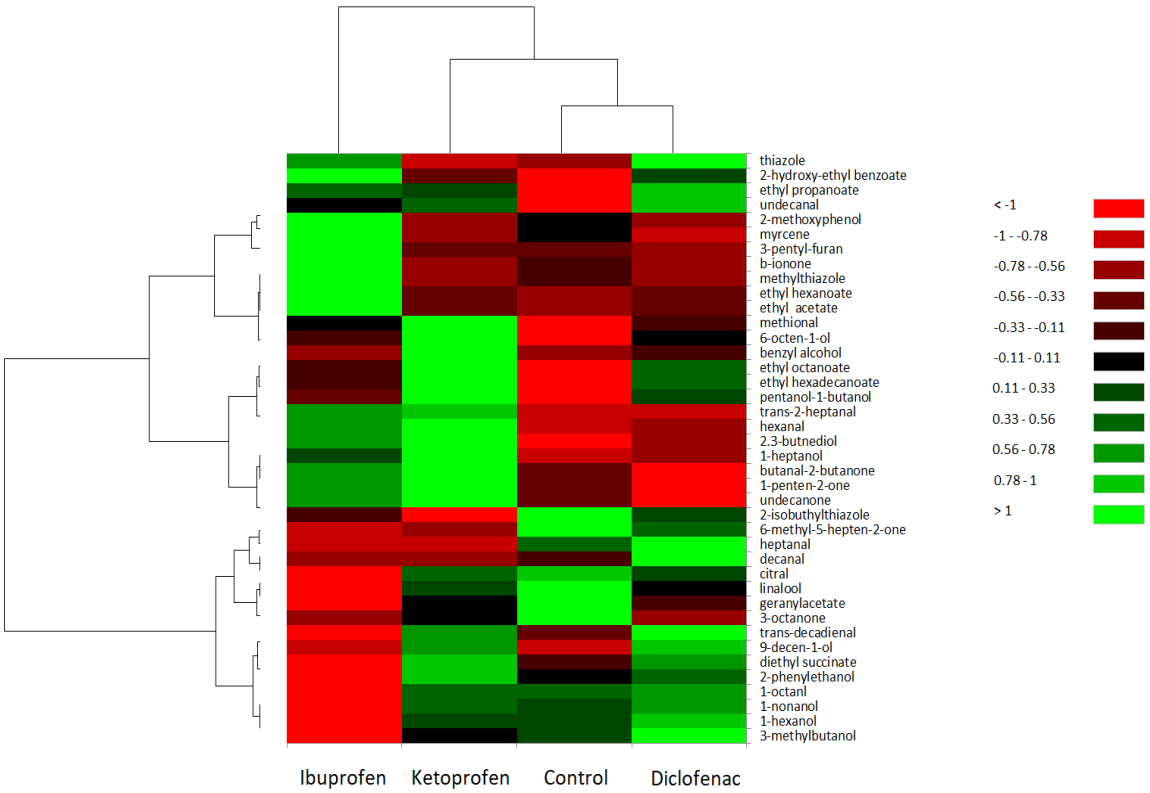


Figure 66. Volatile organic compounds differentiation within tomato fruit grown under exposure at studied NSAIDs

In Figure 66 is presented the heat map differentiation between *Lycopersicon esculentum* fruit volatile organic compounds content reported to control. Significant differentiation was observed in case of contamination with ibuprofen. Decrease up to 34 % and 24 % were identified for 1-hexanol and 3-methylbutanol. Contrary, the presence of these alcohols increased with 13 % when *Lycopersicon esculentum* was exposed at diclofenac. Among ketones, 1-penten-2-one presented minor changes in presence of diclofenac, while in presence of ibuprofen and ketoprofen its amount in tomato fruit increased with 44 % and 65 %, respectively. Oppositely, the amount of 6-methyl-5-hepten-2-one decrease significantly in presence of studied NSAIDs (ibuprofen 70 %, ketoprofen 67 %, diclofenac 27 %). Decrease was identified even in case of representative terpenes.

Carbohydrates content variation in tomato fruit after multi-route exposure experiments: As in case of volatile organic compounds, carbohydrates profile changes when they were grown in different contaminated media. In Figure 67 are presented the changes that were identified for each studied NSAID and route of exposure. Decrease in amount were observed in case of glucose, fructose and xylose especially when tomato plant was in contact

with soil and air contaminated with ketoprofen, soil and water contaminated with diclofenac and soil contaminated with ibuprofen. Fructose content also decreased in presence of soil contaminated with diclofenac and pulverized water contaminated with ketoprofen. Increase of galactose content was observed when tomato plant was in contact with ibuprofen contaminated water. Similarly, sucrose content increased in presence of soil contaminated with ibuprofen.

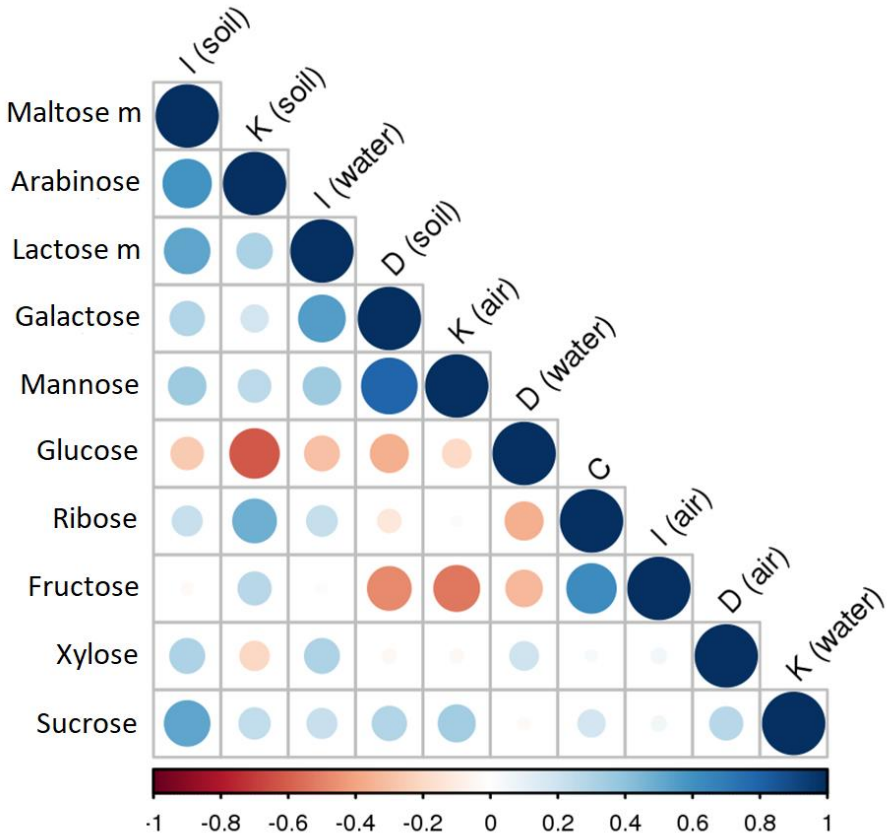


Figure 67. Correlation matrix of carbohydrates variation within tomato fruit grown under different route of exposure at studied NSAIDs

Fatty acid methyl esters (FAMEs) content variation in tomato fruit after multi-route exposure experiments: Among this group of compounds, C14:0 (myristic acid), C16:0 (palmitic acid), C18:1C+T, C18:2C+T (elaidic acid), C18:3n6 (g-linolenic acid), C18:3n3 (g-linolenic acid) and C24:0 (lignoceric acid) were identified in prevalent amount. Heat map correlation (Figure 68) revealed changes of FAME profile when *Lycopersicon esculentum* was in contact with diclofenac, ibuprofen and ketoprofen. Significant changes were observed in presence of ibuprofen if we report the FAME content with that obtained from control sample analysis. In that case the FAME content decreased with approximately 43 % compared control. Although different from control sample outline, the FAME profile of tomato plant exposed to diclofenac and ketoprofen were comparable.

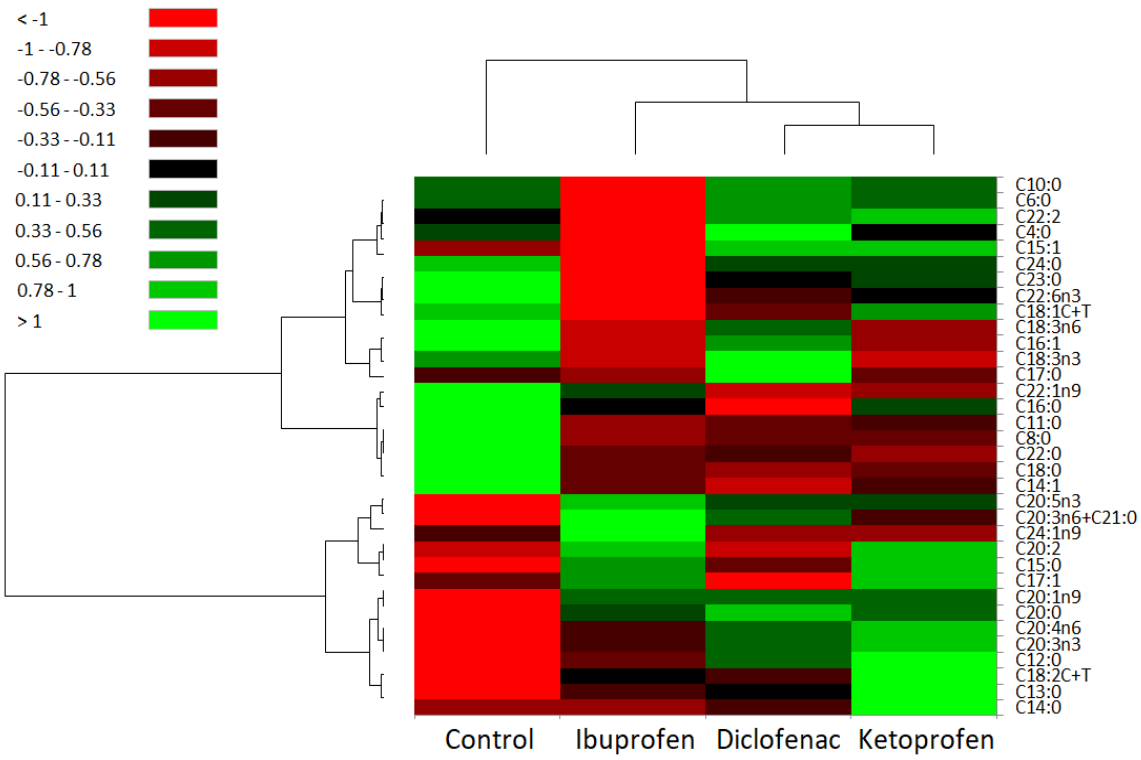


Figure 68. Heat map of fatty acid methyl esters variation within tomato fruit grown under different route of exposure at studied NSAIDs

Amino acids content variation in tomato fruit after multi-route exposure experiments: Glutamic acid, alanine, lysine, valine and leucine were detected in representative amount in tomato fruit. Their amount encountered approximately 60 % from the total content of the identified amino acids. Glutamic acid decreased in case of exposure to ketoprofen while in case of exposure to diclofenac or ibuprofen minor changes were observed. Alanine amount increased in presence of all studied NSAIDs (21 % – exposure to ketoprofen, 18 % – exposure to ibuprofen, and 7 % in case of exposure to diclofenac), especially when the contamination was performed through soil route. Decrease in amount of aspartic acid (7 %) and lysine (5 %) was determined in experiments on tomato when it was exposed at diclofenac contamination. Arginine content increased with approximately 11 % in presence of ibuprofen while in presence of diclofenac it decreased with 9 % compared with control samples.

5.2. Modelling NSAIDs bioconcentration into *Lycopersicon esculentum* fruit considering their growth dynamics

Is a widely accepted assumption by scientific community that pharmaceuticals active ingredient could be uptake by plants. Concerns are amplified by the constant input of these active ingredients in environment all over the world, and because of the potential adverse effect that could result on non-targeted organisms that consume these plants through their common diet. Analytical detection and quantification both of pharmaceuticals active ingredients as well their related TPs is difficult, laborious and involve significant costs. Therefore, numerical modeling could be a solution that allow the high-throughput estimation of NSAIDs active ingredients amount that could reach a plant that is relevant for the diet of a living organism. Further, numerical model that allow estimation of the studied NSAIDs active ingredients bioconcentration into *Lycopersicon esculentum* fruit was implemented based on obtained experimental data.

NSAIDs modelling framework in *Lycopersicon esculentum* fruit

Model concept description: The used model is built on three major parts. The first refer at the partial differential equation (PDE) that describe the dynamic moving boundary of the *Lycopersicon esculentum* fruit "sphere" – tomato fruit growth. In that case NSAIDs diffusion is assumed that occur along with tomato fruit sphere radius. The logistic function proposed by Xiao et al., [385] was applied. This imitates the diffusion of a chemical from a contaminated environment into the edible fruit of a plant (namely potato tuber). Used PDE describe the behavior of an organic chemical in a sphere based on sphere radius, time, moving boundary of studied sphere. This, as a whole has the role to simulate the development in time of a *Lycopersicon esculentum* fruit. Data about NSAIDs uptake by tomato were obtained from experiments performed on tomato plants exposed to diclofenac, ibuprofen and ketoprofen through multiple environmental compartments. These obtained data, were imported further into dynamic sphere model to obtain numerical solutions of the implemented PDE through MATLAB solver (MATLAB, version R2013b).

The second part is the integral form of the proposed model that allow to estimate NSAIDs active ingredients uptake and bioconcentration factor for studied *Lycopersicon esculentum* fruit. In that section the NSAIDs amount were integrated along tomato fruit sphere

assumed radius. That allowed to estimate the amount of studied NSAIDs active ingredients that has diffused with time into tomato fruit.

In the third part of the model the obtained estimated data are compared with those obtained from performing a classic model (diffusion model). The NSAIDs bioconcentration factor for *Lycopersicon esculentum* fruit is mathematically described according with (Eq.90.) where it is defines in relation with time (dynamic) instead of steady-state ratio ones.

$$BCF^{c}(t) \equiv \frac{C^{c}_{Tomato}(t)}{C^{c}_{environment}(t)} \qquad \text{(Eq.90.)}$$

Moving boundary model description: The main assumption of this model is that all fruits (including *Lycopersicon esculentum* fruit) follow a logistic growth function through its development in time until maturity. Because of that, is possible that the uptake process of the NSAIDs active ingredients to be influenced. Therefore, according with those proposed by Xiao et al., [385] the passive diffusion of NSAIDs active ingredients through tomato sphere could be described according with (Eq.91.) and (Eq.92.) which involve the mathematical expression of a simple radial diffusion model:

$$\frac{\partial C_{Tomato}(r,t)}{\partial t} = 0; 0 \leq t \leq t_{contam}; 0 \leq r \leq r_{Tomato}(t) \qquad \text{(Eq.91.)}$$

$$\frac{\partial C_{Tomato}(r,t)}{\partial t} = D^{adjusted}_{Tomato} \cdot \left(\frac{\partial^2 C_{Tomato}(r,t)}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C_{Tomato}(r,t)}{\partial r} \right) - k_{d.Tomato} \cdot C_{Tomato}(r,t); t \geq t_{contam}; ; 0 \leq r \leq r_{Tomato}(t) \qquad \text{(Eq.92.)}$$

The expressions that describe both the tomato as well its potential surrounding contaminated environment are presented in (Eq.93.) and (Eq.94.):

$$C(r,t) = 0; 0 \leq t \leq t_{contam} \qquad \text{(Eq.93.)}$$

$$C(r,t) = \begin{cases} C_{Tomato}(r,t); r < r_{Tomato}(t); \text{this describe what hapen inside of the tomato} \\ \left(\frac{K_{Tomato,environ1}}{K_{environ1,environ2}} \right) \cdot C_{environ}(t); \text{this describe the surface of tomato}; t \geq t_{contam} \\ C_{environ}(t); r > r_{Tomato}(t); \text{this describe what hapens at ouside of the tomato} \end{cases} \qquad \text{(Eq.94.)}$$

Entry data: Numerical data used in this model were either obtained from experiments performed in laboratory (previously described) of collected from literature that is available for public.

NSAIDs diffusion through *Lycopersicon esculentum* fruit dynamic moving-boundary

In Figure 70 are represented the simulated NSAIDs active ingredients concentration in tomato fruit as function of its radius and time after exposure to pharmaceuticals. According to the assumed logistic function, in the horizontal direction the length of the function line increase with time. This indicate that for each NSAIDs the concentration is approximately equally distributed for all assumed growth processes. Analyzing Figure 70 it was observed that in case of tomato exposure to ketoprofen (Figure 70.c.) the active ingredient concentration decreases along the radius of tomato, compared with case when tomato was exposed at ibuprofen for example (Figure 70.b.).

Data revealed that ibuprofen easily diffuse through tomato fruit when plants were growth in contaminated soil or were watered with contaminated water (Figure 69.b.). The reached maximum ibuprofen amount diffused amount of ibuprofen was 0.0586 $\mu g \cdot kg^{-1}$ (growth in contaminated soil). Diclofenac diffusion rate was three-fold higher when *Lycopersicon esculentum* was grown in contaminated soil (0.0284 $\mu g \cdot kg^{-1}$, Figure 70.a.) compared with contamination through watering (0.0087 $\mu g \cdot kg^{-1}$). Ketoprofen has shown a low diffusion rate compared with the other two studied NSAID, the maximum amount was identified to be 0.0174 $\mu g \cdot kg^{-1}$ in case when the contaminated media is the soil (Figure 69.c.). For each NSAID low diffusion rate was determined when contamination was performed through pulverized water. The diffused NSAIDs concentration through tomato fruit varied as follows: 0.0247 $\mu g \cdot kg^{-1}$ (ibuprofen) > 0.0008 $\mu g \cdot kg^{-1}$ (ketoprofen) > 0.005 $\mu g \cdot kg^{-1}$ (diclofenac).

Although the simulated data are close to experimental data (correlation coefficients > 0.688) further experimental data must be collected and used to evaluate the model performance. Experimental data are necessary to correctly establish the component parameters of model as k_d both when we refer at the NSAIDs concentration attenuation in environment as well in living organisms as *Lycopersicon esculentum*. Also, inclusion of transformation potential of the parent active ingredient should be introduced into the model, especially when we refer at pharmaceuticals as NSAIDs that are highly bioactive and undergo to several changes both in

environment as well in living organism. Also, the model should be tested for other contaminants. Such data and models are important to enhance living organisms' health status protection for increased challenges raised from the increased presence and varieties of chemicals in our surrounding environment.

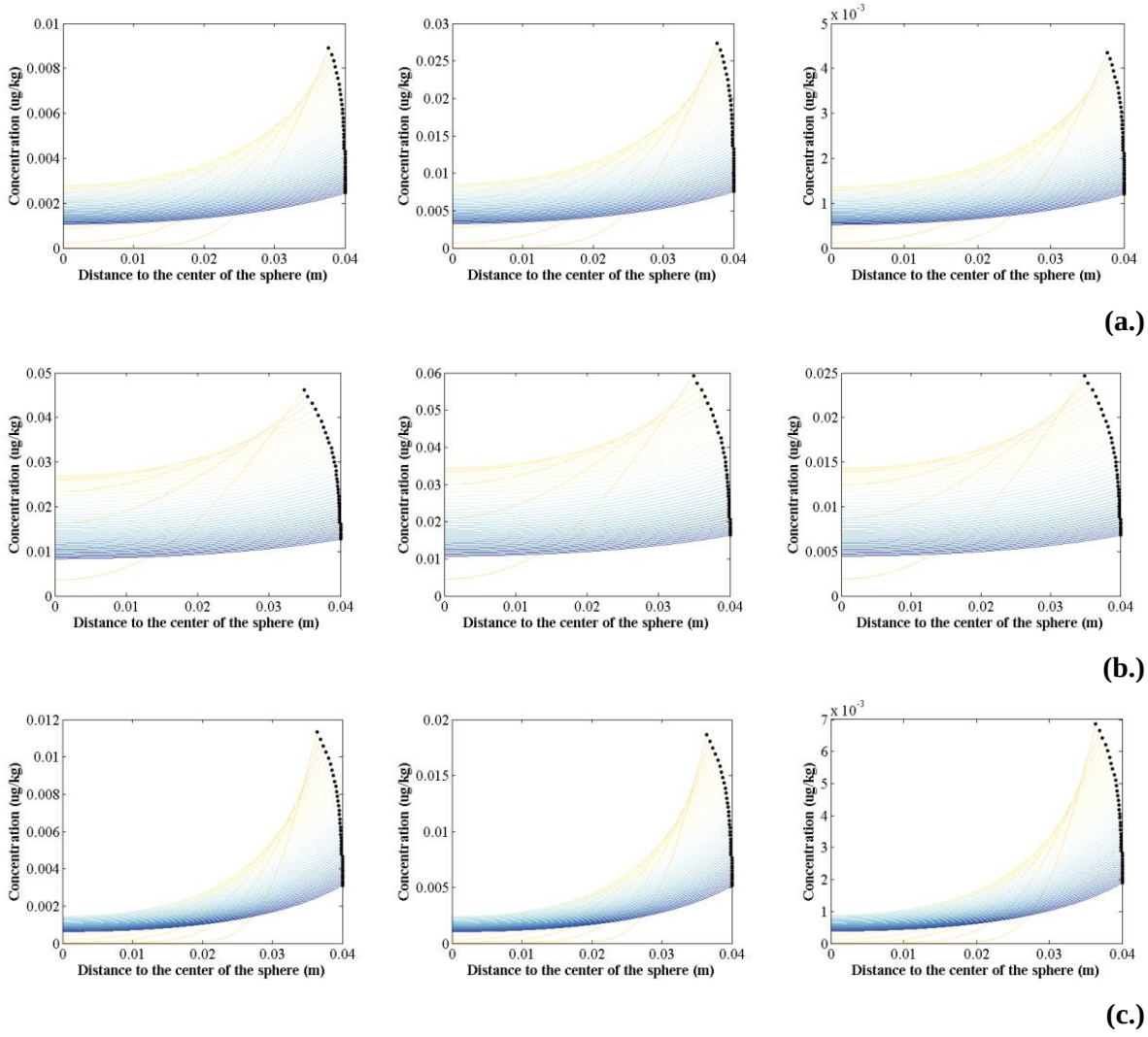


Figure 70. NSAIDs diffusion through *Lycopersicon esculentum* fruit moving-boundary exposed through different media. **(a.)** Diclofenac; **(b.)** Ibuprofen; **(c.)** Ketoprofen

General conclusion

In that thesis pathways of representative NSAIDs as ibuprofen, diclofenac and ketoprofen were assessed within a non-targeted living system as *Lycopersicon esculentum*. With purpose of an accurate understating of these compounds' active ingredients behavior, their pathway in major environmental compartments as soil and water were considered also. This was done in the light that generally a plant could be exposed to pharmaceuticals either through the water used for watering or irrigation purposes, or through soil on that manure or WWTPs resulted sludge were applied.

Through experiments performed on surface water media, influence of biotic, abiotic and sorption processes on diclofenac, ibuprofen and ketoprofen pathway was assessed. Experimental data sets revealed that a pseudo-first order kinetic model describe suitably these pharmaceuticals pathway in surface water environment. Contribution of each process in part to NSAIDs attenuation in water was intensely studied. The obtained experimental data, further were used to numerically rank the participation of each process to NSAIDs attenuation in water environment. As numerical approach structural equation modelling (SEM) combined with partial least squares (PLS) model was applied. After each case a path diagram was proposed. These were tested statistically through multiple goodness of fit parameters.

In experiments on NSAIDs pathway assessment in soil environment correlation between soil physicochemical properties, microbiota phenotypic structure abundance and simulated meteorological related anomalies condition were established. Obtained results revealed negative correlation between NSAIDs attenuation rate under drought condition or contamination with high dose of NSAIDs. Similarly, positive correlation was found between higher abundance of soil microbiota and decrease of NSAIDs active ingredient amount. This was explained by that there are microorganism communities that are able to use these chemicals as carbon source in their metabolic processes. This assumption was sustained by the identified TPs of the studied NSAIDs. Using obtained experimental data, the ibuprofen, diclofenac and ketoprofen pathway mechanism was proposed in terms of mass balance equations, reaction rate constants, etc. Further these were simulated using transport and reaction model of COMSOL solver using Richard's equation for their diffusion through soil layer. In all situations, the proposed numerical solutions were validated comparing the estimated results with those obtained from a new set of experimental data.

Experimental data proved that assumption, revealing that biotic processes are the most representative ones that shape NSAIDs active ingredient persistence and stability both in soil and water environment. This highlights the bioactive potential of these chemicals reported to non-targeted organisms. Experimental data have showed that there were numerous phenotypes that could use these compounds as carbon source in metabolic functions. This was sustained also by the identified TPs. However, both water and soil microbiota are sensitive to changes as those induces by meteorological anomalies due to climate change. These were further felt though a lower attenuation potential of NSAIDs in studied media.

Both active ingredients as well representative TPs accumulation as well potential influence on *Lycopersicon esculentum* was evaluated experimentally and numerically. Experiments were performed considering multiple routes of exposure to NSAIDs, tomato plant development in time (Zadock stages) and anatomical compartments. Data shown differentiated accumulation potential in all considered cases. Among studied NSAIDs diclofenac was more easily retained by the whole anatomy of *Lycopersicon esculentum* while only in fruit part ibuprofen was accumulated significantly. Similar results were obtained also in case of identified representative TPs of NSAIDs. Data also revealed that exposure route could contribute at NSAIDs active ingredients accumulation potential in various anatomical compartment of tomato plant. Of that, soil and water were the representative contributors for increased NSAIDs uptake potential by tomato. After performed experiments it was observed that these NSAIDs active ingredients are differently accumulated in time through tomato plant anatomical compartments. Route of exposure significantly influenced the accumulation potential of studied pharmaceuticals. Active ingredients influence on tomato fruit quality in terms of volatile organic compounds content, amino acids content, carbohydrates content and fatty acid methyl esters content were studied. Experimental results proved that but there were compounds whose amount increased in presence of specific NSAIDs while others decreased.

As collection of experimental data is laborious, difficult, and cost and time consuming this way could be a proper solution. Correlating simulated results with those resulted from a new set of experimental data revealed that use of numerical models could offer answers are accumulation rate of NSAIDs active ingredients in edible part of *Lycopersicon esculentum* or its pathway in fruit once with grown of tomato plant. Xiao model was modified to numerically simulate NSAIDs diffusion in a growing tomato fruit. Obtained estimated data were correlated with those resulted from a classical diffusion model where tomato fruit increase in volume due to growing process was neglected.

Referinte bibliografice (selectie)

[4] Stuer-Lauridsen F., Birkved M., Hansen LP., Lutzhoft HC., Sorensen H. Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere*, 40(7):783-793, **2000**. Doi: 10.1016/S0045-6535(99)00453-1

[5] Tranter D. Pharmaceutical science and technology today: evolving to reflect the modern industrial life-science environment. *Pharmaceutical Science & Technology Today*, 3(12): 399-400, **2000**. Doi: 10.1016/S1461-5347(00)00320-5

[6] Stan HJ., Heberer T., Linkenhagner M. Vorkommen von clofibrinsaure im gewassersystem-ist der humanmediziniche gebrauch urasche fur die contamination von oberflachen, ground-und trinkwasser. *Vom Wasser*, 85:57-68, **1994**.

[7] Waleng NJ., Nomngongo PN. Occurrence of pharmaceuticals in the environmental waters: African and Asian perspectives. *Environmental Chemistry and Ecotoxicology*, 4:50-66, **2022**. Doi: 10.1016/j.enceco.2021.11.002

[8] Ramirez-Morales D., Masis-Mora M., Montiel-Mora JR., Cambronero-Heinrichs JC., Perez-Rojas G., Tormo-Budowski R., Mendez-Rivera M., Briceno-Guevara S., Gutierez-Quiros JA., Arias-Mora V., Brenes-Alfaro L., Beita-Sandi W., Rodriguez-Rodriguez EC. Multi-residue analysis of pharmaceuticals in water samples by liquid chromatography-mass spectrometry: quality assessment and application to the risk assessment of urban-influenced surface waters in a metropolitan area of Central America. *Process Safety and Environmental Protection*, 153:289-300, **2021**. Doi: 10.1016/j.psep.2021.07.025

[9] Jameel Y., Valle D., Kay P. Spatial variation in the detection rates of frequently studied pharmaceuticals in Asian, European and North American rivers. *Science of The Total Environment*, 724:137947, **2020**. Doi: 10.1016/j.scitotenv.2020.137947

[10] Khan KH., Rehman MYA., Malik RN. Fate and toxicity of pharmaceuticals in water environment: an insight on their occurrence in South Asia. *Journal of Environmental Management*, 271:111030, **2020**. Doi: 1.1016/j.jenvman.2020.111030

[11] Li Y., Zhang L., Ding J., Liu X. Prioritization of pharmaceuticals in water environment in China based on environmental criteria and risk analysis of top-priority pharmaceuticals. *Journal of Environmental Management*, 253:109732, **2020**. Doi: 10.1016/j.jenvman.2019.109732

[12] Fekadu S., Alemayehu, Dewil R., Bruggen BV. Pharmaceuticals in freshwater aquatic environment: a comparison of the African and European challenge. *Science of The Total Environment*, 654:324-337, **2019**. Doi: 10.1016/j.scitotenv.2018.11.072

[13] Richardson BJ., Lam KSP., Martin M. Emerging chemicals of concern: pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China. *Marine Pollution Bulletin*, 50(9):913-920, **2005**. Doi: 10.1016/j.1016/j.marpolbul.2005. 06.034

[15] Vane HC., Kim AW., Lopes dos Santos RA., Moss-Hayes V. Contrasting sewage, emerging and persistent organic pollutants in sediment cores from the River Thames estuary, London, England, UK. *Marine Pollution Bulletin*, 175:113340, **2022**. Doi: 10.1016/j.marpolbul.2022.113340

[16] Katsikaros AG., Chrysikopoulos CV. Occurrence and distribution of pharmaceuticals and personal care products (PPCPs) detected in lakes around the world – a review. *Environmental Advances*, 6:100131, **2021**. Doi: 10.1016/j.envadv.2021.100131

[17] Mejias C., Martin J., Santos JL., Aparicio I., Alonso E. Occurrence of pharmaceuticals and their metabolites in sewage sludge and soil: a review on their distribution and environmental risk assessment. *Trends in Environmental Analytical Chemistry*, 30:e00125, **2021**. doi: 10.1016/j.teac.2021.e00125

[18] Quaik S., Embrandiri A., Ravindran B.., Hossain K., Al-Dhabi NA., Arasu MV., Ignacimuthu S., Ismail N. Veterinary antibiotics in animal manure and manure laden soil: Scenario and challenges in Asian countries. *Journal of King Saud University – Science*, 32(2):1300-1305, **2020**. Doi: 10.1016/j.ksus.2019.11.015

[19] Battaglin WA., Bradley PM., Iwanowicz L., Journey CA., Walsh HL., Blazer VS. Pharmaceuticals, hormones, pesticides, and other bioactive contaminants in water, sediment, and tissue from Rocky Mountain National Park, 2012–2013. *Science of The Total Environment*, 643:651-673, **2018**. Doi: 10.1016/j.scitotenv.2018.06.150

[20] Al-Khazrajy OS., Boxall ABA. Risk-based prioritization of pharmaceuticals in the natural environment in Iraq. *Environmental Science and Pollution Research*, 23:15712-15726, **2016**. Doi: 10.1007/s11356-016-6679-0

[21] Wohde M., berkner S., Junker T., Konradi S., Schwartz L., During RA. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. *Environmental Sciences Europe*, 28:23, **2016.** Doi: 10.1186/s12302-016-0091-8

[22] Golbaz S., Yaghmaeian K., Isazadeh S., Zamanzadeh M. Environmental risk assessments of multiclass pharmaceutical active compounds: selection of high priority concern pharmaceuticals using entropy-utility functions. *Environmental Science and Pollution Research*, 28:59745-59770, **2021**. Doi: 10.1007/s11356-021-14693-w

[23] Schwartz S., Gildemeister D., Hein A., Schroder P., Bachmann J. Environmental fate and effects assessment of human pharmaceuticals: lessons learnt from regulatory data. *Environmental Science Europe*, 33:68, **2021**. Doi: 10.1186/s12302-021-00503-0

[24] Zhou H., Zhang Q., Wang X., Zhang Q., Ma L., Zhan Y. Systematic screening of common wastewater-marking pharmaceuticals in urban aquatic environments: implications for environmental risk control. *Environmental Science and Pollution Research*, 21:7113-7129, **2014**. Doi: 10.1007/s11356-014-2622-4

[25] Lin K., Bondarenko S., Gan J. Sorption and persistence of wastewater-borne psychoactive and antilipidemic drugs in soils. *Journal of Soils and Sediments*, 11:1363-1372, **2011**. Doi: 10.1007/s11368-011-0421-9

[26] Araujo L., Wild J., Villa N., Camargo N., Cubillan D., Prieto A. Determination of anti-inflammatory drugs in water samples, by in situ derivatization, solid phase microextraction and gas chromatography-mass spectrometry. *Talanta*, 75(1): 111-115, **2008**. Doi: 10.1016/j.talanta.2007.10.035

[27] Gworek B., Kijenska M., Wrzosek J., Graniewska M. Pharmaceuticals in the soil and plant environment: a review. *Water, Air and Soil Pollution*, 232:145, **2021**. Doi: 10.1007/s11270-020-04954-8

[28] Sun M., Lin H., Guo W., Zhao F., Li J. Bioaccumulation and biodegradation of sulfamethazine in Chlorella pyrenoidosa. *Journal of Ocean University of China*, 16:1167-1174, **2017**. Doi: 10.1007/s11802-017-3367-8

[29] Orias F., Simon L., Perrodin Y. Respective contributions of diet and medium to the bioaccumulation of pharmaceutical compounds in the first levels of an aquatic trophic web. *Environmental Science and Pollution Research*, 22:20207-20214, **2015**. Doi: 10.1007/s11356-015-5243-7

[30] Gomez E., Bachelot M., Boillot C., Munaron D., Chiron S., Casellas C., Fenet H. Bioconcentration of two pharmaceuticals (benzodiazepines) and two personal care products (UV filters) in marine mussels (Mytilus galloprovincialis) under controlled laboratory conditions. *Environmental Science and Pollution Research*, 19:2561-2569, **2012**. Doi: 10.1007/s11356-012-0964-3

[31] Huerta B., Rodriguez-Mozaz S., Barcelo D. Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications. *Analytical and Bioanalytical Chemistry*, 404:2611-2624, **2012**. Doi: 10.1007/s00216-012-6144-y

[152] Madikizela LM., Chimuka L. Simultaneous determination of naproxen, ibuprofen and diclofenac in wastewater using solid-phase extraction with high performance liquid chromatography. *Water SA*, 43(2), **2017**. Doi: 10.4314/wsa.v43i2.10

[250] Wang X., Chen Z., Shen J., Kang J., Zhang X., Li J., Zhao X. Effect of carbon source on pollutant removal and microbial community dynamics in treatment of swine wastewater containing antibiotics by aerobic granular sludge. *Chemosphere*, 260:127544, **2020**.

[363] Beek TAD., Weber FA., Bergmann A., Hickmann S., Ebert I., Hein A., Kuster A. Pharmaceuticals in the environment – global occurrences and perspectives. *Environmental Toxicology and Chemistry*, 35(4):823-835, **2016**. doi:/10.1002/etc.3339

[369] Maculewicz J., Kowalska D., Swiacka K., Tonski M., Stepnowski P., Bialk-Bielinska A., Dolzenek J. Transformation products of pharmaceuticals in the environment: their fate, (eco)toxicity and bioaccumulation potential. *Science of The Total Environment*, 802:149916, **2022**. Doi: 10.1016/j. scitotenv.2021.149916

[371] Klatte S., Schaefer HC., Hempel M. Pharmaceuticals in the environment – a short review on options to minimize the exposure of humans, animals and ecosystems. *Sustainable Chemistry and Pharmacy*, 5:61-66, **2017**. doi: 10.1016/j.scp.2016..07.001

[372] Tena M., Perez M., Solera R. Benefits in the valorization of sewage sludge and wine vinasse via a two-stage acidogenic-thermophilic and methanogenic-mesophilic system based on the circular economy concept. *Fuel*, 296:120654, **2021**. doi: 10.1016/j.fuel.2021.120654

[373] Elmi A., Al-Khaldy A., Alolayan M. Sewage sludge lad application: balancing act between agronomic benefits and environmental concerns. Journal of Cleaner Production, 250:19512, **2020**. doi: 10.1016/j.jclepro.2019.119512

[374] Woltersdorf L., Zimmermann M., Deffner J., Gerlach M., Leihr S. Benefits of an integrated water and nutrient reuse system for urban areas in semi-arid developing countries. *Resources, Conservations and Recycling*, 128:382-393, **2018**. doi: 10.1016/j.resconrec.2016.11.019

[375] Molinos-Senante M., Hernandez-Sancho F., Sala-Garrido R. Cost-benefit analysis of water-reuse projects for environmental purposes: a case study for Spanish wastewater treatment plants. *Journal of Environmental Management*, 92(12):3091-3097, **2011**. doi: 10.1016/j.jenvman.2011.07.023

[376] Hafeez MM., Bouman BAM., Giesen NV., Mushtaq S., Vlek P., Khan S. Water reuse and cost-benefit of pumping at different spatial levels in a rice irrigation system in UPRIIS, Philippines. *Physics and Chemistry of the Earth*, Parts A/B/C, 33(1-):115-126, **2008**. doi: 10.1016/j.pce.2007.04.011

[377] Pei Y., Tong Y., Li H., You J. In-situ biological effects, bioaccumulation, and multimedia distribution of organic contaminants in a shallow lake. *Journal of Hazardous Materials*, 427:128143, **2022**. Doi: 10.1016/j.hazmat.2021.128143

[378] Eysseric E., Gagnon C., Segura P.A. Uncovering transformation products of four organic contaminants of concern by photodegradation experiments and analysis of real samples from a local river. *Chemosphere*, 293:133408, **2022**. Doi: 10.1016/j.chemosphere.2021.133408

[379] Chao H., Sun M., Wu Y., Xia R., Yuan S., Hu F. Quantitative relationship between earthworms' sensitivity to organic pollutants and the contaminants' degradation in soil: a meta-analysis. *Journal of Hazardous Materials*, 429:128286, **2022**. Doi: 10.1016/j.jhazmat.2021.128286

[380] Buell MC., Drouillard K., Metcalfe CD. Ecotoxicological risks from dissolved organic contaminants in a contaminated bay: combining passive sampling with in vivo bioassays. *Journal of Great Lakes Research*, 47(5):1365-1375, **2021**. Doi: 10.1016/j.jglr.2021.07.001

[381] Tian Z., Wark DA., Bogue K., James CA. Suspect and non-target screening of contaminants of emerging concern I streams in agricultural watersheds. *Science of The Total Environment*, 795:148826, **2021**. Doi: 10.1016/j.scititenv.2021.148826

[382] Kovacs ED., Silaghi-Dumitrescu L., Kovacs MH., Roman C. Determination of the uptake of ibuprofen, ketoprofen, and diclofenac by tomatoes, radishes and lettuce by gas chromatography-mass spectrometry (GC-MS). *Analytical Letters*, 54(1-2):314-330, **2021.**

[383] McMullin RM., Chen R., Niu S., Matthews W., Murschell T., Wing SR., Hageman KJ. Organic contaminants in imported salmon feed and their effects on reef ecosystems in New Zealand. Environmemtal Pollution, 298:118853, **2022**.

[385] Xiao S., Gong Y., Li Z., Frantke P. Improving pesticide uptake modelling into potatoes: considering tuber growth dynamics. Journal of Agricultural and Fiid Chemistry, 69(12):3607-0616, 2021. Doi: 10.1021/acs.jafc.1c00151

[425] Mezzelani M., Nardi A., Bernardini A., Milan M., Peruzza L., d'Errico G., Fattorini D., Gorbi S., Patarnello T., Regoli F. Environmental pharmaceuticals and climate change: the case study of carbamazepine in M. galloprovincialis under ocean acidification scenario. *Environmental International*, 146:106269, **2021**. Doi: 10.1016/j.envint.2020.106269

[426] Svecova H., Grabic R., Grabicova K., Stanova AV., Fedorova G., Cerveny D., Turek J., Randak T., Brooks BW. De facto reuse at the watershed scale: seasonal changes, population contribution, instream flows and water quality hazards of human pharmaceuticals. *Environmental Pollution*, 268(Part A):115888, **2021**. Doi: 10.1016/j.envpol.2020.115888

[427] Tarpani RRZ., Alfonsin C., Hospido A., Azapagic A. Life cycle environmental impacts of sewage sludge treatment methods for resource recovery considering ecotoxicity of heavy metals and pharmaceuticals and personal care products. *Journal of Environmental Management*, 260:109643, **2020**. Doi: 10.1016/j.envman.2019.109643

[442] Santos JC., Lopes DRG., Silva LCF., Ramos LLJ., Dias RS., Lima SH., Sousa MP., Waldow VA., Paula SO., Ferreira SO., Silva CC. Characterization of the biofilm structure and microbial diversity of sulfate-reducing bacteria from petroleum produced water supplemented by different carbon sources. *Journal of Environmental Management*, 304:114189, **2022**. Doi: 10.1016/j.jenvman.2021.114189

[443] Cai Y., Yan Z., Ou Y., Peng B., Zhang L., Shao J., Lin Y., Zhang J. Effects of different carbon sources on the removal of ciprofloxacin and pollutants by activated sludge: Mechanism and biodegradation. *Journal of Environmental Sciences*, 111:240-248, **2022**. Doi: 10.1019/j.jes.2021.03.037

[444] Ratnasari A., Zaidi NS., Syafiuddin A., Boopathy R., Kueh ABH., Prasetyo DD. Prospective biodegradation of organic and nitrogenous pollutants from palm oil mill effluent by acidophilic bacteria and archaea. *Bioresource Technology Reports*, 15:100809, **2021**. Doi: 10.1019/j.biteb.2021.100809

[445] Mandal A., Dutta A., Das R., Mukherjes J. Role of intertidal microbial communities in carbon dioxide sequestration and pollutant removal: A review. *Marine Pollution Bulletin*, 170:112626, **2021**. Doi: 10.1019/j.marpolbul.2021.112626

[446] Aguillar-Romero I., Torre-Zuniga JD., Quesada JM., Haidour A., OÇonnell G., McAmmound BM., Hamme JDV., Romero E., Wittich RM., Dillewijn P. Effluent decontamination by the ibuprofen-mineralizing strain, Sphingopyxis granuli RW412: Metabolic processes. *Environmental Pollution*, 274:116536, **2021**. Doi: 10.1019/j.envpol.2021.116536

[447] Waraksa E., Wojtowicz-Zawadka M., Kwiatkowska D., Jaarek A., Malkowska A., Wrzesien R., Namiesnik J. Simultaneous determination of ibuprofen and its metabolites in complex equine urine matrices by GC-EI-MS in excretion study in view of doping control. *Journal of Pharmaceutical and Biomedical Analysis*, 152:279-288, **2018**. 10.1016/j.jpba.2018.02.004

[448] Waraksa E., Wozniak MK., Banaszkiewicz L., Klodzniska E., Ozimek M., Wrzesien R., Bobrowska-Korczak B., Namiesnik J. Quantification of unconjugated and total ibuprofen and its metabolites in equine urine samples by gas chromatography–tandem mass spectrometry: Application to the excretion study. *Microchemical Journal*, 150:104129, **2019**.

[449] Tsikas D., Kayacelebi AA., Hanff E., Mitschke A., Beckmann B., Tiilann HC., Gutzki FM., Muller M., Bernasconi C. GC–MS and GC–MS/MS measurement of ibuprofen in 10-μL aliquots of human plasma and mice serum using [α-methylo-2H3]ibuprofen after ethyl acetate extraction and pentafluorobenzyl bromide derivatization: Discovery of a collision energy-dependent H/D isotope effect and pharmacokinetic application to inhaled ibuprofen-arginine in mice. *Journal of Chromatography* B, 1043;158-166, *2017*. Doi: 10.1016/j.jchromb.2016.06.014

[450] Weigel S., Berger U., Jensen E., Kallenborn R., Thoresen H., Huhnerfuss H. Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromso/Norwaay with emphasis on ibuprofen and its metabolites. *Chemosphere*, 56(6):583-592, **2004**. Doi: 10.1016/j.chemosphere.2004.04.018